



University of  
Zurich<sup>UZH</sup>



CONGRESS CENTRE  
KURSAAL INTERLAKEN



# ICBIC-19



19<sup>th</sup> International  
Conference on  
Biological Inorganic Chemistry

# Book of Abstracts

Interlaken, Switzerland | August 11-16, 2019



Program Overview.....	5
Plenary Lectures.....	11
Keynote Lectures.....	23
Lifelong Achievements Talks.....	69
Invited Lectures.....	85
Oral Presentations.....	245
Young Researchers Presentations.....	337
Poster Presentations.....	369
Artificial Photosystems.....	370
Bioinformatics.....	380
Bioinorganic Chemistry for Nanotechnology.....	384
Bioinspired and Biomimetic Systems.....	387
Bioorganometallic Chemistry.....	429
Environmental and Geochemical Bioinorganic Chemistry.....	452
Metal Homeostasis and Detoxification.....	454
Metalloproteins.....	460
Metal-related Diseases.....	516
Metals in Medicine.....	522
Nucleic Acids.....	586
New Methods and Tools.....	605
Authors Index.....	613

ICBIC-19 runs from Sunday afternoon until Friday night, August 11-16, 2019, with close to 700 registered participants from 43 countries worldwide. The scientific program consists of 5 parallel and 2 poster sessions. 3 SBIC Awards, 10 Plenary, 44 Keynote, 15 Lifelong Achievement and 158 Invited Lectures as well as 89 Oral and 239 Poster Presentations will be given. In addition, 30 Young Researcher and selected Poster Flash Presentations are especially devoted to our young colleagues, the future of our BIC community. We are very much looking forward to five days of exciting science!

- Plenary Lecture
- Keynote Lecture
- Invited Lecture
- Oral Presentation
- Lifelong Achievements Talk
- Young Researcher Presentation
- Poster Flash Presentation
- Social Events

Sunday, August 11	
	Foyer Auditorium
14:00 - 17:30	Registration
	Auditorium
16:30 - 17:00	Opening Ceremony
17:00 - 17:50	Chen P SBIC Award 2017
17:50 - 18:05	Break
18:05 - 18:50	Becker
19:00 - 21:00	ICBIC-19 Welcome Reception

Monday, August 12					
Auditorium					
09:00 - 09:50	Walton (sponsored by RSC Advances)				
	Auditorium	Theatersaal	Kongress-Saal	Ballsaal	Brünig
10:00 - 10:30	Hirota	Schulzke	Hannon	Guo Z	
10:30 - 10:45	Ivancich	Kirk	Massoud	Starha	
10:45 - 11:10	Coffee Break				
11:10 - 11:30	Ikeda-Saito	Tegoni	Miyoshi	Gibson	
11:30 - 11:50	Furtmüller	Peacock	Bombard	Gambino	
11:50 - 12:10	Brzezinski	Pecoraro	Clever	Aldrich-Wright	
12:10 - 12:25	Pinakoulaki	Shengfa	Okamoto	Stephan	
Auditorium					
12:30 - 12:50	Poster Flash Presentations				
12:50 - 14:45	Lunch / Poster Session I (even numbers)				
	Auditorium	Theatersaal	Kongress-Saal	Ballsaal	Brünig
14:45 - 15:05	Kroneck	Kaim	Sigel H	Reedijk	Kozlowski
15:05 - 15:20	Bradley	Gotsbacher	Ruehl	Babak	Bellotti
15:20 - 15:35	Hofbauer	Schwarze	Schönrath	Yaourtis	Perinelli
15:35 - 15:50	Dudek	Henthorn	Amadei	Imberti	Gozzi
15:50 - 16:10	Bröring	Ivanovic-Bur.	Barcelo-Oliver	Jalilehvand	Carver
16:10 - 16:30	Wilks	Duan	Shao	Salifoglou	Yam
16:30 - 16:55	Coffee Break				
16:55 - 17:25	Hayashi	Berners-Price	Leimkühler	Ang	Banci
17:25 - 17:40	Morita	McDonald	Kjendseth	Zhu	Mann
17:40 - 18:00	Obinger	Limberg	Ward T	Che	Zhang Limei
Auditorium					
18:05 - 18:55	Vila				

Tuesday, August 13					
Auditorium					
09:00 - 09:50	Crans				
	Auditorium	Theatersaal	Kongress-Saal	Ballsaal	Brünig
10:00 - 10:30	Chang C	Bren	Herres-Paw.	Bonnet	Brunold
10:30 - 10:45	Wang H	Probst	de Franca	Liu J-G	Xia
10:45 - 11:10	Coffee Break				
11:10 - 11:30	Song W J	Agapie	Swart	Salassa	Seebeck
11:30 - 11:50	Wang J	Shafaat	Riordan	Sadler	Jameson Gu.
11:50 - 12:10	Makris	Fukuzumi	Karlin	Hambley	Krebs C
12:10 - 12:25	Maher	Mathieu	Schindler	Chen Y	Span
12:25 - 13:30	Lunch				
13:30 - 14:00	Einsle	Brudvig	Lu	Hureau	Fahrni
14:00 - 14:20	Hu Y	Sakai	Hu X	Bal	Butler A
14:20 - 14:40	Tatsumi	Chang M	Menage	Quintanar	Duhme-Klair
14:40 - 15:00	Ribbe	Hill	Borovik	Ghosh Dey	Codd
15:00 - 15:20	DeBeer	Wenger	Cho	Bertini	Schalk
15:20 - 15:35	Lebrette	Decroos	Otte	Tan	Zamocky
15:35 - 15:50	Bjornsson	Oohora	Adam	Price	Zhang Lin
15:50 - 16:15	Coffee Break				
16:15 - 16:30	Guo Y	Wei	Lee W-Z	Min	Kennedy
16:30 - 16:50	Daumann	Hess	Comba	Holland J	Outten C
16:50 - 17:10	Jameson Ge.	Wang B	Holland P	Hermann	Datta
17:10 - 17:30	Louro	Marinescu	Sosa-Torres	Alberto	Gumienna-K.
17:30 - 17:50	Giedroc	Luber	Peralta	Xing	Outten F
Auditorium					
17:55 - 18:45	Franz (sponsored by ACS Chemical Biology)				



# Program Overview

Wednesday, August 14					
	Auditorium	Theatersaal	Kongress-Saal	Ballsaal	Brünig
09:00 - 09:30	Rosenzweig	Meyer	Berger	Sun H	Shen J-R
09:30 - 09:45	Smith	André Cunha	Liang Hong	Aureliano	Kieninger
09:45 - 10:05	Splan	Auffinger	Murphy	Griffith	Kräutler
10:05 - 10:25	Green	Sen	Reithofer	Rodriguez R	Brasch
10:25 - 10:45	Haumann	Shionoya	Dyson	Meade	Schatzsch.
10:45 - 11:10	Coffee Break				
11:10 - 11:30	Cavazza	Stulz	Ott I	Sessler	Zelder
11:30 - 11:50	Shoji	Fonseca Guer.	Ronconi	Zobi	Mokhir
11:50 - 12:10	Moura J	Yamamoto	Messori	Policar	Ragsdale
	Free Afternoon				

# Program Overview



Thursday, August 15					
	Auditorium	Theatersaal	Kongress-Saal	Ballsaal	Brünig
09:00 - 09:50	Kepler				
	Auditorium	Theatersaal	Kongress-Saal	Ballsaal	Brünig
10:00 - 10:30	Armstrong	Küpper F	Andrade	Marmion	Högbom
10:30 - 10:45	Sugimoto	Miller	Horn	Sousa	Hersleth
10:45 - 11:10	Coffee Break				
11:10 - 11:30	Neese	Que L	Magyar	Barba-Behrens	Lee Y
11:30 - 11:50	Shaw	Majumdar	Barrios	Heffeter	Ruiz
11:50 - 12:10	O'Hagan	Harrop	Hsu	Da Costa Fer.	Biver
12:10 - 12:25	Magnuson	Majlesi	Dutta	Chen Z-F	Kubeil
	Auditorium				
12:30 - 12:50	Poster Flash Presentations				
12:50 - 14:45	Lunch / Poster Session II (odd numbers)				
	Auditorium	Theatersaal	Kongress-Saal	Ballsaal	Brünig
14:45 - 15:05	Crichton	Meyerstein	Lippert	Farkas	Yamauchi
15:05 - 15:20	Zuccarello	Ségaud	Zima	Frei	Németh
15:20 - 15:35	Ciano	Mondal	Chino	Biancalana	Abdiaziz
15:35 - 15:50	Trindade	Brenig	Cutsail	Chan	Bulos
15:50 - 16:10	Machonkin	Kim	Murthy	Mukherjee C	Dobbek
16:10 - 16:30	Berggren	Bjerrum	Telser	Mao Z	Fontecilla-Camps
16:30 - 16:55	Coffee Break				
16:55 - 17:25	Shima	Tang R	Liu Y	Donelly	Le Brun
17:25 - 17:45	Britt	Turano	Liang Hao	Nowak-Sliw.	Roessler
	Auditorium				
17.50 - 18.40	Lim / SBIC Award 2018				

## Friday, August 16

Friday, August 16					
Auditorium					
09:00 - 09:50	de Cola				
	Auditorium	Theatersaal	Kongress-Saal	Ballsaal	Brünig
10:00 - 10:30	Wong K-B	Kikuchi	Pikramenou	Hartinger	Petering
10:30 - 10:45	Tosha	Pluth	Szalai	Mukherjee A	Heffern
10:45 - 11:10	Coffee Break				
11:10 - 11:30	Ciurli	Hemmingsen	Eliseeva	Chao	Meloni
11:30 - 11:50	Hausinger	Schiemann	Petoud	Romero	Blindauer
11:50 - 12:10	Moura I	Li H	Zhang J-L	Butler S	Gibney
12:10 - 12:30	Varotsis	Harris	Que E	Allen	Yukl
12:30 - 13:30	Lunch / SBIC General Assembly				
13:30 - 13:50	Dawson	Sletten	Ward R	Brabec	Hagen
13:50 - 14:10	Walton	Dominguez-M.	Jiao	Ghosh	Küpper H
14:10 - 14:30	Raven	Hartwig	Stachura	Yang X	Robinson
14:30 - 14:50	Roelfes	Lönnsberg	Masuda	Suman	Michel
14:50 - 15:10	Rosato	Galindo	Stripp	Erleben	Krezel
15:10 - 15:30	Sligar	Thulstrup	Hasnain	Spingler	Dell'Acqua
15:30 - 15:55	Coffee Break				
15:55 - 16:25	Zamble	Müller	O'Halloran	DeRose	Faller
16:25 - 16:45	Rowinska-Zy.	Börner	Chacon	Morrow	Austin
Auditorium					
16:50 - 17:40	Dey A / SBIC Award 2019				
17:40 - 18:15	Closing				
19:30 - 20:00	Apéro				
20:00 - 02:00	Banquet / Disco				

## Plenary Lectures

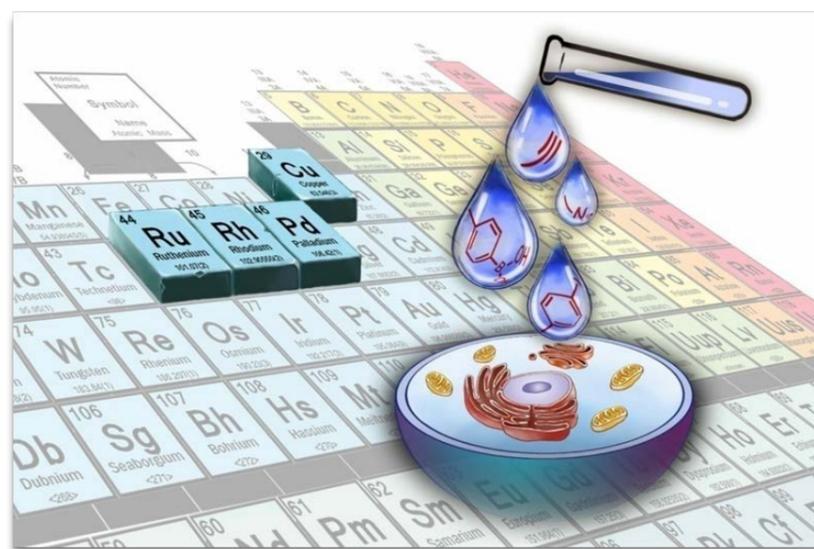
## PL-01

### Transition Metal-Triggered Protein Activation and Signaling in Living Systems

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Employing chemical compounds or reagents to modulate the function of an intracellular protein of interest, particularly in a gain-of-function fashion, remains highly desired but challenging. In this talk, I will first introduce a “genetically encoded chemical decaging” strategy that relies on our developed, transition metal-triggered bioorthogonal cleavage reactions to control protein activation in living systems with high spatial and/or temporal resolution. These reactions exhibited high efficiency and low toxicity, which allowed the gain-of-function study of various enzymes in living cells. These novel reactions were also explored as on-demand pro-drug activation strategies for cancer treatment. In the second part of the talk, I will discuss the signalling roles of transition metals in mediating bacterial transcription responses. We have recently discovered a novel copper signaling pathway that potentiated bacterial antibiotic tolerance through a major antibiotic resistance regulator-MarR in *E. coli*. In contrast to the well appreciated antimicrobial effects of copper from the host immune response, our work reveals that copper was also used by bacteria as a counteracting mechanism to cope with antibiotic stress. A novel zinc-triggered allosteric activation of a transcription factor will also be discussed at the end of the talk. Financial support by NSFC is gratefully acknowledged.



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## PL-02

### How to Inspire with Emotions

Paul-David Becker

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According to most neurologists, 80% of all decisions are made emotionally. In fact, 75% of all buying-decisions are made within the first three to five minutes of an initial contact. In this regard, it is quite astonishing that when we communicate or when we “sell” our ideas, emotions are heavily neglected.

In Paul-David Becker’s Plenary Lecture, you will discover the essence of these emotions. Moreover, you will learn about the significance of three neurological facts to boost your confidence in communicating effectively, to strengthen your non-verbal communication impact, and to help you better differentiate yourself from the rest by conveying more personal profile.

In a nutshell, you will experience the fundamentals of emotional communication, ultimately helping you to make a greater impact on others in the future.

## PL-03

### Gender Equality in Science: Why is it Taking so Long?

**Paul Walton<sup>1</sup>**

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Over nearly all scientific organisations, across every country and across time one finds that the progression of women in research/academia is significantly hindered when compared to men. Such a universal truth represents an enormous loss of talent, including in our very own bioinorganic community.

Recent years have seen some progress in understanding the principal factors behind this phenomenon and there has been some progress in new schemes which are designed to address the lack of women in senior scientific positions. These schemes have also met with some resistance which, in itself, has been revealing of the reasons why there is such a difference in the progression rates of men and women in science. This presentation discusses some of those resistances: why they arise; what they reveal about gender (in)equality in our universities, laboratories, groups; and—most importantly—what can be done about them.

## PL-04

### Metallo- $\beta$ -Lactamases: A Tug of War Between Bacteria and the Immune System for the Available Zn(II)

**Alejandro J. Vila<sup>1</sup>, Guillermo Bahr, Estefania Giannini, Gina Dotta, Antonela R. Palacios, Ma. Agustina Rossi, Juliana Delmonti, Carolina López, Pablo E. Tomatis and Lisandro J. González**

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Metallo- $\beta$ -lactamases (MBLs) are Zn(II)-dependent  $\beta$ -lactamases that constitute the latest resistance mechanism of pathogenic and opportunistic bacteria against carbapenems, considered as last resort drugs. Zn(II) binding is critical in the bacterial periplasm, not only to activate these enzymes and provide resistance, but also to stabilize the protein scaffold. During infection, the immune system elicits a response that scavenges the available Zn(II), impacting in the activity of stability of these proteins, thus compromising bacterial survival. However, the activity and stability of these proteins in vitro does not necessarily correlate with those in the periplasm. Thus, the whole picture must be described by means of an integrated approach.

We developed a strategy aimed to correlate the biochemical and biophysical features in purified enzymes with those in the bacterial periplasm, ultimately leading to the selected phenotype, i.e., resistance to antibiotics. This strategy allows us to dissect the molecular features that are tailored by accumulating mutations during evolution to endure the action of the immune system response. We have applied this approach to in vitro evolved protein in the laboratory, as well as to natural allelic variants selected in clinical strains. This has allowed us to account for the epistatic interactions between mutations at a structural level.

We have also studied the natural evolutionary landscape of allelic variants of a clinically relevant lactamase (NDM), that has been shaped by Zn(II) deprivation conditions. Thus, natural NDM variants with enhanced Zn(II) binding affinity have been selected, overriding the most common evolutionary pressure acting on catalytic efficiency. We also found that this enzyme is being disseminated by being secreted into Outer Membrane Vesicles, that represents an additional evolutionary advantage. Financial support from NIH, ANPCyT and CONICET is gratefully acknowledged.

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PL-05

## Biometal Enhancers of Viral Medicines

**Debbie C. Crans**<sup>1,2</sup>, **Heide A. Murakami**<sup>1</sup>, **Kateryna Kostenkova**<sup>1</sup>, **Anabel Bergeron**<sup>2,3</sup>, **Nouf Alluqmani**<sup>2,3</sup>, **Naveen Haribabu**<sup>3</sup>, **Mohammad Selman**<sup>2</sup>, **Rozanne Arulanandam**<sup>2</sup>, **Jean-Simon Diallo**<sup>2,3</sup>

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Oncolytic viruses are an emerging class of anticancer bio-therapeutics that induce antitumor immunity through selective replication in tumor cells (1). To improve the effects of oncolytic viruses we have introduced a biometal based strategy to boost their efficacy (2). Using immuno-modulating, small molecule protein tyrosine phosphatase inhibitors, specifically vanadium compounds (2,3,4), we are able to enhance the oncolytic virus infection and its anticancer effects in cell lines and animal tumor models. In the following presentation we will describe the results from our research programs on investigations of the mode of action of this enhancement by vanadium compounds, the nature of the vanadium compound exerting the virus-enhancing and anticancer effects, and the development of novel compounds catered to this application. Importantly, these studies are combined with chemical speciation studies underway to investigate the active species (4). How changes in the responses when the different form of vanadium compounds are tested are identified and used to understand the nature of the vanadium-induced activation of oncolytic virus effects. Furthermore, using complementary biochemical screening methods we are establishing a connectivity map pointing to key signaling nodes critical for viral infection and eradication of cancer cells.

Financial support from a Terry Fox Research Institute Program Project grant (to JSD) and by our respective Universities are gratefully acknowledged.

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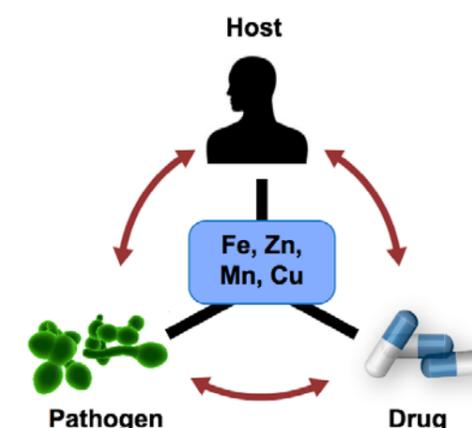
PL-06

## Infectiously Inorganic: A Metallocentric View of Antimicrobial Activity

**Katherine J. Franz**<sup>1</sup>

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Normal and pathogenic cells require a menu of metal nutrients for optimal growth, but also strategies to mitigate toxicity associated with misregulated or excessive levels of metals like Fe, Cu and Zn. Cells adjust metal homeostasis mechanisms depending on cell type, local growth conditions, and in response to stress. These situations present opportunities to manipulate cellular metals as a therapeutic strategy across a number of diseases. Here I will present a metallocentric view on utilizing small molecules and peptides that leverage unique metallobiology associated with bacterial and fungal infections to selectively inhibit growth of pathogenic microorganisms. More broadly, this approach is used to explore how cellular responses at the metallomic level affect and are affected by microbial susceptibility and adaptation to antimicrobial treatment.



Financial support by the National Institutes of Health (Grant GM084176) is gratefully acknowledged.

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PL-07

**Metallo drug Strategies in a Changing Environment of Anticancer Drug Development**

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<sup>3</sup>Institute of Cancer Research and Comprehensive Cancer Center Vienna, Medical University Vienna, Borschkegasse 8a, 1090, Vienna, Austria

The new era of cancer immunotherapy is about to strengthen the role of platinum-based cytotoxics rather than making them obsolete, due to synergies of platinum drugs with immune checkpoint inhibitors. Recent advances in the development of new anticancer platinum drugs are in considerable part based on tumor-targeting approaches. Based on a double-prodrug strategy, we have designed albumin-targeted platinum(IV) complexes, which exert strong activity in various tumor models *in vivo*. The prodrug binds rapidly and specifically to endogenous albumin in the bloodstream, which results in distinctly higher drug levels in the blood and tremendously increased plasma half-life. The prodrug then accumulates in tumor tissue, where platinum(II) species are continuously released by protein degradation and lysosomal reduction of platinum. *In vivo* experiments revealed highly increased efficacy compared to the corresponding (clinically used) free drug in terms of both tumor volume reduction and long-term survival.

The ruthenium complex IT-139 (KP1339) has been studied in a clinical phase I trial, where the compound could be safely administered up to a dose of 625 mg/m<sup>2</sup> to patients whose advanced solid tumors had progressed after failure of established therapies. Drug activity was observed in several tumor entities, most remarkably in gastro-intestinal neuroendocrine tumors [1]. The effects of IT-139 on selected cancer cell lines were analyzed by whole genome expression arrays, where sensitive cell lines showed alterations mainly in gene sets related to response to chemical stimuli and regulation of cell death, while less sensitive cells preferentially activated pathways controlling cell cycle, DNA repair, and metabolism [2]. The relevance of interference with ER homeostasis for the mode of action was substantiated [3], and cellular signals characteristic for the triggering of immunogenic cell death *in vivo* were observed in multicellular spheroid models *in vitro* [4]. A lack of common chemotherapy-related toxicities, unique mode of action and synergies with other anticancer drugs make IT-139 a proper candidate for combination therapies.

The orally bioavailable gallium complex AP-002 (KP46) combines direct anticancer effects with the inhibition of bone breakdown, making it particularly suitable for treatment of cancer metastatic to the bones. The drug causes Ca<sup>2+</sup>-triggered calpain activation and downregulation of focal adhesion proteins, followed by anoikis (cell death due to loss of integrin-mediated cell-matrix interactions), which may outpace induction of classic apoptosis [5]. In addition, damage of mitochondrial morphology and disruption of their functions followed by mitophagy (selective mitochondrial autophagy) has been observed *in vitro* [6]. Clinical evaluation of the drug is expected to resume soon.

**References**

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PL-08

**Bioinorganic Approaches to Study Multiple Facets in Alzheimer's Disease**

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Alzheimer's disease (AD), associated with degeneration of neurons and synapses in the brain, leads to motor impairment and eventual fatality. Neurodegeneration could be related to various interconnected features, including (i) plaque formation from amyloid- $\beta$  (A $\beta$ ) peptides, (ii) metal ion dyshomeostasis and miscompartmentalization, as well as (iii) inflammation and increased oxidative stress due to overproduction of reactive oxygen species (ROS). The inter-relations between some of these pathological factors have been investigated. Metals are found entangled in the A $\beta$  plaque and contribute to A $\beta$ -related toxicity and oxidative stress. ROS have been shown to increase the rate of A $\beta$  plaque formation. Our understanding of the correlation between these elements and AD pathogenesis has been very limited, however. There is currently no cure for AD; therapies are focused on symptomatic relief targeting the decrease in the levels of acetylcholine, only one of the multiple factors causing the disease [1-4]. To identify an effective cure for AD, we require a better understanding of the relationship between the various causative factors of this devastating disease. Towards this goal, we need suitable chemical tools capable of targeting and regulating its multiple underlying factors simultaneously [2-4]. Herein, the rational design and preparation of our chemical tactics will be discussed with detailed molecular-level investigations of their interactions and reactivities with targets *in vitro* as well as their efficacies *in vivo* [3-10].

Financial support by the National Research Foundation of Korea (NRF) grant funded by the Korean government and KAIST is gratefully acknowledged.

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## PL-09

### Self-Assembling Luminescent Complexes: from Understanding to Artificial Virus

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Luminescent molecules that can undergo self-assembly are of great interest for the development of new materials, sensors, biolabels.... The talk will illustrate some of the recent results on soft structures based on metal complexes able to aggregate in fibers, gels and soft mechanochromic materials [1]. The emission of the compounds can be tuned by an appropriate choice of the coordinated ligands as well as of their aggregation in different structures. The formation of soft assemblies allows the tuning of the emission color, by pressure and temperature leading to a new class of materials possessing reversible properties. The monitoring of the different emission properties, used as fingerprint for each of the assembled species, allowed an unprecedented real-time visualization of the evolving self-assemblies [2]. The assemblies can be employed as very sensitive labels for the detection of toxins and drugs [3]. Indeed even though sensing based on fluorescent and luminescent probes are commonly used, the use of aggregates in water allows to distinguish between analytes possessing very similar electronic properties. Sensing can also be done using electrochemiluminescence, ECL. We have recently achieved the first example of aggregation induced ECL showing that assemblies in solution and in the solid state (deposited on the electrode) can generate bright emission [4].

Finally I wish to close my talk showing novel capsules that can be realized using a unique approach to template virus proteins to reconstruct virus-like particles. We use luminescent Pt(II)-complex amphiphiles, able to form supramolecular structures in water solutions, that can act as templates of viruses capsid proteins. The platinum assemblies can have different morphologies and extremely high emission of which the color depends on the assembly. Interestingly we are able to change the size and shape of the particles even though we use the same natural proteins. The obtained virus-like particles can be visualized by their intense emission at room temperature, generated by the self-assembly of the Pt(II)-complexes inside the capsid [5].

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## PL-10

### Managing Protons and Electrons in Small Molecule Activation

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Small molecule activation often requires both protons and electrons. This includes chemical transformations key to sustainable energy and environment e.g. reduction of H<sup>+</sup>, O<sub>2</sub>, CO<sub>2</sub> and N<sub>2</sub>. Similarly, mono-oxygenation of organic molecules using molecular oxygen, a process often described as the Holy grail of chemistry, requires protons and electrons. Erstwhile mechanistic investigations on metallo-enzyme active sites which catalyses these reactions have revealed that the proton and electron delivery often occur in distinct chemical steps and in many cases, coupled, in the same steps. This talk to focus on control of proton and electron delivery in synthetic inorganic molecular catalysts to achieve efficient catalysis using a combination of synthesis, self-assembly, in-situ spectroscopy and electrochemistry. This includes catalysts for hydrogen generation, oxygen reduction, organic substrate oxidation using oxygen and CO<sub>2</sub> reduction; all under aqueous environment and ambient conditions.

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ICBIC-19

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## Keynote Lectures

## KN-01

### Oligomerization of Cytochrome *c*, Myoglobin, and Related Heme Proteins by 3D Domain Swapping

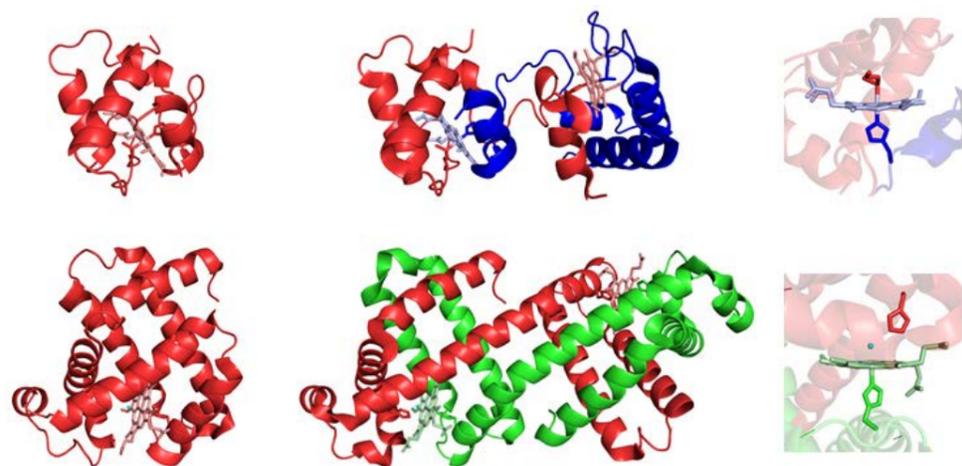
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Supramolecular chemistry is developing remarkably; for example, large metal–organic frameworks with molecular encapsulating properties are expected to be useful for applications such as gas storage, catalysis, and sensing. Heme proteins are responsible for various biological activities, including electron transfer, oxygen transport/storage, substrate oxidation, signal transduction, etc. Thus, oligomerization of heme proteins is useful for construction of new materials with cooperative and systematic functions, and diverse methods have been applied for construction of artificial heme protein oligomers.

3D domain swapping (herein, domain swapping) is a protein oligomerization phenomenon that exchanges the same domain or secondary structural element between molecules. Domain swapping was first reported in 1994 [1]; since then many proteins have been reported to domain swap. Although domain swapping has been observed in a variety of proteins, there had been only a limited number of reports on domain-swapped heme proteins until recently. Our research group has been showing that various heme proteins domain swap [2,3]. We also found that domain swapping of heme proteins occurs at the early stage of protein folding [4] and may occur *in vivo* [5]. We also utilized domain swapping to construct various heme protein assemblies, including nanorings, cages, hetero dimers with different active sites, and a ligand-binding reversible monomer–polymer system [6-9].

Financial support by JSPS is gratefully acknowledged.



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## KN-02

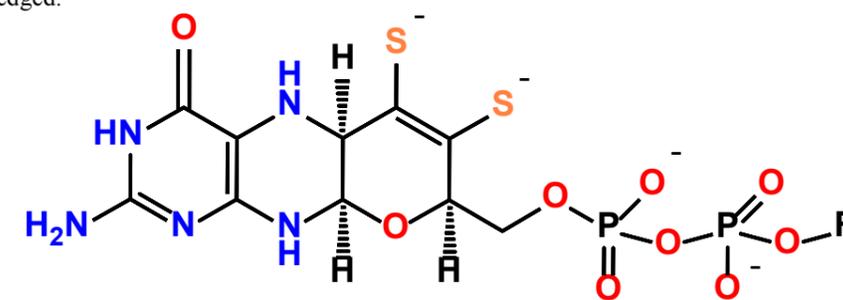
### The Molybdenum Cofactor and the Chemical Challenges it Represents

Carola Schulzke<sup>1</sup>, Christian Fischer<sup>1</sup>, Nicolas Chrysochos<sup>1</sup>, Ivan Trentin<sup>1</sup>, Benedict J. Elvers<sup>1</sup>

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Molybdenum or tungsten containing enzymes are important components of almost any known organism. Except for nitrogenase they typically catalyse the oxygen atom transfer as a two-electron redox process and are involved in the metabolisms of carbon, nitrogen and sulfur. Molybdopterin is a unique ligand in the active sites of these enzymes binding the metal by its dithiolene moiety (see chemical structure below). It was proposed that this ligand actively participates in electron transfer processes. The chemical synthesis of the molybdopterin ligand has proven to be extremely difficult and the possibly nearest model known today was published by Burgmayer and co-workers in 2012 and then investigated further.<sup>1-2</sup> In order to synthesize model ligands for MPT reliably, with good yield and with an acceptable amount of work, typically compounds bearing the coordinating dithiolene function and various different substituents on the double bond are considered to model MPT sufficiently well. Most known models, however, do not take into account the electronic influence MPT might have. In order to find a balance between do-ability and suitability of model ligands, new dithiolenes were developed taking into account several different aspects of the natural molybdopterin.<sup>3-5</sup> Particularly interesting examples comprise dithiolene ligands with unprotected pterin moieties, which have shown strong indications of specific binding to the apo-protein of TMAO reductase. In order to model cofactors of higher developed organisms another fundamental challenge is to synthesize mono-dithiolene molybdenum model complexes which are much more difficult to prepare than bis-dithiolene complexes. In this context we introduced a photosynthetic approach to this type of chemistry. The most promising synthesized structural model complexes were further investigated also as functional mimics in oxygen atom transfer reactions (OAT).

Generous financial support by the ERC (MocoModels) and the DFG (SCHU 1480/4-1) is gratefully acknowledged.



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## KN-03

### Metallo-Supramolecular DNA and RNA Recognition Combined with Nanoscience to Achieve Bio-Activity

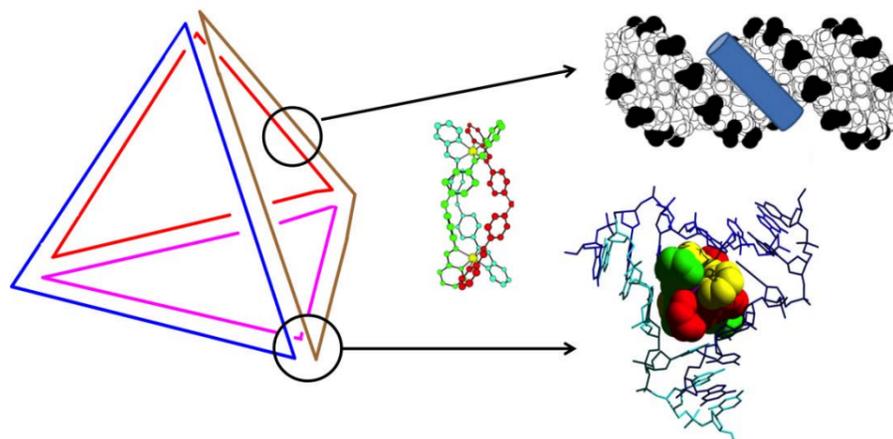
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We have developed a new class of metallo-supramolecular agents that bind strongly and preferentially to DNA and RNA Y-shaped junctions (3-way junctions; forks; bulges) in vitro and prevent DNA transactions. They are taken up readily into cells and rapidly localise in cell nuclei, where the fork-binding agents interfere with the processing of DNA leading to cell cycle arrest followed by apoptosis, without inducing genotoxicity or mutagenicity [1, 2].

Excitingly some agents show potent anti-viral activities through recognition of a specific RNA bulge motif in the HIV genome, and have agents with high anti-viral activity at concentration levels where the compounds are not cytotoxic to mammalian cells [3].

Beyond the genome, DNA can also be used to create interesting synthetic nanostructures. We now show that our cylinders can bind to these nanostructures, and the cylinders can modulate the nanostructure's shape. The cellular uptake and activity of these nanostructure-cylinder composites will be described [4].



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## KN-04

### Mitochondria Targeting Platinum Anticancer Complexes

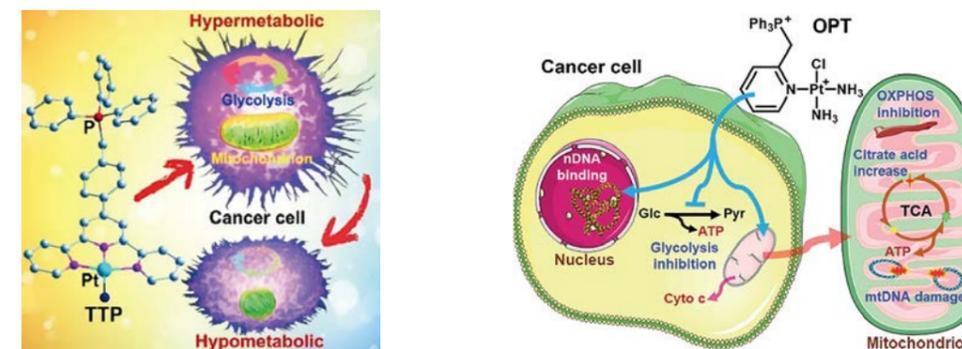
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Platinum-based antitumor drugs play an important role in the treatment of various malignancies such as colorectal and testicular cancers. However, drug resistance and side effects are challenging problems that hinder their wider clinical applications. Mitochondria play central roles in cellular energy conversion, metabolism and apoptosis. Its targeting provides potential alternative for drug design.

In the last few years, our lab has been focusing on the molecular design of platinum-based antitumor complexes with mitochondria targeting potentials. In this context, we have designed a series of triphenylphosphonium-modified monofunctional platinum(II) complex. One of the terpyridine complex (TTP) inhibits thioredoxin reductase and multiple metabolisms of cancer cells. It exhibited enhanced cytotoxicity against cisplatin-insensitive human ovarian cancer cells in a caspase-3-independent manner. In a separate example, we investigated the effect of triphenylphosphonium group on the activity of Pt(II)-pyridine complexes. The *ortho*-complex (OPT) exhibits higher efficacy than the *meta*- and *para*-complexes and cisplatin against A549 lung cancer cells. It also shows a strong inhibition towards the growth of non-small-cell lung cancer in nude mice. Similarly, we have also designed several Pt(IV) prodrugs with mitochondria targeting properties. The results indicate that in addition to DNA binding, bioenergetic pathways also play crucial roles in the antitumor activity of mitochondrion-targeted platinum complexes.

Financial support from the National Science Foundation of China and the Ministry of Science and Technology of China is gratefully acknowledged.



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## KN-05

### Supramolecular Hemoprotein Assemblies

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Supramolecular assemblies using proteins provide unique structures, thermodynamic behaviors, and functions due to reversible interactions between protein-based building blocks. In nature, there are many kinds of unique protein assemblies including rings, sheets, tubes and cages, which are formed by multiple interactions such as electrostatic interactions, hydrophobic contacts, coordination etc. These beautiful composites have encouraged us to generate new nanobiomaterials. Our group has focused on a specific interaction between heme and the heme pocket of a hemoprotein to drive construction of the supramolecular hemoprotein assemblies.

A series of familiar hemoproteins have a cofactor, protoporphyrin IX iron complex (heme *b*), which is bound in the heme pocket via Fe-axial ligand coordination and non-covalent interaction with high affinity ( $K_a = 10^{10} - 10^{15} \text{ M}^{-1}$ ). The cofactor is removable from the heme pocket under acidic conditions to yield a corresponding apoprotein. Furthermore, the incorporation of an artificial cofactor into the apoprotein is capable of providing the reconstituted protein. Over the last decade, we have focused on the heme-substitution with several porphyrinoid metal complexes to generate a biohybrid catalyst.<sup>1</sup> In addition, we have introduced a heme moiety onto the hemoprotein surface via a covalent linkage to construct a supramolecular protein assembly via interprotein heme-heme pocket interaction.<sup>2</sup>

Cytochrome *b*<sub>562</sub> is a small hemoprotein with a 4-helix bundle having no cysteine residue. First, we prepared a cytochrome *b*<sub>562</sub> H63C mutant where the His63 residue was replaced with a cysteine residue. Next, a modified heme containing a maleimide group at the terminal of one heme propionate side chain was linked to the cysteine residue on the protein surface via a covalent bond. After the removal of native heme from the modified protein, the heme-linked apoprotein allowed us to yield the fibrous hemoprotein polymer via the heme-heme pocket interaction. In addition, it is found that the heme-attachment at Cys80 in an N80C mutant provides more rigid and periodic supramolecular structure with heme-heme exciton coupling.<sup>3</sup> Furthermore, we have recently used a naturally occurring assembled hemoprotein, HTHP (hexameric tyrosine-coordinated hemoprotein), as a model of light-harvesting system. The reconstituted HTHP where heme is replaced with zinc protoporphyrin IX or zinc chlorine e6 is found to show the energy migration within the protein. Moreover, an HTHP cluster was also generated by supramolecular interaction.<sup>4</sup>

In this presentation, we will demonstrate two examples of hemoprotein assemblies as shown in Figure 1 and discuss these physicochemical properties.

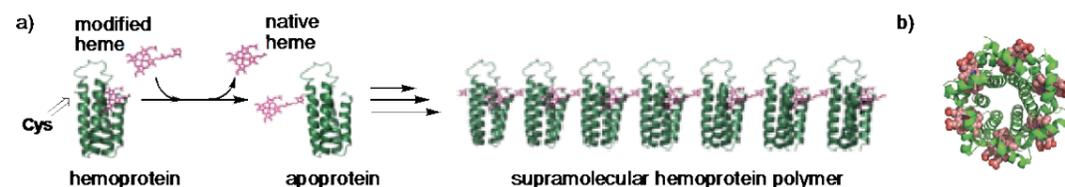


Figure 1. a) Assembly of modified cytochrome *b*<sub>562</sub>. b) Crystal structure of HTHP (2OYY)

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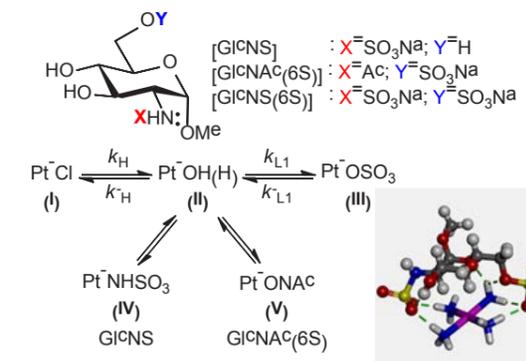
## KN-06

### Glycans as Ligands in Bioinorganic Chemistry

Susan J. Berners-Price<sup>1</sup>, Anil Kumar Gorle<sup>1</sup>, Mark von Itzstein<sup>1</sup>, Nicholas P. Farrell<sup>2,1</sup>

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Metallglycomics – the study of defined coordination compounds with oligosaccharides – opens new areas for bioinorganic chemistry expanding its study to the third major class of biomolecules after proteins and DNA/RNA [1]. One example is the recent demonstration that the strong binding of polynuclear platinum complexes (PPCs) to sulfated-oligosaccharides provides a new approach to glycan-based targeting, based on disruption of the heparan sulfate (HS)/heparanase interaction, through metalshielding of critical sulfate residues involved in recognition [2,3,4]. Understanding these interactions on long, high MW biomolecules requires understanding at the single unique mono/disaccharide level, just as early M-DNA chemistry was concentrated on single purine and pyrimidine chemistry to understand nucleobase/nucleoside/nucleotide preferences. In one approach, we have used [<sup>1</sup>H, <sup>15</sup>N] HSQC NMR to explore the aquation and subsequent covalent binding of the trinuclear clinical agent Triplatin with D-glucosamine residues containing varied *O*-sulfate and *N*-sulfate or *N*-acetyl substitutions, which represent monosaccharide fragments present within the repeating disaccharide sequences of cell surface HS [5]. Comparison of the reactions with monosulfated GlcNS and GlcNAc(6S) reveal the higher reactivity of Triplatin with the 2-*N*-sulfate compared to the 6-*O*-sulfate, but a more rapid liberation.



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## KN-07

### Exploring the Functional Versatility of the Molybdenum Cofactors Present in Molybdoenzymes

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Molybdoenzymes are widespread in all domains of life and catalyze key steps in carbon, sulfur and nitrogen metabolism. In the DMSO reductase family of molybdenum enzymes present only in prokaryotes, the molybdenum coordination sphere generally is composed of two dithiolene groups from two molybdopterin (MPT) guanine dinucleotide (MGD) molecules, one amino acid ligand from the protein backbone and a Mo=O group as sixth ligand. Moco biosynthesis in *E. coli* seems to be more complex than previously suggested, since a novel form of the cofactor has been identified recently in addition to modifications in the ligand sphere of the molybdenum atom. As novel Moco intermediate, bis-Mo-MPT has been revealed, a cofactor that is used by the YdhV protein. Additionally, in recent studies it became obvious that several molybdoenzymes harbor a sulfido-ligand instead of the oxo group at the bis-MGD cofactor. This modification was recently identified to be present in the *Escherichia coli* TMAO reductase TorA when purified under anaerobic conditions. The role of the sulfur ligand for the reaction mechanism of these enzymes is not clear so far. An overview of the large diversity of modifications at Moco in bacteria will be presented.

## KN-08

### Anticancer Platinum(IV) Prodrug Complexes and Their Activation Processes

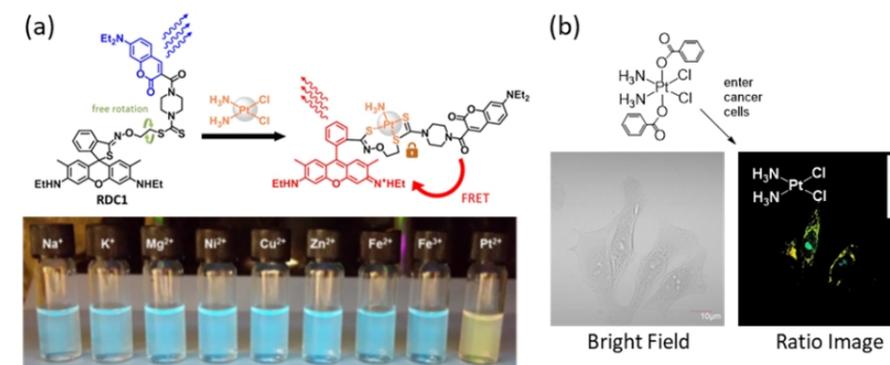
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Platinum(II)-based anticancer drugs, cisplatin, carboplatin and oxaliplatin, are some of the most effective chemotherapies used in clinic. Their cytotoxic activities against cancer cells stems from a combination of processes including cell entry, drug activation, DNA-binding and transcription inhibition, resulting in apoptotic cell death. Due to limitations in platinum-based therapy arising from toxicity, high side-effects and incidence of drug resistance, there have been a renewed interest in harnessing the versatility of platinum(IV) carboxylates as prodrug complexes of platinum(II) anticancer drugs [1]. The platinum(IV) prodrug complexes are engendered with improved aqueous stability and can be functionalised with targeting groups to enhance delivery, thereby overcoming some of the aforementioned limitations. I will be discussing a facile strategy of conjugating peptides to platinum(IV) prodrug complexes with the aim of achieving targeted delivery as well as their use as an adjuvant. In particular, I will also report a rationally-designed platinum(IV)-peptide prodrug conjugate that activates immune cells as immuno-chemotherapeutic agents for cancer therapy. I will also discuss our new efforts towards imaging the reduction of platinum(IV) prodrug complexes in vitro and examine the factors affecting intracellular activation of these compounds [2] (see Figure).

Financial support by the Ministry of Education, Singapore is gratefully acknowledged.



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## KN-09

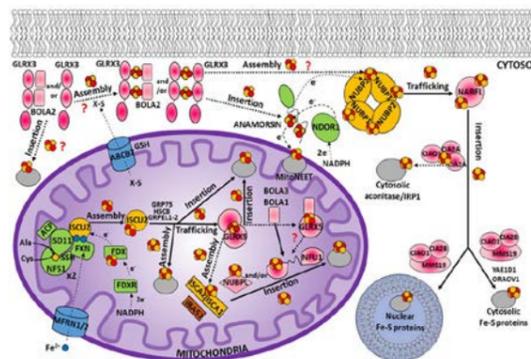
### Metal Trafficking in Cells Through a Cellular Structural Biology Approach

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The description and understanding of metal trafficking processes in the cell require the integration of several approaches, from bioinformatics and computation tools to experimental investigations. Specific bioinformatics tools for genome analysis will allow the identification of metal binding sequences. Metal binding proteins can be then experimentally characterized in terms of structural features, metal coordination properties, interactions with partners and metal transfer processes. On these aspects NMR spectroscopy is a unique tool not only for characterizing the structural and dynamical properties but also for analysing functional processes possibly in a cellular context. Metal transfer processes occur through a series of protein-protein transient interactions<sup>1</sup>. Metal transfer is determined by metal affinity gradients among the various proteins, with kinetic factors contributing to the selectivity of the processes<sup>2</sup>. The characterization of these processes and the understanding of the molecular pathways require their description both at system (e.g. a cell) and at molecular level, (e.g. atomic-resolution characterization of biomolecules). This approach calls for the development of suitable methodologies, capable of addressing multiple, specific, and sometimes non-conventional, aspects and amenable to characterize functional processes in living cells<sup>3</sup>. Specifically, in-cell NMR can provide the description of these processes within living cells<sup>4</sup>. Furthermore, the presence of paramagnetic centers, such as iron-sulfur clusters, which dramatically affects the NMR spectra, requires tailored experiments, possibly integrated with EPR spectra<sup>5-7</sup>.

A few pathways responsible for cellular copper trafficking and for the biogenesis of iron-sulfur proteins will be presented. Through an integrated approach, by increasing the complexity from single protein structures to protein complexes to the functional reaction steps, the processes are described in their cellular context within a molecular perspective.



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## KN-10

### Transition Metal Signaling: Bioinorganic Chemistry Beyond Active Sites

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Metals are essential for all forms of life, and the traditional view of this biological inorganic chemistry is that mobile fluxes of redox-innocent metals like sodium, potassium, and calcium are privileged as dynamic signals while redox-active transition metals like copper and iron must be buried and protected as static metabolic cofactors to prevent oxidative stress. We are advancing a new paradigm of transition metal signaling [1-2], using copper and iron as primary examples to show a broader metabolism/signaling continuum that can influence neural circuitry [3, 6] and metabolism [4,5], with rapid mobilization of exchangeable, labile metal pools and regulation of protein targets by reversible metal binding beyond active sites. This presentation will focus on our latest efforts to decipher new roles for metals in living systems, enabled by chemical technologies such as activity-based sensing.

Financial support by NIH is gratefully acknowledged.

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## KN-11

### Mini-Enzymes for Fuel Production and Small Molecule Activation

**Kara L. Bren<sup>1</sup>, Banu Kandemir<sup>1</sup>, Jennifer M. Le<sup>1</sup>, Jose Luis Alvarez-Hernandez<sup>1</sup>, Jesse R. Stroka<sup>1</sup>, Yixing Guo<sup>1</sup>, Saikat Chakraborty<sup>1</sup>, Emily Edwards<sup>1</sup>, Vincenzo Firpo<sup>2</sup>, Vincenzo Pavone<sup>2</sup>, Angela Lombardi<sup>2</sup>**

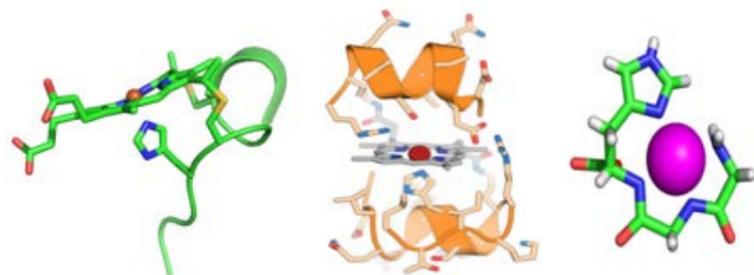
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In nature, energy storage and small-molecule activation reactions are catalyzed by enzymes that facilitate multi-electron, multi-proton reactions. Inspired by nature's catalysts, we are employing simple metalloproteins and metalloporphyrin-peptide assemblies as catalysts of energy-relevant reactions. In this talk, the catalytic activity of these engineered mini-enzymes toward the reduction of aqueous protons and nitrite will be described.

The first of these catalysts is acetylated cobalt-substituted microperoxidase-11 (CoMP11-Ac). CoMP11-Ac is an electrocatalyst for hydrogen production from water at neutral pH with high faradaic efficiency and low sensitivity to oxygen [1]. CoMP11-Ac shows remarkable stability through a range of pH values, and we take advantage of this stability to investigate the mechanism of hydrogen production as a function of pH. We demonstrate that solution pH and the choice of buffer influence the mechanism of the electrocatalytic reaction. A related catalyst is cobalt mimochrome VI\*a (CoMC6\*a), a small synthetic protein consisting of a cobalt deuteroporphyrin and two covalently attached peptides. One of these peptides provides an axial His ligand (proximal peptide) and the other covers the distal face of the porphyrin. The structure of CoMC6\*a can be altered by changing solution conditions, and we show that enhancing folding decreases the overpotential for hydrogen production [2]. Furthermore, through the use of a series of buffer species with varied structures and pK<sub>a</sub> values, we show that the pK<sub>a</sub> and the steric bulk of the buffer both impact the mechanism of the reaction. These results demonstrate that buffer species impact not only solution pH but also can play a direct role in determining reaction mechanism for electrocatalytic hydrogen evolution. Finally, we report results on cobalt complexes of peptides with the sequence XXH, models of the amino-terminal copper- and nickel-binding (ATCUN) motif [3]. The cobalt complex of GGH (CoGGH) catalyzes proton reduction electrocatalytically [4] and in the presence of a photosensitizer. Furthermore, CoGGH shows remarkable activity toward nitrite reduction, yielding ammonium as a major product [5]. Optimization of conditions for catalytic activity and ongoing studies of mechanism will be discussed.

Studies of CoMP11-Ac and CoMC6\*a are supported by the the Chemical Sciences, Geosciences and Biosciences Division, Office of Basic Energy Sciences, Office of Science, U.S. Department of Energy, Grant No. DE-FG02-09ER16121. Studies of CoGGH are supported by the NSF (CHE-1708256) and the University of Rochester.



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## KN-12

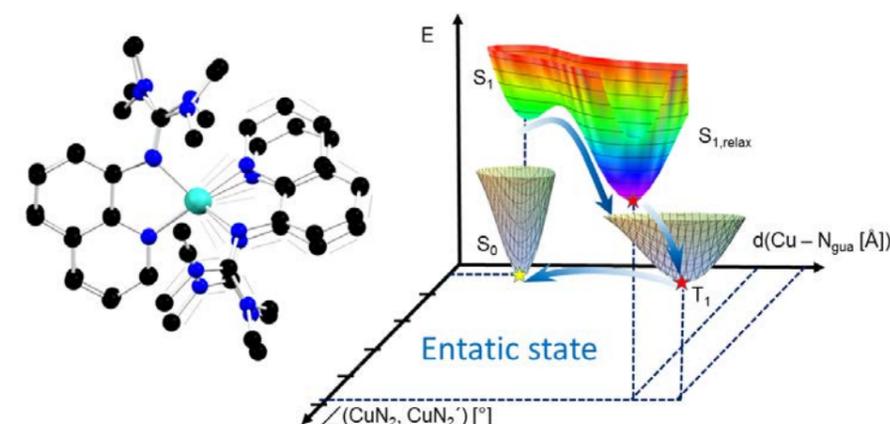
### Biometric Oxygen Transfer and Electron Transfer – Novel Models for Two Archetype Reactions

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Copper proteins mediate oxygen activation and transfer as well as electron transfer in very efficient ways – optimised by millions of years of evolution.[1] With chemical models, we try to harness their superior reactivity. Oxygen transfer is efficiently mediated by tyrosinases to convert phenols to quinones. Numerous model complexes have been reported but only few with catalytic ability.[2] For several years, we have studied bis(pyrazolyl)methanes[3] and guanidines[4] as ligands for tyrosinase models and found subtle ligand influences to be crucial for the catalytic reactivity.

Using guanidinoquinolines, we could model the entatic state found in blue copper proteins.[5] The resulting copper complexes are the fastest pure N-donor electron transfer models[6] and show also entatic behavior during photoexcitation.[7] Latest developments in both fields will be presented.



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## KN-13

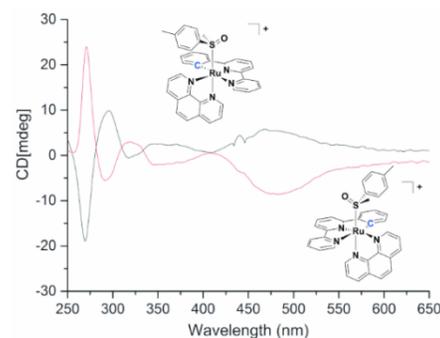
### Cyclometallated Anticancer Compounds in Phototherapy: Different Colors, Different Isomers

Sylvestre Bonnet<sup>1</sup>

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Metal-based compounds show improved light-absorption properties compared to many organic drugs. Still, in anticancer phototherapy shifting the absorption spectrum of light-activated metallodrugs into the photodynamic window (600-1000 nm), where light penetration is good, remains a challenge for inorganic photochemists. One strategy to design light-activated metal complexes that absorb at higher wavelengths is to introduce cyclometallation, i.e. polydentate ligands containing a phenyl group that, upon metallation, generate a metal-carbon bond. The resulting anionic carbon-based ligand strongly shifts the orbitals and energy levels of the metal complex, which in turn influences its photochemistry and phototherapeutic properties. In addition, changing a nitrogen-based ligand into a carbon-based ligand often generates a series of different isomers, the preparation of which can become challenging. In this presentation I will describe the synthesis, photochemistry, and photobiology, of a series of cyclometallated metal complexes based on palladium, ruthenium, and platinum, and discuss the opportunities and challenges generated by cyclometallated metal complexes for anticancer phototherapy.

Financial support by European Council, The Netherlands Organization of Scientific Research (NWO), and the Chinese Science Council, is gratefully acknowledged.



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## KN-14

### Structure/Function Relationships in Cysteine and Cysteamine Dioxygenases

Thomas C. Brunold<sup>1</sup>, Rebeca L. Fernandez<sup>1</sup>, Stephanie L. Dillon<sup>1</sup>, Brian G. Fox<sup>2</sup>

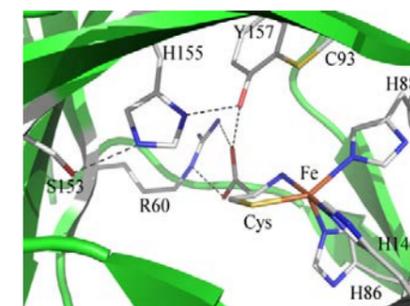
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Cysteine dioxygenase (CDO) and cysteamine dioxygenase (ADO), which are the only known mammalian thiol dioxygenases, play essential roles in the hypotaurine/taurine biosynthesis [1]. The malfunctioning of these enzymes has been implicated in several neurodegenerative diseases (e.g., Parkinson's, Alzheimer's, and motor neuron diseases), as well as autoimmune disorders. Crystal structures of resting and substrate-bound mammalian CDOs revealed two surprising structural motifs in the first and second coordination spheres of the Fe center (Figure below, prepared using PDB code 4JTO [1]). The first is the existence of a neutral three histidine (3-His) facial triad that coordinates the Fe ion, as opposed to an anionic 2-His-1-carboxylate facial triad that is typically observed in mononuclear non-heme Fe(II) dioxygenases. The second is the presence of a covalent crosslink between the sulfur of C93 and an ortho carbon of Y157 (mouse CDO numbering scheme). The exact role that these unusual structural features play in CDO catalysis remains unknown.

Compared to CDO, ADO is very poorly characterized. While ADO has not yet been structurally characterized by X-ray crystallography, a comparison of mammalian ADO sequences with those of homologs present in other eukaryotes revealed that the 3-His facial triad that coordinates the Fe ion in CDO is likely preserved in ADOs [1]. Additionally, a recent study provided evidence for the existence of a similar cysteine-tyrosine crosslink in ADO as the C93-Y157 crosslink in CDO [3]. To explore the structure/function relationships for CDO and better characterize the ADO active site, we have utilized a combination of spectroscopic and computational tools. Importantly, we have successfully performed the first spectroscopic characterization of the ADO active site in the presence of substrate (analogues). Collectively, our results provide significant new insight into the origin of the striking substrate selectivity displayed by CDO and ADO that allows organisms to independently regulate cysteine and cysteamine levels *in vivo*.

Financial support of this research by the NIH (grant R01GM117120 to T.C.B.) is gratefully acknowledged.



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## KN-15

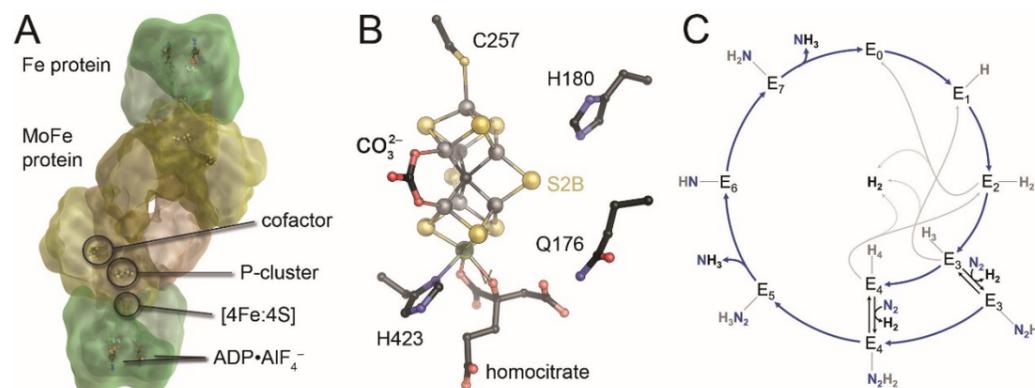
### Biological Nitrogen Fixation at a Dinuclear Active Site in Nitrogenase

Daniel Sippel<sup>1</sup>, Michael Rohde<sup>1</sup>, Christian Trncik<sup>1</sup>, Katharina Grunau<sup>1</sup>, Jakob Gies<sup>1</sup>, Florian Schneider<sup>1</sup>, Ivana Djurdjevic<sup>1</sup>, Susana L.A. Andrade<sup>1</sup>, Oliver Einsle<sup>1</sup>

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In biological nitrogen fixation, chemically inert N<sub>2</sub> gas that amounts for almost 80% of Earth's atmosphere is activated and reduced at ambient conditions on a unique multimetal cofactor, using low-potential electrons from central metabolism and the hydrolysis of ATP as a driving force. The reaction is catalyzed by the enzyme nitrogenase (Fig. 1A), a two-component metalloprotein of which three classes are known, employing either molybdenum, vanadium (Fig. 1B) or only iron at its active site. Nitrogenases catalyze the reduction of N<sub>2</sub> to 2 NH<sub>3</sub>, with a concomitant release of H<sub>2</sub> that occurs at a stoichiometry of at least 1 H<sub>2</sub> per N<sub>2</sub> reduced. In the enzyme, the transfer of four electrons to the active site cofactor is a prerequisite for the binding and activation of N<sub>2</sub>. While a kinetic model by Thorneley and Lowe already described these features in the 1980s (Fig. 1C), it only recently was understood to be due to a reductive elimination of H<sub>2</sub> that leaves the enzyme in a super-reduced state able to break the N<sub>2</sub> triple bond. After the recent reporting of the first ligand-bound structure of nitrogenases, a CO-inhibited complex of the Mo-dependent enzyme, we have now characterized a reaction intermediate bound to the vanadium-containing variant. These data reveal the actual reactive center to be a dinuclear iron site on a highly reduced cluster, providing direct clues for the mechanism of dinitrogen reduction.

Financial support by Deutsche Forschungsgemeinschaft and the European Research Council is gratefully acknowledged.



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## KN-16

### Water Oxidation Chemistry of Photosystem II and Artificial Systems

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Photosystem II (PSII) catalyzes the sunlight-driven oxidation of water during photosynthesis, supplying nearly all the O<sub>2</sub> in our biosphere. In this reaction, Nature has solved the difficult chemical problem of efficient four-electron photochemical oxidation of water to yield oxygen without significant side reactions. It is important to understand the mechanism of the water-oxidation reaction in order to use Nature's solution for the design of materials that split water for solar fuel production. The X-ray crystal structures of cyanobacterial PSII provide information on the structure of the cluster of four Mn and one Ca ions, the redox-active tyrosine called tyrosine-Z, chloride and the surrounding amino acids that comprise the oxygen-evolving complex (OEC). The structure of the OEC and the mechanism of the water-oxidation reaction of PSII will be discussed in the light of the X-ray crystallographic information, biophysical and computational studies of native, site-directed mutated and inhibitor-bound PSII, inorganic chemistry and kinetic isotope effect data. Insights from studies of the natural photosynthetic system are being applied to develop bioinspired materials for photochemical water oxidation and solar fuel production.

## KN-17

### Designing Heteronuclear Metalloenzymes Involved in Multi-Electron Redox Processes: Roles of the Secondary Coordination Spheres for High Enzymatic Activity and Low Overpotentials

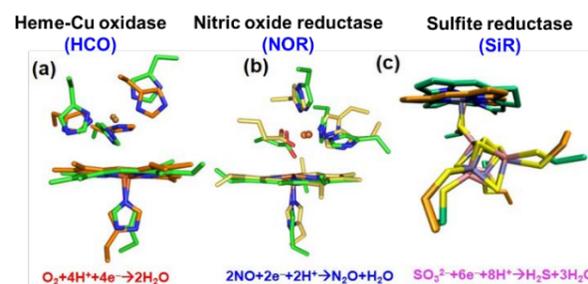
Yi Lu<sup>1,2,3</sup>, Ambika Bhagi-Damodaran<sup>1</sup>, Evan N. Mirts,<sup>2</sup> Chang Cui<sup>1</sup>, Sudharsan Dwaraknath,<sup>1</sup> Avery C. Vilbert<sup>3</sup>

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One of the most exciting developments in bioinorganic chemistry is the discovery of many enzymes containing heteronuclear metal centers that catalyze multi-electron and multi-proton reactions crucial for important functions such as respiration and global nitrogen/carbon/sulfur cycles. Despite their biological importance, most of these enzymes are less well-understood in comparison with homonuclear metalloenzymes, in part due to the inherent complexity from the presence of different metal ions next to each other. Specifically, we seek to investigate why a protein containing a heme-non-heme iron center (as in nitric oxide reductase (NOR)) is effective at 2e<sup>-</sup> reduction of NO, allowing N-N bond formation, whereas a protein containing a heme-copper center (as in heme-copper oxidase (HCO)) is proficient at 4e<sup>-</sup> reduction of O<sub>2</sub>, enabling O-O bond cleavage, yet a protein containing a heme-Fe<sub>4</sub>S<sub>4</sub> center (as in assimilatory sulfite and nitrite reductases (SiR and NiR)) is efficient at 6e<sup>-</sup> reduction of sulfite or nitrite, promoting S-O or N-O bond cleavage, respectively. To achieve this goal, we are designing artificial metalloenzymes that uses stable, easy-to-produce, and well-characterized heme proteins, such as myoglobin and cytochrome c peroxidase, as scaffolds for making structural and functional models of HCO, NOR, SiR and NiR.<sup>1</sup> Using these models, we are elucidating the roles of heme redox potential, electron transfer rates, and hydrogen bonding in conferring the different enzymatic activities, as well as structural features responsible for fine-tuning the specific and cross reactivities between these enzymes. As a result, we have identified several non-covalent interactions in the secondary coordination sphere, such as the presence of Tyr and water, and associated hydrogen bonding networks, in playing key roles in the activities and in tuning the reduction potentials of metal centers with minimal effect on the activities.<sup>1,2</sup> We have also introduced unnatural amino acids and non-native cofactors into the active sites of the models to address issues such as the roles of conserved Tyr and heme redox potential in the enzymatic activities. Financial support by the U.S. National Science Foundation (CHE 17-10241), National Institute of Health (GM06211), and Department of Energy's Center for Advanced Bioenergy and Bioproducts Innovation (DE-SC0018420) is gratefully acknowledged. The research described in this presentation is also part of the Chemical Transformations Initiative at Pacific Northwest National Laboratory (PNNL). It was conducted under the Laboratory Directed Research and Development Program at PNNL, a multiprogram national laboratory operated by Battelle for the U.S. Department of Energy.



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## KN-18

### POMs to Counteract the Effect of Cu(II) – Aβ Interaction in the Context of Alzheimer's Disease

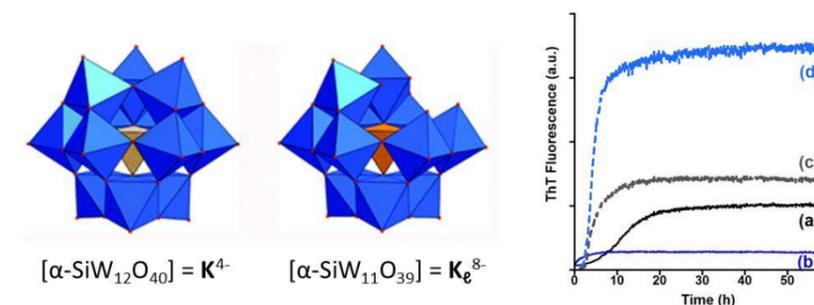
Christelle Hureau<sup>1</sup>, Sébastien Blanchard,<sup>2</sup> Xudong Lin<sup>1</sup>, Elena Atrian-Blasco<sup>1</sup>, Béatrice Mestre-Voegtlé<sup>1</sup>

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Copper ions may play a role in Alzheimer's Disease (AD), because they can modulate the aggregation of the amyloid-β (Aβ) peptides and can catalyze the formation of Reactive Oxygen Species, two events closely related to the aetiology of pathology [1,2]. Hence one therapeutic approach relies on the use of Cu(II) ligands to remove the ion from its interaction with Aβ and lessen associated deleterious effects (ROS and possible stabilization of oligomers regarded as the most toxic species present during the aggregation of the peptide).[2-4] Polyoxometalate (POM) are polyanionic oxoclusters of early transition metal ions which have found applications in various fields from catalysis to material science [5-7]. Controlled basic degradation of POMs can lead to lacunary POMs, which are very efficient all-inorganic ligands [8], making them interesting candidates for chelating Cu(II) out of Aβ. In addition, POMs can modulate the aggregation of amyloidogenic peptides, including Aβ [9] due to electrostatic interactions with positively charged amino-acid residues.

The double ability of two Keggin-type POMs, K<sub>4</sub>[α-SiW<sub>12</sub>O<sub>40</sub>] (K<sub>4</sub>α[K]·10H<sub>2</sub>O) and its monolacunary derivative K<sub>8</sub>[α-SiW<sub>11</sub>O<sub>39</sub>] (K<sub>8</sub>α[K<sub>l</sub>]·18H<sub>2</sub>O) on Cu-induced aggregation of Aβ (see figure below) and ROS production will be shown. In addition, the influence of metal-substituted counterparts, K<sub>M</sub> (M = d-block ions) on the apo- Aβ aggregation will be briefly commented on.



Financial support by the ERC StG-638712 aLzINK is gratefully acknowledged.

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## KN-19

### Probing the Dynamics of Cellular Copper in the Subfemtomolar Regime

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The uptake and efflux of cellular copper occur with surprisingly rapid kinetics, suggesting that cells maintain a labile copper pool that can rapidly exchange with the extracellular medium. At the same time, intracellular copper levels are tightly controlled by an intricate network of transport and storage proteins, which bind copper with subfemtomolar dissociation constants [1]. To explore the dynamics of this kinetically labile yet thermodynamically tightly buffered pool, we developed a suite of ligands and probes that selectively bind to monovalent Cu(I), the prevalent oxidation state of copper within the reducing cellular environment. Built upon phosphine sulfide stabilized phosphine (PSP) donor motifs, the ligands are remarkably resistant towards oxidation and exhibit exceptional Cu(I) affinities with femto- to zeptomolar dissociation constants and a  $10^{17}$ -fold selectivity over other biologically relevant transition metal ions such as Zn(II), Fe(II), or Mn(II) [2]. Biological studies with the high-affinity ligand PSP-2 revealed rapid cellular uptake and demonstrated effective suppression of copper-dependent processes such as the copper-induced trafficking of the Menkes protein (ATP7A) or long-term potentiation of neurons in mouse hippocampal brain tissue. Integration of the PSP ligand motif into a donor-acceptor-substituted fluorophore architecture yielded an emission-ratiometric fluorescent probe, crisp-17, with a low attomolar Cu(I)-dissociation constant [3]. Two-photon excitation microscopy with crisp-17 in live mouse fibroblasts revealed rapid changes in cellular Cu(I) availability upon incubation with the membrane-permeant bis(thiosemicarbazone)Cu(II) complex CuGTSM or the thiol-selective oxidant 2,2'-dithiodipyridine (DTDP). Regardless whether cells were grown in basal or copper-supplemented medium, the probe assumed only a low fractional saturation despite substantial differences in total copper, thus indicating tight buffering at low attomolar levels, likely involving a polydisperse buffer dominated by thiol-containing ligands.

Financial support by the National Institutes of Health (GM67169) is gratefully acknowledged.

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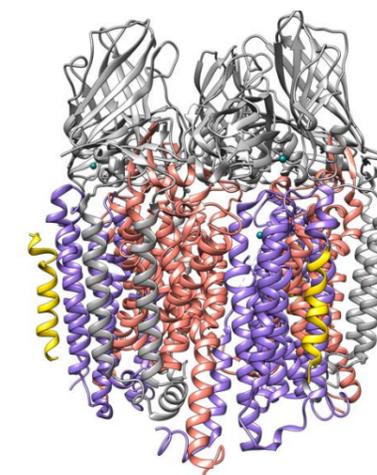
## KN-20

### The Copper Centers of Particulate Methane Monooxygenase

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Methanotrophic bacteria oxidize methane to methanol in the first step of their metabolic pathway. Whereas current catalysts that can selectively activate the 105 kcal mol<sup>-1</sup> C-H bond in methane require high temperatures and pressures, methanotrophs perform this chemistry under ambient conditions using methane monooxygenase (MMO) enzymes. In most methanotrophs, this chemically challenging reaction is catalyzed by particulate methane monooxygenase (pMMO), a copper-dependent, integral membrane enzyme. pMMO is composed of three subunits, PmoA, PmoB, and PmoC, arranged in a trimeric complex [1]. Despite extensive research and the availability of multiple crystal structures, the location and nature of the pMMO copper active site remain controversial. Studies are further complicated by issues with retaining enzymatic activity upon detergent solubilization and purification. Reconstitution of pMMO into bicelles, which mimic the membrane environment, recovers methane oxidation activity, indicating that activity loss is not caused by the removal of catalytic copper ions [2]. Electron paramagnetic resonance (EPR) spectroscopic data acquired on cells of *Methylococcus capsulatus* (Bath) show that the same copper centers are present in vivo and in the isolated pMMO. Moreover, biochemical and advanced EPR characterization is most consistent with the presence of two monocopper sites in pMMO, one in the soluble region of the PmoB subunit and one in the membrane-bound PmoC subunit [3]. Native top-down mass spectrometry (nTDMS) analysis of pMMO in nanodiscs also indicates that PmoB and PmoC each contain a single copper ion [4]. Taken together, these studies provide new insight into the nature and location of the active site.



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## KN-21

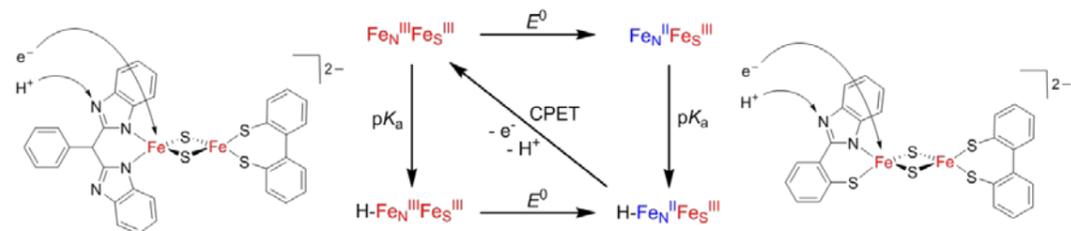
### Biomimetic [2Fe-2S] Clusters with His-type Alternative Ligands: Proton Coupled Electron Transfer, Ligand Rearrangements and Cluster Nitrosylation

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An increasing number of Fe/S clusters with protein-based ligands other than cysteine have been identified, the most common of the so-called alternative cluster ligands being histidine [1]. Non-cysteine ligands are now assumed to play key roles for Fe/S cluster function and reactivity. This is exemplified by the well-known Rieske centers in which His ligands enable proton coupled electron transfer, leading to unusually positive and pH-dependent redox-potentials.

While synthetic analogues with homoleptically coordinated [2Fe-2S] cores are well established in bioinorganic chemistry [2], full characterization of their reduced forms as well as high fidelity synthetic analogues that emulate heteroleptic {N<sub>x</sub>S<sub>y</sub>} coordination as found in the Rieske proteins have been reported only recently [3,4]. In this lecture the effect of protonation on redox potentials, electronic structures and spectroscopic features of the [2Fe-2S] core will be presented, and details of the proton-assisted electron transfer mechanism for first high fidelity synthetic analogues of Rieske centers (see Figure left), the mitochondrial mitoNEET cluster (see Figure right) and related systems will be discussed. The effect of [2Fe-2S] cluster redox and protonation states on ligand rearrangements [5] and on cluster nitrosylation to give dinitrosyl iron complexes (DNICs) [6] will also be addressed.



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## KN-22

### Metal Drugs and the Anticancer Immune Response

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Currently, immunotherapy with checkpoint inhibitor antibodies is revolutionizing clinical oncology even allowing cure of highly aggressive cancer types like melanoma and lung cancer. However, response to these immunotherapies is restricted to patient subgroups and currently conclusive predictive biomarkers are not available. Classically, anticancer metal drugs are considered to target predominantly nucleic acids, hence killing cancer cells by inducing genomic damage and apoptotic cell death. However, during the last years it became clear that metal drugs are not pure cytotoxic agents, but might also strongly interact with the fidelity of anticancer immune responses. Central underlying mechanisms include upregulation of cancer cell immunogenicity or depletion of regulatory immune cell compartments<sup>1</sup>. As one example, we have found that an intraperitoneal colon cancer model can be cured when combining oxaliplatin with bacterial ghosts as adjuvants<sup>2</sup>. Bacterial ghosts are empty envelopes of gram-negative bacteria with a distinct immune-stimulatory potential. In contrast, oxaliplatin alone only retarded tumor growth. Interestingly, animals cured by this immunochemotherapy approach were vaccinated against the original cancer cells making regrowth of the tumor graft impossible. As this vaccination effect was entirely depending on the presence of activated T cells, induction of an immunogenic cell death by oxaliplatin supported by innate immune activation via the adjuvant can be anticipated. This hypothesis was proven by induction of endoplasmic reticulum (ER) stress, calreticulin cell surface exposure, as well as HMGB1 and ATP release by the combination-treated cancer cells. A platinum(IV) prodrug of oxaliplatin targeted for tumor-specific activation based on albumin binding was able to cure CT26 murine colon cancer even without additional adjuvant in immunocompetent but not severe combined immunodeficient (SCID) mice<sup>3</sup>. Comparable observations regarding the upregulation of danger-associated pattern release were also made for the anticancer ruthenium compound KP1339. Accordingly, massive induction of ER stress and caspase 8-mediated apoptosis were detected in KP1339-hypersensitive human colon cancer cells<sup>4</sup>. This anticancer activity was mediated at least in part by KP1339 interference with the central protein chaperon GRP78 in the ER. These preclinical data will be discussed in the light of recent clinical approvals of platinum-based chemotherapy with immune checkpoint inhibitors as first line therapy of lung cancer.

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## KN-23

### Unveiling the Mechanism of Action of Bismuth Drugs by an Integrative Metal-ionic Approach: New Medicinal Applications Beyond *Helicobacter Pylori* Infection

Hongzhe Sun<sup>1</sup>, Hongyan Li<sup>1</sup>, Runming Wang<sup>1,2</sup>, Yuchuan Wang<sup>1</sup>, Pak-Leung Ho<sup>2</sup>, Richard Yi-Tsun Kao<sup>2</sup>,

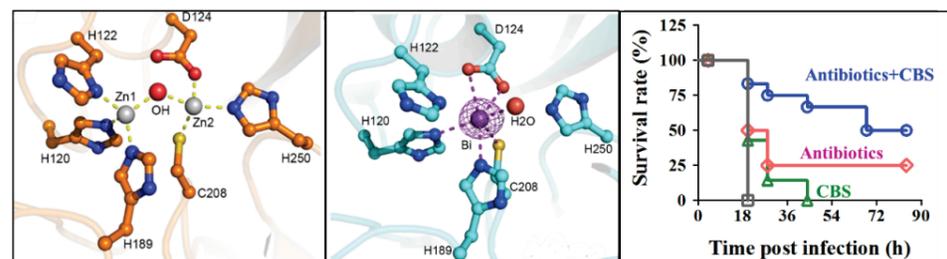
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Metallodrugs have been widely used as either diagnostic or therapeutic agents. It is crucial to understand their mechanisms of action to advance drug design. To achieve this, an integrative approach is required due to the complexity of metal-biomolecule interactions. Bismuth drugs in combination with antibiotics have been used in clinic for decades for the treatment of *Helicobacter pylori* (*H. pylori*) infection including antibiotic resistance strains. We developed a system pharmacology and metalloproteomics approaches consisting of continuous-flow gel electrophoresis and inductively coupled plasma mass spectrometry, LA-ICP-MS, IMAC and fluorescence to identify metal-associated proteins in cells using bismuth drugs as an example (1-3).

The knowledge acquired by these studies enables UreG to be discovered as a new target for the development of urease (4). We have also found that Bi(III) selectively interfere with Zn(II) biochemistry in pathogens (5). Infections caused by metallo- $\beta$ -lactamases (MBLs), e.g., New Delhi metallo- $\beta$ -lactamase 1 (NDM-1) producing bacteria are extremely difficult to treat (4). We show that colloidal bismuth subcitrate (CBS), and related Bi(III) compounds irreversibly inhibit different types of MBLs via the metal displacement mechanism with one Bi(III) displacing two Zn(II) ions. CBS restores meropenem (MER) efficacy against MBL-positive bacteria in vitro, and in animal infection models (6). Therefore, bismuth drugs could be repurposed together with clinically used antibiotics as co-therapy to cope with current antimicrobial resistance crisis.

We anticipate that the methodologies described are generally applicable for understanding the (patho)physiological roles of metals/metalldrugs. Our mechanism-guided discovery of new druggable targets as well as new medicinal applications of bismuth drugs may eventually lead to the development of new effective therapeutics.

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Active site of intact (dinuclear Zn(II), *Left*) and Bi(III)-bound form NDM-1 (*Middle*). Combination of Bi(III) with antibiotics exhibits much better survival rate in mice than Bi(III) and antibiotics respectively (*Right*).

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## KN-24

### Mechanism of Photosynthetic Water Oxidation

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Photosynthetic water oxidation is catalyzed by a Mn<sub>4</sub>CaO<sub>5</sub>-cluster embedded in the protein matrix of Photosystem II (PSII). The water oxidation proceeds through four sequential steps via the S<sub>i</sub>-state cycle (S<sub>i</sub>, i = 0-4). We have solved the structure of the Mn<sub>4</sub>CaO<sub>5</sub>-cluster by both synchrotron radiation X-rays [1] and femtosecond X-ray free electron lasers (XFEL) [2] at atomic resolutions. These studies revealed a “distorted” chair form of the catalytic center and detailed arrangement of each atom, inter-atomic distances within the Mn<sub>4</sub>CaO<sub>5</sub>-cluster, in its dark-stable S<sub>1</sub>-state. In order to fully uncover the reaction mechanism of water oxidation, it is necessary to solve the structures of the catalyst in its intermediate S-states. To this end, we used a pump-probe approach with a combination of “small” PSII crystals and serial femtosecond X-ray crystallography (SFX) using the femtosecond XFELs, to solve the structures of the intermediate S-states. We have reported the structure of 2-flashes induced S<sub>3</sub>-state [3] in which, a new oxygen designated O<sub>6</sub>, was found to be inserted in a position close to O<sub>5</sub>, a unique oxo-bridged oxygen already present in the S<sub>1</sub>-state, resulting a Mn<sub>4</sub>CaO<sub>6</sub>-cluster in the S<sub>3</sub>-state. Our results suggested the possible formation of O=O bond between O<sub>5</sub> and O<sub>6</sub>. Due to the limited resolution, however, there are still uncertainties regarding the distance between O<sub>5</sub> and O<sub>6</sub>, and thus the exact species of O<sub>5</sub>-O<sub>6</sub> and the mechanism of O=O bond formation were still unclear. We have improved the resolution of the intermediate S<sub>3</sub>-state structure, and also solved the 1-flash induced S<sub>2</sub>-state structure. Based on these results, the molecular mechanism for O=O bond formation has now become clear.

Acknowledgments:

I thank all of the collaborators who are involved in the work presented in this talk but whose names are not listed here due to the limited space.

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## KN-25

### Enzyme Catalysis in Electrified Nanospace

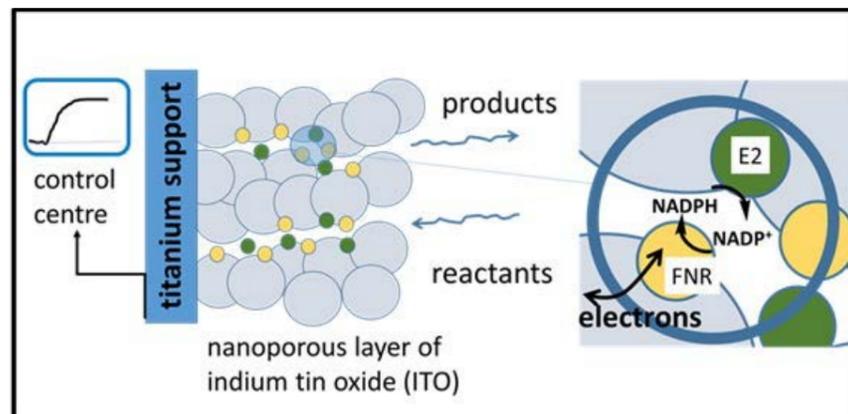
Fraser A. Armstrong<sup>1</sup>, Clare F. Megarity,<sup>1</sup> Bhavin Siritanaratkul,<sup>1</sup> Lei Wan,<sup>1</sup> Giorgio Morello,<sup>1</sup> Beichen Cheng<sup>1</sup>, Adam J. Sills<sup>1</sup>, Sacha Tschen<sup>1</sup>, Nicholas J. Turner<sup>2</sup>, Rachel S. Heath<sup>2</sup>

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In living cells, enzyme reaction sequences are localised in membrane-enclosed nanozones having internal diameters <100 nm – familiar examples being represented by mitochondria, chloroplasts, endoplasmic reticulum etc. Nanoconfinement is essential for life as it results in a massive enhancement in the concentrations of the enzymes and limits the distances across which intermediates and recycled cofactors such as (NAD(P)(H)) must diffuse. Nanoconfinement is now mimicked in bio-inspired catalytic investigations in which two (or more) enzymes are confined within the nanopores (10-100 nm) of a conducting metal oxide layer – one that is quickly and easily deposited to multi-micron depth on a suitable support, such as titanium foil.<sup>1</sup> One of the enzymes is a photosynthetic flavoenzyme ('FNR' = E1) which recycles the mobile nicotinamide cofactor (NADP(H)) using electrons supplied to/from the electrode at a control centre. The second enzyme (E2) may be one of hundreds of oxidoreductases that recycle the NADP(H). Many of these are metalloenzymes, and ones that could be produced by design, including directed evolution. Further enzymes, E3, E4 etc can be included to generate extended nanoconfined cascades, bringing simplicity and control to otherwise very complex reactions. Not only does E1 provide the 'engine' that drives the reactions, but it also provides the 'electrochemical sensor' that continuously measures rate and response to changes along the cascade.

Financial support by the BBSRC and EPA Cephalosporin Fund is gratefully acknowledged.



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## KN-26

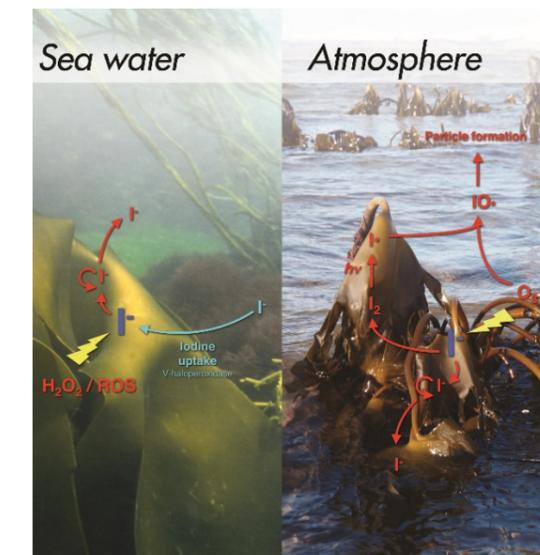
### An Inorganic Antioxidant in a Living System Impacting Atmospheric and Marine Chemistry: Iodide in Seaweeds (Kelp)

Frithjof C. Küpper<sup>1</sup>

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Brown algae of the Laminariales (kelps) are the strongest accumulators of iodine among living organisms. They represent a major pump in the global biogeochemical cycle of iodine and in particular, the major source of iodocarbons in the coastal atmosphere. Nevertheless, the chemical state and biological significance of accumulated iodine have remained unknown. Elucidation of these questions was the objective of this study. Using an interdisciplinary array of techniques, chiefly relying on synchrotron X-ray absorption spectroscopy, we show that the accumulated form is iodide, which readily scavenges a variety of reactive oxygen species (ROS). We propose here that its biological role is that of an inorganic antioxidant, the first ever to be described in a living system. Upon oxidative stress, iodide is effluxed. On the thallus surface and in the apoplast, iodide detoxifies both aqueous oxidants and ozone, the latter resulting in the release of high levels of molecular iodine and consequent formation of hygroscopic iodine oxides leading to particles, which are precursors to cloud condensation nuclei. When kelp thalli are submerged, this process impacts iodine speciation in seawater [1]. In several aspects, iodide is unique as a biological antioxidant. Among the halides, it has by far the best antioxidant properties; yet, bromide complements it for the detoxification of superoxide [2]. This paper will also report on the latest tomography-based findings concerning the subcellular localization of iodine in cells, with implications for its mechanism of uptake, storage and efflux.

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KN-27

## The Coordination Geometry of Ammonium Cations in the Receptor Ks-Amt5

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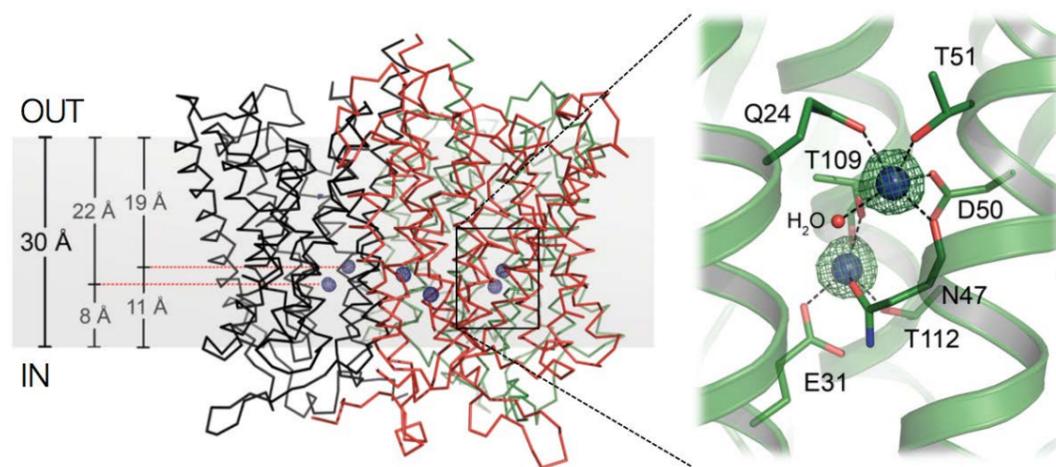
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Within the biogeochemical cycle of nitrogen, complex metalloproteins catalyse the interconversions of various modifications of nitrogen, ranging from the most oxidized nitrate (NO<sub>3</sub><sup>-</sup>) to the fully reduced ammonium (NH<sub>4</sub><sup>+</sup>). While several metabolic pathways use redox reactions for the generation of energy, the particular role of NH<sub>4</sub><sup>+</sup> is defined by the fact that it alone serves as an entry point of inorganic nitrogen into the synthesis of biological macromolecules [1]. NH<sub>4</sub><sup>+</sup> is produced from enzymes such as cytochrome *c* nitrite reductase or nitrogenases, and a distinct class of integral membrane proteins, the Ammonium Transporters (Amt), mediate its transport across biological membranes.

We are currently investigating a novel class of Amts that have retained and use the high selectivity of Amts to reshape their function as NH<sub>4</sub><sup>+</sup> sensors in diverse signalling cascades [2]. A recurrent feature of these receptor Amts seems to be the existence of outer-membrane (transducer) modules, an apparent loss of transport capacity and the development of an internal binding site for NH<sub>4</sub><sup>+</sup>. The characteristics of this site will be presented for the “*Candidatus Kuenenia stuttgartiensis*” sensor histidine kinase Ks-Amt5, providing, to the best of our knowledge, a unique observation of inorganic NH<sub>4</sub><sup>+</sup> interactions with a protein at high-resolution.

Financial support by the DFG is gratefully acknowledged.



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KN-28

## Rational Design and Development of Multi-Modal Metallodrugs: Breaking the Drug Resistance Paradigm?

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Drug resistance is a major health challenge with antimicrobial resistance in particular threatening the very core of modern medicine globally. Cancer drug resistance is fast emerging as another serious threat. The ability to fine-tune the properties of metal complexes has led to the advancement of metallodrugs with wide ranging biomedical applications. While many of these are highly effective, resistance to these metal therapies is fast becoming a major limitation associated with their use. There is therefore an urgent need to rationally design and develop innovative therapeutics that can overcome drug resistance.

With a deeper understanding of cancer at a molecular level, a number of strategies are emerging to overcome drug resistance. These include the exploitation of nanotechnologies for the selective delivery of drugs (e.g. platinum drugs) to tumour cells [1] and the development of multi-functional drugs.[1-2] Our drug design strategy to overcome drug resistance has focused to date on targeting multiple orthogonal biological pathways and, in doing so, block the development of multiple intracellular escape mechanisms essential for tumor survival. With this in mind, we have developed a series of multi-modal platinum, ruthenium and copper complexes, all of which have shown potential to overcome drug resistance.[3-8] The majority of these complexes incorporate clinically used drugs or derivatives thereof as ligands.[9] While some were originally intended to target cancer-resistant tumours only, others were designed to specifically address antibiotic resistance. Other complexes that have been developed offer the potential to act as prophylactic metallo-antibiotics possessing potent anti-cancer and anti-microbial properties.[8,10] A summary of our drug design strategies, our metallodrug candidates and their respective biological properties will be presented.

The following financial support is gratefully acknowledged: Science Foundation Ireland under Grant Nos. [11/RFP.1/CHS/3095], [12/TIDA/B2384] and [17/TIDA/5009], RCSI under the Apjohn Scholarship programme and funding under the Programme for Research in Third-Level Institutions and co-funding under the European Regional Development fund (BioAT programme).

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## KN-29

### Metal-Free Ribonucleotide Reductase Powered by a DOPA Radical

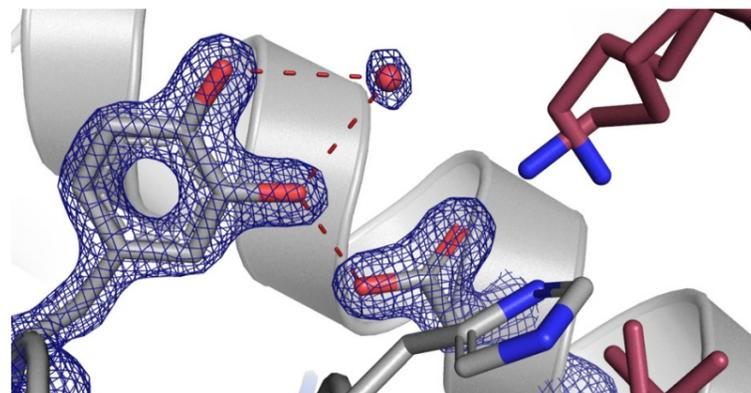
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Ribonucleotide reductase (RNR) catalyzes production of all four deoxyribonucleotides required for DNA synthesis. Class I RNR:s employ protein R2 to generate and store an essential catalytic radical. Over half a century ago it was discovered that protein R2 from *E. coli* requires non-heme iron for function. Since then, it has been established that the radical is generated by a dinuclear metal site in an oxygen dependent reaction [1,2].

Notably, a number of variants of the metal site has been found in different organisms, likely because of adaptations to the metal availability in different growth environments. The metal site can be di-iron (class Ia), di-manganese (class Ib), or heterodinuclear Mn/Fe (class Ic) [1,2]. Classes Ia and Ib generate a stable tyrosyl radical while class Ic forms a radical-equivalent Mn<sup>IV</sup>/Fe<sup>III</sup> high-valent oxidation state of the metal site [3,4]. Class Id, containing a Mn<sup>IV</sup>/Mn<sup>III</sup> cofactor, was also recently proposed [5-7]. The metal sites in classes Ia and Ic perform direct oxygen activation while class Ib requires a flavoprotein, NrdI, to generate superoxide that oxidizes the di-manganese site [8-10].

Recently a new group of metal-free RNR proteins was discovered in several human pathogens [11,12]. Organisms encoding this type of RNR are involved in e.g. diseases of the respiratory, urinary and genital tracts and it potentially developed in response nutritional immunity providing extreme metal restriction. In this group, the R2 protein initiates catalysis using a metal-independent DOPA radical residing on a post-translationally modified tyrosyl residue. The discovery overturns the presumed requirement of a dinuclear metal site in protein R2 and compels completely different mechanisms for radical generation and stabilization. Our current understanding of this remarkable RNR system will be discussed.



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## KN-30

### Catalytic Mechanism of [Fe]-Hydrogenase Based on the Crystal Structure

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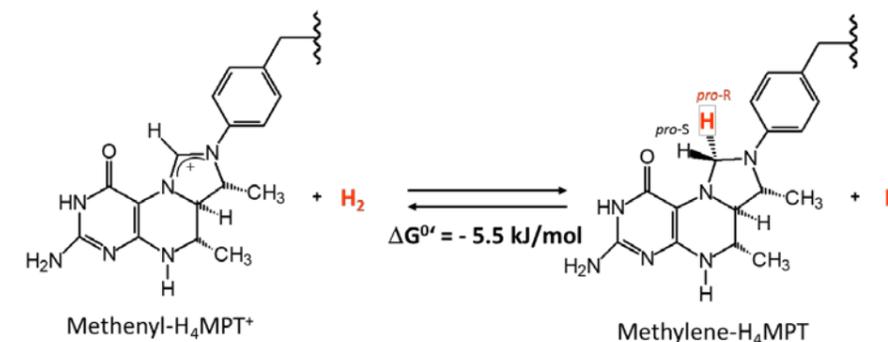
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[Fe]-hydrogenase functions in the methanogenic pathway of hydrogenotrophic methanogenic archaea [1]. It catalyzes the reversible hydrogenation of methenyl-tetrahydromethanopterin (methenyl-H<sub>4</sub>MPT<sup>+</sup>) with H<sub>2</sub> to form methylene-H<sub>4</sub>MPT (Figure). This third type of hydrogenase contains the iron-guanlylpyridinol (FeGP) cofactor, in which the iron atom is ligated by one cysteine sulfur, two CO ligands, one solvent molecule, and a nitrogen and an acyl-carbon from the pyridinol ring. The pyridinol ring of the FeGP cofactor is conjugated with a guanosine monophosphate. Crystal structure analysis indicated that three globular folding units of the [Fe]-hydrogenase homodimer form two active-site clefts. The structural data indicated that the apoenzyme is conformed in a closed form, and the holoenzyme (enzyme with the FeGP cofactor) and the C176A holoenzyme/methylene-H<sub>4</sub>MPT complex in an open form [2-4]. Here, we present a catalytic mechanism based on the 1.06-Å resolution structure of the [Fe]-hydrogenase holoenzyme in a closed form, in which the Fe of the FeGP cofactor is located in front of the hydride-accepting C14a of methenyl-H<sub>4</sub>MPT<sup>+</sup> [5]. The open-to-closed transition generates an unsaturated penta-coordinated Fe upon expulsion of a water-ligand and a deprotonated 2-OH group on the FeGP cofactor. The open Fe site and deprotonated 2-OH group might act as the H<sub>2</sub>-binding site and catalytic base, respectively. Computations based on the experimental model provided pictures of the H<sub>2</sub> activation, heterolytic cleavage and hydride transfer.



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## KN-31

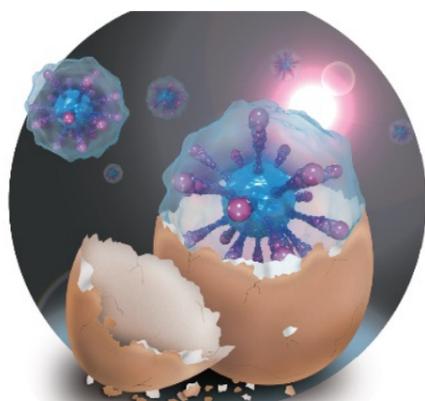
### Biomimetic Mineralization for Biology and Medicine

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Biomimetic mineralization is an important tactic by which biological organisms produce hierarchically structured minerals with marvellous functions. Biomimetic mineralization studies typically focus on the mediation function of organic matrices on inorganic minerals, which helps scientists to design and synthesize bioinspired functional materials. It has been demonstrated that the repair of hard tissues such as bones and teeth can be improved by using biomimetic mineralization. However, the presence of inorganic minerals may also alter the native behaviours of biological organisms and in nature, biomimetic mineralization plays a key role in promoting organism evolution. Accordingly, it represents a new tactic that scientists can utilize to improve biological organisms with functional materials. Typical achievements in this newly emerging research area include biomimetic vaccines, which are “thermostable vaccines that do not need refrigeration”, and biomimetic algae for the biological photosynthesis of hydrogen. Besides, the inorganic mineral phase produced by target cell calcification can be used as an alternative drug-free chemotherapy of cancers. These achievements imply the great potentials of biomimetic mineralization in the biological and medical applications as well as represent the successful biological functionalization by using inorganic materials. We believe the rapid development of biomimetic mineralization studies can enrich our current understanding about biological inorganic chemistry.

This work is supported by the National Natural Science Foundation of China (21625105) and the National Key Research and Development Program of China (2018YFC1105101).



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## KN-32

### Proteins in the Mechanism of Metallo drugs

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Metallo drugs, such as cisplatin, are widely used in clinic for cancer chemotherapy. It is well-known that DNA is the drug target of platinum antitumor agents; however, only small portion of intracellular platinum binds to DNA. Proteins are found to play important roles in the drug uptake, DNA repair and drug efflux, thus determine the drug efficacy and resistance. Sulfur containing proteins are kinetically more favorable than DNA in the reaction to platinum drugs. Copper proteins are highly reactive to cisplatin since Cu(I) and Pt(II) have similar binding preference in coordination chemistry. The copper transport protein Ctr1 has been found to facilitate the cellular uptake of cisplatin. Platinum could transfer from Ctr1 via Atox1 to ATPase. This result confirmed that the proteins associated with the cellular homeostasis of copper could also be involved in metabolic pathway of cisplatin in the view of chemistry.

As copper proteins are involved in the mechanism of platinum drugs, we analyzed the effect of anti-copper agents on the reaction of copper proteins with cisplatin. Tetrathiomolybdate (TM) is used in the clinic for the treatment of Wilson's disease, which is associated with the dysfunction of Wilson's disease protein (WLN, a copper efflux protein). Interestingly, both TM and WLN are associated with the efficacy of cisplatin. We found that TM induces dimerization of the metal-binding domain of WLN4 through a unique sulfur-bridged Mo<sub>2</sub>S<sub>6</sub>O<sub>2</sub> cluster. TM expels copper ions from Cu-WLN4 and forms a copper-free dimer. The binding of Mo to cysteine residues of WLN4 inhibits platination of the protein. Reaction with multi-domain proteins indicates that TM can also connect two domains in the same molecule, forming Mo-bridged intramolecular crosslinks. These results provide structural and chemical insight how TM attenuates the cisplatin resistance mediated by copper efflux proteins.

In addition to platinum drugs, arsenic and ruthenium agents also demonstrate great therapeutic effects in anticancer therapy. We found that zinc-finger proteins (ZFPs) are highly reactive to metallo drugs. Interestingly, different metallo-agents demonstrated different selectivity to various ZFPs, which could be associated their different therapeutic activities. Arsenic trioxide (ATO) is an effective drug used for the treatment of APL; we found that ATO preferentially binds to PML, the drug target of APL. NAMI-A inhibits tumor metastasis; we found that NAMI-A demonstrates higher binding affinity to Sp1, a transcription factor associated with tumor metastasis. The selectivity of NAMI-A is rather different from other metallo drugs on various ZFPs. These findings suggest that the selectivity of metallo drugs is crucial for their therapeutic potency.

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KN-33

## Diagnosis and Therapy with Copper Radiopharmaceuticals

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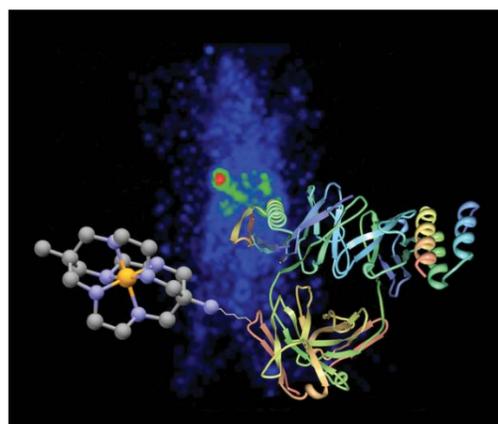
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The principle of using the same molecule for both diagnosis and therapy is called ‘theranostics’. This research will use a ‘matched pair’ of copper radionuclides to develop new theranostic agents. Copper-64 is a positron-emitting radionuclide that can be used for diagnostic imaging while copper-67 is a beta-emitting radionuclide that can be used for targeted radiotherapy. The use of a copper-binding chelator that forms exceptionally stable complexes with copper that is tethered to peptides that bind to receptors over-expressed on tumour tissue will be presented. These new agents can be used for diagnostic imaging with copper-64 and targeted therapy with copper-67.

Specific examples will include the use of peptides that selectively bind to somatostatin receptors that are over-expressed in certain neuroendocrine tumours and prostate specific membrane antigen that is over-expressed in metastatic prostate cancer. The synthesis of the new agents and their pre-clinical evaluation in cancer models will be presented as will first in human clinical trials.

Labelling antibodies with radioactive isotopes can combine the diagnostic and therapeutic possibilities of nuclear medicine with the selectivity of antibody targeting. Antibodies labelled with positron-emitting radioactive isotopes can be used as tracers for PET imaging and are of interest as companion diagnostics to therapeutic antibodies. Strategies to radiolabel antibodies and antibody fragments with copper radionuclides using site-specific enzyme mediated bioconjugation will be presented as will the *in vivo* evaluation of the new constructs in cancer models.

Financial support from the Australian Research Council, National Health and Medical Research Council, and Clarity Pharmaceuticals is gratefully acknowledged.



KN-34

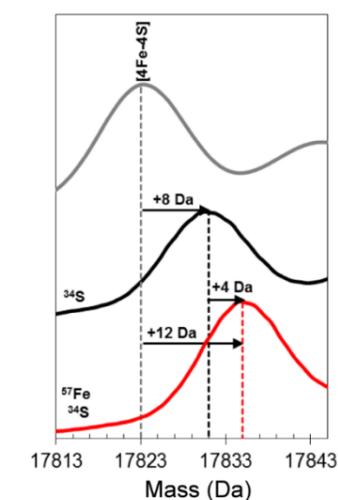
## Iron-Sulfur Cluster Regulatory Proteins as Biological Switches

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Iron-sulfur cluster proteins carry out multiple functions, including as regulators of gene transcription/translation in response to environmental stimuli [1]. In all known cases, the cluster acts as the sensory module, where the inherent reactivity/fragility of iron-sulfur clusters with small/redox active molecules is exploited to effect conformational changes that modulate binding to DNA regulatory sequences. This promotes an often substantial re-programming of the cellular proteome that enables the organism or cell to adapt to, or counteract, its changing circumstances. Here, I will discuss recent progress in the structural and mechanistic characterization of iron-sulfur cluster regulators, focussing on NsrR, RirA and WhiB-like proteins that are involved in sensing e.g. nitric oxide, iron and molecular oxygen in bacteria. In recent years, we have developed the use of mass spectrometry under conditions where iron-sulfur proteins remain folded and the cluster bound [2, 3]; aspects of this work, including time-resolved studies, will be discussed.

Financial support from the UK's Biotechnology and Biological Sciences Research Council (BBSRC) is gratefully acknowledged.



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## KN-35

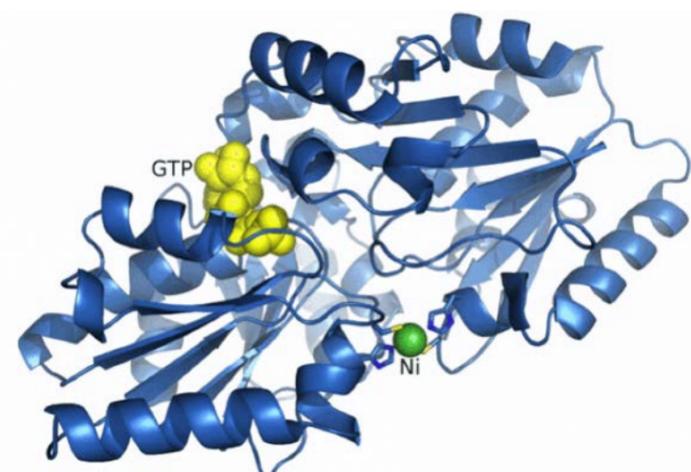
### A Structural View on The Urease Maturation Pathway

**Kam-Bo Wong<sup>1</sup>, Man-Hon Yuen<sup>1</sup>, Yap-Shing Nim<sup>1</sup>, Ivan Y.H. Fong<sup>1</sup>**

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Urease is a metalloenzyme that require nickel ions to become active. Its hydrolysis of urea to ammonia is essential to the survival of *Helicobacter pylori* in acidic human stomach. As nickel is a competitive ion at the top of the Irving-Williams series, one way to ensure correct metallation of urease is through specific protein-protein interactions among metallochaperones along the nickel delivery pathway, which is assisted by four accessory proteins, UreE, UreF, UreG and UreD(H). We have been combining structure determination, mutagenesis and biochemical analysis to understand the urease maturation pathway [1-3]. Our work demonstrated a paradigm on how a metallochaperone UreG couples GTP hydrolysis to allosterically regulate the binding/release of nickel ions and the switching of protein-binding partners, thus providing a mechanism where nickel ions are delivered to the urease without releasing the “free” toxic metal to the cytoplasm.

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## KN-36

### *In Vivo* Chemical Probes for MRI and Fluorescence Imaging

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One of the great challenges in the post-genome era is to clarify the biological significance of intracellular molecules directly in living cells. If we can visualize a molecule in action, it is possible to acquire biological information, which is unavailable if we deal with cell homogenates. One possible approach is to design and synthesize chemical probes that can convert biological information to chemical output. In this talk, molecular design strategies for MR and fluorescence imaging probes are introduced.

MRI (Magnetic Resonance Imaging) is an imaging technique using nuclear magnetic resonance phenomenon. MRI has been clinically used since it yields highly spatial resolution images of deep regions in living animal bodies. A novel <sup>19</sup>F MRI contrast agent, fluorine accumulated silica nanoparticle for MRI contrast enhancement (FLAME) is developed, which is composed of a perfluorocarbon core and a robust silica shell. FLAME has advantages such as high sensitivity, stability, modification of the surface, and biocompatibility. The activatable derivative of FLAME will also be introduced.<sup>[1],[2],[3],[4]</sup>

Intravital imaging by two-photon excitation microscopy (TPEM) has been widely utilized to visualize cell functions. The combination of the rationally designed small molecular probes with a fluorescent protein as a reporter of cell localization enabled quantitation of osteoclast activity and time-lapse imaging of its *in vivo* function.<sup>[5],[6],[7]</sup>

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KN-37

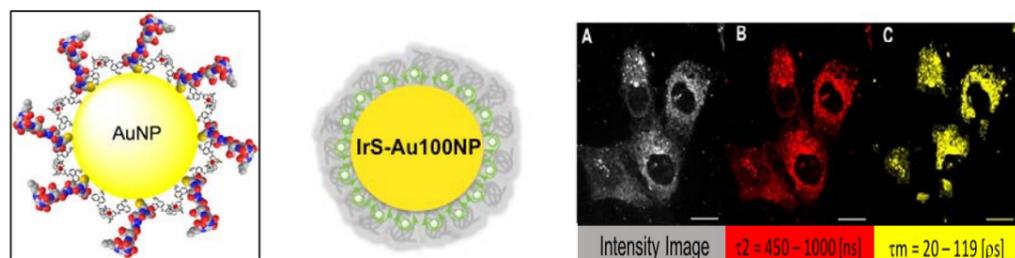
## Luminescent Nanoparticles as Detection Probes in Cancer Cells and Tissues

Zoe Pikramenou<sup>1</sup>, Sunil Claire<sup>1</sup>, Siobhan King<sup>1</sup>, Paul Murray<sup>2</sup>, Nik Hodges<sup>3</sup>, Mike Hannon<sup>1</sup>, Roy Bicknell<sup>2</sup>

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Lanthanide and transition metal complexes are ideal probes for biomolecules based on their photostability, characteristic luminescence with long lifetimes which are important properties in detection and imaging. We have developed different molecular designs based on luminescent metal complexes for biomolecular labeling and surface active groups for attachment to gold surfaces. Gold nanoparticles, AuNP, offer a unique opportunity to incorporate multiple molecular luminescent complexes into a single nanoprobe architecture for signal detection without engaging in lengthy synthetic procedures for the incorporation of multiple labels. Nanoprobes are also ideal as spatially localized cellular probes that can be detected with different imaging modalities. We have employed AuNPs as a scaffold for luminescent coordination complexes so that the nanoprobes bear the distinct optical signature of the luminescent agent, independent of the properties of the particle. Two photon lifetime imaging has revealed that the iridium signal is sensitive to cell environment.<sup>1</sup> Nanoparticles functionalized with lanthanides<sup>2,3</sup> ruthenium<sup>4</sup> or iridium probes<sup>1,5</sup> have been used in monitoring blood flow,<sup>4</sup> imaging in platelets<sup>2</sup> and cancer cell lines.

Financial support by EPSRC and The Leverhulme Trust and the University of Birmingham is gratefully acknowledged.



Metal and peptide coated AuNP    100 nm AuNP    Two-photon Lifetime Imaging

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KN-38

## Organometallic Chemistry and Metallomics in the Design of Anticancer Agents with Non-Classical Modes of Action

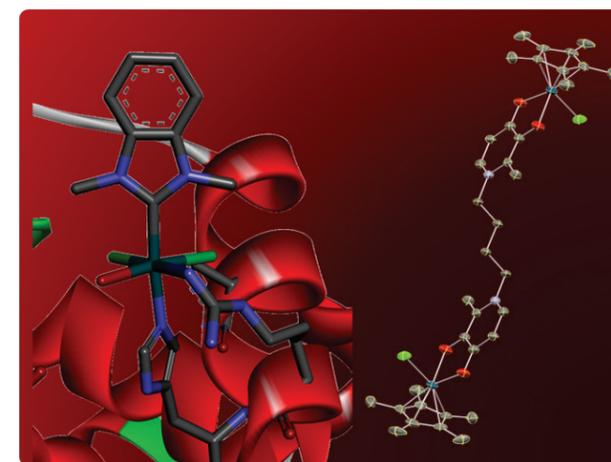
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Resistance of tumors to chemotherapeutics, intrinsic or acquired, is a major limitation in the treatment of cancer patients. To overcome drug resistance, compounds with novel modes of action are required. This represents a major opportunity for metal-based compound design with their unique chemistry and properties very different to organic molecules. The reactivity of labile metal complexes to biological nucleophiles may limit to some extent their design to specifically target a single protein, but may be an advantage as more than one pathway could be affected in cancer cells. In our research, we aim to develop organometallic anticancer agents by using design strategies based on for example polynuclear complexes and multitargeted structures that often feature bioactive ligand systems to harvest a synergistic effect between the metal center and the ligand or allow for selective delivery to tumor tissue [1,2]. By developing new bioanalytical methods, we aim to improve our understanding of the fate of metal-based anticancer agents in a biological environment, especially at the molecular level.

In this lecture, I will present our recent work on organometallic anticancer, with a particular focus on the introduction of novel ligand structures, the preparation of their organometallic complexes, and studies on their reactions with biomolecules [3-5]. The impact of the nature of the ligands on the biological properties of the organometallic anticancer agents will be discussed. This will be complemented by data collected through the application of bioanalytical methods based on separation techniques coupled to mass spectrometry in the analysis of metal complexes in complex matrices, which helps us to gain a deeper understanding of their modes of action and the impact of the nature of the complex on the bioactivity.

Financial support by the University of Auckland, Cancer Research Trust NZ and the Royal Society of New Zealand (Marsden Fund) is gratefully acknowledged.



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## KN-39

### Zinc-Proteomics: Cellular Zn<sup>2+</sup> Trafficking and Its Response to Perturbations of Cell Function

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As many as 3,000 mammalian proteins utilize Zn<sup>2+</sup> as a structural or functional cofactor. Others interact with Zn<sup>2+</sup> during signaling processes. Along with iron deficiency, deficits in dietary zinc constitute a critical nutritional problem for as many as 1.5 billion people worldwide, resulting in widespread, significant health problems. Much has been learned about the organismic and cellular trafficking of zinc, particularly the key role of ZIP and ZnT transporters in controlling access of Zn<sup>2+</sup> to cells and intracellular compartments [1]. Much less is known about mechanisms of Zn<sup>2+</sup> migration within cells and organelles. Because so many Zn-proteins exist, it is difficult to imagine specific chaperone-dependent pathways that direct the insertion of Zn<sup>2+</sup> into each apo-Zn-protein. An alternative hypothesis proposes that the cell's buffer for Zn<sup>2+</sup>, comprised of a plethora of protein and small molecule Zn<sup>2+</sup> binding ligands, supplies the metal ion for the constitution of Zn-proteins.

Components of the zinc buffer include proteomic binding sites, particularly metallothionein (MT), and possibly small molecules such as glutathione (GSH). The cellular dynamics of MT-bound Zn<sup>2+</sup> suggest that it participates in basal Zn<sup>2+</sup> trafficking despite its large affinity for MT ( $K \sim 10^{11-12}$ ) [2]. But MT-null cells also successfully transfer Zn<sup>2+</sup> to its functional binding sites. Thus, the participation of other proteomic and small molecule ligands in Zn<sup>2+</sup> trafficking is required. Based on the use of zinc fluorescent probes and apo-carbonic anhydrase (apo-CA) as monitors of Zn<sup>2+</sup> binding within the proteome, it is clear that the intracellular ligand environment strongly binds Zn<sup>2+</sup> ( $K \sim 10^{10}$ ) and, consequently, that the 'free' Zn<sup>2+</sup> concentration falls into the nM-pM range. In this context, direct ligand substitution mechanisms are hypothesized to mediate Zn-protein constitution and reversible regulatory protein (e.g. MTF-1) activation. To assess the plausibility of this hypothesis, properties of the reconstitution of apo-CA with Zn<sup>2+</sup> from the zinc-buffer, including MT and GSH, will be described. Application of this model to important pathological states, including zinc deficiency and Cd<sup>2+</sup> toxicity, will be considered [3].

Financial support from the NIH National Institutes of Health (ES-024509) and the University of Wisconsin-Milwaukee is gratefully acknowledged.

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## KN-40

### Protein-Directed Nickel Transfer for Biosynthesis of [NiFe]-Hydrogenase in *E. coli*

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[NiFe]-hydrogenase enzymes, which catalyze the reversible formation of hydrogen gas from protons and electrons, are vital components of energy metabolism in many bacteria such as *Escherichia coli* (*E. coli*) and *Helicobacter pylori* (*H. pylori*). The reaction occurs at an intricate, bimetallic center composed of nickel and iron ions coordinated to both protein and non-protein ligands [1]. Biosynthesis of this metalloenzyme requires the cooperative activity of a team of accessory proteins that gather the individual components and assemble the metalcenter in the active site of the precursor protein [2].

Our studies are focused on the delivery of nickel, which is the final component inserted into the hydrogenase active site. Two of the key metallochaperones required for nickel delivery to the *E. coli* hydrogenase 3 precursor protein are HypA and the GTPase HypB. We use a combination of spectroscopic, biochemical, and microbiology methods to examine the activities of the separate proteins and how they impact each other. The metal complexes of both proteins have been extensively characterized, and analysis of nickel in the context of protein complexes provided detailed information about how the metal moves between the proteins. In addition, our results indicate that the GTPase cycle of HypB controls unidirectional and nickel-selective transfer between the proteins primarily by modulating the interaction with HypA. Furthermore, the development and application of a high-throughput hydrogenase assay identified a new component of nickel homeostasis in *E. coli*. Altogether, the results outline a metal-selective pathway for nickel as it moves through the cell to reach the end destination at the active site of the [NiFe]-hydrogenase enzyme.

Financial support from NSERC and CIHR is gratefully acknowledged.

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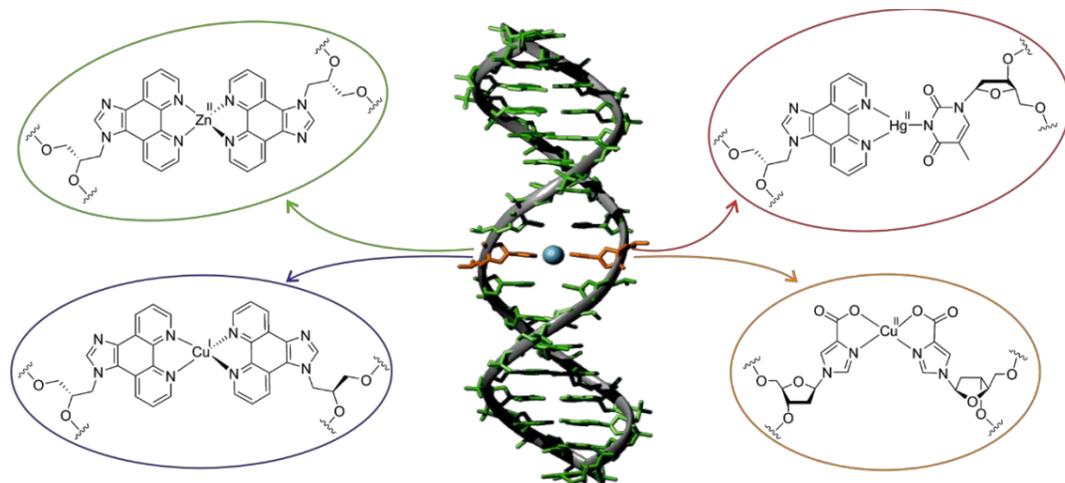
## KN-41

### New Metal-Mediated Base Pairs – Beyond Silver(I)

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Metal-mediated base pairs represent a fascinating area of synthetic bioinorganic chemistry. In these artificial base pairs, metal ions formally replace the hydrogen bonds between complementary nucleobases. In the past decade, several metal-mediated base pairs have been reported [1]. Most metal-mediated base pairs devised by our group contain silver(I) ions. To widen the scope of metal-mediated base pairing, we recently extended our studies towards metal-mediated base pairs bearing metal ions other than silver(I). Here we are reporting the development of new metal-mediated base pairs involving copper(I) [2], copper(II) [3, 4], mercury(II) [5] and zinc(II) [6]. A phenanthroline-based artificial nucleobase was applied for the incorporation of copper(I), mercury(II) and zinc(II) into DNA duplexes. Depending on the experimental conditions, a certain selectivity for these metal ions was achieved. The copper(I)-mediated base pair is the first of its kind [2]. The mercury(II)-mediated base pair forms selectively also in the presence of silver(I), so it was used for the concomitant site-specific incorporation of silver(I) and mercury(II) into the same DNA duplex [5]. Moreover, monoanionic ligands such as 4-carboxylimidazole were used for the binding of copper(II) ions [3]. As a variety of applications have been proposed for metal-containing nucleic acids, including DNA-templated metal nanoclusters [7], the site-specific incorporation of a defined number of distinct metal ions is of high importance. Financial support by the DFG (SFB 858, GRK 2027) is gratefully acknowledged.



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## KN-42

### Inorganic Chemistry Regulating Sperm-Egg Interaction Before and During Fertilization

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Living cells carefully maintain a limited ensemble of transition metals, the concentrations of which are collectively referred to as the *metallome* of the cell. It is becoming increasingly apparent that temporal fluctuations in the zinc metallome, as well as changes in labile zinc concentrations in subcellular compartments, play regulatory roles in gametes across many species. Management of the cellular metallome involves specific *metalloregulatory* proteins that sense metals and regulate discrete processes like transcription, translation and cell division. Here we show how zinc occupancy in some of these factors mediates a diverse array of cellular decisions including the decision of the egg to enter the cell cycle at the time of fertilization. Using protein biochemistry, zinc-protein thermodynamics, single cell quantitative X-ray and small molecule fluorescence microscopy, we show that the egg, must undergo dramatic translocation of zinc, loading and unloading of metalloregulatory proteins and finally exocytosis of zinc in short time frames to ensure successful fertilization. In this talk, zinc fluxes in the sperm will also be delineated and compared with those that occur in the egg at the time of fertilization in events known as 'zinc sparks.'

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## KN-43

### From Peyrone's Salt to Genomics: Tracking Platinum Compounds through Cells

Victoria J. DeRose,<sup>1</sup> Christine McDevitt,<sup>1</sup> Emily S. Sutton,<sup>1,2</sup> Jack Prochnau,<sup>1</sup> Emily Reister,<sup>1,2</sup> Rachael M. Cunningham<sup>1,2</sup>

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Platinum compounds are employed extensively for chemotherapeutic regimes. Despite decades of worldwide use, there is no comprehensive description of cellular targets of these inorganic compounds or convenient method of detecting them in cells [1]. This gap in knowledge inhibits efforts to understand major cell-death pathways, as well as understanding causes of severe side effects and lasting post-treatment damage. While DNA has been investigated as a critical target, platinum adducts on cellular RNA and proteins are also prevalent [2-5]. In an effort to identify, isolate, and visualize cellular targets of platinum compounds we have developed a toolkit of platinum reagents that are modified for post-treatment 'click' cycloaddition reactions [6,7]. Post-treatment fluorescent labeling in mammalian cell culture shows distinct accumulation of Pt compounds in the nucleolus, potentially linking antitumor activity to defects in ribosome assembly [7]. Progress on genome-wide identification of Pt-DNA/RNA adducts as well as protein adducts will be presented. These studies in mammalian cancer cell lines are accompanied by time-dependent differential gene expression analysis. Current results suggest that at least two contributing and reagent-specific pathways involving ER stress and nucleolar stress add to canonical DNA damage, and may explain different treatment outcomes for cancer subtypes.

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## KN-44

### Metallothionein: A Threat for Copper-Drugs

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Cu-metabolism is very well controlled in biological systems. Transporters and chaperons are responsible to bring the Cu to their target, mostly Cu-enzymes. However, Cu could bind to off-target molecules in case of failure of Cu-homeostasis, or upon Cu-overload. Cu bound to off-target biomolecules could be toxic, often due to uncontrolled catalysis of reactive oxygen species (ROS) production. This has been proposed for the peptide amyloid-beta and alpha-synuclein, two proteins that play a key role in Alzheimer's and Parkinson's disease, respectively.

Cu-complexes are also under investigation as potential drugs, like in cancer or as antimicrobials. We are interested in the question of the reactivity of Cu-drugs (Like Cu-thiosemicarbazones, Cu-ATCUN, Cu-phenantroline/bipyridine etc.), in particular their redox activity and the production of reactive oxygen species. Moreover, we investigate which endogenous biomolecules could bind off-target Cu or interact with exogenous Cu-complexes, and if they are able to shuttle these Cu back into the normal Cu circuit.

Glutathione (GSH) and metallothioneins (MT) are known reducing agents and metal chelators, both occurring at quite high concentration in the cytosol and nucleus. They are potentially very active disruptors of Cu(II)-complexes due to their strong reducing and Cu(I)-binding activity.

We show that MTs together with GSH are very potent disruptors of Cu(II)-complexes (endo- or exogenous) and strong competitors for Cu(I)-compounds. Thus MT and GSH can inactivate rapidly inorganic Cu(II)-complexes (like investigated for cancer therapy) and Cu(II)-peptides (e.g. used for artificial nucleases or peptidases), mainly by reducing Cu(II) to Cu(I) and withdrawal of the Cu(I).

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ICBIC-19

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# Lifelong Achievements Talks

## LA-01

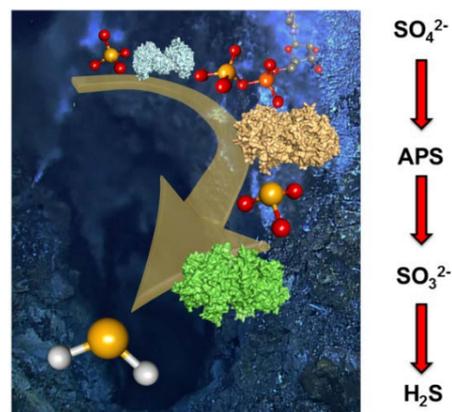
### Ancient Food - How Microbes Reduce Sulfate to Hydrogen Sulfide for Energy Conservation

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Dissimilatory reduction of sulfate to hydrogen sulfide, with sulfite as crucial intermediate, is one of the oldest and most prominent microbial energy conserving pathways on Earth. As life may have originated in hot environments, the occurrences of sulfate reducing hyperthermophilic archaea and deep-branching thermophilic bacteria indicate an early origin of this process. Isotopic data suggest that dissimilatory sulfate reduction began over 3 billion years ago but acquired global significance only after sulfate concentrations had considerably increased in the Precambrian oceans approximately 2.35 billion years ago. Sulfate-reducing microorganisms are ubiquitous and play an imperative role in the global cycling of carbon and sulfur. Sulfate is an energy-rich molecule, however, that is chemically inert. To use this energy source, microorganisms have to invest ATP. Three enzymes are the key players in the dissimilatory reduction of sulfate to hydrogen sulfide: (i) ATP sulfurylase, (ii) adenosine 5'-phosphosulfate reductase, and (iii) dissimilatory sulfite reductase. Because of its low redox potential, sulfate cannot be directly reduced by H<sub>2</sub> or organic acids, it has to be converted to adenosine 5'-phosphosulfate (APS) in a reaction catalyzed by ATP sulfurylase. Hereby, the redox potential E<sup>0</sup> (APS/AMP + HSO<sub>3</sub><sup>-</sup>) is shifted to -60 mV. The formation of APS is endergonic and probably driven by the subsequent hydrolysis of pyrophosphate and the energetically favorable APS reduction. Therefore, the activation of sulfate to APS is assumed to consume two ATP equivalents. APS reductase, a FAD, [4Fe-4S] enzyme, converts APS to sulfite and AMP, followed by the six-electron reduction of sulfite to hydrogen sulfide as the final step (E<sup>0</sup> (HSO<sub>3</sub><sup>-</sup>/HS<sup>-</sup>) = -116 mV) [1 - 3]. The final multi-electron, multi-proton transfer process is catalyzed by dissimilatory sulfite reductase which contains the coupled siroheme-[4Fe-4S] cofactor at the active site. Recently, a protein-based trisulfide was identified as key intermediate that couples the reduction of sulfite to energy conservation [4]. In my talk structural and mechanistic aspects of dissimilatory reduction of sulfate will be discussed briefly. Furthermore, alternative enzymes will be presented which can reduce sulfite to hydrogen sulfide.

My sincere thanks go to all my students, co-workers and collaborators for their valuable contributions. Work in the laboratory was supported by the Deutsche Forschungsgemeinschaft, the Volkswagen-Stiftung, and the University of Konstanz.



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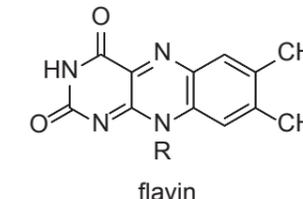
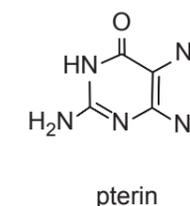
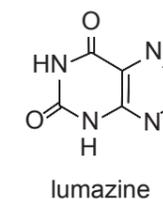
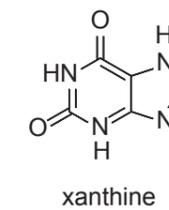
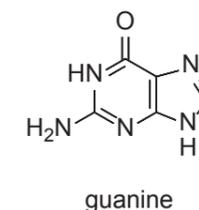
## LA-02

### Metal Coordination by Biorelevant Purine and Pterin Heterocycles

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Using the heterodinuclear cations [(dopf)Cu]<sup>+</sup>, dopf = 1,1'-bis(diorganophosphino)ferrocene, their binding to purine ligands such as guanine or xanthine derivatives [1] or to pterin-based heterocycles such as pterin [2], lumazine [3] or flavin derivatives [4] has been characterized structurally. Additional spectroscopic and electrochemical studies were correlated with the structural results, pointing to the possible function of such ligands in metal binding and interaction.



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## LA-03

### Bioinorganic Chemistry – A Topic for Life – From the Past to the Future

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Bioinorganic Chemistry was and still is a significant part of our common life, though there were other things of great importance to us as well, like travelling to distant places of the globe, skiing or mountain climbing (though in the latter case a day could end with chemistry despite the high altitude [1]). Astrid and I did practically everything together and without her I could not write the following part.

Clearly, I cannot summarize here the research of over 55 years -- only indications can be given: I had my first encounter with nucleotides and derivatives, making me also familiar with "ambivalent" ligands [2], in 1961 during my Ph.D. work with Prof. Dr. Hans Erlenmeyer and PD Dr. Hans Brintzinger (a coworker of Erlenmeyer) at the University of Basel by studying the metal ion-binding properties of 2-aminopyridine N(1)-oxide [3], a model compound of adenosine N(1)-oxide (studied by Perrin [4]), and also of adenosine 5'-monophosphate N(1)-oxide [5]. The situation is related to macrochelate formation which occurs with N7 of the adenine residue by the phosphate-bound metal ion ( $M^{2+}$ ), e.g., in  $M(\text{AMP})$  [6],  $M(\text{ADP})^-$  [7],  $M(\text{ATP})^{2-}$  [8] and related complexes giving rise to an intramolecular equilibrium between a closed (macrochelated) and an open isomer [9,10].

In 1966 in my thesis for the Habilitation it is shown that depending on the conditions in  $M^{2+}$ -ligand-peroxo complexes catalase-like or peroxidase-like reactions can take place which proceed within the coordination sphere of the metal ion ( $\text{Cu}^{2+}$  or  $\text{Ni}^{2+}$ ) and not via free radicals [11,12]. This allowed, e.g., to probe with  $\text{Cu}^{2+}/\text{H}_2\text{O}_2$  the stability of the DNA double helix [11], which is stable in the pH range 6 to 11; by addition of LiCl, NaCl or KCl the helix can be stabilized down to pH 4. -- A few more studies are:

- "Footprinting": Selective degradation of polymyxin-B and angiotensin-II by  $\text{Cu}^{2+}$  or  $\text{Ni}^{2+}/\text{H}_2\text{O}_2$  [12]
- d-Biotin: Metal ions bind stereoselectively to the thioether S of the tetrahydrothiophene ring [13]
- $\alpha$ -Lipoate: Hydrophobic and  $M^{2+}$ -coordinating properties including the disulfide linkage [14]
- Heteroaromatic amines coordinated to 3d  $M^{2+}$  ions discriminate between O- and N-ligands [15]
- $\text{Cu}(\text{ATP})^{2+}$ : Release of N7 from the coordination sphere of  $\text{Cu}^{2+}$  by 2,2'-bipyridine [16]
- Adenosine 5'-triphosphate ( $\text{ATP}^4/\text{NTP}^4$ ): Coordination chemistry of a multi-talented bio-substrate [10]
- Quantification of rare nucleobase tautomers. Acid-base properties of purine residues [17]
- Xanthosinate 5'-monophosphate is not an analogue of guanosine 5'-monophosphate! [18]
- Antiviral acyclic nucleoside phosphonates. Coordination chemistry and isomeric equilibria [19]

Supported by the Department of Chemistry of the University of Basel.

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## LA-04

### The Periodic Table Celebrates its 150<sup>th</sup> Birthday: Still a Major Tool for Bioinorganic Chemistry

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Elements now recognized as belonging to the Periodic Table have been known since ancient times. So, even before elements were as such known and recognized they were already intensely used. No doubt Gold, Silver and Copper were the first of such metals, and these were generally used in jewellery and coins. But in some cases Cu was also in weapons. By the time periodicity in the ordering of the element, initially based on atomic weights, the Periodic System appeared in 1869. Already at that time one was aware that some elements and metal-containing compounds were necessary for life (i.e. Fe). But at the same it was known that other elements are very toxic, like As, Hg.

Already in the ancient times, some of the elements were used to treat diseases, like colloidal gold. The dosage of many of such metallic elements to humans, to cure or prevent diseases has been a subject of study for many decades. In the last 50 years the usage of metal compounds to diagnose or cure diseases has been rapidly grown [1] and has been a repeating main theme at almost all ICBICs. The history of the discovery of metals as trace element, drugs and diagnostics will be briefly described, with a focus on some of our own work [2,3].

#### Elements required for life and/or used in curing and diagnosis

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
	H																	He	
	Li	Be											B	C	N	O	F	Ne	
	Na	Mg											Al	Si	P	S	Cl	Ar	
	K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr	
	Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe	
	Cs	Ba	Ln	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn	
	Fr	Ra	An	Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Cn	Nh	Fl	Mc	Lv	Ts	Og	
	Lanthanoids		La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu		
	Actinoids		Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr		

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LA-05

## What could be Fascinating in Metallopeptides or Modelling of Metalloproteins

**Henryk Kozłowski<sup>1</sup> and co. (>600 coauthors, whose names are depicted on the scheme below)**

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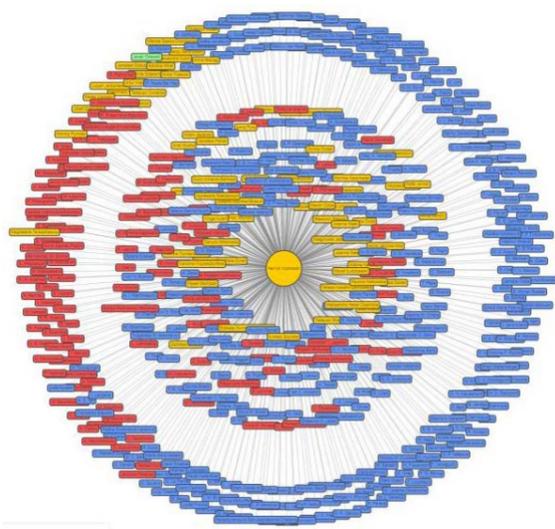
Study of metallo-peptides either as metallo-peptides or as models of larger metalloproteins is a huge area of research. Having hundreds of papers in this field is a simple output of many years of more or less reasonable work. Selecting from all these set of data by the author(s) something which could be of some interest for others is rather hopeless and subjective work, especially when author(s) usually ends his (their) interest when paper is published. Collaboration with many, many different researchers is very exciting, but it is hard to remember all contributions – that is why the selection made in this very short talk will be based on rather accidental criteria.

I may propose based on various feelings the following topics:

1. Neurodegeneration mostly based on the studies on prion proteins and copper [1]
2. His tags and their unusual behaviour in the presence of metal ions [2]
3. Metallophores and possible approach to bacteria resistance [3]

All these topics allowed to work with fantastic researchers and to get invitation to this meeting.

Financial support by the National Science Centre (UMO-2017/26/A/ST5/00363, MAESTRO grant to HK) is gratefully acknowledged.



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LA-06

## Iron Storage Without Ferritin

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Because of its potential to generate toxic hydroxyl radicals via the Fenton reaction, 'free' Fe<sup>2+</sup> must be carefully controlled in living systems. In many unicellular and multicellular organisms, ferritin plays this role, [1] via its ferroxidase centre, while ferritin's structural relative, the Dps protein [2], ubiquitously present in the bacterial and archaeal kingdoms, functions both as an iron scavenger and mechanical protector of DNA. In addition to their ferroxidase centres, both ferritins and Dps proteins also utilize acidic residues located on the inner surface of their protein shells to carry out biomineralization via nucleation of iron to generate iron oxide nanoparticles [1, 3].

However, there are organisms which do not contain ferritin genes in their genomes and so we may ask how they protect against iron toxicity, and how they store iron. Early studies indicated that in *Saccharomyces cerevisiae* iron could be stored in, and mobilized from, intracellular vacuoles [4]. Recently a new type of protein organelle composed of encapsulin nanocompartments has been described which stores iron and protects bacteria from oxidative stress [5]. These nanocompartments are widely distributed in both bacterial and archaeal genomes [6], are able to encapsulate specific ferritin-like cargo proteins attached to their inner surface, have dense iron-rich cores and functionally resemble ferritins, but with a much greater capacity (30,000 iron atoms) to store iron than ferritins. We will compare these ferritin-like proteins with both ferritins and Dps proteins with regard to their ferroxidase activity and their mode of biomineralization.

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LA-07

**Carbonate Anion Radicals as the Source of Oxidative Stress**

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<sup>3</sup> Chemistry Dept., Ben-Gurion University, Beer-Sheva, Israel.

Carbonate anion radicals,  $\text{CO}_3^{\cdot-}$ , are known to be formed via the hemolysis of  $\text{ONOOCO}_2^-$ . This is commonly given as the explanation of the toxicity of  $\text{O}_2^{\cdot-}$ . Furthermore,  $\text{CO}_3^{\cdot-}$  is formed during the enzymatic activity of Cu,Zn-superoxide dismutase and xanthine oxidase. Recent results point out that  $\text{CO}_3^{\cdot-}$  is also formed by the Fenton reaction in the presence of bicarbonate, even at concentrations lower than those present in biological systems. (It should be noted that the reaction  $\text{Fe}(\text{H}_2\text{O})_6^{2+} + \text{H}_2\text{O}_2$  in neutral solutions yields  $\text{Fe}^{\text{IV}}=\text{O}_{\text{aq}}$  and not OH· radicals.) Even in the presence of citrate the Fenton reaction does not yield OH· radicals, and when bicarbonate is present the product is  $\text{CO}_3^{\cdot-}$ . Thus one has to consider  $\text{CO}_3^{\cdot-}$  as the source of oxidative stress. As  $\text{CO}_3^{\cdot-}$  are considerably less reactive than OH· and have a considerably longer lifetime they are expected to be more selective in their reactions in biological systems and be more harmful.

LA-08

**Modelling Metal-Nucleic Acid Interactions: Achievements and Limitations**

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It is a common strategy in chemistry and biochemistry to apply structural and/or functional models in order to understand a novel discovery in more detail. Bioinorganic Chemistry is no exception in this respect. When interactions between metal ions and metal coordination compounds with nucleic acids got into the focus of scientists during the past century, the use of truncated systems, hence of isolated nucleobases or short fragments of the large DNA and RNA molecules with their sometimes complicated tertiary structures, proved distinctly advantageous. These simple models were instrumental in identifying relevant metal binding sites of the common and rare nucleobases and were essential in establishing fundamental aspects of the effects of metal coordination on properties such as acid-base chemistry, tautomerism, and hydrogen bonding. On the other hand, the use of isolated nucleobases rather than large oligomers has distinct limitations in that it cannot provide insight into the importance of neighboring bases and sequence specificity in general, and necessarily fails in establishing potentially crucial secondary interactions. The use of sophisticated spectroscopic techniques, modern X-ray crystallography, as well as computational methods has meanwhile overcome many shortcomings of the “simple” models.

The lecture will focus on findings made in the author’s laboratory over the past four decades and critically examine results which may or may not be of immediate biological relevance, but add to our general understanding of the chemistry of metal species with the heterocyclic nucleobases [1, 2], including topical areas such as Supramolecular Chemistry and Nanotechnology involving nucleic acids or their components and metals.

The author gratefully acknowledges the contributions of several generations of students and postdocs, and fruitful collaborations with colleagues and friends. Financial support has come from Deutsche Forschungsgemeinschaft (DFG) and other institutions.

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## LA-09

### Factors Beyond the Thermodynamic Stability of Complexes Affecting the Metal Ion Selectivity of Hydroxamate-Based Siderophores and Analogous Compounds

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Due to the extremely low bioavailability of iron at physiological pH in the environment, living organisms have developed specific uptake strategies to supply their cells with the adequate amount of this metal ion. Microorganisms solve this problem via low molecular mass high-affinity iron(III) chelators, siderophores, produced under iron-deficient conditions [1]. Hydroxamates are common bidentate chelating groups found in many siderophores, such as in the well-known desferrioxamine B (DFB). These molecules form extremely high stability water-soluble iron(III) complexes and transport this metal to the cells in this form, are able to oxidize even the iron(II) to iron(III) [2].

Although, the high selectivity of the siderophore-based microbial chelators to bind iron works well, siderophores have the ability to bind a variety of metals in addition to iron. The high selectivity for iron(III) is determined significantly, but not exclusively by the very high thermodynamic stability of the iron(III)-siderophore complexes. However, there are numerous additional factors, which affect the interaction of siderophores with metal ions and, as a consequence, determine their relative metal ion preferences under certain conditions. Also these factors might provide us some tools for tuning the order of preferences. During the past few decades in the Bioinorganic Group in Debrecen, some of the factors, e.g. effects of structural elements of siderophores, geometry and kinetical behaviour of the formed complexes, existence of competitive processes (like protonation, hydrolysis) have been investigated and a few interesting results are planned to demonstrate at the conference [3-6].

Out of the siderophores desferrioxamine B (DFB), desferricoprogen (DFC) as well as their exocyclic and endocyclic analogous compounds, desferricrocin (DFR) and triacetylfusarinine C (TAF), respectively, will be mentioned. Regarding the metal ions, in addition to iron(II/III), the following metals will be in the focus of the presentation: molybdenum(VI), copper(II), lead(II) and cobalt(III). Very interestingly the thermodynamic stability of a kinetically inert cobalt(III)-siderophore complex is higher with ca. 5-7 orders of magnitude than that with iron(III). The question arises whether a possible interference between the iron versus cobalt uptake could lead to the development of potential drug for the treatment of some microbial infections relying on a 'Trojan horse' strategy?

Financial support by the EU and by the European Regional Development Fund under the project GINOP-2.3.2-15-2016-00008 and the Hungarian Scientific Research Fund (OTKA K112317) are gratefully acknowledged.

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## LA-10

### From Stability Constants to Enzyme Functions through Elucidation of Ligand-ligand Interactions. Stabilization of the Cu(II)-Phenoxy Radical by Tryptophan Stacking in Galactose Oxidase

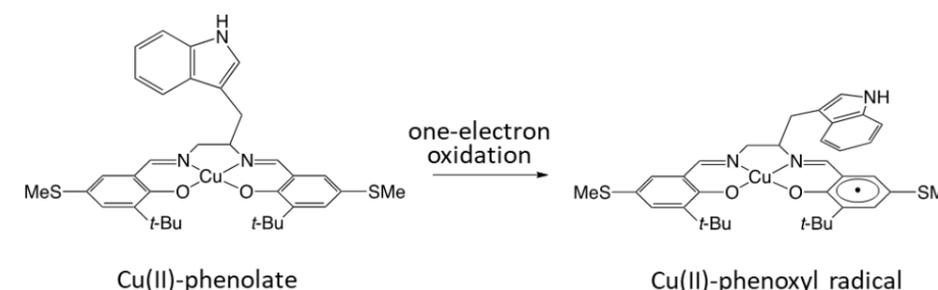
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Weak or noncovalent interactions around the metal center are important for the structures and functions of metal-containing systems [1]. For small metal complexes, solution chemical studies on mixed ligand Cu(II) complexes with aromatic amino acids and aromatic diimine ligands such as 1,10-phenanthroline have established that the stacking interaction between the side chain aromatic ring and the diimine stabilizes the complexes and that the indole ring of tryptophan has a high complex stabilizing effect [2-4]. It was also shown that the indole ring prefers stacking with the pyridine ligand to stacking with the electron-rich phenolate ligand [1,5].

An interesting case of aromatic ring stacking interactions in the metal center of enzymes is the stacking of the Cu(II)-phenoxy radical with the vicinal tryptophan (Trp 290) in the active form of galactose oxidase (GO) [6,7]. Although the radical stabilizing effect of the interaction has long been recognized, relevant chemical details have remained largely unclarified. Recent structural and spectral studies have shown that the indole ring incorporated into the side chain of the Cu(II) complex of a salen-type diphenolate ligand as a GO active site model is involved in the stacking interaction with the Cu(II)-phenoxy radical moiety upon one-electron oxidation and stabilizes the radical [8,9]. The results revealed the details of the stacking interaction and its effect on the radical stability, demonstrating that the weak interactions between the metal center and its environment are essential for the function of metalloenzymes.

The background of the studies and related findings will also be presented and discussed.



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LA-11

**Caught in the Act: Monitoring O-O Bond Cleavage to Form Cytochrome P450 Compound I in Real Time**

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While monitoring the reaction of ferric cytochrome P450cam (Cyp101) with substituted (Cl, CH<sub>3</sub>, OCH<sub>3</sub>) perbenzoic acids using rapid scanning stopped flow (RSSF) spectroscopy, an intermediate appears *en route* to the formation of the high-valent moiety known as Compound I [Fe(IV)=O/porphyrin radical cation] that is thought to be the key catalytic species for oxygen atom transfer to substrate. It was suggested in previous studies (Spolitak, T.; Dawson, J.H.; Ballou, D.P. *J. Biol. Chem.* **2005**, *280*, 20300-20309) that this is an acylperoxy-ferric heme adduct that subsequently undergoes O-O bond cleavage with release of the substituted benzoic acid to generate Compound I. Singular value decomposition analysis of the RSSF data for the formation of this intermediate shows that the energy of its Soret absorption peak is sensitive to the electron donor properties of the aryl substituents. A linear Hammett correlation plot is seen for the energy of the Soret peak vs. the Hammett  $\rho$  constant. This correlation with the nature of the substituents requires that those substituents remain as part of the ligand bound to the heme iron, providing direct evidence that this adduct is indeed a ferric acylperoxy derivative. Further support for this conclusion derives from additional linear Hammett correlation plots for both the rate of formation of the intermediate as well as for its conversion to Compound I. Clearly, the electron donating/withdrawing properties of the substituents affect the donor properties of the binding substrate, changing the observed rate of formation for the acylperoxy intermediate, as well as the propensity and stability of the substituted benzoic acid to serve as the leaving group during O-O bond cleavage yielding Compound I. The ability to directly follow the O-O bond cleavage step in the formation of Compound I from peracids provides a model for how this key step occurs in both P450 enzymes as well as in peroxidases.

Financial support by the US National Institutes of Health is gratefully acknowledged. [GM26730]

LA-12

**Metal Binding to Nucleic Acids – A Journey from the Beginning**

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In the late 1960-ies research on the chemistry of metals in biological systems was mostly focused on amino acids, peptides and proteins. In contrast, the potential role of metal ions in genetic processes attracted less attention. At the time it was commonly accepted that the nucleic acids bind metal ions exclusively at the phosphate groups. However, heterocyclic nucleobases, purine and pyrimidine rings, constitute an interesting group of ligands. In 1967 the first X-ray structure of a metal bonded purine base was published [1], followed by a series of metal-nucleobase structures, including a metal-nucleotide complex (Cu-GMP) which formed an interesting, inverted double helix [2].

The rapid development of automatic synthesis of DNA-sequences and two-dimensional (2D) high-field NMR in the 1980-ies made it possible to determine the conformation of DNA-oligomers in solution. A main topic was to elucidate **sequence-selective binding** of metal ions to DNA-duplexes [3,4]. The dramatic improvement in NMR sensitivity due to the development of high-field cryo-magnets enabled us to study not only structures but also kinetics. Rate constants were determined for reactions between metal ions and double-helical oligodeoxy-ribonucleotides monitored by 2D [<sup>1</sup>H, <sup>15</sup>N] HSQC/HMQC NMR spectroscopy [4,5].

Increasing evidence indicates that sulfur-containing proteins can play important roles in the activity of platinum anticancer drugs. The DNA platination rate was substantially enhanced by methionine (Met) binding, suggesting that the thioether could serve as a catalyst [6].

**Mercury** is regarded as the villain element in biological systems. NMR studies on mercury binding to oligonucleotides for duplex DNA oligomers were carried out using one- and two-dimensional <sup>1</sup>H NMR spectra based on natural abundance correlation spectroscopy [7]. Presently I am engaged in a curious environmental problem: How to remove 67 tons of elemental mercury located in a wreck close to the Norwegian coastline.

**Supramolecular metal complex - DNA interactions.** Having explored the binding of mono-nuclear metal complexes to DNA we moved on to investigate more complex systems as part of an EU project coordinated by Mike Hannon, University of Birmingham. The interactions between a supramolecular di-iron metal complex and a dodecamer, [5'-d(TATGGTACCATA)]<sub>2</sub> turned out to be quite unexpected and spectacular [8].

Looking back at a period of almost 50 years in academia I realize that I have been very fortunate to have collaborated with a lot of wonderful people. I am especially thankful to the late Lyle H. Jensen, Brian R. Reid, Univ. of Washington and Marc Leng, CNRS, Orleans. I also want to thank Gerd LaMar (California), Giovanni Natile (Bari), Peter Sadler (Edinburgh), Helmut Sigel (Basel), Nick Hadjiliadis (Ioannina), Bernard Lippert (Dortmund), Iztok Turell (Ljubljana), J. Kozelka (Brno) and my former student Yangzong Liu, now professor at The University of Science and Technology of China.

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LA-13

### Iron, Inflammation and Parkinson's Disease

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The role played by iron in the aetiology and pathogenesis of Parkinson's disease (PD) remains unclear. In these present studies, in vivo, post-mortem and in vitro studies investigated the connection between iron and inflammation in PD. Early stage PD subjects showed changes in systemic ferritin which associated with a variety of inflammatory plasma markers, ie. hepcidin and IL-6, as well as brain iron content measured with relaxometry imaging and disease severity. Post-mortem analysis of Parkinsonian substantia nigra, divided according to Braak and Braak scores, exhibited microgliosis and increased iron accumulation, the degree of microgliosis correlating with the intensity of nigral iron staining. The response of immortalised glial cells to an inflammatory stimulus showed an increase in the release of inflammatory markers, ie. TNF $\alpha$  and IL-6, in addition to significant elevations of cellular iron as well as changes in hepcidin expression, although there was no evidence for hepcidin release. In conclusion, markers of inflammation and iron metabolism in both systemic and brain systems are closely linked in PD, thus offering a potential biomarker for progression of the disease.

LA-14

### A Subset of New Substitution Inert Platinum Antitumor Agents Kills Cells by a Multimodal Mechanism of Action Involving Changes in the Organization of the Microtubule Cytoskeleton Network

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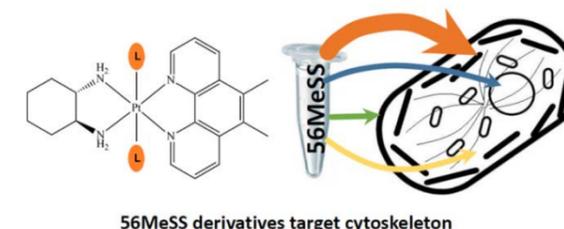
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The substitution inert platinum antitumor agent [Pt(1*S*,2*S*-diaminocyclohexane)(5,6-dimethyl-1,10-phenanthroline)]<sup>2+</sup> (56MeSS) is a very potent cytotoxic metaldrug [1]. It represents along with its derivatives very potent antitumor agents exhibiting sub-micromolar IC<sub>50</sub>s against a variety of cancer cells. In contrast to conventional cisplatin or oxaliplatin, the mechanism of action (MoA) of 56MeSS is fundamentally different. However, the details of the mechanism by which the 5,6-dimethyl-1,10-phenanthroline ligand contributes to the cytotoxicity of the 56MeSS complexes have not been so far sufficiently clarified. Here we show that the 56MeSS compounds exhibit an intriguing potency in the triple-negative breast cancer cells MDA-MB-231 which are known to be highly resistant to clinically used cisplatin; these compounds exhibit antiproliferative activity at the concentrations by several orders of magnitude lower than antitumor platinum compounds used in the clinic. Moreover, we show that the 56MeSS-based Pt(IV) compounds act by multimodal MoA resulting in the global biological effects different from that of the Pt(II) complexes, i.e., that they damage nuclear DNA, reduce the mitochondrial membrane potential, induce the epigenetic processes and last but not least, the data provide evidence that changes in the organization of cytoskeleton networks are functionally important for 56MeSS-based compounds, in contrast to clinically used conventional platinum cytostatics, to kill cancer cells. The latter mechanism has not been described for other antitumor platinum compounds including those used in the clinic. In other words, changes in the organization of cytoskeleton networks is a new mechanism which asserts itself in the antitumor effects of platinum anticancer drugs and might play an important role in conveying preferential and high susceptibility of some cancer cells to the platinum drugs based on 56MeSS. This finding represents a promise of development of new metal-based anticancer drugs that would be active in a spectrum of human tumors different from that attainable by the platinum drugs investigated in the clinical and preclinical research [2].

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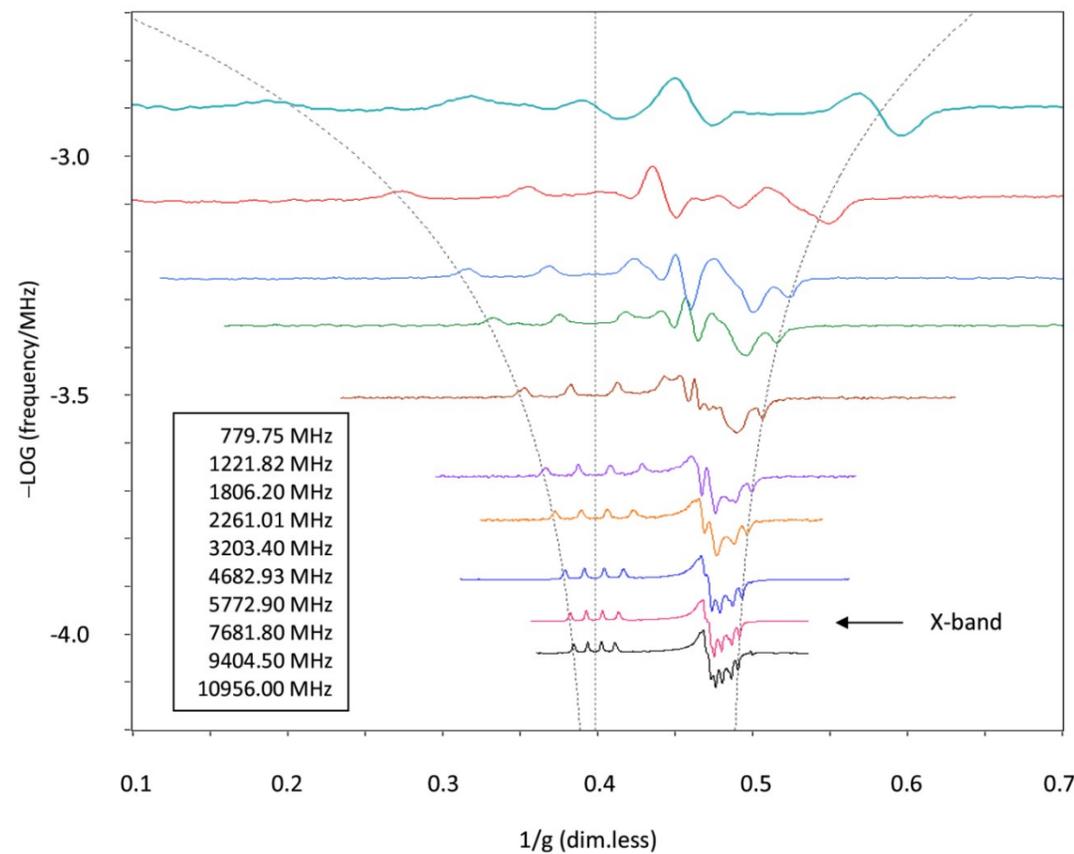
LA-15

**Novel Electronic Structure Determination of Metal Ions in Proteins and Models: Development and Application of a Broadband-Tunable CW-EPR Spectrometer**

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Analysis of the EPR of transition ion complexes requires data taken at different microwave frequencies because the spin hamiltonian contains operators linear in the frequency as well as operators independent of the frequency. In practice data collection is hampered by the fact that conventional EPR spectrometers have always been designed to operate at a single frequency only. Here, a broadband instrument is described and tested that – presently – operates from 0.5 to 12 GHz and whose sensitivity approaches that of single-frequency spectrometers. Multi-frequency EPR from a low-symmetry Cu(II) spectroscopic model (see figure) is globally analyzed to illustrate a novel approach to reliable determination of the molecular electronic structure of transition ion complexes from field-frequency 2D data sets. The methodology is applicable to various classes of metalloproteins.



# Invited Lectures

## IL-001

### Heme Degradation in Pathogenic Bacteria

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Heme degradation is a significant process in bacteria, plants, and mammals. For some pathogenic bacteria iron acquisition from hosts heme is essential for their proliferation and virulence; the heme degradation product biliverdin is the precursor of phytochrome-family of photoreceptors in cyanobacteria and plants; and heme degradation is the initial step of heme iron recovery for iron homeostasis in mammals. All the heme degradation in plants and mammals is attained by heme oxygenase (HO) where the substrate heme activates O<sub>2</sub> molecules, converting hemin to biliverdin with release of carbon monoxide and iron through three consecutive monooxygenase reactions via  $\alpha$ -meso-hydroxyheme and verdoheme intermediates. Mammalian HO has two isozymes, a 33 kDa inducible HO-1 and a 36 kDa constitutive HO-2. Each isozyme has a single C-terminal transmembrane segment which is anchored into the endoplasmic reticulum facing cytosol. HOs from plant, cyanobacteria and pathogenic bacteria are soluble and smaller in size (24 kDa – 29 kDa) than their mammalian counterparts. All the HO enzymes are structurally similar and share the same catalytic mechanism, which is now mostly understood [1]. Recent discovery of new heme degrading enzymes, IsdG and IsdI of *Staphylococcus aureus* and MhuD of *Mycobacterium tuberculosis*, which are structurally and enzymatically distinct from HO, extends the spectrum of biological heme degradation [2]. MhuD degrades hemin to iron and mycobilin where  $\alpha$ -meso carbon retains as a formyl group by sequential mono- and di-oxygenase reactions [3]. The first monooxygenase reaction converts hemin to meso-hydroxyheme intermediates followed by the dioxygenase reaction to form mycobilin and free iron [4]. The IsdG reaction catalyzes conversion of hemin to staphylobilin isomers with the release of iron and formaldehyde [5]. MhuD and IsdG catalytic mechanisms will be discussed at the meeting.

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## IL-002

### Copper(I) and Copper(II) Binding to R1 and R3 Fragments of Tau Protein

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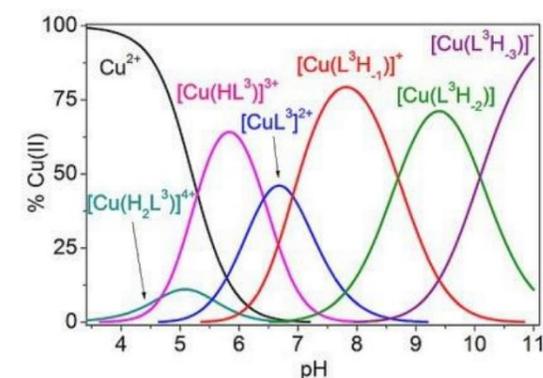
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Tau ( $\tau$ ) is a 441-mer peptide present in significant amounts in neurons, where it contributes to the stabilization of microtubules. Insoluble amyloid aggregates of tau are associated with over 20 neurological disorders known as tauopathies, among which is Parkinson's [1]. In neurons, tau binds tubulin through its microtubule binding domain which comprises four repeats (R1-R4) characterized by the presence of histidine residues. These regions are potential binding sites for metal ions [2]. The elucidation of the binding capacities toward metal ions, especially those redox active such as copper(II), may shed light on the biomolecular processes that underlie the progression of tauopathies [3]. In this contribution we examine the thermodynamic stability of copper(I) and copper(II) adducts with two peptide fragments which are encompassed in the R1 and R3 repeats of tau, namely Ac-<sup>257</sup>VKSKIGSTENLKHQGGG<sup>273</sup>-NH<sub>2</sub> (R1 $\tau$ , HL<sup>1</sup> in its neutral form) and Ac-<sup>323</sup>GSLGNIHKKPGGG<sup>335</sup>-NH<sub>2</sub> (R3 $\tau$ , L<sup>3</sup> in its neutral form).

Copper(II) binding to R1 $\tau$  (HL<sup>1</sup>) at pH 7.4 is dominated by the formation of [Cu(HL<sup>1</sup>)]<sup>2+</sup>, where (L<sup>1</sup>) is tridentate. The copper(II) equatorial coordination positions are occupied by the imidazole ring of His269, two amido nitrogens, and a water molecule. As for the R3 $\tau$  (L<sup>3</sup>) peptide, at pH 7.4 the two most abundant species are [CuL<sup>3</sup>]<sup>2+</sup> and [Cu(L<sup>3</sup>H<sub>2</sub>)]<sup>+</sup> (in a ratio of ca. 1:4, Figure 1, left). While copper(II) coordination mode in [Cu(L<sup>3</sup>H<sub>2</sub>)]<sup>+</sup> is similar to that in [Cu(HL<sup>1</sup>)]<sup>2+</sup>, that of [CuL<sup>3</sup>]<sup>2+</sup> is different and possibly most interesting. Spectroscopic data suggest that in [CuL<sup>3</sup>]<sup>2+</sup> two imidazole donors and one amido nitrogen are equatorially coordinated to copper(II), plus a water molecule (Figure 1, right). The presence of this tandem HisHis fragment makes this peptide interesting in view of the stabilization of copper(I). Indeed, spectroscopic competition titration using a metallochromic indicator clearly showed that copper(I) binds significantly to R3 $\tau$  at neutral pH but not to R1 $\tau$ . The catalytic activity in reactions of oxidation of catecholes and the NMR features of these complexes will be discussed in terms of the speciation of the thermodynamic stability of these complexes with copper in both oxidation states. The authors acknowledge MIUR for financial support through the project "Metal ions, dopamine, and oxidative stress in Parkinson's disease" (PRIN 2015T778JW).



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IL-003

## Molecularly-Targeted Photodynamic Therapy (mtPDT) for a Cancer-Related *RAS* mRNA G-Quadruplex by an Anionic Phthalocyanine with Zinc Ion

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The RAS-targeted cancer therapies should be effective because RAS plays critical roles in malignant degeneration of cancer cells. However, current developments on targeting RAS have not advanced due to structural features of the RAS protein and high affinity of the RAS protein with its ligand. Here, we show that expression of NRAS, a major isoform of RAS, is controllable by photodynamic therapy (PDT) with a new photosensitizer, an anionic phthalocyanine with zinc ion (ZnAPC, Fig. 1a), targeting a G-quadruplex structure (Fig. 1b) formed by 5' UTR of the *NRAS* mRNA.

Photodynamic therapy (PDT) is a low invasion cancer treatment. On the other hand, PDT also causes saviour side effects and mechanism of action remains unclear yet. These problems limit its wide application in cancer treatment. A molecularly targeted PDT (mtPDT) could be promising to overcome these problems. Here we attempted to establish mtPDT for NRAS mRNA which is overexpressed in some cancer cells.

Previously, we have reported that anionic phthalocyanines (APCs) coordinating Ni<sup>2+</sup> or Cu<sup>2+</sup> bound to DNA G-quadruplexes derived from human telomeric DNA.<sup>1,2</sup> According to these results, we hypothesised that an anionic phthalocyanine coordinating Zn<sup>2+</sup> (ZnAPC) can control NRAS expression by photo-irradiation because it has a high photosensitizing ability among phthalocyanine derivatives. Binding studies showed that ZnAPC bound to a G-quadruplex-forming oligonucleotide derived from 5' UTR of the *NRAS* mRNA in a sequence selective manner even in the presence of excess double-stranded RNA oligonucleotides. After photo-irradiation with near IR light, ZnAPC cleaved NRAS RNA G-quadruplex but not other cancer related RNA G-quadruplexes and duplexes. Cell based assays using MCF-7 cells, in which NRAS was overexpressed, demonstrated that ZnAPC reduced expression of *NRAS* mRNA and NRAS protein, and therefore induced cell death.

Noteworthy, it was further shown that ZnAPC was a capable to induce a direct transfer of energy to NRAS mRNA and induced its breakdown upon photo-irradiation even under a low-oxygen condition, which is a typical feature of solid tumours. These results demonstrate that ZnAPC is a promising photosensitizer in the mtPDT targeting *NRAS* mRNA G-quadruplexes in solid tumors and ROS resistance cancers.<sup>3</sup>

Financial support by JSPS KAKENHI (18K19153) and a Grant-in-Aid for Scientific Research on Innovative Areas "Chemistry for Multimolecular Crowding Biosystems" (17H06351).

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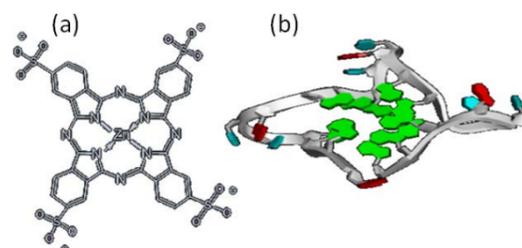


Figure 1. (a) Chemical structure of ZnAPC. (b) Schematic structure of a parallel G-quadruplex.

IL-004

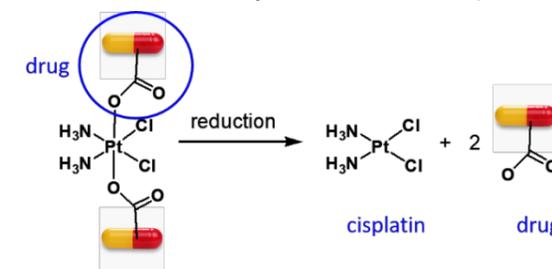
## Novel Approaches to New Classes of Multi-Action Pt(IV) Anticancer Agents

Dan Gibson<sup>1</sup>, Tomer Babu<sup>1</sup>, Subhendu Karmakar<sup>1</sup>, Amrita Sarkar<sup>1</sup>, Thirumal Yempala,<sup>1</sup> Claudia Schmidt,<sup>1</sup> Maisaloon Ishan<sup>1</sup>, Valentina Gandin<sup>2</sup>

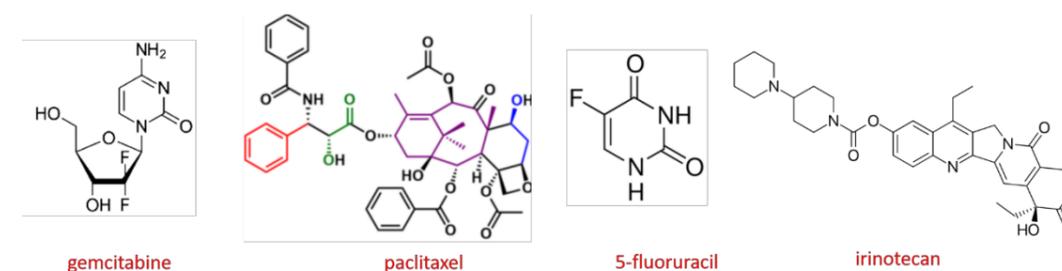
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In attempts to overcome the major drawbacks of the Pt(II) anticancer agents, toxicity and resistance, many researchers have chosen to pursue multi-action Pt(IV) prodrugs. Pt(IV) prodrugs are stable outside the cell and are activated by reduction inside the cell releasing the cytotoxic Pt(II) complex as well as the two axial ligands that can be drugs in their own right. To date, most of the bioactive ligands possess a carboxyl group through which they are tethered to the Pt(IV). This is probably due to the ease of carboxylation of Pt(IV) complexes with axial hydroxido ligands, and to the fact that upon reduction of the Pt(IV), the axial ligands are released immediately in their active form (scheme).



Many well designed and potent dual, triple and quadruple action Pt(IV) prodrugs were described using bioactive molecules with carboxyl groups.<sup>1-3</sup> Yet, we believe that we need to be able to expand the arsenal of bioactive ligands to molecules without carboxyl groups especially since often drugs without carboxyl groups such as gemcitabine, paclitaxel, 5-FU, irinotecan (Figure) and others are administered in combination with Pt drugs in the clinic.



We will describe the approaches we have taken to conjugating anticancer drugs such as gemcitabine or erastmustine, that do not have carboxyl groups, to the axial positions of Pt(IV) complexes in a manner that upon reduction they release the original active form of these drugs.

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## IL-005

### Advancing Metalloprotein Design for New Functions and Applications

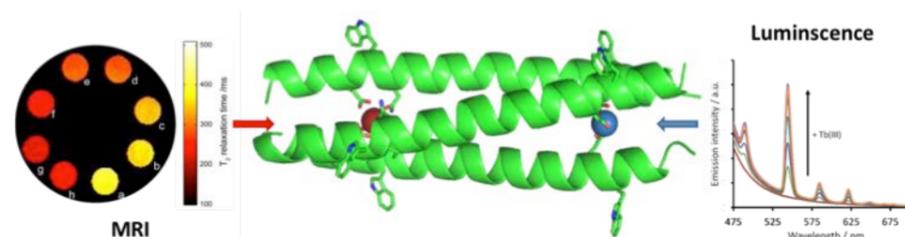
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*De novo* designed miniature protein scaffolds, such as the coiled coil, offer exciting opportunities for metal ion coordination.[1] Not surprisingly due to the protein like nature of the scaffold, the large majority of *de novo* metallocoiled coil examples have focussed their efforts on mimicking the active sites of native metalloenzymes. Our approach is to instead use these artificial proteins as novel ligands for the coordination of xeno metals, with no known biological role, with the view to developing functional systems for valuable applications beyond the scope of nature.

We recently reported the design of the first gadolinium coiled coil, which displayed promise as a potential MRI contrast agent.[2,3] We have since interrogated the coordination of various lanthanide ions to our coiled coils and have for the first time shown that we can selectively discriminate between Ln(III) ions based on size. As a result we have now designed coiled coils capable of binding two different Ln(III) ions selectively to two different sites (see Figure), and at a defined distance from one another. The opportunities this affords and the potential applications of this new class of lanthanide coiled coils, will be discussed.

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## IL-006

### G-Quadruplex Binding Metal Complexes Drugs as Potential Candidate for Radiosensitization

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G-quadruplex structures are secondary structures that form in G-rich repeated sequences of DNA and RNA which are now considered as therapeutic targets. G4-interacting compounds stabilize these structures and can disrupt cellular functions like replication, translation and telomere maintenance. In addition, two of them have shown promising radiosensitizing properties including a derivative of the tetrypyridin platinum complexes [1]. Since an important strategy in cancer therapeutics is to increase tumor sensitivity to radiotherapy, deciphering the mechanism of action for radiosensitization of such complexes is of fundamental concern. In this aim, we screened metal-complexes of different G4 series including terpyridine [2], salphen [3] and platinum conjugates of G4-interacting compounds [4] as well as non-G4-interacting platinum complexes for their radiosensitization potential. They were tested at their subtoxic concentrations in combination with  $\gamma$ -radiation. Four hits, belonging to different series, were found to induce radiosensitization in three cancer cell lines but not in a normal fibroblast cell line. We then focused on exploring their molecular mechanism of radiosensitization. The mandatory need of drugs post-radiation and the presence of delay in DNA damage repair post-irradiation by Pt-tpty (one of the hits), strongly suggests involvement of DNA repair inhibition. Interestingly, telomeric damages pre-or post-irradiation don't seem to be at the origin of radiosensitization by Pt-tpty and reinforce the finding that these metal-complexes could interfere with radiation induced DNA damages repair in a G4-independent manner.

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IL-007

## Pt-Fe Heterobimetallic Ferrocenyl Derivatives Show Cytotoxicity on Highly Proliferative Cells

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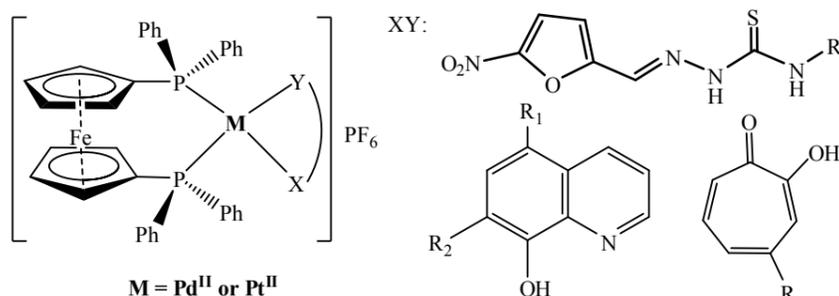
Parasitic diseases, caused by trypanosomatid protozoa, like *Trypanosoma brucei*, mainly affect developing countries and constitute a major health concern. Cancer is also a main public health problem worldwide. Current chemotherapies for the treatment of both types of diseases are inadequate. Trypanosomatid parasites and cancer cells are highly proliferative cells showing metabolic similarities. Based on this, our group is currently focused on the rational design of new organometallic prospective metal-based drugs for the treatment of at least two of these diseases. The strategy involves incorporating bioactive ligands, pharmacologically active metals and selected organometallic cores in the same molecule. In this context, twenty two Pd and Pt 1,1'-bis(diphenylphosphino)ferrocene (dppf) derivatives with different families of bidentate bioactive ligands XY (see Figure) showed interesting activity against *T. brucei*.

Among them, a series of five [Pt<sup>II</sup>(L)(dppf)](PF<sub>6</sub>) compounds, with HL = 8-hydroxyquinoline derivatives, was synthesized and fully characterized. Crystal structures were solved by XRD. The compounds were evaluated on the bloodstream form of *T. brucei*. Selectivity of the compounds towards the parasites was tested using J774 macrophages as a mammalian cell model. The compounds displayed fairly good activity against *T. brucei*, showing IC<sub>50</sub> values in the submicromolar range (IC<sub>50</sub>: 0.14 - 0.93 μM), and good selectivity towards the parasite (SI: 11 - 48). Coordination to the {Mdppf} moiety led in most cases to an enhancement of the activity of the bioactive ligands (11 to 41 fold). Selected compounds could be considered new hits for the development of prospective agents against trypanosomatid parasites.

QSAR studies involving the whole series of twenty two M-dppf-XY compounds showed a correlation between antitrypanosomal activity, lipophilicity and electronic effects of the bioactive ligands.

Cytotoxicity was assessed on A2780/A2780cisR ovarian tumoral cell lines by the MTT assay. Four [Pt<sup>II</sup>(L)(dppf)](PF<sub>6</sub>) compounds were more active (IC<sub>50</sub>: 1.2-4.4 μM) than cisplatin (IC<sub>50</sub>: 26.0 μM) on A2780 cells and also showed far superior activity than cisplatin in the cisplatin resistant A2780cisR cells. The four active compounds induced ROS formation in A2780 cells detected by using the fluorescent probe H<sub>2</sub>DCFDA (dihydro-2'-7'-dichlorofluorescein diacetate). Work is in progress to correlate the cellular uptake with the cytotoxic activity by ICP-MS.

