From NMR spectra to structures

- find conditions under which the protein does not aggregate is resonably stable

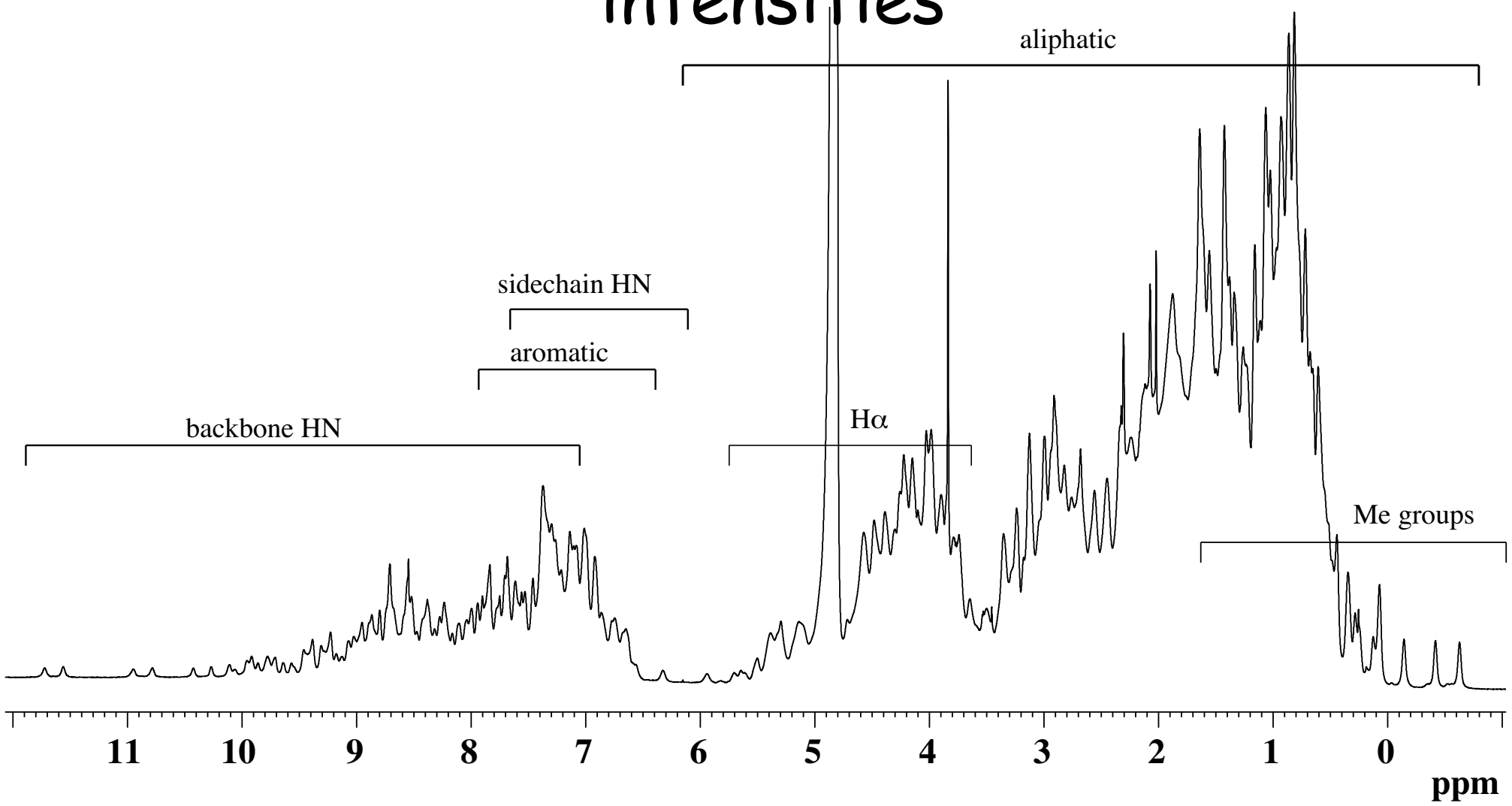
- measure nmr spectra

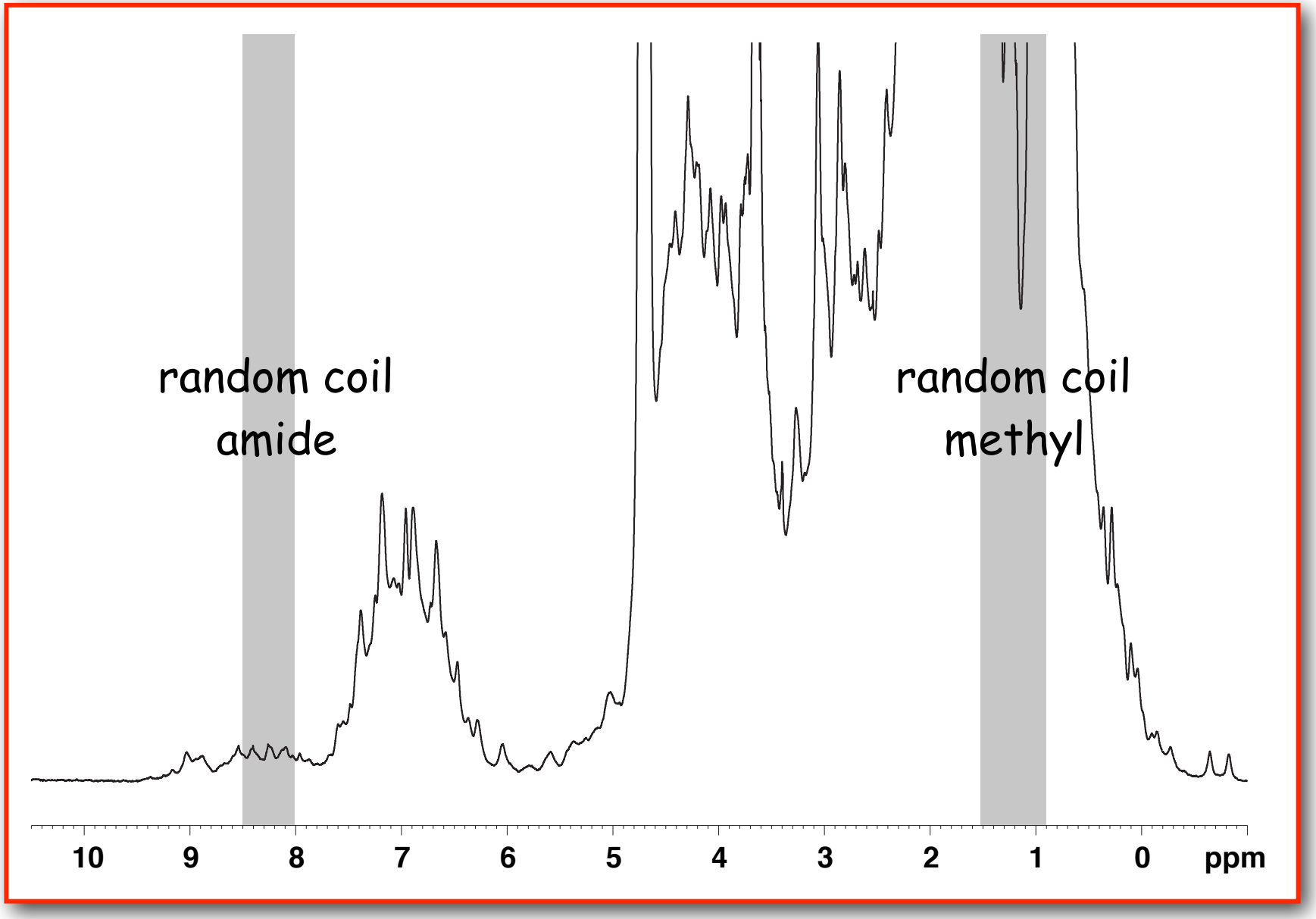
- sequence-specific sequential resonance assignment
- identify spin systems
- link spin systems (sequential assignment)
- (stereospecific assignment of diasterotopic protons)
- fully- interpret NOESY spectrum

- convert NOESY peak amplitudes into distances

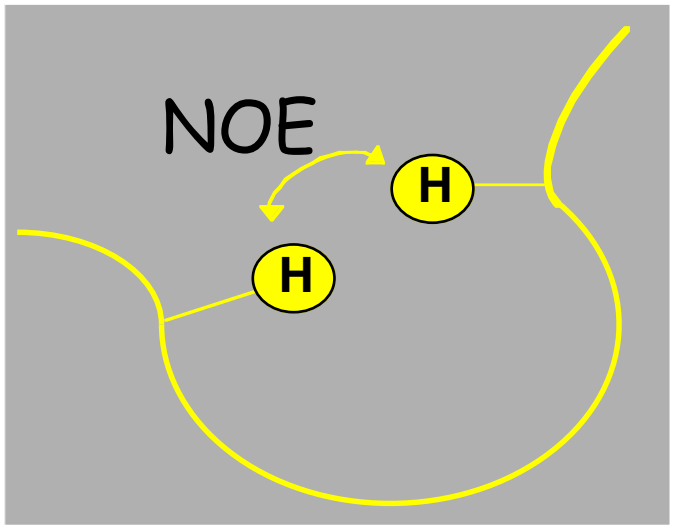
- calculate 3D structure and refine the output

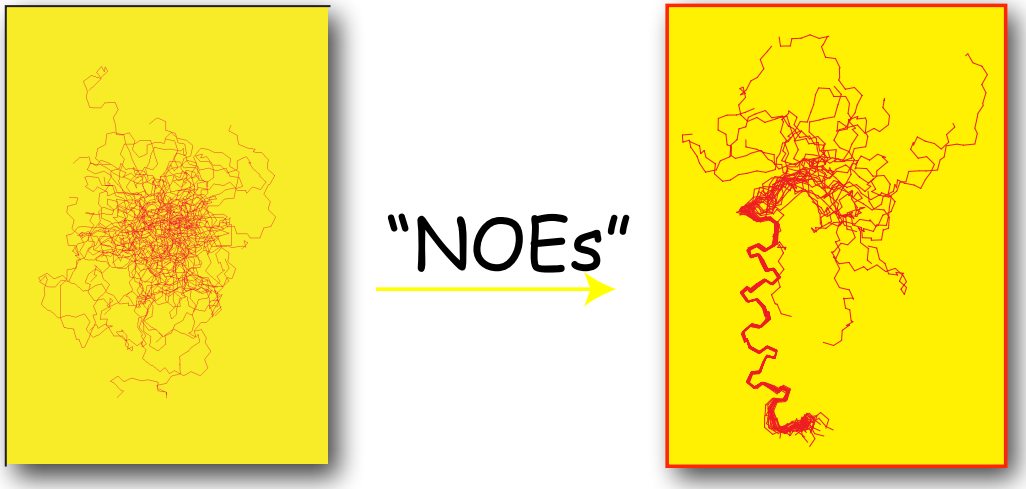
Chemical shifts (frequencies) and line intensities




random coil
amide
random coil
methyl
10
9
8
7
6
5
4
3
2
1
0
ppm

NOE information is used to introduce distance restraints into the structure calculations

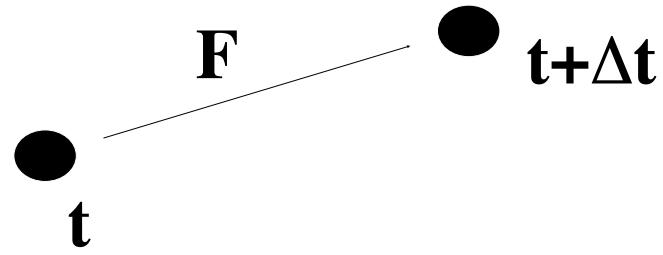




$$\mathbf{NOE} \sim \mathbf{B}_{\mathbf{loc}}^{2} \qquad \mathbf{B}_{\mathbf{loc}} \sim \frac{\gamma_I \gamma_S (3\cos^2\phi - 1)}{r^3}$$

From distances to 3D structures (II)

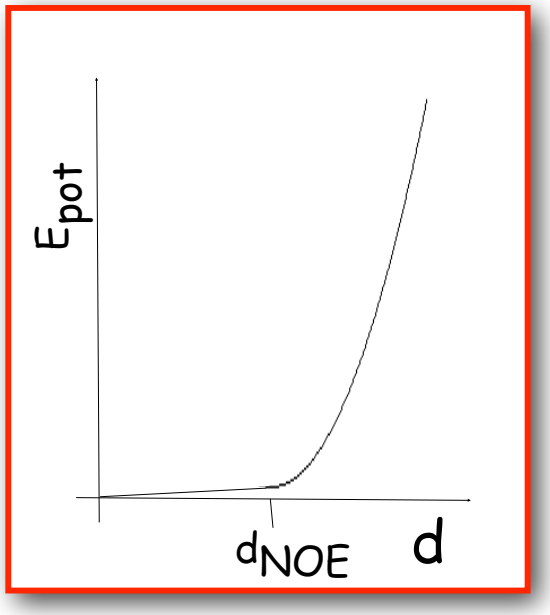
- Restrained molecular dynamics:



$$F = m\frac{\partial^2 r}{\partial t^2} \qquad F = \frac{\partial U_{pot}}{\partial r}$$

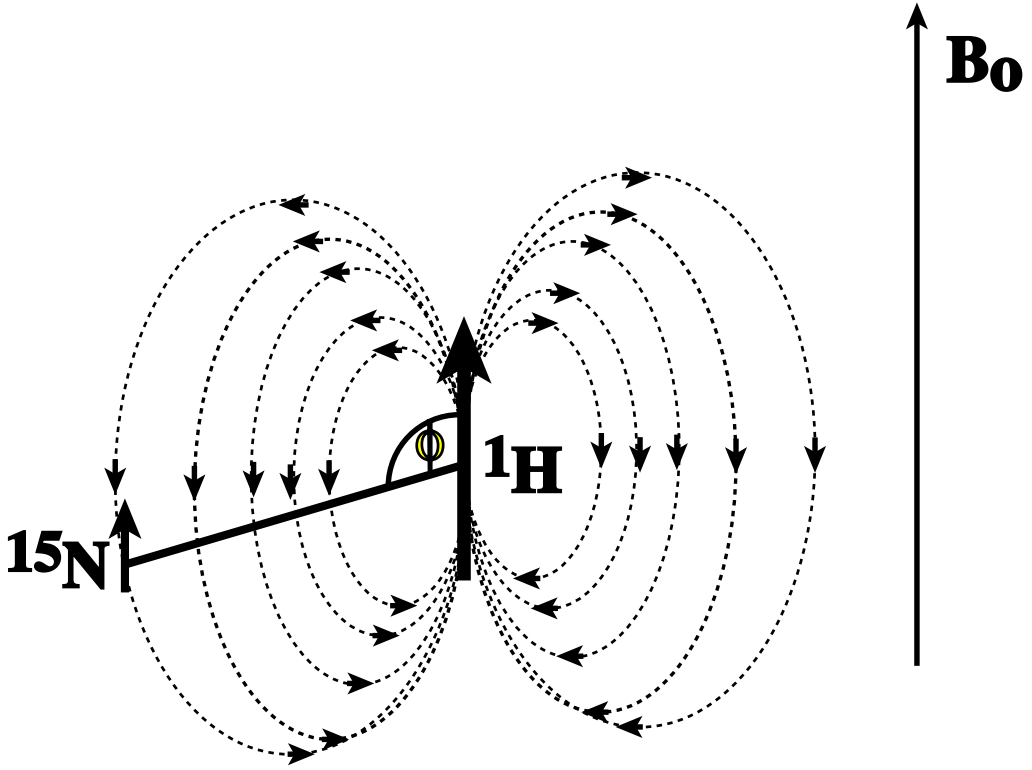
$$U_{pot} = U_{bond} + U_{angle} + U_{dihedral} + U_{chiral} + U_{v.d.Waals}$$

