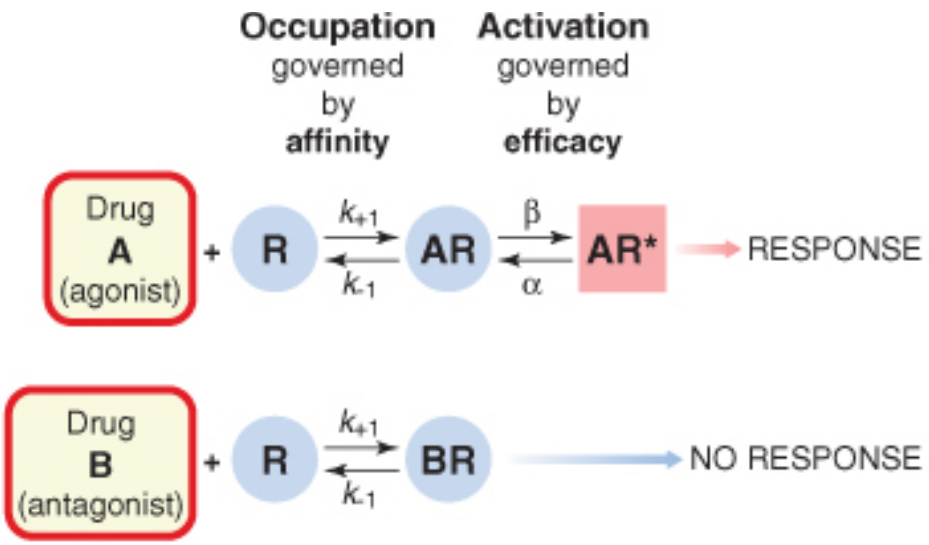
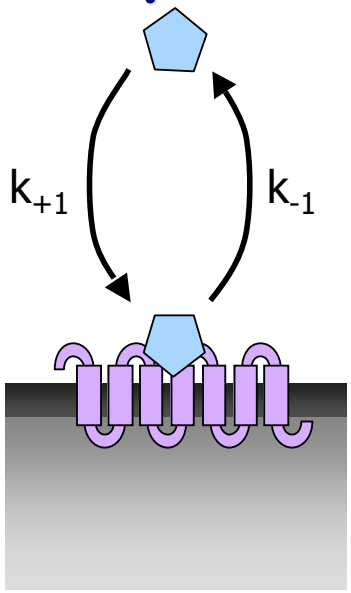
The Targets of the Drugs: Receptors

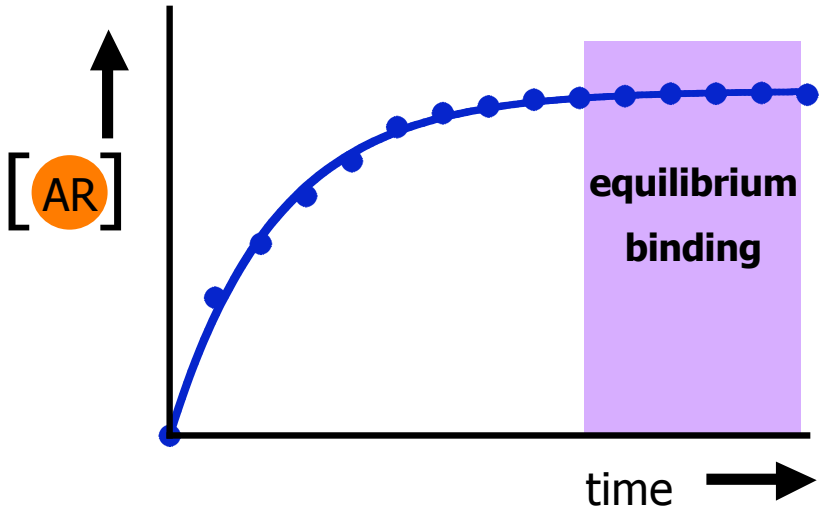
Agonists/Antagonists



- drugs binding to receptors without resulting in receptor activation are called antagonists
- drugs binding to receptors and resulting in activation are called agonists
- receptor affinity describes the tendency for binding
- receptor efficacy describes the potency in receptor activation
- drugs resulting in 100% receptor activation are called full agonists, those causing less than 100% partial agonists

Receptor occupancy – "steady state"





In equilibrium*

association = dissociation

$$k_{+1} \times [A] \times [R] = k_{-1} \times [AR]$$

Equilibrium dissociation constant = affinity

$$K_d = \frac{k_{-1}}{k_{+1}} \qquad K_d = \frac{[A] \times [R]}{[AR]}$$

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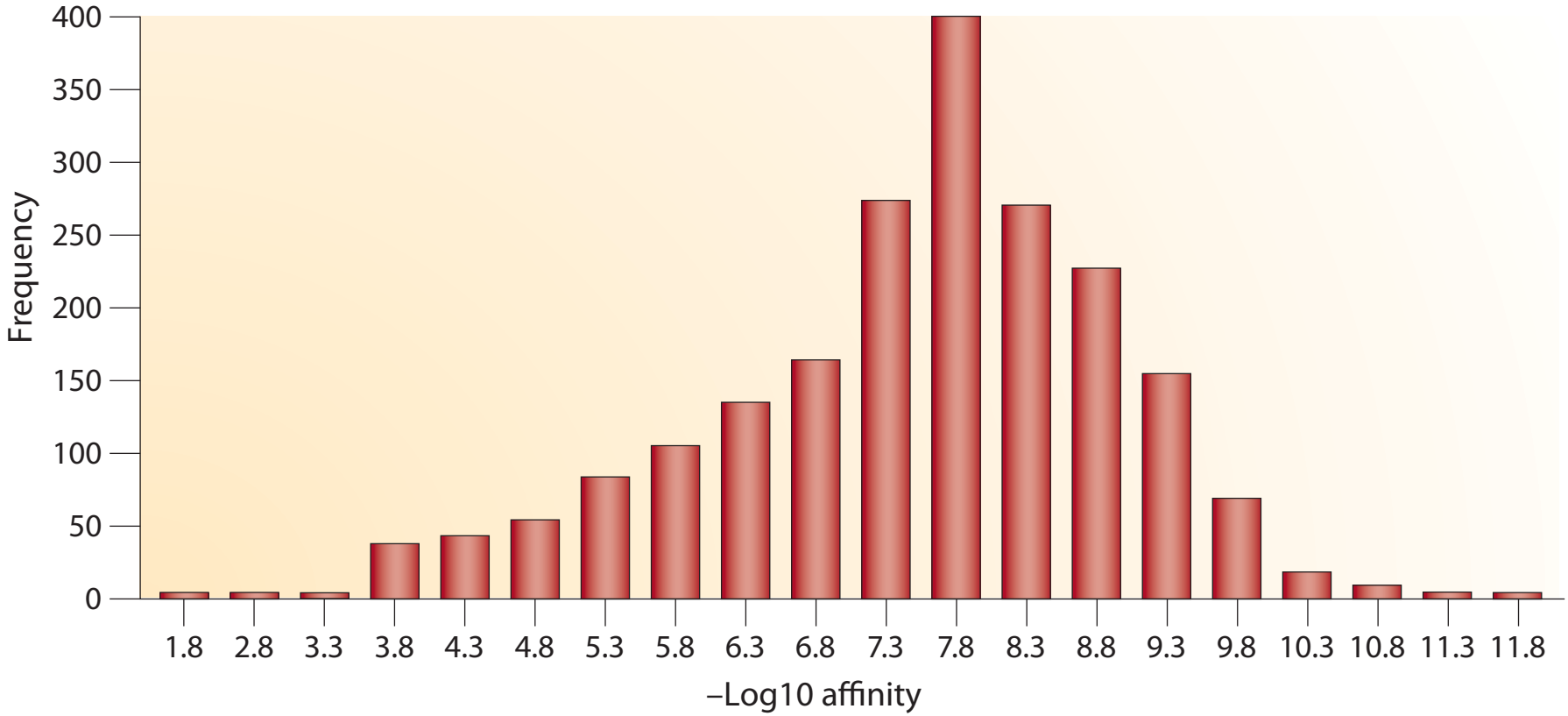
Frequency
400
350
300
250
200
150
100
50
0
1.8
2.8
3.3
3.8
4.3
4.8
5.3
5.8
6.3
6.8
7.3
7.8
8.3
8.8
9.3
9.8
10.3
10.8
11.3
11.8
–Log10 affinity

Drug-Receptor Interactions (Clark occupancy theory)

Fractional Receptor Occupancy

Proportion receptor occupation:

$$p_{AR} = \frac{[AR]}{R_{total}} = \frac{[AR]}{[R]+[AR]}$$

$$[AR] = \frac{[A] \times [R]}{K_d}$$

$$R_{total} = [R] + [AR]$$

$$p_{AR} = \frac{\left(\frac{[A] \times [R]}{K_d}\right)}{[R] + \left(\frac{[A] \times [R]}{K_d}\right)} = \frac{\left(\frac{[A]}{K_d}\right)}{1 + \left(\frac{[A]}{K_d}\right)} = \frac{[A]}{K_d + [A]}$$

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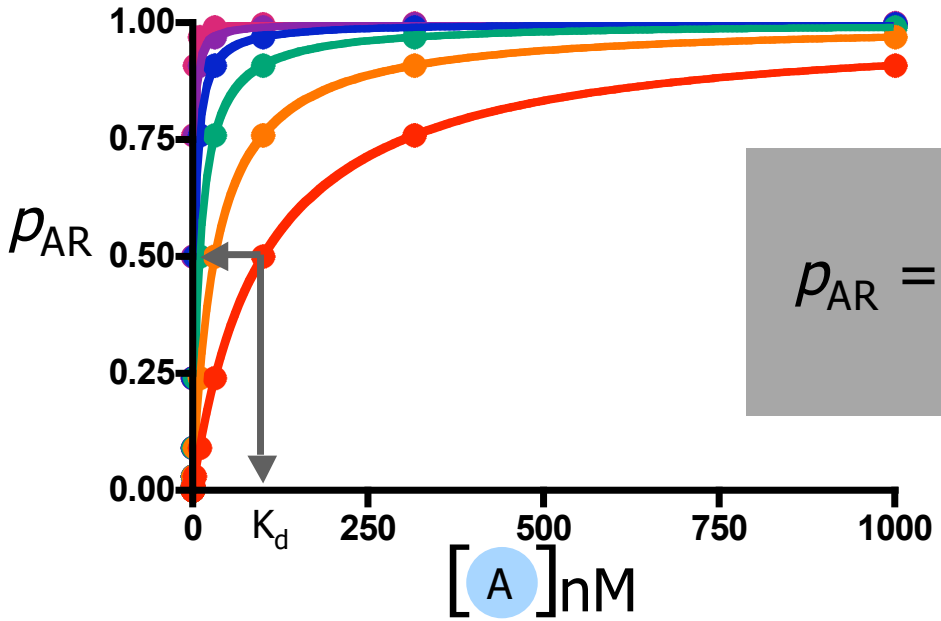
Fractional Receptor Occupancy