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IL-008

## Bacterial and Mitochondrial Respiratory Supercomplexes

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Complexes III and IV of the respiratory chain often form supercomplexes with a III<sub>2</sub>IV<sub>2</sub> or III<sub>2</sub>IV stoichiometry. In *S. cerevisiae* these supercomplexes appear to be dynamic in nature held together by relatively weak interactions. A bound water-soluble cyt. *c* [1, 2] may provide a direct route for electron transfer from CIII to CIV through two-dimensional diffusion. In other organisms such as *M. smegmatis* or *C. glutamicum* the III<sub>2</sub>IV<sub>2</sub> supercomplexes are obligate, stable and establish a direct electron path between the electron donor, QH<sub>2</sub>, and the acceptor O<sub>2</sub> [3]. We recently determined structures of the *S. cerevisiae* [4] and *M. smegmatis* [5] CIII-CIV supercomplexes using cryo-EM. The former shows that the components associate presumably without any changes in structure that would yield differences in functionality of CIII or CIV. Instead, the proximity of CIII and CIV would yield diffusion times of cyt. *c* comparable to the turnover activity of CIII and CIV (~10<sup>2</sup> s<sup>-1</sup>). In *M. smegmatis* the structure of the III<sub>2</sub>IV<sub>2</sub> supercomplex reveals a completely new architecture: (i) a di-heme cyt. *cc* subunit functionally replaces cyt. *c*<sub>1</sub> and the soluble cyt. *c* in canonical electron transport chains. This cyt. *cc* subunit displays two conformations in the two halves of the III<sub>2</sub>IV<sub>2</sub> supercomplex indicating that it can act as an alternating electric switch. In one half it provides a direct electron-transfer pathway between CIII and CIV while in the other half the connection is interrupted. (ii) we identified a novel superoxide dismutase (SOD) subunit that is attached near the cyt. *cc* domain. Assuming a functional role of the SOD in the supercomplex, it may funnel electrons from O<sub>2</sub><sup>-</sup>, via cyt. *cc*, to the CIV catalytic site. Alternatively, the product hydrogen peroxide may be used as a substrate for CIV (i.e. an electron acceptor). (iii) we observed a menaquinone bound in an electron-donating Q<sub>o</sub>-site of CIII, which has not been identified in any other structure. The novel Q<sub>o</sub> site is formed by a cardiolipin, a 200 amino-acid residue extension of the FeS subunit and the cyt. *cc* subunit. (iv) a number of cardiolipin molecules mediate the CIII-CIV interactions and the lipid is also found in an O<sub>2</sub>-conducting channel in CIV. (v) a 150 amino-acid residue extension of cyt. *b* (of CIII) forms a “lid” covering the orifice of the D-proton pathway of CIV. Because this pathway is used for transfer of protons that are pumped across the membrane, the structural arrangement suggests functional interactions between CIII and CIV. Collectively, the data offer new insights into the functional role of respiratory supercomplexes.

Financial support by the Knut and Alice Wallenberg Foundation and the Swedish Research Council.

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## IL-009

### How Heavy Metal Binding to the LINE-1 ORF1p May Influence Human Retrotransposition

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Long interspersed nuclear elements-1 (L1) are autonomous retrotransposons that encode two proteins in different open reading frames (ORF1 and ORF2). The ORF1p, which may be an RNA binding and chaperone protein, contains a three-stranded coiled coil (3SCC) domain that facilitates formation of the biologically active homotrimer. This 3SCC domain is composed of seven amino acid (heptad) repeats as found in native and designed peptides and a stammer that modifies the helical structure. Cysteine residues occur at three hydrophobic positions (2 **a** and 1 **d** site) within this domain. Based on comparison to *de novo* designed peptides that contain cysteine layers in the hydrophobic core of 3SCCs, it is likely that ORF1p binds transition or heavy metals with relatively high affinities, a feature that has not been previously recognized. We have, therefore, investigated the binding of several metals to both a truncated form of the ORF1p and designed peptides, with and without stammers, intended to mimic metal binding. Based on UV-Vis, NMR, XAS spectroscopies and X-ray Crystallography we are able to demonstrate that a variety of metals (Pb(II), Cd(II) and Cu(I)) can bind to these sites. Furthermore, we will provide a hypothesis as to how metal binding may influence retrotransposition in humans depending on whether metals bind upstream or downstream of the stammer found in the coiled coil region.

## IL-010

### Metallo-Protein-Inspired Transition-Metal Coordination G-Quadruplex DNA

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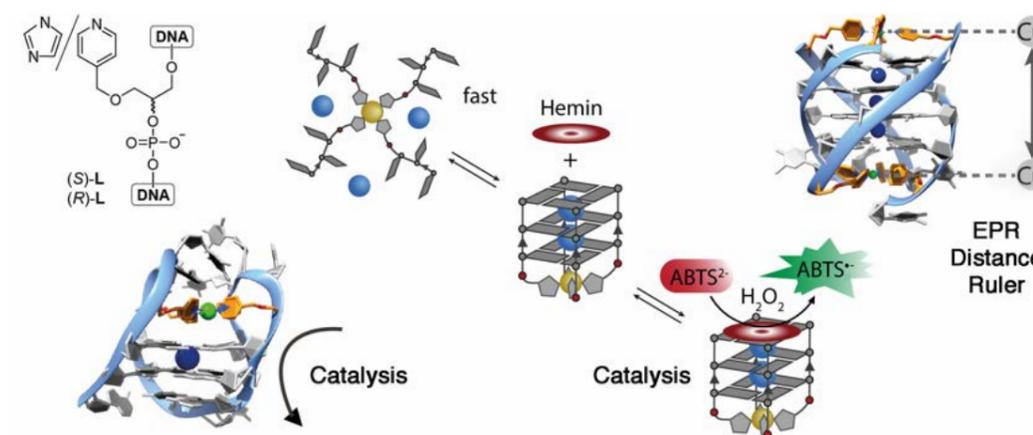
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The concept of metal base pairing, where bonds between transition metal cations and chemically modified nucleobases lead to defined coordination complexes inside oligonucleotides, has been insensitively studied in the context of double stranded DNA [1]. So far, reports about metal-base interactions in other DNA secondary structures remained scarce. We recently introduced a robust and synthetically feasible approach to incorporate ligands such as pyridines or imidazoles into DNA G-quadruplexes [2]. Both tetramolecular as well as unimolecular quadruplex sequences showed to be suited for the incorporation of one or more metal binding sites. By using telomeric sequences containing transition metal binding sites close to the loop region, we demonstrated copper-induced topology switching between two different G-quadruplex folding states [3].

Further, the known DNA-protein interaction between a quadruplex sequence and thrombin could be controlled in a metal-dependent way, as monitored by a fibrinogen clotting assay [3]. Recently, we expanded the scope of ligand derivatives [4] and used the incorporation of paramagnetic copper(II) centers on both remote faces of the guanine stacks to establish an EPR-based distance ruler that contributes to the toolbox of DNA structure elucidation methods [5].

Our current activities are focused on DNase function [6] and enantioselective catalysis with metal-carrying G-quadruplexes, as their straightforward synthetic accessibility and well-defined coordination environments allow for a high degree of fine-tuning the catalytic activity. Furthermore, the single-oligonucleotide nature of the folded telomer sequences allows us to mix different ligands in the pocket created by the loop, thereby opening the potential to mimic metallo-protein activity inside an oligonucleotide environment.



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IL-011

## Multiaction Platinum Anticancer Complexes

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Platinum(II) anticancer drugs are used for approximately half all chemotherapeutic treatments, even though there are significant clinical disadvantages such as acquired resistance, cross-resistance and severe side effects that need to be overcome. This challenge requires innovative design of compounds with different modes of action.<sup>1-3</sup> In this search we have designed, and developed an innovative “proof of concept” Quad-Action Pt(IV) prodrug (cis-DCA-Pt56MeSS-PhB, Figure 1) that releases four different bioactive moieties, simultaneously inside the cancer cell. The cytotoxicity determined in 2D and 3D cancer cells is substantially better than cisplatin. cis-DCA-Pt56MeSS-PhB is 200-450 fold more cytotoxic than cisplatin against KRAS mutated colon and pancreatic cancers (Table 1). This is important because RAS proteins play a key role in regulating cell differentiation, proliferation, and survival and KRAS is mutated in resistant cancers such as 90% of pancreatic adenocarcinomas, 45% of colorectal cancers, and 35% of lung adenocarcinomas. The selectivity index is better than cisplatin by two-fold, suggesting that there is a preferential cytotoxicity towards neoplastic cells. It induces DNA platination, mitochondrial membrane hypopolarization, perturbation of the microtubule cytoskeleton, HDAC inhibition and Cytochrome C release each contributing to effective cell death.

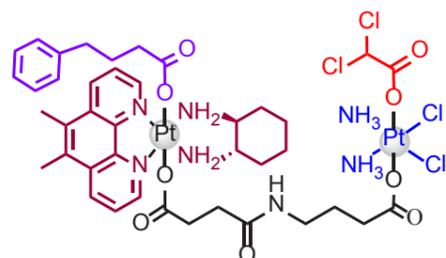


Figure 1 – Chemical Structure

Table 1 – IC<sub>50</sub> of platinum complexes

Complex	IC <sub>50</sub> (μM) ± S.D Wild type			IC <sub>50</sub> (μM) ± S.D. KRAS mutated cell lines		
	HCT-15 colon	A375 melanoma	BxPC3 pancreas	PSN1 pancreas	MIA-PaCa- 2 pancreas	LoVo colon
cisplatin	15.28 ± 2.6	3.11 ± 0.98	6.17 ± 1.37	18.25 ± 3.11	13.45 ± 2.45	9.12 ± 1.35
oxaliplatin	1.15 ± 0.43	6.30 ± 2.01	4.22 ± 0.87	8.25 ± 3.42	-	-
Pt56MeSS	0.69 ± 0.11	1.12 ± 0.42	3.25 ± 0.84	0.25 ± 0.08	1.10 ± 0.23	0.76 ± 0.21
<b>cis-DCA-Pt56MeSS-PhB</b>	<b>0.36 ± 0.08</b>	<b>0.08 ± 0.01</b>	<b>0.19 ± 0.03</b>	<b>0.09 ± 0.02</b>	<b>0.06 ± 0.01</b>	<b>0.02 ± 0.005</b>
<b>Fold Better than cisplatin</b>	42.2	38.8	32.4	202	224	456

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IL-012

## Corroles@Heme Proteins: The Case of the *P. aeruginosa* Heme Acquisition System

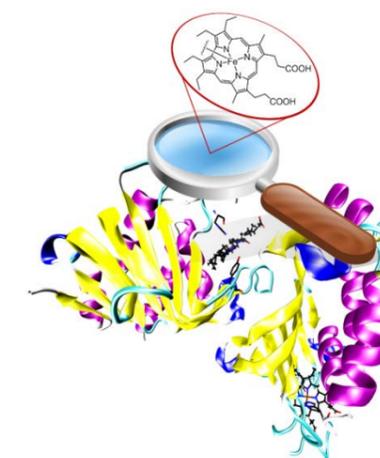
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*Pseudomonas aeruginosa* is a Gram-negative bacterial pathogen which requires iron for growth under aerobic conditions. One of the sources of iron is heme from an invaded host organism. Therefore a hemophore HasA is secreted to scavenge heme and carry it to a membrane bound receptor for internalization.[1] During this process the protein is refolded from an open apo form to a closed holo form, in which the heme iron centre is bound in a *low spin* Fe(III) state by one histidine and one tyrosine axial ligand.[2]

Iron corroles differ from ferric heme by the *intermediate spin* state of the iron atom.[3] The binding of axial ligands is weaker in iron corroles, resulting in a clear preference of five-coordinate over six-coordinate complexes. Therefore iron corroles are suitable probes to investigate details of the heme binding process of wt HasA. The seminar will present the products from such binding studies and discuss the unexpected outcome on the base of bioinorganic model compounds.



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IL-013

**Bioinorganic Redox Signaling and Catalytic Transformations of O<sub>2</sub><sup>-</sup>, NO and H<sub>2</sub>S**

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The mission of our research is in the application of inorganic reaction mechanisms, as well as design and syntheses of redox active molecules for elucidation and modulation of complex (patho)physiological processes involved in redox signalling, paving the way for improvements in human health and the conquering of disease.

This talk will summarize our exciting results in the field of redox signalling induced by manganese complexes and small inorganic species. In particular you will hear about novel strategies for mimicking superoxide-dismutase activity and about non-classical mechanisms for complete dismutation of superoxide to water and oxygen, as well as about bioinorganic chemistry of H<sub>2</sub>S and nitrogen species and a crosstalk between NO and H<sub>2</sub>S.

IL-014

**Effect of the Cobalamin Integrity on the Regulation by the B<sub>12</sub>-Riboswitch from *Klebsiella pneumoniae***

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Riboswitches are RNA molecules situated in the 5'-UTR (untranslated region), which are able to regulate genes expression via changing the RNA conformation upon interaction with a specific metabolite. These molecules consist of two domains; the aptamer where the metabolite is bound and the expression platform, which is the part that finally causes the gene expression regulation. Riboswitches have been also postulated as good antibiotic targets as they regulate crucial metabolic pathways.

A presumable *btuB* riboswitch sequence has been bioinformatically found in the genome of *Klebsiella pneumoniae* and has been proposed as a B<sub>12</sub> riboswitch candidate. In our study, in-line probing experiments have been performed in order to validate the sequence as a riboswitch. A RNA conformational change has been observed in the RNA sequence upon interaction with AdoCbl. In the gene-on conformation, the ribosome binding site (RBS) is free and the *btuB* gene can be expressed. In contrast, after the metabolite interaction, the RNA is shifted to the gene-off conformation in which the RBS is masked in a base paired region. This fact proves the sequence as a riboswitch and suggests a translation inhibition mechanism.

Then, using the isothermal titration calorimetry (ITC) experiments, the thermodynamic parameters of the RNA-metabolite interaction have been recorded. Two different constructs were used: MB01 which contain the aptamer (214 nt.) and JP01 carrying both the aptamer and the expression platform (246 nt.). Titrations using AdoCbl as titrant were performed for both constructs at different temperatures ranging from 15 °C to 30 °C. Taking the results together we could say that the RNA-AdoCbl interaction is exothermic and follows a 1:1 stoichiometry. The enthalpy obtained at 25 °C is - 119±3 kJ/mol for JP01 and -122.7±0.9 kJ/mol for MB01. These enthalpy values are in consonance with a sum of several weak interactions such as H-bond and  $\pi$  stacking. The K<sub>D</sub> values for MB01 (770±90 nM at 25 °C) are higher than those obtained for JP01 (530±50 nM at 25 °C) in the entire temperature range. Thus, we can conclude that the presence of the expression platform in JP01 stabilizes the interaction of the *btuB* riboswitch with the AdoCbl [1].

The inhibition translation mechanism was also proved by coupling the riboswitch to a red fluorescent protein (*mCherry*). The protein synthesis was inhibited after increasing amounts of AdoCbl were added to the culture media. Moreover, it has been also studied the effect of the ancillary ligands' integrity over the translation, by using cyanocobalamin and cobyric acid instead of the entire coenzyme.

Recently, there is a growing research interest in ligand analogues that are tested for antibiotic potential through riboswitch inhibition. With the aim to synthesize an antivitamin of Cbl, we have been able to extract and purify descobaltocobalamin and descobaltocobyric acid from cultures of *Allocromation vinosum*, anaerobically grown in a deficient cobalt medium.

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IL-015

**Iron Homeostasis in Patients with Chronic Obstructive Pulmonary Disease (COPD): Risks of Infection with Administration of Iron (Fe) Products and Transfusions**

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Patients with chronic obstructive pulmonary disease (COPD) experience disrupted iron homeostasis and cellular Fe accumulation, often accompanied by anemia and non-anemic Fe deficiency. Fe content and iron-binding molecules, including ferritin, lipocalin 2, and lactoferrin, are all increased in the lung tissue, sputum, bronchoalveolar lavage fluid, and alveolar macrophages of patients with COPD, and increases are proportional to disease severity and lung function. Increased cellular Fe may be both pathogenic and protective.

We examined the hospital records of all patients admitted with for a non-infectious event, who had an underlying diagnosis of COPD. COPD patients treated with intravenous iron (IV Fe) and/or transfusions of red blood cells (RBC) for iron deficiency anemia (IDA) were compared to a control group of untreated COPD patients. We compared severity of COPD, duration of hospital stay, number and types of infections during hospitalization, and pathogens causing infections. Data collection included demographic information, smoker status, Fe indices (iron, transferrin, ferritin, iron saturation (TSAT), hemoglobin (Hgb), and hematocrit (Hct), hepcidin, Fe products / formulations, and dosages, RBC timing and amount, and administration of Fe chelators. Risk factors for infection were assessed by multivariate logistic regression.

IDA was common in hospitalized patients with COPD. Hgb and Hct increased significantly with IV Fe or RBC (or both) treatment. Correction of the Fe deficiency in COPD patients with IV Fe can improve IDA but may result in an increased risk of infection and an increased hospital stay.

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IL-016

**Extracellular Heme Sensing and Utilization: A Novel Metallotherapeutic Target for the Treatment of *Pseudomonas aeruginosa* Infection.**

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The ability to acquire iron (Fe) is essential to the survival and virulence of bacterial pathogens within the host. In an effort to combat the invading pathogen the innate immune response sequesters iron from the pathogen, a process termed “nutritional immunity”(1). *P. aeruginosa* circumvents these efforts by exploiting a variety of mechanisms to acquire Fe, including the secretion of high affinity Fe<sup>3+</sup>-siderophores (pyoverdine and pyochelin) (2, 3), transport of Fe<sup>2+</sup> by the Feo system (4) and heme acquisition by the heme assimilation (Has) and Pseudomonas heme uptake (Phu) systems (5). Studies in our laboratory have shown *P. aeruginosa* evolves to utilize heme as the primary source of iron, while decreasing its reliance on siderophores (6). Furthermore, we have established the Has and Phu systems play essential but non-redundant roles in heme sensing and transport, respectively (7). Heme sensing is mediated through the interaction of the secreted hemophore HasAp with the HasR receptor that triggers a signalling cascade further upregulating expression of the heme uptake systems (8). Once internalized heme is degraded by heme oxygenase (HemO) to release Fe, CO and biliverdin IX $\beta$  (BVIX $\beta$ ) and BVIX $\delta$  (9). Additionally, the terminal heme metabolite BVIX $\beta$  functions as a positive post-transcriptional regulator of the heme signalling cascade, and a transcriptional activator of several operons encoding systems involved in iron-acquisition (pyochelin) and virulence (proteases and Type III secretion systems). Based on our mechanistic characterization of the heme sensing and transport pathways we will present data on the design, synthesis and activity of novel lead compounds targeting: i) the HasAp-HasR interaction with metallosalophens, and ii) HemO with small molecule inhibitors that target the heme binding site or a recently identified allosteric site (10). Targeting heme uptake at several critical junctures effecting both heme sensing and uptake leads to a global disruption of iron-homeostasis and virulence, while placing less selective pressure on the bacteria to undergo mutation leading to drug resistance. Financial support by the National Institutes of Health (AI102883) is gratefully acknowledged.

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IL-017

## Functional Assembly and Enzyme Mimic of Metal Organic Architectures

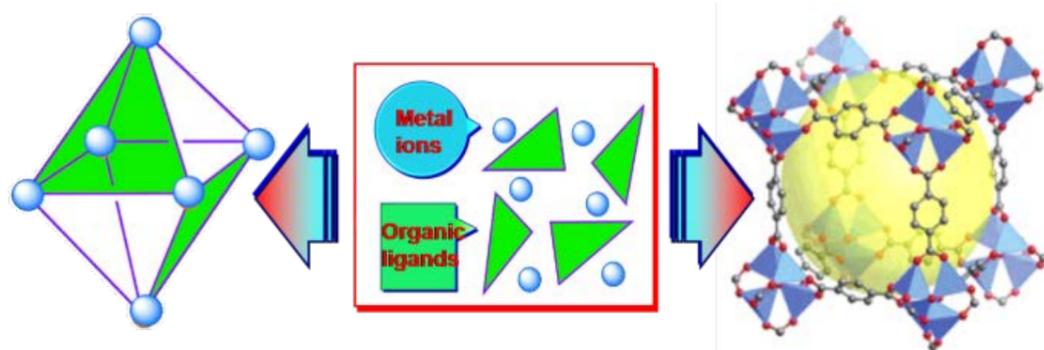
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The highly efficient and selective enzymatic process of life systems is certainly a powerful means to fulfil the need of environmentally friendly chemical activities. An artificial mimetic enzyme, compared to a natural enzyme, is a non-protein molecule and is simpler in structure, and the artificial mimetic enzyme can realize a combination with a substrate and the catalysis of the process. Construction of metal-organic architectures with a unique species of functional molecular containers has obtained increasing attention due to its prominent recognition properties and fascinating reactivity reminiscent of the natural enzymes. These well-defined cavities generated by architectures provided inner void spaces and functional interaction sites for selective encapsulation of specific guest molecules and catalysing related reactions. Because of the promising functionalities of architectures as artificial metalized host platforms, it is possible for these molecular hosts to mimic protein receptors or enzymes and also potential for effectively binding to substrates, stabilizing reactive intermediates or catalysing chemical transformations. The metal-organic hosts with a high activity center structure of enzyme can serve as a carrier for electrons and chemical groups, shorten the distance between reaction substrates so that increase the reaction speed. Herein we reported the synthesis and catalytic properties of several metal-organic hosts to investigate the possibility in the application of enzymatic mimic. These metal-organic architectures could capsule guest molecular through specific inner spaces and bonding sites and realize natural enzymes mimic. Metal-organic polyhedron can retain the enzyme activity and provide a good environment for the catalytic process.

Financial support by the National Natural Science Foundation of China (Grants 21820102001) is gratefully acknowledged.



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IL-018

## [Pt(bpa)]<sup>2+</sup>: a “Light-Switch” for RNA G-Quadruplexes in Live Cells

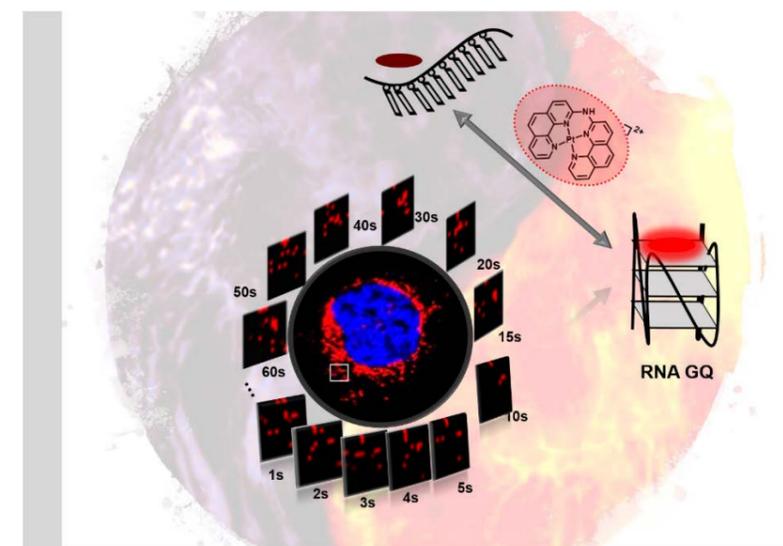
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Though recent studies have shown that RNA G-quadruplexes (GQ) may have pivotal functionalities in many biological and pathological progresses, the transient folding and unfolding of RNA GQ in live cells remain ambiguous, which hampers the understandings of RNA GQ behavior in many disease cells. Recently our group developed a series of [Pt(bpa)]<sup>2+</sup> complexes (bpa: bis(1,10-phenanthroline-2-yl)amine). The complexes with the alkylated *aza*-NH bridge showed unprecedented high stabilizing effects on RNA G-quadruplexes, such as TERRA RNA.<sup>1</sup> Inspired by the duplex-specific fluorogenic mechanism of [Ru(dppz)(bpy)<sub>2</sub>]<sup>2+</sup> complexes, we developed two [Pt(bpa)]<sup>2+</sup> (**1** and **2**) with a free *aza*-NH moiety as a GQ-specific luminescent probe. Both complexes showed high fluorescent intensity (QM~0.45) and large stoke shift upon binding RNA and DNA GQ, while negligible fluorescence was observed in aqueous buffer and on duplex or single stranded DNA/RNA. The planar shape and size of the complexes renders specific binding, but not stabilizing to GQ. A triplet excited state which can be stabilized to have microsecond lifetime due to 1) breaking H-bond network with solvent; 2) restricting thermal vibration/rotation and 3) preventing energy transfer to oxygen when **1** stacks on G-quartets. As fluorogenic probe, **1** was used to stain RNA/DNA GQ in fixed cancerous cells and track the folding dynamics of RNA GQ in live cells. The fluorescent foci of **1** showed HeLa cells have much higher quantity of RNA GQ than the healthy CHO cells at any time during 1 second period. However the fluorescent intensity fluctuated much more significantly in CHO cells, which suggested in general RNA GQs are less stable in healthy cells. By using Pt complexes as fluorogenic probes, the quantity and folding dynamics of RNA GQs are directly compared between cancerous and healthy cells. These inorganic probes hold great potentials in monitoring GQ-targeting molecules in both cancerous and somatic cells, screening the drugs for cancer treatment and evaluating the drug efficiency on targeting GQs.

Financial support by Zhejiang University is gratefully acknowledged.



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IL-019

**Insulin Mimetic Metal-Induced Cellular Events in Metabolic Pathologies. The Case of Diabetes Mellitus II**

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Diabetes is a human pathophysiology, spreading rapidly over the entire world population. It is defined as a group of metabolic abnormalities due to insulin deficiency and/or resistance. A gross distinction of the disease can be made through two types, namely Diabetes mellitus type I and II. Efforts over the years have concentrated on replacing insulin and current therapies or enhancing their activity. To that end, a number of studies support the notion that select metal ions (endogenous or exogenous) could a) enhance or mimic insulin action in vitro and in vivo, b) induce cellular glucose uptake, c) contribute to the control of blood glucose levels, d) induce adipogenesis, and e) interact with essential components of the human body metabolic regulation system [1-4]. Consequently, focusing on early cellular events, culminating with glucose uptake, could shed light onto strategies that could improve considerably current therapeutic protocols or provide new solutions to fighting hyperglycemia. In line with such logic, gaining insight into cellular differentiation of adipose tissue, i.e. the process of adipogenesis, would lead to antidiabetic factors that stand to ameliorate treatment of diabetic patients.

Poised to pursue an investigation of the cellular differentiation of adipose tissue, relying primarily on well-configured complex forms of metal ions (known for their antidiabetic proclivity and new ones), well-defined metal-citrate complexes [Zn(II), Cr(III), Ti(IV), and V(IV,V)] were synthesized in a pH-dependent fashion, isolated and physicochemically characterized in the solid state and in solution. The selection, design and synthetic approaches employed to obtain soluble and bioavailable complex forms of metal-citrates were consistent with the principles of structural speciation, adhered to in all cases of metal ions interacting with the physiological binder of the tricarboxylic acid cycle. The so selected and defined complex species were employed in biotoxicity profile and cellular differentiation studies under physiological conditions and using adipocytes as study models. The biotoxicity profiles of all species were examined in adipocyte cultures to determine which forms should be introduced to the ensuing cell differentiation phase. The process of cell differentiation and maturation of pre-adipocytes to their ultimate mature form was pursued in the presence-absence of insulin (appropriate control). Intimately associated with the process was monitoring closely linked genetic targets, key to adipocyte maturation (PPAR- $\gamma$ , GLUT 1,3,4, Adiponectin (ADIPOQ), Glucokinase (GCK), Insulin receptor (INS-R) and resistin). In parallel, studies were conducted to inquire into the adipogenic potential of the employed binary metal complex forms being suppressed or synergically enhanced by insulin, thereby providing further insight into new mechanistic alternatives in therapeutic administration protocols.

The experimental results a) project distinctly unique biotoxicity profiles for the selected meta-citrate species and commensurably distinct adipogenic biological profiles for the metalloforms involved in a dose-, time- and nature-dependent manner, and b) show clearly metal ion-specific adipogenic response-signals at the same or higher level than insulin toward all selected targets. The adipogenic potential of the structurally select metalloforms, bearing a common hydroxycarboxylic acid chelator substrate, also provides a basis of insulin mimetic comparison toward cellular differentiation of adipocytes, seeking maturation and through it glucose uptake. Collectively, the study model invoked and delved into provides a holistic view of the insulin mimetic metal-induced adipogenicity profile, encompassing well-defined interactions with biomolecular targets known to intervene and effect cell maturation. The so derived knowledge gives rise to reliable molecular biomarkers that support technological advancements toward therapeutic approaches in the fight against Diabetes mellitus II.

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IL-020

**Versatile Metal-Ligand Chromophoric Building Blocks - From Simple Discrete Metal Complexes to Ensembles, Conjugates and Nano-Assemblies for Sensing, Molecular Imaging and Bioassays**

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The major focus of our research is on the molecular design and synthesis of novel inorganic/organometallic metal complexes that may find potential applications as functional metal-based molecular materials. Recent works have shown that novel luminescent metal-based molecular materials could be assembled through the use of various metal-ligand chromophoric building blocks. In this presentation, various design and synthetic strategies together with the successful isolation of new classes of complexes of selected metals will be described. A systematic study of the electronic spectroscopy of the newly synthesized metal complex systems has provided fundamental understanding on the spectroscopic and luminescence origin as well as the structure-property relationship of these complexes. Through a fine control of the interplay amongst various coordination motifs, electrostatic assembly, and non-covalent metal-metal, hydrophobic-hydrophobic and  $\pi$ - $\pi$  interactions, together with the modulation of various photo-induced electron and energy transfer processes, new strategies towards the rational design of luminescent metal-ligand chromophoric ensembles, conjugates and nano-assemblies that would lead to changes in the absorption and emission characteristics and may find potential applications and functions in luminescence sensing, bioassays and molecular imaging have been made.

Financial support from the University Grants Committee Areas of Excellence (AoE) Scheme (AoE/P-03/08) and the General Research Fund (GRF) from the Research Grants Council of the Hong Kong Special Administrative Region, P R China are gratefully acknowledged.

IL-021

## Molecular Mechanism of Enzymatic Chlorite Degradation

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Chlorite dismutases (Clds) are heme *b* containing oxidoreductases found in prokaryotic organisms. They are able to efficiently decompose chlorite (ClO<sub>2</sub><sup>-</sup> or OClO<sup>-</sup>) into harmless chloride (Cl<sup>-</sup>) and dioxygen (O<sub>2</sub>) with chlorite being the sole source of dioxygen. Thereby, a covalent oxygen-oxygen bond is formed, a biochemical reaction that was believed to be unique to the water-splitting manganese complex of photosystem II of oxygenic organisms.

Although structure and steady-state kinetics of Clds have been elucidated [1], many questions remain, e.g. about the mechanism of chlorite cleavage and the pH dependence of the reaction. Computational studies suggested homolytic cleavage of OClO<sup>-</sup> thereby producing chlorine monoxide (ClO<sup>•</sup>) and Compound II [Por...Fe(IV)=O], whereas biochemical studies suggested heterolytic cleavage of chlorite thereby forming Compound I [Por<sup>•+</sup>...Fe(IV)=O] and hypochlorite (HOCl/OCl<sup>-</sup>) as transient intermediates. The pH optimum of chlorite degradation is typically in the acidic pH range and it was postulated that this reflects the protonation state of the strictly conserved flexible distal arginine.

Here, we present high resolution X-ray crystal structures of a dimeric Cld at pH 6.5 and 8.5, its fluoride and isothiocyanate complexes and the neutron structure at pH 9.0 together with the pH dependence of the Fe(III)/Fe(II) redox couple, and the UV-vis and resonance Raman spectral features. We demonstrate that the distal Arg127 cannot act as proton acceptor and is fully ionized even at pH 9.0 ruling out its proposed role in dictating the pH dependence of chlorite degradation. Stopped-flow studies show that Compound I and hypochlorite do not recombine, whereas Compound II is the immediately formed redox intermediate that dominates during turnover. Homolytic cleavage of chlorite is proposed [2].

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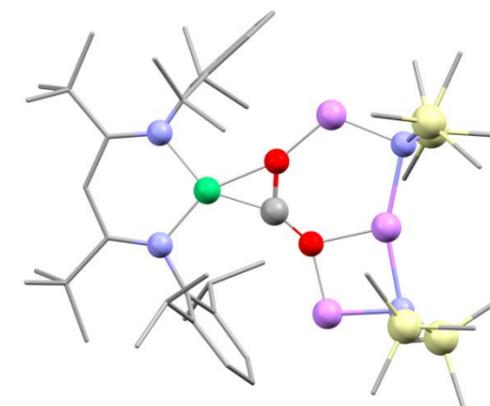
IL-022

## A Biomimetic Nickel Complex with a Reduced CO<sub>2</sub> Ligand Generated by Formate Deprotonation and its Behaviour towards CO<sub>2</sub>

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Reduced CO<sub>2</sub> species are key intermediates in a variety of natural and synthetic processes. In the majority of systems, however, they elude isolation or characterisation due to high reactivity or limited accessibility (heterogeneous systems) and thus formulations often remain uncertain or based on calculations only. We report on a Ni-CO<sub>2</sub><sup>2-</sup> complex (Figure 1) that is unique in many ways.<sup>1</sup> While its structural and electronic features help understanding the CO<sub>2</sub> bound state in Ni,Fe carbon monoxide dehydrogenases,<sup>2</sup> its reactivity sheds light on how CO<sub>2</sub> can be converted into CO/CO<sub>3</sub><sup>2-</sup> by nickel complexes. In addition, the complex has been generated via a rare example of formate β-deprotonation, a mechanistical step relevant to nickel catalysed conversion of H<sub>x</sub>CO<sub>y</sub><sup>z-</sup> at electrodes<sup>3</sup> and formate oxidation in formate dehydrogenases.<sup>4</sup>



**Figure 1** Molecular structure of L<sup>tBu</sup>Ni(OCO κO:κC)Li<sub>3</sub>(N(SiMe<sub>3</sub>)<sub>2</sub>)<sub>2</sub>

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IL-023

## Artificial Metalloenzymes for *in vivo* Catalysis: Challenges and Opportunities

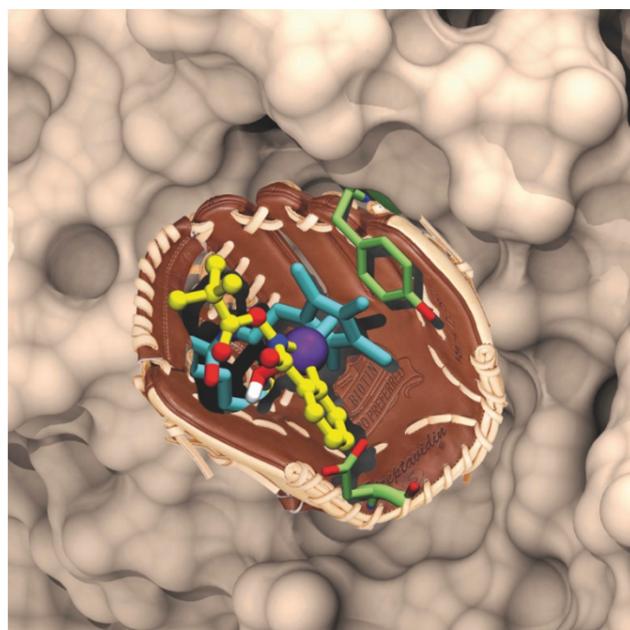
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Artificial metalloenzymes result from the incorporation of a catalytically competent organometallic moiety within a host protein. We and others have been exploiting the potential of the biotin-(strept)avidin technology for the creation of artificial metalloenzymes, Figure. Thanks to the remarkable supramolecular affinity of biotin for either avidin or streptavidin ( $K_D > 10^{-13}$  M), linking of a biotin anchor to a catalyst precursor ensures that, upon stoichiometric addition of (strept)avidin, the metal moiety is quantitatively incorporated within the host protein. Alternatively, human carbonic anhydrase has proven equally versatile for the development of artificial metalloenzymes relying on aryl-sulfonamide anchors to ensure the localization of the abiotic metal cofactors within the host protein.

The resulting artificial metalloenzymes can be optimized either by chemical (variation of the anchor-spacer-ligand moiety) or genetic- (mutation of the host protein) means. These chemo-genetic schemes were applied to optimize the performance for twelve different catalyzed transformations as well multiple reaction cascades in the presence of natural enzymes.<sup>1</sup>

More recently, we have been investigating the potential of artificial metalloenzymes for *in vivo* catalysis to complement metabolic pathways. In this context, *E. coli*'s periplasm as well as surface display have proven particularly versatile to compartmentalize and evolve artificial metalloenzymes while maintaining the critical phenotype-genotype linkage



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IL-024

## Anti-Cancer Platinum(II) Complexes with Non-Canonical Mechanisms of Action Distinct from Cisplatin

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Platinum-based chemotherapy remains standard of care for different types of cancer, with over half of all cancer patients receiving cisplatin, carboplatin or oxaliplatin in chemotherapeutic treatment. The clinically used Pt(II) drugs undergo hydrolysis to form active  $[L_2Pt]^{2+}$  species (L = non-labile amine ligands) which crosslinks with DNA, eliciting DNA damage, inhibiting DNA repair mechanisms and consequently inducing apoptosis. However, cisplatin has significant side effects and frequently evokes the cancer drug resistance. Herein described are a panel of anti-cancer Pt(II) N-heterocyclic carbene (NHC) complexes which exert their activity *via* mechanisms distinct from cisplatin and its derivatives [1]. We have prepared Pt(II) complex with bis-N-heterocyclic carbene (bis-NHC) non-labile ligand and *O,O'* chelating leaving group. The Pt(II) complex overcomes cisplatin resistance in cancer cells and displays significant tumor growth inhibition in mice with higher tolerable doses compared to cisplatin. NanoSIMS imaging of the cellular Pt(II) species showed localization in the cytoplasm but little association with nuclear DNA. The bis-NHC ligated Pt(II) complex formed adducts with cysteine thiols. An unbiased thermal proteome profiling experiment identified asparagine synthetase as a plausible anti-cancer protein target which contains an active site cysteine residue targetable by the Pt(II) complex. We have also designed cyclometalated Pt(II)-NHC complexes which are stable under physiological conditions and engage multiple protein targets via non-covalent interactions. These complexes target proteins involved in cancer cell migration and invasion, and markedly suppress the metastatic tumor growth in mice [2,3]. PEGylation of the Pt(II) complexes further improves the delivery to tumor and biodistribution in mice. Furthermore, the rich luminescent properties of Pt(II)-NHC complexes can be exploited for intracellular tracking and for detection of specific biomolecular structures owing to drastic changes in both emission wavelength and intensity upon subtle change in local microenvironment. We have developed a series of luminescent Pt(II)-NHC complexes for detection of mismatched DNA with potential for analysis of cancers with mismatch repair deficiency [4].

We acknowledge the support from the Innovation and Technology Fund (ITS/488/18).

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IL-025

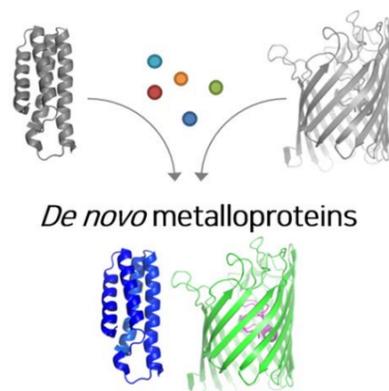
## De Novo Design of $\alpha$ -Helical and $\beta$ -Barrel Metallohydrolases

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De novo design enables us to transform diverse proteins into metalloenzymes, possibly mimicking the emergence and evolution of natural metalloenzymes [1]. Previously, two Zn-dependent metallohydrolases have been created from an  $\alpha$ -helical protein [2, 3]. Although their overall structures and catalytic activities are comparable, the level of sequence optimization is dissimilar, leading us to propose that the location of their metal-active sites might be related to protein evolvability. We have further engineered the less evolvable protein to increase local flexibility, resulting in a series of mutants with enhanced catalytic activities and/or protein stability [4]. In parallel to the studies of  $\alpha$ -helical proteins, we have also employed a  $\beta$ -barrel membrane protein as a novel platform for de novo metalloenzyme design [5]. Although the scaffold is natively neither metalloprotein nor enzyme, structure-based rational design and directed evolution allow us to create metallohydrolases with substantial levels of hydrolytic activities. These studies suggest that catalytically active metal-binding sites can be built on the proteins with few restriction directly related to the secondary structures and/or protein solubility, and therefore, diverse metalloenzymes can be created with desirable structures and functions.

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IL-026

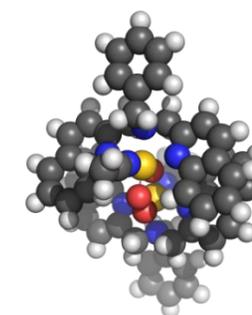
## Linking Spectroscopy to Chemical Structures of Short-Lived Species

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The selective activation of inert molecules and C-H bonds often involves transition-metal complexes in homogeneous solution. However, there is a competition between getting a catalyst as active as possible, and getting it stable enough for characterization: one cannot have both (Que's structure-reactivity paradox). Through joint efforts of experiments and theory, a direct connection can be made between (time-resolved) vibrational and optical spectroscopy, and the chemical structures, as verified by theory and computational spectroscopy. The use of spin-state consistent density functional approximations (like S12g) has been shown to lead to consistent results for (dinuclear) oxoiron, copper, nickel, manganese complexes in oxidation chemistry. Similarities and differences of some examples will be highlighted with an outlook for the future.



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IL-027

## Flavin-Mediated Biorthogonal Catalysis For Drug Activation

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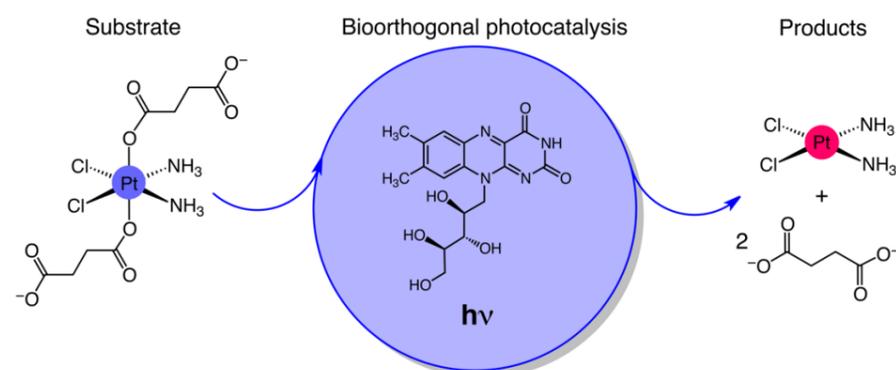
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The light-induced reactivity of transition metal complexes has been successfully tailored to kill cancerous cells by novel mechanisms of action that may help overcoming drug resistance. For this reason, photoactivatable metal complexes have been intensively investigated as agents for photochemotherapy and a Ru polypyridyl photosensitizer has recently entered clinical trials for PDT in Canada.<sup>1</sup>

Over the last few years, my group has focused on the development of new strategies for the photoactivation of Pt(IV) anticancer complexes, one of the most promising class of prodrugs<sup>2</sup> considering the unmatched clinical background available for Pt drugs.

In this contribution, I will discuss how flavin catalysis can be applied to transform Pt(IV) anticancer prodrugs into their biologically active counterparts with high efficiency and bioorthogonal selectivity.

Financial support by the Spanish MINECO (CTQ2016-80844-R) and by the Marie Skłodowska Curie - IF H2020 (grants 746976 and 793702) is gratefully acknowledged.



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IL-028

## Metalloprotein Design Using Genetic Code Expansion

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Photosensitizers, which harness light energy to upgrade weak reductants to strong reductants, are pivotal components of the natural and artificial photosynthesis machineries. However, it has proved difficult to enhance and expand their functions through genetic engineering. Here we report a genetically encoded, 27 kDa photosensitizer protein (PSP), which facilitates the rational design of miniature photocatalytic CO<sub>2</sub>-reducing enzymes. Visible light drives PSP efficiently into a long-lived triplet excited state (PSP\*), which reacts rapidly with reduced nicotinamide adenine dinucleotide to generate a super-reducing radical (PSP dot), which is strong enough to reduce many CO<sub>2</sub>-reducing catalysts. We determined the three-dimensional structure of PSP\* at 1.8 Å resolution by X-ray crystallography. Genetic engineering enabled the site-specific attachment of a nickel-terpyridine complex and the modular optimization of the photochemical properties of PSP, the chromophore/catalytic centre distance and the catalytic centre microenvironment, which culminated in a miniature photocatalytic CO<sub>2</sub>-reducing enzyme (mPCE) that has a CO<sub>2</sub>/CO conversion quantum efficiency of 2.6%. Recently, we have significantly improved the light harvesting efficiency of PSP and TON of mPCE. These unpublished results will be discussed in the meeting.

Financial support by the National Key Research and Development Program of China; the National Science Foundation of China and Chinese Academy of Sciences are gratefully acknowledged.

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IL-029

## Inspired by Nature: Model Metalloenzymes for Energy Conversion

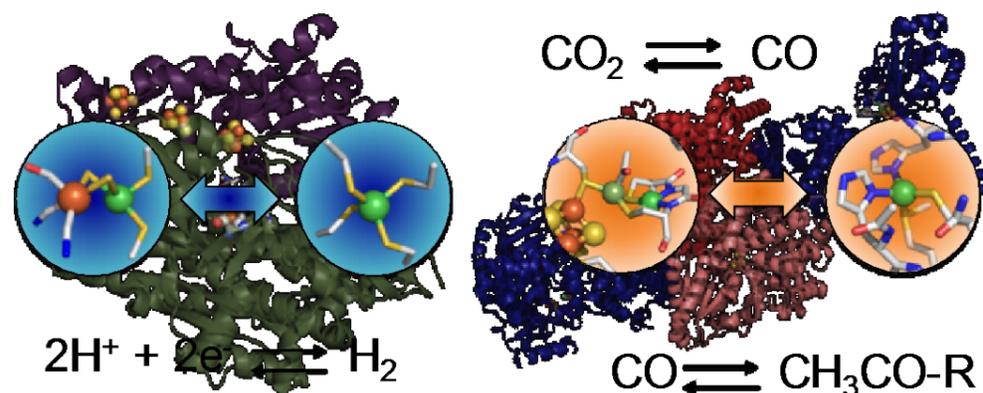
Hannah S. Shafaat,<sup>1,2</sup> Jeffrey W. Slater,<sup>2</sup> Anastasia C. Manesis,<sup>2</sup> Camille R. Schneider,<sup>2</sup> Sean C. Marguet,<sup>1</sup> Regina E. Treviño,<sup>1</sup> Lucas C. Lewis,<sup>1</sup> Alina Yerbulekova<sup>2</sup>

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Metalloenzymes such as hydrogenase, carbon monoxide dehydrogenase, and acetyl coenzyme A synthase use earth-abundant transition metals such as nickel and iron to perform highly efficient energy conversion and storage reactions, such as H<sub>2</sub> evolution and oxidation, CO<sub>2</sub> fixation, and carbon-carbon bond formation. However, the native enzymes are costly to isolate, sensitive to external conditions, and generally poorly suited for large-scale application, rendering anthropogenic utilization impractical. We have approached this problem from a metalloprotein engineering perspective. Using robust scaffolds such as azurin and rubredoxin, we have developed model proteins that mimic the structure and function of these native enzymes. By introducing non-native metals and redesigning the primary and secondary coordination spheres, we have installed novel activity into these simple electron transfer proteins, including catalytic hydrogen evolution and carbon dioxide fixation. Adding key elements from the secondary and tertiary coordination spheres of hydrogenase and CODH enhances both activity and selectivity, pointing to functional roles of specific residues within the natural enzymes. Optical, vibrational, and magnetic resonance spectroscopic techniques have been used in conjunction with density functional theory calculations to probe the active-site structures across different states in order to determine the catalytic mechanisms. These findings will be discussed in the context of identifying the fundamental principles underlying highly active native enzymes and applying those principles towards engineering effective model metalloproteins for energy conversion reactions.

Financial support by the National Science Foundation (NSF, CHE-1454289) and Department of Energy (DOE, DE-SC0018020) is gratefully acknowledged.



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IL-030

## C–H Activation by a Nickel-Dioxygen Complex

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Well-characterized nickel(I) coordination complexes have been shown to react with O<sub>2</sub> under kinetic control yielding a range of nickel-oxygen structure types. Using the archetypal tris(pyrazolyl)borate ligands developed by Trofimenko it is possible to deploy steric control to isolate stable nickel-dioxygen adducts. The species have been fully characterized by a range of spectroscopic, structural and computational methods leading to a detailed understanding of electronic and geometric structures. The resulting nickel(II)-superoxo species activate numerous small molecules. This presentation focuses on the details of intermolecular C–H activation, in particular the mechanistic details of aliphatic oxidations.

Financial support by the US National Science Foundation is gratefully acknowledged.

## IL-031

### Catalytic Organometallic Anticancer Complexes

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Catalytic drugs offer the possibility of carrying out unusual reactions in cells, and hence of introducing new mechanisms of anticancer activity which can fight resistance [1]. I will discuss some of our studies on low-spin d<sup>6</sup> organometallic anticancer catalysts.

Half-sandwich arene iodido Ru(II) and Os(II) azopyridine complexes are relatively inert and activated in cells by catalytic reactions involving the azo bond of the chelated ligand. They kill cells by redox modulation mechanisms, generating superoxide and other ROS [2-4].

Ruthenium(II) arene sulfonyl diamine transfer hydrogenation catalysts can cause reductive redox stress in cancer cells on coadministration with a hydride donor such as formate [5].

An advantage of new osmium(II) arene sulfonyl diamine transfer hydrogenation catalysts is that the active 16-electron catalyst is stable and can be readily isolated, characterised, and used in bio-screening. Asymmetric transfer hydrogenation can be achieved in cancer cells using chiral Os(II) arene catalysts, for example the conversion of pyruvate to unnatural D-lactate [6].

Cyclopentadienyl Ir(III) half-sandwich anticancer complexes can create oxidative stress in cancer cells by converting coenzyme NADH to NAD<sup>+</sup> via formation of Ir-H species [7,8].

Octahedral Ir(III) complexes can transfer spin polarisation to <sup>3</sup>O<sub>2</sub> to generate toxic <sup>1</sup>O<sub>2</sub>, on photoexcitation with one- or two-photon irradiation, an efficient mechanism for killing cancer cells [9]. Interestingly, these photosensitizers can cause specific oxidative damage to proteins, and be delivered to the nucleus by conjugation with serum albumin [10].

We thank the EPSRC, Wellcome Trust, ERC, China Scholarship Council, The Royal Society and Bruker for support, and all our collaborators.

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## IL-032

### Substrate Orientation and Reaction in Thiol Dioxygenases

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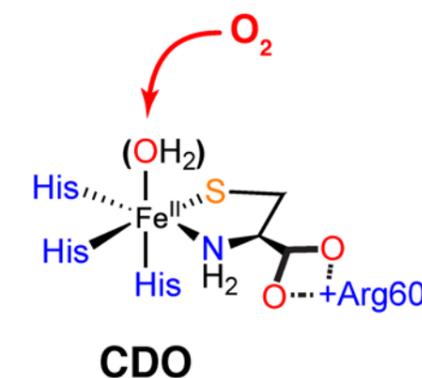
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Thiol dioxygenases are a class of enzymes that catalyse the oxidation of thiols to their corresponding sulfinic acids through the addition of molecular oxygen. Within this class the most well described enzyme is cysteine dioxygenase. The catalytic cycle occurs at a ferrous iron atom coordinated via three histidine residues. Crystal structures, spectroscopic studies and model complexes show cysteine binding via the thiol and amine to leave the sixth coordination site, the dioxygen binding site, partially occupied by a water molecule [1]. The substrate cysteine is held in place through the carboxylate of cysteine forming a salt-bridge with arginine 60. Similar type binding topologies can be proposed for the other carboxylate containing substrates [2]. We have been exploring substrate orientation and its effect on the catalytic cycle through a series of kinetic, spectroscopic and theoretical experiments [3]. We will describe in this presentation the current state of our research.



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IL-033

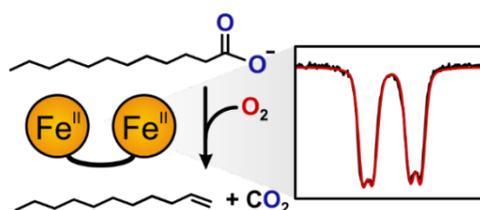
## Emerging Roles of Metalloenzymes for Advanced Biofuel Biosynthesis

Olivia M. Manley<sup>1</sup>, Jose A. Amaya<sup>1</sup>, Ruixi Fan<sup>2</sup>, Yisong Guo<sup>2</sup>, Thomas M. Makris<sup>1</sup>

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Research in my laboratory draws inspiration from the ability of metal-containing enzymes to catalyze diverse and highly selective transformations for the synthesis of molecules with considerable industrial value. This seminar will describe our efforts in understanding the mechanistic basis for two structurally divergent enzymes that convert fatty acids into terminal alkenes via a cryptic decarboxylation reaction. A common theme found in these biocatalysts is the rewiring of iron-containing cofactors that are most often associated with oxygen insertion chemistry to catalyze carbon-carbon cleavage instead. The talk will provide several examples of this functional reprogramming, illustrating how both heme and non-heme iron enzymes can be selectively tuned for new chemical outcomes.



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IL-034

## The Past and Future of Bioinspired Artificial Photosynthesis

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Sustainable and clean energy resources using solar energy are urgently required in order to solve global energy and environmental issues. This lecture focuses on the past and future of bioinspired artificial photosynthesis.[1]

We have developed a variety of photosynthetic reaction center models composed of organic electron donors and acceptors linked by covalent or non-covalent bonding, which undergo efficient charge separation and slow charge recombination.[2] The photocatalytic oxidation of water with O<sub>2</sub> in the air to produce H<sub>2</sub>O<sub>2</sub> has been achieved,[4-6] together with the development of one-compartment H<sub>2</sub>O<sub>2</sub> fuel cells.[7-9] The photocatalytic oxidation of water with O<sub>2</sub> in the air was found to be enhanced significantly in seawater.[10] Thus, the combination of the photocatalytic H<sub>2</sub>O<sub>2</sub> production from seawater and O<sub>2</sub> using solar energy with one-compartment H<sub>2</sub>O<sub>2</sub> fuel cells provides on-site production and usage of H<sub>2</sub>O<sub>2</sub> as a more useful and promising liquid solar fuel than H<sub>2</sub>. [10,11] The solar-driven oxidation of H<sub>2</sub>O by O<sub>2</sub> to produce H<sub>2</sub>O<sub>2</sub> is also combined with catalytic oxidation of benzene by H<sub>2</sub>O<sub>2</sub> to produce phenol, when the overall reaction is solar-driven hydroxylation of benzene by O<sub>2</sub>, which is the greenest oxidant, with H<sub>2</sub>O.[12] Photodriven oxidation of water by p-benzoquinone derivatives (X-Q), which is the first mimicry of Photosystem II, has recently been achieved using a nonheme iron(II) complex ((N4Py)Fe<sup>II</sup>)<sup>2+</sup> to produce O<sub>2</sub> and the corresponding hydroquinone derivatives (X-QH<sub>2</sub>) quantitatively.[13] Financial support by the University of Zurich is gratefully acknowledged.

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IL-035

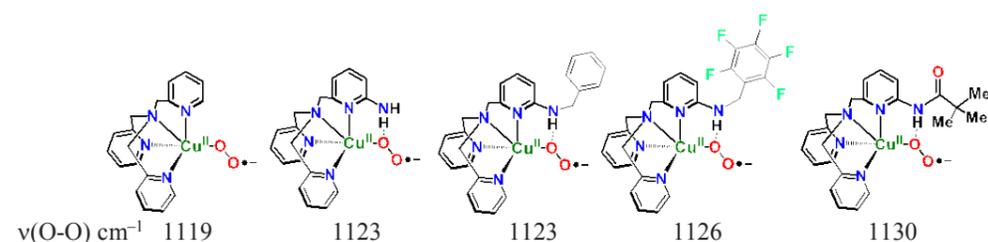
## Cupric-Superoxides, i.e., Primary Copper(I) O<sub>2</sub>-Adducts: Stabilization, Physical-Spectroscopic Properties and Substrate Oxidative Reactivity

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Single copper ion containing copper(I)-dioxygen adducts possessing nitrogen-containing chelating ligands can be best described as cupric-superoxide complexes. Such initial complexes are also found in copper enzymes where they either attack a substrate or are protonated-reduced. Thus, the structures, physical properties and reactivity toward protons-electrons and/or substrates are of fundamental interest in biological and chemical systems where dioxygen-reduction occurs (e.g., also in fuel cells), and where organic substrates are oxidized or oxygenated. Our primary research approach in copper-dioxygen chemistry focuses on ligand design and variation and the use of cryogenic solution handling for the study of new complexes. In these contexts, a description of new (ligand)Cu<sup>II</sup><sub>n</sub>-superoxo complexes (n = 1, 2) will be presented, Tripodal tetradentate ligands with built-in hydrogen-bonding modalities enhance both the stability (i.e., solution lifetime) and reactivity toward O-H (i.e., phenols) and C-H containing substrates. With a binucleating ligand framework, mixed-valent Cu(I)Cu(II) precursors react to form superoxo-dicopper(II) products which can be reversibly reduced to a peroxo-dicopper(II) analogue. For some cases, dicopper(II) superoxo/peroxo reduction potentials have been obtained. Also, superoxo-dicopper(II) complexes can undergo hydrogen-atom abstraction reactions with O-H or C-H substrates, producing a hydroperoxo-dicopper(II) complex product. Aspects of reaction mechanism thermodynamics have been sought, such as the Cu<sup>II</sup><sub>2</sub>-OO-H bond dissociation energy, and will be discussed.

Financial support by the USA National Institutes of Health is gratefully acknowledged.



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IL-036

## Targeted Delivery of Metal Complexes for Precision Oncology

**Trevor Hambley**<sup>1</sup>

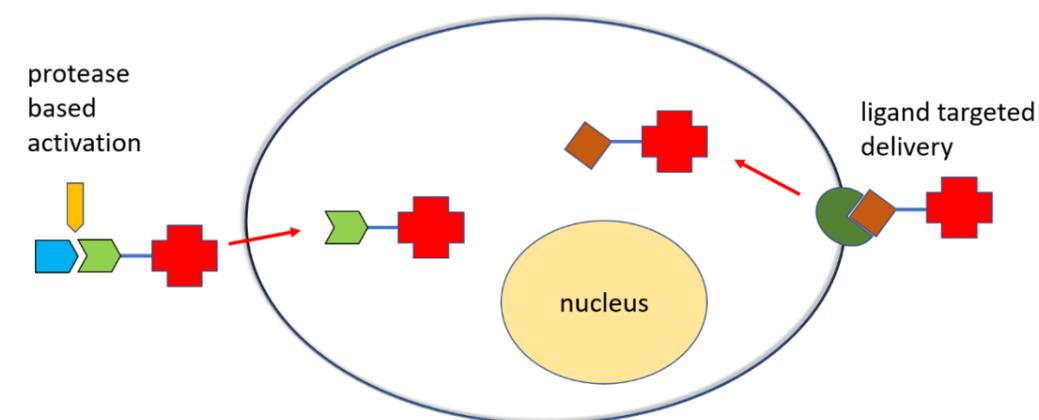
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Precision oncology is a focus of most emerging approaches to cancer treatment. In this presentation, we will argue that for metal-based cytotoxic agents to contribute fully to precision medicine-based approaches to cancer treatment, strategies are required to focus their action, both to tumours themselves and to the various microenvironments that exist within a solid tumour. Such approaches have the potential to reduce the side effects that limit the application and effectiveness of cytotoxic anticancer agents and to generate more durable outcomes.

Precision based approaches to cancer treatment can in principle be based on any features that characterise a tumour and there have been a significant number of studies of using nutrient transporters to selectively deliver platinum complexes to tumour cells that overexpress these transporters [1]. However, there has been little consideration given to using the profile of transporter expression or of protease activity in the tumour environment for developing individualised treatment strategies.

We will report on our work aimed at developing low toxicity pro-drugs which exploit the biological features of different tumour cell properties and the tumour environment with a particular focus on using transporter and protease over-expression to selectively deliver platinum complexes to tumour cells (see Figure). The features required in a complex and the effect of the coordination sphere on the stability and activation of platinum(IV) prodrugs and cobalt(III) based drug delivery vehicles will be described as will examples of strategies for achieving selective uptake of these complexes by cancer cells. We will also discuss the challenges to developing biological systems that are able to replicate the effects of such complexes and establish their potential for use in precision oncology.

Financial support by the Australian Research Council is gratefully acknowledged.



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IL-037

**Use of Stable Vanadyl as a Structural Mimic of the Reactive Ferryl Intermediate in Fe(II)- and 2-(Oxo)-Glutarate-Dependent Oxygenases**

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The Fe(II)- and 2-(oxo)glutarate (Fe/2OG)-dependent oxygenases couple the reduction of O<sub>2</sub> and decarboxylation of 2OG to the generation of the canonical high-spin Fe(IV)-oxo (ferryl) intermediate, which abstracts a hydrogen atom from an aliphatic carbon to initiate its functionalization [1].

While most Fe/2OG enzymes catalyze the hydroxylation of the aliphatic carbon, many other outcomes are known, including halogenation, desaturation, cyclization, epimerization, and endoperoxidation [2].

Current evidence on the Fe/2OG-dependent halogenase SyrB2 and the Fe/2OG-dependent epimerase CarC suggests that the orientation of the C-H bond to be cleaved relative to the ferryl moiety plays a pivotal role in the outcome of the reaction [3,4].

We have recently demonstrated that the stable vanadium(IV)-oxo (vanadyl) ion can be used as a structural model of the reactive C-H-cleaving ferryl by two independent methods (x-ray crystallography and <sup>2</sup>H-HYSCORE spectroscopy) and that the observed spatial disposition is fully consistent with the spectroscopic properties and reactivity of the ferryl intermediate [5,6].

Financial support by the National Institutes of Health (GM-127079 to CK) is gratefully acknowledged

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IL-038

**CO<sub>2</sub> Activation and Reduction by Nitrogenase Fe Proteins and Synthetic [Fe<sub>4</sub>S<sub>4</sub>] Clusters**

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The Fe protein of nitrogenase contains a redox active [Fe<sub>4</sub>S<sub>4</sub>] cluster that plays a key role in electron transfer and substrate reduction. In this work, we show that the Fe protein of *Methanosarcina acetivorans* can reduce CO<sub>2</sub> and CO to hydrocarbons under ambient conditions [1]. Further, we demonstrate that this reactivity is inherent to [Fe<sub>4</sub>S<sub>4</sub>] clusters, showing the ability of a synthetic [Fe<sub>4</sub>S<sub>4</sub>] compound to catalyze the same ambient reaction in solutions. Theoretical calculations suggest a reaction mechanism involving an aldehyde-like intermediate that gives rise to hydrocarbon products upon proton-coupled electron transfer and concomitant removal of water molecules. These results provide a framework for mechanistic investigations of FeS-based activation and reduction of CO<sub>2</sub> and CO while facilitating potential development of FeS catalysts capable of ambient conversion of CO<sub>2</sub> and CO into fuel products.

This work was supported by NSF CAREER grant CHE-1651398 (to Y.H.), a grant-in-aid for scientific research (16H04116) from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT), Hori Sciences and Arts Foundation grant, and Takeda Science Foundation grant (to Y.O.).

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IL-039

### Molecular Catalysis Towards Artificial Solar Generation of Fuels

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Over the past decade, our group has focused on the studies of transition-metal-based molecular systems relevant to the development of artificial photosynthetic molecular devices. The targets of our research involve the studies on (i) water oxidation catalysis in order to uptake protons and electrons required for fuels generation, (ii) catalytic water or CO<sub>2</sub> reduction into sustainable fuels (i.e., H<sub>2</sub>, CO, etc.), (iii) artificial light-harvesting systems towards the effective charge separation and/or migration, and (iv) molecular- and instrumental-level chemical engineering by making hybrid molecular and/or heterogeneous systems using multiple key components. Deeper insights into the mechanism of reaction of interest are always greatly appreciated for the sake of inspiring the rational design strategies towards the more desirable/efficient systems in promoting all relevant processes. In this context, substantial efforts have been devoted to more carefully study the reaction kinetics and equilibria in solution that are relevant to each topic. Various spectrophotometric, electrochemical, and photochemical techniques have been adopted to better understand the mechanistic aspects relevant to all of our systems. Some of the reaction steps of interest are not observable by any experimental techniques, and must be discussed on the basis of our DFT results, which have also greatly helped us understand the mechanism of reactions. Importantly, one of our findings is that, in any catalysis, the reactivity of metal(s) can be rationally tuned by use of redox active ligands that are more or less hybridized with metal(s) in their orbitals. Such issues are often involved in our discussion. One of our interests has concentrated on the molecular Pt-catalyzed hydrogen evolution reactions and their application to fabricate photosensitizer-catalyst hybrid molecular devices [1-3]. Our recent kinetic and electrochemical studies evidence the formation of a hydridodiplatinum(II,III) intermediate when H<sub>2</sub> evolution is catalyzed by a simple mononuclear Pt(bpy)Cl<sub>2</sub> derivative, which is also rationalized by our DFT results. Our studies have also provided new aspects on photo-induced multi-charge separation [4], near-infrared-driven water reduction [5], water oxidation catalysis using various transition metal complexes [6,7], non-precious metal based H<sub>2</sub> evolution catalysis [8], and photoelectrochemical cells for the overall water splitting [9].

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IL-040

### [Mn]-Hydrogenase

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Understanding the choice of Nature in specific metal ions for biochemical reactions is a fundamental subject in biocatalysis. Hydrogenases, enzymes that produce or activate molecular H<sub>2</sub>, use exclusively Fe and Ni. However, other transition metals such as Mn, Co, and Ru are known to activate hydrogen in synthetic systems. Here, we report the development of a Mn-based biomimetic model of [Fe]-hydrogenase. This Mn complex is able to heterolytically cleave H<sub>2</sub> as well as catalyze hydrogenation reactions. Incorporation of the model into an apoenzyme of [Fe]-hydrogenase results in a [Mn]-hydrogenase[1] with enhanced mole-activity over an analogous semi-synthetic [Fe]-hydrogenase[2]. This [Mn]-hydrogenase is the first catalytically active non-native metal hydrogenase. Our findings raise an intriguing question – why does nature choose Fe over Mn?

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## IL-041

### Kinetic Aspects of Cu(II) Exchange and Relevance to Beta-Amyloid Biochemistry

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Cu(II) ions and A $\beta$  peptides coexist in synaptic clefts of glutamatergic neurons, due to involvement of both in neurotransmission. Recently we proposed that N-terminally truncated A $\beta_{4-x}$  peptides rather than “canonical” A $\beta_{1-x}$  peptides may be key Cu(II) targets in this environment. This proposal has been based on our comprehensive studies on Cu(II) binding to A $\beta_{4-x}$  peptides, redox properties of the resulting complexes and their susceptibility to Cu(II) exchange. We found that (i) A $\beta_{4-x}$  peptides bind Cu(II) very tightly ( $K_d = 30$  fM at pH 7.4, 3000 times stronger than A $\beta_{1-x}$  peptides) and compete Cu(II) out from the latter; (ii) CuA $\beta_{4-x}$  complexes are redox inactive in the biologically accessible potential range, cannot be reduced to Cu(I), but undergo irreversible oxidation to Cu(III); (iii) due to a kinetic barrier CuA $\beta_{4-x}$  complexes do not yield copper to metallothionein-3 (MT3), a brain copper scavenger, but such transfer can be enabled by high concentrations of reducing agents [1]. These findings prompted us to formulate a new view on copper/A $\beta$  relations in the brain. A very high stability of CuA $\beta_{4-x}$  and the lack of kinetic barrier between these peptides indicate that A $\beta_{4-x}$  will prevent Cu(II) binding to coexisting A $\beta_{1-x}$  peptides. Thereby, copper will be kept redox silent. The reluctance of CuA $\beta_{4-x}$  to yield copper to MT3 indicates a separate copper transport pathway. Furthermore, we studied the action of neprilysin on A $\beta_{1-x}$  peptides and revealed the formation of the extremely tight CuA $\beta_{4-9}$  complex  $K_d = 6.6$  fM at pH 7.4 [2]. The formation of such small, hydrophilic complex suggests its role in copper clearance. Altogether, A $\beta_{4-x}$  peptides emerge as protecting agents, scavenging synapses from excess of copper and thus enabling continuous neurotransmission.

Our proposal is based on an assumption, shared by all copper/A $\beta$  concepts, that the systems studied are in chemical equilibrium. This assumption is supported by an established view of synaptic cleft as a well-mixed environment. However, neurotransmission may occur on a millisecond scale, comparable to rates of Cu(II) complex formation. Therefore, it is prerequisite to understand the mechanism of Cu(II) association and dissociation from these peptides with respect to copper transporters and reducing agents to elucidate the chemistry underlying synaptic copper recycling of A $\beta_{4-x}$  peptides. From the point of view of Alzheimer’s pathology, it is also important to understand the relation between Cu(II) binding kinetics and seeding of aggregated A $\beta$  species [3]. We established the time scale of several such model processes and discovered the existence of intermediate, partially coordinated complexes in CuA $\beta_{4-x}$  and simpler ATCUN model complexes, with altered reactivity. Stochastic simulations indicate that these species may be involved in fast synaptic Cu(II) scavenging, while the fully formed four-coordinate CuA $\beta_{4-x}$  complexes may present temporary storage for Cu(II), mobilized in slower physiological processes.

Financial support by the National Science Centre (Poland) is gratefully acknowledged, grant 2018/29/B/ST4/01634.

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## IL-042

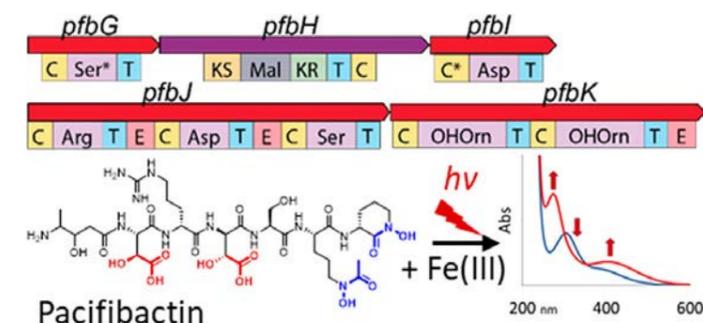
### Microbial Genome Screening for Siderophore Biosynthetic Pathways

Alison Butler<sup>1</sup>, J. Bouvet, J.R. Carmichael, C.D. Hardy, A.M. Jelowicki, C. L. Makris, P. Stow, Z.L. Reitz

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Automated genome mining tools enable high-throughput scanning of bacterial genomes for gene clusters encoding biosynthetic machinery. Natural products produced by nonribosomal peptide synthetases (NRPSs) are particularly amenable to discovery through bioinformatics approaches. A nonribosomal peptide synthetase gene cluster in *Alcanivorax pacificus* encodes the biosynthesis of the new siderophore pacifibactin.<sup>1</sup> The structure of pacifibactin differs markedly from the bioinformatic prediction and contains four bidentate metal chelation sites, atypical for siderophores (see Figure).<sup>1</sup> While adenylation domains specific for hydroxy-ornithine correlate with the presence hydroxamic acid groups in siderophores, adenylation domains specific for aspartic acid correlate with either the presence of L- or D-aspartic acid or the presence of  $\beta$ -hydroxyaspartic acid. In the case of pacifibactin, both aspartic acid residues are hydroxylated, with each present as a different diastereomer. We are particularly interested in the genomics of  $\beta$ -hydroxylation of amino acids which lead to metal binding groups in siderophores,<sup>2</sup> including deciphering the code for the stereochemistry of hydroxylation, as well as the biosynthesis of other metal binding groups in siderophores.<sup>3</sup>

Financial support from the US National Science Foundation is gratefully acknowledged.



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## IL-043

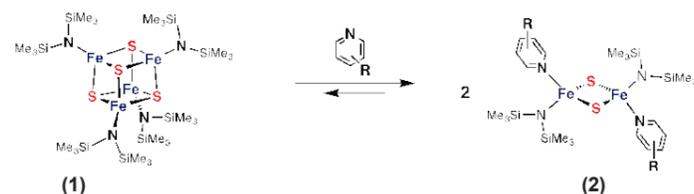
### Electronic and Geometric Flexibility of Iron-Sulfur Clusters: Relevance to the Nitrogenase Active Sites

Kazuyuki Tatsumi<sup>1</sup>, Golam Moula<sup>1</sup>, Kazuki Tanifuji<sup>1</sup>, Yasuhiro Ohki<sup>1</sup>, Tsuyoshi Matsumoto<sup>1</sup>

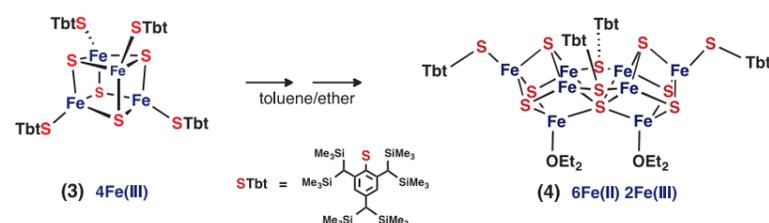
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Iron-sulfur clusters are ubiquitous in nature, and they have been found in various metalloproteins, often exhibiting electron-transfer functions. For instance, the cuboidal  $Fe_4S_4(Cys)_4$  cluster exhibits multiple oxidation states with almost no geometrical changes, and the cuboidal  $[4Fe_4S]$  core structure with the  $2Fe(II)2Fe(III)$  oxidation state is known to be thermodynamically robust. On the other hand, it has been proposed that the  $[4Fe_4S]$  cubane in the fumarate nitrate reductase regulatory (FNR) protein is transformed to dinuclear  $[2Fe_2S]$  cores under  $O_2$ , while the protein dissociates DNA [1]. A similar oxidative decomposition of  $[4Fe_4S]$  in Nif-IscA was postulated to occur generating  $[2Fe_2S]$  cores [2].

We have reported a series of reactions displaying facile interconversion between  $[4Fe_4S]$  and  $[2Fe_2S]$  cores based on the preformed all-ferric cluster,  $[Fe_4S_4\{N(SiMe_3)_2\}_4]$  (**1**) [3]. Treatment of **1** with excess pyridine (py) or pyridine derivatives (py-R) resulted in splitting of the cubane core to  $[Fe_2S_2\{N(SiMe_3)_2\}_2]$  (py-R)<sub>2</sub> (**2**). Conversely, fusion of two  $[2Fe_2S]$  cores of **2** was found to be facilitated by  $B(C_6F_5)_3$ , generating (**1**) and (Py-R) $B(C_6F_5)_3$ . Interestingly reduction of **2** with 1.2 equiv of  $Na[C_{10}H_8]$  in THF afforded  $[Fe_4S_4\{N(SiMe_3)_2\}_4]^{2-}$ , while an analogous reduction of **2** with 0.5 equiv of  $Na[C_{10}H_8]$  gave rise to  $[Fe_4S_4\{N(SiMe_3)_2\}_4]^-$ .



Earlier we reported that the  $[8Fe-7S]$  nitrogenase  $P^N$ -cluster model  $[Fe_8\{N(SiMe_3)_2\}_2(tmtu)_2\{\mu-N(SiMe_3)_2\}_2S_7]$  was synthesized from the reaction of  $Fe\{N(SiMe_3)_2\}_2$ , tetramethylthiourea (*tmtu*), 2, 4, 6-tri-isopropylbenzenethiol (HS-*tip*), and  $S_8$  [4]. A better P-cluster  $[8Fe-7S]$  model complex (**4**) with two bridging Tbt thiolates has been synthesized recently from  $Fe\{N(SiMe_3)_2\}_2$ , HS-*tbt*, and  $S_8$ , by way of all-ferric cuboidal  $Fe_4S_4(S-tbt)_4$  [5,6]. The geometric parameters of the  $[8Fe-7S]$  core of **4** are in fact practically identical to those of nitrogenase  $P^N$ -cluster. This study indicates that the P-cluster  $[8Fe-7S]$  core can be generated from highly-oxidized  $[4Fe-4S]$  cubanes, and that a desulfurization process must be a key to this transformation. Perhaps it is time to seek for new enzymes showing a yet-unknown desulfurization function in nature.



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## IL-044

### Discovery and Application of a Bioinorganic Pathway for the Production of Terminal Alkyne Amino Acids

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Nature has developed biosynthetic routes to the production of a wide range of small molecules. We have been exploring the biosynthesis of a terminal alkyne amino acid made by soil bacteria, and its application to bioorthogonal chemistry. This pathway includes several bioinorganic enzymes that carry out unusual transformations of amino acids, which can be exploited for synthetic purposes.

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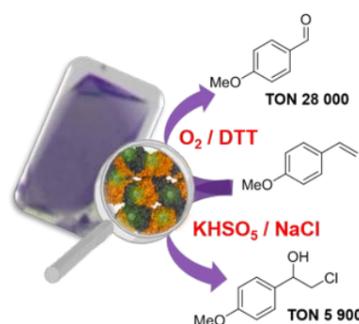
IL-045

## NiKA as Versatile Scaffold for the Design of Artificial Oxidases/Oxygenases

Sarah Lopez,<sup>1</sup> Christine Cavazza,<sup>1</sup> Caroline Marchi-Delapierre<sup>1</sup>, Stéphane Ménage<sup>1</sup>

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Catalysis represents an exciting tool for the development of sustainable chemistry. Among the different approaches, biocatalysis is the most promising strategy but the repertoire of reactions is not reaching yet the one of (in)organic catalysis. Artificial enzymes fulfill then the gap by allowing abiotic reactions with in some cases reaching enzymatic kinetics.<sup>[1]</sup> Our group at LCBM has been involved in the design of artificial metalloenzymes<sup>[2]</sup> for oxidation reaction. Using a protein scaffold, NikA a Nickel transport protein, several chemical transformations have been performed thanks to the embedment of iron and Ru complexes into the protein cavity.<sup>[3]</sup> This lecture will give a special attention to the possible transposition from homogeneous catalysis to heterogeneous catalysis for these objects. Finally, future perspective on in crystallo catalysis will be developed.



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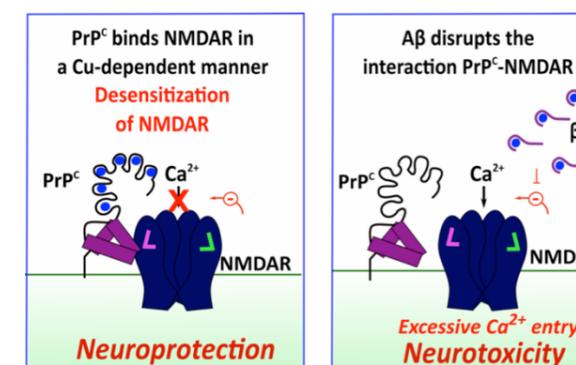
IL-046

## Copper-Protein Interactions at the Synapse: Relevance to Alzheimer's Disease

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Transition metals such as copper, iron, zinc and manganese are essential for brain function, as cofactors of a wide range of metalloproteins. While metal ion trafficking to the brain is highly regulated, alterations in metal ion homeostasis have been associated to neurodegenerative disorders, such as Alzheimer's disease (AD).<sup>1</sup> In this presentation, a brief overview of copper trafficking at the synapse will be provided, followed by a discussion of Cu-protein interactions that are key players in neuroprotective mechanisms and how they might be affected in AD. Recently, it has been proposed that the amyloid-beta peptide ( $\beta$ A) acts as a chelating agent disrupting the function of other Cu-binding proteins, such as the cellular prion protein (PrP<sup>C</sup>).<sup>2,3</sup> Specifically, the Cu coordination properties of PrP<sup>C</sup> and  $\beta$ A will be discussed,<sup>4,6</sup> while a recent spectroscopic study on the competition for Cu ions between these two players will be presented. Our work reveals the formation of a key putative ternary  $\beta$ A-Cu-PrP<sup>C</sup> complex, providing further insights into how  $\beta$ A might alter the Cu-dependent neuroprotective role of PrP<sup>C</sup>. This research has been supported by the National Council for Science and Technology in Mexico (CONACyT grants #221134 and #PN2076).



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IL-047

## Iron-Siderophores as Redox-Reversible Anchors in Artificial Metalloenzymes

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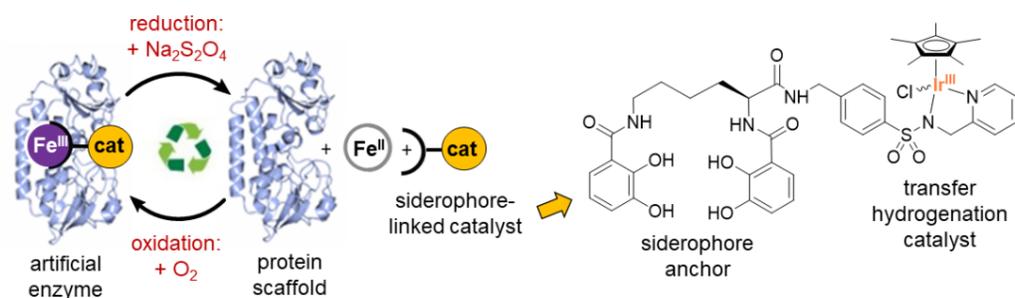
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Artificial metalloenzymes have the potential of combining the wide reaction scope of synthetic catalysts with the selectivity and biocompatibility of proteins [1]. Inspired by the way in which certain pathogenic bacteria use tetradentate siderophores to acquire essential iron [2], we have developed a new siderophore-based anchor to reversibly connect catalysts to protein scaffolds, creating artificial enzymes.

This reversible ‘catch-and-release’ approach is illustrated below (see figure) for the assembly and disassembly of an artificial transfer hydrogenase. In the presence of iron(III), the siderophore-based anchor binds an organometallic imine-reduction catalyst to its cognate protein scaffold, producing the artificial enzyme, but on reduction to iron(II), dissociation takes place and the artificial enzyme disassembles. Since the catalyst produces racemic product in the absence of the protein scaffold, but a reproducible enantiomeric excess if protein bound, the assembly and reductively triggered disassembly of the artificial transfer hydrogenase can be monitored [3]. Importantly, the reversibility of the anchoring system allows the individual components, in particular the protein, to be recovered and reused.

We thank the Engineering and Physical Sciences Research Council (EPSRC, EP/L024829/1) and Biotechnology and Biological Sciences Research Council (BBSRC) for financial support and the Diamond Light Source for access to beamlines I03 and I04 (proposal; mx-13587).



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IL-048

## Nitrogenase M-Cluster Assembly: Tracing the ‘9<sup>th</sup> Sulfur’ of the Nitrogenase Cofactor via a Semi-Synthetic Approach

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The M-cluster is the active site of nitrogenase that contains an 8Fe-core assembled via coupling and rearrangement of two [Fe<sub>4</sub>S<sub>4</sub>] clusters concomitant with the insertion of an interstitial carbon and a ‘9<sup>th</sup> sulfur’ [1]. Combining synthetic [Fe<sub>4</sub>S<sub>4</sub>] clusters with an assembly protein template, we show that sulfite gives rise to the ‘9<sup>th</sup> sulfur’ that is incorporated in the catalytically important belt region of the cofactor after the radical SAM-dependent carbide insertion and the concurrent 8Fe-core rearrangement have already taken place [2]. This work provides a semi-synthetic tool for strategically labeling the cofactor—including its ‘9<sup>th</sup> S’ in the belt region—for mechanistic investigations of nitrogenase while suggesting an interesting link between nitrogen fixation and sulfite detoxification in diazotrophic organisms.

This work was supported by NIH-NIGMS grant GM67626 (to M.W.R. and Y.H.), DOE/BES grant DE-DC0014470 (to M.W.R. and Y.H.), a Takeda Science Foundation grant (to Y.O.) and Grant-in-Aids for Scientific Research (nos 23000007 and 16H04116) from the Ministry of Education, Culture, Sports, Science and Technology, Japan (to K.Tat. and Y.O.).

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## IL-049

### Activity, Selectivity and Stability in Carbon-Free, Molecular Water Oxidation Catalysts

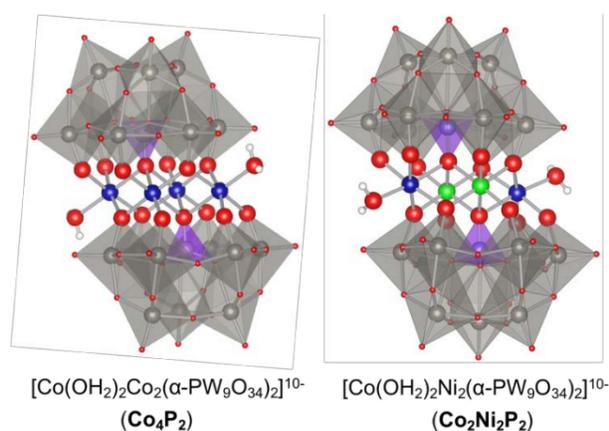
Craig L. Hill<sup>1</sup>, Meilin Liu<sup>1</sup>, Kevin P. Sullivan<sup>1</sup>, Qiushi Yin<sup>1</sup>, Sarah M. Lauinger<sup>1</sup>, Yurii V. Geletii<sup>1</sup>, Tianquan Lian<sup>1</sup>, Djamaladdin G. Musaev<sup>1,2</sup>

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Deeper insights and commensurately better versions of the three interconnected functional units in solar fuel generation systems are needed: water oxidation catalysts (WOCs), H<sub>2</sub>O and CO<sub>2</sub> reduction catalysts and light-absorbing-charge-separating structures. Transition-metal-substituted polyoxometalates (POMs) have been investigated by many groups as WOCs because they combine the advantages of molecular (homogeneous) catalysts (their properties can be studied in depth experimentally and computationally), with the advantages of non-molecular (heterogeneous) catalysts (carbon-free and very robust). Many groups have developed and studied POM WOCs that contain only earth-abundant elements since our report of the first such catalyst “Co<sub>4</sub>P<sub>2</sub>” (X-ray structure in Figure, left).<sup>1,2</sup> A grand challenge in water oxidation and solar fuel production is the development of inexpensive, effective WOCs that also function in strong acid. We will report new earth-abundant-element POMs that are both very good in strong base and, more importantly, in strong acid.

A central challenge in catalytic water oxidation (the OEC or nonbiological catalysts) is to understand at the molecular level the electronic structure and other factors of the WOC that control the rates and selectivities of electron transfer, PCET and oxygen-oxygen bond formation, as well as catalyst stability. Current studies and knowledge will be described. In order to assess the impact of changing the electronic structure of the WOC active site metal (Co blue spheres in the Figure complexes), by changing the internal, solvent-inaccessible, adjacent transition metals (e.g. Ni green spheres in Figure, right) we succeeded in preparing and purifying complexes such as the CoNiNiCo complex (X-ray structure in Figure, right) and confirming both the placement and percent occupancy of similar Z value 3d metals (e.g. Co and Ni) using synchrotron anomalous dispersion diffraction in conjunction with a conventional ensemble of spectroscopic and diffraction techniques. This central metal, like more conventional factors such as proton-accepting proximal ligands, impacts WOC rates and stability.

Financial support by the Solar Photochemistry Program, Office of Basic Sciences, US Department of Energy is gratefully acknowledged.



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## IL-050

### Confinement of Metal Centers within Bioinspired Hosts

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Metalloproteins perform functions not yet achieved in abiotic systems. One reason for this lack of function is the inability of control of the microenvironments about the metal centers. Microenvironments are defined as the volume of space proximal to the metal centers that encompass the secondary coordinate spheres. Results from structural biology point to non-covalent interactions within microenvironments as instrumental in regulating function. Therefore, the function and dysfunction of metalloproteins can be understood within the context of changes within their microenvironments. We are developing systems that allows for the confinement of metal center within hosts to regulate the properties of the immobilized metal centers. Our approaches provide the necessary control to produce new complexes with primary coordination spheres that resemble those found in natural metalloproteins. Moreover, control of the secondary sphere is designed into our systems, including the site-specific incorporation of functional groups that can promote intramolecular hydrogen bonds. The ability to regulate the binding and microenvironment within the host also eliminates unproductive metal-metal interactions and allows for systematic studies into structure-function relationships. This presentation will describe our results in developing systems that confine biological relevant metallocofactors with Fe, Mn, or Cu centers to probe O<sub>2</sub> activation or water oxidation.

Financial support by the National Institutes of Health, USA.

IL-051

## Alzheimer's Disease: A Heme Perspective

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Alzheimer's disease (AD) is a neurodegenerative disorder that has generally been associated with the accumulation of amyloid beta (A $\beta$ ) peptides and formation of partially reduced oxygen species (PROS) catalyzed by Cu bound A $\beta$  active sites in the brain. Heme binding to A $\beta$  peptides has opened up a new direction in this field. The active site environment of heme-A $\beta$  has been defined using several spectroscopic techniques and site directed mutagenesis. The heme-A $\beta$  peptides can act as peroxidases and degrade neurotransmitters like serotonin through high valent reactive intermediates. These entities can interact with physiologically available redox active metals like Cu and signaling molecules like NO and CO. They can use cytochrome c as redox partners and donate the heme to proteins like myoglobin and neuroglobin. Three amino acid residues unique in mammalian A $\beta$  (Arg5, Tyr10 and His13) and missing in A $\beta$  from rodents which do not get affected by AD, play significant roles in the reactivities exhibited by heme-A $\beta$  complexes.

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IL-052

## Assembly of Non-Native Dimeric Macrocyclic Siderophores Using Dual Approaches in Metal-Mediated Synthesis

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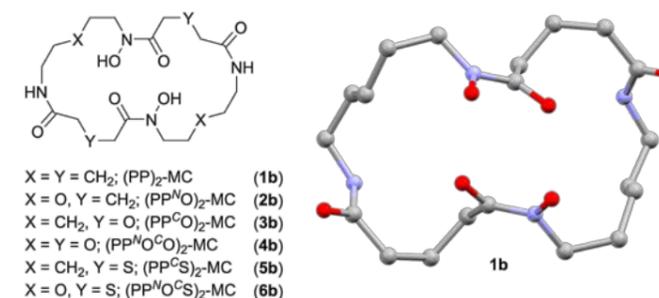
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Siderophores are low-molecular-weight organic compounds produced by bacteria as high-affinity Fe(III) chelates. These macrocyclic or linear natural product ligands play key roles in orchestrating bacterial iron supply, with alternative coordination complexes relevant to medicine, medical imaging and environmental remediation. Native siderophores from all classes (hydroxamic acid, catecholates, citric acid) are produced in bacterial culture in low yield, which provides impetus to develop methods that simultaneously improve access and expand structural diversity.

Access to hydroxamic acid-containing macrocyclic siderophores has been explored using a metal-templated synthesis (MTS) approach [1,2]. In this approach, the reaction between an *endo*-hydroxamic acid amino carboxylic acid monomer and Fe(III) forms a pre-complex with the amino and carboxylic acid groups from contiguous monomers oriented for peptide-based *in situ* ring closure. The architecture of the metal-loaded (holo) macrocycle and the cognate apo-macrocycle, which can be generated following removal of the metal ion template with EDTA or DTPA, can be directed by the monomer, the metal ion, and the reaction stoichiometry [3,4].

Here, we present a new synthetic route toward *endo*-hydroxamic acid monomers with improved yields, time efficiency and structural diversity, compared to established methods [5]. We have used Fe(III)-based MTS to generate a suite of six new non-native dimeric macrocyclic siderophores as Fe(III)-complexes and apo-macrocycles, with variably positioned methylene, ether or thioether atoms in the macrocycle backbone. Two ether-containing isomers showed a remarkable difference in solvation, based on the surrogate measure of RP-HPLC elution time. Density functional theory (DFT) calculations revealed structural distinctions between the two isomers and gave significantly different calculated dipole moments, which supported experiment. MTS proves to be a useful avenue for producing structurally diverse non-native hydroxamic acid macrocycles. On-going studies are focused on developing an alternative metal-mediated synthesis pathway [6] to further improve yields to allow a more complete evaluation of the structure and function of these compounds. Financial support from the Australian Research Council (RC), and the Swiss National Science Foundation (SNSF Professorship PP00P2\_163683) (JPH) and the European Research Council under the Grant Agreement No 676904, ERC-StG-2015, NanoSCAN (JPH), is gratefully acknowledged.



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IL-053

## Beyond the E0 State of Nitrogenase: Spectroscopic Studies of Intermediates in Biological Dinitrogen Reduction

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The conversion of dinitrogen to ammonia is a challenging, energy intensive process, which is enabled biologically by the nitrogenase family of enzymes. The Mo-dependent nitrogenases contain two cofactors, the 8Fe-8S P-cluster and the Mo-7Fe-9S-C iron-molybdenum cofactor, known as “FeMoco”, which is the active site for dinitrogen reduction. FeMoco has long been, and continues to be, an enigmatic cluster. Over 8 years ago the presence of a carbide in the cluster was first revealed. However, the role of the carbide, the role of the Mo heterometal, and the changes which occur at the seven iron sites during the course of catalysis all remain open questions. Herein, we present studies of selenium incorporated FeMoco. High-energy resolution fluorescence detected X-ray absorption spectroscopy (HERFD XAS) at the Se K-edge is utilized to obtain selective information about the electronic structure of FeMoco. These studies reveal a significant asymmetry in the electron distribution within FeMoco, suggesting a much more localized electronic structure than typically assumed for iron sulfur clusters. Further XAS studies of both natively reduced and cryoreduced MoFe protein will be presented. These studies are essential for establishing the nature of the first redox event in the catalytic cycle of nitrogenase. Together, these studies form a basis for unravelling the electronic structural details of this complex catalytic process.

Financial support by the Max Planck Society, the ERC, and the DFG SPP-1927 is gratefully acknowledged.

IL-054

## Combined Enzyme and Photoredox Catalysis and other Adventures in Aqueous Photochemistry

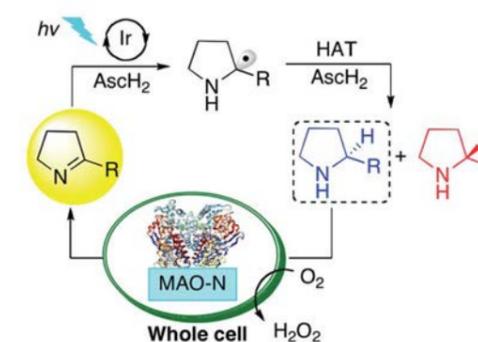
Xingwei Guo<sup>1</sup>, Yasunori Okamoto<sup>2</sup>, Christoph Kerzig<sup>1</sup>, Thomas R. Ward<sup>2</sup>, Oliver S. Wenger<sup>1</sup>

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Building on our recently developed method for reductive amination by photoredox catalysis [1], a joint effort between the Ward and Wenger laboratories led to a new method for the synthesis of enantioenriched amines from imines that relies on catalytic teamwork between a photocatalyst and an enzyme [2]. Using a strongly reducing Ir photocatalyst and ascorbate as an electron and hydrogen atom source, imines were reduced to racemic amines. The enzyme monoamine oxidase (MAO-N-9) was employed to oxidize one of the two amine enantiomers back to the initial imine substrate. Over time, this cyclic reaction network (performed in a one-pot reaction) leads to accumulation of a single amine enantiomer. The concepts developed in this project should be widely applicable to a variety of chemical transformations. This work was generously supported by the NCCR Molecular Systems Engineering.

Using the new water-soluble Ir photocatalyst as well as more common Ru sensitizers, we subsequently explored new photochemistry in water, which could be of interest at the interface between bioinorganic and physical-inorganic chemistry. This includes the demonstration of relatively efficient triplet-triplet annihilation upconversion in water [3], as well as the generation of hydrated electrons using visible light and their use in preparative-scale chemical conversions [4].



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IL-056

## Siderophore-Antibiotic Conjugates – Diverting Iron Uptake to Deliver Drugs inside Bacteria

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Vectorization of bactericide compounds by siderophores (iron chelators produced by bacteria) is a promising Trojan horse strategy able to considerably increase the efficacy of drugs by overcoming the impermeability of the bacterial wall, especially that of Gram-negative bacteria [1]. In such a Trojan horse strategy, the idea is that each time a bacterium internalizes a ferric ion, a molecule of drug is transported as well. To develop such a strategy it is very important to have a good knowledge of bacterial iron acquisition pathways.

Iron is a cofactor of many redox-dependent enzymes and thus essential for growth and virulence. *Pseudomonas aeruginosa*, a human opportunist pathogen, is able to express in order to get access to iron: (i) a ferrous iron uptake pathway, (ii) two haem uptake pathways, (iii) two ferric iron acquisition pathways involving the siderophores pyoverdine and pyochelin (produced by the pathogen itself) and at last (iv) iron acquisition pathways involving siderophores produced by other bacteria (exosiderophores) [2]. The presence of exosiderophores in *P. aeruginosa* environment is sensed by the pathogen, which in response expresses specific transporters for the uptake of their ferric-forms.

Using proteomic and molecular biology approaches, we have investigated how *P. aeruginosa* adapts the expression of its iron acquisition pathways depending on its environment and in different growth conditions: iron restricted growth conditions, epithelial cell infection assay, in the absence or presence of exosiderophores or siderophore-antibiotic conjugates. We have shown that the catechol type exosiderophores or siderophore-antibiotic conjugates were clearly more efficient in inducing the expression of their corresponding transporters than other siderophores because of their very high affinity for iron. In parallel, a significant repression of the expression of the proteins of the pyochelin pathway, and at a lower extent of the pyoverdine pathway, were as well observed, indicating clearly that bacteria opt for the use of the catechol siderophores to get access to iron when such compounds are present in their environment. No effect was seen on the expression levels of the haem or citrate uptake pathways. The data point out that catechol siderophores are the most promising siderophores in Trojan horse strategies where siderophores are used as vectors to transport antibiotics into bacteria.

This work was partially funded by the Centre National de la Recherche Scientifique and grants from the associations Vaincre la Mucoviscidose and Gregory Lemarchal. In addition, these results were generated as part of the work of the Translocation Consortium (www.translocation.com), supported by the Innovative Medicines Joint Undertaking under Grant Agreement no. 115525, through financial contributions from the European Union's Seventh Framework Program (FP7/2007-2013) and contributions in kind from EFPIA companies.

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IL-057

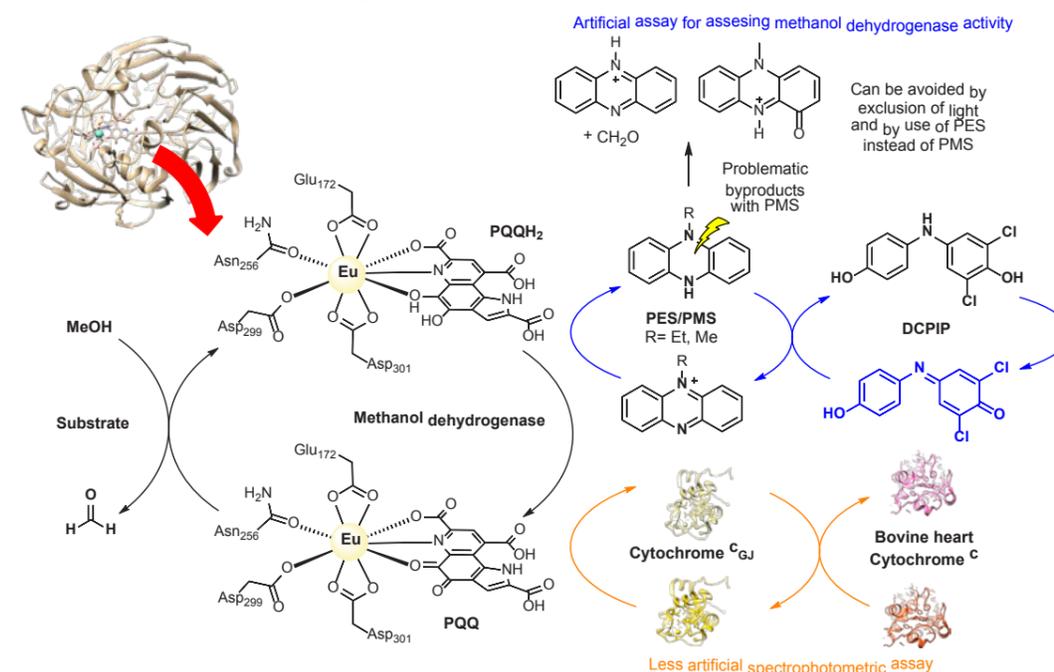
## Essential and Ubiquitous: Lanthanides in Methanol Dehydrogenases

Berenice Jahn<sup>1</sup>, Henning Lumpe<sup>1</sup>, Wouter Versantvoort<sup>2</sup>, Arjan Pol<sup>2</sup>, Huub J.M. Op den Camp<sup>2</sup>, **Lena J. Daumann<sup>1</sup>**

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Lanthanides (Ln) are biologically essential metals. This statement was until recently, unthinkable. This lecture will present the recent developments in the emerging field of lanthanide biochemistry from a coordination chemist's point of view. Why bacteria prefer for example the light Ln has been a subject of debate.<sup>[1,2]</sup> We have previously reported the cultivation of the strictly Ln-dependent methanotrophic bacterium *Methylophilum fumariolicum* SoIV with europium(III), as well as the purification, structural and kinetic analyses of the first Eu-dependent methanol dehydrogenase (MDH).<sup>[3]</sup> Our studies showed, that although lanthanides have similar properties, the differences in ionic radii caused by the lanthanide contraction across the series impact MDH efficiency and even lanthanide uptake by bacteria.<sup>[4]</sup> We further report an investigation of the assay protocol (blue path) that has been used for decades to assess MDH activity and present an alternative method, using the natural electron acceptor of MDH from strain SoIV, cytochrome *c<sub>GJ</sub>*, (orange path).<sup>[5]</sup>



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IL-058

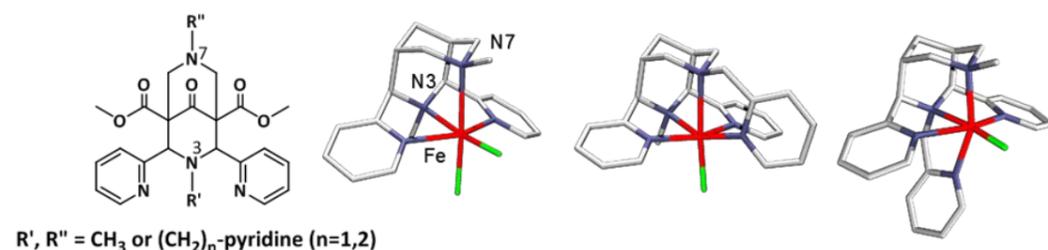
## New Reaction Channels with Nonheme Iron Oxidation Catalysts

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Bispidine ligands are extremely rigid, easy to synthesize and available in a large variety. They enforce coordination geometries derived from *cis*-octahedral, and the two vacant coordination sites with the tetradentate ligand systems are sterically and electronically distinct; with the isomeric pentadentate ligands, the site of the oxo group is enforced by the ligand (see Figure). Coligands coordinated *trans* to N3 generally have strong and short bonds, those *trans* to N7 are more labile. Reasons are analyzed on the basis of computational work as well as experimental structural data, thermodynamics, spectroscopy and reactivities. Implications with respect to the mechanism of formation and decay, the structure and spin state of high-valent iron oxidants and possibilities to tune the spin state, structure and reactivity of these high-valent iron complexes are discussed.[1-3]

The specific example that will be presented is the halogenation of alkanes (the modeling of halogenase activity) and, therefore, based on experimental and computational work, the electronics of the Fe<sup>IV</sup>=O bond enforced by the tetradentate bispidine will be discussed in detail (spin state of iron, bonding of the oxo group and resulting energy barrier and driving force for specific reactions). Financial support by the German Science Foundation.



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IL-059

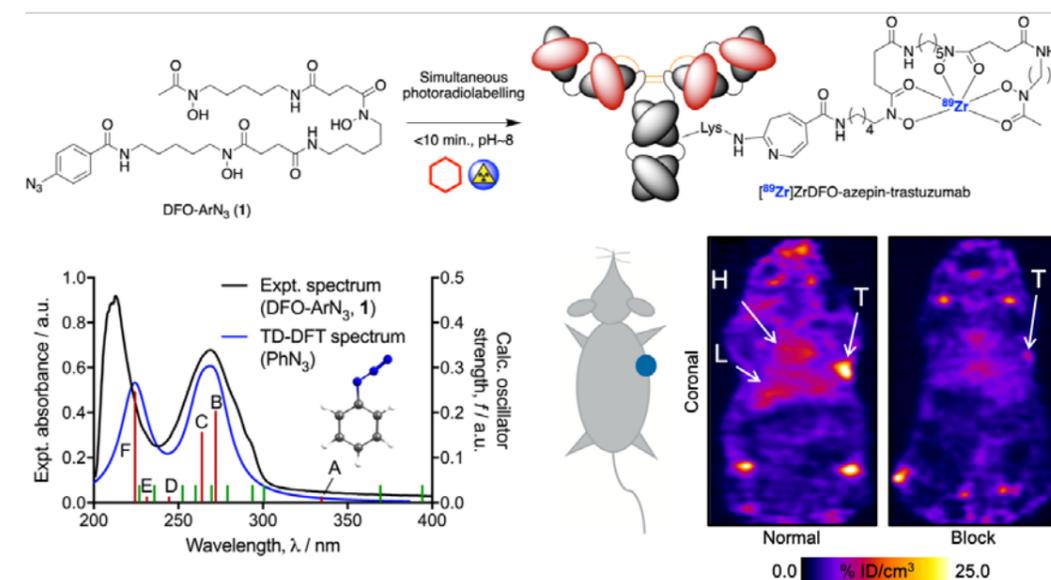
## Radiochemistry in a Flash – Applications of Photochemistry in Radiotracer Design

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Monoclonal antibodies (mAbs), immunoglobulin fragments and other proteins are important scaffolds in the development of radiopharmaceuticals for diagnostic immuno-positron emission tomography (immuno-PET) and targeted radioimmunotherapy. Conventional methods for radiolabelling proteins with metal ions like <sup>68</sup>Ga, <sup>64</sup>Cu, <sup>89</sup>Zr, and <sup>90</sup>Y require multi-step procedures involving pre-purification, functionalisation with a chelate, and subsequent radiolabelling. These coupling chemistries are time consuming, difficult to automate, and involve the synthesis, isolation and storage of an intermediate new molecular entity (the conjugated mAb) whose biochemical properties can differ from those of the parent protein. To circumvent these issues, we developed a *photoradiochemical* approach that uses fast, chemoselective, light-induced protein modification under mild conditions with novel metal ion binding chelates derivatised with arylazide (ArN<sub>3</sub>) groups.[1–3] Kinetic experiments, spectroscopy, radiochemistry, cellular assays and immuno-PET imaging showed that simultaneous photochemical conjugation and radiolabelling of formulated mAbs can be achieved in <20 min. using a range of chelates, radiometal ions and proteins.[4–6] Density functional theory calculations were also used to explore the photoactivation mechanism and aid in the design of more reactive ligands.

JPH thanks the Swiss National Science Foundation (SNSF Professorship PP00P2\_163683), the Swiss Cancer League (Krebsliga Schweiz; KLS-4257-08-2017), and the University of Zurich (UZH) for financial support. This project has received funding from the European Union's Horizon 2020 research and innovation programme / from the European Research Council under the Grant Agreement No 676904, ERC-StG-2015, NanoSCAN. Thank also to all members of the Radiochemistry and Imaging Science group at UZH for helpful discussions.



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## IL-060

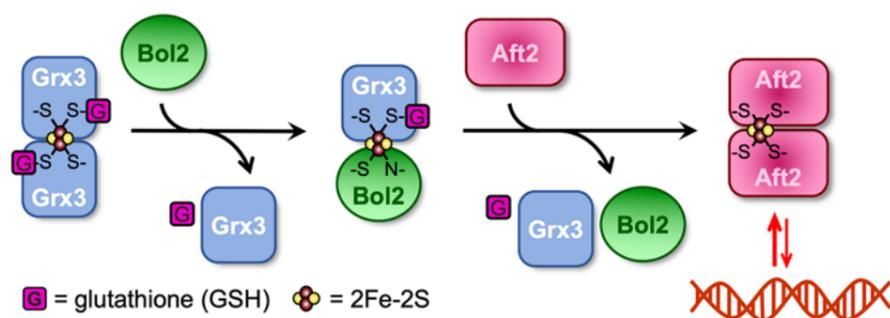
### Iron-Sulfur Cluster Sensors: Roles for Monothiol Glutaredoxins and BolA Proteins in Iron Regulation

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Disruptions in iron metabolism have been implicated in human diseases such as iron overload disorders, neurodegenerative diseases, and mitochondrial dysfunction disorders. To better understand iron regulation at the cellular and molecular level, our research group is teasing out the mechanistic details of iron sensing and regulation using yeast model systems [1, 2]. Our studies are focused on characterizing the roles of monothiol glutaredoxins Grx3/Grx4 that utilize glutathione to bind iron-sulfur (Fe-S) clusters with BolA partner proteins [3]. Monothiol Grxs and BolA proteins have close homologues in bacteria and higher eukaryotes (including humans), but their specific functions have been best characterized in yeast. These binding partners together regulate the function of different yeast transcription factors that control iron uptake, storage, and utilization genes. Using a combination of protein biochemistry, spectroscopy, mutagenesis, and yeast genetics and cell biology, we have demonstrated how Grx3/4 and Bol2 proteins signal and control iron bioavailability in both budding and fission yeast via Fe-S cluster binding and/or transfer reactions [4-6]. We have identified residues in Grx3/4, Bol2, and their transcription factor targets that play key roles in donor-target recognition and influence Fe-S cluster binding and transfer rates. Taken together, these studies provide a detailed picture of the dynamic interactions between these Fe-S binding partners that govern iron regulation in yeast, and demonstrate that monothiol Grxs and BolA proteins have evolutionarily conserved roles in iron regulation.

Financial support by the National Institute of Health Grant GM118164 is gratefully acknowledged.



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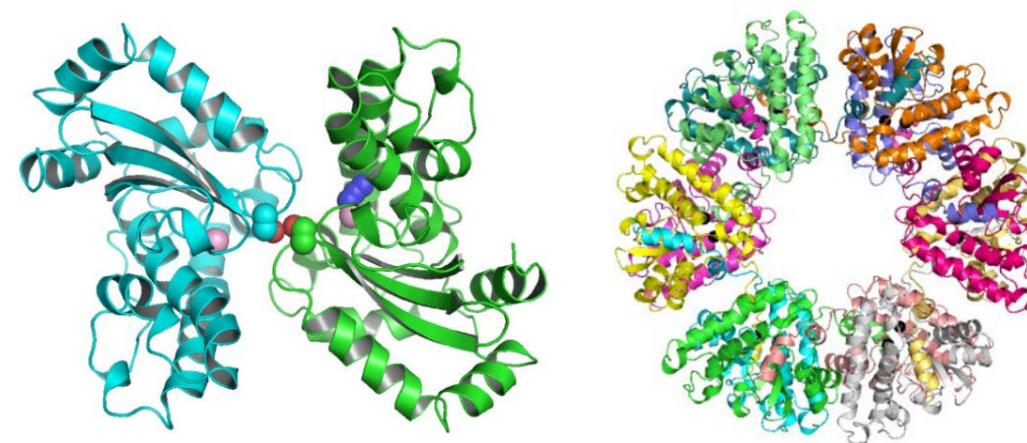
## IL-061

### Subtle Asymmetry in Homo-Oligomeric Metalloproteins – Crystal-Packing Artefacts or Functionally Significant?

**Geoffrey B. Jameson<sup>1</sup>, James R. Salvador<sup>1</sup>, Sarah A. Kessans<sup>1</sup>, Renwick C.J. Dobson<sup>1</sup>, Jatnika Hermawan<sup>1</sup>**

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Many expressed proteins assemble into oligomers comprised of identical subunits. Frequently, when the interface between subunits is examined closely, the apparent non-crystallographic or even the enforced crystallographic symmetry is not perfect. For example, in the manganese superoxide dismutase structure, the two-fold symmetry relating pairs of subunits looks to be very good indeed, at least as concerns the secondary and tertiary structure (left panel below). Indeed, most of the amino-acid side chains maintain essentially identical conformations in both subunits, including the pair of serine residues (Ser126) that are hydrogen-bonded across, in this case, a non-crystallographic two-fold axis. However, the hydrogen atoms of the hydroxyl groups must be disordered and the symmetry less than perfect – barring a here most unlikely deprotonation. Moreover, one manganese ion is unequivocally Mn(II) and five-coordinate whereas the other is Mn(III) and six-coordinate is completed by an azide ion. This and other structures where subtle asymmetry at an otherwise two-fold symmetric interface appears to propagate to substantial asymmetry at the active site will be described and discussed with a view to functional implications and/or crystal packing artefacts.



The consequences of Ser126Asp and Ser126Trp mutations at this dimer interface will also be presented. The former mutation somewhat remarkably preserves the dimer interface to high pH; the latter mutation partially preserves the dimer interface but causes the C-terminal helices (residues 180-205) to fold out and insert in a domain-swapped manner into a neighbouring dimer, forming a dodecamer (right panel below).

Financial support by the Marsden Fund of the Royal Society of New Zealand and the MacDiarmid Institute for Advanced Materials and Nanotechnology is gratefully acknowledged.

IL-062

## Iron-Sulfur Clusters with Unsaturated Iron Sites

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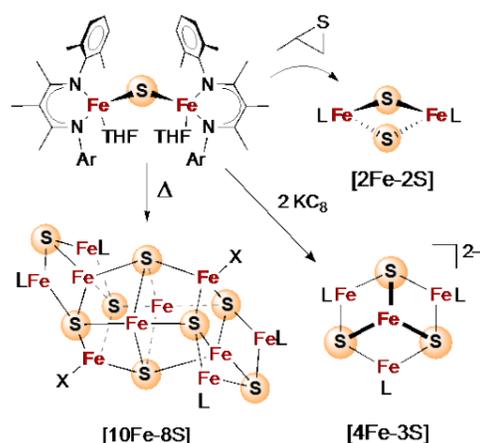
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The four-coordinate iron sites of typical iron-sulfur clusters rarely react with substrates [1-3]. In order to evaluate the feasibility of sulfur-supported three-coordinate iron sites, we have prepared the first compounds with fully sulfide-coordinated three-coordinate iron. We show that a [2Fe-1S] precursor leads to [2Fe-2S] clusters as well as novel [4Fe-3S] and [10Fe-8S] structures with three-coordinate sites or sites with three sulfides and a weakly coordinated solvent molecule. The [4Fe-3S] cluster is particularly interesting, because instead of a high-spin electronic configuration like other iron-sulfur clusters, the planar geometry and short Fe-S bonds of the central iron site lead to a surprising low-spin electronic configuration as determined by spectroscopy and *ab initio* calculations. In a demonstration of biomimetic reactivity, the [4Fe-3S] cluster reduces hydrazine, a natural substrate of nitrogenase, to give the first example of NH<sub>2</sub> bound to an iron-sulfur cluster.

Financial support by the National Institutes of Health (GM-065313) is gratefully acknowledged.



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IL-063

## Solution and Solid-State Studies of Copper(II) Complexes of cb-Cyclams with Phosphorus Acid Pendant Arms: Ligands for <sup>64</sup>Cu-Radiopharmaceuticals

Lucia Pazderová<sup>1</sup>, Přemysl Lubal<sup>2</sup>, Vojtěch Kubíček<sup>1</sup>, Jan Kotek<sup>1</sup>, Tomáš David<sup>1</sup>, Petr Hermann<sup>1</sup>

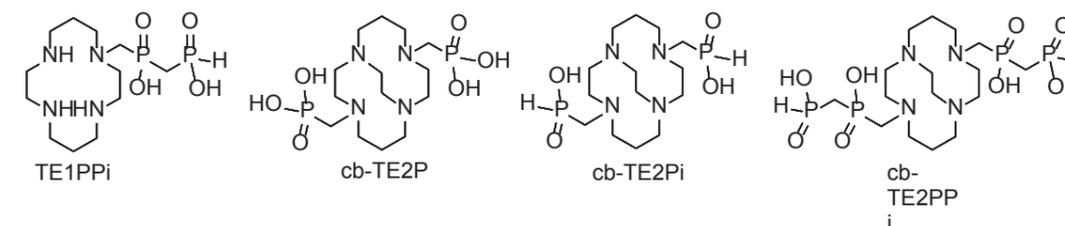
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<sup>2</sup>Department of Chemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic.

Copper radioisotopes (<sup>61/64</sup>-Cu for PET, <sup>67</sup>-Cu for therapy) can be used as a theranostic pair. Suitable chelators for the radioisotopes are commonly based on various macrocycles but they mostly suffer from *in vivo* instability, slow (= not efficient) radiolabeling and/or a low selectivity over competing ions. Currently, the most commonly used have been derivatives of NOTA. Cyclam derivatives offer a high thermodynamic selectivity for Cu(II) and relatively fast complexation. In addition, complexes of cross-bridged (cb-) cyclams are very stable *in-vivo* – however, their radiolabelling is not efficient.

Recently, we have found that bis(phosphinic acid) pendant arm (BPi), bound to cyclam (TE1PPi) highly accelerate *in-cage* Cu(II) complexation and kinetic inertness of the complexes is preserved [1]. Among various pendant arms bound to Me<sub>3</sub>cyclam as a model macrocycle, phosphonic acid and bis(phosphinic acid) pendants were shown as the most efficient ones for fast copper(II) binding [2]. The convenient properties are preserved in bifunctional ligands and in their conjugates, and the labeled conjugates are stable *in-vivo* producing superb PET images [3]. To check if the phosphorus acid pendant arms would also improve properties of cross-bridged cyclams towards copper(II), ligands with two phosphonic acid (cb-TE2P), phosphinic acid (cb-TE2Pi) and bis(phosphinic acid) (cb-TE2PPi) pendant arms were synthesized and studied. Thermodynamic, formation/decomplexation kinetic, the solid-state structural and radiolabelling data were obtained. The ligands behave as proton sponges with the last pK<sub>a</sub> > 14. Thermodynamic stability of their Cu(II) complexes is slightly lower than that of analogous cyclam derivatives. Rate of complexation is the highest for the ligand with the BPi pendants. As expected for cb-cyclam derivatives, the complexes are highly kinetically inert. The phosphonic acid/BPi cb-cyclam derivatives are efficiently radiolabelled with <sup>64</sup>-Cu even at room temperature and small molar excess of the ligands and, thus, with a high specific activity. However, radiolabelling is not efficient if only one phosphorus acid pendant is present in the ligands. Preliminary *in-vivo* data will be also presented, confirming stability *in-vivo*. Thus, the ligands seem to be good chelators for copper-based radiopharmaceuticals.

Financial support by the Grant Agency of the Czech Republic is gratefully acknowledged.



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IL-064

**What's the Catch in Catching Cu: Redox-State Selective Chelators for Alleviating Cu Ion Induced Oxidative Stress**

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Copper ions are essential for biological function yet lead to severe pathophysiological conditions when dysregulated. A major route by which excess copper ions can affect cell physiology is via the catalytic production of hydroxyl radicals that can irreversibly alter essential bio-molecules. Hence, copper selective chelators that can remove excess copper ions and alleviate oxidative stress will help treat copper dysregulation induced diseases. However, most currently available chelators are non-specific, leading to numerous undesirable side-effects. The challenge is to build chelators that can bind to copper ions with high affinity but leave the levels of essential metal ions including protein-bound Cu ions and the benign glutathione bound labile Cu<sup>+</sup> pool unaltered. We have developed novel Cu<sup>2+</sup> selective chelators that exhibit 10<sup>8</sup> times higher conditional stability constants toward Cu<sup>2+</sup> over Cu<sup>+</sup> and other biologically relevant metal ions.<sup>1</sup> The chelators can therefore selectively remove aberrant redox-cycling labile Cu ions that access the Cu<sup>2+</sup> state and relieve metal induced oxidative stress without affecting the essential non-redox cycling labile Cu<sup>+</sup> pool. Importantly, we show that the chelators can alleviate Cu induced oxidative stress in both Menkes disease model cells of Cu overload and a zebrafish larval model of Cu induced oxidative stress. The distinct selectivity of our chelators indicates potential applicability toward the treatment of severe metabolic and neurological disorders associated with abnormal Cu homeostasis including Wilson's disease, Menkes Disease, Alzheimer's disease, and also some forms of cancer. In this talk, I will discuss the current challenges in copper chelation therapy and our approach in designing redox-state selective chelators to address these challenges.

Financial support by the Tata Institute of Fundamental Research, India, is gratefully acknowledged.

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IL-065

**Going across the wall: Characterization of the Terminal Reductase Located on the Surface of the Cell Wall of the Electroactive and Thermophilic Gram-Positive Bacterium *Thermincola Potens* JR**

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Thermophilic Gram-positive organisms were recently shown to be a promising class of organisms to be used in bioelectrochemical systems for the production of electrical energy. These organisms present a thick peptidoglycan wall that was thought to preclude them to perform extracellular electron transfer (i.e. exchange catabolic electrons with solid electron acceptors outside of the cell). Here we describe the structure and functional mechanisms of the multiheme cytochrome OcwA, the terminal reductase of this Gram-positive and thermophilic bacterium *Thermincola potens* JR found at the cell surface of the organism. The structure, determined at 2.2Å resolution shows that the overall-fold and organization of the hemes are not related to other metal reductases, such as MtrC or OmcA, and instead are similar to those of multiheme cytochromes involved in the biogeochemical cycles of nitrogen and sulfur, such as NrfA and HAO. Our data reveal that terminal oxidoreductases of soluble and insoluble substrates are evolutionarily related, providing novel insights into the evolutionary pathway of multiheme cytochromes. They further reveal that OcwA can take the role of a respiratory 'swiss-army knife' allowing *Thermincola* to grow in environments with rapidly changing availability of terminal electron acceptors without the need for transcriptional regulation and protein synthesis.

IL-066

## Oxygen Activation by C-H breaking in the Oxidative Dehydrogenation of Polyamines Coordinated to Iron(III)

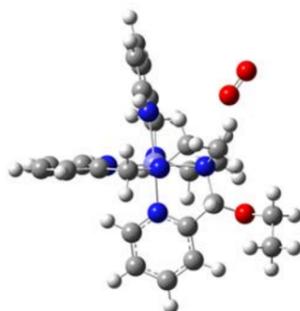
Martha E. Sosa Torres<sup>1</sup>, R. Daniel Páez López<sup>1</sup>, F. Miguel Castro Martínez<sup>2</sup>, Héctor F. Hernández Cortés<sup>2</sup>

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Earlier, the oxidative dehydrogenation of the Fe(III) complex **1**, [Fe(III)L<sup>3</sup>]<sup>3+</sup>, L<sup>3</sup> = 1,9-bis(2'-pyridyl)-5-[(ethoxy-2''-pyridyl)methyl]-2,5,8-triazanonane, has been investigated both in the absence and presence of dioxygen [1, 2]. In this reaction the Fe(II) complex **2**, [Fe(II)L<sup>4</sup>]<sup>2+</sup>, L<sup>4</sup> = 1,9-bis(2'-pyridyl)-5-[(ethoxy-2''-pyridyl)methyl]-2,5,8-triazanon-1-ene, is produced *via* an outer sphere electron transfer process. The reaction in dry ethanol, under dinitrogen, starts with the deprotonation of a ligand N-H bond promoted by the basic EtO<sup>-</sup> anion [1]. In the presence of O<sub>2</sub> the rate determining step was the reduction of dioxygen to the superoxide anion, O<sub>2</sub><sup>-</sup> [2]. To obtain further mechanistic insight, we performed density function calculations on the interaction of O<sub>2</sub> and [Fe(III)L<sup>3</sup>]<sup>3+</sup> which revealed that O<sub>2</sub> induced the cleavage of a C-H bond in [Fe(III)L<sup>3</sup>]<sup>3+</sup> (Figure). This C-H bond is partially polarized by the nearby Fe(III) center. Furthermore, a C-H isotope effect was detected upon deuteration of the L<sup>3</sup> C-H bond which was cleaved in the oxidative dehydrogenation process [3]. These experimental and computational findings strongly suggest that the dioxygen molecule becomes activated via a hydrogen atom transfer (HAT) mechanism. In this coupled proton/electron transfer both the HOO<sup>•</sup> radical and a carbon centered radical [Fe(III)L<sup>3•</sup>]<sup>3+</sup> are produced in the first step of the reaction. In this work I will present and discuss a mechanism of O<sub>2</sub> activation consistent with the new discoveries in the oxidative dehydrogenation reaction of compound **1**.

Financial support by CONACYT is gratefully acknowledged.



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IL-067

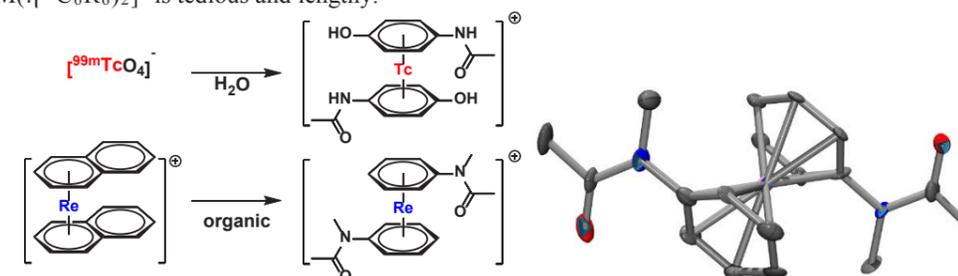
## Direct <sup>99m</sup>Tc Labeling of $\pi$ -Aromatic Ligands in Pharmaceutical Lead Structures

Roger Alberto<sup>1</sup>, Qaisar Nadeem<sup>1</sup>, Giuseppe Meola<sup>1</sup>, Henrik Braband<sup>1</sup>

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For molecular imaging purposes with metallic radionuclides, the bifunctional chelator approach is the common way of combining targeting moieties with an imaging modality. Chelators are often large molecules, bound to a functionality in the targeting structure. This may lead to interferences with the bioactivity, especially for small molecules in which e.g. the molecular weight easily doubles. It would be beneficial, if small biomolecule could be labelled via an integral part of the targeting structure, without additional bonds to one of the functional groups in the basic molecule.

We showed in the past the extraordinary stability of [M( $\eta^6$ -C<sub>6</sub>R<sub>6</sub>)<sub>2</sub>]<sup>+</sup> (M=Re, <sup>99m</sup>Tc) type complexes and a way of preparing the corresponding <sup>99m</sup>Tc complexes, albeit from organic solvents.<sup>1-2</sup> Arenes or phenyls are ubiquitous in pharmaceuticals and might serve as potential ligand sites due to the water and air stabilities of corresponding [M( $\eta^6$ -C<sub>6</sub>R<sub>6</sub>)<sub>2</sub>]<sup>+</sup> complexes. The preparation of these sandwich complexes follows Fischer-Hafner conditions, thus, demands the presence of AlCl<sub>3</sub> as activator. AlCl<sub>3</sub> is however incompatible with many functionalities, which limits the approach. Post-synthetic derivatization of [M( $\eta^6$ -C<sub>6</sub>R<sub>6</sub>)<sub>2</sub>]<sup>+</sup> is tedious and lengthy.



In this presentation, we show that sandwich complexes of the [M( $\eta^6$ -C<sub>6</sub>R<sub>6</sub>)<sub>2</sub>]<sup>+</sup> type with C<sub>6</sub>R<sub>6</sub> being differently substituted arenes such as paracetamol are directly accessible by substituting naphthalene in [Re( $\eta^6$ -C<sub>10</sub>H<sub>8</sub>)<sub>2</sub>]<sup>+</sup> with the corresponding arene. Furthermore, the homologous <sup>99m</sup>Tc complexes are directly accessible from an aqueous solution and [<sup>99m</sup>TcO<sub>4</sub>]<sup>-</sup> in high radiochemical purity and yields depending on the solubilities of the arenes in water. As a perspective, the synthetic concept may even be applicable to more complex organic molecules, which opens a path towards the direct labelling of small molecules with <sup>99m</sup>Tc without the need of bulky bifunctional chelators.

Financial support by the University of Zurich and the Swiss National Science foundation is gratefully acknowledged.

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IL-068

## Iron(II) Clathrochelates as CD-Reporters for Proteins

Vladyslava Kovalska<sup>1,2</sup>, Oleg Varzatskii<sup>2,3</sup>, Andriy Mokhir<sup>4</sup>, Yan Voloshin<sup>5</sup>, Elżbieta Gumienna-Kontecka<sup>6</sup>

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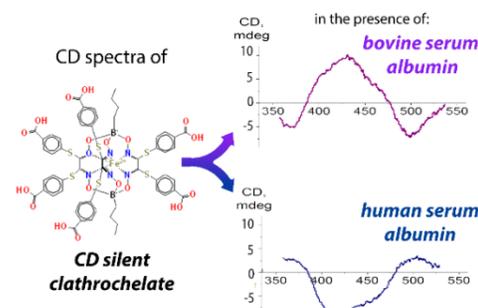
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The specific recognition of protein surface elements seem to be undoubtedly important for the development of various biochemical and biomedical tools, such as sensors, affinity tags, protein-based materials or novel immobilization techniques [1]. Upon interaction, the host protein molecules exhibiting inherent chirality are able to induce asymmetry of achiral organic and coordination compounds, and therefore may cause an appearance of a signal in their CD spectra. These induced CD (ICD) bands are very sensitive to the arrangement of the binding sites of hosting proteins and may reflect both the structural alterations and the conformational transitions of proteins. Various organic compounds and metal complexes have been reported to be ICD probes due to their binding to proteins and sensitivity to their structural alterations. Among them, cage metal complexes = clathrochelates [2], have been recently recognized as prospective biological effectors using their three-dimensional structure as rigid scaffold for macromolecular binding.

Here we will present our recent results on iron(II) clathrochelates as macrobicyclic cage metal complexes able to form multicentered supramolecular interactions in the vacant cavities or at the surface of protein macromolecules and/or their macromolecular complexes [3-4].



The project leading to these results has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 778245.



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IL-069

## Functional and Mechanistic Insights into Zinc-Dependent Metallochaperones

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Members of the zinc-uptake repressor (Zur)-regulated COG0523 subfamily of GTPase metallochaperones strongly impact bacterial transition metal homeostasis under conditions of host-imposed transition metal restriction<sup>1-3</sup>, but the nature of the cognate metal, mechanism of metal transfer, and identification of target protein(s) for metal delivery remain open questions. Here, we explore the multifunctionality of members of the subfamily proposed to deliver Zn<sup>II</sup> to apoprotein targets under these physiological conditions. In this work, we examine two Zur (zinc-uptake repressor)-regulated COG0523 family members, each from a major human pathogen *Acinetobacter baumannii* (*AbZigA*) and *Staphylococcus aureus* (*SaZigA*), in an effort to develop a model for Zn<sup>II</sup> metallochaperone activity. Zn<sup>II</sup> chelator competition experiments reveal one high affinity ( $K_{Zn1} \approx 10^{10}$ - $10^{11}$  M<sup>-1</sup>) metal binding site in each GTPase, while *AbZigA* and *SaZigA* are characterized by 1-2 additional lower affinity ( $K_{Zn2} \approx 10^7$  M<sup>-1</sup>) metal binding sites. High affinity metal binding at the CXCC (C, Cys; X, any amino acid) motif in the  $\beta$ 2 strand<sup>4</sup> activates the GTPase activity of both enzymes with Zn<sup>II</sup> more effective than Co<sup>II</sup>. Neither enzyme coordinates or is regulated by Fe<sup>II</sup>. Both GTPases bind the product, GDP, more tightly in the apo state than in the Zn<sup>II</sup>-bound state, and exhibit what is best described as a "locked" conformation around the GTP substrate. Negative thermodynamic linkage is observed between nucleotide binding and metal binding consistent with a new mechanistic model for COG0523-catalyzed metal delivery. Supported by the US National Institutes of Health (GM118157; T32 GM109825).

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IL-070

## Computational Studies and Design in the Field of Bio-Inspired Solar Light-Driven Water Splitting

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Solar energy is an inexhaustible energy source for a sustainable solution to the global energy consumption. The storage of large amounts of light energy can be achieved by conversion into chemical energy saved in biomass. Artificial photosynthesis permits the splitting of water into molecular hydrogen and oxygen and is therefore a very promising strategy to meet the increasing worldwide need for clean energy. This requires the development of high-performance water- reduction and water-oxidation catalysts where the latter is currently the main bottleneck for efficient photocatalytic water splitting.

Detailed analysis of the catalytic functioning and the factors determining the efficiency of catalysts is a prerequisite for the design of more efficient catalysts. We present our recent research for the in-depth study of water splitting catalysis using forefront computational methods. In particular, we go beyond standard computational approaches using very accurate wavefunction-based electronic structure methods as well as high-performance *ab initio* molecular dynamics. The latter allows for an improved inclusion of solvent and environmental effects at ambient conditions, which have been shown to have a decisive influence on the behaviour of the catalysts, and paves the way for highly sophisticated complete free energy surfaces using enhanced sampling methods.

In close collaboration with experimental groups, we have recently investigated in detail bio-inspired water oxidation catalysts, which feature a cubane core in analogy to the oxygen-evolving complex in nature's photosystem II. Our calculations revealed various factors influencing the water oxidation process and demonstrated certain similarities to nature's oxygen evolving complex. Other projects have dealt with the study of reaction networks and *in silico* design of bio-inspired Ru-based water oxidation catalysts or Co-based water reduction catalysts.

The work has been supported by the University of Zurich, the University Research Priority Program "Solar Light to Chemical Energy Conversion" (LightChEC), and the Swiss National Science Foundation (grant no. PP00P2\_170667).

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IL-071

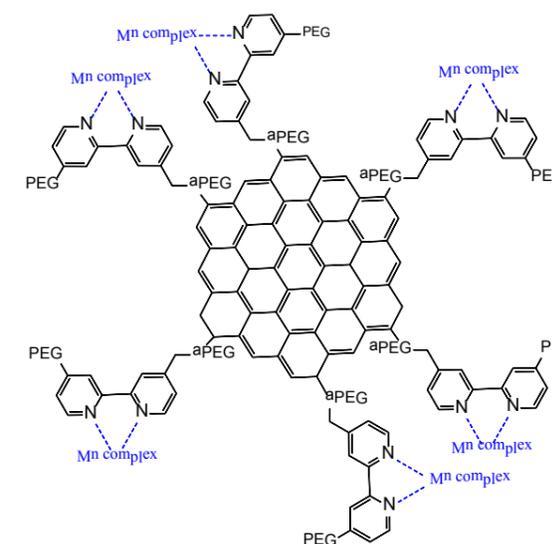
## Photoliberation of CO From Organometallic Compounds Coupled to Functionalized Graphene Oxide

Rosely A. Peralta<sup>1</sup>, Suélen Amorim<sup>1</sup>, Leticia Ilberto<sup>1</sup>, Vitor Weiss<sup>1</sup>, André Amorim<sup>1</sup>

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Carbon monoxide (CO) has been recognized as a toxic gas because of its greater affinity for hemoglobin than oxygen. However, biological studies have revealed an intriguing role for CO as a molecule with therapeutic properties. They have also demonstrated that CO exercises many cellular activities, including anti-inflammatory activities [1]. As a result, there are a large number of reports on the biological applications of CO-releasing molecules (CORMs) in many diseases [1–3]. Controlled release of CO is critical to avoiding the mishaps of using carbon monoxide in free form. One way to control the release of CO from CORMs is to develop photosensitive substances.

The use of graphene oxide as an irradiation converter in chemical energy and CORM transporter allows the photoliberation in regions near infrared (NIR) [4,5]. NIR irradiation penetrates deeper into human tissue and, at the same time, has less phototoxicity to the body than UV light, making the use of CORMs as promising drugs [4]. However, the presence of typical structural defects, system loading, graphene sheet size, degree of aggregation and oxidation are all factors capable of triggering cytotoxicity when cells are exposed to GO [6]. Many polymers have been used to functionalize graphene oxide in order to reduce this problem such as polyethylene glycol (PEG), polyacrylic acid (PAA) and polyetherimide (PEI). The introduction of PEG chains in GO's sheets has shown the best results [6]. Thus, the purpose of this research was the development of organometallic compounds of manganese (Mn) coupled to functionalized graphene oxide that can be used as photoCORMs under NIR irradiation.



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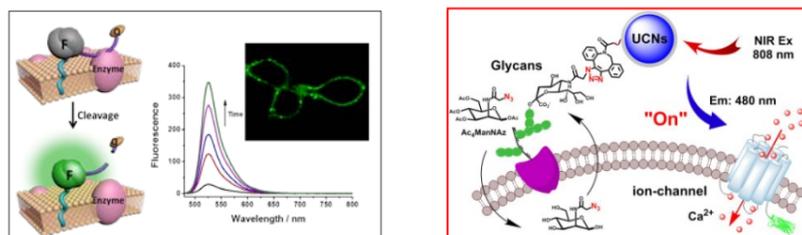
IL-072

## Unique Lanthanide Fluorescent Probes for Specific Manipulation of Cellular Functions and Localized Theranostics

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Generally, optical imaging including fluorescent and bioluminescent imaging enables such rapid, direct and sensitive visualization, mainly due to their high sensitivity, relative safety, and easily handling, and therefore have become robust and reliable tools in monitoring of subcellular protein dynamics and analysis of tumors or pathogen–host interactions *in vitro*, *in vivo* and even in pre-clinical practice. The systematic imaging investigation of biomolecules activities including enzymes or proteins etc in a complicated environment may offer great possibility for the in-depth understanding of the biological basis conferring diseases status, and importantly, for the facilitating of new theranostics *in vitro* and *in vivo*. In our group, a series of simple and specific small molecules or nano-structure based optical imaging probes have been extensively established to real-time visualize cellular function and activities, importantly, the intrinsic mechanisms to involve in potent drug activities and relevant pathways to initiate drug resistance have also been well investigated.



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IL-073

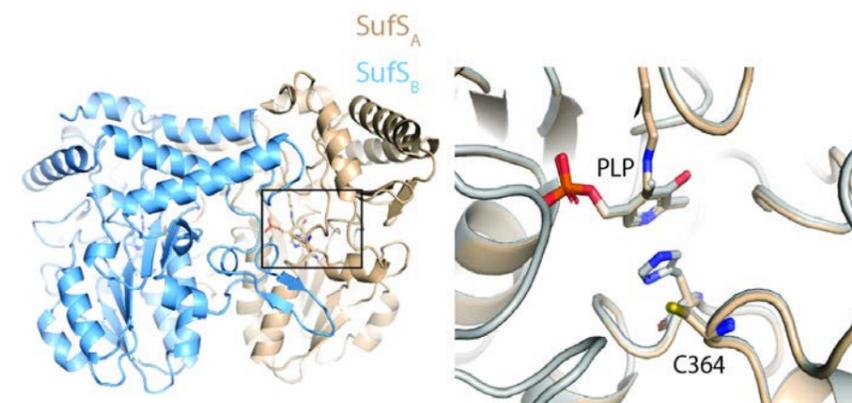
## Direct Observation of SufS Reaction Intermediates Reveals the Functional Role of Conserved Active Site Residues

**Matthew Blahut<sup>1</sup>, Courtney Wise<sup>1</sup>, Michael R. Bruno<sup>2</sup>, Guangchao Dong<sup>1</sup>, Thomas M. Makris<sup>1</sup>, Patrick A. Frantom<sup>2</sup>, Jack A. Dunkle<sup>2</sup>, F. Wayne Outten<sup>1</sup>**

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Fe-S clusters are necessary for the proper functioning of numerous metalloproteins. Isc and Suf are the key biosynthesis pathways responsible for generating these Fe-S cluster prosthetic groups in *E. coli*. While Isc dominates under normal conditions, Suf takes over during periods of iron depletion and oxidative stress.<sup>1,2</sup> Sulfur acquisition via these systems relies on the ability to remove sulfur from free cysteine using a cysteine desulfurase mechanism. For the Suf pathway, which is also found in pathogenic organisms such as *Mycobacterium tuberculosis*, the dimeric SufS protein serves this purpose using the cofactor pyridoxal-5'-phosphate (PLP) to abstract sulfur from free cysteine resulting in the production of alanine and persulfide.<sup>3</sup> For optimal functionality, SufS requires the sulfur shuttle SufE to remove the persulfide and promote turnover.<sup>4</sup> Previous investigation revealed the steady state kinetics of this process with hydrogen-deuterium exchange mass spectrometry (HDX-MS), demonstrating a half-sites model with the two SufS monomers showing distinct qualities for the two PLP active sites.<sup>5</sup> While cysteine desulfurase activity has been investigated, the mechanism by which this PLP-dependent enzyme operates remains a mystery. Here, we have used site-direct mutagenesis and rapid-mix transient kinetics studies, in conjunction with X-ray crystallography, to analyze the pre-steady state behaviors of this process and define several early mechanistic intermediates in SufS catalytic cycle.<sup>6</sup> Preliminary results of the kinetic consequences resulting from interaction of SufS with the SufE effector protein will also be discussed. Financial support by the U.S. National Institutes of Health is gratefully acknowledged (GM112919)



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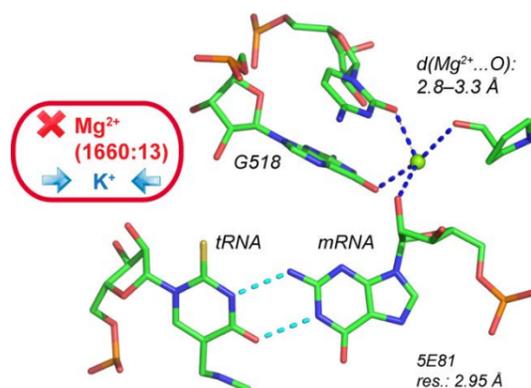
IL-074

## Nucleobase Carbonyl Groups are Poor $Mg^{2+}$ Binders but Excellent Monovalent Ion Binders — Simple Stereochemical Rules Stress the Presence of $K^+$ at the rRNA Decoding Center

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Precise knowledge of  $Mg^{2+}$  binding site properties is vital for our understanding of nucleic acid systems. Unfortunately, the PDB, the main source of  $Mg^{2+}$  binding sites, contains a substantial number of assignment issues that blur our understanding of the functions of these ions. Here, we surveyed nucleic acid X-ray structures with resolutions  $\leq 2.9 \text{ \AA}$  to classify the  $Mg^{2+}$  inner-sphere binding patterns to nucleotide carbonyl, ribose hydroxyl, cyclic ether, and phosphodiester oxygen atoms. We derived a set of "prior-knowledge" nucleobase  $Mg^{2+}$  binding sites and report that crystallographic examples of trustworthy  $Mg^{2+}$  binding sites are fewer than expected since many of those are associated with misidentified  $Na^+/K^+$ . We also emphasize that binding of  $Na^+$  and  $K^+$  to nucleic acids is much more frequent than anticipated. Overall, we provide evidence derived from X-ray structures that nucleobases are poor inner-sphere binders for  $Mg^{2+}$  but good binders for monovalent ions. Based on strict stereochemical criteria, we propose an extended set of guidelines designed to help in the assignment and validation of ions directly contacting nucleobase and ribose atoms. When borderline  $Mg^{2+}$  stereochemistry is observed, alternative placement of  $Na^+$ ,  $K^+$ ,  $NH_4^+$ ,  $Ca^{2+}$  or even  $Zn^{2+}$  must be considered. Among numerous examples, we describe how the application of simple stereochemical rules helps to question the common  $Mg^{2+}$  assignment for the ion located at the rRNA decoding center proximal to  $G_{518}$  and stress the presence of  $K^+$ . Although, at first glance, such assignment issues seem unimportant, it must be envisaged that the  $Mg^{2+}$  stabilization effect is much greater than that of  $K^+$ . Henceforth, a site modeled with one or the other ion behaves differently and affects our perception of the energetics and dynamics of the ribosomal decoding center.



A recurrently assigned  $Mg^{2+}$  with  $K^+$  characteristics in the ribosomal decoding center of several *T. thermophilus* structures with tRNA and mRNA fragments.

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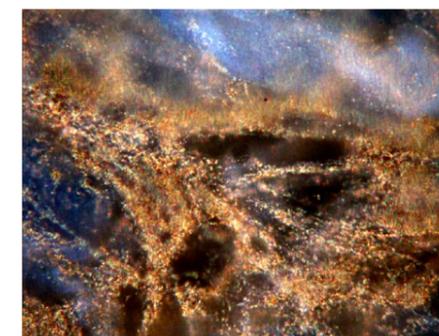
IL-075

## Cascading Biological Effects and Impacts from Gold Nanocrystals

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Gold nanocrystals are well-known for their plasmonic properties, with numerous applications in optical sensing, photothermal heating, metamaterials, and more. In this talk we will discuss how molecules and biomolecules adsorb to these nanocrystals, as a function of particle shape and type. We will also show that there are cascading effects from the initial surface chemistry of the nanocrystals that lead to effects at the molecular, cellular, and ecosystem scales.



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IL-076

## Antimony and Bismuth Hydroxamato/Hydroximato Complexes Targeting Antimicrobial Resistance

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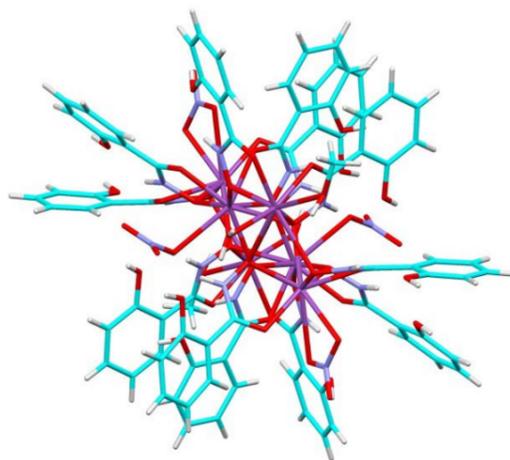
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Antimicrobial resistance (AMR) has emerged as an increasingly serious threat to global public health. It threatens the effective treatment of a range of infections caused by bacteria, parasites, viruses and fungi.

Drug resistant bacterial infections lead to increased healthcare costs, prolonged hospital stays, treatment failures, and increasingly death. By 2050, drug-resistant bacterial infections are projected to annually cause 390,000 deaths in Europe for example. Furthermore there is a startling lack of new effective antimicrobials being developed and brought to market. Significantly bismuth-containing quadruple therapies for *H. pylori* represent a successful strategy for overcoming multidrug bacterial resistance [1].

Leishmaniasis is a neglected tropical disease that is endemic in 98 countries. Approximately 1.3 million new cases of leishmaniasis and 30 thousand leishmaniasis associated deaths are recorded each year. Significantly the incidence of leishmaniasis is increasing due to the failing preventative and therapeutic measures, human migration, global warming and drug resistance [2]. Antimonial-based drugs have been used to treat leishmaniasis since 1910 and are still used in many countries as first choice anti-leishmanial drugs due to their favourable therapeutic indices and relatively low toxicity.

Progress in relation to the development and activity of novel (i) bismuth hydroxamato complexes as potential anti-bacterial agents and (ii) antimony hydroxamato/hydroximato complexes as anti-leishmanial agents will be reported [3-5].



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IL-077

## Vitamin B<sub>12</sub> and the Periodic System

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Replacing the cobalt-center of vitamin B<sub>12</sub> derivatives by other metal ions has been an old challenge for chemists working in the bioinorganic and B<sub>12</sub>-fields [1-6]. Removal of the cobalt-ion from intact cobalamins and other corrinoid vitamin B<sub>12</sub>-derivatives has remained futile [1,5] requiring complex strategies for the preparation of transition metal corrinoids [1,4-6]. Fortunately, the metal-free corrin ligand of vitamin B<sub>12</sub>, hydrogenobyric acid, has become available from biosynthesis by engineered bacteria [7]. This metal-free corrin offers the opportunity to incorporate metal ions chemically and to study the structures and chemical reactivity of the resulting metal-analogues of the cobalt-containing cobyrates. Attachment of the B<sub>12</sub>-nucleotide moiety allows for the direct total synthesis of metbalamins, transition metal-analogues of the cobalamins [4], which are potential B<sub>12</sub>-mimics, e.g. as 'antivitamins B<sub>12</sub>', for use in biological and biomedical experiments [4,8,9]. This lecture will deal with some metal-analogues of natural B<sub>12</sub>-derivatives, their intricate chemical properties and the special role of the corrin ligand of B<sub>12</sub> in binding transition metal-ions.

I would like to thank Martin Warren and his research team at the University of Kent, for a very fruitful collaboration, and Christoph Kieninger and my group for excellent contributions to our B<sub>12</sub>-project, funded generously over the years by the Austrian Science Fund (FWF, current project P-28892).

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IL-078

Compound II — Déjà Vu

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Recent crystallographic reports have suggested that the ferryl form of ascorbate peroxidase (APX) is best described as an iron(IV)hydroxide species. If accurate, this is would be an unusual result. Histidine ligated heme systems are not known to support the iron(IV)hydroxide state. In an effort to provide further insight into this assignment, we have examined the protonation state of the ferryl oxygen in APX compound II using spectroscopic methods. We will present the results from our investigations and attempt to put the recent crystallographic assignments in context, through an examination of previous spectroscopic and crystallographic results.

IL-079

A Catalytic DNA that Promotes Azide-Alkyne Cycloaddition with both Cu<sup>+</sup> and Cu<sup>2+</sup>

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The copper[I] catalysed 1,2,3-triazole forming Huisgen reaction between azides and terminal alkynes (“CuAAC”) has been widely used for bioconjugation reactions owing to its reliability, specificity and biocompatibility. The catalytic copper[I] is usually generated *in situ* by combining a copper[II] salt with a reducing agent. The reduction reaction of copper[II], however, is accompanied by the generation of destructive reactive oxygen species (ROS), and these lead either to oxidative damage to the biomolecules to be conjugated or cytotoxicity if this reaction is carried out within living cells. A few divergent approaches have been reported to address this intrinsic limitation of CuAAC. The present research reports a catalytic DNA (DNAzyme), isolated by *in vitro* selection, that catalyses azide-alkyne cycloaddition with ultra-low concentration of copper[I] *in cis*. What is most interesting is that the DNAzyme also works with low copper[II], such that no extrinsic reducing agent is required. The *in trans* kinetics show that when copper[II] used as the ‘catalytic’ metal ion, there is an induction phase where the reaction rate is slow and then gradually accelerates. This striking result suggests that DNAzyme possesses a limited but in-built reductase activity capable of reducing one or more catalytically relevant Cu[II] Cu[I] *in situ*. When optimized, this DNAzyme will be an excellent addition to the ‘click chemistry’ repertoire and will facilitate studies that have been hampered by the limitations of traditional CuAAC. Financial support by the Natural Sciences and Engineering Research Council of Canada (NSERC) is gratefully acknowledged.

IL-080

## Water-Soluble NHC Stabilized Gold Nanoparticles

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N-heterocyclic carbenes (NHCs) have been investigated as alternatives to thiol-based ligands for the stabilization of metal nanoparticles (NPs) [1,2]. The metal-NHC bond is usually much stronger than the corresponding metal-thiol bond, which should result in more stable NPs, less susceptible to ligand exchange reactions. Although several different types of NHCs have been explored in this regard, the use of NHC ligands to promote water solubility of NPs, is still underdeveloped.

Drawing from the natural amino acid chiral pool, L-histidine was utilized for preparing chiral NHC ligands in the synthesis of water soluble NHC-stabilized gold nanoparticles (AuNPs). For this purpose, *N*-acetyl-L-histidine ethyl ester was converted into its imidazolium salt either using methyl iodide or 2-iodopropane as alkylation agent. Subsequent reaction of the imidazolium salt with [Au(SMe<sub>2</sub>)Cl] yielded the corresponding organometallic gold chloride complex. Histidine-2-ylidene stabilized AuNPs were first generated in organic solvents; the histidine derived capping ligand bore ethyl ester moieties which were saponified, affording water soluble pH-responsive NHC-stabilized AuNPs. Nanoparticles which bear less sterically demanding methyl groups were found to possess pH responsivity (Figure 1) and demonstrated relatively good stability in a variety of biologically relevant solutions (e.g. PBS, NaCl, GSH). In contrast, the isopropyl derivative was much less stable. To evaluate the possibility of biological applications, *in vivo* distribution studies are conducted.

Financial support by the University of Vienna is gratefully acknowledged.

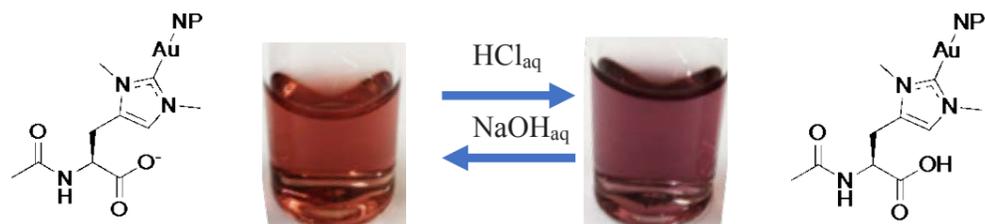


Figure 1. pH responsive gold nanoparticles.

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IL-081

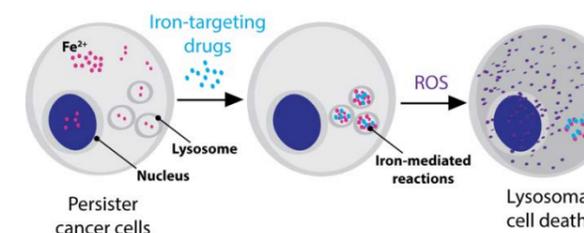
## Reprogramming the Reactivity of Iron in Cancer

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Mesenchymal cancer cells represent a small fraction of solid tumors at a given time point. Typically, these cells are refractory to conventional therapeutic agents. Furthermore, this cell state has been linked to the development of metastasis and cancer relapse. The complex natural product salinomycin has been shown to selectively kill this population of cells across lineages. It was previously proposed that salinomycin mediates its activity by increasing cellular concentrations of alkali metals such as sodium and potassium. To further illuminate mechanisms underlying the selective activity of salinomycin, we used a combination of synthetic organic chemistry, high-resolution microscopy and molecular biology techniques. In particular, we have shown that salinomycin and its synthetic derivatives accumulate in lysosomes and sequester iron in this organelle. As a result, accumulation of iron leads to the production of reactive oxygen species and lysosomal membrane permeabilization, which in turn promotes cell death by means of ferroptosis. These findings revealed the prevalence of iron homeostasis in mesenchymal cancer cells, paving the way towards the development of next-generation therapeutics. Importantly, this work has led to the discovery that iron operates as a master regulator of cellular plasticity in the context of cancer [1].



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IL-082

## Exploring the Mechanism of HNO Release from Photoactive Piloty's Acid Derivatives Incorporating the (Hydroxynaphthalenyl)methyl Phototrigger

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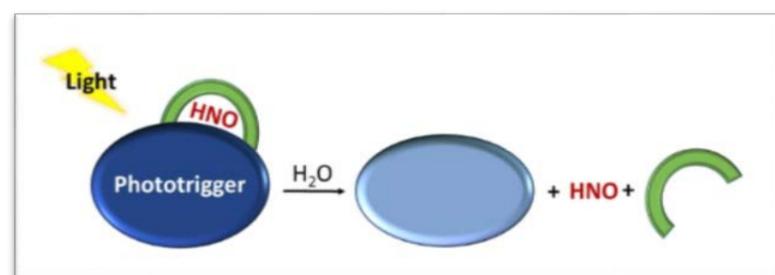
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There is accumulating evidence for the redox cousin of nitric oxide, nitroxyl (HNO, nitrosyl hydride) existing in biological systems. Like nitric oxide, HNO is also a biological signaling molecule. Major biological targets of HNO include transition metal centres of proteins, cysteine side chains of proteins and small molecular weight thiols. Furthermore HNO also shows promise as a therapeutic, particularly in treating heart failure. Identifying the key biochemical mechanisms involving HNO is currently receiving considerable attention. Given that HNO dimerizes rapidly in aqueous solution, molecules which decompose to release HNO (HNO donors) are essential in this emerging field. A major drawback of the currently available HNO donors is the slow rate of HNO release at neutral pH (typically mins to hours). This has prevented us and others from directly determining reaction rates and characterizing intermediates formed for the reactions of HNO with molecules of biological importance. Activation of molecules using light is a highly efficient method to rapidly generate chemical entities upon demand with temporal and spatial control. This talk will focus on the photochemistry of a new class of photoactive HNO donors which combine a (hydroxynaphthalenyl)methyl phototrigger with an N-alkoxysulphonamide HNO generator.



IL-083

## Catalytic Cycle of [FeFe]-Hydrogenase Studied by Spectroscopy and Theory

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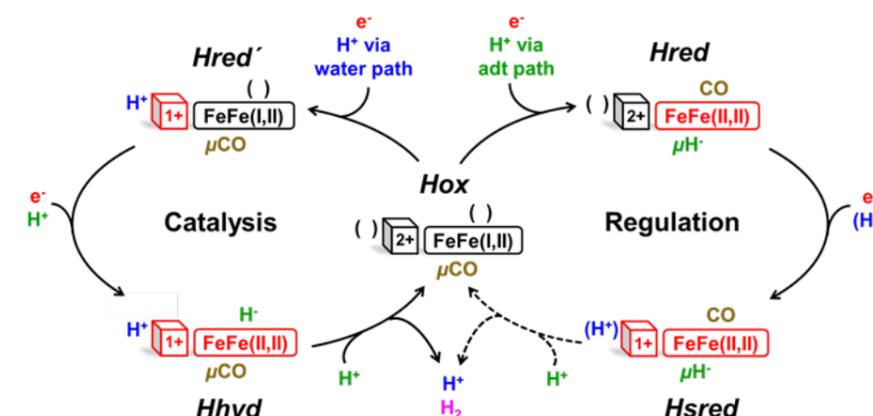
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[FeFe]-hydrogenases are biological hydrogen (H<sub>2</sub>) conversion enzymes with potential for renewable energy applications. The reaction cycle of hydrogen turnover at their catalytic six-iron cofactor, the so-called H-cluster, is controversially debated. We have proposed a novel reaction scheme that attributes the so-far spectroscopically identified H-cluster states either to catalytic or regulatory functions (Fig. 1).<sup>1</sup> These attributions were based on characterization of the H-cluster states using complementary spectroscopic approaches, including infrared and X-ray spectroscopy, as well as quantum chemical and kinetic simulations. A key aspect of the reaction scheme is differential proton-coupled electron transfer that occurs either to the four-iron or diiron sub-complexes of the H-cluster (Fig. 1), which facilitates biasing of the electron localization and redox potential balancing in two sequential cofactor reduction steps as well as rapid H<sub>2</sub> turnover due to avoidance of larger ligand rearrangements at the cofactor. Here, the decisive experimental and computational evidence for this reaction scheme is summarized, derived underlying principles of rapid hydrogen turnover in biology are outlined, and possible implications for improvement of synthetic catalysts are discussed.



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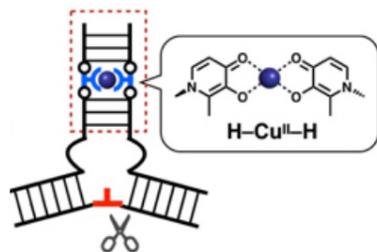
IL-084

## Metal-Responsive DNAzymes Based on Metal-Mediated DNA Base Pairing

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Since we reported the first synthetic metal-mediated DNA base pair in 1999 [1], metal-mediated artificial DNA base pairs, consisting of two or three ligand-type nucleobases and a bridging metal ion, have shown promise as functional units to develop stimuli-responsive DNA materials [2]. Herein we report an enzymatic method to synthesize DNA strands containing a hydroxypyridone (**H**) nucleotide, which forms a Cu<sup>II</sup>-mediated base pair (**H**-Cu<sup>II</sup>-**H**). A two-step reaction using two kinds of polymerases (KF exo- and Dpo4 polymerases) enabled the incorporation of a **H** nucleotide at a desired internal position [3]. The polymerase synthesis was subsequently applied to the development of metal-responsive deoxyribozymes (DNAzymes), whose catalytic activity was expected to be regulated by the formation of a single **H**-Cu<sup>II</sup>-**H** base pair. A metal-responsive DNAzyme was designed based on the sequence of E5 DNAzyme. The RNA-cleaving DNAzyme activity was reversibly switched by the alternate addition and the removal of Cu<sup>II</sup> ions. Furthermore, metal-dependent orthogonal activation of a Cu<sup>II</sup>-responsive **H**-DNAzyme and a Hg<sup>II</sup>-responsive T-DNAzyme was experimentally demonstrated. Accordingly, the facile enzymatic synthesis of artificial ligand-bearing DNAs would significantly expand the toolbox of DNA-based supramolecular chemistry and DNA nanotechnology [3].



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IL-085

## Drug Resistance: What Are the Options for Metal-Based Drugs?

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In this presentation resistance mechanisms to classical metal-based drugs are briefly discussed together with approaches to overcome them. The ability of metal-based drugs to effectively treat intrinsically chemoresistant tumors is then discussed together with the associated mechanisms involved [1]. The presentation concludes with a general outlook on the various approaches that can be used to overcome drug resistance.

Financial support by the EPFL and Swiss National Science Foundation is gratefully acknowledged.

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IL-088

## DNA Based Multi-Porphyrin Systems: From Light Pipes to Membrane Anchors

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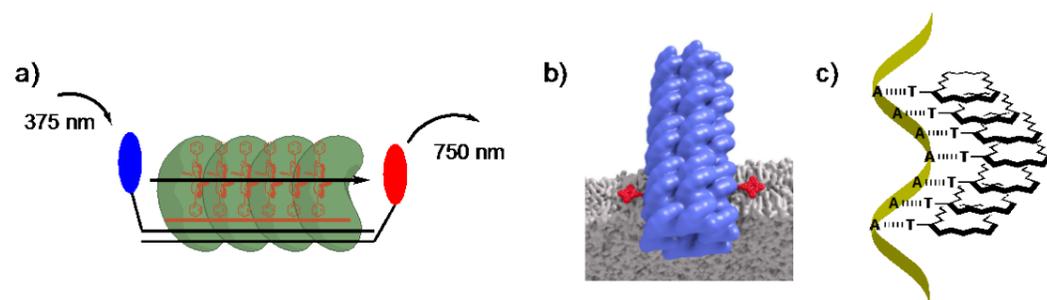
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DNA has become very attractive as scaffold for functional molecules on the nanometre scale. The sequence specific insertion of modified nucleotides using automated DNA synthesis allows for the creation of designer molecules with a wide range of potential applications. We have established a general synthetic route to porphyrin-nucleosides and their subsequent site-specific incorporation into oligonucleotides to create multi-porphyrin arrays [1]. The porphyrin modified DNA is now being used in several applications, spanning optics, electronics, medicinal chemistry and sensors. We will present our latest research in these fields with several selected examples (see Figure):

a) Currently, we are creating a new multi-porphyrin based light pipe which is bio-templated using protein-DNA interactions (RecA), yielding an efficient energy transfer system over a distance of >15 nm.

b) In addition to their photophysical properties, porphyrins are very hydrophobic; we made use of this property to anchor DNA stably into lipid bilayers. In this way, we could create a nano-pore consisting of six DNA helix bundles, where the central cavity of around 2 nm diameter leads to an ionic current [2]. Going small, a DNA duplex with six porphyrins inserted equally well into the membrane, and ionic currents can be observed due to leakage of ions along the DNA. This gives rise to the smallest possible DNA based nano pore [3].

c) The electrochemical properties of the porphyrins are perfect to create genosensors to detect complementary DNA strands of biological and medicinal relevance. We have made both single and multiplex sensors, including microfluidic devices, exploring different designs in the sensor strands [4, 5]. DNA sequences related to H5N1 avian flu virus and to bladder cancer can be detected down to the attomolar concentration, which approaches single molecule detection. Self-assembled porphyrazine systems can be used in sizing forensic short tandem repeat sequences by simple fluorescence readout [6].



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IL-089

## Medicinal Chemistry of Biscarbene Gold(I) Complexes as Anticancer Drugs

Ingo Ott<sup>1</sup>

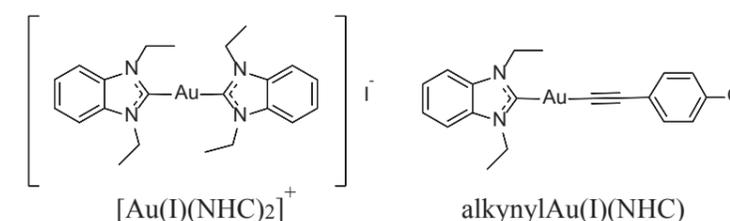
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Organometallic gold complexes have recently attracted mayor attention in inorganic medicinal chemistry based on their high cytotoxic activities and their multiple pharmacological effects against tumor cell proliferation.[1,2] The enhanced stability of their metal-carbon bonds has also been a driving argument regarding the design of potent gold metallodrugs with an improved stability under physiological conditions.

We and many other groups have recently reported on biscarbene gold(I) complexes with two *N*-heterocyclic carbene (NHC) ligands ([Au(I)(NHC)<sub>2</sub>]<sup>+</sup> complexes, see figure for an example) and their potential as anticancer agents.[3-6] The complexes generally triggered high cytotoxic activities, displayed an efficient cellular uptake, and were good inhibitors of the selenoenzyme thioredoxin reductase (TrxR). Other relevant pharmacological effects, such as antimitochondrial properties, could be confirmed additionally. More recently we have developed structurally related neutral biscarbene complexes of the type alkynylAu(I)(NHC).

In this presentation our most relevant current and previous findings on the medicinal chemistry of gold(I) biscarbene species will be summarised with a focus on important early preclinical parameters such as structure-activity-relationships, stability, protein binding, or cellular uptake.

Financial support by the DFG (Deutsche Forschungsgemeinschaft) is gratefully acknowledged.



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IL-090

## Texas Inspired Metal-based Drug Discovery Adventures

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This lecture will present a personal story of a 3x cancer survivor and how with the assistance of great coworkers and collaborators an effort has been made to fight back against this disease by studying the chemistry and anti-cancer biology of texaphyrins and, more recently, gold-carbene redox active systems. Texaphyrins are a specific class of expanded porphyrin (a term we introduced into the literature in 1988 to describe larger homologues of natural blood pigments) that form stable 1:1 complexes with trivalent lanthanide cations, such as Gd(III) and Lu(III). The parent form of the texaphyrins were the founding technology for Pharmacyclics, a company that ultimately developed Imbruvica® (a covalent BTK inhibitor) before being acquired by AbbVie in 2015.

Building on early clinical studies at Pharmacyclics, efforts in the PI's laboratory and that of collaborators have focused on developing Pt(IV) conjugates of texaphyrins as possible drug leads in overcoming platinum resistant colon and ovarian cancer. More recently explorations involving gold-based carbenes have led to the discovery of a set of compounds designed to act as dual targeting agents on the mechanistic level. These agents serve as triggers of immunogenic cell death and show promise as possible cancer vaccines. These parallel drug discovery efforts will be the subject of this presentation.

This work has benefited from support from the U.S. NSF, the NIH, the Cancer Research and Prevention Institute of Texas, and the R. A. Welch Foundation. Collaborations with a number of groups, including those of Profs. Dongho Kim, Jong Seung Kim, Shunichi Fukuzumi, T.K. Chandrashekar, Dirk Guldi, Changhee Lee, Jan Jeppesen, Zahid Siddik, Rick Finch, Zhengrong Cui, and Tomas Torres, are gratefully acknowledged. Thanks also go to Drs. Jonathan F. Arambula and Gregory Thiabaud whose vision has largely driven these projects.

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IL-091

## Interactions in G-Quadruplexes: Quantifying the Interaction of Cations with the Internal Channel Site

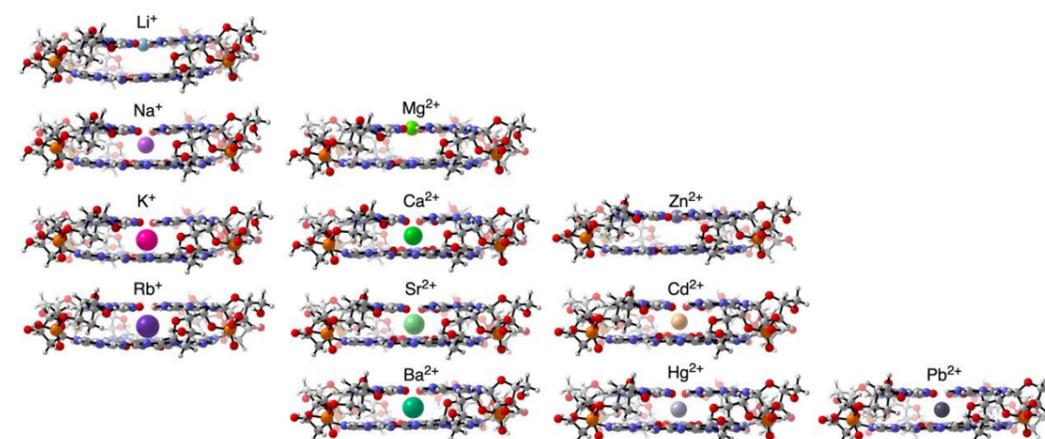
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Coordination of an alkali metal cation is essential for successful guanine quadruplex (GQ) self-assembly in both supramolecular environments and biological conditions. This work investigated computationally the interaction of RNA GQs and DNA GQs with monovalent and divalent cations. New insights on the necessity of the cation for the self-assembly will be presented as well as the higher stability of RNA quadruplexes will be explained. Quantifications of the energetic parameters determining the affinity of these cations by means of an energy decomposition analysis answers why potassium is preferred over sodium in quadruplexes.[1, 2] Simplified model systems of cation-scaffold coordination facilitated the identification of the key donor-acceptor orbital interactions between the metal and the GQ scaffold.[3] Novel findings contribute towards the understanding of lead's ability to induce genomic instability due to its intrinsic physical properties and a particularly high affinity towards quadruplex structures.

Financial support by Netherlands Organisation for Scientific Research (NWO) is gratefully acknowledged.



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IL-092

## Metal-Based Dithiocarbamate Glycoconjugates: A Suitable Strategy to Target Tumor Glycolysis?

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Some metal-dithiocarbamate complexes have shown outstanding *in vitro* and *in vivo* antitumor activity together with negligible systemic and organ toxicity (owing to the intrinsic chemoprotective function of the coordinated dithiocarbamate moiety), although selective tumor targeting is still a major issue.[1]

In order to maximize impact on cancer cells and minimize side-effects, our approach relies on the conjugation of biologically-active molecules to potential metallodrugs. In particular, we aim at designing “Trojan Horse”-type complexes characterized by an improved selectivity provided by selected coordinated ligands targeting specific receptors overexpressed in cancer cells, so as to achieve biomolecular recognition and tumor targeting.[2]

Rapidly dividing tumor cells require higher amounts of nutrients and energy for their fast proliferation, and glucose is no exception (the so-called “Warburg effect”). Consequently, such increased demand of glucose by cancer cells makes it very attractive to selectively target tumor sites. In particular, tailored glucose-like substrates can be conjugated to chemotherapeutics (including metal-containing anticancer agents) to attain the site-specific delivery of drugs into the affected tissues.[3]

Accordingly, we have been focusing on the design of metal-dithiocarbamate glycoconjugates which can combine the antitumor properties and the favorable toxicological profile of the metal-dithiocarbamate scaffold,[1] along with an improved selectivity and cellular uptake provided by the glucose-containing ligands coordinated to the metal center, through the exploitation of the glucose-mediated cellular internalization provided by glucose transporters (GLUTs).[4]

With a view to developing more efficient strategies to the functionalization of metallodrugs with carbohydrates, we here report on an innovative and efficient synthetic route to generate metal-dithiocarbamate glycoconjugates (including Au(I), Au(III), Pt(II) and Mn(I) derivatives) in high yields and purity.[5] Starting from the rationale behind our research work, the main results and preliminary *in vitro* biological studies are illustrated and discussed.

Financial support by NUI Galway (College of Science Scholarship 2014 to AP; Millennium Fund Minor Project 2013 to LR), Irish Research Council (Postgraduate Scholarship GOIPG/2015/2961 to AP), and COST Action CM1105 “Functional metal complexes that bind to biomolecules” is gratefully acknowledged.

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IL-093

## N-Alkylaminoferrocene-Based Prodrugs Activated by Oxidation to Toxic Species: Potential Applications for the Treatment of Cancer and Inflammatory Diseases

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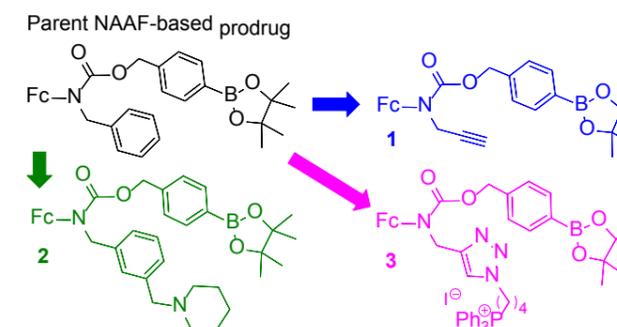
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In contrast to normal cells, cancer cells produce elevated amounts of reactive oxygen species, e.g. hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Based on this difference we developed cancer-specific N-alkylaminoferrocene (NAAF)-based prodrugs. For example, a parent N-benzyl-substituted prodrug (its structure is shown in black in the Scheme) is oxidatively activated in the presence of typical for cancer cells ROS amounts with formation of toxic ferrocenium species that is followed by the amplification of ROS in cancer cells thereby causing their death. However, this compound has some unfavourable properties including proneness to aggregation, moderate ability to generate ROS, low solubility in aqueous buffers and moderate efficacy towards a range of cancer cell lines, primary cells and *in vivo*. In this presentation I will report on our progress in solution of these problems that led to improved prodrugs **1-3** [1-3]. Furthermore, I will discuss the mechanism of action of the NAAF-based prodrugs *in vivo* [4] and present new *in vivo* data indicating that these compounds can be potentially applicable for the treatment of pathologic states characterized by chronic inflammation [5].



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IL-094

## Protein Template Assisted Synthesis of Metal Sites Relevant for Biology and Chemistry

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Synthetic biochemistry and synthetic inorganic chemistry come together in a synergistic manner, in order to elucidate structure and functional aspects of metal sites in enzymes. In particular, small proteins and synthetic peptides involving rich sulfur coordination spheres, as Rubredoxins (Rds) and analogues have been extensively used. The tetra-cysteinyll metal coordination site available in Rd has the surprising capacity of chelating a wide variety of metal ions (beyond Fe) such as Zn, Co, Cd, Ga, In, Hg Ni, Cu, as well as Mo (and W), with particular interest in modeling NiFeHydrogenases and Mononuclear Mo(W) enzymes and other systems. Another case studied is the Orange Protein (ORP), a small bacterial protein, that harbors a unique molybdenum/copper (Mo/Cu) heterometallic cluster, [S<sub>2</sub>MoVIS<sub>2</sub>CuS<sub>2</sub>MoVIS<sub>2</sub>]<sub>3</sub>-, non-covalently bound. We explored the ORP capability of promoting protein-assisted synthesis in order to prepare metal protein derivatives, harboring novel and unique molybdenum heterometallic clusters, such as Mo/Fe-ORP, Mo/Co-ORP, Mo/Ni-ORP, or Mo/Cd-ORP, with distinct magnetic properties. A third case under study concerns a peptide α3DIVL21C, that possesses a four cysteine residues environment (rubredoxin-like) and can bind Mo, another useful model for the sulfur rich environment in molybdenum-bis pyranopterin-containing enzymes (Mo-bis PDG).

The Associate Laboratory Research Unit, for Green Chemistry-Technologies and Processes Clean - LAQV supported this work, also financed by national funds from FCT/MCTES co-financed by the ERDF (PT2020 Partnership Agreement). Thanks are given to many relevant contributions from Isabel Moura, Marta Carepo, Luísa B. Maia, Sofia R. Pauleta, Biplad K. Maiti, Maddalena Elia and Vincent Pecoraro.

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IL-095

## Heme Meets DNA

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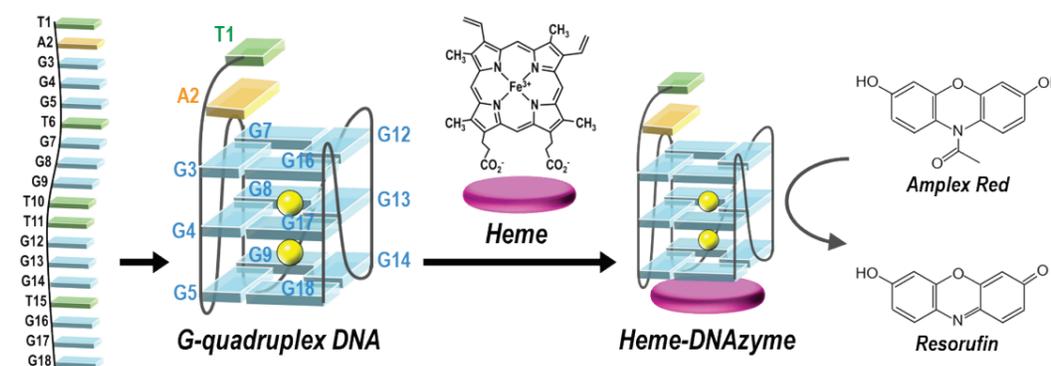
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The RNA world hypothesis [1] has provided a strong stimulus for exploring new catalytic properties for nucleic acids. Heme is a likely player in the postulated RNA world. Heme-bound nucleic acids have been shown to recapitulate at least two of the known catalytic functions of contemporary hemoproteins, lending credence to heme's link to a primordial RNA world [2]. Hence, the heme-bound nucleic acids could be regarded as prototypes for redox-catalyzing ribozymes in the primordial RNA world.

We found that heme binds selectively to the 3'-terminal G-quartet of all parallel-stranded unimolecular G-quadruplex DNAs formed from human telomere related sequences, through a pi-pi stacking interaction between the porphyrin moiety of the heme and the G-quartet, to form 1:1 complexes which exhibit peroxidase activities [3]. In the complexes, a water molecule (H<sub>2</sub>O) at the interface between the heme and G-quartet is coordinated to the heme Fe atom as an axial ligand [4], and possibly acts as an electron-donating ligand that promotes heterolytic peroxide bond cleavage of hydrogen peroxide bound to the heme Fe atom, *trans* to the H<sub>2</sub>O, for the generation of an active species. Unique physicochemical properties of the H<sub>2</sub>O in the complex [5], together with the peroxidase catalytic cycle of the complex, are discussed.



We are indebted Prof. Dipankar Sen (Simon Fraser University, Canada) for the insightful discussion. This work was financially supported by JSPS KAKENHI (No. 16KT0048 and 19H02824 to Y.Y. and 17H03027 to T. K.), and the Bilateral Open Partnership Joint Research Project (No. BBD29011 to Y.Y.).

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IL-096

## Anticancer Gold-Based Drugs: New Mechanistic Insights

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Gold compounds form a promising class of experimental anticancer agents manifesting potent antiproliferative properties *in vitro*. Their mode of action is clearly distinct from Pt drugs and, in most cases, DNA independent. In spite of the large number of studies carried out so far, the mechanisms of action of cytotoxic gold compounds are not fully elucidated and remain largely elusive. We have tried to unveil the molecular mechanisms of a few representative gold compounds with the aid of different investigative approaches. Proteomic methods are particularly effective in disclosing drug induced cellular events opening new horizons in the fields of drug discovery and drug delivery. This type of approach has been applied to 6 structurally diverse gold compounds – both gold(I) and (III) complexes - and to the clinically established gold(I) drug Auranofin in A2780 ovarian cancer cells. Proteins differentially expressed upon treatment were identified through 2D gel electrophoresis and MALDI TOF determinations and their meaning tentatively interpreted. A variety of proteomic alterations were observed for the investigated gold compounds in strict relation to their chemical nature suggesting the occurrence of different modes of action. The cellular processes mostly affected by gold compounds were identified in the various cases. Additional information was obtained by developing a cancer cell line showing acquired resistance to Auranofin. Subsequently, some structure-function relationships were drawn by analyzing the cellular effects of a panel of metal compounds that closely reproduce the main chemical features of AF. Overall, these studies underscore the large amount of mechanistic information that may be gained by applying a variety of investigative methods at the cellular level. Perspectives for future work are delineated.