$$+ U_{coulomb} + U_{NMR}$$



$$U_{NMR} = U_{NOE} + U_J + \ldots$$

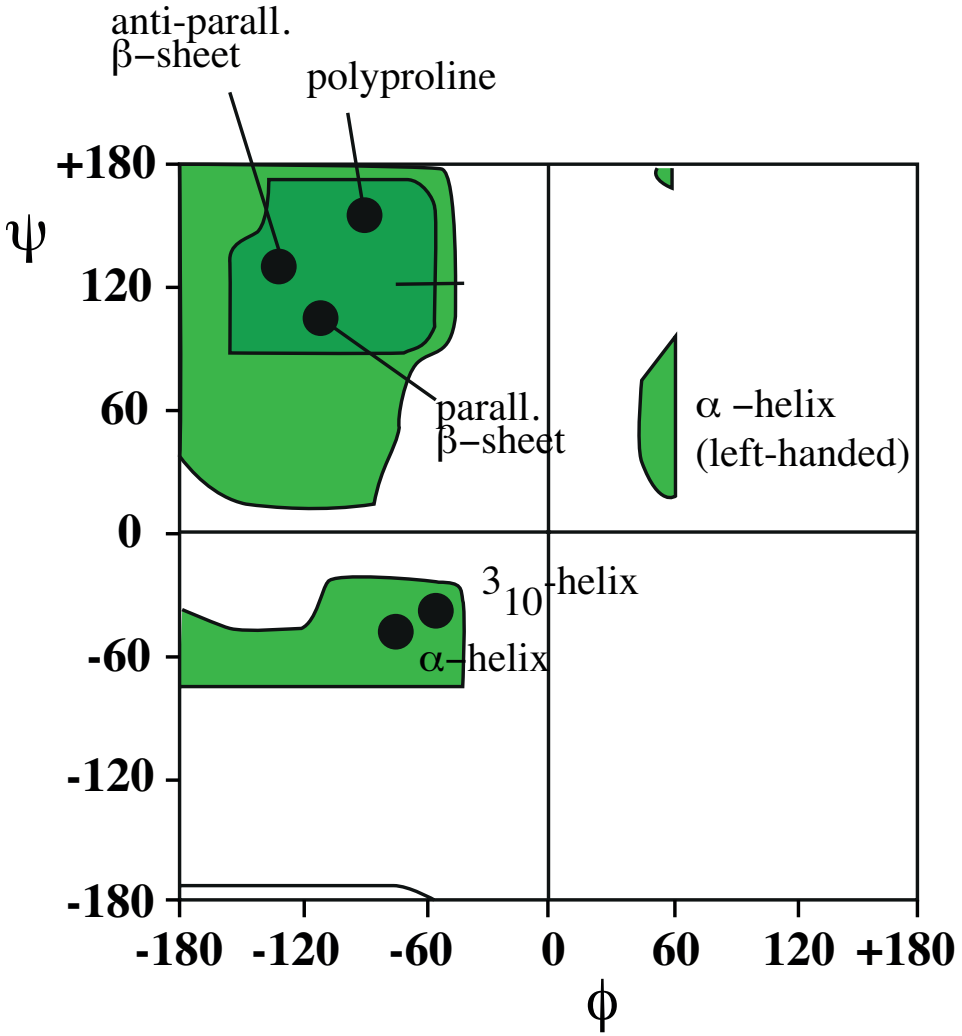
The origin of the NOE is dipolar (through-space) coupling of protons

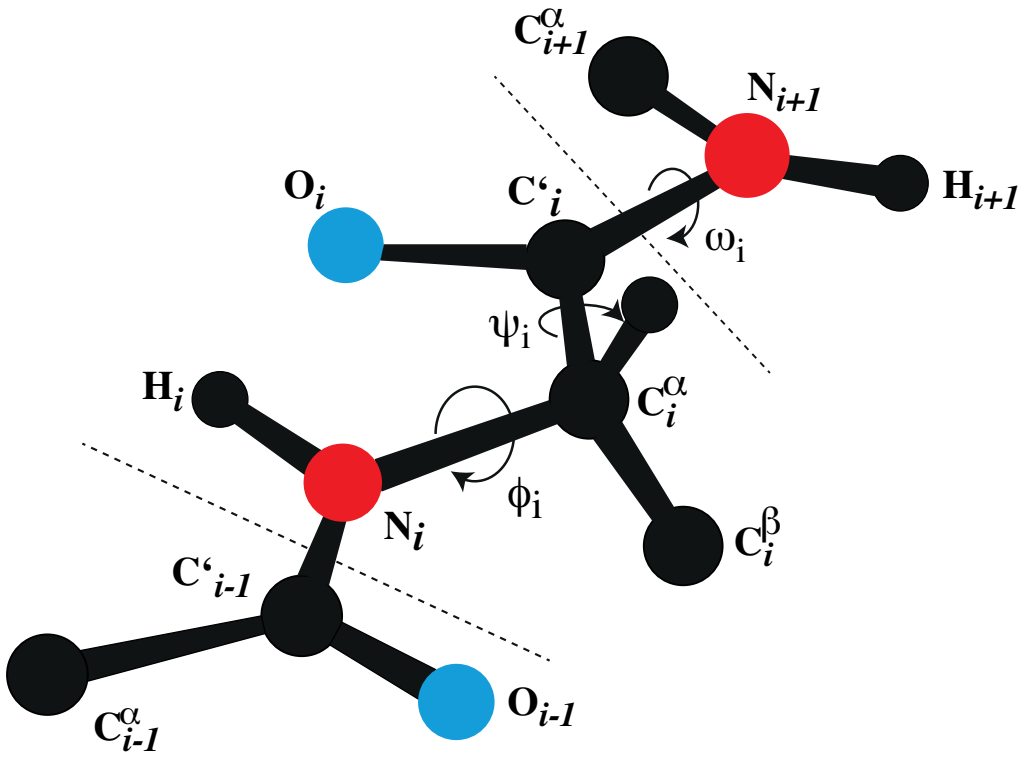


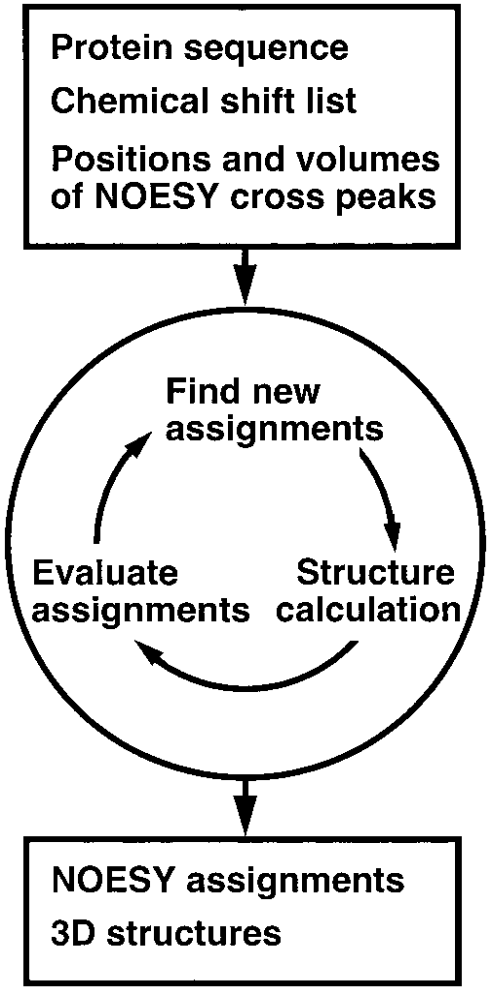
$$B_{loc} \sim \frac{\gamma_I \gamma_S (3\cos^2\phi - 1)}{r^3}$$

$$R2, R1 \sim B_{loc}^2$$

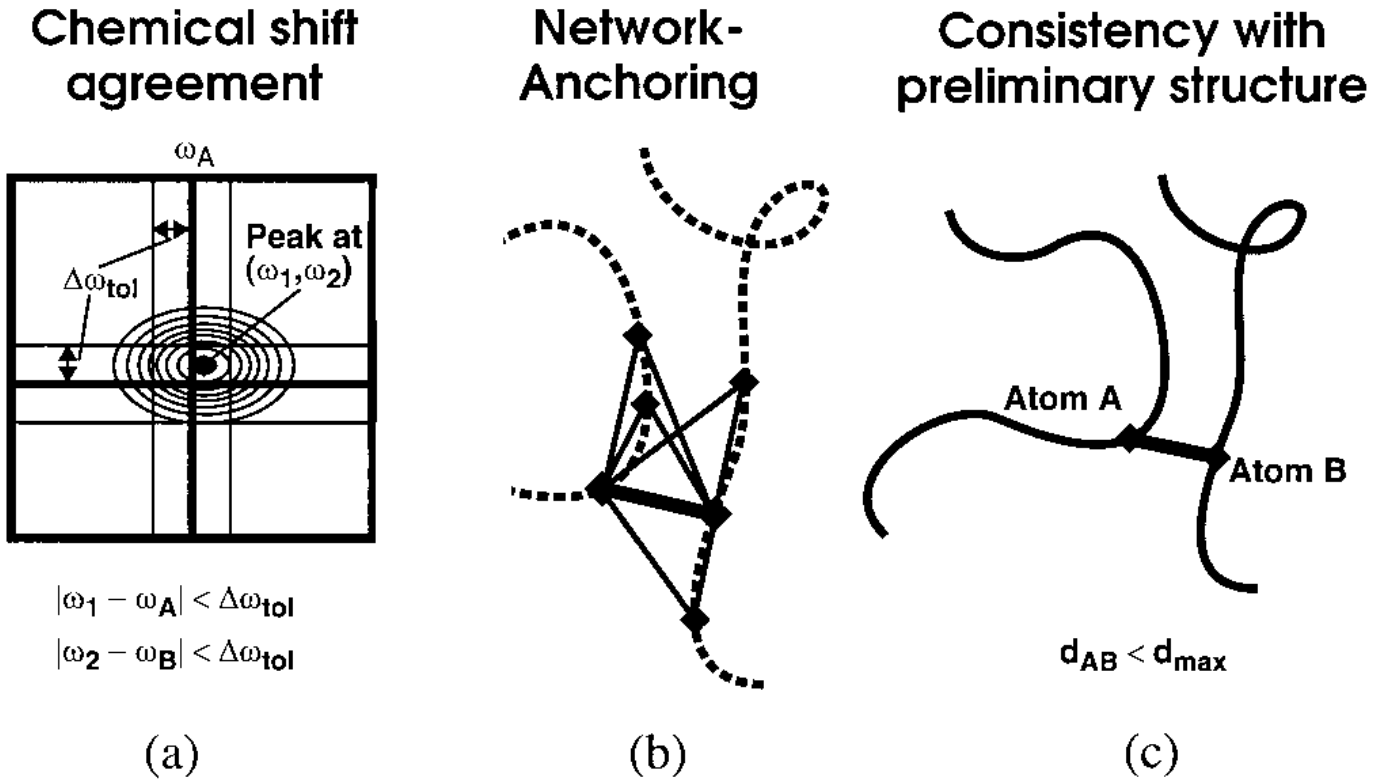
During the structure calculation only rotations about dihedrals are made



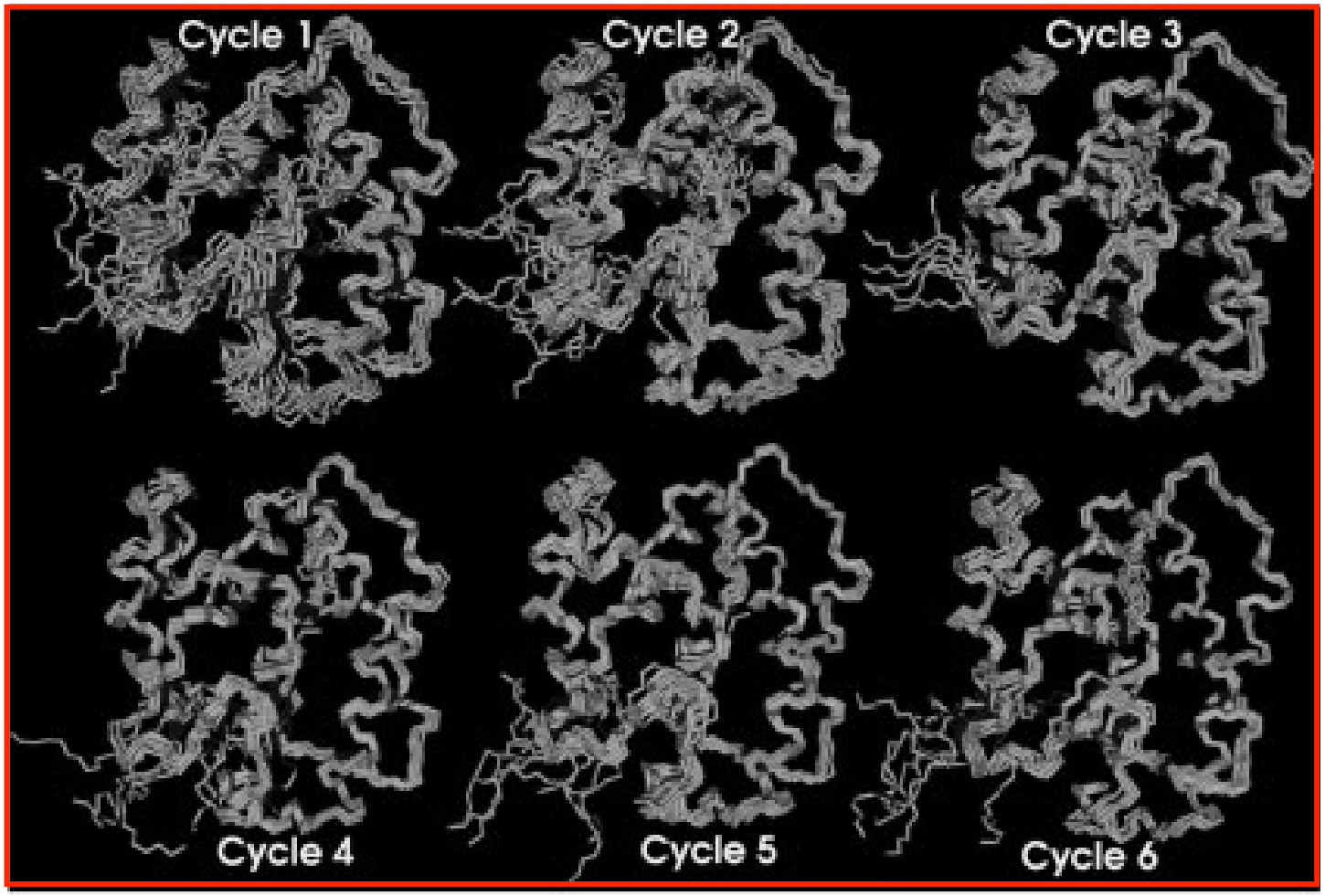



Protein sequence
Chemical shift list
Positions and volumes of NOESY cross peaks
Find new assignments
Structure calculation
Evaluate assignments
NOESY assignments
3D structures