$$p_{AR} = \frac{[A]}{K_d + [A]}$$

When $[A] = K_d$: half the receptors are occupied

$$p_{AR} = \frac{[A]}{[A] + [A]} = 0.5$$

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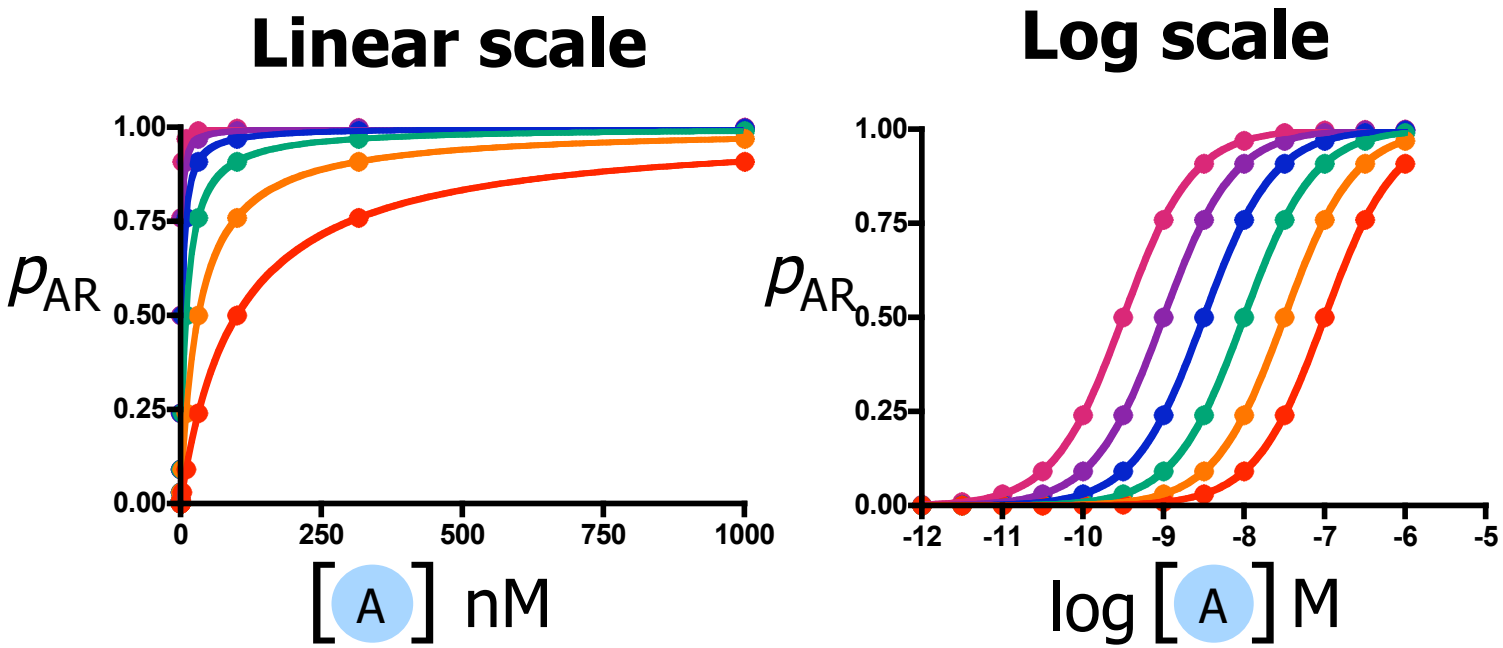
Fractional Receptor Occupancy

$$p_{AR} = \frac{[A]}{K_d + [A]}$$



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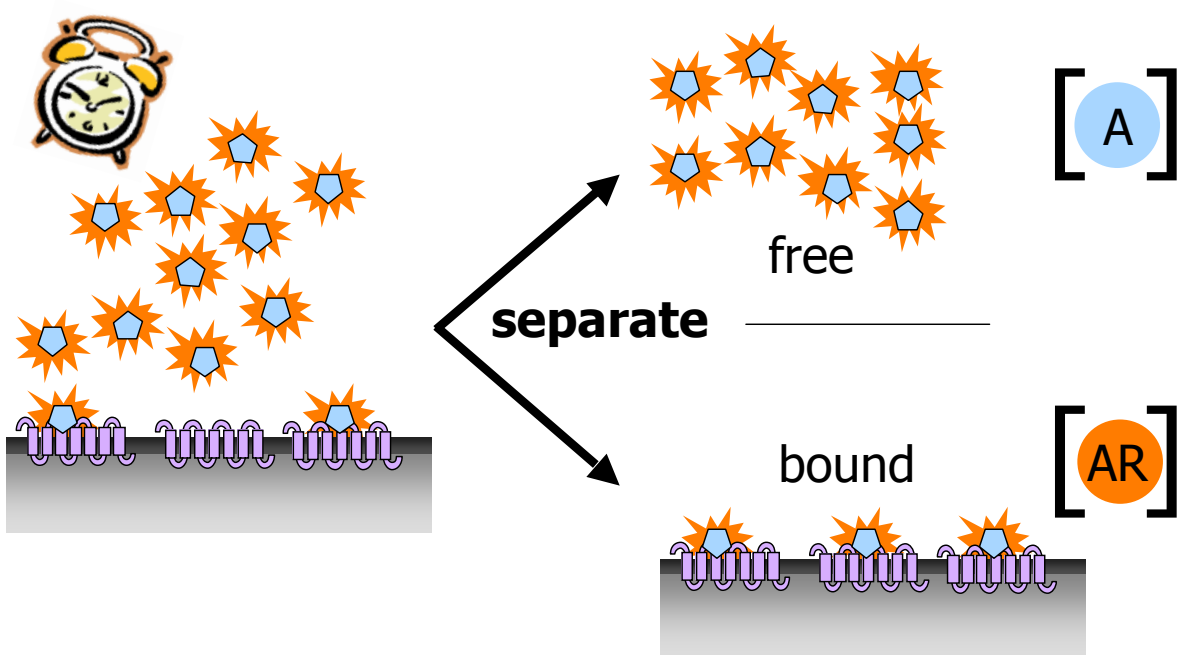
Determining affinities: Radioligand labelling



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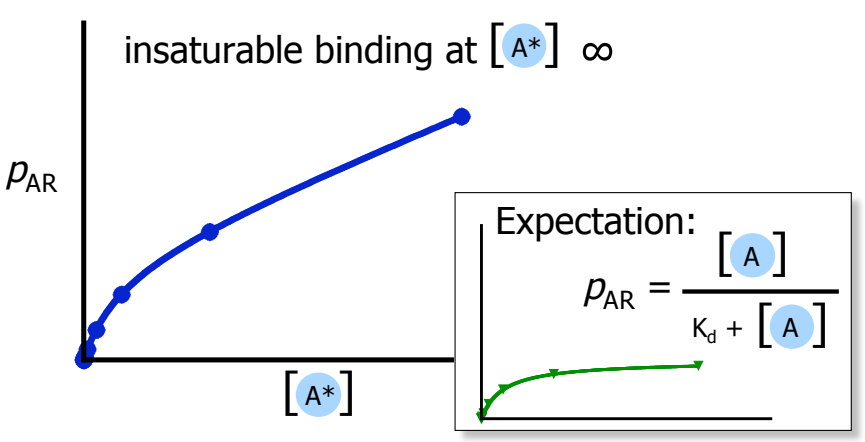
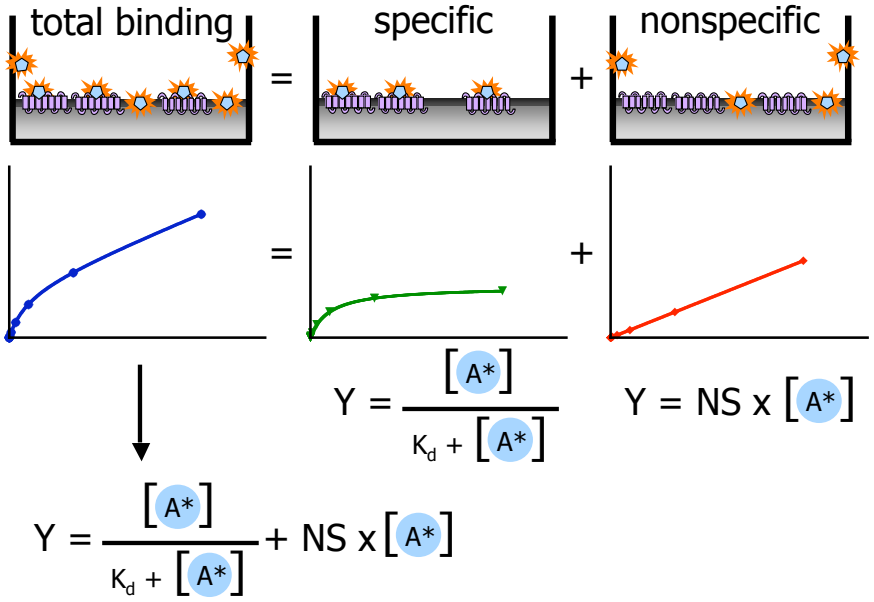
Artifacts from non-specific binding





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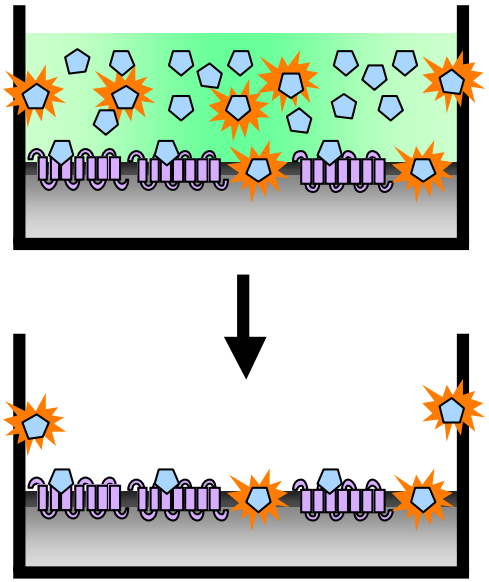
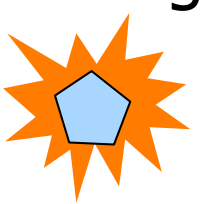
Quantifying non-specific binding



use saturating concentration* of "cold" ligand

prevents radioligand binding to receptor



*concentration "cold":

1 x K_d = 50% occupancy

4 x K_d = 80% occupancy

9 x K_d = 90% occupancy

99 x K_d = 99% occupancy

$$p_{AR} = \frac{[A]}{K_d + [A]}$$

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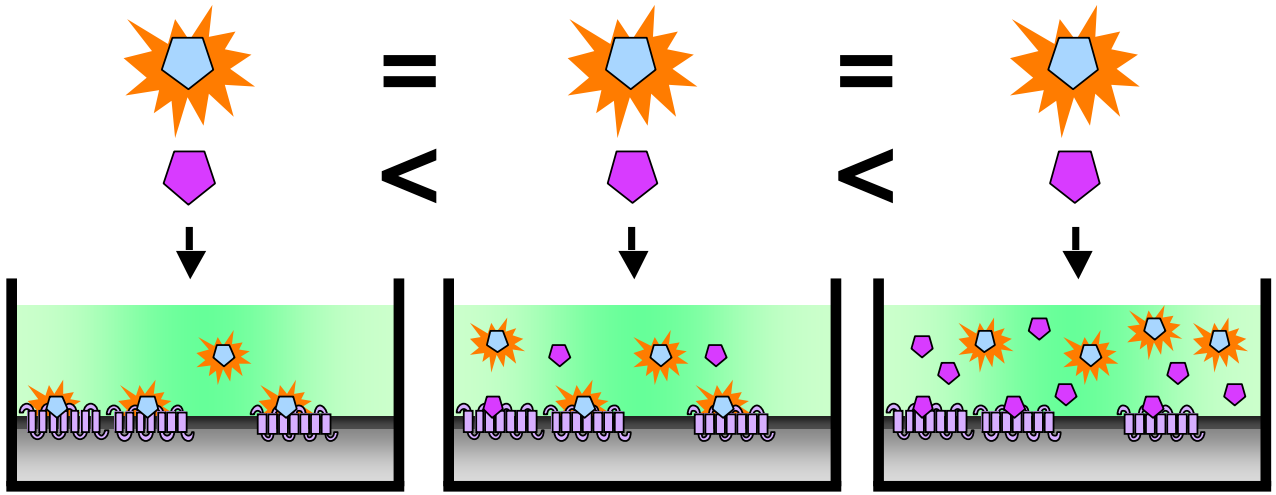
Displacement Assays