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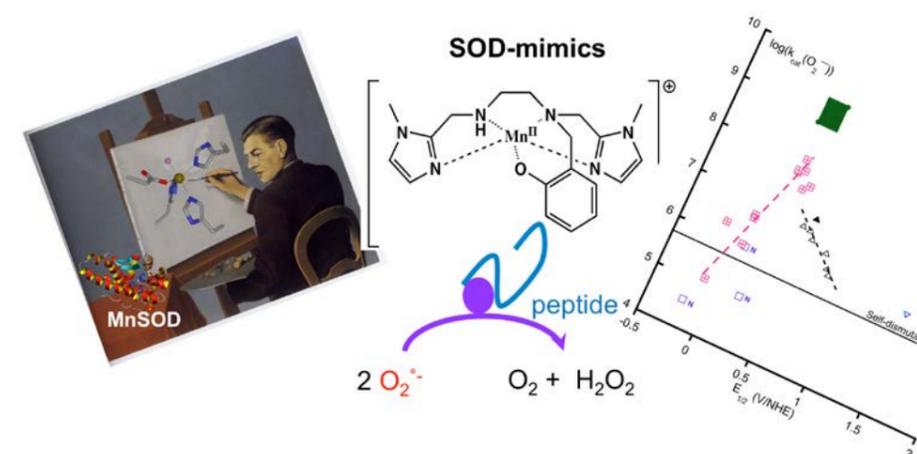
IL-097

## Design of Bio-Inspired SOD Mimics: From Artificial Amine Centered Mn-Complexes to Cu-Peptidomimetics

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Superoxide dismutases are redox active metalloproteins that protect the cell against oxidative stress. These enzymes are highly efficient in catalyzing the dismutation of superoxide with a kinetics close to the diffusion limit. These proteins shows several physico-chemical parameters that have been evolutionarily optimized [1]. The metal cation at the active site of SOD shows a redox potential tuned for the catalysis of the dismutation. There is also efficient electrostatic guidance common to almost all SODs that attract the negatively charged substrate  $O_2^-$ . SOD mimics are low molecular weight complexes designed to reproduce the activity of SOD and that can be interesting for therapeutic applications. In this talk, we will delineate the bio-inspired approach that we have used for the design of SOD mimics [1]. Artificial Mn-complexes based on a central amine or di-amino-ethane [2-5] will be presented and another family of complexes based of peptide developed using a combinatorial approach will also be introduced in the case of a library of Cu-based SOD-mimic peptide [6]. These compounds are interesting candidates to be used in therapeutics as catalytic drugs to fight against oxidative stress.



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IL-098

## Structural Rearrangements and Chemical Coupling Drive the Nickel-Based Organometallic Enzymology of Anaerobic CO<sub>2</sub> and CO Fixation

**Stephen W. Ragsdale<sup>1</sup>, Seth Wiley<sup>1</sup>, Mehmet Can<sup>1</sup>, Peter Eckert<sup>2</sup>, Kevin Kubarych<sup>2</sup>, Ritimukta Sarangi<sup>3</sup>**

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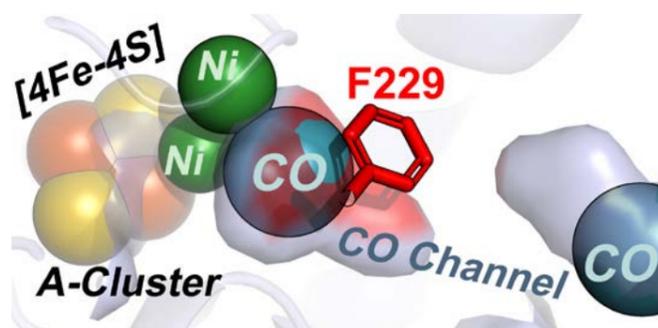
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The Wood-Ljungdahl Pathway (WLP) allows diverse classes of anaerobic microbes to remove and fix CO<sub>2</sub> and CO from the atmosphere. Acetogenic bacteria also couple CO<sub>2</sub> reduction to H<sub>2</sub> oxidation in this pathway as their main source of ATP and reducing equivalents. The major enzyme within this pathway is acetyl-CoA synthase (ACS), part of an enzyme complex that also contains CO dehydrogenase (CODH). CODH catalyzes CO<sub>2</sub> reduction to CO, which travels through a tunnel to the active site A-Cluster of ACS, where CO is condensed with coenzyme A and a methyl group donated by the corrinoid iron-sulfur protein (CFeSP) to form acetyl-CoA. In 1985, the WLP pathway was proposed to involve a series of Ni-based organometallic intermediates (1). The A-cluster is composed of an unusual di-nickel center bridged to an [4Fe-4S] cluster, where the proximal Ni (Ni<sub>p</sub>) forms the bridge between the [4Fe-4S] cluster and the distal Ni (Ni<sub>d</sub>). Ni<sub>d</sub> is the site of CO binding (2) and is proposed also to bind the methyl group and CoA. Photolysis and crystallographic experiments indicated that a hydrophobic alcove directs CO to and keeps CO near the A-cluster (3, 4).

To understand the role of Ni and of the alcove in substrate binding, a residue, which forms a wall of the alcove was substituted with alanine (F229A). Using infrared (IR), electron paramagnetic resonance (EPR), and X-ray absorption (XAS) spectroscopies and kinetic analyses, F229A was shown to disrupt acetyl-CoA synthesis and binding of CO, while methylation and A-cluster composition remain intact. We propose the alcove plays an essential structural role in the ACS mechanism by binding and retaining CO near Ni<sub>p</sub> to facilitate formation of the key Ni<sub>p</sub>(I)-CO, methyl-Ni and acetyl-Ni intermediates in the WLP of acetyl-CoA synthesis. We also propose an essential role of chemical coupling to electron transfer in forming the active Ni<sub>p</sub>(I) species in the WLP.

Financial support by NIH (GM39451) is gratefully acknowledged.



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IL-099

## Spectroscopy, Quantum Chemistry and Reaction Mechanisms: The Case of CO<sub>2</sub> Activation

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The lecture will address how to productively use a combination of spectroscopy and quantum chemistry to obtain insight into chemical reaction mechanisms. Examples will be taken from recent work on CO<sub>2</sub> activation chemistry.[1,2]

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**IL-100**

**In Pursuit of Fe<sup>V</sup>=O Oxidant(s) in Bio-Inspired Nonheme Iron Oxidation Catalysts**

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For the past twenty-five years, we have been interested in the development of nonheme iron catalysts that can use peroxides to oxidize alkanes and olefins in an effort to mimic the fascinating oxidative chemistry carried out by metalloenzymes such as the Rieske oxygenases and methane monooxygenase. Current mechanistic hypotheses invoke mononuclear Fe<sup>V</sup>=O<sup>[1]</sup> or dinuclear Fe<sup>IV</sup>-O-Fe<sup>IV</sup>=O oxidants.<sup>[2]</sup> We have recently found evidence that metastable Fe<sup>III</sup>-OOH intermediates can be activated by Lewis or Bronsted acids to carry out cyclohexane hydroxylation within seconds at -40 °C, suggesting the generation of quite a powerful oxidant.<sup>[3,4]</sup> Furthermore, high alcohol/ketone ratios are observed, excluding the possibility that hydroxyl radicals are involved in these reactions. Our progress in identifying the nature of the oxidant will be discussed.

Financial support from the US National Science Foundation and the National Institutes of Health is gratefully acknowledged.

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**IL-101**

**Cyanobacterial Mn(II), Oxidative Stress, and the Evolution of Photosynthesis**

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Earth was irreversibly changed by the evolution of oxygenic photosynthesis and the rise of atmospheric oxygen in the Great Oxygenation Event (GOE). Life on Earth today uses manganese both for dioxygen production in oxygenic photosynthesis and for protection from deleterious oxidation reactions. The catalytic Mn<sub>4</sub>CaO<sub>5</sub> cluster at the heart of photosystem II is an evolutionary singularity: oxygenic photosynthesis evolved only once, and the same chemistry is used by all known oxygenic photosynthetic organisms, from microbes to plants. In extant organisms, Mn(II) plays an essential role in protecting cells from oxidative stress, not only as a cofactor in antioxidant proteins such as superoxide dismutase and catalase but also in the form of small-molecule inorganic complexes. Prior to the GOE, ~ 2.35 billion years ago, there would have been no evolutionary pressure to develop antioxidant proteins, so pre-GOE life must have depended entirely on inorganic chemistry for protection from oxidation. In order to gain insights into the chemistry of these ancient processes, we have been studying manganese speciation in a variety of extant Cyanobacteria (*e.g.*, *Synechocystis* sp. PCC6803). In modern *Synechocystis*, cellular Mn(II) concentrations are vastly higher than expected based on proteomic requirements, suggesting a continuing role for Mn(II) in antioxidant biology. We are using a combination of fluorescence, magnetic resonance (EPR/ENDOR), XANES, and mass spectrometric techniques to examine manganese concentration, oxidation state, ligand environment, and localization in whole Cyanobacterial cells, providing insights into Mn biogeochemical cycling today and on the early Earth.

## IL-102

### Insight into Non-Covalent Interactions of Biological Active Copper Complexes and Relevance to Their Biological Activity

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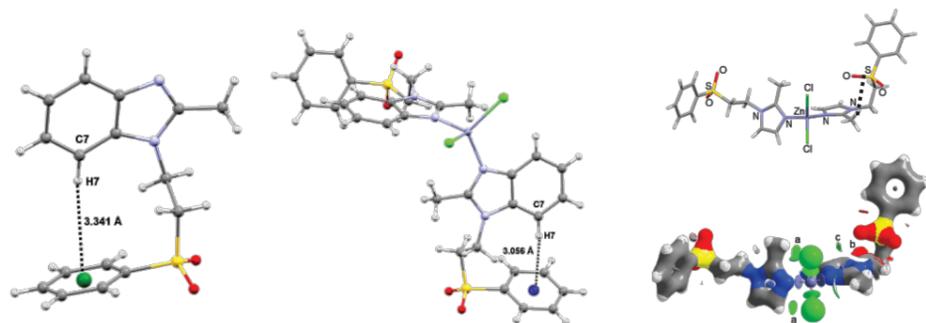
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We have been interested into transition metal coordination compounds of imidazole and benzimidazole derivatives with biological properties [1-2]. Among them, sulfone derivatives are used for the treatment of trichomoniasis, giardiasis, amebiasis and amebiasis [3]. The activity of the ligand may be modify upon coordination to a transition metal ion, either decreasing its side effects, enhancing its biological activity or even showing different activity than the ligand by itself [4,5]. We decided to synthesize transition metal coordination compounds with a series of these derivatives in order to study their cytotoxic and/or antiparasitic properties, as well as to investigate their structural properties and the role of relevant non-covalent interactions (lone pair...pi, pi...pi, H...pi, H...X) into their activity.

Financial support by PAIP 5000-9035 and DGAPA IN223219 (UNAM) is gratefully acknowledged.



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## IL-103

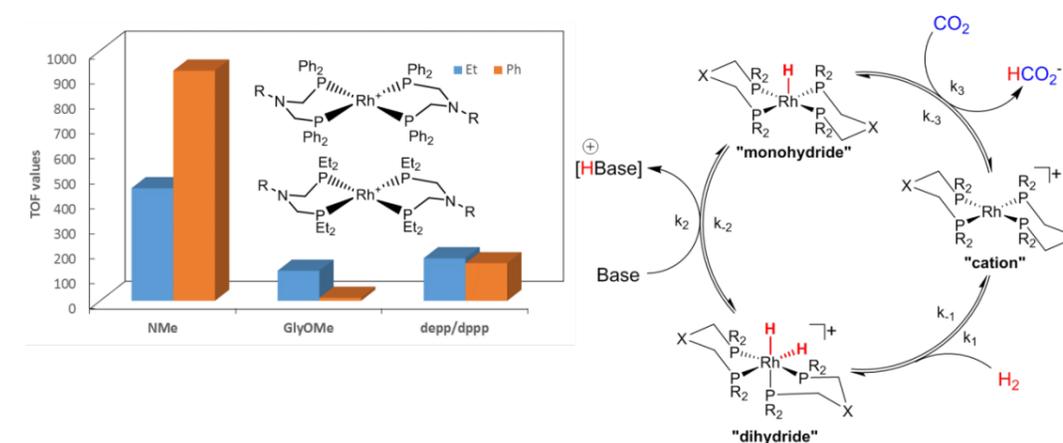
### Influencing Catalytic Reactivity for CO<sub>2</sub> Hydrogenation Using an Amino Acid Scaffold

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In order to achieve enzymatic rates and efficiencies for multi-proton and multi-electron reactions, as well as to understand how enzymes achieve such high performance, we are adding enzyme-inspired outer coordination spheres to thermal catalysts for CO<sub>2</sub> hydrogenation. With a family of amino acid and peptide derived Rh-bis(diphosphine) complexes, we see orders of magnitude differences in rate for certain complexes. Data are consistent with modifications which appear to influence CO<sub>2</sub> addition, as well as modifying the rate by influencing the pK<sub>a</sub> of the pendant amine. Results also show that a modification of the rate limiting step modulates the effect of the scaffold. The clear and significant impact of the outer coordination sphere indicates that it is applicable to many catalysts for small molecule conversion, and provides a platform to understand the role of the scaffold for both enzymatic and synthetic systems.

Financial support by the US Department of Energy, Chemical Sciences, Geosciences, and Biosciences is gratefully acknowledged.



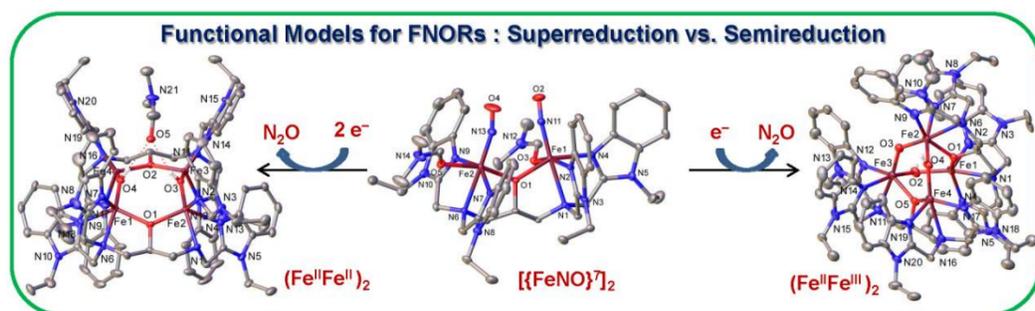
## IL-104

### Functional Mononitrosyl and Dinitrosyl Complexes Generate N<sub>2</sub>O in Relevance with FNORs via a Superreduced and a Semireduced Mechanism Respectively

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The catalytic reduction of NO to N<sub>2</sub>O by flavodiiron nitric oxide reductases (FNORs) involves both mono- and di-nitrosyl diiron(II) species as the key intermediates. [1] While three dinitrosyldiiron(II) complexes were known to mediate the reduction of NO to N<sub>2</sub>O, [2-5] there had been no report for a synthetic mononitrosyl diiron(II) complex, neither was there any direct proof for the identity of the end products after N<sub>2</sub>O generation from the functional model complexes. Based on the redox behavior of a series of thiolate and thiocarboxylate bridged diiron(II) complexes, [5] we have developed an unique synthetic strategy for the synthesis of such an unprecedented mononitrosyl diiron(II) complex [6] and its dinitrosyl analogue. The mono- and di-nitrosyl diiron(II) complexes produce N<sub>2</sub>O upon chemical as well as electrochemical reduction in nearly quantitative yields and thus qualify as the functional models for the two key intermediates of FNORs. Based on the spectroscopic studies, measurement of N<sub>2</sub>O yields and the first ever isolation of the end products after N<sub>2</sub>O generation, it has been established that while the mononitrosyl diiron(II) complex produces N<sub>2</sub>O following an intermolecular superreduced mechanism, the dinitrosyl diiron(II) complex generates N<sub>2</sub>O following an intramolecular semireduced mechanism. [7] Financial support for this work by SERB India and CSIR India is gratefully acknowledged.



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## IL-105

### Thiosemicarbazone Copper Complex Stability and its Influence on Paraptosis Induction

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Due to their high biological activity, Thiosemicarbazones (TSC) are promising candidates in the development of anticancer therapy. Part of their effectivity arises from their ability to chelate metal ions, such as copper and iron, essential for organisms and cells. Triapine, the best studied anticancer TSC, was already tested in several clinical phase I and II trials with promising results against hematological diseases. During the last years, two novel TSCs (DpC and Coti-2) entered clinical trials. This subclass of TSCs with much higher anticancer activity was found to exhibit an additional mode-of-action compared to the less active Triapine. One characteristic is their ability to induce a novel form of programmed cell death, paraptosis, which is characterized by the formation of cytoplasmic vesicles originating from the endoplasmic reticulum (ER), caspase-independent signaling and mitochondrial damage.

The aim of this study was to identify structural and chemical properties associated with paraptosis induction of TSCs. For this purpose, a panel of structurally related TSCs was tested for their Cu(II) complex reduction stability. Strikingly, TSCs with higher anticancer potential and paraptosis-inducing properties, showed higher complex stability, a slower reduction rate by L-glutathione, and consequently less efficient re-oxidation and superoxide generation. Consequently, we hypothesize that only very stable, weakly reducible Cu(II) TSC complexes are able to reach the ER, where they inhibit the protein disulfide isomerase (PDI), an ER-resident protein responsible for the formation and rearrangement of disulfide bonds. Subsequently, this leads to deregulation of the ER thiol homeostasis and paraptosis induction.

## IL-106

### Using Protein Scaffolds to Control the Reactivity of Molecular Catalysts for CO<sub>2</sub> Hydrogenation

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The hydrogenation of CO<sub>2</sub> to formate is an attractive vehicle for energy storage because of the energy density of formic acid. Efficient catalysts for CO<sub>2</sub> hydrogenation are needed for the generation of this fuel. For efficient catalysis to occur, there needs to be efficient interactions between the hydride donor, i.e. metal-hydride catalyst, and hydride acceptor, i.e. the CO<sub>2</sub> substrate. We have investigated using a protein scaffold to help facilitate this interaction. We immobilized a rhodium bisdiphosphine complex within a homo-dimeric protein scaffold, LmrR native to *Lactococcus lactis*.<sup>1</sup> Covalent attachment of a [Rh(PN<sup>δ</sup>lyP)<sub>2</sub>]<sup>+</sup> within LmrR was confirmed using ESI-MS, ICP-OES, UV-Vis, circular dichroism, and nuclear magnetic resonance (<sup>1</sup>H, <sup>13</sup>C, <sup>15</sup>N, <sup>31</sup>P). High pressure Operando NMR spectroscopy was used to measure *in situ* formate production by Rh-LmrR under 34 atm of CO<sub>2</sub>:H<sub>2</sub> (1:1) at varying temperatures in an aqueous solution. The metalloenzyme is most active at room temperature and displayed a turnover frequency of 0.38±0.03 h<sup>-1</sup> at 58 atm and 298 K, and achieved an average turnover number of 14 ± 3. Proposed catalytic intermediates generated and characterized suggest the protein scaffold enables catalysis by facilitating the interaction between CO<sub>2</sub> and the hydride donor intermediate. In contrast, the [Rh(PN<sup>δ</sup>lyP)<sub>2</sub>]<sup>+</sup> complex without the protein scaffold is not a catalyst for formate production under identical conditions. Mutagenesis studies are currently under investigation to understand how the protein scaffold imparts catalytic activity.

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## IL-107

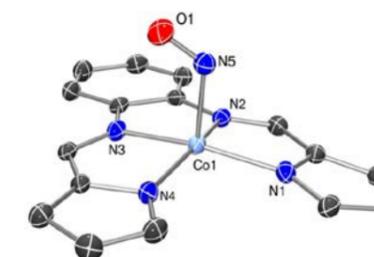
### Synthesis and Reactivity of Metal Nitrosyls in Relation to the Generation of Reduced NO<sub>x</sub> Species

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Nitrogen is an essential element for all forms of life on this planet. As such, nature has evolved metalloenzymes to utilize the N<sub>2</sub> present in the atmosphere and convert it to more bioavailable forms such as ammonia. This process and the chemical reactions transforming inorganic N-containing small molecules in general, is part of the Global Nitrogen Cycle (GNC). The GNC describes the conversions of all environmentally-relevant N-containing species on this planet including nitrate (NO<sub>3</sub><sup>-</sup>), nitrite (NO<sub>2</sub><sup>-</sup>), nitric oxide (NO), nitrous oxide (N<sub>2</sub>O), etc. where Fe-containing enzymes play a central role. Attention in the chemical transformations of select nitrogen oxides (NO<sub>x</sub>) has heightened due to the overuse of nitrogen-rich fertilizers, which has increased the concentration of NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> to toxic levels in water runoff. Additionally, other work emphasizes the role of NO<sub>2</sub><sup>-</sup> as a reservoir of NO for mammals under hypoxic conditions. Indeed, NO<sub>x</sub> compounds participate in a wide range of biological processes. Of interest to our group is the metal-promoted reduction of NO<sub>2</sub><sup>-</sup> to produce NO (and eventually NH<sub>3</sub>) as well as the construction of metal-bound nitroxyl (NO<sup>-</sup> or HNO) coordination complexes. This talk describes our efforts in understanding the fundamental bioinorganic chemistry of NO<sub>x</sub> reduction mediated by non-porphyrin Fe and Co systems [1-6].

Financial support by the United States National Science Foundation (NSF) is gratefully acknowledged.



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## IL-108

### Oxindolimine-Copper(II) Complexes as Potential Antitumor Agents: Main Targets and Cellular Damage Monitored by Raman Microscopy

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Different oxindolimine-copper(II) complexes were designed and prepared, containing *N*, *O* and/or *S* coordinating atoms, with the aim of verifying differences in its cytotoxicity and its possible intracellular targets. These complexes were characterized by different spectroscopic techniques (UV/Vis, IR, EPR) and had its cytotoxicity toward HeLa cells monitored by MTT assays. The determined IC<sub>50</sub> values were in the range 25-90 μM, depending on the structural features of the complexes. DNA is a main target, as attested by comet tests, indicating significant cleavage of the strands. However, ROS formation monitored by flux cytometry (Muse Oxidative Stress Kit, Merck Millipore) was negligible, in contrast to previous studies with similar complexes, also derived from oxindoles, that indicated an oxidative mechanism of damage [1].

Recently, confocal Raman microscopy associated to chemometrics methods (MCR-ALS = multivariate curve resolution asymmetric least squares) have been used to demonstrate distribution of anticancer drugs inside the cell, and consequent cellular damage, [2]. We managed to follow the intracellular spreading of some of these copper(II) complexes in HeLa cells, after 16h incubation at 37°C, through SERS (Surface Enhanced Raman Spectroscopy) technique. The SERS probe was gold nanosphere (AuNS), DTNB as Raman reporter and the complex [Cu(nisatp)] or [Cu(isatp)] (25 μM). Two exciting radiations were used: 785 nm for which AuNS gives huge SERS signals and 532 nm for which AuNS SERS enhancement are absent. For this 532nm excitation only water and high concentrated intracellular components will give Raman signals. Results with only the Raman reporter adsorbed on AuNS showed a pronounced cellular membrane permeabilization, with remarkable water entrance in the cell. In contrast, when cells were treated with AuNS+DTNB+[Cu(nisatp)] or [Cu(isatp)] this effect was surprisingly reduced, and a protective action regarding the membrane cell was observed. In both cases, proteins in the nucleus are also an important target, as visualized in Figure 1. These results are consistent with kinase and topoisomerase proteins inhibition already verified in the presence of this class of metal complexes.

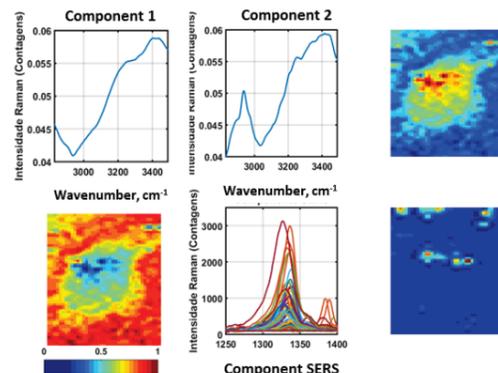


Figure 1 – Chemical images using 532nm exciting radiation for components1 (water) and 2 (protein), using the reporter SERS signal at ~1345 cm<sup>-1</sup> (785 nm exciting radiation). HeLa cells treated with AuNS+DTNB+[Cu(nisatp)].

Acknowledgements: FAPESP, CEPID Redoxoma, CAPES, CNPq.

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## IL-109

### Au(I)-NHC-Anthracenyl and Ag(I)-NHC-Anthracenyl Complexes Binding to Biosubstrates: Some Recent Results with a Focus on Solution Equilibria

Tarita Biver<sup>1,2</sup>, Federica Guarra<sup>2</sup>, Francesca Binacchi<sup>2</sup>, Alessandro Pratesi<sup>3</sup>, Damiano Cirri<sup>3</sup>, Maria Giulia Fabbri<sup>3</sup>, Tiziano Marzo<sup>2</sup>, Chiara Gabbiani<sup>2</sup>, Luigi Messori<sup>3</sup>

<sup>1</sup>Department of Pharmacy, University of Pisa, Via Bonanno 6, 56126 Pisa, Italy.

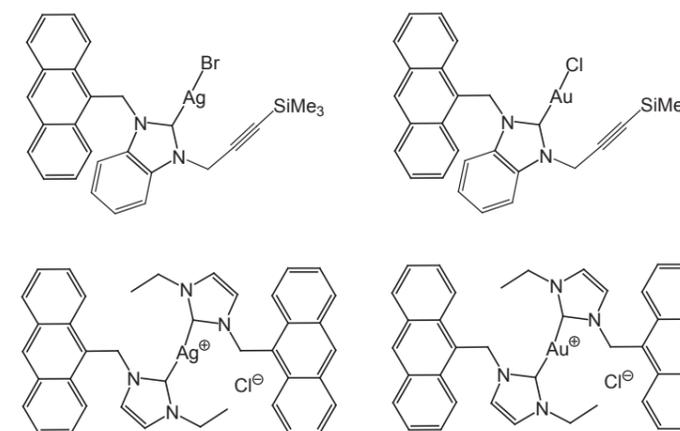
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In 1929, Forestier identified the utility of gold in the treatment of rheumatoid arthritis (RA); later, the well-known orally active gold(I)–phosphine–thiolate complex, Auranofin, was developed to treat RA in a clinical setting [1]. Going beyond the use for RA, there have recently been extensive studies on the therapeutic applications of gold complexes as anticancer agents [2,3]. Most previously reported anticancer gold(I) complexes contain a thiolate group and/or a phosphine ligand but the NHC ligand has emerged as a promising ligand [4]. Also silver(I)-NHC systems have been tested, and recent reports underline an antitumor activity comparable and in some cases greater than that of gold carbenes [5].

We report here on some of our recent results on the biological activity of the Au(I)/Ag(I) – NHC – anthracenyl fluorescent complexes shown in the Figure.

The solution behavior of these complexes and some mechanistic details on their interaction with biosubstrates as DNA were analysed. To this aim ESI-MS experiments, spectrophotometric and spectrofluorometric titrations, melting assays and viscometric tests were done. The results of this analysis will be discussed also in the light of parallel cytotoxicity and thioredoxin reductase activity tests.



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## IL-110

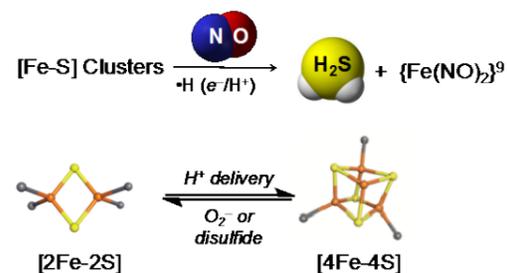
### Synthetic Modeling Chemistry of Iron-Sulfur Clusters Relevant to Redox Signaling Processes

Eunsuk Kim<sup>1</sup>, Kady Oakley<sup>1</sup>, Kevin Sterling<sup>1</sup>, and Ryan Lehane<sup>1</sup>

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Iron-sulfur clusters are common to the most ancient components of living matter. Although the importance of proteins bearing [Fe-S] cofactors has been recognized in electron transfer and in catalysis for several decades, there has been growing interest in Fe-S proteins in recent years with the discovery of their regulatory roles found in bacteria to humans. The common theme in the regulatory activity of Fe-S proteins appears to be that the [Fe-S] clusters respond to specific oxidants such as molecular oxygen, reactive oxygen species, and nitric oxide. We have prepared discrete [2Fe-2S] and [4Fe-4S] complexes to study how [Fe-S] cofactors can be modified and/or assembled by redox signalling molecules including nitric oxide, superoxide, disulphide, and protons.<sup>1</sup> The nature of products produced from such reactions are largely dependent on the reaction environment. Upon exposure to NO in the presence of thiols, [Fe-S] clusters generate another signalling molecule H<sub>2</sub>S with a concomitant formation of dinitrosyl iron complexes (DNICs) or Roussin's red esters (RREs). Controlled delivery of protons, superoxide, or disulfide leads to the cluster interconversion between [2Fe-2S] and [4Fe-4S] cores. The presentation will discuss chemistry underlying this diverse reactivity.

This material is based upon work supported by the National Science Foundation under 1807845.



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## IL-111

### Biomimetic and Catalytic Activities by Transition Metal Complexes of Non-Innocent Ligands

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A non-innocent ligand is capable of changing its redox state in the presence of metal ions. Transition metal complexes of non-innocent ligands have achieved immense importance: I) in understanding enzymatic reactivity *via* model complex study, and II) in catalysis.

Employing transition metal complexes of non-innocent 2-aminophenol derivatives, biomimetic study on 2-Aminophenol-1,6-Dioxygenase (APD) [1]; the activity of intermediate **Q** in Electron Transport Chain (ETC) [2]; and the catalytic conversion of aromatic isocyanate to the corresponding urea compounds under sunlight have been performed and will be presented.

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IL-112

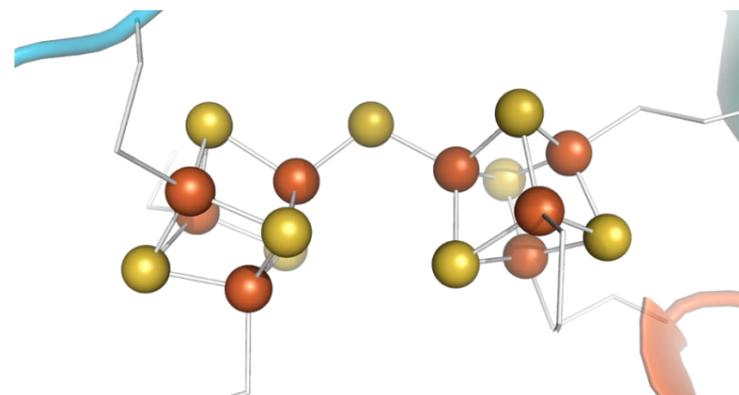
## ATP-Driven Electron Transfer to an [Fe<sub>8</sub>S<sub>9</sub>] Double Cubane Cluster

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Complex metalloenzymes based on iron-sulfur clusters support the conversion of gaseous substrates. Several of the catalyzed reactions are reductions attractive for energy conversion and conservation, such as the reduction of protons, carbon dioxide and dinitrogen. When the reducing power of physiological electrons are not enough to achieve these conversions, electron may be energized beyond the physiological window by coupling ATP-hydrolysis to electron transfer against a redox potential gradient. We study the mechanisms of two classes of electron-transferring ATPases, (I) reducing Co(II) to Co(I), called reductive activators of corrinoid-type enzymes (RACE proteins) [1,2] and (II) reducing low-potential iron-sulfur clusters, catalyzed by ASKHA-type electron transferring ATPases called Archerases [3].

We have recently discovered a novel cofactor associated with the Archerase class of electron transferring ATPase. The cofactor is formed by bridging two cubane-type [Fe<sub>4</sub>S<sub>4</sub>]-clusters by a μ<sub>2</sub>-sulfido-ligand, forming an [Fe<sub>8</sub>S<sub>9</sub>]-cluster [4]. The [Fe<sub>8</sub>S<sub>9</sub>]-cluster requires an electron-transferring ATPase as electron donor and was found capable of reducing acetylene and hydrazine, thus resembling nitrogenases in cofactor structure and reactivity. The sequence motif of six cysteine residues binding the [Fe<sub>8</sub>S<sub>9</sub>]-cluster is wide-spread in nature, apparently occurring in at least three different types of enzymes.



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IL-113

## In Vivo Generation of Semi-Synthetic [FeFe] Hydrogenases

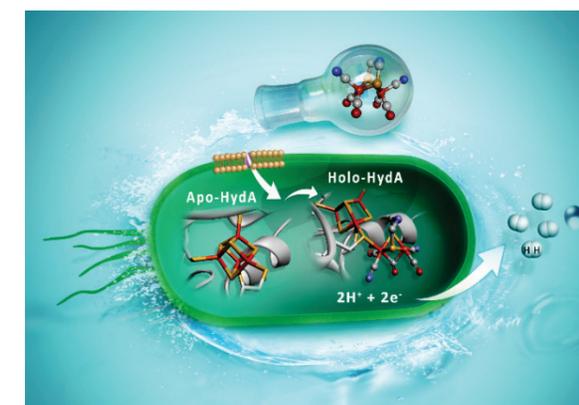
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Hydrogenases catalyze the interconversion of protons and molecular hydrogen with remarkable efficiency. In the case of [FeFe] hydrogenase the reaction occurs at the H-cluster, which contains an organometallic dinuclear [2Fe] subsite. Synthetic chemistry has long been a powerful tool in studies of this cluster via the preparation of biomimetic model compounds, and in 2013 it was shown how such synthetic complexes can be introduced into the enzyme itself under in vitro conditions.[1, 2] This provides a direct link between biomimetic chemistry and biology, and allows us to manipulate the enzyme using synthetic chemistry.

More recently we expanded the concept of “artificial maturation” also to in vivo conditions, thus enabling the preparation of semi-synthetic hydrogenases inside living cells.[3] Here I will provide an overview of how this novel tool can be used for the screening of different host organisms, as well as the in vivo preparation of organometallic mutants with new reactivity and mechanistic studies of [FeFe] hydrogenase.[4, 5, 6]

Financial support by the ERC (StG) and the Swedish Research Council is gratefully acknowledged.



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IL-115

## Theranostic and Targeted Antitumor Metal Complexes

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Cisplatin and its successive analogues mainly exert their activities by causing DNA damage and induce apoptosis in cancer cells. With the development of molecular oncology, biomolecule-targeted anticancer strategies have become the mainstream in the search of new cancer drugs. At the biomolecular level, G-quadruplexes (G4s), histone deacetylases (HDACs), cyclin-dependent kinases (CDKs), and the mammalian target of rapamycin (mTOR) have drawn attention as promising anti-cancer targets. We have developed a series of self-assembled Pt(II) complexes, acting as selective and effective human telomeric G4 binders and stabilizing certain G4s conformations. These Pt(II) complexes exhibit significant telomerase inhibition activity and higher anti-tumor efficacy than cisplatin. We have also focused on the rational design of non-platinum metal complexes acting as enzyme inhibitors, investigated their anti-cancer mechanisms in details and their capability to induce cancer cells apoptosis, para apoptosis, autophagy and cell cycle arrest. Recently, we have a lot of interest in the organelle-targeted multifunctional metallo-anticancer agents. The organelle targets that we investigate mainly include mitochondria, lysosome and nucleus. These metallo-anticancer agents possess excellent phosphorescent properties, allowing the real-time tracking of the morphological, behavioral, and micro-environmental changes in the organelles at the same time as treatment and exhibiting great theranostic potential. To further improve the therapeutic efficacy of metallodrugs, design and development of nanostructured materials is a promising strategy, in which the nanostructure is fabricated by metal complex directed self-assembly or simply functionalized with metallodrugs as anti-cancer agents, thus realizing multimode synergetic therapy. The anticancer properties and the theranostic potential of these nanomaterials and their in vivo anticancer properties have been explored in details for further biomedical applications [1-5]. Financial support by the National Science Foundation of China and the Sun Yat-Sen University are gratefully acknowledged.

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IL-116

## Structure-Function Relationships of the Redox-Sensitive [2Fe-2S]-RsrR Transcription Regulator

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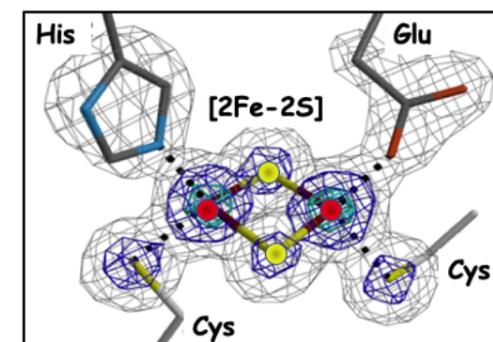
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The recently identified Rrf2 family transcriptional regulator RsrR from *Streptomyces venezuelae* (*Sv*) coordinates a [2Fe-2S] cluster. *Sv*RsrR plays a primary role in regulating the relative concentrations of NADH and NAD(P) in the cell. It also regulates the synthesis of mycothiol, which is the equivalent of glutathione in *Actinobacteria*. Electrophoretic mobility shift assays have shown that the cluster's facile cycling between +2 and +1 states modulates *Sv*RsrR binding to cognate promoter DNA sequences [1]. In order to help understanding how a simple one electron redox process can generate the conformational changes necessary to activate or abolish DNA-binding we have initiated a series of high resolution crystal structure determinations of the *Sv*RsrR dimer [2]. A first observation is that the [2Fe-2S] cluster is asymmetrically coordinated across the *Sv*RsrR monomer-monomer interface by two Cys residues from one subunit and His and Glu residues from the other. This is the first example of a [Fe-S] cluster with three different amino acid side chains as ligands, and of Glu acting as ligand to a [2Fe-2S] cluster.

In addition, we have found that depending on the crystal symmetry and redox state of *Sv*RsrR, Trp9 can adopt two conformations, one exposed to the solvent medium and the other buried in a pre-existing protein cavity not far from the [2Fe-2S] cluster. This conformational change results in a significant shift in the DNA-binding helix-turn-helix region of *Sv*RsrR, which may be significant in modulating DNA binding. Indeed, our very recent chemical modification studies have confirmed that the Trp9 conformation is redox-dependent. We are also in the process of characterizing this change using molecular dynamics calculations. Latest results will be reported at the Conference. Financial support by the French FRISBI (ANR-10-INSB-05-02) and Biotechnology and Biological Sciences Research Council (UK) through grants BB/J003247/1, BB/L007673/1 is gratefully acknowledged.



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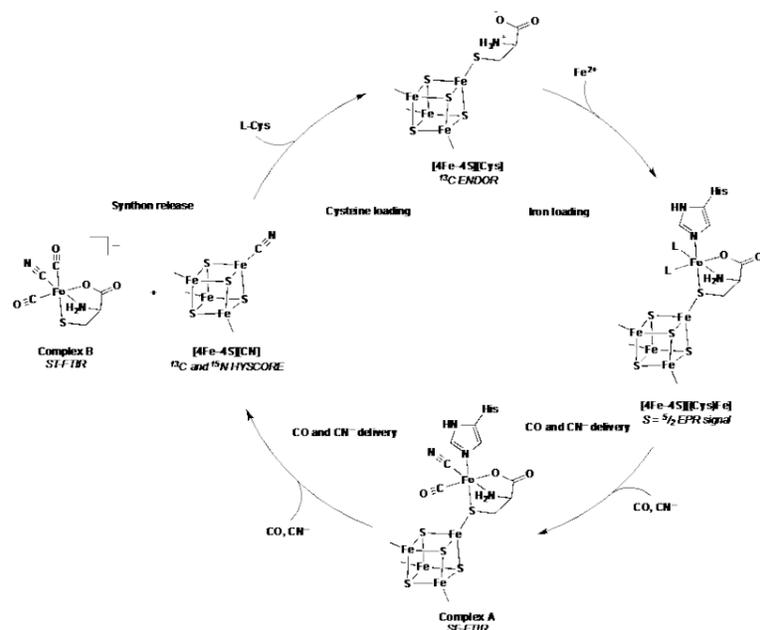
## IL-117

### Biosynthesis of the [FeFe] Hydrogenase H-Cluster

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Radical SAM enzymes (1) use a [4Fe-4S] cluster to cleave S-adenosylmethionine to generate the 5'-deoxyadenosyl radical, which abstracts an H-atom from a given rSAM enzyme's substrate to initiate catalysis. In the maturation of the [Fe-Fe] hydrogenase H-cluster, the radical SAM enzyme HydG lyses tyrosine to generate the CO and CN ligands of the H-cluster of [FeFe] hydrogenase, ultimately building an organometallic Fe(CO)<sub>2</sub>CN(cysteine) moiety that incorporates an iron atom derived from a unique 5-Fe Fe-S cluster (see figure) (2-7). How these "synthons" are pairwise introduced into the formation of the H-cluster, along with the molecular and enzymatic origin of the azadithiolate bridge component of this catalytic cluster, are a current experimental focus of the laboratory. We are also revisiting details of the initial radical SAM tyrosine radical reactions (2) and trying to understand the yet unknown details of CN and CO formation and binding to the "dangler Fe" of the 5-Fe cluster with a combination of experiments and computational chemistry.



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## IL-118

### Ferritin: Iron Biomineralization and Cellular Delivery

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Solution and solid state approaches in wt and mutated cage variants provide insight into the initial steps of iron biomineralization in animal ferritins. Well-defined paths driven by electric gradients established across different areas of the cage direct iron ions from the bulk solution towards the ferroxidase and nucleation centers[1,2]. Along these paths, several transient iron binding sites with peculiar coordination properties are detected.

The use of ferritin as a carrier for targeted cellular delivery is pursued by encapsulating different inorganic species and exploiting the selective ability of cells to internalize ferritin cages rich in L- or H-subunits through specific receptors; the SCARA5 (Scavenger receptor class A type 5) for L-rich cages and TfR1 (Transferrin receptor 1) for H-rich cages. We have observed a significant cytotoxicity associated to iron-loaded, recombinant, human H ferritin uptaken by HeLa cells via the TfR1 receptors and internalized via endocytosis [3]. The process causes release of iron that results cytotoxic. A pool of non-biomineralized iron ions at the catalytic (and accessory) sites of H-ferritin subunits, which is labile at the low endosomal pH, is probably at the basis of the effect.

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## IL-119

### Enhanced Antitumor Activity of RAPTA-C by the Epidermal Growth Factor Receptor (EGFR) Inhibitor Erlotinib

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The organometallic ruthenium(II) compound [Ru( $\eta^6$ -p-cymene)Cl<sub>2</sub>(pta)], where pta = 1,3,5-triaza-7-phosphaadamantane (RAPTA-C) exhibits broad acting anti-tumor efficacy with intrinsic angiostatic activity [1]. Moreover, RAPTA-C exhibits no discernable cytotoxic effects on healthy cells and severe side effects have not been observed in *in vivo* models [2]. We have evaluated activity of RAPTA-C in combination with mechanistically different anti-cancer drugs. Starting with a set of nine drugs we found a synergistic potential of RAPTA-C with the epidermal growth factor receptor (EGFR) inhibitor [3]. This drug combination administrated at low doses strongly inhibited endothelial and cancer cell metabolism *in vitro*. The synergistic activity found in cellular assays also translated to *in vivo* preclinical model of human ovarian carcinoma [4]. RAPTA-C may therefore serve as a safe alternative to platinum-based drugs in combination therapies.

Financial support by the University of Geneva is gratefully acknowledged.

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## IL-120

### The Energy-Coupling Mechanism in Respiratory Complex I: EPR Investigations of Fe-S Cluster and Semiquinone Intermediates

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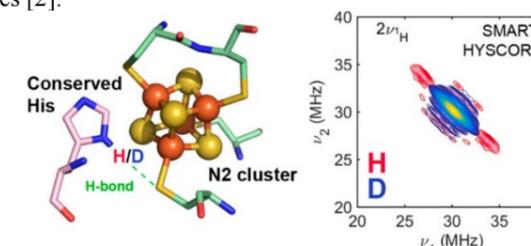
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Energy-transducing respiratory complex I (NADH:ubiquinone oxidoreductase) is one of the largest and most complicated enzymes in mammalian cells and its mechanism is not well understood [1]. In particular, the key step of how electron transfer is coupled to proton translocation across the membrane – the ‘energy coupling mechanism’ – is unknown. Our work is focused on using electron paramagnetic resonance (EPR) spectroscopy to interrogate mechanistically-relevant redox centres in complex I in detail.

We used hyperfine EPR spectroscopic methods, combined with site-directed mutagenesis, to determine the mechanism of a single proton-coupled electron transfer reaction at one of eight iron–sulfur clusters in complex I, [4Fe-4S] cluster N2, the electron donor to ubiquinone. Because of its position and pH-dependent reduction potential, N2 has long been considered a candidate for the elusive ‘energy-coupling’ site in complex I. Using HYSCORE spectroscopy we have shown that a conserved histidine is hydrogen-bonded to N2, tuning its reduction potential. However, the two are not coupled sufficiently strongly to catalyze a stoichiometric and efficient energy transduction reaction. We thus exclude cluster N2 as the energy-coupling site in complex I. Our work demonstrates the capability of pulse EPR methods for providing detailed information on the properties of individual protons in even the most challenging of energy-converting enzymes [2].



Having established that cluster N2 is not directly involved in proton translocation, our recent work has focused on understanding the role of ubiquinone (Q<sub>10</sub>). Despite mounting evidence, primarily from computational work, that the energy-coupling step is driven at the Q<sub>10</sub> site, Q<sub>10</sub> binding, dissociation and semiquinone (SQ) intermediates are difficult to investigate experimentally. We have used a yet-unexplored method of constructing a chimeric respiratory chain using submitochondrial particles (inverted membrane vesicles) and an alternative terminal ubiquinol oxidase to circumvent the misleading effects of soluble quinone analogues to investigate SQ stabilisation during NADH oxidation. Targeted inhibition of individual components of the respiratory chain whilst maintaining complex I turnover, in conjunction with EPR spectroscopy, shows that (i) the dominant SQ radical stabilised in SMPs during NADH:O<sub>2</sub> turnover originates from complex III, (ii) significant contributions arise from off-pathway radicals, (iii) complex I SQ accumulates only transiently [3]. The implications on the energy-coupling mechanism in complex I are discussed.

Financial support by the EPSRC, The Royal Society, The Leverhulme Trust and the Medical Research Council is gratefully acknowledged.

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IL-121

**At Last: The Structure of the Elusive Urease–Urea Complex Unveils the Mechanism of a Paradigmatic Nickel-Dependent Enzyme**

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Urease is most efficient enzyme known, with a rate enhancement of  $10^{15}$ . It was the first enzyme crystallized and proven to be proteinaceous in nature, and it was the first enzyme established to depend on the presence of Ni as an essential element. Urease catalyses the decomposition of urea by two hydrolytic stages: the first, which is strictly enzymatic, produces ammonia and carbamate, while the second involves the uncatalyzed spontaneous decomposition of carbamate to yield another molecule of ammonia and bicarbonate. Urease contains a dinuclear cluster of Ni(II) ions in the active site that causes the formation of a hydroxide ion acting as the co-substrate necessary for the hydrolytic reaction of urea, at near neutral pH, where the enzyme has its maximal activity [1]. The reaction causes a rapid pH increase, with negative consequences for both human health and the environment. The development of urease inhibitors requires knowledge of all steps of the catalytic mechanism at the molecular level.

The crystallographic structure determination of the Ni(II) coordination environment in native urease revealed the molecular framework for the catalytic process, while the structures of several enzyme–inhibitor complexes uncovered the reactivity of the enzyme towards several classes of inhibitors. These structures led to the mechanistic proposal that the Ni-bridging hydroxide acts as the nucleophile in the reaction, attacking the carbonyl carbon of a urea molecule proposed to chelate the two Ni(II) ions [2]. This proposal was alternative to other hypotheses for the catalytic mechanism, which involved a urea molecule terminally bound to Ni(1) through its carbonyl O atom, attacked either by a hydroxide ion terminally bound to Ni(2) [3] or by the bridging hydroxide [4]; a third alternative mechanism, based on biomimetic chemistry [5] or computational methods [6], proposed the occurrence of an elimination reaction to form cyanate, an intermediate that, however, has never been observed in urease-catalysed reactions.

This controversy would be solved only through the determination of the structure of the enzyme–substrate complex; however, the very short lifetime of the urease–urea adduct (ca. 20  $\mu$ s) has so far hampered the determination of its structure. This talk will describe and discuss the determination of the high-resolution (1.42 Å) structure of the urease–urea complex, in which the hydroxide acting as the co-substrate for the reaction has been substituted with an unreactive fluoride, known to inhibit the enzyme by substituting Ni-bound solvent-derived O atoms thus preventing all subsequent catalytic steps from occurring [7,8]. The structure reveals the chelating mode of urea binding to the two Ni(II) ions, using both the O atom and the NH<sub>2</sub> group, which renders the central C atom of urea electron-deficient and poised to undergo the nucleophilic attack by the Ni-bridging hydroxide present in the resting state of the enzyme. The structure sheds light on the initial step of the catalytic mechanism of this paradigmatic metalloenzyme, and resolves the long-standing debate concerning the mechanism of the nickel-driven hydrolysis of urea.

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IL-122

**Nanosecond Dynamics at Protein Metal Sites Explored by PAC Spectroscopy**

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The role of dynamics in protein function has long been appreciated, but while a large number of structures of metalloproteins have been determined, experimental characterization of dynamics at the metal sites is relatively scarce. Many biologically relevant metal ions exhibit nanosecond water exchange dynamics in aqueous solution [1], implying that this may also be a relevant time scale to the function of biomolecular metal binding sites. Perturbed angular correlation (PAC) of  $\gamma$ -rays spectroscopy allows for determination of nanosecond dynamics at the PAC probe site [2-5]. With this presentation I aim to provide a concise introduction to the technique as well as recent examples of application of PAC spectroscopy to elucidate structure and nanosecond dynamics at protein metal sites.

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IL-123

## Challenges and Imaging Applications of Lanthanide(III)-Based Metallacrowns Emitting in the Near-Infrared Range

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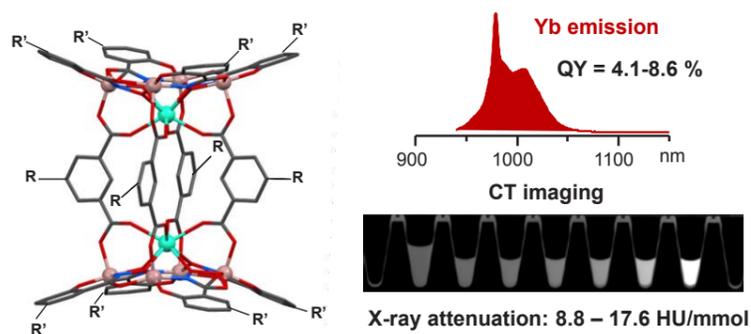
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Characteristic sharp emission bands of lanthanide(III) ions in the UV, visible and near-infrared ranges make them attractive for a broad variety of applications in materials science and biology. However, the design of highly luminescent lanthanide(III)-based compounds requires the optimization of the sensitization processes as well as the minimization of non-radiative deactivation mechanisms of luminescent lanthanide(III) ions.

We have demonstrated that the emission of lanthanide(III) ions, particularly in the near-infrared range, is efficiently sensitized in Zn<sup>II</sup>/Ln<sup>III</sup> and Ga<sup>III</sup>/Ln<sup>III</sup> families of metallacrowns (MCs) [1-3]. In addition, the applicability of Zn<sup>II</sup>/Ln<sup>III</sup> MCs for the labelling of cell necrosis [4] or for the simultaneous cell fixation and counter staining [5] have been envisaged. Herein, we will discuss challenges that have to be addressed to create lanthanide(III)-based MCs emitting in the near-infrared range bearing in mind desired functional properties for applications in optical and computed tomography imaging modalities.



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IL-124

## Mitochondria-Targeting Ru(II)/Ir(III) Anticancer Agents

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Since cancer was identified as one of the leading causes of human death, scientists and researchers worldwide have been motivated by the urgency to find a cure for this disease. By virtue of its role as both the power plant and apoptosis center of cells, the mitochondrion has become an ideal target for anticancer agents. Mitochondria-targeted anticancer drugs that act through disrupting the redox homeostasis of the cell, which leads to the activation of the mitochondrial-dependent cell death signaling pathway, may overcome cancer resistance. Therefore, apoptosis can be triggered efficiently with excess oxidative stress by targeting mitochondria. In this regard, rationally designed anticancer agents that target mitochondria are highly sought. Recently we have focused on Ru(II)/Ir(III) polypyridyl complexes which were found to be excellent candidates owing to their attractive photophysical properties (i.e. high water solubility, large  $\sigma_2$ , high  $^3\text{O}_2$  production, long luminescence lifetime, and excellent chemical- and photo-stability). Our results indicated that these complexes may be considered to be an efficient anticancer candidate.

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IL-125

## Coordination Plasticity Controls Metal Substrate Promiscuity in Transmembrane Primary-Active Zinc Pumps

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P<sub>1B</sub>-type ion pumps constitute a key transmembrane transport system for the selective translocation of essential and toxic transition metal ions across the cell membranes in all the domains of life<sup>1,2</sup>. These primary active transporters exploit the energy from ATP hydrolysis to catalyze ion transport across membranes against their electrochemical gradients. A multidisciplinary structural, biophysical and biochemical strategy is applied to reveal the molecular principles underlying metal substrate selectivity and to address how the energy stored in ATP is transduced into the vectorial flux of specific metal ions across the lipid bilayers<sup>3,4</sup>.

We have investigated the principles controlling metal substrate recognition in a Zn<sup>2+</sup>/Cd<sup>2+</sup>/Hg<sup>2+</sup>/Pb<sup>2+</sup> ATPase (ZntA) to reveal the coordination chemistry that define a unique yet promiscuous metal selectivity towards first-, second- and third-row transition and post-transition metals. Biochemical and X-ray absorption spectroscopic studies on substrate-bound ZntA both in detergent micelles and reconstituted in lipid bilayers reveal, at atomic resolution, the existence of a highly-plastic transmembrane metal-binding site that selects substrates by unique and diverse, yet defined, coordination geometries and ligand-metal distances. Moreover, by coupling time-dependent measurements of metal transport in proteoliposomes with the use of fluorescent sensors we are providing new highlights on the overall mechanism of transport in this class of primary active transporters. The interplay between the nature of the coordinating ligands, the geometry of metal binding in the transmembrane helices, and the unique coordination chemistry determines the mechanism by which substrate selection and translocation occurs in P<sub>1B</sub>-type ATPase zinc pumps.

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IL-126

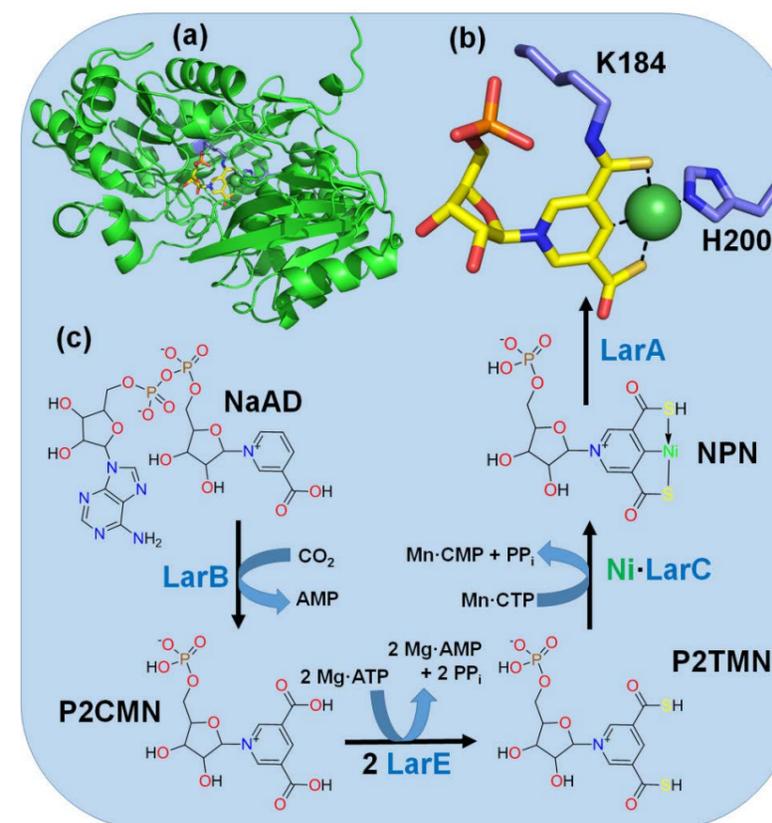
## Biosynthesis of the Nickel-Pincer Nucleotide Cofactor of Lactate Racemase

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Lactic acid, a central metabolic intermediate of many cells, occurs as L- and D-isomers that are interconverted by lactate racemase. The enzyme from *Lactobacillus plantarum*, LarA, harbors a tethered nickel-pincer nucleotide (NPN) coenzyme derived from niacin [1]. This seminar will summarize recent studies related to NPN synthesis [2,3], a process that requires LarB, a carboxylase/hydrolase of nicotinic acid adenine dinucleotide (NaAD); LarE, a Mg-ATP-dependent sacrificial sulfur insertase; and LarC, a CTP-dependent nickel insertase or cyclometallase.

Financial support by the National Science Foundation (CHE-1807073) is gratefully acknowledged.



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## IL-127

### EPR-Based Localization of High- and Low-Spin Metal Ions in Proteins

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Metal ions are important for the structure, folding and catalytic function of biomolecules. It is thus essential to know where the metal ions are bound in the three-dimensional fold of the biomolecule. We devised a method by which paramagnetic metal ions can be localized in analogy to the geo positioning system. Spin labels functioning as the satellites are attached site specifically to the biomolecule, the distances between the labels and the metal center are measured by EPR and the site of the metal ion is determined by trilateration. The type of EPR method one should use for the distance measurements depends on the type of metal ion. For electron spin  $S = 1/2$  ions like Cu(II) Pulsed Electron-Electron Double Resonance (PELDOR or DEER) is very useful, whereas for ions with broad spectral width and/or fast relaxation times like e.g. low-spin Fe(III), Relaxation Induced Dipolar Relaxation (RIDME) is the better method. For the case of high-spin metal ions, we will show on model systems: 1) that a large zero-field splitting changes the usually axial dipolar spectrum to an orthorhombic one, 2) that such a spectrum can be recorded by RIDME and 3) how to analyze such a spectrum. Last but not least the method is applied to the high-spin Fe(III) ion in myoglobin.

Financial support by the DFG within SFB 813, project A6 is gratefully acknowledged.

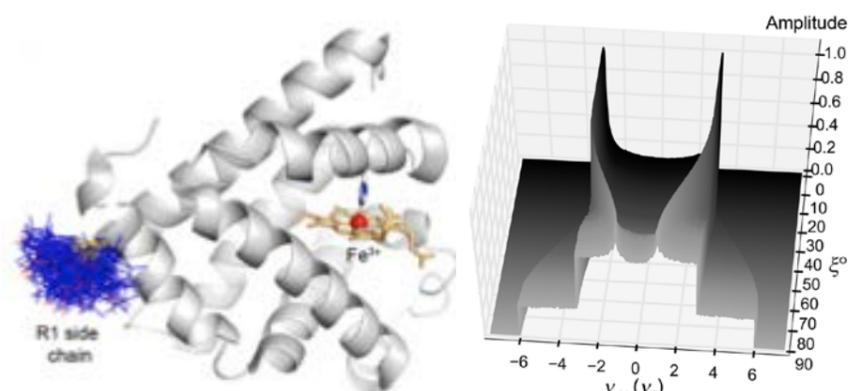


Fig.: Left - Structure of myoglobin with the nitroxide side chain R1 and the haem group highlighted. Right - The orthorhombic "three-horned" dipolar Pake pattern.

## IL-128

### Near-Infrared Emitting Lanthanide Probes for Optical Imaging with Different Sizes: Small Molecules, Metallacrowns and MOF Nanomaterials

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For biological research and medical diagnostic, optical imaging is an increasingly attractive tool for in vitro and in vivo biological imaging due to the high sensitivity of detection, versatility, low cost of instruments and high resolution at the cellular level. A common characteristic of biologic systems is the presence of complex matrices such as blood, cells, tissue and organs. These matrices emit a significant background fluorescence in the visible domain (autofluorescence), strongly limiting the sensitivity of detection.

The luminescence of lanthanide cations possesses several complementary advantages over the fluorescence of organic fluorophores and semiconductor nanocrystals, such as sharp emission bands for spectral discrimination from background emission, long luminescence lifetimes for temporal discrimination and strong resistance to photobleaching.

Several lanthanide cations emit near-infrared (NIR) photons which result in improved detection sensitivity due to the absence of native NIR emission from tissues and cells (autofluorescence). In addition, NIR photons can potentially cross tissues for non-invasive imaging and diagnostic. [1]

The key requirement to take advantage of lanthanide emission is to sensitize them with appropriate chromophores ("antenna effect").

We will present here several complementary NIR emitting systems with increasing sizes associated with their applications for biologic imaging: 1) small monometallic complexes that possess excitation and emission wavelengths within biological diagnostic window, 2) macromolecular metallacrowns possessing high quantum yields [2-4] and 3) nanoMOFs with high density of lanthanide emitters and sensitizers per unit volume. [5]

This research has received funding from the European Community's Seventh Framework Program (FP7/2007-2013) (n° 611488 and n° 316906), the National Science Foundation (CHE-1361799), la Ligue Contre le Cancer, la Région Centre, le Cancéropôle Grand Ouest and INSERM.

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## IL-129

### Ruthenium Redox Modulators with Anticancer Activity

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Platinum complexes, cisplatin, oxaliplatin and carboplatin, are the most widely used anticancer drugs. However, these DNA-targeting treatments are a low selectivity approach with deleterious consequences and a high incidence of resistance. The societal need for new anti-cancer drugs is very substantial, hence, the commercial demand for new anti-cancer agents is very high.<sup>1</sup> Although targeted chemotherapeutics with novel mechanisms of action (for example kinase inhibitors) have been developed more recently, these often have a much narrower clinical utility than platinum-based drugs and generally suffer from rapid onset of resistance, which means that there is still a large unmet clinical need. Hence, there is a wide interest in new metal-based drugs with alternative mechanisms of action.

The chemical scaffold offered by metal-based complexes has significant scope for molecular diversity and has the possibility of accessing chemical reactions beyond reach of organic molecules alone (e.g. Fenton-like reactions and single electron transfers). Furthermore, these complexes could effectively be harnessed to target the redox balance in cancer cells and induce both oxidative and reductive stress, perturbing the cellular balance of reactive oxygen species.<sup>2,3</sup> Cancer cells are primed for this intervention, because they are already redox-hyperactive and in most cases present malfunctioning mitochondria. Dysfunctional mitochondria are unable to control ROS generation efficiently. This allows metal-based complexes, to exert selective toxicity towards cancer cells over normal cells. The design of such drugs is relatively unexplored and, even more so, is their MoA at cellular level.

In this field transition metal complexes have been heavily investigated, particularly half-sandwich or ‘piano-stool’ complexes.<sup>4-6</sup> This work, in particular, describes three families of Ruthenium organometallic complexes which include in their structures a *p*-cymene arene unit and modifications in the *N,N*-chelating ligand. The novel complexes have been physically characterised by means of 2D-Nuclear Magnetic Resonance (NMR), Mass Spectrometry (MS), single crystal X-Rays diffraction and cyclic voltammetry. Their relative hydrophobicity and reactivity under biologically relevant conditions has also been investigated using Ultraviolet and Visible spectroscopy (UV-Vis) and reverse phase HPLC.

These complexes exhibit potent anticancer activity towards ovarian, colorectal and lung carcinoma. The mechanism of action of such complexes has been investigated paying particular attention to their effect on cell cycle, induction of apoptosis, and most importantly generation of ROS and modulation of mitochondrial function.

Financial support by the University of Birmingham is gratefully acknowledged.

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## IL-130

### Balancing Zinc Scarcity and Toxicity in a Marine Cyanobacterium: Sensing by Zur and Tight Binding by BmtA

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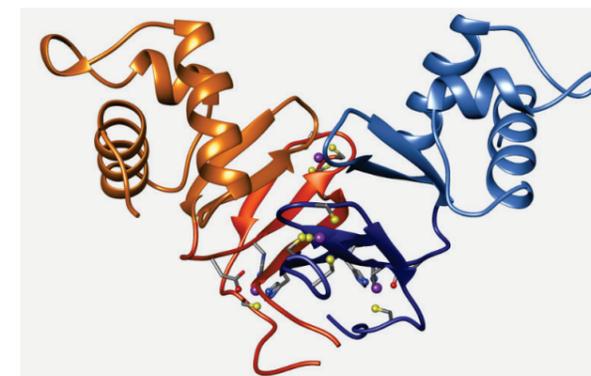
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Marine cyanobacteria are responsible for 25% of global oceanic CO<sub>2</sub> fixation. Many strains dominate regions of the oceans that are characterised by extremely low concentrations of nutrients including trace metals, which are required for photosynthesis (Fe, Mn, Cu) and CO<sub>2</sub> fixation (Zn) amongst other metabolic processes. Indeed, zinc uptake and utilisation in open ocean cyanobacteria is so effective that they do not suffer from zinc limitation even at the lowest possible zinc concentrations [1, 2]. Genome mining has suggested that all cyanobacteria harbour a gene for the zinc uptake regulator Zur [3]. However, since it is not straightforward to predict metal specificity from primary sequence, we have studied the putative Zur protein and its regulon from the oligotrophic marine cyanobacterium *Synechococcus* sp. WH8102 (SynZur).

A *zur* knockout mutant strain displays increased zinc uptake at very low [Zn] as well as decreased zinc tolerance at higher [Zn]. SynZur expressed in *E. coli* was purified with two bound zinc ions, and this metalloform bound as a dimer to specific DNA sequences (Zur boxes) with nanomolar affinity. Dimers were also found in the X-ray crystal structure of Zn<sub>2</sub>SynZur, with a well-conserved structural Cys<sub>4</sub> site, and a novel AspCysHis<sub>2</sub> sensory site unique to cyanobacterial Zur proteins. The dimers show a ‘closed’ conformation similar to that expected in a complex with DNA. Native ESI-MS and electrophoretic mobility shift assays indicate that the removal of Zn<sup>2+</sup> from the sensory site leads to conformational changes, promotes dissociation of the dimers, and abolishes high-affinity DNA binding. In zinc-deficient conditions, this leads to the de-repression of a high-affinity ZnuABC uptake system. A second set of predicted Zur boxes is found upstream of a bacterial metallothionein gene (*bmtA*). Here, Zur binding activates the transcription of *bmtA* at elevated zinc concentrations. We have expressed and purified this BmtA, and found that it binds Zn<sup>2+</sup> with extraordinarily high affinity.

These data taken together suggest a requirement for particularly low free intracellular [Zn<sup>2+</sup>], but also highlight the benefit of having an intracellular zinc-binding protein: at elevated [Zn], the wild-type was able to acquire much more zinc than the *zur* KO mutant, without displaying signs of toxicity. This ability is likely to provide an advantage under both high and low external [Zn].

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## IL-131

### The Tetranuclear Copper-Sulfide Catalytic Center from the Terminal Enzyme of Denitrification

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Denitrification is an important biological pathway with environmental implications. Nitrate accumulation and release of nitrous oxide in the atmosphere due to use of excess fertilizers are examples of two environmental problems, where denitrification plays a central role.

Nitrous oxide reductase the last enzyme of this pathway catalyzes the reduction of nitrous oxide at a new tetranuclear copper center (Cu<sub>Z</sub>) the catalytic center overcoming the high activation energy of this reaction. In this center, each Cu atom is coordinated by two imidazole rings of histidine side-chains, with the exception of Cu<sub>IV</sub>, with only one histidine. This enzyme has been isolated with “Cu<sub>Z</sub>” in two forms Cu<sub>Z</sub>(4Cu<sub>I</sub>S) and Cu<sub>Z</sub>(4Cu<sub>II</sub>S), which differ in the Cu<sub>I</sub>-Cu<sub>IV</sub> bridging ligand, leading to considerable differences in their spectroscopic and catalytic properties. The copper atoms in Cu<sub>Z</sub>(4Cu<sub>I</sub>S) can be reduced to the [4Cu<sup>I+</sup>] oxidation state, and its catalytic properties are compatible with the nitrous oxide reduction rates of whole cells, while in Cu<sub>Z</sub>(4Cu<sub>II</sub>S) they can only be reduced to the [1Cu<sup>2+</sup> - 3Cu<sup>I+</sup>] oxidation state, that has a very low turnover number. The catalytic cycle of this enzyme has been explored and one of the intermediates, Cu<sub>Z</sub><sup>o</sup>, has recently been identified and shown to be in the [1Cu<sup>2+</sup>-3Cu<sup>I+</sup>] oxidation state. Contrary to Cu<sub>Z</sub>(4Cu<sub>II</sub>S), Cu<sub>Z</sub><sup>o</sup> is rapidly reduced intramolecularly by the electron transferring center of the enzyme, Cu<sub>A</sub>, to [4Cu<sup>I+</sup>] by a physiologically relevant redox.

The three-dimensional structure of nitrous oxide reductase with “Cu<sub>Z</sub>” center as Cu<sub>Z</sub>(x partner. 4Cu<sub>I</sub>S) or Cu<sub>Z</sub>(4Cu<sub>II</sub>S) shows that it is a unique functional dimer, with “Cu<sub>Z</sub>” center of one subunit receiving electrons from Cu<sub>A</sub> of the other subunit. The complex nature of this center has posed some questions relative to its assembly, which are only partially answered, as well as which is the active form of “Cu<sub>Z</sub>” *in vivo*.

The structural, spectroscopic and catalytic features of the two forms of “Cu<sub>Z</sub>” center will be addressed here. The understanding of its catalytic features and activation is essential to develop strategies to decrease the release of nitrous oxide to the atmosphere and to reduce its concentration in the stratosphere, as well as to serve of inspiration to synthetic inorganic chemists to develop new models of this peculiar and challenging copper sulfide center.

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## IL-132

### Tracking Endogenous Metalloproteins by Metal Chelation Based Fluorescence Approach

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Metalloproteins, which account nearly a quarter to one third of human proteomes, are involved in various biological functions. In particular, metalloproteins play critical roles in metal homeostasis and disease processes. Moreover, they often serve as drug targets especially for metallodrugs.<sup>1</sup> Despite of numbers of techniques e.g. ICP-MS, or synchrotron-based techniques that have been employed for identification of metalloproteins,<sup>2</sup> conventional method, which involved in the protein separation or purification followed by detection of metals, often results in the loss of metal ions that bind to a protein weakly or transiently.

We report the design and synthesis of a new family of metal-chelation based fluorescence probes, consisting of a metal-binding moiety, a fluorophore and arylazide.<sup>3-6</sup> The metal-binding moiety will guide the probe to bind to proteins in live cells, and arylazide serves as an anchor, upon photoactivation, covalent bonds between the probe and labelled proteins will be formed, enabling downstream protein identification through conventional proteomics. We have developed a series of metal tunable NTA-based fluorescence probes, and identified various metallo-proteomes including Bi(III), Fe(III), Cu(II) and Ni(II) proteomes in various prokaryotic and eukaryotic cells, which provide a basis for further analysis of the biological pathways that these metals participate as well as the mode of action of metallodrugs. Moreover, by conjugation of arsenic binding moiety with a fluorophore and arylazide, an organoarsenic probe was also developed. Using this probe, a number of arsenic-binding proteins have been tracked in both NB4 and HL60 cells. In combination with quantitative proteomics, a new protein target of arsenic trioxide was identified and validated, which extend our knowledge on the mechanism of action of arsenic trioxide. The methodology we developed here combined with other (metallo)proteomics will further advance our understanding on the roles of metals in biology and medicine.

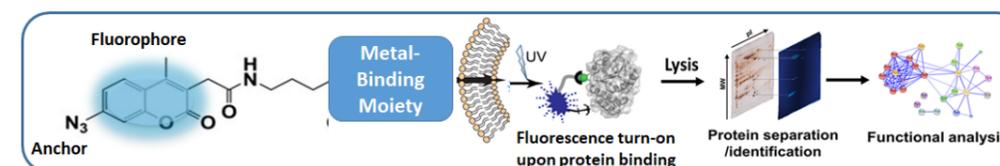


Figure 1. Strategy of tracking metal binding proteins in live cell by metal-chelation based approach

Financial support by the University of Hong Kong, RGC (17333616P, 17307017P) and Norman and Cecilia fund is gratefully acknowledged.

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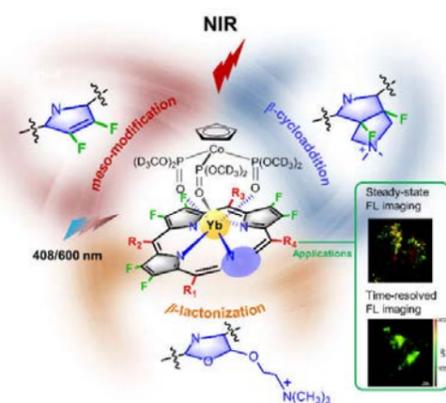
IL-133

## Bioinspired Design of NIR Luminescent Ln Complexes for Bioimaging

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Near-infrared emissive lanthanides attract increasing attention for their promising applications in energy conversion, biomedical imaging and optical materials.<sup>1</sup> Design of novel NIR functional lanthanide coordination complexes with high quantum yields is still urgently to be explored. Recently, we used porphyrins as antenna ligands,<sup>2</sup> and deuterated Kläui ligand as an ancillary ligand to construct sandwiched Yb<sup>3+</sup> complexes by removing the C–H bond close to the Yb<sup>3+</sup> center.<sup>3</sup> These Yb<sup>3+</sup> complexes presented high NIR luminescence (900–1150 nm) with unprecedented quantum yields up to ca. 25% in CH<sub>2</sub>Cl<sub>2</sub> (65% in CD<sub>2</sub>Cl<sub>2</sub>), long decay lifetimes of ca. 200 μs in CH<sub>2</sub>Cl<sub>2</sub> (700 μs in CD<sub>2</sub>Cl<sub>2</sub>) and large extinction coefficients in both visible and red regions (10<sup>5</sup>–10<sup>6</sup> M<sup>-1</sup> cm<sup>-1</sup> for the Soret band and 10<sup>4</sup>–10<sup>5</sup> M<sup>-1</sup> cm<sup>-1</sup> for the Q band). This opens access to the design of biological NIR probes with capability of both steady-state fluorescence and time-resolved fluorescence lifetime imaging.



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IL-134

## Anion Responsive Lanthanide Complexes for Real Time Enzyme Monitoring

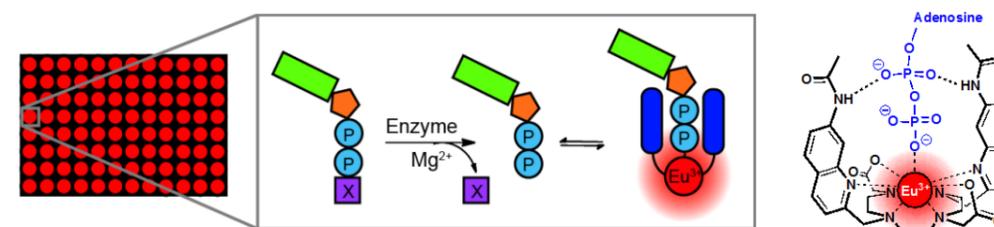
Sarah H. Hewitt,<sup>1</sup> Rozee Ali,<sup>1</sup> Romain Mailhot,<sup>1</sup> Stephen J. Butler<sup>1</sup>

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Nucleoside polyphosphate (NPP) anions, such as ATP and GTP, are some of the most important anions in biological systems. ATP is the universal chemical energy source in cells and is a substrate for a number of pharmaceutically important enzymes. For example, protein kinases convert ATP into ADP during the phosphorylation of proteins and represent one of the largest classes of drug targets in the fight against cancer. The creation of synthetic probes that can bind reversibly and selectively to target NPP anions would enable a range of enzymatic processes to be monitored *in vitro* and in cells, facilitating drug discovery research.<sup>1</sup> However, existing synthetic probes are limited in biological applications because they cannot discriminate effectively between structurally similar NPP anions.

Here we present a new class of luminescent europium(III) complexes (see Figure),<sup>2</sup> capable of binding and discriminating between target NPP anions in aqueous solution. The selective anion binding behaviour of these Eu(III) complexes has been exploited for real-time monitoring of a range of enzyme reactions that generate NPP anions, including kinases and glycosyltransferases.<sup>3</sup> This approach to enzyme monitoring overcomes significant limitations in existing assays, obviating the need for expensive antibodies or equipment, chemically labelled substrates and isolation/purification steps. Our label-free method enables rapid and quantitative analysis of enzyme activities and inhibition, offering a potentially powerful tool for high-throughput screening of new inhibitors. We show how permutations in the ligand structure can allow cell-penetrating probes to be developed, which localise preferentially to the mitochondria, enabling dynamic changes in ATP levels to be monitored in living cells.<sup>4</sup>

Financial support by Loughborough University Centre for Imaging Science is gratefully acknowledged.



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## IL-135

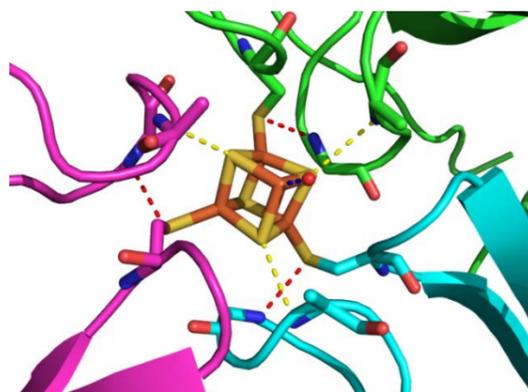
### Insight into Ferredoxins, Metallothioneins, and Zinc Finger Transcription Factors from Designed Iron-Sulfur Proteins

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Our approach to the study of metalloproteins is to engineer and synthesize peptide structures that incorporate metal cofactors as peptide-ligand based synthetic analogues. Herein, we describe the design of a sixteen amino acid peptide which binds a single [4Fe-4S] cluster via four cysteine thiolates in aqueous solution and which possesses all the spectroscopic and electrochemical hallmarks of a natural bacterial ferredoxin [1]. This primitive example of a ferredoxin provides insight into the minimal liganding and non-liganding amino acid requirements for [4Fe-4S] cluster incorporation and stabilization. These results will be discussed as relevant to the iron-sulfur chemistry possible on primordial Earth and in synthetic nanobiochemistry [2]. Furthermore, this tetracysteine peptide binds single Zn(II) ion to generate a synthetic analogue of a metallothionein or a zinc finger transcription factor with exceptionally affinity,  $K_f = 10^{16} \text{ M}^{-1}$ . The Zn(II) affinity of the Cys<sub>4</sub>, Cys<sub>3</sub>His<sub>1</sub> and Cys<sub>2</sub>His<sub>2</sub> versions of this peptide were used to demonstrate that the cost of protein folding in zinc finger transcription factors is typically < +4 kcal/mol [3].



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## IL-136

### X-ray Microscopy and Spectroscopy Combine to Probe Selenium Biology

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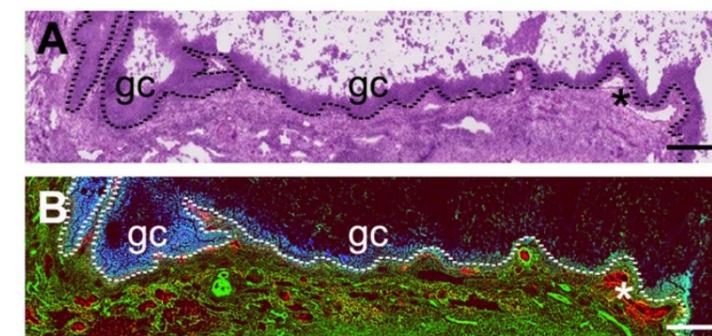
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We have applied the synchrotron-based methods, X-ray fluorescence (XRF) imaging and X-ray absorption spectroscopy (XAS) to track the chemistry of selenium species in both cell culture and animal tissues under both normal physiological and disease model settings.[1] This reveals distinct fates for amino acid forms which can be related to their observed biological effect, a reductive metabolism for selenite linked to delayed production of superoxide radical ions and interactions with copper biochemistry, as well as the discovery of a role for selenium in female reproductive function.[2] In several of these cases, the chemistry of selenium in intact tissue samples provides detail about the redox processes that are involved, while in others, redistribution of other heavy elements is informative. We are then able to link these observations to more traditional biochemical data, which provides valuable context.

XRF imaging of bovine ovary tissues, showed that selenium was transiently recruited to granulosa cells in ovarian follicles just prior to ovulation, and further biochemical studies, indicated that this was in response to a need for redox stress management. These findings suggest that oocytes in older women are exposed to greater levels of oxidative stress as a result of the aging of ovarian antioxidant defense mechanisms, and that the resulting oxidative damage to the egg is a cause of fertility issues. Prior theories had assumed that the oocytes themselves were degrading with age. If the hypothesis is true, it provides the opportunity to improve fertility in older women by boosting antioxidant defense in the ovary. Up-to-date results on research pursuing this opportunity will be covered in the presentation.

Financial support by the Australian Research Council and the National Health and Medical Research Council is gratefully acknowledged.



**Figure 1.** (A) H&E stained serial section of a 15 mm diameter healthy follicle. (B) The corresponding RGB image was generated from XRF elemental distribution maps and depicts the distribution of Zn (green), Fe (red) and Se (blue). \* indicates vasculature; ---- indicates the separation between granulosa layer and the thecal interna; gc indicates granulosa cells. Scale bar: 0.5 mm.

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IL-137

**Harnessing Redox and Coordination Chemistry for  $^{19}\text{F}$  Magnetic Resonance Biosensing**

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$^{19}\text{F}$  Magnetic resonance imaging (MRI) is a promising technique for *in vivo* imaging, showing great promise due to the favorable NMR properties of the fluorine nucleus (high sensitivity, large ppm range) and the lack of detectable fluorine signal in biological systems. Imaging agents can be designed that exhibit either a turn-on or chemical shift response that is selective for a specific biological molecule or event. We are developing a series of metal complexes (transition metals and lanthanides) designed to report on different biological environments associated reductive stress, oxidative stress, changes in pH, metal ion concentration, and enzymatic activity. We will highlight recent progress in metal-ion and enzyme responsive probes in this presentation.

Financial support by the Welch Foundation is gratefully acknowledged.

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IL-138

**Divalent Europium-Based Contrast Agents for Magnetic Resonance Imaging**

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This study explores the properties of europium-based contrast agents as alternatives to gadolinium-based contrast agents and the ability of europium-based agents to image hypoxia. There is a need to study the *in vitro* and *in vivo* properties of europium-based agents in assessing the viability of these molecules to serve as contrast agents. We have studied the physicochemical properties of several europium-containing complexes and performed initial imaging experiments *in vivo*. The data indicate that divalent europium has similar relaxivity to gadolinium and that divalent europium can be used to image hypoxic regions *in vivo*. By taking multiple modalities into account, ratiometric probes have been studied with the intention of removing the need to know europium concentration *in vivo*. The results demonstrate that europium-based contrast agents are a promising alternative to gadolinium-based agents for specific purposes such as in the imaging of hypoxic regions of tumors. Future research is being focused on overcoming chemical issues related to delivery methods *in vivo*.

Financial support by the National Institutes of Health (R01EB013663 and R01EB026453) is gratefully acknowledged.

IL-139

## Spectroscopic Characterisation of a Long-Lived Copper(II)-Tyrosyl in Lytic Polysaccharide Monooxygenases Following Oxidation by Hydrogen Peroxide

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The action of hydrogen peroxide on the copper-containing enzymes lytic polysaccharide monooxygenases (LPMOs) has been shown to enhance the activity of the enzymes on saccharidic substrates, but also lead to rapid inactivation of the enzyme, presumably through protein oxidation.

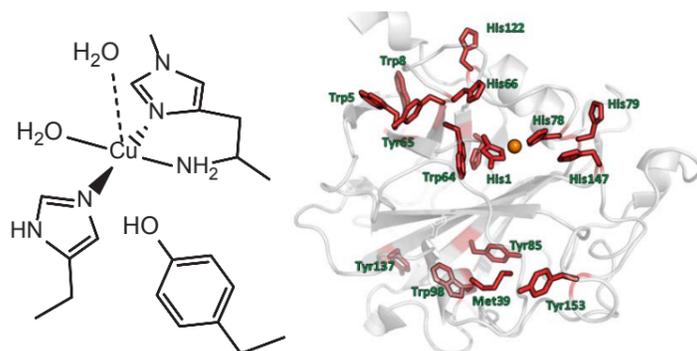
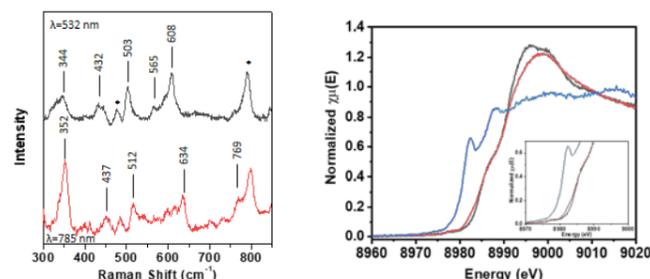


Figure: active site structure of an LPMO and oxidized amino acids (red) following treatment with H<sub>2</sub>O<sub>2</sub>.

Here, through the use of UV/vis, CD, XAS, EPR, MCD, MS and resonance Raman spectroscopies augmented with DFT calculations, we show that one of the products of protein oxidation in a AA9 LPMO is a long-lived ground-state singlet Cu(II)-tyrosyl species, which is inactive for the oxidation of saccharidic substrates. The formation of the stable Cu(II)-tyrosyl species requires the presence of a water molecule in the axial position of the copper coordination sphere, which then allows the d(x<sup>2</sup>-y<sup>2</sup>) SOMO to rotate towards the tyrosyl to form a short Cu-(OTyr) bond. The water molecule is only present in substrate-free conditions, meaning that the binding of substrate prevents Cu(II)-tyrosyl formation and thus protein inactivation during coupled catalytic turnover.



Figure, left resonance Raman spectrum of Cu(II)-tyrosyl, right Cu K-edge X-ray absorption species

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IL-140

## ss-DNA Templated Self-Assembly of Complementary Palladium(II) and Platinum(II) Base-Pairs

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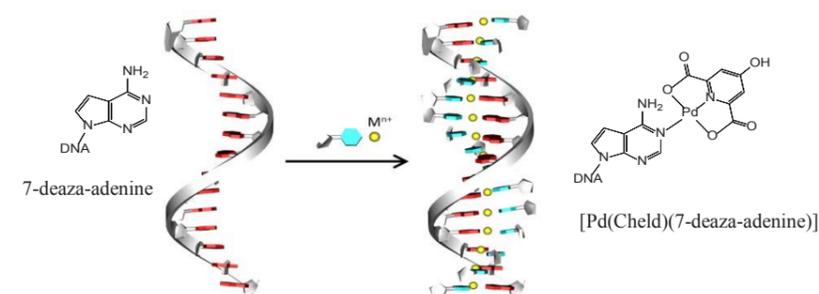
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Over the last decade, DNA molecules have been widely studied looking for future potential applications at the nanoscale [1]. One of the most popular strategies to achieve such new properties in nucleic acids is the design of metal-based DNA molecules by the introducing chelating ligands [2,3] or nucleobases analogues [4] that substitute natural nucleosides. This approach aims to artificially drive metal coordination at our convenience, namely the Watson-Crick face, replacing hydrogen bonding and leading to artificial hybrid metal-ssDNA systems that preserve not only the self-recognition abilities of nucleic acids [5,6] but also the natural double helix structure.

We demonstrate that metal fragments [M(Cheld)(CH<sub>3</sub>CN)] (M = Pd<sup>II</sup>, Pt<sup>II</sup>) can bind to poly(dA) and poly(dX) (X = 7-deazaadenine) oligonucleotides via self-assembly processes leading to the formation of consecutive metal-mediated base pairs [M(Cheld)(adenine)] and [M(Cheld)(7-deaza-adenine)] and therefore giving rise to a novel hybrid metal-DNA systems. The hybrids systems have been widely characterized by different techniques including UV-Vis, CD and NMR spectroscopy, as well as by mass spectrometry and Ab-initio computational studies. The structure of the individual mononuclear metal-base pairs has also been successfully solved by single crystal X-ray diffraction methods. Interesting results and future perspectives of this research will be discussed.

Financial support by the University of Granada and Spanish Ministry of Economy and Competitiveness (CTQ2017-89311-P) is gratefully acknowledged.



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IL-142

### Mechanisms of Sublethal Metal(loid) Toxicity in Contrasting Photosynthetic Organisms

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Many trace metals are essential micronutrients, but their bioavailable concentrations in the environment and agriculture are vastly different (with natural and anthropogenic causes) in various habitats, ranging from deficient to toxic levels. Therefore, research has focused on response of photosynthetic model organisms (algae, bacteria and plants) to trace metals and the metalloids arsenic in terms of uptake, transport, sequestration, speciation, deficiency, toxicity and detoxification. However, numerous studies have used environmentally not relevant extremely high concentrations of the metal(loid)s.

In the short summary of research presented here, we analysed environmentally relevant sublethal stress with a combination of different photosynthetic models incl. purple bacteria (*Rhodospirillum rubrum*) [1], algae (mostly *Euglena gracilis*, [2]) and higher plants (mostly *Ceratophyllum demersum* [3], [4], [5]). We used a combination of various biophysical and biochemical methods for measurements *in vivo* (e.g. photosynthesis biophysics, formation of reactive oxygen species, metal transport), *in situ* (quantitative (sub)cellular metal(loid) distribution and speciation) as well as on isolated proteins (for identification and characterization of metal binding) and metabolites (metabolomics).

These comparisons solved questions about the interdependence of difference mechanisms that diminish the primary productivity of these organisms during sublethal stress by As, Cd, Cu and Cr. Thus, we could show e.g. that metal(loid) concentrations that were previously considered as not toxic actually have a strong impact on photosynthetic cells, with a different sequence of events than observed at the often applied high concentrations. For example, oxidative stress was found to be usually not a primary mechanism of damage, but a consequence of damage to the photosynthetic apparatus. Further, the comparison of the vastly different model organisms and metal(loid)s has shown a lot of similarities, but also important differences between specific organisms and metal(loid)s.

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IL-143

### Heme-Dependent Regulation of Circadian Rhythm

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The circadian clock is an endogenous time-keeping system that is ubiquitous in animals and plants as well as some bacteria. In mammals, the clock regulates the sleep-wake cycle via two basic helix-loop-helix PER-ARNT-SIM (bHLH-PAS) domain proteins - CLOCK and BMAL1. There is emerging evidence to suggest that heme affects circadian control, through binding of heme to various circadian proteins, but the mechanisms of regulation are largely unknown. In this work we examine the interaction of heme with human CLOCK (hCLOCK). We present a crystal structure for the PAS-A domain of hCLOCK, and we examine heme binding to the PAS-A and PAS-B domains. Using UV-visible and EPR spectroscopies, we identify a bis-histidine (His/His) heme species in the oxidised (ferric) PAS-A protein, with evidence for flexibility in the heme pocket. By mutagenesis we identify His144 as a ligand to the heme. Using DNA binding assays, we demonstrate that heme disrupts binding of CLOCK to its E-box DNA target. Evidence is presented for a conformationally mobile protein framework, which is linked to changes in heme ligation and which has the capacity to affect binding to the E-box. Within the hCLOCK structural framework, this would provide a mechanism for heme-dependent transcriptional regulation.

## IL-144

### Redox-Regulation and Zinc Signaling Involved in DNA Damage Response and Genomic Stability

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Zinc-binding structures are common motifs in several transcription factors, DNA repair and tumor suppressor proteins. As one example, poly(ADP-ribose) polymerase 1 (PARP-1) is actively involved in DNA damage signaling, DNA repair and thus maintaining genomic stability. While it is inactive under normal cellular conditions, it gets activated upon the induction of DNA damage; however, the underlying mechanisms are not yet fully understood. PARP-1 contains three zinc finger structures involved in DNA strand break recognition, among which the first zinc finger has a remarkably low affinity towards zinc ions [1]. Therefore, we investigated the impact of the cellular zinc status on PARP-1 activity and on genomic stability in HeLa S3 cells. Significant impairment of H<sub>2</sub>O<sub>2</sub>-induced poly(ADP-ribosyl)ation was detected in case of zinc depletion by the zinc chelator TPEN which reduced the total and labile zinc concentrations, despite an increase of DNA strand breaks under these conditions. On the contrary, cellular zinc overload with respect to total as well as labile zinc concentration provoked by incubation with elevated concentrations of ZnCl<sub>2</sub> lead to an increased H<sub>2</sub>O<sub>2</sub>-induced poly(ADP-ribosyl)ation. Thus, zinc plays an important role in PARP-1 activation and poly(ADP-ribosyl)ation. Furthermore, we investigated the effect of the cellular zinc status on gene expression profiles via a high-throughput RT-qPCR technique comprising 95 genes related to metal homeostasis and genomic stability. Remarkably, genes coding for metallothioneins responded most sensitively towards a broad range of zinc depletion and zinc overload, regulating zinc homeostasis, thereby preventing other stress responses on conditions of mild zinc depletion or moderate zinc overload. The generation of zinc depletion by higher concentrations of TPEN lead to significantly elevated transcript levels of genes coding for DNA repair factors and cell cycle arrest, indicating DNA damage and genomic instability. The impact of zinc overload was less pronounced; here, especially genes related to the oxidative stress response system were up-regulated. Altogether, the results highlight the potential role of zinc signaling for PARP-1 activation and the maintenance of genomic stability.

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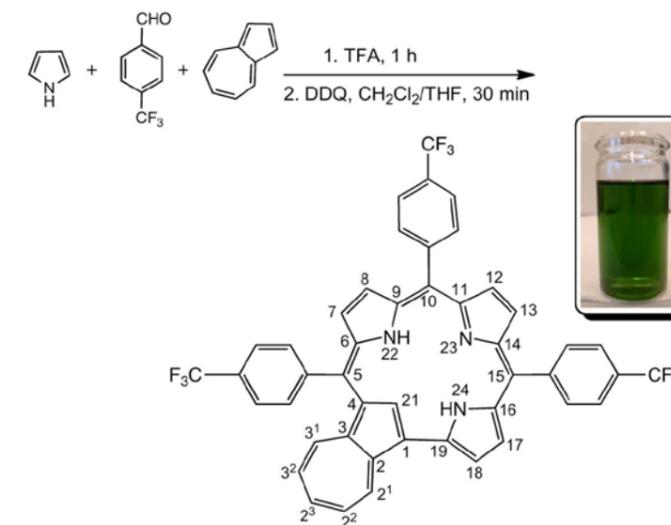
## IL-145

### Metal-Ligand Misfits: Near-IR Phosphorescent 5d Metalloporphyrins in Photomedicine

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The 5d metalloporphyrins are a unique class of complexes that incorporate a large 5d transition metal ion within a sterically constrained macrocyclic ligand [1]. Despite the steric mismatch inherent in their structures, many 5d metalloporphyrins are chemically and photochemically rugged. Many also exhibit near-IR phosphorescence, a property that can be exploited for oxygen sensing and photodynamic therapy, as well as technological applications such as dye-sensitized solar cells [2]. This talk will present the latest advances in this area, including syntheses of new macrocyclic ligands (such as azulicorrole [3], depicted below) and complexes and their applications in photomedicine, particularly photodynamic therapy.



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## IL-146

### Free Energies for Metalation inside Cells Defined by Bacterial Metal-Sensors

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Some metals form more stable complexes with proteins than do others. For first row essential divalent metal ions the binding-order is the Irving-Williams series. This creates a challenge because, of course, some metalloenzymes require the weaker binding metals while other metalloenzymes require the tight binding metals. The challenge can be overcome provided cells maintain the weaker binding metals at greater availabilities than the tighter binding metals: But it has been difficult to define and then measure the availabilities of metals inside cells. Intracellular metal availabilities have recently been determined by measuring the metal sensitivities of a complete set of DNA-binding metal-sensors (1-3). The resulting values for a range of essential metals define the intracellular free energies for metalation. The technical approaches are described in a poster by Andrew Foster. Crucially, intracellular metal availabilities are confirmed to be maintained as the inverse of the Irving-Williams series. These values provide a thermodynamic framework within which it is possible to understand correct protein metalation, exemplified here by a cobalt chelatase for vitamin B<sub>12</sub> biosynthesis.

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## IL-147

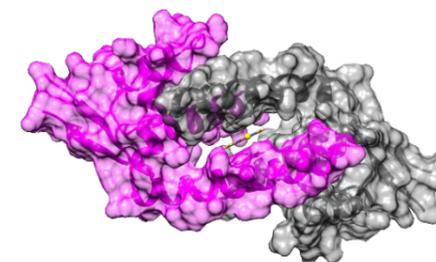
### LmrR: A Privileged Scaffold for Artificial Metalloenzymes

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The catalytic efficiency and high selectivities achieved by natural metalloenzymes are a source of inspiration for the design of novel bio inspired catalysts. A powerful approach for creating artificial metalloenzymes involves incorporating a synthetic transition metal catalysts into a protein. We have developed a new concept for the design of artificial metalloenzymes that involves creation of a novel active site at the dimer interface of the transcription factor LmrR (Lactococcal multidrug resistance Regulator).<sup>[1]</sup> LmrR was selected as the protein scaffold because it contains an unusual large hydrophobic pocket on the dimer interface.

Here, two novel classes of LmrR-based artificial metalloenzymes will be presented, involving either supramolecular anchoring of the metal complex<sup>[2,3]</sup> or biosynthetic incorporation of an unnatural metal binding amino acid using expanded genetic code methodology.<sup>[4]</sup> These artificial metalloenzymes have been applied successfully in catalytic asymmetric C-C bond forming and hydration reactions. Here we will discuss our recent in the evolution and in vivo application of these artificial metalloenzymes.



Structure of an LmrR based artificial metalloenzyme

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## IL-148

### Bifacial Organomercuric Nucleobases

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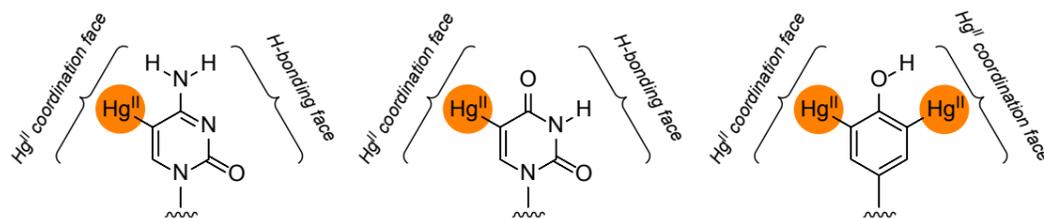
Metal-mediated base pairing between either natural or artificial nucleobases has in many cases been shown to greatly increase the hybridization affinity of oligonucleotides [1-5]. To harness this stabilization for biological applications, we have explored the possibility of replacing one of the coordinative bonds of the metal-mediated base pair with a covalent C-M bond [6-11]. The resulting organometallic nucleobases have the benefit of resisting dissociation even under highly metal-deficient conditions, such as those of the intracellular medium.

Among transition metal ions amenable to metal-mediated base pairing, Hg<sup>II</sup> is attractive because it can readily add to C-H bonds of electron-rich aromatic rings. In the case of cytosine and uracil, addition takes place at the C5 position, resulting in Janus nucleobases with a hydrogen-bonding and a Hg<sup>II</sup> coordination “face”. With phenol, both of the *ortho* carbon atoms can be mercurated, resulting in a nucleobase with two identical Hg<sup>II</sup> coordination “faces”.

Consistent with previously reported results on Hg<sup>II</sup>-mediated base pairing [12-], the Hg<sup>II</sup> centers favour coordination to the thymine-N3 and guanine-N1, with concomitant deprotonation of the nitrogen donor. A good correlation was observed between stabilities of the individual Hg<sup>II</sup>-mediated base pairs (determined by NMR) and thermal stabilities of the respective modified oligonucleotide duplexes (determined by UV melting experiments). In the presence of thiols, Hg<sup>II</sup>-mediated base pairing was abolished and the 5-mercuricytosine and 5-mercuriuracil residues underwent canonical hydrogen-bonded base pairing instead.

In contrast to the monomercurated cytosine and uracil bases, dimercurated phenol can form dinuclear Hg<sup>II</sup>-mediated base triples. The potential of this novel binding mode was demonstrated in a model homothymine•homoadenine•homothymine triplex incorporating the 2,6-dimercuriphenol residue in the middle of the homoadenine strand. Both Hoogsteen and Watson-Crick melting temperatures were increased by more than 10 °C relative to an otherwise identical triplex having the 2,6-dimercuriphenol residue replaced by an adenine residue.

Financial support by the Academy of Finland, the EXPERTS Sustain program, the Finnish National Agency for Education and the Turku University Foundation is gratefully acknowledged.



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## IL-149

### Unique Asymmetric Mixed-Valent Dicopper Complexes as An Active Site Model of pMMO

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The active center of particulate methane monooxygenase (pMMO) that catalyzes the oxidation of methane to methanol under ambient conditions contains dicopper center, each of which is surrounded with different coordination environment [1]. Considering the structural features and strong oxidation ability of pMMO, mixed valent dicopper(II,III) species, ( $\mu$ -oxo)Cu<sup>II</sup>Cu<sup>III</sup> species, is considered to be one of the most possible active species for pMMO enzyme [2].

We have designed and prepared novel asymmetric dicopper complexes as the active intermediate for methane oxidation. Unique dicopper complexes with different coordination environments, [Cu<sup>II</sup><sub>2</sub>(L1)( $\mu$ -PhCOO)] (**1**) and [Cu<sub>2</sub>(L2)( $\mu$ -PhCOO)] (**2**) (L1 = *N*-(3-(bis(pyridine-2-ylmethyl)amino)-2-hydroxypropyl)-3,5-di-*tert*-butyl-2-hydroxybenzamide); L2 = *N*-(3-(bis(pyridine-2-ylmethyl)amino)-2-hydroxypropyl)-3,5-dichloro-2-hydroxybenzamide), were prepared from the reaction of H<sub>3</sub>L1 or H<sub>3</sub>L2, Cu<sup>II</sup>(PhCOO)<sub>2</sub>, and NaH in DMF. They were characterized by using elemental, UV-vis/NIR spectroscopic, ESR spectroscopic, and X-ray structure analyses and cyclic voltammetry. The addition of 1 eq. of acetylferrocenium and CAN to complexes **1** and **2**, respectively, gave one-electron oxidized species, whose ESR and UV-vis/NIR spectroscopic measurements demonstrated that they are copper(III/II) species. Chemical and electrochemical reductions of complexes **1** and **2** were also studied.

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## IL-150

### New Biological Activities of Vanadium Compounds Revealed by Molecular Interactions and Intracellular Signaling

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Vanadium compounds are promising agents in the therapeutic treatment of diabetes. In the past decades, investigations on the mechanism of hypoglycemic action of vanadium have revealed key targets [1] including: (i) the heat shock protein family such as Hsp60, grp75, grp78 and Hsc70 etc., with which the interactions involves regulation of PPAR $\gamma$ -AMPK signaling, unfolded protein responses, mitochondrial function and Cyclin D signaling; (ii) Protein phosphatases, with which the interactions regulates the activity of a variety of protein kinases. However, the great challenge is how to achieve highly specific inhibition of certain kind of phosphatases, such as PTP1B for insulin enhancement. Besides the anti-diabetic effects, the molecular interaction of vanadium with the targets and subsequent intracellular signal transduction would indicate a variety of new biological/pharmacological activities of vanadium compounds such as anti-Alzheimer's disease (AD), anti-neural cancer (neuroblastoma), anti-autism and anti-aging. Therefore, discovery and development of novel vanadium-based medicine would again be an exciting and promising research field. Recently, we demonstrated [2] that anti-diabetic vanadyl complexes could improve the viability of mouse primary cortex neurons; in the APP/PS1 transgenic AD model mice, vanadium treatment could well preserve the cognitive function and attenuated neuron loss without affecting brain A $\beta$  plaques or the distribution. The molecular mechanism involve (i) improving glucose and energy metabolism via activation of the PPAR $\gamma$ -AMPK signal transduction and (ii) suppressing p53-mediated neuronal apoptosis via up-regulation of grp75 after A $\beta$  plaque formation. Thus, vanadyl complexes are of potential to develop disease-modifying therapeutic agent for future treatment of AD.

This work was supported by National Natural Science Foundation of China (#21571006 and #21771010)

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## IL-151

### Signaling Zinc: Deducing the Roles of Zinc fingers, H<sub>2</sub>S and Toxic Metals in Modulating the NF- $\kappa$ B Inflammatory Pathway

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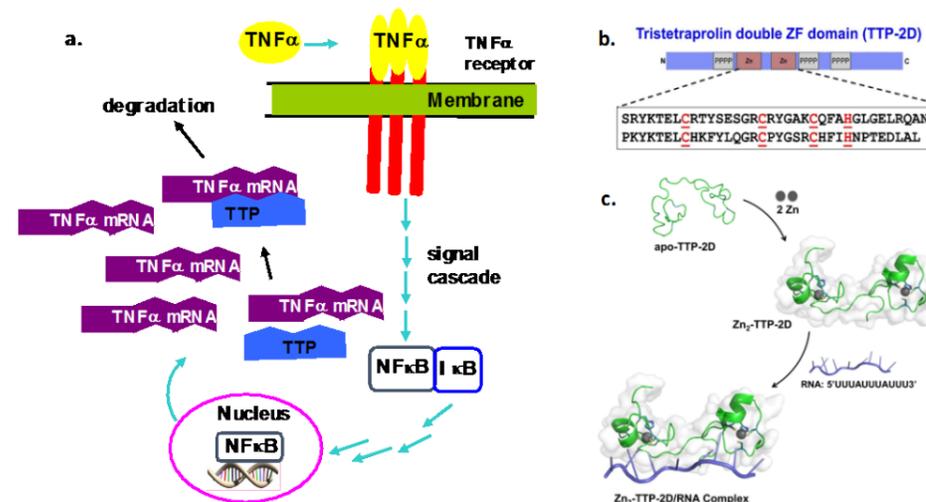
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Zinc has long been known to play crucial biological roles, including functioning as a co-factor for enzymes and for structural proteins, and more recently, as a signal to modulate specific cellular pathways. One key biological response for which zinc plays an important role is in the regulation of inflammation via the NF- $\kappa$ B pathway. There are multiple zinc co-factored and putative zinc co-factored proteins in the NF- $\kappa$ B pathway; however, the molecular level details of how zinc modulates this pathway, which proteins within the pathway are bona fide zinc co-factored proteins, and whether zinc plays a signaling role are not well established. We are combining biochemical, cellular, and proteomics approaches to understand how zinc modulates the NF- $\kappa$ B pathway and how exogenous molecules (i.e. toxic metal ions, beneficial metal ions and signaling molecules) target this pathway. Our work has focused on the protein tristetraprolin (TTP), an RNA binding zinc finger protein that is regulated by NF- $\kappa$ B and which serves as a biomarker of inflammation. Studies aimed at understanding how Zn(II), Pb(II), Au(I) and H<sub>2</sub>S modulate TTP activity in vitro and in cells will be presented, along with preliminary proteomics data. These studies are providing insight into the roles of native and xenobiotic metal ions as well as signaling molecules in inflammation, and have the potential to lead to novel anti-inflammatory strategies. Financial support by the NSF (CHE1708732) and FDA (UO1FD005266) are gratefully acknowledged.



a. Zinc mediated NF $\kappa$ B signalling pathway, b. Cartoon diagram of TTP and its accompanying amino acid sequence, c. Structures of TTP bound to zinc and RNA

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IL-152

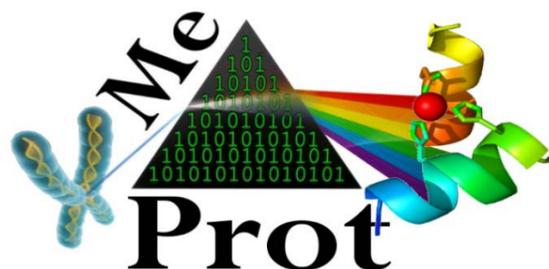
## Bioinformatics of Metalloproteins

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Metalloproteins are essential to life and widespread in organisms from all kingdoms of life. In this presentation, we will describe the bioinformatics tools developed by our group to facilitate the understanding of the structure-function relationship in metalloproteins. The central resource is the MetalPDB database of metal sites in biological macromolecules (<http://metalweb.cerm.unifi.it/>) [1]. In the latest update of MetalPDB, we specifically addressed the identification of potential MFSs in 3D structures lacking the metal cofactor. In addition, updated statistical analyses on the database contents are available on the web site. We are currently introducing functional annotations for all sites, based on manual curation via a dedicated, password-protected annotator interface. To better exploit the contents of MetalPDB, the MetalS2 [2] and MetalS3 [3] servers allow users to compare pairs of metal sites and to search for structurally similar metal-binding sites within the database, respectively. We will show how these tools have been applied to the computational characterization of the human iron-proteome [4]. Finally, we will present a newly developed on-line resource, entitled hMeProt (for human metalloproteome). hMeProt is a database of all human metalloproteins, predicted or experimentally validated, that aggregates information from multiple sources ranging from tissue expression and subcellular localization to functional features such as involvement in disease. The main usage scenarios of hMeProt will be discussed.



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IL-153

## Tuning the Conducting and Photoluminescence Properties of Molecules Comprising Continuous Cytosine-Ag<sup>I</sup>-Cytosine Base Pair

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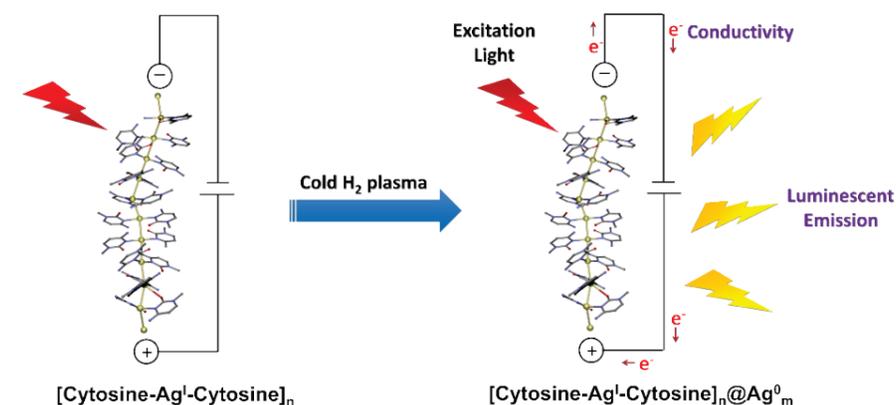
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The concept of manipulating the self-organizing properties of DNA to create nanowires, and ultimately nanocircuits, can be exploited using a new way to precisely control the stoichiometry and the position of the metal ions; the use of DNA molecules containing so-called metal-mediated base pairs [1]. Following this strategy continuous 1D linear chains of Ag<sup>I</sup> ions can be generated inside DNA duplex [2], thus demonstrating that new metallo-DNA nanowires could be rationally prepared. However, the conducting properties of these systems remains unclear. The reduction of the Ag<sup>I</sup> inside the duplex could considerably improve the conductivity of these systems, but this process should not modify the native Ag-DNA structure in order to support the formation of metallic silver wires.

In an effort to explore new reduction methodologies that could be applied for Ag-DNA systems based on silver-mediated base pairs, we have synthesized and characterized a helical compound containing stacked silver-mediated cytosine base pairs [Ag(mC)<sub>2</sub>]BF<sub>4</sub> (mC = N1-methylcytosine), that contain uninterrupted polymeric Ag<sup>I</sup> chains that run through the center of the helices, comparable to related silver-DNA structures [2b]. The exposure of nanostructures of [Ag(mC)<sub>2</sub>]BF<sub>4</sub> to cold hydrogen plasma stimulates the reduction of the prearranged Ag<sup>I</sup> polymeric chains to metallic silver along the material. This solvent-free reduction strategy lead to compound [AgI(mC)<sub>2</sub>]X@Ag<sup>0</sup><sub>n</sub> that contains uniformly well-distributed silver metallic nanostructures that are responsible for the new conducting and photoluminescence properties of the material [3].



This methodology could be employed for the generation of multifunctional silver-DNA related materials with tailored properties.

Financial support by the Spanish MINECO (CTQ2017-89311-P), Junta de Andalucía (FQM-2293) and University of Granada is gratefully acknowledged.

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IL-154

## Biological Studies and Antidotal Efficacy of a Molybdenum Based Cyanide Poisoning Antidote

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Cyanide poisoning antidotes function to prevent cyanide from permanently inhibit cytochrome c oxidase who is primary lethal target [1]. Although these antidotes are efficacious, their downsides prevent their use for emergency treatment of cyanide poisoning [2]. Since cyanide is an endogenous molecule [3], a physiological mechanism to transform cyanide into a non-toxic thiocyanate involves the rhodanase enzyme [4].

Molybdenum sulfur complex salts are capable of converting cyanide to thiocyanate [5]. Selected compounds were evaluated for cytotoxicity in three different cancer cell lines [6]. The compounds proved to have low cytotoxicity compared to cisplatin and even less so for alkali salts compared to tetraalkylammonium salts. Uptake and distribution in cells showed the compounds enter the cells and distribution was confirmed in cytosol, nucleus and mitochondria [5]. Toxicity *in vivo* was studied in a mouse model showing the compounds are relatively safe [7]. Pharmacokinetic data revealed a selected compound enters the bloodstream rapidly and exits in a timely manner [7]. Inhalation study in a mouse model showed modest efficacy against a challenge [7]. The presentation will summarize these findings and discuss their relevance for an emergency antidote to treat cyanide poisoning.

Financial support by the Icelandic Technology Development Fund (Rannís Tæknipróunarsjóður) grant nr. 164784 and by The Icelandic Centre of Research grant nr 140945 is gratefully acknowledged.

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IL-155

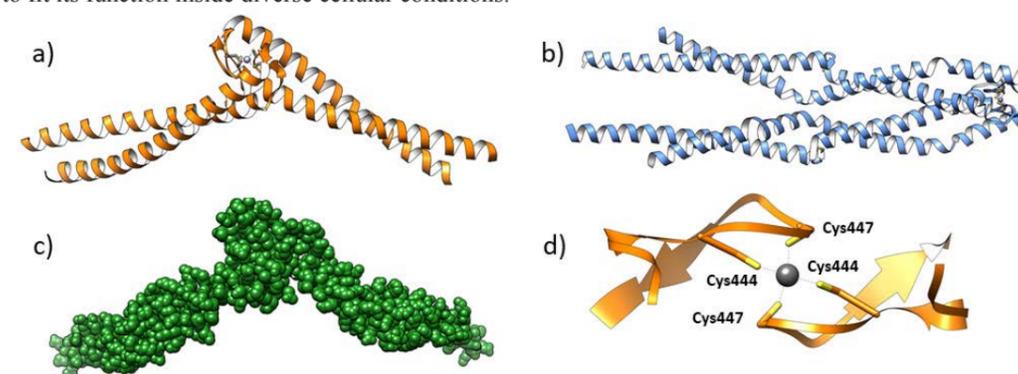
## Zinc Hook Domain – Puzzling Rad50 Interprotein Zn(II) Binding Site with Many Faces

Józef Tran<sup>1</sup>, Olga Kerber<sup>1</sup>, Michał Padjasek<sup>1</sup>, Maciej Maciejczyk<sup>2</sup>, Marek Łuczowski<sup>1</sup>, Tomasz Kočańczyk<sup>1</sup>, Artur Krężel<sup>1</sup>

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<sup>2</sup>Division of Physics and Biophysics, University of Warmia and Mazury, Oczapowskiego 4, 10-719 Olsztyn, Poland

Zinc hook domain is the central fragment of Rad50 protein present in every cell containing genomic DNA. Rad50, as a component of conserved MR(N/X) complex, plays central role in double-strand DNA break repair, maintenance of telomere integrity, meiosis and recombination [1]. Suggested key function of Rad50 is to bind DNA ends and place them in close proximity to facilitate downstream repair and signalling procedures. This role can be performed by means of an appropriate structure; a globular domain whose halves are divided by long (500 Å), anti-parallel, superhelical fragment at centre of which resides unique β-type structure, i.e. the zinc hook. Hook domain contains the intermolecular binding site for Zn(II) ion: (C)CXXC (adjacent Cys residue is distinctive for eukaryotes), responsible for the dimerization of the entire MR(N) complex [2]. Although highly conserved within and alongside zinc binding site, minor alterations in Rad50's amino acid sequence is responsible for different affinity towards Zn(II) as well as different dimer assemblies of Rad50 representatives throughout organisms from different domains of life. By using various physicochemical techniques, i.e. UV-Vis, homo- and hetero-FRET detection, spectropolarimetry, potentiometry, ITC, NMR and X-ray we were able to thoroughly investigate the relationship between Zn(II) affinity, zinc-coupled folding and overall quaternary structure of Rad50 dimers. Affinity towards Zn(II) ions varies for different orthologs of Rad50 protein, which seems to be correlated with Zn(II) availability in cell - *P. furiosus* Rad50 with  $-\log K_{ML2} = 21$ , prokaryotes ( $\log K_{ML2} \sim 20$ ), eukaryotes (*H. sapiens* with  $\log K_{ML2} \sim 19.8$  and *S. cerevisiae* with  $\log K_{ML2} \sim 19$ ) [3,4]. Moreover, zinc hook domains from different organisms consist of dimers with different compositions and arrangements, which may be related to the mechanism of DNA damage response [4]. This systematic overview illustrates how evolution shaped Rad50 protein in terms of its fold and Zn(II) binding properties to fit its function inside diverse cellular conditions.



Structures of zinc hook domains from different Rad50 orthologs a) *P. furiosus* (1L8D), b) *H. sapiens* (5GOX), c) *S. cerevisiae* (SAXS-based), d) zinc binding site from *P. furiosus* hook domain.

Financial support by the NCN under Opus grant no. 2016/21/B/NZ1/02847 is gratefully acknowledged.

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## IL-156

### Solvent Isotope Effects Reveal Non-Canonical Cytochrome P450 Mechanisms

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Since the pioneering work by Groves in the 1970s, the mechanisms of aliphatic carbon oxidation by the cytochrome P450s is envisioned to proceed via the generation of a high valent heme-oxo "Compound I" state generated by heterolytic oxygen-oxygen bond cleavage of the iron-hydroperoxo intermediate. Suggestions of other "active oxygen" reactive states, such as the precursor iron-peroxoanion and the hydroperoxo itself have been proposed, particularly for reactions other than aliphatic hydroxylation. We have examined the carbon-carbon bond scission involved in the generation of androgens by human P450 CYP17A1. We discovered that this reaction is characterized by an *inverse* solvent isotope effect that is explained by nucleophilic attack of the heme-peroxo anion on the 20-carbonyl of 17-OH pregnenolone and 17-OH progesterone. With Professors Kincaid and Mak (Marquette) we confirm this reactivity by low temperature trapping of a six membered hemi-ketal and characterization by resonance Raman spectroscopy. QM/MM calculations by Olsen and co-workers show this intermediate to be the favorable transition state in this C-C lyase reaction and note that hydrogen abstraction from an aliphatic alcohol, an alternate proposal, is not possible. We have analyzed the complete kinetic description of the P450 reaction cycle to establish the inverse solvent isotope effect as an easily measured marker for non-canonical P450 reaction chemistry using the peroxoanion.

Supported by NIH R35 GM118145

## IL-157

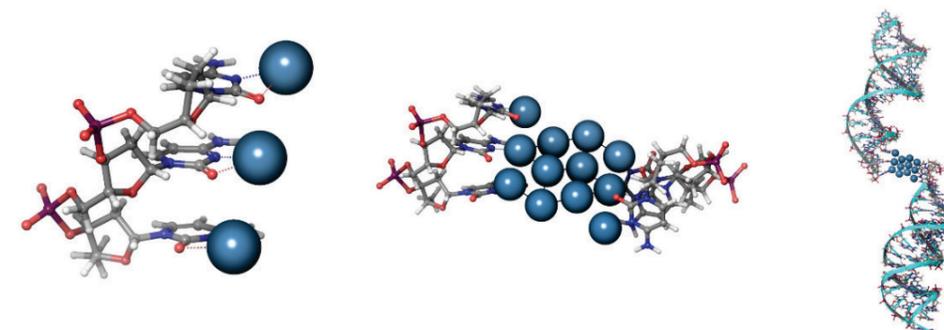
### Characterization of Luminescent Silver Nanoclusters formed at Interfacial Binding Sites Facilitating DNA Oligomerization

Reka Geczy<sup>1</sup>, Niels Johan Christensen<sup>1</sup>, Pratik Shah<sup>2</sup>, Seong Wook Yang<sup>2</sup>, Morten J. Bjerrum<sup>1</sup>, Peter W. Thulstrup<sup>1</sup>

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Nucleic acids can interact with silver ions but also bind silver after partial reduction and formation of few-atom clusters of Ag/Ag<sup>+</sup> [1]. Such Ag-nanoclusters can display very interesting optical properties such as luminescence in the visible and near-infrared regions [2]. These DNA-stabilized silver nanoclusters (DNA-AgNCs) are applied in a range of nanoscientific applications including in the efficient detection of proteins and nucleic acids [3]. However, the diverse optical properties of DNA-AgNCs, their mechanism of formation and aspects of their composition remain unexplored, making the intelligent design of nanocluster probes challenging. Here we present a synthetic procedure for obtaining highly emissive DNA-AgNCs based on a probe design with a C-loop structure in a hairpin with duplex base-pairing, which has been applied in microRNA detection [4]. Purification through size-exclusion chromatography allowed the isolation of the emitting species on a preparatory scale. Through a combination of gel electrophoresis, ICP-MS, and small-angle X-ray scattering (SAXS) in conjunction with the systematic study of various DNA sequences, the low-resolution structure and mechanism of the formation of AgNCs with a C-loop hairpin DNA sequence was investigated. It is proposed that the DNA-AgNCs self-assemble via a head-to-head binding of two DNA hairpins, bridged by the silver nanocluster, resulting in the modelling of a dimeric structure, harbouring an Ag<sub>12</sub> cluster.



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## IL-158

### Multi-Action Anticancer Metallodrugs: Synthesis, Cytotoxicity and Mechanistic Studies

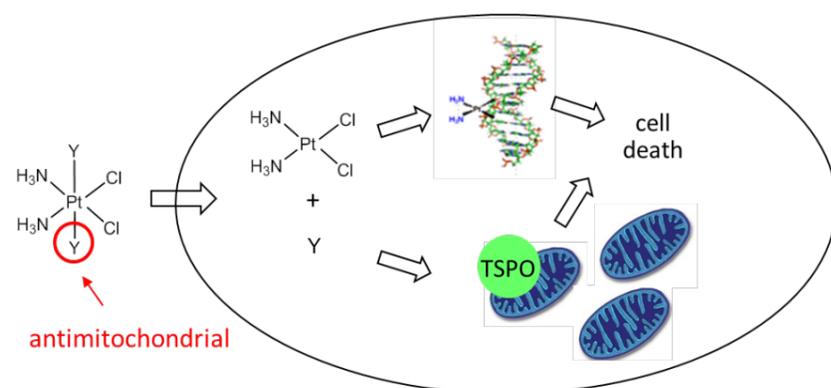
**Andrea Erxleben<sup>1</sup>**

<sup>1</sup>*School of Chemistry, National University of Ireland Galway, University Road, Galway, Ireland.*

Despite significant progress and the continuous discovery of ever more efficient cancer therapies, chemoresistance has remained a formidable challenge in the treatment of cancer. Besides toxicity and adverse side-effects, inherent and acquired resistance towards platinum-drugs are the major limitations of the leading anticancer drug cisplatin. A common strategy to reduce the risk of cancer cells developing resistance is the application of combination therapy, i.e. the co-administration of different drugs with distinct targets and independent modes of action. Metallodrugs allow the combination of two (or more) anticancer entities, i.e. a bioactive metal and bioactive ligand(s), into a single molecule, thus ensuring the simultaneous delivery and activation at the target site.

Octahedral Pt(IV) derivatives of cisplatin are pro-drugs that release the DNA-targeting Pt(II) species and the axial ligands on intramolecular reduction. Our group is interested in bioactive ligands that target the mitochondria [1]. So-called ‘mitocans’ (the acronym for mitochondria and cancer) are an emerging class of anticancer agents that attack the energy-producing machinery of the cancer cell [2].

This lecture will present a series of new Pt(IV) complexes based on the cisplatin and oxaliplatin scaffolds and a bioactive ligand that interacts with the translocator protein on the outer mitochondrial membrane. The complexes show micromolar activity against MCF-7 breast cancer cells. Detailed mechanistic studies including DNA damage, cell death pathway, effect on the mitochondrial membrane potential and on the mitochondrial morphology will be discussed. The combination of antimitochondrial ligands with metal ions other than Pt will also be briefly described.



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## IL-159

### Antimicrobial Peptide – Metal Interactions – Relationship between Coordination Chemistry, Structure, Thermodynamics and Mode of Action

**Magdalena Rowińska-Żyrek<sup>1</sup>, Dorota Dudek<sup>1</sup>, Denise Belotti<sup>1</sup>, Adriana Miller<sup>1</sup>, Aleksandra Mikolajczyk<sup>2</sup>, Agnieszka Matera-Witkiewicz<sup>2</sup>**

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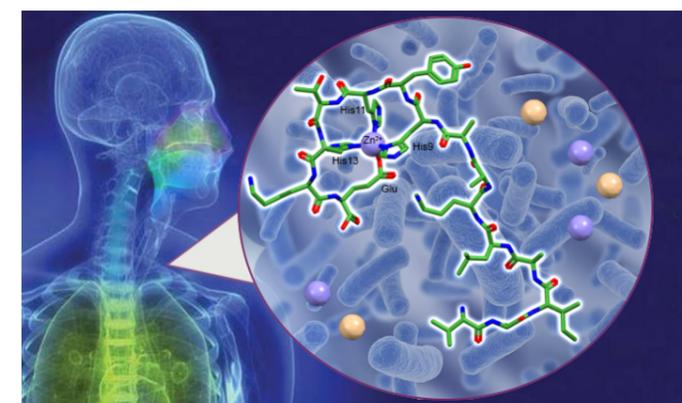
Increasing bacterial and fungal drug resistance makes novel, effective antimicrobial treatments actively sought. Because of the general lack of resistance towards AMPs, they are being relied on as a novel class of therapeutics aimed to conquer drug-resistant bacteria and fungi [1].

Biologically indispensable metal ions have a dual effect on the activity of antimicrobial peptides: (i) AMPs bind them, so that microbes cannot get enough metals essential for their life and virulence (withdrawal of metal ions, nutritional immunity) or (ii) AMPs need the given metal ion as a booster of their antimicrobial activity (metal ions affect the AMP charge and/or structure) [2].

The presence of Zn(II) and Cu(II) significantly enhances the antimicrobial activity of calcitermin, an antimicrobial peptide from the fluid of the human airways (a C-terminal cleavage fragment of calgranulin C), SAAPs – anionic peptides from sheep and clavanins – His-rich, cationic peptides from tunicates. MIC breakpoints of several of these complexes are much lower than the ones for commonly used antibiotics and antifungal agents.

We discuss the details of the coordination mode, structure and stability of the studied complexes, in order to understand the relationship between their bioinorganic chemistry and mode of action.

Financial support by the National Science Centre (UMO-2017/26/A/ST5/00364, SONATA BIS grant to MRZ) is gratefully acknowledged.



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## IL-160

### Secret Handshakes: Tracking Protein-to-Protein Metal Ion Transfer by Rapid X-ray Absorption and Intrinsic Fluorescence Techniques

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The challenge of biochemically characterizing spectroscopically “silent” metals such as Cu(I), Ag(I) and Zn(II) in proteins can be tackled by techniques such as extended x-ray absorption (EXAFS) and fluorescence spectroscopy. Here I will be describing my laboratory’s success in extending these techniques to monitor metalloprotein metal transfer in real time *via* native tryptophan stopped flow fluorescence as well as rapid freeze quench EXAFS. I have also been able to use both techniques complementarily in order to obtain both structural and kinetic data on important and challenging silent metal efflux and transfer proteins. As a professor at a primarily undergraduate institution in the U.S., my lab’s work is best when combined with collaboration, so in this unique talk I will be discussing three collaborative projects<sup>1,2</sup> and the progress we have been able to make by combining our individual labs’ skills toward answering abiding metallobiochemical questions. In particular, I will discuss our work to determine the mode of Cu(I)/Ag(I) efflux in the Cus C(F)BA pump in *E. coli*, and Zn(II) metallotransfer in the Azt Zn chaperone pathway in *P. denitrificans*. I will also discuss our recent spectroscopic work to identify and understand Fe-S clusters in interesting and pathogenically relevant iron uptake systems.

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## IL-161

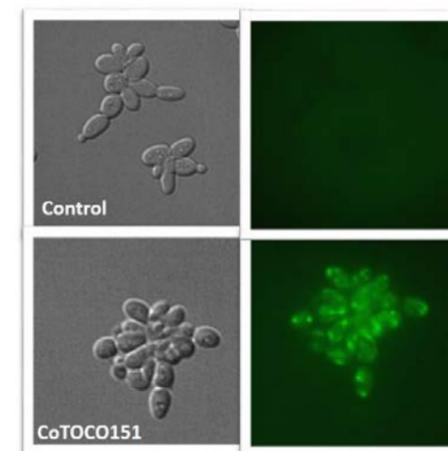
### Cell Labeling with Bimodal Paramagnetic Transition Metal Complexes

Janet Morrow<sup>1</sup>, Akanksha Patel<sup>1</sup>, Didar Asik<sup>1</sup>, Paul Cullen<sup>2</sup>

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Cells labeled with paramagnetic metal complexes produce a MRI signature that facilitates cell tracking in vivo.<sup>1</sup> While mammalian stem cells have been the focus of MRI cell tracking studies to date,<sup>2</sup> it is also of interest to image fungi by using MRI. Fungal infections have gained attention due to the ubiquitous presence of yeast in the human body and the increasing number of immunocompromised patients that develop infections. Our laboratory is involved in the development of transition metal contrast agents<sup>3-5</sup> based on Co(II), Fe(II) and Fe(III) for cell tracking studies. Complexes of Co(II) and Fe(II) produce highly shifted ligand and water proton resonances, producing asymmetric z-spectra when loaded into cells or into liposomes as shown by NMR and MRI studies. Fe(III) complexes enhance T<sub>1</sub> or T<sub>2</sub> relaxation effects in cells. In efforts described here, bimodal imaging agents of Co(II) or Fe(III) that contain a paramagnetic center and a fluorophore will be presented. The fluorescent tag enables identification of the cellular compartmentalization of the probe by using fluorescence microscopy. Studies on *Saccharomyces cerevisiae*, and the infectious yeast, *Candidiasis*, will be presented. Pinocytosis, electroporation and heat shock uptake have been studied, resulting in different cellular compartmentalization of the probe. In some cases, a unique MRI signature is observed that is characteristic of the metal complex interaction with the yeast cell wall. MRI signatures based on type of contrast agent will be presented.



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IL-162

**Disentangling the Cellular Biology of Metallothionein-3 (MT3)**

**Rahma Elsiey<sup>1</sup>, Mehrose Ahmad<sup>1</sup>, Laura Arbelaez<sup>1</sup>, Matthew R. Mehlenbacher<sup>2</sup>, Sarah Lubin<sup>1</sup>, Clarey Kaseke<sup>1</sup>, Joanly Sanchez<sup>1</sup>, Alexa Pinsky<sup>1</sup>, Carla Hachicho, Dean E. Wilcox<sup>2</sup>, Christina L. Vizcarra<sup>1</sup>, Mary Sever<sup>1</sup>, Rachel N. Austin<sup>1</sup>**

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<sup>2</sup> Department of Chemistry, Dartmouth College, Hanover NH USA

Metallothionein-3 (MT3) is a member of the mammalian metallothionein family primarily expressed in the central nervous system. Initially identified for its ability to inhibit the growth of neurons, it remains uncertain whether MT3 plays a role in the cellular biology of metals in the brain. We have measured binding constants for MT3 and several metal ions and showed that it binds lead more tightly than it binds zinc. We have also shown that MT3 mRNA levels are not elevated by exposure to metal ions in mammalian neuronal cell cultures, despite the presence of several metal response elements in the mt3 promoter region. In this work, we will discuss results from experiments designed to elucidate the functionality of the mt3 metal response elements as well as experiments that probe the response of MT3 mRNA levels to hypoxia, another cellular stressor associated with MT3 activity. We also present results from experiments that examine MT3-actin interactions as a function of MT3 metallation status in an effort to explore cellular processes that can explain the mechanism by which MT3 inhibits the growth of neurons. We will also present results from ITC studies of the thermodynamic of copper binding to MT3.

## Oral Presentations

### OP-01

#### A Spectroscopy-Based Approach to Understand Resistance of *M. Tuberculosis* to Aactivate the Prodrug Isoniazid via Mutations in the KatG Heme Enzyme

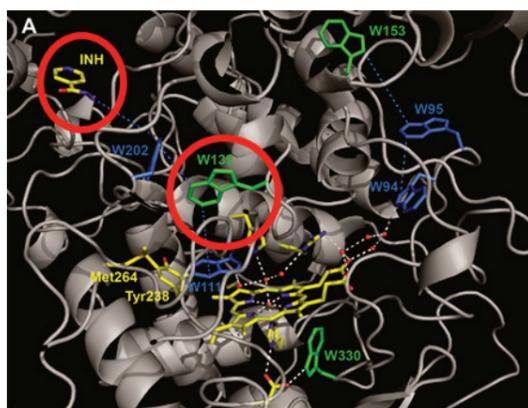
Estelle Capton<sup>2</sup>, Jacek Switala<sup>3</sup>, Peter C. Loewen<sup>3</sup>, Wladimir Sougakoff<sup>2</sup>, **Anabella Ivancich<sup>1</sup>**

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<sup>3</sup>Department of Microbiology. University of Manitoba. Winnipeg, Canada.

Mycobacterium tuberculosis (TB) re-emerges as a major Public Health risk due to appearance of multidrug-resistant strains. The first-line antituberculosis treatment is based on isoniazid (INH), a prodrug that is activated by the pathogenic bacteria itself and via a heme-containing enzyme (*M. tuberculosis* catalase-peroxidase, MtKatG) that mediates the formation of the INH-NADH adduct. However, the description of the molecular mechanism for the enzyme-catalyzed activation of INH by MtKatG is yet under debate (1). The oxidation of INH at the heme site and using the conventional peroxidase-like mechanism fails to explain the role of the frequently found mutations in KatG isolated from INH resistant patients, called missense-type, and which are far from the heme site (2). Clearly such mutations are uncorrelated to the heme-edge oxidation of INH as substrate, and involving the [Fe(IV)=O Por<sup>+</sup>] catalytic intermediate. Based on our previous findings on KatG catalytic intermediates (3,4), the INH binding site being far from the KatG heme site (5), and the INH oxidation occurring via a [Fe(IV)=O Trp<sup>\*</sup>] species formed subsequently to the [Fe(IV)=O Por<sup>+</sup>] intermediate and involving catalytically-relevant long-range electron transfers (6), we have further identified an allosteric effect of the various mutations allowing to rationalize, for the first time, the induced inactivation effect of the mutations on the capability of MtKatG to oxidize INH. Specifically, our detailed EPR spectroscopy characterization of the various mutants well-agree with perturbations (long-range allosteric effects) on the extended H-bonding network (see Figure), that we have previously shown to be crucial for the formation of the Trp-based intermediates in KatGs (7). Our unprecedented rationalization of the TB resistance provides a new tool to design INH analogs to overcome the resistance induced by the various mutations uncorrelated to the heme site.



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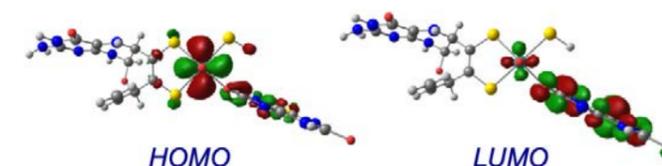
### OP-02

#### Molybdenum Cofactor Construction and Novel Cofactor Contributions to Catalysis

**Martin L. Kirk<sup>1</sup>**, Jing Yang<sup>1</sup>, Khadanand KC<sup>1</sup>, Laura Ingersol<sup>1</sup>, Amrit Pokhrel<sup>1</sup>

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Pyranopterin molybdenum enzymes are ubiquitous in Nature and play fundamental roles in human health and global C, N, and S cycles [1]. All pyranopterin containing Mo enzymes require a unique and properly synthesized molybdenum cofactor (Moco) [2] for function. We will discuss how molybdate is incorporated into molybdopterin (MPT) to form Moco [3], in addition to our latest results relating to how Mo insertase proteins abstract a S from cysteine and incorporate it into Moco to form a modified cofactor for insertion into xanthine oxidase family apoenzymes. We will discuss new results from our laboratory that analyze new enzyme-product complexes, and how they provide detailed insight into C-H bond activation and electron transfer reactivity in xanthine oxidase (see Figure) [4-5]. Other topics for discussion include the Mo-containing methionine sulfoxide reductase (MsrP), which appears to be an unusual enzyme that has been purported to employ two-electron ligand redox and not Mo-based redox to catalyze the reduction of oxidized methionine residues by oxygen atom transfer. A combined spectroscopic approach has been employed to define the geometric and electronic structure of paramagnetic *as-isolated* and *variant forms* of MsrP in order to provide critical new information regarding an alternative mechanism of MsrP activity. We will also detail our most recent spectroscopic and electronic structure studies of the DMSO reductase *high-g split* intermediate. We have synthesized the first small molecule analogs for *high-g split*, which have allowed for new insight into key relationships between the geometric and electronic structure of this catalytically relevant intermediate. M.L.K. acknowledges the National Institutes of Health (GM-057378) for financial support.



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### OP-03

#### DNA Cleavage and Cytotoxicity of Five-Coordinate Cu(II) Complexes Based on Piperazine Bearing Pendant Pyridyl Arms

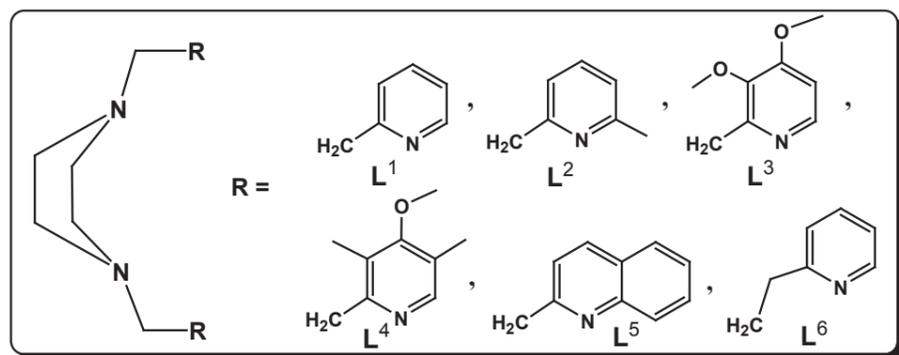
Salah S. Massoud<sup>1</sup>, Yvonne Nossol<sup>2</sup>, Sebastian D. Kettenmann<sup>2</sup>, Bailey Williams<sup>1</sup>, Andrew Milner<sup>1</sup>, Febee R. Louka<sup>1</sup>, Franz A. Mautner<sup>3</sup>, Nora Kulak<sup>2</sup>

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A novel series of five-coordinate chlorido and perchlorato Cu(II) complexes based piperazine bearing symmetrically two pendant pyridyl derivatives, [Cu(N4)X]ClO<sub>4</sub>/PF<sub>6</sub> were synthesized and structurally, where X = ClO<sub>4</sub> or Cl and N4 = L<sup>1</sup> - L<sup>6</sup> (Scheme 1). In aqueous acetonitrile solution, the complexes react instantaneously with H<sub>2</sub>O with color change which is associated with strong blue shift in the visible region where [Cu(N4)(H<sub>2</sub>O)X]<sup>+</sup> exists in equilibrium with [Cu(N4)(H<sub>2</sub>O)]<sup>2+</sup> ion. The cleavage activity of the complexes towards pBR322 plasmid DNA and the mechanistic pathway of the cleavage process were investigated under physiological conditions. The *in vitro* cytotoxicity of the complexes were tested against cancer cell lines A2780 (ovarian cancer) and Fibroblasts (cells found in connective tissue). Complexes [Cu(N4)ClO<sub>4</sub>]ClO<sub>4</sub> (N4 = L<sup>1</sup>, L<sup>5</sup>) showed significant cytotoxicity for the cancer cells. Financial support by University of Louisiana at Lafayette and Freie Universität Berlin is gratefully acknowledged.



Scheme 1. Structures and abbreviations of piperazine ligands used in this study

### OP-04

#### Half-Sandwich Tantalum Complexes as a New Scaffold for Bioinorganic Chemistry

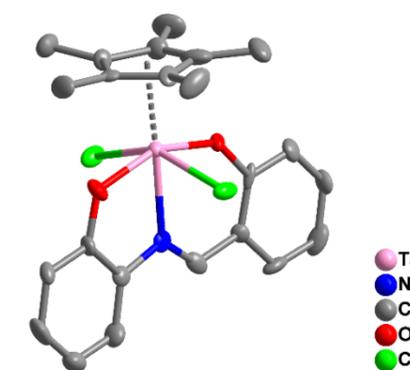
Pavel Štarha, Zdeněk Trávníček

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Tantalum is well-known to have multiple utilization in medicine, specifically for the construction of surgical instruments and implants for reconstructive surgery. Surprisingly, the development of biologically active tantalum complexes have been ignored by bioinorganic chemists from the end of last century [1]. In the meantime, many pharmacologically prospective complexes of various transition metals (including niobium) have been reported to date. In our opinion, it has been of great interest to investigate whether tantalum complexes could be a challenge for bioinorganic chemistry, following the similar story as other transition metals, such as platinum, ruthenium osmium or iridium.

Since the tantalum coordination chemistry is quite rich, we had to choose carefully the structural type to be studied. We chose, as an analogue of many highly cytotoxic Ru, Rh, Os and Ir agents, the half-sandwich structural type and we prepared a series of the [Ta(η<sup>5</sup>-Cp\*)Cl<sub>2</sub>(nL)] complexes; Cp\* = pentamethylcyclopentadienyl, nH<sub>2</sub>L = 2-[(E)-(2-hydroxyphenyl)imino]methylphenol (H<sub>2</sub>L) or its derivative. The first representative of this series, [Ta(η<sup>5</sup>-Cp\*)Cl<sub>2</sub>(L)] (**1**; see figure below), was markedly more active *in vitro* (*p* < 0.005) at various human cancer cell lines with different sensitivity towards *cisplatin*, implying its ability to kill cancer cells with both the intrinsic and acquired resistance towards the therapeutic effect of the conventional *cisplatin* [2]. In contrast to the cancer cells, the biological effect of **1** at the used non-cancerous cells (e.g. primary culture of hepatocytes) was negligible. The cell death is induced via apoptosis, most likely through the mitochondrial pathway, as suggested by the results of various flow cytometry experiments (e.g., induction of apoptosis or a release of the pro-apoptotic protein cytochrome c into the cytosol) performed on the treated cancer cells. Within the contribution, an attention will be paid to the effect of the H<sub>2</sub>L ligand substitution on the biological activity of the [Ta(η<sup>5</sup>-Cp\*)Cl<sub>2</sub>(nL)] complexes, and to several [Ta(η<sup>5</sup>-Cp\*)(nL)X<sub>2</sub>] complexes with both the chlorido ligands being replaced by a bioactive ligand (X = e.g., pyruvate dehydrogenase kinase (PDK) inhibitor dichloroacetate) [3]. The details of solution behaviour in various water-containing media as well as the results of the interaction studies with various relevant biomolecules will be also discussed.

This work was supported by the projects IGA\_PrF\_2019\_012, LO1305 and CZ.1.05/2.1.00/19.0377.



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### OP-05

#### Posttranslational Modification of Heme in Peroxidases – Impact on Structure and Function

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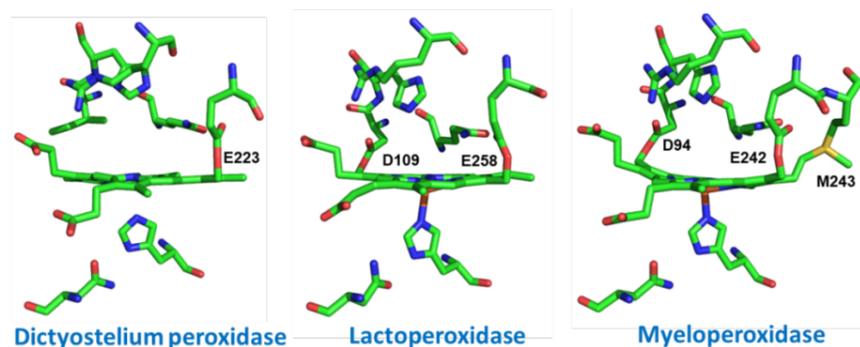
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Four heme peroxidase superfamilies arose independently in evolution [1]. Only in the peroxidase-cyclooxygenase superfamily the prosthetic group is posttranslationally modified (PTM). As a consequence these peroxidases can form one, two or three covalent bonds between heme substituents and the protein. This include ester bonds between heme 1- and 5-methyl groups and glutamate and aspartate residues as well as a sulfonium ion link between the heme 2-vinyl substituent and a methionine.

Here the phylogeny and physiological roles of representatives of chordata peroxidases (family 1) [2], peroxidasins (family 2) [3,4], and peroxidockerins (family 6) [5,6] of this superfamily, their occurrence in all kingdoms of life, the relevant sequence motifs for definite identification and the available crystal structures are presented. We demonstrate the autocatalytic posttranslational maturation process and the impact of the covalent links on spectral and redox properties as well as on catalysis, including Compound I formation and reduction by one- and two-electron donors. Finally, we discuss the evolutionary advantage of these PTMs with respect to the proposed physiological functions of the metalloenzymes that range from antimicrobial defence in innate immunity to extracellular matrix formation and hormone biosynthesis. Financial support by the FWF (W1224) is gratefully acknowledged



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### OP-06

#### Nitrite Coordination and Associated Spin Transitions in Heme-Globins as Revealed by Resonance Raman Spectroscopy

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Nitrite that is present at micromolar levels in tissues and nanomolar levels in blood can be reduced to the bioactive nitric oxide molecule during hypoxia and mediate physiological signaling. Although no dedicated mammalian nitrite reductase has been identified up to date, a growing number of proteins are reported to be able to reduce nitrite to nitric oxide, among which are hemoglobin, myoglobin and heme-copper oxidase. In this work, we present the resonance Raman structural characterization of the hemoglobin heme-nitrito and heme-nitrito/nitrovinyl species and outline the differences in the properties of these species compared to those of the previously described myoglobin-nitrite adducts [1-3]. Variations in the spin state of the hemoglobin- versus myoglobin-nitrite adducts along with the observation of spin transitions are discussed. We propose that the proximal heme Fe-N<sub>His</sub> bond is a determining factor in controlling the electronic structure and reactivity of the heme-nitrito species.

Financial support by the European Regional Development Fund and the Republic of Cyprus through the Research Promotion Foundation (Grant No. EXCELLENCE/1216/0477) is gratefully acknowledged.

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### OP-07

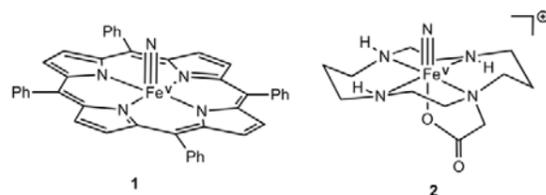
#### Electronic Structures and Reactivity of Iron(V)-Oxo and Nitrido Complexes

Shengfa Ye<sup>1</sup>, Hao-Ching Chang<sup>1</sup>, Bhaskar Mondal<sup>2</sup>, Huayi Fang<sup>2</sup>, Frank Neese<sup>1</sup>, Eckhard Bill<sup>2</sup>

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High-valent iron species has attracted much interest in bio-inorganic chemistry, because they play key roles in important biological as well as industrial processes. Oxo-iron species have been identified as a pivotal intermediate in the catalytic cycle of a range of O<sub>2</sub>-activating heme and nonheme iron enzymes. Nitrido-iron complexes are proposed to implicate in biological N<sub>2</sub> fixation and the Haber-Bosch process. Herein we present a combined spectroscopic and computational study on the electronic structures of [Fe<sup>V</sup>(N)(TPP)] (**1**, TPP<sup>2-</sup> = tetraphenyl porphyrinate), the nitrido congener of compound I, the C-H cleaving agent in cytochrome P450. Specifically, wavefunction based ab initio calculations predict a low spin d<sup>3</sup> electron configuration for **1**. Experimentally, complexes **1** elicits an exceedingly anisotropic EPR spectrum ( $g_{\perp} \sim 1.7$  and  $g_{\parallel} \sim 1$ ), nearly identical to that detected for nonheme iron(V)-nitrido complex [Fe<sup>V</sup>(N)(cyclam-ac)]<sup>+</sup> (**2**, cyclam-ac = cyclam-1-acetate). Both findings evidence that complex **1** is a genuine iron(V)-nitrido species, in contrast to compound I, which is best described as an iron(IV)-oxo unit antiferromagnetically coupled to a porphyrin radical.



As analyzed by the ligand field theory coupled to theoretical calculations, the origin of the large  $g$ -anisotropy of complexes **1** and **2** is a manifestation of their unusual electronic structure having  $S = 1/2$  orbitally nearly degenerate ground states. On the basis of that, characteristic EPR features of low spin tetragonal iron(V)-nitrido and -oxo complexes was proposed.

Starting from the experimentally validated electronic structure, the reactivity of a range of iron(V)-nitrido and -oxo complex is discussed.

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### OP-08

#### Inorganic Reactions for Analysis of Epigenetically Modified DNA

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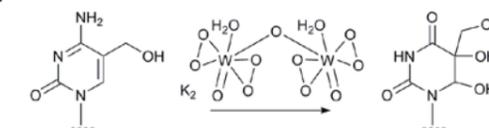
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Gene expression is regulated by the epigenetic modification of DNA and histone proteins, independent of their primary sequences. In particular, methylation/demethylation of cytosine (C) in DNA is an important epigenetic modification of the genome, and this process plays a crucial role in the regulation of developmental gene expression. The mechanism of DNA methylation, i.e., formation of 5-methylcytosine (5mC) has been studied very well, whereas for DNA demethylation recent studies revealed that 5-hydroxymethylcytosine (5hmC) is an important intermediate in the active demethylation process. Much effort has gone into developing a simple reaction that can be used for the detection of 5mC and 5hmC. Although bisulfide methods or immunoprecipitation methods are often used for 5mC analysis, they are not suitable for sequence-specific labeling of 5mC. The ability to distinguish 5mC and 5hmC from C, i.e., to detect the existence of only one methyl group in a long DNA strand, is still a chemically and biologically challenging subject.

We have developed the 5mC-selective osmium complex formation for effective detection of 5mC.[1] We synthesized a bipyridine-attached adenine derivative for sequence-specific osmium complex formation. Sequence-specific osmium complex formation was achieved by the hybridization of a short DNA molecule containing this functional nucleotide to a target DNA sequence, and resulted in the formation of a crosslinked structure. The interstrand crosslink clearly distinguished 5mC from C, which was applied to quantification of the degree of methylation at a specific cytosine in a whole genome and to visualization of methylation regions in chromosomes.

We have also developed a detection method of 5hmC, which is a newly discovered natural nucleobase that may play an important intermediary role in the active DNA demethylation pathway.[2-4] We found that the oxidation using dinuclear peroxotungstate  $K_2[W(=O)(O_2)_2(H_2O)]_2(\mu-O) \cdot 2H_2O$  is useful for the discrimination of 5hmC from its epigenetic precursors, C and 5mC in DNA. The dinuclear peroxotungstate was added to a DNA solution in a pH 7 sodium phosphate buffer and the mixture was incubated at 50 °C for 5 h. The reaction proceeded 5hmC-selectively. The mass spectrum of the oxidation product obtained from the 5hmC-containing DNA indicated the formation of trihydroxylated thymine (<sup>th</sup>T) in DNA. The tungsten oxidation products induced the incorporation of adenine into the complementary site in a primer extension, and made it possible to detect 5hmC in a DNA sequence of interest in a conventional DNA sequencing analysis. Using the tungsten oxidation, we analyzed the distribution of 5hmC in human brain genome.

These inorganic analyses of epigenetically modified DNA will provide beneficial information on the design of a powerful method for 5mC/5hmC scanning and typing to solve the mystery of the initialization of gene function through methylation/demethylation.



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### OP-09

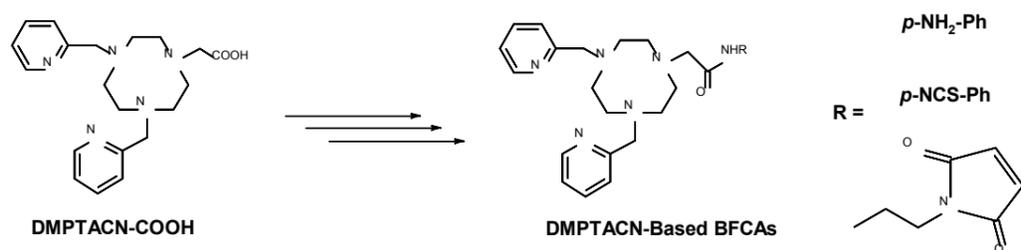
#### 1,4,7-Triazacyclononane Ligands as Bifunctional Radiocopper Chelating Agents

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The design of tailor-made bifunctional chelating agents (BFCAs) for radioactive transition metals in view of nuclear medical applications as well as acquisition of reliable information about the biodistribution of different materials represents an intensive and rapidly developing field of research [1]. In this context, the tridentate macrocycle 1,4,7-triazacyclononane (TACN) is of special interest since it forms stable complexes with transition metal ions particularly with Cu(II) [2]. Further, the introduction of donor groups, such as pyridyl units, on the TACN scaffold, significantly enhances the thermodynamic stability as well as the kinetic inertness of the Cu(II) complexes formed. Furthermore, the ligand structure offers various possibilities to introduce biological vectors and suitable linkers for tuning the lipophilicity, overall charge and aqueous solubility of the final bioconjugates. For example, TACN ligands with two pyridylmethyl side-arms (DMPTACN derivatives) rapidly chelate copper(II) radionuclides under ambient conditions and the resulting complexes show high *in vivo* stability. One such derivative, 2-[4,7-bis(2-pyridylmethyl)-1,4,7-triazacyclononan-1-yl]acetic acid (**DMPTACN-COOH**), containing two coordinating picoline groups, not only exhibits excellent *in vivo* stability after <sup>64</sup>Cu radiolabeling, but also allows for direct attachment of vector molecules as well as easy introduction of bioconjugatable functionalities (e.g., maleimide, isothiocyanate) via the carboxylate pendant. This makes DMPTACN-COOH and its derivatives promising BFCAs for radiocopper (**DMPTACN-based BFCAs**), facilitating the preparation of radiolabeled targeting molecules and bio(nano)materials.

Examples of target-specific peptides and bio(nano)materials equipped with DMPTACN ligands for labeling with <sup>64</sup>Cu as an ideal positron emitter are discussed. This enables tumor imaging and the biodistribution of the materials to be studied over a period of days *via* positron emission tomography (PET).



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### OP-10

#### Cellular Uptake and Reactivity with Sulfur-Containing Biomolecules of Antitumor Active Dirhodium(II) Tetraacetate

Farideh Jalilehvand<sup>1</sup>, Alejandra Enriquez Garcia<sup>1</sup>, Valerie Brunskill<sup>1</sup>, Benjamin S. Gelfand<sup>1</sup>, Carrie S. Shemanko<sup>2</sup>, Hugh H. Harris<sup>3</sup>

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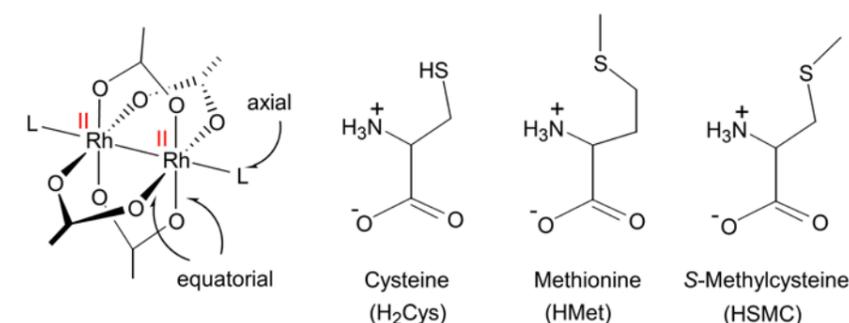
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Dirhodium(II) carboxylates,  $\text{Rh}_2(\mu\text{-RCOO})_4$ , show remarkable antitumor properties. Possible mechanisms of action include inhibition of DNA replication by direct DNA binding, and inhibition of transcription due to redox reactions with thiol groups on T7-RNA polymerase [1]. Earlier *in-vitro* studies showed irreversible inhibition of enzymes containing thiol (-SH) groups at or near their active site, leading to breakdown of the paddlewheel structure around the Rh(II)-Rh(II) bond and oxidation of the Rh(II) ions. But the cage structure with four bridging carboxylate groups (see the figure below) remained intact in reactions with human serum albumin or methionine, since both the imidazole group of the histidine residue and the thioether of methionine could bind reversibly to its axial positions [2].

Recent studies in our group demonstrated that aerobic reactions of  $\text{Rh}_2(\text{CH}_3\text{COO})_4$  with glutathione and *L*-cysteine led to multi-nuclear complexes with two or three thiolate bridges between Rh(III) atoms. We also found that methionine (HMet) at  $\sim 40^\circ\text{C}$  could form a stable dirhodium(II) complex,  $\text{Rh}_2(\text{CH}_3\text{COO})_2(\text{S},\text{N},\text{O-Met})_2$ , where two of the bridging acetate groups were replaced by tridentate methionine ligands also occupying the axial sites [3]. *S*-methylcysteine, however, formed mononuclear Rh(III) species, showing that the ring size of the chelating ligand is important for the stability of the complex [4]. Moreover, X-ray fluorescence microscopy (XFM) and cell viability studies showed no toxicity and poor uptake of the  $\text{Rh}_2(\text{CH}_3\text{COO})_2(\text{S},\text{N},\text{O-Met})_2$  complex, as compared to  $\text{Rh}_2(\text{CH}_3\text{COO})_4$ , emphasizing that the antitumor activity depends on availability of the axial positions of these dirhodium(II) compounds [4]. Our results on the reactions of  $\text{Rh}_2(\text{CH}_3\text{COO})_4$  with the above sulfur-containing biomolecules will be presented during this conference.

Financial support by the Natural Sciences and Engineering Council of Canada, and beam times by the Stanford Synchrotron Radiation Lightsource and the Advanced Photon Source are acknowledged.



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### OP-11

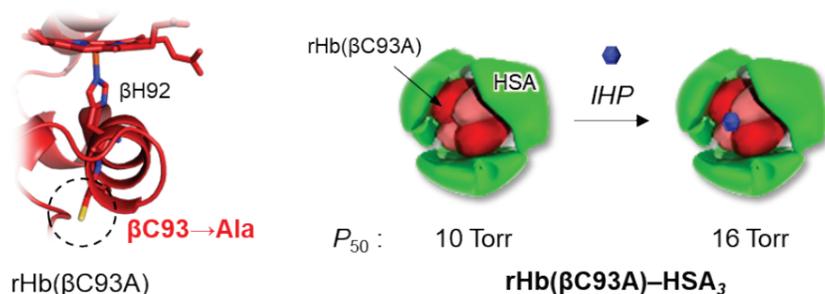
#### Hemoglobin( $\beta$ C93A)-Albumin Cluster with Moderate Allosteric Effect by Inositol Hexaphosphate

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Hemoglobin (Hb)-based O<sub>2</sub>-carriers of several kinds have been developed as red blood cell (RBC) substitutes because of worldwide blood supply shortage.<sup>[1]</sup> We previously synthesized a core-shell structured protein cluster composed of Hb at nucleus and human serum albumin at periphery (Hb-HSA<sub>3</sub>),<sup>[2,3]</sup> which has long circulation lifetime in blood stream and superior biocompatibility.<sup>[4]</sup> The Hb-HSA<sub>3</sub> cluster shows higher O<sub>2</sub> affinity and lower cooperativity than those of native Hb because the bindings of the crosslinker,  $\alpha$ -succinimidyl- $\omega$ -maleimide, to the Cys- $\beta$ 93 and surface Lys residues of Hb inhibit the quaternary structural motion from the relaxed (R) state to tense (T) state.<sup>[5]</sup> Inositol hexaphosphate (IHP), a strong allosteric effector, stabilizes the T-state structure of Hb and reduces the O<sub>2</sub> affinity. However, the chemical modification of Cys- $\beta$ 93 in Hb weakens the IHP effect.<sup>[6]</sup> In this paper, we present the new protein cluster composed of recombinant Hb (Cys- $\beta$ 93 $\rightarrow$ Ala) variant, rHb( $\beta$ C93A)-HSA<sub>3</sub>, and its unique O<sub>2</sub> binding property.<sup>[7]</sup>

First, rHb( $\beta$ C93A) in which Cys- $\beta$ 93 is genetically replaced with Ala was expressed using *Pichia* yeast and purified sequentially using cation-exchange column chromatography and anion-exchange column chromatography. To wrap rHb( $\beta$ C93A) with HSAs, we used *N*-succinimidyl 3-maleimidopropionate (SMP) as a crosslinking agent. The O<sub>2</sub> affinity ( $P_{50}$ : O<sub>2</sub> pressure where Hb is half-saturated with O<sub>2</sub>) and Hill coefficient ( $n$ ) of rHb( $\beta$ C93A) and rHb( $\beta$ C93A)-HSA<sub>3</sub> cluster were determined from their O<sub>2</sub> dissociation curves in PBS solution at 37°C. As expected, rHb( $\beta$ C93A)-HSA<sub>3</sub> cluster showed almost the same O<sub>2</sub> binding parameters ( $P_{50} = 10$  Torr,  $n = 1.3$ ) as those of naked rHb( $\beta$ C93A) ( $P_{50} = 11$  Torr,  $n = 1.5$ ). Remarkably, the O<sub>2</sub> affinity of rHb( $\beta$ C93A)-HSA<sub>3</sub> cluster decreased ( $P_{50}$ : 10 $\rightarrow$ 16 Torr) upon addition of IHP. It rather contrasts against the fact that IHP effect was not observed in Hb-HSA<sub>3</sub> cluster. The complexation of IHP induced the moderate reduction of the O<sub>2</sub> affinity in much the same way as native Hb.



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### OP-12

#### Mimicking Class Ib Dimanganese Ribonucleotide Reductase

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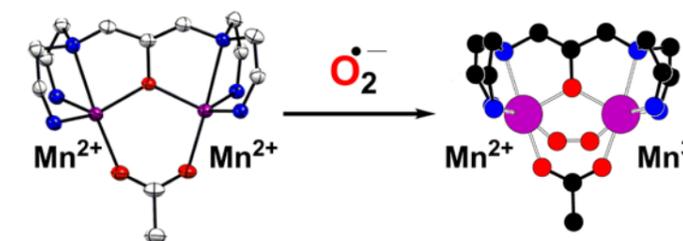
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A fascinating facet of ribonucleotide reductase's (RNRs) Chemistry has been the identification of a dimanganese (Mn<sub>2</sub>) active site in class Ib RNRs that requires superoxide anion (O<sub>2</sub><sup>•-</sup>), rather than dioxygen (O<sub>2</sub>), to access a high-valent Mn<sub>2</sub> oxidant via a Mn<sup>II</sup>Mn<sup>III</sup>-peroxide intermediate. We have identified two Mn<sup>II</sup><sub>2</sub> complexes that, upon exposure to KO<sub>2</sub>, yield Mn<sup>II</sup>Mn<sup>III</sup>-peroxide adducts, providing insight into the proposed class Ib Mn<sub>2</sub> RNRs reactivity. The Mn<sup>II</sup>Mn<sup>III</sup>-peroxide complexes displayed electronic absorption features ( $\lambda_{\max} \sim 450, 600$  nm) typical of a Mn-peroxide species, and either a 29- or 22-line EPR signal typical of a Mn<sup>II</sup>Mn<sup>III</sup> entity. Mn K-edge X-ray absorption near-edge spectroscopy (XANES) suggested formal oxidation states of Mn<sup>II</sup>Mn<sup>III</sup> for both, while electrospray ionisation mass spectrometry (ESI-MS) confirmed the elemental composition of the Mn<sup>II</sup>Mn<sup>III</sup>-peroxides. Both complexes were unreactive towards weak C-H bonds, and were only reactive with ferrocene and weak O-H bonds upon activation with proton donors. In contrast, one of the complexes was found to be an efficient nucleophilic oxidant. Our findings provide support for the postulated mechanism of O<sub>2</sub><sup>•-</sup> activation at class Ib Mn<sub>2</sub> RNRs and the activation of the Mn<sup>II</sup>Mn<sup>III</sup>-peroxide core with electrophiles.

#### Mimicking Class Ib Mn<sub>2</sub> RNRs



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### OP-13

#### Mechanism of the Chitinolytic Peroxygenase Reaction

Bastien Bissaro<sup>1</sup>, Bennett Streit<sup>2</sup>, Ingvild Isaksen<sup>1</sup>, Vincent G.H. Eijnsink<sup>1</sup>, Gregg T. Beckham<sup>3</sup>, Jennifer DuBois<sup>2</sup>, Åsmund K. Røhr<sup>1</sup>

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Lytic polysaccharide monooxygenases (LPMOs) are a recently discovered class of monocopper enzymes, broadly distributed across the Tree of Life [1]. Recent reports indicate that LPMOs can use H<sub>2</sub>O<sub>2</sub> as an oxidant, and, thus, carry out a novel type of peroxygenase reaction involving unprecedented copper chemistry [2].

Here, we present a combined computational and experimental analysis of the H<sub>2</sub>O<sub>2</sub>-mediated reaction mechanism [3]. *In silico* studies, based on a model of the enzyme in complex with a crystalline substrate [4], suggest that a network of hydrogen bonds, involving both the enzyme and the substrate, brings H<sub>2</sub>O<sub>2</sub> into a strained reactive conformation, and guides a derived hydroxyl radical towards formation of a copper-oxyl intermediate. The initial cleavage of H<sub>2</sub>O<sub>2</sub> and subsequent hydrogen atom abstraction from chitin by the copper-oxyl intermediate are the main energy barriers. Stopped-flow fluorimetry experiments demonstrated that the priming reduction of LPMO-Cu(II) to LPMO-Cu(I) is a fast process compared to the re-oxidation reactions.

Using single turnover conditions, we found that re-oxidation of LPMO-Cu(I) is 2000-fold faster with H<sub>2</sub>O<sub>2</sub> than with O<sub>2</sub>, the latter being several orders of magnitude slower than rates reported for other monooxygenases. In accordance with the peroxygenase nature of the LPMO, the presence of substrate accelerated re-oxidation by H<sub>2</sub>O<sub>2</sub>, whereas re-oxidation by O<sub>2</sub> became slower. These novel insights into the peroxygenase nature of LPMOs will aid in the development and application of enzymatic and synthetic copper catalysts and may contribute to a further understanding the roles of LPMOs in Nature, varying from biomass conversion to chitinolytic pathogenesis-defense mechanisms.

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### OP-14

#### Development of Pt(IV) Anticancer Prodrugs that can be Controllably Activated by Visible Light

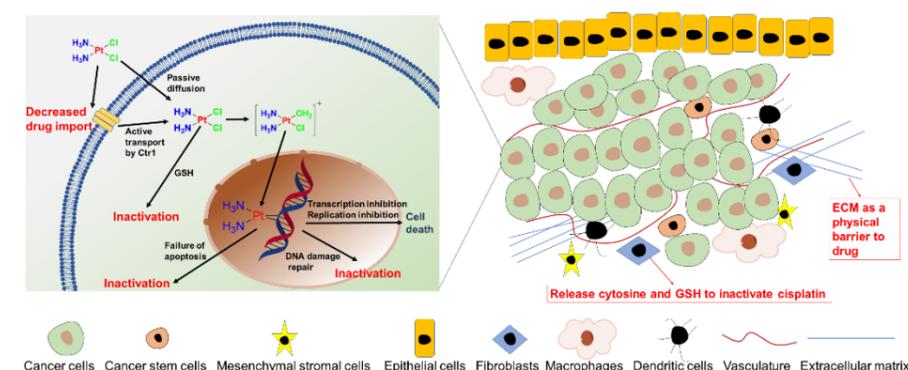
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Despite the broad clinical applications of platinum-based anticancer drugs including cisplatin, their side-effects and resistance issues have encouraged researchers to look for novel metal-based anticancer complexes. Non-traditional platinum compounds especially Pt(IV) complexes have been extensively studied and they hold great promise to be further developed as the next-generation platinum drugs.[1] Selective activation of prodrugs within a tumor is particularly attractive because of their low damage to normal tissue. In this presentation, I will introduce the design, photoactivation mechanism, and antitumor activity of a visible light-activatable Pt(IV) prodrug.[2] This small-molecule prodrug has controllable activation property: it is shown to be inert in the dark but under short-period irradiation with low intensity of visible light, without the need of any external catalyst, the prodrug is rapidly reduced. The prodrug displays superior antitumor activity both in vitro and in vivo in human carcinoma models. The controllable activation property and superior antitumor activity of this prodrug may suggest a novel strategy for the design of visible light-activatable platinum prodrugs to reduce the adverse effects and conquer drug resistance of traditional platinum chemotherapy.



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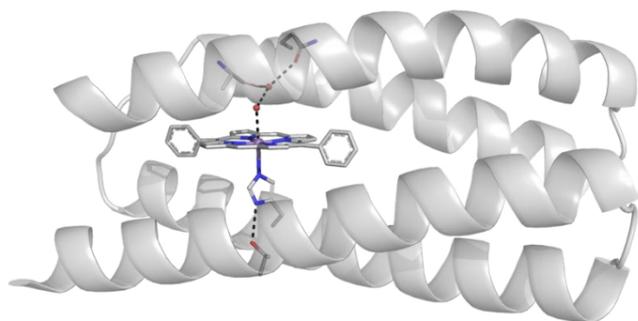
## OP-15

### Toward the De Novo Design of Functional Metalloporphyrin-Containing Proteins

Samuel I. Mann<sup>1</sup>, Yibing Wu<sup>1</sup>, Nicholas F. Polizzi<sup>1</sup>, and William F. DeGrado<sup>1</sup>

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Metalloproteins perform chemical transformations with rates and selectivities that have yet to be achieved in synthetic or designed systems. These differences in reactivity are directly linked to the environment produced by the protein matrix that cannot be easily reproduced in synthetic constructs. To test our understanding of how metalloproteins function, we aim to design *de novo* metalloproteins from scratch. Proteins that bind metalloporphyrins are of particular interest, as heme proteins are known to perform a variety of reactions. Only recently have we been able to design proteins that bind a cofactor with sub-Å accuracy.<sup>1</sup> This opens the door to expand on the utility of natural proteins by incorporating synthetic inorganic cofactors into proteins that lack pre-evolved function. This research seeks to elucidate the design features necessary to bind M-diphenylporphyrin (M-DPP; M= Fe, Mn) complexes in close proximity to a substrate binding pocket. I have successfully designed a four-helix bundle that binds M-DPP complexes with nanomolar affinity and is one of the few *de novo* cofactor-binding proteins that is well structured. Structural characterization with XRD showed that the structure matches the design with 1 Å RMSD. First- and second-shell interactions will be engineered to control orientation, electronic structure, and reaction pathway of the cofactor and substrate. The selectivity of these metalloenzymes will also be tested and the protein scaffold redesigned to elicit regioselectivity. Financial support by the NIH-GMS is gratefully acknowledged.



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## OP-16

### Structural and Mechanistic Investigation on Iron-Sulfur Cluster-Bound Transcription Factors in *Mycobacterium Tuberculosis*

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The abundance and versatility of iron-sulfur (Fe-S) cluster-bound transcription factors in all forms of life. This vividly contrasts with the scarcity of information on their structural and molecular mechanism, as exemplified by the White B-like (Wbl) proteins found in the notoriously persistent bacterial pathogen *Mycobacterium tuberculosis* (*Mtb*). *Mtb* Wbl proteins are a group of [4Fe-4S] cluster-bound monomeric transcription factors, and they play versatile roles in the redox stress response and persistence of the pathogen. Previous studies indicate *Mtb* Wbl proteins regulate gene expression by interacting with the sigma-70 primary sigma factor SigA in the RNA polymerase holoenzyme of *Mtb*. However, the underlying mechanism of action for Wbl proteins remain conjecture due to lack of atomic structural information of how a Wbl protein binds to SigA. To fill this critical knowledge gap, we have structurally characterized the interaction between a Wbl protein and region 4 of SigA (Wan, *et. al.*, unpublished). Our atomic resolution structure revealed unusual characteristics of the interaction between the two proteins. Combining our biochemical and site-directed mutagenesis studies, the results from the structure-function analysis support that the Wbl protein regulates gene expression by a noncanonical mechanism relative to other transcription activators bound to region 4 of the primary sigma factor.

### OP-17

#### Deciphering Molecular Mechanism of Silver by Integrated Omic Approaches Enables Enhancing its Antimicrobial Efficacy in *E. Coli*

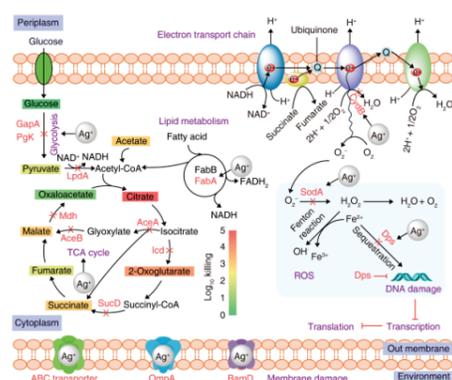
Haibo Wang<sup>1</sup>, Hongyan Li<sup>1</sup> and Hongzhe Sun<sup>1</sup>

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Despite the broad-spectrum antimicrobial activities of silver, its internal usage is restricted owing to the toxicity. Strategies to enhance its efficacy are highly desirable but rely heavily on the understanding of its molecular mechanism of action [1]. Silver has long been deemed to bind thiols in enzymes and thereby inactivates their functions, however, up to now, no direct silver-targeting proteins have been mined at a proteome-wide scale owing to the lack of appropriate techniques, which hinders systemic studies on the biological pathways interrupted by silver [2]. Herein, we build up a unique system, namely LC-GE-ICP-MS, and mapped out Ag<sup>+</sup>-binding proteins (Ag<sup>+</sup>-proteome) in *E. coli* for the first time. By using integrated omic approaches including metalloproteomics, metabolomics, bioinformatics and systemic biology, we delineated the first dynamic antimicrobial actions of Ag<sup>+</sup>, i.e., it primarily damages multiple enzymes in glycolysis and TCA cycle, leading to the stalling of the oxidative branch of the TCA cycle and an adaptive metabolic divergence to the reductive glyoxylate pathway. It then further damages the adaptive glyoxylate pathway and suppresses the cellular oxidative stress responses, causing systemic damages and death of the bacterium [3]. The silver-targeting residues in the identified proteins were further illustrated by crystal structures of silver-bound GAPDH and MDH at the atomic level [4].

Based on the molecular mechanism derived from our investigation, the therapeutic effect of Ag<sup>+</sup> could be enhanced by supplementation of metabolites from TCA cycle and glycolysis, thereby reducing its dosage. Our mouse model of *E. coli* infection successfully demonstrates a proof-of-principle that fundamental knowledge and molecular understanding of silver stress can be translated into new anti-bacterial approaches in clinic. Our study resolves long-standing questions on how Ag<sup>+</sup> exerts its antimicrobial activity at the molecular level and may also open up new horizons for in-depth exploration of silver (and nanosilver) toxicology in other bacteria. The integrated omic approaches approach we describe here can be widely applied to explore the molecular targets and mechanisms of action of other metal-based antimicrobial and anticancer drugs, and thereby facilitating the development of new therapeutics [5].

We gratefully acknowledge the University of Hong Kong for financial support.



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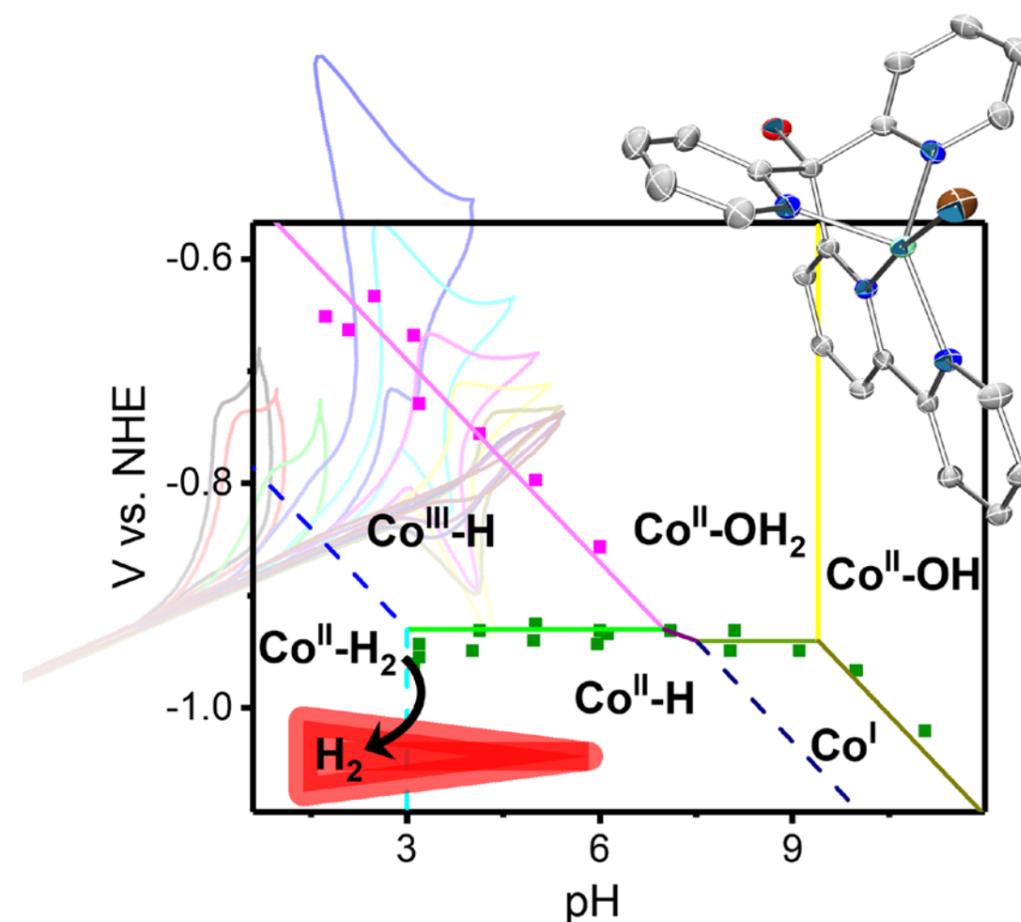
### OP-18

#### Understanding Hydrogen Evolution by Cobalt Polypyridyl Catalysts in Water: Pourbaix Diagram for Catalysis by Co<sup>II</sup>(tpy)

Stephan Schnidrig, Cyril Bachmann, Peter Müller<sup>1</sup>, Miguel Guttentag, Alexander Rodenberg, Roger Alberto<sup>1</sup>, Benjamin Probst<sup>1</sup>

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A detailed mechanistic picture is obtained by combining electrochemistry, spectroscopy, and photocatalysis for a cobalt complex of the tetradentate methanol-bridged bispyridyl–bipyridyl ligand [Co<sup>II</sup>Br(tpy)]Br.<sup>[1-2]</sup> In the acidic branch, a proton-coupled electron transfer, assigned to formation of Co<sup>III</sup>-H, is found upon reduction of Co<sup>II</sup>, in line with a pK<sub>a</sub>(Co<sup>III</sup>-H) of approximately 7.25. Subsequent reduction (-0.94 V vs. NHE) and protonation close the catalytic cycle. An upper limit for the second relevant protonation, eg the pK<sub>a</sub> of Co<sup>II</sup>-H, is found at a pH of 3. Methoxy substitution on the bipyridyl scaffold results in the expected cathodic shift of the reduction, but fails to change the pK<sub>a</sub>(Co<sup>III</sup>-H). A mechanistic proposal is formulated based on the Pourbaix diagram along with an outlook for design criteria for new generations of catalysts.