Automated calculation of NMR structures

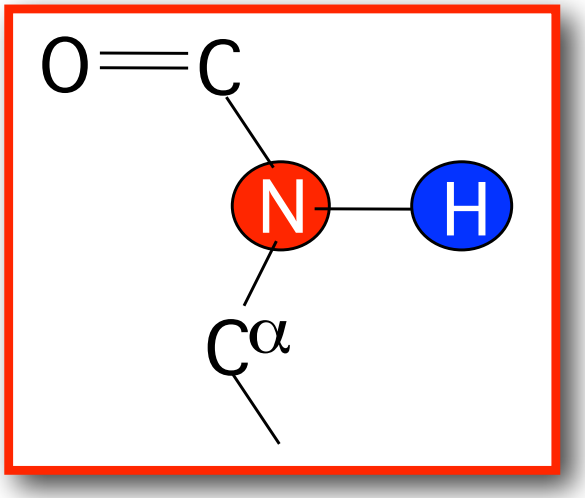


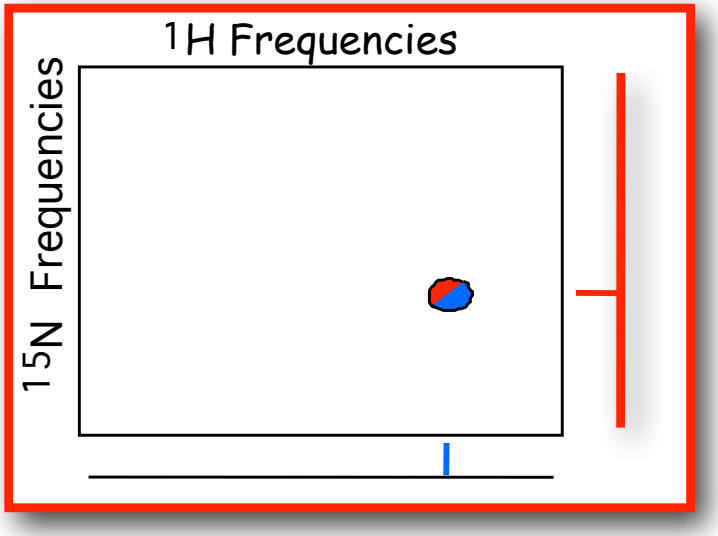
Güntert, Quarterly Reviews of Biophysics ??31 (1998), 145-

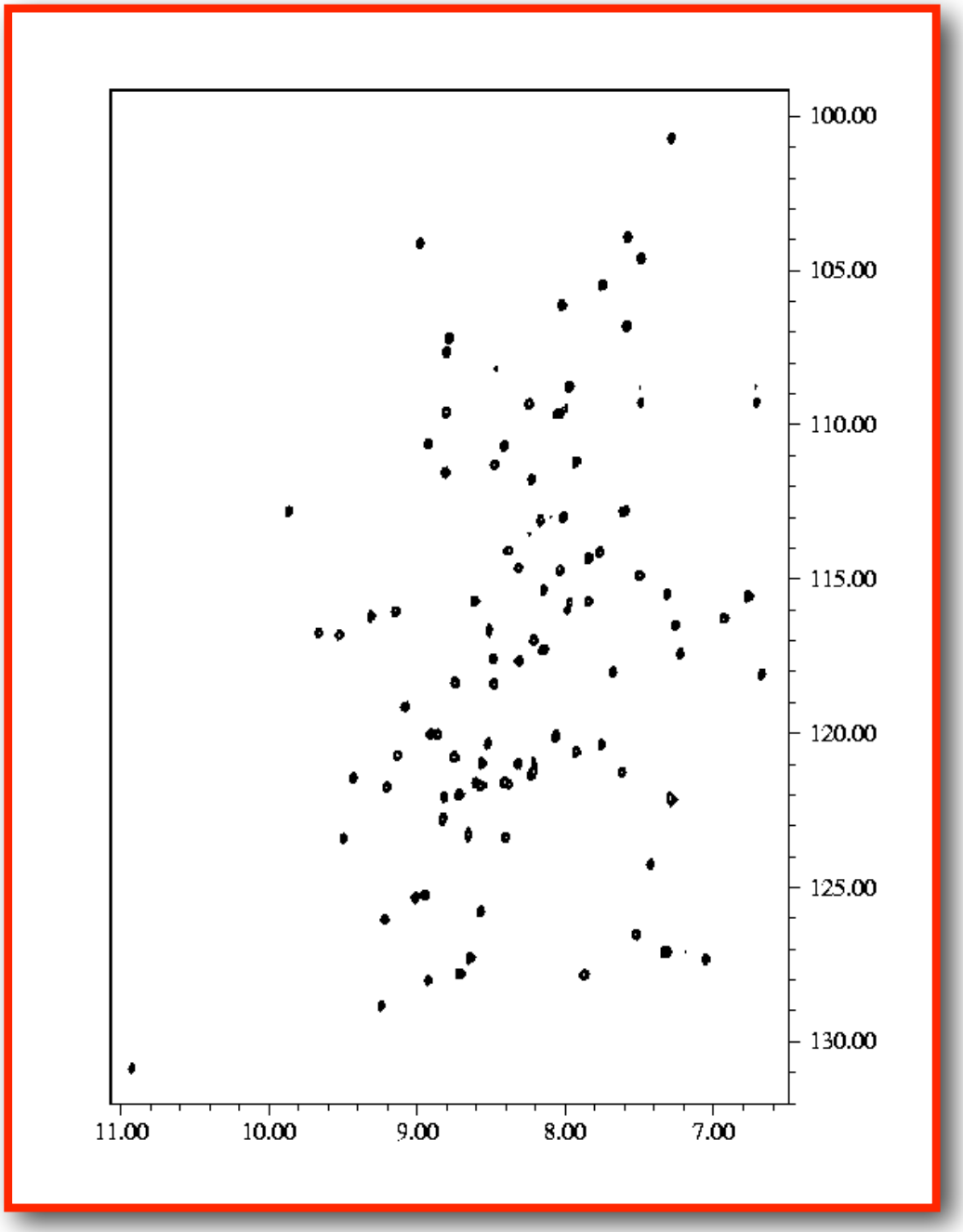

Cycle 1
Cycle 2
Cycle 3
Cycle 4
Cycle 5
Cycle 6

Methods for assigning larger proteins

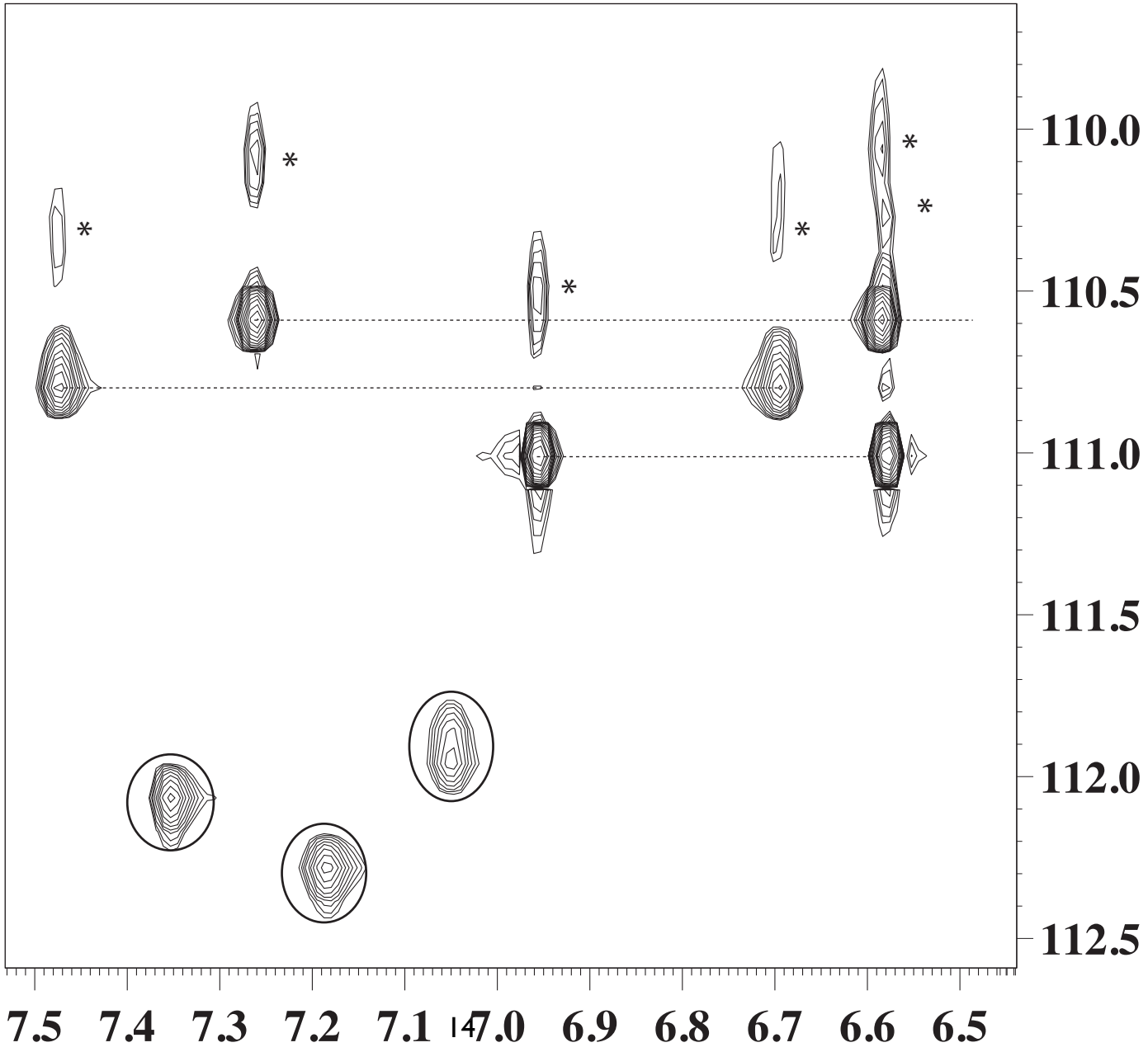
$^{15}N,^{1}H$ HSQC spectra are fingerprints of proteins



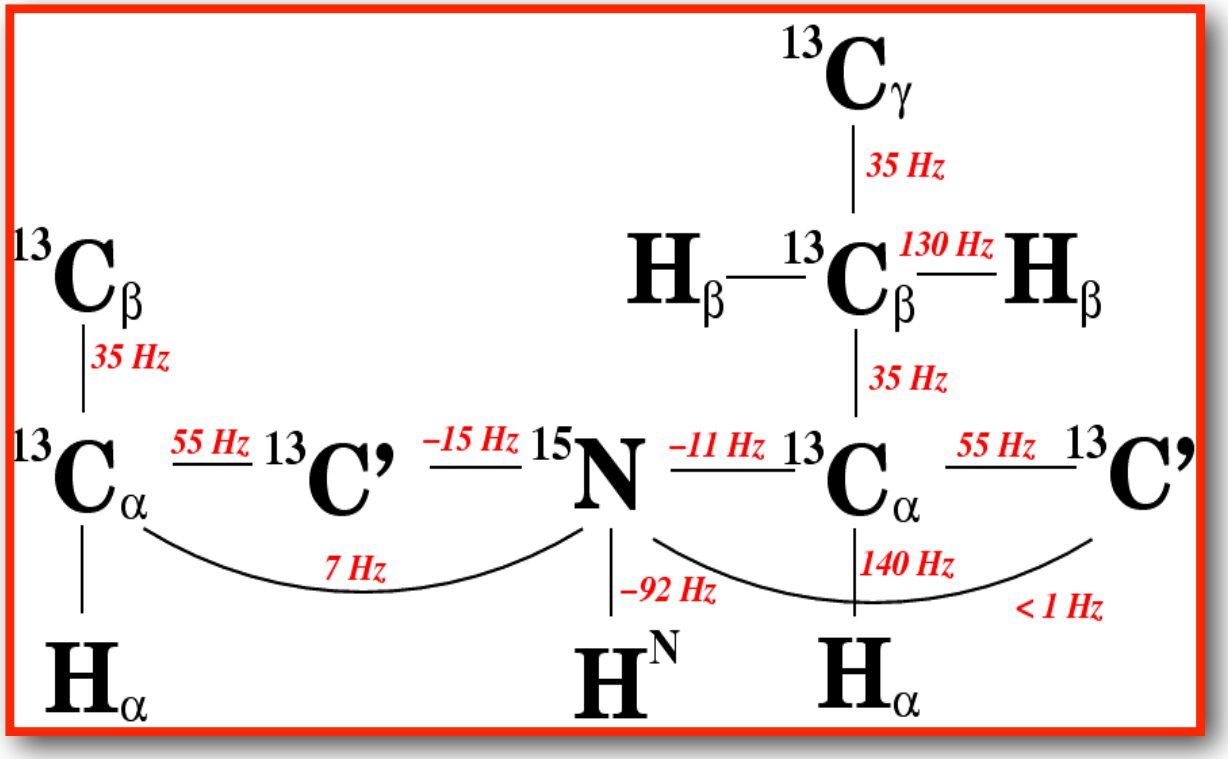




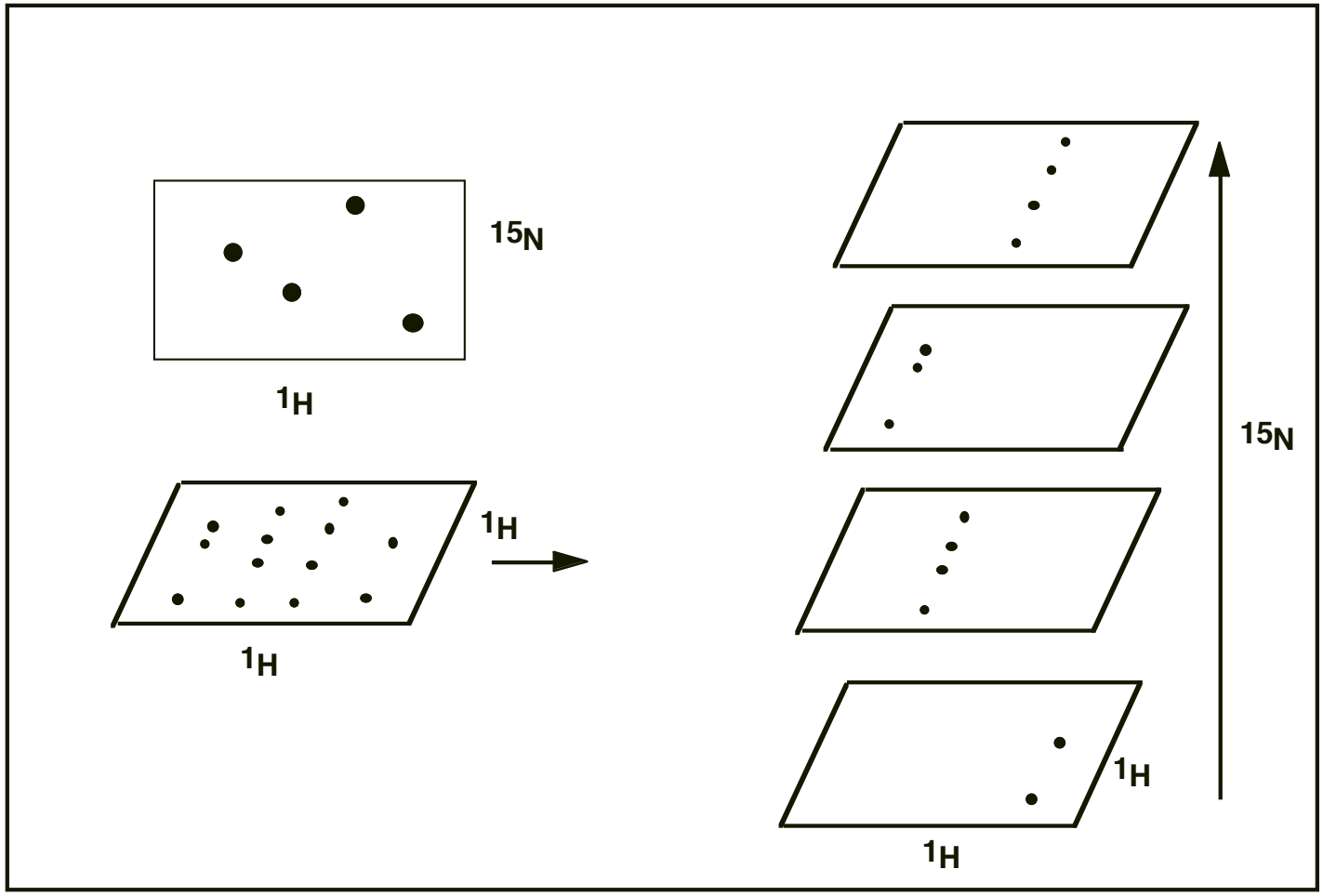
^{15}N spectra of proteins: Sidechain peaks