Indirect measure of affinity of **unlabeled** ligands

Displace fixed [radioligand] from receptor by increasing concentrations unlabeled ligand



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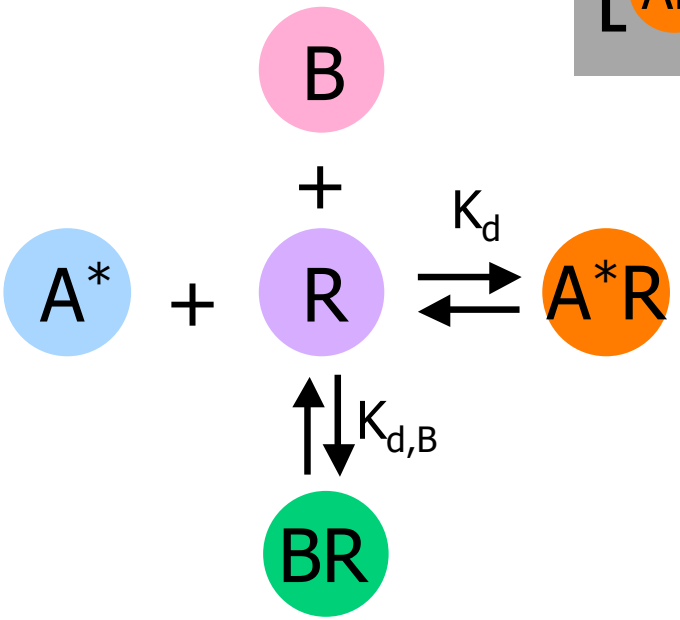
Displacement Assays

$$[AR] = \frac{[A^*] \times [R]}{K_d} \qquad [BR] = \frac{[B] \times [R]}{K_{d,B}}$$



$$p_{AR,B} = \frac{[AR]}{[R] + [AR] + [BR]}$$

$$p_{AR,B} = \frac{\left(\frac{[A^*] \times [R]}{K_d}\right)}{[R] + \left(\frac{[A^*] \times [R]}{K_d}\right) + \left(\frac{[B] \times [R]}{K_{d,B}}\right)}$$

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Displacement Assays

Relation IC_{50} and Kd,B

$$P = \frac{p_{AR,B}}{p_{AR,0}} = 0.5 \quad \text{when:} \quad [B] = IC_{50}$$

(herein, $p_{AR,0}$ denotes the concentration of AR *in absence* of the competitor B)

$$P = \frac{1 + \frac{[A^*]}{K_d}}{1 + \left(\frac{[A^*]}{K_d}\right) + \left(\frac{IC_{50}}{K_{d,B}}\right)} = 0.5$$

$$K_{d,B} = \frac{IC_{50}}{1 + \left(\frac{[A^*]}{K_d}\right)}$$

Cheng-Prusoff equation

In the experiment, B is added and the displaced radioligand A* is quantified.

At IC_{50} half of A* is replaced by B. You must know the K_d of A at the receptor R. Since A* is a commercial radioligand, this is usually known.

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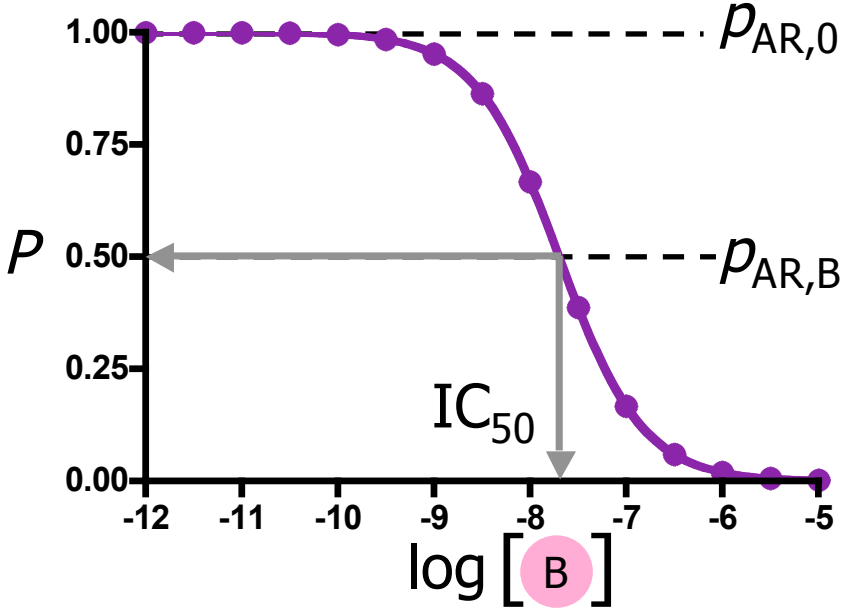
Displacement Assays

IC_{50} is [B] giving half $p_{AR,0}$



$$p_{AR,B} = 0.5 \times p_{AR,0}$$

$$P = \frac{p_{AR,B}}{p_{AR,0}} = 0.5$$

IC_{50} measure of the potency of B to displace A*

IC_{50} ≠ affinity of B ($K_{d,B}$)

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Displacement Assays

$$K_{d,B} = \frac{IC_{50}}{1 + \left(\frac{[A^*]}{K_d}\right)} \rightarrow IC_{50} = K_{d,B} + \left(\frac{K_{d,B} \times [A^*]}{K_d}\right)$$

IC_{50} determined by:

- Affinity of competing ligand B ($K_{d,B}$)
- Affinity of radioligand A* (K_d)
- Concentration of radioligand A*

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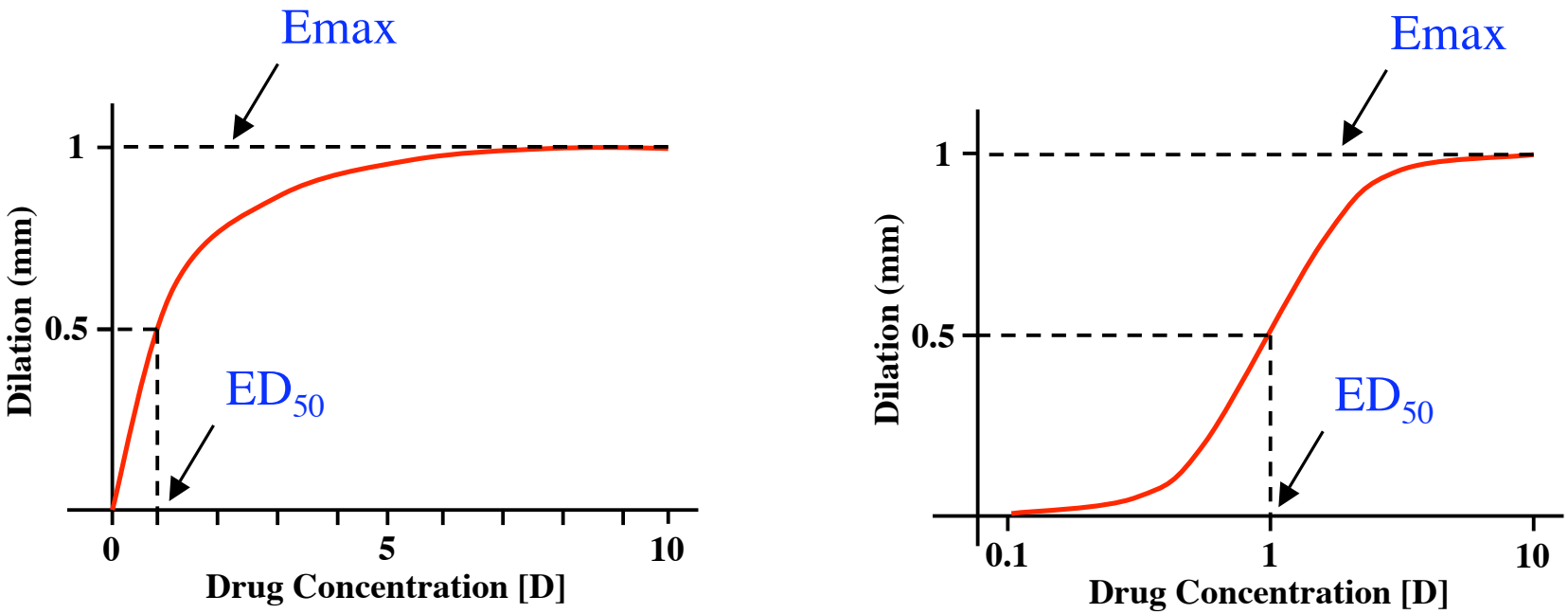
Dose-response curves



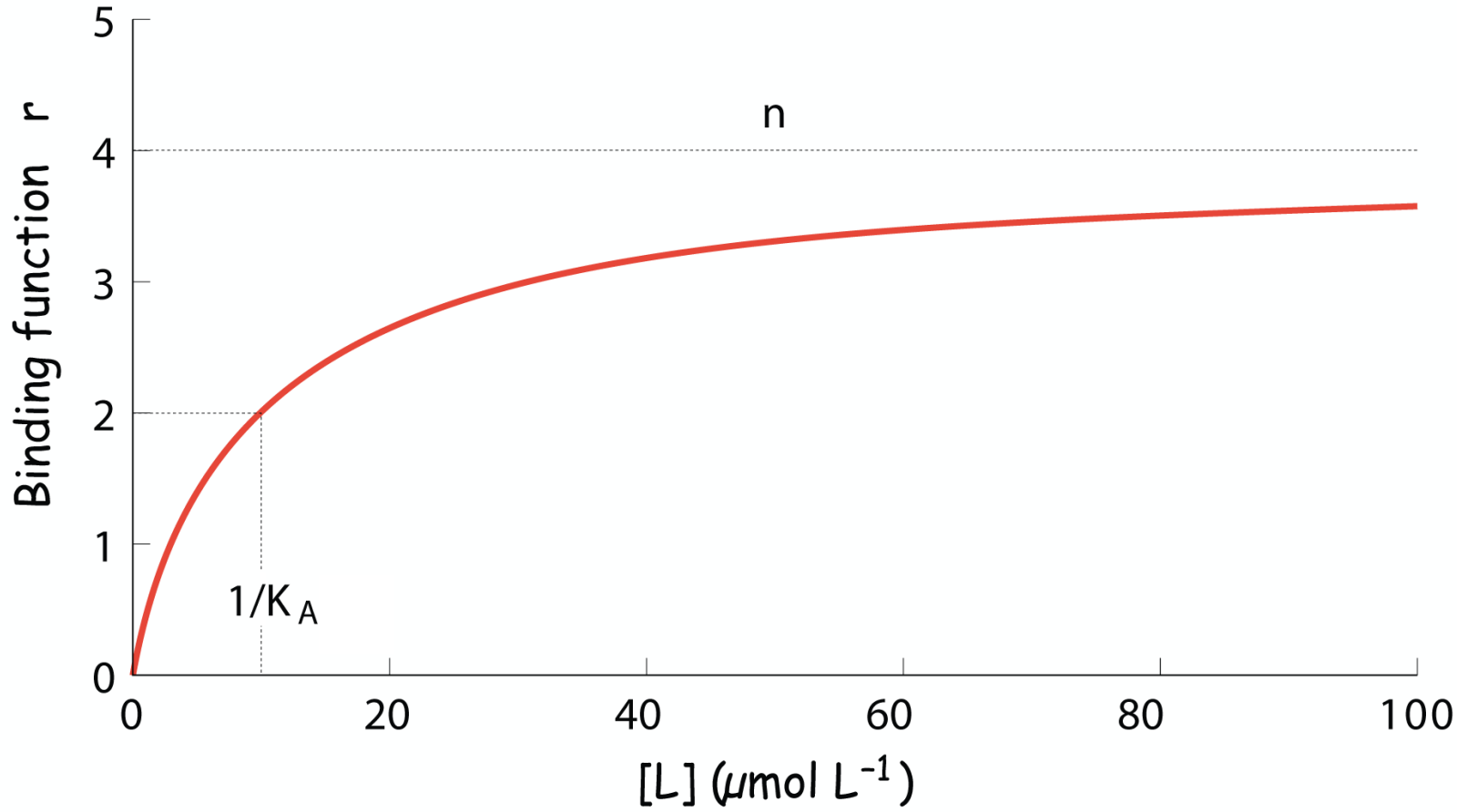
E_{max} the maximum response achieved by an agonist
also referred to as drug efficacy