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### OP-19

#### Comparison in the Kinetics of Vasodilation of Sodium Nitroprusside and Ruthenium Nitrosyl Complexes

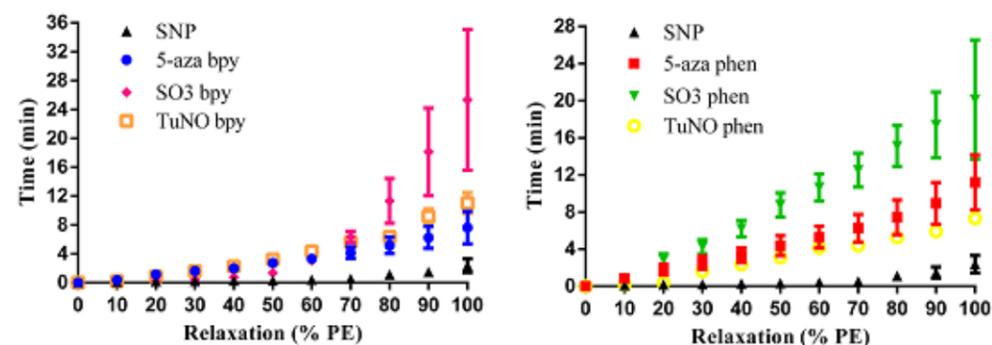
Iury A. Paz<sup>1,2</sup>, Carlos Daniel S. Silva<sup>3</sup>, Eduardo H. Sousa<sup>1</sup>, Nilberto R. F. Nascimento<sup>2</sup>, Luiz G. F. Lopes<sup>1</sup>

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Cardiovascular diseases are one of the leading causes of death in the world, often caused by endothelial dysfunction. In hypertensive crises, sodium nitroprusside (SNP),  $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]$ , is frequently used, which causes undesirable side effects. One of them is the release of cyanide in the body, and also a strong hypotension caused by fast vasodilation kinetics due to NO release. Therefore, ruthenium compounds containing a nitrosyl group and molecular formula  $\text{cis-}[\text{Ru}(\text{NO})(\text{L}_1)(\text{L}_2)_2]^{n+}$ , where  $\text{L}_1 = \text{sulphite} (\text{SO}_3^{2-})$ , 5-azaindole (5-aza) or thiourea (Tu) and  $\text{L}_2 = \text{bipyridine} (\text{bpy})$  or phenanthroline (phen), were synthesized as an alternative to SNP. In order to compare the kinetic effect of vasodilatation of ruthenium and nitroprusside complexes, vasodilation tests were performed using aortic rings of wistar rats. Nitroprusside showed extremely rapid vasodilation kinetics to relax 100% of the aorta ring (198 s), while the bipyridine ruthenium complexes containing  $\text{L} = 5\text{-aza}$ , Tu and  $\text{SO}_3^{2-}$  ligands have a time of 450 s; 670 s and 1535 s, respectively. For the phenanthroline ruthenium complexes containing  $\text{L} = 5\text{-aza}$ , Tu and  $\text{SO}_3^{2-}$  ligands the average time to reach the maximum relaxation effect was 665 s; 451 s and 1213 s, respectively. The ruthenium complexes with sulphite showed a kinetic of vasodilatation approximately 10-fold slower than sodium nitroprusside, while the other complexes were 3- to 5-fold slower as well. These results support ruthenium nitrosyl complexes promising dynamics for promoting more controlled vasodilation, while not having any issue on releasing cyanide.

Financial support by the CAPES/CNPq/FUNCAP/UFC



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### OP-20

#### Multi-Functional Nanoplatfoms for Photo-Controlled Targeted Nitric Oxide Delivery

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Nitric oxide (NO) not only plays critical roles in various physiological processes but also has been implicated in cancer biology. The potential therapeutic applications of NO in regulation of vascular tone, anticancer, antibacterial, anti-inflammatory, and wound healing has ignited explosive research interest in NO donor compounds and their relating materials that is capable of releasing NO.<sup>[1-2]</sup> Given the biological functions of this free radical gas are related to its timing, location, and dosage, it is highly imperative to develop nanoplatfoms that enables exogenous delivery of NO spatiotemporally to a targeted site for realizing NO-mediated therapy.

Targeted delivery of a photoactivable prodrug using light is a promising approach for minimizing the side effect of chemotherapy.<sup>[3]</sup> Ruthenium nitrosyls are photolabile NO donors from which NO releasing can be manipulated by light. In this talk, we will present our efforts to develop photo-controlled NO delivery using ruthenium nitrosyls grafted nanoplatfoms, focusing on how to sensitize the ruthenium nitrosyls with visible and near infrared (NIR) light for targeted cellular NO delivery in combination with photodynamic therapy, photothermal therapy and chemotherapy for anticancer treatment.<sup>[4-10]</sup>

Financial support by the National Nature Science Foundation of China (no. 21571062, 21271072) and the program for professor of special appointment (Eastern Scholar) at shanghai institutions of higher learning is gratefully acknowledged.

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### OP-21

#### The Molecular Mechanism of Gallium Uptake by *Pseudomonas Aeruginosa*

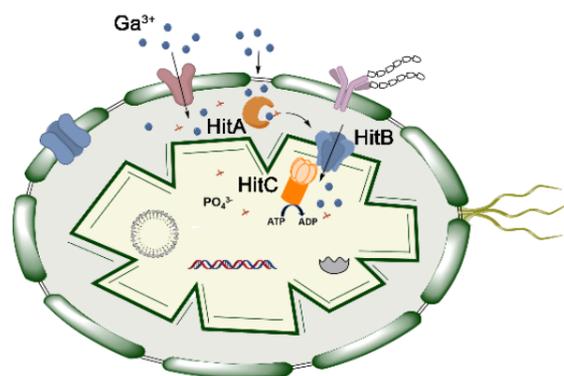
Yu Guo<sup>1</sup>, Hongyan Li<sup>2</sup>, Hongzhe Sun<sup>2</sup> and Wei Xia<sup>1</sup>

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*Pseudomonas aeruginosa* (*P. aeruginosa*) is an important opportunistic human pathogen [1]. The infections caused by *P. aeruginosa* are usually resistant to treatment of multiple antibiotics due to the bacterial intrinsic drug resistance and formation of biofilm [2]. Recent studies demonstrated that Ga(NO<sub>3</sub>)<sub>3</sub> exhibited excellent bactericidal activity against *P. aeruginosa* by interfering bacterial iron metabolism [3]. Although it is indicated that *P. aeruginosa* utilized the same transport pathway to uptake iron and gallium, the exact mechanism of gallium uptake remains unknown. Herein, we utilized CRISPR-Cas9 to inactivate 5 identified iron uptake pathways in *P. aeruginosa* individually. And we demonstrate that a ferric transporter (HitABC) is the major pathway for gallium uptake in *P. aeruginosa*. In contrast, the two siderophore-mediated (pyochelin and pyoverdine) iron uptake pathways are not involved in gallium uptake. *In vitro* biochemical data confirmed that the periplasmic iron binding protein, HitA could bind gallium tightly. The structure of metal-bound HitA further revealed that gallium and ferric ion shared the same metal-binding site of HitA.

Financial supports by the the National Natural Science Foundation of China, Science and Technology Program of Guangzhou, RGC of Hong Kong, the Ministry of Education of China, the Fundamental Research Funds for the Central Universities are gratefully acknowledged.



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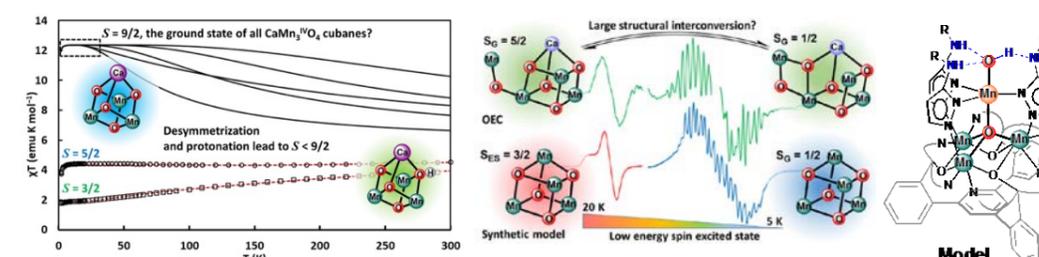
### OP-22

#### Synthetic Cluster Models of the Biological Oxygen Evolving Complex: Insights Regarding Function and Spectroscopy

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Complex inorganic active sites perform challenging catalytic transformations in biological systems, such as water oxidation by Photosystem II and nitrogen reduction in Nitrogenase. The effect of cluster structure on the physical and chemical properties of these active sites is not well understood. We have developed methodologies for the rational synthesis of tetra- and pentanuclear homo- and hetero-metallic cluster models of the Oxygen Evolving Complex in Photosystem II, which allow for systematic structure-property studies. Clusters displaying the cubane motif found in the OEC have been prepared, and the redox inactive metal was found to impact redox chemistry. Tetranuclear clusters that structurally model the dangler motif have also been synthesized, with open coordination sites for water coordination. Distal redox changes have been demonstrated to have a substantial effect on the reactivity of aquo, hydroxide, and oxo ligands relevant to water oxidation. Upon incorporation of second coordination sphere hydrogen bonding interactions, water oxidation catalysis was observed. Spectroscopic studies of models with structures or redox states relevant to the OEC provide benchmarking for the biological system. Implications for the function and spectroscopy of the OEC will be discussed. Financial support by the NIH is gratefully acknowledged.



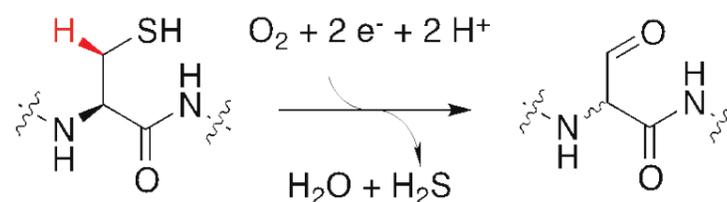
## OP-23

### Structure of Formylglycine-Generating Enzyme in Complex with Copper and Substrate Reveals an Acidic Pocket for Binding and Activation of Molecular Oxygen

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Copper is a versatile catalyst for oxygen-dependent reactions. Combined with appropriate ligands, copper can cycle between the oxidation states I, II and III to activate molecular oxygen (O<sub>2</sub>) and to form reactive oxygen species that can initiate very difficult reactions.<sup>[1]</sup> Mononuclear copper enzymes usually bind copper in histidine-dominated tetrahedral or square planar coordination spheres. The formylglycine generating enzyme (FGE) is unique in this regard, because it binds a single Cu (I) by linear bis-cysteine coordination.<sup>[2]</sup> FGE catalyzes the oxidative conversion of specific peptidyl-cysteines to formylglycine via abstraction of the pro-(R)-β-hydrogen atom. The crystal structure of FGE from *Thermomonospora curvata* (*tcFGE*) in complex with Ag (I) (*tcFGE\_Ag*, PDB: 5NXL), and most recently, the crystal structure of FGE from *Streptomyces coelicolor* in complex with Cu (I) (*scFGE\_Cu*, PDB: 6MUJ), combined with biochemical characterization showed that two active site cysteines are the only metal ligands and that the Cu (I) bound state is the catalytic resting state.<sup>[3]</sup> In this presentation we describe the crystal structure of *tcFGE* in complex with Cu (I) and a 17-residue substrate analog. Based on this structure with atomic resolution (1.04 Å) together with NMR spectroscopy and kinetic characterization, we identify an acidic O<sub>2</sub>-binding pocket juxtaposed to the copper center as key determinant for efficient O<sub>2</sub>-activation.



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## OP-24

### Structural Snapshots of Manganese Uptake in *Streptococcus Pneumoniae*

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Bacterial infection involves a constant tug-of-war between host and pathogen for the essential nutrients of life. The acquisition of the first-row transition metal ions (for example, iron, zinc, and manganese) from the host is crucial for the survival and propagation of pathogenic bacteria. The primary transporters used by bacteria to scavenge these essential trace metals are the ATP-binding cassette (ABC) permeases. Accordingly, any loss or disruption to the function of these transporters severely impairs bacterial virulence. Despite this, how ABC permeases recognise and acquire their cognate metal ion cargo remains poorly understood. Given the essential role of ABC permeases in bacterial virulence and their absence from eukaryotic genomes, knowledge of the molecular details of these transporters will provide novel opportunities for antimicrobial exploitation. *Streptococcus pneumoniae* is a Gram-positive human bacterial pathogen that is responsible for more than 1 million deaths every year. The *S. pneumoniae* ABC permease, PsaBCA, is a manganese-specific uptake transporter that is essential for growth and *in vivo* virulence. This presentation will describe our work in understanding how transition metals are bound by the metal-recruiting protein PsaA and delivered to the PsaBC transporter, in addition to our recent determination of the high resolution crystal structure of the entire PsaBC transporter.

### OP-25

#### Photoinduced Electron Transfer in Lanthanide Complexes

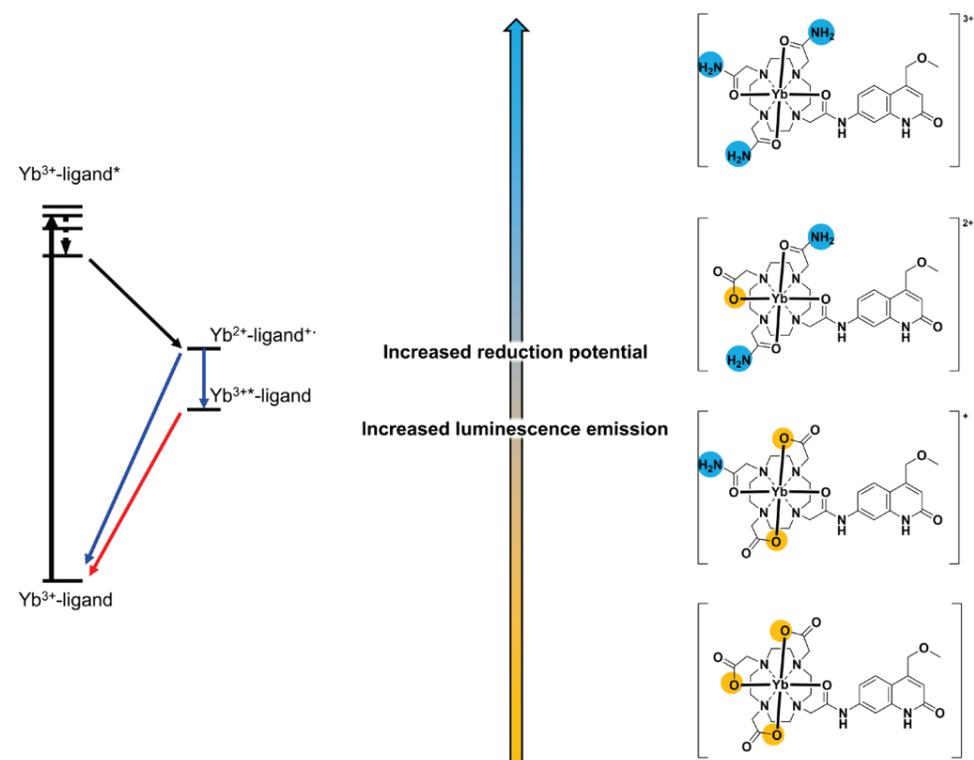
Emilie Mathieu<sup>1</sup>, Daniel Kovacs<sup>1</sup>, K. Eszter Borbas<sup>1</sup>

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Photoinduced electron transfer (PeT) has been observed in lanthanide complexes upon excitation of the light-harvesting antenna of the ligand [1-3]. This process is dependent on the reductive power of the antenna in its excited state, and on the reduction potential of the lanthanide(III) ion. PeT leads temporarily to the formation of  $\text{Ant}^+-\text{Ln(II)}$  species. For most lanthanide complexes PeT results in the quenching of luminescence. However, for Yb-complexes photo-induced electron transfer, followed by a back electron transfer can yield an excited state Yb(III) species, and thus sensitize luminescence emission [4].

In this work, a series of Yb(III)-complexes based on a cyclen scaffold were designed and synthesized. The pendant arms were substituted with either carboxylate or amide groups in order to modulate the reduction potential of the metal centre. The redox properties of the complexes, as well as their luminescent properties were investigated. Changes of the ligand-based and metal-based emissions were observed, that correlated with the tuned reduction potential of Yb(II)/Yb(III). Control of the reduction potential of the metal centre could thus modulate Yb emission. This strategy represents an alternative to altering the light-harvesting antenna.

Financial support by the Carl Tryggers Stiftelse is gratefully acknowledged.



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### OP-26

#### Interaction of Dioxygen with Copper and Iron Complexes with a New Tripodal Imine Ligand

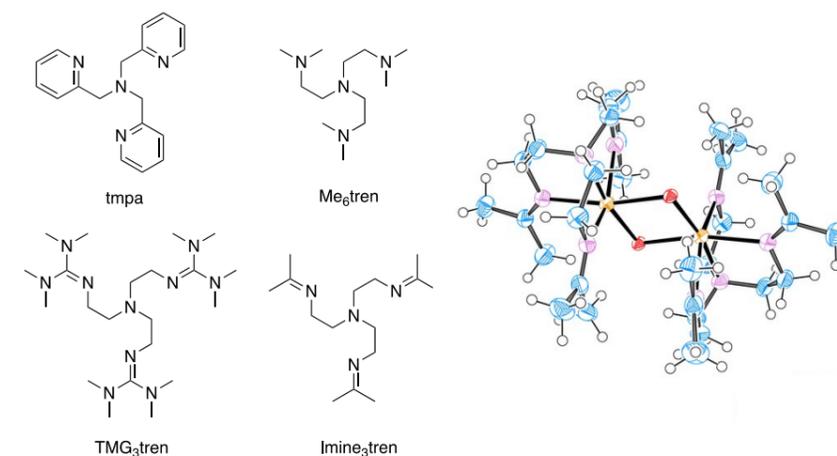
Siegfried Schindler<sup>1</sup>, Lars Schneider<sup>1</sup>, Janine Will<sup>1</sup>

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Tripodal ligands (e.g. tmpa or  $\text{Me}_6\text{tren}$ , see Figure) have been used previously to successfully study interaction of dioxygen with iron and copper complexes allowing full characterization of "dioxygen adduct" complexes (see for example references [1, 2]). By applying one of these ligands, a tren guanidine derivative ( $\text{TMG}_3\text{tren}$ , see Figure), we have been able to structurally characterize the first and up to now only *end-on* superoxido copper complex that can be regarded as a suitable model compound for the enzyme peptidylglycine alpha-hydroxylating monooxygenase (PHM). [3, 4]

Modification of the tripodal ligand system allows "tuning" of the reactivity of the corresponding copper or iron complexes towards dioxygen and their potential for oxidation/oxygen transfer (see for example reference [5]). A missing link in the series of amine, pyridine or guanidine based tripodal ligands is an imine tren system derived from tren and acetone,  $\text{Imine}_3\text{tren}$  (see Figure). This ligand has been difficult to synthesize for different reasons however, we recently achieved the synthesis of its corresponding copper and iron complexes. Our results on the investigation of the reactivity of these complexes towards dioxygen will be reported. Currently we already could characterize a binuclear oxido-bridged iron(III) complex (see Figure), a structural unit that is quite rare and we are only aware of two other examples reported so far. [6, 7]

Financial support by the Justus-Liebig University of Gießen is gratefully acknowledged.



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## OP-27

### Nucleus-Targeting Ru(II) Complexes as Topoisomerase I/II Dual Inhibitors

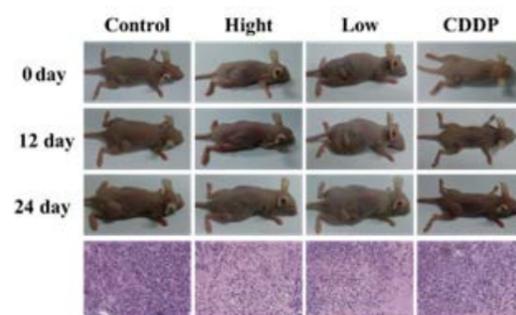
Yu Chen<sup>1</sup>, Liting He<sup>1</sup>, Kai Xiong<sup>1</sup>, Liangnian Ji<sup>1</sup>, Hui Chao<sup>1</sup>

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As the crucial nuclear enzymes that control the topological state of DNA, topoisomerase I (Topo I) and topoisomerase (Topo II) are the promising drug target in oncology.<sup>[1]</sup> However, several negative consequences were observed when using single inhibitor or even simultaneous treatment.<sup>[2]</sup> Therefore, Topo I/II dual inhibitors have attracted increasing interest. On the other hand, poison and catalytic inhibitor are two type of topoisomerase inhibitor. Poison refers to inhibitors that selectively affect the religation of DNA and form stable enzyme-DNA-inhibitor ternary complexes. In contrast, catalytic inhibitor is the term used for those agents that prevent the binding of topo enzymes and DNA, which is resulted from inhibitor-DNA interaction and/or inhibitor-enzyme interaction. It should be noticed that, the majority of topo inhibitors, including clinical used drugs camptothecin and etoposide, are designated topo poisons. Reports on topo I and topo II dual catalysis inhibitors are rare. This may be due to the structural differences between the two enzymes, resulting in design difficulties. To the best of our knowledge, none of catalytic inhibitor have been proved for clinical treatment.

Our previous reports indicated that some Ru(II) complexes, such as [Ru(bpy)<sub>2</sub>(pscl)]<sup>2+</sup> and [Ru(bpy)<sub>2</sub>(psbr)]<sup>2+</sup>, could efficiently inhibit both of Topo I and Topo II.<sup>[3-4]</sup> However, they are all designated topo poisons and their nuclear accumulation remains to be confirmed. Herein, Ru(II) complexes (**1-7**) were developed. In vitro study indicated that **1-7** are efficient Topo I/II dual inhibitors with similar activity (IC<sub>50</sub> < 1 μM), which is resulted from DNA-intercalating and Topo I/II protein binding. However, their cytotoxicity is inconsistent with the inhibit activity. Due to their poor membrane penetration, the IC<sub>50</sub> value of complex **1-5** for cisplatin resistance cancer cell A549/DDP are more than 150 μM. The introduction of auxiliary ligand dip can improve cellular uptake and cytotoxicity (IC<sub>50</sub> ≈ 80 μM) but fail to localize nuclear. The nuclear targeting was successfully achieved by cyclometallation. The IC<sub>50</sub> value for cyclometalated complex **7** is about 2 μM and its ability as anticancer agent is further confirmed by in vivo experiment.

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## OP-28

### Impact of Cu(I) on Structural Metal-Binding Sites in Zinc Metalloproteins and Peptides

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Proper metalloprotein structure is maintained, in part, by the coordination geometry of the endogenous metal, and substitution of the native metal with one that displays different coordination preferences is likely to alter protein structure and function. While metal-ion substitution has clear ramifications in the context of metal-ion toxicity, changes in protein function upon metal-ion substitution may also constitute a functional strategy. Cu(I) exhibits high affinity for thiolate ligands, suggesting that thiol-rich metalloprotein binding sites may be subject to disruption under elevated copper concentrations. In this context, we report spectroscopic studies that characterize copper(I) binding to several zinc-binding peptides and proteins, including both classical and non-classical zinc finger domains and multiple domains of the X-linked Inhibitor of Apoptosis Protein (XIAP). We demonstrate that both synthetic zinc finger peptides and recombinantly expressed zinc metalloproteins are susceptible to Cu(I) substitution at the zinc binding sites, and that copper substitution results in both structural and functional changes. The results of this work suggest that Cu(I)-substituted zinc finger domains might be relevant in the context of both copper toxicity mechanisms and copper-responsive transcription factors.

Financial support by Macalester College and the National Science Foundation (CHE# 1807773) is gratefully acknowledged.

### OP-29

#### On the Propagation of the OH Radical Produced by Cu(II) A $\beta$ (1-16). The Intramolecular A $\beta$ Oxidation Processes from the Molecular Modelling Point of View.

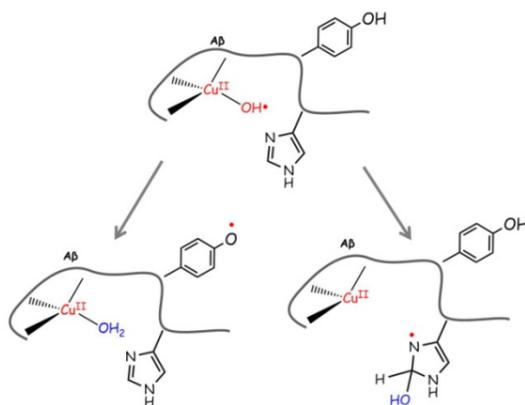
Luca Bertini<sup>1</sup>, Federica Arrigoni<sup>1</sup>, Luca De Gioia<sup>1</sup>, Fabio Rizzi<sup>1</sup>, Renata Tisi<sup>1</sup>, Jacopo Vertemara<sup>1</sup>, Giuseppe Zampella<sup>1</sup>.

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The Oxidative stress appears to be one of the major determinants of the pathogenesis and progression of Alzheimer's disease (AD). According to the oxidative stress hypothesis in AD, the interactions of redox active Cu(II) ions with A $\beta$  peptide is linked to production of toxic reactive oxygen species (ROS).

While the Cu(II)A $\beta$  ascorbate-assisted redox chemistry toward OH radical production is well characterized, much less is known about the following OH propagation processes. There is a number of possible/plausible intramolecular A $\beta$  oxidative processes [1] in which the OH radical propagates oxidizing the side chain residues or the peptide bonds. Among them, we focus on the tyrosine oxidation [1,4] that favours the Tyr-Tyr crosslinks, and the histidine oxidation[1]. We adopted two levels of theory. First by simulating the Cu(II)-A $\beta$ -OH product that comes from the O<sub>2</sub> ascorbate-assisted reduction cycle to successively investigate the thermodynamics of the OH propagation.

We investigate at Density functional theory level (DFT) [2,3] the OH propagation exploring the intramolecular electron transfer of the three most plausible redox competent Cu(II)-A $\beta$ -OH coordinations. Then we consider a number of full peptide Cu(II)-A $\beta$ (1-16)-OH structures to unveil in better detail the energetic and peptide dynamic of the OH propagation. For this latter, long MD simulations of the Cu(II)-A $\beta$ (1-16)-OH have been carried out suggesting that OH propagation is Cu(II) coordination dependent.



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### OP-30

#### Mn/Fe Ribonucleotide Reductase Pictured by Femtosecond Crystallography

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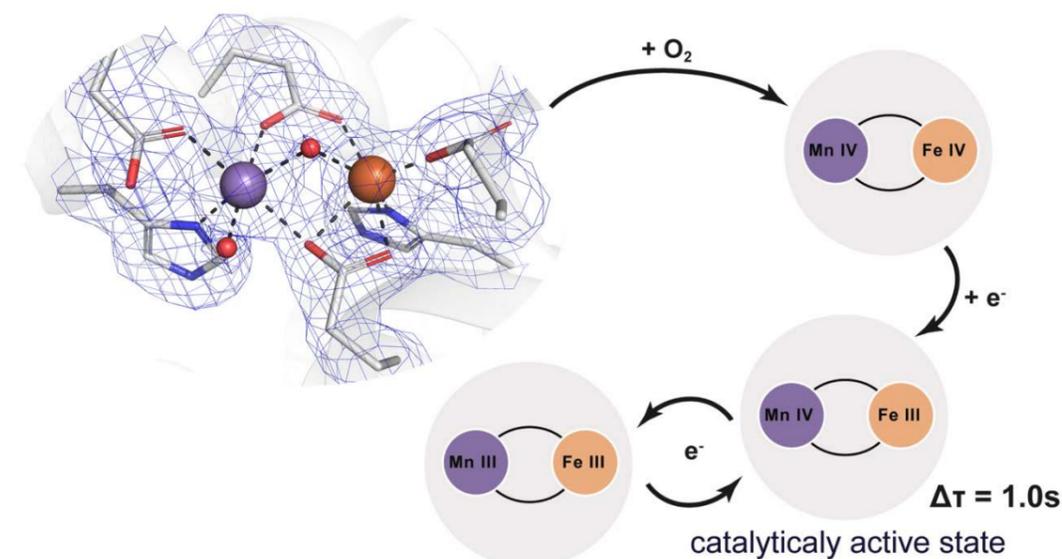
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All domains of life require the essential enzyme ribonucleotide reductase (RNR), which represents the only pathway for *de novo* synthesis of deoxyribonucleotides from ribonucleotides. Class I RNR depends on a ferritin-like R2 subunit to produce a catalytic radical, subsequently shuttled via proton-coupled electron transfer to the R1 subunit performing the ribonucleotide reduction.

Except in the recently discovered metal-free subclass Ie [1], R2 utilises a dinuclear metal centre to generate the radical upon O<sub>2</sub> activation, via the formation of high-valent intermediates. Distinct subclasses of R2 proteins specifically rely on different dinuclear Mn and/or Fe cofactors. In subclass Ic R2 (R2c), a Mn<sup>II</sup>/Fe<sup>II</sup> heterodinuclear centre initially provides all four electrons required for complete O<sub>2</sub> reduction. It results in a Mn<sup>IV</sup>/Fe<sup>IV</sup> intermediate which undergoes a one-electron reduction to form the Mn<sup>IV</sup>/Fe<sup>III</sup> active state of the protein. Although still debated, the inorganic cores of R2c directly following O<sub>2</sub> exposure are to date believed to be Mn<sup>IV</sup>( $\mu$ -O)<sub>2</sub>Fe<sup>IV</sup> and Mn<sup>IV</sup>( $\mu$ -O)( $\mu$ -OH)Fe<sup>III</sup>.

Here, we present our attempts to structurally characterise high-valent states of the R2c Mn/Fe cofactor using serial femtosecond X-ray crystallography and simultaneous X-ray emission spectroscopy (XES) at room temperature at X-ray free-electron laser (XFEL) sources. Using a drop-on-demand sample delivery protocol [2], we recently obtained high-resolution R2c XFEL crystal structures coupled with Mn and Fe XES data after different times of O<sub>2</sub>-exposure. We will discuss how these new structural insights compare with the existing data from the literature.

Financial support was provided by the Swedish Research Council, the Knut and Alice Wallenberg Foundation and the European Research Council.



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### OP-31

#### Unusual Second Coordination Sphere in the Copper Active Site of some Bacterial Lytic Polysaccharide Monooxygenases

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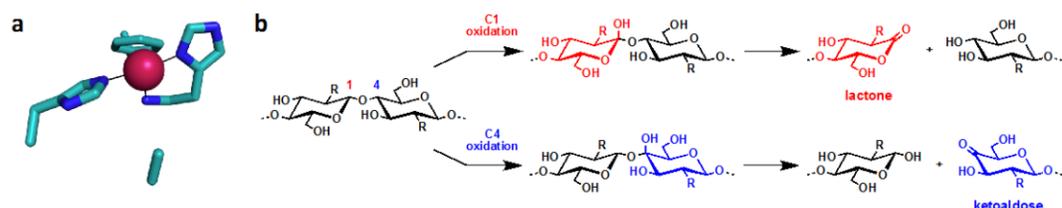
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Fungal and bacterial lytic polysaccharide monooxygenases (LPMOs) are a recently discovered class of secreted mononuclear copper monooxygenases. LPMOs participate in the degradation of recalcitrant polysaccharides such as cellulose or chitin in synergy with hydrolytic carbohydrate-active enzymes [1]. The topology of their solvent-exposed active site is unique among copper-containing oxygenases. The mononuclear copper(II) ion is coordinated by both the side chain nitrogen and the main-chain amino group of the *N*-terminal histidine, a motif known as "histidine brace", and by another histidine side chain (Figure a, copper active site of a bacterial chitin-active LPMO) [1]. From a molecular point of view, LPMOs catalyze the oxidative cleavage of glycosidic bonds using dioxygen or hydrogen peroxide as co-substrate and an electron donor (Figure b, reactions catalyzed by LPMOs leading to lactone or ketoaldose products that are in equilibrium with the corresponding *gem*-diol or aldonic acid (not shown), respectively, in solution. Cellulose (R = -OH)-active LPMOs oxidize their substrate in position C1 and / or C4, whereas C1 oxidation is only observed for chitin (R = -NHAc)-active LPMOs [1,2].

In bacterial LPMOs, a conserved second coordination sphere alanine residue (Figure a) was proposed to control the access of the co-substrate (dioxygen or hydrogen peroxide) to the axial coordination site on the copper ion [3]. Sequence analysis of bacterial LPMOs classified in the CAZy database (www.cazy.org) revealed some variability at this position. We are currently investigating the structure-function relationship of these LPMOs harbouring another residue in place of the alanine. Current efforts will be reported to investigate the potential role of this active site residue. In particular, we characterized a new chitin-active LPMO from the entomopathogenic bacterium *Photorhabdus luminescens* (with an isoleucine in place of the conserved alanine) [4] using a combination of biochemical, biophysical, and structural techniques.

Financial support by the CNRS and Aix Marseille Universit   is gratefully acknowledged.



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### OP-32

#### Bio-Inspired Complexes Using an Endo-Functionalized Organic Cage as Ligand

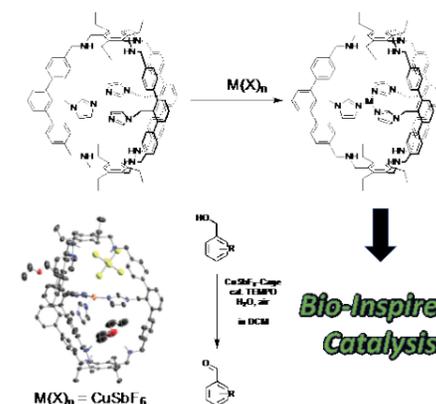
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Mimicking the reactivity and selectivity of many histidine coordinated non-heme enzymatic active sites is a challenge for synthetic catalysts. Approaches to address this issue have mostly been devoted towards the first coordination sphere, resulting in coordination complexes of rather low molecular weight. In comparison, the design of cavity-based metal complexes has received way less attention.

Among others, we aim to overcome these challenges via the development of endo-functionalized cavities. Previous examples reported by us are organic macrocycles and cages that show cooperativity between their functional groups [1,2]. Recently, we developed an imidazole-functionalized cage (see figure), which can act as a ligand for Cu(I) [3]. This results in a T-shaped coordination of copper by the cage ligand reminiscent of the coordination geometry found in copper based enzymatic active sites. This copper complex is able to oxidize benzylic alcohols to aldehydes in the presence of water, employing oxygen as the terminal oxidant.

In this talk, the mentioned systems will be discussed. Furthermore, the use of the imidazole-functionalized cage as ligand to other metals and investigation of their reactivity will be shown. Financial support by the Deutsche Forschungsgemeinschaft (DFG) is gratefully acknowledged.



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### OP-33

#### Exploring the Coordination Environment of Phosphorescent Metal Complexes for Potent Multifunctional Anticancer Agents

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As anticancer agents, metal complexes have many characteristics compared with small organic molecules. For example, metal complexes have outstanding optical, electrical and magnetic properties, which can be used to prepare multifunctional metal-based anticancer agents. The research of new metal anticancer drugs is of great significance not only for the development of novel anticancer drugs, but also for deepening our understanding of coordination chemistry in life system. These complexes may also be used as a new research tool in cell biology and molecular biology.

Based on these considerations, our work is concentrated in developing new non-traditional metal-based anticancer drugs in recent years. We mainly carry out research in the following two aspects: (1) The construction of molecular/subcellular organelle-targeted multifunctional phosphorescent metal-based anticancer complexes. Based on the coordination structure and optical properties of phosphorescent metal complexes, and the three-dimensional structure of targeted biomolecules, a series of molecular targeted antitumor metal complexes have been designed. The antitumor mechanism and optical properties of these complexes are organically combined to achieve satisfactory anticancer properties and multifunctionalities. Selective inhibition of the targets at the cellular level, and real-time induction and monitoring of the intracellular physiological processes have been simultaneously achieved by using the strategy. For example, by introducing the organelle-targeting or microenvironment-sensitive groups into the structures of phosphorescent metal complexes, we have realized real-time damaging and tracing of mitochondria/lysosomes simultaneously.<sup>[1-6]</sup> Moreover, we have designed multifunctional metal-based anticancer agents targeting histone deacetylase incorporating phosphorescent imaging, photodynamic therapy (PDT) and subcellular targeting properties.<sup>[7]</sup> (2) Exploration of the mechanism of programmed cell death induced by metal-based anticancer agents. By means of cell biology and molecular biology, we have investigated the mechanisms of programmed cell death induced by anticancer metal complexes in detail.<sup>[8-9]</sup>

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### OP-34

#### Discovering Gene Clusters Coding for Cephalochordata Heme Peroxidases

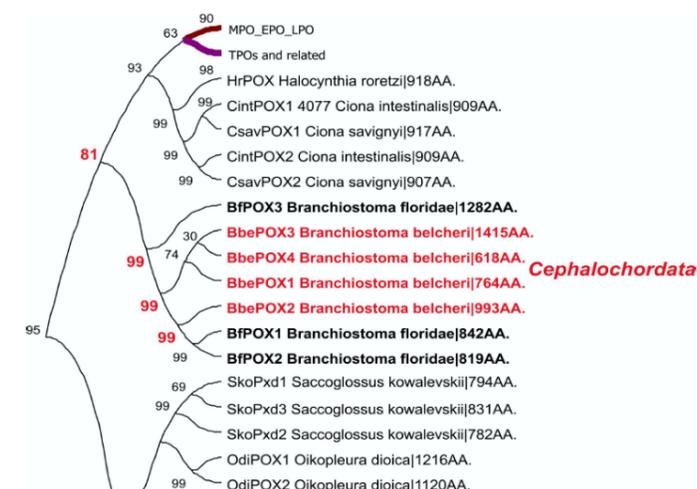
Marcel Zamocky<sup>1,3</sup>, Oleg Simakov<sup>1</sup>, Paul G. Furtmüller<sup>2</sup>, Christian Obinger<sup>2</sup>

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The peroxidase-cyclooxygenase superfamily represents one of four independently evolved heme peroxidase lineages [1]. It is annotated as IPR019791 in the specialized InterPro database containing numerous representatives from all domains of life. Among them the most intensively investigated are the animal heme peroxidases significantly contributing to innate immunity. It is expected that all members of this large superfamily possess a typical and unique posttranslational modification within a conserved structural fold that results in peculiar covalent bonds between the heme prosthetic group and highly conserved amino acids of the protein [2]. We have performed a detailed phylogenomic analysis of peroxidase gene complement and genome evolution across metazoans. For this purpose we analysed all available cephalochordate genomes and compared the regions with detected peroxidase genes with similar regions in other metazoan genomes, in particular urochordate and vertebrate genomes. We have identified two gene clusters in the genome of *Branchiostoma belcheri* containing co-localized genes coding for thyroid-peroxidase-like proteins and for peroxidasins. Discovered gene clusters revealed synteny with corresponding genomic regions in the tunicate *Ciona intestinalis*, cephalopod *Euprymna scolopes*, branchiopod *Daphnia pulex*, chilopod *Strigamia maritima*, and insects being represented by the genome of *Drosophila melanogaster*. We could follow the synteny pattern of one described cluster up to the human genome where it is located on chromosome #2. All discovered synteny regions contain closely evolutionary related peroxidase genes of the same superfamily IPR019791. Further, the phylogenetic analysis performed by us with Maximum Likelihood method on 586 complete protein sequences revealed a basal position for seven heme peroxidases from cephalochordate (Figure) thus representing an important turning point in the complex evolution between vertebrate thyroid peroxidases and peroxidasins. Selected synthetic genes of *Branchiostoma* peroxidases were constructed and heterologously expressed in various hosts. Structure-function relationships of affinity purified heme proteins are currently being studied. Financial support from the Austrian FWF project P 31707-B32 is gratefully acknowledged.



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OP-35

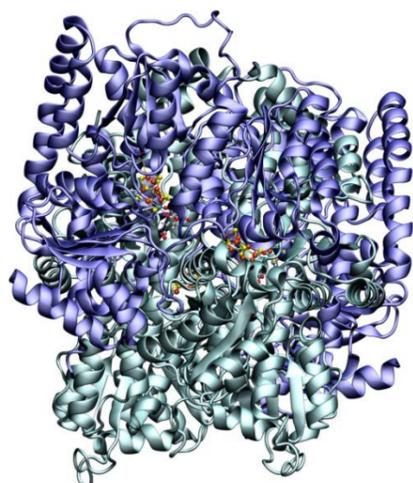
### Calculations of the Redox States of Mo Nitrogenase

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Nitrogenase is one of the most fascinating enzymes in Nature, being responsible for all biological nitrogen reduction. Despite decades of research the mechanism remains poorly understood. The MoFe protein of nitrogenase contains a metal-sulfur cluster, FeMoco, where N<sub>2</sub> reduction takes place. The resting state of FeMoco (E<sub>0</sub>) has been characterized by crystallography, multiple spectroscopic techniques (XAS, Mössbauer, EPR) and theory (BS-DFT) and its molecular structure can be considered complete while the complex electronic structure, involving 8-metal spin coupling, delocalized electrons and weak metal-metal bonding, is not completely understood yet[1-3]. Much less is known about the other redox states (E<sub>1</sub>-E<sub>8</sub>) where no crystal structures are available but hydrides have been proposed to be present in the early redox states.

We are studying the system via QM/MM models of FeMoco in order to properly incorporate protein environmental effects[4]. Broken-symmetry DFT calculations are used to describe the QM region using ORCA. Our calculated structures are in excellent agreement with the high-resolution crystal structure but also reveal a strong sensitivity with respect to electronic structure method. We will present new calculations that go beyond the resting state of the cofactor and will discuss our recent work on characterizing the E<sub>1</sub>-E<sub>4</sub> states of the cofactor and dinitrogen binding.



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OP-36

### Hemoprotein Reconstituted with Manganese Porphycene toward a Catalyst for Alkane Hydroxylation

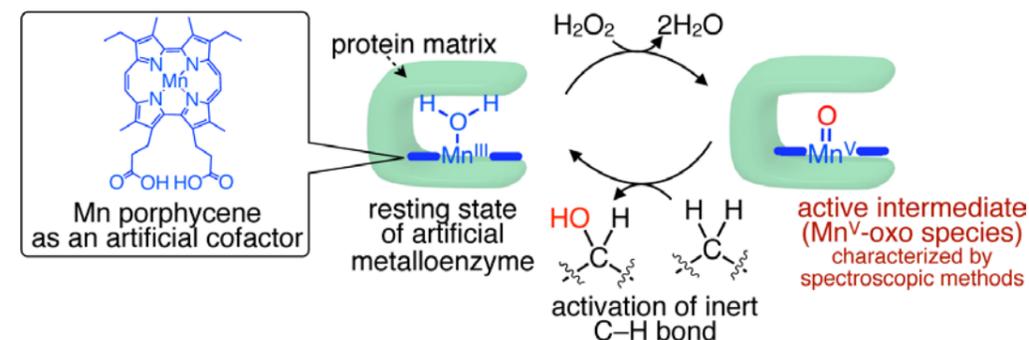
Koji Oohora<sup>1,2</sup>, Tomoya Tanaka<sup>1</sup>, Yoshiyuki Kagawa<sup>1</sup>, Natsuno Chiba<sup>1</sup>, Takashi Hayashi<sup>1</sup>

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For sustainable chemistry, protein is one of the promising materials due to the fine-tuned functions accompanied by the sophisticated structures. In this context, our group has demonstrated the chemical approach of modification of hemoproteins toward artificial metalloenzymes. A heme (porphyrin iron complex) cofactor in hemoprotein is replaced with designed artificial metal complex to modify the reactivities and physicochemical properties of the hemoproteins [1,2]. In this presentation, artificial metalloenzymes for C–H bond hydroxylation will be introduced as shown in Figure [2,3].

Myoglobin is a well-known hemoprotein for oxygen storage, whereas no catalytic activity toward C–H bond hydroxylation is reported in contrast to native hemoenzymes such as cytochrome P450. Recently, our group found that manganese porphycene, an artificial metal porphyrinoid, serves as an active site toward catalytic C–H bond hydroxylation in the reconstituted myoglobin (Figure). The reconstituted protein is capable of hydroxylation of ethylbenzene, toluene and cyclohexane using hydrogen peroxide as a terminal oxidant under mild conditions at pH 7.0 and 25°C. Interestingly, the manganese(V)-oxo species is spectroscopically detectable as an active species in this system and this species was characterized by EPR. Detailed mechanistic investigation suggests the catalytic hydroxylation by the rate-determining H atom abstraction and following OH-rebound, which is similar to the mechanism found in cytochrome P450. This is the first example for myoglobin-based artificial metalloenzyme catalyzing inert C–H bond hydroxylation of external substrate. Further optimization of the protein matrix by the mutations has achieved the significant enhancement of enantioselectivity of the product and turnover number of the hydroxylation reactions of the more inert alkane substrates.



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OP-37

## Manganese Reactive Oxygen Complexes in C-H bond Activation and Oxygen Atom Transfer Reaction

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High-valent manganese oxygen species have been widely considered as a key intermediate in various oxidative reactions of biological systems. Herein, we present the structure and reactivity of two high-valent manganese reactive oxygen complexes bearing a tetraaza macrocyclic ligand composed of two amine and two pyridine. These novel intermediates would be prominently valuable for expanding the chemistry of transition metal catalysts.

Naphthalene oxidation is one of the most difficult reactions in environmental and biological chemistry. We report that a Mn<sup>IV</sup>-bis(hydroxo) complex, [Mn<sup>IV</sup>(TBDAP)(OH)<sub>2</sub>]<sup>2+</sup>, which was fully characterized by various physicochemical methods such as UV-vis, ESI-MS, resonance Raman, EPR, X-ray and XAS, shows the naphthalene oxidation in the presence of acid to afford 1,4-naphthoquinone. Redox titration of the Mn<sup>IV</sup>-bis(hydroxo) complex exhibits one electron reduction potential of 1.09 V, which is the most positive potential for the previously reported nonheme Mn<sup>IV</sup>-bis(hydroxo) species as well as Mn<sup>IV</sup>-oxo analogues. Kinetic studies including kinetic isotope effect suggest that the naphthalene oxidation by the Mn<sup>IV</sup>-bis(hydroxo) complex in the acid-promoted reaction occurs via a rate-determining electron transfer process.

Transition metal-iodosylarene complexes have been proposed to be key intermediates in the catalytic cycles of metal catalysts with iodosylarene. We report the first X-ray crystal structure and spectroscopic characterization of a mononuclear nonheme manganese(III)-iodosylarene complex with a tetradentate macrocyclic ligand, [Mn<sup>III</sup>(TBDAP)(OIPh)(OH)]<sup>2+</sup>. The manganese(III)-iodosylarene complex is capable of conducting various oxidation reactions with organic substrates, such as C-H bond activation, sulfoxidation and epoxidation. Kinetic studies including isotope labeling experiments and Hammett correlation demonstrate the electrophilic character on the Mn-iodosylarene adduct.

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OP-38

## Synergistic Use of Computational, Spectroscopic, and Radiochemical Methods to Design the Next-Generation of Radiometal Chelators

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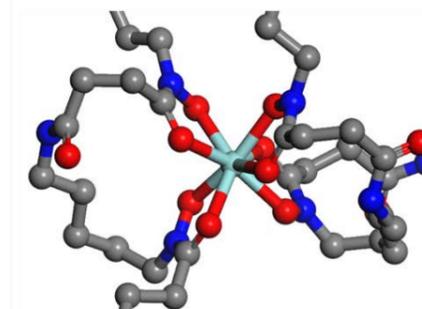
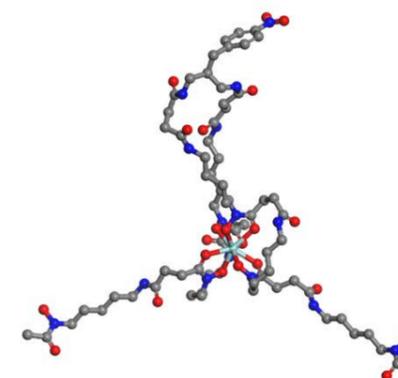
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Molecular imaging with positron emission tomography (PET) is a non-invasive technique that provides detailed, quantitative, and clinically power information on cellular and metabolic events. A variety of radiometals with differing nuclear properties have been produced and studied to date, with most of them requiring a different bifunctional chelator (BFCs) to provide optimal radiolabeling and stability properties. We have designed a new family of high-denticity (maximum CN=12) BFCs based on the “gold standard” for zirconium-89, the bacterial siderophore desferrioxamine (DFO). This new family of chelators includes those we’ve named DFO2 (DFT structure of Zr-DFO2 pictured below), DFO2glu, DFO2lys, and DFO2’, with a synthetic platform that features simple and rapid synthesis of new derivatives with different properties to enable binding to Zr(IV), Ti(IV), Nb(V), and potentially other radiometals. Although several successful new BFCs for zirconium-89 have been published in recent years, we are designing new BFCs with not only enhanced stability, but a focus on easy synthesis and a modular design for fast synthetic iteration. More importantly – we are investigating the use of EXAFS spectroscopy and DFT computations with the goal of validating and then integrating these methods with our rapid synthesis platform and traditional radiochemical stability assays to predict the most promising structures.

We have performed DFT calculations via ComputeCanada (SharcNet, Graham) and compared results obtained from combinations of the functionals B3LYP and PBEPBE – and the basis sets LanL2DZ/6-31G+(d,p), Def2TZVP, and SDD – and used experimentally determined bond-length values from EXAFS spectroscopy as standards to select the most accurate computation method for our application (PCM used to model solvent effects). We have also compared these results to calculations performed on our own local computer system running Materials Studio (DMol3/GGA/PBE). The purpose of this work is to determine the most accurate computation method for predicting the metal-ligand bond lengths of these compounds using the very accurate bond length values determined via EXAFS spectroscopy as references.

Preliminary zirconium-89 radiolabeling and stability assays have demonstrated that complexes of several of our new chelators are more stable than the “gold standard” chelator desferrioxamine (DFO). Conjugation of bifunctional derivatives to the model antibody trastuzumab and *in vivo* studies in mice are currently ongoing to determine the [<sup>89</sup>Zr]Zr-chelate stability by monitoring bone uptake over time. We hope to be able to correlate trends between EXAFS/DFT/radiochemical stability results with this first generation of chelators and use this information to more efficiently design new chelators in the future. Financial support from the University of Saskatchewan, the NSERC Discovery Grant program, the Canada Research Chairs program, and the CFI JELF program is greatly appreciated.



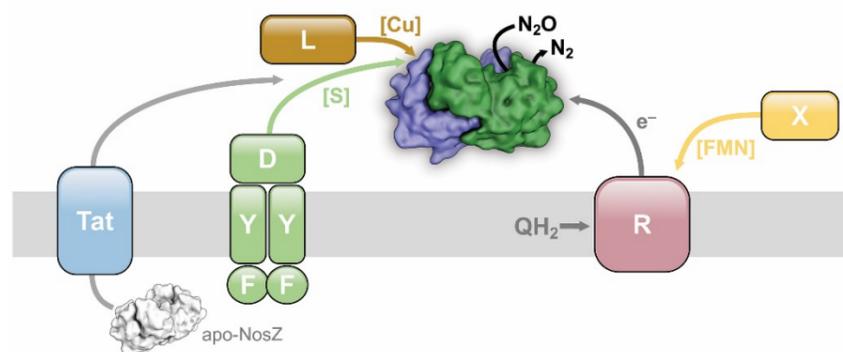
### OP-39

#### Functional Assembly of a Recombinant Nitrous Oxide Reductase in *Escherichia Coli*

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Nitrous oxide (N<sub>2</sub>O, 'laughing gas') is a potent greenhouse gas whose 100-year global warming potential exceeds the one of carbon dioxide (CO<sub>2</sub>) by a factor of 300 [1]. The biological reduction of N<sub>2</sub>O that completes denitrification is catalyzed by nitrous oxide reductase (N<sub>2</sub>OR), a periplasmic, homodimeric metalloprotein of 130 kDa and contains two copper centers, Cu<sub>A</sub> and Cu<sub>Z</sub>, in each monomer [2]. Its structural gene, *nosZ*, in most cases forms part of a gene cluster of architecture *nosRZDFYL* [3]. NosR is a transmembrane iron-sulfur flavoprotein which involved in electron transfer to active N<sub>2</sub>OR[4], while the ABC transporter NosFY and the periplasmic protein NosD are candidates of sulfur donation for Cu<sub>Z</sub> assembly. The outer membrane anchored protein NosL, is thought to be involved in copper trafficking to apo-N<sub>2</sub>OR. The maturation of the two metal centers require an elaborate assembly machinery that so far has precluded heterologous production as a prerequisite for bioremediatory applications. Here we report on the heterologous generation of active holo-enzyme in *Escherichia coli* and its application to gain deeper insight into the roles of individual assembly factors. Financial support by the European Research Council is gratefully acknowledged. [ERC grant no. 310656]



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### OP-40

#### The Choreography of the Oxygen Atom Transfer in the Epoxidation Reaction Catalyzed by the Non-Heme Iron Enzyme, AsqJ

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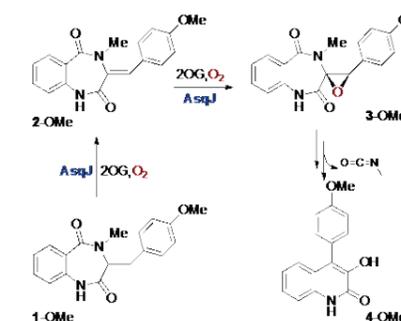
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Epoxide (oxirane) functional group is widely distributed in numerous nature products, which display a broad spectrum of biological activities, including antibacterial, antifungal, antiviral, and antitumor activities [1,2]. In nature, two strategies are utilized to install epoxide: 1) a formal dehydrogenation by cleaving a C-H and an O-H bond on two adjacent carbons, and 2) an oxygen atom transfer (OAT) reaction onto an olefin moiety of the substrate. Mechanistic understandings of the latter strategy (OAT) are largely derived from studies of chloroperoxidases (CPOs), cytochrome P450s, high-valent heme-iron model complexes, and high-valent non-heme iron model complexes [3-5]. It is generally accepted that the OAT reactivity exhibited by high-valent iron species is strongly electrophilic in nature, and epoxide ring could be formed in a stepwise manner via a discrete radical or cation species, or in a concerted mechanism. However, the mechanistic details on how non-heme iron enzyme catalyzed epoxidation through the OAT strategy is less well understood.

AsqJ from *Aspergillus nidulans* is a recently discovered iron(II)- and 2-oxoglutarate-dependent (Fe(II)/2OG) enzyme, which catalyzes a stepwise oxidation (desaturation and epoxidation) in the biosynthesis of a quinolone-type fungal alkaloid, 4'-methoxy-viridicatin [6]. Here we present our recent studies on the mechanism of the epoxidation catalyzed by AsqJ. Using transient state kinetics and Mössbauer spectroscopy, we demonstrate that an Fe(IV)=O intermediate is responsible for the oxygen installation to the C3-C10 double bond of the AsqJ substrate. However, this Fe(IV)=O species does not exhibit strong electrophilicity towards the electron rich olefin moiety of the substrate, a reactivity behavior in contrast with many high-valent iron species. More interestingly, crystallographic data reveal a unique iron-alkoxide species between the iron center and the C10 of the substrate. With additional DFT calculations and molecular dynamic simulations, a detailed mechanism of AsqJ catalyzed epoxidation is revealed.

Financial support by the National Institute of Health (NIH GM125924) is gratefully acknowledged.



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### OP-41

#### Structural Insights and Biomimetic Application of Heavy Metal Proteins

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The solar-to-chemical production by artificial and bioinspired photosynthetic systems is of tremendous interest to help solve current global energy and environmental problems. We developed a bioinorganic hybrid system for photocatalytic hydrogen production under aerobic conditions by combining light-harvesting semiconductors, hydrogenase catalysis, and self-aggregation of whole bacterial cells. We induced hydrogen production via self-photosynthesis in engineered *Escherichia coli* cells, which were originally designed for bioremediation, with in situ biosynthesis of biocompatible cadmium sulfide nanoparticles using a surface-display system. This biohybrid catalytic approach may serve as a general strategy for solar-to-chemical production.

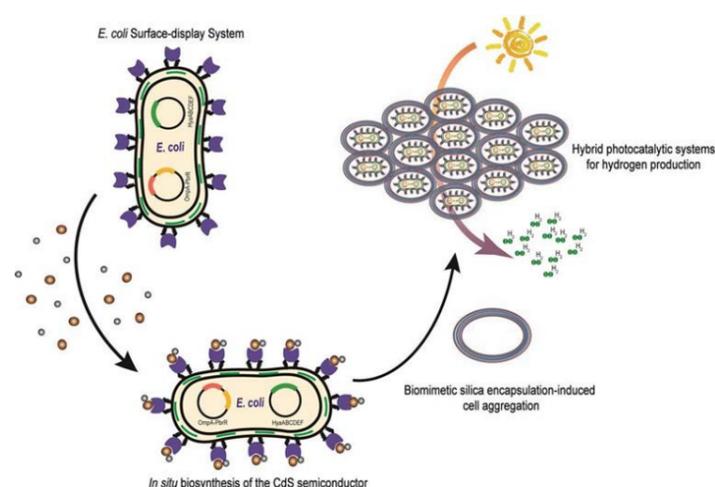


Figure 1. Surface-display biohybrid approach to light-driven hydrogen production in air.

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### OP-42

#### Reactivity Study of Nonheme Manganese(III)-Superoxo Complex

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Nature has developed a variety of enzymes that contain transition metal cofactors to activate O<sub>2</sub>; thus, metalloenzyme-catalyzed reactions typically proceed with unrivaled efficiencies under ambient conditions. The majority of O<sub>2</sub>-activating wild-type enzymes utilize Fe and Cu in their active sites, whereas a few of them feature Mn. Two forms of homoprotocatechuate 2,3-dioxygenases with the respective active-site metal ions being Fe<sup>II</sup> and Mn<sup>II</sup> have been isolated, namely, Fe-HPCD from *Brevibacterium fuscum* [1] and Mn-MndD from *Arthrobacter globiformis* [2]. Surprisingly, the native enzymes, Fe-HPCD and Mn-MndD, and their artificial variants, Mn-HPCD, Fe-MndD and Co-HPCD generated by reconstituting the native enzymes with nonphysiological metal ions, all exhibit comparable catalytic activity [3,4]. Therefore, it has long been believed that transition metal-catalyzed oxidative transformations share common mechanistic features, in which O<sub>2</sub> initially binds to a reduced metal center to form an oxidized metal-superoxo species, which functions as the reagent to perform subsequent chemistry [5,6]. Upon mixing of O<sub>2</sub> to Mn-HPCD, two intermediates were trapped and characterized by EPR spectroscopy. They were assigned to a Mn<sup>III</sup> ion coordinated by an unidentified radical, assumed to be a superoxo, and a Mn<sup>II</sup>-alkylperoxo species, but the conclusive evidence remains elusive [7]. In addition, well-defined Mn<sup>III</sup>-superoxo complexes remain rather rare. Recently, two Mn<sup>III</sup>-superoxo complexes, Mn(BDPP)(O<sub>2</sub><sup>-</sup>) (2) and Mn(BDP<sup>Br</sup>P)(O<sub>2</sub><sup>-</sup>) (2') were prepared. Herein, we would like to present the reactivity of the Mn<sup>III</sup>-superoxo complexes.

Financial support by the Ministry of Science and Technology, Taiwan is gratefully acknowledged.



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**OP-43****Nanomedicine for Improving Chemoradiotherapy, Real-time Evaluating Drug Efficacy, and Enhancing Immunotherapy in Cancer Treatment****Yuanzeng Min<sup>1</sup>**<sup>1</sup>*Department of Chemistry, University of University of Science and Technology of China, Hefei, Anhui, China minyz@ustc.edu.cn*

Cancer, a major public health problem worldwide, is a death causing disease. Chemotherapy and radiotherapy are main treatments for cancer. However, their side effects and resistance limited their clinical use. On the other hand, nanomedicine, the area of interdisciplinary science, applies nanotechnology to health and medicine [1]. First, we demonstrate that how nanotechnology overcomes platinum drug resistance [2]. The platinum prodrug was designed and tethered onto surface of gold nanorods. The designed delivery system can overcome resistance through increasing drug uptake and decreasing drug deactivation. We also used nanomedicine strategy to deliver chemotherapy drugs, such as Histone deacetylase (HDAC) inhibitor, to sensitize radiotherapy through inhibiting DNA repair [3]. Second, we developed a multifunctional platform with controlled activation of platinum prodrug using near infrared (NIR) light and with real-time evaluation of drug efficacy [4]. The ultraviolet (UV) light sensitive platinum prodrug was synthesized and conjugated onto surface of upconversion nanoparticles (UCNPs). Upon absorbing NIR light, UCNPs convert it into UV light, which activates platinum prodrug, release active components and then induce cancer cells apoptosis. Meanwhile, an apoptosis-imaging probe was also conjugated onto UCNPs. Once NIR light irradiated UCNPs, cancer cells apoptosis was triggered, which unleashed the release of enzyme, caspase-3 and subsequently activate the imaging probe. Therefore, the direct imaging of apoptosis in living cells can be for real-time evaluation of drug efficacy. Finally, yet importantly, nowadays immunotherapy generates promising clinical data that have not seen before. It showed that combining radiotherapy and immunotherapy can enhance immune responses and can boost 'abscopal effect'. Unfortunately, response rates for this strategy remain low. Herein we utilizes antigen-capturing nanoparticles (AC-NPs) to improve this strategy [5]. Our engineered AC-NPs can capture a set of protein antigens released post radiotherapy and it helped antigen presentation, which eventually induce enhanced immune responses and enhance 'abscopal effect'.

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**OP-44****Quantifying the Biological Fate of Nanosilver****David Kennedy<sup>1</sup>, Valerie Gies<sup>1</sup>**<sup>1</sup>*Metrology – National Research Council of Canada, 100 Sussex Drive, Ottawa, Canada*

With a growing number of high precision tools for studying biological systems, it is important to develop traceable quantitative methods that result in accurate measurements. Because biological systems are both complex and fluxional, context is vitally important for such measurements in order for them to be accurate. Correlation of measurements through space and time can provide such quantitative assessments. Metallic nanoparticles pose many challenges for measurement in cellular systems. The metal can interfere with the detection method and the particles can change in size and shape over time and in association with different biological molecules.<sup>1</sup>

At the National Research Council we seek to correlate detailed physical characterization of silver nanoparticles with biological measurements to generate methods for measuring the impact of nanosilver on different cell types and quantifying the specific interactions of nanosilver with biological molecules.<sup>2,3</sup> Correlating changes in nanoparticles over time in biological fluids helps to provide an understanding of nanoparticle behaviour and results in higher reproducibility of observed biological endpoints. Surface coatings play a pivotal role in recognition of the particles by cellular receptors suggesting active transport plays a critical role in the nanosilver life cycle.<sup>2</sup>

Physical and chemical differences between silver nanoparticles and changes that occur in biological test media can be correlated to toxicity, and different mechanisms for toxicity are apparent. Uptake rates and localization is also different between different cell lines. Uptake and localization of particles provides evidence that nanosilver should not be treated as a single material but should be studied as an array of materials with different properties in different biological systems.

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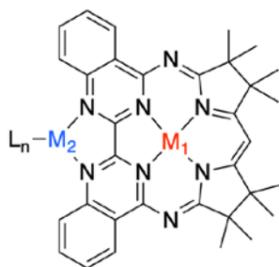
OP-45

## Earth-Abundant Mono- and Bi-Metallic Mabiq Complexes for Photocatalysis

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The redox cascades and water splitting reaction of Photosystem II are instigated via light absorption by chlorophylls. Artificial photosystems that catalyze H<sub>2</sub>O splitting and solar fuel production (e.g. H<sub>2</sub> evolution), similarly rely on molecular photosensitizers to initiate the electron transfer processes. However, noble metal complexes based on Ru or Ir are commonly employed as the photoactive molecules in such systems. An important aim in the field of photocatalysis is to replace these noble metal complexes with earth-abundant alternatives, as found in PSII. Our own work focuses on studies with a series of late, first-row transition metal complexes coordinated by a macrocyclic ligand, Mabiq. The ligand shares features with the biologically relevant porphyrins and corrins. The series of mono- and bimetallic Mabiq complexes are photoactive, and the M<sup>II</sup> complexes can be photoreduced. The electronic structure, photochemical properties and reactivity of the M<sub>n</sub>-Mabiq compounds will be presented.



OP-46

## Construction and Function of Biomimetic Diiron Nitrogen Fixation Systems

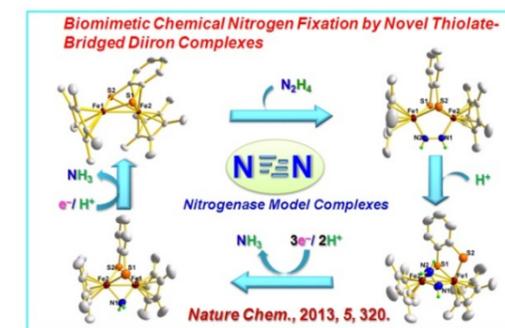
Dawei Yang<sup>1</sup>, Baomin Wang<sup>1</sup>, Jingping Qu<sup>1</sup>

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Nitrogen fixation, which converts inert dinitrogen (N<sub>2</sub>) to ammonia (NH<sub>3</sub>), is a process of fundamental importance both in nature and for modern society. The Haber-Bosch ammonia synthesis process as one of the greatest inventions of the 20<sup>th</sup> century poses many serious environment and energy problems because of its harsh reaction conditions. In sharp contrast, nitrogenase can catalyze N<sub>2</sub> reduction to NH<sub>3</sub> under ambient conditions. Hence, it is effective to adopt biomimetic strategy for developing mild ammonia synthesis technology.

Based on the belt diiron structure of FeMo-cofactor as the potential active center of nitrogenase, we designed and constructed a series of novel thiolate-bridged diiron complexes as nitrogenase mimics. By cooperative activation effect between the two iron centers, we mainly focused on functional transformation of small molecules, especially biomimetic chemical nitrogen fixation. Firstly, catalytic N–N cleavage of hydrazine compounds was realized to release corresponding amines and ammonia[1]. Secondly, we presented a detailed biological nitrogen fixation process from diazene (NH=NH) to ammonia in biomimetic diiron scaffold [2]. Based on these findings, we firstly proposed a new biological nitrogen fixation mechanism in the diiron active center. Besides, we also extend our work to biomimetic catalytic transformation of other inert small molecules [3-6].

Financial support by “111” project of the Ministry of Education of China is gratefully acknowledged.



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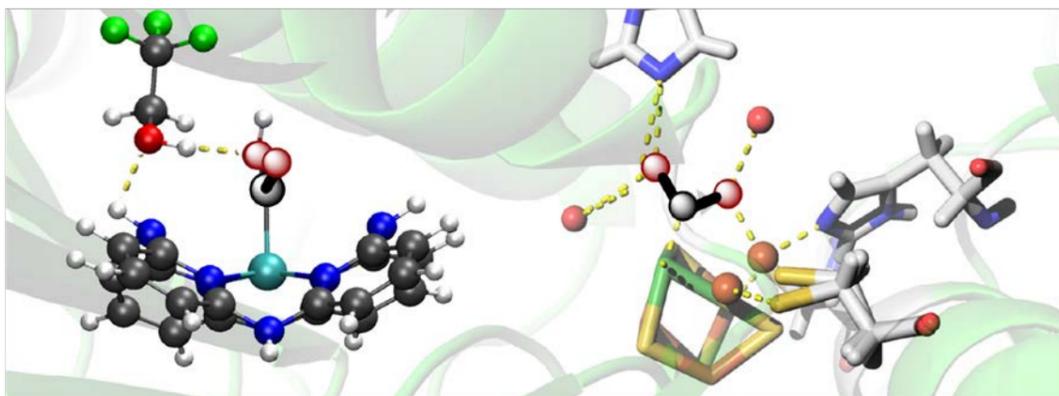
## OP-47

### Efficient CO<sub>2</sub> Reduction by Cobalt Catalysts with Pendant Hydrogen-Bond Donors

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Energy harvested directly from sunlight offers a desirable approach toward fulfilling the global need for clean energy with minimal environmental impact. Marinescu group focuses on fundamental research to understand, design, and synthesize novel catalytic systems essential to the development of efficient *solar-to-fuel* technologies. Inspired by biological systems, we develop molecular catalysts that involve hydrogen-bonding networks capable of small molecule activation, through multiple proton and electron transfers. We have shown that cobalt complexes with pendant secondary amines (NH groups) act as highly efficient catalysts for the reduction of CO<sub>2</sub> to CO in comparison to the alkylated versions. This results suggests that the presence of the pendant NH moiety is crucial for catalysis. We have recently synthesized a series of cobalt-centered CO<sub>2</sub>-to-CO reduction catalysts, with 0 to 4 pendant secondary amines. We have demonstrated that the rate of catalysis has a direct correlation with the number of pendant protic moieties. Theory and experiment suggest that the pendant secondary amines (NH groups) do not directly transfer protons to CO<sub>2</sub>, but instead bind acid molecules from solution. Taken together, these results suggest a mechanism in which noncooperative pendant amines facilitate a hydrogen-bonding network that enables direct proton transfer from acid to the activated CO<sub>2</sub> substrate, providing a relevant model for biological systems.



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## OP-48

### *Escherichia Coli* FeoC Binds an Oxygen-Sensitive, Redox-Active [4Fe-4S] Cluster

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The acquisition of iron is an essential process for the establishment of virulence among virtually all pathogens [1,2]. Under acidic and/or anaerobic conditions, such as those found in the stomach, the intestines, and within biofilms, the majority of pathogens utilize the ferrous iron (Fe<sup>2+</sup>) uptake (Feo) system to import Fe<sup>2+</sup> in order to fulfill their requirement for iron. The Feo system is the most widely distributed prokaryotic Fe<sup>2+</sup> import pathway, and its presence is required for normal growth of most unicellular organisms [3]. The Feo system is poorly understood at the atomic, molecular, and mechanistic levels, which has prohibited the targeting of this system for bacterial exploits and represents a pressing and urgent gap in the field of bacterial metal homeostasis. While the main membrane component of the Feo system (FeoB) is essential for the translocation of ferrous iron, the small, cytosolic proteins FeoA and FeoC are postulated to function as accessories to this process, but their roles remain poorly defined. In this work, we are the first to demonstrate that *Escherichia coli* FeoC (*EcFeoC*) binds an [Fe-S] cluster, and we spectroscopically and biophysically characterize the nature of this cluster. Under strictly anaerobic conditions, we demonstrate that *EcFeoC* binds a redox-active and rapidly oxygen-sensitive [4Fe-4S]<sup>2+/+</sup> cluster, in distinct contrast to the only previous study on FeoC from *Klebsiella pneumoniae* [4]. Importantly, we show that this cluster binding is associated with modest conformational changes of the polypeptide but not protein dimerization. We posit a working hypothesis in which the cluster-binding FeoCs may function as *in vivo* iron sensors to fine-tune ferrous pathogenic iron acquisition.

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### OP-49

#### An Atomistic View over Mg<sup>2+</sup> Induced Kinetic Heterogeneity in the Group II Intron-Exon Recognition Site

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The driving factors of nucleic acids folding are the stability of base-base and cation-mediated electrostatic interactions. In particular, Mg<sup>2+</sup> ions strongly bind to RNA molecules promoting the formation of tertiary contacts and even specific functional motifs. As an example, a recurring tertiary contact of the group II intron family the 5'-exon-intron binding site interaction (EBS1-(d)IBS1), can only be stably formed upon addition of Mg<sup>2+</sup> ion or under very high concentration of monovalent cations of the RNA cognate only. Although not in the same way, but the effect is observed for both RNA-RNA and RNA-DNA pairs. FRET and NMR studies suggest that cations interfere not only with the stability of the EBS1-(d)IBS1 interaction but also with the kinetics by inducing heterogeneity. In other terms, there is a multi-state folding regulated by Mg<sup>2+</sup> specific binding site occupancy [1-3]. In this work we utilize molecular dynamics simulations powered by a combination of many state-of-the-art enhanced sampling techniques to characterize the structural and thermodynamics effects of Mg<sup>2+</sup> and K<sup>+</sup> binding on the homo and heteroduplex binding motifs. This simulation allows for the determination of relative binding affinities of divalent metal ions such as Mg<sup>2+</sup> and the effects of ion competition. The highest affinity binding sites identified in the simulations corroborate with the proposed by NMR experiments. Preliminary results indicate that there are specific ion-induced conformational changes in the backbone of the RNA-RNA pair that further stabilize the tertiary contact formation while the same doesn't happen for its RNA-DNA counterpart. The combination of simulations and experiments provide a multi-lateral description in an atomistic level of the 5'-exon-intron binding ranging from its thermodynamics to the kinetics including non-trivial effects arising from RNA-ions binding. Financial support by the University of Zurich is gratefully acknowledged.

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### OP-50

#### Traditional Chinese Active Ingredients Metal-based Anticancer Agents

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Alkaloids are active ingredients of traditional Chinese medicines, and possess wide range of bioactivity. We put forward an thinking of using the coordination regulating effect of Chinese medicine active ingredients and metal active center to design metal-based drugs, and gave rise a new mode to develop metal-based anticancer agents. Targeting G4-DNA, telomerase, DNA, we synthesized a series of antitumor metal complexes of traditional Chinese medicines active ingredient alkaloids matrine, oxoaporphine, oxoisoaporphine, and tetrahydroisoquinoline as well as their derivatives. We have obtained a series of TCMAI-metal complexes (including ten leading compounds) with high in vivo anticancer activity and good in vivo safety [1-25]. These leadings exhibited multi-targeting and multi-mechanism features, which provide the possibility to overcome the resistance of metal-based antitumor drug [26]. Financial support by the NNSF of China (Nos. 21401031, 21431001), IRT\_16R15, and NSF of Guangxi Province (No. 2016GXNSFGA380005) as well as "BAGUI Scholar" program of Guangxi of China, is gratefully acknowledged.

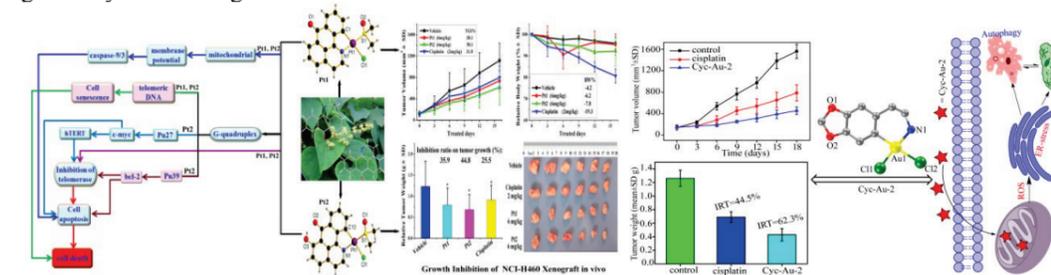


Fig.1 Oxoisoaporphine Pt(II) complexes.

Fig. 2 Tetrahydroisoquinoline Au(III) complex

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## OP-51

### Polyoxovanadates with Antibacterial Activity: Are P-type ATPases the only Target?

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Polyoxometalates (POMs) and POM-based hybrid and nanocomposite structures have become of increasing interest in medicine due to their antibacterial and anticancer activities [1,2]. Herein, we compare the antibacterial activity of three polyoxovanadates (POVs) namely MnV<sub>11</sub>, MnV<sub>13</sub> and V<sub>10</sub> against *Escherichia coli* growth. It was observed that MnV<sub>11</sub> presents the lowest growth inhibition (GI<sub>50</sub>) value followed by MnV<sub>13</sub> compound and being about 2 times lower than V<sub>10</sub>, respectively the values obtained were 0.21, 0.27 and 0.58 mM. All three compounds were more effective than vanadate alone (GI<sub>50</sub>=1.1 mM) and also than decaniobate, Nb<sub>10</sub> (GI<sub>50</sub>>10 mM), a isostructural POM of V<sub>10</sub>. However, POVs exhibiting the highest antibacterial activity (MnV<sub>11</sub>) shows to have the lowest Ca<sup>2+</sup>-ATPase inhibitor capacity (IC<sub>50</sub> = 58 μM) whereas decavanadate, which was also very active against this P-type ATPase (IC<sub>50</sub>=15 μM), was the less active against *Escherichia coli* growth. Thus, for the analyzed POVs it was observed a reverse correlation between the Ca<sup>2+</sup>-ATPase IC<sub>50</sub> values and the *Escherichia coli* GI<sub>50</sub> values suggesting that decavanadate and others POVs inhibitors of ion pumps cannot be directly associated with the inhibition of *Escherichia coli* growth. Nevertheless, although the biological processes affected in bacteria by each POMs are yet to be clarified, ion pumps as well known molecular targets for drugs and polyoxometalates should be taken in consideration once they are directly or indirectly involved in bacterial cell death [3,4].

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## OP-52

### Swapping Cobalt for Nickel in Vitamin B<sub>12</sub>: Nibalamin

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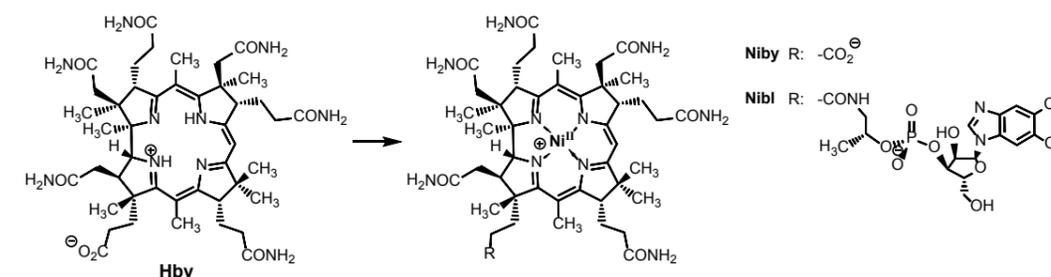
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The exchange of the cobalt center of vitamin B<sub>12</sub> to create metal analogues has been a long-held aspiration of B<sub>12</sub>-chemistry.[1] Unfortunately, all attempts to remove the cobalt ion from natural corrinoids to yield intact corrins have failed.[2] Alternative semi-synthetic approaches to substitute Co for Ni in vitamin B<sub>12</sub> derivatives, by breaking and reconstructing a C-C-bond of the macrocycle, have been attempted,[3] and the partial synthesis of 5,6-dihydroxy-nibalamin starting from vitamin B<sub>12</sub> has recently been achieved.[4]

We have taken a different approach through the development of a crafted biosynthesis of hydrogenobyric acid (**Hby**), which serves as a metal-free template for the construction of metal analogues of B<sub>12</sub>. [5] Herein we describe the construction of the nickel analogue of vitamin B<sub>12</sub>, nibalamin (**Nibl**). We have selected **Nibl**, with a Ni<sup>II</sup>-central atom, as an attractive target to create a stable mimic of the structurally uncharacterized and highly reactive cob(II)alamin, a key intermediate in B<sub>12</sub>-biocatalysis.[6] **Nibl** was synthesized from **Hby** and characterized structurally by detailed NMR-analysis. In the course of our investigations we synthesized the Ni-complex of **Hby**, nibyric acid (**Niby**), which was further characterized by single crystal X-ray diffraction. The coordination of the Ni<sup>II</sup>-ion in **Niby** shows remarkable structural similarities to the first synthetic Ni<sup>II</sup>-complex of a model corrin.[7-8]



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OP-53

### Sulfide Protects [FeFe] Hydrogenases from Oxygen

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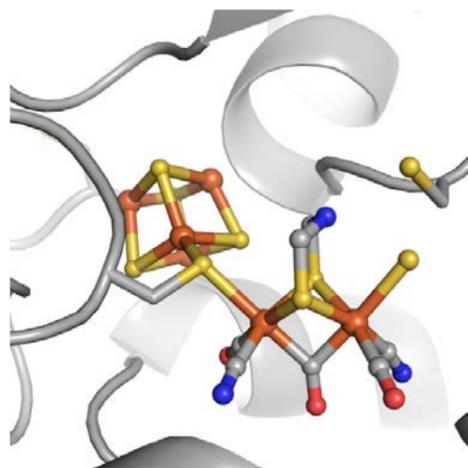
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Hydrogenases are enzymes that catalyze the oxidation and generation of molecular hydrogen in a highly efficient and reversible manner. While [NiFe] hydrogenases are the most common in nature, it is the [FeFe] hydrogenases that are the most active, but at the same time they are the most oxygen-sensitive.[1,2] The active site of [FeFe] hydrogenases consists of the H-cluster, which is constructed from a canonical [4Fe-4S] sub-cluster coupled to a unique [2Fe] sub-cluster containing a unique bridging azapropane dithiolate as well as carbonyl and cyanide ligands.[3-5] Exposure of the active enzyme to O<sub>2</sub> results in rapid irreversible activity loss.[6]

The enzyme from the sulfate-reducing bacterium *Desulfovibrio desulfuricans* (DdHydAB) is one of the most active and bidirectional [FeFe] hydrogenases. An additional interesting feature of DdHydAB is that it can be purified aerobically in an oxygen-stable inactive state called H<sub>inact</sub>. This state can be converted into the active oxidized state H<sub>ox</sub>, ready to bind H<sub>2</sub> and enter the catalytic cycle. Recently, we have been able to generate the H<sub>inact</sub> state under oxidizing conditions in the presence of sulfide, suggesting that sulfide coordinates to the [2Fe] sub-cluster. To investigate the structure of the enzyme in the H<sub>inact</sub> state, we have crystallized DdHydAB and solved its structure to 1.5 Å. The structure clearly shows electron density in the open coordination site of the distal Fe, demonstrating the presence of sulfide. These data are supported by resonance Raman and nuclear resonance vibrational spectra of samples using natural abundance and <sup>34</sup>S isotopically labelled sulfide sources. The formation of the oxygen-stable state *in vitro* allows the handling of hydrogenases in air, making their implementation in biotechnological applications more feasible.

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OP-54

### A Peculiar Ni(II)-Binding Site in the Carbon Monoxide Dehydrogenase Accessory Protein CooT

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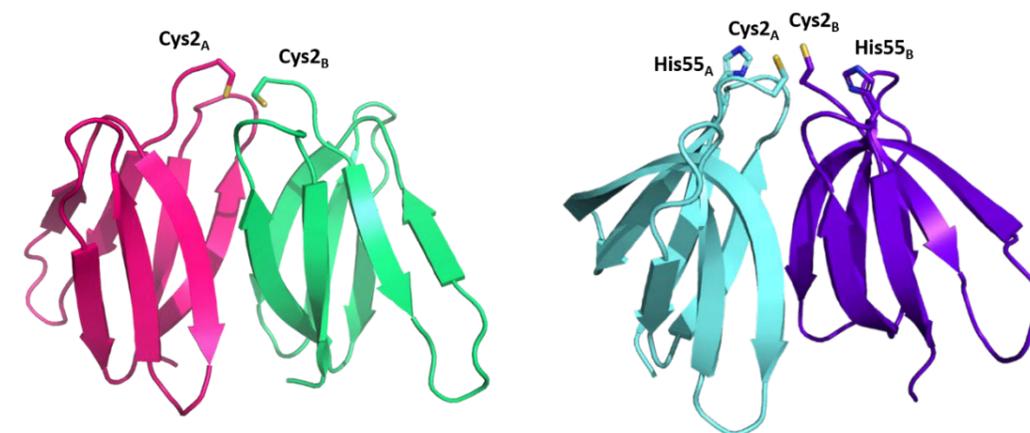
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CODH reversibly oxidized CO into CO<sub>2</sub> and plays a central role in the carbon metabolism of anaerobic microorganisms. In hydrogenogenic carboxydrotrophs, such as *Rhodospirillum rubrum* or *Carboxydotherrmus hydrogenoformans*, CO can be used as a sole energy source via the water-gas shift (WGS) reaction. In this process, the conversion of CO and H<sub>2</sub>O into CO<sub>2</sub> and H<sub>2</sub> is catalyzed by two enzymes: a monofunctional carbon monoxide dehydrogenase (CODH) coupled to an energy-conserving hydrogenase. The crystal structures of CODH from *C. hydrogenoformans* [1] and *R. rubrum* [2] revealed a unique active site: a [NiFe<sub>3</sub>S<sub>4</sub>] cubane coordinated to a mononuclear iron site, known as C-cluster. CODH activation relies on the insertion of Ni into the C-cluster and requires the intervention of several accessory proteins. Among them, we recently characterized CooT from *R. rubrum* (RrCooT) and reported the ability of this protein to bind 1 Ni(II) per dimer with high affinity (K<sub>d</sub>=9 nM) [3]. In addition, our phylogenetic analysis revealed the existence of a CooT family, regrouping CooT homologs present in anaerobic archaea and bacteria. In this family, two different Ni(II)-binding modes were identified from their sequences and from the X-ray structures of apoRrCooT [3] and the putative CooT from *C. hydrogenoformans* (ChCooT) [4].

Here we present the characterization of holo-RrCooT. The only cysteine, present in second position in sequence, has been recognized as the main protagonist in Ni(II) coordination, highlighting the importance of the N-terminal amino acids. A binding mode is proposed, characterized via CD, EXAFS, NMR and computational studies. This work revealed a peculiar Ni(II)-binding mode triggered by the protein dimerization, via two solvent exposed cysteines at the N-termini.



X-ray structures of RrCooT dimer (A) & ChCooT dimer (B), with their putative Ni(II)-coordinating ligands in sticks.

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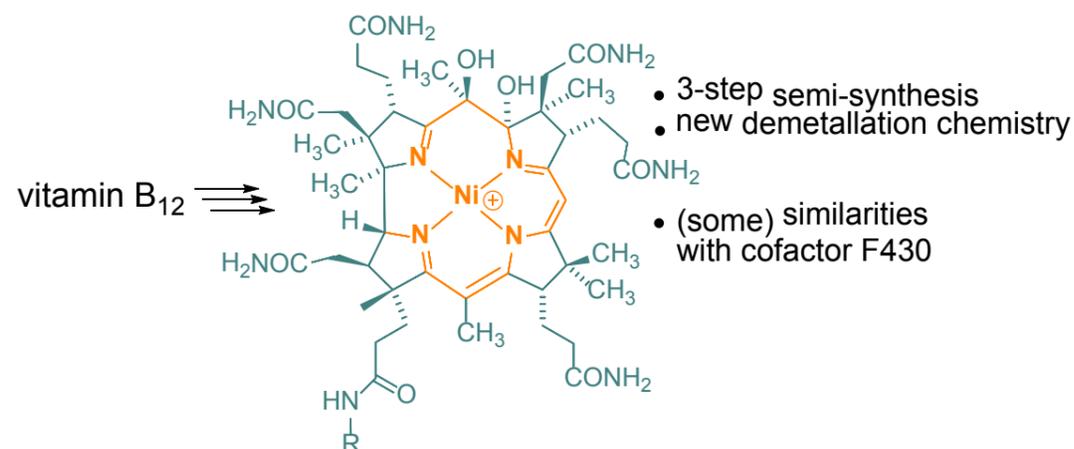
OP-55

### A Nickel Containing Vitamin B<sub>12</sub> Derivate – Similarities and Differences with Cofactor F430

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Metal-porphyrinoids are among the most fascinating enzymatic cofactors with functions ranging from light harvesting to the catalysis of difficult organic transformations. Cobalamins combine a corrin ligand with a cobalt ion and catalyse methyl transfer and [1, 2] rearrangement reactions, whereas cofactor F430 represents a Ni-corrin complex used during enzymatic conversion of CO<sub>2</sub> to methane [1]. Recently my group described the partial synthesis of a Ni-containing cobalamin derivative from vitamin B<sub>12</sub> [2]. In the presentation, the synthesis, properties as well as similarities and differences of the “nibalamin” with cofactor F430 and vitamin B<sub>12</sub> will be discussed. These findings will be compared to pioneering studies with Ni-corrin and Co-corrin model compounds developed in the Eschenmoser group [3]. Financial support by the University of Zurich and the “Forschungskredit” of the University of Zurich is gratefully acknowledged. We acknowledge generous gifts of B<sub>12</sub> from DSM Nutritional Products AG (Basel/Switzerland) as well as from Prof. em. Bernhard Jaun (ETH Zurich).



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OP-56

### Hydroxylation of Non-Native Substrates by Cytochrome P450BM3 Exploiting Decoy Molecules

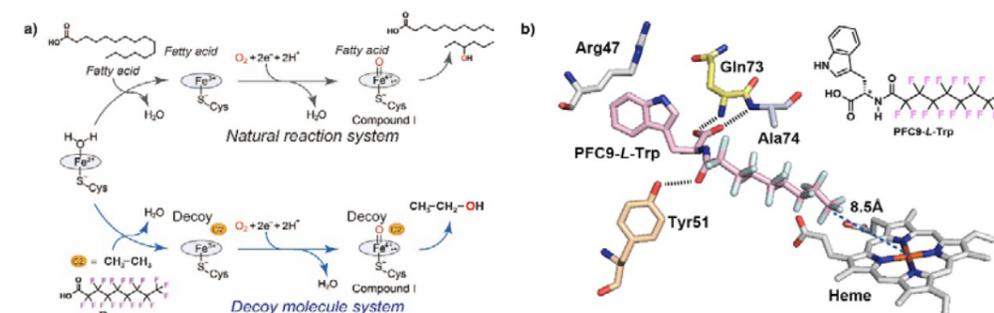
Osami Shoji<sup>1,3</sup>, Shinya Ariyasu<sup>1,3</sup>, Yuichiro Aiba<sup>1</sup>, Chie Kasai, Joshua Kyle Stanfield, Kazuto Suzuki, Keita Omura, Masayuki Karasawa<sup>1</sup>, Yusaku Kodama, Ayaka Matsumoto, Kai Yonemura<sup>1</sup>, Yoshihito Watanabe<sup>2</sup>

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Cytochrome P450s (P450s) are regarded as potential candidates for the development of biocatalysts because of their high catalytic activity in the hydroxylation of unactivated C–H bonds. Among the reported P450s, CYP102A1 (P450BM3) isolated from *Bacillus megaterium* has garnered much attention because of its high monooxygenase activity. In general, P450BM3 displays a high substrate specificity, exclusively catalyzing the hydroxylation of long-alkyl-chain fatty acids while remaining inactive for small non-native substrates such as propane and benzene. However, it was observed that P450BM3 can be “fooled” into initiating hydroxylation of non-native substrates in the presence of perfluorinated carboxylic acids (PFCs), which function as inert dummy substrates (decoy molecules). PFCs initiate the activation of molecular oxygen in the same manner as with long-alkyl-chain fatty acids and induce the generation of compound I, but the compound I hydroxylates gaseous alkanes and benzene because the C–F bonds of PFCs are not oxidizable.<sup>1,2</sup> We have succeeded in developing the next generation of decoy molecules by modifying the carboxylate of PFCs with amino acids and succeeded in enhancing the catalytic activity for gaseous alkanes.<sup>3</sup> Amongst the next generation of decoy molecules examined, *N*-perfluorononanoyl-*L*-leucine (PFC9-*L*-Leu) was the most effective for hydroxylation of propane (256/min/P450). Furthermore, we have succeeded in crystallizing the *N*-perfluorononanoyl-*L*-tryptophan (PFC9-*L*-Trp)-bound form of P450BM3. The crystal structure analysis of PFC9-*L*-Trp-bound form of P450BM3 (PDB code: 3WSP) showed that the terminal of alkyl chain does not reach to the active site owing to the multiple hydrogen bonding interactions between the carboxyl and carbonyl groups of PFC9-*L*-Trp and amino acids (Tyr-51, Gln-73, and Ala-74) located at the entrance of P450BM3. More recently, we have demonstrated that various carboxylic acids modified with amino acids (*N*-acyl amino acids) as well as amino acid dimers having a completely different structure from fatty acids can serve as decoy molecules.<sup>4</sup> We also have confirmed that wild-type cytochrome P450BM3 (P450BM3) expressed in *E. coli* can be activated by adding amino acid derivatives as decoy molecules to the culture medium and benzene was hydroxylated without supplementing with NADPH.<sup>5</sup> The yield of phenol reached 59 % when *N*-heptyl-*L*-prolyl-*L*-phenylalanine (C7-*L*-Pro-*L*-Phe) was employed as the decoy molecule.<sup>5</sup>



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OP-57

### Antiplasmodial Activity and *In Vivo* Bio-Distribution of Chloroquine Molecules Released with a 4-(4-ethynylphenyl)-Triazole Moiety from Organometallo-Cobalamins that Accumulate in Both Erythro- and Hepatocytes

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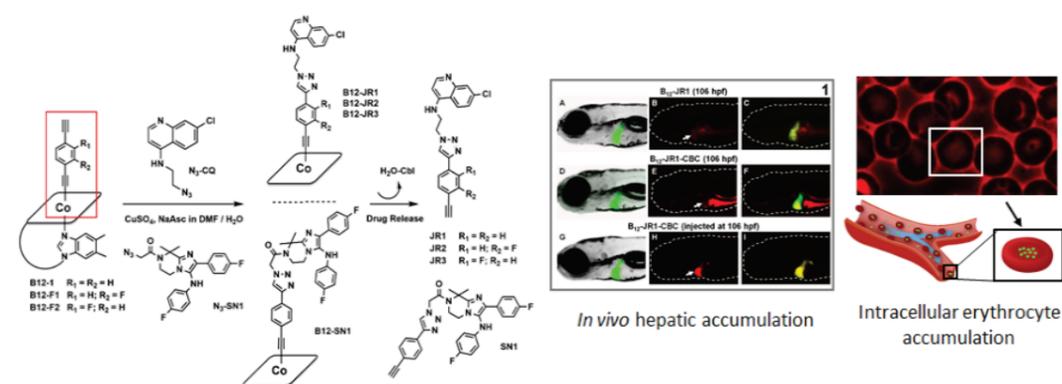
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We have explored the possibility of using organometallic derivatives of cobalamin as a scaffold for the delivery of the same antimalarial drug to both erythro- and hepatocytes. This hybrid molecule approach [1], intended as a possible tool for the development of multi-stage antimalarial agents [2], pivots on the preparation of azide-functionalized drugs which, after coupling to the vitamin, are released with a 4-(4-ethynylphenyl)-triazole functionality. Three chloroquine and one imidazolopiperazine derivative (based on the KAF156 structure) [3] were selected as model drugs. One hybrid chloroquine conjugate (B12-JR1) was extensively studied via fluorescent labelling for *in vitro* and *in vivo* bio-distribution studies and gave proof-of-concept for the design. It showed no toxicity *in vivo* (zebrafish model) as well as no hepatotoxicity, no cardiotoxicity or developmental toxicity of the embryos. All 4-(4-ethynylphenyl)-triazole derivatives of chloroquine were equally active against chloroquine-resistant (CQR) and chloroquine-sensitive (CQS) *P. falciparum* strains. Spectrophotometric titrations of the drugs into a solution of strictly monomeric ferriprotoporphyrin IX indicates that the molecules might exert their antiplasmodial activity by promoting ferriprotoporphyrin  $\mu$ -oxo dimer formation, thereby shifting the haematin/ $\mu$ -oxo ferriprotoporphyrin IX dimer equilibrium.

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OP-58

### Structural Basis of Iron Reduction by Human Duodenal Cytochrome *b* (Dcytb) Involved in Intestinal Iron Absorption

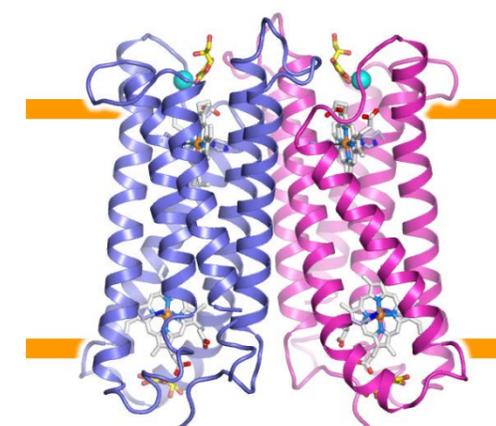
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Duodenal cytochrome *b* (Dcytb) is an Fe<sup>3+</sup> reductase that was identified in the duodenal brush border. Since the metal transporter DMT-1 favors the absorption of divalent metal including Fe<sup>2+</sup>, the reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> by Dcytb in the duodenum is essential for effective intestinal iron absorption. Dcytb is an integral membrane protein that catalyzes reduction of nonheme Fe<sup>3+</sup> by electron transfer from ascorbate across the membrane. Here we report the crystallographic structures of human Dcytb and its complex with ascorbate and Zn<sup>2+</sup> [1]. Each monomer of the homodimeric protein possesses six transmembrane helices and cytoplasmic and apical heme groups, as well as cytoplasmic and apical ascorbate-binding sites located adjacent to each heme. Zn<sup>2+</sup> coordinates to two hydroxyl groups of the apical ascorbate and to a histidine residue. Biochemical analysis indicates that Fe<sup>3+</sup> competes with Zn<sup>2+</sup> for this binding site. The identification of the metal binding site on Dcytb that is located adjacent to the apical binding site for ascorbate provides structural evidence for cytochrome *b*<sub>561</sub> family involvement in metal ion reduction. The cooperative binding of ascorbate and Zn<sup>2+</sup> to the apical substrate binding site in the Dcytb structure reveals key mechanistic insight into Dcytb function.



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### OP-59

#### Exploiting the Unique Properties of Transition Metals in the Fight against Antibiotic Resistance

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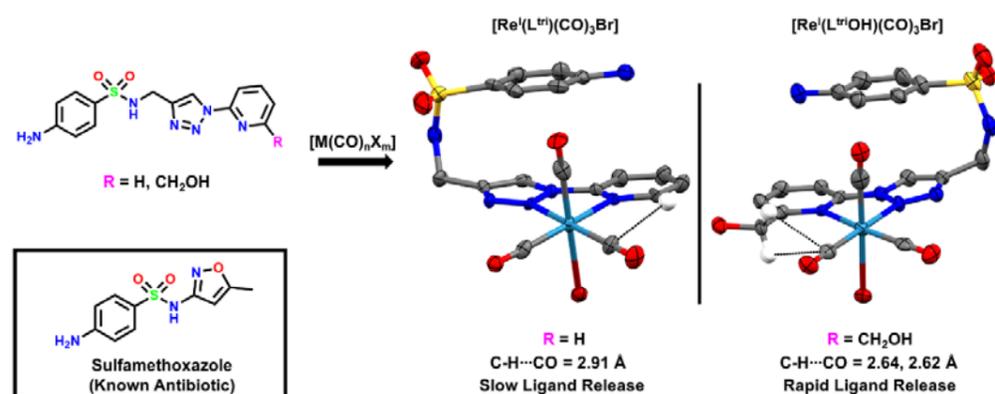
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Antibiotic resistance is one of the biggest challenges facing healthcare in the developed world. The development of novel antibiotics is being outpaced by the development of bacterial strains with resistance to them, including the often lethal, hospital ‘superbug’ MRSA. This is a problem that will continue to be exacerbated due to increasing global population and high density living facilitating the spread of contagious diseases. Therefore, an alternative approach, which impedes the development of resistance, is required [1].

The development of new antibiotics has been mostly focussed on the discovery and modification of purely organic molecules. However, metallodrugs may possess distinct advantages over conventional organic antibiotics, including new targets, new modes of action, a greatly increased range of available geometries, and targeted activity through light or redox activation [2,3]. We are particularly interested in the use of transition metals for targeted drug delivery to bacterial cells. A major advantage of such targeted delivery is the ability to achieve a higher local concentration in the target cells, despite maintaining a lower systemic concentration. A higher local concentration reduces the chance of partially resistant organisms remaining viable and reproducing, while a lower systemic concentration could reduce possible side-effects as well as combating the spread of resistance through other mechanisms such as horizontal gene transfer from non-pathogenic bacteria.

In this work, CuAAC click chemistry was used to synthesise sulfonamide-derived pyridyl-triazole ligands as well as ruthenium and rhenium complexes of them (See figure). Interestingly, the inclusion of a hydroxymethyl group ortho to the nitrogen atom on the coordinating pyridine ring increased the rate of ligand exchange up to 100-fold relative to the unsubstituted ligand. Subsequent structural characterisation indicated the increased (photo)lability is primarily due to steric factors. Furthermore, the metal complexes showed improved antibacterial activity relative to a clinical analogue (sulfamethoxazole) in MRSA strains which are resistant to sulfonamide-based antibiotics [4].



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### OP-60

#### Phosphatase Activity of a New Dinuclear Zinc Complex Containing an Unsymmetrical Heptadentate N<sub>4</sub>O<sub>3</sub>-Donor Ligand

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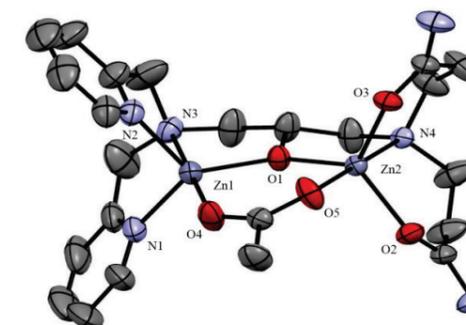
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Phosphatases are enzymes that carry out the hydrolysis of phosphate esters. Some of them show metal ions in the active sites, and, therefore, are called metalloenzymes. Examples of such metalloenzymes include purple acid phosphate, alkaline phosphatase and organophosphate degrading agent [1,2]. The latter show zinc ions in their active sites.

Aiming the development of new mimetic compounds for phosphatase enzymes, we are describing herein the synthesis of a new heptadentate unsymmetrical ligand and its first dinuclear zinc complex. The ligand was built on the 1,3-diaminopropan-2-ol backbone and contains two methylpyridyl and two propylamide groups as pendant arms. Both ligand and complex were characterized by IR, <sup>1</sup>H, <sup>13</sup>C, <sup>15</sup>N NMR. The x-ray molecular structure revealed the formation of a dinuclear zinc complex. Both zinc centres are pentacoordinate, showing a trigonal-bipyramidal geometry. The zinc ions are bridged by an acetate and an alkoxo bridge. Zn(1) is also coordinated by one tertiary amine and two pyridine groups. On the other hand, Zn(2) is coordinated by one tertiary amine and two amide groups. Although the ligand is unsymmetrical, the bond distances around Zn(1) and Zn(2) are very similar. The longest bond distances are Zn(1)-N(3) (2.222(5) Å) and Zn(2)-N(4) (2.186(4) Å), while the shortest ones are Zn(1)-O(1) and Zn(2)-O(2), which show the same bond lengths (1.994(3) Å). The Zn(1)-N(1) and Zn(1)-N(2) bond lengths are 2.034(5) and 2.040(5) Å, while the Zn(2)-O(2) and Zn(2)-O(3) bond distance are 2.024(4) and 2.028(4) Å, respectively. The Kinetic studies carried out at different pH values (6-11) showed that the optimal activity is at pH 9.0, and, therefore, kinetic studies were carried out at this pH employing bis(2,4-dinitrophenyl)phosphate as substrate, at 25°C. The data were fitted to the Michaelis-Menten equation. The kinetic studies resulted in V<sub>max</sub> = 2.52 ± 0.42 x 10<sup>-6</sup> M<sup>-1</sup> min<sup>-1</sup>, K<sub>m</sub> = 5.7 ± 1.6 mM, k<sub>cat</sub> 5.06 s<sup>-1</sup> and k<sub>cat</sub>/K<sub>m</sub> = 0.89 M<sup>-1</sup> min<sup>-1</sup>. This new compound was less active than the symmetrical one previously reported by us [3], which contains four methylpyridil groups attached to 1,3-diaminepropan-2-ol backbone, indicating that the unsymmetrical ligand did not improve the catalytic activity of the zinc complex.

Financial support by the Brazilian agencies CNPq and CAPES is gratefully acknowledged.



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### OP-61

#### An Isoniazid-Conjugated Ruthenium Complex as a New Strategy for Phototherapy

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Candidates for new drugs have faced many drawbacks, particularly due to their toxic side effects, calling for new strategies for selectivity. Among the options available, the use of light as a controlled trigger stands out[1]. Photo-uncaging of compounds have been widely explored with the release of biological active molecules, generation of ROS/RNI species, among others [2-5]. Here, we prepared a metal complex by conjugating a trisbipyridine ruthenium(II) moiety to isoniazid through an acyl hydrazone linkage. Since 50s, isoniazid has been used as the first choice drug in a cocktail for the treatment of tuberculosis[6]. However, isoniazid-resistant strains of *Mycobacterium tuberculosis* have emerged mainly by disrupting isoniazid activation step. This process of enzymatic activation requires an oxidative step with production of a key species, an isonicotinoyl radical [6]. Our proof-of-concept complex was designed to work by photochemically generating a strong oxidative reactive species (singlet oxygen) that would disrupt the acyl hydrazone bond. This process would lead to the formation of the isonicotinoyl radical, bypassing the enzymatic route of activation. Thus, an isoniazid conjugated ruthenium complex was prepared by mixing isoniazid and cis-[Ru(bpy)<sub>2</sub>(mbpy-COH)]<sup>2+</sup> (mbpy-COH = 4'-methyl-2,2'-bipyridine-4-carboxaldehyde), whose product was characterized by HPLC, elemental analysis and mass spectrometry confirming its formulation and purity. Interestingly, this complex was stable in the dark in a broad range of pHs (3-10) for at least 15 h at 25 °C. However, blue light irradiation caused a quick disruption of that linkage originating isonicotinic acid and a trisbipyridine ruthenium(II) derivative complex as final products, as monitored by HPLC and MS. EPR measurements using PBN trap upon blue light irradiation showed a profile consistent to a isonicotinoyl radical. Singlet oxygen production was also measured by using DPBF probe. In addition to that, DNA electrophoresis assay showed a singlet-oxygen-dependent cleavage of DNA. Biological assays were carried out using four bacterial strains (*S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. coli*), whereas no activity was observed in the dark, but blue light irradiation led to a promising microcidal activity (e.g. *S. aureus* +light MIC = 7.6 μmol L<sup>-1</sup>). Cytotoxicity assays also showed low EC<sub>50</sub> (> 50 μg mL<sup>-1</sup>), which increased upon shining blue light only in cancer cell line (LNCAP, prostate adenocarcinoma). Altogether, these results support our approach, validating an acyl hydrazone linkage as a promising strategy to photochemical generation of activated species.

Financial support by CNPq (L. G. F. Lopes 303732/2014-8; E. H. S. Sousa 312030/2015-0, Universal 403866/2016-2) and FUNCAP (PRONEX PR2 0101-00030.01.00/15 SPU N°: 3265612/2015)

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### OP-62

#### Activation of the Class Ib Ribonucleotide Reductase by a Flavodoxin Reductase in *Bacillus cereus*

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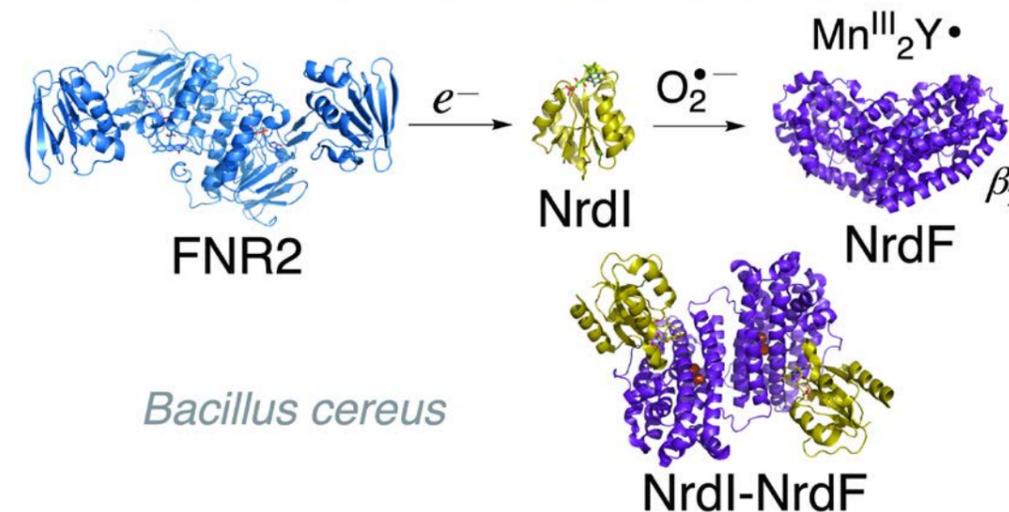
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The ribonucleotide reductases (RNRs) reduce ribonucleotides to deoxyribonucleotides by employing radical chemistry. In class Ib RNRs, reduced NrdI, a flavodoxin-like protein, is essential for the activation of a dimanganese center in the radical generating RNR β-subunit (NrdF) [1,2]. It had been proposed that NrdI is recycled *in vivo* by an NrdI reductase, but no NrdI reductase had been identified.

Ferredoxin/flavodoxin-NADP<sup>+</sup> oxidoreductases (FNRs) are probable reductants of NrdI. We identified three, thioredoxin-like flavodoxin reductases in the genome of *Bacillus cereus* and carried out structural and functional studies in order to characterise their ability to reduce NrdI. Binding studies showed that all three FNRs bind NrdI with similar affinities, however, steady-state kinetics revealed that one FNR reduces NrdI at a much higher rate than the other two FNRs. Using this FNR as an NrdI reductase, we were also able to activate the RNR β-subunit under aerobic conditions, mimicking cellular conditions. Altogether, our observations suggest that this FNR might be the superior NrdI reductase *in vivo* [3].

The *B. cereus* also contains two flavodoxins (Fld). We have investigated the whole FNR-Fld redox network that can be used by several metalloproteins for activation. This has identified that one FNR-Fld pair is more efficient than the others when there are several FNR-Fld pairs available [4].

#### Ribonucleotide reductase class Ib



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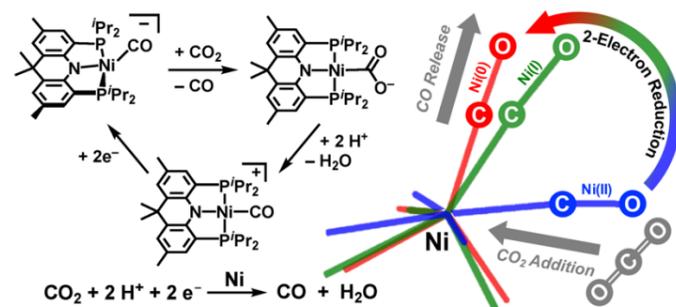
## OP-63

### Selective Transformation of CO<sub>2</sub> to CO at a Single Nickel Center Inspired by CODH

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Carbon dioxide conversions mediated by transition metal complexes continue to attract much attention due to its potential utilization as a C1 source for the future industry. One of the main challenges in transition metal-based CO<sub>2</sub> catalysis is to accomplish the high selectivity in producing a desired product. This may rely on the interaction of CO<sub>2</sub> with a metal center. Given the presence of nickel in natural systems that allow for extremely efficient catalysis, studies that focus on selective CO<sub>2</sub> conversion to CO with synthetic nickel species are currently of considerable interest in our group. The chemistry is inspired by an efficient enzymatic CO<sub>2</sub> catalysis occurring at the active site of the carbon monoxide dehydrogenase (CODH). Since the binding and reactivity toward CO<sub>2</sub> is controlled in part by the geometry of a L<sub>3</sub>Ni scaffold, we have explored the chemistry of low-valent nickel supported by pincer systems (E = P or N), in which a pseudo-tetrahedral or square planar geometry is accommodated. The central donor atom is P for a PP<sup>Me</sup>P ligand (PP<sup>Me</sup>P = P<sup>Me</sup>[2-P<sup>i</sup>Pr<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>]<sub>2</sub>) and N for PNP (PNP<sup>-</sup> = N[2-P<sup>i</sup>Pr<sub>2</sub>-4-Me-C<sub>6</sub>H<sub>3</sub>]<sub>2</sub><sup>-</sup>) and <sup>acri</sup>PNP ligands (<sup>acri</sup>PNP<sup>-</sup> = 4,5-bis(diisopropylphosphino)-2,7,9,9-tetramethyl-9H-acridin-10-ide). Two isolated nickel-CO<sub>2</sub> adducts, (PP<sup>Me</sup>P)Ni(η<sup>2</sup>-CO<sub>2</sub>-κC) and {Na(12-C-4)<sub>2</sub>}{(PNP)NiCO<sub>2</sub>}, demonstrate that the geometry of a nickel ion is crucial in the binding of CO<sub>2</sub> and its level of activation. In the case of a square planar nickel center, a series of bimetallic metallacarboxylate Ni-μ-CO<sub>2</sub>-κC, O-M species (M = H, Na, Ni or Fe) were synthesized and studied. Protonation cleaves the C-O bond in the nickel(II)-carboxylate species resulting in the formation of a nickel(II) monocarbonyl complex. By sequential reduction, the corresponding mono- and zero-valent Ni-CO species were generated. In particular, a (PNP)Ni(0)-CO species shows immediate reactivity toward CO<sub>2</sub> but displays the formation of multiple products. With a structurally rigidified <sup>acri</sup>PNP ligand, the Ni(0)-CO species reveals the selective addition of CO<sub>2</sub> to give a nickel(II)-carboxylate species with the expulsion of CO. The closed synthetic cycle for CO<sub>2</sub> reduction to CO was finally established with a (<sup>acri</sup>PNP)Ni system.



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## OP-64

### Investigating the Catalytic Promiscuity of Metallohydrolases and Metallohydrolase Mimics

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Metalloenzymes are biological catalysts responsible for carrying out critical chemical reactions in the cell. Usually, metalloenzymes are highly selective in their reactivity. However, some metalloenzymes are catalytically promiscuous and are capable of catalyzing several different reactions. While numerous synthetic mimics of metalloenzymes active sites have been developed and their ability to catalyze biomimetic reactions investigated, the scope of their catalytic promiscuity has often not been extensively characterized. In order to obtain greater insight into the catalytic promiscuity of metalloenzymes and their synthetic mimics, we have assembled a panel of fluorogenic substrates and profiled the hydrolase activity of a series of metalloenzymes and metalloenzyme mimics against the panel. Using this approach, we find that, for example, a zinc-binding three-helix bundle peptide designed as a mimic of carbonic anhydrase also exhibits protease activity on a small peptide substrate. We anticipate that the approach outlined here will be useful for investigating the catalytic activities of a wide variety of metallohydrolase mimics.

### OP-65

#### New Half-Sandwich Metal Complexes as Proteosynthesis Inhibitors in Cancer Cells

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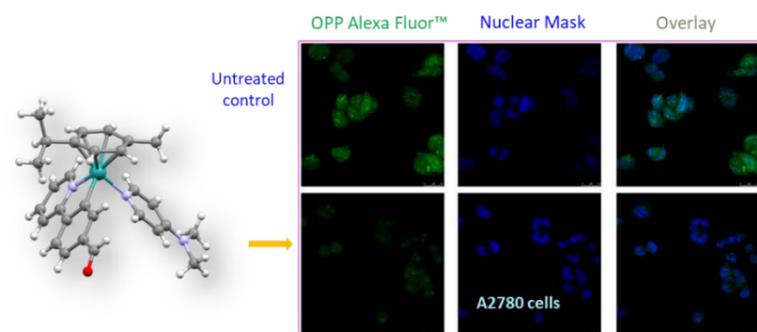
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In spite of the huge advancement achieved in drug research and technology development for cancer treatment during the past decades, cancer is currently the second leading cause of death, imposing a huge socio-economic burden on humankind. Due to their high growth rate, cancer cells are exposed to a constant demand for newly synthesized proteins, which are required for proliferation. Translation, one of the most energy-consuming activities within the cell, is the central regulator process that permits gene expression and the overproduction of the translation apparatus is commonly associated with tumorigenesis.<sup>1,2</sup> Half-sandwich ruthenium(II) and osmium(II) complexes  $[(\eta^6\text{-p-cymene})\text{M}(\text{C}^{\wedge}\text{N})(\text{X})]^{0/+}$  (M= Ru, Os; X = Cl, py or 4-NMe<sub>2</sub>-py) have been synthesized to achieve selective cytotoxicity to cancer cells, some of the compounds acting as proteosynthesis inhibitors; this is a new mode of action for half-sandwich metal complexes.<sup>3</sup>

Financial support by the Spanish Ministry of Economy and Competitiveness and FEDER funds (Project CTQ2015-64319-R) and Fundación Séneca-CARM (Projects 20277/FPI/17 and 20857/PI/18) is gratefully acknowledged.



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### OP-66

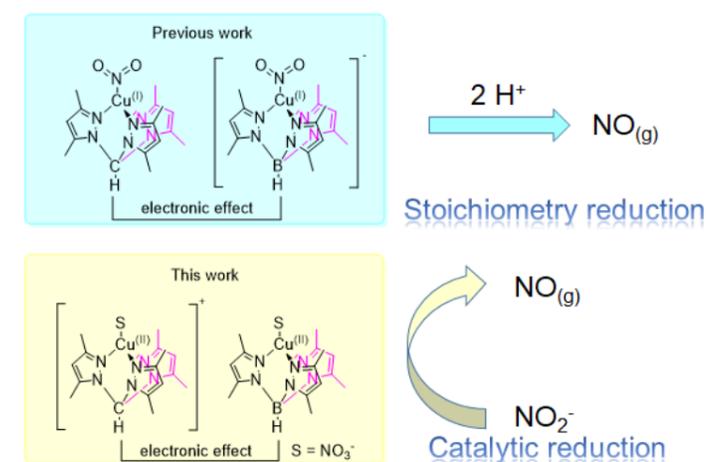
#### Bio-Inspired Copper Nitrite Complexes for Understanding the Nitrite Reduction of Copper Nitrite Reductase

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Copper-containing nitrite reductases (Cu-NIRs) catalyze the reduction of nitrite to nitric oxide (NO), a key step in denitrification. Due to the complexity of the Cu-NIRs enzyme structures, chemists have tried to use some relatively simple ligands to model the environment of a type 2 copper center. Because of the proposed key role they play in catalysis by the Cu-NIRs, copper(I)-nitro or -nitrito species have been important targets of structural and chemical studies. In order to understand how electronic issue will affect the nitrite reduction ability in the bio-inspired copper(I)-nitro model of Cu-NIRs, we first reported and examined the electron-rich anionic  $[\text{Tp}^{\text{Me}_2}\text{CuNO}_2]$  complex which is more effective than neutral  $[\text{Tp}^{\text{Me}_2}\text{CuNO}_2]$  analogue in nitrite reduction.[1] However, the detail mechanism of copper mediated nitrite reduction still remains unknown. In order to understand the catalytic mechanism of nitrite reduction, a series of copper complexes have been synthesized as potential catalytic agents.[2] Initial catalytic investigations reveal the binding mode of key Cu(I)-NO<sub>2</sub> intermediate may exist as nitrito binding rather than nitro binding.

Financial supports by the Ministry of Science and Technology of Taiwan and Kaohsiung Medical University is gratefully acknowledged.



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OP-67

### Tuning Redox Potentials of the FeS-Cluster Chain in the Cyanobacterial Uptake Hydrogenase Small Subunit (HupS)

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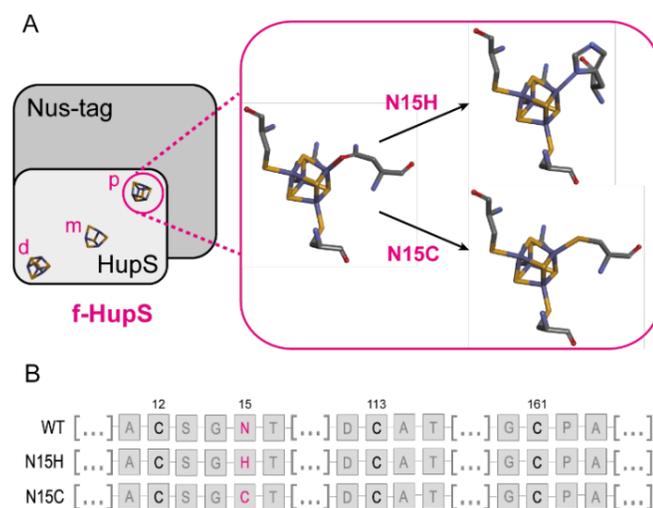
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The filamentous cyanobacterium *Nostoc punctiforme* (*N. punctiforme*) is used as a model system for developing photobiological production of hydrogen and other products, using solar energy. *N. punctiforme* expresses the NiFe uptake hydrogenase HupSL under nitrogen-fixing conditions. HupSL is composed of two subunits: HupL, containing the NiFe active site, and HupS, comprising three iron-sulfur (FeS) clusters [1,2]. HupS possesses unique non-cysteiny FeS coordinating amino acids in both the distal and proximal [4Fe-4S] clusters: Asn in the proximal cluster and Gln in the distal one. The effect of these unusual motifs on the properties of the clusters is to this date unknown.

We have previously isolated and characterized HupS as the fusion protein f-HupS by expression in *E. coli* [1]. By modifying the proximal [4Fe-4S] cluster by introducing the designed mutation C12P, we found a new paramagnetic species at the proximal cluster site consistent with a [4Fe-4S] to [3Fe-4S] cluster conversion [2]. When C12P-HupSL was expressed in *N. punctiforme*, the strain had a consistently higher H<sub>2</sub> production than the background  $\Delta$ hupSL mutant. We concluded that the increase in H<sub>2</sub> production was due to the modification of the proximal iron-sulfur cluster, leading to a turn of the electron flow in the enzyme [2].

In the present work, we replaced Asn15 with histidine and cysteine, respectively. Electron paramagnetic resonance (EPR) spectroscopy of the modified N15H and N15C versions of f-HupS shows that while all three [FeS] clusters are present in the protein, the proximal cluster has acquired a different structure. To assess the reduction potentials of the FeS clusters in these mutant variants, we used protein-film voltammetry. The cyclic voltammogram, in a span of -600 – +200 mV, showed two electrode reactions close to 0 V vs. SHE. While we reproducibly detected the reduced [4Fe-4S] clusters by EPR spectroscopy, we did not record any currents at more reducing potentials. The voltammogram for the N15H mutant displayed a shift of the oxidative peak current, indicating that this mutation had an effect on electron transfer in f-HupS.

By engineering the electron transfer chain in HupSL it is possible to tune the redox potentials of the three FeS clusters in the small subunit. Our aim is to understand and control the direction of the electron transfer to and from the active site, in order to design and create robust H<sub>2</sub>-producing cyanobacterial strains.



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OP-68

### Interaction of GLDA with Molybdenum(VI) at Different Ionic Strengths

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The biodegradable ligand, L- glutamic acid N,N-diacetic acid tetrasodium salt (GLDA) is a green, safe and readily biodegradable and strong chelating agent that can be used as alternative for conventional complexing agents. GLDA is the only complexing agent with green carbon atoms which is due to the biobased source of the carbon atoms. It is used in different areas of applications such as food preservation, oil industry, industrial cleaning, metal plating and electronics, agriculture, gas sweetening, building and construction, food fortification, cleaning and detergents, feed additives. In this work the results of the study on the interaction of GLDA with molybdenum (VI) were reported. This metal is essential for nitrogen fixation, normal growth and health of animals, plants and microorganisms. It is a key component of the active site in an extensive range of enzymes that catalyse important reactions in the carbon, nitrogen, sulphur, selenium and arsenic cycles of the biosphere. Human metabolism involves several molybdenum enzymes, making it an essential trace element in our diet.

In particular the interaction of GLDA with molybdenum (VI) was studied at pH = 6.00,  $T = 298.15 \text{ K}$ , and different ionic strengths ( $0.1 < I / \text{mol} \cdot \text{dm}^{-3} < 2.5$ ) of sodium chloride by using UV spectrophotometric method. Experimental data were modelled according to the extended Debye-Hückel and specific ion interaction models and for the formation constant ( $\text{MoO}_3\text{GLDA}^{4-}$ ) in pure water we found a value of 18.96 in the molal concentration scale. Also a case study has been done in which the concentration of  $10^{-6} \text{ mol} \cdot \text{dm}^{-3}$  has been used for the modelling of the molybdenum speciation in urine. [1] When we consider the simultaneous presence of Mo (VI) ( $0.000001 \text{ mol} \cdot \text{dm}^{-3}$ ) and GLDA ( $0.000001 \text{ mol} \cdot \text{dm}^{-3}$ ) in the urine model at pH = 6, we have the 100% of the Mo(VI) complexed as ( $\text{MoO}_3\text{GLDA}^{4-}$ ) species. [1] Only few data of formation constants with molybdate are present for some aminopolycarboxylates. A correlation between  $n_N^* n_{\text{COOH}}$  involved in the protonation with the formation constants (ionic strength 0.1-0.2  $\text{mol} \cdot \text{dm}^{-3}$ ) of ligands-Mo(VI) was done and a fairly good correlation  $Y = 17.95 + 0.2083 \cdot X$  with a  $R^2 = 0.9213$ , ( $Y$  and  $X$  represents the values of formation constants and  $n_N^* n_{\text{COOH}}$  respectively) was found. [1] If we apply the correlation for instance in our case with GLDA, we found a value of 18.78 (at  $\sim 0.1 \text{ mol} \cdot \text{dm}^{-3}$ ) that is completely acceptable with the experimentally found value 18.38. [1]

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OP-69

### Enzyme-Inspired Synthetic Auxiliary Proton Channel Enhances Electrocatalytic H<sub>2</sub> Production of Cobaloximes in Water

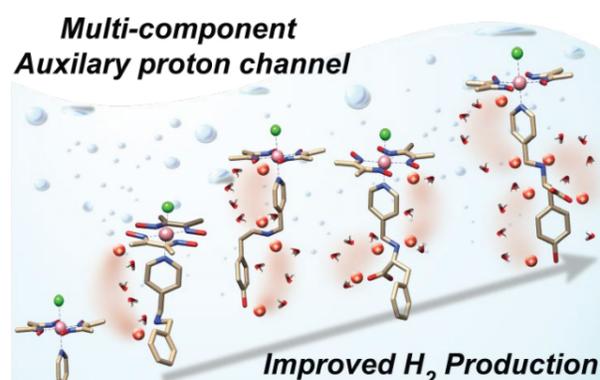
Arnab Dutta<sup>1</sup>, Dependu Dolui<sup>1</sup>, Shikha Khandelwal<sup>1</sup>, Althaf Shaik<sup>1</sup>, Vijay Thiruvengadam<sup>2</sup>

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The Cobaloxime complexes containing axial pyridines typically exhibit moderate H<sub>2</sub> production catalysis. The hydrogen-bonded oxime-functionality present in the ligand framework acts as the primary proton channel to tune its catalytic reactivity. However, this proton channel collapses under acidic conditions (pH < 5) and limits the H<sub>2</sub> production by Cobaloximes only under neutral condition (pH ~7) in water. Here, in this work, we have included variable combinations of peripheral protic groups (such as secondary amine, carboxylic acid, and phenolic-OH) around the same Cobaloxime core that were appended through an axial pyridine ligand. The strategic incorporation of these protic groups surrounding the cobalt center significantly improved (~2.0-9.5 times) their electrocatalytic H<sub>2</sub> production while expanding the active chemical space for H<sub>2</sub> production even into the acidic aqueous conditions (pH 3-7). The three dimensional crystallographic data along with the detailed one- and two-dimensional NMR studies have revealed the formation of an intricate hydrogen-bonding network between the peripheral basic functionalities and specifically positioned water and chloride molecules. This hydrogen bonding nexus generated a water mediated auxiliary proton channel around the Cobaloxime skeleton to play a pivotal role in their significantly improved catalytic activity. These results highlight that the catalytic activity of the artificial catalysts can be fine-tuned by the proper incorporation of enzyme-inspired, outer coordination sphere features around a synthetic catalyst framework.

Financial supports by IIT Gandhinagar and DST-SERB are gratefully acknowledged.



OP-70

### Discovery of $\beta$ -Carboline Copper(II) Complexes as Novel Mcl-1 Inhibitors and *In Vitro* and *In Vivo* Activity in Cancer Models

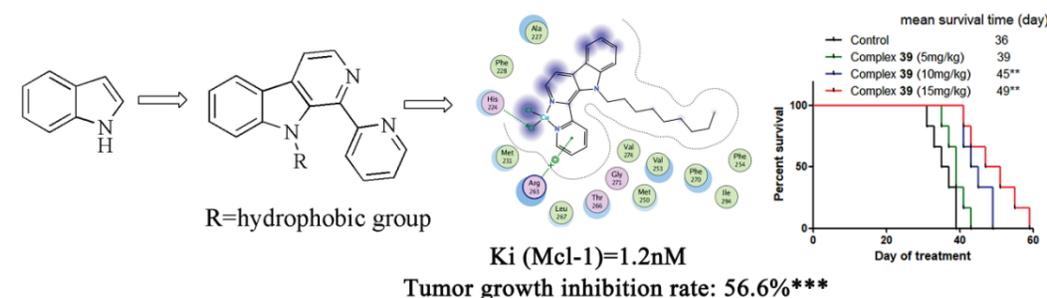
Xing Lu<sup>1</sup>, Zhen-Feng Chen<sup>1</sup>, Yan-Cheng Liu<sup>1</sup>, Chris Orvig<sup>2</sup>, Hong Liang<sup>1</sup>

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Mcl-1 (myeloid cell leukemia 1) is an anti-apoptotic member of Bcl-2 family of proteins [1,2]. It plays an important role in the survival of a variety of cancer cells. However, the development of inhibitors of Mcl-1 has been challenging. Up to now, no metal-based Mcl-1inhibitor has been reported [3-5]. To develop metal-based Mcl-1inhibitors, twenty three 9-substituted  $\beta$ -carboline derivatives **2–24** were designed and synthesized. In addition, twenty two corresponding copper(II) complexes **25–46** were synthesized and characterized. Complexes **38** and **39** showed higher cytotoxicity than the corresponding ligands or cisplatin *in vitro*. The most potent complex **39** selectively inhibited Mcl-1 with and killed tumor cells by Bax/Bak mediated apoptosis. Complex **39** showed a profile for selectivity binding to Mcl-1 with 795-fold versus Bcl-2, 698-fold versus Bcl-x1, 520-fold versus Bcl-w, and 356-fold versus A1/Bfl-1, respectively. Complex **39** showed an excellent safety profile in mouse model. Moreover, complex 39 significantly inhibited the tumor growth in NCI-H460 tumor bearing model with an inhibitory rate of 56.6% (P<0.001). Complex **39** prolonged the survival time of the tumor bearing mice. Thus, complex 39, the first metal-based Mcl-1 inhibitor, may have the potential to be further developed as an antitumor agent with high efficacy and low toxicity.

Financial support by the National Natural Science Foundation of China (Grants 81473102, 21431001), IRT\_16R15, Natural Science Foundation of Guangxi Province (Grant2016GXNSFGA380005), and Innovation Project of Guangxi Graduate Education (YCBZ2017029) as well as “BAGUI Scholar” program of Guangxi Province of China, is gratefully acknowledged.



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OP-71

### Photodecarbonylation and *In Vitro* Studies of Dicarbonyl Ruthenium Complexes

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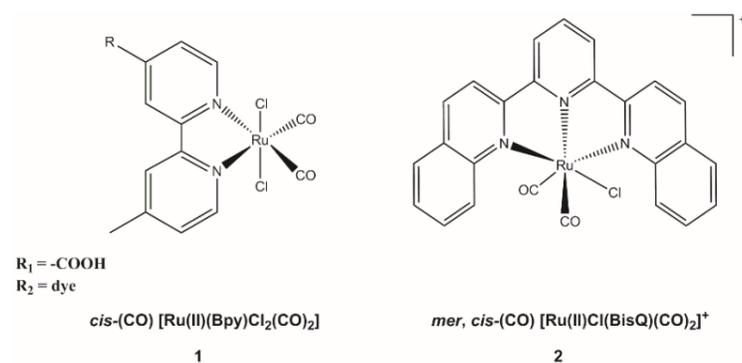
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Carbon monoxide has been demonstrated to exhibit several beneficial effects on biological targets (anti-inflammatory, anti-proliferative, anti-apoptotic effects, causes vasodilation, etc.).<sup>1</sup>

Consequently, the development of CO releasing molecules (CORMs) that allows a controlled release of CO under physiological conditions has therefore become a major field of scientific and medical interest.<sup>2</sup> Considerable research interest has been drawn on light-activated CORMs (photoCORMs) which only release CO upon radiation with certain wavelengths. However, despite a large number of photoCORMs reported, relatively little information is available on the precise mechanism of CO release from most photoCORMs and even less compounds have been tested as anti-cancer agents in cells so far.

Herein, we report the synthesis of ruthenium(II) carbonyl complexes functionalized with (fluorescent) bidentate pyridyl (**1**) and tridentate diquinolyl ligands (**2**) and investigate the mechanism of CO release in aqueous media (before and after light-activation). The photo-induced CO release kinetics of the Ru(II) photoCORMs, as well as *in vitro* studies in cancerous and healthy cell lines will be presented [3].

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OP-72

### Detection and Characterization of a Novel Copper-Dependent Intermediate in a Lytic Polysaccharide Monooxygenase

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Lytic polysaccharide monooxygenases (LPMOs) are copper-containing enzymes capable of oxidizing crystalline cellulose and the enzyme has large practical application in the process of refining biomass. Crystal structures have shown the presence of copper(II) ion in a characteristic flat, solvent exposed, active-site in the AA9 family of LPMOs [1].

The function of LPMO has been the subject of debate. In the established model, this class of enzymes was considered to be monooxygenases. However, this view is now challenged by new data indicating that both O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> may function as co-substrates [2].

Here, we report a long-lived intermediate ( $t_{1/2} = 6 - 8$  minutes) observed in an LPMO from *Thermoascus aurantiacus* (TaLPMO9A). The intermediate has a strong absorption peak around 420 nm and is formed when reduced LPMO-Cu(I) reacts with H<sub>2</sub>O<sub>2</sub>. UV-vis absorption spectroscopy, electron paramagnetic resonance (EPR), and stopped-flow spectroscopy indicate that the observed long-lived intermediate involves the copper site and a nearby tyrosine (Tyr175). Addition of sub-equimolar amount of H<sub>2</sub>O<sub>2</sub> to reduced TaLPMO9A boosts oxidation of phosphoric acid swollen cellulose which suggests that the long-lived copper-dependent intermediate is part of the catalytic mechanism for LPMOs.

We propose that the reaction with H<sub>2</sub>O<sub>2</sub> first generates a highly reactive short-lived Cu(III)-intermediate which is subsequently transformed into the observed long-lived copper-dependent intermediate. The proposed mechanism offers new perspectives in the oxidative reaction mechanism of copper enzymes and hence for the biomass oxidation and the reactivity of copper in biological systems.

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### OP-73

#### Facile Oxidative O-Demethylation Reaction by Iron(II) Complexes of *o*-Anisole-Appended Bis(2-picoly)amine Ligand

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Heme and nonheme iron monooxygenases enzyme active sites catalyse the oxidative N-dealkylation of O-demethylation of organic substrates by employing dioxygen as oxidant [1]. Model studies to elucidate mechanistic features and development of catalysts have been considerably explored [2]. High-valent oxo-iron species have been invoked as the key intermediates in heme systems. Analogous studies with nonheme iron model complexes are limited and those that effect O-demethylation of aryl methyl ethers are rare [3]. We have recently reported [4] a facile and selective O-demethylation of anisole-append at the iron(II) centre of complex, [Fe(L-OMe)Cl<sub>2</sub>] (**1**) (where L-OMe = *o*-[bis(2-picoly)amino]anisole) using *t*-BuO<sub>2</sub>H as co-oxidant [1]. It provided a rare terminal phenoxide-ligated mononuclear iron(III) complex, [Fe(L-O)Cl<sub>2</sub>] (**2**) and oxo-bridged unsymmetrical diiron(III) complex, [Fe(L-OMe)Cl-O-(FeCl<sub>3</sub>)](FeCl<sub>4</sub>)<sub>2</sub> (**3**) with no change in the ligand structure. In contrast, an analogous reaction with O<sub>2</sub> has furnished only the usual oxo-bridged symmetrical diiron(III) complex. The structural, spectroscopic and electrochemical studies of these complexes have been studied. The results of these investigations along with a plausible mechanism for the oxidative demethylation reaction will be highlighted in this seminar.

Financial support by the IIT Madras is gratefully acknowledged.

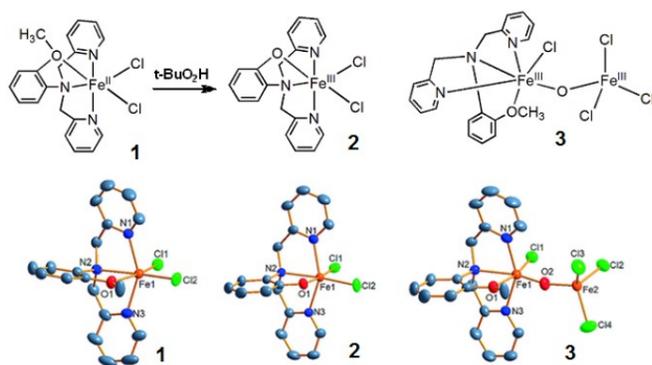


Figure 1. Iron(II) complex used and oxidation products studied

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### OP-74

#### Field- and Frequency-Domain Paramagnetic Resonance Spectroscopic and Computational Studies of Highly Oxidized Iron Complexes of Biological Relevance

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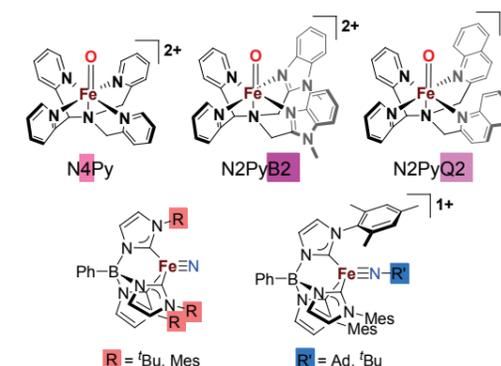
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<sup>6</sup>Helmholtz-Zentrum für Materialien und Energie, Berlin, D-12489, Germany.

Oxidoiron(IV) (ferryl) intermediates are involved in the catalytic cycles of non-heme iron oxygenases.[1] Formally Fe(IV) (3d<sup>4</sup>) species can exist in both spin triplet (*S* = 1) and quintet (*S* = 2) ground states. While the majority of oxidoiron(IV) model complexes are characterized in the *S* = 1 ground spin state, many oxidases have been shown to employ only the *S* = 2 ground spin state ferryl motif. The different spin ground and excited states have been implicated in the reactivity of both enzymes and model compounds.[2] High oxidation state iron complexes with nitrogen donor ligands, nitrido (N<sup>3-</sup>) and imido (NR<sup>2-</sup>) are also of interest, which are relevant both to the nitrogenase enzyme and to bio-inspired small molecule activation in general.[3,4]

We describe advanced spectroscopic studies on oxido complexes of Fe(IV) supported by pentadentate nitrogen (monoamino/tetraimino) donor ligands, and on nitride/imido complexes of Fe(IV) supported by tridentate carbon donor (imidazol-2-ylidene – “NHC”) ligands. These complexes are shown in the scheme below, wherein the top row depicts the oxido and the bottom row the nitrido/imido complexes.



Both field-domain, high-frequency and -field electron paramagnetic resonance (HFEP) and frequency-domain, far-infrared magnetic resonance (FIRMS) spectroscopic techniques are employed, which provide the complete set of spin Hamiltonian parameters for these integer spin systems, which are essentially “silent” to conventional field and frequency EPR methods. This information is used, in conjunction with quantum chemical theory, to understand the electronic structure of these complexes in relation to their molecular structure and reactivity, in particular, the role of ground and excited spin states. Financial support by U.S. National Science Foundation (CHE-1665391 to L.Q.) and the U.S. Department of Energy – Basic Energy Sciences (DE-FG02-08ER15996 to J. M. S.) is gratefully acknowledged. The NHMFL is supported by NSF through Cooperative Agreement DMR-1644779 and the State of Florida. The computational studies were supported by the Slovak Grant Agency VEGA (contract no. 1/0598/16).

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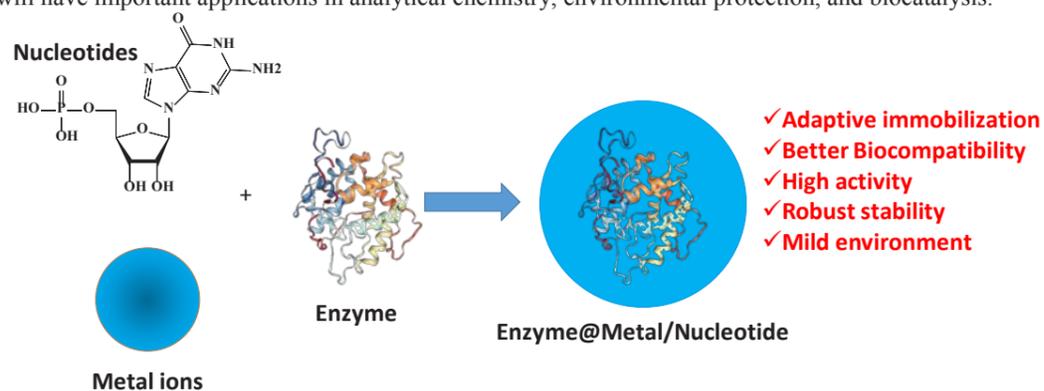
OP-75

### Nucleotide Coordination Polymers as a Potential Platform for Immobilizing Enzymes with High Activity and Robust Stability

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Biocoordination polymers (BCPs), formed by metal ions and bridging bio-organic ligands, have recently received considerable attention and are considered as new functional composite materials. Not only as the constituents of nucleic acids, nucleotides can be used to construct metal-coordination complexes with adaptive inclusion ability and high biological compatibility, and they can also regulate the morphologies, properties and functions of supramolecular assemblies and nanomaterials as modulators. Because of their mild polymerization conditions, porosity, and high guest entrapment efficiency, nucleotide-based BCPs could play important roles in enzyme catalysis and immobilization. We tested different metal ions with different nucleotides, and different morphology and structure of metal-nucleotide nanomaterials could be obtained by selecting various nucleotides and controlling the reaction condition. The nucleotide-based BCPs showed a good adaptive encapsulating ability by encapsulating a diverse range of guests including water-soluble small dyes, proteins, and nanoparticles. Furthermore, we used the nucleotide-based BCPs to immobilize some enzymes, such as SAM synthetase, GOx, HRP, CPO and CRL. It displayed high catalytic efficiency, high selectivity and enhanced stability due to the protecting effect of the framework. As a result, the relative activity of some immobilized enzymes increased more than 10-fold compared to free enzyme. In summary, the nucleotide-based BCPs will have important applications in analytical chemistry, environmental protection, and biocatalysis.



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OP-76

### Mechanism of P450nor-Catalyzed NO Reduction Proved by Time-Resolved Spectroscopic and Crystallographic Analyses

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Cytochrome P450nor from *Fusarium oxysporum* is a member of heme-thiolate containing P450 enzymes. Although P450 enzymes generally catalyze a monooxygenation of wide variety of substances including hydrocarbons, steroid hormones and fatty acids, P450nor catalyzes reductive coupling of two nitric oxide (NO) molecules using NAD(P)H to produce nitrous oxide (N<sub>2</sub>O) according to the following equation; 2NO + NADH + H<sup>+</sup> → N<sub>2</sub>O + H<sub>2</sub>O + NAD<sup>+</sup>. The mechanism of NO reduction by P450nor is proposed to consist of three processes. First NO molecule binds to resting ferric P450nor to form a NO-bound form. Subsequent hydride transfer from NADH produces reaction intermediate called intermediate *I*. Finally, second NO molecule attacks the protonated NO ligand in intermediate *I* to produce N<sub>2</sub>O and ferric resting enzyme. To completely understand the reaction mechanism, it is necessary to elucidate the atomic structure and the electronic state of each intermediate.

In this study, we applied recently developed time-resolved (TR) X-ray crystallography using X-ray free electron laser and TR spectroscopy to structural characterization of the reaction intermediates in the P450nor-catalyzed NO reduction. For the pump-probe experiments, we utilized a photosensitive caged-NO compound (BNN5a) which quantitatively releases NO within μs time domain upon UV illumination [1] as a trigger for the photo-induced reaction and a NO source for the P450nor-catalyzed reaction [2]. TR visible absorption measurements showed that the UV illumination to the crystalline P450nor soaked with caged-NO and NADH could induce the formation of the NO-bound form at ~10 ms time domain and the formation of intermediate *I* at ~s time region. With use of this reaction system, we carried out TR serial femtosecond crystallography (SFX) in which micro-crystals are continuously supplied to the XFEL irradiation spot, and solved the structure of the NO-bound form during the enzymatic reaction. The structure indicated the slightly bent conformation of the FeNO unit in the NO-bound form [2]. Since the formation of intermediate *I* in micro-crystals required ~5 s, we performed freeze-trap method to characterize this species. The structure of intermediate *I* which was determined by fixed-target SFX showed that the FeNO unit would be highly bent conformation. In order to get more information on the electronic structure of each intermediate, we measured TR infrared (IR) spectra of P450nor since the NO stretching frequency (νNO) is sensitive to the structure of the FeNO unit. In the TR-IR spectra, formation of the νNO at 1330 cm<sup>-1</sup> with decay of νNO at 1852 cm<sup>-1</sup> of the NO-bound form was observed, indicating that the band at 1330 cm<sup>-1</sup> is assignable to the νNO of intermediate *I*. This observation suggests that a Fe<sup>3+</sup>-NHO species is possible state for intermediate *I*. On the basis of current finding, we will discuss possible mechanism of NO reduction by P450nor.

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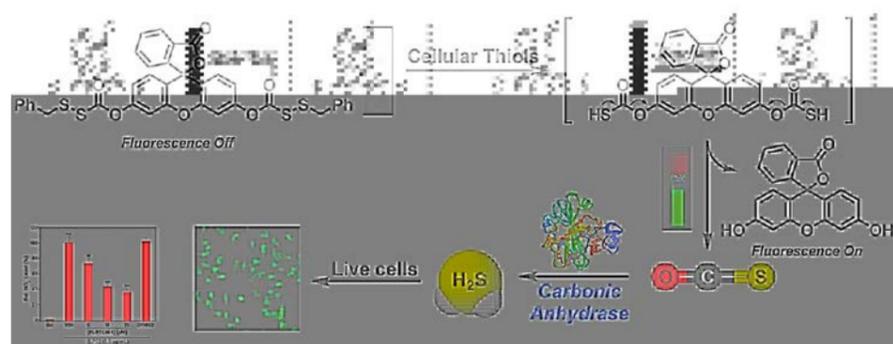
OP-77

## Chemical Tools for Detection and Delivery of Biological Reactive Sulfur Species

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Reactive sulfur species, such as H<sub>2</sub>S and sulfane-sulfur compounds, play key roles in different (patho)physiological processes.<sup>1</sup> Aligned with this importance, our lab has recently developed a palette of new fluorescent reporters and responsive donors motifs for H<sub>2</sub>S and related reactive sulfur species. One key strategy in the development of these compounds focuses on the release carbonyl sulfide (COS), which is quickly converted to H<sub>2</sub>S by the ubiquitous enzyme carbonic anhydrase (CA). This design strategy enabled the first examples of analyte-replacement fluorescent probes for H<sub>2</sub>S,<sup>2</sup> which we further leveraged to development of responsive COS/H<sub>2</sub>S donors. In these systems, the donor is activated or triggered by specific stimuli, such as reactive oxygen species,<sup>3</sup> thiols,<sup>4</sup> pH changes,<sup>5</sup> enzymes,<sup>6</sup> etc. We have also developed both colorimetric<sup>4</sup> and fluorescent<sup>3</sup> COS/H<sub>2</sub>S donor motifs, which enable real-time tracking of H<sub>2</sub>S by fluorescence microscopy. This presentation will focus on recent sensor and donor constructs developed in our lab, cytoprotective effects of developed motifs, and new traceable donors that enable H<sub>2</sub>S donation to be monitored directly.



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OP-78

## Instrumentation and Measurement Development for Electron Paramagnetic Resonance (EPR) Spectroscopy of Biomacromolecular Systems

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Recent advances in nanofabrication and biotechnology rely on biomacromolecules, which are often used to marry bottom-to-top self-assembly with top-down lithographic methods. In biotechnology, biomacromolecules are either themselves therapies (monoclonal antibodies) or are used in combination with nanoscale drug delivery vectors. Structural measurements on biomacromolecules enable design and engineering of robust, reliable systems. Electron paramagnetic resonance (EPR) spectroscopy is a structural biology method that is particularly powerful for nanoscale systems lacking long-range (crystalline) order, a regime in which many biomacromolecules fall. We present an overview of our work at NIST to advance pulsed EPR measurements on biomacromolecules, ranging from synthetic model systems to biomacromolecular complexes. This presentation highlights our work to measure distances between cationic copper porphyrins bound to guanine quadruplex DNA structures of increasing length<sup>1</sup> as well as our newest efforts in instrumentation development.<sup>2</sup>

Financial support from the Cooperative Research Agreement between the University of Maryland and the National Institute of Standards and Technology Center for Nanoscale Science and Technology, Award 70NANB10H193 (MPD, NA, AA), the Summer High School Intern Program at NIST (SM), NIH P41 EB002034 (BE), and NIH R50 CA211408 (BE) are gratefully acknowledged.

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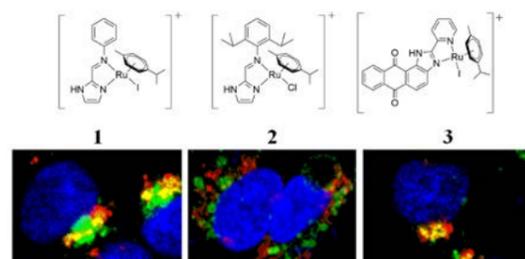
### OP-79

#### ATP7B Mediated Transport of Ru<sup>II</sup>-*p*-Cymene Complexes: Effect of Steric Hindrance and the Halide Coordination in Controlling the Efflux

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The resistance against chemotherapeutic drugs (viz. cisplatin, carboplatin, oxaliplatin) has been assigned to various factors including binding to thiols and efflux proteins (viz. Pgp, MDRs, ATPases) leading to the excretion from cells. The ATPase proteins ATP7A and ATP7B are among those held responsible for the efflux of platinum drugs outside cell and mediate resistance. It is known that both the proteins have more than 50% sequence identity and in response to cisplatin accumulation in cancer cells mobilizes from the golgi towards the membrane upon binding cisplatin to their thiol containing motifs CXXC. [1-2] There are reports which states that there is more accumulation of Pt drugs in cells overexpressing ATP7A or 7B but the toxicity is reduced due to vesicles formed upon the binding of the ATPases with Pt drugs. These vesicles isolate the drugs in compartments inside cells thus prohibiting them from acting on their main target DNA. Ru(II/III) complexes has emerged as excellent candidates against cisplatin resistant metastatic cancer cells as per *in vitro* and *in vivo* studies. The charge/radius ratio and the known interaction of Ru(II) with thiols suggests that Ru(II) may be equally or more susceptible to binding by the CXXC motif of the ATPases. In the proposed presentation I would discuss our progress in this regard and also disseminate how the Ru coordination atmosphere and steric hindrance around the Ru(II) centre plays a role in inhibiting the transport. Recently it was shown that Ru uptake by ATP based transporters may be affected by depletion of ATP and this may have dependence on the Ru(II) coordinated halide.[3] Here in we show that ATP7B is a potential transport protein that can deactivate and transport Ru(II) anticancer agents or it can isolate them in vesicles depending on the type of complexes and stop them from acting against their targets in cell. The complexes with variation in steric hindrance and the coordinated halide **1**, **2** and **3** [4,5] when studied for transport by ATP 7B we found that the highest transport was for the most sterically hindered chloro coordinated **2**. In contrast, **1** and **3** were quite resistant to efflux by ATP 7B. The results would be discussed in the presentation.



**Figure 1.** (A) Chemical structure of **1-3**. (B) ATP7B mobilization in HepG2 cells in response to the drug, yellow fluorescence demonstrates co-localization of ATP7B (green) in golgi (red).

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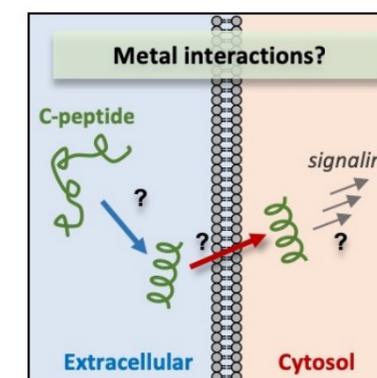
### OP-80

#### Investigating the Dependence of Proinsulin C-Peptide on Metal Micronutrients

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C-peptide is a 31-residue bioactive peptide of growing interest for its potential therapeutic benefits. It is generated as part of proinsulin, the precursor to the hormone insulin, which regulates glucose levels in the bloodstream. In proinsulin, C-peptide connects the A- and B-chains of insulin and is cleaved in the secretory vesicles during the maturation of insulin. As such, it is released in equimolar amounts of insulin by the pancreas. While initially believed to be biologically inert, increasing studies suggest the potential for therapeutic applications of C-peptide in ameliorating illnesses such as kidney disease and diabetes. Further works reaffirm the beneficial role of C-peptide in diabetic patients, when coupled with metal ions such as Zn(II), Fe(II) and Cr(III), however the molecular mechanisms that govern these metal-dependent activities remain unclear. In addition, C-peptide has also been shown to internalize homogeneously into cells and may interact with proteins located in the cytoplasm. To this end, we sought to untangle the metal-mediated functions of C-peptide behind these interactions. Specifically, we used spectroscopic techniques to assess and understand binding properties between C-peptide and transition metal ions and the effect on the structure of C-peptide after binding. In addition to the metal-bound characterization of C-peptide, we determined the effects that metal ions have in either facilitating or inhibiting cellular internalization. Taken together, these results suggest that metal ions mediate structural changes and the internalization of C-peptide, thereby giving the peptide its biological activity. Financial support by the University of California, Department of Chemistry is gratefully acknowledged.



## OP-81

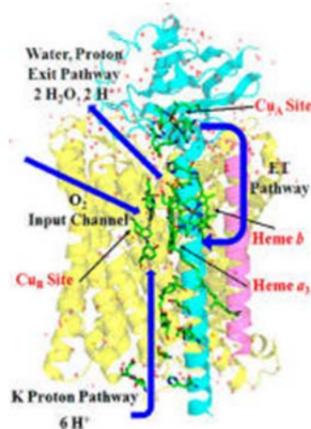
### Discrete Ligand Binding and Electron Transfer Properties of *ba*<sub>3</sub>-Cytochrome *c* Oxidase from *Thermus thermophilus*: Evolutionary Adaption to Low Oxygen and High Temperature Environments

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Cytochrome *c* oxidase (CcO) couples the oxidation of cytochrome *c* to the reduction of molecular oxygen to water and links these electron transfers to proton translocation. The redox-driven CcO conserves part of the released free energy generating a proton motive force that leads to the synthesis of the main biological energy source ATP. Cytochrome *ba*<sub>3</sub> oxidase is a B type oxidase from the extremely thermophilic eubacterium *Thermus thermophilus* with high O<sub>2</sub> affinity, expressed under elevated temperatures and limited oxygen supply and possessing discrete structural, ligand binding, and electron transfer properties. The origin and the cause of the peculiar, as compared to other CcOs, thermodynamic and kinetic properties remain unknown. Fourier transform infrared (FTIR) and time-resolved step-scan FTIR (TRS<sup>2</sup>-FTIR) spectroscopies have been employed to investigate the origin of the binding and electron transfer properties of cytochrome *ba*<sub>3</sub> oxidase in both the fully reduced (FR) and mixed valence (MV) forms. Several independent and not easily separated factors leading to increased thermostability and high O<sub>2</sub> affinity have been determined. These include (i) the increased hydrophobicity of the active center, (ii) the existence of a ligand input channel, (iii) the high affinity of Cu<sub>B</sub> for exogenous ligands, (iv) the optimized electron transfer (ET) pathways, (v) the effective proton-input channel and water-exit pathway as well the proton-loading/exit sites, (vi) the specifically engineered protein structure, and (vii) the subtle thermodynamic and kinetic regulation. We correlate the unique ligand binding and electron transfer properties of cytochrome *ba*<sub>3</sub> oxidase with the existence of an adaption mechanism which is necessary for efficient function. These results suggest that a cascade of structural factors have been optimized by evolution, through protein architecture, to ensure the conversion of cytochrome *ba*<sub>3</sub> oxidase into a high O<sub>2</sub> affinity enzyme that functions effectively in its extreme native environment. The present results show that *ba*<sub>3</sub>-cytochrome *c* oxidase uses a unique structural pattern of energy conversion that has taken into account all the extreme environmental factors that affect the function of the enzyme and is assembled in such a way that its exclusive functions are secured. Based on the available data of CcOs, we propose possible factors including the rigidity and nonpolar hydrophobic interactions that contribute to the behavior observed in cytochrome *ba*<sub>3</sub> oxidase.

Financial support by the Cyprus University of Technology is gratefully acknowledged.



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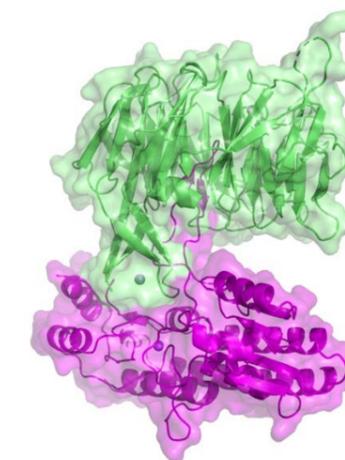
## OP-82

### The Role of the Metallochaperone AztD in Bacterial Zinc Homeostasis

Erik Yukl<sup>1</sup>, Durga Prasad Neupane<sup>1</sup>, Stephanie Fullam<sup>1</sup>

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As an essential element that is toxic in excess, zinc homeostasis is a critical function for all organisms. Among bacteria, it is generally maintained by transcriptional regulation of zinc import and export proteins by zinc-responsive transcriptional regulators. Disruption of zinc homeostasis in bacterial pathogens can result in dramatic attenuation of virulence, making these systems attractive targets for the development of novel antibiotics. In particular, zinc importers of the ATP binding cassette (ABC) type are critical for the survival of many bacteria under zinc limited conditions. Bacterial ABC transporters rely on a periplasmic or lipoprotein solute binding protein (SBP) to bind zinc with high affinity and specificity and deliver it to the membrane permease for import into the cytoplasm. Recent data also indicates the participation of other extracellular zinc-binding proteins (e.g. metallochaperones) in metal import through ABC transporters in some species. We are studying zinc homeostasis in *Paracoccus denitrificans*, an organism with two zinc-specific ABC transporter operons *znuABC* and *aztABCD*. These are under transcriptional control of the zinc uptake regulator Zur [1], are highly upregulated during zinc deprivation, and are required for growth under these conditions [2]. Further, both operons are highly conserved in several human pathogens, including carbapenem Enterobacteriaceae (CRE) species such as *Klebsiella pneumoniae*. The *aztABCD* operon encodes a periplasmic metallochaperone (AztD) capable of transferring zinc to the associated SBP (AztC) through a specific, associative mechanism that can be followed *in vitro* using intrinsic tryptophan fluorescence [3]. Crystal structures of AztD and AztC [4] combined with kinetic data and mutational studies are beginning to reveal the function of AztD in zinc homeostasis and the molecular mechanism of zinc transfer. The observation that AztD is conserved in more than 500 genomes from diverse bacterial taxa suggests that this protein is an important component of zinc homeostasis and a newly identified family of zinc metallochaperones.



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OP-83

### Construction of Cell Membrane Targeted Sensors and Their Fluorescent Imaging

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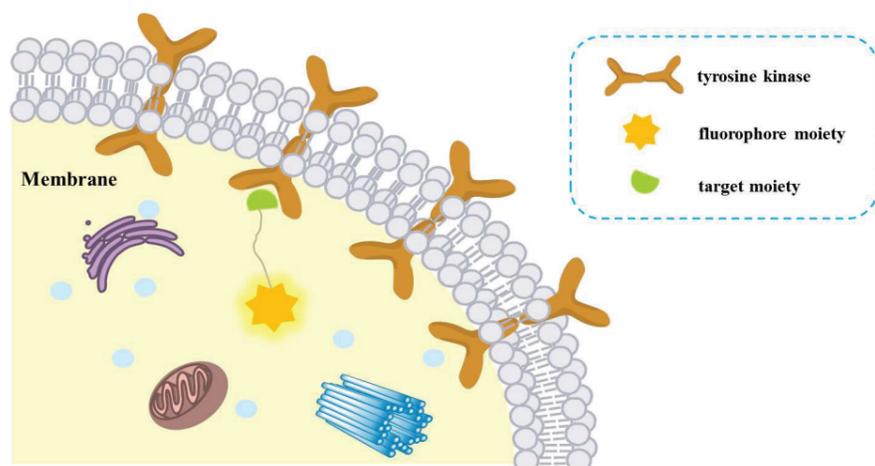
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The fluorescent imaging techniques have rapidly used in diagnosis and treatment of cancer. Receptor tyrosine kinases are a kind of important cell membrane receptor. The aberrant expression of receptor tyrosine kinases could result in a series of downstream signaling pathways and leading to cell proliferation regulation disorders. Receptor tyrosine kinases are overexpressed in many tumors, and the identification and detection of receptor tyrosine kinases have prominently research significance for early diagnosis, prevention and prognosis of tumors. Herein, a series of receptor tyrosine kinases sensors were designed and synthesized, which were consisted of the fluorophore, linker and target moiety. The fluorescence signal is selectively and quickly generated by interaction with receptor tyrosine kinases accumulating on the cell membranes of cancer cells. The sensors are fluorescence quenching in the body fluids while the cell membrane of the tumor cells exhibit fluorescence due to conformational conversion of the fluorescent sensors. Moreover, they have good biological compatibility and permit the rapid, highly selective and sensitive identification of cancer cells via imaging of the tumor cell membranes. These sensors have high sensitivity and selectivity towards receptor tyrosine kinases on cell membranes and achieve *in vivo* and *in vitro* imaging, and they provide an effective means of early diagnosis of tumors. Financial support by the National Natural Science Foundation of China (Grants 21820102001) is gratefully acknowledged.



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OP-84

### Applications of $\beta$ -NMR Spectroscopy in Bioinorganic Chemistry

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$\beta$ -radiation detected nuclear magnetic resonance ( $\beta$ -NMR) spectroscopy is an ultrasensitive NMR technique which is based on the detection of  $\beta$ -particles emitted anisotropically by spin polarized nuclei. The combination of nuclear spin polarization and high detection efficiency of  $\beta$ -particles gives rise to a billion fold ( $10^9$ ) or higher increase in sensitivity as compared to conventional NMR, and allows for interrogation of elements which are otherwise difficult to access.  $\beta$ -NMR has been applied in nuclear and solid state physics for over six decades, and here we present recent advances of the technique for bioinorganic and medicinal chemistry.

$\beta$ -NMR has already been applied multiple times in measurements on  $Mg^{2+}$  and  $Li^+$  in ionic liquid solutions at TRIUMF, Canada's particle accelerator centre [1-3]. In contrast to any previously reported measurements for  $Mg^{2+}$  ions, 25 mM  $MgCl_2$  in 1-ethyl-3-methylimidazolium acetate (EMIM-Ac) and in 1-ethyl-3-methylimidazolium dicyanamide (EMIM-DCA) compare favourably with conventional  $^{25}Mg$  NMR, but do not suffer from line broadening due to quadrupole interactions ( $I=1/2$  for  $^{31}Mg$  while  $I=5/2$  for  $^{25}Mg$ ), as shown Fig. 1. All spectra exhibit very high resolution, with line widths of about 3 ppm, allowing for discrimination of species with oxygen and nitrogen coordination. Furthermore, just recently  $\beta$ -NMR has also been applied to study of  $Mg^{2+}$  binding to ATP – the energy currency of life – in ionic liquid solution.

To demonstrate the potential of the technique, recent advances of  $\beta$ -NMR measurements, including experiments with ultra-trace (pM) amounts of  $^{31}Mg^{2+}$ , will be presented and discussed. Furthermore, in this contribution we will also highlight the advances made towards  $\beta$ -NMR measurements on Cu and Ac isotopes, and discuss future plans in detail.

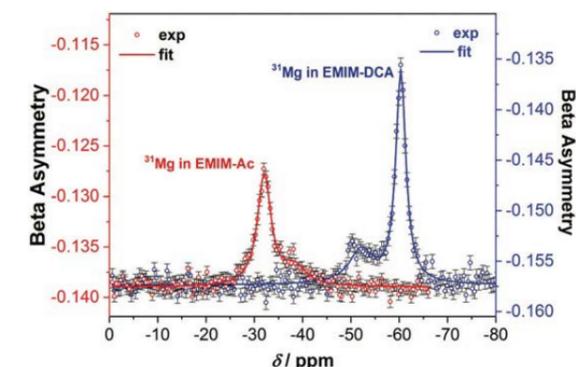


Fig.1.  $^{31}Mg$   $\beta$ -NMR spectrum of 25 mM  $MgCl_2$  in EMIM-Ac (red) and EMIM-DCA (blue) [1].

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OP-85

### The Catalytic Hydrogen Bonding Network of [FeFe]-Hydrogenases

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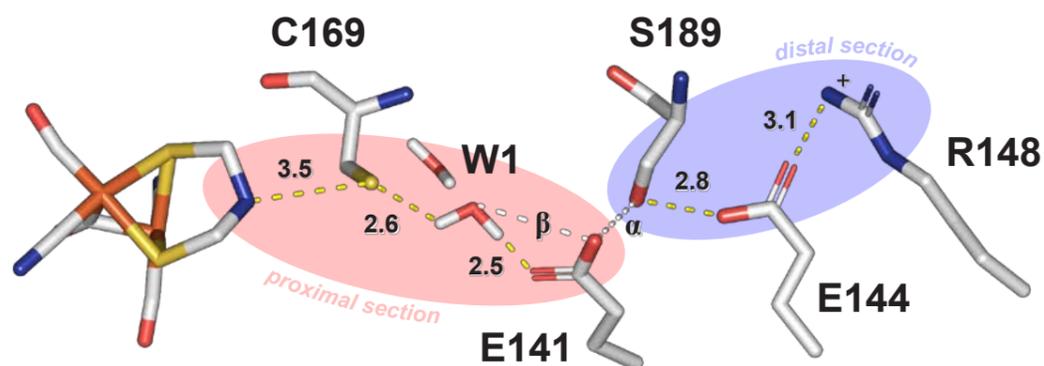
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For the last three years, we evaluated the molecular proceedings of hydrogen turnover with [FeFe]-hydrogenases making use of in situ ATR FTIR spectroscopy under gas and electrochemical control. [1] The active site cofactor is a structurally flexible iron-sulphur centre that comprises a [4Fe-4S] cluster tethered to the eponymous diiron site. We found that the redox chemistry of [4Fe-4S] cluster and diiron site is modulated by individual proton transfer (PT) pathways. [2,3] This allows [FeFe]-hydrogenases stabilizing the “rotated geometry” of the oxidized H-cluster (Hox) throughout one- and two-electron reduction (Hred’ and Hhyd, respectively). Recently, we identified the amino acid residues involved in catalytic PT from bulk water to the diiron site.[4] However, the dynamics of PT and hydrogen bonding (HB) remained elusive.

Upon reduction of the diiron site, a proton binds in Fe-Fe bridging position and arrests the cofactor in a conformation that significantly differs from the “rotated geometry”. [5] This redox state, Hred, can be accumulated very efficiently. Reduction render the diiron site more alkaline, leads to the formation of a bridging hydride ( $\mu\text{H}$ ), and includes changes in the HB network. I will present in situ ATR FTIR difference spectroscopy on the transition from Hox to Hred. For the first time, the infrared regime from 1750 - 1550  $\text{cm}^{-1}$  was addressed to analyse the dynamics of HB changes in the catalytic PT pathway of [FeFe]-hydrogenases directly. I will discuss the impact on hydrogen turnover.



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OP-86

### Optimal Macromolecular Crowding Conditions Maximize Ribozymes Cleavage Activity at Lower [Magnesium(II)]

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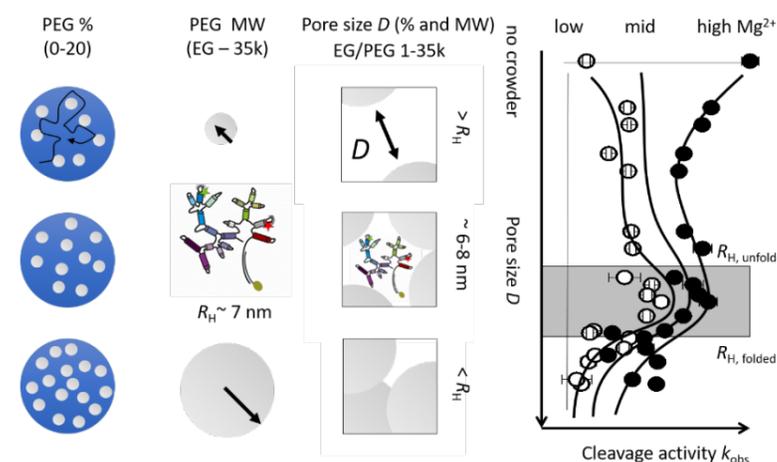
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Ribozymes are catalytic active RNAs requiring a high millimolar magnesium(II) concentration to show folding and function *in vitro* [1,2]. In contrast, *in vivo* conditions are characterized by a highly crowded cellular environment and a low millimolar divalent ion concentration. Molecular crowding agents are used to mimic the cellular environment. However, how the physicochemical properties of co-solutes or macromolecular crowders influence the folding and function of RNAs is poorly understood. We screened volume fraction (%) and molecular weight (MW) of different crowding particles by bulk activity assays, smFRET and NMR diffusion experiments. In this way, we gain new insights on how polymer properties like dielectric constant, viscosity, diffusion, and pore size influence the activity and folding of a large non-coding RNA, the group IIB intron ribozyme ai5 $\gamma$  from *S. cerevisiae* [3,4]. Interestingly, smFRET experiments show that the most compact state, the putative active state of the ribozyme, becomes more abundant with increasing PEG concentration, although dense crowding becomes detrimental for activity. We found that the ribozymes activity is maximized when the pore size, *i.e.* the average distance of the crowding particles in solution, matches the hydrodynamic radius of the RNA [4].

Financial support from the European Research Council (MIRNA N° 259092 to RKOS), the Swiss National Science Foundation (RKOS), the UZH Forschungskredit (FK-14-096 and FK-15-095 to RB) and the University of Zurich are gratefully acknowledged.



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OP-87

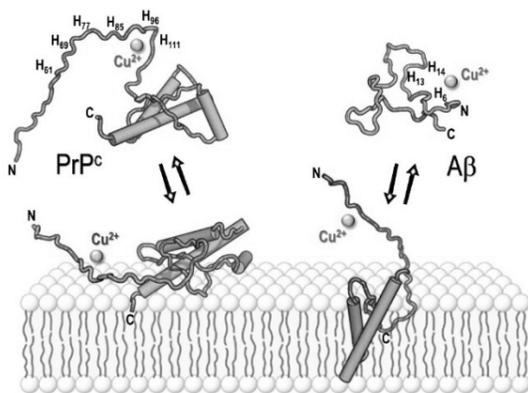
### Copper-Prion and Copper-A $\beta$ Peptide Complexes in Membrane-Like Environment Strongly Affects Dopamine Toxicity

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Dysregulation of catecholamine neurotransmitters, and in particular their accumulation, plays a yet poorly understood role in the pathogenesis of neurodegenerative diseases, primarily Parkinson's disease but also Alzheimer's disease and other forms of dementia [1]. The toxic effects of catecholamines depend on their relatively easy oxidation to quinone species under oxidative stress conditions, which are typically promoted by endogenous redox active metal ions such as copper and iron [2-4]. In the present study we report a comparative study of the oxidative reactivity of Cu-complexes with  $\beta$ -amyloid (A $\beta_{40}$ ) and the prion peptide fragment 76-114 (PrP<sub>76-114</sub>), containing the high-affinity binding site, towards dopamine and 4-methylcatechol, in aqueous buffer and in SDS micelles, as model membrane environment. In particular, our aim was to assess the efficiency in the oxidation of external substrates by the Cu-peptide complexes and evaluate the competitive oxidative and covalent modifications undergone by the peptides. The presence of SDS micelles has strong impact on the reactivity of the Cu-peptide complexes, because the peptides bear strong binding affinity to lipid membranes. The effect of SDS is to strongly reduce (A $\beta_{40}$ ) and quench (PrP<sub>76-114</sub>) the oxidative reactivity of Cu-peptide complexes. This behavior is due to the inclusion of PrP peptide in the lipid phase, whereas for Cu-A $\beta$  complexes the binding site for both Cu<sup>II</sup> and Cu<sup>I</sup> is in the polar N-terminal portion of the peptide and remain essentially outside of the membrane (Scheme 1). The reactivity of Cu-A $\beta$  complexes decreases in SDS because a significant fraction of the substrate may stick on the micelle surface hindering its binding to the Cu center in the aqueous phase. These results improve our understanding of physio- and pathological effects associated with PrP as its strongly Cu binding and stress-protective properties can only be exerted when bound to membranes.



Scheme 1. Schematic representation of the binding equilibria of PrP<sup>C</sup> and A $\beta$  to membranes, showing the different position of the copper sites for the membrane-bound peptides, within the membrane for PrP<sup>C</sup> and outside the membrane for A $\beta$ .

The authors acknowledge MIUR for financial support through the project "Metal ions, dopamine, and oxidative stress in Parkinson's disease" (PRIN 2015T778JW).

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OP-88

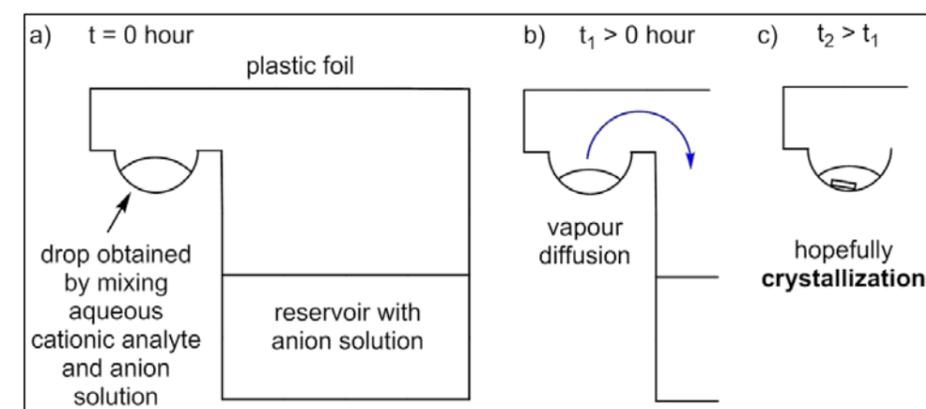
### Single Crystal Growth of Water-Stable Coordination Complexes

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We have recently designed a crystallization screen consisting of 77 different anions and successfully applied the screen for the crystallization of six out of seven very diverse organic cations.<sup>1,2</sup> The 96 different solutions of the screen are either mixed with the help of a pipetting robot<sup>1</sup> or just with a cheap multi-channel pipette under oil<sup>2</sup>. Vapour diffusion of water from the crystallization drops either in the bigger reservoir<sup>1</sup> (see Figure) or through the oil into the air<sup>2</sup> induces the growth of the crystals. In this presentation, we want to show our promising results of growing single crystals of various positively charged coordination compounds with the help of this crystallization screen. The first coordination complex crystallized by this technique has just been reported.<sup>3</sup>

Financial support by the University of Zurich, the R'Equip programme of the Swiss National Science Foundation (project No. 206021\_164018 to BS), the Czech Science Foundation (grant No. 16-10035S) and the Specific University Research (MSMT No. 21-SVV/2018) is gratefully acknowledged.



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### OP-89

#### Recent Structural Highlights of Copper Nitrite Reductase and a Quinol-Dependent Nitric Oxide Reductase

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We would provide a highlight<sup>1</sup> from our structural and mechanistic work on enzymes catalysing the formation of NO via copper nitrite reductases (CuNiR) and further metabolism of nitric oxide (NO) by membrane integrated nitric oxide reductases (NORs) to form nitrous oxide (N<sub>2</sub>O). CuNiRs catalyze the one-electron reduction of nitrite to nitric oxide (NO) [NO<sub>2</sub><sup>-</sup> + e<sup>-</sup> + 2H<sup>+</sup> ⇌ NO + H<sub>2</sub>O] are homotrimeric have been well studied providing clear example of proton coupled ET that is gated by the substrate binding at the T2Cu catalytic site<sup>2</sup>. ‘Molecular Movies’ of CuNiR have been constructed at a variety of temperatures using MSOX (Multiple structures collected serially from one crystal), recording *in crystallo* enzyme conversion of NO<sub>2</sub> to NO in the 2-domain AcNiR with a ‘side-on’ NO product prior to returning to the resting state. Combining this structural movie with damage-free structures obtained using X-ray Free Electron Laser (XFEL) of AcNiR in the resting state, nitrite bound and chemically-reduced state has enabled us to establish the salient features of catalysis.