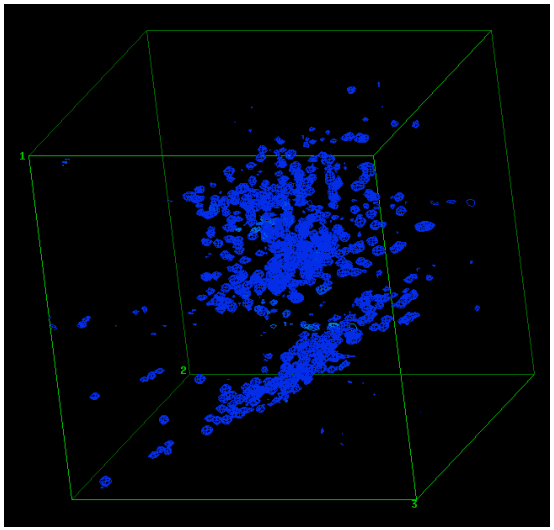


Sequence-specific resonance assignment in $^{13}C,^{15}N$ labelled proteins

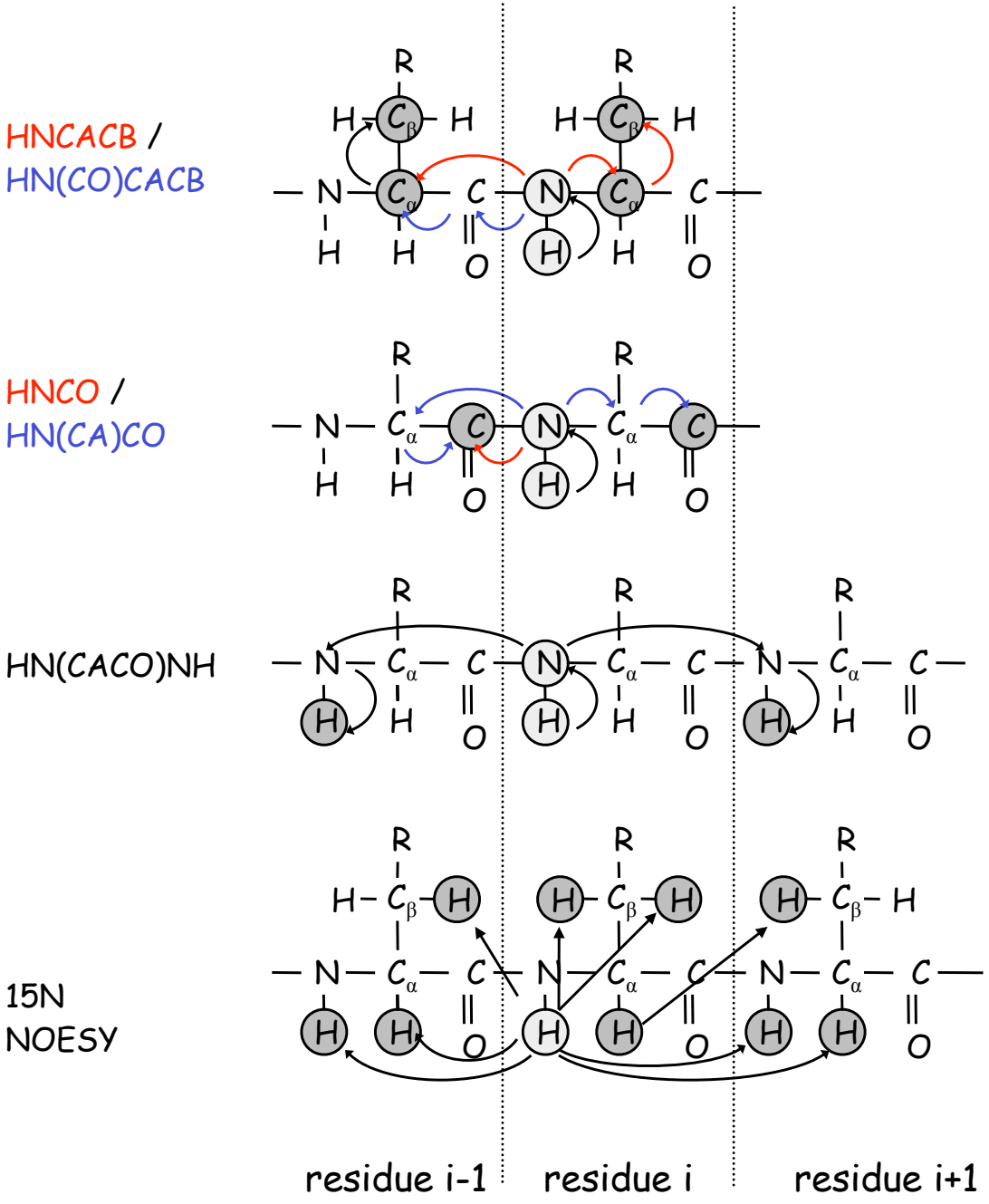


>>Use of 3-dimensional (tripleresonance) experiments

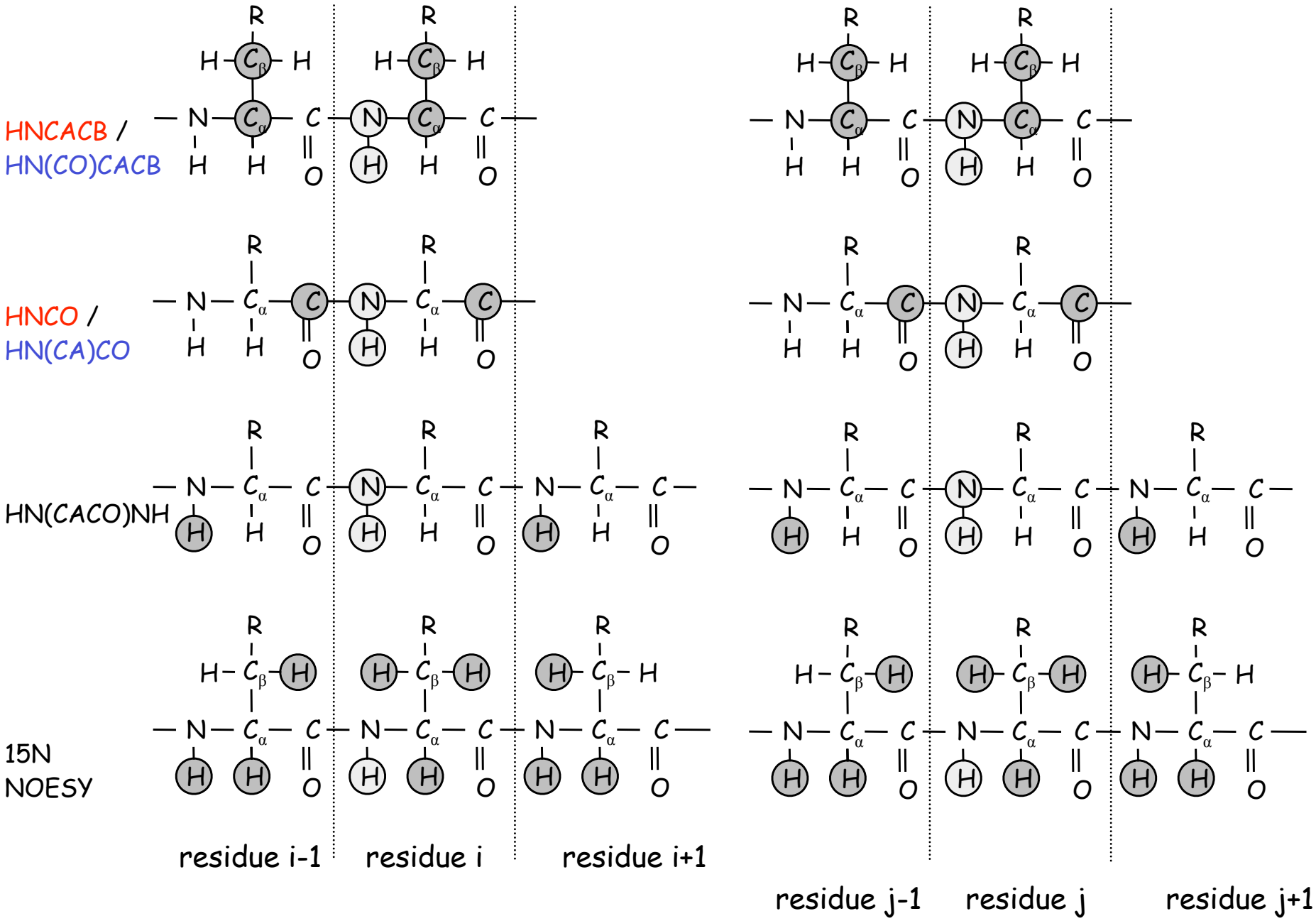




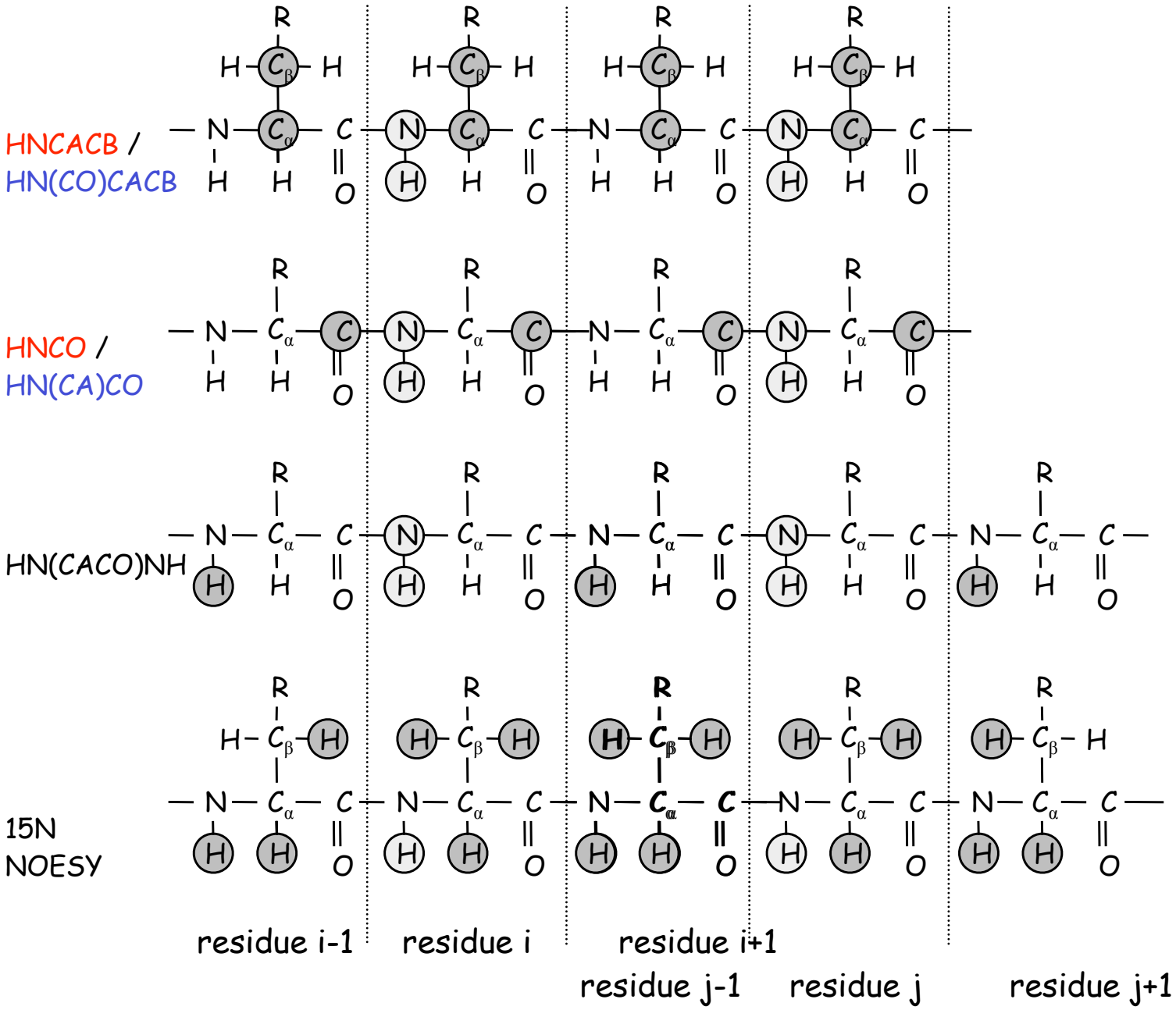
Backbone Assignment – 3D Experiments



Backbone Assignment – 3D Experiments

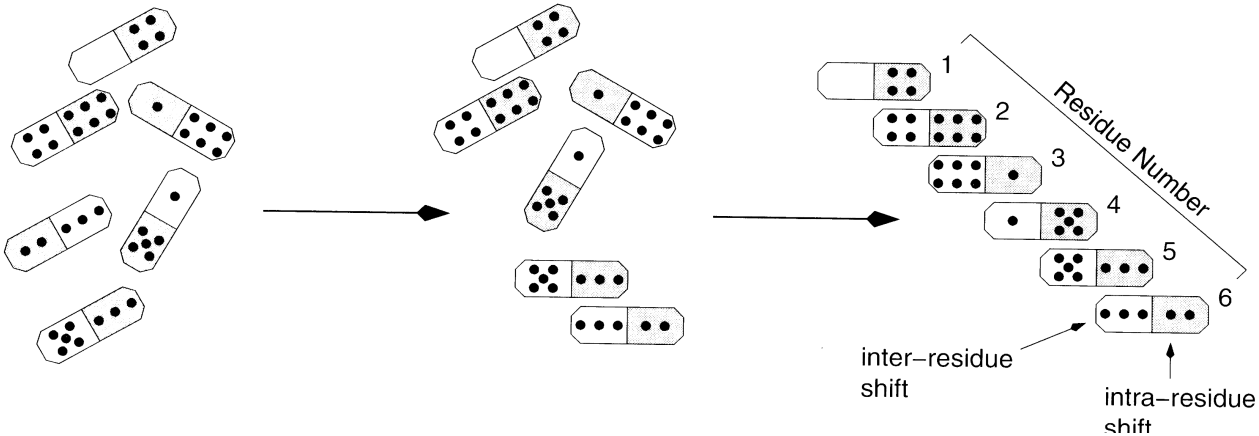


Backbone Assignment – 3D Experiments



sequential assignment strategy: building of fragments

1. picking of HN-C peaks -> spin systems (numbers 1-125)
2. alignment of ^{13}C dimension for linking of correct successors/ predecessors