ED_{50} the drug concentration (or does) at which 50% of Emax is achieved
also referred to as drug potency

Many binding sites

$$r = \frac{[L_{tot}] - [L_{free}]}{[R_{tot}]} = \frac{nK_A[L_{free}]}{1 + K_A[L_{free}]}$$

$$r = \frac{[L_{bound}]}{[R_{tot}]}$$



D. J. Winzor, Encyclopedia of Life Sciences, 2001

Scatchard Plot

$$L + R \rightleftharpoons LR$$

$$K_a = \frac{[L_{bound}]}{[L_{free}][R_{free}]}$$

$$r = \frac{[L_{bound}]}{[R_{tot}]} \Rightarrow [L_{bound}] = r \bullet [R_{tot}]$$

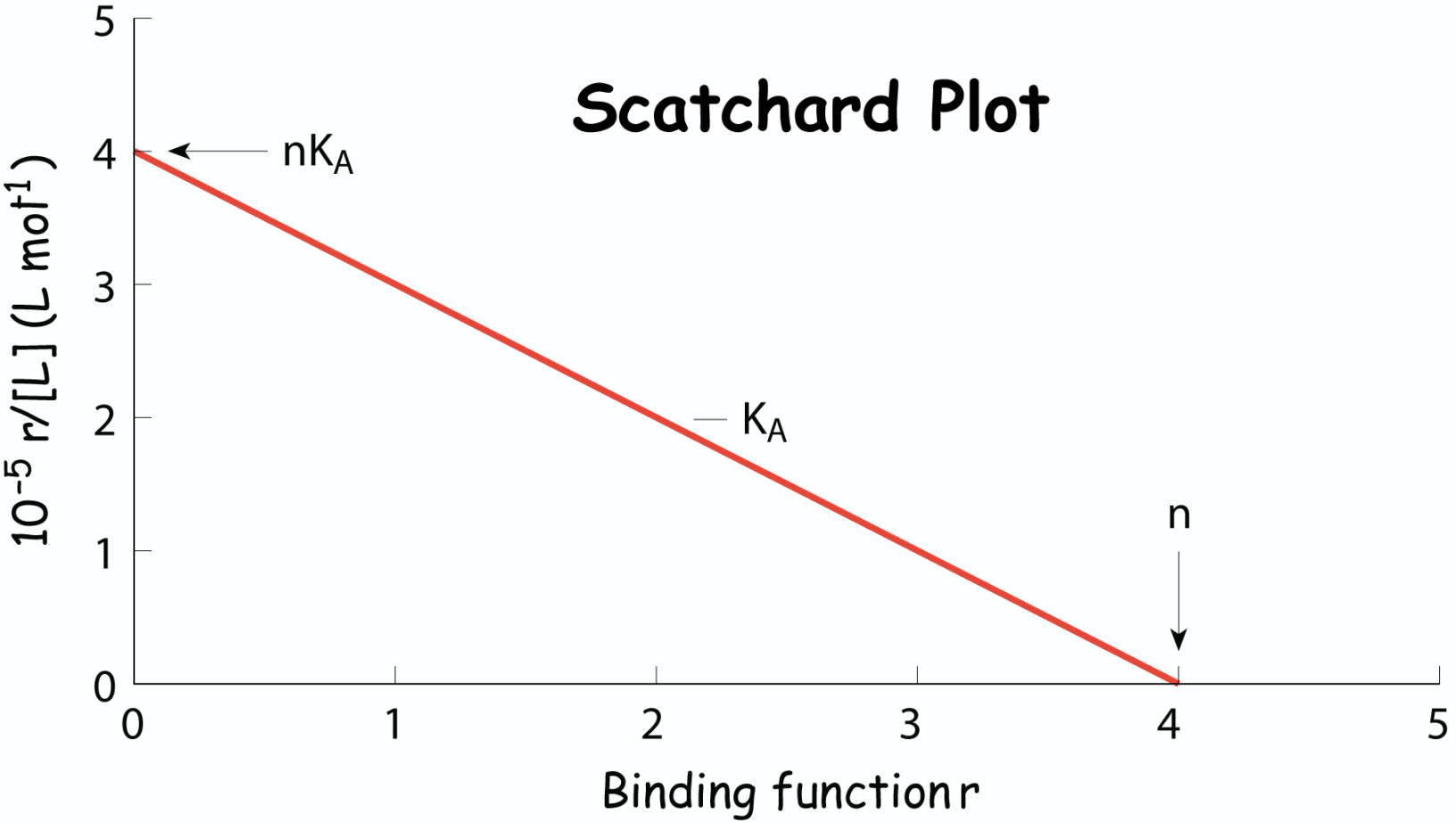
$$[R_{free}] = n[R_{tot}] - r[R_{tot}] = (n - r)[R_{tot}]$$

(n number of binding sites)

$$K_a = \frac{r \bullet [R_{tot}]}{[L_{free}](n - r)[R_{tot}]}$$

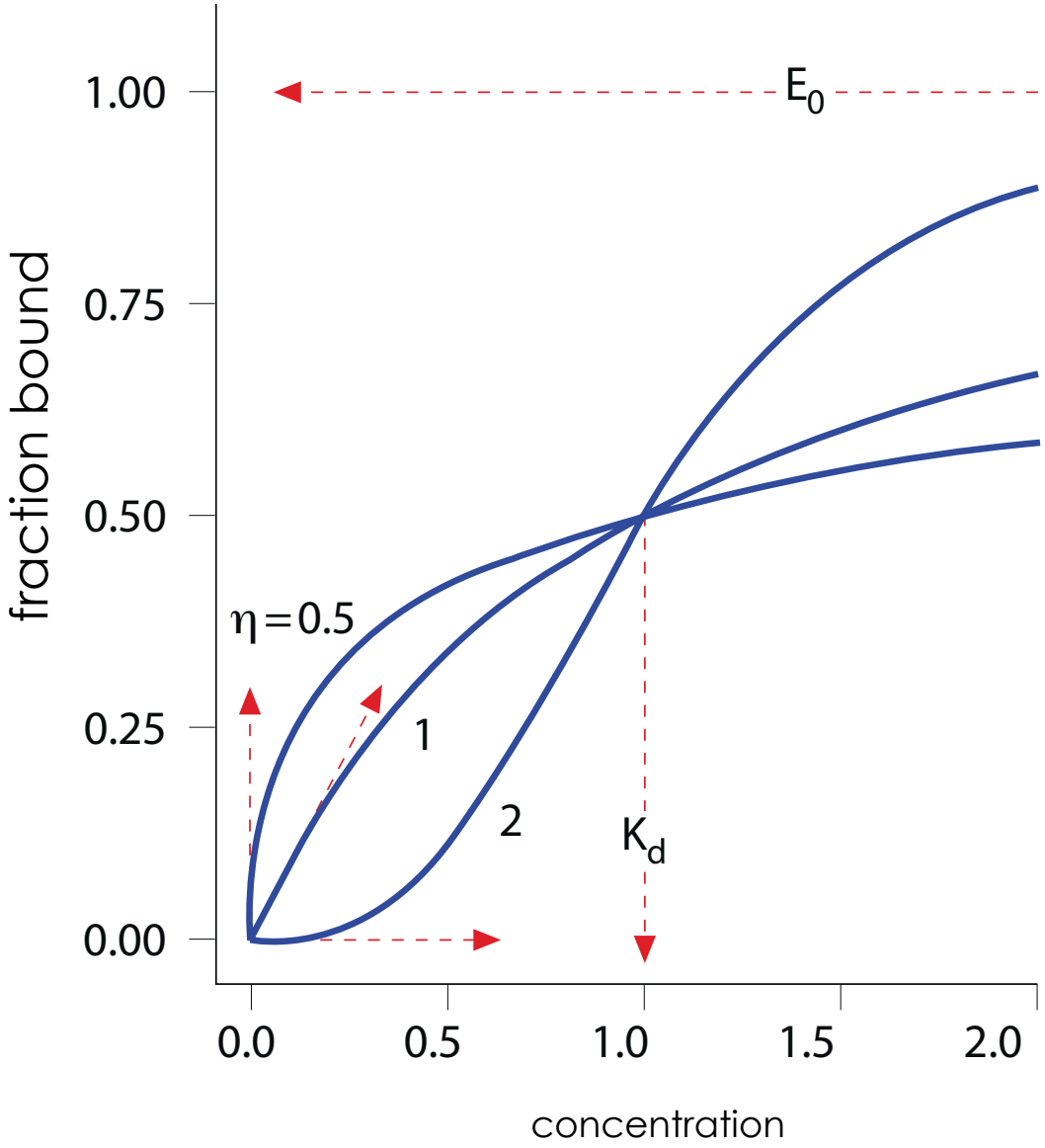
$$\frac{r}{[L_{free}]} = \frac{K_a(n - r)[R_{tot}]}{[R_{tot}]} = nK_a - rK_a$$

$$\frac{r}{[L_{free}]} = nK_A - K_A r$$



D. J. Winzor, Encyclopedia of Life Sciences, 20(

The Hill coefficient and cooperativity of binding



$$[L_{bound}] = \frac{[R_{tot}] \bullet [L_{free}]^{\eta}}{K_d^{\eta} + [L_{free}]^{\eta}}$$