We have used Cryo-electron microscopy to determine the structure of Quinol-dependent Nitric Oxide Reductases (qNOR) from *Alcaligenes xylosoxidans*, in the native and an activity-enhancing mutant to 3.7 and 3.2 Å, respectively. They unexpectedly reveal a dimeric conformation (also confirmed for qNOR from *Neisseria meningitidis*), define the active site configuration, with a clear water channel from the cytoplasm. Structure-based mutagenesis has identified key residues involved in proton transport and substrate delivery to the active site of qNORs. The importance of dimeric structure is discussed in the context of functionality of the enzyme.

Financial support by the UK’s Biotechnology and Biological Science Research Council is gratefully acknowledged.

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[1] This is a summary highlight of our work during the last five years. Authors and teams that contributed to this are acknowledged via the following references.

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### OP-90

#### Characterization and Reactivity of Heterobimetallic Complexes for Bioinspired CO<sub>2</sub> Chemical Valorization

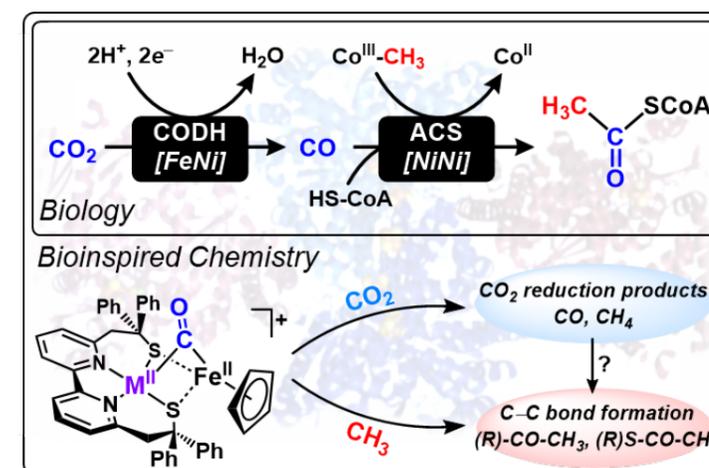
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Interest in catalytic CO<sub>2</sub> reduction derives from several overarching goals in environmental and bio-inorganic chemistry. One promising strategy to approach this objective is to learn from the biological systems which carry out such chemistry.<sup>1</sup> Two nickel- and iron-containing metalloenzymes, carbon monoxide dehydrogenase (CODH) and acetyl-coA synthase (ACS), are responsible for the reduction of CO<sub>2</sub> to CO and the generation of acetyl-coA from CO, coenzyme A, and methylcobalamin, respectively.<sup>2</sup> The work presented herein covers investigations of the reactivity of heterodinuclear [NiFe] and/or [CoFe] models towards CO<sub>2</sub> and CO in terms of redox cooperativity of metal centers, structural and electronic ligand effects, and mechanistic details. We outline the synthesis and spectroscopic characterization of such complexes, which have been designed based on several known mono- and dinuclear biomimetic catalysts.<sup>3-5</sup> The known relative affinities of Ni<sup>I</sup>/Ni<sup>II</sup> for CO<sub>2</sub> vs. CO as well as the alkylating potential of Co<sup>II</sup>(N<sub>2</sub>S<sub>2</sub>)<sup>6</sup> are each exploited in tandem with a low-valent iron center in order to provide suitable conditions for CO<sub>2</sub> reduction or C<sub>(methyl)</sub>-C<sub>(C=O)</sub> bond formation, respectively. The reactivities of these heterobimetallic catalysts have been investigated using electrochemical and spectroscopic techniques as well as theoretical methods, and the results are analyzed and discussed in the context of their implications in developing comprehensive systems for CO<sub>2</sub> valorization.

Financial support from Labex ARCANE and the Institute of Metals in Biology of Grenoble (IMBG) is gratefully acknowledged.



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**OP-91**

**A Comparison of Two Hydroquinone Ring-Cleaving Dioxygenases**

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The hydroquinone dioxygenases (HQDOs) are members of a large family of non-heme Fe(II)-containing enzymes that catalyze the oxidative cleavage of an aromatic ring, of which the catechol extradiol dioxygenases are by far the best known and best studied. Like the catechol extradiol dioxygenases, the HQDOs are found in two unrelated structural classes, Type I and Type II, yet share the same 2-His-1-Glu facial capping triad. The enzyme PcpA, from *Sphingobium chlorophenicum* L-1, is a Type I HQDO and is structurally related to the major class of extradiol catechol dioxygenases. We have shown that PcpA is specific for *ortho*-disubstituted hydroquinones (but *not* catechols) with chloro- or bromo- (but not fluoro-) substituents greatly preferred at the *ortho* positions, and that ring cleavage occurs between the OH and *ortho* substituent.[1] Several lines of evidence indicate that halogen polarizability is a major factor that determines the specificity of this enzyme. Substrate binding titrations show only a small shift in  $pK_a$  between the free vs. enzyme-bound substrate, suggesting that PcpA lacks an active site base needed to deprotonate the substrate, which is a key difference from the structurally homologous catechol extradiol dioxygenases. PnpC1C2, from *Pseudomonas putida* DLL-E4, is a Type II HQDO and displays a completely different pattern of substrate specificity, showing a preference for unsubstituted and monosubstituted hydroquinones, and a different ring cleavage regioselectivity. This points to important differences in the second coordination sphere that define how the hydroquinone substrate binds and is set up to be attacked by dioxygen.

Financial support by the National Science Foundation (CHE-0951999, CHE-1506458, MRI-0922775) is gratefully acknowledged.

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# Young Researchers Presentations

## YR-01

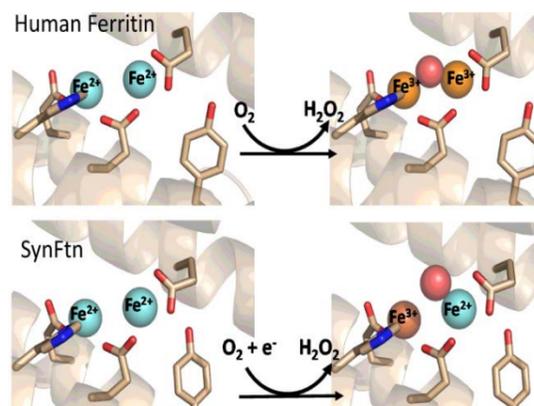
### The Di-Iron Catalytic Center of *SynFtn* Carries out hitherto Uncharacterized Iron-Oxygen Chemistry

Justin Bradley<sup>1</sup>, Jacob Pullin<sup>2</sup>, Natalie Hill<sup>1</sup>, Rhona Stuart<sup>3</sup>, Brian Palenik<sup>3</sup>, Michael Wilson<sup>2</sup>, Geoffrey Moore<sup>1</sup>, Dimitri Svistunenko<sup>2</sup>, Andrew Hemmings<sup>1</sup>, Nick Le Brun<sup>1</sup>

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Ferritins are a superfamily of proteins involved in iron storage and detoxification. They are widely distributed, examples being found in all domains of life<sup>[1]</sup>. The genome of the marine cyanobacterium *Synechococcus sp.* CC9311 encodes three predicted ferritins. One of these, *SynFtn*, is unusual in that protein expression is upregulated in response to copper rather than iron<sup>[2]</sup>. We have recently shown that in addition to this unusual transcriptional response *SynFtn* contains structural features that distinguish it from all previously characterized ferritins. One consequence of these structural variations is a change in the mechanism of the iron-oxygen chemistry catalysed by the active site. The first intermediate formed in the reaction between iron and oxygen at the catalytic sites of other ferritins is a diferric-peroxo species<sup>[3]</sup>, a feature that they share with the vast majority of di-iron oxygenases. The reaction centres of *SynFtn* also generate peroxide as the product of oxygen reduction but the first observable intermediate formed by the di-iron sites is a mixed valent Fe<sup>II</sup>/Fe<sup>III</sup> species. We have interrogated the iron-oxygen chemistry of *SynFtn* using a combination of magnetic- and optical- spectrokinetic studies, protein crystallography and site directed mutagenesis. Our data indicate that charge balance is achieved during this reaction via an unprecedented mechanism in which two centres separated by  $\geq 24$  Å both provide a single electron for the reduction of a shared oxygen substrate bound at only one. The long range electron transfer implicit in the proposed mechanism is supported by the transient oxidation of aromatic amino acid sidechains<sup>[4]</sup>.

Multiple sequence alignments of annotated ferritins in genome databases revealed > 100 homologues of *SynFtn*. These occur almost exclusively in the marine picocyanobacteria suggesting that *SynFtn* may represent the first characterised example of a subset ferritins distinct from the established system of classification comprising animal-, prokaryotic- and phyto-ferritins.



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## YR-02

### Azido-Desferrioxamine B for Generating Chemical Probes Using *In Situ* Copper-Free Click Chemistry

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The trihydroxamate siderophore desferrioxamine B (DFOB) is biosynthesised by *Streptomyces sp.* to sequester growth essential environmental iron. The biosynthesis, sequestration and re-uptake rely on the enzyme/transporter cluster DesABCDEF, with parts of this cascade still under debate [1,2]. Using the *S. pilosus* system as a model, we semisynthetically modified DFOB by chemical diazo-transfer to yield azido-DFOB as a click-chemistry-amenable analogue. We used azido-DFOB to undertake strain-promoted azide-alkyne cycloaddition to generate biotinylated DFOB as a chemical probe [3,4]. In parallel, diazo-transfer performed directly within the semi-purified supernatant of *S. pilosus* yielded further azide-functionalised metabolites that could have potential use as chemical probes. Precursor-directed biosynthesis in the culture medium of *S. pilosus* with non-native, asymmetric (“blunt-end”) substrates competing against endogenous 1,5-diaminopentane during the biosynthesis of DFOB showed substrates were subject to *N*-acetylation but not *N*-hydroxylation, suggesting that DesC or a functionally equivalent *N*-acetyl transferase, but not DesB or DesD, are capable of turning over “blunt-end” substrates *in vitro* [5]. Despite no further evidence of biosynthetic incorporation of the exogenous substrates into monomeric or dimeric precursors or the trimeric DFOB-analogues natively produced by *S. pilosus*, this approach opens up the opportunity to directly tag growth-essential secondary metabolites, such as siderophores, and investigate biosynthetic processes in native systems of bacterial pathogens. This expedited and less invasive path facilitates biochemical interrogation by probing native metabolites and circumvents other labour-intensive approaches to produce chemical probes. Deepening our understanding of bacterial biosynthesis may ultimately lead to identification of novel drug targets and therapeutics. Financial support by the Australian Research Council and the University of Sydney is gratefully acknowledged.

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YR-03

## Exploring a New Binding Mode of Octahedral Cobalt Complexes to G-Quadruplex DNA

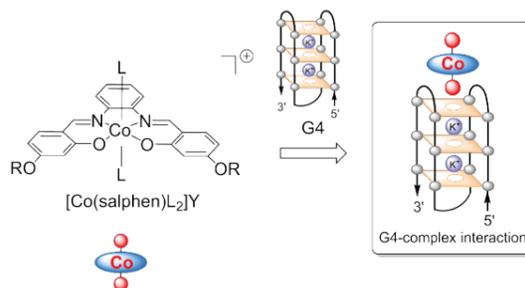
Carmen L. Ruehl<sup>1</sup>, Aaron H. M. Lim<sup>1,2</sup>, Timothy Kench<sup>1</sup>, David J. Mann<sup>2</sup> and Ramón Vilar<sup>1</sup>

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Guanine-rich DNA sequences have the ability to form four stranded helical structures named G-quadruplexes (G4) under physiological conditions. Stabilisation of G4 is induced by potassium or sodium ions that are located in the central ion channel. These DNA structures have gained interest due to their role in biological processes, such as chromosomal maintenance and regulation of gene expression, and have been proposed as an attractive target for the development of anticancer drugs [1-2]. Thus, it is of interest to develop small molecules that are able to stabilise G-rich sequences; ideally, these should have both a high affinity for G4 and a high selectivity over duplex DNA. To date, a number of compounds including organic molecules and metal complexes have shown to be good G4 binders, e.g. metal complexes with Schiff base-derived ligands. Almost all complexes stabilise these non-canonical structures through  $\pi$ - $\pi$  interactions via end-stacking with the G4 tetrad. While excellent G4 binders have been developed using this approach, the G4 motif features a number of structural features that are not being exploited to develop more selective binders [3-4]. In particular, the interaction of small molecules with the central channel of the G4 tetrad is practically unexplored [5]. It was proposed that moving from a square planar system to an octahedral complex could achieve extra stabilisation of G4 through the axial ligands interacting with the central ion channel. To strengthen this hypothesis, I designed and synthesised a series of novel octahedral cobalt(III) salphen complexes,  $[\text{Co}(\text{salphen})\text{L}_2]\text{Y}$ , with different amine-based ligands (L) in the axial positions. After investigating their stability in buffered media, the binding affinities of the complexes to different telomeric and oncogenic DNA sequences as well as duplex DNA were examined *in vitro* using a variety of different techniques, including Förster resonance energy transfer, fluorescent indicator displacement assay, UV-Vis spectroscopy, circular dichroism and fluorescence lifetime indicator displacement assay. Moreover, the affinities of the cobalt complexes to G4 in cell-mimicking conditions were tested by investigating the unwinding activity of helicase, a protein that is able to unfold G4, in the presence and absence of the complexes. Lastly, cell experiments were performed to prove the uptake of cobalt complexes in different cancer cell lines as well as their cytotoxicity.

In summary, we report the first known octahedral salphen complex expected to bind in a barely explored binding mode via the central ion channel of G4. High G4 binding affinity and selectivity, inhibition of helicase activity and templation of G4 formation at low concentrations are the key results. Successful cell experiments strengthen its application as potential candidate as anti-cancer drug. Financial support by the Engineering and Physical Sciences Research Council (EPSRC) is gratefully acknowledged.



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YR-04

## Dual-Targeting Dual-Action Platinum(IV) Platform for Enhanced Anticancer Activity and Reduced Nephrotoxicity

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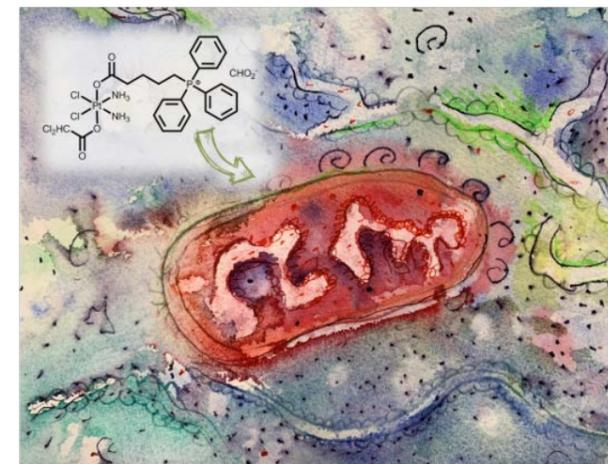
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In this work, we designed a novel highly efficient Pt(IV) platform to ensure targeted *in vivo* delivery of dual-action Pt(IV) prodrugs. The dual targeting was established by liposomal encapsulation of Pt(IV) complexes, thereby utilizing the enhanced permeability and retention (EPR) effect as the first stage of targeting to attain high accumulation of the drug-loaded liposomes in the tumor. After the release of the Pt(IV) prodrug inside cancer cells, a second stage of targeting directed a portion of the Pt(IV) prodrugs to the mitochondria. Upon intracellular reduction, these Pt(IV) prodrugs released two bioactive molecules, acting both on the mitochondrial and on the nuclear DNA.

Our Pt(IV) system showed excellent activity *in vitro* and *in vivo*, characterized by enhanced mitochondrial damage and complete tumor remission, respectively. Notably, marked *in vivo* activity was accompanied by reduced kidney toxicity, highlighting the unique therapeutic potential of our novel dual-targeting dual-action platform. This study provides an exciting strategy for improving on the clinical success of Pt-based anticancer drugs.



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## YR-05

### Zn(II) and Cu(II) Binding Ability of ZinT – A Highly Conserved Periplasmic Protein Expressed by Different Bacterial Species

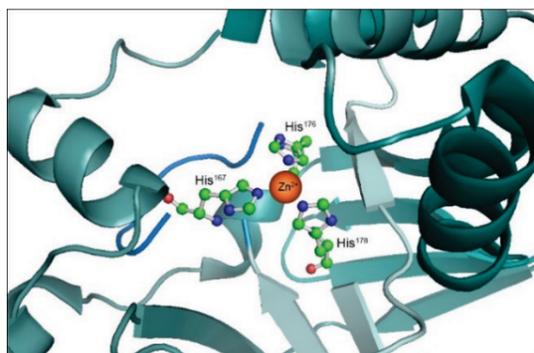
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The necessity of new antimicrobial agents is unarguable, since current therapeutic treatments are not always effective due to the development of bacterial drug resistance. Nevertheless, the mechanism of metal trafficking at host/pathogens interface can provide a fertile ground for the design of new effective antibiotic therapies. To prevent infections, humans restrict the access to essential micronutrients by means of an innate immune response termed "nutritional immunity", on the contrary bacteria rely on sophisticated systems (e.g. metallophores) to overcome the scarce metal bioavailability. In the attempt to shed light on the host nutritional immune response (e.g. deepen the way of action of antimicrobial peptides and other metal-sequestering proteins) and to develop novel highly specific antibiotics, it is crucial to investigate not only the host-mediated defence but also the pathogenic metals acquisition processes [1]. Among several proteins involved in the mechanism of metals recruitment, we recently focused on ZinT, a 216-amino acid protein found in the cytoplasm of several bacterial species, which undergoes translocation to the periplasm in order to express its task of Zn(II) uptake under severe zinc-limited conditions, then shuttling the metal to ZnuABC transporter. The most probable metal-binding site of ZinT corresponds to three highly conserved histidine residues (His167, His176 and His178). Additionally, ZinT possesses a highly conserved N-terminal histidine-rich loop (HGHHXH), whose role is unclear, although it has been suggested its participation in Zn(II) uptake [2-4]. The above results prompted us to deeply investigate thermodynamics and coordination chemistry of ZinT complexes with Zn(II) and Cu(II), two endogenic and competing metal ions.

For this purpose, we studied the protected peptides Ac-<sup>166</sup>DHIIAPRKSSHFH<sup>178</sup>-Am and Ac-<sup>166</sup>DHIIAPRKSAHFH<sup>178</sup>-Am, corresponding to the 166-178 amino acid sequence of ZinT in *Escherichia coli* and *Salmonella enterica* and *typhimurium*, respectively. The N-terminal His-rich sequences of ZinT-*E.coli* (Ac-<sup>124</sup>HGHSH<sup>129</sup>-Am) and ZinT-*S.typhimurium* (<sup>124</sup>HGHHAH<sup>129</sup>-Am) have been also considered, along with the His-rich fragment of YrpE metal-binding protein from *Bacillus subtilis* (Ac-<sup>57</sup>HTHEHSHDHS<sup>69</sup>-Am), which shows a remarkable similarity with ZinT. The characterization of the complexes has been achieved by means of mass spectrometry, potentiometry, UV-Vis spectrophotometry, circular dichroism (CD) and nuclear magnetic resonance (NMR). Financial support of the National Science Centre (UMO-2017/26/A/ST5/00364) is gratefully acknowledged.



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## YR-06

### Molecular Mechanism of Coproheme to Heme *b* Conversion

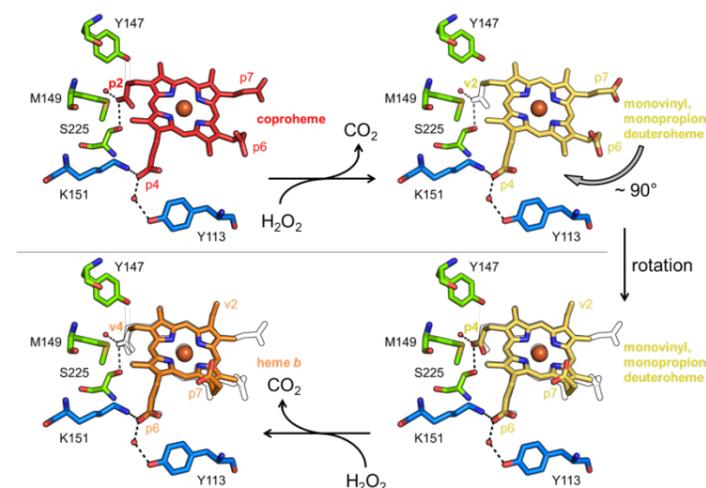
Stefan Hofbauer<sup>1</sup>, Lisa Milazzo<sup>2</sup>, Thomas Gabler<sup>1</sup>, Dominic Pühringer<sup>3</sup>, Vera Pfanzagl<sup>1</sup>, Kristina Djinović-Carugo<sup>3</sup>, Paul G. Furtmüller<sup>1</sup>, Christian Obinger<sup>1</sup>, Giulietta Smulevich<sup>2</sup>

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Coproheme decarboxylase (ChdC) catalyzes the ultimate step in the coproporphyrin-dependent heme biosynthesis pathway of monoderm bacteria with coproheme acting both as redox cofactor and substrate [1]. Hydrogen peroxide mediates the stepwise decarboxylation of propionates 2 and 4 of coproheme [2,3]. Here we present structural data of coproheme-loaded ChdC from *Listeria monocytogenes* (LmChdC) and the three-propionate intermediate, in which the propionate at position 2 (p2) is converted to a vinyl group and is rotated by 90° compared to the coproheme complex structure.

The design of single, double and triple mutants of LmChdC [4,5], with impaired H-bonding interactions from the protein moiety selectively to propionates 2, 4, 6 and 7, allowed us to obtain the assignment of all the coproheme propionate bands by resonance Raman spectroscopy and to follow the H<sub>2</sub>O<sub>2</sub>-mediated conversion of coproheme to heme *b*. Substitution of H<sub>2</sub>O<sub>2</sub> with chlorite allowed us to monitor Compound I formation in the inactive Y147H variant which lacks the catalytically essential Y147. This residue demonstrated to be oxidized during turnover, forming an essential tyrosyl radical, by using the spin-trap 2-methyl-2-nitrosopropane. Based on these findings we propose a reaction mechanism for the stepwise decarboxylation of coproheme that includes a 90° rotation of the intermediate three-propionate redox cofactor. Financial support by the FWF (P29099, W1224) is gratefully acknowledged.



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YR-07

## Development of a New Class of Potent Cytotoxic Bioinorganic Agents: Molybdacarboranes Bearing Versatile 2,2'-Bipyridine-Modified Tamoxifene-Type Vectors

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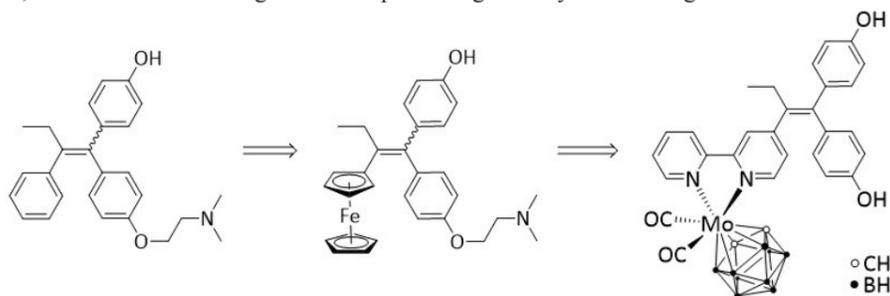
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While the chemistry of carboranes has been studied extensively since the 1960ies, recent developments focus mainly on design and principles for application. One aspect includes applications of metallacarboranes in biomedical chemistry.[1] Isosteric replacement strategies are applied linking the concepts of phenyl- or cyclopentadienyl rings to carborane clusters (*closo*-1,2-C<sub>2</sub>B<sub>10</sub>H<sub>12</sub>, [*nido*-7,8-C<sub>2</sub>B<sub>9</sub>H<sub>12</sub>]<sup>-</sup> or the fragment [3-M-1,2-C<sub>2</sub>B<sub>9</sub>H<sub>11</sub>]) in drug design. In that context, the carborane cluster offers unique features, which are often recognised as superior to those of its organic counterparts (phenyl ring and cyclopentadienyl ligand), in view of a specific application, such as hydrophobicity, larger steric demand, special electronic structure, or site-specific derivatisation pattern.[2]

Our group is specialised in metallacarborane chemistry with a special focus on half- and mixed-sandwich ruthena- and molybdacarboranes as pharmacophores for the design of antitumour agents. This approach allows a symbiosis of the carborane features, the versatility of metals in biological systems and specific ligands which can be selected according to their targets. We report here on the design, synthesis and biological evaluation of a hydroxytamoxifen derivative as a versatile ligand and vector system, which can be combined with a variety of transition metal complex fragments. Thus, incorporation of a molybdacarborane modulates the biological activity towards breast cancer, glioma and glioblastoma cell lines compared to other successfully applied redox-active selective estrogen receptor modulators (SERMs), like ferrocifen.[3]

Flow cytometric analysis of MCF-7 cells revealed that the molybdacarborane derivative has an influence on the generation of reactive oxygen species (ROS), the nitric oxide (NO) levels and autophagy processes.[4] Biological studies were performed with our new bovine serum albumin (BSA)-based nano-carrier system for metallacarboranes which was shown to significantly improve the cytotoxic activity of the studied half-sandwich molybdacarborane structures.[5]

Financial support from the Fonds der Chemischen Industrie, the Graduate School Leipzig School of Natural Sciences – Building with Molecules and Nano-objects (BuildMoNa) and the Serbian Ministry of Education, Science and Technological Development is gratefully acknowledged.



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YR-08

## 1,3-Diaza-2-Oxophenoxazine as a Luminescent Cytosine Analog in Silver(I)-Mediated Base Pairing

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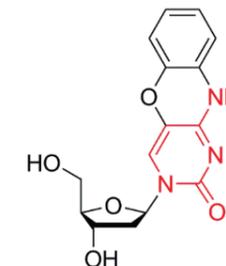
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Recently, metal-mediated base pairing has become a convenient approach to functionalize nucleic acids site-specifically [1]. Parallel-stranded DNA which only rarely occurs in nature even expands the possible binding geometries for transition metal ions due to the transoid orientation of the glycosidic bonds [2]. Fluorescent nucleobase analogues are of special interest as they could be used as sensitive metal ion sensors when the incorporated metal ion tailors the fluorescence intensity and absorption maxima [3].

In parallel-stranded DNA duplexes, 1,3-diaza-2-oxophenoxazine (**X**) forms stabilizing **X**–Ag(I)–**X** and **X**–Ag(I)–**C** base pairs as was confirmed by temperature-dependent UV spectroscopy and luminescence spectroscopy. The homo base pair **X**–Ag(I)–**X** stabilizes the examined duplex by 5.3 °C whereas the incorporation of silver ions into the hetero base pair leads to a thermal stabilisation of 7.9 °C (**X**:**C**) or 3.6 °C (**C**:**X**), respectively. Upon silver(I)-binding to the **X**:**X** or **X**:**C** base pairs, the luminescence emission maximum experiences a red-shift from 448 to 461 nm upon excitation at 370 nm. Importantly, the luminescence of the 1,3-diaza-2-oxophenoxazine ligand is not quenched significantly upon binding a silver(I) ion. In fact, the luminescence intensity even increases upon formation of a **C**–Ag(I)–**X** base pair. As a consequence, the silver(I)-mediated phenoxazinone base pairs represent the first strongly fluorescent metal-mediated base pairs.

In addition, theoretical calculations have been accomplished: DFT calculations of the silver(I)-mediated base pairs suggest the presence of a synergistic hydrogen bond. MD simulations of entire DNA duplexes nicely underline the geometrical flexibility of these base pairs, with the synergistic hydrogen bond facing either the major or the minor groove.

Funding by the Deutsche Forschungsgemeinschaft (SFB 858) and the Russian Science Foundation (project no. 18-74-00051 - phenoxazine phosphoramidite and modified oligonucleotides synthesis) is gratefully acknowledged.



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## YR-09

### Metals In Medicine: Phenotypic Changes Induced In Breast Cancer Cells Treated With Metal-Complexes

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Following the discovery of cisplatin in 1965, there has been a considerable interest in the area of metal complexes as anti-cancer treatments. Nevertheless, cancer metastasis remains to be the leading cause of death amongst cancer patients and it is predicted that 13 million cancer-related deaths will occur annually by 2030 [1]. Treatment of MDA-MB-231 cells (a highly aggressive human breast cancer cell line *in vitro*) with certain anti-cancer metal-complexes induce morphological and phenotypical changes in these cells, shifting them from a metastatic phenotype to a potentially less aggressive phenotype. Transdifferentiation of cancer cells from one cell type to another is reported to reduce malignancy and tumorigenicity [2]. The aim of our research was to characterise and quantify observed morphological changes in cells after treatment with the metal anti-cancer complexes, and to investigate the intracellular biochemical changes that are associated with and/or contribute to such phenotypic changes.

Cells were treated with sub-toxic concentrations of metal anticancer drugs, and after 72 hours digital holographic microscopy revealed remarkable changes in cell morphometric parameters including significant increases in cell area, optical volume, cell eccentricity and irregularity, as well as a decrease in cell motility and migration in treatment groups. Cell enlargement and surface flattening in treated cells was further confirmed with scanning electron microscopy. Scratch wound assay was also performed demonstrating that treatment with certain metal anti-cancer drugs inhibits wound healing and invasion despite having no effect on cell confluency. Further, capillary isoelectric focusing determined phosphorylation of extracellular regulated kinase (ERK) protein, indicative of changes in cell signaling induced by such drugs. This study provides promising anti-cancer metallodrugs that have the potential to change the phenotype of aggressive metastatic cancer cells to a less invasive one. Development of such drugs targeting cancer metastasis will have profound clinical implications in the treatment of highly aggressive cancers.

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## YR-10

### Mal\_MT3: The First Iron-Binding Metallothionein

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Metallothioneines (MTs) are a well known super-family of small, cysteine-rich metalloproteins. The thiolate ligands confer these proteins with a great binding capacity towards divalent metal ions, with a preference for those with  $d^{10}$  electron configuration such as Zn(II) and Cd(II). MTs feature a wide set of functions such as metal ion homeostasis and detoxification as well as an important role in fighting against oxidative stress and reactive oxygen species formation.

Many different metal ions have been studied, but so far only Cd(II), Zn(II), and Cu(I) have been isolated *in vivo*, and only the last two seem to have a biological relevance in the native organism [1]. In 1986, *Vasak and Good* managed for the first time to obtain an Fe(II)-MT complex [2] but, in the following twenty years, only hints regarding the involvement of MTs in iron metabolism were found [3,4].

Only in 2011, *Hayashi et al.* have been able to reconstitute a stable tetranuclear Fe(II)-cysteine cluster in a MT. This cluster was proven to have electron-transfer ability, being able to promote the reduction of met-myoglobin [5].

With this work we want to present, to our knowledge for the first time, an iron-binding MT (mal\_MT3). This MT3 protein, found in apple (*malus domestica*), is produced in *Escherichia coli* in its iron-metalated form and, against any expectation, shows a strong preference to Fe(II) rather than Zn(II) *in vivo*. Furthermore, the intense red color of the protein sample (see *Figure*) and the profile of the absorption spectra suggest the formation of an [2Fe-2S] cluster, comparable to the one found in anamorsin [6].

This discovery shades a new light on MTs studies, opening an entire new chapter about the possible roles and functions of this proteins in biological systems.

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## YR-11

### How the Coordination of Zn<sup>2+</sup> and Cu<sup>2+</sup> Ions Affects the Antimicrobial Properties of Non-Aggregating Analogues of Amylin

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Amylin, also known as the islet amyloid polypeptide (IAPP) is a 37 residue peptide, which is co-secreted from pancreatic  $\beta$ -cells together with its synergic partner, insulin [1]. It plays a metabolic role by reducing glucose levels in plasma. However, the increased aggregation of hIAPP is connected with type II diabetes and may aggravate the disease [2]. Recently it has been indicated that hIAPP shows also antimicrobial activity towards Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli* [3].

The aim of this work is to understand how zinc(II) and copper(II) ions can interact with: (i) the 1-19 fragment of human amylin, which exhibits almost identical membrane disruptive abilities as full-length protein, but does not form amyloid fibers [4,5]; (ii) rat amylin, which amino acid sequence differs from human one only by 6 residues, and the most significant changes in the sequence are the lack of histidine residue in position 18 and the presence of structural stabilizing prolines in positions 25, 28 and 29 (Table 1), while it has no tendency to form fibrils and (iii) pramlintide – a synthetic amylin analogue, a drug commercially used to treat type II diabetes. In order to reduce fibrilization, three amino acids (Ala-25, Ser-28, Ser-29) were replaced by proline, as in rIAPP [6]. The histidine residue (His18), which can be crucial in the formation of complexes with metal ions such as zinc(II) and copper(II), remains present in two of the studied peptides: hIAPP and pramlintide, while it is absent in rIAPP (amino acid sequences are listed below in Table 1). Experiments were carried out both in water and in a membrane-mimicking environment. We aimed to check whether the formation of such complexes affects the structure and antimicrobial activity of each peptide and elucidate the mode of antimicrobial action and the connection between amyloid and antimicrobial peptides. Experiments of mass spectrometry, potentiometry, NMR, UV-Vis and CD spectroscopy, determination of MIC and liposome leakage assay were performed. The results allowed us to determine the stoichiometry, the exact binding site of zinc(II) and copper(II) ions, geometry of complexes and possible mode of antimicrobial action.

The work was supported by the National Science Centre (no.UMO-2017/26/E/ST5/00364) and Ministry of Science and Higher Education (0420/2915/18)

**Table1.** Amino acid sequences of studied peptides. Differences marked in green.

Human amylin (hIAPP)	KCNTATCAT	QRLANFLVHS	SNNFGAILSS	TNVGSNTY
Rat amylin (rIAPP)	KCNTATCAT	QRLANFLVRS	SNNLGPVLPP	TNVGSNTY
pramlintide	KCNTATCAT	QRLANFLVHS	SNNFGPILPP	TNVGSNTY

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## YR-12

### Selenium as a (Non-)Innocent X-ray Spectroscopic Probe in FeS Clusters: Characterization of Diiron Dichalcogenide Complexes Relevant to Se-Substituted Nitrogenase

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Selenium has frequently been employed as a structural and spectroscopic surrogate for sulfur in both biological and synthetic FeS clusters, demonstrated perhaps most intriguingly by Se incorporation in the FeMo cofactor of nitrogenase.[1,2] While the electronic perturbation of Se-for-S substitution in FeS clusters has generally been assumed to be negligible, quantitative and systematic spectroscopic studies are relatively rare.[3,4] Synthesis of novel [Fe<sub>2</sub>S<sub>2</sub>]<sup>n+</sup>, [Fe<sub>2</sub>SSe]<sup>n+</sup>, and [Fe<sub>2</sub>Se<sub>2</sub>]<sup>n+</sup> complexes supported by bulky beta-diketiminato ligands allows a thorough spectroscopic investigation of the Se-for-S substitution in FeS dimers. Through a combination of Mössbauer, SQUID, and X-ray spectroscopic and magnetic studies the electronic effect of Se substitution in these diiron dichalcogenide systems is explored, revealing intriguing delocalized electronic structures not previously observed in FeS dimers. The unusual electronic structure of these diiron dichalcogenide complexes are demonstrated and quantified via Fe and Se X-ray absorption and emission spectroscopies and contrasted with more traditional FeS dimers, revealing the extent to which Se can be employed as an innocent X-ray spectroscopic probe of larger FeS clusters such as the FeMo cofactor of nitrogenase.

Financial support from Alexander von Humboldt Foundation (JTH and GEC) and the Max-Planck-Gesellschaft are gratefully acknowledged.

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## YR-13

### Hunting for the Identity of the Missing Metabolite of the Moco Riboswitch in the Moco Biosynthetic Pathway

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Molybdenum cofactor (Moco) is an essential metabolite for almost all living organisms consisting of a molybdenum center coordinated to a tricyclic pyranopterin. Moco-dependent enzymes use of the metal redox properties to catalyze fundamental metabolic reactions. Moco is biosynthesized through a highly conserved 4-step pathway starting from GTP, which is consecutively converted into cPMP, MPT and MPT-AMP prior to the insertion of the molybdenum(VI) deriving from molybdate [1]. This pathway involves a variety of different enzymes, whose expression seems to be controlled by an ncRNA regulatory element, the Moco riboswitch [2].

Riboswitches are involved in the control of gene expression and their activity is usually triggered by changes in the cellular concentration of a specific metabolite. This molecule binds to the RNA causing structural rearrangements and leading to a change in the gene expression by regulatory mechanisms that can occur both at the transcriptional or translational level. The Moco riboswitch presents classical characteristics common to this kind of systems, such as sequence conservation, nucleotide covariation and sparse conserved nucleotides alternate to structured base-paired elements. Moreover, studies on the Moco riboswitch from *E. coli*, which is located upstream of genes involved in the Moco biosynthesis, proved its implication in their regulation [2]. However, no evidence of direct interactions between the Moco riboswitch and Moco or any of its biosynthetic precursors have ever been observed. This is due to the scarce availability and high instability of these molecules, which share a peculiar organic oxygen-sensitive scaffold that makes them unsynthesizable. However, protocols were established to isolate cPMP from bacteria and to obtain Moco inserted in a Moco carrier protein (MCP) from *Chlamydomonas reinhardtii* [3,4].

Our goal is to determinate the identity of the metabolite that causes a conformational change in the Moco riboswitch testing the possible *in vitro* interaction between the Moco riboswitch and all available metabolites along the Moco biosynthetic pathway including cPMP and Moco in the form of Moco-MCP. For this purpose, we performed native gel electrophoresis and different footprinting assays under strict oxygen-free conditions. We proved that GTP, molybdate and cPMP are not specific Moco riboswitch binder.

Moreover, both the apo-MCP and the Moco loaded MCP from *C.reinhardtii* cause conformational changes in the RNA structure. However, when the MCP is loaded with Moco, the protein has a higher affinity to the RNA compared to the apo-MCP. Our findings suggest that Moco plays a role in the RNA-protein interaction enhancing the affinity between the two species. These results led to the hypothesis that Moco Carrier Proteins are not only involved in the Moco transfer but that they might have an active role in the RNA gene regulation.

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## YR-14

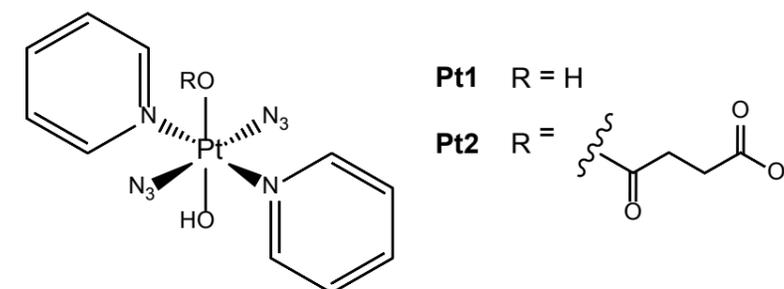
### Targeting Photoactive Pt(IV) Azido Anticancer Complexes by Protein Conjugation

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Pt(IV) azido complexes represent a promising class of photoactivatable anticancer agents. They are inert in the dark, but are reduced to cytotoxic Pt(II) species upon irradiation with visible light, with concomitant release of the azido ligands. [Pt(N<sub>3</sub>)<sub>2</sub>(OH)<sub>2</sub>(py)<sub>2</sub>] (**Pt1**) is the most potent amongst these complexes with higher cytotoxicity than cisplatin and active in cisplatin-resistant cell lines [1]. **Pt1** can be derivatised at the axial hydroxyl species to introduce a second cytotoxic species or cancer targeting agent. Previously reported examples of such derivatisation have exploited reactions with succinic anhydride to generate a succinic acid derivative **Pt2** that was then tethered to amino groups in peptides and small molecules in the presence of coupling reagents [2, 3]. However, these coupling conditions are unsuitable for other targeting vectors such as proteins, whose conjugation requires mild aqueous conditions.

Here we present new Pt(IV) photoactivatable azido complexes for efficient conjugation to proteins by targeting amines (lysine residues) or free thiol groups (cysteine residues). The complexes were synthesised and fully characterised and their photoactivation pathways, upon irradiation with blue light, investigated. Conjugation with proteins including antibodies in aqueous solution are being investigated. Future work will include evaluation of the cytotoxicity of the new complexes and their conjugates in cancer and healthy cell lines in comparison with the parent compound **Pt1**.



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YR-015

## On the Combination of a Ruthenacarborane Fragment with Quinoline-Based Autophagy Inhibitors

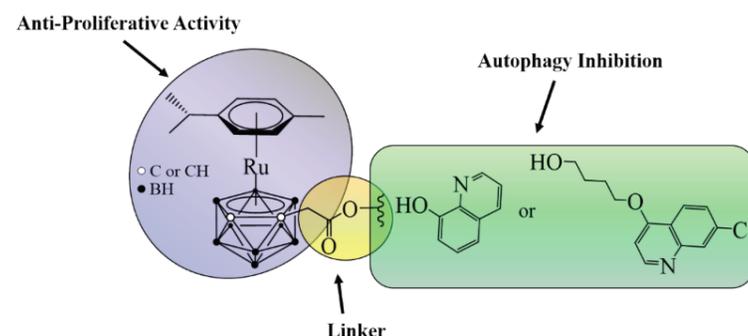
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In the field of medicinal inorganic chemistry, the most widely studied icosahedral metallocarboranes are those of type [com<sub>3</sub>-3,3'-Co(1,2-C<sub>2</sub>B<sub>9</sub>H<sub>11</sub>)<sub>2</sub>], commonly known as COSAN species. COSAN-type complexes were in fact discovered in 2005 by Cíglar *et al.* to act as potent and selective HIV protease inhibitors, thanks to the properties of the carborane cluster *per se*. [1] The latter possesses great chemical stability under biological conditions, it is highly hydrophobic and has low systemic toxicity, which are properties that strongly motivate its use as scaffold in drug design, particularly for target-vector recognition approaches. [2] In our group, we focus our attention on metallocarborane complexes of type *closo*-[3-L<sub>n</sub>-3,1,2-MC<sub>2</sub>B<sub>9</sub>H<sub>11</sub>] (L<sub>n</sub> = arene, CO, *N,N*-bidentate ligands; M = Ru<sup>II</sup>, Mo<sup>II</sup>), as pharmacophores for the design of antitumor agents, following a mechanism-based approach. Our first investigations revealed that the metallocarborane fragment spontaneously forms self-assemblies in aqueous solutions, in the nanometer range, whose size can be efficiently controlled upon formulation with bovine serum albumin (BSA), in a 10:1 molar ratio of BSA over metallocarborane. [3] These self-assembled nanoparticles might provide a selective drug delivery system, via exploitation of the well-known "enhanced permeability and retention" (EPR) effect. Here, we present our results on the design and *in vitro* biological evaluation of quinoline-conjugated ruthenacarborane complexes, against human glioblastoma cells (LN229). The drugs combine a ruthenacarborane fragment with known bioactive groups (quinoline derivatives), already in use as scaffold for chemotherapeutic agents against glioblastomas, for its activity as autophagy inhibitor. The aqueous solution behavior of the complexes was investigated via UV-vis spectroscopy and Nanoparticle Tracking Analysis (NTA). Flow cytometric analysis on the LN229 cells revealed that one conjugate drug effectively acted as autophagy inhibitor, and as potent inhibitor of cell proliferation, and was therefore identified as promising drug candidate for further *in vivo* studies.

Financial support by the Saxon Ministry for Sciences and the Arts (SMWK), the Graduate School Leipzig School of Natural Sciences – Building with Molecules and Nano-objects (BuildMoNa) and the Serbian Ministry of Education, Science and Technological Development is gratefully acknowledged.



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YR-16

## Effect of Cysteine Axial Ligation in c-Type Cytochromes with Native His/Cys Heme Coordination Studied by FTIR Difference Spectroscopy

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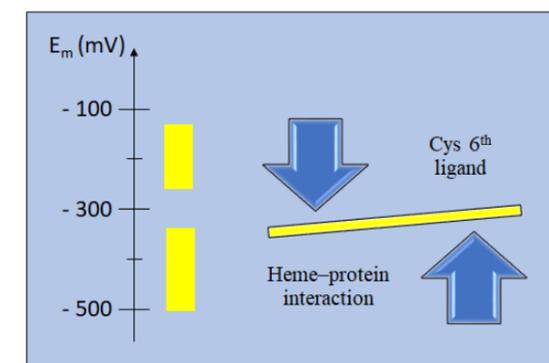
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Cytochromes are hemoproteins containing iron-porphyrin as cofactor. They are involved in a large variety of reactions, including electron transfer in bioenergetics chains, storage and transport of molecular oxygen, hydroxylation or oxidation of various organic substrates. In this work we have investigated the structure – properties relationship of two newly purified c-type cytochromes with His/Cys coordination of the thermophilic cyanobacterium *Thermosynechococcus elongatus* PsbV2 [1] and TII0287 [2], using Mid- and Far- FTIR difference spectroscopy coupled to electrochemistry.

The properties of PsbV2 and TII0287 were compared to those of a Met58Cys mutant (M58C) of cytochrome c6, used as model for the specific His/Cys axial coordination [3]. The cysteine axial ligand determines the very negative redox potentials ( $E_m$ ) in His/Cys c-type cytochromes, i.e. -255 mV [4], -370 mV and -500 mV, for TII0287, M58C and PsbV2, respectively. However, as compared to M58C, cytochromes TII0287 and PsbV2 have significantly different redox potentials. The role of the heme environment and protein structure in modulating the redox properties was demonstrated using FTIR difference spectroscopy. Protonation of the cysteine 6<sup>th</sup> axial ligand was observed upon M58C reduction. For TII0287, and PsbV2, Cys remains in the thiolate form. Stabilization of the axial ligands in an electrophilic environment in TII0287 or the presence of a highly structured water molecule in PsbV2 are proposed to modulate the redox properties of the cytochromes with native His/Cys axial coordination.

A new signal in the Far-IR region between 289 cm<sup>-1</sup> and 303 cm<sup>-1</sup> is proposed as a marker of the His/Cys axial coordination of the heme. Its frequency was correlated with the  $E_m$  value, suggesting that this signal could be a marker for the stability of the oxidised cytochrome.



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YR-17

## N-Heterocyclic Carbene Ligand in Copper Enzymes Tunes Redox Properties

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Copper enzymes are efficient catalysts for electron transfer and dioxygen activation. Histidine is the common amino acid in the different active site types (Type 1, 2, 3 and CuA), and binds copper through a well-established N-bonding mode.<sup>1</sup> In synthetic complexes, C-bonding to different metal centers has been observed by tautomerization processes.<sup>2</sup>

We are interested in inducing C-bonding rather than N-bonding of an imidazole and in investigating its effect on the activity of selected copper enzymes. For this purpose, N,N'-dimethylimidazolium-2-carboxylate (DMI-CO<sub>2</sub>) has been used as an N-heterocyclic carbene (NHC) precursor.<sup>3</sup> A variety of synthetic copper(II)-NHC complexes have been thus prepared as model complexes for C-bonding, to study the coordination chemistry of such challenging complexes.<sup>4</sup>

Moreover, the Type 1 copper active site of natural redox-active enzymes has been mutated to replace a histidine residue for a glycine, in order to bind an exogenous ligand. The displacement of the copper-coordinated water molecule in these mutants has been monitored spectroscopically (UV-vis, EPR), where binding of N-methylimidazole or NHC has been observed. Thus, the effect of C-bonding (NHC ligand) to copper on catalysis activity has been compared to N-bonding (N-methylimidazole ligand), where an effect on the redox processes was observed.<sup>5</sup>

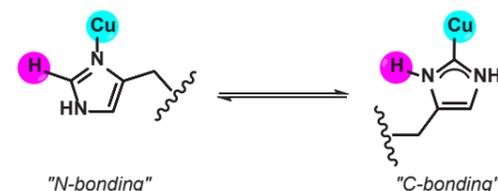


Fig. 1. Possible bonding modes of histidine coordinating to copper in natural enzyme active sites.

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YR-19

## Light-Activated Rhenium Complexes against Gram(+) and Gram(-) Bacteria

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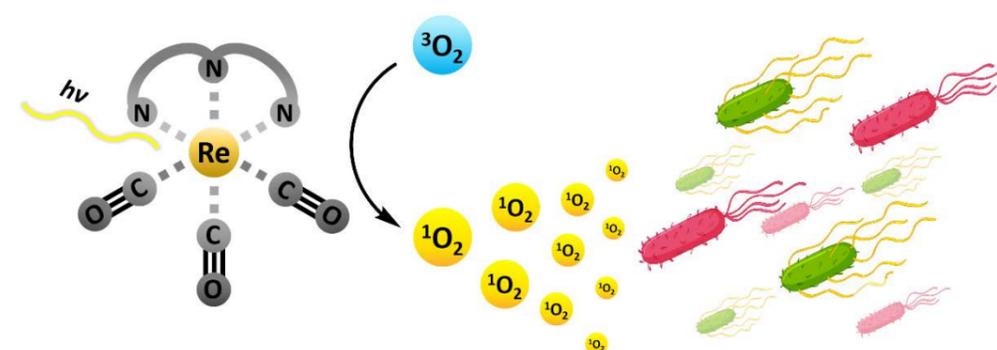
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Of the 42 small molecule antibiotic drug candidates that are in clinical trials in 2019 most represent slightly modified versions of already approved compounds. Only a quarter of these 42 represent new compound classes with new modes of actions. Even worse, only one of these is active against Gram-negative pathogens, which are notoriously harder to eradicate.<sup>[1]</sup>

Metal complexes have proven themselves very promising in the search for new cancer treatments, with multiple compounds in clinical trials.<sup>[2]</sup> Surprisingly, most of the attention seems to be on antitumour applications, while reports on metal-based antibiotic compounds are rather sparse. Photodynamic Therapy (PDT)-based approaches where a photosensitizer is excited by light irradiation to generate reactive oxygen species has been shown to be an effective antibacterial approach.<sup>[3]</sup> Recent studies found that bacteria are slow if not unable to develop resistance against the broad-spectrum effects of antimicrobial PDT, further highlighting the potential of this strategy.<sup>[4]</sup> While some organic photosensitizers have been studied extensively for aPDT, only little is known about metal-based photosensitizers against bacteria.

We have prepared rhenium tricarbonyl-based photosensitizers that show both light-dependent and independent activity against several Gram-positive and Gram-negative pathogens. By comparing the activity of these complexes in different resistant and mutated strains as well as determining their bacterial cell uptake we are able to make some conclusions about their mode of action which will aid in the preparation of targeted aPDT agents.

Financial support by the Swiss National Foundation is gratefully acknowledged.



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## YR-20

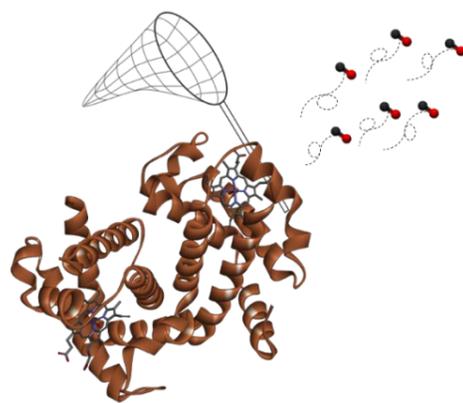
### The Birth of the Hydrogenase: Study of the Quaternary Structure and the Redox Chemistry of the FeFe Hydrogenase Maturation

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The active site of the FeFe hydrogenase, the “H-cluster”, is composed of a canonical [4Fe4S] cluster coupled to a diiron complex decorated with carbonyl and cyanide ligands.<sup>1</sup> The biosynthesis and insertion of the, in biology unique, diiron complex requires at least three hydrogenase specific auxiliary proteins or maturase enzymes.<sup>2</sup> Two of these enzymes (HydG and HydE) belong to the radical SAM enzyme family and are responsible for the synthesis of the precatalyst on the scaffold protein HydF<sup>3,4,5</sup>, which delivers the pre-catalyst to the FeFe hydrogenase (HydA)<sup>6</sup>.

The biological synthesis of the H-cluster is an intensively studied process. Nevertheless, there is still a lack of information concerning the stages of this process and HydF-HydA interaction on both quaternary structural and cofactor level. To a large extent this is due to difficulties in obtaining sufficient quantities of homogenous and active HydF. Recent studies have shown that biomimetic model compounds can be introduced into HydF<sup>7,8</sup>. Critically, these semi-synthetic forms of HydF can mimic the reactivity of native HydF and transfer their synthetic cargo to apo-HydA, to generate fully active forms of HydA indistinguishable from the native hydrogenase enzyme. Here I will show how we can take advantage of this new found chemistry, which allows the preparation of significantly larger quantities of active HydF, and use it to elucidate the mechanism by which HydF activates HydA.



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## YR-21

### Spectroscopic Investigations of Protein-Substrate Interactions in Lytic Polysaccharide Monooxygenases (LPMOs)

Luisa Ciano<sup>1</sup>, Gideon J. Davies<sup>2</sup> and Paul H. Walton<sup>2</sup>

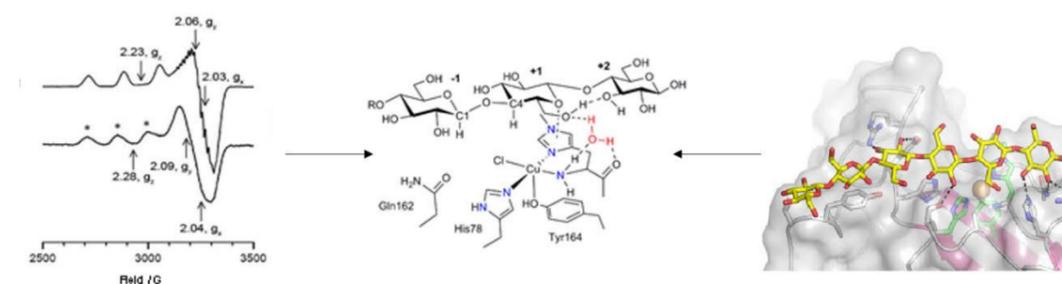
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The effective use of biomass is crucial in addressing the global need for sustainable energy sources. However, polysaccharides are highly recalcitrant to degradation, which poses a major challenge in their utilisation for the production of biofuel and commodity chemicals from renewable sources. A major breakthrough in the field was achieved by the discovery of lytic polysaccharide monooxygenases (LPMOs), copper-dependent enzymes found in a variety of organisms [1,2]. LPMOs break down polysaccharide chains *via* an oxidative mechanism in the presence of a reducing agent and molecular oxygen, thus boosting the action of classic enzymatic cocktails for industrial polysaccharide degradation.

Understanding the mechanism of action of these enzymes and their interaction with the substrates is essential for the development of this field. Comprehensive structural, kinetic and spectroscopic investigations of an oligosaccharide-active LPMO from the AA9 family have recently been reported [3,4], giving the first insight into the changes caused by the arrival of substrate to the Cu active site of the enzyme. <sup>1</sup>H HYSCORE experiments in the presence of celohexaose and chloride demonstrated the involvement of the N-terminus in a hydrogen bond network which ‘connects’ the copper active site to the oligosaccharide *via* a ‘pocket’ water molecule [3]. Electron paramagnetic resonance (EPR) spectroscopy studies revealed differences in Cu-coordination upon binding of glucans and xylan [4]. We have now furthered these studies by analysing the interaction between the LPMO and its natural crystalline substrate through the development of a semi-oriented EPR spectroscopy method. Furthermore, we have expanded the analysis of the LPMO-substrate interaction using a chitin active enzyme belonging to the AA10 family. In this talk, we will highlight these collective data in LPMO chemistry and the implications for their effective utilisation in biomass degradation.

Financial support by the Biotechnology and Biological Sciences Research Council (BBSRC) and the Engineering and Physical Sciences Research Council (EPSRC) is gratefully acknowledged.



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YR-22

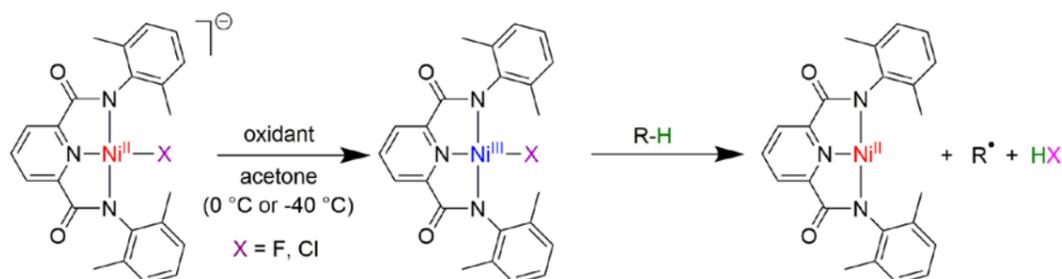
## Hydrocarbon Oxidation via Hydrogen Atom Transfer Mechanism by Well Characterized High Valent Nickel Halide (F, Cl) Complexes

Prasenjit Mondal<sup>1</sup>, Paolo Pirovano<sup>1</sup>, Ankita Das<sup>1</sup>, Erik R. Farquhar<sup>2</sup>, A. R. McDonald<sup>1</sup>

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The selective oxidative functionalization of the inert C-H bonds in hydrocarbon is an economically and environmentally valuable chemical transformation process. Nature has evolved a number of metalloenzymes that perform such hydrocarbons oxidation, forming hydroxylated, halogenated and desaturated hydrocarbons.<sup>1</sup> It has been proposed that such reactions involve hydrogen atom transfer (HAT) from an inert C-H bond to a high valent metal based oxidants such as M=O and M-OX (OX = OH, OR, O<sub>2</sub>C-R, ONO<sub>2</sub>).<sup>2-3</sup> However, the HAT reactivity of the metal-bound halide in C-H activation has not been studied before this work.<sup>4</sup> To get an idea about the HAT reactivity toward the hydrocarbon oxidation by metal-bound halide, two high-valent nickel-halide complexes [Ni<sup>III</sup>(X)(L)] (X = F, Cl, L = *N,N'*-(2,6-dimethylphenyl)-2,6-pyridinedicarboxamide) have been prepared by one-electron oxidation of the [Ni<sup>II</sup>(X)(L)]<sup>-</sup> precursors (Scheme 1) and characterized using electronic absorption, EPR, XAS and mass spectrometry.<sup>4</sup> Oxidative reactivity through kinetics study of these complexes was investigated with a series of organic substrates containing phenolic O-H and hydrocarbon C-H bonds (Scheme 1). Analysis of the Hammett, Evans-Polanyi, Marcus plots, KIE and oxidative product study confirm that oxidation reactions were proceed through a hydrogen atom transfer (HAT) mechanism. These are the first high valent nickel-halide oxidants that perform HAT reaction and hence open new routes for oxidative halogenation catalyst design without involvement of metal-oxo group.



**Scheme 1.** Preparation of high valent nickel-halide complexes and their oxidative reaction.

Financial support by the European Research Council is gratefully acknowledged.

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YR-23

## Multiple Metal Sites in De Novo Designed Metalloproteins: From Isolated Cofactors to Bridged Metal Clusters

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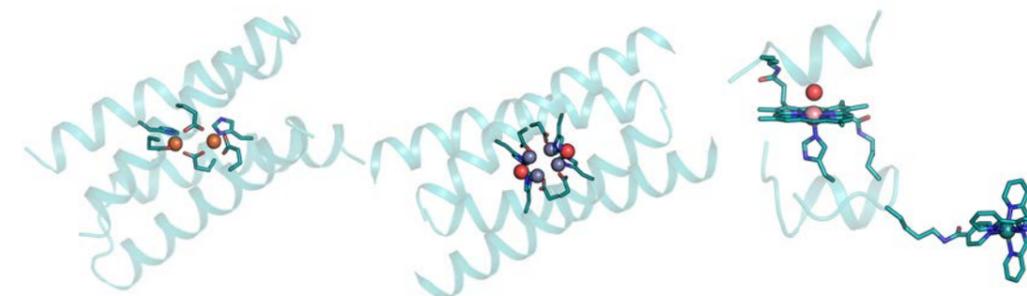
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Metalloproteins bind a single or multiple metal ions to perform their functions, either as different interacting cofactors or as multinuclear metal clusters. Some of the most difficult transformations are achieved by the subtle interplay of these metal units in many different ways such as magnetic coupling, charge transfer or sacrificial/protective roles [1]. Together with deep structural and spectroscopic studies of natural proteins, de novo protein design aims in disentangling the interactions between the protein matrix and the metal cofactors [2]. By different approaches, we designed small, yet functional, models bearing multiple metal sites: (i) a carboxylate bridged diiron site, (ii) an abiotic tetranuclear zinc cluster, (iii) a cobalt porphyrin coupled to a ruthenium photosensitizer. We first show that by asymmetrization of the four-helix bundle scaffold, called DF-C1, and precise positioning of the residues at the binding site, we reached unprecedented selectivity in the oxidative coupling of aromatic dyes, performing a net 4-electron reduction of dioxygen [3]. Then we show how very small changes of a similar scaffold may alter the number of bound metals and most importantly the geometry of a tetrazinc cluster in the 26-mer peptide homotetramer, called QF [4]. Finally, we describe our efforts to couple a photoactive polypyridyl ruthenium complex with a cobalt-substituted porphyrin/peptide conjugate, CoMC6\*a, active in hydrogen evolution [5], by direct ligation to the peptide moiety. In perspective, our designed metalloproteins will be ultimately combined to get new catalysts of ever growing complexity.

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YR-24

## Iron-Based Drugs: Exploring the Properties of Diiron Vinyliminium Complexes as Anticancer Agents

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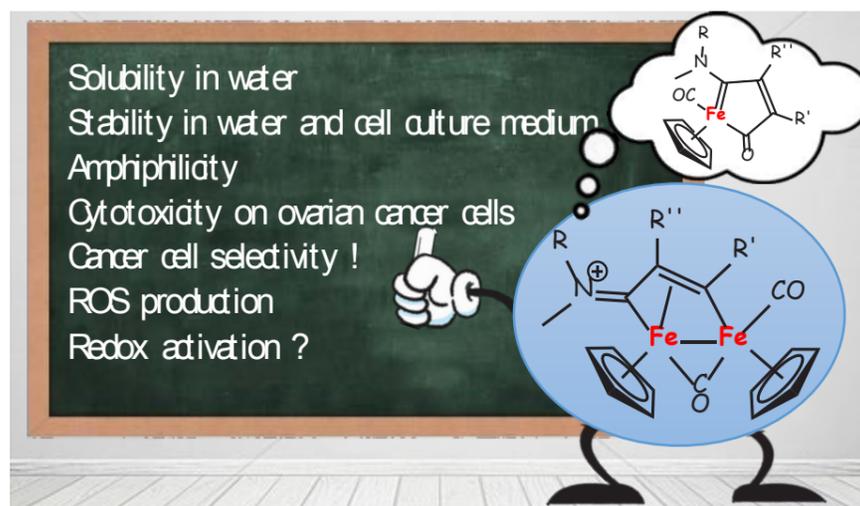
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Iron represents an appealing choice to develop new metal-based drugs, being a cheap, bio-essential and relatively nontoxic element. Indeed several mononuclear iron complexes have been investigated for their anticancer behaviour and substituted ferrocenes have emerged as the most promising candidates [1]. On the other hand, a large number of dinuclear iron cyclopentadienyl complexes deriving from  $[\text{Fe}_2\text{Cp}_2(\text{CO})_4]$  have been synthesized over the last 35 years [2] and yet their pharmacological properties remained unexplored.

Bridging vinyliminium ligands can be installed on the diiron scaffold by stepwise assembly of isocyanide and alkyne units, thus giving access to a family of compounds with considerable structural diversity. These compounds are soluble in water and display good stability both in water and in cell culture medium. The combination of a net positive charge and non-polar fragments endow such complexes with an amphiphilic character ( $\text{Log } P_{\text{ow}}$ ).

Diiron vinyliminium complexes exhibit  $\text{IC}_{50}$  values in the low/medium micromolar range against ovarian cancer cell lines (A2780 and cisplatin-resistant A2780cisR), together with an appreciable selectivity towards non-tumorigenic cells (HEK-293). Ovarian cancer cell lines incubated with  $\text{Fe}_2$  complexes show an increased production of reactive oxygen species (ROS), which could be associated to the cytotoxic activity. The diiron compounds may behave as pro-drugs, being activated by reduction to mononuclear Fe-cyclopentadienyl species and atomic iron, thus stimulating ROS formation.

Financial support by the University of Pisa (PRA\_2017\_25) and the EPFL is gratefully acknowledged.



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YR-25

## Protein Film Electrochemical EPR Spectroscopy as a Technique to Investigate Redox Reactions in Biomolecules

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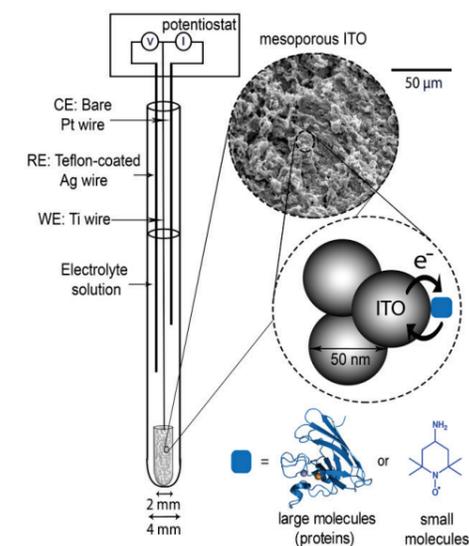
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The combination of electron paramagnetic resonance (EPR) spectroscopy with protein film electrochemistry (PFE) provides a novel platform for the investigation of redox-active species including metalloproteins. The development of a PFE-EPR system enables the detection of paramagnetic species with direct and accurate potential control, providing new mechanistic insight into redox-based processes in biomolecules. Current EPR spectroelectrochemical setups are based on generating radical species in solution via electrolysis and/or require mediators<sup>1</sup> and are therefore limited by diffusion. Accurate potential adjustment and concomitant investigation of paramagnetic intermediates, is thus not possible with large redox active complexes, including many proteins. We are developing a generally applicable PFE-EPR method to study biomolecules irrespective of their size.

Here, we prove the feasibility of a combined PFE-EPR method using small paramagnetic molecules and proteins, 4-amino TEMPO and Cu-Zn superoxide dismutase (16 kDa dimer), respectively. We demonstrate that hierarchical indium tin oxide (ITO) structures<sup>2</sup> are suitable working electrode materials, offering good conductivity and a large surface area for protein immobilisation. The mesoporous structure (meso-ITO) is compatible with commercially-available EPR instrumentation (Figure 1) and remarkable sensitivity for EPR spectroelectrochemical applications was achieved with only 1 mm<sup>3</sup> of electrode inside the EPR cavity. EPR spectra of 4-amino TEMPO and SOD not only indicate successful reduction and detection of the paramagnetic species but also demonstrate the direct, accurate and fast potential control offered by our PFE-EPR method.<sup>3</sup> Further developments of the PFE-EPR method with greater surface area<sup>2</sup> to investigate the mechanism of large metalloproteins such as respiratory complex I are also presented.



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## YR-26

### Shining Light on Photoferrotrophic Iron Oxidation: The Structure & Function of Metalloproteins in *Rhodospseudomonas Palustris* TIE-1

Inês B. Trindade<sup>1</sup>, Michelle Invernici<sup>2</sup>, Nazua L. Costa<sup>1</sup>, Ivo Saraiva<sup>1</sup>, Bruno M. Fonseca<sup>1</sup>, Francesca Cantini<sup>2</sup>, Mario Piccioli<sup>2</sup>, Ricardo O. Louro<sup>1</sup>

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*Rhodospseudomonas palustris* TIE-1 is a Gram-negative bacterium with a very versatile metabolism, including the remarkable capacity of harvesting energy from sunlight while oxidizing iron II to iron III, one of the most ancient metabolisms on Earth.

This was the first photoferrotroph ever isolated and during anaerobic growth it expresses a cytochrome *c*<sub>2</sub> and two HiPIPs (PioC and Rpal\_4085). Both HiPIPs and soluble c-type cytochromes are known to donate electrons to the reaction centers of anoxygenic photosynthesis, but the apparent redundancy of this pathway has yet to be fully clarified. In *R. palustris*, the cytochrome *c*<sub>2</sub>, as previously seen for other purple bacteria, acts in cyclic electron flow to support photosynthetic growth. PioC is an HiPIP with specialized role in phototrophic iron oxidation. It delivers electrons directly to the light capturing reaction center, but it does not support cyclic electron flow and deletion mutants of PioC are still able to maintain residual reductive activity. Rpal\_4085 has similar electrochemical properties to PioC and the two HiPIPs coexist in the periplasm, but Rpal\_4085 is unable to functionally replace PioC, appearing to be involved in divalent metal sensing [1].

In this work we used NMR spectroscopy to investigate the structural basis for the discrimination of the function of these small redox-active metalloproteins in the versatile anoxygenic phototrophic pathways of *R. palustris*. These results not only illuminate the molecular aspects underpinning this ancient metabolism, but also guide the development of biotechnological applications of this versatile microorganism [2]

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## YR-27

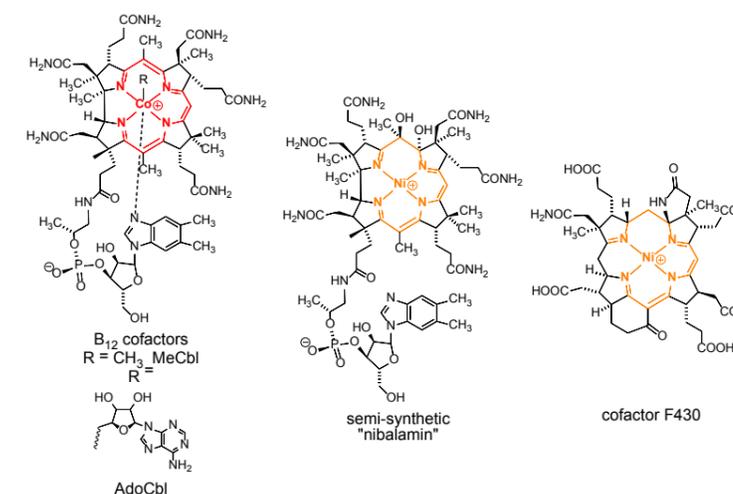
### A Novel Ni-containing B<sub>12</sub> Derivative – Elucidating the Origin of Corrinoid and Corphinoid Cofactors

Christopher Brenig<sup>1</sup>, Lucas Prieto<sup>1</sup>, René Oetterli<sup>1</sup>, Felix Zelder<sup>1</sup>

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For a better understanding of biological processes catalyzed by transition metal complexes with porphyrinoid ligands, it is important to discern why different structural alterations of one ligand system were chosen by nature in order to accommodate a specific metal center. Some important porphyrinoid cofactors that display a high structural complexity are the B<sub>12</sub> cofactors methylcobalamin (MeCbl) and 5'-deoxyadenosylcobalamin (AdoCbl), as well as cofactor F430. While the two former cofactors, derived from vitamin B<sub>12</sub>, play important roles in the metabolism of methionine and folate, nucleotide synthesis<sup>[1]</sup> and the breakdown of odd fatty acids,<sup>[2]</sup> the latter ("F430") is found only in methanogenic bacteria where it catalyzes the formation – as well as the oxidation – of methane.<sup>[3]</sup> Recently we accomplished the first partial chemical synthesis of a cobalamin derivative starting from vitamin B<sub>12</sub>, that contains a central nickel ion instead of cobalt. Key step of the synthetic route was the simultaneous demetallation and ring closure of a ring-opened 5,6-secocobalamin. The resulting 'nibalamin' derivative shares spectroscopic features with F430 that are attributed to structural similarities of the tetradentate ligand framework.<sup>[4]</sup>

The coordination chemistry of this hybrid F430-B<sub>12</sub> species, however, supports pioneering work of the Eschenmoser group regarding why nature has combined Ni with a corphin rather than a corrin ligand in F430 to allow for challenging biochemical transformations.<sup>[5]</sup> Semi-synthetic metallocorrins can shed new light on the old question of corrin vs. corphin ligand in nature but might as well bear potential for applications in analytical and medicinal science. Financial support by the Forschungskredit of the University of Zurich (grant FK-17-088) is gratefully acknowledged.



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YR-28

## Spectroscopic Characterization of Intermediate Spin-States in Mixed-Valent Diiron Dichalcogenide Complexes

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Iron-sulfur clusters exhibit a breadth and variety in their molecular and structural composition matched only by that of their biological function. The electronic structure of these clusters is integral to their function and reactivity, and as such studying these systems necessitates a detailed understanding of the underlying electronic states. Nearly all known biological mixed-valent  $[\text{Fe}_2\text{S}_2]^+$  clusters exist in the localized  $S = 1/2$  ground state, arising from strong antiferromagnetic coupling between the locally high spin Fe(III)  $S = 5/2$  and Fe(II)  $S = 2$  centers. Fully delocalized  $[\text{Fe}_2\text{S}_2]^+$  isolated dimers exhibiting a  $S = 9/2$  ground spin-state has only been observed for the Cys60Ser mutant ferredoxin of *C. pasteurianum*.<sup>[1]</sup> However, all known synthetic  $[\text{Fe}_2\text{S}_2]^+$  models possess  $S = 1/2$  ground-states. A single well-characterized  $[\text{Fe}_2(\mu\text{-OH})_3]^{2+}$   $S = 9/2$  synthetic complex is known, with a diiron distance much shorter than typical  $[\text{Fe}_2\text{S}_2]^+$  clusters.<sup>[2-3]</sup> In all cases where  $S = 9/2$  ground states have been observed, the iron ions remain anti-ferromagnetically coupled in a classic exchange coupling mechanism,  $J < 0$ , and a Heisenberg double-exchange (sometimes referred to as resonance delocalization),  $B$ , is invoked to explain the fully delocalized ground state.<sup>[3-4]</sup> The interplay of these two mechanisms allows for the stabilization of an antiferromagnetically coupled  $S = 9/2$  ground state when  $|B/J|$  is sufficiently large, but more interestingly predicts the possibility of stabilizing intermediate-spin ground states  $S = 3/2$ ,  $5/2$ , or  $7/2$  for certain balanced  $|B/J|$  ratios.

The recent isolation of novel  $[\text{Fe}_2\text{S}_2]^+$ ,  $[\text{Fe}_2\text{SSe}]^+$ , and  $[\text{Fe}_2\text{Se}_2]^+$  complexes with bulky bidentate ligands have yielded new and intriguing electronic properties not observed in other diiron biomimetic complexes. These complexes are the first known examples of intermediate spin-states ( $1/2 < S < 9/2$ ) in diiron dichalcogenide mixed-valent complexes. In-depth analysis of the Mössbauer, EPR, and ENDOR spectroscopic data of these complexes complemented with magnetic susceptibility measurements clearly establish the atypical electronic structure observed. These synthetic complexes provide insight into the mechanism of electronic delocalization in biological clusters, a fundamental concept for the understanding of electronic structure and reactivity of larger, more complex FeS clusters.

Financial support from Alexander von Humboldt Foundation (GEC and JTH) and the Max-Planck-Gesellschaft are gratefully acknowledged.

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YR-29

## Targeted Delivery of Metal Complexes Across the Blood-Brain Barrier Using Focused Ultrasound for the Treatment of Alzheimer's Disease

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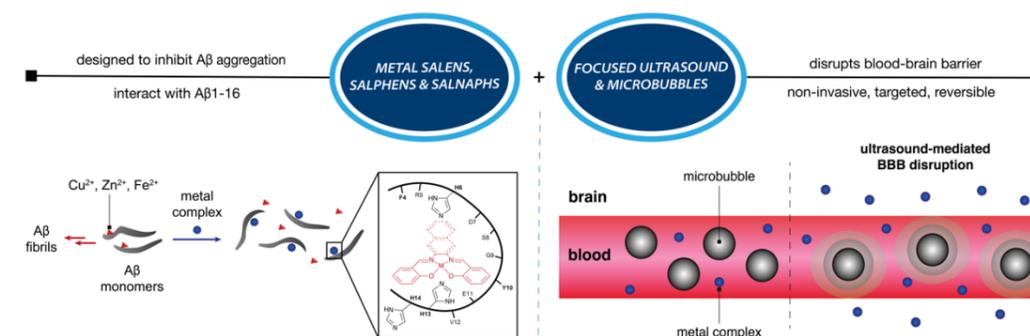
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**Background:** Alzheimer's disease is the most common form of dementia, affecting ~30 million people worldwide. One of the hallmarks of Alzheimer's disease is the aggregation of amyloid beta ( $\text{A}\beta$ ) to form fibrils, a process that is promoted by the binding of  $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cu}^{2+}$  ions to its N-terminal (amino acids 1-16). To date, a variety of metal complexes, including platinum phenanthroline derivatives and cobalt Schiff base complexes, have been developed to bind to the N-terminal of  $\text{A}\beta$  to prevent  $\text{A}\beta$  aggregation; however, whilst many of these complexes have shown promise *in vitro*, they are ineffective *in vivo* due to an inability to cross the blood-brain barrier (BBB).<sup>1,2</sup> As of now, the only method to disrupt the BBB in a non-invasive, targeted and reversible manner is with focused ultrasound and microbubbles.<sup>3</sup> In this work, we have synthesised a series of metal salens, salphens and salnaphs as potential inhibitors of  $\text{A}\beta$  aggregation, investigated their binding mode to  $\text{A}\beta$  and show that they can be delivered across the BBB *in vivo* using focused ultrasound.

**Methods:** Metal complexes were synthesised and assessed for their ability to inhibit  $\text{A}\beta$  aggregation using the Thioflavin-T assay. Binding interactions with  $\text{A}\beta$  were probed using fluorescence and <sup>1</sup>H NMR spectroscopy. Metal complexes were delivered to the left hippocampus of mice using focused ultrasound, with the right hippocampus used as the non-sonicated control. Brain sections were analysed using laser ablation inductively-coupled plasma mass spectrometry (LA-ICP-MS) or stained with haematoxylin and eosin (H&E) to detect the presence of the complex within the brain and assess safety respectively.

**Results:** All metal complexes tested show an ability to inhibit  $\text{A}\beta$  aggregation, with greater inhibitory activity observed for complexes with vacant coordination sites. <sup>1</sup>H NMR and fluorescence experiments suggest that these complexes interact with  $\text{A}\beta$  *via*  $\pi$ - $\pi$  stacking interactions with aromatic residues such as Tyr-10 and coordination with histidine residues (His-6, His-13 or His-14). Using focused ultrasound and microbubbles, metal complexes were successfully delivered across the BBB to the left hippocampus of mice. No signs of tissue damage were observed in H&E-stained brain sections.



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## YR-30

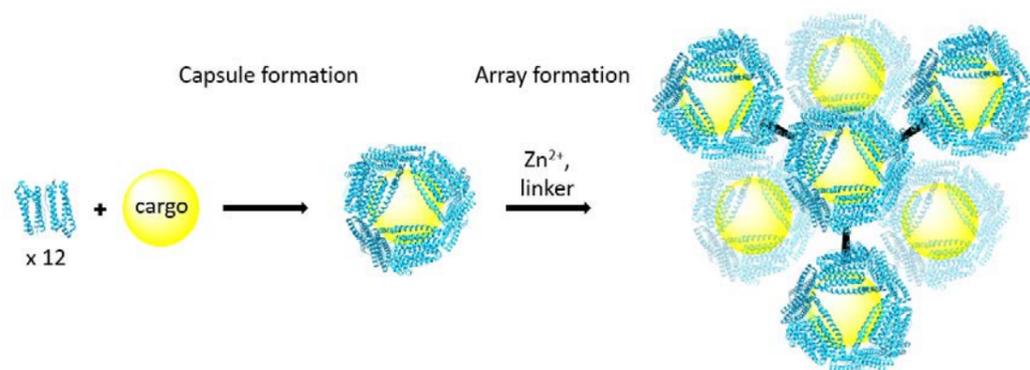
### Ferritin Capsules for Arraying Nanoparticle and Enzyme Cargo

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Constructing nanostructures with tunable higher-order architectures remains a challenge. Nanomaterials assembled using complementary oligonucleotides are costly, and it is challenging to attach oligonucleotides at well-defined sites and uniform coverage densities. Defects in nanoparticle size and shape further increase the polydispersity of these assemblies. However, protein cages, such as ferritin, provide atomically precise, nanoscale building blocks that are monodisperse and allow for specific attachment of different linkers. Ferritins provide stable protein cages, amenable to many modifications via site-directed mutagenesis and synthetic chemistry. We previously demonstrated efficient encapsulation of inorganic nanoparticles[1,2] within thermophilic ferritin (AfFtn) from *Archaeoglobus fulgidus*. More recently, we and others have shown encapsulation of supercharged protein cargo within AfFtn[3,4]. Here, we introduce a Zn<sup>2+</sup>-binding site to the small, 3-fold symmetric channels of AfFtn, which can be further modified with bishydroxamate linkers to prepare arrays of cargo-templated protein capsules with tunable distances and geometries.

Financial support by the University of Pennsylvania, LRSM-MRSEC, VIEST and the NSF are gratefully acknowledged.



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## YR-31

### Biomimetic Catalytic Systems Based on the Iron Complexes for Aromatic C–H Bond Oxidation: Electronic Structure of the Oxoiron(V) Intermediates Dependence on Carboxylic Acid Structure

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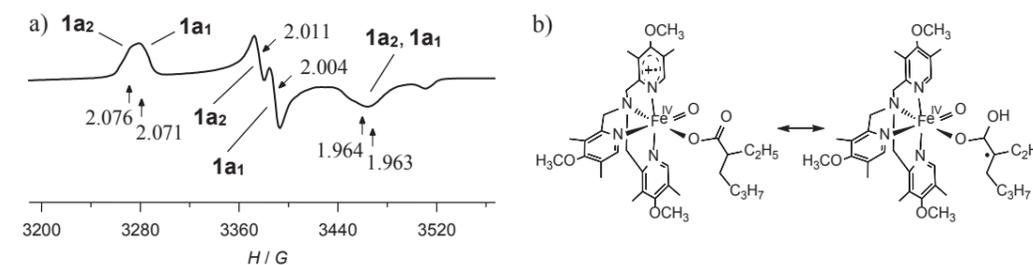
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The development of selective and efficient catalysts for oxidation of various organic substrates has become an important task in pharmaceuticals and chemical industry. A great challenge of the fine organic synthesis is the selective epoxidation of C=C and hydroxylation of strong aliphatic C–H bonds. Furthermore, it is very important to use environmentally friendly and nonexpensive reagents.

Natural metalloenzymes catalyze the oxidation of multifunctional organic substrates with very high regio- and stereoselectivity under mild conditions inspiring researchers to design biomimetic catalysts (synthetic models of nonheme iron oxygenases). The systems based on iron complexes with tetradentate N-donor ligand and H<sub>2</sub>O<sub>2</sub> as an oxidant were found to be one of the best biomimetic catalyst systems for alkenes epoxidation and alkanes hydroxylation [1]. Nowadays for all preparatively useful catalyst systems Fe<sup>II</sup>, Fe<sup>III</sup>/H<sub>2</sub>O<sub>2</sub>/RCOOH, the Fe<sup>V</sup>=O species have been proposed to be active oxidizing intermediates.

However, to date, only a few examples of selective hydroxylation of aromatic substrates are known. In 2010, Rybak-Akimova and coworkers obtained convincing indirect evidence in favor of the participation of oxoiron(V) species in aromatic hydroxylation [2]. So far no direct studies have been carried out on the reactivity of nonheme oxoiron(V) species towards arenes.

Herein, we report EPR spectroscopic and reactivity studies of the catalyst systems [(L)Fe<sup>III</sup>(μ-OH)<sub>2</sub>Fe<sup>III</sup>(L)](CF<sub>3</sub>SO<sub>3</sub>)<sub>4</sub>/H<sub>2</sub>O<sub>2</sub>(CH<sub>3</sub>CO<sub>3</sub>H)/RCOOH (L = tris(3,5-dimethyl-4-methoxyphenyl)-2-methylamine (complex **1**) or bis(3,5-dimethyl-4-methoxyphenylmethyl)-(S,S)-2,2'-bipyridine (complex **2**)). It has been shown that the electronic structure of the detected oxoiron(V) intermediates depends on the structure of the added carboxylic acid RCOOH. So, catalyst systems with the additive of branched carboxylic acids 1/H<sub>2</sub>O<sub>2</sub>(CH<sub>3</sub>CO<sub>3</sub>H)/(for example, 2-ethylhexanoic acid) exhibit EPR spectra of two unstable Fe<sup>V</sup>=O intermediates, **1a1** and **1a2** (Figure 1a), whereas catalyst systems 1/H<sub>2</sub>O<sub>2</sub>(CH<sub>3</sub>CO<sub>3</sub>H)/(linear carboxylic acid) display EPR spectrum of one unstable Fe<sup>V</sup>=O intermediate **1a**. In the case of systems with 2-ethylhexanoic acid additive the presence of an equilibrium between two tautomeric species has been proposed (Figure 1b).



**Figure 1.** EPR spectrum (−196 °C) of the sample 1/CH<sub>3</sub>CO<sub>3</sub>H/2-ethylhexanoic acid ([Fe]:[CH<sub>3</sub>CO<sub>3</sub>H]:[2-ethylhexanoic acid] = 1:3:10, [Fe] = 0.04 M) frozen (a) 2 min after mixing the reagents at −70 °C, and storing the sample at −80 °C for 12 min; (b) proposed equilibrium between **1a1** and **1a2**.

It was found that the direct reactivity of the oxoiron(V) intermediates **1a** and **2a** toward substituted benzenes increases in the following order: nitrobenzene < acetophenone < chlorobenzene < benzene < toluene, in accordance with the growth of the electron-rich properties of the substrate, which is consistent with the mechanism of electrophilic substitution in the aromatic ring. The iron complex **2** catalyzes aromatic hydroxylation by H<sub>2</sub>O<sub>2</sub> or peracetic acid in acetonitrile, performing up to 36.5 catalytic turnovers per Fe atom. Also, high selectivity toward aromatic oxidation products (up to 91%) has been documented.

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## Poster Presentations

P001

### Synthesis of a Photosensitized Artificial Metalloenzyme for Hydrogen Evolution

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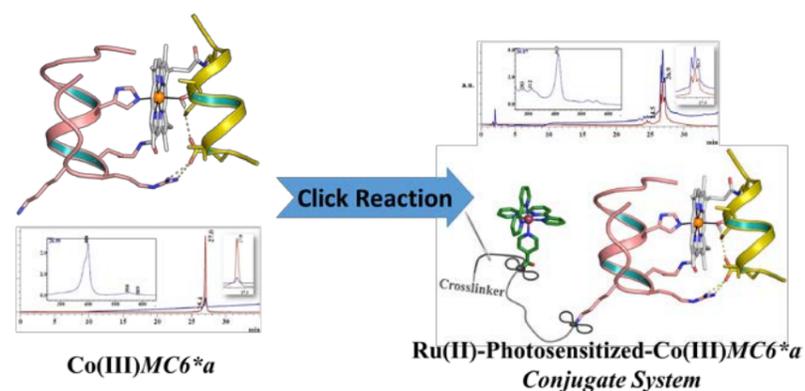
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<sup>3</sup>Institute of Biostructures and Bioimages, National Research Council, Via Mezzocannone, 80134 Napoli, Italy.

The hydrogen evolution reaction (HER) from water splitting is a substantial process to generate a precious alternative to fossil fuels. However, the high overpotential is its main constraint. Different approaches have been developed to overcome this limitation [1]. Accordingly, the development of innovative bio-inspired systems to catalyze the HER is considered a promising solution [2]. Previously, we have rationally designed porphyrin-peptide conjugates, named "Mimochromes", a family of peptides mimicking natural heme proteins, with diverse catalytic activities [3]. The iron(III) derivative of *mimochrome VI\*a* (Fe(III)MC6\*a), the most active analogue, has shown a peroxidase activity that approaches natural peroxidases [4].

Cobalt(III) derivative (Co(III)MC6\*a) has shown promising catalytic-activity, longevity and stability for electrochemical HER under mild conditions [5]. Nevertheless, the high overpotential (~680 mV) hampered its potential application. A stable, effective and stand-alone photo-sensitizing system may represent a viable solution to bias such thermodynamic requirement. On this respect, some criteria should be taken into account in the choice of (i) the photosensitizing moiety (e.g. solubility, quantum yield, reduction potential), (ii) the conjugation method (e.g. orthogonality, modularity, scalability), and (iii) the optimal distance between the active centers.

Herein, we are introducing the design and synthesis of our first prototype of Ru(II)-based photosensitized Co(III)MC6\*a system. First, a new ruthenium-based photosensitizer has been designed and synthesized to achieve the chemical stability and photo-activity requirements. Spectroscopic and theoretical characterizations of the photoactive complex support the followed approach. Next, a derivatizable carboxyl moiety allows the facile conjugation to the peptide scaffold of Co(III)MC6\*a. Azide-alkyne cycloaddition reaction has been adopted to couple the Ru-photosensitizer to Co(III)MC6\*a, by adopting a tunable PEG crosslinker.



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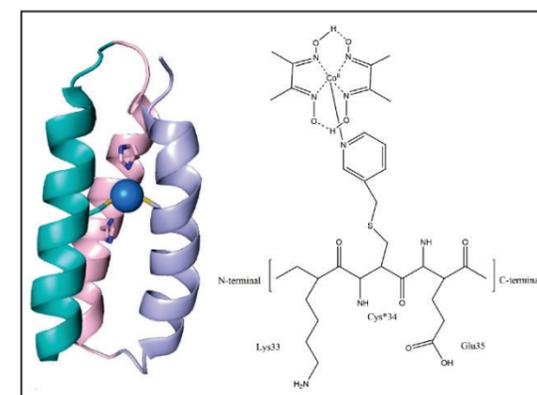
P002

### Construction and Characterization of De Novo Metalloenzymes for Production of Solarfuels

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Inventing novel approaches to creating sustainable energy is an important cause in a world where the atmosphere temperature is rising, conflicts over fossil fuels are ongoing and options are sparse or inaccessible. The aim with this project is to develop biological catalysts that can sustainably and efficiently produce solar fuels such as hydrogen gas or methane through the oxidation of water or reduction of carbon dioxide. Using transition metals for this type of redox-chemistry has been an attractive target for some time, and it is our hope that applying it to a biological scaffold will help overcome problems such as oxygen-sensitivity, solubility and stability, as well as being able to use cheaper, first row transition metals.<sup>1</sup> The  $\alpha_3\text{x}$ -protein designed by Tommos et al. is currently the scaffold being used for this project, and several metal-binding sites have been planned.<sup>2</sup> Current work is ongoing to create binding sites containing four histidines, four cysteines and a mixture of the two using site directed mutagenesis (right part of figure for an example with two cysteines and two histidines). This scaffold will bind several of the interesting redox-active transition metals (Mn, Fe, Co, Ni, Cu). With this approach we can, with spectroscopic techniques, screen for what metal-protein complex will most readily form, and also perform redox chemistry most efficiently. The redox potentials and catalytic properties and mechanisms will be studied with protein film voltammetry and transient absorption laser spectroscopy. We aim to find a scaffold/metal system, which is active for both reducing and oxidizing chemistry, depending on, among other things, the substrate and the metal ion. It is our aim that this project lead to novel and applicable catalysts for solar fuel production, but also that they will act as a stepping stone for other solar-driven catalysis using pure proteins.



This project will hopefully contribute to the bioinorganic chemistry-community as a whole with new insights on metal-protein interactions and the chemistry that can be achieved with this type of complexes. We also aim to incorporate non-water soluble molecular catalysts such as cobaloxime to the  $\alpha_3\text{x}$  scaffold (left part of figure), which would provide a non-polar environment, replacing non-polar solvents. This way the molecular catalyst could be indirectly solvated in water, by being surrounded by a non-polar protein core. One of the problems with molecular catalysts for e.g. hydrogen evolution is that they are often not water soluble, and so their usability in industrial settings is limited as it is an important goal for the energy sector to reduce not only their CO<sub>2</sub> emissions, but also their environmental impact in general. Water is more environmentally friendly and abundant than many of the organic solvents that are used for molecular catalysts, and so being able to show a proof of concept of using proteins instead of organic solvents for these types of molecules would be a great development in the field of energy research.

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P003

**A Multi-Application-Toolkit: One POM for Water Splitting, Plastic Waste Recovery and Anti-Bacteria under Light Irradiation**

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POMs, as one class of unique metal-oxo clusters with well-defined structures and nano size, are composed of W, Mo, V, Nb, and Ta centers with their highest oxidation states [1]. The unique redox activity, strong Brønsted acidity and unmatched range of molecular structures are key to the reactions that feature prominently in many applications [2]. They feature a remarkably wide application potential in the design of multifunctional POM materials with the ability to address different environmental issues, such as energy transformation, waste recovery, and sterilization of water [3–5]. At present, materials chemistry has evolved to the point where rational design of one multi-functional POM with synergistic capabilities is possible. Thus, designing a low-cost multi-application-POM will be undoubtedly promising. Financial support by the University of Zurich is gratefully acknowledged.

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P004

**Development of Crystal Biohybrid Photocatalysts for Asymmetric Heterogeneous Oxidation**

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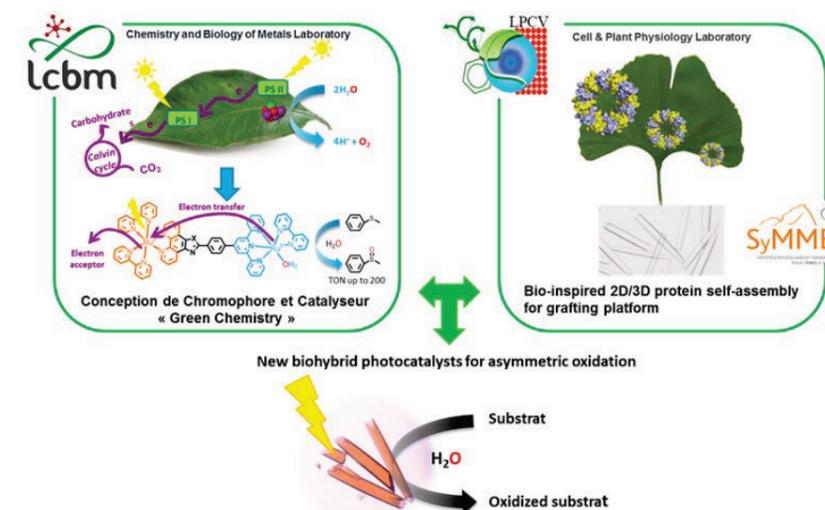
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For the last decades the development of sustainable chemistry became a priority for our society. In this field, catalysis plays an important role into chemical processes but, still remains challenging for oxidation reactions using renewable oxygen atom sources. In our laboratory Ru-Ru dyads were developed enabling photocatalytic oxidation of organic substrates using H<sub>2</sub>O as oxygen atom sources respectively [1]. Here, we focus on another challenge related to the introduction of chirality in order to perform asymmetric photocatalysis. Biocatalysis is an interesting strategy due to enzymes abilities to perform various reactions in high yield, high selectivity and high efficiency. In this context, a new way of research combining metal-based catalysts and protein scaffold affording artificial metalloenzymes is emerging [2]. This approach is very attractive due to system modularity (protein mutation, various inorganics complexes and substrates).

In this project, we aim to develop bio-hybrid photocatalysts combining photosensitizers and catalysts into a well-characterized protein crystal. The selected protein is oligomerisation domain of the LEAFY protein from *Ginkgo biloba*. It was observed that such a protein is able to generate porous structures by self-assembling [3]. Inside the tubes, a peptidic chain of about 30 amino-acids, per monomer, can be found and will served as grafting platform for both Ru-based catalysts and photosensitizer for asymmetric photocatalytic oxidation of organic substrates. It is expected that such an environment would afford high selectivity (chimo and stereo) in addition to a high stability. Our strategy is based first on the study (selectivity, quantification) of each partner separately inside the crystal to determine the optimized conditions. Then, the full system will be generated and studied before evaluation of its photocatalytic activity during oxidation reactions.



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**P005**

**Solar-Driven Enzymatic Carbon Dioxide Fixation**

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Since industrialisation the CO<sub>2</sub> concentration in the atmosphere has increased continuously. The elevated CO<sub>2</sub> level leads to the acidification of the sea and contributes significantly to global warming. Thus, it is desirable to minimise the release of carbon dioxide and utilise the atmospheric CO<sub>2</sub> chemically by building base chemicals and fuel. Some enzymes, including Carbon monoxide dehydrogenase (CODH) and Formate dehydrogenase (FDH), are capable of fixing CO<sub>2</sub> despite its highly inert character under mild conditions and use the products of the reduction for their metabolism. Inspired by these natural systems, there are already first studies published in the area of CO<sub>2</sub> reduction by ribosomally expressed and purified enzymes attached to dye-sensitised semiconducting nanoparticles [1,2]. In these systems it has been shown that the solar-driven reduction of CO<sub>2</sub> to formate and carbon monoxide respectively as well as the accumulation of the reduction product is possible. The necessary reduction equivalents are delivered by sacrificial electron donors *via* a photocatalytic systems.

In this work the focus is on the transformation of CO<sub>2</sub> to methanol, an important base chemical, by a cascade of enzymes consisting of FDH, Aldehyde dehydrogenase and Alcohol dehydrogenase. The chosen biohybrid approach is particularly elegant, since solar power is used to convert the climatically and environmentally malign atmospheric CO<sub>2</sub> into an energy-dense fuel *via* solar power.

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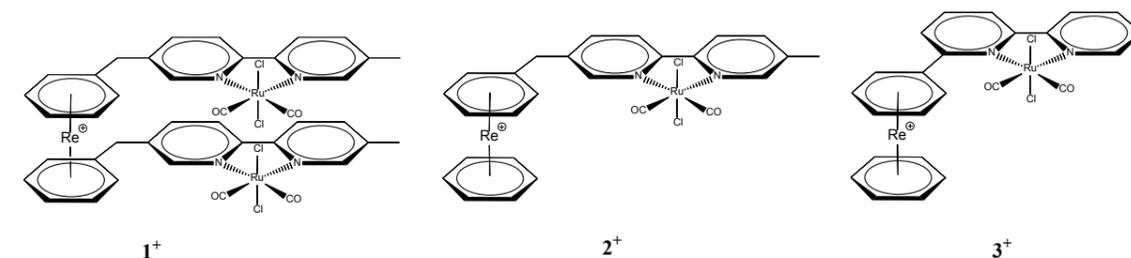
**P006**

**A Highly Efficient and Selective CO<sub>2</sub> to CO Photocatalytic System Based on Re(I) Bis-Arene Frameworks**

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Converting selectively and efficiently CO<sub>2</sub> into valuable chemical compounds using solar energy is a potential strategy to solve current environmental and energy problems. Transition metal-based molecular catalysts have been the focus of many homogeneous CO<sub>2</sub> reduction processes<sup>1</sup>. Reduction of CO<sub>2</sub> involves a multi-electron process. Dinuclear complexes offer advantages over mononuclear systems for more efficient reductions. Coupling two metal complexes by a flexible linker is required. The extraordinary stability and the structural flexibility of Re(I) bis-arene complexes offers this opportunity<sup>2,3</sup>. Rotation around the rhenium axis and tilting of pendent functionalities are possible for such sandwich complexes without a notable energetic barrier. Combination of one or two identical or different complexes, separated by ring-ring distance is also possible. Those properties make [Re(η<sup>6</sup>-arene)<sub>2</sub>]<sup>+</sup> complexes ideal frameworks for the development of CO<sub>2</sub> reduction photocatalysts.



Rhenium bis-arene complexes with one or two pendent ruthenium dicarbonyl photocatalysts were synthesized. High efficiency and selectivity for CO for the photoreduction of CO<sub>2</sub> to CO is observed for all catalysts. Conjugation of the catalysts via a CH<sub>2</sub> bridge (complexes 1<sup>+</sup> and 2<sup>+</sup>) shows better photocatalytic performance as compared to the catalyst electronically connected (3<sup>+</sup>) to the sandwich framework. Comparison in catalysis between 1<sup>+</sup> and 2<sup>+</sup> shows a substantial increase in catalysis for 1<sup>+</sup>. This result supports a possible synergistic effect between the two mononuclear catalytic units. Confirmation of electronic communication between the two Ru atoms as well as the elucidation of the mechanism is currently under study by advanced spectroscopic tools.

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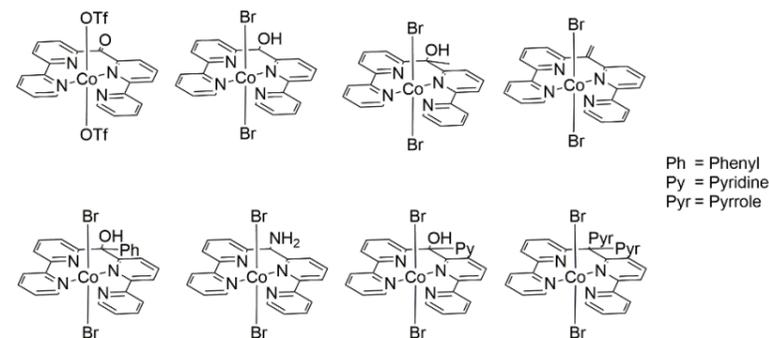
P007

### Derivatisation of Cobalt Polypyridyl Based Water Reduction Catalysts: Influence on Performance and Stability in Photocatalysis

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In a world where energy consumption raises more and more, the need of a renewable energy source as well as a possibility to store this energy is crucial<sup>1,2</sup>. An already existing, inexhaustible energy source is the sun. Besides harvesting sunlight, the energy must be stored in a suitable material. In this context, artificial photosynthesis is one of the most promising approaches for both, harvesting and storage of solar energy. A complete water splitting system would contain a hydrogen evolving catalyst (HEC), an oxygen evolving catalyst (OEC) and a photosensitizer (PS) where electrons from the oxidative half reaction are transferred efficiently to the reductive half reaction where protons get reduced to hydrogen. To study the activity or mechanistic aspects of a HEC, the oxidative half reaction is replaced by a sacrificial electron donor (SED) to rapidly deliver electrons to the PS ground state<sup>3</sup>. For water reduction, Co(II)-polypyridyl HEC's emerged as quite promising, stable and well performing systems in aqueous media. Cobalt as catalytic centre is a very suitable first row element due to its ability of supporting multiple oxidation states and coordination geometries<sup>4</sup>. The ligand systems themselves are crucial in catalysis.



To assess the role of the ligand systems for catalytic performance of HEC's, our group synthesized a series of catalysts with different functionalities on the CH<sub>2</sub>-bridge. We will present a benchmarking of photocatalysis and differences in electrochemical behaviour for catalysts shown in the scheme<sup>5</sup>. These derivatisation of the bridging group of the ligand system allowed us to understand the mechanistic role of these functional groups attached to the bridge.

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P009

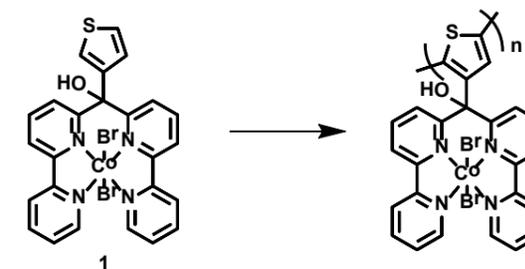
### Water Reduction Catalyst Embedded in a Conductive Polymer

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Homogeneous photocatalytic water reduction is investigated by our group with polypyridyl-based cobalt containing water reduction catalysts (WRC) and rhenium- or ruthenium-based photosensitisers (PS).[1] As for a whole water splitting system the oxidation and reduction have to be separated, different approaches to immobilise water reduction catalysts and photosensitisers are currently followed. One approach is the preparation of a photocathode consisting of polymerised PS and polymerised WRC on a conductive support. We present here the progress with one WRC monomer.

A thiophene was added to the basic structure of selected and highly active polypyridyl ligands. With this thiophene, a polymerisable unit was introduced to the ligand that can form a conductive polymer. After complexation to cobalt, the monomeric WRC **1** was obtained. Polymerisation of thiophene and its derivatives with Fe(III) in presence of different doping anions has been described.[2] This method was successfully applied to **1** and resulted in a water soluble, catalytically active polymer. It is possible to precipitate this poly-cation with the poly-anion polystyrene sulfonate from the aqueous solution. Alternatively, electropolymerisation allows a direct grafting onto an electrode. Direct electropolymerisation on glassy carbon (GC) was not achieved. However, when a small amount of 2,5-di-(2-thienyl)-pyrrole was first polymerised on GC, subsequent electropolymerisation of **1** onto this modified electrode was successful.



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P010

### Modeling Artificial Water Oxidation – Insights into Reaction Mechanism and Catalyst Design

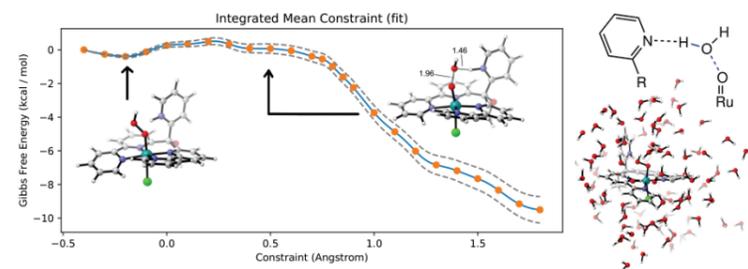
Mauro Schilling<sup>1</sup>, Sandra Lubert<sup>1</sup>

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Burning fossil fuels has potentially contributed significantly towards man made climate change – reverting or at least limiting its effects is among the biggest challenges of the 21st century. The development of renewable energy sources appears to be a key requirement in order to reduce the dependence on fossil fuels and reducing greenhouse gas emissions. Solar light driven water splitting, a process inspired by nature's photosynthesis, promises to be a valuable source of renewable energy. While there has been considerable success in developing catalysts to facilitate this process, so far there is no commercial viable setup available. In order to further improve existing catalysts, as well as to design novel catalysts, an in-depth understanding of the underlying reaction network is necessary. Since the complexity of bio-mimetic catalysts is often found to be overwhelming due to their sheer size, simpler model systems such as mononuclear Ru-based catalysts have been developed. The latter offer the opportunity for a detailed study of their physicochemical properties as well as for targeted design.

Recently we proposed a viable reaction mechanism for a bio-inspired mononuclear Ru-based water oxidation catalyst. A key feature of the latter is the non-coordinating covalently linked pyridine which was found to be in an optimal position to facilitate the oxygen-oxygen bond formation by interacting with the approaching nucleophile. Our theoretical study further showed that by tuning the basicity of the pyridine the barrier for the O-O bond formation could be significantly lowered. Moreover, ligand design was carried out *in silico* for the development of more active catalysts.

In the current study, we use forefront *ab initio* molecular dynamics simulations for a sophisticated inclusion of solvent effects. In order to get an accurate picture of the free energy surface, we apply enhanced sampling methods such as *Blumoon* and *Metadynamics* – those allow us to characterize the reactive species under ambient conditions. Thereby we are not limited to the active site of the catalyst, but we are able to get a complete picture including the dynamics of the hydrogen bonding network. As a part of this study we also evaluated the applicability of well known protocols based on the *Blumoon* ensemble to predict the pK<sub>a</sub> value of the ligand of said transition metal based water oxidation catalyst. The work has been supported by the University of Zurich Research Priority Program "Solar Light to Chemical Energy Conversion" (LightChEC) and the Swiss National Science Foundation (grant no. PP00P2\_170667).



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P011

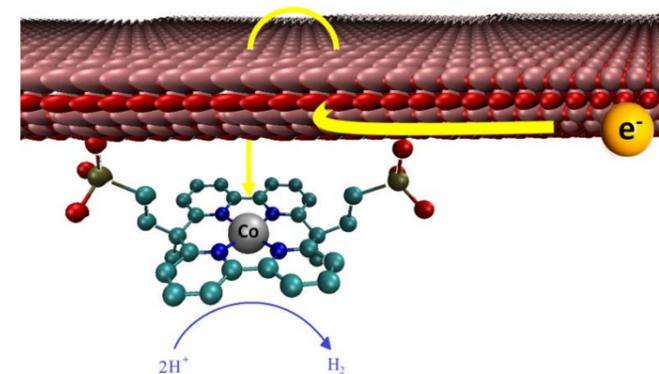
### A Molecular Water Reduction Catalyst in a Heterogeneous System: Combining Molecular Efficiency with Surface-Stability

Nicola Weder<sup>1</sup>, Nora Grundmann<sup>1</sup>, Benjamin Probst<sup>1</sup>, Roger Alberto<sup>1</sup>

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The ongoing research on photocatalytic water splitting can be roughly subdivided into molecular catalysis, mostly complexes inspired by natural photo- and redox active systems,<sup>[1,2]</sup> and surface catalysed approaches. Although molecular catalysts outperform any surface in terms of turnover frequency and electrochemical tunability of the catalytic centre, they bear some crucial disadvantages compared to surface catalysed systems, especially regarding long-term stability and applicability in a full water splitting system. The lack of separation of the two half-reactions in a homogeneous system leads to electron short-cuts, which quench the catalytic cycle. Separation would lead to control of the direction of electron flow, as it is the case in a PEC cell.

To integrate the high catalytic rate of molecular catalysts in a two-cell system, a new, active and stable cobalt-polypyridyl water reduction catalyst (WRC), with phosphonic acid anchoring groups was synthesized and covalently bound to TiO<sub>2</sub>, as this substrate is often used as passivation layer with a large bandgap in PEC cells. As cobalt-porphyrin complexes have already been shown to be both, fast and stable in homogeneous systems,<sup>[3]</sup> the anchoring-groups were introduced at the bridging-position of the cyclic ligand framework.



The molecular footprint of the WRC on TiO<sub>2</sub> nanoparticles as well as pH dependent binding stability was determined. Photocatalytic H<sub>2</sub>-evolution measurements were performed as well as kinetic studies of the electron transfer from the photosensitizer to the WRC, both with the focus on the comparison between *in-solution* and *on-particle* systems. Finally, the WRC was adsorbed on a TiO<sub>2</sub>-coated FTO-cathode and subjected to electrocatalysis at different pH values. Simultaneous VIS-absorption measurements of the working electrode served to detect the reduced Co(I)-WRC at the specific reduction potential and in-line CG measurements allowed to find the effective overpotential for H<sub>2</sub>-production.

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P020

## Synthesis, Characterization and DNA Binding of 5-Chloro-2-Hydroxy-3-(Pyrazin-2-ylidiazanyl) Benzaldehyde with Platinum Complex

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The complex of [L<sub>1</sub>Pt(DMSO)]Cl, where DMSO= dimethyl sulfoxide & L<sub>1</sub>= 5-chloro-2-hydroxy-3-(pyrazin-2-ylidiazanyl)benzaldehyde(C<sub>11</sub>H<sub>7</sub>ClN<sub>4</sub>O<sub>2</sub>), has been synthesized and characterized by IR, NMR, UV-Visible, molar conductance and mass spectra. The molar ratio and mass spectra have approved that the complex format to ligand 1:1. IR and NMR assured the coordination tridentate ligand to the metal from N, N and O. The conductance measurement of complex indicated a chloride ion outside the coordination. DNA binding of complex affected by hyperchromic with blue shift. Molecular docking displayed that complex has better CT-DNA docking than ligand.

P021

## MASH-FRET: A Software Package for Next Generation Analyzing of Single-Molecule Fluorescence Data

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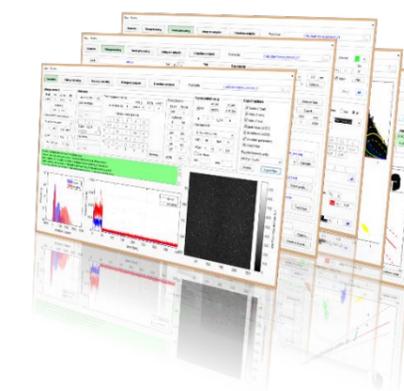
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Single-molecule Förster resonance energy transfer (smFRET) is a powerful technique to probe biomolecular structure and dynamics. A popular implementation of smFRET consists in recording fluorescence intensity time traces of surface- or vesicle immobilized, chromophore-tagged molecules, such as nucleic acids or proteins [1].

We developed MASH-FRET, a MATLAB-based Multifunctional Analysis Software for Handling smFRET data that allows to analyze and simulate camera-based single-molecule videos (SMV) [2-6]. In brief, our software extracts fluorescence trajectories from SMVs, allows sorting the molecules according to their dynamics and photophysics and analyzes the resulting FRET or fluorescence intensity state populations both thermodynamically and kinetically [2,4]. To validate experimental distributions of FRET states and their interconversion rates, MASH-FRET additionally allows the user to simulate realistic SMV [3]. The software is freely available for download on GitHub at <https://github.com/RNA-FRETtools/MASH-FRET> and documented with the help of step-by-step tutorials that are available at <https://ma-fretools.github.io/MASH-FRET/>.

Here, we provide a presentation of our software package with a standard analyzing strategy for SMV. We further explain the basic concepts of smFRET and how to get the most out of your intensity-based smFRET data in terms of thermodynamics and kinetics.

Financial support by the Swiss National Science Foundation (RKOS), the UZH Forschungskredit (MCASH, DK, FDS, SLBK, SZP and RB) and the University of Zurich (RB and RKOS) is acknowledged.



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P022

### Integrative Transcriptome and Proteome Analysis Identifies Anti-Tumor Gold(III) Porphyrins as Aminopeptidase Inhibitor

Di Hu<sup>1</sup>, Wai-Lun Kwong<sup>2</sup>, Bei Cao<sup>2</sup>, Chun-Nam Lok<sup>1</sup>, Chi-Ming Che<sup>1,2</sup>

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The unique geometric and electronic properties of metal complexes make them exciting structural scaffolds for protein bindings that may lead to enzyme inhibition. Some metal complexes have been found to exert their anti-cancer activity by targeting proteins directly and inhibiting cancer-associated enzymatic activity. Gold(III) meso-tetraphenylporphyrin ([Au(TPP)]Cl, denoted **gold-1a**) is chemically stable under physiological conditions, and it has been reported to exist high cytotoxicity towards a wide range of cancer cell lines including cisplatin-resistant cancer cells and display promising *in vivo* tumor growth inhibition in multiple animal tumor models. Identification of target engagement of anti-cancer metal complexes has always been a difficult task. Previously, a clickable photo-affinity probe of **gold-1a** was used to identify heat shock protein 60 (Hsp60) as one of the molecular targets of gold(III) porphyrins and the inhibition of **gold-1a** on the chaperone activity of Hsp60 was observed [1]. However, there could be additional molecular targets escaping detection with chemical probes, as structure modifications may affect the activity spectra of original compounds.

Owing to the advancements in multiple omics technologies and bioinformatics resources, methodologies have been developed to predict drug targets and reveal the mechanisms of action *in silico*. Understanding the regulatory behaviour of both transcriptome and proteome of cells response to drug treatment is necessary, which information can provide useful insights into the mechanisms of action. In this work, a joint analysis of transcriptomic and proteomic data was performed to produce unbiased perspectives on the mRNA and protein expression status of colorectal cancer cells with **gold-1a** treatment. The genome-wide transcriptome profiling in combination with bioinformatics analysis including Library of Integrated Network Based Cellular Signatures (LINCS) and Gene Set Enrichment Analysis (GSEA) identified **gold-1a** as an aminopeptidase inhibitor. The transcriptional and proteomic responses of cancer cells to **gold-1a** were found to be similar to those of tosedostat (CHR-2797); the latter has been proven effective against a wide range of cancers in clinical trials and could inhibit intracellular aminopeptidases. Aminopeptidases play significant roles in cancer progression. Thus, targeting aminopeptidase is a viable strategy in the development of new anti-cancer agents. **Gold-1a** inhibits the activity of intracellular aminopeptidases and subsequently induces a series of downstream amino acid deprivation response, such as up-regulating the expression of amino acid synthetic and transporter genes, modulating mTOR signalling pathway, disrupting protein turnover and inducing autophagy. In addition, computational calculation reveals the spatial construction of protein-small molecule complex, in which **gold-1a** fills the enzyme active site of aminopeptidase N. Our findings demonstrate that gold(III) porphyrin is a potent scaffold for the development of novel aminopeptidase inhibitors.

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P023

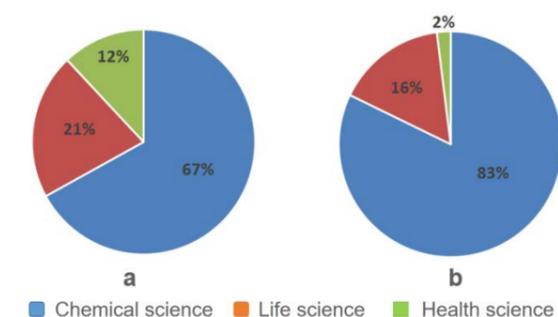
### Major Research Plans of Chemical Biology in NSFC

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In the past decades, chemical biology has evolved into a global community from an idea [1]. The division of chemical biology was listed in 2004 in the department of chemical of Natural Science Foundation of China (NSFC). However, the independent academic committee of chemical biology division was organized and valued research projects in 2017. Bioinorganic chemistry is one important part in the division. Looking back the developments of chemical biology in China, the funding supporting from Major Research Plan (MRP) of NSFC has occupied important role. The first MRP entitled "Investigations on Signal Transduction Process Utilizing Small Chemical Probes" was initiated in 2007 [2]. Projects of four specific themes have been encouraged: 1) Generation of chemical probes for studying signal transduction; 2) Development of new techniques and methods for detecting the information of signaling processes; 3) Signaling mechanisms of cellular functions based on chemical small molecules; 4) Biomarker, target and lead discovery based on signal transduction processes. 189 Million Chinese Yuan with 155 research projects related to chemical biology have been supported until the MRP was completed in December 31, 2015. Funded project investigators came from chemical science, life science and health science (Fig 1, a). And the most of the project participants with interdisciplinary background.

Subsequently, the second MRP entitled "Dynamic Modifications of Biomacromolecules and Chemical Intervention" was approved and initiated in 2017. Three main specific research fields were encouraged: 1) Chemical labeling and detection technology of dynamic modifications of biomacromolecules; 2) Regulation mechanism and function analysis of dynamic modifications of biomacromolecules; 3) Chemical intervention and application of dynamic modifications of biomacromolecules. 90 Chinese Yuan Million with 89 research projects have been funded from 2017-2018 (Fig 1, b). Most of the projects cover the research theme 1 and 2. And theme 3 would be enhanced to attract more scientists with different background. With the operation of this MRP, ties between chemistry and biology would be further strengthened to advance the development of chemical biology in China.



**Fig. 1** NSFC Funding budget distribution for Major Research Plan: a) Investigations on Signal Transduction Process Utilizing Small Chemical Probes (2007-2015); b) Dynamic Modifications of Biomacromolecules and Chemical Intervention (2017-2018)

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P031

### A Dual Stimulus-Responsive Copper Sulfide-Polydopamine Nanopatform for Activatable In Vivo Cancer Imaging and Synergistic Therapy

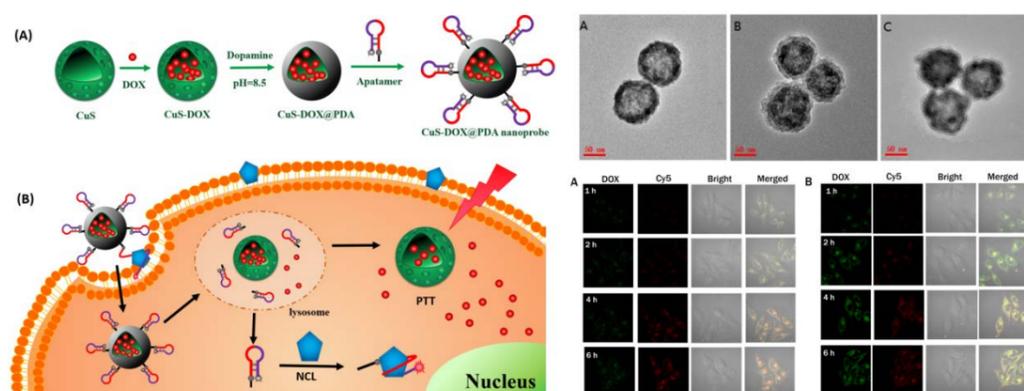
Jing Hua<sup>1</sup>, Lifang Yao<sup>1</sup>, Yong Huang<sup>1</sup>, Jiayao Xu<sup>1</sup>, Xin Li<sup>1</sup>, Ying Li<sup>1</sup>, Danni Zhang<sup>1</sup>, Hong Liang<sup>1</sup>

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Due to their controlled-release pattern and enhanced physiological specificity and therapeutic efficacy, stimuli-responsive drug delivery systems is showing increasing promising for cancer treatment. To date, a number of stimuli-responsive drug delivery systems have been developed, such as biomolecules, pH, temperature, photoirradiation, and redox [1-4]. In particular, the integration of multiple stimulus offers new opportunities to further fine-tune their response to each stimuli as well as precisely regulate release profile. Several multi-stimuli responsive drug delivery systems have been reported, such as pH/temperature, temperature/host-guest interaction/redox, and enzyme/photoirradiation [5-7]. Despite the progress made, the development of more efficient drug release system that can respond to multiple stimulus is still highly desirable for cancer treatment.

Herein, we rationally designed a novel dual stimulus-responsive drug delivery system for combining activatable in vivo cancer imaging with chemo-photothermal synergistic therapy. In this system, hollow mesoporous copper sulfide nanoparticles (HMCuS NPs) were used to encapsulate anticancer drug doxorubicin (DOX) and then capped with polydopamine (PDA) simultaneously as smart gatekeeper as well as pH-responsive moiety. After that, the HMCuS NP@PDA hybrids were functionalized with aptamer beacons (AS1411) as both tumor targeting moiety and activatable fluorogenic agent. By aptamer-mediated recognition, the fluorescence is activated and HMCuS NP@PDA hybrids selectively enter into the target cancer cells. Subsequently, the coated PDA molecules could be degraded in acidic endosome/lysosome, leading to the release of DOX. Additionally, by taking advantage of the excellent photothermal property of HMCuS NPs, HMCuS NPs could accelerate the release of DOX and enhance therapeutic efficacy upon near-infrared (NIR) irradiation. HMCuS NP@PDA hybrids could effectively kill cancer cells in vitro and in vivo with minimal side effect, which provides a promising strategy for cancer theranostics.

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P032

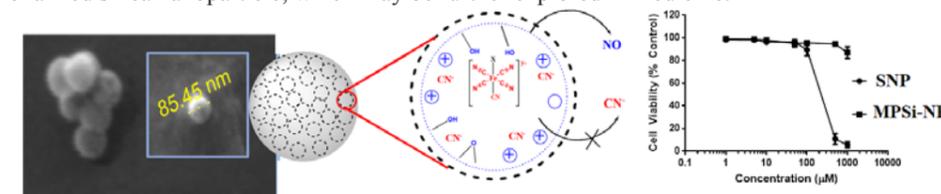
### Nitroprusside on Silica-Based Nanoparticles - Cyanide Retention and Cytotoxicity Assay

Elisane Longhinotti<sup>1</sup>, Pedro M. Silva Filho<sup>1</sup>, Iury A. Paz<sup>1</sup>, Nilberto R. F. Nascimento<sup>2</sup>, Cláudia F. Santos<sup>2</sup>, Valdevane R. Araújo<sup>2</sup>, Luiz G. F. Lopes<sup>1</sup>, Eduardo H. S. Sousa<sup>1</sup>

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Nanoparticles have attracted much interest in medicine as systems for controlled delivery of drugs. These systems must be biocompatible, cause no immunogenic reactions, and promote controllably release of drugs at the target sites without altering its therapeutic effects. Among the major problems pointed out for the application of nanoparticles in medicine are their easy aggregation in solution and toxicity to the organism. However, these problems can be bypassed with a functionalization step using biocompatible linker molecules or polymer chains [1-2]. Our research group has synthesized and functionalized mesoporous silica particles (MPSi) with amino groups [3], recently, amino functionalized nanoparticles (MPSi-NH<sub>2</sub>), with a spherical shape and uniform size distribution between 80 and 110 nm, were successfully obtained. MPSi-NH<sub>2</sub> was used to anchor sodium nitroprusside (SNP) with an incorporation of 320 μmol of SNP per gram of nanoparticle, originating MPSi-NP. A series of experiments using MPSi-NP to evaluate NO releasing were conducted, which showed very efficient ability to release NO. Once the major drawback in the clinical use of nitroprusside still resides on the amount of cyanide released simultaneously with NO [4], this work aimed to investigate the ability of the nanoparticles to minimize cyanide toxicity. We performed these measurements investigating the potential of retention of cyanide on the MPSi-NH<sub>2</sub> right after NO release either induced by chemical (ascorbic acid) or photochemical (blue LED at 450-495 nm) processes. Additionally, other experiments were performed to evaluate the cytotoxicity of the MPSi-NP according to standardized method using Vero cells (mammalian kidney epithelial cells). The results showed that NO was efficiently released from MPSi-NP by using ascorbic acid and blue LED, where the amount of free CN<sup>-</sup> in solution was only *ca.* 31.9% and 6.6 %, respectively, in comparison to 100% of CN<sup>-</sup> release by SNP. The relatively larger release of cyanide for ascorbic acid induced, we suggest it might be due to a competition between cyanide and ascorbate ions for the positively charged surface of the material (zeta potential value of 20 ± 0.5 mV). The assays with kidney cells indicated a much lower cytotoxicity for MPSi-NP in comparison to SNP. Actually, the calculated CI<sub>50</sub> for SNP was 228.5 μM, while for MPSi-NP it could not be obtained even at the highest concentration used (1000 μM). The maximal decrease in cell viability induced by MPSi-NP was 13.1 ± 3.2 %. In summary, these studies supported the promising properties of the nitroprusside anchored on a functionalized silica nanoparticle, which may be further explored in medicine.



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P033

## Increasing Cytotoxicity toward SKMEL-147 Melanoma Cells Using Modified-POSS Nanoparticles – A New Drive for Cell Death

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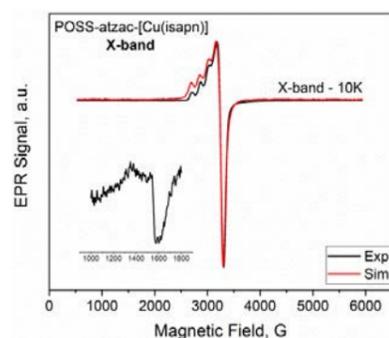
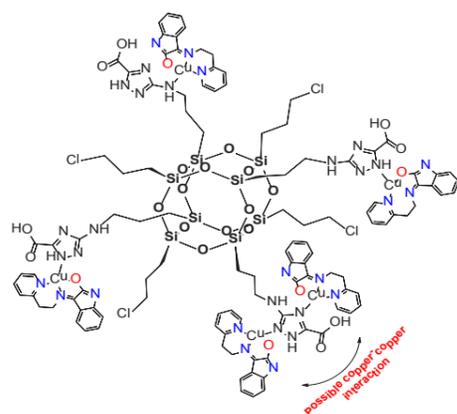
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Modified-POSS nanoparticle is a class of new biomaterials that have been extensively studied as drug carriers of a diversity of therapeutic agents [1]. The interest in this kind of compounds applied as host of active molecules, given a bioconjugated material, is due to the need for prolonged and improved control of drug administration, as well as the potentialization of metallopharmaceuticals. Therefore, the aim of this research was the preparation of new modified-POSS materials based on silsesquioxane to carry potential antitumor metal complexes. Targeting biological applications, on the POSS were immobilized two oxindolimine copper(II) complexes forming some new materials. Morphological and structural characterizations were accomplished. Electron paramagnetic resonance (EPR) showed that both copper(II) complexes were linked to matrices modified, and it was evidenced on X-band a signal at forbidden transition, ( $M_S \pm 2$ ) exactly at the half-field of the g-factor (G) at different temperature, which may be owing to interaction between two copper(II) nuclei with ferromagnetic behavior. This possible interaction between two copper and dimeric minority formation may be the reason for the increase in cytotoxicity. This initial idea is in agreement with one of the papers published in our group in Brazil, where binuclear complexes showed higher/better results compared to mononuclear systems [2].

The anticancer activity of the new compounds was then tested toward SKMEL-147 melanoma cell lines, in comparison to non-tumor fibroblasts. The results displayed high toxicity of these materials after both copper(II) complexes be immobilized on the POSS surface towards SKMEL-147, and show lower cytotoxicity against fibroblasts. In contrast, the pure POSS and complexes were non-toxic against the same melanoma cell lines proving the high performance of the bioconjugated systems.

Cytotoxicity assays on SKMEL-147 human melanoma cells revealed that bioconjugated materials are at least two to five times more active against melanoma cells compared to non-tumor cell line (Fibroblast P4). Cell apoptosis may be related to increased production of reactive oxygen species (ROS) in SKMEL-147 cells in relation to control.



**Acknowledgements:** FAPESP, CEPID Redoxoma, CAPES, CNPq.

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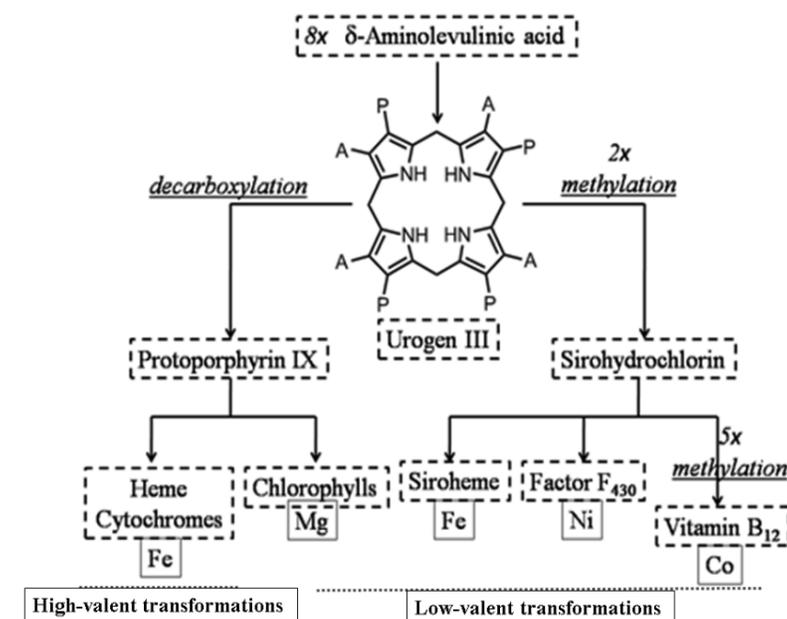
P040

## Understanding Nature's Choice of Macrocyclic Porphyrinoids for Low Valent Chemistry

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Tetrapyrrole macrocycles play a central role in most of the present living organisms. The importance of them stem from the unique attributes of tetrapyrroles for diverse bioenergetic processes (including photosynthesis) and have been further encouraged by the versatility, concision and molecular logic of the tetrapyrrole biosynthetic pathway. All naturally occurring corrinoid, porphyrin and (bacterio)chlorin macrocycles known as the pigments of life, investigated so far are biosynthetically derived from the common precursor, uroporphyrin III. Porphyrins have been utilized for high-valent transformation utilizing  $O_2$ , while other saturated porphyrins (porphyrinoids) are assigned for low-valent transformations like  $NO_2^-$ ,  $SO_3^{2-}$  reduction etc. Fe-Porphyrins have been synthesized and the redox potentials have been tuned by introducing electron withdrawing groups (EWGs) to the  $\beta$ -pyrroles<sup>1</sup>. Two EWG can afford facile  $O_2$  reduction while four EWG increases the over-potential- indicates the obvious choice of heme *a* in cytochrome *c* oxidase. It has been selectively saturated utilizing 1,3-cycloaddition reaction. These porphyrins and Chlorins possess very interesting electronic structure<sup>2</sup> and their  $NO_2^-$ ,  $SO_3^{2-}$  reductase activity is underway investigation.



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P041

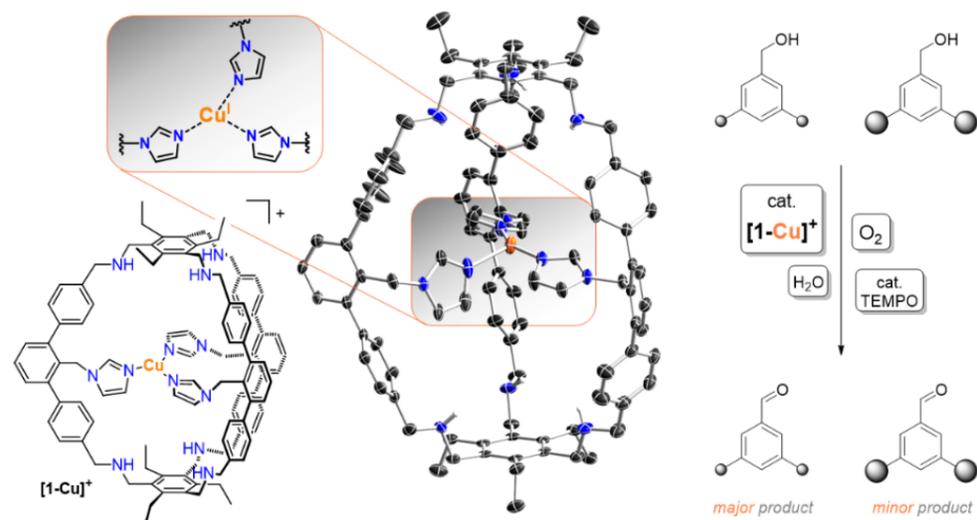
### A Biomimetic Imidazole-Functionalized Copper Cage Complex

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The coordination of histidine imidazole moieties to copper ions is found in many enzyme active sites such as in particulate methane monooxygenases, lytic polysaccharide monooxygenases and tyrosinases [1]. These coordination motives allow for their fascinating reactivities and selectivities that inspire chemists to develop appropriate model compounds. The biomimetic approach has mostly considered the primary coordination sphere only, whereas cavity based metal complexes have been shown to mimic the active site pocket as well [2].

Examples of molecular cages that offer a well-defined ligand sphere to mimic non-heme enzymatic active sites via imidazole coordination are yet unprecedented. Here, the endohedrally functionalized cage ligand 1, derived by DCC, acts as a tridentate ligand for a single copper ion. X-ray analysis reveals a T-shaped coordination geometry of copper as it is also found nature [1]. [1-Cu]<sup>+</sup> can catalyze the oxidation of benzylic alcohols to benzaldehydes with oxygen as the terminal oxidant (Figure 1) [3].



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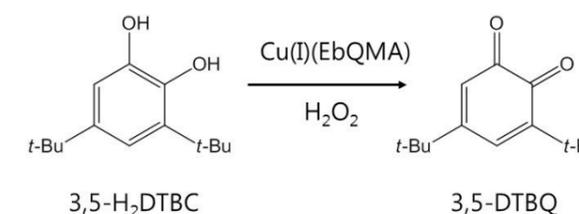
P042

### Catechol Oxidase-like Activity of a Mononuclear Copper(I) Complex

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Copper can be found in many important biological processes as a constituent of metalloenzymes. Copper-containing metalloenzymes are classified into Type I, Type II, and Type III. Type III copper enzyme has dicopper center which is surrounded by three histidines. Catechol oxidase, one of the type III enzymes, catalyzes the oxidation of catechol to quinone [1]. Quinone-like compounds can polymerize for allomelanin [2], that protects UV radiation damage at human skin [3]. Several copper complexes mimicking catechol oxidase have been developed [4]. But only few of them shows mechanism of catechol oxidase reaction that starts with copper(I) state. In this study, we have synthesized new mononuclear copper(I) complexes, [Cu(EbQMA)]ClO<sub>4</sub> and [Cu(EbQMA)]BF<sub>4</sub> with quinoline-based tetradentate schiff base ligand. Catechol oxidase-like activity of the complexes were explored by monitoring the oxidation of 3,5-di-*tert*-butylcatechol (3,5-H<sub>2</sub>DTBC) to 3,5-di-*tert*-butylbenzoquinone (3,5-DTBQ) in the presence of hydrogen peroxide. We discuss here the details of the reactions. Financial support by BK21 program of Ministry of Education of Korea is gratefully acknowledged.



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P043

### Synthetic Model Systems for Alkene Dioxygenase

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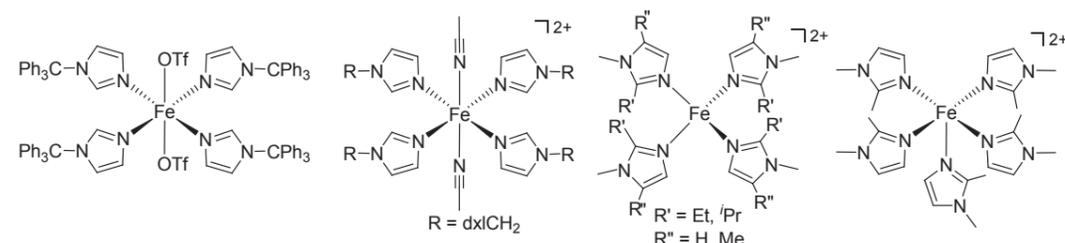
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Carotenoids carry out a variety of critical functions in all forms of life. In plants they operate as accessory light-harvesting components (antenna complex) of photosynthetic systems, light induced antioxidants, membrane fluidity regulators, and pigments (yellow to red-orange; important in attracting pollinators). In animals, carotenoids yield essential precursors necessary for vision, embryonic development, cellular homeostasis, and immunity (Vitamin A). Since vitamin A is not synthesized de novo in animals, it is necessary to consume pre-formed vitamin A (retinyl esters) from other sources or as provitamin A compounds found in vegetables and fruits. Carotenoid Cleavage Dioxygenases (CCDs) are enzymes capable of producing carotenoid derivatives (apocarotenoids). They are found in all forms of life. They generally catalyze the cleavage of carotenoid carbon-carbon double bonds. CCDs possess a nonheme group in their active sites. The ferrous ion is coordinated to 4 histidine nitrogens in a “see-saw” geometry. In this work, we will probe the structure-spectroscopic relationship for the CCD active sites using small molecule model compounds. We have synthesized a series iron(II) compounds coordinated to 4 histidine mimics (imidazole) in various geometries. The complexes have been characterized by X-ray crystallography, Mössbauer spectroscopy, electrochemistry, and DFT.

Financial support by National Institutes of Health R15GM112395, National Science Foundation Grants CHE-0748607, CHE-0821487, CHE-1213440. WWB acknowledges an NSF MRI Grant (CHE-1725028). A REF grant from Oakland University is also acknowledged.



Structures of (a)  $[\text{Fe}(\text{TrIm})_4(\text{OTf})_2]$ , (b)  $[\text{Fe}(\text{Im-dxlCH}_2)_4(\text{CH}_3\text{CN})_2]^{2+}$ , (c)  $[\text{Fe}(1\text{-Me-2-R}'\text{-5-R}''\text{Im})_4]^{2+}$ , and (d)  $[\text{Fe}(1,2\text{-Me}_2\text{Im})_5]^+$ .

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P044

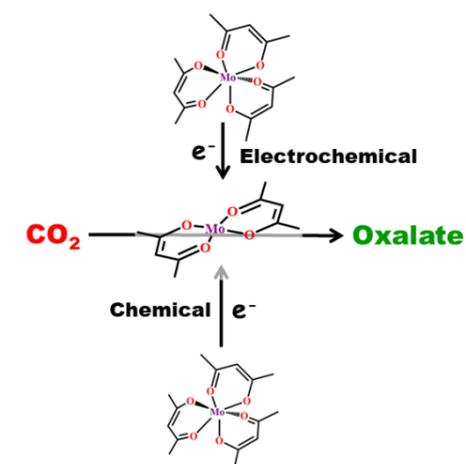
### Electrochemical Activation of CO<sub>2</sub> by Mo(acac)<sub>3</sub> Complex

Subal Dey<sup>1</sup>, Marc Fontecave<sup>2</sup>, Victor Mougel<sup>1</sup>

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<sup>2</sup>Laboratoire de Chimie des Processus Biologiques, Collège de France, 11 place Marcelin Berthelot, 75005 Paris, France. marc.fontecave@college-de-france.fr

Low valent molybdenum complexes, coordinated with anionic ligands are rare and supposed to be highly reactive for small molecule activation. Here, we present a modified synthesis of Mo(acac)<sub>3</sub> complex with substantial high yields. Then a low coordinated Mo(acac)<sub>2</sub> complex is synthesized and characterized from the Mo(acac)<sub>3</sub> precursor. The effect of ligands on the coordination geometry is established by addition of various exogenous neutral ligands (e. g – pyridine, imidazole etc.). The high reactivity of this Mo(acac)<sub>2</sub> complex was investigated for small molecule activation e.g. CO<sub>2</sub>. Electrocatalytic CO<sub>2</sub> reduction by the electrogenerated Mo(II) species was also been investigated. Combined spectroscopic data and x-ray crystallographic structural analysis provide a detailed information about the active catalyst and possible intermediates involved in CO<sub>2</sub> reduction pathway. This catalyst belongs within the few members of selective CO<sub>2</sub> to oxalate reduction catalysts.



P045

### A Ni-SOD Mimic with a S3O Coordination Sphere Based on a Tripodal Cysteine-Rich Ligand: pH Tuning of the SOD Activity

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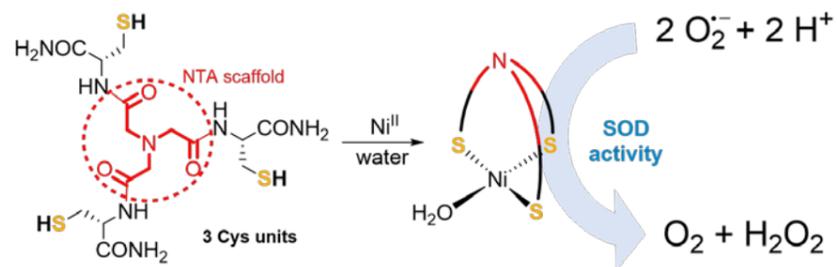
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The superoxide radical anion,  $O_2^{\cdot-}$ , is generated by many life processes. Its radical properties make it a highly reactive species able to damage all macromolecules contributing to the pathogenesis of many diseases including neurodegenerative disorders.<sup>[1]</sup> In order to protect cells against  $O_2^{\cdot-}$ , Nature uses superoxide dismutases (SODs) which catalyze the dismutation of  $O_2^{\cdot-}$  into hydrogen peroxide and oxygen. The last discovered SOD contains a nickel cofactor (Figure 1).<sup>[2,3]</sup> Importantly the NiSOD is found in several pathogenic bacteria but not in humans. Therefore targeting the NiSOD is a promising approach to develop antibiotics. Secondly, the development of novel SOD mimics may have potential uses as therapeutic agents in oxidative stress-related diseases.

Our project aims at developing innovative active NiSOD mimics, based on the use of peptide like ligands (see Figure),<sup>[4]</sup> with two main objectives: (i) to develop efficient SOD like catalysts, active in water, displaying antioxidant properties and (ii) to contribute to the full understanding of the catalytic mechanism of the NiSOD to highlight the specific key elements that differ NiSOD from the human MnSOD.

Two NiSOD mimics based on a sulphur-rich pseudo-peptide ligand, derived from nitrilotriacetic acid (NTA) with the three acid functions grafted with cysteines ( $L^{3S}$ ), have been investigated. In the presence of  $Ni^{II}$  and  $L^{3S}$  in a 1:1 ratio, two Ni complexes are formed with a similar S3O coordination sphere, which have been fully characterized by a combination of spectroscopic techniques including  $^1H$  NMR, UV-vis, CD and XAS, together with theoretical calculations. Three thiolates originating from the cysteines of the  $L^{3S}$  ligand bind the  $Ni^{II}$  ion. The square planar geometry is completed by either a water molecule,  $[NiL^{3S}(OH_2)]^-$ , at physiological pH (pH range 5-8), or a hydroxo molecule,  $[NiL^{3S}(OH)]^{2-}$ , in more basic conditions (pH>8). The overall set of data evidences that a key structural feature to obtain a catalytic activity is the presence of a labile ligand in the coordination sphere of the  $Ni^{II}$  ion.



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P046

### Charge Transfer Studies of 7-Deaza-6-Pyrazolylpurine-Containing DNA Films on Gold Surfaces

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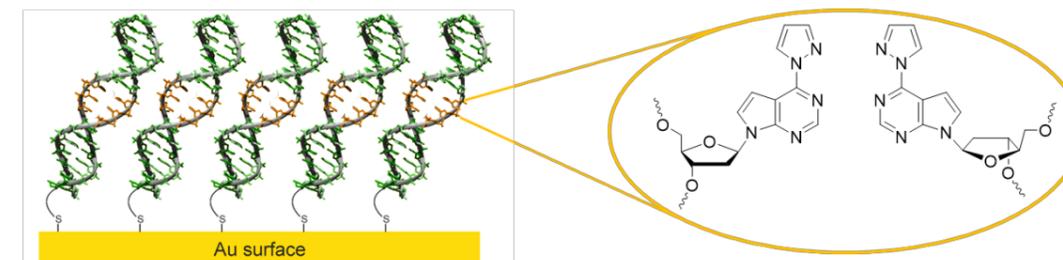
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7-Deaza-6-pyrazol-1-yl-purine ( ${}^D6PP$ ) is an artificial nucleobase which can be incorporated into DNA strands. In duplexes composed of these DNA strands, metal-mediated base pairs can be formed upon metal ion treatment. In contrast to canonical base pairs, the hydrogen bonds between complementary nucleobases are formally replaced by coordinate bonds to one or more transition metal ions [1]. Since these metal ions are located inside the double helix, a modification of the charge transfer properties of this DNA is anticipated.

In this work, the charge transfer resistance ( $R_{CT}$ ) of DNA films containing artificial homo and hetero base pairs of  ${}^D6PP$  has been investigated by electrochemical impedance spectroscopy (EIS) to study if charge transfer through DNA can occur. In prior studies, DNA films including artificial Im:Im base pairs have been investigated, which showed an increase in charge transfer resistance upon Ag(I) treatment (Im = imidazole) [2]. This behaviour was not according to expectations since the presence of Hg(II)-mediated T:T base pairs (T = thymine) leads to a decreasing charge transfer resistance for the corresponding DNA film [3]. It was assumed that the Im:Im mismatches within the DNA destabilize the duplex structure, resulting in a higher flexibility and bulged loops, thus leading to a thinner DNA film on the gold surface. It was speculated that this effect has a bigger impact on the charge transfer resistance than the formation of the metal-mediated base pairs.

To prove this assumption, DNA films with metal-mediated homo and hetero base pairs of  ${}^D6PP$  have been studied towards their  $R_{CT}$  change upon metal ion treatment. This artificial base was chosen since melting studies of  ${}^D6PP$ -containing DNA revealed a relatively high thermal stability in the absence of Ag(I). This behaviour can be explained by an expanded  $\pi$ -surface, leading to more rigid duplexes even in absence of metal ions. Therefore, the  ${}^D6PP$  was a promising candidate for charge transfer resistance studies on gold surfaces. The results of these studies will be presented here.

Financial support by the DFG (GRK 2027) is gratefully acknowledged.



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P047

### Mechanistic Insight into the Olefin Epoxidation and Alkane Hydroxylation Reaction of Non-heme Iron(III)-Peroxido Complexes

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Non-heme iron-based compounds act as intermediates in many metabolic and bio-synthesis pathways. Instead of the naturally occurring intermediates, well-defined bio-inspired molecules are often studied to understand the structure and reactivity of related metallo-enzymes. The reactivity of iron(III)-peroxido complexes has remained a riddle to inorganic chemists owing to their insufficient thermal stability and impotency towards organic substrates. These iron-oxygen adducts have been labeled as sluggish oxidants towards oxidative electrophilic and nucleophilic reactions.

High-valent iron(IV/V)-oxido species have been shown to be strong oxidants, capable of oxidizing e.g. olefins at very low temperatures, but they lag in stereoselectivity. It has also been shown, that high-spin iron(III)-peroxido species are capable of these oxidations as well, because they are in equilibrium with a high-valent iron(V)-oxido species. Non-heme high-spin iron(III)-peroxido complexes were presented with high reactivities in olefin epoxidation and alkane hydroxylation reactions with high stereo- and enantioselectivities in olefin epoxidation and high regio- and stereoselectivities in alkane hydroxylation.<sup>[1]</sup> Herein, we report the computational analysis of the reaction of olefin epoxidation and alkane hydroxylation with high-spin iron(III)-peroxido complexes. The main focus of this work is to explore the mechanistic details of such reactions (olefin epoxidation and alkane hydroxylation) and identify the reason for their high regio- and stereoselectivities. The computed results show that the C-H bond activation by the iron(III)-peroxido species is the rate-determining step, and this correlates well with the reaction rates and bond dissociation energies of alkanes. The epoxidation is asymmetric but reacts faster to the product than it rotates around the weakened single bond. The hydroxylation reacts *via* transition states and an intermediate with a radical C-atom that still binds the H-atom, so no movements, proton-transfers etc. are possible, leading to a stereoselective hydroxylation. On the basis of our computational mechanistic studies, the iron(III)-peroxido complexes behave as strong oxidants, capable of the oxygenating of hydrocarbons prior to their conversion into the corresponding high-valent iron(IV)/iron(V)-oxido species via O-O bond cleavage.

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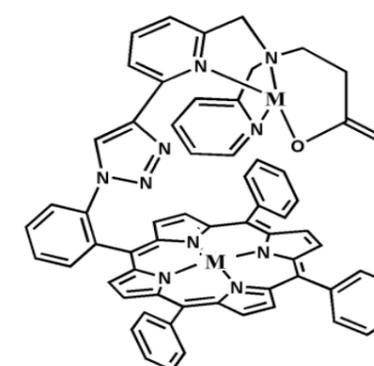
P048

### Development of Synthetic Model Systems for Bacterial NO Reductase

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Bacterial NO Reductase (NorBC) is a membrane-bound enzyme found in denitrifying bacteria that is involved in catalyzing the two-electron reduction of NO to N<sub>2</sub>O and water. The mechanism under which NorBC operates is highly debated, due to the fact that the protein is difficult to work with. There are three proposed mechanistic pathways, involving the unique diiron heme b<sub>3</sub>/non-heme Fe<sub>B</sub> active site. In the Fe<sup>III</sup>Fe<sup>III</sup> resting state, the heme b<sub>3</sub> is ligated by a proximal His, and the non-heme Fe center is coordinated by three His residues and a Glu. Also, an oxo bridge connects the two metal centers, making the Fe-Fe distance only 3.5 Å. Synthetic model systems provide the opportunity to give insight into the mechanism of action of this enzyme. We have developed a synthetic model system of NorBC, consisting of a tetraphenylporphyrin-derivative clicked to a modified BMPA-Pr ligand, as shown in the figure. This complex has been characterized by NMR and Uv-Vis spectroscopy. Armed with this synthetic model, we are now investigating the systems interaction with NO, and examine some of the different factors that influence the reaction mechanism.



P049

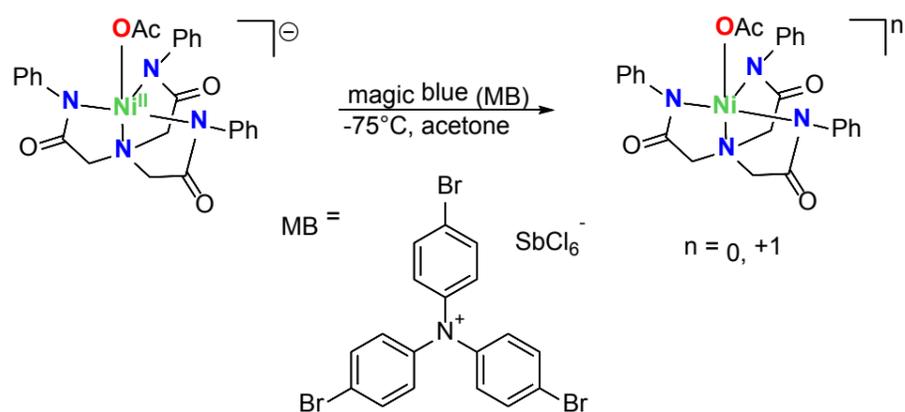
### Nickel Complex in Trigonal Bipyramidal Geometry Supported by Tripodal amidate Ligand System Displays Chemically Accessible +3 and +4 Oxidation States

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High valent transition metal ions are natural mediators of oxidative reactions in biological systems.<sup>[1]</sup> The petrochemical industry relies heavily on homogeneous catalysts to convert hydrocarbon feedstock to high valuable oxidised products.<sup>[2]</sup> Little emphasis has been placed on high valent metal oxidants based on late transition metals, namely cobalt, nickel and copper.<sup>[3]</sup> The high oxidation and reduction potentials of Ni ions necessitates the modulation the ligand system, to make the high oxidation states more accessible. The lower redox potentials of early-mid first row transition metals gives precedent for the wealth of high valent manganese and iron based oxidants in biological systems, with only few examples of enzymes that rely on nickel.<sup>[4]</sup> The low valent Ni<sup>II</sup> complexes [Ni<sup>II</sup>L<sup>Ph</sup>(OAc)]<sup>-</sup> supported by the nascent tripodal amidate ligand system has been generated in trigonal bipyramidal geometry and structurally characterized. [Ni<sup>II</sup>L<sup>Ph</sup>(OAc)]<sup>-</sup> displays electrochemically accessible +3 and +4 oxidation states. The reaction of up to two equivalents of the one electron oxidant magic blue (MB) (Figure below), generated an electronically characterized Ni<sup>III</sup> and a potential Ni<sup>IV</sup> entity that have been trapped at low temperature. The hydrogen atom abstracting ability of these oxidants has been investigated with phenols, with promising preliminary results. With few natural examples based on well characterized high valent nickel complexes, we have shown the suitability of the tripodal amidate ligand system is in lowering the redox potential of Ni<sup>II</sup> ions, stabilizing high oxidation states for further reactivity with suitable substrates.



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P050

### Copper(II) Complexes with Simultaneous Superoxide Dismutase- and Catalase-like Activities Probed by EPR and Kinetic Studies

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From the bioinorganic point of view, there is growing interest in the development of mimetic compounds for superoxide dismutase (SOD) and catalase (CAT) due to the antioxidant activities presented by them.<sup>[1]</sup> Thus, we present herein the synthesis, physicochemical characterization and kinetic studies of four copper(II) complexes containing the ligand 2-(pyridin-2-ylmethylamino)ethanol (HL1) or 1-(pyridin-2-ylmethylamino)propan-2-ol (HL2): Cu(HL1)Cl<sub>2</sub> (**1**), [Cu(HL2)Cl<sub>2</sub>] (**2**), [Cu(HL1)<sub>2</sub>](ClO<sub>4</sub>)<sub>2</sub> (**3**) and [Cu(HL2)<sub>2</sub>](ClO<sub>4</sub>)<sub>2</sub> (**4**). Compounds **1** and **2** have been previously published<sup>[2]</sup> whereas compounds **3** and **4** are new. Structural information obtained by X-ray diffraction studies revealed mononuclear compounds. The mimetic activity of CAT was evaluated using the Clark-type oxygen electrode technique and the evaluation of the mimetic activity of SOD was performed through electronic spectroscopy (superoxide anion was generated in enzymatic (xanthine/ xanthine oxidase) system in the presence or absence of test complex, and O<sub>2</sub> production was determined by monitoring the reduction of NBT to monoformazan dye at 560 nm, at 298 ± 0.1 K). All compounds presented dual activity, however, the most active against H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub><sup>-</sup> respectively, was compound **1**, presenting the velocity constant (**k**) of 1.717.10<sup>7</sup> dm<sup>3</sup> mol<sup>-1</sup> s<sup>-1</sup> and IC<sub>50</sub> of 0.099 μmol dm<sup>-3</sup>. IC<sub>50</sub> value of bovine erythrocyte SOD is 0.042 ± 0.01 μmol dm<sup>-3</sup>.<sup>[3]</sup> Due to the high dismutation rate of hydrogen peroxide and the superoxide radical promoted by compound **1**, the interaction of this compound with H<sub>2</sub>O<sub>2</sub> was investigated by RPE and ESI-(+)-MS and the interaction with superoxide radical was investigated by RPE (the superoxide radical was produced employing KO<sub>2</sub> in DMSO). Suppression of the characteristic signal of the superoxide radical immediately after interaction with compound **1** reinforces the high dismutation activity verified in the kinetic studies. Thus compound **1** presents potential for future pharmacological studies due to the significant dismutation activity against hydrogen peroxide and superoxide radical.

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P051

### Functionalized Bis(pyrazolyl)methane Ligands for the Biomimetic Transition Metal Coordination

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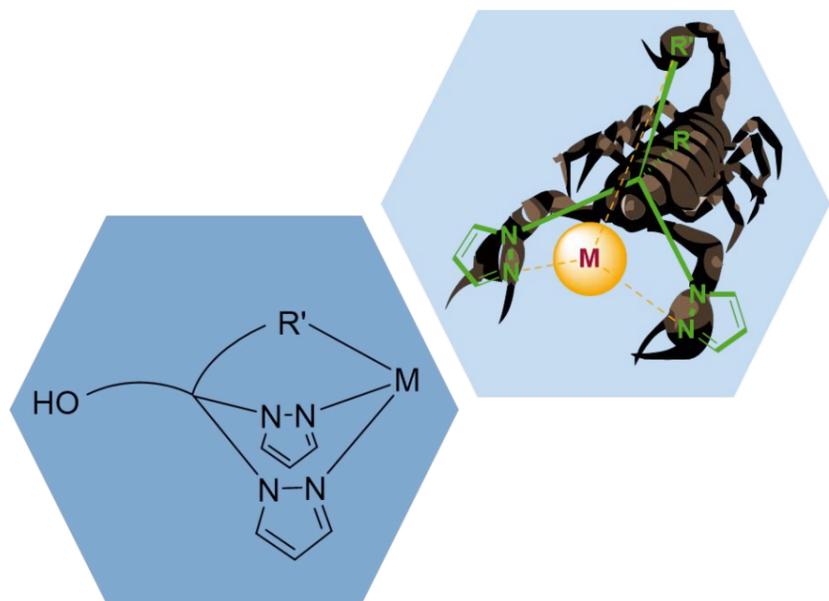
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Scorpionates are tridentate ligands which are classically tris(pyrazolyl)borates. Derived from this ligands bis(pyrazolyl)methane ligands have been developed. As part of the scorpionate ligand family bis(pyrazolyl)methanes are known as versatile ligands for the coordination of various transition metals.[1,2]

Due to the similar structures of pyrazole and the amino acid histidine bis(pyrazolyl)methane ligands are used for biomimetic complexes. For example, they serve as promising ligands for copper in the field of tyrosinase model complex systems. In general, complexes with iron, copper or zinc are interesting as biomimetic model complexes.[2-4]

Herein, we report backbone functionalized bis(pyrazolyl)methane ligands which possess now an additional hydroxyl group. These OH-functionalized bis(pyrazolyl)methane ligands have been tested in terms of their coordination behaviour for several transition metal salts. With iron(II) and iron(III) salts an *N,N,N* coordination seems to be preferred. On the other hand copper(II) ions accepts *N,O* as well as *N,N,N* coordination. Additionally, copper tends to build bridged complexes.

Furthermore, the hydroxyl group is a possible starting point for further functionalization or anchoring the complexes.



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P052

### Proton-Enhanced Reactivity of a Manganese(IV) Bis(hydroxo) Complex with Naphthalene

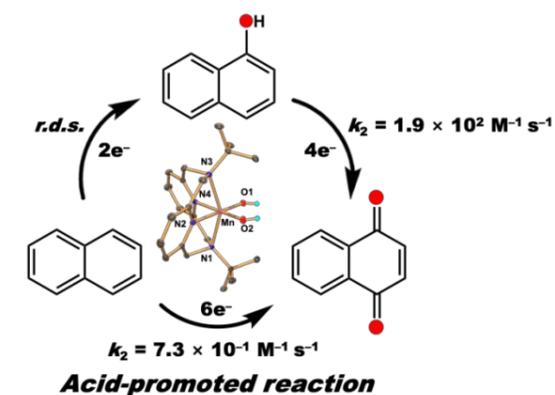
Donghyun Jeong<sup>1</sup>, James J. Yan<sup>2</sup>, Hyeonju Noh<sup>1</sup>, Britt Hedman<sup>2,3</sup>, Keith O. Hodgson<sup>2,3</sup>, Edward I. Solomon<sup>2,3</sup>, Jaeheung Cho<sup>1</sup>

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Naphthalene oxidation with metal-oxygen intermediates is a difficult reaction in environment and biological chemistry. Herein, we report the first oxidation reaction of naphthalene with a mononuclear Mn<sup>IV</sup> complex with terminal hydroxide ligands, [Mn<sup>IV</sup>(TBDAP)(OH)<sub>2</sub>]<sup>2+</sup> in the presence of acid, together with various characterization including ESI-MS, UV-vis, EPR, X-ray diffraction and XAS as well as detailed kinetic studies. To the best of our knowledge, this is the first report on acid-promoted oxidation with a high-valent metal hydroxo species.[1]



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P053

### A Functional Iron(IV)Oxo Model Complex Showing Activity Reminiscent of TET Enzymes

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Ten eleven translocation methyl cytosine dioxygenases (TET) play a key role in epigenetics by oxidizing the epigenetic marker 5-methyl cytosine (5mC) to 5-hydroxymethyl cytosine (5hmC), 5-formyl cytosine (5fC), and 5-carboxy cytosine (5cC).<sup>[1,2]</sup> An iron(IV)-oxo species has been proposed as the active species in the catalytic cycle of such iron(II)- $\alpha$ -ketoglutarate dependent enzymes (Fig. 1A).<sup>[3]</sup> Discrepancies between the observed catalytic activity of TET enzymes and calculated bond-dissociation energies of the substrates have been noted.<sup>[4]</sup> We report a biomimetic iron(IV)-oxo complex capable of oxidizing 5mC exclusively to 5hmC, 5fC, and 5cC reminiscent of the activity of TET enzymes (Fig. 1A) to shed light on this matter.

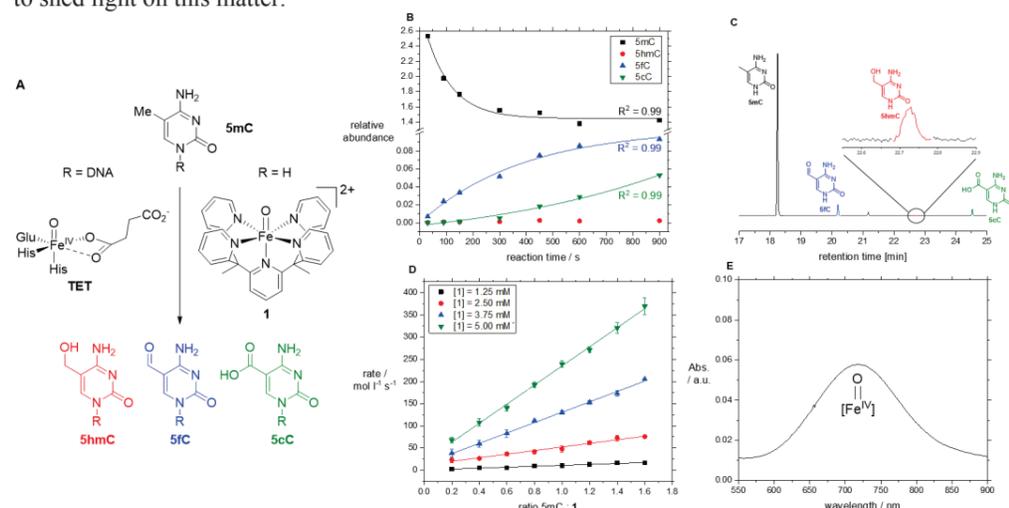


Figure 1A: Reaction of 5mC to the metabolites 5hmC, 5fC, and 5cC, in the case of TET enzymes (left side of the reaction arrow) cytosines are coupled to DNA, in the case of the model complex (1, previously published by Chang *et al.*<sup>[5]</sup>) cytosines are used as free nucleobases (R = H, right side of the reaction arrow). B: Product distribution of the reaction shown in A on the right side. C: GC/MS trace of the same reaction. D: UV-Vis kinetics of the reaction of 1 with 5mC. E: Excerpt of an UV-Vis spectrum of 1 showing the characteristic iron(IV)-oxo band.

Kinetic studies using GC/MS and UV/Vis allowed us to determine the rate law governing the reaction of both 5mC and 5hmC with 1 and show that 5hmC is preferentially turned over compared to 5mC and 5fC (Fig. 1B, D). Experiments with deuterated substrates ( $d_3$ -5mC and  $d_2$ -5hmC) showed large kinetic isotope effects elucidating the role of the hydrogen atom abstraction step in the mechanism. Financial support by the CRC1309 Chemical Biology of Epigenetic Modifications is gratefully acknowledged.

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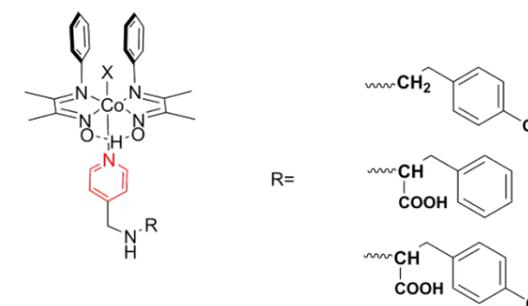
P055

### Tuning the Peripheral Electronic and Basic Properties of Co-Bis-(Oxime-Imine) Complexes to Improve Electrocatalytic H<sub>2</sub> Production

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Energy efficient and economically viable H<sub>2</sub> production is crucial for successful utilization of renewable energy resources [1]. In nature, the hydrogenase enzyme provides the gold-standard for efficient H<sub>2</sub> evolution catalyst [2]. Several synthetic catalysts were developed by mimicking the enzyme active site over the past few years but most of them failed to attain the enzymatic efficiency [3,4]. The poor activity of these synthetic mimics indicates that the active site -surrounding protein scaffold should be considered during the design of synthetic catalyst. Shaw *et al.* have included amino acids as bio-inspired outer coordination sphere (OCS) ingredient around nickel-bis-phosphine core to improve its reactivity [5, 6]. In this work we have, we have chosen Co-diimine-dioxime complex as a core and modified it by including two important features. First, a redox-active ligand was incorporated and secondly, natural amino acid-based basic groups were decorated in the periphery using pyridine as an axial ligand. A series of four complexes were developed following this strategy and examined electrochemically in aqueous solution displaying variable H<sub>2</sub> production activity. The peripheral basic groups provided additional protonation site as well as stability to the complex to work across the broad pH ranges (pH 1-7) while the redox active ligand scaffold reduced the overpotential requirement. Thus this work highlights the importance of inclusion of enzyme-inspired features in artificial catalysts design for tuning their reactivity.



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P056

### A Unique Fe<sup>II</sup>-S<sup>\*</sup> Complex: Synthesis, Structure, Spectroscopy and Reactivity

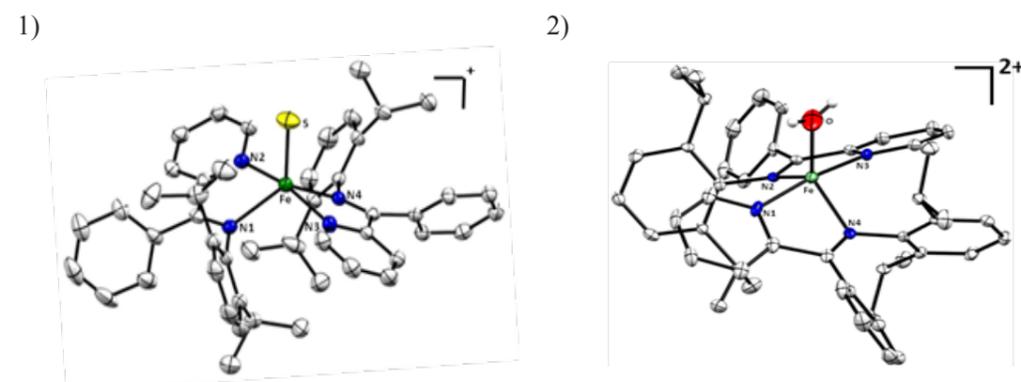
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There are many examples of non-heme Fe(IV)=O model systems, and their spectroscopic properties and reactivity have been studied in much detail. Only little is known about Fe(III)-oxo compounds, although they are important intermediates in iron-catalyzed oxidation reactions. Also the corresponding iron-sulfur systems have not found much attention although sulfido transition metal complexes are of importance for enzymatic and industrial hydrodesulfurization. Here we report a unique Fe(II)-S<sup>\*</sup> complex of ligand L (see Figure), together with its structural and electronic properties and reactivity. In solution, the triflate salt of the iron(II) complex of ligand L is transformed quantitatively to the corresponding Fe(II)-S<sup>\*</sup> complex, characterized by a crystal structure, various spectroscopies and supported by DFT. Electronic structure of this complex 1a was compared to its analogous Fe-aqua structure (Complex 2) with same ligand L, Complex 2 is electronically same with complex 1a except water molecule attached to Fe-centre in complex 2 instead of sulfido. The reactivity of the Fe(II)-S<sup>\*</sup> complex was tested with tris-cyclohexyl phosphine and yielded selectively the novel diphosphine product Cy<sub>3</sub>P=S=PCy<sub>3</sub>, characterized by <sup>31</sup>P-NMR and UPLC-MS.



Ortep diagram of Fe(II)-S<sup>\*</sup> complex (1) and Fe(II)-aqua complex (2) (H-atoms omitted for clarity).

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P057

### Installing a Copper Site Onto De Novo Peptide Fibers

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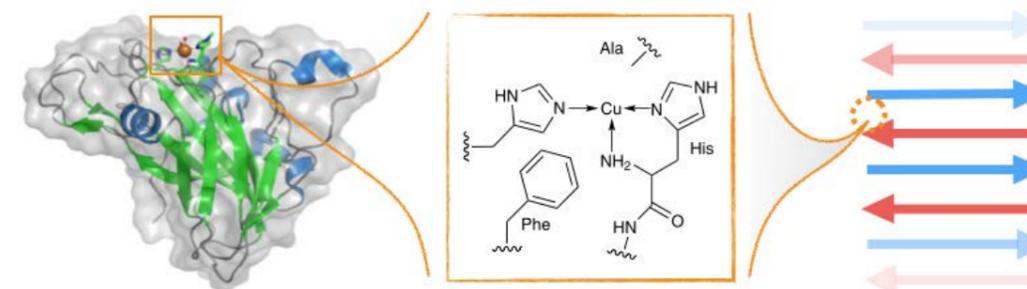
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The chemistry of metals plays an essential role in biology.<sup>[1]</sup> Indeed, metal ions are often responsible for the catalysis of difficult reactions by metalloenzymes (eg N<sub>2</sub> reduction, H<sub>2</sub>O oxidation). As an example, the Lytic Polysaccharide MonoOxygenases (LPMOs) use copper ion to catalyse the oxidation of C-H bonds.<sup>[2]</sup> It significantly accelerates the degradation of biomass, such as chitin or cellulose. This process being of interest for human societies (biomass conversion), it is of importance to be able to reproduce such reactivity with catalysts that are simpler and cheaper to produce than full-length proteins.<sup>[3]</sup> In that regard, several groups have focused their effort into developing very short peptides that assemble into fibres and can perform catalysis (with or without metal). Recent reviews showcase the potential of this approach.<sup>[4,5]</sup>

Here, we present a very naïve design inspired by LPMOs. The type of coordination of copper in the native enzyme is termed “histidine brace”. It is composed of two histidines, one binding copper via its side chain only, and the other via both its N-terminal amine and its side chain. Therefore, we based our design on two de novo peptides, that were designed and described as forming fibres only when mixed together.<sup>[6]</sup> Each peptide was modified to bear one part of the ligands, in order to form the full histidine brace coordination sphere upon hetero assembly. This poster presents the characterization and results obtained with these peptides upon copper binding.

Financial support by the CNRS, the FRC and the University of Strasbourg is gratefully acknowledged.



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P058

### The Reactions of Cu<sup>II/I</sup>ATP Complexes with Methyl Radicals

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Copper complexes in biological systems act as electron transport agents and as oxygen carriers, processes that exploit the Cu<sup>II/I</sup> easy redox processes. As such, copper ions can act as both antioxidant and pro-oxidant species. Radicals occur naturally in the human body and can damage cell walls, interact with genetic material, and contribute to the development of a number of health problems. Alkyl radicals are usually formed in biological systems via the reaction between hydroxyl radicals and organic molecules. Methyl radicals are the simplest alkyl radicals and are therefore used in mechanistic studies.

This study focuses on the interaction of ATP with both Cu(II) and Cu(I) ions and the redox activity of the latter formed complexes in general, and their reactions with methyl radicals. The methyl radicals were produced *in vitro* by continuous radiolysis of N<sub>2</sub>O saturated aqueous solutions containing DMSO. Cu(I)ATP reacts with ·CH<sub>3</sub> in a process which involves the formation of a transient with a metal-carbon σ bond ATPCu(II)-CH<sub>3</sub> which decomposes heterolytically to give methane and Cu(II)ATP as the major products. Its di-valent analogue forms ATPCu(III)-CH<sub>3</sub>, which decomposes heterolytically to give methanol and Cu(I)ATP. Rate constants for these reactions have been determined.

Detailed data and mechanisms of reactions will be presented.

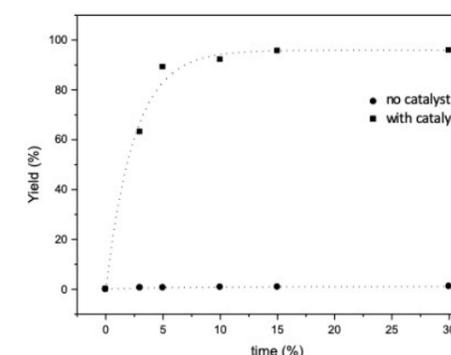
P059

### Mild Working Potential Hydrogen Production Catalyzed by Fe-S Complexes in the Presence of Decamethylferrocene

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The mechanistic study of hydrogen production by iron thiolate catalyst is reported. Complex [TBA][μ,κ<sup>2</sup>-bdt)(μ-PPH<sub>2</sub>)Fe<sub>2</sub>(CO)<sub>5</sub>]<sup>-</sup> was protonated to the formation of Fe-hydride species first and Fe-hydride-S-protonated species eventually in the presence of acids. The title complex exhibited catalytic events at -1.16 and -1.57 V (vs Fc<sup>+/0</sup>) in the presence of trifluoromethanesulfonic acid and trifluoroacetic acid, respectively. The former and latter catalytic events were originated from the Fe-hydride and Fe-hydride-S-protonated intermediate species, respectively. When anilinium acid was used, the catalysis occurred at -1.16 V, identical with the working potential of the trifluoromethanesulfonic acid catalysis, although the employment of anilinium acid was only capable of achieving the Fe-hydride state on the basis of the spectral and calculated results. The thermodynamics and kinetics of individual steps of the catalysis were analyzed by density functional theory calculations and electroanalytical simulations. The results suggest that the involvement of anilinium acid most likely initiated a proton-coupled electron transfer pathway that avoided the disfavored intermediate after the initial protonation. Via the proton-coupled electron transfer pathway, the heterogeneous electron transfer rate was increased and the overpotential was decreased by 0.4 V in comparison with the stepwise pathways involving trifluoromethanesulfonic acid or trifluoroacetic acid. It is found that the Fe-hydride-S-protonated species reacted with decamethylferrocene to produce hydrogen, the Fe-hydride species, and decamethylferrocenium cation as the final products. The yield of hydrogen production reached 75%. Compared to the reduction potential of decamethylferrocene at -0.59 V, hydrogen evolution from the reduction of the Fe-hydride-S-protonated species by decamethylferrocene was thermodynamically unfavored. According to the results of the computational investigations, it is suggested that decamethylferrocene molecules stayed in the near proximity of the sulfur proton with the presence of possible hydrogen bonding. It facilitated proton coupled electron transfer for the formation of hydrogen. When Fe<sub>2</sub> analogs with bulky substituents were employed in the catalysis, the hydrogen evolution was proceeded at a slower rate and a lower yield was observed, suggesting the steric hindrance retarded the catalytic reaction.



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P060

### Understanding the Rebound/Dissociation Dichotomy in H-Atom Abstraction through Reactive Mode Composition Analysis

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Our investigation addresses the selectivity displayed by bioinspired iron-oxo oxidants performing C-H bond activation. Upon H-atom abstraction (HAA), the nascent C-centered radical and iron-hydroxo complex may follow different pathways: (a) dissociation of the products, (b) rebound hydroxylation or (c) desaturation. Although studied for over 40 years, the physicochemical factors governing the outcome of these reactions is still not fully understood and up to this day cannot be predicted.

By applying density functional theory to a set of eight experimentally well-characterized systems showcasing HAA followed by either rebound or dissociation, we studied this post-HAA reactivity in the search for clues on this conundrum. First, we applied the standard computational approach: characterize all minima and saddle points in the relevant spin surfaces for each process, sketching a general energetic overview. To distill the most out of this endeavor, we applied a methodology “Reactive Mode Composition Analysis”, providing the Kinetic Energy Distribution [1] in the transition state mode. With this, we built a coherent picture showing that, besides the energetic considerations, there is a dynamic component embodied in the reactive mode that has been neglected so far in the study of rebound and dissociation.

Our ongoing work is projected to broaden the understanding of C-H activation reactivity, provide a simple tool to diagnose rebound vs dissociation reactivity and inspire the analysis of reactive modes in general reaction mechanisms.

Financial support by the Ministry of Education, Youth and Sports of the Czech Republic is gratefully acknowledged.

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P062

### A Versatile Non-Heme Iron Complex Capable of Structurally Modelling Different Classes of Mononuclear Non-Heme Iron Enzymes

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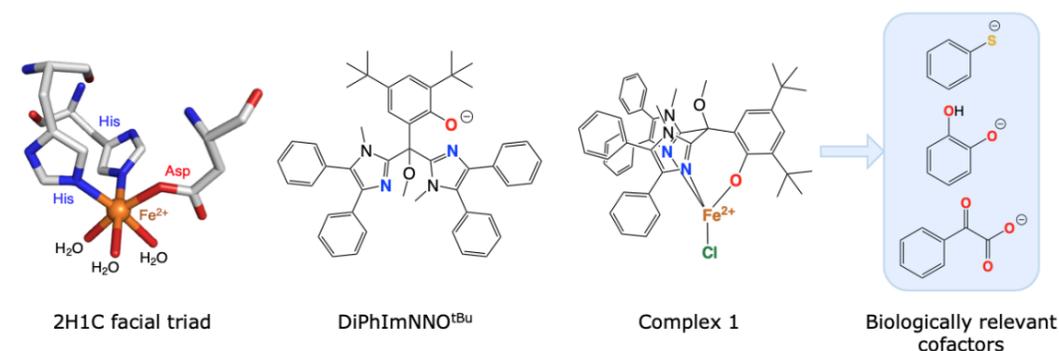
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Mononuclear non-heme iron enzymes that contain the 2-His-1-Carboxylate facial triad (2H1C) are able to activate molecular oxygen to catalyse a broad and diverse range of oxidative transformations, rivalling those mediated by their heme counterparts [1]. However, non-heme enzymatic active sites are generally more difficult to study as they do not bear the strong spectral features typical of the heme porphyrin ligand. Therefore, developing synthetic structural models of non-heme iron enzymes through biomimetic inorganic chemistry is an attractive means with which to gain further spectroscopic and mechanistic understanding of non-heme iron enzymes.