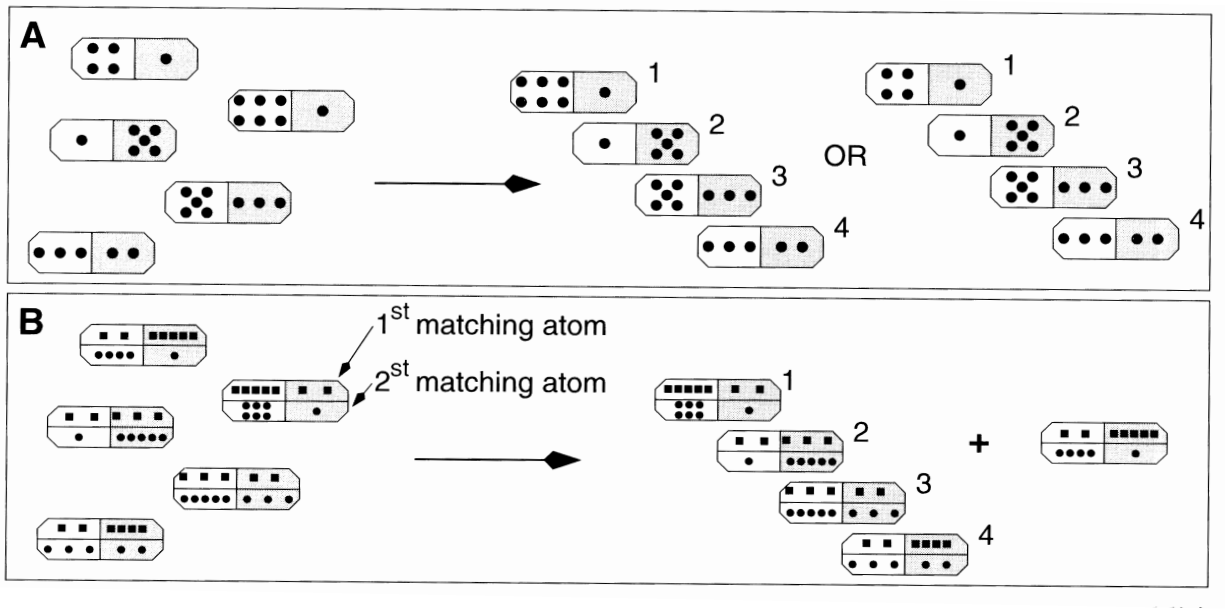


CA / COCA

CACB / COCACB

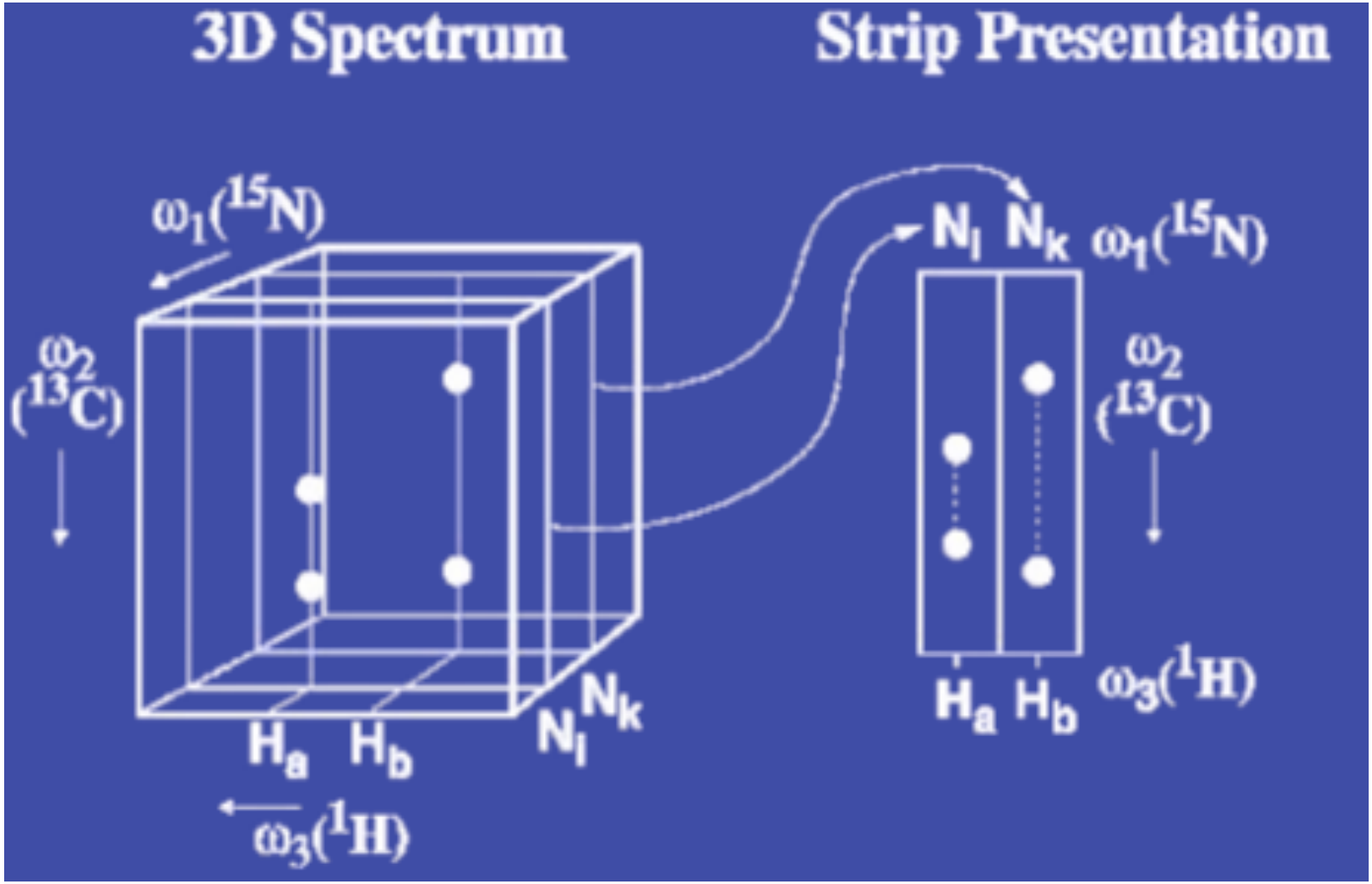
CG / COCG

NN



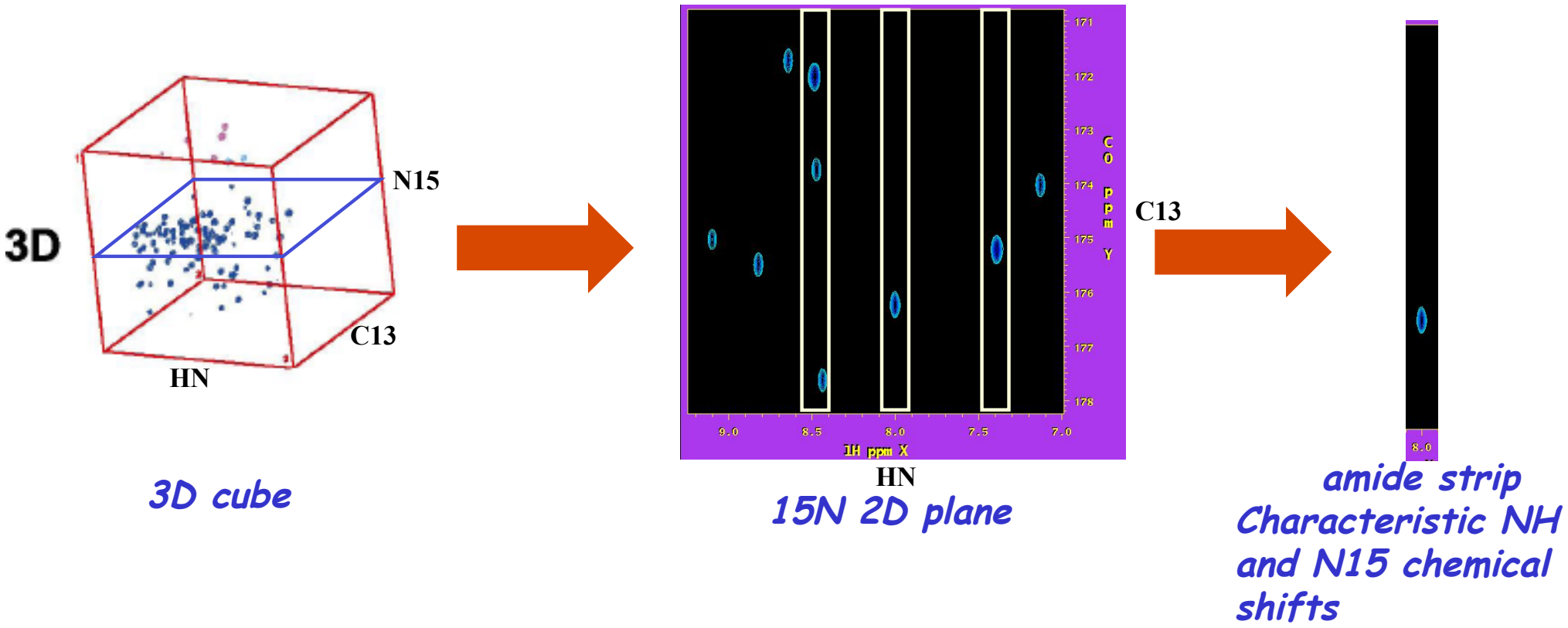
3. try to match fragments on amino acid sequence

view of the 3D spectrum in CARA



2D ^{1}H-^{15}N HSQC is the root experiment of most of the standard *triple-resonance* (^{1}H, ^{13}C, ^{15}N) NMR experiments used for backbone assignment.

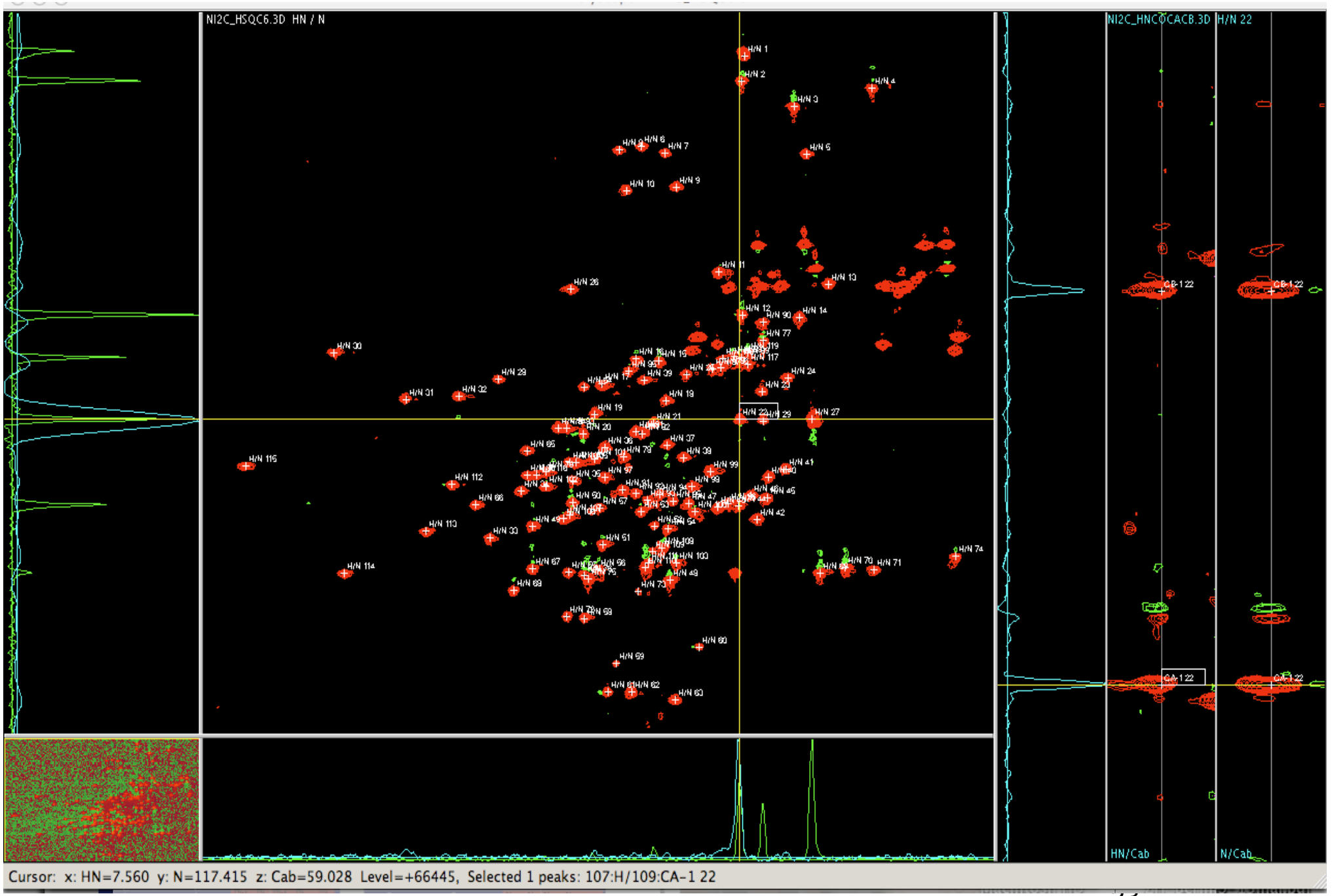
All the 3D triple resonance experiments are related by the common ^{1}H,^{15}N chemical shifts of the HSQC spectra: **AMIDE STRIP**



3D cube

15N 2D plane

amide strip Characteristic NH and N15 chemical shifts


NI2C_HSQC6.3D HN / N
NI2C_HNCOCACB.3D H/N 22
HN/Cab
N/Cab
Cursor: x: HN=7.560 y: N=117.415 z: Cab=59.028 Level=+66445, Selected 1 peaks: 107:H/109:CA-1 22