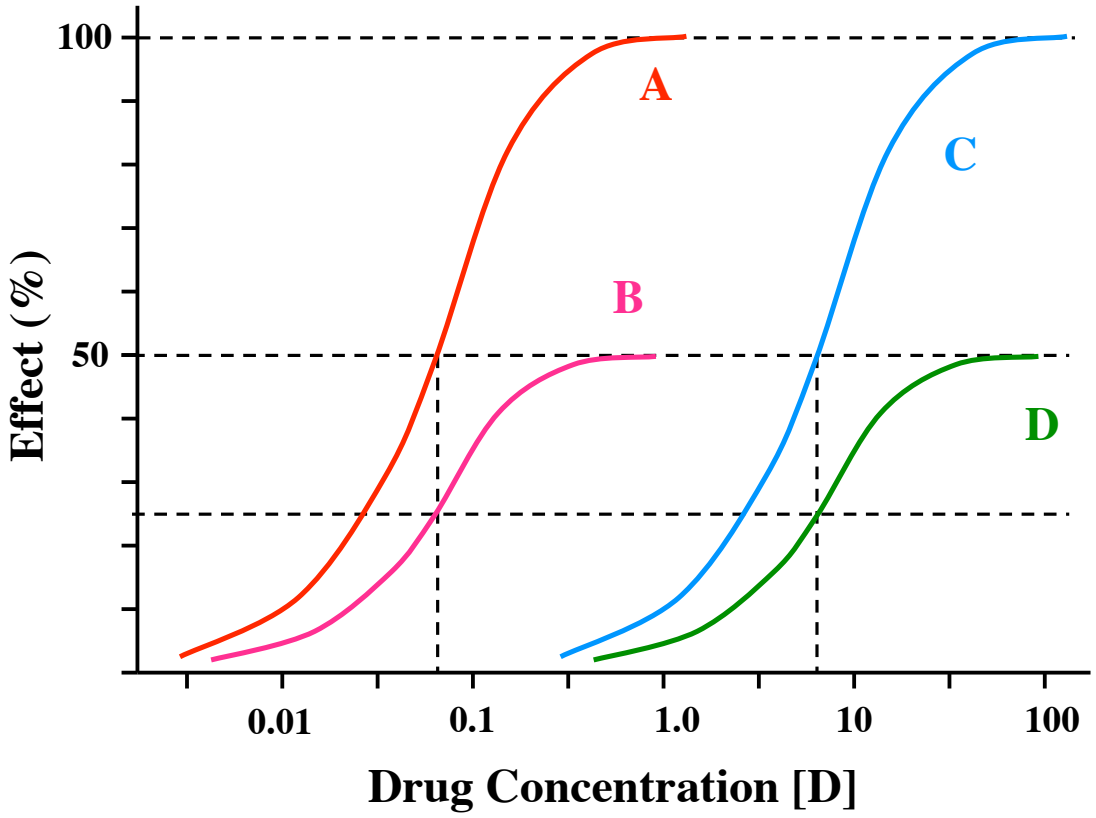
(η is the Hill coefficient)

positive cooperativity: binding to another site enhances affinity to the site

negative cooperativity: binding to another site decreases affinity to the site

V. Pliska Encyclopedia of Life Sciences, 2001

Various Types of Agonists



A: full agonist
maximum potency
maximum efficacy

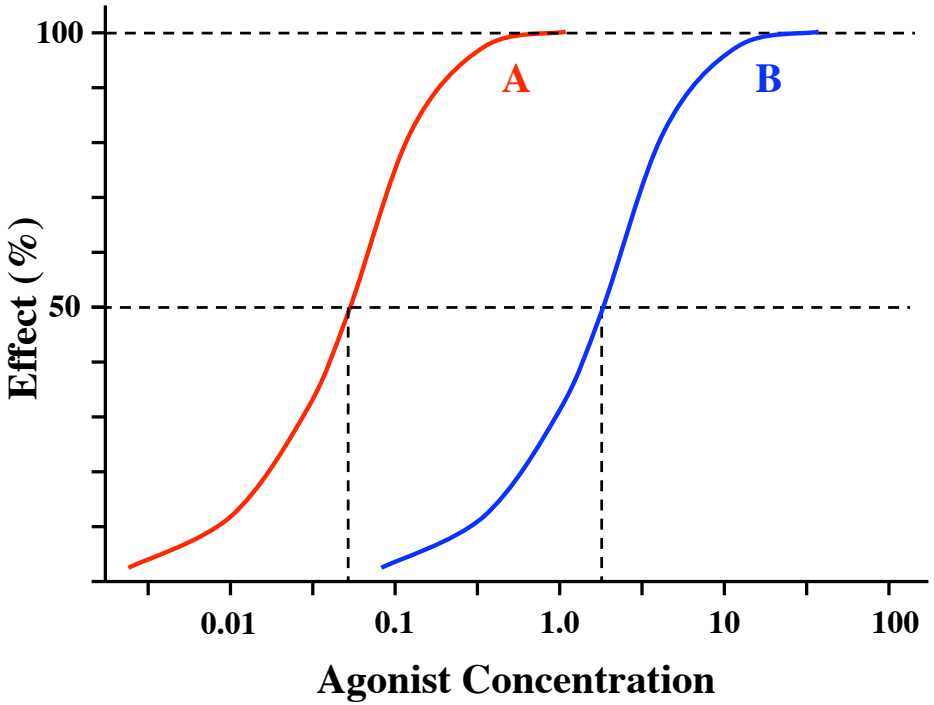
B: partial agonist
maximum potency
reduced efficacy

C: full agonist
reduced potency
maximum efficacy

D: partial agonist
reduced potency
reduced efficacy

Various Types of Antagonists

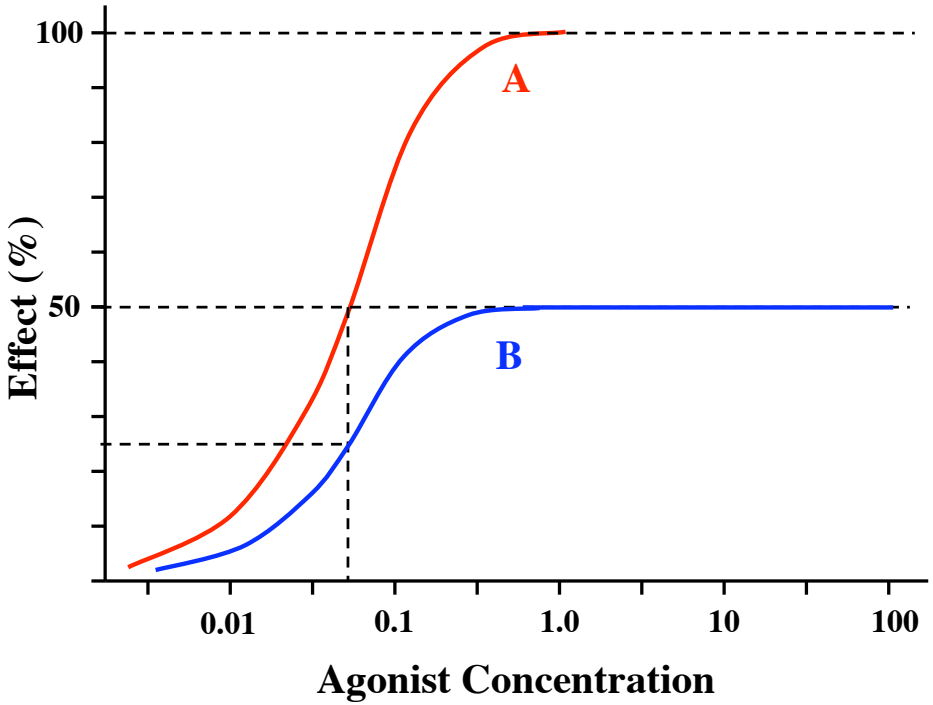
• competitive



A: agonist -antagonist
agonist has max potency
max efficacy

B: agonist + competitive antagonist
agonist has red. potency
but max efficacy

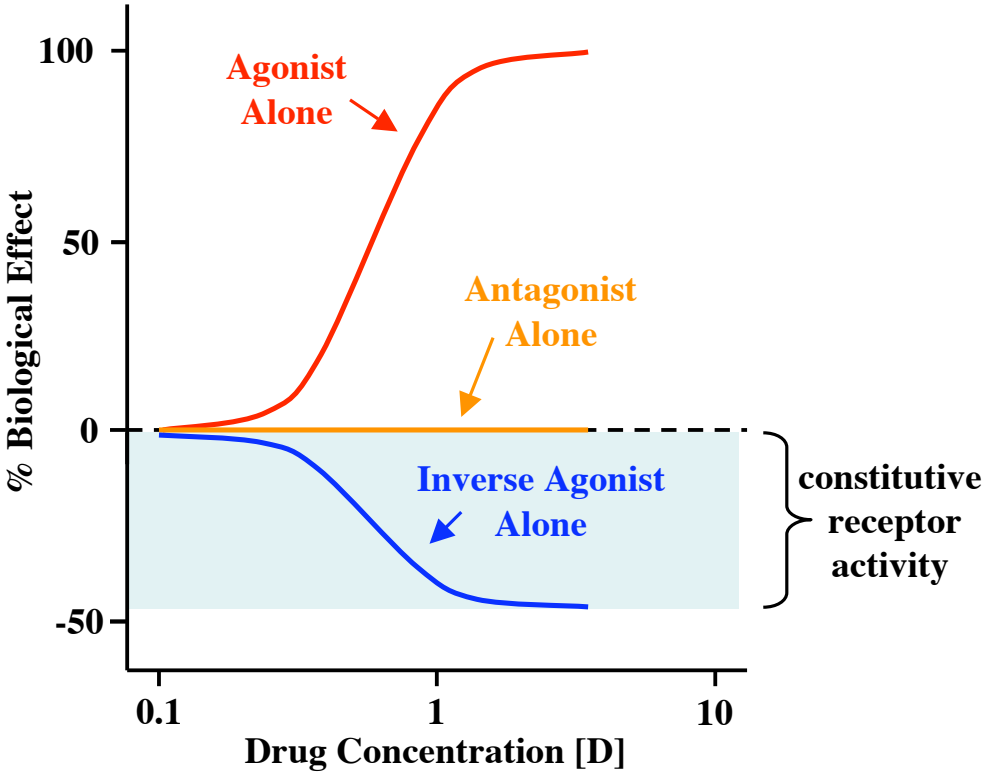
• non-competitive



A: agonist - antagonist
agonist has max potency
max efficacy

B: agonist + non-competitive antagonist
agonist has max. potency
but red. efficacy