In this work, a novel phenolate ligand (DiPhImNNO<sup>tBu</sup>) is demonstrated as being one of the most faithful structural mimics of the 2H1C to date. The ligand comprises two biorelevant imidazole groups and a phenolate moiety that together form an anionic *N,N,O* facial triad, mimicking the two histidine residues and the carboxylate group of the 2H1C [4]. Complexation of the ligand to iron(II) chloride produces a high-spin mononuclear non-heme iron(II) complex of tetrahedral geometry (Complex 1). The ligand's *N,N,O* facial triad occupies three of the coordination sites, and the fourth is occupied by a substitutionally labile chloride ion.

The substitution of the chloride anion in Complex 1 is a highly attractive means with which to model the coordination of exogenous ligands (e.g. cofactors or substrate molecules) to the enzymatic resting state. Indeed, it is these exogenous ligands that enhance the dioxygen binding affinity of the enzyme active site, from which all oxidation catalysis is derived from. In this study, the substitutional lability of the chloride ligand is explored with biologically relevant cofactors of varying denticity, e.g. monodentate thiolate, bidentate catechol and bidentate benzoylformate ligands. In this way, Complex 1 is established as a highly convenient and versatile synthon from which a range of different biomimetic complexes can be created, whose spectroscopic properties accurately model the active sites of their metalloenzyme counterparts. The subsequent oxidative transformations of these complexes upon exposure to dioxygen are also under investigation.



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P063

### A Molecular Model System for Redox Coupling via Electron Bifurcation

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Electron bifurcation (EB) describes a process wherein an endergonic redox reaction is driven by the negative free energy change of a coupled exergonic redox reaction, limiting the free energy lost as heat. EB is emerging as a fundamental mechanism of biological energy conservation and is operative in the mitochondrial Q-cycle as well as in metabolic transformations in many anaerobic microorganisms. Despite its importance in metabolism, fundamental mechanistic understanding of EB is still very limited. The focus of this work is to design and study tunable molecular model systems for EB to gain insight into the kinetic and thermodynamic requirements for efficient bifurcation. Results from transient absorption spectroscopy indicate that these model systems do engage in redox coupling, thus offering a rich arena for mechanistic exploration.

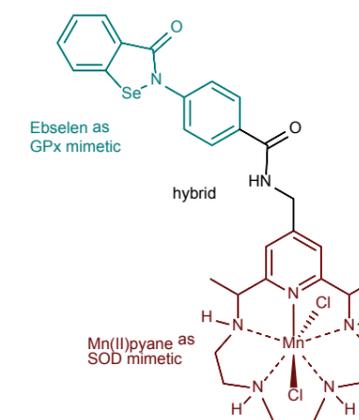
P064

### Synthesis of a Hybrid between SOD Mimetic and Ebselen to Target Oxidative Stress

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Oxidative stress emerged as target in drug discovery due to its diverse role in various diseases. The superoxide anion radical is considered to be a reactive oxygen species (ROS).[1] Although it naturally occurs in the body, an imbalance of superoxide is linked to aging processes, cancer, cardiovascular and neurodegenerative diseases.[1-2] The enzyme superoxide dismutase (SOD) should balance its steady-state concentration in living organisms by catalyzing superoxide decomposition to hydrogen peroxide and oxygen. Hydrogen peroxide in general can damage biomolecules. [1, 3] Hence, the presence of peroxide cleaving enzymes like glutathione peroxidase (GPx) utilizing glutathione (GSH) as reductant is crucial for maintaining healthy ROS levels in the body.[4] The organoselenium compound ebselen has gained large interest, not merely due to its GPx mimicking abilities, but furthermore, since it is widely applicable as biological antioxidant in the treatment of neurodegeneration, bipolar disease, diabetes, cancer and due to its antibacterial, antimycotic and antiviral effects.[5] Among the superoxide dismutase mimetics (SODm), Mn(II)pyane as a prototype of manganese(II) pentaazamacrocycles (MnPAMs) was observed to have remarkable SOD activity and beneficial biological effects.[6] The combination of two or more active agents covalently bonded is long known as the concept of hybridization and represents a sophisticated approach aiming at several targets.[7] To eliminate oxidative stress and its accompanying damage, we designed a hybrid molecule of Mn(II)pyane-CH<sub>2</sub>NH<sub>2</sub> and ebselen (see figure) to target both superoxide, with the former, and hydrogen peroxide, with the latter, potentially resulting in water and oxygen as products, i.e. complete ROS removal.



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P065

### Synthesis and Comparative Reactivity Study of Diiron(II)-Hydrosulfide and Thiolate Complexes towards Nitric Oxide

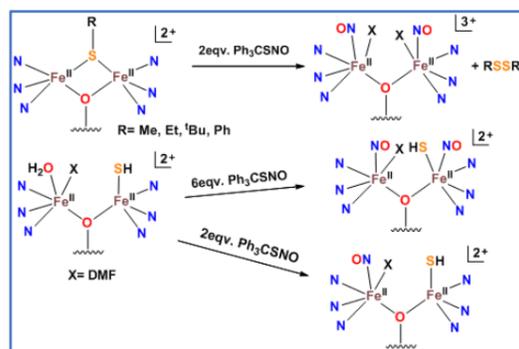
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As compared to Carboxylate-bridged nonheme diiron(II) complexes, the diiron(II)-hydrosulfide and thiolate complexes are very rare in Literature. In this context, complexes  $[\text{Fe}_2(\text{N-Et-HPTB})(\text{R}^1\text{S})](\text{BF}_4)_2$  ( $\text{R}^1 = \text{Me, Et, } ^i\text{Bu, Ph}$ ), N-Et-HPTB is the anion of N,N,N',N'-tetrakis[2-(1-ethylbenzimidazolyl)]-2-hydroxy-1,3-diaminopropane) could be synthesized directly from the addition of sodium salt of thiolate into a mixture of  $\text{Fe}(\text{BF}_4)_2 \cdot 6\text{H}_2\text{O}$ , HN-Et-HPTB, and  $\text{Et}_3\text{N}$ . Reaction of  $(\text{Cp}_2\text{Fe})(\text{BF}_4)$  with 1b yielded  $[\text{FeII}_2(\text{N-Et-HPTB})(\text{DMF})_3](\text{BF}_4)_3 \cdot \text{DMF}$  (4) (when crystallized from DMF/diethyl ether), which might indicate the formation of a transient ethanethiolate bridged  $\{\text{FeIIFeIII}\}$  species, followed by a rapid internal redox reaction to generate diethyldisulfide and the solvent coordinated diiron(II) complex, 5. This possibility was supported by a comparative cyclic voltammetric study of 1a–1c and 5. Reaction of  $[\text{Fe}_2(\text{N-Et-HPTB})(\text{R}^1\text{S})](\text{BF}_4)_2$  with  $\text{Ph}_3\text{CNO}$  produces  $[\text{Fe}_2(\text{N-Et-HPTB})(\text{NO})_2(\text{DMF})_2](\text{BF}_4)_3$ .

While attempted synthesis of diiron(II)-hydrosulfide complexes using  $\text{HS}^-$  produced insoluble precipitate, Reaction of  $\text{Fe}(\text{BF}_4)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Et}_3\text{N}$  and HN-Et-HPTB with RSH ( $\text{R} = \text{PhCH}_2, ^i\text{Bu}$ )/NaS<sup>t</sup>Bu in DMF at RT yielded the desired complex,  $[\text{Fe}_2(\text{N-Et-HPTB})(\text{SH})(\text{H}_2\text{O})](\text{BF}_4)_2 \cdot \text{DMF}$ . Treatment of 2a with 1 eq. of  $(\text{Cp}_2\text{Fe})(\text{BF}_4)$  resulted into the isolation of an unprecedented, mixed-valence diiron(II, III)-hydrosulfide complex,  $[\text{Fe}_2(\text{N-Et-HPTB})(\text{SH})(\text{H}_2\text{O})(\text{DMF})_2](\text{BF}_4)_3$ . Reaction of 2a with 6 eq.  $\text{Ph}_3\text{CNO}$  produces  $[\text{Fe}_2(\text{N-Et-HPTB})(\text{SH})(\text{NO})_2(\text{DMF})](\text{BF}_4)_2$ .

Financial support by the DST/SERB and CSIR, India is gratefully acknowledged.



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P066

### A Catalytically Active [Mn]-Hydrogenase Incorporating a Non-Native Metal Cofactor

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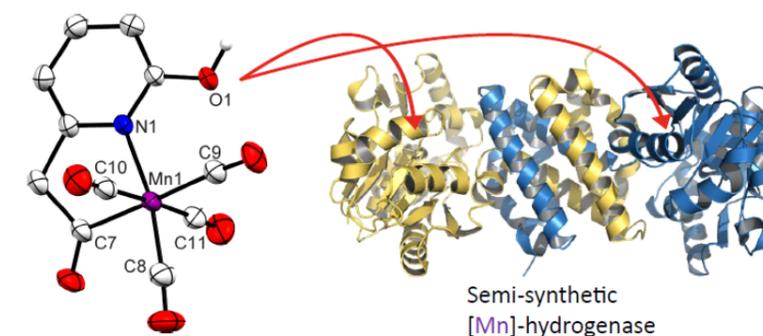
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Nature carefully selects specific metal ions for incorporation into the enzymes that catalyze the chemical reactions necessary for life. Hydrogenases, enzymes that activate molecular  $\text{H}_2$ , exclusively utilize Ni and Fe in  $[\text{NiFe}]$ -,  $[\text{FeFe}]$ -, and  $[\text{Fe}]$ -hydrogenases.<sup>1</sup> However, other transition metals are known to activate or catalyze the production of hydrogen in synthetic systems.<sup>2</sup> Here, we report the development of a biomimetic model complex of  $[\text{Fe}]$ -hydrogenase that incorporates a Mn, as opposed to a Fe, metal center. This Mn complex is able to heterolytically cleave  $\text{H}_2$  as well as catalyze hydrogenation reactions. Incorporation of the model into an apoenzyme of  $[\text{Fe}]$ -hydrogenase results in a  $[\text{Mn}]$ -hydrogenase with enhanced occupancy-normalized activity over an analogous semi-synthetic  $[\text{Fe}]$ -hydrogenase. These findings represent the first instance of a non-native metal hydrogenase showing catalytic functionality and demonstrate that hydrogenases based on a manganese active site are viable.



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P067

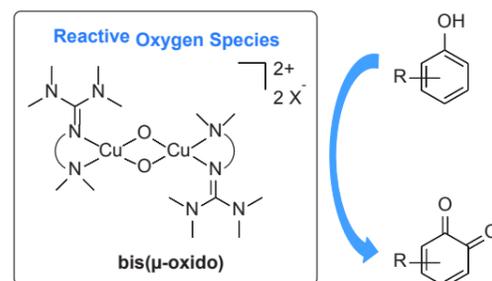
### Hybrid Guanidine-Stabilized Copper Complexes for the Activation of Dioxygen: Synthesis, Properties and Hydroxylation Catalysis

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The use of dioxygen as a readily available oxidizing agent is crucial for many biological and biomimetic oxygenation processes.<sup>[1]</sup> While natural copper enzymes, e.g. tyrosinase or particulate methane monooxygenase, efficiently activate molecular dioxygen the nature of copper-dioxygen species is still not fully understood. The type 3 copper protein tyrosinase is essential in living organisms for catalytic phenol oxidation in melanin biosynthesis. Using nature as an archetype, the activity and reactivity of copper-dioxygen intermediates are investigated by designing molecular copper complexes. Synthetic model systems involve N-donor ligands mimicking histidine sphere in protein backbone. Hybrid guanidine ligands combine strong N-donating abilities of a guanidine moiety<sup>[2]</sup> and small steric demand of an amine-function to promote the formation of bis( $\mu$ -oxido) copper complexes with appropriate substrate accessibility as well as stoichiometric hydroxylation activity of phenols.<sup>[3]</sup>

Now, copper complexes stabilized by novel aromatic hybrid guanidine ligands are presented which show high catalytic hydroxylation activity of a variety of phenolic substrates up to crossing enzymatic substrate limitations. In addition, copper-dioxygen chemistry has already proven to be a promising tool for multi-phase flows offering reaction systems to investigate hydrodynamic behavior in reactive bubbly flows.<sup>[4]</sup>



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P068

### Mössbauer Studies of Model Complexes for Intermediates in the Catalytic Cycle of Mononuclear Non-Heme Iron Dioxygenases

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Microbial biodegradation of aromatic compounds is an important process for the carbon biogeochemical cycle and for biotechnology.<sup>[1]</sup> Ring-cleaving dioxygenases are a diverse class of mononuclear non-heme enzymes that catalyze the oxidative cleavage of aromatic compounds, which may be manmade or natural in origin. Within this class, aminophenol dioxygenases (APDO) catalyze key steps in the degradation pathways of nitrophenol and p-chloronitrobenzene.

In this study, model complexes based on the ligand hydrotris(3,5-dimethylpyrazol-1-yl)borate ( $R^2Tp$ ) are used to probe the catalytic cycle of APDO.<sup>[2]</sup> The complexes model the facial coordination geometry of monoanionic 2-His-1-Carboxylate coordination motif of most dioxygenases and allow coordination of a bidentate substrate ligand, namely 4,6-di-tert-butylaminophenolate (DTBAP). The focus of this poster is the initial characterization of the ferrous  $Fe^{II}(Tp^{Me2})(DTBAP)$  resting state and of the intermediates which have been obtained by reacting the Fe(II) complex with dioxygen in THF.

The complex was synthesized with partial enrichment to enable collection of Mössbauer spectra in frozen solution. We will present the results of our monitoring the reaction with  $O_2$  at  $-80^\circ C$  in THF at times between a few second and minutes. The Fe(II) complex has an isomer shift,  $\delta$ , of 1.15 mm/s, typical for ferrous ions with N/O coordination. Upon reaction with  $O_2$  at low temperature ( $-70^\circ C$ ), color changes were observed by electronic absorption spectroscopy and the corresponding changes were monitored by Mössbauer spectroscopy in frozen solution. Preliminary results indicate that a transient species trapped within 10 s is paramagnetic and quickly forms an integer-spin species with  $\delta = 0.3$  mm/s and a large quadrupole splitting reminiscent of Fe-O-O intermediates trapped in studies in HPCD.<sup>[3, 4]</sup> In turn, this second species forms a decay product, which has a Mössbauer spectrum typical for high-spin ferric complexes. Details will be presented regarding the possible electronic structures of these species. Financial support from The College of Arts and Sciences at University of St. Thomas is gratefully acknowledged.

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P069

### Protonation versus Hydrogen Bonding in Fe(OEP)(NO)<sup>-</sup> Complexes

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Previous work in our laboratory has shown that the reaction of Fe(OEP)(NO)<sup>-</sup> (and other porphyrins) with substituted phenols forms the Fe(OEP)(HNO) complex [1]. This complex has previously been studied using visible and infrared spectroelectrochemistry [1]. <sup>1</sup>H NMR spectroscopy of this species in the presence of excess 3,5-dichlorophenol as a function of temperature will be reported in this work. The chemical shift of the proton in the HNO moiety showed an unusual behaviour as a function of temperature, and was consistent with an equilibrium between the Fe(OEP)(HNO) complex and a hydrogen bonded Fe(OEP)(NO)<sup>-</sup> complex. Low temperatures favored the hydrogen bonded complex. In addition to <sup>1</sup>H NMR, <sup>2</sup>H NMR was also used with fully deuterated 3,5-dichlorophenol, and similar results were observed. Stopped flow visible spectroscopy was also used in order to verify that the complex remained in the {Fe<sup>II</sup>} oxidation state after addition of the weak acid. Stopped flow and variable temperature visible spectroscopy showed that the Fe(OEP)(HNO) and the Fe(OEP)(NO)<sup>-</sup> hydrogen bonded complex have similar visible spectra, which were significantly different from Fe(OEP)(NO)<sup>-</sup> in the absence of hydrogen bonding. These results emphasize the importance of verifying that the HNO complex remains protonated as the temperature is lowered for spectroscopic techniques that need to be carried out at low temperatures.

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P070

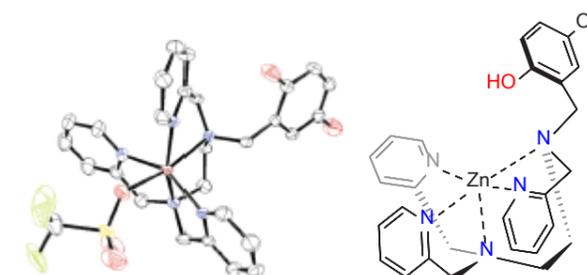
### Superoxide Dismutase Activity Enabled by a Redox-Active Ligand rather than Metal

Andreas Scheitler<sup>1</sup>, Meghan B. Ward<sup>2</sup>, Meng Yu<sup>2</sup>, Laura Senft<sup>1</sup>, Annika S. Zillmann<sup>1</sup>, John D. Gorden<sup>2</sup>, Dean D. Schwartz<sup>3</sup>, Ivana Ivanović-Burmazović<sup>1</sup> and Christian R. Goldsmith<sup>2</sup>

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Reactive oxygen species are integral to many physiological processes. Although their roles are still being elucidated, they seem to be linked to a variety of disorders and may represent promising drug targets. Mimics of superoxide dismutases, which catalyse the decomposition of O<sub>2</sub><sup>-</sup> to H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub>, have traditionally used redox-active metals, which are toxic outside of a tightly coordinating ligand. Purely organic antioxidants have also been investigated but generally require stoichiometric, rather than catalytic, doses. Here, we show that a complex of the redox-inactive metal zinc(ii) with a hexadentate ligand containing a redox-active quinol can catalytically degrade superoxide, as demonstrated by both reactivity assays and stopped-flow kinetics studies of direct reactions with O<sub>2</sub><sup>-</sup> and the zinc(ii) complex. The observed superoxide dismutase catalysis has an important advantage over previously reported work in that it is hastened, rather than impeded, by the presence of phosphate, the concentration of which is high under physiological conditions.



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P071

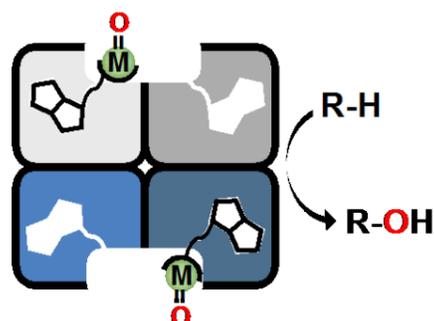
### Artificial Metalloenzymes for C-H Oxidation

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Artificial metalloenzymes (ArMs) result from the anchoring of a catalytically competent abiotic cofactor within a protein scaffold.<sup>[1,2]</sup> They have emerged as an attractive approach during the last decade: in a sense, these systems provide a bridge between homogeneous and enzymatic catalysis. In a biomimetic spirit, the well-defined secondary sphere coordination around the metal cofactor provided upon its incorporation within the protein cavity offers fascinating perspectives to optimize metal-catalyzed transformations with unprecedented selectivities.<sup>[3]</sup>

Herein we explore the development of novel ArMs based on the streptavidin-biotin technology to serve as artificial alkane monooxygenases. Following a bioinspired approach, we anchor manganese- and iron-based alkane hydroxylation catalysts inside an engineered streptavidin host and use environmentally friendly oxidants.



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P072

### The Effect of $\pi$ - $\pi$ Stacking Interaction in the One-Electron Oxidized Metal(II)-Diphenolate Complexes Containing a Side Chain Indole Ring

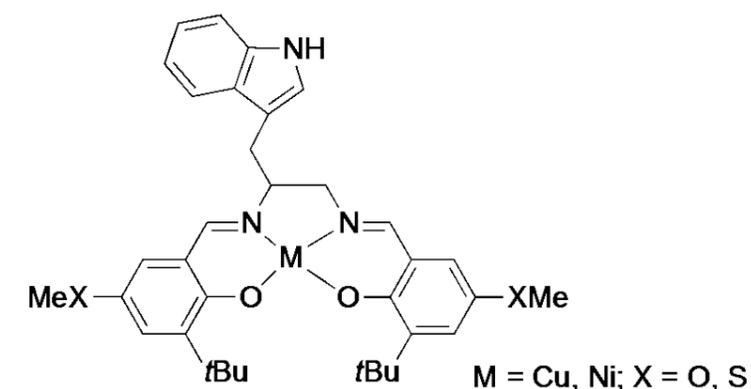
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Galactose oxidase (GO) is a copper containing enzyme, which catalyzes an oxidation of primary alcohol by oxygen. It has phenol and phenolate moieties of tyrosine (Tyr), and two imidazole rings of histidine in the first coordination sphere of the copper ion. In the course of oxidation of the substrate, Cu(II)-phenoxyl radical species originated from Tyr 272 is generated as an active form of GO. The Cu(II)-phenoxyl radical species is considered to be stabilized by the  $\pi$ - $\pi$  stacking interaction by indole ring of tryptophan (Trp) 290 in the second coordination sphere, and the  $\pi$ - $\pi$  stacking interaction influences for the stability of phenoxyl radical. However, the effects of the second coordination sphere, the C-S bond of the phenoxyl radical and  $\pi$ - $\pi$  stacking interaction of the indole ring, have not been fully clarified. In order to understand the effects of the  $\pi$ - $\pi$  stacking interaction with indole ring for the electronic structures and properties of phenoxyl radical, we have synthesized M(II)-diphenolate complexes (M = Cu, Ni) of Schiff base ligands having a side chain indole ring (Figure 1) and characterized their one-electron oxidized complexes.

The X-ray crystal structures of the oxidized Cu<sup>II</sup> and Ni<sup>II</sup> complexes exhibited the  $\pi$ - $\pi$  stacking interaction of the indole ring mainly with one of the two phenolate moieties. The phenolate moiety in close contact with the indole moiety showed the characteristic phenoxyl radical structural features, indicating that the indole ring favors the  $\pi$ - $\pi$  stacking interaction with the phenoxyl radical. However, the effects of the  $\pi$ - $\pi$  stacking interaction to the oxidized Cu<sup>II</sup> and Ni<sup>II</sup> complexes were different due to the electronic structure difference of these complexes. In this presentation, the effect of the  $\pi$ - $\pi$  stacking interaction of the indole ring with the phenoxyl radical will be discussed.



**Figure 1.** Structure of M(II)-diphenolate complexes (M = Cu, Ni) of Schiff base ligands having a side chain indole ring

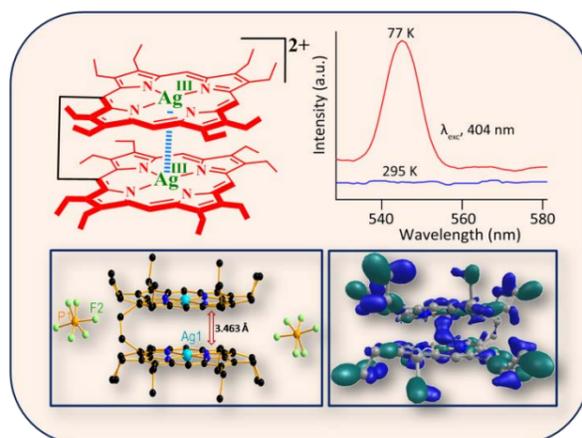
P073

### Oxidation Triggered Intermacrocylic Interactions in a Metalloporphyrin Dimer: Metal vs Ring Oxidation

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Oxidation triggered electronic interactions of closely spaced tetrapyrrolic macrocycles determine the unique properties in several enzymes such as the reaction centre in photosynthetic light-harvesting complex, etc. Highly oxidized metalloporphyrins have been identified as intermediates in the catalytic cycles of a number of heme proteins, including catalase, peroxidase, and cytochrome P-450. The biological significance, unusual electronic properties, and unique reactivities of these intermediates have recently generated much interest. These attractive features have prompted us to further investigate the intermacrocylic interactions based redox behavior of various metalloporphyrin dimers. Oxidation of a metalloporphyrin occurs either at the coordinated metal center or at the porphyrin macrocycle. However, in most of the cases, oxidation produces porphyrin  $\pi$ -cation radicals at the lower potentials. The covalently linked metalloporphyrin dimers (M: 2H, Zn<sup>2+</sup>) upon 1e<sup>-</sup> oxidation orient the two porphyrin macrocycles closer and cofacial to each other while 2e<sup>-</sup> oxidation forces them to be away as far as possible.<sup>[3-7]</sup> However, in case of Ag(II) porphyrin dimers, stepwise oxidations generate Ag(III) dimer, which exhibits significant metallophilic interaction in the form of a close unprecedented Ag<sup>III</sup>...Ag<sup>III</sup> contact that anchors the two porphyrin rings even more cofacial, contrary to porphyrin ring oxidation where the repulsion between both the positively charged porphyrins move them apart. Such Ag<sup>III</sup>...Ag<sup>III</sup> metallophilic interaction leads to interesting photophysical properties which could be exploited to design several light responsive materials and molecular semiconductors.



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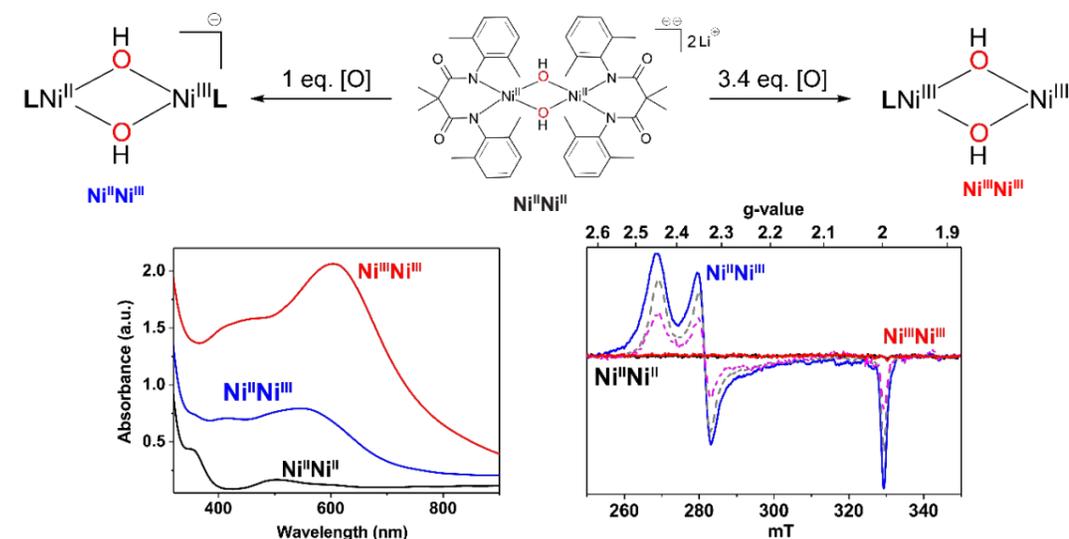
P074

### Mimicking MMO's: High Valent Bis( $\mu$ -Hydroxo) Complexes Capable of Hydrogen Atom Transfer

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Soluble and particulate methane monooxygenases (MMO's) are enzymes capable of oxidising strong C-H bonds such as those found in methane (C-H bond dissociation energy = 105 kcal/mol). High valent Cu and Fe oxo- or hydroxo- adducts are postulated to be the active oxidants in the MMOs.<sup>[1, 2]</sup> Several examples of high valent dinuclear Mn, Fe, Cu and Ni bis( $\mu$ -oxo) species have been reported. However, to the best of our knowledge, there is a dearth of high valent bis( $\mu$ -hydroxo) complexes. Herein we report the synthesis and characterization of the first examples of high valent bis( $\mu$ -hydroxo)M<sup>III</sup>2 and bis( $\mu$ -hydroxo)M<sup>II</sup>M<sup>III</sup> complexes (M = Ni, Cu). The complexes were prepared from low-valent precursors supported by a novel dicarboxamidate ligand and were characterised using X-ray absorption and electron paramagnetic resonance spectroscopies, mass spectrometry, and DFT calculations. These techniques confirmed the oxidation states of the metal sites in the complexes and showed the retention of the bis( $\mu$ -hydroxo) diamond core in the high valent species. Reactivity studies were performed, demonstrating that both high valent bis( $\mu$ -hydroxo)Ni<sup>III</sup>2 complexes were powerful oxidants towards oxidative activation of phenolic O-H bonds. Thorough kinetic analysis of these reactions showed the high-valent complexes to react through a hydrogen atom transfer mechanism.



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**P075**
**On the Role of Asynchronicity in C-H Bond Activation**
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We will present our methodology [1] recently developed for the analysis of various contributions to C-H bond activation reactivity including the contribution from asynchronicity between H<sup>+</sup>/e<sup>-</sup> transfers, which stems from the disparity between redox and acidobasic thermodynamic forces. To demonstrate the power of the approach, we will show and discuss H-atom abstraction reactivity of several biomimetic complexes and organic radicals. In the second part of the presentation, we will also correlate asynchronicity with a so-called 'reactive mode composition factor' and we will discuss the importance of this factor for rebound vs. dissociation selectivity of several experimentally well-characterized ferryl complexes.

Financial support by the Grant Agency of the Czech Republic (Grant No. 18-13093S) is gratefully acknowledged.

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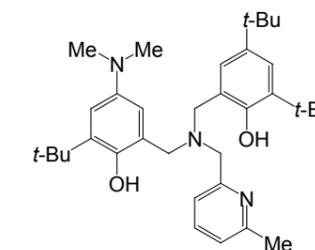
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**P076**
**Formation of the Cu<sup>II</sup>-Phenoxy Radical Complex by O<sub>2</sub> from the Cu<sup>II</sup>-Phenolate and its Mechanism**
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Galactose oxidase is a Cu-containing enzyme, which catalyzes oxidation of primary alcohols to give corresponding aldehydes.<sup>[1]</sup> In the catalytic cycle, Cu<sup>II</sup>-phenoxy radical formed by oxygen molecule acts as two-electron oxidant for the primary alcohols to generate the corresponding aldehyde. The active site of GO is converted to Cu<sup>I</sup>-phenol in the course of the alcohol oxidation, but the Cu<sup>I</sup>-phenol can be oxidized by O<sub>2</sub> to reproduce the Cu<sup>II</sup>-phenoxy radical. In order to understand the detailed properties and reactivities of GO, many metal(II)-phenoxy radical complexes have been reported,<sup>[2]</sup> while the formation of the Cu<sup>II</sup>-phenoxy radical from Cu<sup>I</sup>-phenol with O<sub>2</sub> is still rare.<sup>[2,3]</sup> In this connection, some studies have been reported, showing that Cu<sup>II</sup>-phenolate complexes activated O<sub>2</sub> in the catalytic reaction as models of GO.<sup>[2,4]</sup> However, the detail mechanisms for alcohol oxidation with Cu<sup>II</sup>-phenolate complexes and its redox behavior have not been clarified.

On the other hand, in the catalytic cycle of copper amine oxidase (CAO), Cu<sup>I</sup>-phenoxy radical described as a valence tautomer of the Cu<sup>II</sup>-phenolate has been proposed as the one of the important intermediates in the pathways for formation of the Cu<sup>II</sup>-quinone species by O<sub>2</sub>.<sup>[5]</sup> However, direct observation of formation of the stable phenoxy radical from the Cu<sup>II</sup>-phenolate complex with O<sub>2</sub> is yet to be reported. In these points in mind, we have investigated formation of Cu<sup>II</sup>-phenoxy radical complex with tetradentate 2N2O-tripodal ligand containing two phenolate moieties by O<sub>2</sub>-oxidation of Cu<sup>II</sup>-phenolate complex and its mechanism.

Reaction of Cu(ClO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O with H<sub>2</sub>(Me<sub>2</sub>NL) (right figure) in the presence of two equivalents of triethylamine in 1:1 mixture of CH<sub>2</sub>Cl<sub>2</sub>/MeOH under inert gas atmosphere gave Cu<sup>II</sup>-diphenolate complex [Cu(Me<sub>2</sub>NL)(H<sub>2</sub>O)] (**1**) as crystals. Reaction of complex **1** with O<sub>2</sub> caused color change to purple, and the UV-vis absorption spectral change revealed that the 515-nm band derived from *N,N*-dimethylamino phenoxy radical gradually increased. The resulting species showed EPR silent at 77 K, while the copper ion was still +II state from the results of Cu-K-edge XANES. Therefore, the reaction product of complex **1** with O<sub>2</sub> assigned to the Cu<sup>II</sup>-dimethylaminophenoxy radical species, which could be isolated to [Cu(Me<sub>2</sub>NL)(MeOH)]ClO<sub>4</sub> (**2**) as crystals. X-ray crystal structures of complexes **1** and **2** are similar geometry, while the detail structural features of **2** are different from those of complex **1** exhibiting dimethylaminophenoxy radical structural characteristics in complex **2**. The EPR and UV-vis absorption spectra of CH<sub>2</sub>Cl<sub>2</sub> solution of complex **1** clearly exhibited temperature-dependent equilibrium between Cu<sup>II</sup>-diphenolate and Cu<sup>I</sup>-*N,N*-dimethylaminophenoxy radical. From these results, formation of the Cu<sup>II</sup>-phenoxy radical complex **2** was formed by the reaction of complex **1** with O<sub>2</sub> via the Cu<sup>I</sup>-phenoxy radical intermediate.


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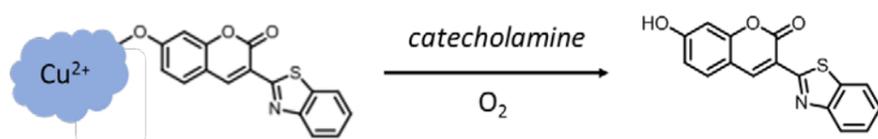
P078

### Selective Recognition and Detection of Catecholamines in Living Cells

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Catecholamines (e.g. dopamine, adrenaline and noradrenaline) are small molecule neurotransmitters that involve in vital physiological processes and are tightly regulated in the human body. Catecholamine dysregulation is implicated in various neurodegenerative diseases such as Parkinson's disease, schizophrenia, psychosis and attention deficit hyperactivity disorder (ADHD). Selective recognition and detection of catecholamines is exceedingly challenging due to their small size, non-specific molecular shape and trivial chemical properties. We report here the development of a bioinspired, copper-based oxidative bond cleavage for the catecholamine-triggered release of a fluorescent reporter for selective and sensitive catecholamine fluorescent detection. Live cell imaging with the new probe successfully visualized the accumulation and depletion of dopamine in cellular models of neuron differentiation and Parkinson's disease. Complemented by morphological studies in the same experiment, catecholamine imaging using the new probe provides for the first time an integrative approach for studying important neuronal processes.



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P079

### Metal-Ligand Cooperative Activation of CO<sub>2</sub>

Yu-Ting Tseng<sup>1,2</sup>, Wen-Feng Liaw<sup>1</sup>, Tsai-Te Lu<sup>2,3</sup>

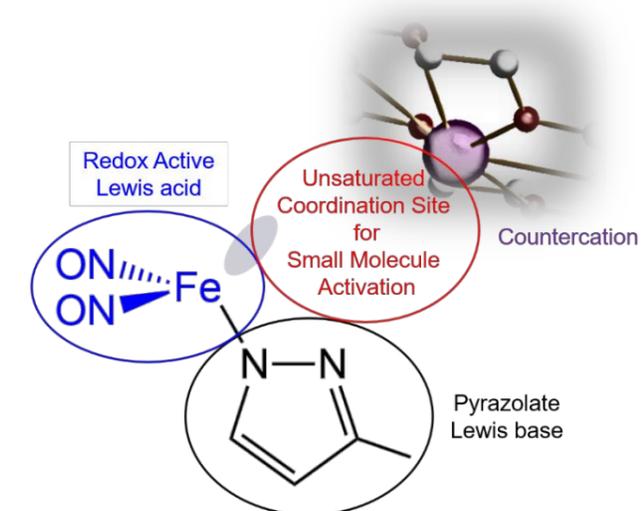
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Carbon dioxide, the waste from human activity, is expected to be employed as an inexpensive and potential feedstock of C1 sources for the regeneration of valuable chemicals and fuel. Relying on one-electron redox activity of the [Fe(NO)<sub>2</sub>]<sup>+0</sup> core, herein, we report the development of dinuclear complexes [Fe<sub>2</sub>(μ-<sup>R</sup>Pyr)<sub>2</sub>(NO)<sub>4</sub>]<sup>0/1-/2-</sup> (**1-3**) (<sup>R</sup>Pyr = pyrazolate) for the metal-ligand cooperative binding, activation, and transformation of CO<sub>2</sub>. The dianionic reduced DNIC [Fe<sub>2</sub>(μ-<sup>R</sup>Pyr)<sub>2</sub>(NO)<sub>4</sub>]<sup>2-</sup> (**3**), featuring planar and butterfly [Fe(μ-<sup>R</sup>Pyr)<sub>2</sub>Fe] core regulated by interaction among nitrosyl ligands, pyrazolate ligands, and [K-18-crown-6-ether] counteraction, can serve as frustrated Lewis pairs for CO<sub>2</sub> activation via formation of CO<sub>2</sub>-bond DNIC [Fe(<sup>R</sup>Pyr-COO)(NO)<sub>2</sub>]<sup>-</sup> (**4**). Furthermore, the CO<sub>2</sub> reduction reactivity was triggered by redox-inactive Lewis acid/redox-active Lewis acid/oxophilic borane reagents to result in the generation of new Fe-NO complexes. Finally, investigation of the subsequent transformation of the bound CO<sub>2</sub> in complex [Fe(<sup>R</sup>Pyr-COO)(NO)<sub>2</sub>]<sup>-</sup> is ongoing to demonstrate the metal-ligand cooperation as a novel strategy for the regeneration of value-added chemicals from CO<sub>2</sub>.

We gratefully acknowledge the financial support from the Ministry of Science and Technology (Taiwan).



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P080

### Nickel(II) Complexes of Peptides Containing Cysteinyl Residues

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Nickel is an essential element for a lot of plants and bacteria. It plays a role in the metabolism of higher organisms [1]. For nickel(II), the terminal amino group and side chain imidazole-N, thiolate-S and carboxylate-O of proteins serve as main binding sites in the metalloproteins.

In our previous research we have synthesized and studied the zinc(II), cadmium(II) and lead(II) complexes of a series of peptides containing two cysteine residues [2, 3]. One group of the ligands have a free N-terminal amino group and an amide group on the C-termini: Ser-SerCysSerSerAlaCysSer-NH<sub>2</sub> (SSCSSACS-NH<sub>2</sub>), AlaCysSerSerAlaCysSer-NH<sub>2</sub> (ACSSSACS-NH<sub>2</sub>) and CysSerSerAlaCysSer-NH<sub>2</sub> (CSSSACS-NH<sub>2</sub>), while the other group consists of terminally protected peptides: Ac-AlaAlaAlaCysSer-NH<sub>2</sub> (Ac-AAAC-NH<sub>2</sub>), Ac-SerAlaAlaCysSer-NH<sub>2</sub> (Ac-SAAC-NH<sub>2</sub>) and Ac-CysSerSerAlaCysSer-NH<sub>2</sub> (Ac-CSSSACS-NH<sub>2</sub>).

We have continued this work with the investigation of nickel(II) complexes of aforementioned peptides and completed them with the terminally protected tetrapeptides containing cysteine on the N-termini: Ac-Cys-GlyAlaAla-NH<sub>2</sub> (Ac-CGAA-NH<sub>2</sub>), Ac-CysGlyAlaLys-NH<sub>2</sub> (Ac-CGAK-NH<sub>2</sub>), Ac-CysGlyAlaAsp-NH<sub>2</sub> (Ac-CGAD-NH<sub>2</sub>) and Ac-CysGlyAlaHis-NH<sub>2</sub> (Ac-CGAH-NH<sub>2</sub>).

The stoichiometry and stability constants of the metal complexes were determined by potentiometry, while their structures were supported by means of CD-, UV-Vis, MS- and NMR-spectroscopy.

In the case of three peptides containing free terminal amino group, the N-terminal part of the molecules is the primary binding site and (NH<sub>2</sub>, S<sup>-</sup>), (NH<sub>2</sub>, N<sup>-</sup>, S<sup>-</sup>) or (NH<sub>2</sub>, N<sup>-</sup>, N<sup>-</sup>, S<sup>-</sup>) donor sets coordinate the metal ion, but the coordination of C terminal cysteine-S<sup>-</sup> contributes to the formation of stable complexes. At high pH-range, however, the C terminal thiolate group is able to behave as an anchor group, and coordination isomers are present in equimolar solution, while dinuclear species were detected at excess of nickel(II) ion.

In the case of the terminally protected tetra- and hexapeptides, the C-terminal thiolate and the amide groups are the primary binding sites. For tetrapeptides containing cysteine on the N-termini the thiolate group is the primary binding site only, when there are no side chain donor groups with strong coordination ability in the peptide (Ac-CGAA-NH<sub>2</sub>, Ac-CGAK-NH<sub>2</sub> and Ac-CGAD-NH<sub>2</sub>).

The presence of C-terminal histidine in the Ac-CGAH-NH<sub>2</sub> peptide however, takes over the role of primary binding site, and the (N<sup>-</sup>,N<sup>-</sup>,N<sup>-</sup>, N<sub>im</sub>) coordinated nickel(II) complexes are formed.

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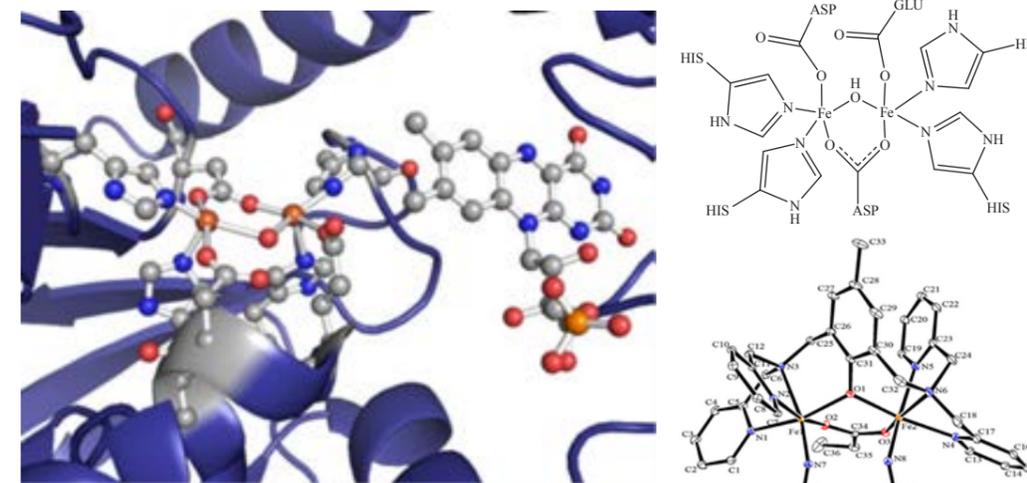
P081

### Modeling Flavodiiron Nitric Oxide Reductases: The Semi-Reduction Pathway for N-N Coupling

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Flavodiiron nitric oxide reductases (FNORs) are a subclass of flavodiiron proteins (FDPs) capable of preferential binding and reduction of NO to N<sub>2</sub>O. FNORs are found in certain pathogenic bacteria, equipping them with resistance to nitrosative stress, generated as a part of the immune defense in humans. Despite the significance of these enzymes in bacterial pathogenesis, the mechanism of NO reduction is not unambiguously defined. The complex [Fe<sub>2</sub>(BPMP)(OPr)(NO)<sub>2</sub>](OTf)<sub>2</sub> was synthesized as a model for FNORs, and its NO reduction was spectroscopically characterized. Using UV-Vis spectroscopy, cyclic voltammetry, and spectro-electrochemistry, we show that one reductive equivalent is in fact sufficient for the quantitative generation of N<sub>2</sub>O, following a *semi-reduced* reaction mechanism. This reaction is very efficient and produces N<sub>2</sub>O with a first order rate constant  $k > 10^2 \text{ s}^{-1}$ . This reaction proceeds at -80°C, allowing for the direct observation of the mixed-valent product of the reaction.



P082

### Molecular Interactions of Pt(II)-Aromatic Diimine-Polyaminopolycarboxylate Complexes with Aromatic Biomolecules

Tatsuo Yajima<sup>1</sup>, Shoichi Hinakura<sup>1</sup>, Atsushi Ito<sup>1</sup>, Yasuo Nakabayashi<sup>1</sup>, Osamu Yamauchi<sup>1,2</sup>

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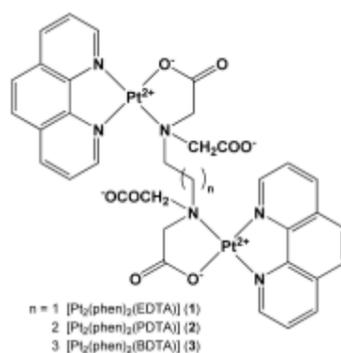
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Non-covalent interactions such as electrostatic interactions, hydrogen bonds and aromatic ring stacking interactions play important roles in biological systems for molecular recognition and regulation of reaction. Galactose oxidase is one of the mononuclear copper enzymes, which catalysed oxidation of a primary alcohol and has a phenoxyl radical coordinated to the Cu<sup>II</sup> ion in the active form. The indole ring of a tryptophan (Trp), which has a  $\pi$ - $\pi$  stacking interaction with the phenol radical moiety, is considered to stabilize the active form of GO and a study of Cu-phenoxyl radical complexes having  $\pi$ - $\pi$  stacking interactions supports the notion.[1] Metal-coordinated aromatic diimine ligands such as 1,10-phenanthroline (phen) interact to aromatic moieties with stacking interactions as seen in [Cu<sup>II</sup>(phen)(Trp)], and interactions with uncoordinated aromatic molecules lead to formation of adducts.[2-4]

We studied syntheses and characterizations of binuclear complexes of Pt<sup>II</sup>(phen) moieties bound to EDTA and its analogs. [PtCl<sub>2</sub>(phen)] reacted with EDTA, 1,3-diaminopropane-*N,N,N',N'*-tetraacetate (PDTA), and 1,4-diaminobutane-*N,N,N',N'*-tetraacetate (BDTA) to form [Pt<sub>2</sub>(phen)<sub>2</sub>(EDTA)] (1), [Pt<sub>2</sub>(phen)<sub>2</sub>(PDTA)] (2), and [Pt<sub>2</sub>(phen)<sub>2</sub>(BDTA)] (3), respectively, where the Pt(phen) units are bound to the both ends of EDTA, etc. with a 3N1O donor set, as revealed by X-ray crystal structure analyses. Crystal structures of them also revealed that the Pt(phen) moieties of the EDTA and PDTA complexed interact each other intramolecular and intermolecular, while in the BDTA complex the Pt(phen) moiety has no intramolecular  $\pi$ - $\pi$  stacking interaction but only intermolecular stacking interactions, which implies that the BDTA complex may interact with other aromatic molecules through two stacking interactions.

Adduct formations of the complexes with aromatic biomolecules such as indoleacetate and 5-hydroxytryptamine (serotonin) were studied by <sup>1</sup>H NMR measurements and calorimetry methods. Financial support by the Kansai University is gratefully acknowledged.



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P083

### Highly Selective Fluorescent Probes for Ascorbate Detection in Living Cells

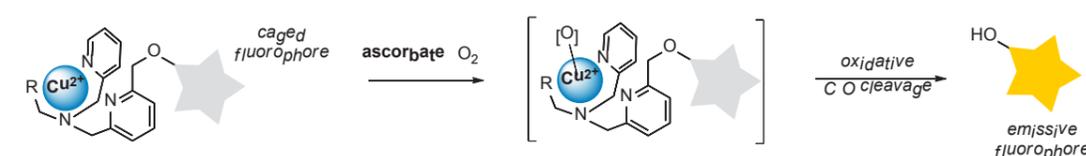
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As an essential biological reducing agent, ascorbate plays numerous roles from being an antioxidant to mediating electron transfer in enzymatic processes. Bioanalytical tools for direct, redox-state specific, intracellular ascorbate detection are therefore indispensable for understanding ascorbate-related processes in cells. However, conventional approach such as HPLC, electrochemical methods and optical assays for ascorbate analyses are far from satisfactory. They either lack the selectivity or require isolation with chemical pre-treatments and specific handling conditions for samples.

We report here the development of a new reaction-based ascorbate probes inspired by the LMPOs copper monooxygenase.<sup>1</sup> With the new ascorbate-triggered copper-mediated oxidative cleavage, ascorbate detection can be achieved with a turn-on fluorescence signal. The probes are very sensitive and selective towards ascorbate against a broad range of reducing agents and biological antioxidants. We also successfully apply the probes in sensing ascorbate in commercial drink samples, human plasma, serum and in live cells for confocal imaging and flow cytometry.

#### Bioinspired Design of Ascorbate Probe



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P084

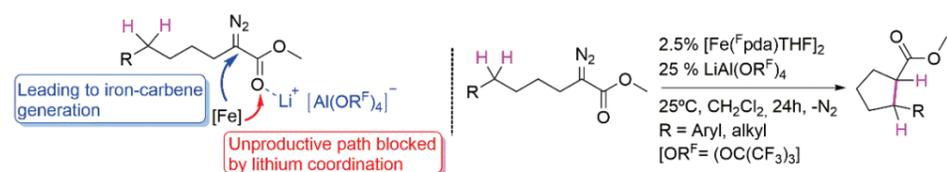
### Intramolecular C<sub>sp</sub><sup>3</sup>-H alkylation using low-coordinate iron complexes via metallocarbene intermediates

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The functionalization of non-activated C-H bonds to form new C-C bonds is a reaction of high interest since it becomes a starting point for the synthesis of value-added compounds. Among all transformations, metal-catalyzed carbene transfer reaction from diazocompounds to inert C-H bonds is a promising methodology due to its grater atom economy when compared with the most extended cross-coupling procedures. [1] Until now, copper and rhodium complexes have been found to be the most efficient metals to perform this transformation. However, the iron-catalyzed carbene transfer reactions have been less explored and they are limited to less demanding processes. [2]

In this work we present the combination of an electrophilic iron complex with pre-activation of  $\alpha$ -alkyl substituted  $\alpha$ -diazooesters reagents by the Lewis acid LiAl(ORF)<sub>4</sub> [ORF= (OC(CF<sub>3</sub>)<sub>3</sub>) provides unprecedented access to selective iron catalyzed intramolecular functionalization of strong alkyl C(sp<sup>3</sup>)-H bonds. Reactions occur at 25°C, via  $\alpha$ -alkyl-metallocarbene intermediates, and with activity/selectivity levels similar to rhodium carboxylate catalysts. Mechanistic investigations reveal a crucial role of the lithium cation in the rate determining formation of the electrophilic iron-carbene intermediate, which then proceeds via concerted insertion into the C H bond. [3]



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P090

### Crystallographic Features and Photocytotoxicity of Ternary Thiosemicarbazone Complexes of Oxovanadium(IV) and copper (II)

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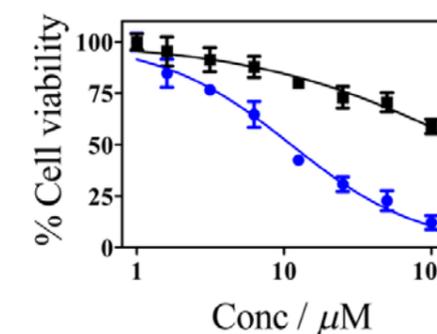
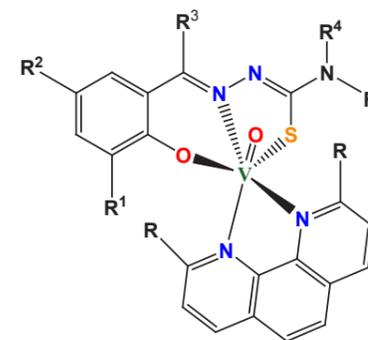
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Thiosemicarbazones continue to draw attention as an important multifunctional class of ligands for multifarious reasons, including variable donor properties, structural diversity and biological applications. Their biological activities are enhanced when these ligands are complexed with bioactive main-group and transition-metal ions. Thiosemicarbazones display coordination versatility due to their tendency to undergo tautomerisation. They exist as thione-thiol tautomers, and can bind to a metal center in the neutral or the anionic forms. Thiosemicarbazones bind iron selectively and exhibit marked antitumor activity *in vitro* and *in vivo*.<sup>1-3</sup> These ligands also find applications in the treatment of Fe-overload disorders such as  $\beta$ -thalassemia major.<sup>1</sup> Moreover, a variety of transition-metal complexes of thiosemicarbazones have been reported to possess DNA cleavage behavior.<sup>2,4-6</sup>

In this work we have designed, synthesized and structurally characterized novel ternary complexes of oxovanadium(IV) comprising thiosemicarbazones. These complexes are of both physicochemical and biological interest. They represent the first examples of crystallographically elucidated complexes of their kind. In this study we compare and contrast the X-ray structures and the photocytotoxicity of the ternary thiosemicarbazone complexes of oxovanadium(IV) with those of the corresponding copper(II) analogues. The cytotoxicity investigation has been conducted using HeLa and MCF-7 cancer cells. The oxovanadium(IV) complexes show remarkable photodynamic behavior. Structurally, the *trans* influence of the oxo group in the vanadium(IV) complexes is compared with the tetragonal distortion of the copper(II) (d<sup>9</sup>) centers that leads to the asymmetric axial-equatorial coordination mode of phen and its derivatives.



**Figure 1:** General structure of ternary thiosemicarbazone complexes of oxovanadium(IV) and photo-induced cytotoxicity

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P091

### Ligand Rearrangement in Ru(II) Arene Complexes Coordinating to Simple Proteins

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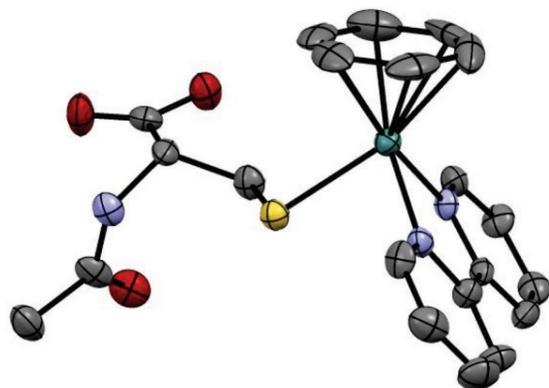
Artificial Metalloenzymes (ArMs) result from the introduction of an abiotic metal cofactor into a protein framework. ArM's combine the selectivity and control of enzymatic processes with the wide-range of synthetically useful transformations catalysed by transition metals. The substrate specificity and regioselectivity provided by the protein framework then enables their use in complex mixtures.<sup>1</sup>

Attaching the metal complex with designed ligands to a specific place on the protein, remains a key challenge in the development of efficient ArM's. To date, the most successful ArM's either involve; a linker,<sup>2</sup> substitution of an endogenous metal,<sup>3</sup> or artificial amino acids.<sup>4</sup>

Our approach looks to introduce a catalytically inert Ru(II) arene scaffold directly to a cysteine residue which upon protein coordination the catalytic activity of the ruthenium cofactor is unmasked. Direct coordination is beneficial as it has the potential to be applied to a variety of protein scaffolds and doesn't rely on linker technology, which means there can be direct influence of protein energetics on the metal centre. Using a relatively inert pre-catalyst means that there are fewer possible side reactions, and any catalytic activity can be attributed to the metal-protein hybrid.

We have observed intriguing reactivity with the Ru(II)(arene)(bipyridine) fragment and the K63C mutant of human ubiquitin. The [Ru(II)(Arene)(H-Bipy)Cl] complex binds relatively slowly to the cysteine residue, and the bipyridyl ligand remains coordinated, however, the fluorinated complex [Ru(II)(Arene)(F<sub>2</sub>-Bipy)Cl] binds rapidly at equimolar Ru:Protein concentration, and involves loss of the difluoro-bipyridyl ligand. The reactivity can be attenuated by using differently donating arene ligands.

Our goal is to be able to directly coordinate ruthenium pre-catalysts to polypeptides and control the coordination environment around the metal, just as biology does with Co in vitamin B<sub>12</sub> and Fe in heme, for example. We hope to be able to develop this methodology into a range of different protein scaffolds and observe catalytic activity.



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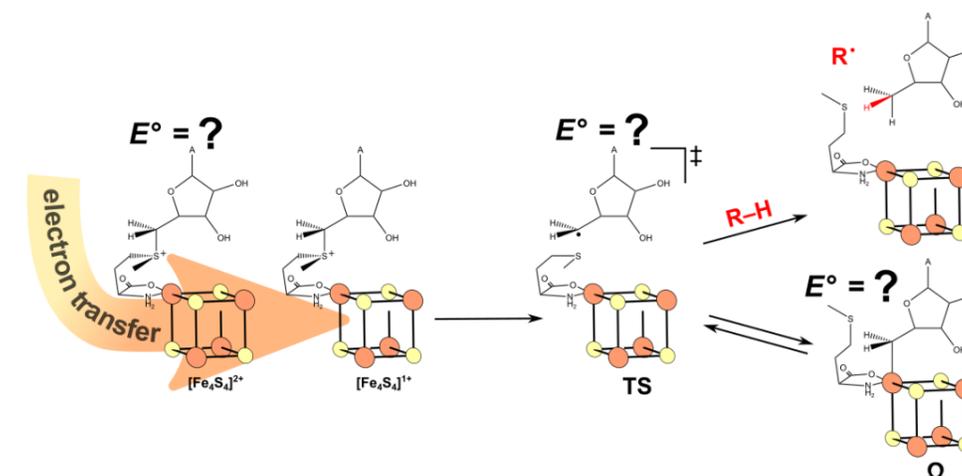
### Redox Properties of Multinuclear Iron Enzymes

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Iron is the most abundant redox active metal in biology. It appears in diverse functional forms, such as Fe/S, S-adenosylmethionine (SAM) radical, heme iron, and mononuclear and binuclear nonheme iron enzymes. An important common theme for all of these species is that their chemistry is closely related to their redox properties. Herein, we present a computational methodology for calculation of reduction potentials of multinuclear iron enzyme active sites, including SAM radical enzymes. First, the methodology is validated on a large series of biomimetic compounds, including non-heme iron complexes and iron-sulfur [Fe<sub>4</sub>S<sub>4</sub>] clusters [1,2]. Then, it is extended to calculate the reduction potentials of particular multinuclear iron enzymes to associate redox properties with their reactivity.



Radical SAM enzymes catalyze diverse reactions through a homolytic SAM cleavage. Recently, the organometallic intermediate  $\Omega$  was suggested to be an integral part of the catalytic cycle of pyruvate formate-lyase activating-enzyme (PFL-AE) [3] Herein, we provide a detailed insight into the mechanism of reductive cleavage of SAM by calculating the reduction potentials of all key intermediates along the reaction coordinate, including the  $\Omega$  intermediate.

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P093

**AuMF, A New Promising Inhibitor of Thioredoxin Reductase**

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Thioredoxin reductase (TrxR) is one of important enzymes of thioredoxin system, which regulates partial of cellular redox metabolism. TrxR is often overexpressed in tumor cells and plays a critical role in cancer cell growth and development. These make it an attractive target for new drugs designed for treatment of cancer. Gold (III) fluorinated porphyrin AuMF our group recently synthesized may be a promising selenoproteins inhibitor. Au (III) fluorinated porphyrin shows a dose-dependent increase effect on TrxR activity of NCI H460 cells (Figure 1) in 2h, with an IC<sub>50</sub> value of 9.7 μM by using DTNB with the substrate.

Financial support by the The University of Hong Kong is gratefully acknowledged.

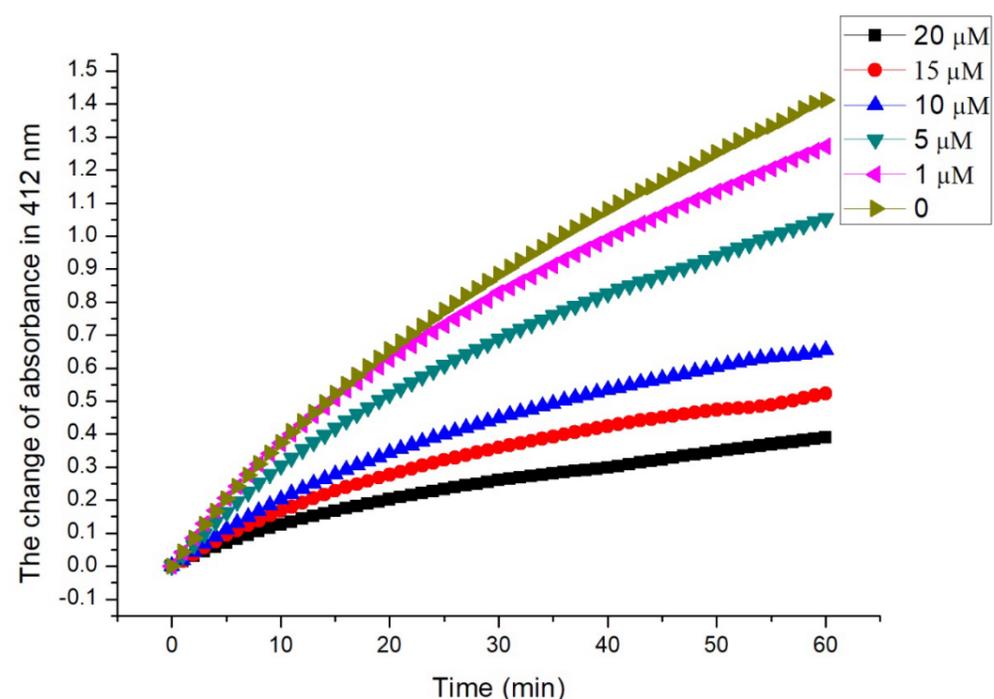


Figure 1: Kinetic analysis of effect of AuMF on TrxR activity of NCI H460 cells using DTNB substrate. NCI H460 cells was incubated with various concentrations of AuMF for 2 h and the enzyme activities were measured as increases in absorbance at 412 nm after adding DTNB.

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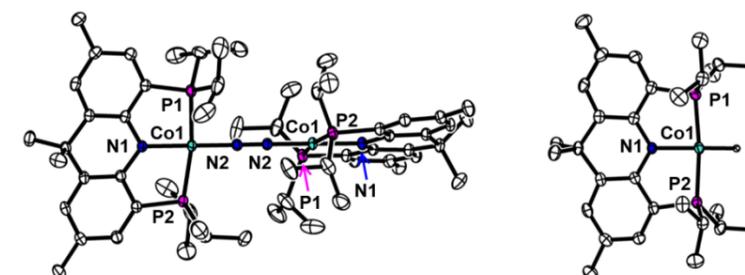
**Reactivity of a Low-Spin Low-Coordinate Cobalt Species Towards Dihydrogen and Silane**

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Nonclassical dihydrogen species of cobalt have been proposed as key intermediates in homogeneous hydrogenation, dehydrogenation and hydrogen evolution reaction. Such species typically undergo oxidative addition to generate cobalt dihydride species. Computational studies suggested that the formation of the dihydride species may be initiated by the high spin state of the cobalt center, while the low spin state leads to the production of a nonclassical dihydrogen complex [1]. A T-shaped cobalt complex with the strong ligand field may generate a low-spin electronic configuration. The low-spin three-coordinate cobalt species could allow the exploration of the nonclassical dihydrogen cobalt complex.

Here, we will present syntheses and characterization of cobalt complexes supported by an acridane adapted <sup>acri</sup>PNP ligand (<sup>acri</sup>PNP<sup>-</sup> = 4,5-bis(diisopropylphosphino)-2,7,9,9-tetramethyl-9H-acridin-10-ide) [2]. A monovalent cobalt-dinitrogen complex {(<sup>acri</sup>PNP)Co}<sub>2</sub>(μ-N<sub>2</sub>) was synthesized from the chemical reduction of corresponding cobalt bromide species. By applying, a low spin three-coordinate cobalt complex was prepared. Upon addition of dihydrogen, a square planar cobalt monohydride species was generated via a homolytic cleavage of H<sub>2</sub>. During the conversion, a nonclassical cobalt-dihydrogen species was detected spectroscopically. As a model study of the cobalt-H<sub>2</sub> species, cobalt-η<sup>2</sup>-silane compound was exploited. Further investigation on the transformation of cobalt-H<sub>2</sub> and cobalt-silane species will be discussed in detail.



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P095

### Coumarin-Tagged Dinuclear Trithiolato-Bridged Ruthenium(II)-Arene Complexes – Photophysical Properties and Antiparasitic Activity

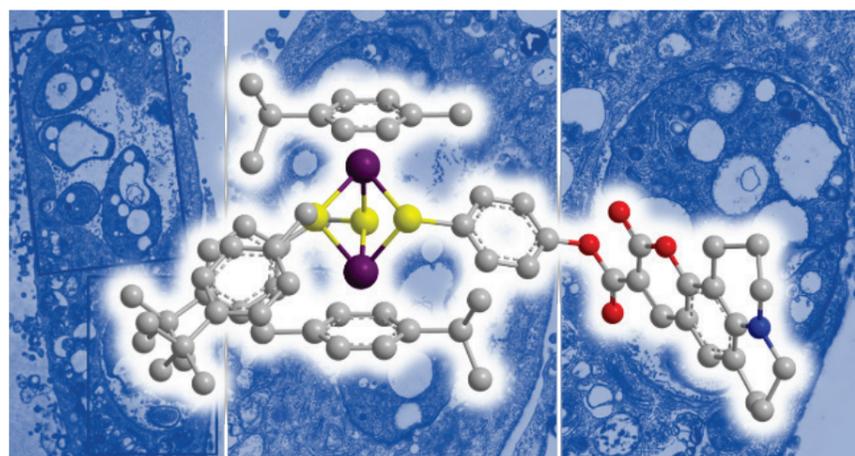
Oksana Desiatkina<sup>1</sup>, Emilia Păunescu<sup>1</sup>, Julien Furrer<sup>1</sup>, Nicoleta Anghel<sup>2</sup>, Ghalia Boubaker<sup>2</sup>, Yosra Amdouni<sup>2</sup>, Andrew Hemphill<sup>2</sup>

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Symmetric and ‘mixed’ cationic trithiolato-bridged dinuclear ruthenium(II)-arene complexes (general formula  $[(\eta^6\text{-arene})_2\text{Ru}_2(\mu_2\text{-SR})_3]^+$  and, respectively,  $[(\eta^6\text{-arene})_2\text{Ru}_2(\mu_2\text{-SR}^1)(\mu_2\text{-SR}^2)]^+$ ) were identified as potential antiparasitic agents against apicomplexan parasites such as *Toxoplasma gondii*<sup>1</sup> and *Neospora caninum*<sup>2</sup> and were shown to target the mitochondrion. Investigating the possible mechanism of action of this type of compounds, and their traffic after cell uptake, has become a priority.

Confocal fluorescence microscopy of fluorophore-labelled conjugates is one of the methods allowing the study of sub-cellular localization of metalorganic complexes. Coumarin-tagged ruthenium complex conjugates were shown to be versatile tools for bioimaging.<sup>3</sup> A small library of seven trithiolato-bridged dinuclear ruthenium(II)-*p*-cymene conjugates modified with coumarin fluorophores was synthesized. We aimed to assess how various structural features influence the photophysical and antiparasitic properties. Fluorescence measurements revealed that anchoring the organometallic moiety to the dye led to an important fluorescence quenching for all conjugates. Hindering the free rotation of the amine in the coumarin dye, connection *via* amide bond and the presence of a linker between the organometallic complex and the fluorophore led to higher quantum yields. Human foreskin fibroblast (HFF) host cells and *T. gondii* tachyzoites grown in HFF monolayer cultures were exposed to 1 and 0.1  $\mu\text{M}$  of each compound of interest (coumarin-labeled conjugates, non-modified thiolato-bridged dinuclear ruthenium(II)-arene complexes and free coumarin dyes). This preliminary evaluation led to the selection of two compounds for further biological studies. These conjugates, both modified with butterfly coumarin, inhibited *T. gondii* proliferation with 50% inhibitory concentrations ( $\text{IC}_{50}$ s) of 0.032 and 0.118  $\mu\text{M}$ , respectively, and they did not affect HFF at dosages of 2.5  $\mu\text{M}$  and above, resulting in good selectivity indexes. Transmission electron microscopy showed that within the first 24 h of treatment, both conjugates caused important ultrastructural alterations in the parasite mitochondria, resulting in pronounced destruction of tachyzoites.



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P096

### Development of Novel Bioreductive Co<sup>III</sup> Hydroxamate Compounds Exploiting Localised Tumour Hypoxia

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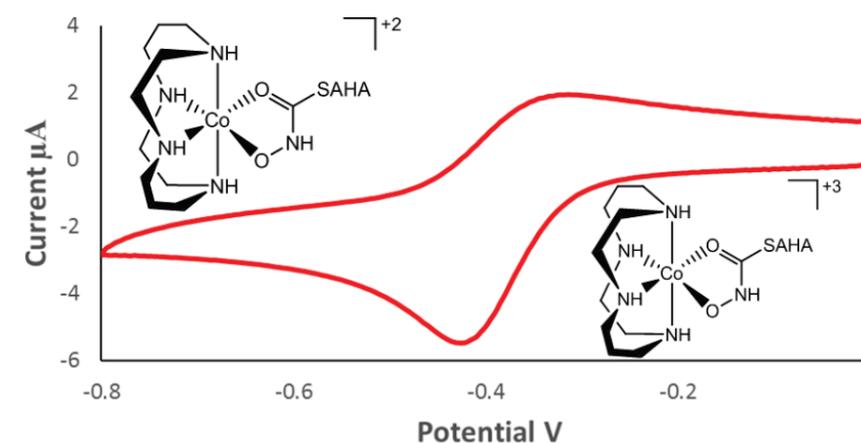
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Stemming from the serendipitous discovery of cisplatin as a potent anticancer chemotherapeutic, the field of metal-derived anticancer agents has flourished. In attempts to improve the activity and toxicity profile of metal-based drugs, transition metal redox coupling between higher and lower charged states has been employed to activate drugs within hypoxic, reducing, tumour environments. This redox ability modulates the physicochemical properties of the metal centre especially with respect to the aqueous stability of the complexes. This results in higher oxidation state metal centres typically having slower ligand exchange kinetics when compared to the lower oxidation state equivalents. With this in mind, Co<sup>III</sup> drug complexes have been developed utilising the hypoxia induced reduction to Co<sup>II</sup> as an activation mechanism for site selective ligand release.<sup>1</sup>

Hydroxamic acids are bidentate ligands which coordinate to metal centres through an O,O'-binding motif forming a 5-membered chelate ring.<sup>2</sup> Hydroxamic acid-derived drugs generally target metalloenzymes by coordination to the central metal ion such as the binding of vorinostat to the Zn<sup>II</sup> ion within histone deacetylase.<sup>3</sup> In order to generate stable Co<sup>III</sup> complexes which are only reduced *in situ*, they require ancillary ligands to optimise the redox potential of the metal centre to ensure site specific reduction by cellular reductants with oxygen acting as a sacrificial electron donor in normoxic conditions.<sup>4</sup> The use of the tetraazamacrocycles, such as cyclen and cyclam, have been the most promising for delivering O,O'-hydroxamic acid-based drugs to tumours as they successfully stabilise the +3 oxidation state, are stable long-term in aqueous solution and cyclam being the first ancillary ligand to generate a quasi-reversible Co<sup>III</sup>/Co<sup>II</sup> couple by cyclic voltammetry. Financial support from The University of Auckland is gratefully acknowledged.



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P097

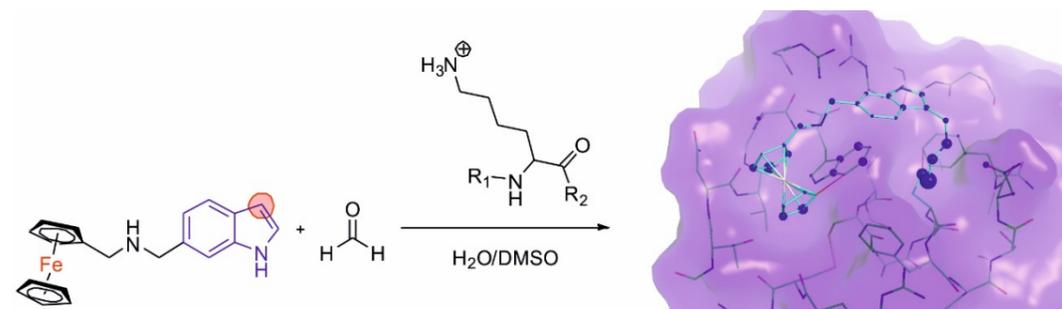
### Conjugation of Organometallic Indole Derivatives to Lysozyme

Dominic Graf<sup>1</sup>, Kevin Lüken<sup>1</sup>, Christoph A. Sotriffer<sup>2</sup>, Ulrich Schatzschneider<sup>1</sup>

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Artificial protein modification with organic or organometallic groups allows the introduction of new functionalities to such biomacromolecules. This requires bioorthogonal reactions which do not undergo side reactions with other functional groups present in a particular biological system [1-3]. Recently, an interesting new method was reported for the modification of the  $\epsilon$ -amino groups of lysine residues in lysozyme in a Mannich-like reaction with indole derivatives under retention of the native charge [4]. To extend this method to organometallic protein labelling, we have synthesized a family of indole conjugates with cymantrene ( $\text{CpMn}(\text{CO})_3$ ), cyrhetrene ( $\text{CpRe}(\text{CO})_3$ ), and ferrocene ( $\text{Cp}_2\text{Fe}$ ) groups attached to different positions on the 6-membered ring of indole to study their conjugation efficiency with lysozyme and compare it to that of commercially available organic indoles. Conjugate formation was analyzed with high-resolution ESI mass spectrometry. In addition, covalent docking simulations were carried out to determine the most accessible lysine side chains and to elucidate the steric influence of the protein surface as well as that of the indole derivatives through the empirical scoring functions ASP and DSX in GOLD.



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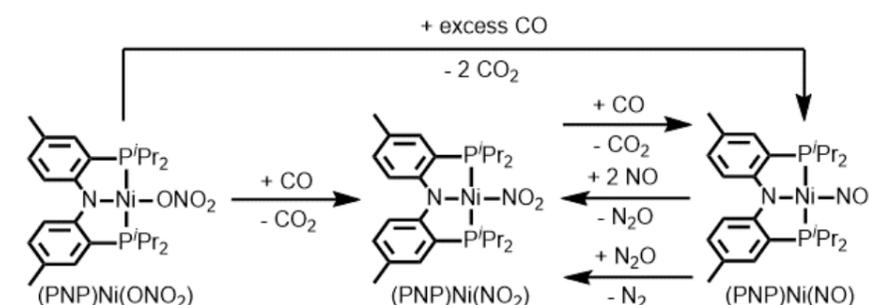
P098

### Stepwise Reduction of $\text{NO}_3^-$ to $\text{N}_2$ at a Single Nickel Center

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$\text{NO}_x$  conversion involving multi-step reduction processes to produce  $\text{N}_2$  is recently receiving much attention due to environmental and social issues. According to the substantial use in industry and agriculture, nitrates has become a critical water pollutant and it demands harsh reaction conditions to be reduced. As a main detrimental components of exhaust gas,  $\text{NO}_2$ ,  $\text{NO}$  and  $\text{N}_2\text{O}$  also should be converted effectively under the flue gas conditions. In nature, such conversion known as denitrification occurs via a multi-step process to reduce nitrate to dinitrogen ( $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$ ) as a part of biological nitrogen cycle. Although the efficient enzymatic reactions attractively operate under mild conditions, four different metalloenzymes are needed to convert a series of intermediate  $\text{NO}_x$  species. In other words, their applications to the  $\text{NO}_x$  conversion catalyst are limited due to the complications of reaction path that comes from the inherent biological purpose. Herein, a stepwise reduction from nitrate to dinitrogen at a single nickel center will be presented. A PNP nickel scaffold ( $\text{PNP}^- = \text{N}[2\text{-P}^i\text{Pr}_2\text{-4-Me-C}_6\text{H}_3\text{]}_2$ ) emerged as a universal platform for the deoxygenation of  $\text{NO}_x$  substrates. In these reactions carbon monoxide acts as the oxygen acceptor and forms  $\text{CO}_2$  to provide the necessary chemical driving force. Whereas the first two oxygens are removed from the Ni-nitrate and Ni-nitrite complexes with  $\text{CO}$ , the deoxygenation of  $\text{NO}$  requires a disproportionation reaction with another  $\text{NO}$  molecule to form  $\text{NO}_2$  and  $\text{N}_2\text{O}$ . The final deoxygenation of nitrous oxide is accomplished by the Ni- $\text{NO}$  complex and generates  $\text{N}_2$  and Ni- $\text{NO}_2$  in a relatively slow, but clean reaction. This sequence of reactions is the first example of the complete denitrification of nitrate at a single metal-site and suggests a new paradigm of connecting  $\text{CO}$  and  $\text{NO}_x$  as an effective reaction pair for  $\text{NO}_x$  removal.



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P099

### Novel Copper Guanidine Complexes as Entatic State Models for Electron Transfer

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Guanidine ligands are strong neutral *N*-donor ligands. There are various types of guanidine ligands that differ in the structure of their backbone and their further coordination units. Therefore, guanidines are versatile ligands that can be used in various applications with different metals.

One type of guanidine ligands are the guanidine quinoline ligands that were used in copper guanidine quinoline complexes as entatic state models for fast electron transfer [1-3] and as charge transfer complexes. [4] By now, these complexes exhibit the highest self-exchange rates  $k_{11}$  in the electron transfer of all pure *N* donor ligands. [1] But all used guanidine quinoline ligands are similar in their structure. Hence, the influence of the structure of different guanidine ligands in the electron transfer is of interest.

Herein, we report the properties of three novel copper guanidine complexes as entatic state models in the electron transfer. Guanidine ligands that differ in the structure of their backbone and their further coordination units were used. The self-electron exchange rates  $k_{11}$  of the copper complexes were determined using the Marcus cross relation.

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P100

### Stability Tests of Alkynylgold(I)(NHC) Complexes in Solution

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Gold organometallic compounds have been extensively investigated as potential anticancer metallodrugs and have shown a high potential regarding antiproliferative effects [1-4]. Enhanced stability is a driving argument for the design of gold organometallics, the more surprising is the lack of methods for pharmaceutical analytics of their stability and solution chemistry. Such analytical methods are important key elements for future pharmacokinetic studies and will help to ensure a better understanding of their biological behavior [5].

We selected complexes of the type of alkynylgold(I)(NHC) for detailed stability studies by HPLC-DAD-MS, in comparison to the well-known antirheumatic agent Auranofin. A RP-based chromatographic method was established to separate possible degradation products of alkynylgold(I)(NHC) complexes. The stability studies were performed at 37°C over 24h using dimethylformamide (DMF), dimethyl sulfoxide (DMSO), water and Dulbecco's modified eagle medium (DMEM) solutions of each compound. Furthermore, interaction experiments of alkynylgold(I)(NHC) complexes with acetylcysteine are under way with the same set-up as the stability tests [6].

ESI (+) and (-) ionisation with a quadrupole analyser was used for mass spectrometry. The first results indicate that alkynylgold(I)(NHC) complexes are stable over extended periods in the mentioned solvents with no significant changes in their AUCs [Fig 1].

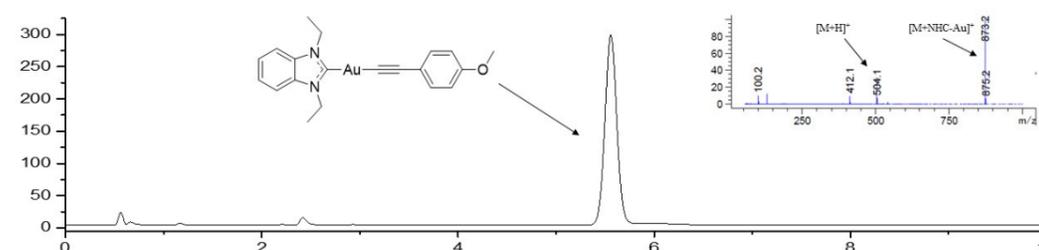


Figure 1: Chromatogram of a selected complex in DMEM after 24h. The insert shows the ESI (+) mass spectrum of the main peak.

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P101

### Synthesis, Characterization and Antimicrobial Activities of Supramolecular Cobalt-Peptide Conjugates

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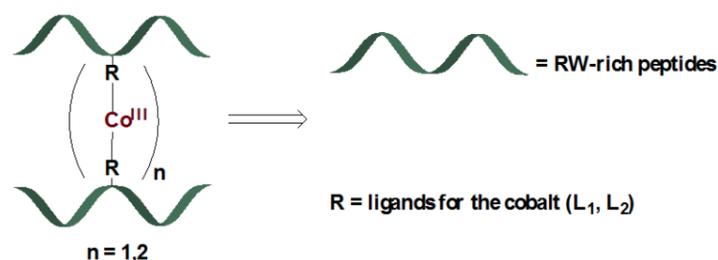
In the past decade, only a few new antibiotics have come onto the market, while the emergence and spread of highly resistant bacteria has continued to increase. As a result, the need for the effort to develop and discover new natural and synthetic antibiotic agents has increased recently.<sup>[1]</sup>

One promising new antibiotic agent is cationic amphipathic hexapeptide RWRWRW-NH<sub>2</sub>. This peptide exhibits low hemolytic activity and toxicity against human cell lines, has a bactericidal effect on Gram-positive bacteria such as MRSA.<sup>[2]</sup> The short cationic peptide interacts with or even partly cover the cell membrane. Unfortunately, the first case of resistance against a membrane-targeting antimicrobial agent was recently discovered.<sup>[3]</sup>

Albada *et al.* tried to modify membrane-targeting peptide by an insertion of metalorganic moieties such as metallocene into the RW-rich peptide structure. Thereby, the deceleration of enzymatic degradation and the fast destruction of bacteria strains were established. Unfortunately, the mechanism of action by insertion of metallocene with redox active metals into RW-rich peptides were unsuccessful. However, the activity against several Gram-negative and Gram-positive bacteria increased significantly.<sup>[4]</sup>

The essential transition metal cobalt (Co) exhibits favourable redox chemistry (Co(II/III)) and might act as prodrugs.<sup>[5]</sup> Therefore, the cobalt species itself can act an effector and cause oxidative stress in cells, which might lead to changes in the mechanism of action of membrane-targeting peptides.<sup>[5-6]</sup> Additionally, the Co(III)-peptide conjugates might decelerate enzymatic degradation of peptides through their stability.

In this work, the different supramolecular cobalt(III)-peptide conjugates of different RW-rich peptides are synthesized (figure). To glean information on the size and shape, as well as to probe intermolecular complex formation diffusion ordered spectroscopy (DOSY) was used. Thereby, the intermolecular formation of only one product after complexation with cobalt was indicated. Next, the minimum inhibitory concentration (MIC) was determined to provide the first data about the biological activity of the prepared cobalt-peptide conjugates. Our poster will show formation and structure of complexes, which was confirmed based on the obtained diffusion coefficients from DOSY NMR experiments. Therefore, our poster will show the general trend in the observed MIC values of peptides and their cobalt-conjugates.



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P102

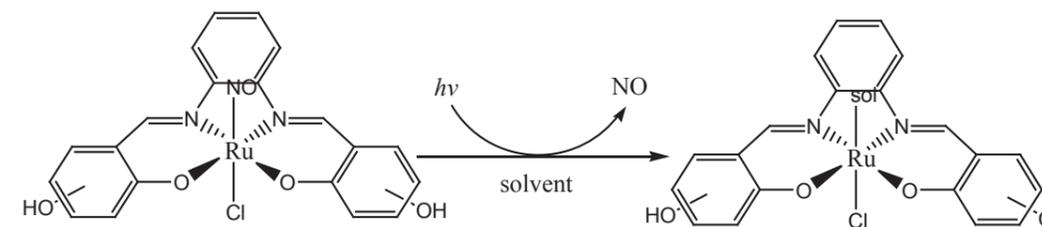
### Syntheses of Water-Soluble Ruthenium Nitrosyl Complexes with Schiff Base Ligand

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Since the discovery of Nitric Oxide (NO) as one of the major signal-transduction molecules in cells, there have been many attempts to devise acute NO-delivering systems for the purpose of developing disease therapies as well as studying cell functions. Metal-nitrosyl complexes are often releasing NO by light activation. This ability can be adapted to killing cancer cells with high specificity because high concentration of NO in cells induces apoptosis. Among them, ruthenium nitrosyl complexes have been proposed as attractive photodynamic therapeutic agents in biomedicine and in tumor treatment. We researched ruthenium nitrosyl complex with salophen ligand, [Ru(III)(NO)(salophen)Cl]. This research aims at developing ruthenium nitrosyl complexes which absorb long wavelength visible light to release NO and are dissolved in water. We introduce new ligands with hydroxyl group on the positions of ortho, meta and para of the salophen ligand in order to increase the solubility in water. In this study, we show the NO-releasing properties of the complexes monitored by UV-VIS, IR spectroscopy and EPR which can be interpreted as that the diamagnetic [Ru-NO]<sup>6</sup> electronic state of the complex becomes low-spin Ru(III) (d<sup>5</sup>, S=1/2) state upon losing NO by photoactivation.

Financial support by BK21 program of Ministry of Education of Korea is gratefully acknowledged.



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P103

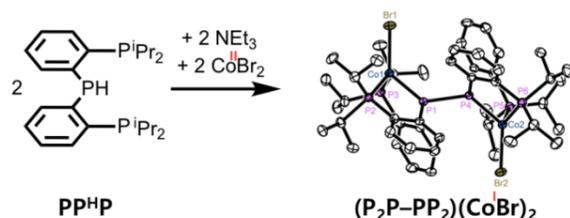
### Redox-Active Behavior of a Tridentate Bis(phosphinophenyl)phosphido Ligand at a Cobalt Center

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Metal-ligand cooperation (MLC) that the metal center and the ligand participate in the key transformations has been studied widely. Our group recently established a distinctive MLC of a tridentate bis(phosphinophenyl)phosphido ligand (PPP =  $^{-}P[2-P^iPr_2-C_6H_4]_2$ ) with a nickel center. The phosphide-nickel(II) alkoxide complex converts into phosphinite-nickel(0) by the introduction of a  $\pi$ -acidic ligand such as CO(g). [1] The two-electron redox change at the nickel center comes along with the geometric change from the square planar to pseudo-tetrahedral. In the case of a nickel(II) anilido complex, an anionic phosphido radical is generated upon the addition of CO(g) followed by the P–P bond coupling to form a dinuclear complex and the isocyanate generation. [2]

The unique MLC of a PPP ligand is attributed to the HOMO possessing a dominant central phosphorus atom character based on the density functional theory analysis. The high covalency between a ligand and a metal center can induce an efficient electron transfer process. Thus, the cobalt was introduced to the PPP ligand to increase the covalency of the central phosphorus and the metal center. The metalation of a (PPP)H ligand with CoBr<sub>2</sub> under basic condition generates a dinuclear cobalt(I) complex, (P<sub>2</sub>P–PP<sub>2</sub>)(CoBr)<sub>2</sub>. This unusual P–P bond coupling reaction can occur through a single electron transfer between the PPP ligand and the cobalt center. Detailed redox activity of a PPP ligand cooperated with the cobalt center through a reversible P–P bond formation and cleavage will be discussed.



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P104

### Platinum(IV) Complexes Coupled to dGC for Tumour Targeting

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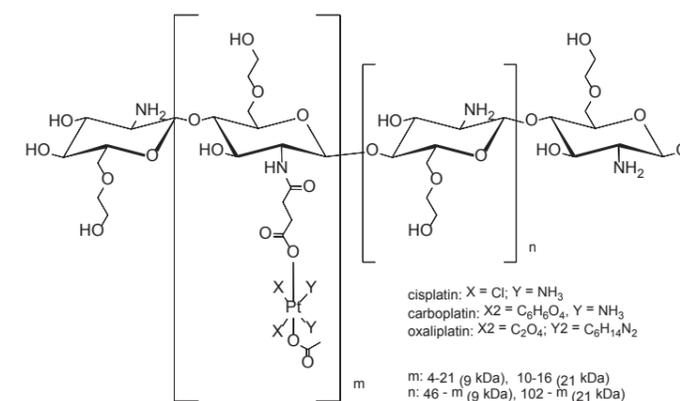
Up to now, platinum(II) complexes such as cisplatin, carboplatin and oxaliplatin have been an indispensable part of chemotherapy. However, platinum-based therapy is accompanied by severe side effects such as nephrotoxicity, neurotoxicity and ototoxicity as a result of their lability and non-selectivity towards tumour tissue. [1][2]

In order to decrease toxicities, asymmetrically carboxylated platinum(IV) analogues of cisplatin, carboplatin and oxaliplatin were synthesised. Besides an optimised reduction potential, the additional axial ligands lead to kinetically more inert prodrugs and allow the introduction of targeting moieties. [3] Consequently, the platinum(IV) complexes were coupled to drug carrier polymers to exploit the enhanced permeability and retention (EPR) effect of tumour tissue and enable the accumulation of the cytotoxic agent. [1]

The choice of chitosan polymers is based on their biocompatibility. Additionally, the chitosan polymers may be derivatised for targeting purposes. [4][5]

Starting from glycol chitosan, degraded glycol chitosan polymers (dGC) of two different chain lengths (21 and 9 kDa) were synthesised *via* acidic degradation. The molecular weight was determined by gel permeation chromatography (GPC). Subsequently, the functionalised platinum(IV) complexes were coupled to dGC *via* amide bond formation.

The obtained platinum(IV)-dGC conjugates were characterised by NMR spectroscopy and ESI-MS. The successful coupling was further proven by diffusion-ordered NMR spectroscopy (DOSY). The average loading rates of platinum(IV) complex per dGC-polymer were established by inductively coupled plasma MS (ICP-MS) measurements and were found between 10 and 50 %. Finally, cytotoxicity was investigated by MTT-assays in human cancer cell lines CH1/PA-1, A549 and SW480. In general, IC<sub>50</sub>s of low micromolare to nanomolare range were recorded whereby the cisplatin-dGC conjugate (8 kDa, 27 %) showed the lowest IC<sub>50</sub> value of 0.053 ± 0.007 μM in CH1/PA-1 ovarian teratocarcinoma cells.



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P105

### Transition Metal Catalyzed Biorthogonal Reactions inside Bacteria Cells

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Since the discovery of the first antibiotics in the early 20<sup>th</sup> century, these agents were the first choice for the treatment of bacterial infections. However, the widespread use and the partial misuse of antibiotics in both humans and livestock has led to an increasing number of resistant bacteria. Therefore, the development of novel antibiotic drugs with new modes of action is urgently necessary.<sup>[1]</sup>

In the last decade, biorthogonal chemistry has become a promising tool for applications in imaging, drug development, and biotechnology. The term biorthogonal chemistry describes the performance of chemical modifications in living biological systems with the aim of not interfering with the host's biochemistry.<sup>[2]</sup> However, the performance of such modifications in living biological systems is challenging since suitable biorthogonal functional groups and catalysts need to fulfill certain requirements: the functional groups should be non-existent in nature and the catalyst should be stable in the presence of water, air, and small concentrations of thiols and other nucleophiles.<sup>[3]</sup>

Meggers *et al.* initially demonstrated that the organoruthenium complex (shown in figure below) can catalyze the conversion of biorthogonal allyl carbamates into their respective amines inside living mammalian cells by deprotecting of an inactive anticancer prodrug inside HeLa cells.<sup>[3]</sup>

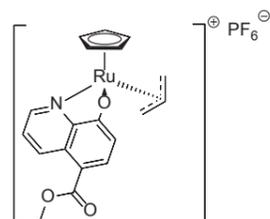
Despite the promising results for the uncaging of an anticancer prodrug inside HeLa cells, this principle has not yet been tested for antibiotics in living bacteria cells.<sup>[4]</sup> Therefore, the aim of the ongoing project is to develop a suitable biorthogonal catalyst/substrate pair which can be used to perform catalytic deprotection reactions inside bacteria cells. This strategy could help to deal with the issue of resistant bacteria.<sup>[5]</sup>

For this strategy, inactive allyl carbamate prodrugs of the well-known antibiotics Puromycin and Seromycin were synthesized. Next, the minimum inhibitory concentrations of the protected prodrugs were determined to demonstrate that the allyl carbamate derivatives had lost their antibacterial activity.

Parallel to the synthesis of the protected antibiotics, allyl carbamate derivatives of the well-known fluorescent dyes coumarin and naphthalene were synthesized which had yielded non-fluorescent protected dyes.

The organoruthenium complex shown in the figure below can convert the inactive prodrugs and the non-fluorescent protected dyes under biological conditions (presence of water, air, and thiols) into their respective amines yielding either active antibiotics or fluorescence dyes. Our poster will show the optimized reaction conditions for the uncaging reactions under these conditions which were determined by using nuclear magnetic resonance spectroscopy and liquid chromatography - mass spectrometry technique.

The uptake of the catalyst inside the bacteria cells is crucial for the strategy planned in the ongoing project and therefore our poster will show the catalyst's cellular uptake which was determined by inductively coupled plasma optical emission spectrometry measurements.



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P106

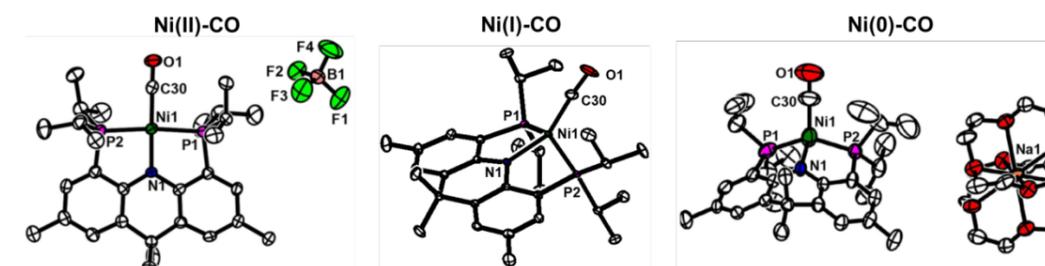
### Low-Valent Nickel Chemistry Supported by Pincer-Type Amido Diphosphine Ligands

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Low-valent nickel complexes play an important role in biological systems as well as in chemical applications. In particular, nickel(0), nickel(I) and nickel(II) species have been proposed as intermediates in several enzymes. Among the Ni-containing enzymes, carbon monoxide dehydrogenase (CODH) is of significant environment interest as it can provide a concept of developing an efficient catalyst for the selective conversion of CO<sub>2</sub>. Despite the recent structural and spectroscopic advances, however, details of the mechanism, including the oxidation states of nickel active site during CO<sub>2</sub> binding, remain unclear. Herein, reactivity of nickel complexes with various oxidation states (+2, +1 and 0) was described to understand organometallic principles in the CODH active site.

Our group exploited the low-valent nickel chemistry supported by pincer-type amido diphosphine ligands to study the CODH active site having a 4-coordinate nickel center. Protonation of nickel(II) carboxylates Ni-μ-CO<sub>2</sub>-κC,O-M (M = H, Na, Ni, Fe) cleaves the C–O bond, resulting in a formation of nickel(II) monocarbonyl complexes [1, 2]. By sequential reduction, the corresponding mono- and zero-valent nickel carbonyl species were obtained, and their reactivity toward CO<sub>2</sub> was explored. Especially, the reaction of a nickel(0) carbonyl complex supported by a PNP ligand (PNP<sup>-</sup> = [2-P'Pr<sub>2</sub>-4-Me-C<sub>6</sub>H<sub>3</sub>]<sub>2</sub><sup>-</sup>) with CO<sub>2</sub> displays multiple products including a nickel-carbamate species [3]. However, a nickel(II) carboxylate complex and CO gas are selectively generated from the reaction of a nickel(0) carbonyl species involving a structurally rigidified <sup>acri</sup>PNP ligand (<sup>acri</sup>PNP<sup>-</sup> = 4,5-bis(diisopropylphosphino)-2,7,9,9-tetramethyl-9H-acridin-10-ide) with CO<sub>2</sub>, to demonstrate the synthetic cycle for the selective reduction of CO<sub>2</sub> to CO [4]. Furthermore, the <sup>acri</sup>PNP ligand scaffold allows the synthesis and isolation of a T-shaped nickel(I) metalloradical species, (<sup>acri</sup>PNP)Ni [5], and a corresponding nickel(0)-N<sub>2</sub> species, {(<sup>acri</sup>PNP)Ni-(N<sub>2</sub>)-Na(THF)<sub>2</sub>}<sub>2</sub>, while (PNP)Ni(I) and (PNP)Ni(0) species are not stabilized without π-acidic ligands. The three-coordinate monomeric nickel(I) complex exhibits open-shell reactivity having a half-filled d<sub>x<sup>2</sup>-y<sup>2</sup></sub> orbital [5], and the dimeric nickel(0) complex is able to activate C–H bonds in unfunctionalized arenes under ambient conditions.



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P107

### Hybrid Compounds Constructed on a Trithiolato-Bridged Dinuclear Ruthenium(II)-Arene Scaffold - Synthesis, Structural and Spectroscopic Characterization

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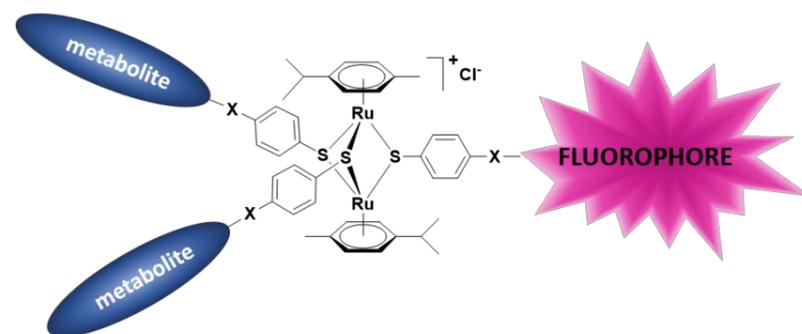
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The high cytotoxicity against human cancer cells<sup>1</sup> of cationic trithiolato-bridged dinuclear ruthenium(II)-arene complexes (general formula  $[(\eta^6\text{-arene})_2\text{Ru}_2(\mu_2\text{-SR})_3]^+$ ) makes them interesting frameworks for the development of new compounds with potential biological activity.

The fate of this type of ruthenium complexes in cells and how this relates to the anticancer effect is yet unknown. Confocal fluorescence microscopy of BODIPY<sup>2</sup> and coumarin<sup>3</sup>-tagged ruthenium conjugates proved to be an efficient tool for the investigation of sub-cellular localization of ruthenium(II)-arene compounds.

Tethering a bioactive molecule to the organometallic scaffold is one approach used to modulate the medicinal properties of ruthenium(II)-arene complexes. This type of intramolecular combination can lead to compounds with possible new modes of action or can help directing the organometallic moiety to a specific sub-cellular target. The attachment of various metabolites onto anticancer organometallic complexes, as strategy aimed to enhance the activity and selectivity, was already reported.<sup>4,5</sup>

On this background, we have designed and synthesized a series of new trithiolato-bridged dinuclear ruthenium(II)-arene organometallic hybrid molecules in which metabolite units, as well as fluorophore moieties were anchored as pendant arms on the bridge thiols. In a first step, disubstituted compounds were isolated from the coupling reaction of trithiolato complexes with the general formula  $[(\eta^6\text{-arene})_2\text{Ru}_2(\mu_2\text{-S-C}_6\text{H}_4\text{-XH})_3]\text{Cl}$  (where  $\text{-XH} = \text{-OH}, \text{-NH}_2$ ) with metabolites containing carboxylic acid groups. These intermediates allowed further modification with judiciously functionalized fluorophores in a second step. As proof-of-concept, first a short library of hybrid molecules containing two units of the same metabolite (i.e. lipoic acid) and various BODIPY and coumarin fluorophores were synthesized. Subsequently, a series of compounds containing the same coumarin dye (i.e. (7-(diethylamino)-2-oxo-2H-chromene-3-carboxylic acid) but various metabolite moieties were obtained.



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P108

### Thioredoxin Reductase Inhibiting Alkynylgold(I)NHC Complexes as Potential Antitumor Agents

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In consequence of spread and severity of cancer there is need for novel drugs. Thioredoxin reductase (TrxR) has been identified as a potential target in the treatment of cancer burden. With its chemical property as a soft Lewis acid, gold(I) has a high affinity to thiols and selenols, which are present in the active site of TrxR. [1,2]

Inspired by the disease-modifying antirheumatic drug Auranofin, several types of ligands have been used to form gold(I) complexes as TrxR inhibitors [2]. Beside halide, thiolate and phosphane based ligands, the focus has more recently shifted towards organometallic complexes due to their higher chemical stability. Recent published results show that alkynes and *N*-heterocyclic carbenes (NHCs) as ligands to gold(I) display both described effects (forming stable complexes and good activity against TrxR). [1-3]

NHC-gold(I) complexes demonstrated their potential as strong inhibitors of the TrxR in combination with cytotoxic activity against several tumor-cell-lines, such as MCF-7 (breast adenocarcinoma) and HT-29 (colon adenocarcinoma) [3,4]. Alkynyl-gold(I)-phosphanes have shown a similar strong activity against the same cell lines with additional anti-angiogenic effects in zebrafish embryos [5].

A synthesis and characterization procedure for complexes with an alkynyl and a NHC ligand was developed [Figure 1]. Ongoing biological tests deal with the cytotoxicity and TrxR inhibition of this type of gold organometallics. First experiments confirmed strong cytotoxicity against several tumor cell lines and efficient inhibition of TrxR. The current results will be presented on the poster.

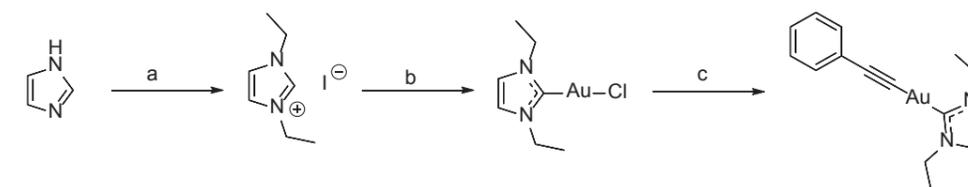


Figure 1: Synthesis procedure for an example of an alkynyl-gold(I)-NHC-complex. a) a) alkylation with ethyl iodide, b) reaction with  $\text{Ag}_2\text{O}$  and transmetalation with  $\text{SMe}_2\text{AuCl}$ , c) reaction with phenylacetylene under basic conditions

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P109

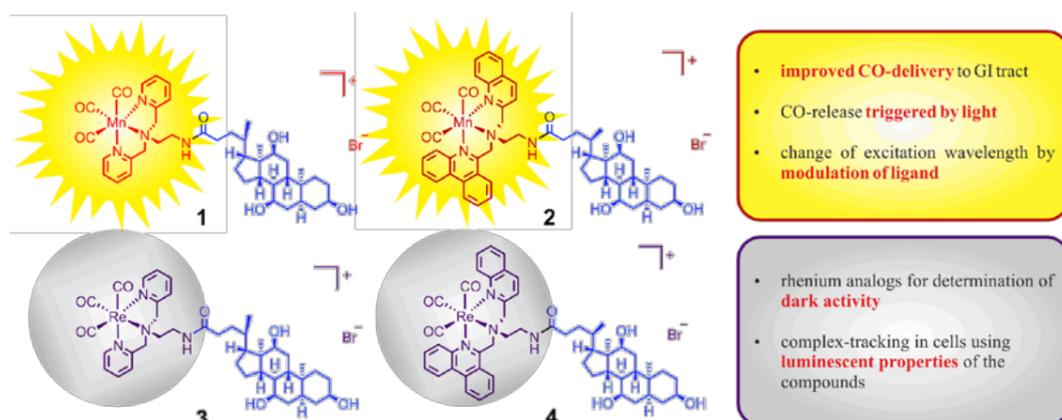
### Novel Highly Functionalized CO-Releasing Molecules (CORMs) Containing Cholic Acid for Improved Bioavailability and Targeting

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As a colorless and odorless gas, carbon monoxide (CO) is generally known to the public only as the “silent killer”. However, it is also endogenously generated in living organisms by the degradation of heme by heme oxygenase (HO) enzymes. As a small signalling molecules, it dilates blood vessels and possesses anti-inflammatory and anti-apoptotic as well as cytoprotective properties.

The key problem in the therapeutic application of CO is to find novel delivery systems for specific tissue accumulation. *CO-releasing molecules* (CORMs) are metal carbonyl complexes which have the potential for facile CO delivery that can be controlled in a spatial and temporal manner. As a novel strategy to increase tissue targeting, highly functionalized photoactivatable CORMs (PhotoCORMs) conjugated to biomolecular carrier systems were developed and characterized for multitude of different interesting applications. [1-5]



In this context, bis(pyridin-2-ylmethyl)amine (bpa) and *N*-(phenanthridin-6-ylmethyl)-*N*-(quinolin-2-ylmethyl)amine (pqa) manganese(I) tricarbonyl complexes were functionalized with the primary bile acid cholic acid (Fig. 1) which is a known detergent for adsorption and transport of nutrients, fatty acids, vitamins and drugs and plays an important role in the lipid, glucose and energy metabolism. [6] The corresponding rhenium analogs were also prepared as controls to determine potential non-CO mediated dark activities.

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P110

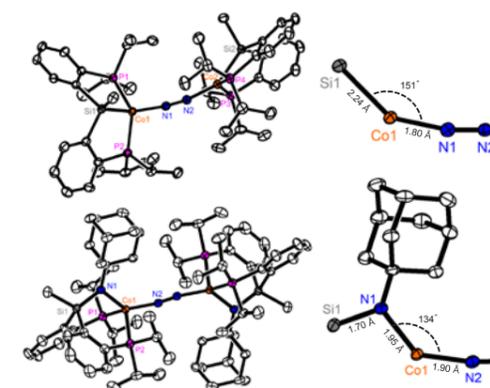
### Ligand-Assisted Nitrene Group Transfer of a Cobalt Complex Supported by a Diphosphinosilyl Ligand

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Transition metal complexes having a metal-nitrogen multiple bond are important reactive intermediate species of the nitrene group transfer. However, only limited catalytic nitrene transfer reactions mediated by metal-nitrene species have been reported due to the high reactivity of late transition metal-nitrene species. In order to overcome such limitations, metal-ligand cooperation can be one possible strategy. Our approach is to introduce a silyl moiety to assist the metal center in stabilizing a nitrene group, since several examples of group transfer to silyl groups have been reported.

Here, we will present cobalt complexes supported by an anionic diphosphinosilyl ligand MeSiP<sub>2</sub> (MeSiP<sub>2</sub><sup>-</sup> = MeSi[2-P'Pr<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>]<sub>2</sub><sup>-</sup>). We found that a silyl anchor allows reversible metal-ligand bond formation, resulting in group transfer reactivity. A 4-coordinate dicobalt N<sub>2</sub> species {(MeSiP<sub>2</sub>)Co}<sub>2</sub>(μ-N<sub>2</sub>) was generated from the reduction of (MeSiP<sub>2</sub>)CoBr [1] and its characterization was established by various spectroscopic and XRD data. The reaction of the cobalt dinitrogen species with adamantyl azide generates a Co-silylamido complex {(MeSiN<sup>Ad</sup>P<sub>2</sub>)Co}<sub>2</sub>(μ-N<sub>2</sub>) via the nitrene group insertion into the Co-Si bond. Interestingly, such compound can produce the adamantyl isocyanate (AdNCO) from the reaction with CO by forming the bis-carbonyl cobalt species (MeSiP<sub>2</sub>)Co(CO)<sub>2</sub>. Further catalytic isocyanate generation reaction of (MeSiP<sub>2</sub>)Co(CO)<sub>2</sub> with excess amount of CO and AdN<sub>3</sub> was investigated by photolysis. The detailed characterizations of cobalt complexes and the proposed catalytic cycle will be discussed.



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P111

### Exploring Plectin as a Target for Organometallic Compounds

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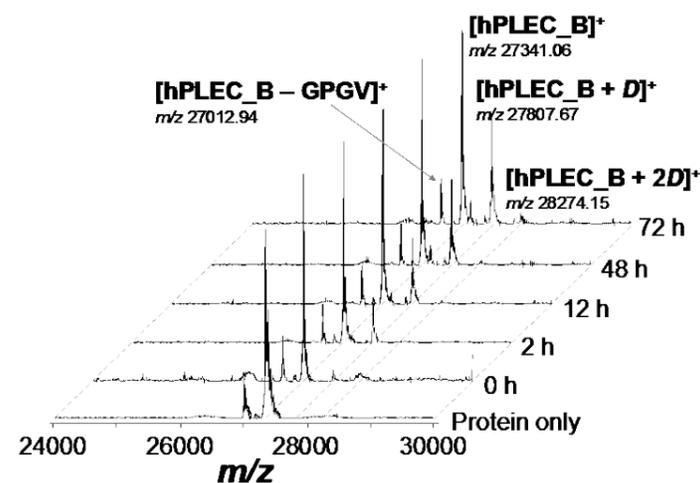
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Platinum-based chemotherapeutics play an important role in the clinic for cancer treatment. For the further development of novel metal-based anticancer agents it is of utmost importance to elucidate the modes of action using bioanalytical methods. Promising novel anticancer agents are based on the piano-stool scaffold which confers a number of favourable properties and the biological activity can be modulated through the choice of metal centre and/or ligands. This allows the design of complexes with specific functions through selective interaction with biological targets.

*N*-Substituted 2-pyridinecarbothioamides (PCA) are bidentate ligands with an S,N donor system. The attachment of a bidentate PCA ligand to an organometallic piano-stool scaffold led to the discovery of a promising chemotherapeutic with low micromolar activity in cancer cells [1]. Oral administration to mice reduced tumour growth more efficiently in an invasive melanoma than in a colon cancer xenograft. To explore the mode of action of these complexes, a proteomics-based target-response profiling approach revealed very high selectivity (160-fold) for the scaffold protein and cytolinker plectin [2].

To study the interaction of the PCA complexes with plectin with our established bioanalytical toolkit [3], the large scaffold protein of 151 kDa was split into nine constructs consisting of approximately two domains each. These were expressed and purified with initial protein crystallographic hits achieved. ESI-MS was allowed identifying four out of eight constructs to interact with [Ru(cym)(PCA)Cl]Cl over a period of three days to form [construct + *nD*] adducts {*D* = Ru(cym)(PCA)}. The initially formed mono adducts transformed over time into bisadducts (Figure).

Financial support from the Cancer Research Trust NZ and the University of Auckland is gratefully acknowledged.



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P113

### Ultra-Low Dose Near-Infrared Light Activated Mitochondria-Targeting Photosensitizer for PDT Cancer Therapy

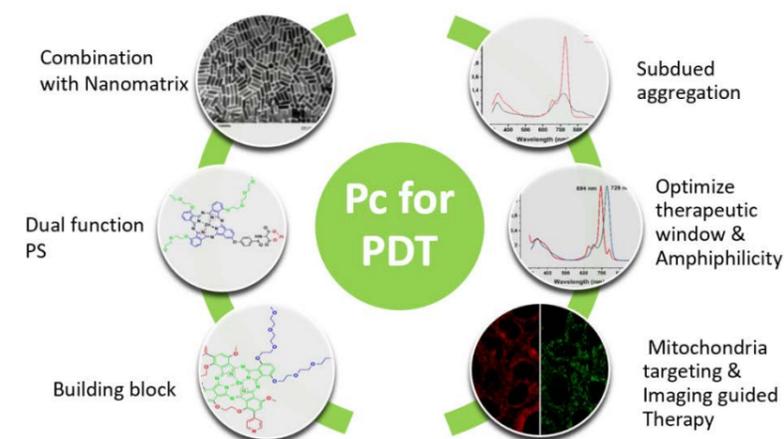
Wenyu Wu<sup>1</sup>, Nadine Giger<sup>1</sup>, Christine König<sup>2</sup>, Patrick Spielmann<sup>3</sup>, Roland Wenger<sup>3</sup>, Stefano Ferrari<sup>2</sup>, Bernhard Spingler<sup>1</sup>

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Owing to the near infrared absorption and photoinduced reactive oxygen production properties, phthalocyanine could provide an ideal therapeutic window for PDT treatment.<sup>1,2</sup> Yet the high aromatic core may lead to aggregation and then undesired quenching of reactive oxygen species, therefore we have designed short PEG-chain functionalized A3B type phthalocyanine (Pc) based photosensitizer, with not only increased solubility but also decreased aggregation. In this vein, we report the synthesis, structure determination, optical properties, cellular localization and cytotoxicity of a family of expanded phthalocyanine analogues. The stepwise functionalization of the peripheral moieties effects the solubility, cell uptake, cell localization and then cytotoxicity. A follow up structure-activity relationship analysis investigating the impact of lipophilicity modification revealed the presence of malonic ester functionality as indispensable to confer cytotoxicity to the Pc series. The mitochondria targeting MLC31 gave a desired photo-index (PI = 748) in cisplatin-resist A2780/CP70 cell line, with very low light toxicity (IC<sub>50</sub> = 157 nM), and barely no dark cytotoxicity (IC<sub>50</sub> = 117 μM). In addition, we carried out a preliminary investigation of aspects of cytotoxicity and present evidence that, upon near infra-red (NIR) light irradiation, MLC31 disrupt the mitochondrial membrane potential, and induce apoptosis. Furthermore, it remained similar cytotoxicity even under hypoxic condition. In conclusion, MLC31 may serve as an effective photosensitizer for NIR light mediated PDT in antitumor therapy.



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P121

### Effects of the CdTe Quantum Dots Exposure to Herb Plants (Rosemary and Peppermint)

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Various types of nanoparticles have been developed for many purposes. However environmental problems are also pointed out in these days with respect to increased consumption of the nanoparticles [1]. For the reason, studying environmental behaviors of nanoparticles is important issue to expect the possible problems to the living things. Among the organisms, plants are regarded as good study model to monitor effects caused by nanoparticles since they have high chance to be exposed to nanoparticles and presented as predator in the food-chain [2,3].

In here, we studied physiological effects of quantum dots (QDs) to plants in order to evaluate risk assessment as food. As known, QDs is nanoparticles highly applied for the industry due to its noteworthy optical and electronic properties. However it have been showed high toxicity to living organisms because of released heavy metal ions from the nanoparticles. To study phytotoxic effects caused by QDs, herb species (rosemary and peppermint) were cultivated for 45 days in the nutrient solution which was containing 5 and 10 mg L<sup>-1</sup> of CdTe QDs. In order to confirm physiological effects, we analyzed enzyme activities (SOD and CAT), concentration of pigments (chlorophylls and carotenoid), and growth rate. Furthermore, photosynthetic efficiency was measured to suggest possible effects on photosynthesis on the leaf with a portable IR gas exchange analyzer [4].

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P123

### Accumulation and Intracellular Speciation of Arsenic in Ferns

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Arsenic (As) is highly toxic metalloid that poses serious hazards to virtually all livings. Treatments of As-polluted soils by using a phytoremediation approach with hyperaccumulators may provide an eco-friendly means to prevent environmental and health disturbances caused by As. Studies indicated that certain *Pteris* spp./varieties can hyperaccumulate As, sparking the research interest in the biology of As in (hyper)accumulating ferns.

Here we report on accumulation and intracellular As species in three ferns, *P. cretica* var. *Parkerii*, *P. cretica* var. *Albo-lineata* and *P. straminea*. Following the sampling (fronds and roots separated) of plants grown in soils amended with 20, 100 and 250 mg As kg<sup>-1</sup> dwt for 30, 90, and 180 days, the As analyses revealed striking hyperaccumulation trait in the var. *Albo-lineata* (up to 3.7 and 1 g kg<sup>-1</sup> dwt of frond and root tissues, respectively, after 90 days), but not the var. *Parkerii* or *P. straminea* (respectively, frond and root As after 90 days of 0,19 and 0,12 or 0,22 and 0,15 g kg<sup>-1</sup> dwt). Irrespective of the accumulation status, the analysis of the tissue extracts revealed virtually all As unbound with any peptidaceous ligands, such as phytochelatins (PC) peptides ( $\gamma$ -Glu-Cys)<sub>n</sub>Gly, PCn, known to bind with thiophilic As species. Although the levels of PC2 increased in a As dose-dependent manner in all ferns (minute PC3 was detected only in *P. straminea*) the molar levels of potential As-binding (PC) were three orders of magnitude lower than the concentrations of intracellular As, indicating that PCs may have only minor, if any, role in handling of As in the investigated ferns. Current data thus indicate that hyperaccumulation trait of *P. cretica* var. *Albo-lineata* does not rely on its distinct ability to sequester As as or biotransform it into a “hyperaccumulator-specific” species. To follow up in a search for As tolerance determinants in var. *Albo-lineata*, the coding sequence of its predicted 134-amino acid arsenate reductase (88% identical to the hyperaccumulating *P. vittata* enzyme) was obtained from the frond transcriptome for further studies. Financial support by the Czech Science Foundation, Grant No. 17-10591S is gratefully acknowledged.

**P130****Effect of Zinc on Mouse Spermatozoa Function****Isabella Borgula<sup>1,2</sup>, Andrew Nowakowski<sup>1,2</sup>, Teresa K. Woodruff<sup>2,3</sup>, Thomas O'Halloran<sup>1,2</sup>**<sup>1</sup>Department of Chemistry, Northwestern University, IL, 60201, USA.<sup>2</sup>Chemistry of Life Processes Institute, Northwestern University, IL, 60201, USA.<sup>3</sup>Department of Obstetrics and Gynecology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA.

After fertilization or chemically-induced egg activation, mammalian eggs release billions of zinc ions in events known as 'zinc sparks'. This discharge of zinc has been shown to harden the zona pellucida by crosslinking its glycoprotein coat [1], and is believed to be one of several mechanisms required to prevent supernumerary sperm from entering a fertilized egg. Whether there is a direct effect of zinc on sperm has not been explored. Once in the female reproductive tract, ejaculated sperm undergo a number of maturation processes, including capacitation, to become activated for fertilization. Fertilization occurs after the sperm undergoes the acrosome reaction, which is a process by which a capacitated sperm releases proteolytic enzymes from the acrosome organelle to break down the glycoprotein matrix of the zona pellucida. Because the acrosome reaction occurs in close vicinity to the egg and facilitates fertilization, we hypothesize that the high local zinc levels inhibit the acrosome reaction of sperm that may have bound immediately after the fertilizing sperm and thus further prevent polyspermy. To investigate this question, the acrosome reaction was induced by calcium ionophore A23187 or progesterone, and multiple concentrations of zinc sulfate were added to simulate the conditions after the zinc spark. The treated sperm were stained with Coomassie blue to highlight whether the acrosome reaction occurred and analyzed under a bright field microscope to visually determine the frequency of acrosome reactions under these conditions. Preliminary data suggests sperm treated with zinc have a lower likelihood of undergoing the acrosome reaction and losing their stained acrosome, which indicates a repressed acrosome reaction. Additionally, acrosome reaction frequency varies inversely with concentration of zinc. These data support our hypothesis that one function of zinc in the extra-zygotic environment is to signal suppression of the acrosome reaction in the subordinate sperm that bind the zona pellucida.

Financial support by the Chemistry of Life Processes Institute at Northwestern University is gratefully acknowledged.

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**P131****Visualizing the Internalization of Histatin-5 in *Candida Albicans*****Joanna Campbell<sup>1</sup>, Katherine Franz<sup>1</sup>**<sup>1</sup>Department of Chemistry, Duke University, 124 Science Drive, Durham, North Carolina 27708, United States of America.

Histatin-5 (Hist-5) is a polycationic, histidine-rich antimicrobial peptide with potent antifungal activity against the opportunistic fungal pathogen *Candida albicans*.<sup>1-3</sup> Hist-5 has the ability to bind metals in vitro.<sup>2</sup> The goal of this work is to further elucidate the mechanism of action of Hist-5 and how that mechanism may be affected by biologically relevant metals. Toward this goal, we prepared a fluorescently-labeled Hist-5 called Hist-5Mca-ABD. Hist-5Mca-ABD was observed to bind Cu(II) in vitro and retained its antifungal activity against *C. albicans*. Antifungal susceptibility assays showed that Hist-5Mca-ABD's activity was potentiated by copper, lowering the minimum inhibitory concentration (MIC) from 12.5  $\mu$ M with Hist-5Mca-ABD alone to 3.1  $\mu$ M with supplemental copper. Internalization of the fluorescent peptide was observed through confocal fluorescence microscopy. These preliminary data indicate that fluorescent Hist-5Mca-ABD is a good model to investigate cell internalization of Hist-5 and how the metal environment affects uptake and intracellular movement of Hist-5.

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P132

**Bacterial Sensors Define Intracellular Free Energies for Correct Enzyme Metalation**Deenah Osman<sup>1,2</sup>, Maria Alessandra Martini<sup>1</sup>, Andrew W. Foster<sup>1,2</sup>, Junjun Chen<sup>3</sup>, Andrew J. P. Scott,<sup>1</sup> Richard J. Morton<sup>4</sup>, Jonathan W. Steed<sup>2</sup>, Elena Lurie-Luke<sup>1</sup>, Thomas G. Huggins<sup>3</sup>, Andrew D. Lawrence<sup>5</sup>, Evelyn Deery<sup>5</sup>, Martin J. Warren<sup>5</sup>, Peter T. Chivers<sup>1,2</sup>, Nigel J. Robinson<sup>1,2</sup><sup>1</sup>Department of Biosciences, Durham University, UK.<sup>2</sup>Department of Chemistry, Durham University, UK.<sup>3</sup>Procter and Gamble, Mason Business Center, Cincinnati, OH, USA.<sup>4</sup>Department of Mathematics, Physics and Electrical Engineering, Northumbria University, Newcastle-upon-Tyne, UK.<sup>5</sup>School of Biosciences, University of Kent, Canterbury, Kent, UK.

About a half of enzymes are metalloenzymes. Intracellular metal availabilities are important because some metals form more stable complexes with proteins than do others. Correct metalation can be achieved if cells 'level the playing field' by maintaining the tighter binding metals at lower availabilities than the weaker binding ones but it has been challenging to define and measure intracellular metal-availabilities. We previously established that DNA-binding, metal-sensing, transcriptional regulators are tuned to the available buffered levels of the metals that they detect [1]. This means that metal sensors provide a window through which to observe metal availabilities inside a cell, provided we can determine their tuning. We have determined the tuning of a complete set of metal sensors for different essential metals from *Salmonella* [2]. This data set can be used to explain the correct metalation of metalloproteins. The various metal affinities of the cobalt chelatase, CbiK, involved in vitamin B<sub>12</sub> biosynthesis have been determined and compared against the series of metal availabilities. Within the metal controlled environment of the *Salmonella* cytosol CbiK will selectively acquire cobalt in preference to other metals, thus explaining the correct metalation of sirohdrochlorin during vitamin B<sub>12</sub> biosynthesis. Buffering of metals is essential to prevent mis-metalation of sirohdrochlorin and under this regime a chelatase is required for cobalt acquisition by sirohdrochlorin.

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P133

**Copper Toxicity Damage to the Photosynthetic Apparatus of *Rhodospirillum Rubrum***Noelia Jaime-Pérez<sup>1</sup>, David Kaftan<sup>2,3</sup>, David Bina<sup>2,4</sup>, Syed Nadeem Hussain Bokhari<sup>1</sup>, Hendrik Küpper<sup>1,5</sup><sup>1</sup>Department of Plant Biophysics & Biochemistry, Biology Centre of the Czech Academy of Sciences, Branišovská 31/1160, 370 05 České Budějovice, Czech Republic.<sup>2</sup>Institute of Chemistry, University of South Bohemia, Branišovská 1760, 370 05 České Budějovice, Czech Republic.<sup>3</sup>Institute of Microbiology CAS, Centre Algatech, 37981 Třeboň, Czech Republic.<sup>4</sup>Department of Photosynthesis, Biology Centre of the Czech Academy of Sciences, Branišovská 31/1160, 370 05 České Budějovice, Czech Republic.<sup>5</sup>Department of Experimental Plant Biology, University of South Bohemia, Branišovská 31/1160, 370 05 České Budějovice, Czech Republic.

The mechanism of function of light harvesting complexes has been a subject of intense scientific research. Chlorophyll (Chl) in plants and bacteriochlorophyll (BChl) in photosynthetic bacteria are the major light harvesting pigments to carry out the desired energy converting photochemical reactions. Among all metallochlorophylls, the complexes with Mg<sup>2+</sup> have the highest tendency to bind axial ligands [1], which are essential for the correct binding of the pigment [2] and for the correct folding of the pigment-protein complexes [3]. For this and other reasons, heavy metals (such as Cu, Zn, Ni and Mn) can be toxic to photosynthetic organisms, as they can replace the central magnesium (Mg<sup>2+</sup>) present in Chl and BChl, being this replacement non-functional in photosynthesis [4].

In the present study, using the anoxygenic purple bacterium *Rhodospirillum rubrum* (*R. rubrum*), we investigated the influence of relatively low Cu<sup>2+</sup> concentrations (that can occur under current environmental conditions) on the photosynthetic apparatus of this organism. This was accomplished by a combination of *in vivo* measurements of flash photolysis and fast fluorescence kinetics, combined with the analysis of metal binding to pigments and pigment-protein complexes isolated from Cu<sup>2+</sup>-stressed cells by HPLC-ICPMS (ICP-sfMS).

Our results suggest that Cu<sup>2+</sup> is incorporated in the BChl and this shift of metal centres in BChl from Mg<sup>2+</sup> to Cu<sup>2+</sup> can occur *in vivo* in the RCs of *R. rubrum* under environmentally realistic Cu<sup>2+</sup> concentrations (already at 2 μM Cu<sup>2+</sup>), leading to a strong inhibition of the function of these photosynthetic RCs. The inhibition of growth and of RCs activity was related to the formation of Cu-containing BChl degradation products, that occurred much more in the RC than in LH1. This finding is important because only very few earlier studies dealt with copper toxicity in phototrophic bacteria and in those, either much higher copper concentrations were applied, which led to entirely different effects, or inhibition of photosynthesis was not investigated [5].

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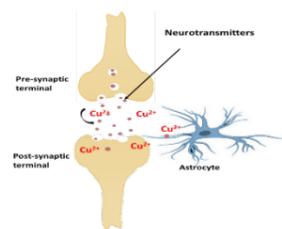
P134

### Characterisation of NKB-NK3R Receptor Mediated Intracellular Trafficking as a Novel Copper Uptake Mechanism

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Copper is an indispensable co-factor of several ubiquitous bio-chemical mechanisms in living organism. Among many proteins known to be involved in copper metabolism in cells, CTR1 predominantly accounts for the majority of metal uptake into cells. However, studies demonstrated that CTR1 inactivation does not completely inhibit copper uptake into cells hinting at the existence of alternate uptake pathways within cells. We have been exploring the ability of neuropeptides, particularly neurokinin B (NKB), to bind copper. (1) The effect of copper on neuropeptide structure can have significant impact of cellular function, and for some neuropeptides the complex can alter cellular signalling pathways. (2) The binding of copper by NKB can lead to increases in intracellular copper, yet little is known about the mechanisms and pathways by which neurokinin B can traffic copper. To begin to address this, we used fluorescein-labelled apo-NKB (apo-FNKB) and copper-bound FNKB to track the peptide in the cell, and immunofluorescence to follow trafficking of the NKB cognate receptor (NK3R) on ligand activation. We show that copper-bound FNKB is taken up into the cell in dense endosomal vesicles and localises to the perinuclear region more rapidly than apo-FNKB. Studies also demonstrated that from these dense sorting endosomes, the copper-NKB-NK3R complex relocates into either Rab 5 regulated early endosomes enabling recycling of both metal and peptide near to the plasma membrane. More vesicles undergo late endosomal fusion with LAMP 1 regulated lysosomes in the perinuclear region. We speculate that this lengthy transport mechanism facilitates the copper dissociation from the NKB to metal-binding motifs on NK3R which facilitates metal shuttling into nuclear compartments. We predict that receptor mediated endocytosis accounts for the increased cellular copper concentrations by copper-bound NKB and mimics an uptake mechanism only previously observed for iron. (3)



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P135

### Metal Release from *Xenopus Laevis* Zygotes Following Fertilization

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Zinc fluxes are important for mammalian oocyte meiosis. Total intracellular zinc significantly increases between prophase I and metaphase II. Following fertilization, zinc is released from the zygote's cortical granules in a process described as "zinc sparks". The released zinc hardens the zona pellucida, acting as a block to polyspermy. We sought to determine if the release of zinc at fertilization is conserved in *Xenopus laevis*, the African Clawed Frog. *X. laevis* is a model organism with many applications in developmental biology and is amenable to biochemical interrogations. Using confocal microscopy, we found that the zinc efflux is conserved in *Xenopus* at the time of fertilization. A single zinc spark travels around the circumference of the zygote, taking around 3 minutes to reach the point opposite its origin. Proportionally less zinc is released from the frog zygote than the mouse zygote: ~20% of total intracellular zinc is released from a mouse zygote, while >1% is released from the frog. In addition to zinc, we monitored the overall elemental transitions during the time before and after sperm addition. Elemental change was quantitated using inductively coupled plasma mass spectrometry (ICP-MS). Zinc could not be detected by this method, likely due to a sensitivity issue, but we did detect an unexpected drop of intracellular manganese by 70%. To determine if the zinc and other metals exocytosed at the time of fertilization were within cortical granules, we analyzed egg slices via X-ray fluorescence spectroscopy (XRF). Similar to mice, zinc is stored in cortical vesicles of frog eggs, albeit at a lower concentration (15 mM vs. 200 mM in mouse eggs). XRF images show that significantly more metal-containing vesicles are stored in the animal pole, the half of the egg containing the chromosomes and the site of sperm entry, than the vegetal pole, the half of the egg that serves as yolk storage. A higher-resolution analysis of these vesicles using scanning transmission electron microscopy energy dispersive X-ray spectroscopy confirms our XRF results and shows that the diameter of the vesicles is around 500 nm. These are larger than mouse cortical granules, which average 260 nm in diameter. Surprisingly, not only zinc but copper, manganese, cobalt, and nickel are also stored in these vesicles. A subset of vesicles contains a single metal, while most contain multiple metals stored at high micromolar to low millimolar concentrations. Some of these metals have biological roles in amphibians (zinc, copper, and manganese), while others have few or no known roles (cobalt and nickel). Each of these elements was quantitated in the frog tank water and were present at nanomolar levels. These results indicate that zinc is released at the time of fertilization and stored in cortical vesicles, similar to other zinc fluxes that take place around vertebrate fertilization. The frog differs from mouse and other mammals in the unexpected drop of intracellular manganese during this time interval and the presence of individual and mixed metals in cortical bodies. It is not clear why amphibians accumulate metals like cobalt and nickel but given their physical environment, the egg may have adapted mechanisms that partition toxic elements that are present in the environment (water) away from genetic material. Further studies on the full spectrum of metal management in meiotic transitions and the specific role of zinc in meiotic progression and transition to mitotic embryos are ongoing.

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P140

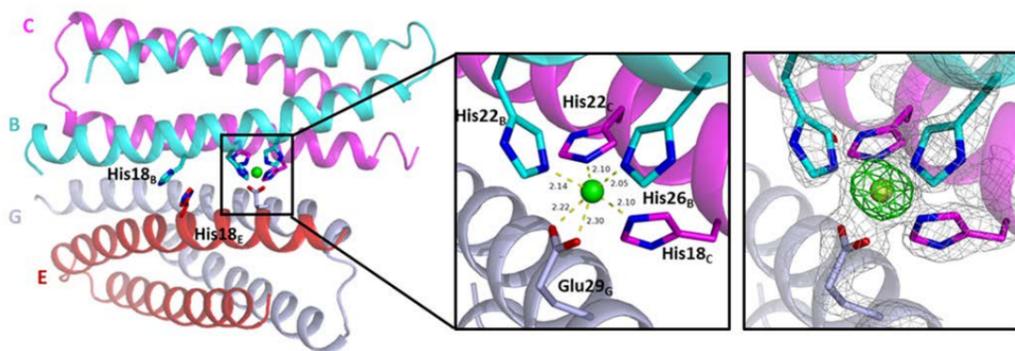
### Biochemical and Structural Characterization of the Carbon Monoxide Dehydrogenase Accessory Protein CooJ: An Unexpected High Affinity Nickel Binding Site

Alfano M<sup>1</sup>, Pérard J<sup>1</sup>, Carpentier P<sup>1</sup>, Basset C<sup>1</sup>, Zambelli B<sup>2</sup>, Timm J<sup>1</sup>, Crouzy S<sup>1</sup>, Ciurli S<sup>2</sup>, Cavazza C<sup>1</sup>

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<sup>2</sup>Laboratory of Bioinorganic Chemistry, University of Bologna, 40127 Bologna, Italy.

CODH reversibly oxidized CO into CO<sub>2</sub>, playing a central role in carbon metabolism of anaerobic microorganisms. The crystal structures of CODH from *C. hydrogenoformans* [1] and *R. rubrum* [2] revealed a unique active site: a [NiFe<sub>3</sub>S<sub>4</sub>] cubane coordinated to a mononuclear iron site, known as C-cluster. While the reaction mechanism of CODH is well established [3], very little is known about the activation of the C-cluster. Nickel insertion is essential for the enzyme activation and requires the intervention of the accessory proteins CooC, CooJ and CooT, able to supply the nickel into the CODH. Previously, CooJ from *R. rubrum* (RrCooJ) has been described as a Ni chaperone with 16 histidines and 2 cysteines at its C-terminus [4].



Here we present our recent biochemical and structural characterization of CooJ, reporting the X-ray structure for a truncated version, combined with small-angle X-ray scattering (SAXS) data and a modelling study of the full-length protein. Using isothermal calorimetry, we characterized several metal-binding sites (four per dimer), involving the His-rich motifs and having similar metal affinity (K<sub>D</sub> = 1.6 μM). Remarkably, biophysical approaches, site-directed mutagenesis and X-ray crystallography uncovered an additional Ni-binding site at the dimer interface. Although RrCooJ was initially thought to be a unique protein, a proteome database search identified at least 46 bacterial CooJ homologs. These homologs all possess two spatially separated nickel-binding motifs: a variable C-terminal histidine tail and a strictly conserved “H-(W/F)-X2-H-X3-H” motif, identified on our truncated version [5], suggesting a dual function for CooJ both as a Ni chaperone and as a Ni storage protein.

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P141

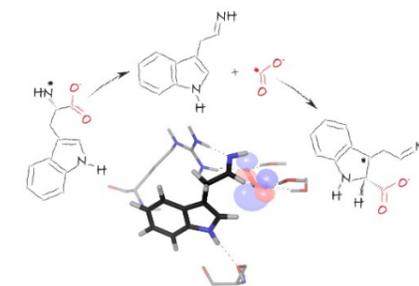
### Carboxyl Radical Migration in the Radical SAM Tryptophan Lyase (NosL) Mechanism

Patricia Amara<sup>1</sup>, Jean-Marie Mousesca<sup>2</sup>, Maxime Bella<sup>1</sup>, Lydie Martin<sup>1</sup>, Claire Saragaglia<sup>1</sup>, Serge Gambarelli<sup>2</sup>, Yvain Nicolet<sup>1</sup>

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The radical *S*-adenosyl-L-methionine tryptophan lyase [1] converts L-tryptophan into 3-methyl-2-indolic acid, a fragment in the biosynthesis of the thiopeptide antibiotic nosiheptide. This complex reaction involves i) the activation by a specific hydrogen-atom abstraction, ii) an unprecedented •CO<sub>2</sub><sup>-</sup> radical migration, iii) a cyanide fragment release [2] and iv) the termination of the radical-based reaction. *In vitro* study of this reaction is made more difficult because the enzyme produces a significant amount of a shunt product instead of the natural product. We combined X-ray crystallography, electron paramagnetic resonance spectroscopy and quantum and hybrid quantum mechanical / molecular mechanical calculations to investigate the •CO<sub>2</sub><sup>-</sup> radical migration step. QM/MM calculations with L-tryptophan and analogs reproduce the EPR species previously observed [3] and suggest a tight control by the protein matrix [4].



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P142

**Investigation of Hg(II) Binding of CueR Protein: A Possible Role of C-Terminal Cysteines in Selective Operation of the Protein**Ria K. Balogh<sup>1</sup>, Béla Gyurcsik<sup>1</sup>, Éva Hunyadi-Gulyás<sup>2</sup>, Lars Hemmingsen<sup>3</sup>, Peter W. Thulstrup<sup>3</sup>, Attila Jancsó<sup>1</sup><sup>1</sup>Department of Inorganic and Analytical Chemistry, University of Szeged, Dóm tér 7, 6720 Szeged, Hungary. baloghr@chem.u-szeged.hu<sup>2</sup>Laboratory of Proteomics, Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Temesvári krt. 62, 6726 Szeged, Hungary.<sup>3</sup>Department of Chemistry, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen, Denmark.

Selective recognition of toxic metal ions may attract wide interest due to its potential medical and environmental applications. Understanding the details of bacterial metal ion regulatory mechanisms may forward the design of selective metal ion binding molecules or develop sensitive metal ion detection techniques.

Our study focused on the metal ion selective transcription regulator CueR protein that controls the intracellular concentration of Cu(I) ions in *Escherichia coli* [1]. The regulation is based on a conformational change induced by the cognate metal ion coordinated into the metal binding loop influencing the structure of the protein-bound DNA and ultimately initiating the transcription of downstream genes [1]. The metal binding loop of the protein is followed by a short two turns helix continued in a disordered C-terminal region containing two cysteines in a CCHH motif. This so-called C-terminal helix has an allosteric role in the activation process. Upon metal ion binding of CueR the loop docks into a hydrophobic pocket resulting in a small “scissor-type” movement of the protein and the stabilization of the activator conformation [2].

Although CueR is one of the most thoroughly characterized protein in the MerR family, the mechanism of how it discriminates between mono and divalent metal ions is still not fully understood. Surprisingly, Hg(II) does not trigger the activation of transcription by CueR [1], despite the protein possesses a bis-thiolate coordination environment highly preferred by Hg(II). Furthermore, the role of the well-conserved cysteines of the C-terminal CCHH motif is still unclear.

In this work we aimed to investigate the molecular details of the metal ion recognition process and the role of the C-terminal CCHH motif focussing on the binding of the non-cognate Hg(II) ion to CueR. We performed studies both with the native CueR protein and its truncated variant, lacking seven C-terminal residues (including the CCHH fragment) with native mass spectrometry and <sup>199m</sup>Hg perturbed angular correlation of  $\gamma$ -rays (PAC) spectroscopy. The results presented will elaborate the effects of pH, metal ion to protein ratio on the coordination characteristics of the metal ion, and most importantly a possible role of metal ion binding amino acids outside the loop region in the protein function.

Support for the beam time by ISOLDE/CERN, financial support by EURONS, NICE and Hungarian National Research, Development and Innovation Office (GINOP-2.3.2-15-2016-00038 and K\_16/120130) are gratefully acknowledged.

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P144

**Investigating the Iron Sulfur Cluster (Isc) Assembly System Using Non-Denaturing Electrospray Ionisation Mass Spectrometry**Sophie Bennett<sup>1</sup>, Rita Puglisi<sup>2</sup>, Jason Crack<sup>1</sup>, Annalisa Pastore<sup>2</sup>, Nick Le Brun<sup>1</sup><sup>1</sup> Centre for Molecular and Structural Biochemistry, School of Chemistry, University of East Anglia, Norwich Research Park, Norwich, UK. Sophie.bennett@uea.ac.uk<sup>2</sup> The Wohl Institute, King’s College London, London, UK.

Iron sulfur (Fe-S) cluster cofactors are located in proteins involved in a diverse range of cellular processes including respiration, central metabolism and DNA repair. This versatile prosthetic group performs multiple functions including electron transport, transcriptional regulation, redox catalyst, and as a sulfur donor. An increasing number of diseases are associated with impairment of Fe-S associated proteins and their maturation, including neurodegeneration. Therefore, an understanding of this machinery is essential.

In bacteria, three systems responsible for the post-transcriptional Fe-S cluster generation and insertion into apo-protein *in vivo* are well known: the *nif* operon encodes the system responsible for maturation in nitrogenase, while proteins from the *suf* and *isc* operons generate housekeeping Fe-S clusters under stress and optimal conditions, respectively. The Isc assembly machinery, encoded by the *iscRSUA-hscBA-fdx* operon in *E. coli*, is the most widespread system and has direct orthologues in the eukaryotic mitochondrion. Studying the assembly of Fe-S clusters in this system will have direct implications in developing our understanding of human Fe-S cluster biogenesis. Among the Isc apparatus is IscS, a pyridoxal phosphate (PLP) containing desulfurase which converts L-cysteine to alanine, generating sulfide that, along with iron, is used to build a [2Fe-2S] cluster on the scaffold protein, IscU. IscS is a dimeric protein (~90 kDa), with each monomeric unit binding able to bind a single IscU protein [1]. Reconstitution of a [2Fe-2S] cluster on IscU using a source of cysteine, iron and reducing agents has been demonstrated [2,3]. Two further accessory proteins, IscX and CyaY (the bacterial orthologue to the eukaryotic protein frataxin) bind iron and also bind to the same surface on IscS, thereby acting as regulators of Fe-S cluster biogenesis in response to accessible iron [4]. The exact source of iron is unknown. Further proteins are involved in the transfer of the cluster to the receiving apo-protein, namely IscA, HscA and HscB.

Non-denaturing ESI-MS (electrospray ionisation mass spectrometry) has been used to investigate proteins in a folded state in which non-covalently bound cofactors, such as Fe-S clusters, remain intact. This technique has been used to demonstrate the mechanism of O<sub>2</sub> induced [4Fe-4S] cluster degradation of FNR [5] and has also been used to study the association state of IscS and IscX [4]. This work employs the same technique to analyse the individual steps in Fe-S cluster biogenesis, characterising the pivotal proteins IscS and IscU by ESI-MS as a complex and analysing mass shifts upon the introduction of iron and sulfur species. Subsequent introduction of further assembly proteins, to identify complexes, will offer further insight into the process of Fe-S cluster biosynthesis. This work aims to address major remaining questions, including the nature of intermediates of Fe-S cluster assembly on IscU, the complex associations of Isc proteins, and the mechanism of transfer to accepting apo-proteins.

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P145

**Activation of CO by Carbon Monoxide Dehydrogenase Using QM and QM/MM Methods**

**Dalia Biswas<sup>1</sup>, Tao Large<sup>1</sup>, Morgan Dienst<sup>1</sup>, Marius Retegan<sup>2</sup>, Frank Neese<sup>3</sup>**

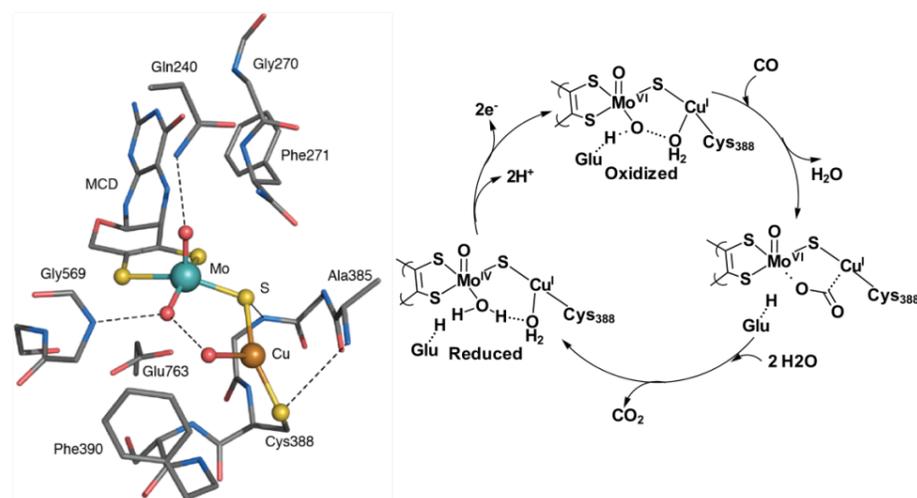
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Carbon monoxide dehydrogenase (CODH) containing a unique Mo-Cu active site from the aerobic bacterium *Oligotropha carboxidovarans* catalyzes the following reaction:  $\text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + 2\text{H}^+ + 2\text{e}^-$ . The catalytically active state is thought to be the Mo(VI)-Cu(I) state, and is reduced to Mo(IV)-Cu(I) state during catalysis. Despite the close to atomic resolution structure (1.1 Å) for both oxidation states, major uncertainties remain with regard to the active site composition and geometry: equatorial oxo vs. hydroxo ligands coordinated to Mo, Mo/Cu oxidation and spin states, substrate binding, and intermediates during the catalytic cycle [1]. To address these ambiguities, various QM and QM/MM models of CODH were developed systematically, and were used to examine various oxidation/spin states, as well as the protonation states of the Mo-coordinated equatorial ligand ( $\text{O}^{2-}$ ,  $\text{OH}^-$ ,  $\text{H}_2\text{O}$ ), and point mutations in key residues surrounding the CO binding pocket. Our QM model revealed important second sphere interactions that are essential for maintaining a unique geometry of the CO binding cavity [2]. Study of catalytic transformation of CO and  $\text{H}_2\text{O}$  are currently underway using our QM and QM/MM models. We will provide insights of the involvement of Mo and Cu, and other important active site residues during the catalytic conversion of CO and  $\text{H}_2\text{O}$ .

Financial support by Murdock Charitable Trust, National Science Foundation, and Whitman College is gratefully acknowledged.



Active site and the proposed catalytic cycle of CODH

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P146

**Creating a Toolbox for Tuneable Artificial Metalloenzymes**

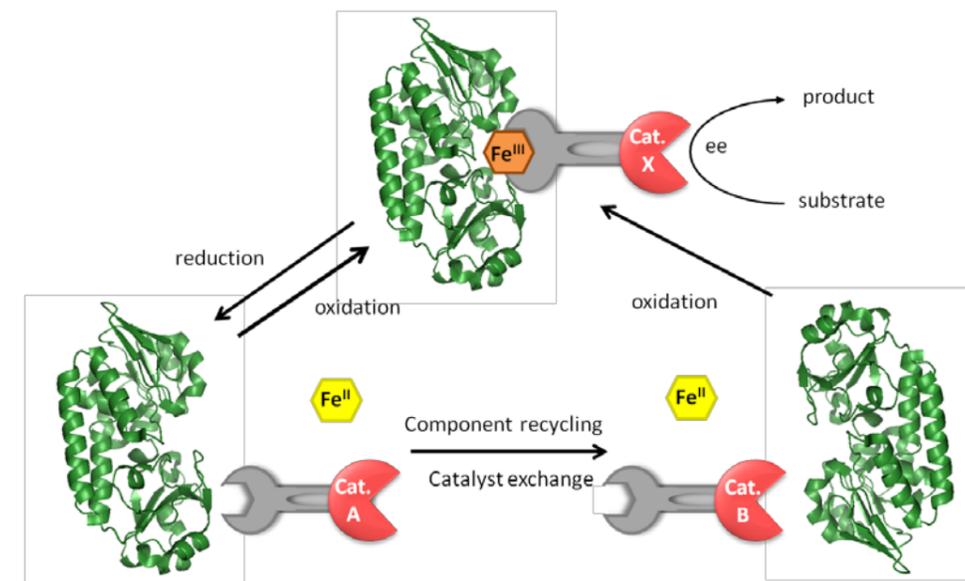
**Rosalind L. Booth<sup>1,2</sup>, Daniel J. Raines<sup>1</sup>, Justin E. Clarke<sup>1,2</sup>, Keith S. Wilson<sup>2</sup>, Gideon J. Grogan<sup>2</sup>, Anne-Kathrin Duhme-Klair<sup>1</sup>**

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Artificial metalloenzymes combine the high catalytic rate and diverse reaction scope of synthetic catalysts with the selectivity provided by a secondary coordination sphere from the protein component [1]. The design of this artificial enzyme is inspired by iron(III)-chelating siderophores used by bacteria to scavenge iron from their environment. In anchoring the synthetic catalyst to a siderophore, we can bury the catalyst inside a protein with a high-affinity for the Fe(III)-siderophore conjugate [2]. A variety of synthetic catalysts can be used depending on the transformation the enzyme is required to perform. The non-covalent nature of the binding between the synthetic catalyst and the protein component in this design allows for reversible binding of the synthetic catalyst. The natural mechanism bacteria use to release iron inside the cell is reduction of Fe(III) to Fe(II), triggering dissociation [3]. This release mechanism can be exploited to allow the recovery and recycling of the components of the artificial enzyme, including valuable protein. Designing a toolbox of synthetic catalysts with redox-reversible anchoring groups allows the construction of artificial metalloenzymes tuned for specific synthetic transformations.

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P148

**Principles of Metal Selectivity in Human Metallothioneins Metal–Thiolate Clusters**

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Metallothioneins (MTs) are small, cysteine-rich proteins that bind d<sup>10</sup> metal ions with high affinity, thereby playing major roles in metal storage and detoxification. Of the four human MT isoforms (MT-1/4), only MT-3, an isoform expressed in the brain, exhibits growth inhibitory activity and protects neurons from amyloid- $\beta$  toxicity. Despite the conservation of all 20 cysteines which coordinate Zn(II) or Cu(I) in metal thiolate clusters in all isoforms, MT-3 shows a copper-thionein character while MT-2 possesses higher zinc selectivity—features that underlie these isoforms' specific functions. In this work, we used a combination of spectroscopic and biochemical techniques to investigate the structural features in MT-3 fundamental to its unique Cu-thionein character. We reveal, by studying rates of Cu(II) reduction and Cu(I) binding, that Zn<sub>7</sub>MT-3 binds Cu(I) significantly faster than Zn<sub>7</sub>MT-2. Using competition assays with metal-selective chelators, we show that Cu(I)<sub>4</sub>Zn<sub>4</sub>MT-3 possesses a higher affinity for Cu(I) than MT-2 in its N-terminal  $\beta$ -domain, whereas Zn<sub>7</sub>MT-2 shows higher selectivity for Zn(II) than MT-3. From mutational studies, we showed that the Thr-Cys-Pro-Cys-Pro motif unique in MT-3 is critical for its metal selectivity bias for Cu(I). We also dissected key roles of other residues in modulating cluster dynamics and metal exchange rates, in increasing the Cu(I)-affinity in MT-3 and/or modulating the higher stability of the Zn(II)-thiolate cluster in MT-2  $\beta$ -domain. We thus engineered MT-3 variants in which the copper-thionein character is converted into zinc-thionein. Thus, we have identified isoform-specific non-coordinating residues in MT-3 that impart its copper selectivity and reactivity which are fundamental for its roles in copper homeostasis and controlling aberrant Cu-protein interactions.

P149

**Inner-Sphere and Outer-Sphere Reorganization Energies of Ht Cytochrome c: Role of Two States**

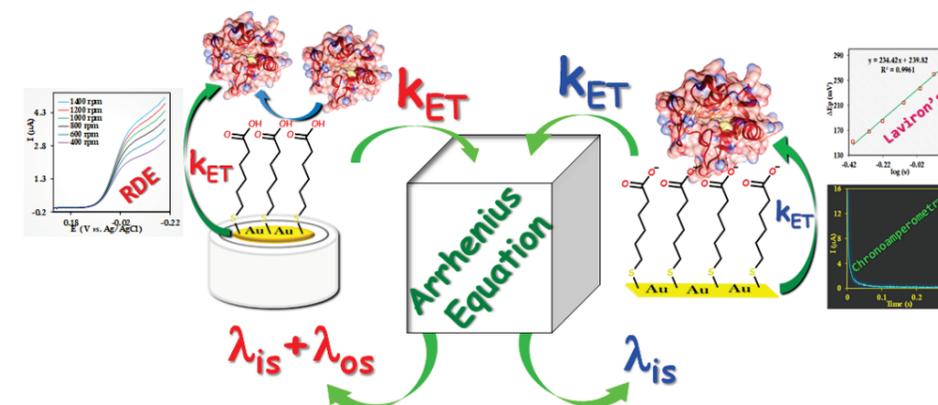
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Electron transfer (ET), the act of moving an electron from one place to another, is the simplest chemical process yet certainly crucial in most biological processes. It also plays key role in the reduction of molecular O<sub>2</sub> to H<sub>2</sub>O in mitochondria where cytochrome *c*, a mitochondrial membrane bound protein drives electrons from complex III (coenzyme Q – cytochrome *c* reductase) to complex IV (cytochrome *c* oxidase) during the process. The kinetics of ET depends on several factors like the driving force ( $\Delta G$ ) of the process, reorganization energies ( $\lambda$ ) of the involved species and the ET coupling element between donor and acceptor ( $H_{DA}$ ). Reorganization energy,  $\lambda$ , is summation of inner-sphere  $\lambda$  and outer-sphere  $\lambda$  where the former part can be calculated from the change in equilibrium bond distance upon ET and the energy associated with the change in the solvent sphere around the electroactive species during ET represents the later part.<sup>1</sup>

Among the cytochrome *c* family, Hydrogenobacter thermophilus cytochrome *c* (HtWt cyt *c*) is one of the most studied monoheme proteins due to its spontaneous cytoplasmic maturation when expressed in *E. coli*. The main feature of HtWt Cyt *c* is the fluxionality of its axial methionine group ligated to the heme iron which makes it unique for scientific studies.<sup>2</sup> Diversity in the orientation of the histidine-methionine ligand to the heme iron is believed to play a major role in the ET mechanism of cytochrome *c* family and therefore recent researches focus on the effect of such fluxional orientations of methionine on their ET. Single point mutation on the wild type (WT) protein shows that the 64th amino acid residue, glutamine is responsible for such fluxional orientation of the axial methionine group. Mutation of the 64th residue with valine (HtQ64V) and asparagine (HtQ64N) restricts the orientation of that methionine group and provides two different conformations, R and S in the HtQ64V and HtQ64N, respectively. In spite of a decade of dedicated research, neither  $\lambda$  value of these mutants nor the effect of methionine conformation upon  $\lambda$  is reported. We have optimized rotating disc electrochemistry (RDE), a dynamic electrochemical technique to measure the total  $\lambda$  of HtWt and two of its mutants in solution. We have also determined the inner-sphere  $\lambda$  of these proteins using chronoamperometry and cyclic voltammetry by immobilizing the proteins on top of Au electrodes modified with self-assembled monolayer of thiol carboxylic acid. Our studies suggest that HtQ64V has the smallest  $\lambda$  value whereas the HtWt has the highest value of  $\lambda$  which is directly related to the conformations of the axial methionine in the proteins. The theoretical calculations also shed light into the factors responsible for the differences in the value of  $\lambda$  upon single point mutation.



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P150

### Structural Insights into the Electron/Proton Transfer Pathways in the Quinol:Fumarate Reductase Complex from *Desulfovibrio Gigas*

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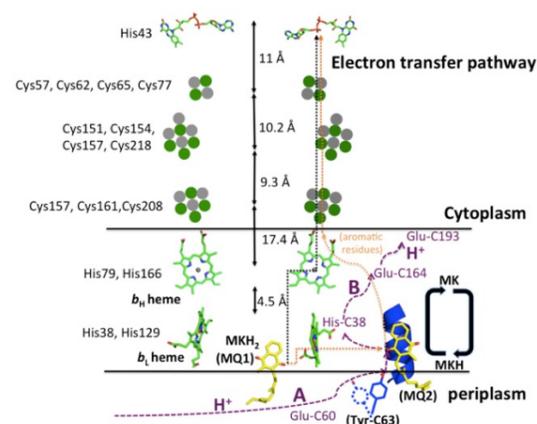
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The membrane-embedded quinol:fumarate reductase (QFR) in anaerobic bacteria catalyzes the reduction of fumarate to succinate by quinol in the anaerobic respiratory chain. A consensus on the mechanism of electron/proton transfers remains elusive because of the lack of essential knowledge on the complete redox cofactors and substrates distributed in the QFR structure. To clarify the mechanism of QFR, we have determined the crystal structure of QFR purified directly from large amounts of the cell membranes of the bacterium *Desulfovibrio gigas* under anaerobic conditions. Here we present the crystal structure of QFR from the anaerobic sulphate-reducing bacterium *Desulfovibrio gigas* (*D. gigas*) at 3.6 Å resolution. The structure of the *D. gigas* QFR is a homo-dimer, each protomer comprising two hydrophilic subunits, A and B, and one transmembrane subunit C. In our structure, a bound menaquinone and all the redox cofactors including two *b*-hemes are clearly revealed. One menaquinone molecule is bound near heme *b*<sub>L</sub> in the hydrophobic subunit C [1]. This location of the menaquinone-binding site differs from the menaquinol-binding cavity proposed previously for QFR from *Wolinella succinogenes*. The observed bound menaquinone might serve as an additional redox cofactor to mediate the proton-coupled electron transport across the membrane. On the basis of these new structural insights and comparison with the structures of QFRs from other organisms including both prokaryotes and eukaryotes, it is possible to clarify the roles of the two *b* hemes in the electron-bifurcation pathway in the menaquinol oxidation, and we have delineated the mechanism of proton-coupled pathways from the periplasm across the membrane during the transfers of the reducing equivalents from the quinol to fumarate in *D. gigas* QFR [1].

Financial supports by the NSRRC and MOST are gratefully acknowledged.



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P151

### Structural and Functional Characterization of *Wolinella Succinogenes* Flavocytochrome *c* Complex

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The *Wolinella succinogenes* flavocytochrome *c* FccA subunit is a 56 kDa periplasmic flavoprotein that displays amino acid sequence homologies to flavocytochrome *c* fumarate reductases and to the catalytic subunit of the membrane-bound succinate:quinone oxidoreductase (respiratory complex II)[1]. Interestingly however, its active site cofactor, a flavin adenine dinucleotide does not reduce fumarate but methacrylate [2]. Methacrylate, a common monomer in polymer plastics and resins, is strictly a man-made molecule and is a significant environmental contaminant. FccA is the second described protein to reduce methacrylate [3]. FccA is co-transcribed with FccB (a 14 kDa protein with homologies to the tetraheme domain of fumarate reductase) and FccC (a 24 kDa membrane anchored tetraheme *c*-type cytochrome with homologies to the NirT/NapC family) [4]. Therefore, it is likely that FccBC functions as a mediator for the electron transfer from the membrane quinone pool to the terminal reductase, FccA for methacrylate reduction resulting in a periplasmic oxidoreductase complex.

We have cloned FccA, FccB, FccC and FccABC, and heterologously expressed the proteins in *E.coli*. The periplasmic protein FccA and the membrane anchored FccBC complex has been successfully purified. We have determined the three-dimensional structure of FccA to 1.85 Å and investigated the kinetics of methacrylate reduction by FccA. Purification of the remaining proteins for X-ray structural evaluation is currently being pursued. Our goal is to characterize the complex in atomic detail to understand the molecular determinants that modulate the functionality of the protein and the exact role of the FAD cofactor during catalysis.

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P152

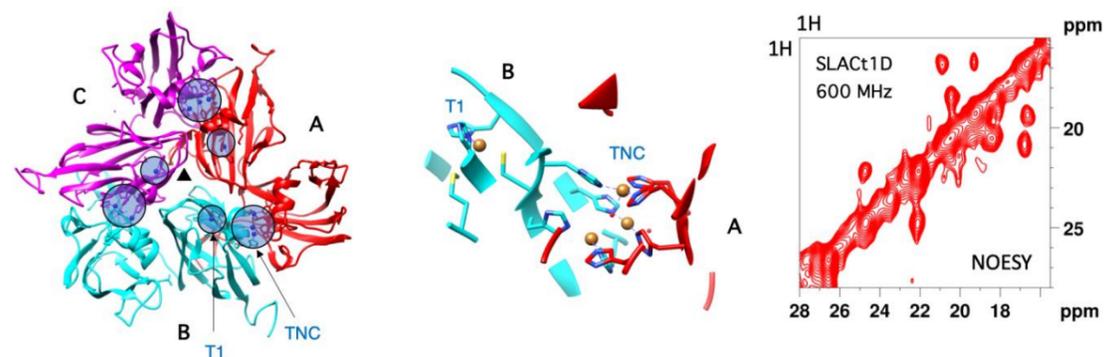
### First 2D NMR Correlation Spectra of Paramagnetically Shifted Resonances from Small Laccase Type 1 Depleted Mutant (SLAct1D)

Rubin Dasgupta<sup>1</sup>, Karthick B.S.S. Gupta<sup>1</sup>, Huub J.M. de Groot<sup>1</sup>, Marcellus Ubbink<sup>1</sup>

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Laccases, due to their high reduction potential of the T1 site and efficient 4e<sup>-</sup> & 4H<sup>+</sup> oxygen reduction process at the tri-nuclear copper centre (TNC), are known for wide variety of industrial applications. It is known that the protein matrix plays important role in directing the electrons and protons to their respective positions with very little or no overpotential [1,2]. This project is aimed at identifying specific motions at the TNC, that result in decreasing the overpotential for oxygen reduction reaction. Small laccase with the copper in the T1 centre removed (mutant T1D), expressed and isolated from *E.coli*, was used as the preferred system to study the dynamics at the TNC. Using 1D WEFT NMR experiment and samples prepared in 90% H<sub>2</sub>O/10% D<sub>2</sub>O buffer, buffer exchanged to 100% D<sub>2</sub>O and perdeuterated with complete proton exchange, exchangeable and non-exchangeable protons were identified. We report that most of the paramagnetically shifted resonances are from exchangeable protons, which suggests that the efficiency of proton transfer arises due to this property. We also report the first high resolution <sup>1</sup>H-<sup>1</sup>H NOESY NMR spectra of paramagnetically shifted proton resonances using mixing time of 3 ms to 8 ms and the first solid state NMR proton spectra of the paramagnetically shifted resonances, that will lead to the assignment of the resonance and extracting associated dynamical property. The high resolution and sensitivity of the resonances can be attributed to the strong Cu-Cu coupling at the TNC as reported previously from temperature dependence of the 1D NMR spectrum, a result in line with EPR results [3,4]. We hope that such NMR study will shed some light on the effects of dynamics of the protein matrix in modulating the oxygen reduction mechanism at the TNC of SLAct1D.

Financial support by the Netherlands' Magnetic Resonance Research School (NWO-BOO 022.005.029)



is gratefully acknowledged

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P153

### Mimicking “Group C” Nitrogenase Cofactor Environment by Mutagenesis in *A. Vinelandii*

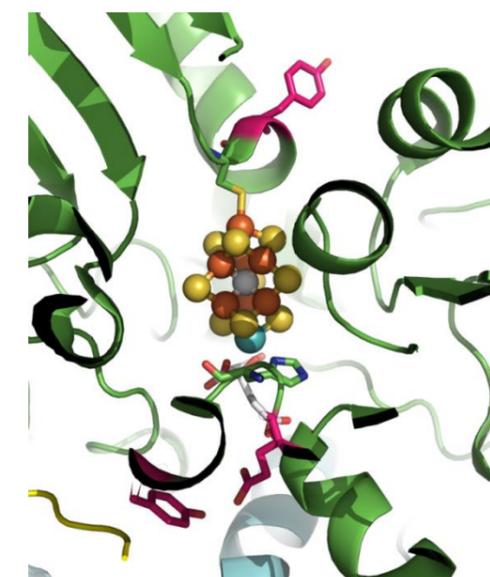
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Biological nitrogen fixation relies on nitrogenases, the only enzymes capable of reducing dinitrogen (N<sub>2</sub>) into ammonium (NH<sub>4</sub><sup>+</sup>). Nitrogenases can be divided in four groups according to their protein sequences: groups A, B and C are molybdenum nitrogenases, and group V represents the “alternative” vanadium- and iron nitrogenases [1]. Molybdenum nitrogenases contain a [7Fe:9S:C:Mo]:homocitrate cofactor termed FeMo-co, where the reaction takes place. This cofactor is coordinated by a cysteine residue at position 275, and a histidine at position 442 [2]. Among molybdenum nitrogenases, although group A (e.g. of *Clostridium pasteurianum*) and group B (e.g. of *Azotobacter vinelandii*) nitrogenases have been thoroughly characterized, group C nitrogenases remain understudied. These nitrogenases are mostly found in Archaea and thermophilic Firmicutes. In contrast to other nitrogenases, group C nitrogenases contain a glutamine at position 276 and an asparagine at position 440, and lack a residue corresponding to the aromatic residue found at position 444. All three positions are occupied by conserved residues in each group of nitrogenases.

To investigate the influence of these conserved residues on nitrogenase structure, maturation and catalysis, we generated strains of *A. vinelandii* bearing mutations at these positions, and isolated the resulting nitrogenase variants.

Growth experiments and activity assays (acetylene reduction) on whole cells revealed that mutated *A. vinelandii* grew slower and were less able to reduce acetylene than wild-type cells. Isolated variants also showed lower acetylene reduction activity. Finally, EPR spectroscopy on whole-cells and on the isolated enzymes showed new signals in the *g* = 3/2 region, suggesting that these nitrogenase variants contain isoforms of FeMo-co.



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P154

### Single Mutation Distal from the Active Site Optimized Zn(II) Affinity in the NDM Carbapenemase

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The New Delhi metallo-β-lactamase (NDM) is a periplasmic Zn(II)-dependent enzyme capable to hydrolyze carbapenems, the last resort antibiotic against multiresistant bacteria. During infection the host immune response withholds nutrient metal ions from microbial pathogens by releasing metal-chelating proteins such as calprotectin. This impacts directly on periplasmic Zn(II) levels. In metal limitation conditions, metallo-β-lactamases (MBL), and in particular NDM, lose activity against β-lactams by dissociation of the Zn(II) cofactor, and the periplasmic accumulation *in vivo* of the enzymes decreases.

Since its discovery in 2008, NDM has shown a fast worldwide spread with 27 natural variants reported in the clinic. NDM variants differ by a few mutations outside the active site, with the substitution M154L being the most frequent. Studies in our group have shown that the tolerance to Zn(II) starvation is selected during the evolution of NDM. It has been reported that the mutation M154L increases the resistance under zinc deprivation conditions *in vivo* without imparting protein stabilization. Moreover, all double mutants containing M154L substitution have shown the highest resistance under Zn(II) deprivation conditions. Between them, the variant NDM-15 with mutations M154L and A233V was the least susceptible to metal depletion.

In this way, we focus on the substitution M154L present on the natural variant NDM-4 as a single mutation and more than the half of the alleles. We aim to assess the biochemical and biophysical features that are tailored by the mutation to endure the action of the immune system response. First, we measured the stability of the purified variants and their apo (non-metallated) derivatives by thermal shift analysis. Alleles NDM-4 and NDM-15, presented slightly higher *T<sub>m</sub>* values than NDM-1. Nevertheless, they all displayed the same *T<sub>m</sub>* gap between holo (metallated) and apo forms. These results indicate that the mutation M154L does not increase significantly protein stability. We performed competition experiments with Zn(II) chromophoric chelators. We proved that substitution M154L impacts significantly on the first binding event, without changing the affinity of the second Zn(II) ion. By UV-Vis spectroscopy and paramagnetic NMR studies with Co(II) substituted variants we demonstrated that the increment in Zn(II) affinity is not due to changes on metal coordination in the active site.

<sup>15</sup>N-<sup>1</sup>H HSQC spectra of holo NDM-4 and NDM-1 were almost superimposable, indicating that the mutation does not affect significantly the backbone structure in solution as expected from the reported X-ray crystal structures. However, the HSQC spectrum of apo NDM-4 showed a lower amount of signals due to line broadening compared to apo NDM-1, reflecting more structural flexibility of apo NDM-4.

Overall, these results suggest that mutation M154L might be altering the dynamic or accessibility of residues near the active site in the apo-form consequently affecting metal affinity of the protein, mainly changing the first Zn(II) binding event.

Acknowledgements: ANPCyT, NIH and CONICET.

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P155

### Heme-Based Sensor DevS from *Mycobacterium Tuberculosis*: A Redox versus Oxygen Sensor

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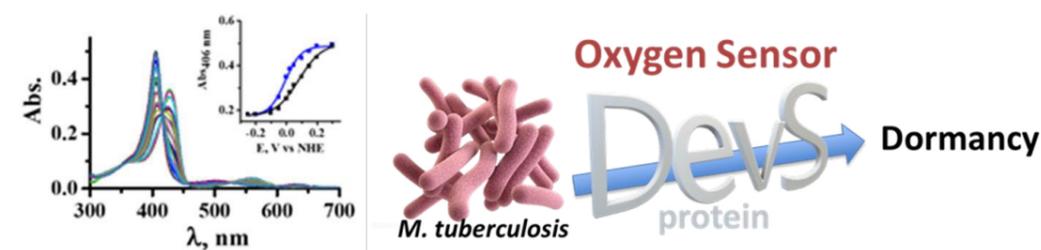
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Heme-based sensors are a large family of proteins involved in the regulation of several relevant biological processes such as biofilm, chemotaxis, blood pressure, circadian events, symbiosis, virulence, among others [1]. DevS is a heme-based sensor protein found in the *Mycobacterium tuberculosis* (Mtb), the pathogen causing tuberculosis, which is a major cause of death around the world. This sensor is part of a key system involved in regulating the entrance into dormancy which is, *per se*, a crucial strategy to make the success of this bacterium as a human pathogen. In 2007, right after this protein was shown to be a heme-based regulated sensor [2,3], a conflict on the actual physiological signal emerged if it was either a redox or an oxygen sensor. Aiming to shed light on this issue and further study the electrochemistry behavior of this protein, we carried out a series of spectroelectrochemistry measurements of the full-length holo DevS in anaerobic conditions as well as bound to CO, NO, imidazole (Imz), CN<sup>-</sup> and O<sub>2</sub>. Figure shows the electronic spectra of the non-liganded form of DevS at different applied potentials along with the curves of Soret band vs potential in both the reductive (blue) and oxidative (black) directions. Interestingly, the midpoint redox potential (*E<sub>m</sub>*) as determined by spectroelectrochemistry correlates well with the HOMO energies calculated for the GAF domain of the non-liganded DevS and liganded to CO, Imz and CN<sup>-</sup> (linear least squares fitting,  $E_{\text{HOMO}} = -5.91 - [8.8 \times 10^{-4} \text{ mV} \times E_{\text{m}}]$ ). This result enabled us to estimate the value of *E<sub>m</sub>* for DevS-O<sub>2</sub> as +170 mV, which is fully consistent with the experimental data, i.e. an *E<sub>m</sub>* value within the range +10 to +195 mV.

Another interesting feature of this protein is the ability to bind imidazole even in the ferrous state, with measured dissociation constants of 112 and 0.300 mM for the ferrous and ferric states, respectively. Nonetheless, an *E<sub>m</sub>* value of +10 mV (vs NHE) for DevS as measured under anaerobic conditions is much higher than the expected cytosolic potential for Mtb or even within stimulated macrophages (*ca.* -270 mV vs NHE). This result, along with its great oxygen affinity and very slow auto-oxidation rate, discarded DevS as a redox sensor and further validated its biological function as an oxygen sensor directly involved in the dormancy/latency of Mtb (see below the cartoon representation of the DevS function).



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P157

**Redox-Mediated on/off Switch of Zinc Finger Binding to DNA****Moritz Durtschi<sup>1</sup>, Silke Johannsen<sup>1</sup>, Roland K. O. Sigel<sup>1</sup>**<sup>1</sup>*Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland.*

Zinc finger (ZnF) domains are one of the most physiologically relevant examples of metalloproteins, as they are present in about 3% of all genes in the human genome. They are part of transcription factors that regulate diverse processes such as transcription, translation, or apoptosis. Beyond these roles, ZnF proteins can also act as redox switches, sensing the cell redox status and triggering appropriate responses. This is possible because Zn(II)-coordinating cysteine residues are readily oxidized, leading to dissociation from the stabilizing Zn(II) ion along with loss of structure and loss of DNA-binding activity.

In order to efficiently act as regulatory transcription factors under normal physiological conditions, ZnFs need to associate but also to dissociate readily from DNA. However, no dissociation mechanism is currently known. Dissociation under simultaneous Zn(II) release seems unlikely, since the level of free Zn(II) in the cell is virtually zero and no Zn(II) chaperones are known, and since one-time use of ZnFs would be highly inefficient. We therefore hypothesize that the dissociation of ZnFs from DNA involves oxidation-induced formation of an alternative structure in which Zn(II) remains bound. Thus, subsequent reduction, of the ZnFs would easily restore its DNA-binding activity.

Our central aim is to prove the existence of such an oxidized Zn(II)-binding structure under physiological conditions and to characterize it by NMR and X-ray crystallography. The structural role of alternative Zn(II) ligands is of particular interest, and can be directly addressed if the structure is characterized. Similar oxidized Zn(II)-binding structures have been shown to exist e.g. in the nucleocapsid protein of HIV-1<sup>1</sup>, and in *in vitro* oxidized ZnF models<sup>2</sup>, although the latter involves the formation of sulfinate moieties, which might not be formed *in vivo*. Overall, this study is of fundamental interest in obtaining a structural and mechanistic understanding of the dissociation process of zinc fingers in general, and of their function as redox switches in particular.

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P158

**Determination of Local and Global Dynamics in Proteins on the Nanosecond Timescale Using PAC Spectroscopy****Rasmus Fromsejer<sup>1</sup>, Vincent L. Pecoraro<sup>2</sup>, Lars Hemmingsen<sup>1</sup>**<sup>1</sup>*Department of Chemistry, University of Copenhagen, Universitetsparken 5, 2100 K benhavn  , Denmark. wbv564@alumni.ku.dk*<sup>2</sup>*Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109-1055, United States.*

Dynamics at metal sites is essential to protein function, but rarely characterized experimentally. Many biologically relevant metal ions exhibit nanosecond water exchange dynamics in aqueous solution [1], implying that this is a central time scale also at biomolecular metal binding sites. In addition, rotational diffusion of macromolecules occurs on the ns time scale. Perturbed angular correlation (PAC) of  $\gamma$ -rays spectroscopy allows for determination of nanosecond metal site dynamics [2,3] as well as rotational correlation times of the entire protein. We compare literature data on rotational correlation times,  $\tau_c$ , determined by PAC spectroscopy with a simple theoretical model for rotational diffusion [4]. The simple model displays good qualitative agreement with the experimental data. Interestingly, deviation of the experimental data from the simple model may indicate either pronounced local metal site dynamics or deviation from spherical shape of the protein. Finally, we present a series of PAC experiments on a model protein [5] in solution, to determine  $\tau_c$ 's correlation with the viscosity. Extrapolating to infinite viscosity, this may provide a tool to elucidate whether local metal site dynamics on the ns timescale contribute to the experimentally determined  $\tau_c$ .

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P159

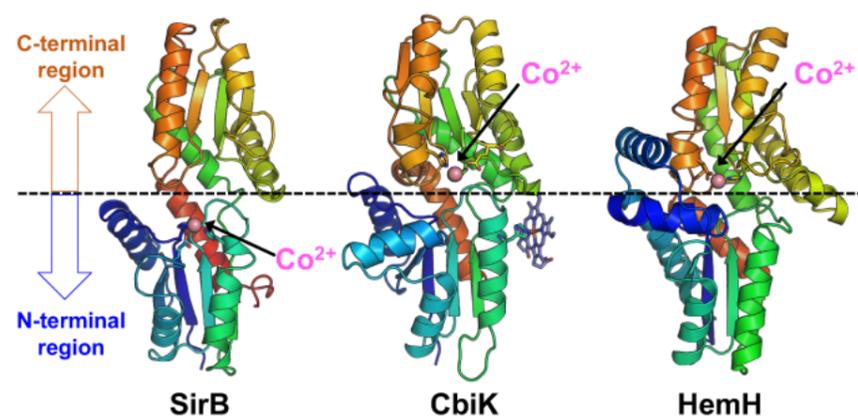
### Structure-Function Relationship of Sirohydrochlorin Ferrochelatase SirB

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SirB is one of the class II chelatase enzymes, that catalyze insertion of divalent metal ions to macrocyclic tetrapyrroles. SirB is involved in biosynthesis of siroheme using Fe<sup>2+</sup> and sirohydrochlorin as substrates in the cells.[1] It is also known that SirB is able to use other types of divalent metals (e.g. Co<sup>2+</sup>) as substrates with sirohydrochlorin for synthesis the corresponding metallated sirohydrochlorins *in vitro*.[2] However, the structure of SirB and its catalytic mechanism has been unclear so far, although those of other types of class II chelatases (e.g. CbiK[3] and HemH[4]) are well-studied. Here, we report the first X-ray crystal structure of Co<sup>2+</sup>-bound SirB.[5] The overall structure of SirB is similar to those of the other class II chelatases such as CbiK and HemH despite their low primary amino acid sequence identities (15 % or less). More interestingly, the binding site for Co<sup>2+</sup> in SirB is distinct from those in CbiK and HemH: SirB uses its N-terminal region with His10, Glu43 and His76 as ligands to Co<sup>2+</sup>, whereas CbiK and HemH use their C-terminal regions with His and/or Glu as ligands. Structure-function relationship of SirB was further investigated in a model reaction for the siroheme biosynthesis by using Co<sup>2+</sup> and uroporphyrin I and protoporphyrin IX, which are commercially available macrocyclic tetrapyrroles. As a result, uroporphyrin I, that is more structurally similar to sirohydrochlorin, was consumed as a substrate by SirB, but protoporphyrin IX was not. Docking simulations of these two tetrapyrroles as well as sirohydrochlorin to SirB revealed that the substrate specificity of SirB was likely to be subject to the hydrophobic/hydrophilic interactions between the tetrapyrroles and some hydrophobic residues inside the SirB active site cavity.

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P160

### Principles of Transition Metal Selectivity in a Primary Active Transmembrane Zinc Pump from *P. Aeruginosa*

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Transition metal selectivity in P<sub>1B</sub>-type ATPase primary active transporters is determined by conserved amino acid motifs on transmembrane helices embedded in the lipid bilayer that are responsible for metal selection and transport across membranes. We report on the coordination chemistry of the transmembrane metal binding site in a Zinc P-Type ATPase from *Pseudomonas aeruginosa* (ZntA) to reveal the coordination principles which govern the defined, yet promiscuous, metal selectivity of the pump. In this study, we generated wild-type and truncated ZntA in which the N-terminal metal binding domain (N-MBD) has been deleted and characterized them in detergent micelles and reconstituted in artificial lipid bilayers. Truncation of the N-MBD preserves the metal selectivity and functionality of the Zn-pump in agreement to the proposed catalytic regulatory role of the N-MBD. We showed that the pump possesses promiscuous substrate selectivity toward both essential (Zn<sup>2+</sup>) and toxic metals (Cd<sup>2+</sup>, Hg<sup>2+</sup> and Pb<sup>2+</sup>). We generated Zn<sup>2+</sup>/Cd<sup>2+</sup>/Pb<sup>2+</sup>/Hg<sup>2+</sup>-bound forms of N-MBD-truncated ZntA and performed biochemical and X-ray absorption spectroscopic studies to reveal the coordination chemistry underlying its substrate promiscuity. The results reveal the existence of an unprecedented highly-plastic transmembrane high-affinity metal-binding site that confers metal selection by different coordination geometries and ligand-metal distances but relying on common coordination chemistry properties.