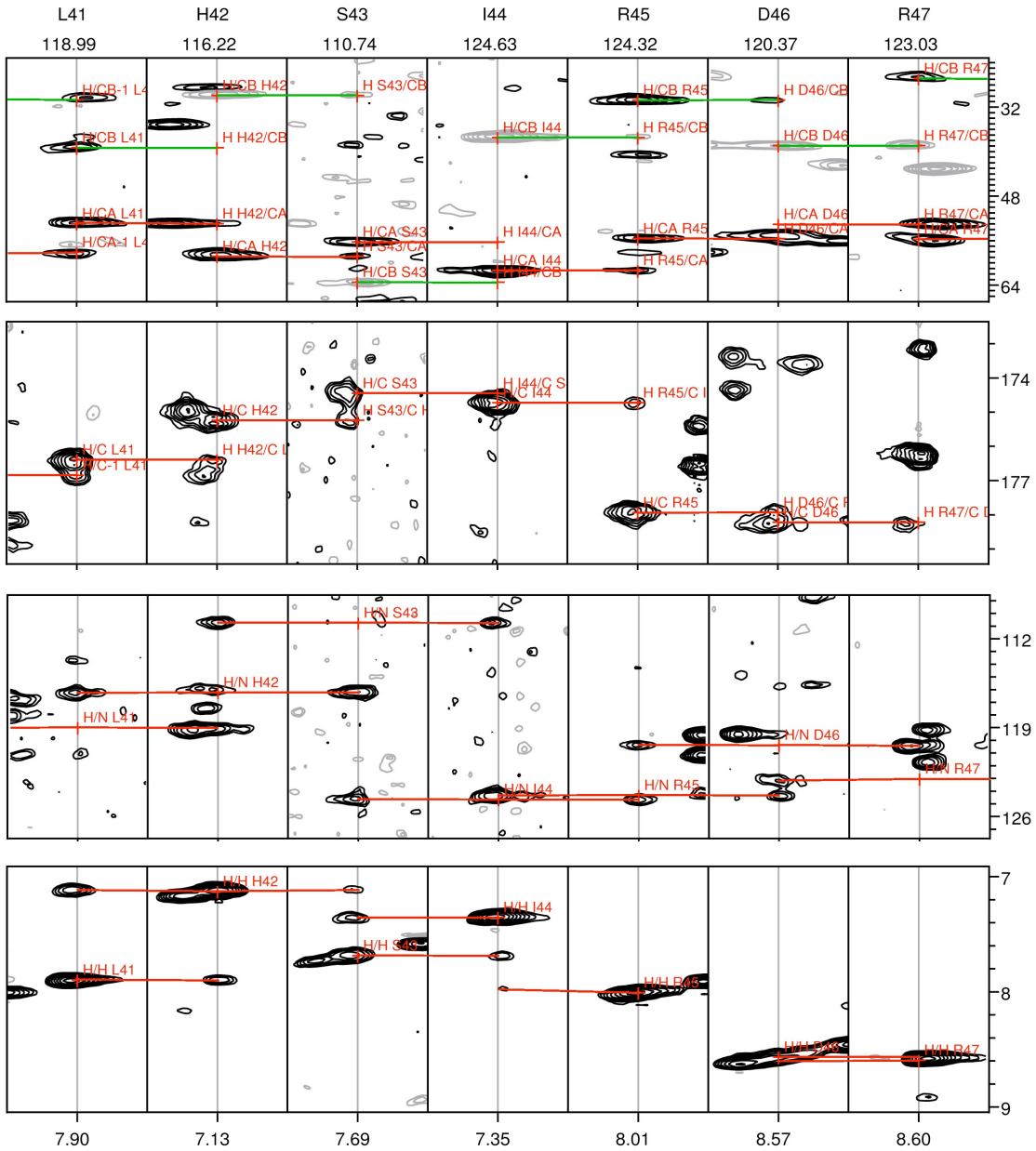
Backbone Assignment – 3D Experiments

HNCACB /
HN(CO)CACB

HNCO /
HN(CA)CO

HN(CACO)NH

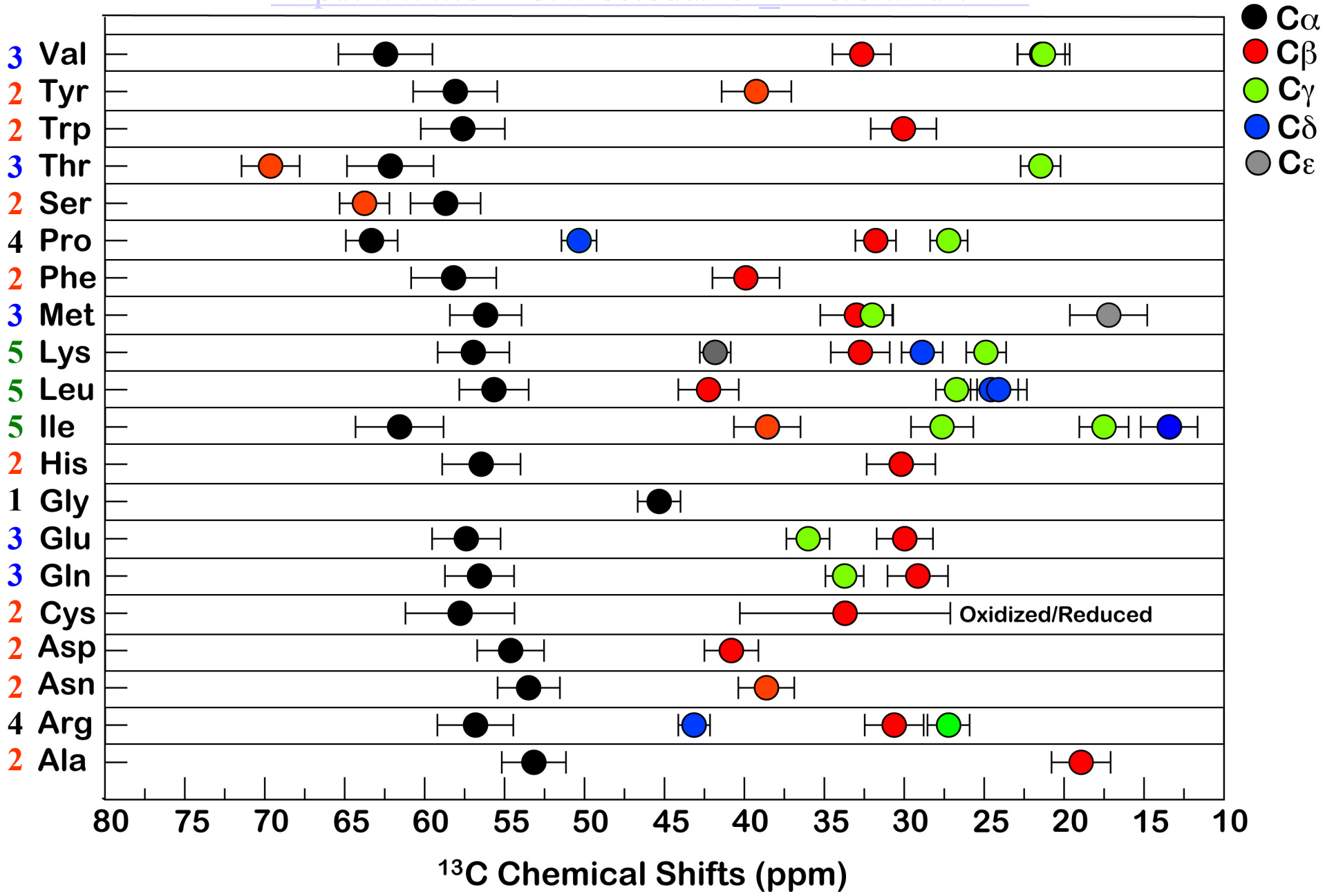
15N
NOESY



Standard Carbon Chemical shifts

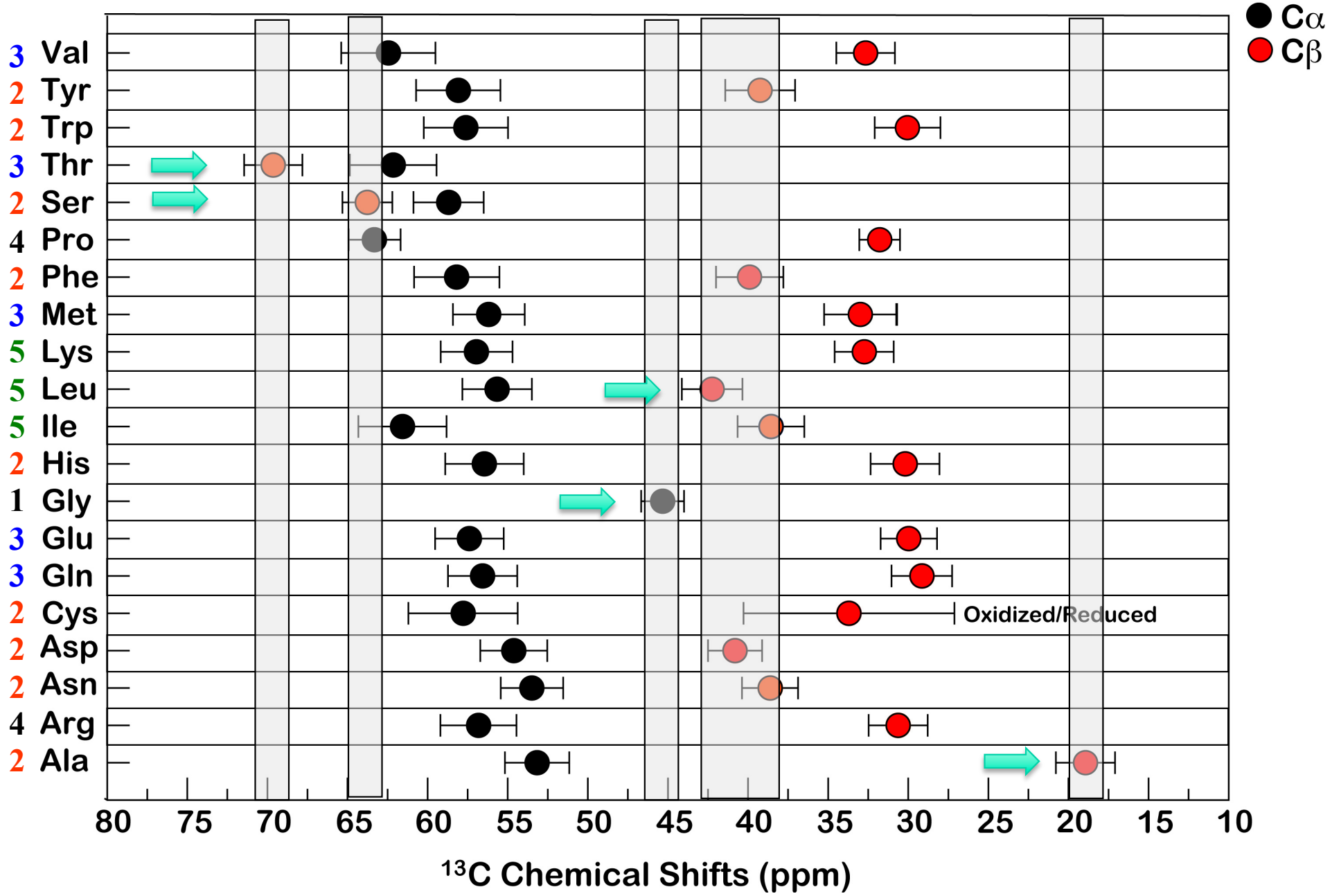
Biological Magnetic Resonance Databank (Diamagnetic Shifts - 03/09/2007)

http://www.bmrb.wisc.edu/ref_info/statful.htm



Standard Carbon Chemical shifts

Biological Magnetic Resonance Databank (Diamagnetic Shifts - 03/09/2007)



Identifying Residue type from chemical shifts

Can easily identify Gly, Ser/Thr and Ala from CB,CA shifts: Gly Cα ~45 ppm; Ser and Thr can be distinguished by Cβ shifts: Thr Cβ ~70 ppm ; Ser Cβ ~ 63 ppm. Ala Cβ chemical shifts is around ~18 ppm.
Can group Leu, Tyr, Phe, Asn, Ile and Asp based on their Cβ shifts ~> 35 ppm.

Differentiate between the residues having two (Asp, Asn, Trp, Tyr, Cys, His, Phe) carbons sidechains and those having 3 or more carbons in the sidechain by using CC(CO)NH.

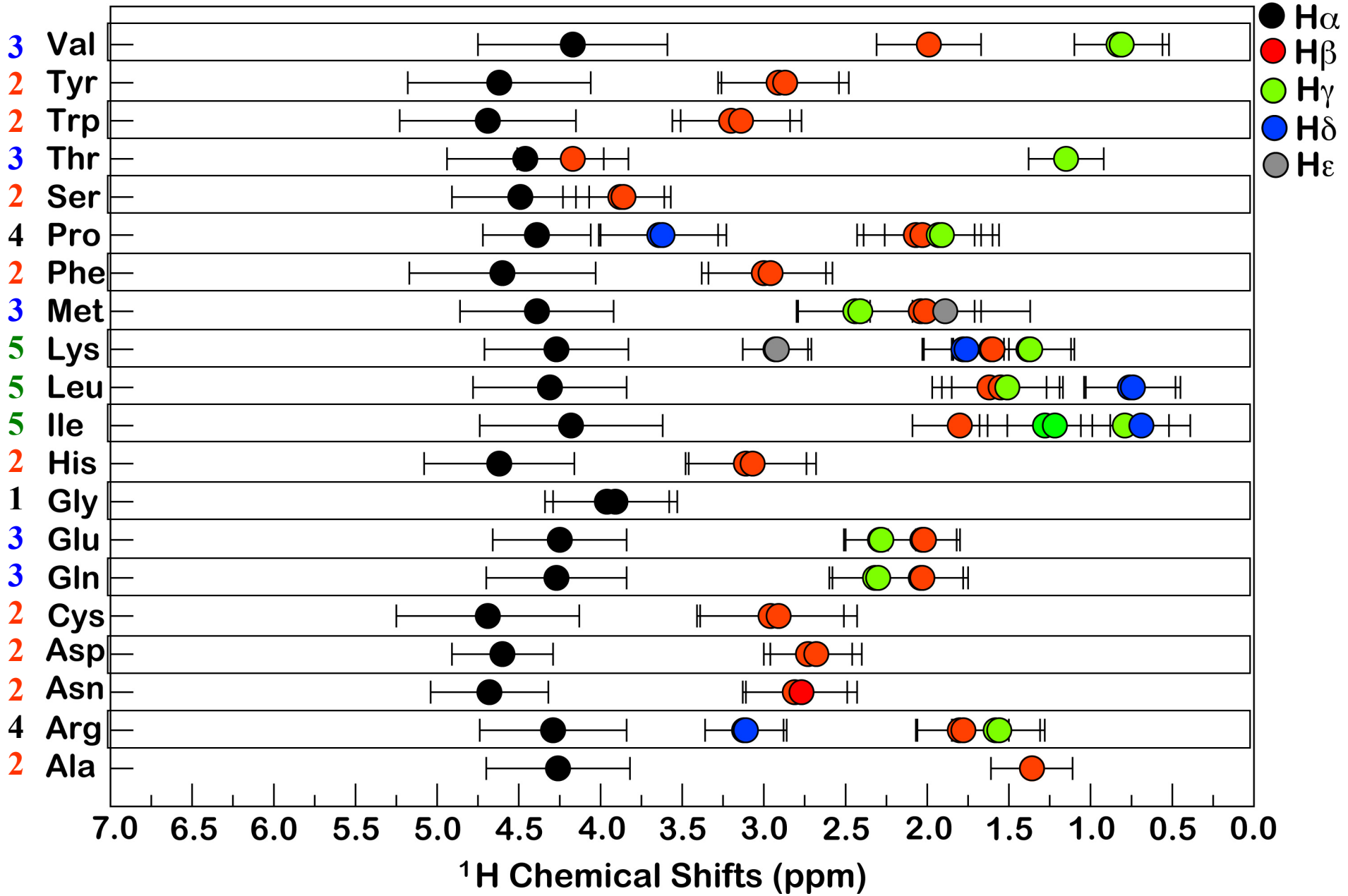
Among the residues having three carbon sidechain: Val, Met, Thr, Glu and Gln, Val has most upfield Cg chemical shifts. Ser/Thr can be distinguished Cg shift. Glu and Gln can be identified by their Cg shifts, Glu Cγ > 35 ppm and Gln Cγ < 35 ppm.

Residues with four carbons sidechain: Pro and Arg can easily be distinguished by their Cδ shifts, for Pro Cδ ~ 50 ppm whereas for Arg Cδ ~43 ppm.

Among the residues having five carbons side chain: Leu, Lys and Ile. Ile has the most upfield Cδ shifts ~10 ppm whereas Leu has Cβ ~ 43 ppm and Lys will have Cε ~43 ppm.

Standard Proton Chemical shifts

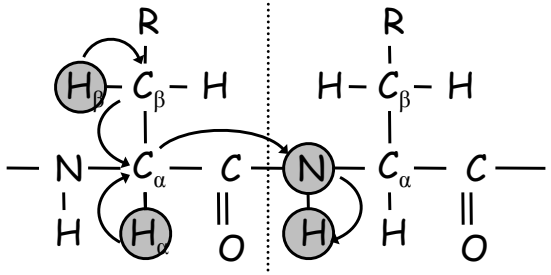
Biological Magnetic Resonance Databank (Diamagnetic Shifts - 03/09/2007)



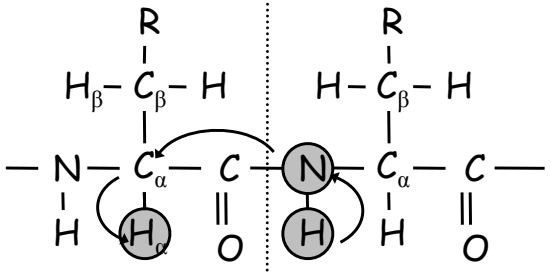
Side Chain Assignment Strategies

Identification of backbone protons:

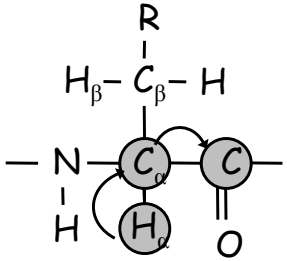
HBHA(CACBCO)NH



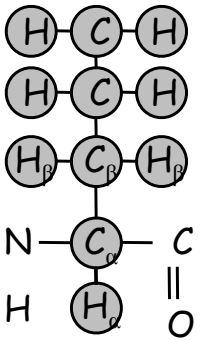
HN(COCA)HA

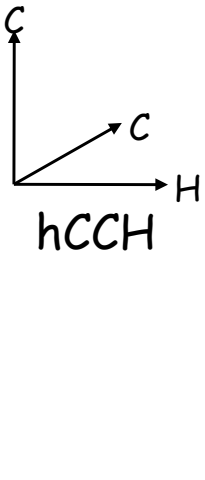


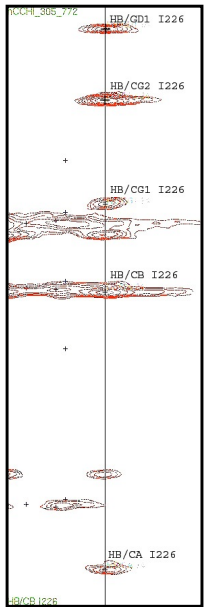
HACACO

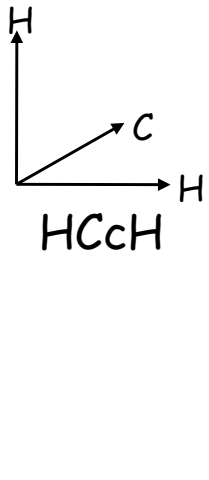


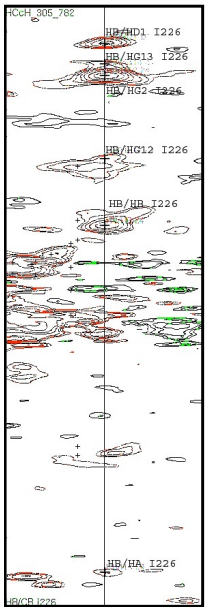
Side chain assignment:



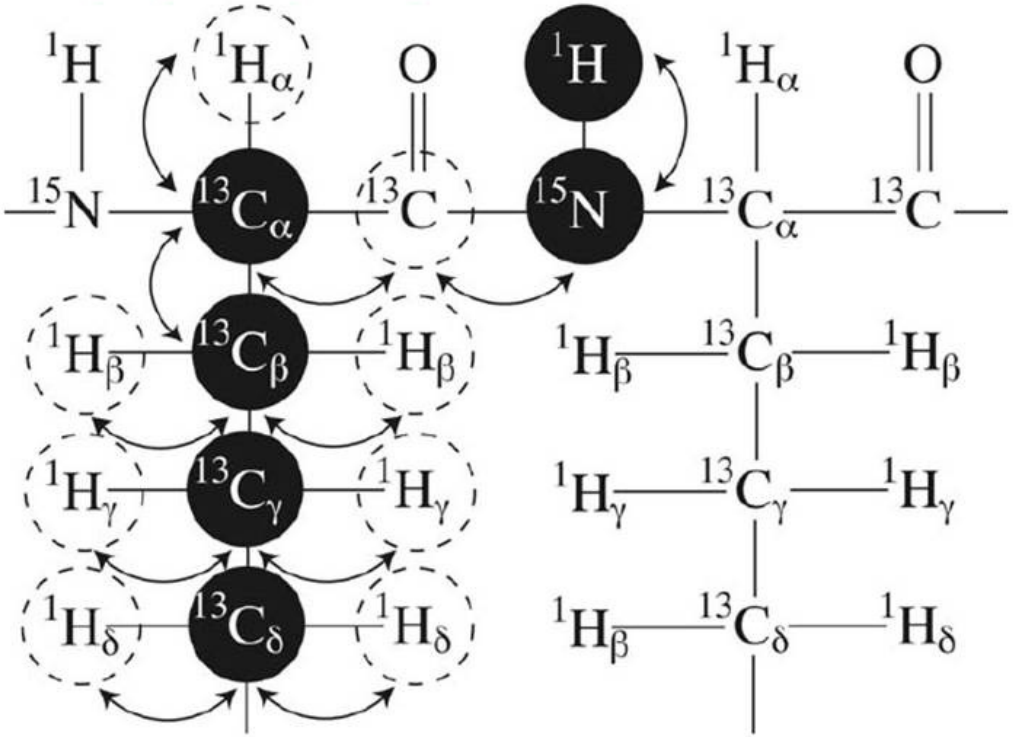




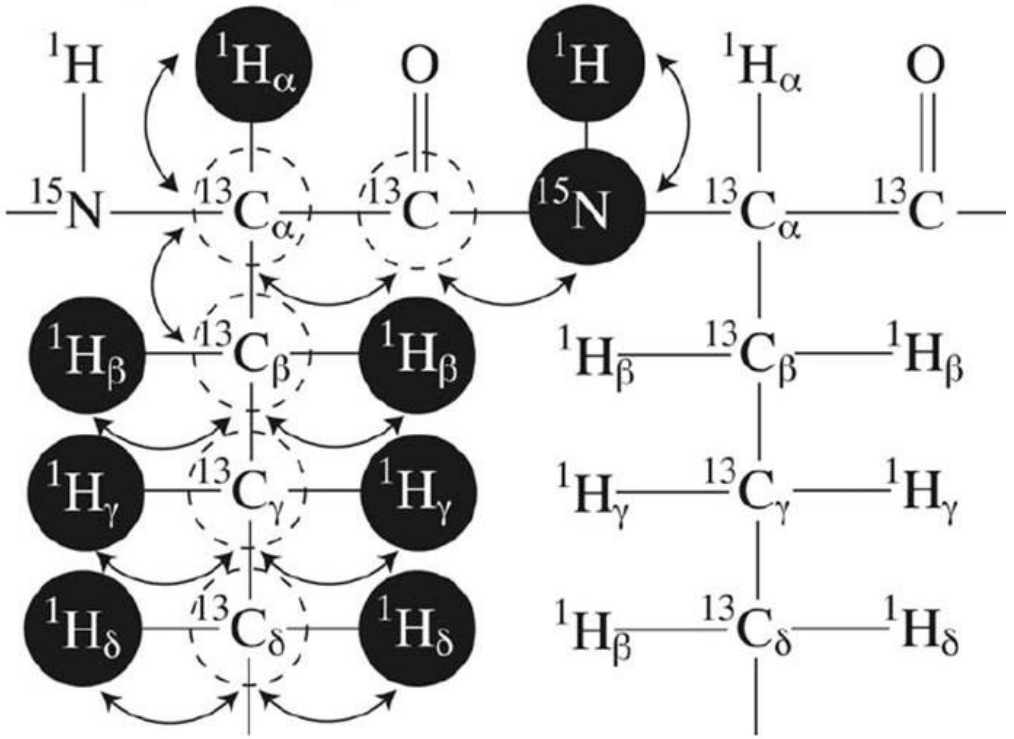




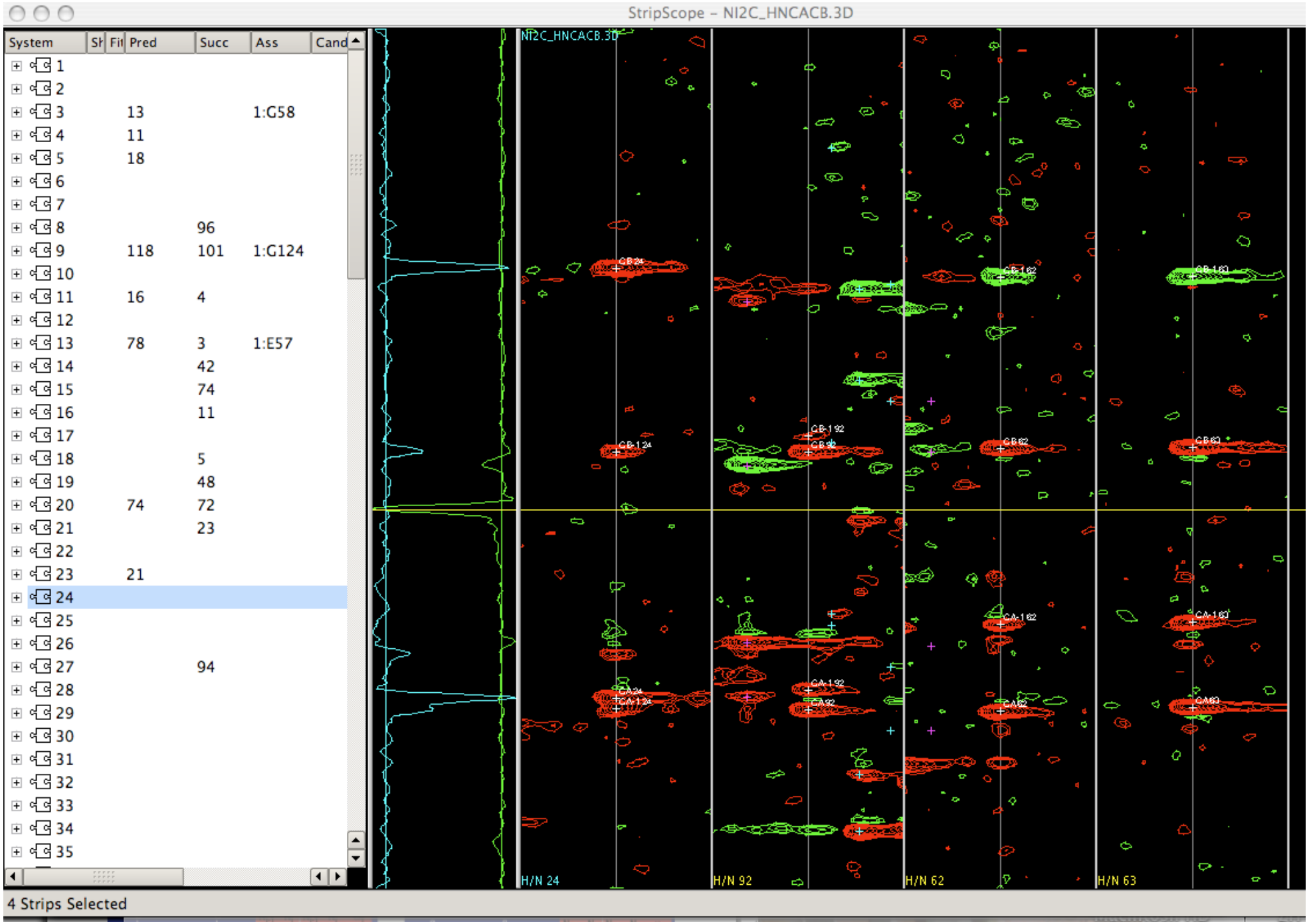
(H)CC(CO)NH



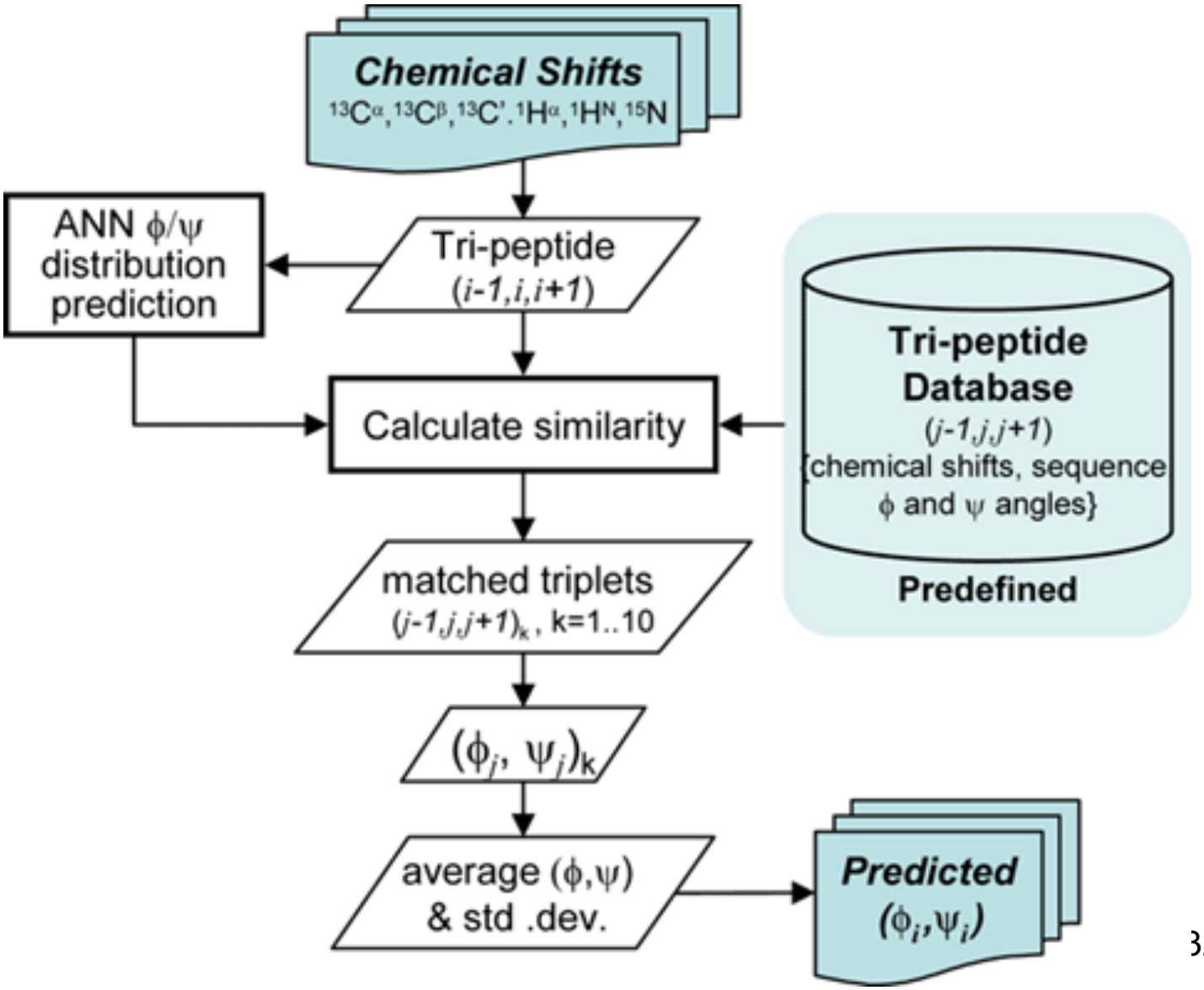
H(CCCO)NH



^{13}C dimension strips of single spin systems for linking

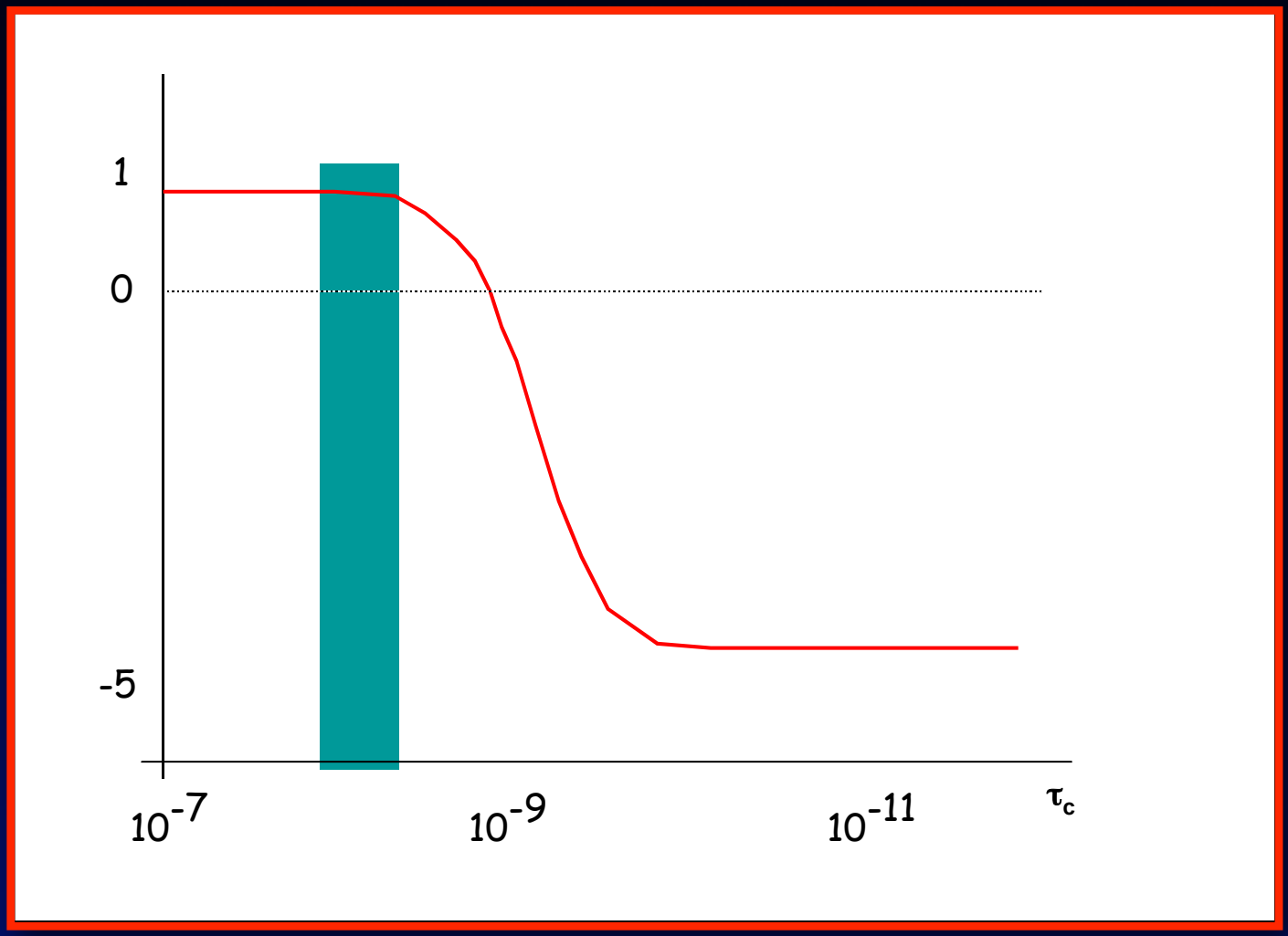


Prediction of secondary structure using chemical shifts using TALOS



The magnitude of the $^{1}H\{^{15}N\}$-NOE depends on the motional properties

$^{15}N\{^{1}H\}$ -NOE

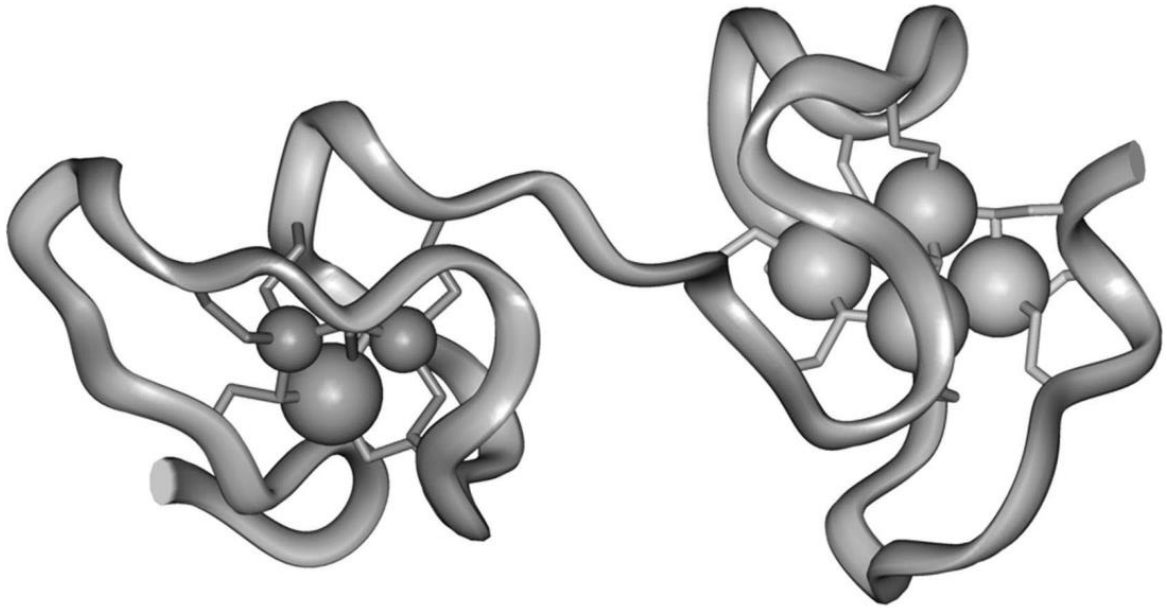


Correlation time

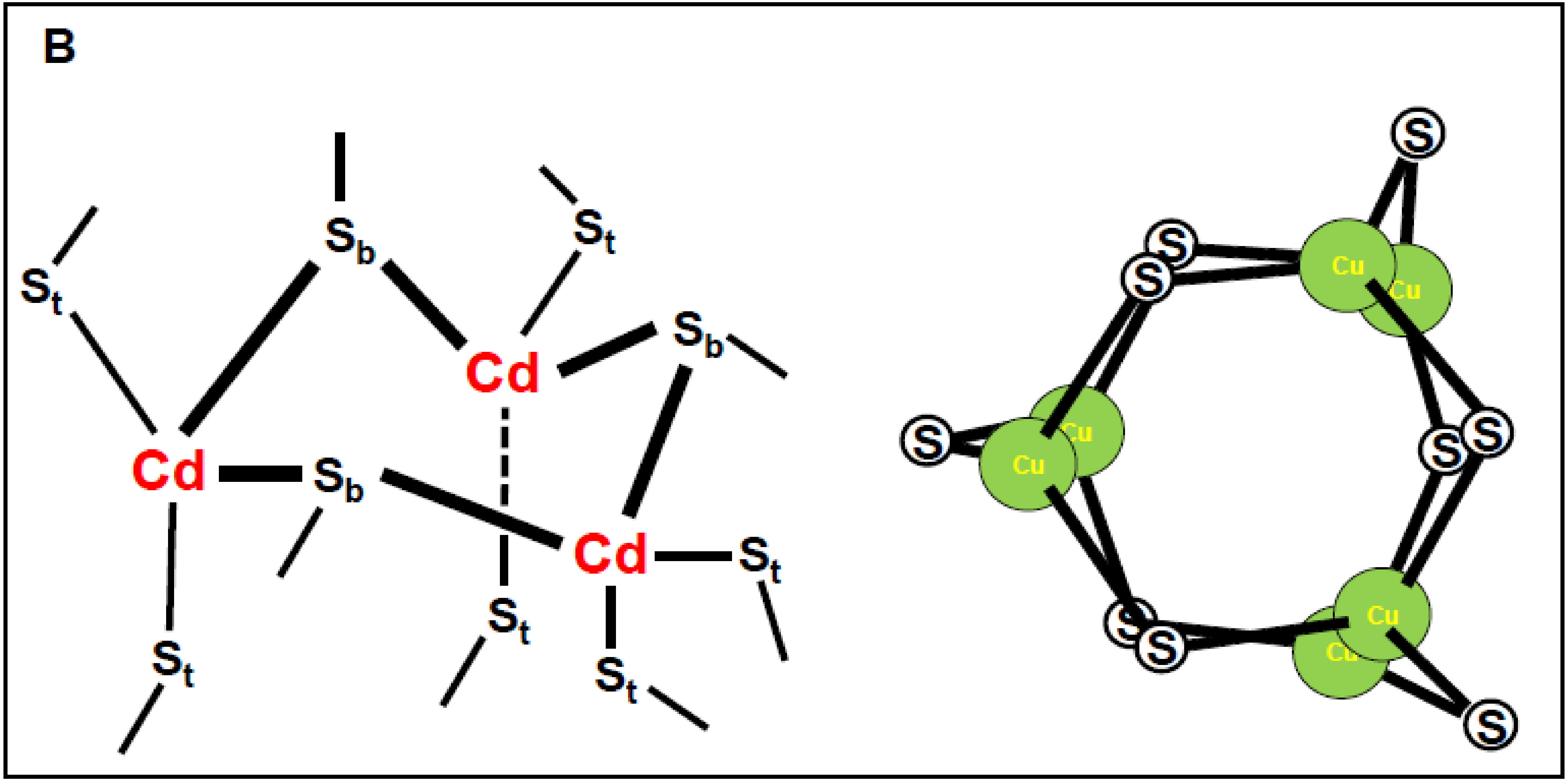
NMR of metallothioneins

Metallothioneins

- Small proteins: ~60 aa with ~30% cysteine residues
- Coordinate metal ions
- No secondary structure elements