Inverse Agonists



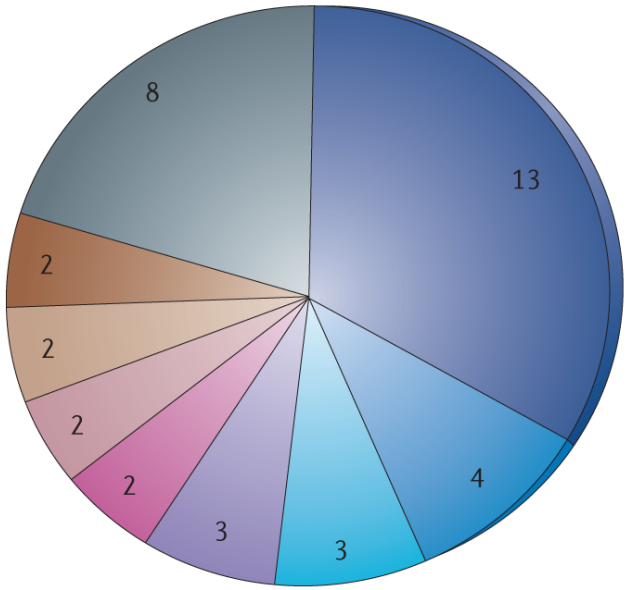
Inverse Agonist

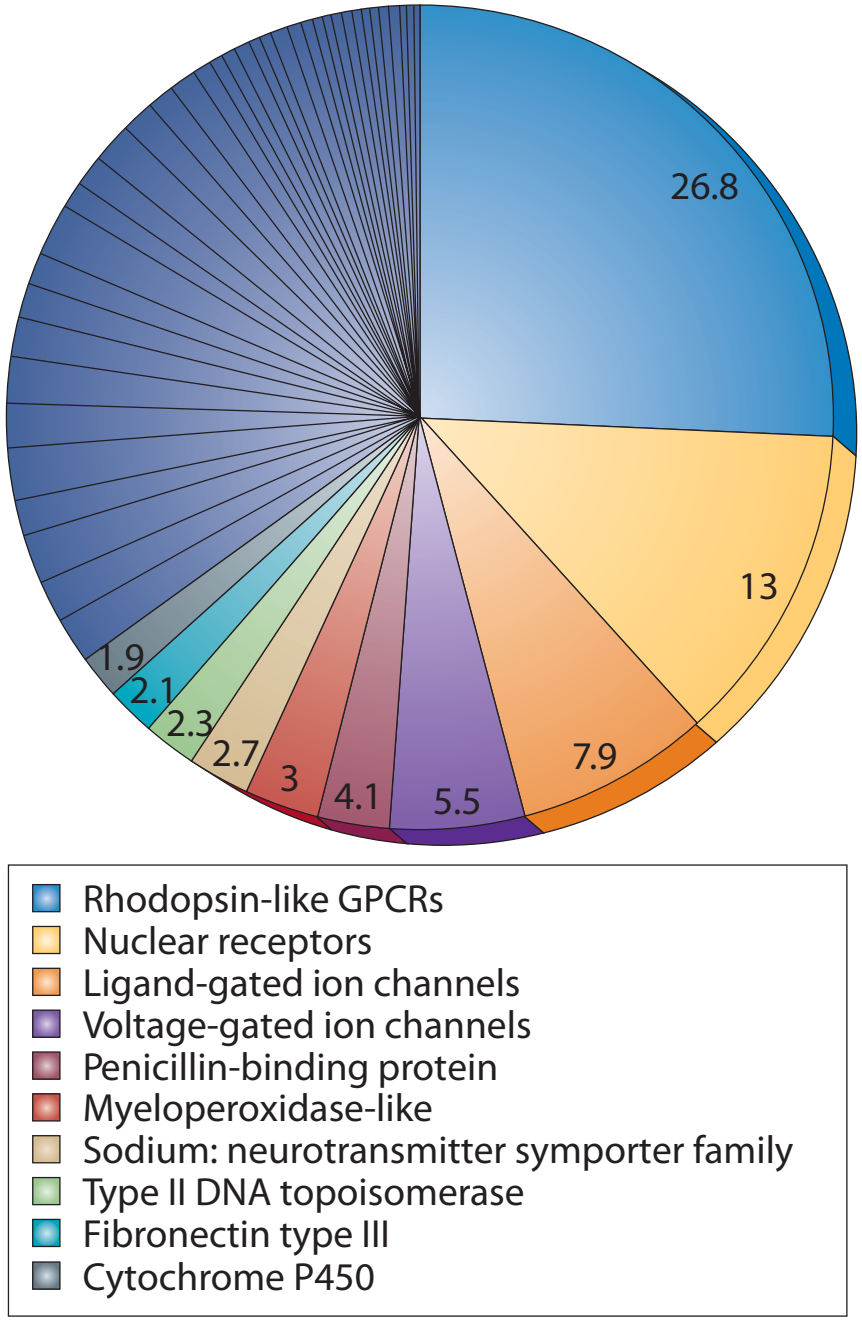
- a ligand that produces an effect opposite to that of an agonist
- binds to the same receptor as agonist

Types of receptors

- ligand-gated ion channels
- G-protein coupled receptors
- kinase-linked receptors
- nuclear receptors
- nucleic acids
- enzymes

New Drugs 2012





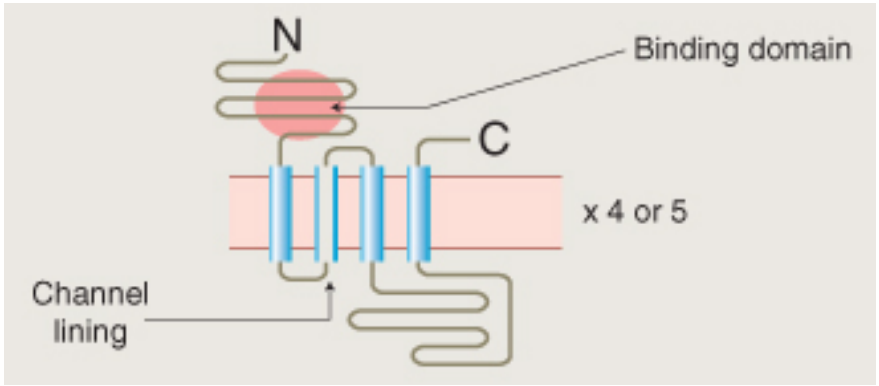
Enzymes

class	enzyme	function
Oxireductase		
	dehydrogenases	transfer of H from substrate to cofactor
	reductases	addition of H to substrate
	oxidases	transfer of H from substrate to oxygen
Transferases		
	aminotransferases	transfer of amino groups
	transacetylases	transfer of acetyl groups
	phosphorylases	transfer of phosphate groups
Hydrolases		
	glycosidases	hydrolysis of glycosidic bonds
	esterases	hydrolysis of ester bonds
	peptidases	hydrolysis of peptide bonds
Lyases		
	hydratases	addition of water to double bonds
	decarboxylases	removal of CO2 from substrate
	aldolases	aldol condensations
Isomerases		
	racemases	D/L interconversions
	cis/trans isomerases	cis/trans interconversions
	mutases	intramolecular group transfer
Ligases		
	synthetases	condensation of two molecules
	carboxylases	addition of CO2 to substrate

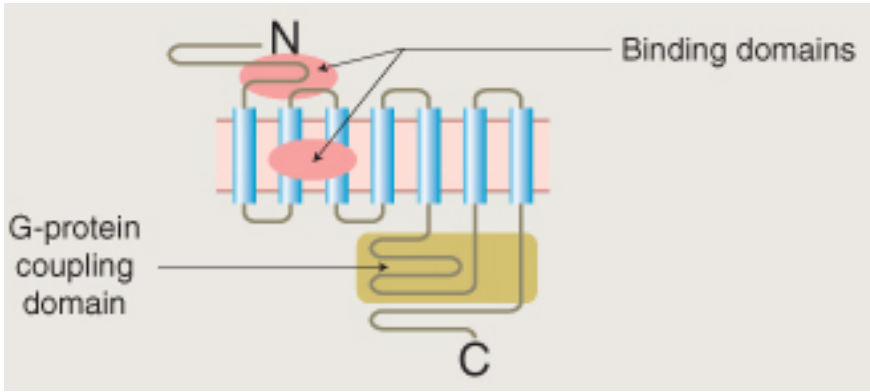
Types of receptors (II)

	ligand-gated ion channel	**GPCR**	**kinase-linked**	**nuclear receptors**
location	membrane	membrane	membrane	intracellular
effector	ion channel	channel or enzyme	enzyme	gene transcription
coupling	direct	G-protein	direct	via DNA
examples	nicotinic acetylcholine rec. GABA	adrenoreceptors	insulin, growth factors, cytokine receptors	steroid, thyroid rec.
structure	oligomeric assembly of subunits surrounding central pore	heptahelical structure	single TM helix lining intra- and extracellular domains	monomeric structure with separate receptor and DNA-binding domains