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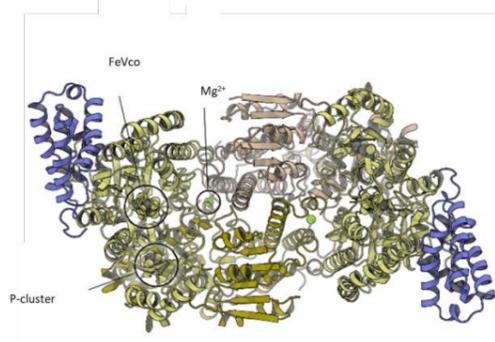
P161

### Structure and Unique Properties of the Vanadium Nitrogenase

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Nitrogenases are the only enzymes capable of breaking the triple bond of dinitrogen at ambient temperatures and pressures in an ATP-dependent reduction of dinitrogen to ammonia [1]. The Mo-nitrogenase consists of two components, the MoFe- and the Fe proteins. The catalytic MoFe component NifD<sub>2</sub>K<sub>2</sub>, harbors the unique FeMo-cofactor ([Mo-Fe<sub>7</sub>-S<sub>9</sub>-C]) as its active site. Although the structure of this most active and best studied Mo variant has been solved [2], the mechanism of nitrogen fixation remains unclear. Two alternative enzymes, the vanadium [3] and iron-only [4] variants have been characterized. The vanadium variant of nitrogenase is also able to reduce CO to hydrocarbons, comparable to the Fischer-Tropsch process [5]. Our recently described method allows for the production and purification of vanadium-nitrogenase from wildtype *Azotobacter vinelandii* using Mo-depleted bacterial cultures [6]. This led to the structure determination of the catalytic component VnfD<sub>2</sub>K<sub>2</sub>G<sub>2</sub> of the vanadium nitrogenase [7]. The 240 kDa protein contains an additional  $\delta$ -subunit VnfG that is absent in the Mo-variant. Interestingly, Fe V cofactor (FeVco) also replaces one sulfide with a bridging ligand, making it a [V-Fe<sub>7</sub>-S<sub>8</sub>-C] cluster. The different chemical character of the vanadium nitrogenase can help rationalize the altered chemical properties of this unique N<sub>2</sub> and CO reducing enzyme.



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P162

### Applications of Ni(II)-Induced Peptide Bond Cleavage

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It is known that Ni(II)-ions can induce peptide bond hydrolysis that occurs at the N-terminal side of Ser or Thr residues in peptides or proteins bearing X-(Ser/Thr)-X-His-X sequences (X being any amino acid except of Pro before or after Ser/Thr) [1-3]. Our current research focuses on the possible applications of this hydrolytic reaction.

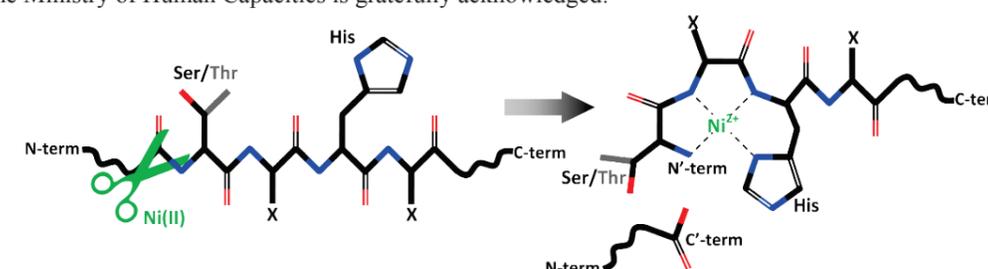
(i) Ni(II)-induced peptide bond hydrolysis was applied to establish an ATCUN motif in situ at the N-terminal end of a zinc finger protein. The ATCUN motif is able to cleave DNA non-specifically [4]. We examined whether it can be turned into a specific artificial metallonuclease, in which the zinc finger domain recognizes the selected DNA sequence, while the ATCUN motif cleaves it.

(ii) We optimized a protein purification method including the Ni(II)-induced hydrolysis reaction [5]. For simple protein purification a C-terminal affinity-tag is attached to the protein of interest. A new carrier DNA vector, allowing for complete removal of the tag after the affinity purification step was constructed. The gene coding for the native protein sequence was inserted directly before the DNA code of the SRH hydrolytically sensitive sequence followed by the code of the affinity tag. Thus, Ni(II) ions can be used to cleave the affinity tag instead of expensive and sensitive proteases. Our method is optimised for the expression and purification of proteins with precise native sequences.

(iii) We found that the Ni(II)-induced cleavage reaction can also be suitable for activation of allosterically inhibited enzymes. The Ni(II)-sensitive overhang was fused to otherwise catalytically active NCoIE7-type nucleases, and the DNA cleavage was monitored.

The results obtained by UV and CD spectroscopy, ICP and ESI mass spectrometry, isothermal titration calorimetry, recombinant DNA technology, agarose and SDS-PAGE electrophoresis will be presented.

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P163

**New Thiosemicarbazone Derivatives and their Copper(II) Complexes as Potential Inhibitors against Mammalian Ribonucleotide Reductase**

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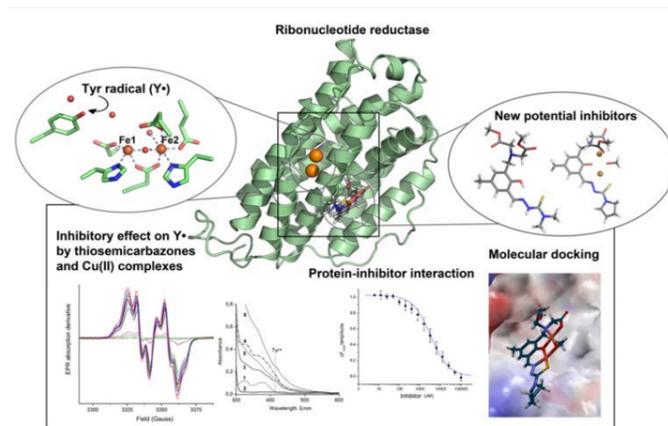
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Ribonucleotide reductases (RNRs) catalyze the conversion of ribonucleotides to their corresponding deoxyribonucleotides, providing building blocks for DNA replication and repair. The R2 subunit of the mammalian RNR houses a  $\mu$ -oxo-bridged diferric tyrosyl radical (Fe(III)<sub>2</sub>-Y•) cofactor, essential for initiating the reaction mechanism in the catalytic R1 subunit. As RNR plays a crucial role in nucleic acid metabolism, it is subject for several clinical drugs, and the understanding of, and search for new RNR inhibitors is important for further optimization and development of new anticancer agents. Thiosemicarbazones are known for their versatile coordination chemistry, biological activity and theranostic applications. Triapine, e.g., an efficient R2-inhibitor, has entered several clinical trials. Thiosemicarbazones exert their biological effects through effectively binding transition metal ions, in particular, Fe(III). Dicopper(II) complexes have also shown great cytotoxicity in several cancer cell lines. In our studies, proligands able to form transition metal complexes, as well as dicopper(II) complexes were synthesized, and their antiproliferative effects were examined in various human cancer cell lines, where several of the compounds showed high cytotoxicity. Based on this, potential candidates were tested *in vitro* as potential R2 inhibitors. Their interaction with R2 and effect on the Fe(III)<sub>2</sub>-Y• cofactor was characterized with microscale thermophoresis and various spectroscopic techniques such as EPR, rRaman and UV/vis spectroscopy. Our findings suggest that the newly-synthesized proligands and Cu(II) complexes could serve as effective antiproliferative agents in several cancer cell lines, targeting RNR, deserving further investigation as potential anticancer drugs [1]. Through ongoing studies, we also aim for a deeper explanation of the observed spectra by including DFT calculations for prediction of UV/vis and Raman spectra. In addition, ongoing and future studies on newly synthesized, potential R2 inhibitors are initiated.



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P164

**Optimization of Crystallization Conditions of Metalloenzymes for Serial Crystallography and XFEL**

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Developments in the recent years have made serial crystallography approaches more readily available to more users, both at synchrotrons and x-ray free electron lasers (XFEL). This allows design of novel experiments and new questions can be asked in the field of structural biology. By collecting serial crystallography datasets at synchrotron or XFEL it is possible to design time resolved experiments with induction of a reaction e.g. via a pump laser or oxygen-exposure. Room temperature experiments are possible and allow mixing of the mother liquor with substrate, so that different time points can be recorded. Especially XFELs with their high brilliance allow the usage of crystals as small as a few micrometres in size and make atomic resolution accessible to biological systems that grow no sufficiently large crystals. For systems that are prone to radiation damage in steady state synchrotron data collection, like metalloenzymes, serial crystallography is a great opportunity, since synchrotron or XFEL serial crystallography can reduce or even eliminate the problem of radiation damage altogether.

But serial crystallography also comes with its own set of challenges. In most cases large amounts of microcrystals are needed. They cannot be too fragile and have to survive at room temperature for a longer period of time. The crystallization condition, crystal size and concentration need to be considered for the specific experimental setup. The optimization of sample for a serial crystallography experiment differs therefore drastically from a single crystal synchrotron experiment.

Working with metalloenzymes additionally requires to control the occupancy and redox states of the metal cofactors involved in order to be able to start the experiment with the right conditions.

In this study experiences from sample preparation of metalloenzymes for serial crystallography will be presented and some challenges and their solutions will be discussed.

P165

### Activating Bacteria by External Additives: Direct Conversion of Benzene to Phenol Catalyzed by P450BM3-Based Whole-Cell Biocatalyst

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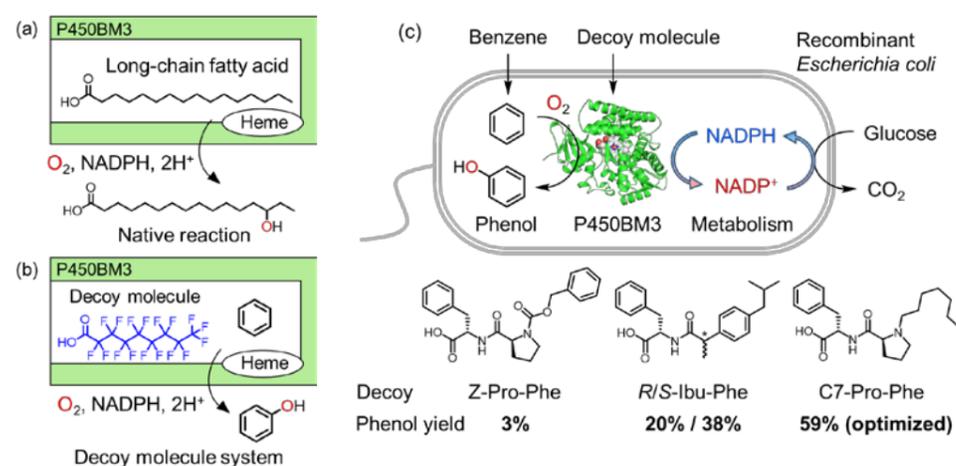
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Functionalization of unactivated carbon-hydrogen bonds remains a worthwhile goal in synthetic chemistry. Cytochrome P450BM3 (P450BM3), a heme enzyme isolated from *B. megaterium*, has thus gained prominence owing to its high monooxygenase activities toward long-chain fatty acids. However, since P450BM3 recognizes substrates by the carboxylate group in long-chain fatty acids, small molecules such as propane and benzene are not hydroxylated by P450BM3. To expand the potential substrate applicability of P450BM3, we reported a technique to remodel the active site of P450BM3 with shorter fatty-acid analogs (decoy molecule, Figure b), instead of established mutagenesis strategies<sup>[1, 2]</sup>. Decoy molecules bind to P450BM3 as a dummy substrate and thus initiates the catalytic cycle of the enzyme. On the other hand, shorter carbon chain can create the new reaction site inside the enzyme, permitting P450BM3 to oxidize non-native substrates accommodated in the heme active site<sup>[3]</sup>.

Unfortunately, P450BM3 has yet another serious problem of requiring expensive NADPH as electron donor, which limits the further application of decoy molecule system. To overcome this limitation, we endeavored to develop a whole-cell reaction system, wherein the P450BM3-catalyzed hydroxylation is supported by intracellular NADPH supply through inexpensive sugar metabolism. We herein report the direct conversion of benzene to phenol catalyzed by recombinant *Escherichia coli* expressing wild-type P450BM3 by the simple addition of decoy molecules to the culture medium (Figure c).

In the absence of decoy molecules, benzene hydroxylation was scarcely proceeded with a phenol yield of only 0.4% at 10 mM substrate after 5 h reaction. However, addition of decoy molecules significantly enhanced the benzene hydroxylation depending on the structure of decoy molecules, indicating that decoy molecules traversed cell membrane and thus activated P450BM3 even expressed in bacteria. Examined decoy molecules showed negligible cytotoxicity toward *E. coli*, as judged from the growth inhibition activities of these molecules. Finally, reaction conditions were optimized with C7-Pro-Phe as the decoy molecule, yielding up to 59% of phenol from benzene<sup>[4]</sup>.



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P166

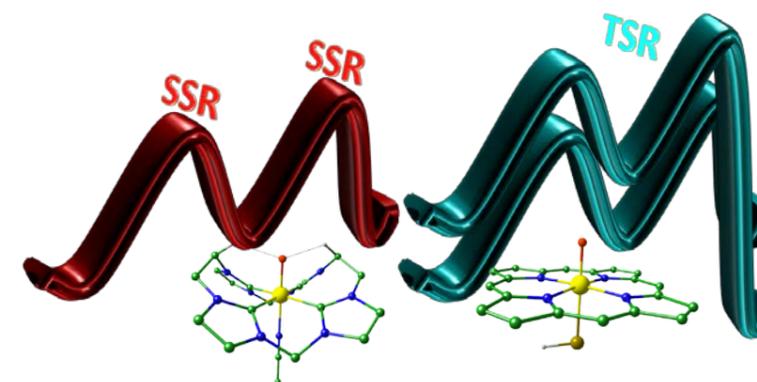
### Can a Strong Equatorial Ligation Suppress the Two-State Reactivity Of Biologically Active Iron(IV)-Oxo Species?

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High-valent iron-oxo species are known for its very high reactivity in biological system and this aspect has been studied in detail over the years. Particularly the role of axial ligands in fine-tuning the reactivity of the iron(IV)-oxo species are studied in detail. The role of equatorial ligands in fine-tuning the reactivity of such species is rarely explored and is of prime importance in the development of non-heme chemistry. Here, we have undertaken a detailed DFT calculations on  $[(L^{NHC})Fe^{IV}(O)(CH_3CN)]^{2+}$  (**1**) species ( $L^{NHC}=3,9,14,20$ -tetraaza-1,6,12,17-tetraazoniapentacyclohexanecosane-1(23),4,6(26),10,12(25),15,17(24),21-octaene) in comparison to compound II of cytochrome P450  $[(Porphyrin)Fe^{IV}(O)(SH)]^-$  (**2**) to probe this aspect. The electronic structures of **1** and **2** are found to vary significantly and this has led to a large variation in the reactivity. Particularly, strong equatorial ligand present in **1** destabilizes the quintet states significantly as compared to species **2**. To fully understand the reactivity pattern of this species, we have modelled the hydroxylation of methane by species **1** and **2**. Our calculations reveal that species **1** reacts via low-lying  $S=1$ ,  $\pi$ -pathway and generally available  $S=2$ ,  $\sigma$ -pathway is not energetically accessible. In addition to possessing a significant barrier for C-H bond activation, the -OH rebound step is also computed to have a large barrier height leading to a marked difference in reactivity between these two species. Of particular relevance here is the observation of pure triplet state reactivity for species **1**. Besides we have also attempted to test the role of axial ligands in fine-tuning the reactivity of species **1** and our results demonstrate that in contrast to heme systems, the axial ligands in **1** do not significantly influence the reactivity. This highlights the importance of designing the equatorial ligands to fine-tune reactivity of high-valent iron(IV)-oxo species.



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P167

### A Dual Role for C2H2 Zinc Fingers in both DNA Binding and Protein Binding in Nuclear Import by Transcription Factor Sp1

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Cys<sub>2</sub>His<sub>2</sub> (C2H2) zinc finger is a typical DNA binding motif and can also binds to RNA. The motif possesses tandem repeats of zinc binding module which contains two pairs of conserved Cys and His residues and forms a compact β-β-α structure (Fig.1) and is known to be quite abundant in human genome. Because of their unique features, we have been intrigued by versatility of these motifs and have been searching a novel role for them, especially protein interaction. One of these functions is nuclear localization signal (NLS).

Bidirectional traffic between the cytoplasm and the nucleus is routed through the nuclear pore complex (NPC) embedded in the nuclear envelope. Nuclear import of globular proteins greater than ca. 60 kDa in size requires a suitable NLS. Classical NLS consists of one or two short basic clusters. Nuclear transport of a classical NLS-containing protein is mediated by a ternary complex with importins α and β. Most of classical NLS can be recognized by an adaptor protein importin α, whereas a few NLS is the target of importin β.

Sp1 is a ubiquitous transcription factor involved in the early development of an organism. The protein comprises three tandem repeats of C2H2 zinc finger motif at its carboxyl terminus, which binds to a GC-rich element of DNA (GC box)(1) and activates reasonably large subset of mammalian genes containing GC box upstream promoter elements. Like other nuclear proteins, Sp1 is supposed to be actively transported into the nucleus due to its molecular mass (95 kDa).

We identified that three C2H2 zinc fingers can serve as a 'bona fide' NLS of Sp1 [2,3]. Then, we analyzed several factors affecting nuclear localization. Overall tertiary structure formed by zinc binding as well as basic amino acids dispersed in the entire zinc finger region was required for its complete NLS function. Solid-phase binding assay indicated that Sp1 zinc fingers can directly interact with at least one of transport factors. A series of basic amino acids in zinc finger region play pivotal role for specific DNA recognition. Those may also contribute to the interaction with transport protein because classic NLS has one or two basic clusters important for importin binding. Then, we tried to use GC box DNA as a probe to evaluate comprehensively the role for basic amino acids dispersed in the entire zinc finger region on interaction with transport factors. Furthermore, interaction of Sp1 zinc finger with importins was elucidated by biophysical method. The mode of protein interaction via the zinc finger domain will be discussed.

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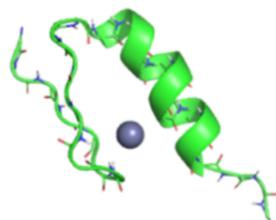


Fig.1 C2H2 zinc finger.

P168

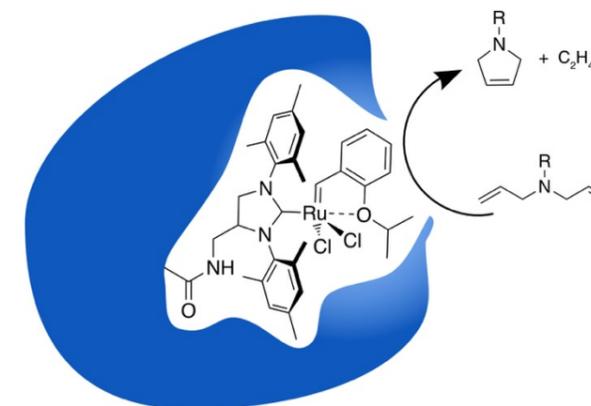
### Alternative Protein Scaffolds for Artificial Metalloenzymes

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Enzymes and small-molecule synthetic catalysts offer distinct catalytic advantages. Enzymes provide high turnover and specificity. In contrast, synthetic catalysts afford access to reactions not found in nature with high substrate scope. Artificial metalloenzymes (ArMs) are created by anchoring a synthetic catalyst within a protein.[1] By their nature, ArMs offer the opportunity to create catalysts that combine the advantages of both enzymes and synthetic catalysts.[2,3] Here, we report the creation of an ArM for metathesis by fusion of a ruthenium catalyst to a new protein scaffold. Through directed evolution, we show that the catalytic turnover of the ArM can be improved drastically. We propose that the close proximity of the ruthenium and the scaffold surface allows for the dramatic effects observed in directed evolution.

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**P169****A Comparison of Two Hydroquinone Ring-Cleaving Dioxygenases****Timothy E. Machonkin<sup>1</sup>, Julia E. Burrows<sup>1</sup>, Madeleine C. Maker<sup>1</sup>**<sup>1</sup>Department of Chemistry, Whitman College, 345 Boyer Ave., Walla Walla, WA, USA.

The hydroquinone dioxygenases (HQDOs) are members of a large family of non-heme Fe(II)-containing enzymes that catalyze the oxidative cleavage of an aromatic ring, of which the catechol extradiol dioxygenases are by far the best known and best studied. Like the catechol extradiol dioxygenases, the HQDOs are found in two unrelated structural classes, Type I and Type II, yet share the same 2-His-1-Glu facial capping triad. The enzyme PcpA, from *Sphingobium chlorophenicum* L-1, is a Type I HQDO and is structurally related to the major class of extradiol catechol dioxygenases. We have shown that PcpA is specific for *ortho*-disubstituted hydroquinones (but *not* catechols) with chloro- or bromo- (but not fluoro-) substituents greatly preferred at the *ortho* positions, and that ring cleavage occurs between the OH and *ortho* substituent.[1] Several lines of evidence indicate that halogen polarizability is a major factor that determines the specificity of this enzyme. Substrate binding titrations show only a small shift in pK<sub>a</sub> between the free vs. enzyme-bound substrate, suggesting that PcpA lacks an active site base needed to deprotonate the substrate, which is a key difference from the structurally homologous catechol extradiol dioxygenases. PnpC1C2, from *Pseudomonas putida* DLL-E4, is a Type II HQDO and displays a completely different pattern of substrate specificity, showing a preference for unsubstituted and monosubstituted hydroquinones, and a different ring cleavage regioselectivity. This points to important differences in the second coordination sphere that define how the hydroquinone substrate binds and is set up to be attacked by dioxygen.

Financial support by the National Science Foundation (CHE-0951999, CHE-1506458, MRI-0922775) is gratefully acknowledged.

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**P170****Carrier-Driven Crystallization of E<sub>c</sub>-1****Alejandro Marquez Espinoza<sup>1</sup>, Eva Freisinger<sup>1</sup>**<sup>1</sup>Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland.

Metallothioneins (MTs) are ubiquitous small, cysteine-rich proteins that are mostly involved in Zn(II) and/or Cu(I) homeostasis and heavy metal detoxification. The early cysteine-labeled protein (E<sub>c</sub>-1) from *Triticum aestivum* (bread wheat) embryos was the first MT from higher plants to be isolated and characterized [1]. Spectroscopic data including structure determination with NMR spectroscopy showed that E<sub>c</sub>-1 is a two-domain protein that coordinates up to six Zn(II) ions via cysteine and histidine ligands [2-4]. However, as NMR spectroscopy is only able to give indirect information about metal ion coordination, and this information is based on experiments using <sup>113</sup>Cd nucleus to mimic Zn(II) ions, additional structural data from crystallography is highly desirable. Since previous trials to crystallize the full-length protein and separated domains (γ and β<sub>E</sub>) were not successful, we present here our first attempts with carrier-driven crystallization. In this approach, a crystallization chaperone such as glutathione s-transferase (GST) or the maltose-binding protein (MBP) can provide additional surface area to promote crystal contact formation [5]. We obtained several crystals of fusion-proteins that could bring complementary information of the E<sub>c</sub>-1 architecture to understand its biological functions. In addition, this approach could be extended to others MTs with intrinsically disordered regions that make difficult structural investigations.

Financial support from the Swiss National Science Foundation and the Faculty of Science of the University of Zurich is gratefully acknowledged.

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P171

### Structural Insights into Catalytic Mechanism of Formylglycine Generating Enzyme

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Nature utilizes different approaches to solve oxygen binding and activation problem. To overcome these complications many enzymes were evolved to use copper as a cofactor. Being appropriately coordinated, copper can switch between the oxidative states I, II and III and activate molecular oxygen (O<sub>2</sub>) via electron transfer. The formed reactive oxygen species are able to initiate subsequently very complicated reactions. [1, 2]

The formylglycine generating enzyme (FGE) is copper dependent oxidase, which activates pro- and eukaryotic sulfatases through oxygen-dependent conversion of specific cysteine residue to formylglycine [3]. Recently revealed linear bis-cysteine coordination of copper in FGE resting state is unprecedented for mononuclear copper oxidases, which predominantly bind metal in histidine-rich tetrahedral or square planar coordination geometry. In contrast to copper trafficking enzymes, FGE active site binds and activates O<sub>2</sub> to abstract the peptidyl-cysteine pro-(R)-β-hydrogen. [4-6]

In this report we discuss new insights into FGE-catalytic cycle, based on NMR spectroscopy and the high-resolution crystal structure of FGE from *T. curvata* in the complex with Cu(I) and 17-residue substrate analog (1.04 Å).

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P172

### An Actinobacterial Representative Sheds a New light on the Reaction Mechanism of Coproheme Decarboxylases

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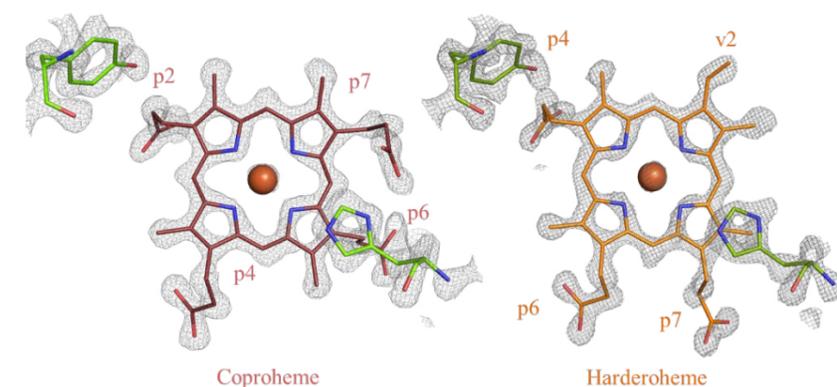
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Coproheme decarboxylase (ChdC) catalyses the last step in the recently discovered coproporphyrin dependent heme *b* biosynthesis pathway [1]. In a hydrogen peroxide dependent reaction, it cleaves off two propionates from the substrate coproheme to form heme *b*. This decarboxylation reaction has been studied thoroughly since its' discovery but is not fully understood yet. However, the enzymes under study are exclusively originating from organisms belonging to the phylum of Firmicutes. A recent study showed that actinobacterial ChdC from *Corynebacterium diphtheriae* (CdChdC) exhibits higher activity and efficiency compared to previously described representatives from other clades [2].

In this study, potentially relevant amino acid residues were selected for mutational studies based on the first actinobacterial ChdC crystal structure. It has been shown that Tyr135 is crucial for Compound I\* formation, elimination of this residue allowed us to uncover CdChdC Compound I using UV-vis spectroscopy. His118 was found to be in very close proximity to the heme iron, no such amino acid with an active group is found at this position in Firmicutes ChdC. The H118A variant required four times more hydrogen peroxide for conversion of coproheme than the wildtype. The reaction was also found to be about 20 fold slower than that of the wildtype. These observations support the assumption that H118 is involved in Compound I formation.

Finally, the crystal structures of the enzyme with either the substrate or the reaction intermediate revealed with great clarity, that the three-propionate species harderoheme is reoriented in the active site pocket before the second decarboxylation reaction takes place. Financial support by the FWF (P29099) and the International PhD program in protein biotechnology – BioToP (W 1224) is gratefully acknowledged.



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P173

**Optimization of Crystallization and Biochemical Studies of KanJ Protein****Beata Mrugala<sup>1,2</sup>, Anna Milaczewska<sup>1</sup>, Ewa Niedzialkowska<sup>2</sup>, Przemyslaw Porebski<sup>2</sup>, Wlodek Minor<sup>2</sup>, Tomasz Borowski<sup>1</sup>**<sup>1</sup>*Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Niezapominajek 8, 30239 Krakow, Poland.*<sup>2</sup>*Department of Molecular Physiology and Biological Physics, University of Virginia, 1340 Jefferson Park Avenue, Charlottesville, VA 22908, USA.*

Kanamycin A, from *Streptomyces Kanamyceticus* is one of the most important representatives of aminoglycoside antibiotics with a strong anti-bacterial effect against gram negative bacteria, used as a second-generation drug against tuberculosis. The mechanism of kanamycin biosynthesis is unknown, however based on biochemical studies and mutational biosynthesis it was possible to isolate proteins that enter the synthetic pathway [1,2,3].

In our study, we highlighted the first step of biosynthesis kanamycin by using the KanJ enzyme belonging to the 2-ketoglutarate depend dioxygenases from a new group of proteins involved in antibiotic biosynthesis.

We have expressed recombinant KanJ in *Escherichia coli*, and optimized the purification of active protein. Thermofluor Shift Assays were used to screen for conditions that facilitate protein crystallization. The enzyme activity was tested using LC-MS to detect kanamycin A in the post-reaction mixture ITC were also done to study biomolecular interactions and check the binding of potential ligands. Biochemical measurements were carried out in three different pHs: 6.3, 7.5 and 8.2 in buffers with different ionic strength. Determination of structure and protein activity will allow to get to know the reaction mechanism and design modifications, that may be important in the design of new drugs.

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P174

**How Does CYP Sequence Affect CYP:CPR Complexation in a Phospholipid Bilayer and the Transfer of Electrons?****Goutam Mukherjee<sup>1,2</sup>, Prajwal P. Nandekar<sup>1,3</sup>, Ghulam Mustafa<sup>1</sup>, Rebecca C. Wade<sup>1,2,3</sup>**<sup>1</sup>*Molecular and Cellular Modeling Group, Heidelberg Institute of Theoretical Studies, Germany.*<sup>2</sup>*Interdisciplinary Center for Scientific Computing (IWR), Heidelberg University, Germany.*<sup>3</sup>*Center for Molecular Biology (ZMBH), DKFZ-ZMBH Alliance, Heidelberg University, Germany.*

Cytochrome P450 (CYP) is the cardinal xenobiotic-metabolizing superfamily of enzymes. CYPs require two electrons for their catalytic cycle. For microsomal CYPs, these electrons are transferred by their redox partner, NADPH-cytochrome P450 oxidoreductase (CPR) to the CYP active site heme cofactor. CYP and CPR are membrane-anchored proteins and the association of the two proteins to form a complex, CYP-CPR, is driven by electrostatic interactions. However, so far, no crystal structure has been solved of their full length complex.

Previously, we developed and reported a protocol to build and simulate CYPs in a phospholipid bilayer.<sup>1-2</sup> Here, we have developed a transferable multiresolution computational approach to build and simulate a full length CYP-CPR-membrane complex using Brownian dynamics and all-atom molecular dynamics simulations. Application of this approach yielded multiple arrangements of CPR around CYP that are electron transfer (ET) competent. The modelled complexes allow identification of contacting residues in CYP1A1-CPR and CYP17A1-CPR complexes and the determinants of electron transfer rates, which agree well with available experimental data.<sup>3-8</sup>

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P175

### Structural Analysis of HypX Responsible for CO Production in the Maturation of a [NiFe]-Hydrogenase

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Hydrogenases are metalloenzymes that catalyze the oxidation of H<sub>2</sub> into electrons and protons and the reduction of protons into H<sub>2</sub> reversibly. The active site in a [NiFe]-hydrogenase consists of a dinuclear NiFe cluster (Figure 1), in which a Fe atom binds two cyanide (CN) molecules and one carbon monoxide (CO) as intrinsic ligands. In the maturation of the [NiFe] hydrogenase, Fe(CN)<sub>2</sub>CO unit is biosynthesized and inserted into the large subunit of the [NiFe] hydrogenase. CN ligand is produced from carbamoyl phosphate by HypE and HypF protein complex. Although the detail mechanism of biosynthesis and assemble of CN ligand has been elucidated, that of CO ligand remains unknown. HypX was recently identified as the enzyme responsible for CO biosynthesis during the maturation of [NiFe] hydrogenase. Here, we report the structural analysis of the novel enzyme HypX and discuss the reaction mechanism of HypX.

We determined the crystal structure of HypX from *Aquifex aeolicus* (Figure 2). HypX consists of the N- and C-terminal domains linked by a loop with the C-terminal tail. A continuous cavity connecting the two domains is present in the interior of HypX. The N-terminal domain of HypX has a structural homology to the hydrolase domain (FDH-h) of N10-formyl-tetrahydrofolate (N10-formyl-THF) dehydrogenase, which catalyzes the formyl-group transfer from N10-formyl-THF. In the crystal structure of HypX soaked with THF, THF is bound in the corresponding position in FDH-h. The structure and sequence comparison of HypX and FDH-h indicates that HypX catalyzes the formyl-group transfer from N10-formyl-THF as is the case of FDH-h. The C-terminal domain of HypX has a structural homology to enoyl-CoA hydratase/isomerase using the CoA derivatives as substrates. The structural analysis of HypX variants, MD simulation and native MS analyses reveal that CoA is constitutively bound in the C-terminal region of the cavity and that CoA can change the conformation in the cavity. Taken together, we propose the reaction mechanism of CO biosynthesis by HypX, in which the internal CoA mediates the conversion of the formyl-group in N10-formyl-THF to CO.

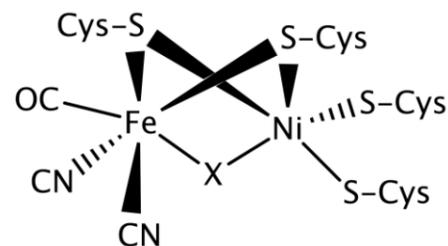


Figure 1. NiFe cluster in [NiFe]-hydrogenase.

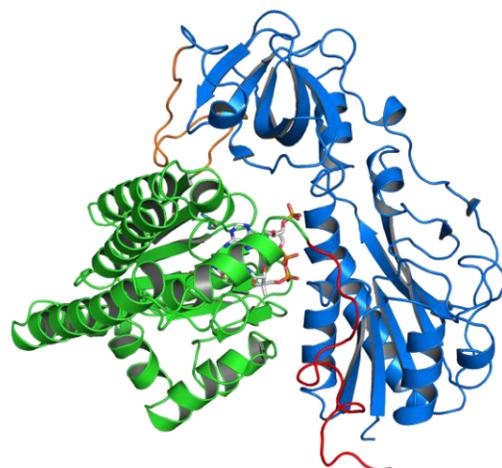


Figure 2. Crystal structure of HypX

P176

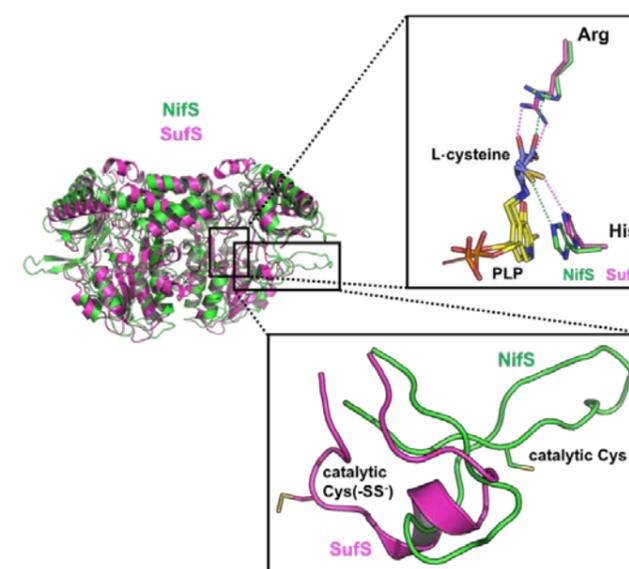
### X-Ray Crystallographic Snapshots of Catalytic Intermediates of Cysteine Desulfurases

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The iron-sulfur (Fe-S) cluster is an ancient metallo-cofactor and utilized by Fe-S proteins for their multifarious functions (e.g. electron transfer and radical generation) [1]. In most organisms ranging from bacteria to animals and plants, Fe-S clusters are biologically synthesized by dedicated biosynthesis machineries. The machineries are classified into three groups (NIF, ISC and SUF) in which the mechanism of Fe-S cluster assembly differs from each other. However, the machineries utilize a “cysteine desulfurase” in common that is termed NifS, IscS or SufS. Cysteine desulfurases is a pyridoxal-5'-phosphate (PLP)-dependent enzyme that extracts inorganic sulfur (S<sup>0</sup>) from L-cysteine for sulfur-mobilization through a mechanism involving traditional PLP chemistry and C-S bond cleavage [2]. It is proposed that the substrate L-cysteine is bound to PLP and attacked by a catalytically essential cysteine residue for generation of S<sup>0</sup> via C-S bond cleavage. The generated S<sup>0</sup> is stored on the cysteine residue as a persulfide (Cys-SS<sup>-</sup>), and transferred to sulfur-acceptor proteins. Despite the critical role of the catalytic cysteine residue, the local structure of the cysteine-containing regions are different between type I (NifS and IscS) and type II (SufS) cysteine desulfurases. For understanding the mechanistic difference in detail, the catalytic intermediates of the desulfurizing reactions of NifS and SufS were investigated by X-ray crystallography. In both NifS and SufS, a PLP-L-cysteine adduct was captured as an intermediate that is stabilized by electrostatic interaction and hydrogen bonding to arginine (Arg) and histidine (His), respectively, at the PLP site, thereby defining the orientation of the substrate suitable for nucleophilic attack by the catalytically essential cysteine residue. However, the catalytic mechanism is different between NifS and SufS after PLP-L-cysteine binding. In desulfurizing reaction of SufS, the catalytic cysteine residue moves only slightly with a rotation of the side chain. By contrast, the local structure containing the catalytic cysteine residue of NifS undergoes a large conformational change via secondary structural dynamics, allowing the cysteine to move over 23.5 Å. This mechanistic difference should be inversely associated with the conformational flexibility of the partner proteins (SufU or NifU) that accept the sulfur from cysteine desulfurases.



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P177

### Effect of Heme on Target RNA-Binding in Cold Shock Protein, CspD

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Iron is important for proteins as active sites or regulators for electron transport, chemical reactions, and gene regulation, etc. in organisms. On the other hand, excess intracellular iron can generate reactive oxygen species, leading to organ dysfunctions such as cancer. Therefore, iron homeostasis is essential for cells. Cellular iron level is regulated by iron itself and iron-protoporphyrin IX complex, heme. Typical “heme-regulated” proteins, iron regulatory proteins (IRPs) and iron response regulator (Irr), have a short consensus sequence containing Cys-Pro, the heme regulatory motif (HRM). Recently, many HRM containing proteins not directly related to cellular iron homeostasis have been identified, suggesting that heme can function as a global signalling molecule for various kinds of biological processes. Cold shock protein (Csp) from a pathogenic bacterium, *Vibrio cholerae*, is one of such HRM containing proteins, functioning as RNA chaperones to stabilize RNA structures in cellular adaptation at temperature changes. Among four *V. cholerae* Csp (CspA, CspD, CspV, VCA0184), only CspD has a HRM (Cys22-Pro23), but the heme binding to the HRM in CspD has not yet been confirmed and functional significance of the heme binding to CspD is unclear. Here, we examined the heme binding properties of *V. cholerae* CspD and its functional significance in the RNA chaperone.

To clarify the signalling function of heme for CspD, we followed the effects of the heme binding on the target RNA binding to CspD by the fluorescence quenching of Trp near the RNA binding site, and a single strand DNA (ssDNA) was used as a stable model substrate for the target RNA. By the addition of ssDNA, the fluorescence intensity at 340 nm was decreased (Fig. 1: closed circle), indicating that CspD binds to ssDNA. On the other hand, such a decrease in the fluorescence intensity was not observed by the addition of ssDNA in the presence of heme (Fig. 1: open circle), suggesting that heme inhibits the ssDNA binding to CspD. To determine the stoichiometry of heme binding to CspD, spectrophotometric heme titration was performed. One equivalent of heme was found to bind to CspD and the Soret peak of the heme-CspD complex appeared at 417 nm, corresponding to a Cys/His coordination. We mutated Cys22 in the HRM of CspD to identify the heme ligand, but the Cys22 mutated CspD (Cys22Ala) was able to bind one equivalent of heme with lower affinity than that of the wild-type protein. In the presence of heme, however, the fluorescence intensity at 340 nm of the Cys22Ala mutant was diminished by adding ssDNA (Fig. 1: open diamond), showing that the heme binding to the Cys22Ala mutant did not inhibit the ssDNA binding. The heme binding at Cys22 is, therefore, essential for inhibition of the target RNA binding in CspD, and the mutation at Cys22 resulted in the shift of the heme ligand from Cys22 to other amino acid residue. Inhibition of the formation of the CspD-ssDNA complex by the heme binding to Cys22 is supported by the crystal structure of *N. meningitidis* Csp showing 60% identity with *V. cholerae* CspD. Based on the heme environmental structure of *N. meningitidis* Csp (Fig. 1: right), Cys22 of *V. cholerae* CspD would be positioned close to the RNA binding site (Phe20) and the heme binding at Cys22 would sterically interfere the target RNA binding. Thus, we can propose that CspD uses heme as a signalling molecule and Cys22 is the heme binding site to inhibit the target RNA binding.

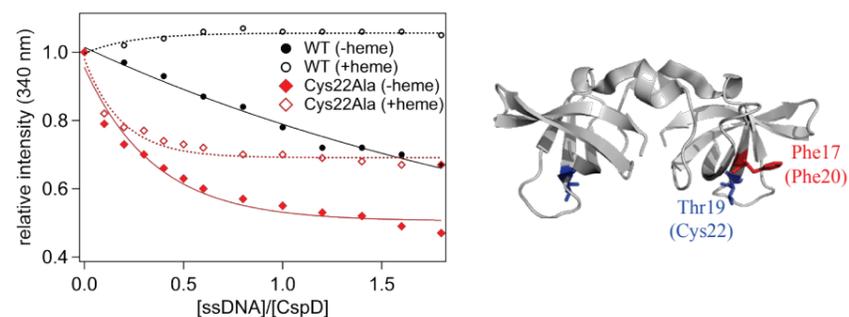


Fig. 1 Fluorescence quenching of Trp by addition of ssDNA (left) and crystal structure of *N. meningitidis* Csp (PDB: 3CAM) (right)

P179

### Oxygen Activation by Self-sufficient Cytochrome P450 Reconstituted with Manganese Porphyrin

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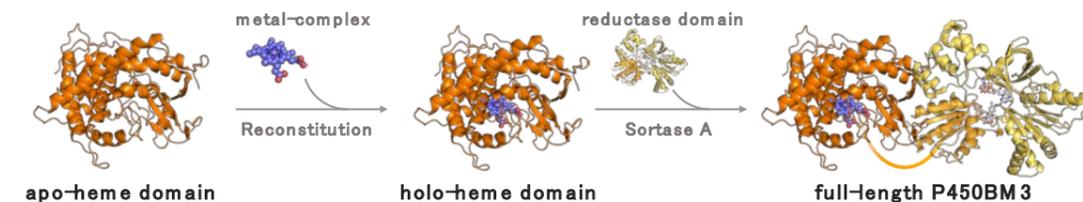
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CYP102A1 (P450BM3) isolated from *Bacillus megaterium* has been regarded as a promising biocatalyst, possessing a very high catalytic activity among cytochrome P450s [1]. In general, P450BM3 catalyzes the hydroxylation of long-chain fatty acids while it is inactive for small non-native substrates. In our previous research, we have successfully altered the substrate specificity of P450BM3 by using natural-substrate analogs, which we call ‘decoy molecules,’ and achieved hydroxylation of unnatural substrates such as benzene, cyclohexane, propane, etc. without any mutagenesis [2]. This P450BM3/decoy system is also useful for stereoselective hydroxylation of small organic substrates and we have succeeded in controlling the stereoselectivity of products hydroxylated by wild-type P450BM3 only by changing decoy molecules [3].

Herein we accomplished the heme substitution of P450BM3 with artificial metal-complexes, to modify its catalytic activity [4]. As a conventional method, acid/organic solvent has been used to remove the heme from target hemoproteins. However, this method is not applicable to every CYPs, and P450BM3 is irreversibly denatured under the condition prohibiting the reconstitution with artificial complexes. In our laboratory, a mild heme substitution method was developed by utilizing Fe-limiting medium, and we successfully reconstituted hemoproteins with artificial metal-complexes [5]. However, even this mild method was not applicable to full-length BM3, due to its structural complexity. We focused on transpeptidase Sortase A from *Staphylococcus aureus* which recognizes specific peptide sequences and catalyzes the peptide-peptide ligation reaction, and we finally succeeded in the reconstitution of full-length BM3 by ligating its heme domain with the reductase domain. By this heme substitution method with Sortase A, we prepared Mn-substituted BM3 with manganese protoporphyrin IX, and investigated its catalytic properties.



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P180

**Time-Resolved Serial Femtosecond Crystallography of the Isopenicillin N Synthase Reaction with ACV and O<sub>2</sub>**

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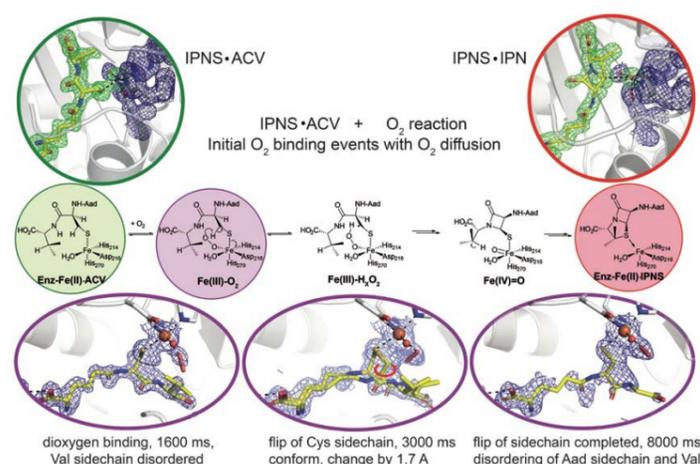
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The use of femtosecond pulses at an X-ray free electron laser (XFEL) allows one to probe reactions to yield atomic and electronic structures, without X-ray radiation-induced changes to sensitive sites such as an active site metal centre. To this end, our collaboration has developed a drop-on-demand sample delivery system that enables simultaneous collection and correlation of time-resolved X-ray diffraction data and X-ray emission spectroscopy.[1] Crystallographic data yields atomic models throughout the asymmetric unit, spectroscopy monitors the changes to the electronic structure of an active site metal ion, and both are correlated with time resolution between  $\mu$ s through several seconds.

Here we present structural and spectroscopic results from isopenicillin N synthase (IPNS), which catalyses the nonheme iron-dependant, four electron oxidation of the linear tripeptide  $\delta$ -(L- $\alpha$ -aminoadipoyl)-L-cysteiny-D-valine (ACV) into isopenicillin N.[2] The work involved microcrystal slurry optimization, synthesis of isotopically labelled ACV and new methods in sample delivery. A unique feature of the proposed reaction mechanism is the role of two high-valent iron species - an Fe(III)-superoxo and a high-spin Fe(IV)=O species that promote the first and second ring closures of the  $\beta$ -lactam, respectively. We present results for these intermediates obtained during O<sub>2</sub>-catalyzed turnover of the IPNS•ACV complex. High valent iron intermediates are of exceptional importance throughout biology where they function as key intermediates, including those that form antimicrobial compounds and in human enzymes, including in hypoxia sensing/response and DNA damage repair.[3]

Financial support in part, by a Wellcome Trust Investigator Award in Science to AMO (210734/Z/18/Z).



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P181

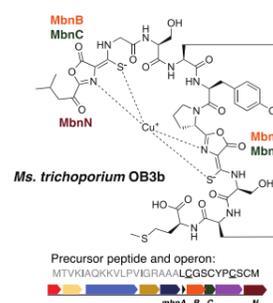
**Understanding Biosynthesis of Methanobactins**

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Methanobactins (Mbns) are copper-chelating peptidic natural products produced by methanotrophs.<sup>1</sup> Copper is necessary for their primary metabolic enzyme, particulate methane monooxygenase (pMMO).<sup>2</sup> In order to meet their high necessity for copper, some methanotrophs produce Mbns to acquire copper from the environment under copper starved conditions.<sup>1</sup> Due to their high affinity for copper, Mbns have been investigated as potential drug candidates for Wilson disease, a human genetic disorder of copper metabolism that causes toxic copper accumulation.<sup>3</sup>

The Rosenzweig laboratory has been interested in how Mbns are synthesized in methanotrophs.<sup>4,5</sup> Mbn is a ribosomally-synthesized and post-translationally modified peptide that is produced from a precursor peptide, MbnA.<sup>6</sup> MbnA comprises a leader peptide that is eventually cleaved off and a core peptide that is post-translationally modified to form mature Mbn. The post-translational modifications occur at two cysteine residues in the MbnA core peptide, resulting in the formation of the copper binding ligands, oxazolone and thioamide groups. Two core proteins, MbnB and MbnC, form a heterodimeric iron-containing enzyme complex, MbnBC, that catalyzes formation of the oxazolone/thioamide groups from the cysteine residues.<sup>4</sup> The iron site in MbnB is believed to interact with O<sub>2</sub> and MbnA to modify the cysteine side chain. However, in vitro reaction stalled and generated an intermediate rather than mature Mbn. Another biosynthetic enzyme, MbnN (a PLP-dependent aminotransferase), encoded in the *Methylosinus (Ms.) trichosporium* OB3b and *Ms. sp.* LW4 operons performs a transamination reaction that produces a carbonyl group as the last step of Mbn biosynthesis.<sup>5</sup> The final chemical structure of the copper-bound Mbn from *Ms. trichosporium* OB3b as well as its precursor peptide and operon are depicted in the figure below.<sup>1,4</sup> In this presentation, current efforts to investigate how to complete the Mbn biosynthesis in vitro and to determine its reaction mechanisms will be discussed. This work provides important insights into novel enzymatic chemistries performed by the Mbn biosynthesis enzymes and will impact the development of Mbns and Mbn-like molecules as therapeutics.



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P182

### X-Ray Induced Reduction Kinetics of Heme Metal Centers is Protein-Independent – Implications for Structural Studies of Redox Sensitive Proteins

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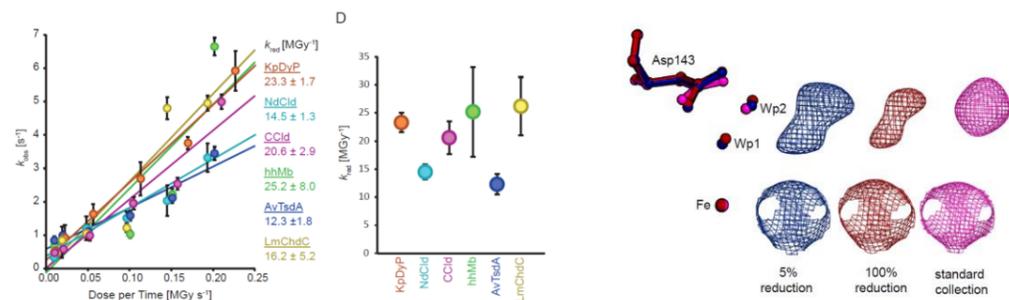
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X-ray crystallography is one of the main resources to obtain information on the coordination of molecules within the active site of metalloproteins on an atomistic level. Based on ligand coordination, interatomic distances and relative positioning of catalytic amino acids enzymologists try to understand the underlying electronic reaction mechanism. Therefore the exact redox status and conformation of the cofactor in question is of utmost importance. Unfortunately the redox active nature of metal cofactors makes them especially susceptible to irradiation induced photoreduction, making structural information obtained by photo-reducing X-ray sources the least trustworthy [1,2].

Here we present a study of the pre-steady state reduction kinetics of X-ray induced photo-reduction of six different model heme proteins to identify a reasonable dose-limit for the collection of non-reduced datasets for redox-active metallo enzymes. Using online-UV-vis spectroscopy we examine the reduction kinetics of six heme proteins in order to understand the impact of sample-derived variables (protein, crystallization conditions, crystal morphology, and cofactor) and irradiation-derived variables (dose and dose rate). Our results clearly show that the reduction kinetics solely depend on the dose, irrespective of the sample-derived variables. We can therefore define a protein-independent dose-limit of 25 kGy, which corresponds to a 50% reduction of the desired initial redox state. Furthermore we can present a method of data collection and processing that allows for the collection of time-resolved low dose structures using standard macromolecular crystallography tools. Finally we present structures of a model heme protein (KpDyP) in different defined redox states. These structures show photoreduction induced rearrangements in water coordination and conformation of the catalytically relevant residue Asp 143 [3]. The observed effects of photo-reduction highlight that care has to be taken when in-solution data of ferric proteins are rationalized by structural constraints derived from crystal structures of reduced enzymes. Financial support by the FWF (W1224) is gratefully acknowledged.



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P183

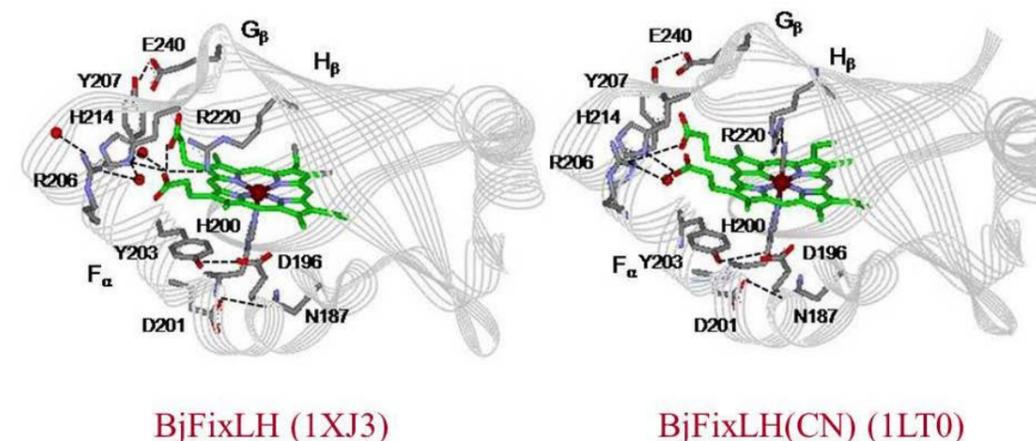
### Spectroscopic Studies of the SmFixL Protein from S. Meliloti Reveal that Conserved Residues in the Heme Domain Play Crucial Roles in the Oxygen Sensing Mechanism

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SmFixL is part of the heme-PAS histidine kinase families of biological sensors that regulate a wide variety of important cellular processes in nature.<sup>1,2,3</sup> Oxygen binding to the Heme-PAS domain of SmFixL turns off the histidine kinase domain.<sup>4</sup> We have examined the role of conserved F<sub>α</sub>-helix residues in the heme domain of SmFixL\* using site-directed mutagenesis, small molecule binding studies with NO, O<sub>2</sub>, CO and CN<sup>-</sup>, resonance Raman, CD and EPR spectroscopic studies and kinase assays. Our results indicate that the conserved residues R200, R214 and Y197 play important roles in oxygen sensing in the heme domain and may play a crucial role in signal transduction to the kinase domain. A detailed understanding of the oxygen sensing mechanism of SmFixL may lead to a better understanding of the Heme-PAS and histidine kinase families.

Financial support by ACS-PRF, Research Corporation and Merck is acknowledged.



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P184

### Structural Studies of Trigonal Pyramidal Pb(II)S<sub>3</sub> Centers in *De Novo* Three-stranded Coiled Coils to Model Lead Binding in the Human LINE-1 Retrotransposon

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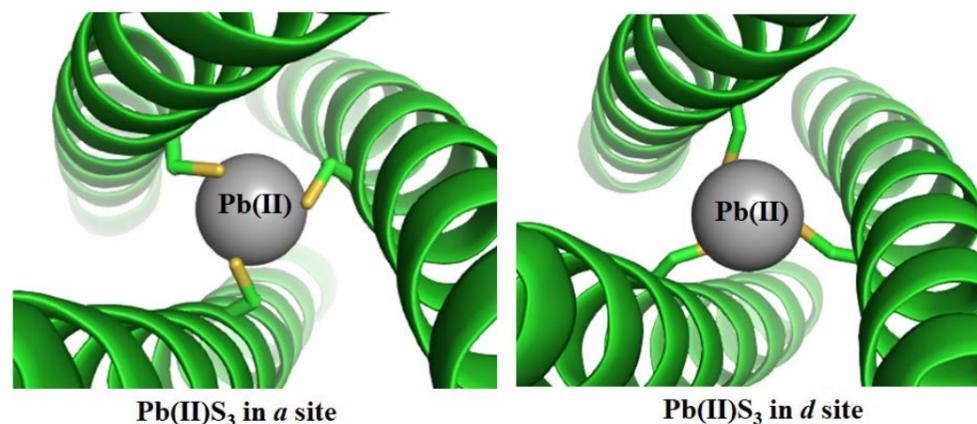
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Human LINE-1 retrotransposons are genetic elements responsible for an increase of human genome variability. The ORF1p sequence is one of the two open reading frames that encodes highly conserved N-terminal trimeric coiled coil domain proteins that connect to a central RNA recognition motif. The three-stranded coiled coil (3SCC) region utilizes a heptad repeat where hydrophobes occupy in *a* and *d* positions. Cysteine residues are found to locate in three different layers (one in a *d* position and two in *a* positions), making these thiolate-sites potential to bind heavy metals in an MS<sub>3</sub> geometry due to the enthalpically strong M-S bond. Though the structural details of heavy metals bound to the ORF1p have not been recognized, here we employ *de novo* 3SCC peptides, GRAND-CS, to understand how leads bind into a 3SCC. Structurally characterized from the dual tri-thiolate sites in the (GRAND-CSL16CL23C)<sub>3</sub> crystal structure, both Pb(II) centers simultaneously adopt a trigonal pyramidal geometry with an *endo* conformation, where the metal is located below the cysteine plane and downward to the C-termini. The Pb(II)-S bond lengths are within 2.57 – 2.62 Å, depending on the sites and the conformers of cysteine ligands. The binding of Pb(II) in the *d* site is demonstrated from the Pb(II)(CSL12C)<sub>3</sub> structure. Similar to the *a* sites Pb(II) binds in a trigonal pyramidal geometry with an *endo* conformation (average Pb(II)-S distance = 2.60 Å); however, the metal is surprisingly located above the thiol plane (toward the N-termini of the 3SCC). Further analysis with the apo-peptides has shown that the *a* sites are preorganized, while the *d* site is predisposed for the metal complexation. Based on these crystallographic observations using the designed peptides, we provide a potential model for the unrecognized Pb(II) binding sites that could happen in the 3SCC region of the ORF1p encoded proteins. Such perturbation may result in a drastically mutagenic effect to the human genome in a long-run.

Financial support from KMUTT, Thailand is gratefully acknowledged.



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P185

### Preliminary Characterisation of a Novel *o*-Aminophenol Oxidase from *Streptomyces* sp. Produced in *Escherichia Coli*

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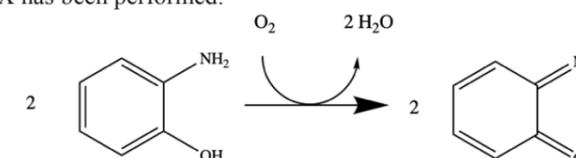
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*o*-Aminophenol oxidases (EC 1.10.3.4) catalyse the oxidation of *o*-aminophenol to the corresponding quinone imine using molecular oxygen. The quinone imine further polymerises under non-enzymatic and enzymatic oxidative reactions and contribute to the formation of melanin pigmentation. Some, but not all, of the characterised *o*-aminophenol oxidases belong to the coupled binuclear copper (CBC) enzymes family, where two copper ions are antiferromagnetically coordinated [1].

*o*-Aminophenol oxidase activity has been reported for other CBC enzymes, such as tyrosinase (EC 1.14.18.1) from *Agaricus bisporus* [2] and catechol oxidase (EC 1.10.3.2) from *Aspergillus oryzae* (AoCO4) [3]. Thus far, the most well-characterised *o*-aminophenol oxidase is from *Streptomyces griseus* subsp. *griseus* (GriF) [4].

In this work, we present a novel *o*-aminophenol oxidase from *Streptomyces* sp. (AMINOX). AMINOX was heterologously expressed in *Escherichia coli* cells BL21(DE3) and purified by affinity chromatography on a HisTrap HP column charged with CuSO<sub>4</sub>. The substrate specificity of AMINOX on *o*-aminophenol, di-phenols, and three-phenols was tested by means of a qualitative colorimetric screening. L-tyrosine, L-DOPA, and phenol were also tested as substrates. AMINOX showed clear activity on *o*-aminophenol and pyrocatechol. Similarly to GriF and AoCO4, AMINOX did not present activity on L-tyrosine, which seems to be a substrate specific of tyrosinase enzymes only.

Thus far, a scientific explanation for the reason why only certain CBC enzymes shows activity on L-tyrosine has not yet been presented. With the aim of contributing to this research field, a crystallisation screening on AMINOX has been performed.



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P186

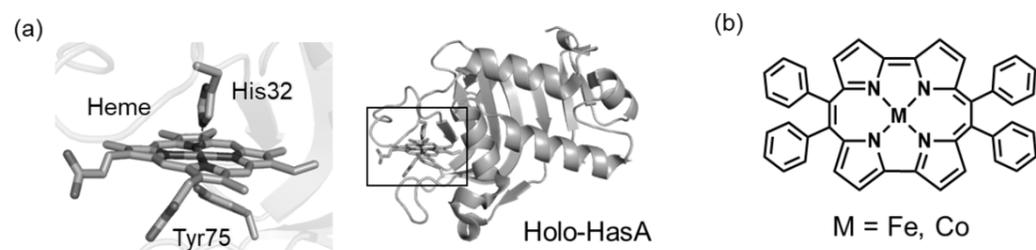
### Construction of Hemoprotein HasA Incorporating Bulky Metal Complexes

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Heme substitution is one of the powerful approaches for control of protein functions, and has been widely examined to produce novel biocatalysts, biosensors, and supramolecular structures. There are many reports that heme protein functions have been enhanced by substituting heme for its analogues that are modified with functional groups and/or replaced for metals other than iron. However, high bulky metal complexes compared with heme generally cannot be incorporated into native heme proteins, since the heme binding site is suitable for the accommodation of heme molecule. Recently, we have reported that the heme protein HasA can accommodate various artificial metal complexes that are totally different structures from heme. HasA is a heme-acquisition protein secreted by a certain type of pathogens, such as *Pseudomonas aeruginosa*, under iron deficiency conditions. After acquiring heme from its hosts, HasA transfers the heme to the HasA-specific receptor, HasR as an iron source for its survival. HasA captures heme using its two loops with coordination of His32 and Tyr75 to the heme iron (Figure 1a). The heme-binding position is highly exposed to solvent, thereby the moiety of the captured haem can be seen from outside of HasA in the crystal structure of HasA incorporating heme (Holo-HasA). This unique heme-binding fusion allows the incorporation of synthetic metal complexes with structures different from heme such as iron-salophen, iron-phthalocyanine, and iron-diphenylporphyrin.<sup>[1][2]</sup> The crystal structures of HasA incorporating metal complexes revealed that HasA can bind these metal complexes in the same binding fashion as that of heme without any structural perturbation suggesting that a wide variety of the metal complexes would be accommodated by HasA.

In this study, we demonstrated that a native heme protein HasA can accommodate metallo-tetraphenylporphycenes (Figure. 1b) with bulky substituents, while their structures are significantly different from heme. We also have succeeded in crystal structure analysis of HasA capturing cobalt(III)-9,10,19,20-tetraphenylporphycene (Co-Ph<sub>4</sub>Pc). The X-ray crystal structure analysis of HasA with Co-Ph<sub>4</sub>Pc revealed that HasA can accommodate Co-Ph<sub>4</sub>Pc in its heme-binding position by locating four bulky phenyl groups on Co-Ph<sub>4</sub>Pc at the outside of HasA. Additionally, we found that HasA-loops forming the heme-binding pocket possess satisfactory flexibility. The appropriate structural changes of its loops allowed incorporation of bulky phenyl groups on metallo-Ph<sub>4</sub>Pcs into HasA.



**Figure 1** (a) Crystal structure of heme-bound HasA (Holo-HasA), (b) Structure of metallo-tetraphenylporphycene

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P187

### Structural Assembly of a Plant Metallothionein 2 Protein – Secondary Structural Elements in the Flexible Linker Region

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Metallothioneins (MTs) are a heterogenous group of a small cysteine-rich proteins that have been found in most phyla. Their cysteine residues have a high affinity toward metal ions preferably with d<sup>10</sup> electron configuration, such as Zn<sup>II</sup>, Cd<sup>II</sup>, and Cu<sup>I</sup>. Metal ions are bound in characteristic thermodynamically stable metal-thiolate clusters which are the predominant elements of their tertiary structure.<sup>1,2</sup>

Plant MTs are far from being investigated intensively, and there is scarce information about their structural and functional aspects. Our main focus is set on the investigation of structural characteristics of the plant MT2 protein (cicMT2) from chickpea (*Cicer arietinum*) and the importance of its central Cys-free linker region. However, it is obvious that this central region has a significant impact on the structure of plant MTs in comparison to members of the other families. cicMT2 is a typical member of the plant MT2 subgroup featuring an N-terminal Cys-rich region with eight Cys residues and a C-terminal region with six, separated by a 41 amino acids long Cys-devoid linker region. The binding of five divalent metal ions to protein thiolate groups leads to the formation of a single metal-thiolate cluster.<sup>3</sup> So far, approaches to determine the structure of the full-length protein both with crystallographic methods and NMR spectroscopy were not successful due to its flexible nature. Hence, we focus our attempts on the evaluation of the secondary structural elements, their specific localization, and their stabilization by metal ions, as well as the influence of plant MT-specific linker region on the overall stability of the protein. Financial support from the Swiss National Science Foundation, the Institute of Chemistry, and the Faculty of Sciences of the University of Zurich is gratefully acknowledged.

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P188

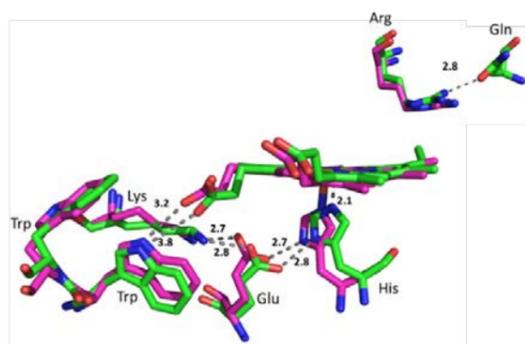
### Manipulation of Heme Environment in the Active Site of Chlorite Dismutases and its Impact on the Enzymatic Activity

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Chlorite dismutases (Clds), are heme *b*-dependent oxidoreductases that were originally discovered in chlorate- and perchlorate-reducing bacteria (PCRB). However they are also found in many further bacterial and archaeal phyla [1]. These enzymes are able to catalyse the degradation of the cytotoxic respiratory chain product chlorite (ClO<sub>2</sub><sup>-</sup>) to harmless chloride (Cl<sup>-</sup>) and molecular oxygen (O<sub>2</sub>). This catalytic function turns Clds into a highly interesting enzyme for bioremediation, since this harmful anthropogenic compound is a serious environmental concern and increasing concentrations of chlorite have been detected in soil, ground- and drinking water. Furthermore it is very interesting from a biochemical point of view, as it is the only known enzyme system which efficiently catalyses the formation of a covalent O=O bond, beside the water-splitting manganese complex of photosystem II. Functional Clds (i.e. chlorite degrading Clds) can be divided into two phylogenetically distinct clades, 1 and 2, which differ in subunit size, stability and oligomerisation. Clade 1 mainly consists of hexameric or pentameric proteins, whereas dimeric Clds appear in clade 2 [2].

It was shown that several functional Clds possess a flexible arginine in a more or less hydrophobic distal heme pocket that can adopt two distinct conformations. Either it is pointing towards the substrate entry channel (“out”) or towards the heme iron (“in”) [3]. Furthermore, the X-ray crystal structure of a dimeric chlorite dismutase from *Cyanothece* sp. PCC7425 (CCld, pdb: 5MAU, clade 2) reveals that there is a glutamine residue that acts as hydrogen bonding partner and thereby putatively stabilizes the out-conformation of the catalytically important distal arginine [4]. This glutamine is not present in pentameric Clds (e.g. chlorite dismutase from *Nitrospira defluvii* (NdCld, pdb: 3NN2, clade 1). This work focuses on the dimeric chlorite dismutase CCld and the role of the distal glutamine in the heme pocket with respect to chlorite reactivity and accessibility of the active site. We replaced the distal Gln74 by valine to mimic the active site architecture of NdCld. Here, we show the latest experimental results comparing kinetic data of wild type CCld and the variant Gln74Val. Financial support by the FWF (P30979) and the International PhD program in protein biotechnology – BioToP (W 1224) is gratefully acknowledged.



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P189

### Specific Photosterilization of *Pseudomonas aeruginosa* Exploiting Its Heme Acquisition System

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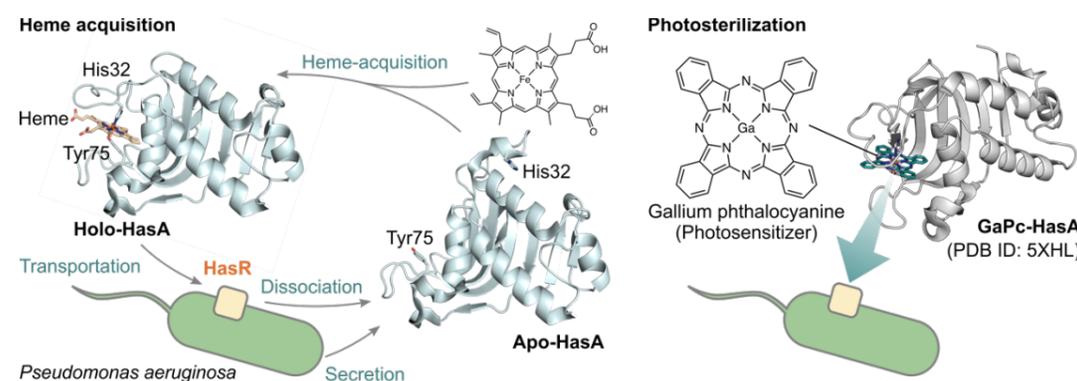
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The development of target-selective photosensitizers is sought to accomplish the efficient and selective photosterilization of serious pathogens, such as *Pseudomonas aeruginosa*, whilst reducing damage done to surrounding tissues. To selectively deliver photosensitizers to *P. aeruginosa*, we focused on the heme acquisition system (Has) of *P. aeruginosa*, which permits the scavenging of extracellular heme as an iron source. Under iron-limiting conditions, *P. aeruginosa* secretes the heme acquisition protein HasA (apo-HasA). Apo-HasA pirates extracellular heme from its host to yield the heme-bound form of HasA (holo-HasA). Heme scavenged by HasA is then delivered to the HasA-specific outer membrane receptor HasR. After formation of the high affinity holo-HasA-HasR ternary complex, heme is transferred from HasA to HasR followed by dissociation of heme-free HasA (apo-HasA) from HasR ready for a further round of heme acquisition. We have previously demonstrated that HasA is rather promiscuous, capturing several synthetic metal complexes other than heme, such as iron phthalocyanine (FePc). HasA incorporating FePc (FePc-HasA) has been shown to potently inhibit HasA-mediated heme acquisition (IC<sub>50</sub> approx. 24 nM), resulting in the suppression of *P. aeruginosa* growth [1]. We reasoned that FePc-HasA may irreversibly interact with HasR, thus blocking further binding of holo-HasA and concomitant transfer of heme, essentially starving the bacteria of vital iron [1, 2]. These findings support the hypothesis that the outer membrane receptor HasR possesses the ability to selectively accept photosensitizers captured by HasA, encouraging us to utilize the aforementioned HasA-HasR interaction for antimicrobial photodynamic therapy (aPDT).

Herein we report the specific photosterilization of *P. aeruginosa* by utilizing HasA coordinating a photosensitizer gallium phthalocyanine (GaPc-HasA). We demonstrated that GaPc-HasA can be used as a selective photosensitizer, enabling us to efficiently eliminate *P. aeruginosa* including multidrug-resistant strains by irradiation with near-infrared (NIR) light irrespective of antibiotic-resistance type.



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P190

**Thermal Unfolding and Refolding of a Lytic Polysaccharide Monooxygenase from *Thermoascus Aurantiacus***

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Lytic polysaccharide monooxygenases (LPMOs) are copper-containing enzymes which catalyze the oxidation of cellulosic biomass and promote the degradation of recalcitrant polysaccharides like cellulose or chitin [1,2]. However, stability of LPMO has not been major focus of investigation considering its crucial role in industrial application [3]. Here, We have investigated the thermostability of an LPMO from *Thermoascus aurantiacus* (TaLPMO9A). TaLPMO9A was found to retain most of its initial activity after incubating at 100 °C while its apparent melting temperature (T<sub>m</sub>) is 69 °C at neutral pH. Uniquely, our studies show that TaLPMO9A can fold back to its original conformation with an intact copper site upon lowering the temperature. The thermal unfolding and refolding of TaLPMO9A was measured spectroscopically utilizing the two-state model and detailed thermodynamic parameters were obtained for the *holo*TaLPMO. ESI-MS analysis of TaLPMO9A revealed that the enzyme is glycosylated in its native form and the thermostability of the deglycosylated form was also investigated. Activity of the TaLPMO was studied by an optimized. Amplex® Red assay as well as by TaLPMO9A catalysed oxidation of phosphoric acid swollen cellulose (PASC). These studies confirm the functional regain of TaLPMO9A activity upon going through one cycle of unfolding and refolding.

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P191

**Metal-Free Ribonucleotide Reduction Powered by a DOPA Radical in Mycoplasma Pathogens**

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Conversion of ribonucleotides to deoxyribonucleotides is an essential biochemical reaction in all living organisms that use DNA as their genetic material. Under aerobic conditions, class I Ribonucleotide reductase (RNR) proteins perform this complex reaction via a di-metal cofactor that undergoes oxygen activation to generate and stabilize a radical critical for catalysis. Different subclasses of RNRs ranging from Ia to Id rely on specific combinations of Fe and/or Mn ions to generate a tyrosine radical or a radical-equivalent high valent metal center. A di-metal site was believed to be an absolute requirement for function across all known class I aerobic RNRs [1].

In this study we describe a new subclass of class I RNR, class Ie, that by modifying a tyrosine amino acid into a 3,4-dihydroxyphenylalanine (DOPA), overcomes the explicit requirement of a di-metal cofactor for radical generation and stabilization. Studies using X-ray crystallography, EPR and UV-vis spectroscopy, TXRF, mass spectrometry, RNR in-vivo and in-vitro assays describe this metal independent radical species and the new RNR subclass [2]. A metal-independent RNR could possibly impart an evolutionary advantage to pathogens residing under metal limiting conditions e.g. induced by the immune system. Further characterization of the mechanisms for covalent modification and radical generation are ongoing.

Financial support by the Swedish Research Council, European Research Council and the Knut and Alice Wallenberg Foundation is gratefully acknowledged.



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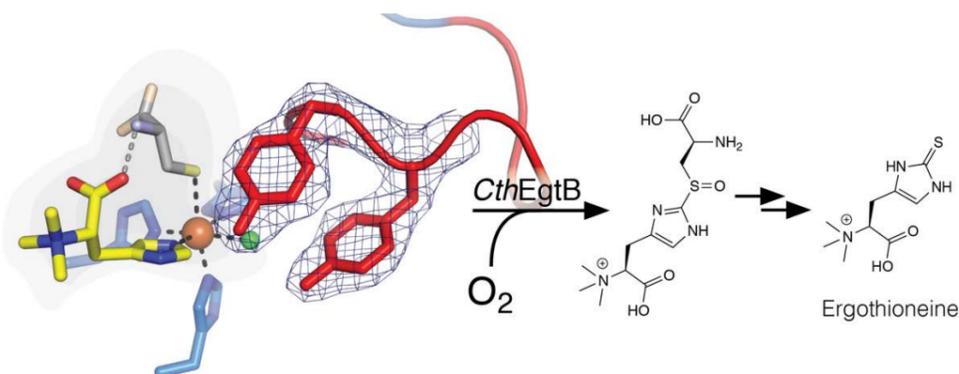
P192

### The Role of Dynamic Active Site Loops in Oxygen Activation in an Iron-dependent Sulfoxide Synthase, EgtB

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EgtB is an iron-dependent mono-oxygenase that catalyses oxidative C-S bond formation as a key step in the biosynthesis of ergothioneine. The structure and mechanism of this enzyme is distinct from other known oxygenases [1]. We have recently characterised a new class of EgtB (type II), which revealed several key differences to characterised homologues, despite catalysing an almost identical reaction [2]. The most intriguing differences are associated with the oxygen-binding site, with type II EgtB's having a completely different configuration of active site residues that are involved in oxygen binding and activation. Two mobile active site loops that appear to fold in a substrate dependent manner replace the rigid active site observed in other types. These two active site loops play a dynamic role in catalysis. In this presentation we will discuss our efforts to elucidate the role of these catalytic loops.



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P193

### WhiB-Like Proteins and their Fe-S Dependent Protein-Protein Interactions

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WhiB-like (Wbl) proteins are iron-sulfur (Fe-S) cluster proteins unique to Actinobacteria, a phylum of Gram-positive bacteria that includes medically important pathogens such as *Mycobacterium tuberculosis*, and soil bacteria belonging to the *Streptomyces* genus, the source of over half of all clinically useful antibiotics. Wbl proteins are important for the ability of *M. tuberculosis* to persist in an antibiotic resistant state in the host for prolonged periods of time, and for sporulation in *Streptomyces* species.

Mutagenesis studies of the four conserved cysteines which coordinate the Fe-S cluster show them to be crucial for Wbl protein function. Furthermore, at least some Wbl proteins appear to be sensors of nitric oxide (NO), and WhiD from *Streptomyces coelicolor* and WhiB1 from *M. tuberculosis* have been previously shown to undergo a rapid, complex reaction with NO, reaching completion after the sequential addition of 8 NO molecules per cluster. This appears to give rise to a mixture of protein-bound iron-nitrosyl complexes with similarities to well-known inorganic small molecule iron-nitrosyls [1,2].

The mechanisms by which Wbl proteins function have yet to be clearly demonstrated. They appear to act as transcriptional regulators, but in some cases at least this occurs via an interaction with another protein. For example, in *S. coelicolor*, the WhiB regulon has been shown to be identical to that of the WhiA protein (a regulator required for sporulation), indicating that the proteins cooperate to effect transcriptional control [3]. WhiA is not itself an Fe-S cluster protein, it is nevertheless unusual in that it has an N terminal domain related to a class of homing endonucleases, but lacks the residues required for catalysis. The link between Wbl proteins and a number of sigma factors has also been documented, the implications of these interactions is currently unknown.

The aim of this work is to gain insight into the Wbl proteins from *S. venezuelae*, in terms of both their reaction with NO and interactions with potential partner proteins, and the mechanisms for signal transduction. To do this, both non-denaturing and LC-MS methodologies are being employed alongside more traditional bioanalytical and spectroscopic approaches.

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P194

### The Interaction between Nitrite and the Ferric Globin Domain of GLB-33, a Unique Chimeric Globin in *Caenorhabditis Elegans*

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Besides the fact that *Caenorhabditis elegans* is an excellent model organism itself, it is highly suitable to study globin function because its genome consists of 34 globin genes, which are all expressed throughout different cell types [1]. One of them is GLB-33 that is mainly expressed in the neuronal tissues of the nematode and from homology modeling it is suggested to consist of a 7  $\alpha$ -helical transmembrane domain and a heme-containing globin domain (GLB-33GD). The first is similar to a Phe-Met-Arg-Phe-amide neuropeptide receptor whereas the latter is a myoglobin-type molecule. Interestingly, GLB-33GD shows faster nitrite reductase activity than any other reported globin [2]. Here, we use the combination of optical, vibrational and electron paramagnetic resonance spectroscopy to reveal that nitrite does not easily bind the ferric heme iron since it remains strongly hydroxo ligated. Mildly acidic conditions were needed to obtain maximal nitrite coordination and the effect of acidity on recombinantly overexpressed GLB-33GD and on the GLB-33GD[NO<sub>2</sub><sup>-</sup>]-complex was studied. Furthermore, we conclude from resonance Raman spectroscopy with <sup>14/15</sup>NO<sub>2</sub><sup>-</sup> and mass spectrometry that nitrovinyl heme is formed at low *pH*-values via the substitution of a vinyl proton with NO<sub>2</sub>. Distal amino acids in the vicinity of the heme play a major role in the stabilization of small ligands and more specifically in the binding mode of nitrite in hemoproteins [3]. The distal side of the heme in GLB-33GD consists of an exceptional hydrophobic pocket including the following crucial amino acids linked to globin function: Leu-41(CD3), Ile-69(E7), Arg-72(E10), and Ile-73(E11). From this set, only Arg-72 is capable of ligand stabilization and Ile-69 is responsible for pentacoordination of the iron in the reduced state. In order to study nitrite stabilization and the catalytic roles played by the lack of a distal His-69 residue in GLB-33GD, Arg-72-Val and Ile-69-His mutants are currently under investigation.

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P195

### Biochemical Study of UbiU and UbiV, Two New [4Fe-4S] Proteins Involved in Anaerobic Ubiquinone Biosynthesis in *E. Coli*

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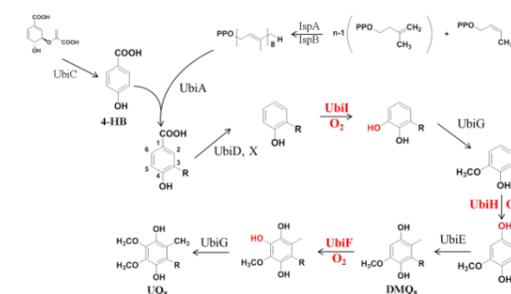
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Ubiquinone (coenzyme Q or UQ<sub>8</sub>) is a redox active lipid that plays a crucial role in the mitochondrial electron transport chain. The aerobic UQ<sub>8</sub> biosynthesis pathway in *E. coli* requires at least twelve *ubi* genes, most of them encode enzymes that decorate the aromatic ring of 4-hydroxybenzoate (4-HB) <sup>1,2,3,4</sup>. The three aerobic hydroxylases UbiI, UbiH and UbiF which are involved in C5, C1 and C6 hydroxylation steps, are all O<sub>2</sub> and FAD-dependent monooxygenases <sup>4</sup>. Thus they are not involved in the anaerobic UQ<sub>8</sub> biosynthesis pathway <sup>5</sup>. In *E. coli*, the *yhbU* (renamed *ubiU*) gene is located next to *yhbV* (renamed *ubiV*) gene, all two being likely to constitute an operon, and they appear to be important for the biosynthesis of UQ<sub>8</sub> in the absence of oxygen, as the knock-outs of these genes abolish the UQ<sub>8</sub> content in *E. coli* in anoxic conditions. Here, we report for the first time the detailed biochemical and biophysical properties of UbiU and UbiV from *E. coli* and demonstrate the important role of four conserved cysteine residues that each protein possesses. UV-visible absorption spectra of aerobically purified UbiV show the presence of a [2Fe-2S] cluster, whereas the electron paramagnetic resonance (EPR) spectra of anaerobically reconstituted protein show the presence of a [4Fe-4S] cluster. Next we show that UbiU and UbiV form a heterodimer complex and the EPR spectra of anaerobically reconstituted complex show the presence of two [4Fe-4S] clusters whose environments are not identical. Although reconstitution of [4Fe-4S] cluster in UbiU alone was unsuccessful, we are able to show that UbiU carries the second [4Fe-4S] cluster through studies of the UbiU-V complex.

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P196

### The Presence of An Alcove Facilitates Substrate Binding in Acetyl-CoA Synthase (ACS): Geometry, Methylation, & Carbonylation

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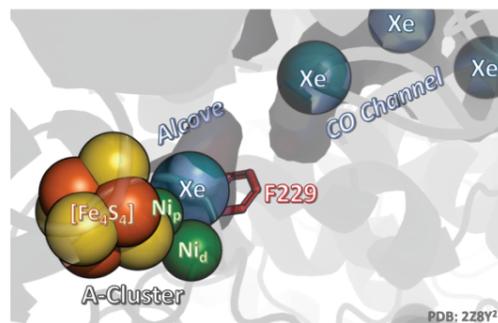
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The Wood-Ljungdahl pathway is native to a diverse range of anaerobic microbes for incorporating CO<sub>2</sub> into acetyl-CoA, where it becomes energy or material in the cell [1]. A linchpin enzyme within this pathway is acetyl-CoA synthase (ACS), part of an enzyme pair containing CO dehydrogenase (CODH). CODH reduces CO<sub>2</sub> to CO, which travels through a tunnel to ACS, where CO condenses with CoA and a methyl group donated by corrinoid iron-sulfur protein (CFeSP) into acetyl-CoA [1,2]. A previous study probing CO binding to ACS indicated the presence of an alcove keeping CO near the ACS A-cluster active site, which composed of an unusual di-nickel center proximal to an [Fe<sub>4</sub>-S<sub>4</sub>] cluster [3]. At the A-cluster, the proximal Ni (Ni<sub>p</sub>) is the site of CO, methyl, and CoA binding, and forms a bridge between the [Fe<sub>4</sub>-S<sub>4</sub>] cluster and the distal Ni (Ni<sub>d</sub>) [1,2]. Crystallographic studies of CODH/ACS under high pressures of Xenon further identified a gas-binding site, or hydrophobic “box” at the edge of the CO channel near Ni<sub>p</sub> [2].

To understand the alcove’s role in CO binding, a key residue forming a wall of the alcove, phenylalanine 229 (F229), was mutated to alanine (F229A), effectively removing the alcove’s structure, and to a tryptophan (F229W). Using numerous spectroscopic techniques, including IR, EPR, and XAS, the alanine variant’s ability to bind CO is significantly altered, while the ability to methylate is not. This disrupted CO binding is evident through significant EPR shifts, absent Ni-CO XAS, and lack of a characteristic Ni-CO infrared band at 1995 cm<sup>-1</sup> [3,4]. The F229A variant’s disturbed ability for carbonylation seems to be solely due to the missing phenylalanine 229 residue, which appears uniquely crucial to binding and maintaining CO near the ACS active site while leaving methylation at levels and rates similar to those of the wildtype.

We propose the alcove plays an essential role in the ACS mechanism by binding and retaining CO near Ni<sub>p</sub> to facilitate formation of the key Ni<sub>p</sub>(I)-CO, methyl-Ni and acetyl-Ni intermediates in the Wood-Ljungdahl pathway of acetyl-CoA synthesis. which is further supported by a biomimetic A-cluster model’s ability to bind CO and methylate in the Ni<sub>p</sub>(I), while as-isolated ACS in the Ni<sub>p</sub>(II) state cannot [5,6]. Financial support from the University of Michigan Rackham Graduate School and Department of Biological Chemistry is gratefully acknowledged.



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P197

### The Hydroxylation/Desaturation Selectivity of Clavaminc Acid Synthase – A QM/MM Study

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Clavaminc acid synthase (CAS) from *Streptomyces clavuligerus* is an Fe(II)/2-oxoglutarate dependent dioxygenase that catalyses three steps in biosynthesis of the β-lactamase inhibitor clavulanic acid [1]. The three oxidative reactions, i.e. hydroxylation, cyclisation and desaturation, lead to formation of the clavam nucleus and proceed in a single binding cavity of CAS [2]. The aim of this QM/MM study is to elucidate the factors that determine hydroxylation/desaturation selectivity and to explain how the versatility of the reaction selectivity is achieved.

For both the native substrates of the enzyme, i.e. deoxyguanidinoproclavamate (DGPC) and dihydroclavamate (DHC), the difference between the strength of the C-O bond (formed during radical rebound) and the O-H bond (formed during desaturation) favours the hydroxylation reaction as demonstrated with use of valence bond theory [3]. For the former substrate (DHC), the interactions with the binding cavity of CAS compensate for the electronic properties of the reactants and alter the selectivity towards desaturation. The QM/MM results indicate that the key to enzyme versatility might be the residues that interact with the charged terminal group of the substrate (guanidine group of DGPC and amine group of DHC) and thus impose such a position of the substrate that facilitates hydroxylation in case of DGPC and desaturation for DHC.

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P198

### Exploratory Research of Transition Metal Ions and Complexes Inhibiting the Fibril-Formation of Collagen Proteins

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Collagens, which are characterized by the unique triple-helical structure, are the most dominant proteins in the extracellular matrix of the animal body. Fibril-forming collagens play crucial roles in maintaining the integrity of tissues and organs, where triple-helical molecules of the fibril-forming collagens have intrinsic propensity to self-assemble with forming well-ordered fibrils. In the fibril, 300-nm long collagen molecules are placed parallel to one another with a regular staggering of one-fourth of the molecular length, and then the collagen fibrils further form thick fibres. However, the complete mechanism of this well-organized self-assembly has not been elucidated [1].

Excessive deposition of collagen fibres in tissues and organs causes fibrotic diseases such as liver cirrhosis, pulmonary and renal fibrosis, keloid and scleroderma. Therefore, inhibition of collagen overproduction or excessive deposition is recognized as a promising mechanism for the research and development of antifibrotic agents. On the basis of the inhibition of collagen fibril-formation, an anti-telopeptide monoclonal antibody has been developed for treatment of keloid [2]. However, small-molecule candidates with low molecular weights have not been discovered to date.

Recently, *cis*-diamminedichloroplatinum(II) (cisplatin), a well-known anticancer agent, was discovered as a potential inhibitor of collagen fibril-formation, using an *in vitro* random screening of small-molecule compounds [3]. Because the inhibitory effect was found only in dimethylsulphoxide (DMSO) solution of cisplatin, the active species were suggested to be cisplatin derivatives formed in the DMSO solution. Then, mass spectrometry of cisplatin derivatives formed in DMSO was analysed, indicating that the active compound is identified to be [Pt(NH<sub>3</sub>)<sub>2</sub>(Cl)(DMSO)]<sup>+</sup> [4].

In this study, the foregoing results motivate us to investigate and explore the biological transition metal ions and complexes with potent inhibiting activity against the formation of collagen fibrils, such as Cr<sup>3+</sup>, Cr<sup>6+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Ag<sup>+</sup>, Au<sup>3+</sup>, and Gd<sup>3+</sup>, where Cu<sup>+</sup> was obtained by reduction of Cu<sup>2+</sup> with ascorbic acid. We monitored the degree of fibril-formation of porcine atelocollagen in phosphate buffered saline (pH7.4) by measuring the increase of absorbance due to turbidity at 313 nm using Infinite®200PRO plate reader at 37°C [3]. The ratio of fibril-formation was calculated from the difference in absorbance from 5 to 120 min, and the 50% inhibitory concentrations (IC<sub>50</sub>) of metal ions were estimated from the inhibition-titration curves. Additionally, the triple-helical conformation structure of collagen molecules was confirmed by measuring the specific CD spectra of positive cotton effects at 225 nm.

From the results, only Cu<sup>+</sup>, Cu<sup>2+</sup> and Au<sup>3+</sup> were found to inhibit the formation of collagen fibrils *in vitro* in the metal concentration-dependent manner without affecting the triple-helical conformation of the collagen molecules. While, other metal ions had little or no effects on the fibril-formation of collagen. Cu<sup>2+</sup> and Au<sup>3+</sup> were further revealed to interact with specific sites on the collagen triple helix by competition assay using the mixed solution of metal ion and each amino acid such as Gly, His, Arg, Lys, Glu, Asp, Hyp, and Met, and the binding sites of Cu<sup>2+</sup> and Au<sup>3+</sup> were suggested to contain His and Met residues, respectively.

Interestingly, Au<sup>3+</sup>-Met mixture exhibited stronger inhibition against the fibril-formation of collagen than Au<sup>3+</sup> ion in the complex concentration-dependent manner, which was speculated to occur by the counter effect of Au<sup>3+</sup> due to coordination with lone-pair electrons of R-S-CH<sub>3</sub> group.

These results for transition metal ions and complexes in this study will be useful for both investigating molecular mechanisms of collagen self-assembly and drug development for the treatment of fibrotic diseases with lower toxicity than cisplatin.

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P199

### Screening of N-Substituted Dipeptides for Activation of Cytochrome P450BM3

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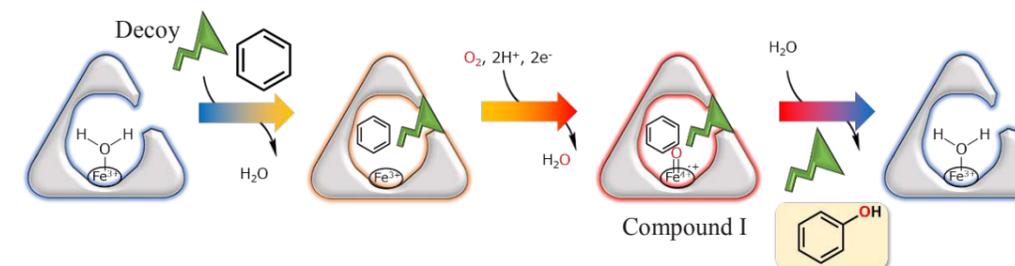
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Cytochrome P450s (P450s) are heme enzyme which catalyze hydroxylation of inert C-H bonds under mild conditions. Their beneficial enzymatic activity has attracted much interest of researchers thus they have been studied intensively over the last few decades aiming application for industrial use. P450BM3 isolated from *Bacillus megaterium* catalyzes hydroxylation of long chain fatty acids at sub-terminal positions ( $\omega$ -1,  $\omega$ -2,  $\omega$ -3) and its catalytic activity is the highest among P450s reported before. The heme-domain of P450BM3 is fused to the reductase-domain. Such a unique structure enables efficient electron transfer from NADPH to heme cofactor, resulting in high catalytic activity. Despite its advantageous catalytic activity, P450BM3 does not catalyze hydroxylation of non-native substrates such as benzene because binding of long chain fatty acid is essential for activation of P450BM3 and generation of reactive species, Compound I. Recently, our research group reported that hydroxylation of non-native substrates is catalyzed by P450BM3 in the presence of a series of perfluorocarboxylic acid (PFCs)<sup>[1][2]</sup>. We named these functional molecules “Decoy molecules” from their function. Decoy molecules are misrecognized as native substrates by P450BM3 and activate P450BM3 because they bind to P450B3 in a similar manner to native substrates. However, decoy molecules themselves are not hydroxylated because of shortage of chain length therefore various non-natural substrates are hydroxylated instead<sup>[3]</sup>. The structure of decoy molecules has been improved to achieve more efficient activation of P450BM3 and the latest study demonstrated that a variety of amino acid derivatives serves as decoy molecules<sup>[4]</sup>. Among such decoy molecules, ((benzyloxy)carbonyl)-L-prolyl-L-phenylalanine (Z-L-Pro-L-Phe) showed high P450BM3-activation potency for benzene hydroxylation (Turn Over Frequency = 229 ± 9 min<sup>-1</sup>)<sup>[4]</sup>, suggesting that *N*-substituted dipeptide scaffold is a promising framework for development of the novel decoy molecules.

Herein, we prepared over 600 *N*-substituted dipeptides and examined their P450BM3-activation potency in benzene hydroxylation. All dipeptides and their derivatives were prepared by parallel or split-and-mix solid-phase synthesis. We achieved discovering the most potent decoy molecule among all the decoy molecules reported before. Moreover, we succeeded in crystallization of P450BM3 with novel decoy molecules. Binding of the decoy molecules was confirmed and interactions between P450BM3 and decoy molecules were elucidated. Based on the results of screening, we discuss the structure-activity relationships of decoy molecules.



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P210

### Effect of the N-Truncation of A $\beta$ Peptides on the ROS Production by their Copper Complexes – Friends or Enemies?

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Alzheimer's disease (AD) is the most common neurodegenerative disease and the major cause of dementia throughout the world that makes it one of the biggest challenges of the 21<sup>st</sup> century in public health. The prevalence of this disease is expected to increase rapidly in the coming decades. Although the mechanisms underlying this complex pathology are not yet fully understood, a broad consensus attributes the early development of AD to a so called amyloid cascade. This deleterious process relies on the disturbed equilibrium between the production of a peptide called amyloid- $\beta$  (A $\beta$ ) and its degradation by other proteases. This results in a significant increase in extracellular concentration, leading to its aggregation *via* the formation of oligomers, protofibrils and fibrils.[1] These assemblages come together to form amyloid plaques, a distinctive post-mortem marker of the disease. Different forms of A $\beta$  peptides are found in the senile plaques, such as the “full-length” A $\beta$ <sub>1-40/42</sub> peptide and the N-truncated peptides A $\beta$ <sub>n-40/42</sub> (pos. 1-11).[2-5] Strong evidences have associated the high toxicity of Cu-containing aggregates to their ability to promote the oxidative stress observed in AD *via* the catalytic production of reactive oxygen species (ROS).[6-7] A lot have been done regarding the studies of the ROS production with the full length A $\beta$  peptide.[8] nevertheless N-truncated forms which are little considered are of interest because it's thought that there are present in huge quantity in the brain and senile plaques.[3, 9] Moreover it's admitted that they possess a protective effect against ROS production thank to their ATCUN (Amino-Terminal Copper and Nickel binding) type coordination site.

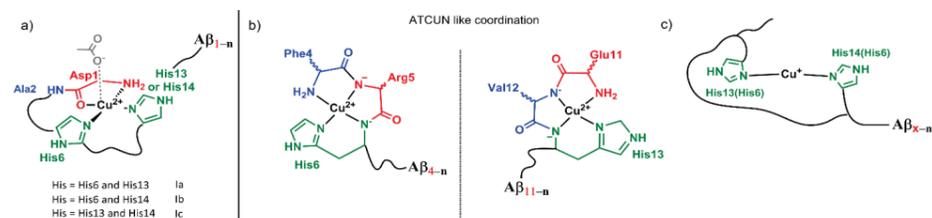


Fig. 1: Proposed coordination sites (major form) of a) Cu<sup>II</sup>A $\beta$ <sub>1-n</sub>, b) Cu<sup>II</sup>A $\beta$ <sub>4-n</sub> and Cu<sup>II</sup>A $\beta$ <sub>11-n</sub> and c) Cu<sup>I</sup>A $\beta$ <sub>1/4/11-n</sub>.

Indeed, the coordination site of the N-truncated peptides is different from the one found in A $\beta$ <sub>1-40/42</sub> (Figure1) which confers them different properties regarding the ROS production.[10] Here we present the results of the study of the ROS production obtained with two N-terminal A $\beta$  isoforms found in the brain: A $\beta$ <sub>4-n</sub> and A $\beta$ <sub>11-n</sub>. This study aims to clarify/investigate the following points: (i) the ROS production of Cu<sup>II/I</sup>A $\beta$ <sub>4/11-16</sub> in our experimental conditions, and (ii) the effect of the presence of A $\beta$ <sub>4/11-n</sub> on the ROS production by Cu<sup>II/I</sup>A $\beta$ <sub>1-16</sub>. Funding from the ERC (StG-638712) and the PRESTIGE program are gratefully acknowledged.

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P211

### Modifications of Metal-Bound Amyloidogenic Peptides by a Chemical Modulator

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The dysregulation of transition metals could contribute to neurodegeneration [1]. In the brain of Alzheimer's disease, transition metal ions are observed to be bound to A $\beta$  forming metal-A $\beta$  complexes [2]. Complexes of redox-active transition metal ion and A $\beta$  peptides can stabilize toxic A $\beta$  oligomers and induce oxidative damage under cellular environments [1-3]; however, no chemical reagent or strategy is currently available to control both metal-A $\beta$  complexation and the oligomerization of the resultant metal-free A $\beta$  upon removal of metal from the complexes. In this presentation, we will present a novel tactic to modify metal-A $\beta$  complexes. Our overall studies demonstrate the feasibility of developing effective strategies to regulate metal-bound aggregation-prone peptides found in neurodegenerative disorders.

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P212

### Rational Strategy of Designing Small Molecules with Multi-Reactivity against Free Radicals and Metal-Free and Metal-Bound Amyloid- $\beta$ Peptides

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Multiple pathogenic elements, including reactive oxygen species (ROS), amyloid- $\beta$  ( $A\beta$ ) peptides, and transition metal ions, are implicated in the pathogenesis of Alzheimer's disease (AD) [1,2]. In addition, these pathological elements show their inter-connections which can aggravate the progression of AD [1-3]. For instance, redox-active transition metal ions can interact with  $A\beta$  to form metal-bound  $A\beta$  (metal- $A\beta$ ) that can overproduce ROS [1-4]. Therefore, the regulation of these multiple factors using multifunctional molecules is an effective approach towards attenuation of AD pathology [2]. In this presentation, we will report a rational strategy of designing small molecules with multi-reactivity towards free radicals, metal-free  $A\beta$ , and metal- $A\beta$ .

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P213

### Development of Fluorescent Probes for Cellular Imaging of Cu(I) and Colocalisation Studies with Alpha-Synuclein

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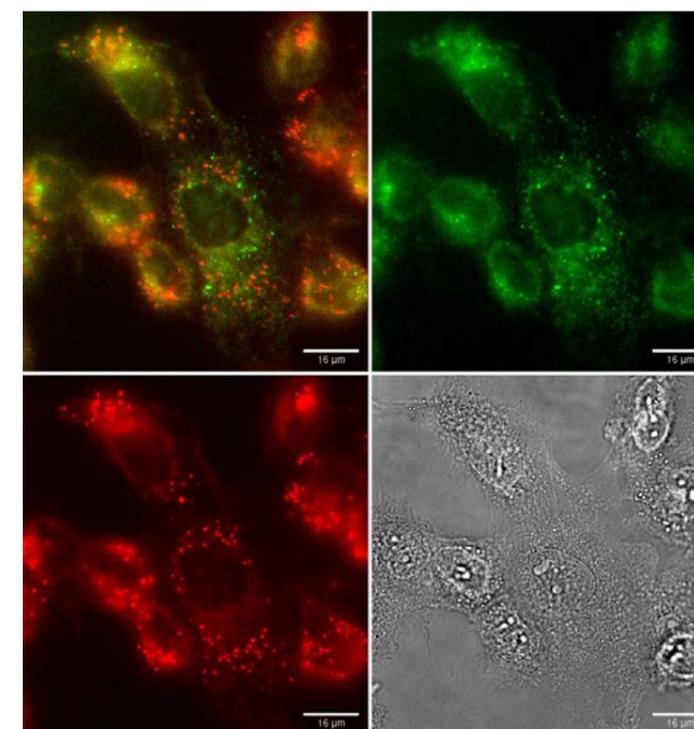
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Copper is essential for life and a faulty cellular copper homeostasis has been associated with the development of Parkinson's disease. In vitro studies have shown that even at physiological concentration copper may accelerate the rate of  $\alpha$ S aggregation.<sup>1</sup> Therefore, it has been proposed that misregulation of copper can lead to the aggregation of the protein  $\alpha$ -synuclein which causes cellular damage in neurons.<sup>2</sup>

Here we report the synthesis and characterization of a novel BODIPY based near-infrared fluorescent copper(I) probe. This probe has good photophysical properties for cellular imaging due to a selective 4-fold fluorescent turn-ON response to Cu(I) ( $\Phi = 0.65$ ) over other metal ions. It shows an absorption peak at 647 nm, a fluorescent emission at 661 nm. This new probe (as well as a previously reported one<sup>3</sup>) has been used to study the colocalization of copper(I) and  $\alpha$ -synuclein in N27 and SH-SY5Y cells. Financial support from the EPSRC is gratefully acknowledged.



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P214

### Copper-Ion Mediated Oxidation of $\beta$ -Amyloid

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Age-related neurodegenerative disorders affect millions of people worldwide, and Alzheimer's disease (AD) is the most frequent of this family of diseases. According to Alzheimer Disease International, every 3.2 seconds one new case is adding worldwide. However, no molecular level understanding of pathogenesis has been established, and there is neither cure nor reliable methods for early diagnosis. Oxidative stress coupled with metal-ion dyshomeostasis is of major mechanistic and physiological significance due to its direct involvement in reactive oxygen species (ROS) production and oxidative stress [1,2]. Oxidation products of the  $\beta$ -amyloid (A $\beta$ ) peptide, as well as metal-ions, are found in the senile plaques that are hallmarks of AD, leading to the hypothesis that redox-active metal ions may constitute a key element in the disease etiology [3].

We hypothesize that common pathogenesis exists for familial and sporadic AD, both depending on the chemical (amino acid mutations or oxidation, respectively) modification of A $\beta$ . In this study we report a "controlled metal-ion catalyzed oxidation [4]" to probe early molecular events related to oxidatively induced damages to A $\beta$ 16 wild-type, and two variants (A $\beta$ 16-A2T, and A $\beta$ 16-A2V). We have examined time-dependent oxidative events using Cu<sup>2+</sup>/AsCH/A $\beta$  resulting in mild extents of oxidation. We show that within 15 minutes of oxidative reaction, ~ 95% of parent peptides are oxidized. ESI-MS analyses showed distinct oxidized A $\beta$  species under mild oxidation within 3 minutes. LC/MS analysis demonstrated that the copper-coordinating histidine residues as a major target of oxidation. Importantly, N-terminal cleavage, oxidative cleavage, and oxidative decarboxylation, as well as up to 3 oxygen atoms adducts with parent peptides, were also identified as the products of Cu<sup>2+</sup>/AsCH<sup>+</sup> oxidation.

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P215

### Tunable Regulatory Activities of 1,10-Phenanthroline Derivatives towards Acid Sphingomyelinase and Zn(II)-Amyloid- $\beta$

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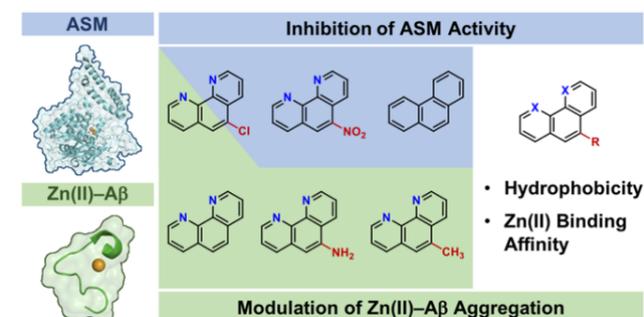
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Amyloid- $\beta$  (A $\beta$ ) peptides are proposed to be involved in the development of Alzheimer's disease (AD). For the greater control of A $\beta$ -involved pathology in AD, the regulation of both A $\beta$  clearance and metal-A $\beta$  complexation would be valuable. In this presentation, we will report a new series of small molecules able to achieve the tunability of modulatory activities against acid sphingomyelinase (ASM) and Zn(II)-bound A $\beta$  [Zn(II)-A $\beta$ ], two pathological targets found in the brain affected by AD. Rational tuning of the hydrophobicity and Zn(II) binding affinity of the 1,10-phenanthroline (**phen**) framework successfully yielded compounds as chemical modulators for ASM (**4** and **5**), Zn(II)-A $\beta$  (**phen**, **1**, and **2**), or both (**3**). Our overall approaches and findings will show promise for rationally engineering simple and small molecules as chemical modulators for single or dual pathological features (especially, metalloenzymes or metal-related amyloidogenic peptides) implicated in neurodegenerative diseases [1].



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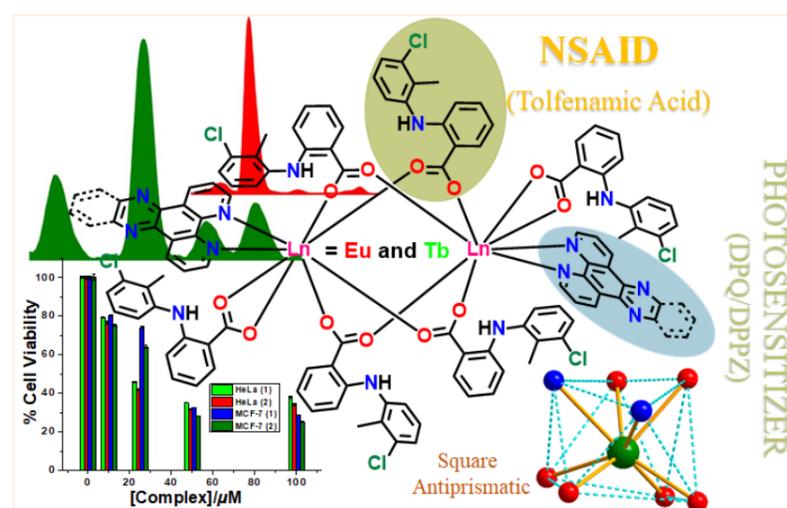
P220

### Isostructural Bimetallic Lanthanide Complexes of N,N'-Heterocyclic bases and Tolfenamic Acid: Structures, Photophysical Aspects and Biological Activity

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Lanthanide coordination complexes have widely been admired as emissive optical probes that could have the potential to be utilized for the better understanding of biological phenomena with minimal damage because of their fascinating optical properties from sharp emission spectral bands; large Stokes' shift and longer luminescence lifetimes ( $\mu\text{s}$ -ms) and photobleaching resistance. Eu(III) and Tb(III) has been the choice among most brightest visible light emissive Ln(III) complexes with longer lifetime [1]. Due to very low absorptivity of Ln(III), a sensitizing antenna of suitable energy needed to be used as a ligand or to be appended in the ligand structure. The N,N' donor heterocyclic bases have been well recognized as effective sensitizing antenna moiety for Ln(III) ions results in highly emissive Ln(III) bioprobes for investigating *in cellulo* distribution of various bioactive species (such as citrate and lactate anions) [2][3]. Non-steroidal anti-inflammatory drugs (NSAIDs) are the one among readily used analgesic, anti-inflammatory and antipyretic agents [4]. In the view of clinical utility of tolfenamic acid and photosensitization of heterocyclic bases, herein we deliberately synthesized four Eu(III) and Tb(III) complexes namely [Eu<sub>2</sub>(DPQ)<sub>2</sub>(TFA)<sub>6</sub>] (1), [Tb<sub>2</sub>(DPQ)<sub>2</sub>(TFA)<sub>6</sub>] (2), [Eu<sub>2</sub>(DPPZ)<sub>2</sub>(TFA)<sub>6</sub>] (3) and [Tb<sub>2</sub>(DPPZ)<sub>2</sub>(TFA)<sub>6</sub>] (4) with tolfenamic acid (TFA), a NSAID and N,N' donor ligands (dpq=dipyrido[3,2-d:2',3'-f]quinoxaline and dppz = dipyrido[3,2-a:2',3'-c]phenazine) and characterized with physicochemical and spectroscopic techniques. All the complexes have been structurally characterized by X-ray crystallography, showing an eight coordinated square anti-prismatic geometry around Ln(III) ion. We have tested these complexes for their biological utility with various biological targets. Cellular uptake and cytotoxicity studies in HeLa and MCF-7 were carryout to evaluate the potential use of these complexes for bio-imaging and therapeutic applications.



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P221

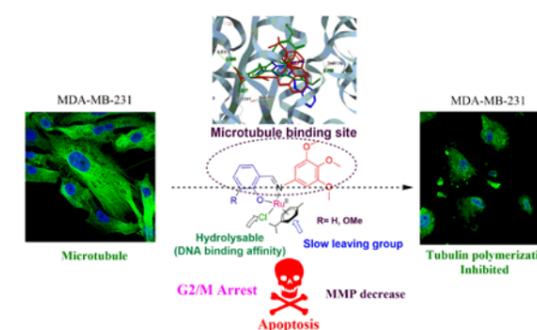
### Organometallic Ru<sup>II</sup> Complexes of Trimethoxyaniline Containing Schiff Bases: Synthesis, Structure, Stability and Inhibition of Tubulin Polymerization

Sourav Acharya<sup>1</sup>, Moumita Maji<sup>1</sup>, Raturaj<sup>2</sup>, Kallol Purkait<sup>1</sup>, Arnab Gupta<sup>2</sup>, Arindam Mukherjee<sup>1</sup>

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Ruthenium and Gallium have been two promising metals after Pt in successful development of metal based anticancer drugs. Among the ruthenium complexes tetrahedral organometallic half sandwiched Ru<sup>II</sup>-*p*-cymene complexes are widely studied because of the structural flexibility that allows development of metal complexes by tuning the bidentate ligands, arenes and halides[1,2]. The recent advances show the use of tubulin binding agents to Ru and Pt complexes to make them efficient inhibitor of tubulin polymerization[3,4]. However, ligands can be designed such that the overall complex itself inhibits tubulin polymerization. Microtubules, which are dynamic polymers of  $\alpha\beta$ -tubulin, play an essential role in mitosis, forming the dynamic spindle apparatus. Disruption of microtubule dynamics prevents microtubule function that ultimately leads to cell death thus making them efficient target for anticancer drugs. We designed a ligand keeping in mind the aromatic trimethoxy motif in colchicine (a microtubule polymerization inhibitor) to suppress the dynamics of the mitotic spindle leading to arrest in mitotic phase and cell death. We synthesized four N-O coordinating Ru<sup>II</sup> complexes, (1-4) bearing formula [Ru<sup>II</sup>(*p*-cym)(L)Cl], that have the affinity to bind at the tubulin active site that could distort or inhibit microtubule polymerization. All the complexes showed *in vitro* antiproliferative activity (IC<sub>50</sub> range 10-20  $\mu\text{M}$ ) against various cancer cells including the difficult to treat triple negative human metastatic breast adenocarcinoma (MDA-MB-231). The ESI-MS and <sup>1</sup>H NMR data shows that the complexes are stable in aqueous solution at pH 7.4 (4 mM NaCl) with less than 10% hydrolysis for at least 24 h. The immunofluorescence studies show that the complexes inhibit tubulin polymerization at IC<sub>50</sub> dose in the metastatic MDA-MB-231 cells and induce apoptosis by arresting the cell cycle in G2/M phase.



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P222

### Synthesis and Biochemical Study of some Heterocyclic-Azo Compounds and their Copper (II) Complexes

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Biologically active azo dye ligands, L<sub>1</sub> “1-[4-Amino-3-(benzothiazol-2-ylazo)-phenyl]-ethanone, 2-(Benzothiazol-2-ylazo)-4-methyl-phenylamine” and L<sub>2</sub> “2-(Benzothiazol-2-ylazo)-4-nitro-phenylamine”, have been synthesized by diazo-coupling reaction at 0-5 °C. The synthesized azo dye ligands were employed for complexation with Cu (II) metal ion. The synthesized compounds were studied theoretically and characterized by physical, spectral and analytical analysis. The thermal studies have been undertaken as an additional information to confirm the structure of the studied complexes and to study its thermal stability. Molar conductivity of the synthesised complexes in DMSO has indicated the non-electrolytic nature of ligands and their Cu (II) complexes. Molecular docking and binding studies of all the synthesized complexes against CT-DNA have been performed.

Financial support by the Qassim University is gratefully acknowledged.

P223

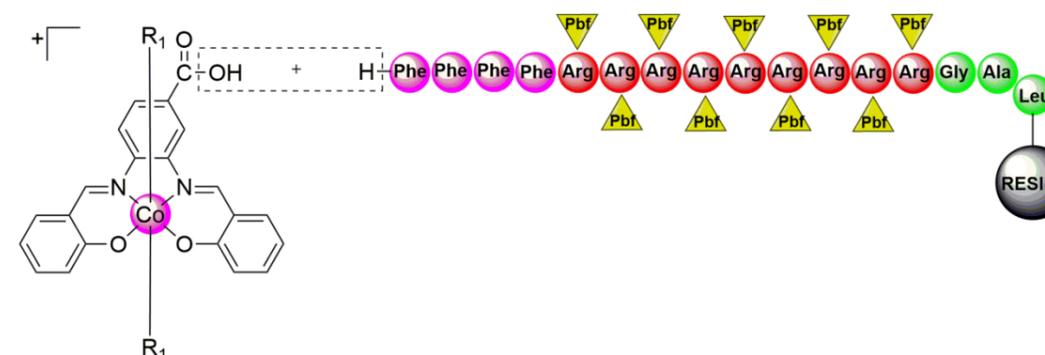
### Co(III)-Peptide Bioconjugates as Promising Anticancer Agents

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Recently, cobalt complexes have gained increased attention as promising alternatives in comparison to platinum-based drugs, which often result in severe side effects. From a chemical and physical point of view cobalt, similar to platinum, adopts a wide variety of coordination numbers, geometries, oxidation states, and ligand binding affinities, which has led to an interest in cobalt chemistry in the pursuit of alternative anticancer agents. Interestingly, cobalt(III) complexes exhibit different mode of action than platinum(IV) prodrugs. A relatively inert oxidised cobalt(III) state is reduced to very labile cobalt(II) facilitating exchange of axial ligands. Binding of the complexes to important histidine residues in active sites of proteins cause irreversibly inhibition of activity. Cobalt(III) complexes inhibit histidine-containing proteins and enzymes including zinc finger transcription factors (TFs) and metalloendopeptidases [1,2].

In this work, promising Co(III) complexes were coupled to cell penetrating peptides in order to increase the cytotoxic potential (See figure). The Schiff base ligand H<sub>2</sub>salophen was used for complexation to CoCl<sub>2</sub>·6H<sub>2</sub>O under nitrogen. In the next step, six-coordinate Co(III) complexes were synthesized with imidazole, 2-methylimidazole and N-Boc-L-histidine methyl ester in axial positions. All Co(III) complexes were characterized by multinuclear NMR spectroscopy (<sup>1</sup>H, <sup>13</sup>C and <sup>59</sup>Co NMR), FT-IR, mass spectrometry and HPLC. The Co(III) complexes were conjugated to three different cell penetrating peptides: FFFF, RRRRRRRRRGAL and FFFFRRRRRRRRRGAL. Standard solid-phase peptide chemistry was used for the synthesis of cell penetrating peptides [3,4]. Coupling of N-terminal peptides with the cobalt complexes, possessing carboxylic group on tetradentate Schiff base ligand, afforded Co(III)-peptide bioconjugates, which were purified by semi-preparative HPLC and characterized by analytical HPLC, ESI-MS and MALDI. The anticancer activity of the synthesized compounds was studied against different human tumor cell lines: lung cancer A549, liver cancer HepG2 and normal human fibroblasts GM5657T, in comparison with the activity of cisplatin as a reference drug.



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P224

**A New Entry into Multi-Action Platinum(IV) Anticancer Agents: Gemcitabine as an Example for Attachment of Hydroxyl Groups via a Carbonate Linkage**

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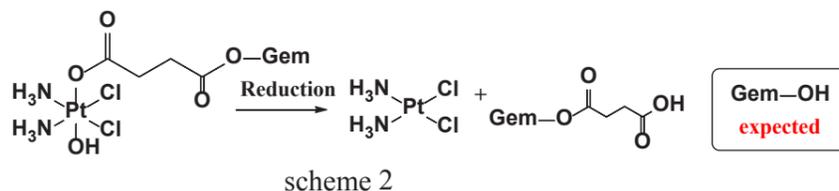
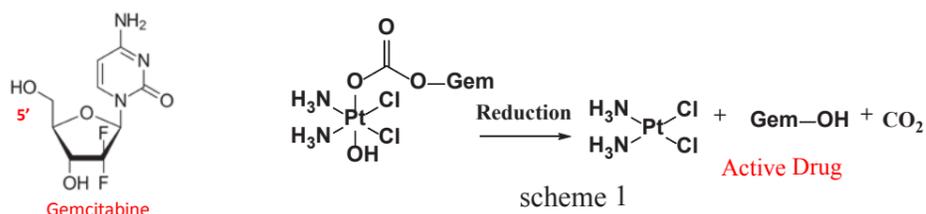
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Despite the progress that was made in the chemistry of Pt(IV) anti-cancer prodrugs [1,2], most of the ligands which are reported in multi action Pt(IV) prodrugs are carboxylic acids, which are tethered to the axial position of the Pt(IV) via a carboxylate [3]. Gemcitabine (Gemzar – figure) is a nucleoside analog that acts as an antimetabolite and is frequently administered with Cisplatin in the clinic as treatment against various tumors. Since it does not possess any carboxylic group, attaching it to the Pt(IV) complex in a way that will release the active form of the drug, is not straightforward. [4].

Here, we describe a novel approach for attaching an OH containing drug (in this case via the 5'-OH of gemcitabine) to the axial position of Pt(IV) via a carbonate linkage. Shortly after reduction of the Pt(IV) complex, the carbonate linkage is cleaved from the complex followed by rapid loss of CO<sub>2</sub> (decarboxylation) generating the active drug (scheme 1).

For comparison, we also linked the Pt(IV) complex with gemcitabine via a succinate linkage (traditional way for attaching ligands that lack carboxylic group). Following reduction, the succinate linkage is cleaved from the Pt(IV) complex. However, in contrast to the carbonate linkage, only the Gem-succinate conjugate was observed with no free gemcitabine detected (scheme 2).

The carbonate bridged triple-action ctc-[Pt(NH<sub>3</sub>)<sub>2</sub>(PhB)(O<sub>2</sub>C-Gem)Cl<sub>2</sub>] was significantly more cytotoxic than the succinate bridged ctc-[Pt(NH<sub>3</sub>)<sub>2</sub>(PhB)(O<sub>2</sub>CCH<sub>2</sub>CO-Gem)Cl<sub>2</sub>] in a pair of ovarian cancer cell lines (A2780 and A2780cisR). In vivo data shows that the carbonate bridged complex was more potent and less toxic than gemcitabine or cisplatin. This new synthesis approach can pave the way for new unthought-of ligands that can be attached to the Pt(IV) complex.



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P225

**Improvement of Bioanalytical Sensitivity and COX-2-Selective Antitumor Activity of Cobalt Alkyne Complexes Due to Ligand Fluorination**

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[(Prop-2-ynyl)-2-acetoxybenzoate]dicobalthexacarbonyl (Co-ASS) is a metal complex that demonstrated promising growth-inhibitory potency against certain tumor cell lines [1]. The inhibition of both cyclooxygenase isoenzymes (COX-1 and COX-2) is assumed as probable mode of action. However, the selective inhibition of COX-2 is aimed because it plays a significant role in carcinogenesis, while COX-1 mainly regulates homeostatic functions [2].

With the objective of generating COX-2-selective compounds, the approach to introduce a fluorine substituent within the aromatic moiety of Co-ASS was followed as a frequently applied strategy in medicinal chemistry [3]. Moreover, it was intended to exploit the fluorination for the purpose of bioanalytical labeling.

The influence of the fluorine substituent on the cytotoxic and the antimetabolic properties against colon (HT-29) and breast cancer cell lines (MDA-MB-231, MCF-7) was determined. The inhibition of both cellular and isolated COX as well as the induction of the apoptosis-related caspases 3/7 were evaluated. Additionally, cellular uptake studies were conducted based on the sequential analysis of both cobalt and fluorine performing high-resolution continuum-source atomic respectively molecular absorption spectrometry (HR CS AAS / MAS).

The compounds exhibited a remarkably less activity in the COX-1/2-negative MCF-7 cell line, while they reduced the cell biomass in the COX-1/2-positive HT-29 and MDA-MB-231 cells in the low micromolar range and showed a concentration-dependent antimetabolic activity. Fluorination effected a preferential inhibition of COX-2 compared to Co-ASS. Furthermore, it was demonstrated in cellular uptake studies that an about 5-fold higher bioanalytical sensitivity (HR CS MAS technique) can be achieved when using fluorine as tracer instead of cobalt.

Fluorinated cobalt alkyne complexes derived from Co-ASS were synthesized and characterized as promising antitumor active agents. The outcomes confirm that the interaction with the COX cascade can be assumed as mode of action and fluorination shifted the selectivity towards COX-2. Moreover, the measurement of fluorine using HR CS MAS was proved as a highly sensitive method for the quantification of fluorinated cobalt complexes in biological samples.

Financial support by the “Tiroler Wissenschaftsfonds (TWF)” (GZ: UNI-0404/1982) is gratefully acknowledged.

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P226

### Insights Into the Anticancer Therapeutic Properties of Cu(II) Complexes of Phenanthroline and Histidine Containing Ligands

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Cancer has been the second cause of death in the world for men and women. [1] The best-known anticancer metaldrug is Cisplatin, a planar Pt<sup>2+</sup> complex approved by the FDA in 1978. Since then the community of bioinorganic chemistry has intensify the research on anticancer therapy in order to overcome the limitations of the platinum based metalodrugs, mainly the high level of toxicity of these systems that leads to undesired side-effects. [2] Copper has become an alternative to complexes of platinum and platinum group metals as anticancer drugs. [3,4,5] This versatile biometal has borderline Lewis acid properties [6] and therefore can bind to different donor atoms forming complexes with diverse coordination numbers and geometries, and distinct redox properties.

Two copper complexes based on phenanthroline and histidine containing ligands were synthesized and characterized by different methods (potentiometry, spectroscopy, mass spectrometry, electrochemistry and DFT calculations). [7] These complexes have high stability in aqueous solution and are redox active (Cu<sup>2+</sup>/Cu<sup>+</sup>). DNA cleave studies indicate low redox based nuclease activity and cytotoxic studies using MCF7 and A2780 cancer cell lines reveal moderate IC<sub>50</sub> values. To further understand their properties and optimize the systems, uptake studies and production of reactive oxygen species inside the cancer cells were carried out. These data will be presented and correlated with the mild cytotoxic activity against different cancer cell lines.

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P229

### Nanofocussed X-Ray Mapping of Organo-Osmium Anticancer Complexes in Cancer Cells

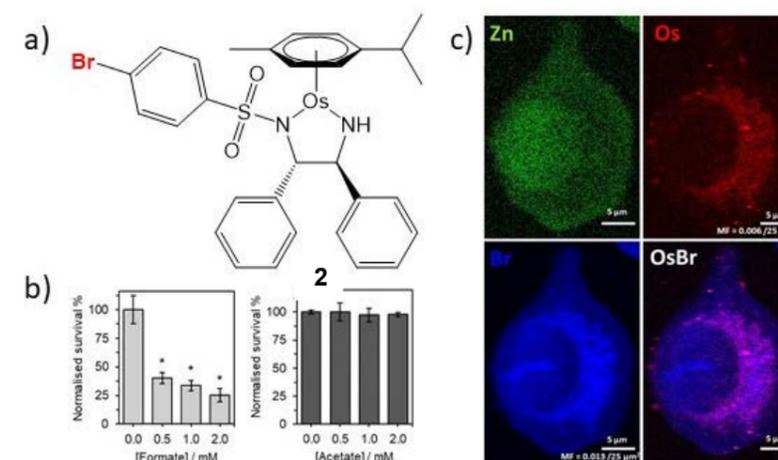
Elizabeth M. Bolitho<sup>1,2</sup>, James. P. C. Coverdale<sup>1</sup>, Hannah E. Bridgewater<sup>1</sup>, Guy J. Clarkson<sup>1</sup>, Paul Quinn<sup>2</sup>, Peter J. Sadler<sup>1</sup>, Carlos Sanchez-Cano<sup>3\*</sup>

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Organo-osmium asymmetric hydrogenation catalysts show promising anticancer activity. [1-3] Complex **1** [Os(n<sup>6</sup>-*p*-cym)(TsDPEN)], where *p*-cym = *para*-cymene and Ts(Me)DPEN = *p*-methyl tosyl diphenylethylenediamine can catalyze the intracellular enantioselective reduction of pyruvate (a key intermediate in cell metabolism) to unnatural D-lactate, in the presence of non-toxic concentrations of formate (**Fig. 3**), and is highly dependent on the chirality and coordination of the TsDPEN ligand.<sup>[1]</sup> This provides an opportunity for selectivity through catalytic modes of action by exploiting the inherent redox vulnerabilities of cancer cells. The efficiency of the intracellular catalysis, the complex stability and the mechanism(s) of action of this drug candidate have been explored by incorporation of a chemical probe (Br) to a peripheral site of **1** (**2**, **Fig. 1a**) and use of synchrotron x-ray fluorescence to map simultaneously the concentration-dependent intracellular localization and quantification of osmium (L<sub>3</sub>-edge) and bromine (K-edge) in A549 human lung cancer cells. Osmium co-localizes with bromine in the cytoplasm, implying that some of the chelated-ligand is coordinated to the osmium (**Fig. 3c**). Hence it appears that osmium does not target nuclear DNA, in contrast to clinical platinum drugs, for which resistance and selectivity are major clinical concerns. Interestingly, bromine also co-localized with zinc in the nucleus, implying that partial dissociation of chiral TsDPEN from osmium and potential binding to biomolecules in the nucleus, may occur. Time-dependent accumulation of osmium and bromine in lung cancer cells treated with **2** has been investigated using an alkaline digestion method and ICP-MS.



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P230

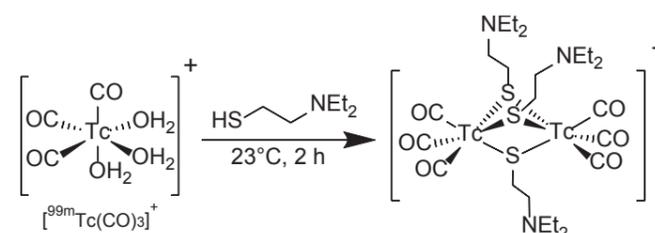
### Dinuclear $^{99m}\text{Tc}$ complexes do exist!

Robin Bolliger, Henrik Braband, Giuseppe Meola, Roger Alberto

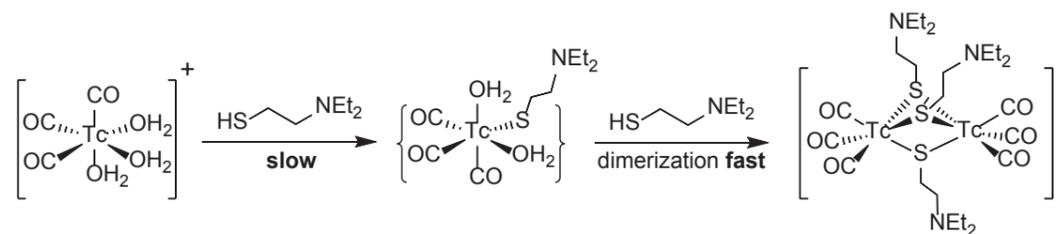
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$^{99m}\text{Tc}$  (half-life time  $t_{1/2} = 6\text{h}$ ), the metastable form of the  $^{99}\text{Tc}$ -isotope ( $t_{1/2} = 221\text{ky}$ ), is one of the most widely used radionuclide in nuclear medicinal diagnostic. Due to its nearly ideal decay properties, such as half life time and decay energy of 140 keV, it is perfectly suited for Single Photon Emission Computed Tomography (SPECT).<sup>[1]</sup>

All  $^{99m}\text{Tc}$  applications start with  $[\text{}^{99m}\text{TcO}_4]^-$  from the elution of a  $^{99}\text{Mo}/^{99m}\text{Tc}$  generator with saline. The  $^{99m}\text{Tc}$ -concentrations are very small ( $10^{-7} - 10^{-9}\text{M}$ ) and therefore the formation of di- or polynuclear complexes is kinetically unfavoured and there was no report about the formation of a dinuclear  $^{99m}\text{Tc}$ -complex.<sup>[2]</sup> All reported  $^{99m}\text{Tc}$ -compounds are mononuclear, despite numerous reports of polynuclear  $^{99}\text{Tc}$ -compounds.<sup>[3,4]</sup> Different reaction conditions on the tracer ( $^{99m}\text{Tc}$ ) and on the macroscopic level ( $^{99}\text{Tc}$ ) might be responsible. Ligand concentrations are usually in  $10^3 - 10^6$  fold excess over  $^{99m}\text{Tc}$ , while equimolar concentrations with  $^{99}\text{Tc}$  apply. As there was no report of a di- or polynuclear  $^{99m}\text{Tc}$  complex, such complexes are believed to be synthetically inaccessible.



With appropriate bridging ligands (herein thiols HS-R) and a suitable  $^{99m}\text{Tc}$ -precursor  $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ , we demonstrate that dinuclear  $^{99m}\text{Tc}$ -complexes are formed at room temperature and at nanomolar concentrations. The dinuclear nature of  $[\text{}^{99m}\text{Tc}_2(\mu_2\text{-SR})_3(\text{CO})_6]^-$  was assessed by chromatographic comparison with its rhenium homologue and with its true  $^{99}\text{Tc}$  analogue. Kinetic studies showed that the rate limiting step is not the dimerization, but the formation of a mononuclear  $^{99m}\text{Tc}$ -thiol complex, which rapidly dimerizes.<sup>[5]</sup>



Financial support by the University of Zurich is gratefully acknowledged.

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P231

### Cytotoxic Properties of Novel $\eta^6$ -p-Cymene Ruthenium(II) Complexes Containing a N,S-type Ligand and their Structural Characterization

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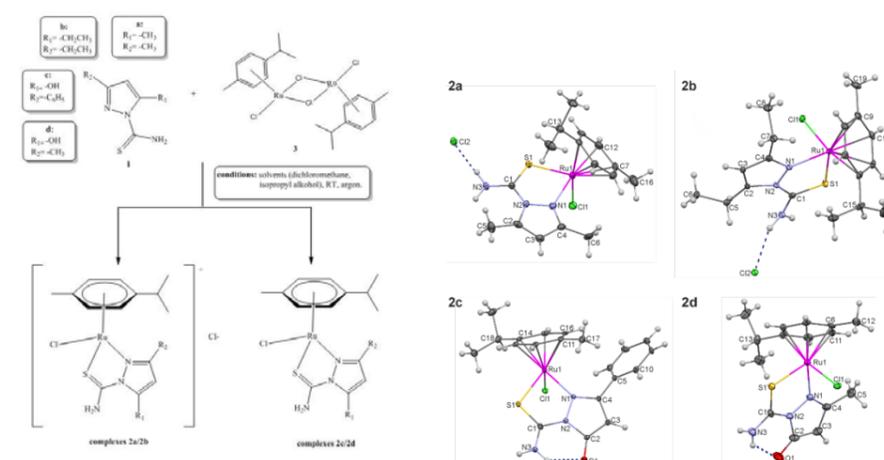
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In this study new pyrazole carbothioamide derivatives and their four arene-ruthenium(II) complexes were synthesized. The title compounds were characterized by IR,  $^1\text{H}$  NMR, mass spectrometry, elemental analysis and X-ray diffraction.

The cytotoxic effect against three cancer cell lines: HL-60, NALM-6, WM-115 and normal human foreskin fibroblasts (HFF-1) was investigated using MTT assay. The highest antitumor activity has been observed for complex **2d** against WM-115 cell line ( $\text{IC}_{50} = 7.99 \pm 0.87\ \mu\text{M}$ ), while complex **2c** was the most active against NALM-6 cell line ( $\text{IC}_{50} = 11.71 \pm 1.62\ \mu\text{M}$ ). Moreover, it has been proved that chosen ruthenium(II) complexes **2b**, **2d** can play similar role as nucleases by cleavage the DNA Form I into Form II and Form III. It seems that both complexes are able to cut the DNA strand at two positions.



Financial support from Medical University of Lodz (grant No 503/3-066-02/503-31-001 to E. Budzisz).

P232

### An Anthracenyl-Pendant Ruthenium(II) Complex Conjugated to Biotin as a Biological Bait for Pharmacological Applications

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Ruthenium polypyridyl complexes have shown promise as agents for photodynamic therapy (PDT) [1], but they usually lack site selectivity or a better cellular uptake. Biotin has been conjugated to drugs as a strategy to provide selective delivery to cancer cell [2]. Previously, our lab prepared a new ruthenium(II) polypyridyl complex with dual action, an enhanced singlet oxygen production along with strong DNA binding [3]. These properties were achieved by combining an anthracenyl-pendant bipyridine ligand and a phenazine ligand (dppz), which originated the complex  $[\text{Ru}(\text{bpy-anth})(\text{bpy})(\text{dppz})](\text{PF}_6)_2$  (bpy-anth = 4-amidoanthracenyl-4'-methyl-2,2'-bipyridine; dppz = dipyrido[3,2-a:2',3'-c]phenazine). Aiming to improve activity against bacteria and cancer cells, we further incorporate biotin to a bipyridine ligand, and prepared  $[\text{Ru}(\text{bpy-anth})(\text{bpy-biot})(\text{dppz})](\text{PF}_6)_2$  (bpy-biot = 4-(N-((2-biotinamido)ethyl)amido)-4'-methyl-2,2'-bipyridine). This compound was fully characterized and its photophysical and electrochemical properties investigated. Photophysical studies showed that upon excitation, the complex exhibited a metal-to-ligand charge transfer (<sup>3</sup>MLCT) emission at 631 nm with quantum yield of 0.07 in acetonitrile. Interestingly, this compound exhibited a quantum yield for singlet oxygen production of 0.75, along with very high DNA binding ( $\text{Log}K_b = 6.80$ ), supporting biotin incorporation was not deleterious. This photochemical behavior could be ascribed to the lower triplet state involving the anthracenyl-modified bipyridine associated to an easier oxygen quenching. Moreover, this complex showed a high lipophilicity ( $\text{log } P = 1.32$ ), which is close to the range assumed for oral and intestinal absorption. Altogether, these results make this complex as a promising cytotoxic agent.

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P235

### Supramolecular Cylinders and Pillarplexes: Their DNA Binding Properties and Biological Effects

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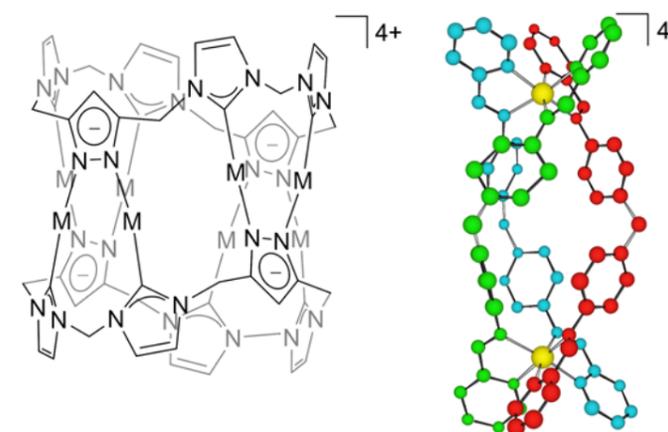
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Tetracationic metallo-supramolecular cylinders' target non-canonical Y-shaped DNA and RNA structures, through their core cylindrical structure,  $\pi$ -stacking interactions and electrostatic interactions. [1] These cylinders have been shown to have a substantial impact on biological systems. [2] The effects of varying the metal centres and organic framework of these cylinders on their DNA/RNA binding and biological effects have been investigated and are described.

Pillarplexes are tetracations with a similar shape and dimensions to the metallo-supramolecular cylinders but are shorter in length (1 nm wide by 1 nm length c.f. 1 nm wide by 2 nm in length). [3] They also have the ability to form similar intermolecular interactions such as hydrogen bonding,  $\pi$ -stacking and electrostatic interactions. The canonical and non-canonical DNA binding of these pillarplexes to DNA are described using LD, CD, Agarose/PAGE gel electrophoresis, DLS, AFM, UV-Vis and Emission. We will also present analysis of their biological effects.



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P236

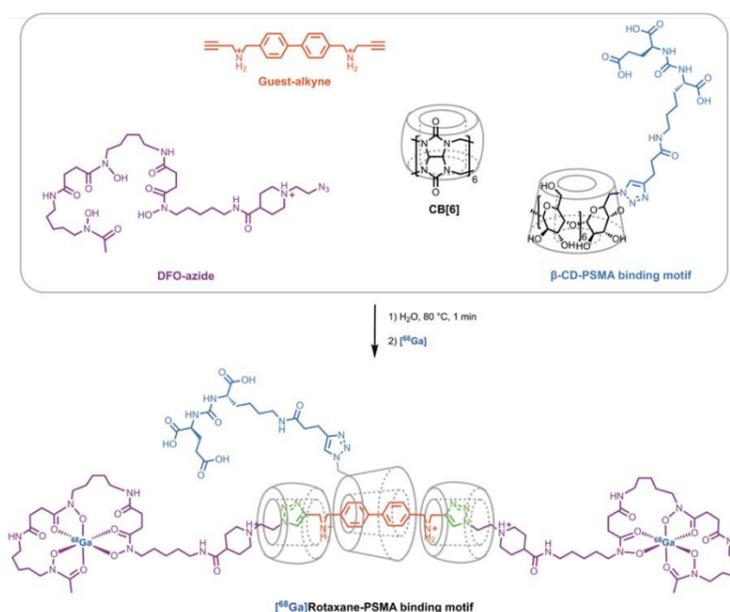
### Synthesis of Targeted Supramolecular Radiotracers by Cooperative Capture

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$\beta$ -cyclodextrins ( $\beta$ -CD) and cucurbituril (CB[6]) are two supramolecular host molecules that have the ability to trap molecules through molecular recognition in their unique hydrophobic cavities. These macrocycles are involved in a cooperative capture approach used for the development of rotaxanes (mechanically interlocked molecules) in a one-pot reaction strategy.[1] Taking advantage of the ability of CB[6] to catalyse the azide-alkyne cycloaddition (CB-AAC) [2], we aim to develop an efficient and modular synthetic tool for the synthesis of targeted non-covalently bound radiotracers. For our purpose, the advantage of adding  $\beta$ -CD is two-fold: (i)  $\beta$ -CD acts as a co-catalyst in the CB-AAC by forming a hydrogen bonding network with two adjacent CB[6] rings that accelerates the cycloaddition step,[1] and (ii)  $\beta$ -CD can be derivatised to introduce a targeting vector, such as the prostate membrane specific antigen (PSMA) binding motif, Gly-NH-C(O)-NH-Lys. Using this strategy, we successfully synthesised a rotaxane within one minute in H<sub>2</sub>O by mixing DFO-azide, CB[6], guest-alkyne and  $\beta$ -CD-PSMA binding motif in a 2:2:1:1 ratio at 80 °C. Radiolabelling reactions led to the preparation of [<sup>68</sup>Ga]rotaxane as a unprecedented, non-covalently bound targeted radiotracer in high radiochemical yield and purity. Further optimisation of the one-pot cooperative capture and radiolabelling method as a rapid approach to construct supramolecular radiotracers is advancing and the biological properties of targeted [<sup>68</sup>Ga]rotaxanes are under evaluation in cellular assays and *in vivo*.

We thank the Swiss Government Excellence Scholarship scheme for a student award (FdO), the Swiss National Science Foundation (SNSF Professorship PP00P2\_163683), the Swiss Cancer League (Krebsliga Schweiz; KLS-4257-08-2017), and the University of Zurich (UZH) for financial support.



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P237

### Recognising Unusual DNA Structures

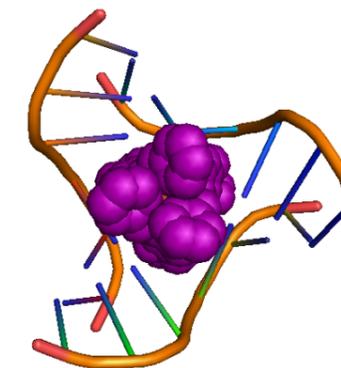
Ross Egan<sup>1</sup>, Michael Hannon<sup>1</sup>

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Non-canonical DNA targeting is an exciting way to disrupt DNA behaviour. Three-way junctions have been successfully targeted with the use of a triple stranded helicate, that owes its effectiveness to its accommodating dimensions and ability to  $\pi$ - $\pi$  stack with the DNA bases.<sup>1</sup> Use of ruthenium in the metal centres has resulted in improved stability upon the original iron cylinder.

Further developments on improving the stability of the cylinder, specifically a new ligand and consequently a new collection of complexes will be discussed, with interesting revelations for nickel centered complexes.

Quadruplex DNA often occurs near to Y-shaped DNA forks which are very similar in structure to the Y-shaped DNA three-way junction.<sup>2</sup> Could we take advantage of this locality by targeting in tandem?



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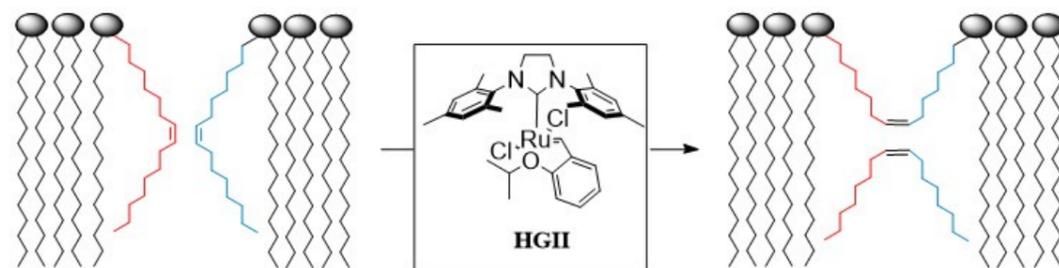
P238

### Olefin Metathesis in Antibiotic Research

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A recent WHO report reveals an alarming degree of resistance of common bacteria against antibiotics, which also includes “last resort” antibiotics. This could lead to some life-threatening diseases, caused by infections of e.g. *K. pneumonia*, becoming incurable.<sup>1</sup> In addition to raising awareness of the proper use of these pharmaceuticals and prevention of (hospital-acquired) infections, the development of new antimicrobial and antibiotic agent is inevitable to solve this problem. In this project the search for new antibacterial agents is approached by catalytic conversion in or on cells. The cell membrane was chosen as the main target with phospholipids as substrates. *Cis*-unsaturated fatty acids contribute to the fluidity of the cell membrane, which holds important enzymes e.g. of the respiratory chain. These double bonds were chosen for modification reactions with the intention to promote a disruption of the fluidity and therefore a loss of vital enzymes.<sup>2</sup> In olefin metathesis the residues of a double bond undergo an exchange reaction with a metallocyclobutane intermediate. When this reaction is performed on double bonds in lipids, a shortened lipid dimer would be formed, that will have a reduced mobility compared to the substrate. A major advantage of this reaction is, that it can be performed at moderate temperatures and in different solvents by a series of Ru-based catalysts (Grubbs-type catalysts). The Hoveyda-Grubbs catalyst of the 2<sup>nd</sup> generation (**HGII**) is the most suitable catalyst for biological application due to its water and oxygen stability as well as its high activity.<sup>3</sup> The catalyst will be modified at its NHC ligand to enable conjugation reactions with a membrane-targeting peptide (RWRWRW) that is selective to bacteria.<sup>4</sup> We have been able to prove the activity of **HGII** on several phospholipids in methanol in inert atmosphere. On the poster, we will discuss various metathesis experiments both on lipids in general and on liposomes. These studies are performed on a molecular level in terms of substrate conversion as well as a macroscopic level regarding the size of the liposome and the fluidity of the membrane.



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P239

### Synthesis, DNA-Binding and Anticancer Properties of Enantiomerically pure Ru (II) Helicates

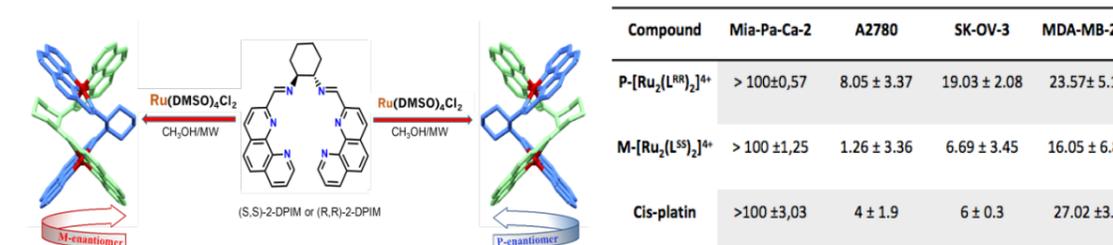
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Medicinal inorganic chemistry has made a great effort in designing new synthetic agents for the treatment of diseases such as cancer, taking advantage of the diverse geometries and coordination numbers of metals and the wide range of available ligands. [1] Helicates have shown interesting structural properties for DNA modification, due to their cationic charge and intrinsic chirality. The “topological” character that the recognition phenomena between helicate and DNA has, is based on the establishment of specific non-covalent interactions.[2] However, most of the helical complexes already reported are racemic, and they can be separated into their enantiomers by difficult processes. Chiral ligands having at least one optically active center, make possible the formation of enantiopure and stable  $\Delta\Delta$  or  $\Lambda\Lambda$  helicates. [3]

In this work, we present the synthesis of two enantiopure Ru(II) helicates, M-[Ru<sub>2</sub>(L<sup>SS</sup>)<sub>2</sub>]<sup>4+</sup> and P-[Ru<sub>2</sub>(L<sup>RR</sup>)<sub>2</sub>]<sup>4+</sup>, which were prepared using bis-phenanthroline ligands bearing (S,S) or (R,R)-*trans*-1,2-diaminocyclohexyl as chiral spacers. The effect of the chirality of the enantiomers on the affinity to bind DNA was studied using canonical B-DNA and non-canonical 3WJ DNA.



Differences in the interaction mode between the enantiomers and DNA were studied by gel electrophoresis, UV-vis., NMR, molecular dynamics, T-melting, CD and LD. The junction of those techniques is clear to show the enantioselective character in the binding phenomena. Consequently, the M-enantiomer has a stronger affinity for DNA, compared to the P-enantiomer. To verify if enantioselectivity has an impact on the cellular viability, the helicates were tested on four cancer cell lines by the MTT assay: A2780, SK-OV-3 (ovarian), MD-MBA-231 (breast) and MIA-PACA-2 (pancreas). Consistently, the M-[Ru<sub>2</sub>(L<sup>SS</sup>)<sub>2</sub>]<sup>4+</sup> helicate showed the highest inhibitory effect on ovarian cancer cells, according to results of ROS induction and cell cycle by flow cytometry.

Acknowledgments: Fondecyt Regular # 1190763

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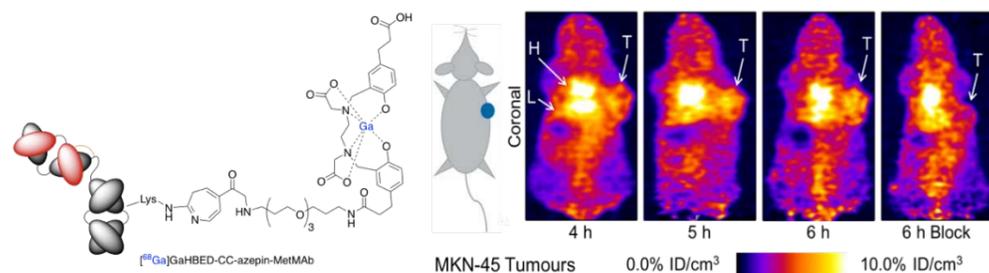
P241

### Photochemical Conjugation of HBED-CC-ArN<sub>3</sub> to MetMab for PET Imaging of c-MET Receptor Expression

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Using a non-traditional method of photochemical conjugation, a c-MET targeting <sup>68</sup>Ga radiotracer was designed and studied. First, a photoactive aryl-azide derivative of the hexa-dentate <sup>68</sup>Ga coordinating chelate, HBED-CC, was synthesised. The modified aryl-azide chelate (HBED.CC-ArN<sub>3</sub>) was photoconjugated to the anti c-MET engineered monoclonal antibody, MetMab, with a photochemical conversion of 18.5 ± 0.5% (*n* = 2). The purified bioconjugate was radiolabelled in acidic conditions (pH 4.4) with <sup>68</sup>Ga. The [<sup>68</sup>Ga]GaHBED-CC-azepin-MetMab radiotracer retained its radiochemical purity upon incubation with human serum albumin and the immunoreactivity of the radiotracer was determined in different c-MET expressing cell lines (MKN-45 [high expression] and PC-3 [low/moderate expression]). [<sup>68</sup>Ga]GaHBED-CC-azepin-MetMab was investigated as a c-MET PET imaging agent *in vivo* in both MKN-45 and PC-3 tumour models. Tumour specific uptake of [<sup>68</sup>Ga]GaHBED-CC-azepin-MetMab was observed in c-MET-positive MKN-45 (high-expression) tumours with tumour-associated activities at 6 h post-administration of 10.3±1.3 %ID/g (*n* = 5). In competitive blocking experiments in MKN-45 tumour models, tumour uptake decreased by approximately 55% (4.6±0.7 %ID/g (*n* = 5), *P*-value < 0.001 compared with normal group) confirming the specific radiotracer binding to c-MET *in vivo*. In conclusion, we demonstrated that a novel photoactive chelate HBED-CC-ArN<sub>3</sub> is a viable reagent for photoradiochemical synthesis of <sup>68</sup>Ga<sup>3+</sup> radiotracers based on engineered proteins.



JPH thanks the Swiss National Science Foundation (SNSF Professorship PP00P2\_163683), the Swiss Cancer League (Krebsliga Schweiz; KLS-4257-08-2017), and the University of Zurich (UZH) for financial support. This project has received funding from the European Union's Horizon 2020 research and innovation programme / from the European Research Council under the Grant Agreement No 676904, ERC-StG-2015, NanoSCAN.

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P242

### Anticancer Mechanistic Study of Auranofin

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Auranofin (AuRF) is an orally administered gold(I) therapeutic, approved by the US Food and Drug Administration (FDA), used for treatment of rheumatoid arthritis. In 1979, AuRF was shown to exhibit anticancer properties [1]. In the past few years, AuRF entered clinical trials as anticancer drugs for ovarian cancer, lung cancer and leukemia. However, as up to now, the mechanism of actions (MoAs) of AuRF has not been clearly realized. In the present study, we employed an optimized subcellular fractionation (SCF) [2] method in combination with HPLC-MS/MS technique for proteomics analysis [3] of AuRF-treated and vehicle-treated cancer cells. SCF technique was used to separate and enrich organellar proteins prior to proteomic analysis. This SCF-proteomics approach increased proteome coverage by enrichment of low-abundance organellar proteins, while simultaneously providing information on protein subcellular location. The nuclear sub-proteomes obtained by SCF were subjected to transcriptional regulators (TRs) analysis; while the merged global proteome data were used for KeyNode-mediated pathway analysis [4] by using bioinformatics tools to help understanding the MoAs of AuRF. The findings from SCF-proteomics and bioinformatics analysis will be discussed. Financial support by the Special Equipment Grant from the University Grants Committee of the Hong Kong Special Administrative Region, China (Project Code: SEG\_HKU02), The Hong Kong Jockey Club Charities Trust (HKJCCT) and Department of Health, HKSAR is gratefully acknowledged.

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P244

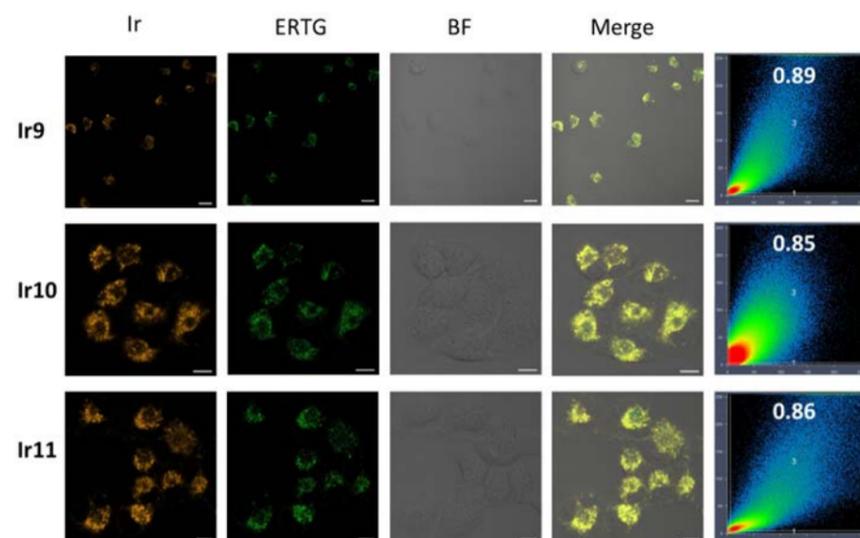
### Multi-Organelle Dysfunction by Endoplasmic Reticulum-Targeting Iridium(III) Complexes

Ruilin Guan<sup>1</sup>, Lina Xie<sup>1</sup>, Yu Chen<sup>1</sup>, Liangnian Ji<sup>1</sup>, Hui Chao<sup>1</sup>

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Endoplasmic Reticulum (ER) is a universal organelle served in the synthesis, folding and transportation of proteins and lipids. Turbulences in ER could cause ER stress and initiate a unique type of apoptosis, which could be transferred and affect other organelles due to ER-related organelle interactions through redox imbalance, ion-flux and protein pathways. Herein, we present a series of tridentate iridium(III) complexes as ER-targeting agents and study their anticancer properties in drug-resistant lung cell line A549R. These compounds localized in the endoplasmic reticulum preferentially, leading to a redox disorder termed ER stress and the leakage of calcium ions, which cascaded in the circulation of ER and mitochondria. The accumulated reactive oxygen species (ROS) during the assaults by the compounds could cause oxidative stress in mitochondria and affect lysosomes and cellular membrane. The fatal multi-organelle dysfunction induced by **Ir1-Ir3** suggested their potential application as anti-cancer drugs to drug-resistant cancer.

This work was supported by the National Science Foundation of China (No. 21525105, 21778079), the 973 Program (No. 2015CB856301), and the Ministry of Education of China (No. IRT-17R111).



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P246

### <sup>89</sup>ZrDFO-azepin-MetMAB for Imaging c-MET Expression in Gastric Cancer

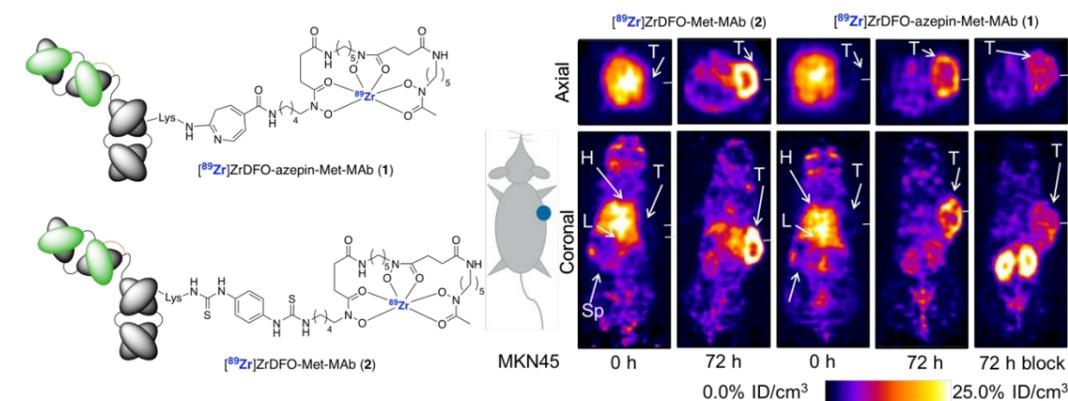
Melanie Gut<sup>1</sup>, Simon Klingler<sup>1</sup>, Rachael Fay<sup>1</sup>, Jason P. Holland<sup>1</sup>

<sup>1</sup>Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland.

The zirconium chelate deferoxamine (DFO) was conjugated to the anti-c-Met antibody MetMAB (onartuzumab) using two bioconjugation strategies. A recently developed photochemical conjugation method [1–3] was used to couple DFO to the antibody in less than 15 min. with a photoradiochemical conversion of ~50%. The biological properties of <sup>89</sup>ZrDFO-azepin-MetMAB (**1**) were compared in a head-to-head study <sup>89</sup>Zr-DFO-Bn-NCS-MetMAB (**2**) produced via a conventional thermochemical conjugation route using DFO-Bz-NCS.

Both radiotracers were purified, formulated and their properties were assessed *in vitro* and *in vivo*. Similar immunoreactivity against c-MET positive MKN45 cells was detected and the radioactive compounds were found to be stable for up to 72 h in human serum stability assays. PET imaging and biodistribution studies confirmed the specific tumour uptake in mice bearing subcutaneous MKN45 xenografts. By 72 h post-administration accumulation of compounds **1** and **2** in the tumours was 15.4±5.2 (*n* = 4) and 21.4±11.6 (*n* = 4) %ID/g, respectively. At the same time point, a competitive blocking study of the photochemically conjugated DFO-azepin-MetMAB confirmed specific binding of the radiotracer to c-Met with ~59% lower tumour uptake (*P*-value < 0.001 compared with non-blocked experiments). Overall, *in vivo* and *in vitro* experiments confirmed that photochemical conjugation methods do not alter the biochemical viability or pharmacokinetic properties of the radiotracer when compared with conventional bioconjugation and radiolabelling technology. tumour uptake of the tracer.

We thank the Swiss National Science Foundation (SNSF Professorship PP00P2\_163683), the Swiss Cancer League (Krebsliga Schweiz; KLS-4257-08-2017), and the University of Zurich (UZH) for financial support. This project has received funding from the European Union's Horizon 2020 research and innovation programme / from the European Research Council under the Grant Agreement No 676904, ERC-StG-2015, NanoSCAN.



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P247

### Unsaturated Macrocyclic Complexes of Zr(IV) as Fluoride Carriers

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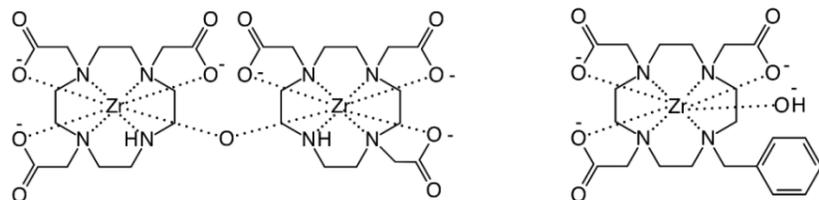
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Positron Emission Tomography is a medical imaging technique utilizing positron-emitting radionuclides. The most common positron emitter is <sup>18</sup>F due to its good radiochemical properties and accessibility. The most utilized fluorinated agent is <sup>18</sup>F-fluorodeoxyglucose. Despite its almost universal use there is an effort to prepare new labelled antibodies. These substances have the prior position among <sup>18</sup>F radiotracers, however, their preparation is often quite laborious and time consuming. The time required for tracer production reduces the specific activity of the administered sample. In recent years attention is focused on attachment of fluorine to the vector through high-energy bonds, such as Si–F, B–F or Al–F. In our research we concentrate on compounds with Zr–F bond.[1][2][3]

In order to increase the efficiency of radiolabelling and radiochemical yield we study the behaviour of zirconium with unsaturated coordination sphere. These complexes bind fluoride anions from the solution quickly and effectively. We have prepared two different ligands suitable for complexation of Zr(IV) cation – 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (DO3A) and its *N*-benzyl derivative. Structures of Zr(IV) complexes are different for each of the ligands (both structures are displayed in the picture below). Surprisingly, Zr-DO3A exists in a form of dimer complex which is quite uncommon for Zr(IV)-complexes.

Two different methods of are used for investigation of the studied systems. Potentiometry with fluoride selective electrode is used for kinetic studies. <sup>19</sup>F-NMR is used for stability studies. We have shown that the rate of creating Zr–F bond strongly depends on the reaction temperature, pH, complex-to-fluoride ratio and concentration of the component.

Financial support by the Charles University Grant Agency No. 1306119 is gratefully acknowledged.



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P248

### Cu, Fe, and Zn Isotopic Compositions are Altered in Serum and Whole Blood of Roux-en-Y Gastric Bypass (RYGB) Patients

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Bariatric surgery is an effective medical treatment to achieve weight loss in obese patients. The most common procedure is a Roux-en-Y gastric bypass (RYGB), in which the duodenum and upper intestine are bypassed. Malnutrition often occurs after bariatric surgery due to a reduced intake and malabsorption. In this study, we assessed the isotopic compositions of Cu, Fe, and Zn in the serum and whole blood of obese patients who underwent RYGB. Premenopausal female patients (body mass index (BMI)  $\geq 40$  kg/m<sup>2</sup>) who underwent a laparoscopic RYGB were selected. Samples were collected before surgery and at 3, 6, and 12 months post-surgery. The Cu isotopic composition was lighter in the post-surgery serum and whole blood samples compared to the corresponding pre-surgery samples. The difference between the Zn isotopic composition in serum and that in whole blood was larger after surgery. A correlation between Fe isotopic composition and glycaemic parameters was established. To conclude, the altered metal homeostasis in post-bariatric surgery patients is reflected in changes in the isotopic compositions of Cu, Fe, and Zn, even one year after the surgery.

P249

### A Ru(II) Polypyridyl Complex Bearing Aldehyde Functions as a Versatile Synthetic Precursor for Photodynamic Therapy Photosensitizers

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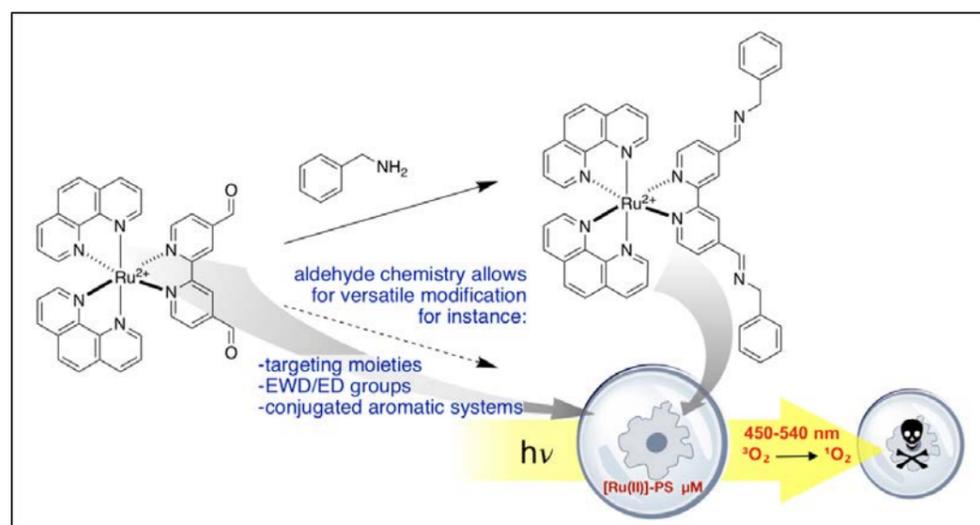
<sup>3</sup>Chimie ParisTech, PSL University, CNRS, Institute of Chemistry for Life and Health Sciences, Theoretical Chemistry and Modelling, 75005 Paris, France.

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<sup>a</sup>These authors have contributed equally to the work.

Photodynamic Therapy (PDT) has been gaining attention over the last decades. It is used as treatment for different types of cancer and infections of bacteria, fungi and viruses. Photosensitizers already used in clinics still have a number of side-effects that make PDT unpleasant to use, namely slow clearance from the body and corresponding long photo sensitivity of the patient, low water solubility and photobleaching. Presented is a Ruthenium polypyridyl complex (**1**) with carbonyl functionalized bipyridine ligand. This shall be used to attach other chemical or biological molecules to it which ideally leads to altered chemical and biological characteristics. Namely, targeting moieties to increase selectivity and accumulation in the pathologic tissue. Enlarging the conjugated pi-system and adding EWG/EDG might lead to a changed absorption. Compared to its version without an aldehyde substituted bipyridine (**2**) the compound is characterized by a red shift in absorption towards the spectral therapeutic window and a high <sup>1</sup>O<sub>2</sub> yield under light irradiation. As a proof of concept, complex **1** was further modified with benzylamine forming an imine bond and leading to compound **3**. The stability was examined in DMSO, PBS and cell media did not hamper the use as PDT agent. In fact, **3** shows great characteristics including no dark toxicity and photocytotoxicity against HeLa cells in a range of 450-540 nm at low micromolar concentrations. Additionally, DFT calculations were performed for both structures.

Financial support by the Paris Sciences & Lettres Research University and the European Research Council is gratefully acknowledged.



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P250

### In Vitro and in Vivo Evaluation of Platinum(II)-Oxalato Complexes with Adenine Derivatives

Jan Hošek<sup>1</sup>, Ján Vančo<sup>1</sup>, Pavel Štarha<sup>1</sup>, Marta Chalupová<sup>2</sup>, Pavel Suchý Jr.<sup>2</sup>, Zdeněk Trávníček<sup>1</sup>

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Platinum(II) complexes represent some of therapeutic standards in the treatment of different types of cancer [1]. However, these complexes also show significant negative side effects, such as neurotoxicity, renal toxicity, and hepatotoxicity. These facts support further research of platinum-based complexes with less severe adverse effects.

We focused on two platinum(II) oxalato complexes [Pt(ox)(L<sub>1</sub>)<sub>2</sub>] (**1**) and [Pt(ox)(L<sub>2</sub>)<sub>2</sub>] (**2**) involving N6-benzyladenine derivatives, where L<sub>1</sub> = 2-(1-ethyl-2-hydroxyethylamino)-N6-(4-methoxybenzyl)-9-isopropyladenine (roscovitine) and L<sub>2</sub> = 2-chloro-N6-(2,4-dimethoxybenzyl)-9-isopropyladenine. These complexes involve cyclin-dependent kinase (CDK) inhibitor roscovitine (L<sub>1</sub>) and its derivative (L<sub>2</sub>) as N-donor ligands and showed up to 7-times higher *in vitro* cytotoxicity than cisplatin [2-4]. With respect to the above-described features of the studied complexes, we decided to study their anticancer activities and mechanisms more deeply, evaluating their cellular and molecular effects against the selected cancer cells, and by the *in vivo* studies to elucidate their antitumor activity on the mice bearing the L1210 lymphocytic leukaemia.

The effects of complexes (**1**) and (**2**) on the cell cycle were evaluated on human A2780 and MCF7 cell lines. Complex (**1**) increased G2/M cell cycle phase population by ca 1.5-times in both cell lines, but complex (**2**) did not affect cell cycle. On the other hand, cisplatin showed apparent increase of sub-G1 cell population. The further analysis revealed that complex (**2**) had similar ratio between apoptosis and necrosis as cisplatin. Complex (**1**) induced preferentially apoptosis than necrosis. Positive results from *in vitro* experiments prompted us to test both complexes further *in vivo*. The best result was observed for complex (**1**), which prolonged mean survival time of mice, whereas cisplatin and complex (**2**) shortened it. However, cisplatin dramatically decreased the body weight (in average up to 30 % of the initial body weight); this effect was not observed for both the tested complexes. Complexes (**1**) and (**2**) also caused less severe side effects, such as total systemic failure, apathy, loss of appetite, cirrhosis, and colon endothelial damage, than cisplatin. Both tested complexes activated caspase-3 more effectively than cisplatin thus confirming that they preferentially induced apoptosis than necrosis.

The obtained results indicated that the shown platinum(II) oxalato complexes represent promising cancer therapeutics with less severe side effects than cisplatin revealing different mechanism of action. This research was funded by the Ministry of Education, Youth and Sports of the Czech Republic (project LO1305).

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P252

### Luminescent Re(I) Complexes as Probes for Synaptic Imaging

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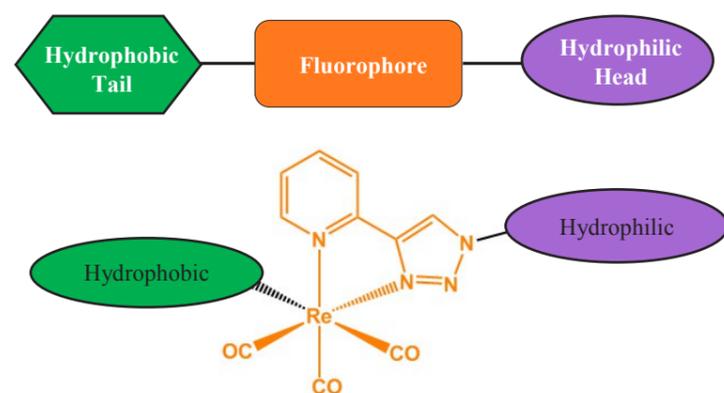
Current methods for imaging synaptic vesicles favour the use of amphiphilic styryl dyes (FM dyes) due to their ability to partition into and out of membranes; however, the organic nature of these dyes means that they are susceptible to photobleaching, even when irradiation is limited.<sup>1</sup> In addition, poor electron density makes them unsuitable for use in electron microscopy (EM) unless treated with OsO<sub>4</sub> in a time consuming process.<sup>2</sup>

Luminescent transition metal complexes (TMs) exhibit many favourable photophysical properties, such as large Stokes shifts and long emission lifetimes; in particular, d<sup>6</sup> TMs afford promising biological probes due to their kinetic inertness.<sup>3</sup> By employing a Re(I) tricarbonyl core, the fluorophore is significantly more resistant to photobleaching, whilst the TM centre may provide the electron density needed for EM. This not only overcomes the main disadvantages of the FM dyes but also presents the opportunity to employ correlative light and electron microscopy (CLEM).

Chelating pyridyl triazole (pyta) ligands were accessed via Sonogashira coupling, diazo transfer and Cu(I) azide-alkyne cycloaddition. Their rhenium complexes are only weakly emissive when excited at 405 nm – the wavelength commonly used in confocal microscopy; however, to overcome this limitation, a series of substituted pyta ligands were synthesised with the aim of tuning the excitation (and emission) wavelength of their Re(I) complexes. In addition to alteration of the photophysical properties, the inclusion of these functional groups also provides a synthetic handle for conjugation to a drug molecule for targeted therapy or photoswitchable moiety.

The inclusion of ligands with structural similarities to that of the FM dyes should therefore mimic their lipophilicity and amphiphilic nature. Naturally occurring amines are water-soluble, and closely resemble the hydrophilic head of FM dyes; they also carry the distinct advantage of facile incorporation into the pyta. Further modification of the complex can be achieved through synthetic manipulation of the monodentate ancillary ligands to introduce hydrophobic character. The results of these studies will be presented.

Financial support by the University of Leicester is gratefully acknowledged.



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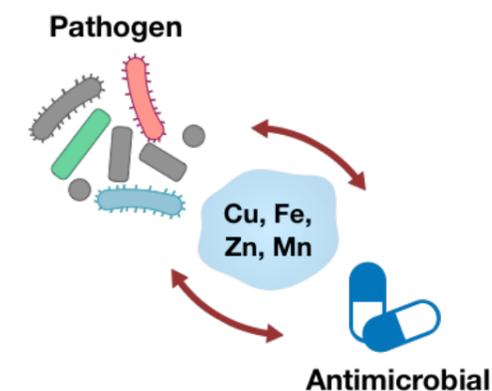
P253

### Fungal Pathogen *Candida Albicans* Reprioritizes Metal Handling During Fluconazole Stress

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To survive, fungal pathogens acquire nutrients from their environment to support basic biological processes. These nutrients include transition metals like copper and iron, but due to their redox activity, these metals can become toxic if misappropriated. The host immune system exploits this delicate balance by manipulating metal availability at the host-pathogen interface. In turn, pathogens have developed mechanisms for tolerating metal starvation or excess and are well-equipped to survive these harsh territories. We posited that when faced with antifungal drug stress, a pathogen's access to essential metals could impact its ability to survive. In this work, we describe how metal availability impacts the response of fungal pathogen *Candida albicans* to treatment with the antifungal drug fluconazole, a mainstay in antifungal therapy. Overall, we establish that fluconazole both affects and is affected by cellular Cu machinery, and that the availability of Cu in the growth environment further modulates these effects, resulting in metal-mediated outcomes of drug treatment.



P254

### Replacement of Equatorial Chlorides of Satraplatin by Acetates: Dual & Triple Action Water-Soluble Satraplatin Derivatives

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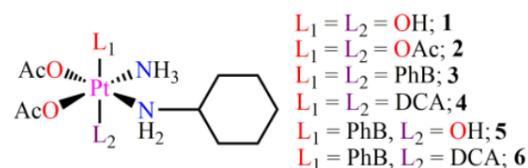
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The platinum (Pt)-based cancer chemotherapy became popular after the landmark discovery by the Rosenberg and his co-workers [1]. The three FDA-approved Pt(II) drugs cisplatin, carboplatin and oxaliplatin are still helping cancer patients worldwide in eradication of the disease. Serious side effects [2] and acquired drug resistances [3] are two of the major drawbacks of treatment associated with cisplatin. These drawbacks encouraged extensive efforts to design platinum drugs with improved therapeutic outputs. At the moment, one of the most popular approaches to overcome these problems is to prepare octahedral Pt(IV) derivatives of the square planar Pt(II) drugs to minimize side effect, increase activity and moreover to make orally bioavailable.

Recently, it is popular to conjugate bioactive ligands to the axial positions of the Pt(IV) complex to attain “dual action” or “triple action”. However, the reported “dual action” Pt(IV) prodrugs have limited solubility in water. Replacing the equatorial chlorides by acetates can enhance solubility. It is evident from the recent literature that Pt(IV) complexes having fixed equatorial am(m)ines and axial ligands, equatorial acetates are more stable in aqueous solution than chlorides [4]. Thus, we chose to prepare “dual” and “triple action” derivatives of satraplatin, the most successful Pt(IV) drug in clinical trials, where the chlorides were replaced by acetates, derivatives and studied the cytotoxicity and mode of action against several cancer cell lines.

Our hypothesis is that there are two stages of activation for the Pt(IV) prodrugs; the first is the reduction to the Pt(II) complex and the second is the activation by aquation of the Pt(II). Since both stages occur inside the cancer cell, it may be advantageous to replace the chlorides with acetates that aquate rapidly and might be efficient DNA platinators. Based on our previous experience [5], we used phenylbutyrate (PhB, HDAC inhibitor) & dichloroacetate (DCA, PDK inhibitor) as the bioactive ligands and hydroxides & acetates as the innocent ones.

Herein, we report on the synthesis, characterization and solution stability of the compounds as well as their biological properties including anticancer activity and DNA damage induction potential.



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P255

### Half-Sandwich Os(II) and Ru(II) Complexes with Unusual Activity Profile in Highly Invasive Triple-Negative Breast Cancer Cells

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In motivation for finding new chemotherapeutics displaying selective toxicity for aggressive triple-negative breast cancer (TNBC) cells, the effect of two, recently developed metal-based half sandwich complexes  $[\text{Os}(\eta^6\text{-pcym})(\text{bphen})(\text{dca})]\text{PF}_6$  (Os-dca) and  $[\text{Ru}(\eta^6\text{-pcym})(\text{bphen})(\text{dca})]\text{PF}_6$  (Ru-dca) [pcym = 1-methyl-4-(propan-2-yl)benzene (p-cymene); bphen = 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline); dca = dichloroacetate] on triple-negative breast cancer cells MDA-MB-231 was studied. The complexes show high and selective toxicity in several tumor cells, and a prominent effect has been observed for Os-analogue. The lower potency of Ru-dca in comparison with Os-dca has been shown to be connected with a relatively quick release of dca ligand due to hydrolysis of Ru-dca before this complex enters the cells [1]. Remarkably, both Os-dca and Ru-dca reduced successfully metastases related properties of the triple-negative breast cancer cells such as migration, invasion, and re-adhesion. The anti-metastatic effects of Os-dca and Ru-dca have been found to be associated with their ability to suppress matrix metalloproteinase activity and/or production and reduce expression of aquaporins. Further studies reveal that Os-dca reverts Warburg's effect and oncosis seems to be a prominent mode of cell death that predominates over the apoptosis. As such, Os-dca can efficiently overcome the resistance of cancer cells to clinically used apoptotic inducers cisplatin and carboplatin. The cytostatic and anti-metastatic properties of Os-dca in MDA-MB-231 provide a strong impetus for the development of new metal-based compounds to target hardly treatable human TNBC cells and displaying different mode of action compared to the antitumor metallodrugs in clinical use [2].

This work was supported by the Czech Science Foundation, Grant No. 17-05302S.