- Two metal-thiolate clusters per protein:
  - α-domain: 11 Cys coordinating 4 divalent metal ions
  - β-domain: 9 Cys coordinating 3 divalent metal ions



Crystal structure of MT-2 isolated from rat liver. (Romero-Isart N, Vasák M. *J Inorg Biochem.* Feb 2002)



B
S_t
S_b
S_t
Cd
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Cd
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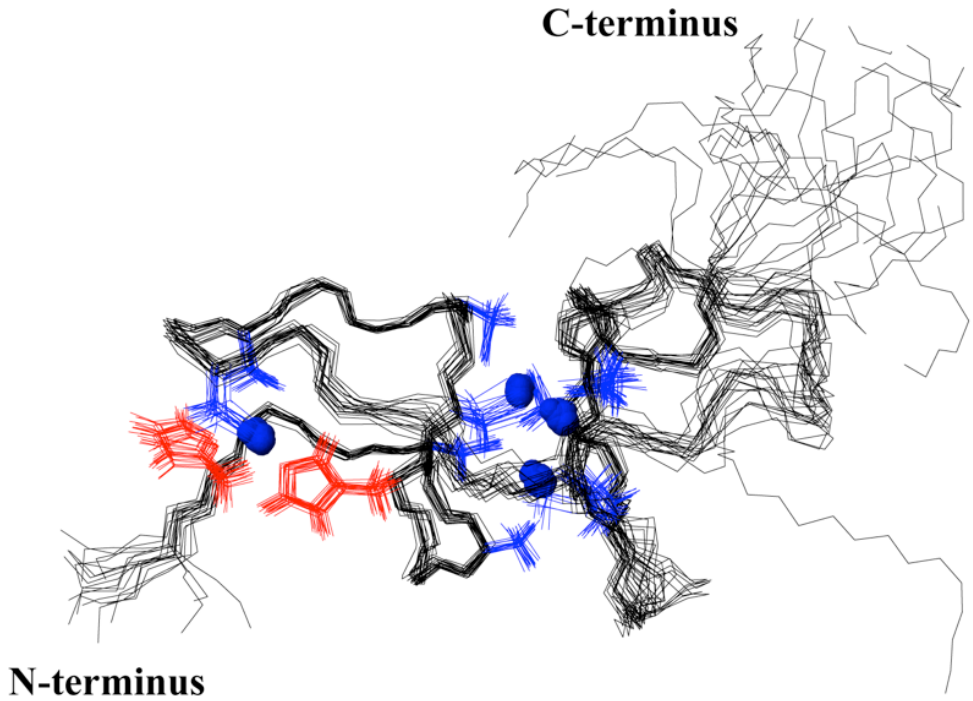
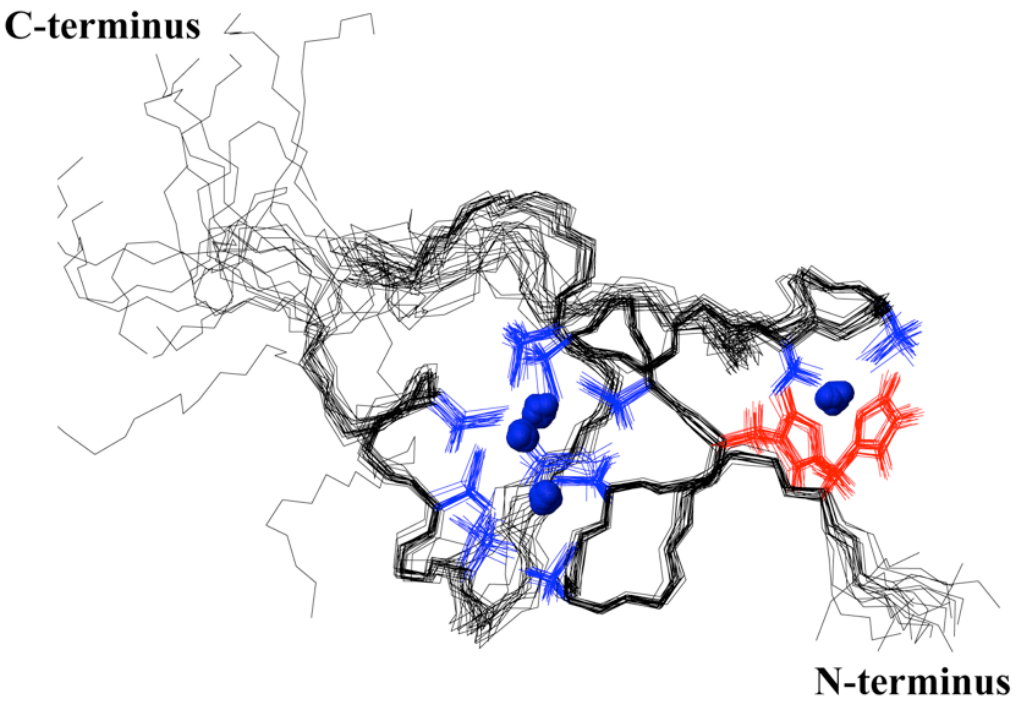
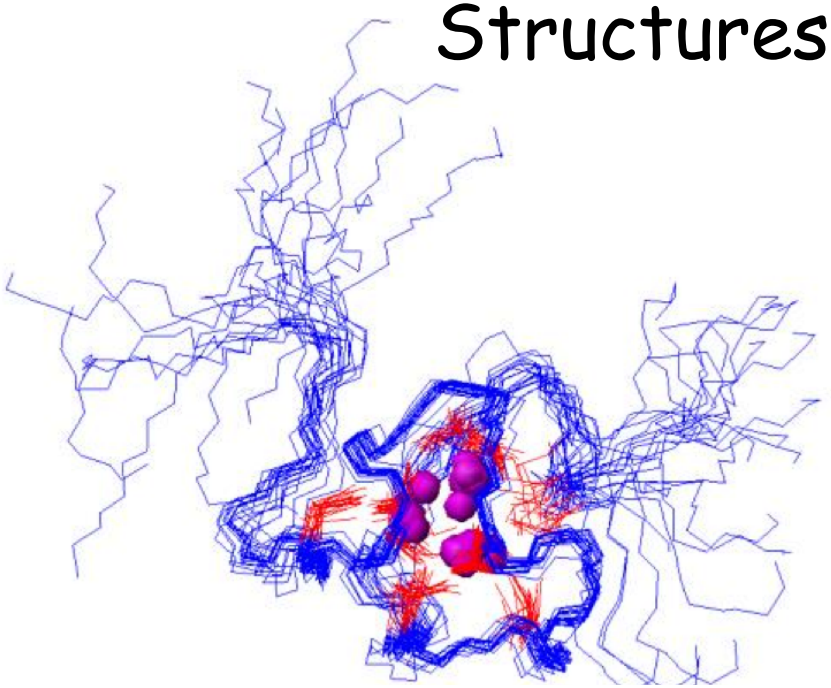
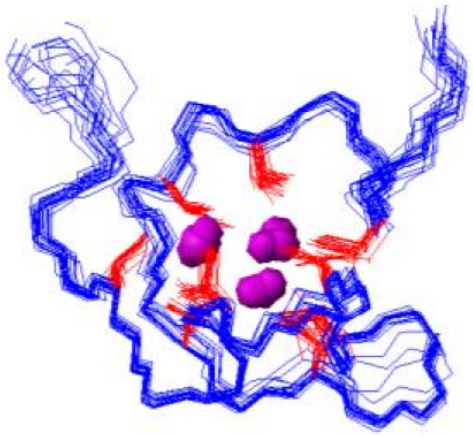

C-terminus
N-terminus


C-terminus
N-terminus

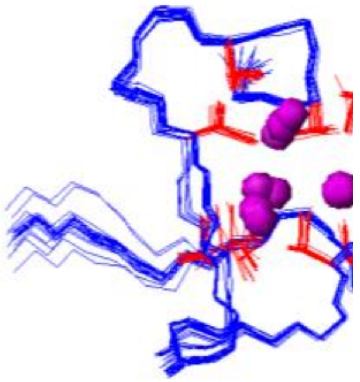
Structures of Littorina littorea MT



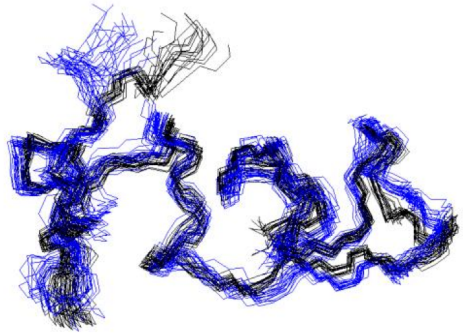
N-term



center



C-term



center and C-term