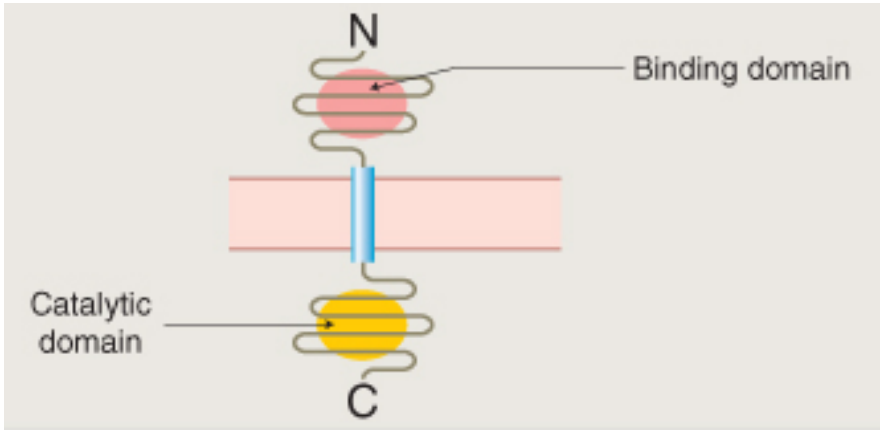
ligand-gated ion channels



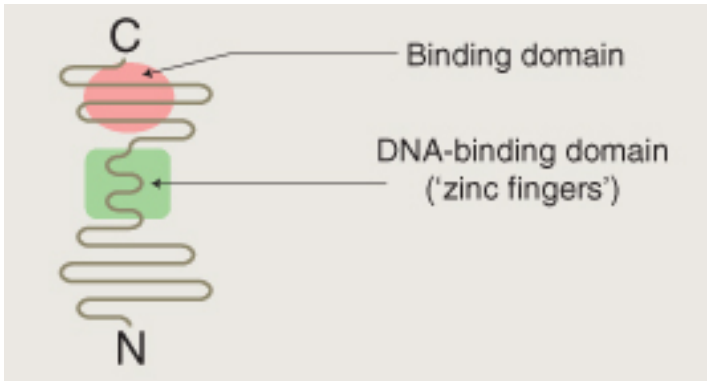
GPCRs

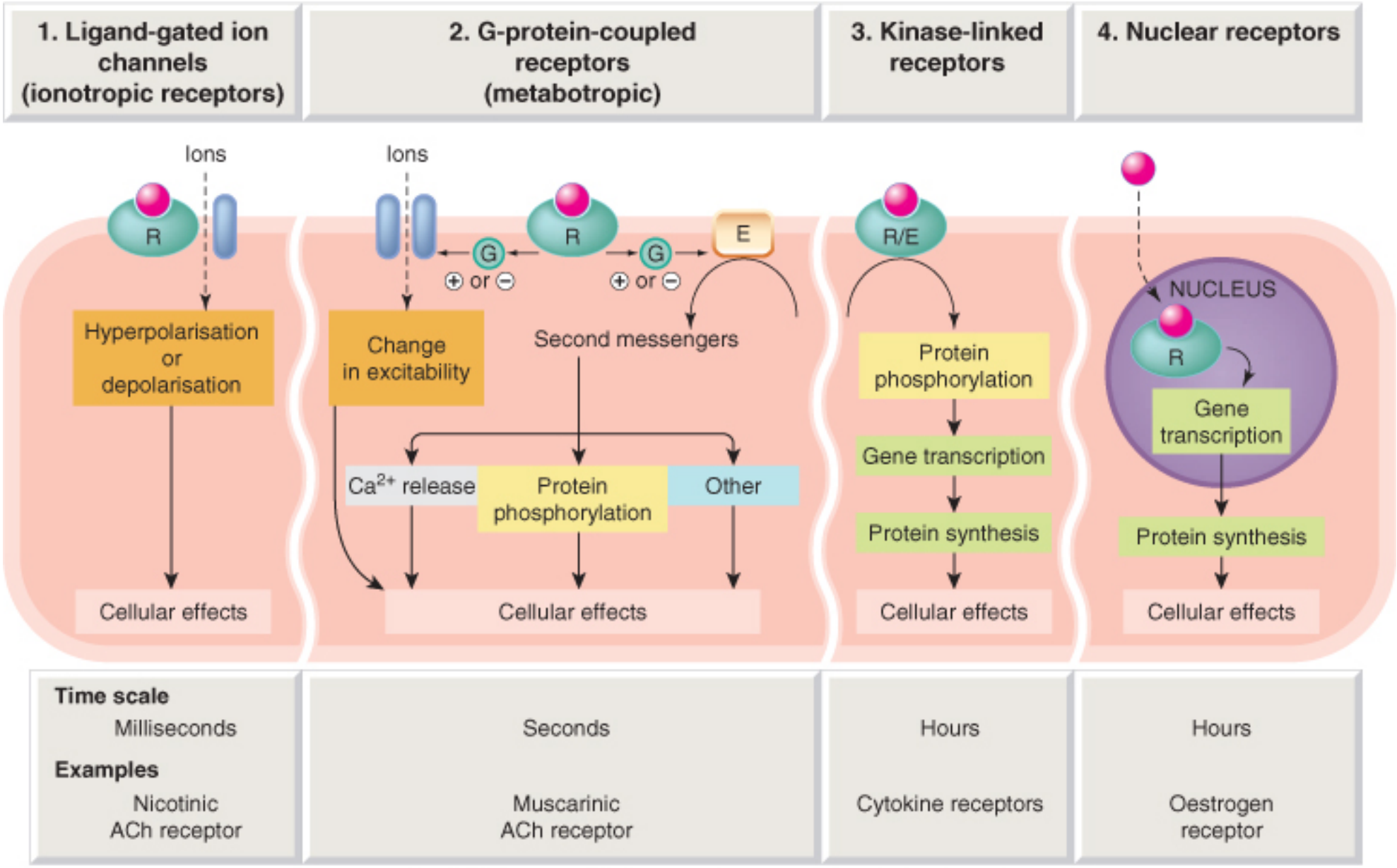


kinase-linked receptors

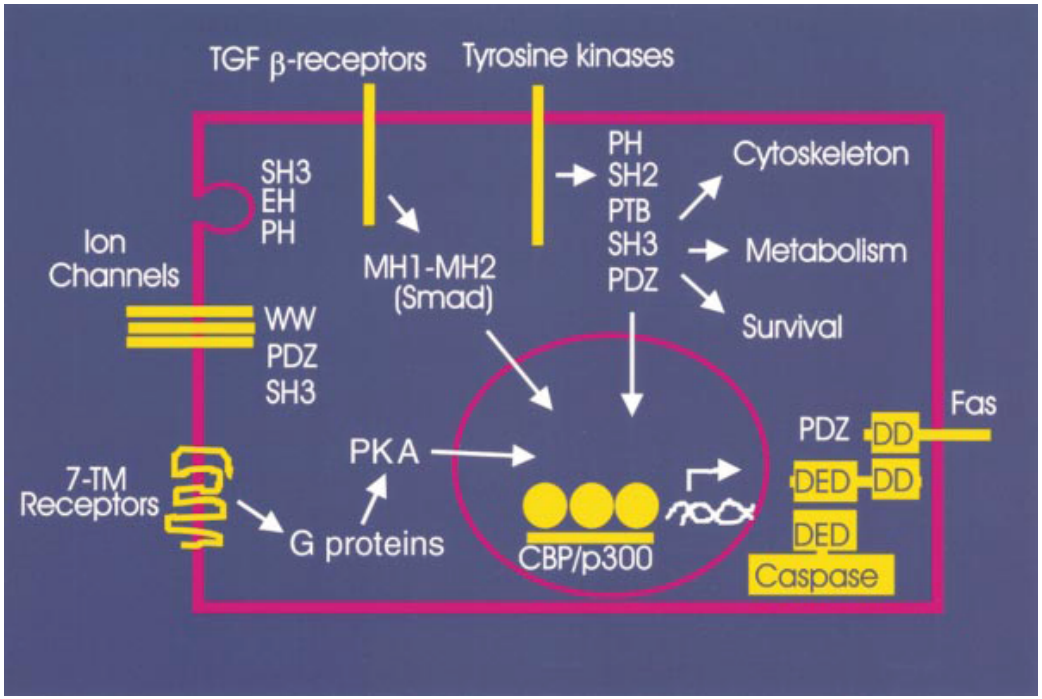


nuclear receptors



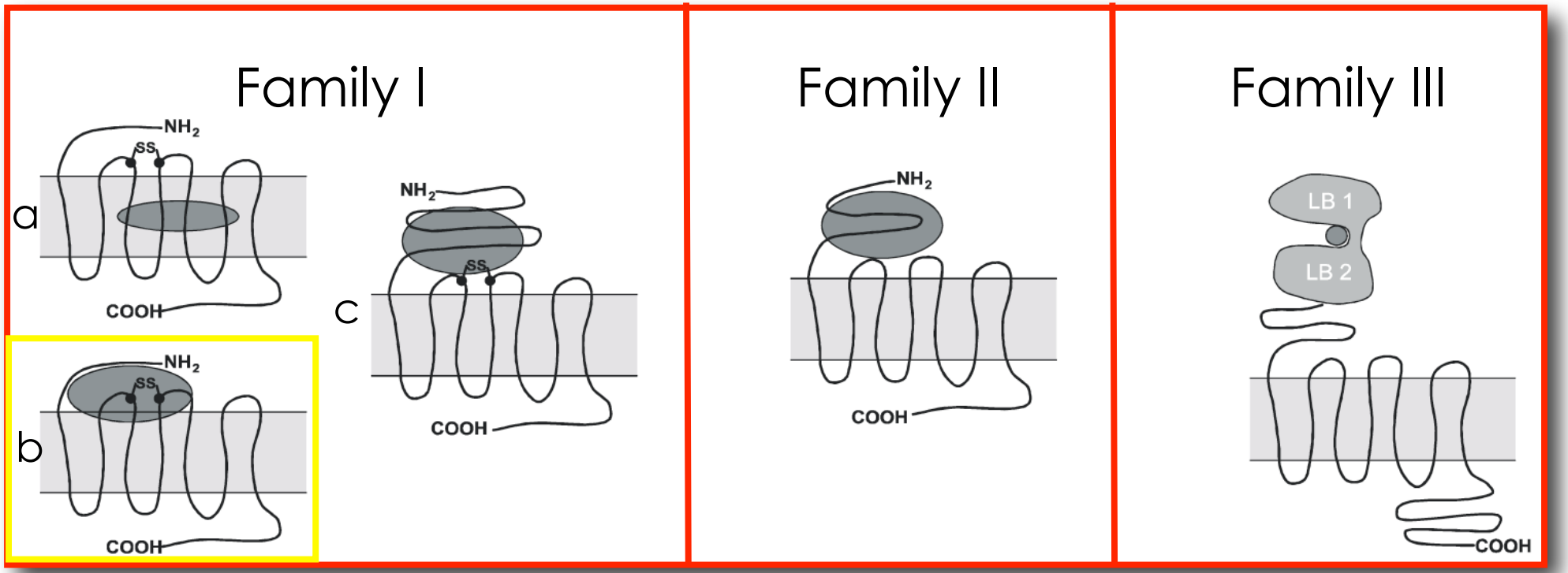


Modules for recognition of signaling molecules



Module	Rationale for name	Recognition motif
SH2	Src homology-2	P-tyrosine
PTB	Phosphotyrosine binding	P-tyrosine
SH3	Src homology-3	Pro-rich sequence
WW	WW denotes 2 conserves Trp	P-P-X-Y, P-P-L-P, P-R/p-S/T-P
PH	Pleckstrin Homology	phospholipids,
FYVE	present in **F**ab1,,**Y**GLD23, **V**PS27 + EEA1 proteins	PtdIns(3)P
PDZ	present in **P**SD-95, **D**igA, **Z**O-1 proteins	C-term. Val/Leu

GPCR classification

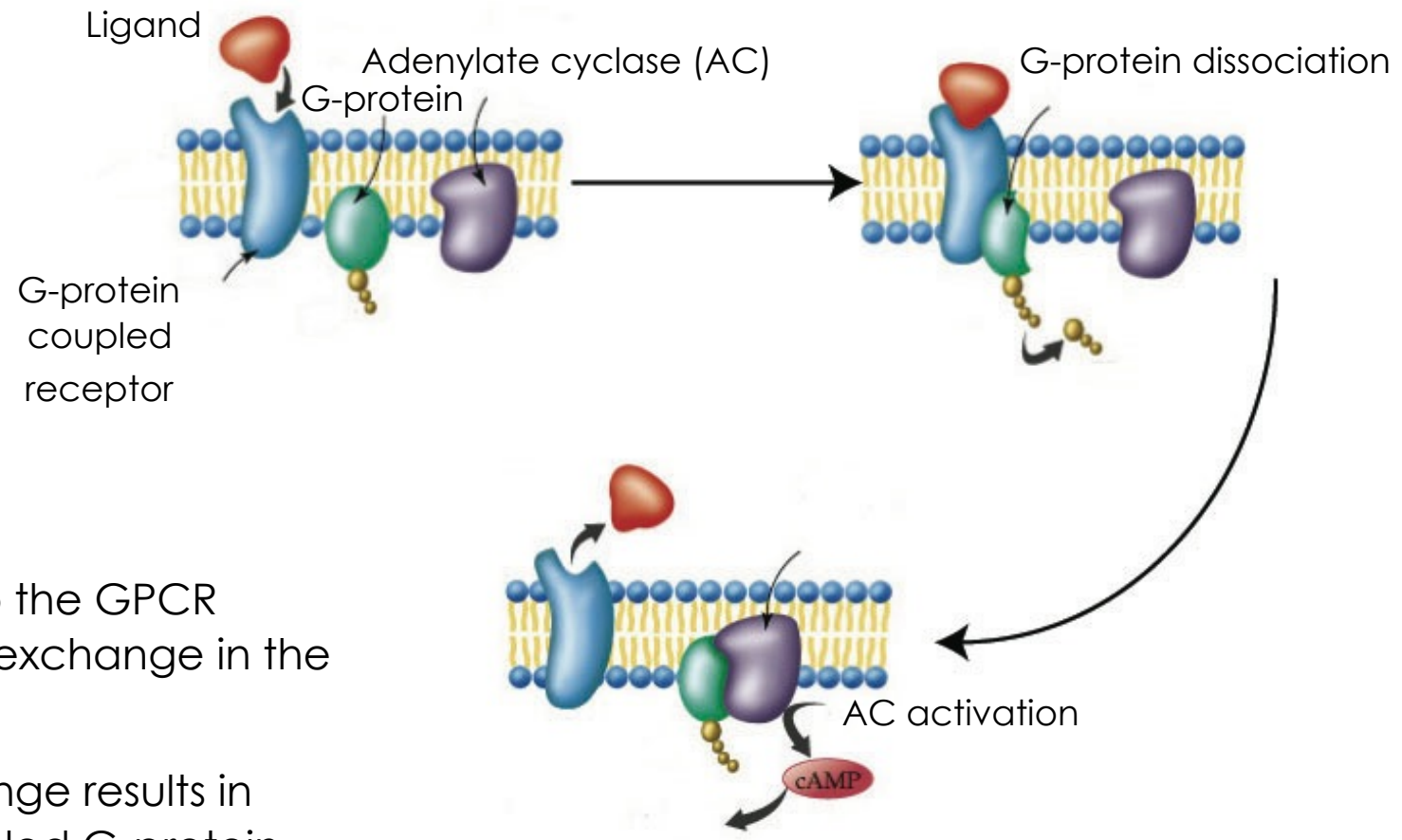


1a) rhodopsin, opoid receptors, β-adrenergic rec.
1b) NPY (hormones)
1c) glycoprotein receptors