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P256

### Development of Unconventional Cytotoxic Platinum(II) and Platinum(IV) Anticancer Agents

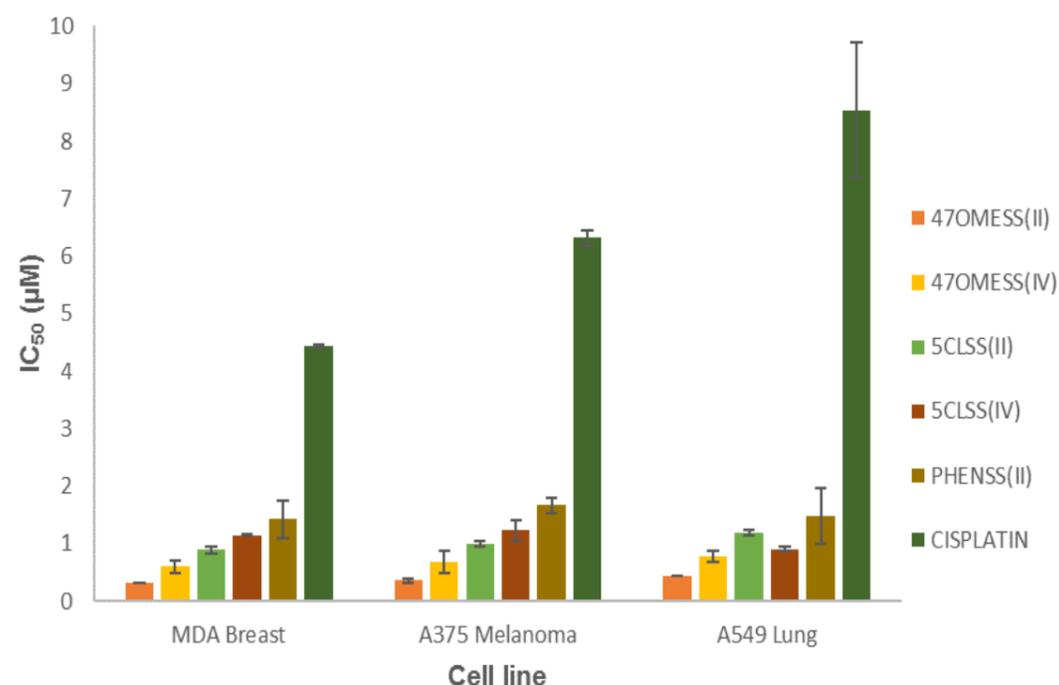
Aleen Khoury<sup>1</sup>, Robin Taleb<sup>2</sup>, Jennette Sakoff<sup>3</sup>, Jayne Gilbert<sup>3</sup>, Janice Aldrich-Wright<sup>1</sup>

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Cancer is a leading cause of death with over 14 million new cancer cases occurring each year worldwide. Many cancer treatments involve platinum(II)-based drugs like cisplatin, carboplatin and oxaliplatin, which illicit their potency by binding covalently to DNA. The clinical use of these drugs is limited due to resistance and severe toxic side effects that are caused by reactions with off target molecules.[1,2] To address these limitations, we have developed unconventional platinum(II) and platinum(IV) complexes, that exhibit a different mechanism of action to cisplatin and its analogues.[3] Platinum(IV) complexes are kinetically inert, reducing the possibility of reactions with extracellular biomolecules. These unconventional complexes are in the form  $[Pt(P_L)(A_L)]^{2+}$  (where  $P_L$  is a polyaromatic ligand and  $A_L$  is an ancillary ligand) and demonstrated significantly better cytotoxicity than cisplatin as indicated in the figure below.



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P257

### Exploiting the Gallium-Fluoride Bond in the Design of New Radiopharmaceuticals: Potential Application in Diagnostic Imaging of Alzheimer's Disease

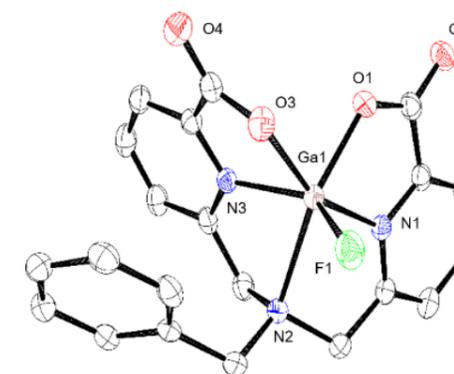
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Positron emission tomography (PET) is a molecular imaging technique that produces three-dimensional images in high resolution used to assist diagnosis and the monitoring of disease. Fluorine-18 is a positron-emitting isotope ( $t_{1/2} = 109.8$  min) that is widely used in PET to radiolabel small molecules. Conventional radiolabelling with fluorine-18 involves the formation of covalent C-F bonds. The development of late stage fluorine-18 radiolabelling using metal complexes has emerged as an exciting method to synthesise PET imaging agents.<sup>1,2</sup> As fluoride has a small ionic radius and high electronegativity, it binds strongly to group 13 metal ions such as  $Al^{3+}$  and  $Ga^{3+}$  (bond dissociation energies of Al-F and Ga-F are estimated at 665 and 557 kJ/mol respectively). Coordinate bond formation between  $Ga^{3+}/Al^{3+}$  and  $F^-$  may lead to complexes of sufficient stability compared to covalent C-F bond formation for imaging applications. In this work, we explore the potential of  $Ga^{3+}$ -F complexes as an alternative method for rapid incorporation of fluorine-18 into molecules.

Lipophilic and stable  $[^{18}F]F^-Ga^{3+}$  complexes with the potential to cross the blood brain barrier could be used as brain perfusion imaging agents for PET to assist in the diagnosis of Alzheimer's disease. With the aim of synthesising charge neutral  $Ga^{3+}$ -F complexes, pentadentate ligands were synthesised to coordinate to  $Ga^{3+}$ . Fluorination of  $Ga^{3+}$  complexes was achieved using KF. The formation of the Ga-F bond was confirmed by  $^{19}F\{^1H\}$  NMR spectroscopic studies and single-crystal X-ray crystallography. In one example, to develop new methods for the incorporation of fluorine-18 into  $Ga^{3+}$  complexes that bind to protein deposits associated with Alzheimer's disease, a stilbene derivative conjugated to 1,4,7-triazacyclononane-1,4-diacetic acid was coordinated to  $Ga^{3+}$ . The binding properties of the compounds to extracellular  $\beta$ -amyloid ( $A\beta$ ) plaques in human tissue was evaluated. The coordination chemistry and radiolabelling studies will be presented.



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P258

### An Anticancer Pt<sup>IV</sup> Prodrug That Acts by Mechanisms Involving DNA Damage and Different Epigenetic Effects

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Dual- or multi-action Pt<sup>IV</sup> prodrugs represent a new generation of platinum anticancer drugs. The important property of these Pt<sup>IV</sup> prodrugs is that their antitumor action combines several different mechanisms owing to the presence of biologically active axial ligands. Here we present some biological properties of a “tripleaction” prodrug that releases in cancer cells cisplatin and two different epigenetically acting moieties, octanoate and phenylbutyrate [1], [2].

It is demonstrated, with the aid of modern methods of molecular and cellular biology and pharmacology, that the presence of three different functionalities in a single molecule of the Pt<sup>IV</sup> prodrug results in high potency in tumor cells including those resistant to cisplatin [the IC<sub>50</sub> values in the screened malignant cell lines ranged from as low as 9 nM (HCT-116) to 74 nM (MDA-MB231)]. It is also demonstrated that cellular activation of the Pt<sup>IV</sup> prodrug results in covalent modification of DNA through the release of the platinum moiety accompanied by inhibition of the activity of histone deacetylases caused by phenylbutyrate and by global hypermethylation of DNA by octanoate. Thus, the Pt<sup>IV</sup> prodrug acts as a true “multi-action” prodrug, which is over two orders of magnitude more active than clinically used cisplatin, in both 2D monolayer culture and 3D spheroid cancer cells [3].

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P259

### Organoruthenium Anticancer Agents: Evaluation of Speciation, Photo-Activity, Biological Interactions and Glioblastoma Cell Inhibition

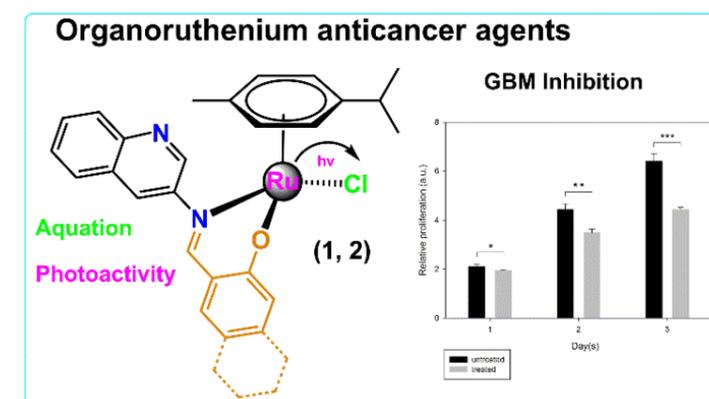
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Ruthenium complexes have been identified as potential therapeutic drug candidates and explored for their utility towards anticancer, antimicrobial, antifungal and antimetastatic agents in last few decades [1, 2]. The anticancer properties exhibited from the interaction with diversified targets such as DNA, protein and enzyme. Ru-complexes displayed significant non-cross resistant with the platinum drugs and identified to overcome multi-drug resistance [3]. The unique features of Ru-complexes originate from diverse complexation ability with wide range of ligands, redox modulation under physiological condition, luminescence properties, interesting ligand exchange and photo-induced ligand dissociation processes. Ruthenium complexes potentially target specific cellular and subcellular component, evaluated for their diagnostic and therapeutic properties and therefore termed as next-generation anticancer drugs [4].

Herein, we will present the design, structures, photophysical properties of two *p*-cymene Ru(II) complexes: [Ru(aqsa)Cl] (1) and [Ru(aqna)Cl] (2), where aqsa and aqna are respective Schiff bases derived from 3-aminoquinoline with salicylaldehyde and 2-hydroxy naphthalaldehyde. The complexes have been studied for their aquation, photo-induced activation, interactions with DNA and HSA. Further, the cytotoxicity results showed that the complexes are effective towards very aggressive human glioblastoma (LN229) cells which is of - great clinical challenge of prognosis for chemotherapy and radiotherapy by virtue of its drug resistant and neurotoxic side effects. Our results showed a significant cytotoxicity and inhibition of GBM cell proliferations, diminished clonogenic potential, inhibition of migration potential, nuclear fragmentations of LN229 cells at μM concentrations indicated their promising therapeutic potential.



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P260

### Multi-Functionalised Graphene Nanoflakes as Tumour-Targeted Theranostic Agents

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Graphene nanomaterials hold potential as scaffolds for building multi-functionalised drug molecules. In this work, graphene nanoflakes (GNFs) were used in the design of 'theranostic' agents for applications as targeted drug delivery vehicles and in positron emission tomography (PET) imaging. GNFs are a unique material with a pristine aromatic system and polycarboxylated edges. [1-2] GNFs were multi-functionalised with derivatives of (i) a prostate-specific membrane antigen (PSMA) binding motif based on the Glu-NH-C(O)-NH-Lys unit, (ii) a potent anti-mitotic drug (*R*)-ispinesib, (iii) the chelate desferrioxamine B (DFO), and (iv) an albumin-binding tag to extend pharmacokinetic half-life *in vivo*. [3-4] All materials were evaluated using a combination of radiochemistry, *in vitro* and *in vivo* experiments to test the potential of using GNFs in theranostic design.

Experiments demonstrated that GNFs can be functionalised by standard coupling chemistry and radiolabelled with <sup>68</sup>Ga<sup>3+</sup> ions. DFO-constructs were radiolabelled with radiochemical conversions (RCC) >97% and specific activities (A<sub>s</sub>) ~9 GBq mg<sup>-1</sup>. Antimitotic and cellular binding assays provided evidence of (*R*)-ispinesib and Glu-NH-C(O)-NH-Lys derivatisation. Dose-response curves, FACS analysis and confocal microscopy confirmed that GNFs loaded with (*R*)-ispinesib induced G<sub>2</sub>/M-phase cell cycle arrest. Cellular binding and blocking assays demonstrated a degree of specificity of the Glu-NH-C(O)-NH-Lys containing constructs towards the PSMA expressing LNCaP cell line. Studies in athymic nude mice bearing subcutaneous LNCaP tumours found that functionalised GNFs have rapid blood pool clearance and renal excretion. Time-activity curves extracted from dynamic PET scans showed that circulation times could be extended *via* introduction of the addition of the albumin-binding tag. Modulation of the pharmacokinetic profile is encouraging evidence that the chemical and biological properties of functionalised GNFs can be tuned for use in theranostic drug development.

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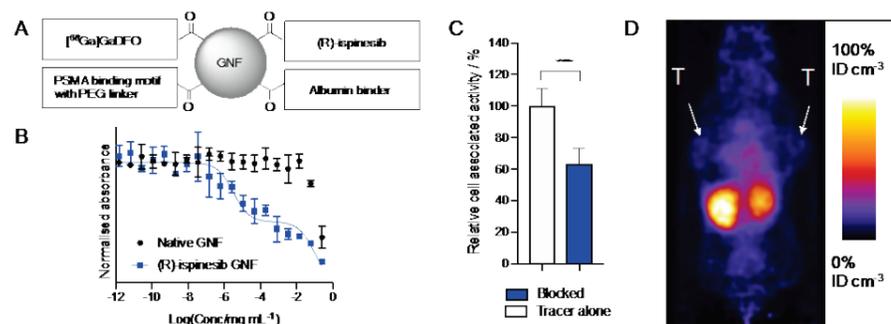


Figure 1: A) Simplified schematic of the modified GNF structure. B) Cellular anti-proliferation (MTT) curves showing the response of LNCaP (PSMA +ve) cells to treatment with modified GNF. C) Blocking assay with the LNCaP (PSMA +ve) cell line pre-treated with free Glu-NH-C(O)-NH-Lys ligand (5 μM) before addition of radiotracers. D) 2 h coronal PET image recorded following injection with radiolabeled modified GNF.

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P261

### Using 6-Amino-1,4-Diazepine Triacetate to Create a Novel Targeted Radiotracer for the Detection of Activated Platelets in Acute Thrombosis

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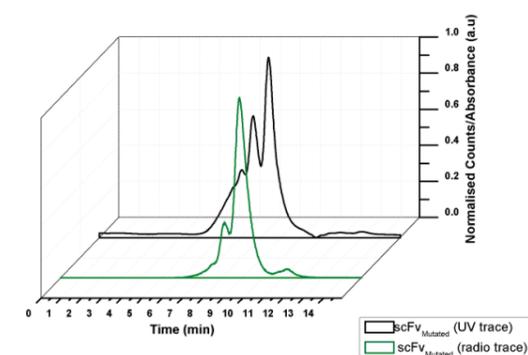
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Through the advent of self-contained generators and favourable properties ( $t_{1/2} = 68$  minutes, decay via 89% positron emission) radioactive gallium has become widely available as a promising Positron Emission Tomography isotope for clinical use. Antibodies hold great promise for more specific diagnosis and therapy of disease. One challenge for labelling antibodies has been the availability of gallium specific chelators which can radiolabel under mild conditions without requiring elevated temperatures. This would allow the creation of kit-based systems wherein labelling is achieved through direct addition of generator eluate to a preformulated antibody-chelator construct. Here we report an innovative gallium chelator, the 6-amino-1,4-diazepine triacetate (DATA) ligand that can form single-species complexes with <sup>68</sup>Ga and be quantitatively labelled at room temperature over a range of pH's.<sup>1</sup>

Through attachment of DATA to a single-chain antibody (scFv) targeting activated platelets, a thrombosis imaging agent can be implemented in a 1-pot kit-based system. The recombinant scFv<sub>anti-GPIIb/IIIa</sub> specifically binds to ligand induced binding sites on the integrin complex GPIIb/IIIa only when it is activated.<sup>2</sup> Detecting activated platelets has wide utility as a diagnostic agent for thrombosis and inflammation. The DATA ligand was attached to the targeting agents using site-specific immobilisation via the Sortase A transpeptidase from *Staphylococcus aureus*. This ensures specific C-terminal post-translational modification without compromising the active site of the antibody.<sup>3</sup>

We attached the Sortase A substrate DATA-PEG<sub>4</sub>-Gly<sub>3</sub> to the scFv<sub>anti-GPIIb/IIIa</sub> and the non-binding control scFv<sub>Mut.</sub>. Confirmation of successful conjugation and preserved binding was demonstrated by LC/MS and FACS. Next, SEC and radio-SEC with <sup>68</sup>Ga was performed showing stability of the novel construct. The radiolabelling protocol led to successful radiolabelling of the [<sup>68</sup>Ga]Ga-DATA-scFv complexes in 5 minutes, at ambient temperature at pH 5. After purification, 95% radiochemical purity was achieved.



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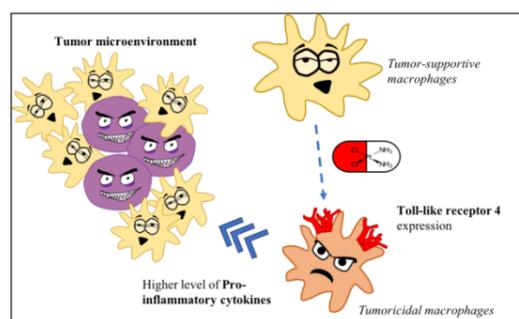
P262

### Immuno-Modulation of Platinum-Based Complexes on RAW246.7 Macrophages

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Platinum-based agents have been widely used in cancer therapeutics with the formation of Pt-DNA adducts as considered as their principle mode of action.<sup>[1]</sup> However, it is now revealed that their binding to off-target molecules also contributed to their tumoricidal efficacy through activating immune responses to cancer.<sup>[2]</sup> Herein, we reported several Pt agents acting as immuno-stimulants using macrophage model which plays a key role on supporting tumor progression.<sup>[3]</sup> In this project, we hypothesized that the Pt-based agents can activate macrophages and therefore enhance their tumoricidal efficiency. Using RAW264.7 cells, we first screened the library of drugs which induced Toll-like receptor 4 (TLR4), a surface receptor that can initiate the production of pro-inflammatory cytokines. Results showed that among all complexes tested, cDDP and JM118 induced higher levels of TLR4 surface expression, leading to the higher production of pro-inflammatory cytokines like TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-12. cDDP later was chosen a main agent for further experiments due to its long history of clinical treatment. The level of PRAT4A protein, a chaperone that transport TLR4 to cell surface,<sup>[4]</sup> was also enhanced under treatment of cDDP, thus confirming the regulation of cDDP on TLR4 activation. The cDDP-treated macrophages showed a higher anti-cancer effect compared to control ones. Supernatants collected from activated cells were more toxic to L929 fibrosarcoma cells than controls. Results from macrophages-mediated cytotoxicity assay also indicated the killing superior of activated macrophages, about 10% higher than controls (where controls killed 70% of L929 cells). The difference is not big due to reduction of phagocytosis rate of RAW264.7 cells by cDDP, suggesting the killing ability of activated cells is mainly via cytokine secretion. Next, non-cell-to-cell-contact assay showed that the tumoricidal efficacy of activated cells was about 20% while one of controls was approximately 3%. The activated macrophages induced more cancer cells arrested at G1 and more necrotic and apoptotic cells. Further experiments need to be carried out to find out biological targets of cDDP.



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P263

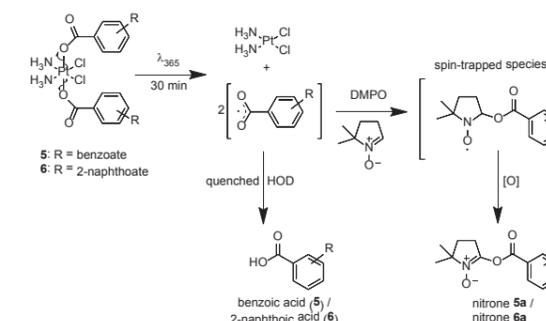
### Design and Investigation of Photoactivatable Platinum(IV) Prodrug Complexes of Cisplatin

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Platinum(IV) carboxylate scaffolds have garnered considerable research interest as they can be engineered to function as prodrugs of clinical platinum(II) anticancer drugs. These platinum(IV) prodrug complexes are stable, tunable, and activated by reduction to release their cytotoxic platinum(II) cargo. Cisplatin is one of the most effective and widely used chemotherapeutic drug to treat various cancers but cisplatin treatment is limited by toxic side effects due to poor selectivity.<sup>1,2</sup> Here we propose new platinum(IV) prodrug complexes designed to release cisplatin via photoreduction upon UV irradiation. The central strategy is to utilise aryl carboxylate ligands on the axial positions of the platinum(IV) scaffold as they confer significant UV absorption and would stabilise carboxyl radical formation, thus favouring homolytic Pt-O bond cleavage. We isolated and identified aryl carboxyl radicals via spin-trapping and showed that the photoreduced platinum species mirror cisplatin reactivity toward DNA bases, thereby validating the efficacy of this approach.<sup>3</sup>



Financial support by Singapore Ministry of Education and NUS Graduate School of Integrative Sciences and Engineering are gratefully acknowledged.

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P264

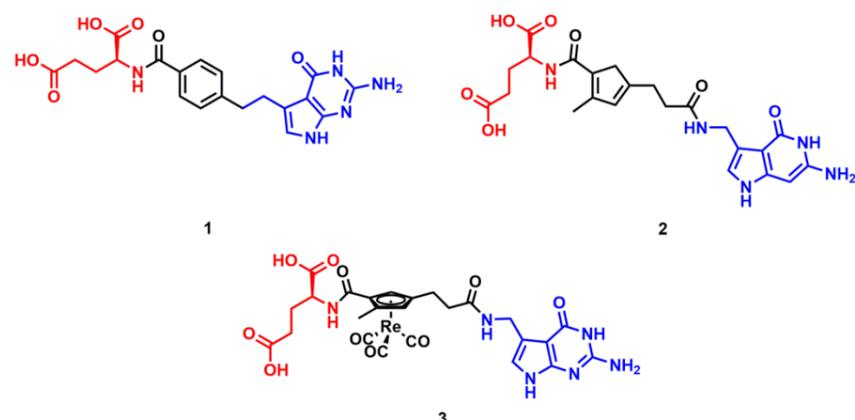
**Development of a Cyclopentadienyl Based Mimic for the Anti-Cancer Drug Pemetrexed**

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Pemetrexed (**1**) is a chemotherapeutic drug that acts as an anti-folate and is used in the treatment of non-small cell lung cancer. Since folate receptors, which can increase the uptake of both folates and anti-folates, are overexpressed in certain tumours, **1** offers an interesting starting point for the development of novel metal-based radiopharmaceuticals.<sup>1</sup>

Recently, our group developed a bifunctional cyclopentadiene,<sup>2</sup> which can replace the phenyl ring in **1**, and thereby generating the Pemetrexed mimic **2**. The Cyclopentadiene in the structure of **2** can act as a strong ligand for Re and <sup>99m</sup>Tc. Compound **3** in which the [Re(CO)<sub>3</sub>]<sup>+</sup> moiety coordinates to the integrated cyclopentadienyl ring, has been synthesised and characterized. The biological behaviour was assessed for the rhenium compound **3** and the synthesis of the corresponding <sup>99m</sup>Tc compound is on its way. Further bioorganometallic pharmacomimetics following the same concept will be presented.



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P265

**Cyclometalated Ir(III) Chaperone Complexes of Curcumin as Light-Activated Prodrugs for Cancer Therapy**

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Curcumin has been widely reported as potential anticancer candidate drugs that can inhibit the clonogenicity of cancer cells and induce anti-proliferative and apoptotic effects on drug-resistant cancer cells. However, the clinical trials involving curcumin is hindered by its poor bioavailability. To tackle this issue, herein we present a novel series of cyclometalated Ir(III) complexes that incorporate the advantages of Ir(III) metal complexes with curcumin serving as light-activatable prodrugs. Current results indicated that the combination of curcumin with Ir(II) chaperone complexes was an effective way in promoting bioavailability, and imparted the complexes with photo-induced curcumin release property. Intriguingly, the post-activation Ir(III) complexes were meanwhile endowed with photosensitizing capability. Thus, we can realize dual modes (chemotherapy and PDT) of cancer therapy by a photocage manner. Further evaluations on the photo-induced chemotherapy are in progress. We hope that by this study we can fully document the photo-activation mechanism and cytotoxicity profiles of the Ir(III) complexes, and assess the potential of these complexes as prodrugs for cancer therapy.

Financial support by the National Science Foundation of China (No. 21525105, 21778079, and 21807119) is gratefully acknowledged.

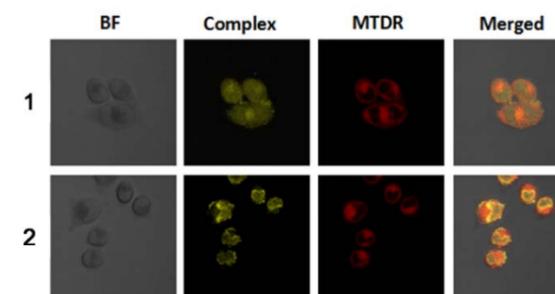


Fig 1. The colocalization study of Ir(II) chaperone complexes of curcumin with MitoTracker Deep Red (MTDR).

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P266

### Functionalisation of G-quadruplex Binders to Enable Theranostic Applications

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G-quadruplexes (G4) are higher-order DNA and RNA structures which contribute to regulation of gene expression, telomeric maintenance and DNA replication.<sup>1</sup> G4 stabilisation leads to modulation of some oncogenes' transcription and inhibition of telomerase. Consequently, there is significant interest in developing small molecules which target and stabilise G4, and in doing so act as potential anticancer therapeutics.<sup>2,3</sup> To further explore the potential of G4 binders both *in vitro* and *in vivo* we adapted G4 binders for use in a range of applications, such as imaging or targeting, using click chemistry. We report the synthesis and characterisation of novel nickel, zinc and platinum salphen complexes bearing "clickable" backbones. Phosphorescence properties of the platinum(II) series ( $\lambda_{EM} = 582$  nm) were exploited for further G4 characterisation. G4-binding was evaluated using circular dichroism melting studies and emission titrations comparing G4 DNA (*i.e.* oncogenic (*c-Myc*) and telomeric (*H.Telo*)) with duplex DNA (*ds26*). Several novel metal salphen complexes were functionalised with either alkyne, azide or *trans*-cyclooctene (TCO) to "click-on" probes suitable for optical or nuclear imaging (*e.g.* DOTA for radiometal labelling) or for specific targeting (**Fig.1**). This general, modular strategy enables the production of different probes for multiple applications derived from a single precursor. By utilising this synthetic route we conserve the planar ligand system necessary for G4 binding, as evaluated by various biophysical techniques. Thermal stabilisation of G4 and binding affinities for unmodified *versus* modified platinum(II) salphen complexes were similar indicating the presence of the clickable group did not impair G4 DNA binding.

Financial support by the EPSRC, via the CDT in Medical Imaging, is gratefully acknowledged.

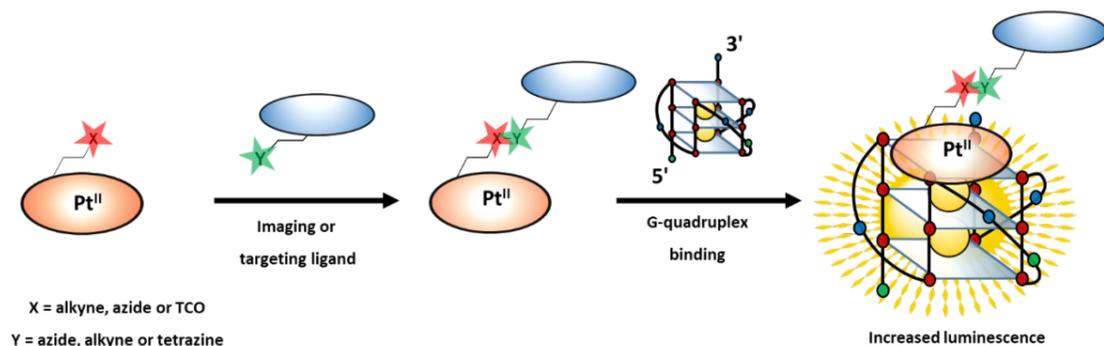


Figure 1. Schematic route for multi-purpose platinum(II) G-quadruplex binders.

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P267

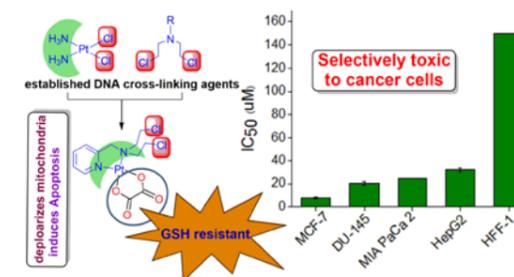
### Oxamusplatin: A Glutathione Resistant Nitrogen Mustard Based Pt(II) Complex with Enhanced Selectivity towards Cancer

Moumita Maji<sup>1</sup>, Subhendu Karmakar<sup>1</sup>, Raturaj<sup>2</sup>, Arnab Gupta<sup>2</sup>, Arindam Mukherjee<sup>1</sup>

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The Nitrogen mustards (*viz.* mechlorethamine, Chlorambucil, Bendamustine) are among the first generation of chemotherapeutic drugs against cancer. However, they are highly toxic to even normal cells due to their high reactivity. The reactivity has been controlled by engaging the lone pair on the nitrogen and development over the years has led to drugs like bendamustine and chlorambucil which are continued in clinic due to their ability to increase the life of a cancer patient in spite of the severe side effects of these drugs. Metal complexation is an efficient way to engage the lone pair on the nitrogen of the mustard responsible for the aziridine formation.[1] Metals like Co and Cu have shown promises in pre-clinical experiments.[2-3] However, the most successful metal in clinic Pt(II) has been relatively less investigated in this regard in spite of the fact that 50% of cancer chemotherapy in clinic involves a platinum drug (*viz.* cisplatin, oxaliplatin, carboplatin) either in combination or as standalone.[4] The clinical Pt-drugs also suffer from dose limiting side effects and resistance towards chemotherapy. The platinum drugs and nitrogen mustards are DNA cross-linking agents and hence their combination may lead to increase in steric hindrance at the Pt site thus reducing deactivation by glutathione or prevent pre-mature hydrolysis. In our attempt to generate a stable complex and study the effect of complexing a nitrogen mustard with platinum(II) we previously made a bis(2-chloroethyl)pyridylmethylamine bound *cis*-dichloroplatinum(II) complex (**1**) which was found to be more active than cisplatin in human lung adenocarcinoma (A549) and human breast adenocarcinoma (MCF-7) cell line. However, **1** is also cytotoxic to normal cells.[5] Motivated by the desire to design a more stable but active Pt-mustard complex we have made two more Pt(II) mustard complexes using **1** as a precursor. Among them the oxalate bound 'oxamusplatin'(3) (Figure) is 7 times more toxic towards metastatic prostate cancer cells (DU145,  $IC_{50} = 20.51$   $\mu$ M) and 18 times more toxic to the hormone sensitive breast cancer cell line (MCF-7,  $IC_{50} = 8.15$   $\mu$ M) over normal foreskin fibroblast (HFF-1,  $IC_{50} > 150$   $\mu$ M). The accumulation of the complex is similar to cisplatin inside cancer cells as per the ICP-MS data and it strongly resists binding to glutathione at pH 7.4 even when 15 molar equivalent excess glutathione is used.



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P268

### A Silver(I) Complex with 4-Fluoroanthranilic Acid: Synthesis and Biological Activities

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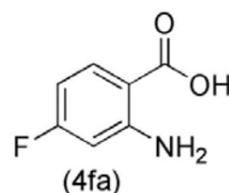
The growth of multidrug-resistant bacterial strains to the existing antibiotics is a global case of concern. Tuberculosis (TB) which is caused by the bacteria *Mycobacterium tuberculosis*, is the most lethal of all infectious diseases [1] and the cases of extensively drug resistant-TB have called attention of the World Health Organization. Anthranilic acid derivatives are known since the 1950's for inhibiting the tryptophan synthesis pathway in bacteria [2]. It is suggested that the mechanism of inhibition occur by counter-selection of genes that codify proteins responsible for the conversion of anthranilic acid in tryptophan [3]. Fluoroanthranilic acid isomers are active against the *M. tuberculosis* variant capable of forming biofilms, with minimum inhibitory concentration (MIC) of 19.4 mg/L, while the antibacterial activities of pyrazinamide (MIC > 1000 mg/L), isoniazid (MIC > 256 mg/L), streptomycin (MIC = 125 mg/L) and amikacin (MIC = 1000 mg/L) against the same strain are much lower [2].

Silver compounds have also been considered in the synthesis of antibacterial agents due to the remarkable activities of silver ions. One of the best examples is silver sulfadiazine, which is widely used for the treatment of skin burns and wounds. In this case, sulfadiazine slowly releases silver ions, which are responsible for the antibacterial activity [4].

In the present work we describe the synthesis of a new silver(I) complex with 4-fluoroanthranilic acid (Ag-4fa) and its antibacterial activities over Gram-positive and Gram-negative bacterial strains. The complex was synthesized by the reaction of an aqueous solution of AgNO<sub>3</sub> (0.60 mmol) with the potassium salt of 4fa (0.60 mmol) in ethanol under stirring. After 2 hours, the white solid formed was collected by filtration and washed with water. The yield was 92%. Elemental analysis suggests a 1:1 metal:ligand composition. Infrared and NMR spectroscopic analyses indicate coordination of the ligand to silver(I) by the nitrogen atom of the amine group and the oxygen of the carboxylate moiety.

The silver(I) complex showed antibacterial activities with MIC = 62.25 mg/L against four strains of bacteria: *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 14579), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27583), while the ligand alone did not show activity under the same experimental conditions. Preliminary biophysical assays were performed to assign possible biomolecular targets for the Ag-4fa complex. Agarose gel electrophoresis assays suggest that DNA is not a target for this compound.

Financial support: This study was supported by the Brazilian Agencies FAPESP (Grants 2017/25995-6; 2018/12590-0 and 2018/12062-4) and CNPq (Grant 407012/2018-4). This study was also financed in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brazil (CAPES)-Finance Code 001.



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P270

### Synthesis, Characterization and HSA Binding Studies of Quinizarin Containing Ternary Co(III) Complexes

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In order to overcome the problems associated with the lack of selectivity of anticancer drugs, inert cobalt(III) complexes may provide a remarkable platform as they are capable of being selectively reduced under the hypoxic conditions of cancer cells. Drug molecules which are released from the less stable and much more labile Co(II) complex may selectively act only in the tumour [1].

Our previous research has shown, how the functionalization of the 2O donors and modifications of various tripodal 4N donors influence the redox properties of mixed ligand Co(III) complexes [2]. Based on these results we have used tris(2-methylpyridyl)amine (tpa) and tris(2-aminoethyl)amine (tren) as one of the building blocks of ternary complexes in our recent experiments [3]. For drug molecules, anthracyclines could be a good choice. Many compounds bearing this scaffold (e.g. doxorubicin) have antitumor properties and are also used in cancer chemotherapy. Their aromatic moiety can intercalate between the DNA base pairs, however their antitumor action is mainly attributed to topoisomerase-II inhibition. The efficacy of these drugs are limited by their side effects, they may cause chromosomal damage [4]. In our work hydroxy-derivatives of anthraquinones were selected, because the 1,4-dihydroxyanthraquinone (quinizarin) and its 2-sulphonate derivative (quinizarin-2-sulphonate) are the simplest molecules, which represent the basic structure of anthracyclines.

Our hypothesis was that, the ternary complexes would be able to increase the selectivity and decrease the side effects. To explore this field novel [(Co(III)(4N))<sub>2</sub>(OO)]<sup>n+</sup> type complexes have been designed, synthesized and characterized, incorporating quinizarin and sodium quinizarin-2-sulphonate. This contribution will summarize the results obtained in solution by various (NMR, IR, CV) methods on the novel Co(III) complexes. The lipophilicity and binding ability of free ligands and their complexes to human serum albumin (HSA) were also studied to predict their pharmacokinetic properties.

The research was supported by the EU and co-financed by the European Regional Development Fund under the projects GINOP-2.3.2-15-2016-00008, GINOP-2.3.2-15-2016-00038 and the Hungarian Scientific Research Fund (OTKA K112317, FK 124240).

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P271

### Synthesis, Characterization and Biological Applications of a Palladium(II) Complex with a 4-(2-Aminoethyl)Benzenesulfonamide Schiff Base

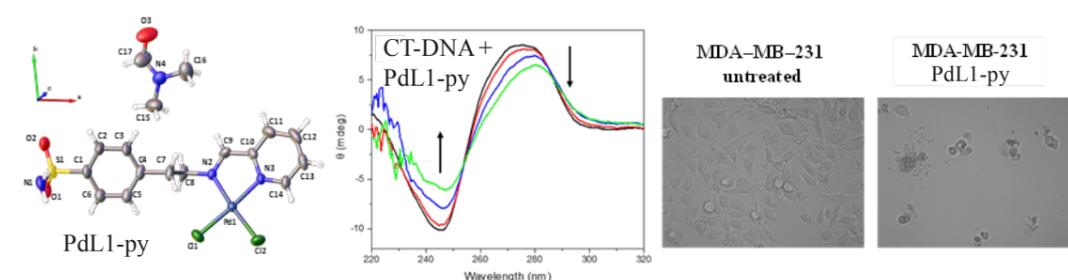
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Palladium has received much attention as an alternative to platinum in the development of metallodrugs. The palladium(II) ion shares many properties with platinum(II), since both have the d<sup>8</sup> electronic configuration and adopt square planar geometry. Several research groups have focused on the evaluation of the biological properties of palladium(II) complexes of the type PdX<sub>2</sub>L<sub>2</sub> as potential anticancer agents [1]. In medicinal inorganic chemistry, Schiff bases have been considered due to their anti-inflammatory, analgesic, antimicrobial, anticonvulsant, anticancer and antioxidant properties [2]. Moreover, Schiff base metallodrugs have also been investigated in the development of new anticancer and antimicrobial chemotherapeutics [3]. The aim of this work was the synthesis and characterization of a new palladium(II) complex with a Schiff base and the evaluation of its biological activities. The selected Schiff base was prepared by the reaction of the 4-(2-aminoethyl)benzenesulfonamide (**L1-NH<sub>2</sub>**) with 2-pyridinecarboxaldehyde, resulting in the **L1-py** ligand. The sulfonamide (**L1-NH<sub>2</sub>**) was chosen due to its specific carbonic anhydrase IX inhibition activity [4]. Elemental analysis of the complex indicates the composition C<sub>14</sub>H<sub>15</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub>PdS. The crystal structure of the palladium(II) complex confirmed the formation of the chelate of the **L1-py** ligand to palladium(II) by the imine and pyridyl nitrogen atoms. Spectroscopic measurements showed that the complex does not undergo ligand replacement in solution even after 24 hours. Circular dichroism (CD) spectroscopy indicates alterations on the secondary structure of calf-thymus DNA after incubation with the palladium(II) complex, which is consistent with coordination of the nucleobases to the metal centre. Competitive fluorescence displacement experiment with ethidium bromide resulted in quenching of the fluorescence after titration with the complex. This quenching is in accordance with ethidium bromide displacement after the alterations in secondary structure of DNA verified by CD. The cytotoxicity of the complex was evaluated over the MDA-MB-231 cell line. The cell morphology after treatment with the compound indicate loss of cell integrity and presence of cellular debris, along with hampering of proliferation.

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P272

### Antitumor Activity of Polypyridine Co(III) Complexes against Hypoxia Tumor and their Solution Reaction with Ascorbic Acid

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The development of hypoxia-activated prodrugs is important for cancer treatment, since hypoxic cancer cells are resistant to conventional radiotherapy and chemotherapy. In hypoxic cancer cell, there is a reductive atmosphere. So far, bioreductive Co(III) complexes can be used for prodrug toward hypoxic regions. Some Co(III) complexes undergo bioreduction to the more labile Co(II) species by ubiquitous one-electron reductase in cells. In hypoxic cells, the labile Co(II) complexes dissociate to release its bioactive ligands, and the high toxic ligands can induce apoptosis of cancer cells. To the best of our knowledge, there is no report about cytotoxicity of Co(II) species which have released their ligands. Therefore, we have focused on Co(III) with polypyridine ligand such as 2,2'-bipyridine (bpy), 1,10-phenanthroline (phen), and 4-methyl-1,10-phenanthroline (mephen) and have investigated cytotoxicities against hypoxic cancer cells.

According to the previous literatures, [Co(bpy)<sub>2</sub>(CO<sub>3</sub>)]Cl, [Co(phen)<sub>2</sub>(CO<sub>3</sub>)]Cl, [Co(mephen)<sub>2</sub>(CO<sub>3</sub>)]Cl, [Co(bpy)<sub>2</sub>(acac)]I<sub>2</sub>, and [Co(phen)<sub>2</sub>(acac)]I<sub>2</sub> (acac<sup>-</sup> = acetylacetonate) were synthesized. The redox potentials of the all Co complexes are within -0.4 V, and these are enough of reduction potentials in hypoxia tumor. The cytotoxicities of these complexes against HeLa cells were investigated by MTT assay. The cytotoxicities of the all complexes in hypoxia condition were higher than those in aerobic condition. However, [Co(bpy)<sub>2</sub>(CO<sub>3</sub>)]Cl and [Co(phen)<sub>2</sub>(CO<sub>3</sub>)]Cl have strong cytotoxicities in aerobic condition compared with other Co(III) complexes, because of their low reduction potential. [Co(mephen)<sub>2</sub>(CO<sub>3</sub>)]Cl has the highest selectivity for hypoxia tumor of these complexes. In addition, these Co(III) complexes have low cytotoxicities against human normal cells (MRC-5).

In order to investigate the chemical species of these Co(III) complexes in the reductive solution, we measured UV-vis. and ESI-MS spectra of these Co(III) complexes with ascorbic acid. The UV-vis. spectral changes and ESI-MS spectra of carbonate Co(III) complexes suggested that the carbonate ions disassociated from the Co(III) complexes. However, the UV-vis. and ESI spectra of the Co(III) complexes having acac ligands suggested the acac<sup>-</sup> ligands dissociated from the Co(III) complexes. The reaction rate of [Co(mephen)<sub>2</sub>(CO<sub>3</sub>)]Cl was higher than those of the Co(III) complexes having acac ligands. These results suggested that the high hypoxia selectivity of [Co(mephen)<sub>2</sub>(CO<sub>3</sub>)]Cl was due to both appropriate redox potential and high reactivity with ascorbic acid, and this Co(III) complex was expected as the efficient anti-hypoxia drug.

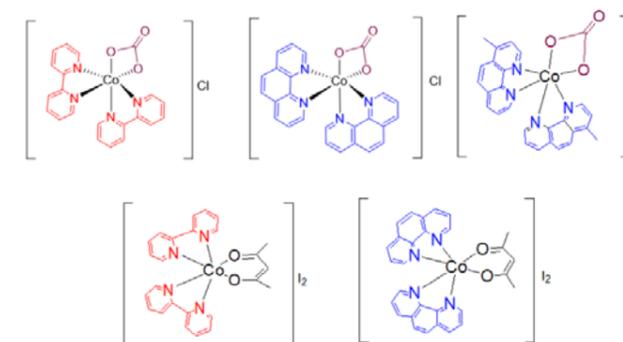


Figure. The polypyridine complexes in this study.

P273

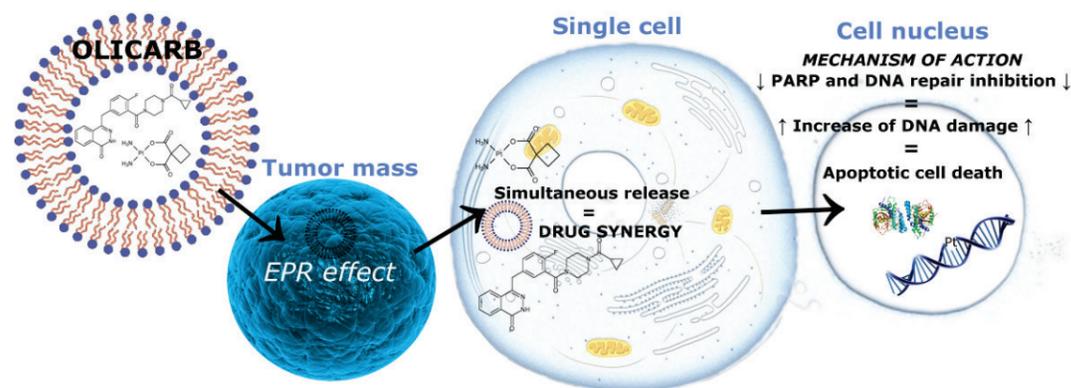
**Simultaneous Delivery of Olaparib and Carboplatin in PEGylated Liposomes Imparts this Drug Combination Hypersensitivity and Selectivity for Breast Tumor Cells**

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Combination regimens involving platinum anticancer drugs and agents with unrelated mechanisms of action are a subject of widespread interest. Here, we show that synergistic toxic action in cancer cells of combinations of antitumor platinum drug carboplatin and effective PARP inhibitor olaparib is considerably improved if these combined drugs are encapsulated into liposomes. Notably, the formation of such nanoformulations, called OLICARB, leads to a marked enhancement of activity in human cancer cell lines (including those resistant to conventional platinum antitumor drugs) and selectivity towards tumor cells. We used immunofluorescence analysis of  $\gamma$ H2AX expression and examined DNA damage in cancerous cells treated with the investigated compounds. We find that the synergistic toxic effects in cancer cells of both drugs used in combination, nonencapsulated or embedded in the OLICARB nanoparticles, positively correlates with DNA damage. These results also suggest that the enhancement of the toxic effects of carboplatin by olaparib in cancer cells is a consequence of an accumulation of cytotoxic lesions in DNA due to the inhibition of repair of platinated DNA augmented by the synergistic action of olaparib as an effective PARP inhibitor. Our findings also reveal that the combination of olaparib with carboplatin encapsulated in the OLICARB nanoparticles is particularly effective to inhibit the growth of 3D mammospheres. Collectively, the data provide convincing evidence that the encapsulation of carboplatin and olaparib into liposomal constructs to form the OLICARB nanoparticles may represent the viable approach for the treatment of tumors with the aim to eliminate the possible effects of acquired resistance. [1] Clinical case study for the non-encapsulated drug combination has been reported and indicate a great durable effectivity on heavily pretreated patient with stage IV triple-negative invasive ductal carcinoma. [2]

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P274

**Probing Intracellular Reduction of Anticancer Platinum(IV) Prodrug Complexes**

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Platinum(II) anticancer drugs such as cisplatin, carboplatin and oxaliplatin constitute some of the most widely used chemotherapeutic agents. Despite their clinical success, their uses are limited due to high toxicity and incidences of drug resistance. The application of platinum(IV) prodrug strategy has provided an excellent alternative to overcoming drawbacks associated with conventional platinum(II) drug therapy and is a promising strategy to obtain next-generation platinum drugs. Although many platinum(IV) complexes have been developed, researchers still do not fully comprehend how this new class of platinum drugs are processed at the cellular level. Current approaches to study platinum compounds such as elemental techniques and fluorescence-tagging have their limitations. The use of exogenous platinum-selective fluorescent sensors could potentially help to overcome challenges associated with these methodologies. These platinum-selective fluorescent sensors would provide a direct and efficient method of probing platinum(IV) complexes, allowing us to acquire better understanding of these compounds. Previously, our group developed a platinum-selective rhodamine-based fluorescent turn-on probe equipped with a diethyldithiocarbamate recognition motif to demonstrate the intracellular reduction of a series of platinum(IV) carboxylate prodrugs in cells.<sup>[1]</sup> Subsequently, through structure-activity relationship studies, the detection capability of the turn-on probe was enhanced by incorporation of a thiospirolactam.<sup>[2]</sup> Herein, we report the first ratiometric probe based on FRET for selective platinum sensing to investigate platinum(IV) anticancer complexes in biological systems.<sup>[3]</sup> The ability of the probe to distinguish between platinum(II) species and their parental platinum(IV) prodrug complexes allowed us to apply the probe in cells to study the intracellular activation of platinum(IV) prodrug complexes. The correlation between the amount of active platinum(II) species available after intracellular reduction of platinum(IV) complexes to their cytotoxicity and the role glutathione plays in the reduction of platinum(IV) complexes were investigated.

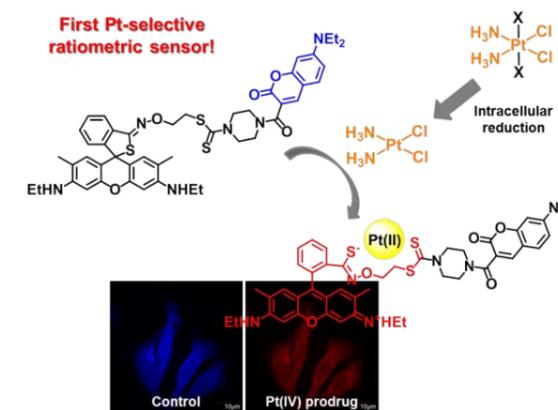


Figure 1. Ratiometric probe based on FRET applied to study the intracellular reduction of platinum(IV) prodrug complexes.

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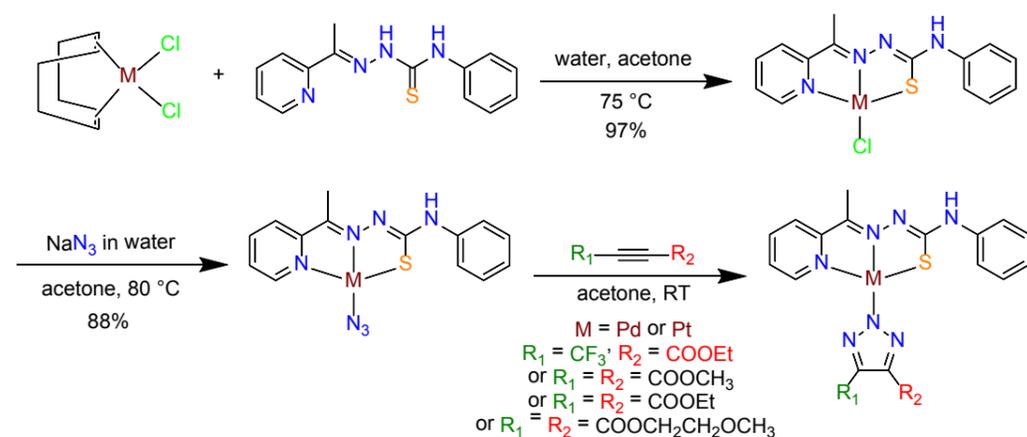
P275

**The Modular Synthesis of Cytotoxic Pd(II) and Pt(II) Triazolate Complexes through iClick Reactions: Reaction Progress, Isomerization and Kinetic Studies**

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Inspired by the success of cisplatin in the clinical treatment of various types of malignancies, the chemistry of metal-based drugs has received enormous attention in recent years. However, the effectiveness of metal-based compounds as antitumor agents is often hampered by their poor water solubility, low bioavailability, and lack of target specificity. Inorganic click (iClick) reactions [1], which allow easy tuning of the bioavailability and ADMET (absorption, distribution, metabolism, excretion, toxicity) properties of novel compounds for bioactivity studies, can be utilized for a modular access to molecular diversity in transition metal complexes with novel structural motifs. In this work, a series of Pd(II) and Pt(II) triazolate complexes was successfully synthesized from the corresponding azide compounds and electron-deficient alkynes at room temperature by the catalyst-free iClick [3 + 2] cycloaddition reaction.



The trifluoromethyl group of the alkyne applied can serve as a sensitive <sup>19</sup>F NMR marker to monitor the progress of the reaction and allows studies of reaction kinetics and stereochemical preference [2]. Thus, the progress of the iClick reaction was tracked by <sup>19</sup>F NMR spectroscopy, which allowed identification of key intermediates as well as N1→N2 isomerization of the triazolate-metal bond. Kinetic studies by NMR spectroscopy demonstrated that the Pd-azide compound is much more reactive than the Pt-azide analogue, and iClick reactions involving alkynes with stronger electron-withdrawing groups react faster than the other ones. In addition, the resulting triazolate complexes show generally high cytotoxicity towards a human brain tumor cell line (GaMG), and Pt-triazolate complex is more active than its Pd-triazolate analogue. Interestingly, the cytotoxicity was also enhanced by increasing the length of chains from the triazolate substituents.

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P276

**Synthesis, Spectroscopic Characterization, Molecular Modeling and *In Vitro* Biological Assays of a Silver(I) Complex with Amantadine**

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Silver compounds have been explored as antimicrobial agents for centuries. After the first report of the antibacterial activities of silver sulfadiazine, used in the treatment of bacterial infections in skin burns and wounds in the 1960's, the interest in the synthesis of new silver-based compounds increased [1]. The success of a silver complex as a metallodrug will depend on the choice of ligand, because it can play an important role in the properties of the compounds, including stability, solubility and release of ions Ag(I) ions [2]. Amantadine (atd) is an amine derivative of adamantane known for its action against the replication of the *influenza A* virus. The mechanism of inhibition of the viral replication is related with the direct interaction of the drug with the ion channel of the M2 viral protein [3]. So, the aim of this work was the synthesis and characterization of a novel silver(I) complex with amantadine (Ag-atd) and the study of its biological activities. The silver(I) complex was synthesized as follows: amantadine, in its neutral form (0.90 mmol), was dissolved in a solution containing 5.0 mL of water and 1.0 mL of a 1.25 mol L<sup>-1</sup> aqueous solution of nitric acid. After one hour of stirring at room temperature, an aqueous solution containing 0.90 mmol of silver nitrate was added to the solution of the ligand (molar composition 1:1 metal/ligand). Then, a solution of potassium hydroxide (0.44 mol L<sup>-1</sup>) was added dropwise to the reaction mixture, with precipitation of a light-grey solid. The solid obtained was collected by filtration, washed with water and dried in a desiccator over P<sub>4</sub>O<sub>10</sub>. Elemental, thermogravimetric and mass spectrometric analyses indicated a 1:2 metal/ligand ratio, with the molecular composition AgC<sub>20</sub>H<sub>34</sub>N<sub>2</sub>·NO<sub>3</sub>. The crystal structure was determined by single crystal X-ray studies and confirmed the coordination of amantadine to the Ag(I) ion by the nitrogen atom. The spectral analysis by infrared and <sup>1</sup>H, <sup>13</sup>C and {<sup>15</sup>N,<sup>1</sup>H} nuclear magnetic resonance reinforced the coordination by the NH<sub>2</sub> group. Computational studies revealed the vibration modes similar to those found experimentally. The *in vitro* antibacterial assays showed the activity of the Ag-atd complex over *Staphylococcus aureus* (Gram-positive), *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative) bacterial strains in the concentration ≤ 31.25 µg mL<sup>-1</sup>. Biophysical assays based on fluorescence spectroscopy indicated that the silver(I) complex interacts weakly with bovine serum albumin, while agarose gel electrophoresis analysis revealed that the compound does not interact with plasmid DNA.

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P277

### Pyriplatin-BODIPY Conjugates for Mitochondria-Targeted Photodynamic Therapy: A Dual Action Photo-Chemotherapeutic Agents

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In effort to find a new drug that circumvent resistance to conventional bifunctional platinum-based drugs, designing complexes for mitochondria-targeted therapy is of importance as the nucleotide excision repair (NER) mechanism is absent for mt-DNA [1]. Photodynamic therapy (PDT) has emerged as an alternative method for its a non-invasive nature to treat selectively cancer using red light activation of the drug in presence of oxygen [2]. We have combined these two methodologies into one by designing a new class of monofunctional platinum(II) complexes having photoactive BODIPY (boron dipyrromethene) ligand for PDT activity in visible light on a pyriplatin motif. The pyriplatin-B complexes, *cis*-[Pt(NH<sub>3</sub>)<sub>2</sub>(L)Cl](NO<sub>3</sub>) (**1-3**) having BODIPY pendants (L) with 1,3,5,7-tetramethyl-8-(4-pyridyl)-4,4'-difluoroboradiazaindacene moieties, were designed and synthesized, and their photocytotoxic properties studied. The activity has been shown to be tunable on incorporation of one or two heavy iodine atoms to the BODIPY core thus transforming a highly fluorescent non-iodo BODIPY core to non-fluorescent but high singlet oxygen generating diiodo-BODIPY core as evidenced from the DPBF titration experiments, DCFDA and DNA photocleavage activity. The mono-iodo BODIPY core retained both the properties and acted as a fluorescent photosensitizer showing both the effects. Complex *cis*-[Pt(NH<sub>3</sub>)<sub>2</sub>(4-Me-py)Cl](NO<sub>3</sub>) (**4**) was used as a control for determining the structural aspects by X-ray crystallography. The cytotoxicity of the pyriplatin-B complexes was tested against A549 (human lung cancer), MCF-7 (human breast cancer), and HaCaT (human skin keratinocyte) cells in visible light with IC<sub>50</sub> values in nanomolar (IC<sub>50</sub> ≈ 0.05 μM, light of 400-700 nm, power: 10 J cm<sup>-2</sup>). The complexes were nontoxic (IC<sub>50</sub> > 100 μM) in dark. The emissive bands of **1** and **2** near 535 nm in 1% DMSO/DMEM were used for cellular imaging by confocal microscopy study, which showed their mitochondrial localization. This was further supported by platinum estimation from isolated mitochondria and mitochondrial depolarization through a JC-1 assay. The photo mediated apoptotic cell death was evidenced from flow cytometric assays, annexin-V/FITC-PI and cell cycle arrest in sub-G1 and G2/M phases. The complexes showed binding to 9-ethylguanine as a model nucleobase to form monoadducts. The combination of photodynamic therapy with DNA cross-linking property enhanced the anticancer potential of the monofunctional BODIPY-conjugates, namely, pyriplatins. This work provides new insights and directions in the chemistry of monofunctional platinum(II) complexes and highlights the synergistic effect of the photoactive prodrugs towards the development of next generation of photo-chemotherapeutics agents [3].

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P278

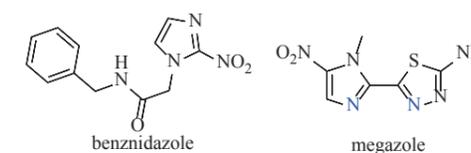
### Re and <sup>99m</sup>Tc Complexes with Megazol Derivatives: Potential Multifunctional Agents for Chagas Disease Diagnostics or Treatment

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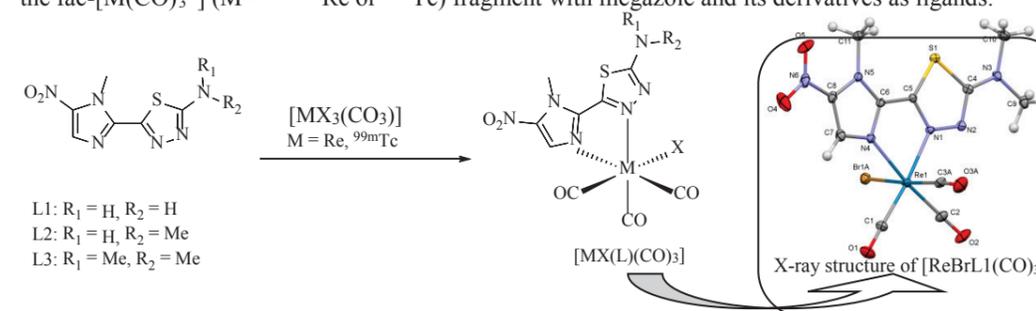
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Chagas disease causes 14000 deaths annually, afflicting around 7 million people from which less than 1% have access to a diagnosis and clinical treatment according to the Drugs for Neglected Diseases initiative (DnDi) organization.[1] Only one drug is currently known to treat the disease: the nitroheterocyclic compound called benznidazole. The drug is effective only in the acute phase of the disease and no drug has been discovered since 7 decades. In addition, the diagnostics of Chagas is complex, mainly during the chronic phase, due to the lack of symptoms and the low parasitemia.[2] Therefore, considering the similarity between the metabolism of eukaryotic parasites and that of neoplastic cells,[3] an interesting alternative for the diagnosis and evaluation of the clinical evolution of Chagas disease is radiodiagnosis based on <sup>99m</sup>Tc. Moreover, due to the comparable chemical properties of Re and Tc [4], non-radioactive Re complexes could be employed as model of comparison to the technetium analogue complexes as well as in chemotherapy of Chagas disease. In this sense, the choice of a good ligand system is important in order to stabilize the metal center as well as to acquire the desired biological activity. However, benznidazole itself is not a choice since it is able to act as a monodentate ligand only. On the other hand, megazol, another nitroheterocyclic derivative, presents *in vivo* activity against *T. cruzi* and *T. brucei* strains, acting as an inhibitor of essential enzymes for the parasites such as trypanothione reductase and NADH furamate reductase,[3] besides being a potential chelating ligand.



This work thus deals with the synthesis and characterization of megazol and new complexes containing the fac-[M(CO)<sub>3</sub>]<sup>+</sup> (M = <sup>185/187</sup>Re or <sup>99m</sup>Tc) fragment with megazol and its derivatives as ligands.



Once this objective is achieved, the products will be compared with those obtained by reactions with the <sup>99m</sup>Tc isotope. Beside eventual therapy with the rhenium complexes, the aim is to apply the homologue compound containing <sup>99m</sup>Tc isotope for *in vivo* biodistribution.

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P279

### A New Bisdipyrrin-Based Ligand as a Potential Probe for Imaging Prostate Cancer

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Stable copper(II) complexes are interesting as potential PET imaging agents through incorporation of the copper-64 radioisotope. Prostate-specific membrane antigen (PSMA) is a well-known target on the surface of prostate cancer (PC) cells that is often targeted as a diagnostic disease marker and can be indicative of cancer metastasis and disease progression. Imaging of this surface protein has previously been explored through the use of radiotracers coordinated to a bifunctional chelator (BFC) to incorporate the PSMA targeting group.

This work describes the synthesis and purification of a bisdipyrrin chelator functionalized with a PSMA-targeting group, the ureido-dipeptide denoted [Lys ureido-glu] (Figure 1). This ligand has been shown to undergo facile radiolabelling with copper-64 under mild conditions. Preliminary pre-clinical investigations will be presented.

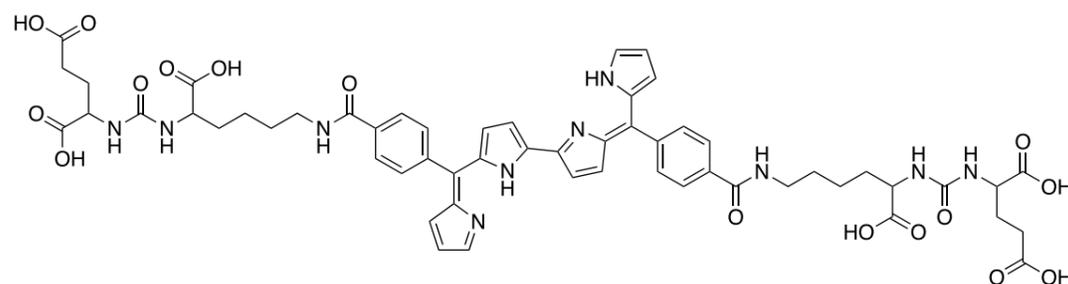


Figure 1. Structure of bifunctional chelator,  $H_2L^1$ .

P280

### A Novel Approach in Cancer Therapy: Induction of Ferroptosis by Iron(III) Salophene Complexes

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Ferroptosis is a currently discovered oxidative and iron-dependent form of regulated cell death, which is driven by the accumulation of lipid-based reactive oxygen species (ROS) [1]. Since many cancer cells are sensitive to ferroptosis, this type of cell death appears as a novel approach in the treatment of cancer [2]. Hence, it was of interest, if iron(III) salophene complexes are also competent to induce ferroptosis.

For this purpose a series of chlorido[*N,N'*-disalicylidene-1,2-phenylenediamine]iron(III) complexes with varying phenylenediamine cores was synthesized and investigated concerning their impact on different leukemic cell lines (HL-60, K-562, SD-1). Particularly, the participation of ferroptosis in the mode of action was evaluated.

The proliferation and the metabolic activity were diminished concentration-dependently upon incubation of the leukemic cells with the iron(III) salophene complexes. They caused the formation of ROS. As the antimetabolic activity of the compounds was not completely abolished by *N*-acetyl-L-cysteine, a scavenger of mitochondrial ROS, the effects were related to lipid-ROS as part of ferroptosis.

Intriguingly, the antimetabolic effect was fully revoked upon concomitant administration of either the ferroptosis and/or necroptosis inhibitor Ferrostatin-1 and Necrostatin-1, respectively. Therefore, the extent of ferroptosis and/or necroptosis is dependent on the substituents of the 1,2-phenylenediamine moiety. The regulation of both ferroptosis and necroptosis by simple substituents may allow a synergistic adjustment of these cell death modalities for the treatment of cancer.

Due to the strong binding of the complexes to the iron transport protein apo-Transferrin, a cellular uptake mediated by apo-Transferrin is assumed. Additionally, the uptake into leukemic cells was confirmed by cellular uptake studies.

To exclude the release of iron from the complexes, the stability under physiological-like conditions as well as in the presence of Ferrostatin-1 and the iron specific chelator Deferoxamine was investigated. No decomposition of the iron(III) salophene complexes was affirmed. Herewith we demonstrate for the first time that, besides free iron ions, intact iron salophene complexes are capable to induce ferroptosis.

Our findings, that iron(III) salophene complexes serve as ferroptosis inducers represent a promising perspective for the future development of anti-cancer active metallodrugs.

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P281

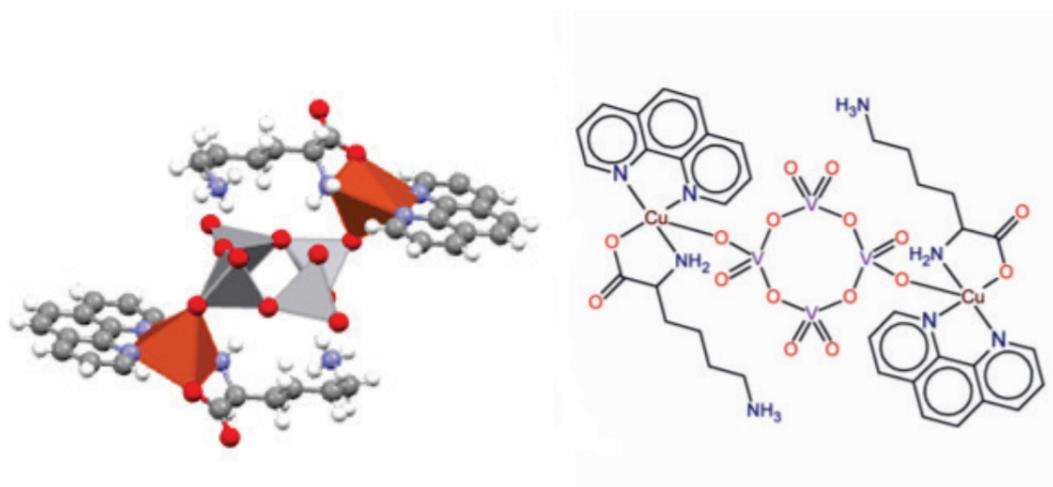
### Cyclo-Tetравanadate Bridged Copper Complexes as Potential Double Bullet Prometallo-drugs for Cancer Treatment

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Over the last decade, Copper and Vanadium complexes have shown promising properties for the treatment of several types of cancer. In particular, the group of copper-based complexes named Casiopeinas® have received much attention and their mechanism of action has been extensively studied since their structure is simple and they can be inexpensively synthesized (1,2). On the other hand, Vanadium-containing compounds both in the form of complexes or simple polyoxovanadates have also been studied as potential antitumor agents (3). Here we report potential prodrugs that would release the two metals, V and Cu, in usable form to act in tandem against cancer cells. The new series of Casiopeinas bridged by a cyclo-tetравanadate ion allows the synthesis of stable compounds with a generic formula  $(N,N' Cu AA)_2 (V_4O_{12})$ , where  $(N,N')$  represent bipyridine and phenanthroline, and AA are the aminoacidate ions (Lysine, Ornithine, and Glycine). Importantly, the structure allows compounds that can be easily crystallized and characterized by various methods.

Financial support by VIEP-BUAP and CONACYT.



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P282

### Combretastatin A4 based Dual and Triple Action Platinum(IV) Complexes for the Application in the Treatment of Cancer

Claudia Schmidt<sup>1</sup>, Tomer Babu<sup>1</sup>, Dan Gibson<sup>1</sup>

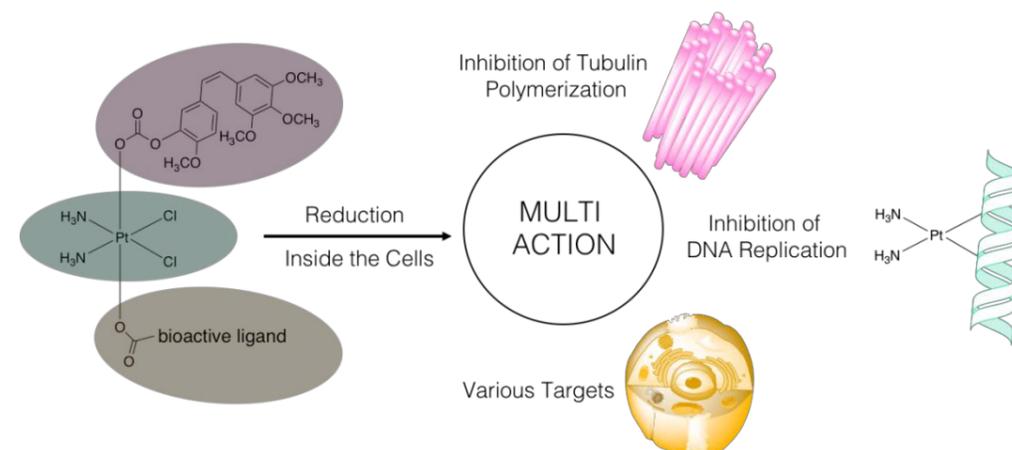
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Square planar platinum(II) based anticancer agents were developed into a class of well-established drugs, used worldwide in the treatment of various cancerous diseases for the last 40 years. Cisplatin, the most widely used drug, has two major drawbacks; toxicity (ototoxicity and nephrotoxicity) and inherent and acquired resistance. Clinicians try to overcome resistance by combination chemotherapy administering several drugs with different modes of action and different cellular targets. This approach is difficult to manage because each drug has its own pharmacokinetics, biodistribution etc. [1,2]

Octahedral platinum(IV) complexes allow us to combine two additional bioactive molecules with the cisplatin, in a way that they are all released inside the cancer cell following reduction of the Pt(IV) thereby forming "multi-action" prodrugs. These complexes are more stable and therefore less reactive in physiological systems until they reach the cells which is expected to lead to fewer side effects.

Combretastatin A4 (CA4) is an antimetabolic and antiangiogenic natural product in clinical trials for the treatment of anaplastic thyroid cancer. It acts by inhibiting tubulin polymerization which leads to cell cycle arrest and apoptosis. It is a potent cytotoxic agent exhibiting antiproliferative effects in various cell lines in the low micromolar to low nanomolar range. [3] CA4 was successfully attached to the platinum(IV) via a carbonate linkage for the first time. One advantage of this new method is that directly following reduction of the complex, CA4 is released without any structural alterations of the active drug through decarboxylation of the carbonate bond. [4] Different bioactive ligands were attached to the other axial position to gain a dual or triple action complex.

Here, we discuss the activity of these new potential anticancer drugs towards several cancer cell lines, investigating reduction rates and stability in cell culture medium versus human serum.



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P283

### Insights Into Biochemical Targets and Changes Induced by Ru(II) Arene Anticancer Complexes

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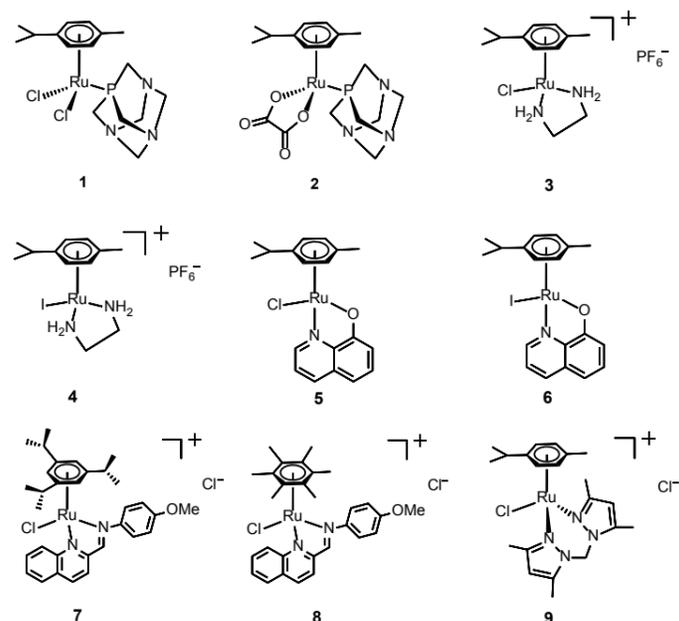
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Ruthenium complexes have emerged as promising alternatives to existing platinum drugs as cancer chemotherapeutics, with the Ru(II) arene complexes being particularly attractive owing to the number of properties and antitumour effects they can possess depending on ligand choice [1]. In addition, many metal-based drugs have been recently recognized for exerting immunogenic effects, a promising new research direction [2]. However in the case of Ru(II) arene complexes, these effects, along with mechanisms of action and biological targets, are not fully understood.

Extracellular vesicles (EVs) are membrane-enclosed particles excreted by all known cell types. Understanding of key roles they play in a number of biological processes including intercellular communication, disease progression and drug action mechanisms is currently emerging [3]. They offer unique, multifaceted opportunities to study both cancer as a disease, and Ru(II) arene complexes as potential treatments. Additionally, the role of interactions between proteoglycan species and metal-based drugs remains an underdeveloped, yet highly exploitable, research direction in chemotherapy [4].

In this project, a library of Ru(II) arene complexes (**1-9** in the figure) covering a variety of ligands and modes of antitumour activities has been prepared, and investigated in breast cancer cell lines and their secreted EVs through spectroscopic techniques and biochemical assays. Distinct differences in the biochemical profiles of EVs sourced from cells treated with the complexes were observed, with implications for impacts on cell signalling, drug resistance and the development of disease mechanisms. Our recent metalloglycomics studies on the interactions of the complexes with proteoglycans will also be discussed.



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P284

### Novel Metallodrugs Targeting the Translocator Protein as Anticancer Agents

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Cisplatin is the most important and effective anticancer drug widely used clinically. In spite of the great success of cisplatin, its clinical use is associated with side-effects such as ototoxicity, neurotoxicity, nephrotoxicity, inherited or acquired resistance, and a narrow spectrum of activity. A new class of anticancer chemotherapeutics has been developed to target the translocator protein (TSPO) which is overexpressed in certain cancers including brain, liver, breast, ovarian, and colorectal cancer, with the aim to overcome the limitations of the clinically used platinum drugs. In this work, Pt(IV) complexes have been designed as new anticancer metallodrugs that selectively target the mitochondria of cancer cells by combining the favourable cytotoxic properties of Pt with the capability of TSPO binders to act as delivery vehicles for antimetastatic agents. The complexes have been fully characterized. Cytotoxic and mechanistic studies of these complexes will also be reported.

Financial support by the European Commission (Marie Curie, H2020-MSCA-IF scholarship to L.T.) is gratefully acknowledged.

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P285

### Glucose-Directed Self-Assembly and Anti-Cancer Properties of Amphiphilic Platinum(II) Terpyridine Complexes

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A panel of platinum(II) terpyridine complexes containing glycosylated acetylde ligands was synthesized and characterized. Coordination of strong  $\sigma$ -donating arylacetylde ligands renders the Pt(II) complexes stable against ligand substitution with physiological thiols and hydrolysis. Spectroscopic studies revealed that the planar Pt(II) terpyridine scaffold enables the complex **1a** to bind to DNA *via* intercalation with greater binding affinity compared with **2a** bearing bulky *tert*-butyl substituted terpyridine ligand [1]. Tethering the hydrophilic glucose unit to the alkynylplatinum(II) complexes has been shown in assisting the self-assembly of particle-like nanostructures in aqueous media. The results of UPLC/MS indicated that the glucosidase-triggered cleavage of the glycosidic bond of **1a** gives rise to the release of **1d**, which exhibits different molecular assemblies in buffer solution, as supported by TEM imaging. Cellular uptake experiments and TEM analysis revealed the accumulation of **1a** into the cancer cells through glucose-dependent endocytosis. The electron-dense nanostructures in **1a**-treated cells demonstrated that the complex localized in late endosomes/lysosomes and mitochondria, triggered the increased lysosomal membrane permeability, accumulation of autophagosomes, mitochondrial dysfunction, cell cycle arrest, and ultimately induced cell death.

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P286

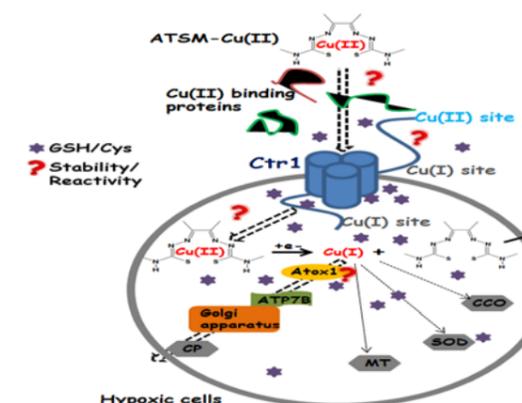
### Reactivity of ATSM-Cu<sup>II</sup> a Hypoxic Biomarker in the Human Cellular Copper Cycle

**Gulshan Walke<sup>1</sup> and Sharon Ruthstein<sup>1</sup>**

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Hypoxia is an oxygen-deprived condition of cells commonly observed in cancer and neurodegenerative diseases like Alzheimer's and Parkinson's disease. [1] Identification of hypoxic cells plays a critical role for early diagnosis of diseased conditions hence to prevent the progression of the disease. <sup>64</sup>Cu<sup>II</sup>-tracers such as <sup>64</sup>Cu<sup>II</sup>-diacetyl-bis(N<sup>4</sup>-methylthiosemicarbazone), ATSM-<sup>64</sup>Cu<sup>II</sup>, has been proposed to become one of the most promising PET agents for hypoxia imaging. [2] It was suggested that ATSM-<sup>64</sup>Cu(II) tracer is actively uptake by the cell and it is incorporated in the cellular copper cycle by 3 proteins: Ctr1, Atox1, and ATP7B. However, the mechanism of cellular uptake of ATSM-<sup>64</sup>Cu<sup>II</sup> remains unclear.

We studied the interactions of ATSM-Cu<sup>II</sup> complex with human copper-binding peptides/proteins under strong reducing conditions using UV-Visible spectroscopy and Electron Paramagnetic Resonance spectroscopy. ATSM-Cu<sup>II</sup> remains intact against extracellular high-affinity Cu<sup>II</sup> binding peptides, DAHK (amino-terminal Cu<sup>II</sup> and Ni<sup>II</sup> binding (ATCUN) motif of HSA) [3] and NCtr1 (extracellular N-terminal of Ctr1 protein, also an ATCUN motif) [4]. The 100-fold excess of physiological reductants, ascorbate (AA), L-Cysteine (Cys), and glutathione (GSH) can reduce ATSM-Cu<sup>II</sup> complex incompletely till 120 min, in order of AA > Cys > GSH (very sluggish). The use of bichinchonic acid (BCA) as Cu<sup>I</sup> detector helped to follow the reduction reaction of ATSM-Cu<sup>II</sup> in the presence of intracellular Cu<sup>I</sup> sinks, GSH and Atox1. Interestingly, BCA enhances the rate of ATSM-Cu<sup>II</sup> reduction, detected as a chromogenic (BCA)<sub>2</sub>-Cu<sup>I</sup> complex; however, ATSM ligand may also efficiently competes for BCA for Cu<sup>I</sup> binding. Atox1 significantly decrease the rate of ATSM-Cu<sup>II</sup> reduction. Even in the presence of BCA, the Cu<sup>I</sup> may bind Atox1 instead of BCA as no (BCA)<sub>2</sub>-Cu<sup>I</sup> was detected. Similar results were observed in the case of GSH however the low amount of (BCA)<sub>2</sub>-Cu<sup>I</sup> was identified which confirms that GSH has a little ability to bind Cu<sup>I</sup> than Atox1. Overall, this study suggests that the extracellular Cu<sup>II</sup> binding peptides do not affect the stability of ATSM-Cu<sup>II</sup>. However, the intracellular copper protein-Atox1 (Human) and GSH may affect the reduction of ATSM-Cu<sup>II</sup> through Cu<sup>I</sup> binding but shows low activity. Financial support by the ERC-STG (ERC-STG 754365) is gratefully acknowledged.



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P287

**Anticancer Cyclometalated Platinum(II) Complexes Containing N-Heterocyclic Carbene Ligands: Vimentin Targeting, Metabolism and Nanof ormulation****Pui-Ki Wan<sup>1</sup>, Ka-Chung Tong<sup>1</sup>, Chunlei Zhang<sup>1</sup>, Chun-Lam Lok<sup>1</sup>, Chi-Ming Che<sup>1</sup>**<sup>1</sup>State Key Laboratory of Synthetic Chemistry, Department of Chemistry and Chemical Biology Center, The University of Hong Kong, Pokfulam Road, Hong Kong, China. kikiwan@connect.hku.hk

A stable cyclometalated platinum(II) complex containing N-heterocyclic carbene (NHC) ligand, Pt1a, was shown to display promising *in vitro* cytotoxicity and *in vivo* antitumor activity [1]. Nevertheless, the molecular target and metabolism of Pt1a remain unexplored. In our recent work, we have identified that Pt1a engages with vimentin. The binding interactions and associated cellular responses were validated comprehensively by different techniques. Computational docking studies further demonstrate the possible binding modes of Pt1a with vimentin. Regarding vimentin as a mesenchymal marker in epithelial-to-mesenchymal transition during tumor progression [2], the anti-metastatic property of Pt1a was examined using mouse model of metastasis. Pt1a significantly inhibited the tumor growth of lung metastasis without apparent body weight loss over a month of treatment.

In metabolism studies, Pt1a was found to undergo cytochrome P450 phase I and phase II metabolism, generating different hydrophilic metabolites. The hydroxylated derivatives of Pt1a were then synthesized and characterized, serving as the standards for verifying the metabolites in rat liver microsomal extract and mouse urine by using UPLC-QTOF-MS. The metabolites were shown to be less cytotoxic towards a panel of cancer cell lines compared with the parent Pt1a, presumably due to the lower extent of cellular uptake. Furthermore, PEGylation [3] was employed to alter the *in vivo* biological response of Pt(II)-NHC complex. The PEGylated Pt(II) conjugate exhibited potent antitumor activity in lung cancer xenograft model with prolonged plasma retention and improved overall biodistribution of platinum content in mice.

These results demonstrate that the target engagement of Pt1a with cellular vimentin and the resultant biological consequences, as well as the importance of metabolism and the feasibility of structural modification of the pincer platinum(II)-NHC scaffold for anticancer application.

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P288

**How Does Thiosulfate-Binding Protein CysP Efficiently Transport S<sub>2</sub>O<sub>3</sub><sup>2-</sup>****Qi Zhang<sup>1</sup>, Hongyan Li<sup>1</sup>, Hongzhe Sun<sup>1</sup>**<sup>1</sup>Department of Chemistry, The University of Hong Kong, Pokfulam Road, Pok Fu Lam, Hong Kong, P. R. China. u3004182@connect.hku.hk

Sulfur, one of major elements, constitutes lots of functional organic molecules *in vivo*[1]. Its content is strictly controlled by many transport systems. In Gram-negative bacteria, sulfur/thiosulfate can be efficiently transferred into cell by sulfate/thiosulfate transport system which consists of ATP dependent membrane protein CysU/CysW, inner member binding protein CysA and system unique protein (thiosulfate-binding protein CysP or sulfate-binding protein Sbp). Although function overlapping, the two systems show obvious substrate specificity. Thiosulfate transport system has stronger binding affinity with thiosulfate than sulfate. Its unique protein CysP shows highly sequence conservation (>90%) among lots of pathogenic gram-negative bacteria. Thus, CysP has been suggested to be a good candidate of vaccine antigen.

Here, we elaborate its exact maturing progress. It presents as a dimer in cytoplasm with a signal peptide and then shows as the trimer in periplasmic space once its signal peptide is deleted. Interestingly, our thermal shift data indicate that, its native substrate S<sub>2</sub>O<sub>3</sub><sup>2-</sup>, unlike SO<sub>4</sub><sup>2-</sup>, doesn't show obvious effect on strengthening the thermal stability of immature CysP although both S<sub>2</sub>O<sub>3</sub><sup>2-</sup> and SO<sub>4</sub><sup>2-</sup> can enhance mature CysP thermal stability, which may come from different binding affinity as revealed from SPR data which show that the deletion leads to the enhancement in its substrate specificity, i.e. the binding affinity (*K<sub>d</sub>*) is enhanced about 8 times (3.9 and 32.6 nM) between mature CysP and its potential substrate ions, S<sub>2</sub>O<sub>3</sub><sup>2-</sup> or SO<sub>4</sub><sup>2-</sup>, instead of comparable binding (17.43 and 11.1 nM) when the signal peptide is still located at the N-terminal of CysP. Furthermore, we show that the native CysP bind 8-fold S<sub>2</sub>O<sub>3</sub><sup>2-</sup>, whereas without obvious binding to Mo and Se. Our crystallography data further support these arguments, that substrate ion S<sub>2</sub>O<sub>3</sub><sup>2-</sup> is compactly filled into a 7.6Å cavity by interaction with Y<sub>12</sub>, D<sub>13</sub>, S<sub>48</sub>, N<sub>67</sub>, S<sub>134</sub>, N<sub>136</sub>, and F<sub>196</sub>. This work is supported by a grant from Research Grants Council of Hong Kong (170461P).

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P289

### A Platinum(IV) Prodrug Bearing Toll-Like Receptor 7 Agonist for Enhanced Chemo-Immunotherapy

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Immunotherapy complemented platinum drugs-based chemotherapy could significantly boost its antitumor efficacy especially in resistant cancer.[1] It has been shown that oxaliplatin could stimulate immune response by inducing tumor immunogenetic cell death (ICD).[2] Here, an oxaliplatin prodrug containing a toll-like receptor 7 (TLR7) agonist was prepared and well characterized. Within tumor, the prodrug was expected to release oxaliplatin and TLR7 agonist upon reduction. Oxaliplatin will induce ICD to stimulate immune response, while TLR7 agonist will improve phagocytosis and antigen presentation of dendritic cells (DC) which will further activate tumor-specific T cell cytotoxic lymphocytes (CTL) to kill cancer cells. *In vitro assay* showed that the prodrug activate TLR7 and significantly promote the secretion of cytokines of DC. The prodrug induced apoptosis and ICD in murine mammary carcinoma cell. The prodrug is also active *in vivo* against the growth of murine mammary tumor. Mechanism studies suggested that increased activation of CTL contribute to the enhanced *in vivo* activity of the prodrug. This work sheds light on the development of more potent platinum-based anticancer drugs by combination with immunomodulator.

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P291

### Gallium-Based Agents as an Inhibitor of Metallo- $\beta$ -Lactamase

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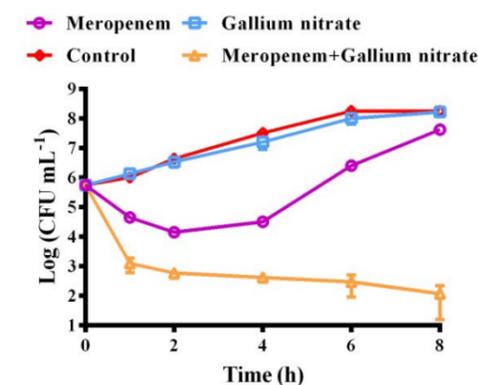
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Being the top ten threats to global health in 2019 as enumerated by the World Health Organization (WHO), antimicrobial resistance (AMR) empowers bacteria the ability in confronting existing antimicrobial agents. Metallo- $\beta$ -lactamase (MBLs), exemplifying with New Delhi metallo- $\beta$ -lactamase 1 (NDM-1), which is a subclass B1 MBL and able to hydrolyze almost all  $\beta$ -lactam antibiotics, including the last resort carbapenems.

Combination therapy is regarded as one of the promising approaches in combating AMR. We previously found that metallo-agents are potential MBL inhibitors [1]. Despite the fact that gallium-based agents are well-known for their therapeutic activity in cancers, infections and inflammatory conditions [2], previous studies demonstrated that clinically approved gallium compounds exhibited antibacterial activity against several human pathogens, including ESKAPE species [3,4], aligning with our findings.

Here, we found that gallium-based agents exhibited good antimicrobial activity against MBL-positive bacteria. We show that gallium complexes had synergistic effect with meropenem (MER), could reduce the minimum inhibitory concentration (MIC) of MER by 16-folds, with a fractional inhibitory concentration (FIC) index of 0.375. Co-treatment of gallium complexes could reduce bacterial density by over 10,000-folds in comparison with any single component after 8 hrs, indicative of remarkably enhanced bactericidal activity of MER, while gallium itself showed no inhibition to bacterial growth. Enzyme assay showed that enzymatic activity was almost completely retarded in the presence of gallium, with a half-maximum inhibitory concentration (IC<sub>50</sub>) of 262.9  $\mu$ M ( $\pm$ 44.5  $\mu$ M). We therefore conclude that gallium-based agents may serve as promising antimicrobial candidates to combat AMR caused by MBL producing bacteria.

We thank the Research Grants Council of Hong Kong (R7070-18) and the University of Hong Kong for their support of this work.



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P294

### Activation of Potent Organometallic Iridium(III) Anticancer Complexes

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Precious metal-based anticancer agents offer novel mechanisms of action which might address acquired resistance and undesired side effects of platinum drugs.[1] Organometallic iridium complexes have emerged as effective catalytic agents for the potential treatment of cancer.[2] For example, the half-sandwich organometallic iridium(III) complex  $[\text{Ir}(\eta^5\text{-Cp}^{\text{xbiph}})(\text{phpy})\text{py}]\text{PF}_6$  containing tetramethyl(phenyl)cyclopentadienyl ( $\text{Cp}^{\text{xph}}$ ) and phenylpyridine chelating ligands, exhibits nanomolar activity in a wide range of cancer cell lines in the NCI-60 screen, and is an order of magnitude more potent than cisplatin. The mechanism of action appears to involve catalytic hydride transfer from coenzyme NADH to oxygen to produce the ROS  $\text{H}_2\text{O}_2$ . [3]

We report here the synthesis, characterization and anticancer activity of a new family of half-sandwich organometallic iridium(III) complexes which are relatively inert towards ligand exchange but exhibit potent anticancer activity, and investigations of their mechanism of activation. Density functional theory (DFT) calculations on their hydrolysis and binding to biomolecules have provided insights into their activation. The complexes have interesting catalytic activity, which appears to involve ligand-based reactions.

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P295

### Anticancer Activities of Au(III) Thiosemicarbazone Complexes

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In this study, a series of gold(III) complexes containing thiosemicarbazone ligand have been synthesized and characterized. The stability of complex 1 towards glutathione (GSH) was studied by UV-Vis, ESI-MS and <sup>1</sup>H NMR spectroscopy. The cytotoxicity of these complexes against a panel of human cancer cell lines has been investigated and these complexes demonstrated strong antiproliferative activity. Enzyme inhibition studies showed these complexes can inhibit thioredoxin reductase (TrxR). Interestingly, complex 4 induced a significant increase in intracellular levels of reactive oxygen species (ROS). Further in vivo studies of complex 4 reveal that it can significantly inhibit tumor growth in nude mice bearing *NCI-H460* xenograft.

Financial support by The University of Hong Kong is gratefully acknowledged.

**P300****Tracing the Self-Splicing of a Group II Intron at the Molecular Level Via smFRET**Esra Ahunbay<sup>1</sup>, Susann Zelger-Paulus<sup>1</sup>, Richard Börner<sup>1</sup>, Roland K. O. Sigel<sup>1</sup><sup>1</sup>Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland.  
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The dynamics of biomolecules are extensively studied by means of Förster Resonance Energy Transfer (FRET). To disentangle the ensemble average and to visualize distinct conformational states and state-transitions, their fine surveillance is achievable at the single-molecule (sm) level by smFRET [1]. In this study, we take advantage of this technique to monitor the splicing events of a long non-coding RNA, namely the group II intron ai5γ, found in the mitochondria of *Saccharomyces cerevisiae*. Recent advances in the field of splicing by *in vitro* studies under near-physiological conditions especially focus on the nature of ligation of the coding regions catalyzed by the group II intron itself [2]. In addition, these endeavors are greatly complemented with conformational state analysis by employing smFRET [3]. Nevertheless, to fully capture the complex self-catalytic mechanism, it is crucial to couple the changes in the ribozyme structure with function.

Oriented towards multi-color fluorescence imaging, here, we propose a highly precise and stable intermolecular labeling strategy, which is promising to site-specifically tag the particularly long intron, without compromising the ribozyme activity. This bioorthogonal approach involves phosphoramidate activation of the 5' terminus of the RNA and its further functionalization with an amine modified fluorophore, as well as the periodate oxidation of the 3' end and its subsequent modification with a hydrazide attached dye [4]. Our choice of FRET pair is Cy3 and Cy5 dyes, donor and acceptor, respectively. Thereby, dual fluorescent labeling of the flanking exons grants the visualization of the folding pathways and the self-splicing mechanism of the intron.

Financial support by the Swiss National Science Foundation (RKOS) and the University of Zurich and the Graduate School of Chemical and Molecular Sciences Zurich (CMSZH) is gratefully acknowledged.

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**P303****Synthesis, Molecular Properties and Comparative Docking and QSAR of New 2-(7-Hydroxy-2-Oxo-2H-Chromen-4-yl)Acetic Acid Derivatives as Possible Anticancer Agents**Elhenawy, A.A.<sup>1</sup>, Al-Harbi, L.M.<sup>2</sup>, El-Gazzar, M.A.<sup>3</sup>, Khowdiary, M.M.<sup>3</sup>, Moustfa, A.<sup>3</sup><sup>1</sup>Chemistry Department, Faculty of Science, Al-Azhar University (Boys Branch), Nasr City, Cairo, Egypt.<sup>2</sup>King Abdulaziz University, P.O. Box 80203, Jeddah, 21589, Saudi Arabia.<sup>3</sup>Egyptian Petroleum Research Institute, Applied Surfactant Laboratory, Nasr City, Cairo 11727, Egypt.

Cancer multi-drug resistance (MDR) is a challenge problem for cancer treatment [1,2]. Coumarin has been selected due to the widely presence in various natural products [3], as well as their extensive properties as antibacterial, antioxidant, anti-inflammatory and anticancer [4–6]. Coumarin acetic acid connected with amino acid fragment has been considered a useful strategy for design new bioactive compounds with promising biological properties [7].

Novel coumarin amino acid derivatives were synthesized. The structure of synthesized compounds has established on basis of different spectral data. The optimization geometry, frontier molecular orbitals (FMOs), thermodynamic parameters and chemical reactivity, were discussed using DFT\B3LYP by 6-311G\* basis set, to identify a clear view for inter and intramolecular interaction of tested compounds. The molecular electrostatic potential (MEP) has plotted to investigate a recognition manner of synthesized compounds upon COX-2 receptor. All tested compounds showed a promising (NLOs) nonlinear optical properties. Bond dissociation energy (BDE) has studied to investigate a potency of these molecules against autoxidation mechanism Polynomial molecular docking logarithms have performed into the COX-2 active site for tested compounds. The docking protocol that has low RMSD has selected for discussion the binding affinity. The compounds with a high docking score 3,4,6–8,10 and 11 were selected for additional study against ADMET insilico, which showed that these compounds are a good oral bioavailability without observed carcinogenesis affect. The compounds (3,4,6–8,10 and 11) which that passed through docking and ADMET profile have examined their potency against (MCF-7) breast cancer cell *in vitro*. The compound 7 showed a highest potency against MCF-7 with IC 50 value 0.39 μM. The QSAR model has created to discover the structural necessity inhibition of MCF-7. The derived QSAR model has a statistically significant with a good predictive power.

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P304

### Xanthosinate 5'-Monophosphate (XMP – H)<sup>3-</sup>: Its Intriguing Acid–Base and Metal Ion-Binding Properties

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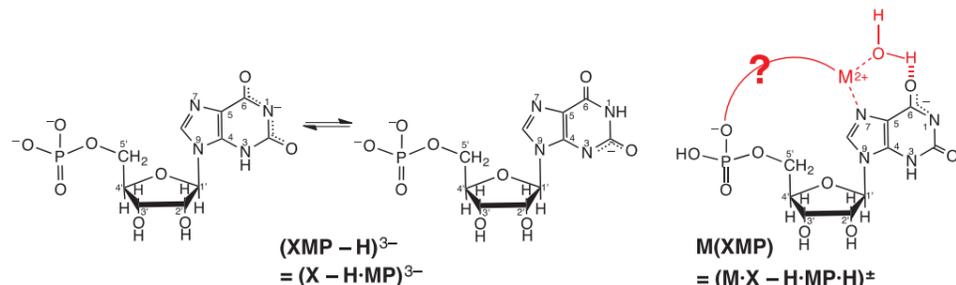
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Fifteen years ago it had been pointed out that in the physiological pH range of 7.5 the xanthine residue of xanthosine nucleotides exists in a monodeprotonated form, i.e., the (N1)H/(N3)H sites have lost one H<sup>+</sup> [1,2]. Considering that xanthosine (Xao) and its nucleotides are important metabolic intermediates [1,3,4], it is surprising that the indicated acid–base property is often overlooked [3,5,6]. For example, in the literature [3,5,6], the structure of XMP is often shown in analogy to guanosine 5'-monophosphate, which is not correct [4] and thus, revisions will be needed, e.g., in calculations concerning thermodynamics [5].

Monoprotonated XMP<sup>2-</sup>, i.e., H(XMP)<sup>-</sup>, is deprotonated at the xanthine moiety (pK<sub>a</sub> = 5.30 ± 0.02) [2,4], with an H<sup>+</sup> still at the phosphate, hence, XMP<sup>2-</sup> should preferably be written as (X – H·MP·H)<sup>2-</sup>. Indeed, XMP<sup>2-</sup> is a minority species (ca 12%), the dominating tautomer being (X – H·MP·H)<sup>2-</sup> with about 88%. This species loses H<sup>+</sup> from P(O)<sub>2</sub>(OH)<sup>-</sup> (pK<sub>a</sub> = 6.45 ± 0.02) [2,4] and exists thus at pH 7.5 mainly as (X – H·MP)<sup>3-</sup>. In 9-methylxanthine deprotonation occurs to over 99% at (N3)H; in xanthosinate already about 30% are (N1)H-deprotonated and for (X – H·MP)<sup>3-</sup> it is concluded [4] (see the tautomers below) that (N1)H deprotonation is further favored, especially upon metal ion (M<sup>2+</sup>) coordination at N7.

The anionic xanthinate moiety has a pronounced M<sup>2+</sup> affinity [7]; the resulting complexes, formed with (X – H·MP·H)<sup>2-</sup>, are best written as (M·X – H·MP·H)<sup>±</sup>; they all have M<sup>2+</sup> at the N7/[(C6)O] site and H<sup>+</sup> at the phosphate [4,7]. In the tentative structure shown below, (C6)O participates via H-bonding. Interestingly, M<sup>2+</sup> forms macrochelates with P(O)<sub>2</sub>(OH)<sup>-</sup>; their average formation degree is 64 ± 9% (3σ) for Ba<sup>2+</sup>, Sr<sup>2+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, and Cd<sup>2+</sup> [4,7]. This result is best explained by an outer-sphere interaction between M<sup>2+</sup> and P(O)<sub>2</sub>(OH)<sup>-</sup>. Note, the 64% refer to an intramolecular equilibrium, "open" ⇌ "closed" (chelated), and are thus independent of the absolute stabilities of the complexes [4,7].

Release of H<sup>+</sup> from P(O)<sub>2</sub>(OH)<sup>-</sup> in (M·X – H·MP·H)<sup>±</sup> leads to a shift of M<sup>2+</sup> from the nucleobase to the phosphate, the latter now being the primary binding site [4,7]. In the resulting M(X – H·MP)<sup>-</sup> species the M<sup>2+</sup> ions behave as individuals, i.e., the macrochelates involving N7 and possibly also C(6)O, e.g., with Ca<sup>2+</sup>, Mn<sup>2+</sup>, Cu<sup>2+</sup>, and Zn<sup>2+</sup> form to about 0, 50, 95, and 88%, respectively [4,7]. Finally, with regard to biological systems one may add that the negatively charged xanthinate residue is able to undergo aromatic-ring stacking as proven for Cu(1,10-phenanthroline)(X – H·MP)<sup>-</sup> complexes [8].



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P305

### Platinum(II) and Palladium(II) Complexes of Tridentate Hydrazone-Based Ligands as selective Guanine Quadruplex Binders

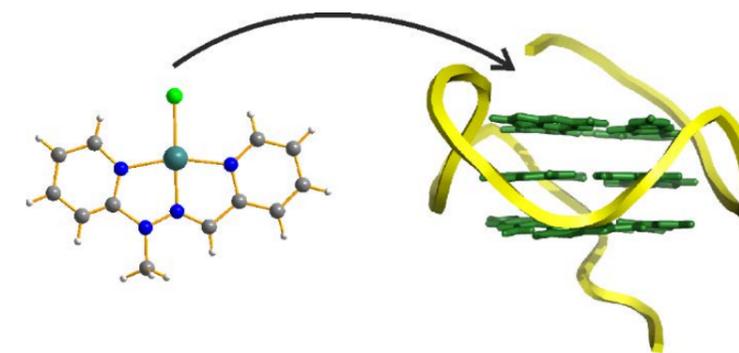
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Guanine quadruplexes (G4) represent a polymorph of DNA consisting of tetra-stranded helices formed upon stacking of two or more G-tetrads. The G-tetrad consists a planar arrangement of four guanine residues involved in H-bonding interactions *via Watson-Crick* and *Hoogsteen* faces. These structures are further stabilised by the presence of alkali-metal cations such as K<sup>+</sup>, Na<sup>+</sup>. Guanine-rich sequences are found at telomeric regions and near the promoter region of several oncogenes (*c-myc*, *c-kit*). Formation of G-quadruplex structures in these regions inhibit telomerase and regulate the transcription of certain oncogenes. Thus small molecules capable of inducing G-quadruplex formation are of great interest for the development of anticancer drugs. Metal complexes with a broad range of structural and electronic properties can be exploited for the design of G4 binders [1, 2].

Here, we present the interaction of nine Pd(II) and Pt(II) complexes bearing hydrazone-based tridentate ligands with quadruplex-forming sequences, H-telo and *c-myc*. Circular dichroism (CD) spectroscopy, temperature-dependent CD spectroscopy, UV-Vis spectroscopy and a fluorescent intercalator displacement (FID) assay were employed to study the binding interactions. All the metal complexes were found to interact with the quadruplex sequences and increase the melting temperature of the G-quadruplexes. According to the FID assay, some of the complexes belong to the highest-affinity metal-containing quadruplex binders reported so far. Their affinity towards quadruplex DNA is up to 80-fold higher than to a reference double helix, which confirms the selective interaction of the complexes with quadruplex sequences [3].

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P306

### Sequence-Specific DNA Damage Induced by Metal-Complex-PNA Conjugate

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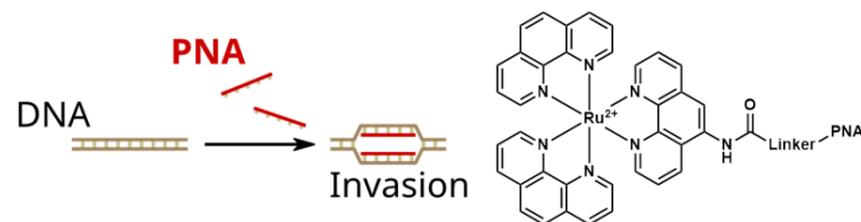
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We developed a metal-complex-conjugated double-stranded DNA (dsDNA) binder which can sequence-specifically cause damage to the target DNA. Recent developments of artificial nucleic acid analogues improved sequence specificity and DNA recognition affinity of oligonucleotides and those improvements have widened their applicability, for example, to DNA sensing, DNA nanoarchitecture, and gene expression control. Although hybridization-based DNA binder mainly targets single-stranded DNA, the recognition of dsDNA is attracting much attention in order to target genomic DNA, which exists as a double-stranded form in cellular. Peptide nucleic acid (PNA), one of the artificial nucleic acid analogues, was reported to directly invade into dsDNA and form a pair of PNA/DNA duplexes [1]. This invasion of PNA selectively recognizes sequences in double-stranded DNA and is expected to be utilized for intracellular DNA recognition, but the invasion efficiency is decreased at physiological salt concentrations.

Here, we developed a conjugate of PNA with Ru complex (Ru-PNA). The Ru complex is known as a minor-groove binder of DNA [2] and should reinforce the DNA binding affinity of PNA by tightly anchoring PNA to DNA [3]. In addition, the Ru complex was also reported to break DNA strands by photoreaction [4]. This Ru-PNA conjugate is expected to recognize and cleave the target DNA sequence specifically and should be applied to genome editing.

Because PNA is synthesized by standard solid-phase peptide synthesis (SPPS), the Ru complex with a carboxylic acid linker was synthesized to be introduced via the amino group on the PNA during SPPS. After the optimization of the introduction manner, the Ru complex was modified to the side chain of diaminopropionic acid. The optimized Ru-PNA formed a more stable duplex with DNA than the PNA without Ru complex modification (non-Ru PNA) and showed higher  $T_m$  values even under physiological condition. The invasion efficiency of Ru-PNA was evaluated by electrophoresis mobility shift assay (EMSA), and the Ru-PNA showed much higher invasion efficiency than non-Ru PNA. After the formation of the invasion complex, blue light was irradiated to the solution containing the invasion complex to evoke the DNA breaks by Ru-complex photoreaction. Although non-Ru PNA caused almost no change in EMSA results, the band of invasion complex with the Ru-PNA disappeared by this light irradiation. The DNA damage was also observed even on super-coiled plasmid DNA and gave an open circular DNA. These phenomena were not observed with single-base-mismatched target DNA, and thus the Ru-PNA can sequence-specifically cause damage to the target DNA. In addition to the Ru complex, the investigation of other metal complexes is currently underway in our laboratory.



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P308

### Magnesium(II)-Dependent RNA Folding of a Group II Intron Investigated by Single Molecule FRET

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Group II introns are a class of self-splicing ribozymes and belong to the largest ribozymes in nature [1]. *In vitro*, they fold into their native structure in a stepwise process, yet  $Mg^{2+}$  dependent manner [2]. For studying the folding mechanism, the model system D135-L14, derived from the group II intron *Sc.ai5γ* of *Saccharomyces cerevisiae* is widely used. The construct possesses all necessary domains (D) for catalytic activity and has two additional loops (L) for labelling with fluorescence dyes, thus suitable for single-molecule Förster resonance energy transfer (smFRET). Previous studies assumed an overall compaction of the construct from the unfolded to the native state [2-4]. For further insights into the folding mechanism, knowing the positions of the flanking exons during the folding process gives information about the catalytically relevant 5'- and 3' splice sites and thus of the catalytic core of the ribozyme. For this reason, we developed a new construct eD135-L14e that comprises short flanking exons serving as additional labeling platforms [5]. Hence, together with L1 and L4 labeling this enables various FRET and distance trajectories to study the folding in a comprehensive manner [5]. To determine a FRET network, i.e., the combination of multiple FRET trajectories to attribute distinct FRET values of different labelling positions to the same conformational state, smFRET experiments under various  $Mg^{2+}$  concentrations will be performed. We assume, that FRET histograms will change and reveal the magnesium(II) dependent population of conformational states ranging from the unfolded to the native fold of the ribozyme. The Cy3/5 labelled construct is investigated, using both total internal reflection microscopy (TIRM) and confocal microscopy smFRET, to ensure data integrity on all time scales. With the FRET network at hand, we will be able to solve the dynamic pathway from the pre- to the post catalytic state of the ribozyme.

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P309

Artificial Nuclease and Anticancer Activity of a Mononuclear Copper(I) Complex and a Related Binuclear Double-Stranded Helicate

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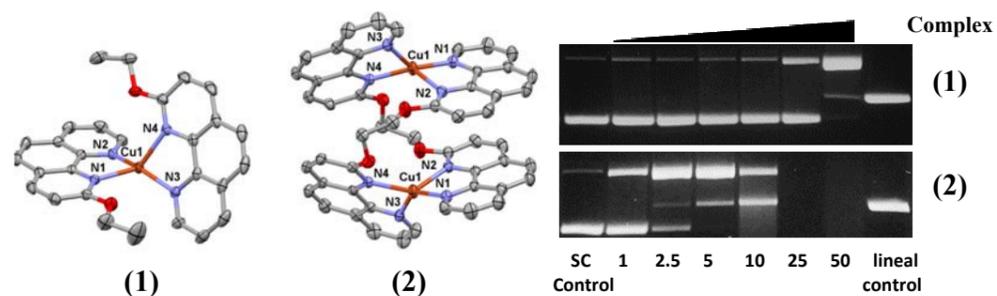
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Artificial nucleases have potential applications in the field of biotechnology and medicine. [1,2] In particular, coordination compounds with nuclease activity are candidates as chemotherapeutic agents in the treatment of cancer [3]. Here, we report the synthesis and characterization of a new mononuclear copper (I) complex derived of phenanthroline, [Cu(L<sup>1</sup>)<sub>2</sub>](ClO<sub>4</sub>), (1), and a related binuclear double-stranded helicate, [Cu<sub>2</sub>(L<sup>2</sup>)<sub>2</sub>](ClO<sub>4</sub>)<sub>2</sub>, (2). The structure of both complexes was confirmed in solid and solution phase through NMR, UV-Vis, elemental analysis, electrochemistry and X-ray diffraction (fig. 1).

Interestingly, both complexes can operate as artificial nucleases against pBR322 plasmid DNA, with no addition of any oxidant agent such as hydrogen peroxide. The results of DNA cleavage show that the binuclear helicate has a higher activity, with a moderate activity at 1 μM (~30% DNA cleavage), while complete conversion from the supercoiled form to nicked and lineal form at 5 μM. The mononuclear complex shows only moderated activity at 25 μM (~44% DNA cleavage). Mechanistic studies by electrophoresis technique using ROS scavengers, showed that HO• radical and H<sub>2</sub>O<sub>2</sub> are involved in the damage of DNA by oxidative process. [4] Differences in the activity of the mono and bimetallic complexes are discussed in terms of their nuclearity, cationic charge, size and geometrical distortions from the copper atoms.

Both copper(I) complexes had been studied as cytotoxic agents in human bone (MG-63), breast (MCF-7) and colon (HT-29) cancer cell lines, presenting activity at the sub-micromolar concentration level. Results are discussed in base an oxidative mechanism induce by copper(I) complexes, through intracellular ROS generation.



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P311

Copper(II) Complexes of Tripodal N-donors Pyridyl Ligands as Anticancer Agents

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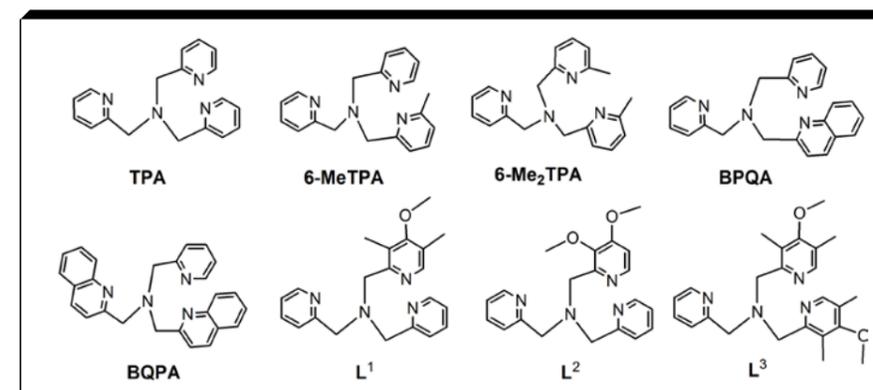
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The complexes [Cu(L)Cl]ClO<sub>4</sub>/PF<sub>6</sub> (1-ClO<sub>4</sub>, L = TPA[1]; 2-ClO<sub>4</sub>/2-PF<sub>6</sub>: L = 6-MeTPA; 3-PF<sub>6</sub>, L = Me<sub>2</sub>TPA; 4-ClO<sub>4</sub>/4-PF<sub>6</sub>, L = BPQA; 5-ClO<sub>4</sub>/PF<sub>6</sub>, L = BQPA; 6-ClO<sub>4</sub>/6-PF<sub>6</sub>, L = L<sup>1</sup>; 7-ClO<sub>4</sub>, L = L<sup>2</sup>; 8-ClO<sub>4</sub>, L = L<sup>3</sup>) have been synthesized and structurally characterized (Scheme 1). The *in vitro* cytotoxicity studies of the complexes against A2780 (ovarian), A2780R (cisplatin-resistant variant) and MCF7 (breast cancer) human cancer cell lines revealed moderate-to-significant effects compared to the reference *cisplatin* drug. Interestingly, the 5-ClO<sub>4</sub> and 5-PF<sub>6</sub> compounds showed very high cytotoxicity, with the lowest IC<sub>50</sub> values about 5–10 μM. The DNA cleavage studies demonstrated that the complexes are effective in causing the DNA damage by means of the minor direct hydrolytic cleavage and major oxidative mechanism (associated probably with the formation of oxidative metal-based intermediates, similar to those formed in the enzymatic mechanism of mono-copper monooxygenases, such as CuO<sup>+</sup>, CuO<sup>+</sup>• or [CuOOH]<sup>+</sup>).

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Scheme 1. Structures and abbreviations of tripod N-donors pyridyl ligands used in this study

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P312

### Determination of the NMR Solution Structure of the CPEB3 Ribozyme

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This work is focused on the investigation of the solution structure and folding mechanism of the cytoplasmic polyadenylation element binding protein 3 (CPEB3) ribozyme by nuclear magnetic resonance (NMR). Ribozymes are RNA molecules that act as chemical catalysts in cells. The discovery of ribozymes was a milestone in RNA research and revealed the unique role of RNA in a multitude of cellular reactions. The CPEB3 ribozyme is until now the only confirmed small ribozyme in mammals and its role remains still unknown. [1] As RNA function is directly linked to structure, structural studies are the basis to understand RNA function.

The CPEB3 ribozyme belongs to the Hepatitis Delta Virus (HDV)-like family of self-cleaving ribozymes that have a nested double pseudoknot fold and a 5'-end cleavage activity in common. [2] The best studied ribozyme in this family is the HDV ribozyme itself, whose three dimensional structure was solved by X-ray crystallography. [3] Even though, the number of newly discovered HDV-like ribozyme is continuously increasing, the HDV ribozyme is still the only one with a known structure. Therefore, solving the structure of the CPEB3 ribozyme will not only help to enlighten its biological role but also to expand the knowledge on the HDV-like ribozyme family in general.

NMR structure determination of such a large RNA is a challenging task due to heavy spectral overlap and large line widths of the proton resonances. To overcome this issue, we use different labeling schemes. For example, we use partially deuterated nucleotides, <sup>13</sup>C, <sup>15</sup>N labeling techniques and apply up-to-date multinuclear and multidimensional NMR spectroscopy. Financial support by the University of Zurich and the Swiss National Science Foundation is gratefully acknowledged.

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P313

### Light-Induced Formation of Thymine-Containing Hg(II)-Mediated Base Pairs

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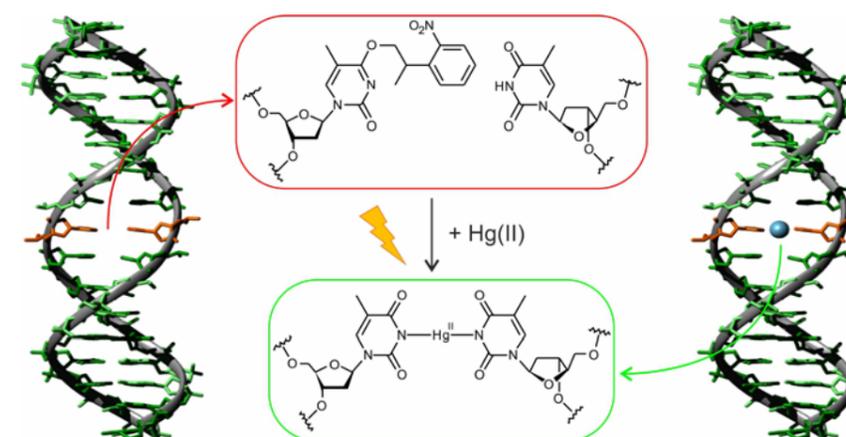
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DNA is a self-assembling supramolecule with a highly specific molecular recognition between two complementary oligonucleotides during the formation of a double helix. The double-helical structure of DNA is stabilized by two non-covalent interactions, the  $\pi$  stacking of the planar aromatic nucleobases and the hydrogen bonding within the complementary base pairs.

DNA can be used as a ligand system for the coordination of metal ions, involving the concept of metal-mediated base pairing. Here, the hydrogen bonds, as present in a canonical duplex, are formally replaced by metal-ligand coordinate bonds [1, 2].

In the work presented here, we developed the concept of using light as an external trigger to control the formation of a thymine-based metal-mediated base pair [3]. We introduced a photo-labile protecting group [4] in a position important for the formation of the metal-mediated base pair so that the duplex is unable to bind the metal ion unless it has been irradiated with light. Using this caged thymidine residue, the formation of a T-Hg(II)-T base pair was successfully triggered by light. In contrast, when applying a bidentate artificial nucleoside analog complementary to the caged thymidine, the duplex experiences a minor metal-induced thermal stabilisation already prior to irradiation, which is intensified upon irradiation. This indicates that the nucleobases involved in metal-mediated base pairing need to be chosen properly to achieve an optimal outcome. The possibility of using light as an external trigger for metal-mediated base pair formation significantly extends the scope of this type of DNA modification. In combination with DNA that switches its topology upon metal-mediated base pair formation, interesting applications are anticipated.

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P314

**Imine-, Thiosemicarbazone- and Pyridine-Based Ligands for Copper(II): Consequences on DNA Binding and Nuclease Activity**

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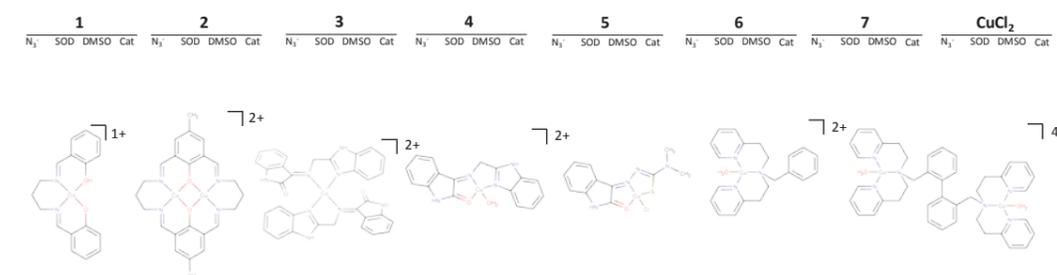
Copper is an essential metal for mammals, being a fundamental component for redox-active metalloenzymes (such as cytochrome C oxidase, tyrosinase and superoxide dismutase). Due to being redox-active, copper transport is tightly controlled in the cells, from uptake (hCTR1 in humans) to distribution (Cox17 transports copper to the mitochondria; CCS to SOD and Atox1 to ATPases [1]). These very same properties that make copper a “threat under control” to living systems are explored for the development of copper-based anticancer compounds. Here we describe a series of copper(II) compounds with imine (**1-4**), thiosemicarbazone (**5**) and pyridine-containing (**6** and **7**) ligands.

Artificial nucleases are metal compounds capable of promoting the cleavage of the phosphodiester bond in nucleic acids. [Cu(phen)<sub>2</sub>]<sup>2+</sup> was the first copper compound to have its nuclease activity identified [2]. Nuclease activity was observed for all compounds evaluated here but **2** (which contains the macrocyclic imine). The imine compounds **1** and **4** demonstrated nuclease activity even in the absence of added reducing agent (ascorbic acid). In the presence of reducing agent, the most potent nucleases were compounds **4**, **6** and **7**, which led to 100% plasmid cleavage at 2.5, 1.25 and 1.25 μM respectively. The cleavage mechanism varies widely among the compounds studied here, but it can be grouped consistently based on the nature of the ligand. For compounds **1**, **3** and **4** (imine-containing), the nuclease activity is based on singlet oxygen and/or peroxide. For the thiosemicarbazone-containing **5**, the nuclease activity has contribution from singlet oxygen, superoxide and peroxide. Finally, for the pyridine-based compounds **6** and **7**, singlet oxygen and hydroxyl radicals are present, but the most prominent species is peroxide.

Conformational changes on calf thymus (CT) DNA induced by the model compounds were also followed by circular dichroism (CD). All compounds studied here were found to interact with CT-DNA to some extent. Specifically, **2** (containing the cyclic imine) is less reactive than the analogue **1**; **4** (single imine) is more reactive than **3** (two imines); and the dinuclear **7** is more reactive than the mononuclear **6**.

The potent nuclease activities observed for compounds **3**, **4**, **6** and **7** are directly translated to their anticancer properties. Compounds **3** and **4** were tested against HeLa, exhibiting IC<sub>50</sub> values of 21.2 and 13.1 μM respectively. Compounds **6** and **7** were previously assayed against two melanoma cell lines (TM1MNG3 and B16F10). Compound **6** was active against B16F10 only (IC<sub>50</sub> = 54.5 μM), while compound **7** was active against both cell lines (IC<sub>50</sub> = 63.5 and 10 μM, respectively) [3]. The compounds are currently being evaluated against a wider panel of tumorigenic cell lines.

Acknowledgements: FAPESP 2018/21537-6, 2013/07937-8, CEPID Redoxoma, CNPq, CAPES



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P315

**Control and Regulation of S-Adenosylmethionine Biosynthesis by the Regulatory β Subunit and Quinolone-Based Compounds**

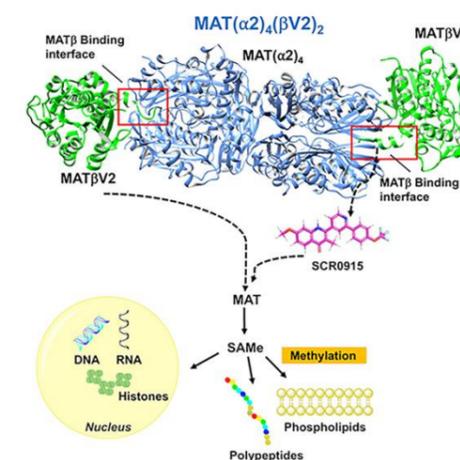
Panmance J<sup>1</sup>, Bradley-Clarke J<sup>1</sup>, Mato JM<sup>2</sup>, O'Neill PM<sup>3</sup>, Antonyuk SV<sup>1</sup>, Hasnain SS<sup>1</sup>

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Methylation is an underpinning process of life and provides control for biological processes such as DNA synthesis, cell growth and apoptosis. Methionine Adenosyltransferases (MAT) produce the cellular methyl donor, S-Adenosylmethionine (S-AdoMet). Dysregulation of S-AdoMet level is a relevant event in many diseases, including cancers such as hepatocellular carcinoma and colon cancer. In addition, mutation of Arg264 in MATα1 causes isolated persistent hypermethioninemia, which is characterized by low activity of the enzyme in liver and high level of plasma methionine. In mammals, MATα1/α2 and MATβV1/V2 are the catalytic and the major form of regulatory subunits respectively. A gating loop comprising residues 113-131 is located beside the active site of catalytic subunits (MATα1/α2) and provides controlled access to the active site. Here, we provide evidence of how the gating loop facilitates the catalysis and define some of the key elements that control the catalytic efficiency. Mutation of several residues of MATα2 including Gln113, Ser114 and Arg264 lead to partial or total loss of enzymatic activity, demonstrating their critical role in catalysis. The enzymatic activity of the mutated enzymes is restored to varying degree upon complex formation with MATβV1 or MATβV2, endorsing its role as an allosteric regulator of MATα2 in response to the levels of methionine or S-AdoMet. Finally, the protein-protein interacting surface formed in MATα2:MATβ complexes is explored to demonstrate that several quinolone-based compounds modulate the activity of MATα2 and its mutants, providing a rational for chemical design/intervention responsive to the level of S-AdoMet in the cellular environment. This article is protected by copyright. All rights reserved.



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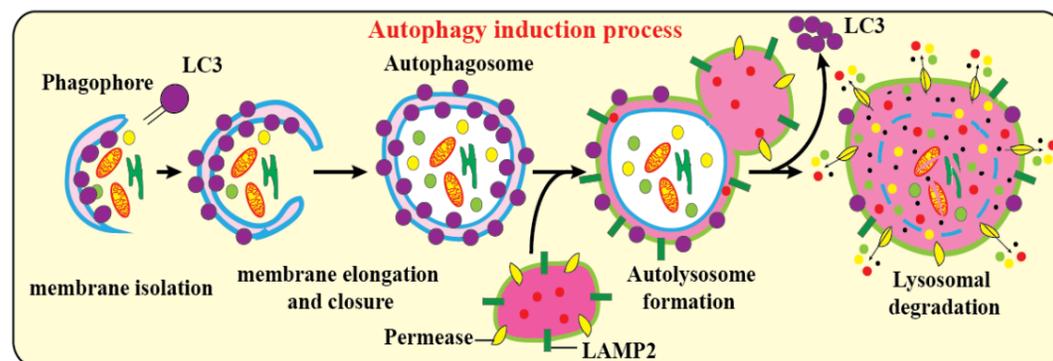
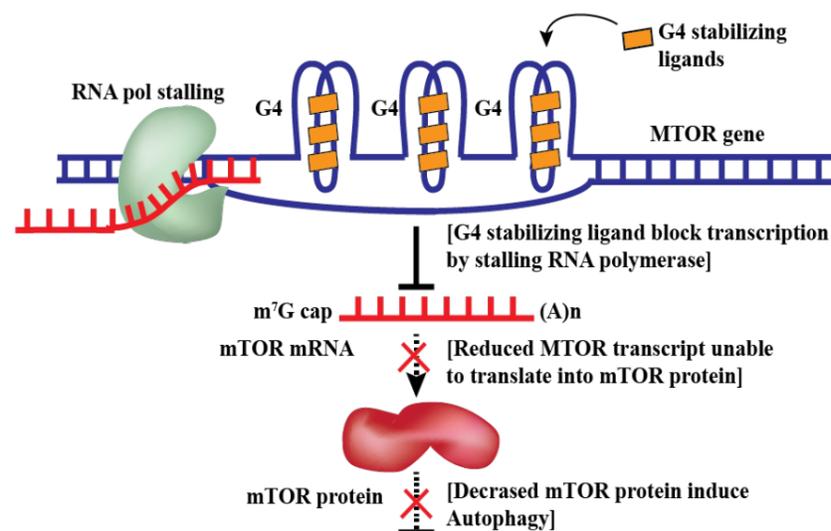
P316

### The Effects of Small Molecule-Mediated G-Quadruplex Stabilization on Induction of Autophagy

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G-quadruplex (G4) structures have emerged as therapeutic targets for many human diseases such as cancer and neurodegeneration. Autophagy is a housekeeping cellular homeostatic mechanism and deregulation of autophagy is common in cancer and in neurodegenerative diseases. We identified the presence of 46 putative G4 sequences in the *mTOR* gene by use of GQRS mapper tool. Our group has developed dimeric carbocyanine based ligands that exhibit G4 selective binding and stabilization. The interaction of these G4-targeting ligands with putative G4 sequences is likely to affect the replication, transcription as well as translation of the *mTOR* gene. In this study, the induction of autophagic pathway via *mTOR* gene regulation was monitored based on interaction with one particular dimeric carbocyanine ligand (Bis 4,3) treated Hela and SHSY-5Y cell lines. The effect of Bis 4,3 was compared with TMPyP4, a known G4-stabilizer. Notably, mTOR being the key negative regulator of autophagy, treatment with G4-selective ligand downregulates mTOR activity and leads to the induction of excessive autophagy. The current work suggests potential of G4 stabilizing ligands towards induction of autophagy through the downregulation of mTOR.



P317

### Solving the 1<sup>st</sup> Structure from the HDV-Like Ribozyme Family: The CPEB3 Ribozyme

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Discovered in the human pathogen Hepatitis delta virus (HDV), the HDV ribozyme is one of the best studied small ribozymes. It is the fastest known naturally occurring self-cleaving ribozyme with a cleavage rate of more than 1 per second at 65°C [1]. Structure determination by X-ray crystallography in 1998, revealed a complex fold into a nested double pseudoknot and disclosed the essential role of a cytosine in the catalytic core for its cleavage activity [1]. In 2006, a genome-wide search identified four ribozymes in the human genome, one of those being the cytoplasmic polyadenylation element-binding protein 3 ribozyme (CPEB3). The CPEB3 ribozyme, which is highly conserved in all mammals, adopts not only the same double pseudoknot fold like the HDV ribozyme, but probably also follows a very similar self-cleaving mechanism [2,3]. Structural based searches have identified more of such HDV-like ribozymes in all kinds of organisms like bacteria, virus or metazoan [4]. Even though the sequences of these ribozymes exhibit great diversity (with the exception of a few nucleotides including the catalytic cytosine) the complex fold into a double pseudoknot is well conserved.

In order to better understand the complex folding of HDV-like ribozymes and to locate the metal ions involved in that particular fold, we would like to solve the three-dimension structure of the CPEB3 ribozyme by X-ray crystallography. However, many factors affect the successful crystallization process of RNA, such as sample purity and homogeneity, structural dynamics as well as ligand binding. Based on the accomplished crystallization of the HDV ribozyme we designed a number of different CPEB3 constructs. All of them include a binding site for the U1A protein as crystallization module to stabilize the CPEB3 structure.

Our preliminary results reveal: (i) All CPEB3 constructs comprising the U1A binding sites are still catalytic active indicating that the internal folding of the ribozyme is not disturbed, (ii) the U1A protein is binding to the CPEB3 constructs, (iii) CPEB3 is crystallizing and preliminary scattering data until a resolution of 2.8 Å were achieved, and (iv) non-native gel analysis of unused crystals prove the absence of degraded RNA.

Financial support by the Forschungskredit from the University of Zurich (to A.I.P.-M.) is gratefully acknowledged.

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P318

### Imidazole-Modified Metal-Binding DNA G-quadruplexes: A Step towards Artificial Metalloenzymes

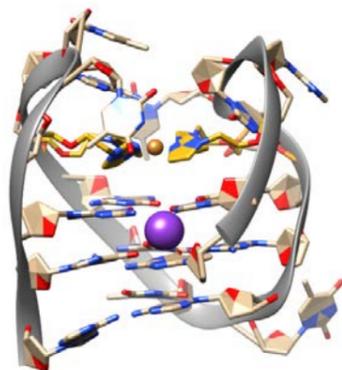
Philip M. Punt<sup>1</sup>, Guido H. Clever<sup>1</sup>

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Apart from the widely known duplex structure DNA can fold into various secondary structures such as hairpin loops, triplexes and G-quadruplexes.<sup>1</sup> The latter self-assemble from guanine rich strands by Hoogsteen base pairing to form stacked guanine tetrads. Since their discovery, G-quadruplexes became increasingly interesting in the field of chemical biology due to their property to control the elongation of telomeres and the expression of oncogenes. Furthermore, first examples of a DNAzyme activity in G quadruplexes were shown.<sup>2</sup> Drawback of those systems was that the exact position and coordination environment of the catalytic active transition metal ion was largely unknown. In this context, the incorporation of covalently bound ligands into the backbone of G-quadruplex structures would be a next step in the development of DNAzymes. Recently, our group reported a first example of this strategy by incorporation of pyridine ligands, allowing the Cu(II) and Ni(II) mediated folding of G-quadruplex structures.<sup>3</sup> This was then exploited to trigger the folding of a thrombin inhibiting aptamer upon Cu(II) addition.

Now, we incorporated – inspired by the natural amino acid histidine – an imidazole based ligand into G-quadruplex structures. We were able to show a tremendous acceleration of G-quadruplex formation as well as a stabilization by addition of different transition metals such as Cu(II), Ni(II) and Zn(II). By varying the number of incorporated imidazole units, we were able to finetune metal affinities in accordance with their preferred coordination number. The concept of metal mediated GQs was then expanded to design an active peroxidase with the cofactor Hemin which is activated by Cu(II) addition.<sup>4</sup>

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P319

### Structural Characterization of DNA-Mediated Catalysis

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DNAzymes are single-stranded DNA molecules that are capable of catalyzing chemical transformations. They are isolated from randomized sequence libraries by *in vitro* selection methods. DNAzymes can be applied in gene silencing, in biosensor technology, in diagnostics or as synthetic tools. Structural information on the architecture of the catalytic site are scarce, resulting in a poor understanding of the mechanisms of DNA-mediated catalysis.

Our main focus is to obtain structural and functional information of the 10-23 DNAzyme in complex with its RNA target in a catalytically active conformation by x-ray crystallography. The success of crystallization of nucleic acids highly depends on the properties of the biological sample, rather than screening conditions. Therefore, we put great effort in searching for a suitable sample: The RNA-binding protein U1A will be used as a crystallization module to compensate the negatively charged surfaces of nucleic acids, which lead to a poor long-range order. To capture the complex in a catalytically active conformation, the 2'OH group at the RNA cleavage site will be substituted by a fluorine atom. DNAzyme variants with single point mutations within a self-complementary core sequence will be screened to prevent the formation of a catalytically irrelevant duplex structure.

P322

### Let It Go: Kinetics of Exon Unbinding in Group II Introns by Single-Molecule FRET and Molecular Dynamics

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Formation of stable RNA tertiary contacts is inextricably linked to metal cations to compensate the electrostatic stress and guide RNA folding. Self-splicing group II introns establish long-range tertiary interactions between domain 1 and the upstream exon to accurately position the 5'-splice site within the ribozyme's active core. [1] Here we use single-molecule FRET and computer simulations on a model RNA hairpin to monitor the kinetics of exon recognition and release in response to K<sup>+</sup> and Mg<sup>2+</sup>. [2] We find that exon unbinding rates are heterogeneous as a result of degeneracy in the FRET states which in turn originates from the presence or absence of specifically coordinated Mg<sup>2+</sup> ions. [2-5] We solve the rate system by hidden Markov modeling and identify the structural origin of the kinetic heterogeneity. While metal ion binding locks the RNA tertiary contact in a rigid conformation, molecular dynamics simulation show that strain on the sugar phosphate backbone is alleviated through fast sugar puckering. Switching between pucker conformations is pronounced in DNA exons which explains their lower affinity towards group II introns.

Financial support by the Swiss National Science Foundation (RKOS), the UZH Forschungskredit (FDS and RB) and the University of Zurich is gratefully acknowledged.

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P323

### Paramagnetic Metal-Tetrads in Higher-Order DNA G-Quadruplex Structures as EPR-active Probes for Distance Measurements and Structure Elucidation

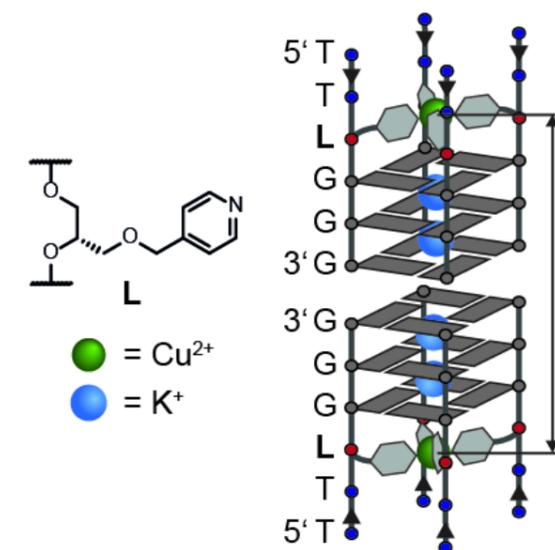
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G-quadruplexes are classic DNA secondary structures formed by Hoogsteen base pairing in guanine-rich oligonucleotides resulting in stacked guanine tetrads. They have been found to regulate the expression of oncogenes and human telomeric properties *in vivo*. [1] Recently in our group, the concept of metal-mediated base pairing, where the exchange of the canonical nucleobases by ligands allows for the incorporation of transition metals inside DNA duplexes, thereby imparting a higher stability and conferring unique metal-based properties like magnetism, charge transfer, catalysis, sensing etc. to it, [2] has been successfully extended for G-quadruplexes.

The folding of oligonucleotide sequences containing covalently incorporated pyridine donors into defined G-quadruplex structures, leads to a pre-arranged ligand environment suitable for binding transition metal ions like Cu(II) or Ni(II). A substantial thermal stabilization of the metal ion-bound secondary structure has been observed, which has been probed by UV/Vis and CD spectroscopy. [3] In a recent study, we demonstrated that the system undergoes a metal-induced control in its folding topology and protein binding. [4]

Furthermore, the paramagnetic nature of bound Cu(II) ions in G-quadruplex structures has been exploited as EPR-active probe. In the first step, intramolecular Cu-Cu distances were quantified with the help of pulsed EPR PELDOR and RIDME measurements in structures bearing metal pyridine tetrads at both 5' and 3' ends with potential application as EPR-based ruler. [5] This concept is now extended to investigate intermolecular distances in higher-order G-quadruplex structures like dimers (Figure 1), sandwich complexes and other adducts with binding ligands e.g. metalloporphyrins.



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P323

### Photophysical Study on the Interaction between RNA G-quadruplex and Pt(II) Complexes

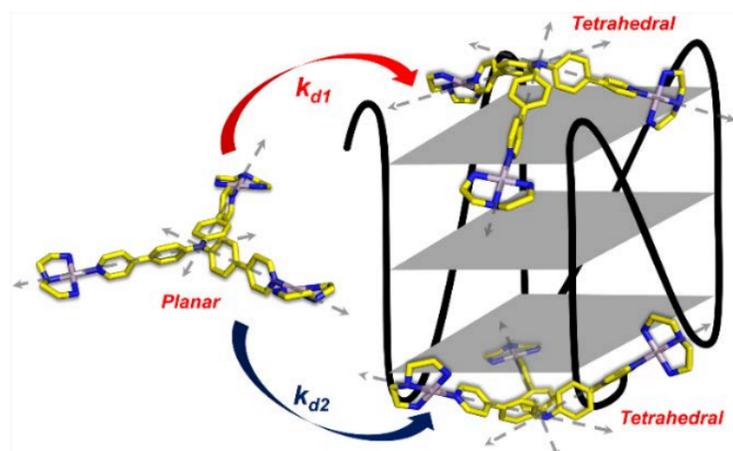
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As non-canonical nucleic acid structures, G-quadruplexes (G4s) play crucial roles in gene expression and regulation [1]. Small molecules interacting with G4s could possibly interfere and regulate the biological function of G4s. Therefore, a detailed understanding on how these small molecules interact with G4s is crucial for binding optimization and selectivity, but very rarely available till date [2, 3].

We applied a combination of several photophysical methods to investigate the interaction between an RNA G4 and two fluorescent Pt(II) complexes. With fluorescence lifetime and dynamic fluorescence anisotropy studies, the binding stoichiometry and binding affinity between the metal complexes and the RNA G4 were successfully determined. Indicated by fundamental anisotropy, we unexpectedly observed a 'planar-to-tetrahedral' conformational change for the Pt(II) complexes upon binding to the RNA G4. The binding is also confirmed by induced chirality to the complexes. Combination with MS data, we confirmed that two binding sites exist on the RNA G4 with different affinities for the Pt(II) complexes. To the best of our knowledge, this work is the first study on the interaction between RNA G4 and metal complexes. These findings thus provide insights into the rational design for small molecules as RNA G4 binders. Furthermore, the photophysical methods used here have shown an extremely high sensitivity and informative on binding strength, stoichiometry, dynamics and associated structural changes, having thus fundamental implications for further studies of such systems.



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P331

### Valence to Core XES Study on Molybdenum and its Application to Molybdenum-Iron-Sulfur Clusters

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In this work we present for the first time a systematic x ray emission spectroscopy study done on a second row transition metal (Molybdenum) and a comprehensive analysis of the origins of the transitions that gives intensity to this region of the spectrum.

Transition metal (TM) K-edge X-ray absorption spectroscopy (XAS)<sup>1,2</sup> has been extensively used for the study of metal sites in the bioinorganic chemistry field as they can probe lowest unoccupied molecular orbitals, providing hence information of the geometric and electronic structure of several metalloenzymes (i.e. XAS on MMOQ)<sup>3</sup> and small molecules complexes with great success. X ray emission spectroscopy (XES) in the so-called valence-to-core region<sup>4</sup> (VtC) provides information about the highest occupied electronic levels and can serve as a direct probe of ligand ionization potentials.

While VtC XES has demonstrated being tremendously useful<sup>4</sup>, capable of giving metal-ligand information, in some cases also overcoming the EXAFS limitations, the studies are only limited to the first row of transition metal. We present the first systematic XES study done on a second row transition metal: Molybdenum. The importance of Mo in catalytic processes motivates interest in selective spectroscopic probes of Mo. Mo K-edge XAS has had a significant impact on our understanding of the geometric and electronic structure of Mo-containing active sites (i.e. Nitrogenase), however Mo K $\beta$  XES has yet to be explored in bioinorganic chemistry. The presence and identity of the central atom in Nitrogenase Fe-Mo cofactor was found to be a Carbon atom via Fe valence-to-core XES<sup>5</sup>. We propose the use of Mo XES technique to achieve similar important findings about the identity of ligands on Mo centers in several metalloenzymes. Therefore, the applications of the technique have been explored in synthetic Mo-Fe cubane clusters as effective models for investigations in MoFe protein (with regard to metrical parameters, distribution of oxidation states, and heterometal bonding) as a practical example of the efficiency of this technique for elucidating the nature of not only Mo but also any other 2nd row TM center in biological systems.

In addition to this project, K $\beta$  Resonant XES (RXES)<sup>6</sup> was also measured. In these measurements the core hole -lifetime is now depending on the RIXS state for the K $\beta$  Resonant XES, revealing hidden features in the non-resonant XES spectrum. DFT calculations are also on process and would be coupled with the experimental data (Resonant and Non Resonant XES) for a better understanding of the origin of the transitions than give shape to the valence to core spectra in TM in the second row.

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P332

### ESUO - The European Synchrotron and FEL User Organisation: Aims and activities

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<sup>1</sup>Institute for Molecules and Materials, Faculty of Science, Radboud University, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands, m.feiters@science.ru.nl. List of other delegates limited to ESUO Executive Board members: <sup>2</sup>University of Mons, Mons, Belgium. <sup>3</sup>Polish Academy of Sciences, Warsaw, Poland. <sup>4</sup>University of Lund, Lund, Sweden. <sup>5</sup>Sapienza University of Rome, Rome, Italy. <sup>6</sup>Trinity College Dublin, Dublin, Ireland. <sup>7</sup>Helmholtz-Zentrum Dresden-Rossendorf, Dresden, Germany. <sup>8</sup>University of Siegen, Siegen, Germany

The European Synchrotron and free-electron laser (FEL) User Organisation (ESUO) was established in 2010 [1] and today represents about 22.000 users of European synchrotrons and FELs from over 30 member states of the European Union and associated states, each represented within ESUO by up to four national delegate(s) per country [2]. ESUO's vision is to support a thriving European synchrotron and free-electron laser (FEL) user community with equal opportunities of access and participation for all scientists based solely on the scientific merit of their ideas.

ESUO's mission statements are:

- to represent interests and needs of all Europe-based Synchrotron Radiation and FEL users
- to support the facilities in their ambitions to create equal access opportunities for Europe-based scientists solely on the basis of scientific merit and to make this access as simple as possible
- to enable future strategies/funding schemes for equal (transnational) access [3] by European scientists independent of their financial resources
- to foster contacts with users in Widening and European neighbour Countries, sharing knowledge/expertise
- to strengthen cooperation with National User Organisations
- to find collaborations/synergies with user organisations of other analytical facilities.

ESUO was founded in the framework of the project ELISA and took up its work in the project CALIPSO (respectively funded from the 7th Framework Program of the EU under the Grant Agreement 226716 and 312284) followed by the post-CALIPSO bridging agreement, and continues and expands its activities with the support of CALIPSOplus which is financially supported by the EU under the Grant Agreement 730872 from the EU Framework Programme for Research and Innovation HORIZON 2020.



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P333

### Heat Triggering of Plasticized Shape Memory Polymer for Biomaterials

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Shape memory polyurethane (SMPU) have been considered for biomedical applications [1-2] because of their two interesting unique features that are particularly suited for the medical field. First, SMPUs are non-toxic and biocompatible, which makes them suitable for usage inside the human body. Second, the shape of SMPUs can be controlled by temperature. The weight ratio of SMP to plasticizer used to prepare the gel film was adjusted from 1:0.10, 1:0.30, 1:0.50 (S-10DBA, S-30DBA, S-50DBA). Young's modulus became smaller with increasing amount of plasticizer content. Furthermore, strain of gels found to be improved. Therefore, the softness of SMP film has been enhanced. As the expected application of SMPU would be potential for self-healing bandage. The glass transition temperature (T<sub>g</sub>) was investigated by Dynamic mechanical analysis (DMA) from -20 to 100 °C. After filling plasticizer, T<sub>g</sub> of gels could be switched from 65 °C to 39 °C. The switching T<sub>g</sub> area is very comfortable to transform the shape of material under human body temperature since human. The shape memory effect was observed by thermomechanical analysis with the tensile testing method at different temperatures and stress. This analysis was performed in five steps programming. Additionally, samples were observed cell experiment in vitro by culturing with cells for 6 h. Surface of the samples were captured by a scanning electron microscope. reveal that the samples contained cells adhered on the sample surface. the cells on sample surfaces were flat and adhered to neighboring cells. This is a sign of cell activation. This study has been successfully innovated smart material to used not only friendly with human body but also could be actuated by heat with human body temperature. The advantage of memorial and recovery shape would be potential for bio smart materials.

Financial support by Advanced Leading program of Shinshu university is gratefully acknowledged.

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P335

## Stable Isotope Mass Spectrometry Investigations of Zn-Altered Amyloid- $\beta$ Aggregation

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The Zinc isotope composition in brain tissue from healthy mice tends to become lighter with aging, whereas the effect is significantly less obvious with transgenic mice affected by the Alzheimer disease (AD) [1]. There is thus a Zn isotopic signature potentially marking this disease in mice brain. Although interesting, this global approach makes it difficult to understand the exact molecular mechanism responsible for such Zn isotope dynamics.

We therefore investigated this isotopic effect at the molecular scale to understand the biochemical reactions at play. Amyloid Beta ( $A\beta$ ) is a peptide ranging from 40 to 42 amino acids, coming from the degradation of the Amyloid Protein Precursor. The aggregation of  $A\beta_{40-42}$  leads to plaques in the brain of AD patients. Transition metals such as Zn and Cu are involved in this aggregation process, but the exact origin of these metals and the exact chemical reactions involved remain obscure [2].

We have conducted aggregation experiments on  $A\beta_{28}$  peptides, a shorter form of  $A\beta_{40}$  keeping the coordination and aggregation ability of the full peptide, in the presence of Zn. Experiments were ran using different ratio of Zinc/ $A\beta$  and with the presence or not of different amino acids with different functional groups (including Histidine, Glutamate and Cysteine). Aggregation was monitored by fluorescence and when it did not evolve anymore, fibrils and supernatant were separated and Zinc concentration analysis were performed after the mineralization process. It reveals different distributions of Zinc between aggregated and supernatant depending on the nature and the amount of the amino acid added.

Subsequent Zn purification and isotopic analyses by MC-ICP-MS revealed  $\delta^{66}\text{Zn}$  fractionation between peptide and supernatant ranging from nil to  $\sim 0.4\%$ . These preliminary results illustrate that  $\delta^{66}\text{Zn}$  can inform on the Zn coordination chemistry at play during  $A\beta$  aggregation.

Financial supports by the European Research Council (StG - 638712 aLzINK) and CNRS MITI Défi-ISOTOP are gratefully acknowledged.

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P337

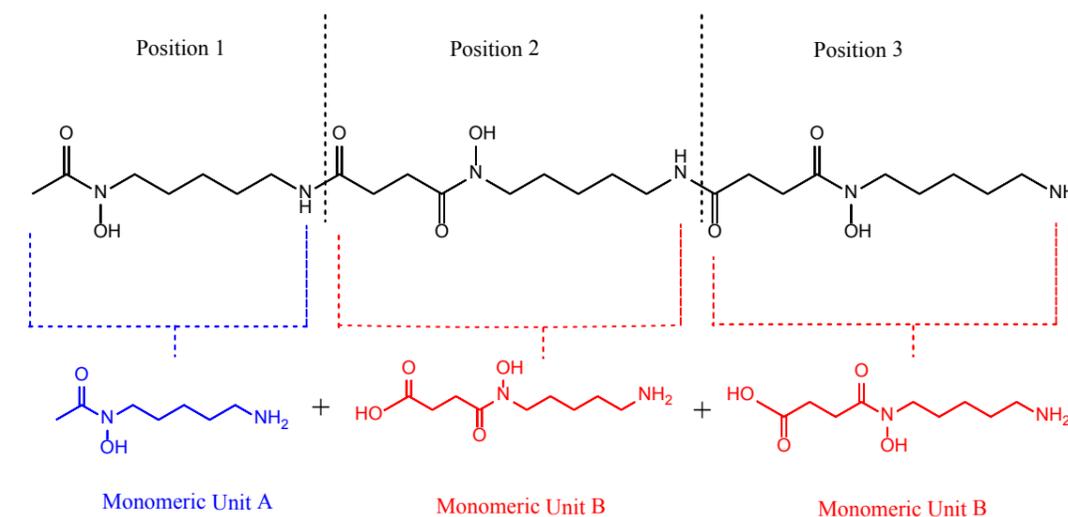
## A Novel Total Synthetic Approach to Desferrioxamine B Analogues

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Desferrioxamine B (DFOB) is a hydroxamic acid-based bacterial metabolite essential in guaranteeing cellular iron supply. DFOB is a linear ligand which forms stable hexacoordinate, octahedral complexes with Fe(III) through three bidentate hydroxamic acid functional groups. The exquisite affinity for Fe(III) indicated the clinical use of DFOB as a scavenger for excess iron in patients with transfusion-dependent haemoglobin disorders. The potential clinical utility of DFOB is under scrutiny, which warrants further study into new DFOB analogues with new properties and function.

Studies have used biological and semisynthetic approaches to produce analogues of DFOB, with other work focusing on total synthesis [1,2,3]. The proposed synthesis in the current work is predicated on the trimeric structure of DFOB consisting of one monomeric unit A linked to two monomeric unit Bs. Synthesis of native and ether containing monomeric units A and B allowed for the production of a suite of eight analogues of DFOB, including native DFOB, with ether subunits inserted into positions 1 and/or 2 and/or 3. These constitutional isomers are predicted to co-elute with the compounds generated in our group using biosynthetic methods. The proposed synthetic scheme is highly flexible and can be adapted to produce various structural analogues. The synthetic route provides access to trihydroxamic acid adducts as well as dihydroxamic acid and other analogues. Improved access to structural variety may reveal nuances in the relationship between DFOB structure and properties that may inspire further therapeutic use.



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**P338****Bioinorganic Chemistry of Molybdenum Compounds****Vinay Kumar Srivastava<sup>1</sup>**<sup>1</sup>Department of Chemistry, D.S. College Aligarh, GT Road Aligarh, 202001 Aligarh, India.

The Chemistry of compounds containing Transition metals bound to sulfur ligands has been actively studied. Interest in these compounds arises from the identification of the biological importance of Iron-sulfur containing proteins as well as the unusual behaviour of several types of synthetic metal-sulfur complexes. Molybdenum multinuclear Complexes of Bioinorganic relevance were investigated. The complexes  $[M(M'S_4)_2]^{2-}$  were prepared with high yield and purity as salts of the variety of organic cations. The diamagnetism and spectroscopic properties of these complexes confirmed that their structures are essentially equivalent with two bidentate  $M'S_4^{2-}$  ligands coordinated to the central  $d^8$  metal in a square planar geometry. The interaction of the complexes with CT-DNA was studied. Results showed that metal complexes increased DNA's relative viscosity and quenched the fluorescence intensity of EB bound to DNA. In antimicrobial activities all complexes showed good antimicrobial activity higher than the ligands against gram positive, gram negative bacteria and Fungi. The antitumor properties have been tested *in vitro* against two tumor human cell lines, Hela (derived from cervical cancer) and MCF-7 (derived from breast cancer) using a metabolic activity test. Results showed that the complexes are promising chemotherapeutic alternatives in the search of anticancer agents.

Financial Support by the University grant commission New Delhi, India is gratefully acknowledged.

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**P339****Bioorthogonal Chemistry: Developing Novel Bioorthogonal Reactions****Cheng Weng<sup>1</sup>, Wee Han Ang<sup>1,2</sup>**<sup>1</sup>Department of Chemistry, National University of Singapore, 3 Science Drive 2, 117543 Singapore.<sup>2</sup>NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, 21 Lower Kent Ridge Rd, 119077 Singapore.

Bioorthogonal reactions, serving as a powerful technique to dissect native biological processes, have been a thriving research area in recent years. Currently, there are generally four different types of well-defined reactions with excellent biocompatibility in the repertoire of bioorthogonal chemistry: ligation reaction, cleavage reaction, transfer hydrogenation, and thiol oxidation. These reactions have extremely expanded our toolkit to enable an array of exciting new biological applications ranging from biomolecule labeling, metabolite detection and intracellular probe release, to in situ enzyme and prodrug activation. However, there is a highly formidable challenge in developing or discovering novel and efficient bioorthogonal reaction due to the interference of complex cellular environment [1, 2, 3]. Here we highlight three well-developed reactions under physiological conditions all of which are promising to involve as bioorthogonal reaction, Ru-arene Schiff-base (RAS) complex-catalyzed [4] transfer hydrogenation of aryl azide, RAS complex-catalyzed glutathione (GSH) oxidation via molecular oxygen as oxidant and finally novel click ligation reaction inspired by native chemical ligation [5].

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P340

## A Disassembly Approach for Imaging Endogenous Pyrophosphate in Living Cells using Fe<sup>III</sup>-Salen Complexes

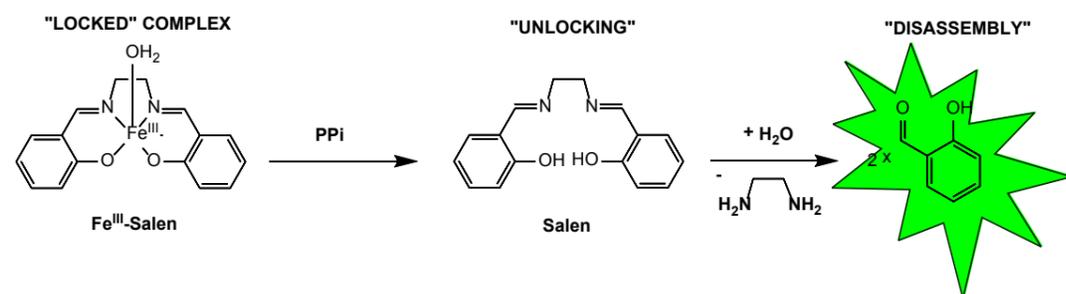
Prerna Yadav<sup>1</sup>, Marta Jakubaszek<sup>2</sup>, Gilles Gasser<sup>2</sup>, Felix Zelder<sup>1</sup>

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A stimulus-induced disassembly approach is presented for detection of analytes in living cells [1]. Particularly, we demonstrate that Fe<sup>III</sup> salen complexes fluorometrically detect endogenous pyrophosphate (PPi), an important diagnostic marker for cancer and many other diseases [1-3]. In our approach, PPi sequesters the Fe<sup>III</sup> centre from the salen-complex and the “unlocked”, metal-free ligand then subsequently hydrolyses into its molecular subunits ethylenediamine and salicylaldehyde [3]. Initially, the intrinsic fluorescence of the salicylaldehyde subunit is quenched by the paramagnetic metal ion, but turns on back during the disassembly of the complex. Our group studies show that the selectivity and reactivity of the Fe<sup>III</sup> complexes can be affected by incorporating modifications in the salen ligand structure.



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## A

Abbas, Mohammad Zafar	P220
Abdiaziz, Kaltum	YR-25
Acharya, Sourav	P221
Adam, Suzanne	OP-90
Agapie, Theodor	OP-22
Ahunbay, Esra	P300
Al-Harbi, Laila	P303
Al Salmi, Iman	P090
Alamri, Mona	P222
Alberto, Roger	IL-067, P006, P007, P009, P011, P230, P264, P278
Aldrich-Wright, Janice	IL-011, P256
Alenezi, Mona	P020
Alfano, Marila	P140
Allen, Matthew	IL-138
Amadei, Fabio	YR-13
Amanullah, Sk	P040
Amara, Patricia	P141
André Cunha, Richard	OP-49
Andrade, Susana	KN-27, P151
Ang, Wee Han	KN-08, P262, P263, P274, P339
Aono, Shigetoshi	P175
Aref, Diaa	P001
Armstrong, Fraser	KN-25
Auffinger, Pascal	IL-074
Aureliano, Manuel	OP-51
Austin, Rachel	IL-162

## B

Babak, Maria	YR-04
Babu, Tomer	P224, P282
Bäcker, Daniel	P225, P280
Bal, Wojciech	IL-041, P162
Balderrama-Martinez-Sotomayor, Raúl	P226
Balogh, Ria Katalin	P142, P162

Banci, Lucia	KN-09
Barba-Behrens, Norah	IL-102
Barceló-Oliver, Miquel	IL-014
Barrios, Amy	OP-64
Becker, Paul-David	PL-02
Bellotti, Denise	YR-05
Bennett, Sophie	P144
Berger, Walter	KN-22
Berggren, Gustav	IL-113
Berglund, Sigrid	P002
Berners-Price, Sue	KN-06
Bertini, Luca	OP-29
Bete, Sarah	P041
Biancalana, Lorenzo	YR-24
Biggs, George	P091
Bím Daniel	P060, P075, P092
Biswas, Dalia	P145
Biver, Tarita	IL-109
Bjerrum, Morten J.	OP-72, P190, P214
Bjornsson, Ragnar	OP-35
Blindauer, Claudia	IL-130
Bolitho, Elizabeth	P229
Bolliger, Robin	P230
Bombard-Caucat, Sophie	IL-006
Bonnet, Sylvestre	KN-13
Booth, Rosalind	P146
Börner, Richard	OP-86, P021, P300, P308, P322, P324
Borgula, Isabella	P130
Borovik, Andrew	IL-050
Borowski, Tomasz	P173, P197
Brabec, Viktor	LA-14, P255, P258, P273
Bradley, Justin	YR-01, P315
Brasch, Nicola	IL-082
Bren, Kara	KN-11, P149
Brenig, Christopher Niklas	YR-27
Britt, R. David	IL-117
Bröring, Martin	IL-012

Brudvig, Gary	KN-16
Brunold, Thomas	KN-14
Brzezinski, Peter	IL-008
Budzisz, Elzbieta	P231
Bulos, Joshua	YR-30
Butler, Alison	IL-042
Butler, Stephen	IL-134

## C

Calvo, Jenifer	P148
Campbell, Joanna	P131
Carvalho, Idalina	P232
Carver, Peggy	IL-015
Cavazza, Christine	OP-54, P140
Chacon, Kelly	IL-160
Chae, Eun Su	P042
Chang Michelle	IL-044
Chan, Tiffany	YR-29
Chang, Christopher	KN-10
Chao, Hui	IL-124, P244, P265
Chattopadhyay, Samir	P149
Chavez, Ferman	P043
Che, Chi-Ming	IL-024, P022, P093, P242, P285, P287, P295
Chen, Yu	OP-27, P244
Chen, Chun-Jung	P150
Chen, Peng	PL-01
Chen, Jing	P093
Chen, Hang	P003
Chen, Zhen-Feng	OP-70
Chiari, Lucile	P004
Chino, Marco	YR-23, P001
Cho, Jaeheung	OP-37, P052
Choi, Jonghoon	P094
Ciano, Luisa	YR-21
Ciurli, Stefano	IL-121, P140
Clever, Guido	IL-010, P318, P323
Codd, Rachel	IL-052, P337

Comba, Peter	IL-058, P047, P056
Craig, James	P235
Crans, Debbie	PL-05
Crichton, Robert	LA-06
Cutsail, George	YR-28

## D

Da Costa Ferreira, Ana Maria	IL-108, P033, P314
Dabhade, Prachi	P151
Dasgupta, Rubin	P152
Datta, Ankona	IL-064
Daumann, Lena	IL-057, P053
Dawson, John	LA-11
De Cola, Luisa	PL-09
De Franca Lopes, Luiz Gonzaga	OP-19
DeBeer, Serena	IL-053, P153, P331
Decamps, Laure	P153
Decroos, Christophe	OP-31
Dell'Acqua, Simone	OP-87
Delmonti, Juliana	P154
DeRose, Victoria	KN-43
Desiatkina, Oksana	P095, P107
Dey, Subal	P044
Dey, Abhishek	PL-10, P040, P149
Diogenes, Izaura	P155
Dobbek, Holger	IL-112
Domínguez-Martín, Alicia	IL-140, P324
Domergue, Jérémy	P045
Donnelly, Paul	KN-33, P257, P261, P279
d'Orchymont, Faustine	P236
Duan, Chunying	IL-017
Dudek, Dorota	YR-11
Duhme-Klair, Anne-Kathrin	IL-047, P146
Durtschi, Moritz	P157
Dutta, Arnab	OP-69, P055

Dyson, Paul	IL-085
-------------	--------

## E

Egan, Ross	P237
Eichert, Pina	P238
Einsle, Oliver	KN-15, P161
Eisenschmidt, Annika	P005
Eliseeva, Svetlana	IL-123
Erxleben, Andrea	IL-158, P284
Escher, Daniela	P046
Escudero Pérez, Dayra	P239
Esmieu, Charlène	P210

## F

Fahrni, Christoph	KN-19
Faller, Peter	KN-44, P057
Farkas, Etelka	LA-09, P270
Fay, Rachael	P241, P246
Feiters, Martin	P332
Fonseca Guerra, Celia	IL-091
Fontecilla-Camps, Juan Carlos	IL-116
Foster, Andrew	P132
Franz, Katherine	PL-06, P131, P253
Frei, Angelo	YR-19, P264
Freisinger, Eva	P170, P187, P317
Fromsejer, Rasmus	P158
Fujishiro, Takashi	P159, P176
Fung, Yi Man Eva	P242
Fukuzumi, Shunichi	IL-034
Furtmüller, Paul	OP-05, P172, P188

## G

Galindo, Miguel A.	IL-153
Gallenito, Marc	P160
Gambino, Dinorah	IL-007
Ghosh, Abhik	IL-145
Ghosh Dey, Somdatta	IL-051
Gibney, Brian	IL-135

Gibson, Dan	IL-004, P224, P254, P258, P282
Giedroc, David	IL-069
Gies-Elterlein, Jakob	P161
Gomez Castillo, Rebeca	P331
Goodman, David	P096
Gotsbacher, Michael	YR-02
Gozzi, Marta	YR-15
Graf, Dominic	P097
Green, Michael	IL-078
Griesser, Rolf	P304
Griffith, Darren	IL-076
Grundmann, Nora	P011
Guan, Ruilin	P244
Guha, Rweetuparna	P305
Gumienna-Kontecka, Elzbieta	IL-068
Gunasekaran, Velmurugan	P047, P056
Guo, Zijian	KN-04
Guo, Yisong	OP-40
Gust, Ronald	P225, P280
Gut, Melanie	P241, P246
Gwak, Jinseong	P098

## H

Hacaperkova, Eliska	P247
Hagen, Wilfred	LA-15
Hajdu, Bálint	P162
Hambley, Trevor	IL-036
Hammerstad, Marta	P163
Han, Jiyeon	P211, P212, P215
Hannon, Michael	KN-03, P235, P237, P239
Harland, Jill	P048
Harris, Hugh	IL-136
Harrop, Todd	IL-107
Hartinger, Christian	KN-38, P096, P111
Hartwig, Andrea	IL-144
Hasnain, S. Samar	OP-89, P315

Hastuti, Agustina Ari Murti Budi	P248
Haumann, Michael	IL-083
Hausinger, Robert	IL-126
Hayashi, Takashi	KN-05
Heck, Joshua	P099
Heffern, Marie	OP-80
Heffeter, Petra	IL-105, P254
Heim, Philipp	P049
Heinemann, Franz	P249
Hemmingsen, Lars	IL-122, P142, P158
Henthorn, Justin	YR-12, P331
Hermann, Petr	IL-063, P247
Hernández Valdés, Daniel	P006
Herres-Pawlis, Sonja	KN-12, P051, P067, P099
Hersleth, Hans-Petter	OP-62, P163
Hess, Corinna	OP-45
Hibino, Masaki	P306
Hill, Craig	IL-049
Hirota, Shun	KN-01
Högbom, Martin	KN-29, P164, P191
Hofbauer, Stefan	YR-06, P172, P182, P188
Hoffmeister, Henrik	P100
Holland, Jason	IL-059, P236, P241, P246, P260
Holland, Patrick	IL-062
Horn, Christiane	P050
Horn Junior, Adolfo	OP-60, P050
Hošek, Jan	P250, P311
Hsu, Sodio	OP-66
Hu, Di	P022
Hu, Yilin	IL-038
Hu, Xile	IL-040, P066
Hua, Jing	P031
Humphreys, Lucy	P252
Hunsaker, Elizabeth	P253
Hüppe, Henrika	P051

Hureau, Christelle	KN-18, P210, P335
--------------------	-------------------

## I

Ikeda-Saito, Masao	IL-001
Imberti, Cinzia	YR-14
Ivancich, Anabella	OP-01
Ivanovic-Burmazovic, Ivana	IL-013, P064, P070

## J

Jaime-Pérez, Noelia	P133
Jalilehvand, Farideh	OP-10
Jameson, Geoffrey	IL-061
Jameson, Guy	IL-032
Janzen, Liudmila	P101
Jeong, Donghyun	P052
Jiao, Yang	OP-83
Johannsen, Silke	P157, P312, P317
John, Juliane	P164
Jonasson, Niko	P053

## K

Kaim, Wolfgang	LA-02
Karasawa, Masayuki	P165
Karlin, Kenneth	IL-035
Karmakar, Subhendu	P254, P267
Kasparkova, Jana	P255, P258, P273
Kennedy, David	OP-44
Keppler, Bernhard	PL-07, P104
Khandelwal, Shikha	P055
Khoury, Aleen	P256
Kieninger, Christoph	OP-52
Kikuchi, Kazuya	KN-36
Kim, Eunsuk	IL-110
Kim, Mingeun	P212
Kim, Minyeong	P102
Kim, Seji	P103
Kirk, Martin	OP-02
Kjendseth, Åsmund Røhr	OP-13

Ko, Jung Ah	P121
Koay, HuiJing	P257
Kostrhunova, Hana	P255, P258
Kozłowski, Henryk	LA-05
Kräutler, Bernhard	IL-077
Kraft, Kevin	P308
Krebs, Carsten	IL-037
Kreith, Yvonne	P104
Krezel, Artur	IL-155
Kroneck, Peter M. H.	LA-01
Kubeil, Manja	OP-71
Kumar, Ravi	P166
Kumar, Priyaranjan	P259
Kumar, Pardeep	P056
Küpper, Frithjof	KN-26
Küpper, Hendrik	IL-142, P133
Kuwahara, Jun	P167

## L

Lamb, Jennifer	P260
Lange, Jaclyn	P261
Le, Hai Van	P262, P274
Le Brun, Nick	KN-34, P144, P193
Lebrette, Hugo	OP-30, P164, P191
Lebrun, Vincent	P057
Lee, Way-Zen	OP-42
Lee, Eng Yee Violet	P263
Lee, Yunho	OP-63, P094, P098, P103, P106, P110
Leimkühler, Silke	KN-07
Lengacher, Raphael	P264
Lerner, Ana	P058
Levín, Pedro	P309
Li, Hongyan	IL-132, P288, P291
Liang, Alexandria	P168
Liang, Hao	OP-75
Liang, Hong	OP-50, P031
Lim, Mi Hee	PL-08, P211, P212, P215

Limberg, Christian	IL-022
Lippert, Bernhard	LA-08
Liu, Jiangping	P265
Liu, Jin-Gang	OP-20
Liu, Yu-Chiao	P059
Liu, Yangzhong	KN-32
Lo, Rainbow	P266
Longhinotti, Elisane	P032
Lönnberg, Tuomas	IL-148
Lorenz, Nicole	P105
Louka, Febee	P311
Louro, Ricardo	IL-065
Lu, Yi	KN-17
Luber, Sandra	IL-070, P010

## M

Machonkin, Timothy	OP-91, P169
Magnuson, Ann	OP-67
Magyar, John	IL-101
Maher, Megan	OP-24
Maji, Moumita	P221, P267
Majlesi, Kavosh	OP-68
Majumdar, Amit	IL-104, P065
Majumder, Piyali	P316
Makris, Thomas	IL-033
Maldonado-Dominguez, Carlos Mauricio	P060, P075
Mann, Samuel	OP-15
Manzano, Carlos	P268, P276
Mao, Zong-Wan	IL-115, P324
Marinescu, Smaranda	OP-47
Markova, Irina	P312
Marmion, Celine	KN-28
Marquez, Alejandro	P170
Massoud, Salah	OP-03, P311
Masuda, Hideki	IL-149
Mathieu, Emilie	OP-25
McDonald, Aidan	OP-12, P049, P074
Meade, Thomas	IL-086

Meloni, Gabriele	IL-125, P148, P160
Menage, Stephane	IL-045
Menon, Resmi	P134
Messori, Luigi	IL-096
Meyer, Franc	KN-21
Meyerstein, Dan	LA-07, P058
Miarzlou, Dzmitry	P171
Michel, Sarah	IL-151
Michlits, Hanna	P172, P182
Miller, Reece	OP-59, P101
Min, Yuanzeng	OP-43
Min, Sehye	P106
Miyoshi, Daisuke	IL-003
Mokhir, Andriy	IL-093
Mondal, Prasenjit	YR-22
Monkcom, Emily	P062
Morita, Yoshitsugu	OP-11
Morrow, Janet	IL-161
Mösching, Martin	P107
Moura, Isabel	IL-131
Moura, Jose	IL-094
Mrugala, Beata	P173
Mukherjee, Arindam	OP-79, P221, P267
Mukherjee, Goutam	P174
Mukherjee, Chandan	IL-111
Müller, Jens	KN-41, P046, P305, P313
Müller, Peter	P007
Muraki, Norifumi	P175
Murphy, Catherine	IL-075
Murthy, N. Narasimha	OP-73

## N

Nagy, Sándor	P270
Nakahata, Douglas	P271, P276
Nakai, Misaki	P272
Nakamura, Ryosuke	P159, P176
Nam, Dayeon	P177
Naskar, Shuvankar	P313

Németh, Brigitta	YR-20
Neese, Frank	IL-099, P145
Nichols, Eva	P063
Novohradsky, Vojtech	P255, P273
Nowak-Sliwinska, Patrycja	IL-119

## O

Obinger, Christian	IL-021, P172, P182, P188
O'Hagan, Molly	IL-106
O'Halloran, Thomas	KN-42, P130, P135
Okamoto, Akimitsu	OP-08
Omura, Keita	P179
Ong, Jun Xiang	P274
Oohora, Koji	OP-36
Orth, Nicole	P064
Orville, Allen	P180
Ott, Sandro	P007
Ott, Ingo	IL-089, P100, P108
Otte, Matthias	OP-32, P041
Outten, Caryn	IL-060
Outten, Franklin W	IL-073

## P

Paiva, Raphael	P314
Pal, Nabhendu	P065
Pan, Hui-Jie	P066
Panmanee, Jiraporn	P315
Park, Yun Ji	P181
Paul, Melanie	P067
Peacock, Anna	IL-005
Pecoraro, Vincent	IL-009, P158, P184
Peng, Kun	P275
Peralta, Rosely	IL-071
Pereira, Anna	P276
Perinelli, Monica	YR-10
Petering, David	KN-39
Petoud, Stephane	IL-128
Pfanzagl, Vera	P172, P182

Pikramenou, Zoe	KN-37
Pinakoulaki, Eftychia	OP-06
Pluth, Michael	OP-77
Policar, Clotilde	IL-097
Popescu, Codrina	P068
Popov, Marek	P123
Prause, Andre	P108
Price, Eric	OP-38
Priessner, Martin	P213
Pringpromsuk, Suphassa	P333
Probst, Benjamin	OP-18, P006, P009, P011
Przytula-Mally, Anna Ilaria	P317
Punt, Philip	P318

## Q

Que, Emily	IL-137
Que, Lawrence	IL-100
Quintanar, Liliana	IL-046

## R

Ragsdale, Stephen	IL-098, P196
Rahn, Franziska	P009
Raven, Emma	IL-143
Raza, Md Kausar	P277
Reedijk, Jan	LA-04
Reithofer, Michael	IL-080
Resende Gonçalves, Ana Cristina	P278
Reynolds, Mark	P183
Ribbe, Markus	IL-048
Riordan, Charles	IL-030
Robinson, Nigel	IL-146, P132
Rodriguez, Raphaël	IL-081
Roelfes, Gerard	IL-147
Roessler, Maxie	IL-120
Romero Canelon, Isolda	IL-129
Ronconi, Luca	IL-092

Rosato, Antonio	IL-152
Rosenbach, Hannah	P319
Rosenzweig, Amy	KN-20, P181
Roth, Patrick	P109
Rowan, Jacob	P279
Rowińska-Żyrek, Magdalena	IL-159
Ruckthong, Leela	P184
Ruehl, Carmen	YR-03
Ruiz, Jose	OP-65
Rutanen, Chiara	P185
Ryan, Michael	P069

## S

Sabater, Laurent	P335
Sadler, Peter	IL-031, P229, P294
Sagasser, Jessica	P280
Sakai, Ken	IL-039
Sakakibara, Erika	P186
Salassa, Luca	IL-027
Salifoglou, Thanos	IL-019
Salim, Alma	P187
Sánchez-Lara, Eduardo	P281
Schalk, Isabelle	IL-056
Schatzschneider, Ulrich	IL-087, P097, P109, P275
Scheitler, Andreas	P064, P070
Schiemann, Olav	IL-127
Schilling, Mauro	P010
Schindler, Siegfried	OP-26
Schmidt, Daniel	P182, P188
Schmidt, Claudia	P282
Schoenrath, Isabell	YR-08
Schulzke, Carola	KN-02
Schwarze, Benedikt	YR-07
Seebeck, Florian	OP-23, P171, P192
Seeler, John	P135
Ségaud, Nathalie	YR-17
Sen, Dipankar	IL-079

Serrano-Plana, Joan	P071
Sessler, Jonathan	IL-090
Shafaat, Hannah	IL-029
Shao, Fangwei	IL-018
Shaw, Wendy	IL-103
Shen, Jian-Ren	KN-24
Shengfa, Ye	OP-07
Shima, Seigo	KN-30, P066
Shimazaki, Yuichi	P072, P076
Shionoya, Mitsuhiko	IL-084
Shisaka, Yuma	P186, P189
Shoji, Osami	OP-56, P165, P179, P186, P189, P199, P306
Sigel, Roland K. O.	P021, P157, P300, P308, P312, P317, P322, P324
Sigel, Astrid	P304
Sigel, Helmut	LA-03, P304
Singh, Akhil	P073
Singh, Raushan Kumar	P190
Sletten, Einar	LA-12
Sligar, Stephen	IL-156
Śmiłowicz, Dariusz	P223
Smith, Aaron	OP-48
So, Jongho	P110
Song, Woon Ju	IL-025
Sosa-Torres, Martha Elena	IL-066
Sousa, Eduardo H. S.	OP-61, P032, P155, P232
Span, Ingrid	OP-53, P319
Spedalotto, Giuseppe	P074
Spingler, Bernhard	OP-88, P006, P113, P249
Splan, Kathryn	OP-28
Sresutharsan, Athavan	P337
Srinivas, Vivek	P164, P191
Srivastava, Vinay Kumar	P338
Srnc, Martin	P060, P075, P092
Stachura, Monika	OP-84

Stampfli, Anja	P192
Starha, Pavel	OP-04, P250, P255
Steffen, Fabio	P021, P322, P324
Stephan, Holger	OP-09
Stewart, Melissa	P193
Stewart, Thomas	P283
Stratmann, Lukas	P323
Stripp, Sven	OP-85
Stulz, Eugen	IL-088
Sugimoto, Hiroshi	OP-58, P179, P186, P189, P199
Sullivan, Matthew	P111
Suman, Sigridur	IL-154
Sun, Hongzhe	KN-23, P288, P291
Swart, Marcel	IL-026
Szalai, Veronika	OP-78

## T

Tabrizi, Leila	P284
Takashi, Suzuki	P076
Tan, Cai-Ping	OP-33
Tang, Ruikang	KN-31
Tatsumi, Kazuyuki	IL-043
Tegoni, Matteo	IL-002
Telser, Joshua	OP-74
Thulstrup, Peter	IL-157, P142
Tiwari, Manish Kumar	P214
Tong, Ka Yan	P078
Tong, Ka-Chung	P285, P287
Tosha, Takehiko	OP-76, P189
Travnicek, Zdenek	P250, P255, P311
Trindade, Inês	YR-26
Tseng, Yu-Ting	P079
Turano, Paola	IL-118

## U

## V

Várnagy, Katalin	P080
Van Brempt, Niels	P194

Varotsis, Constantinos	OP-81
Vieira, Eduardo	P033
Vila, Alejandro	PL-04, P154
Vo, Chau Duy Tam	P195

## W

Walke, Gulshan	P286
Walton, Paul	PL-03, IL-139
Wan, Pui-Ki	P285, P287
Wang, Baomin	OP-46
Wang, Haibo	OP-17
Wang, Jiangyun	IL-028
Wang, Zenghui	P324
Wang, Zhigang	P289
Ward, Thomas	IL-023, P071, P168
Ward, Roberta	LA-13
Wei, Wei	OP-41
Weng, Cheng	P339
Wenger, Oliver	IL-054
Wettstein, Lionel	P006
White, Corey	P081
Wiley, Seth	P196
Wilks, Angela	IL-016
Wojdyła, Zuzanna	P197
Wong, Kam-Bo	KN-35
Wong, Yuen Ting	P291
Wu, Wenyu	P113

## X

Xia, Wei	OP-21
Xing, Bengang	IL-072

## Y

Yadav, Prerna	P340
Yajima, Tatsuo	P082
Yam, Vivian Wing Wah	IL-020
Yamamoto, Yasuhiko	IL-095
Yamauchi, Osamu	LA-10, P082
Yang, Xiaoda	IL-150

Yano, Shigenobu	P272
Yaourtis, Andria	YR-09
Yasui, Hiroyuki	P198
Yi, Yelim	P215
Yonemura, Kai	P199
Yu, Zuo Hang	P083
Yukl, Erik	OP-82

## Z

Zamble, Deborah	KN-40
Zamocky, Marcel	OP-34
Zelder, Felix	OP-55, P340
Zelger-Paulus, Susann	P021, P300, P308
Zhang, Jun-Long	IL-133
Zhang, Limei	OP-16, P160
Zhang, Lin	OP-39
Zhang, Qi	P288, P291
Zhang, Wenying	P294
Zhang, Yan	P023
Zhang, Zhifeng	P295
Zhu, Guangyu	OP-14
Zima, Alexandra	YR-31
Zobi, Fabio	OP-57
Zuccarello, Lidia	YR-16



# ICBIC-19

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# ICBIC-19

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