2) glucagon, secretine, PACAP, PTH

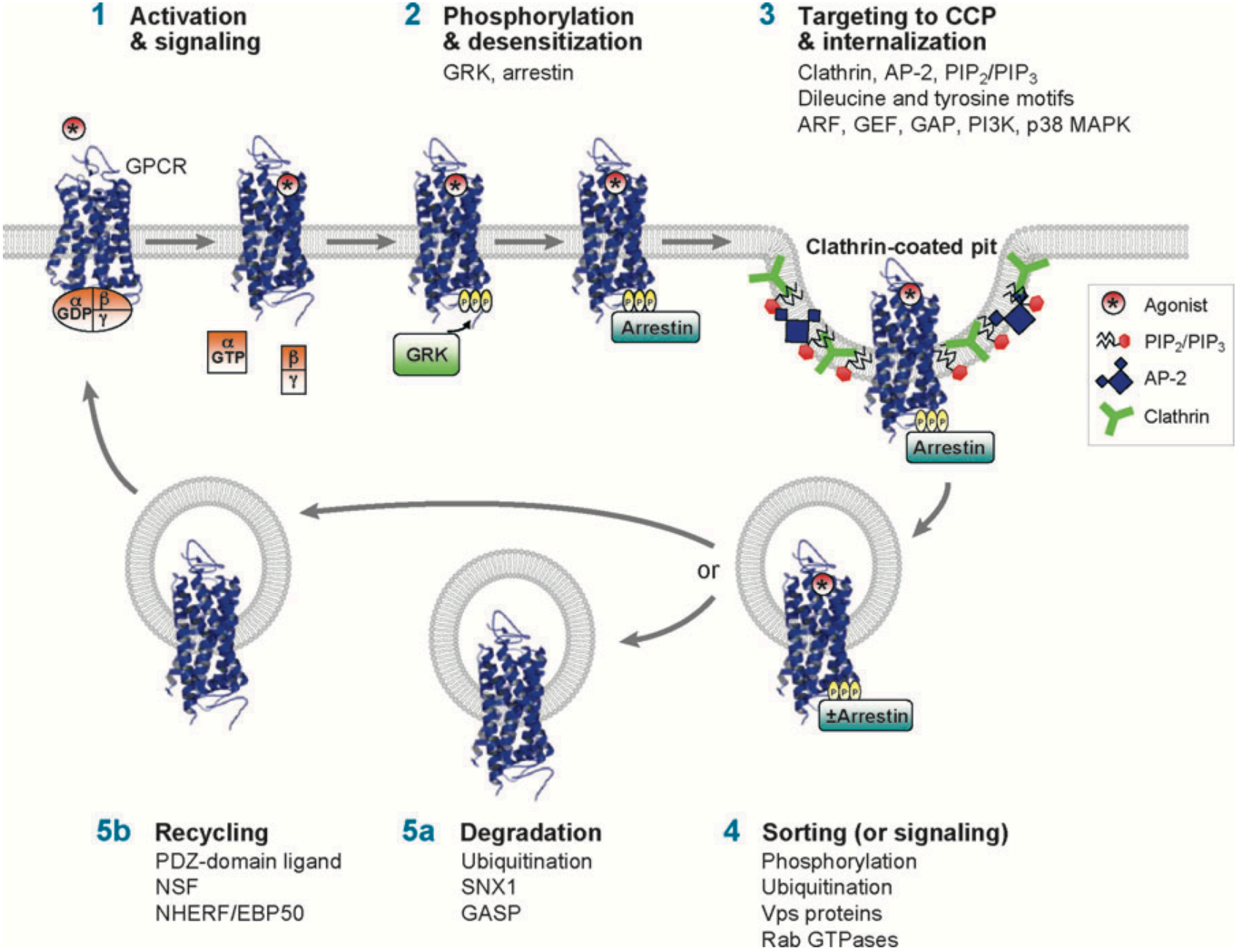
3) glutamate receptors GABA receptors

GPCR signaling



- Binding of the ligand to the GPCR triggers the GDP to GTP exchange in the bound G-protein
- The GDP to GTP exchange results in dissociation of the coupled G-protein into the a and β/γ subunits
- Both, the a and the β/γ subunit can activate downstream processes
- The a and β/γ subunits can reassociate to form an inactive G protein again

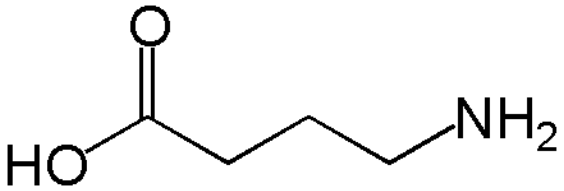
Receptor Desensitization



Benovic, Ann. Rev. Physiol. 2007

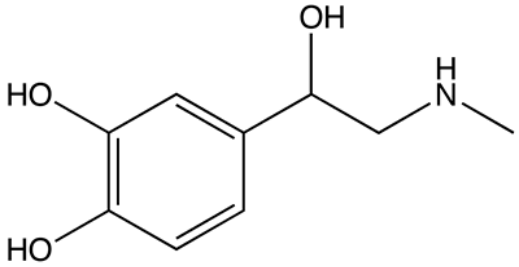
GPCR Ligands

Aminoacids



GABA

Biogenic amines



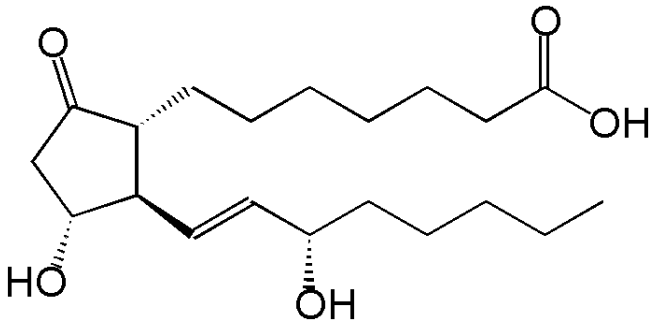
Adrenaline

Peptides/Proteins

H_3N-Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-Asp-Met-Ala-Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH_2

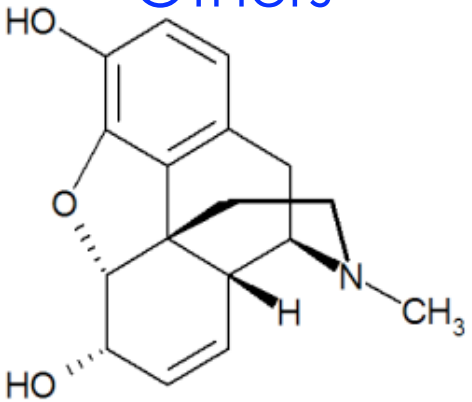
Neuropeptide Y (NPY)

Lipids



Prostaglandin E1

Others

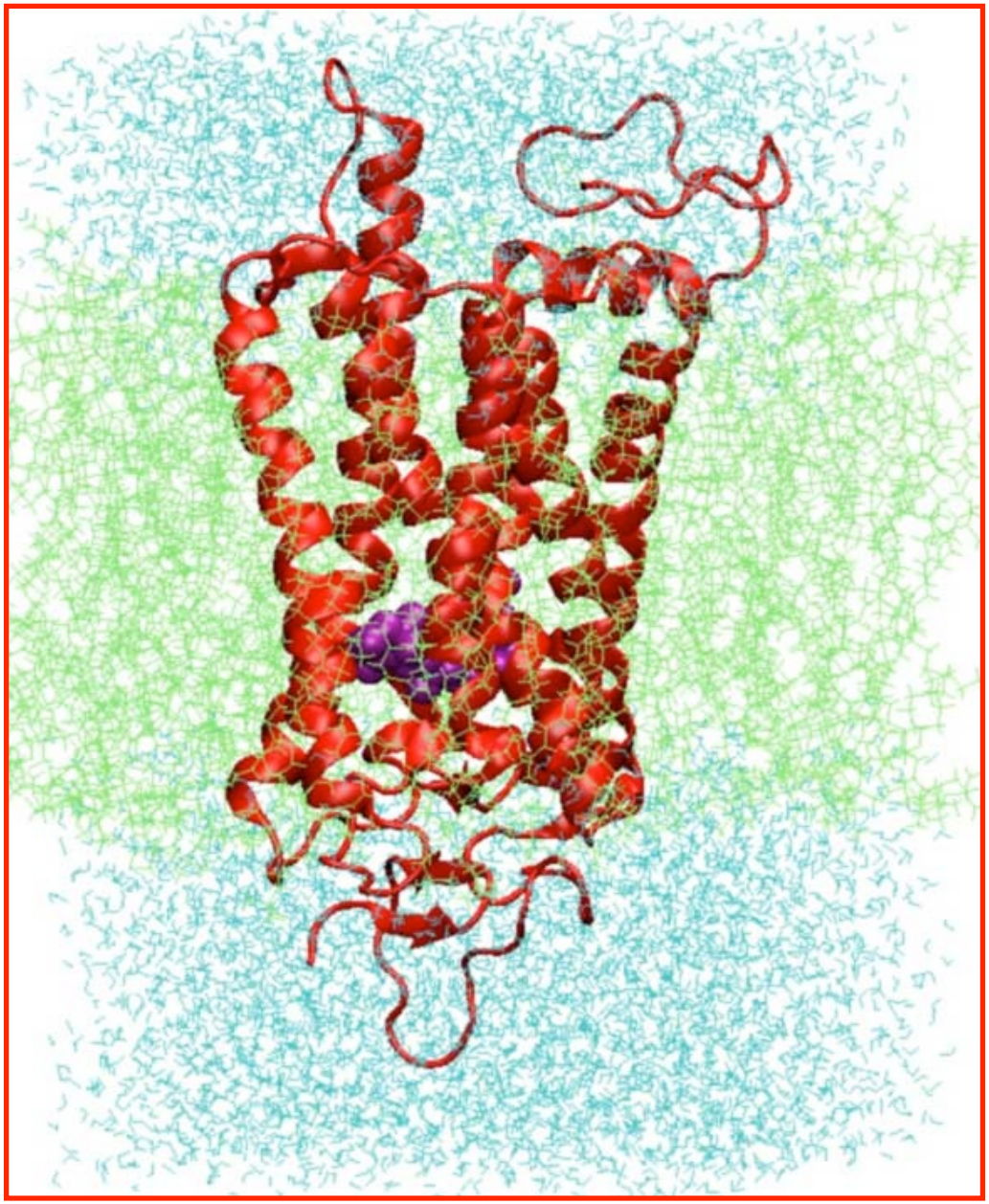


Morphine (opioids)

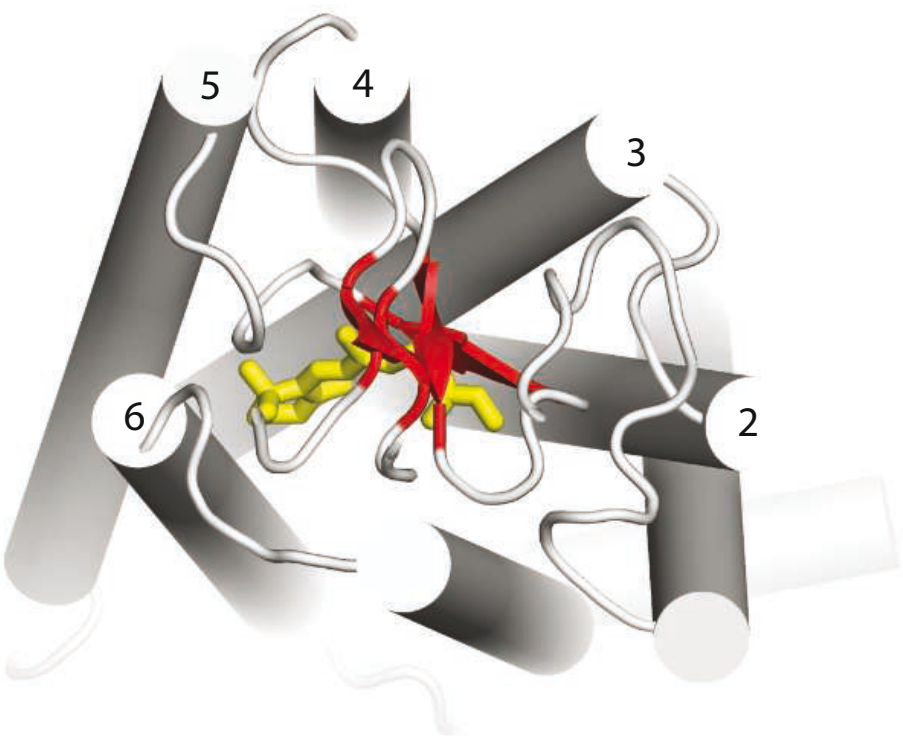
Light

Ions

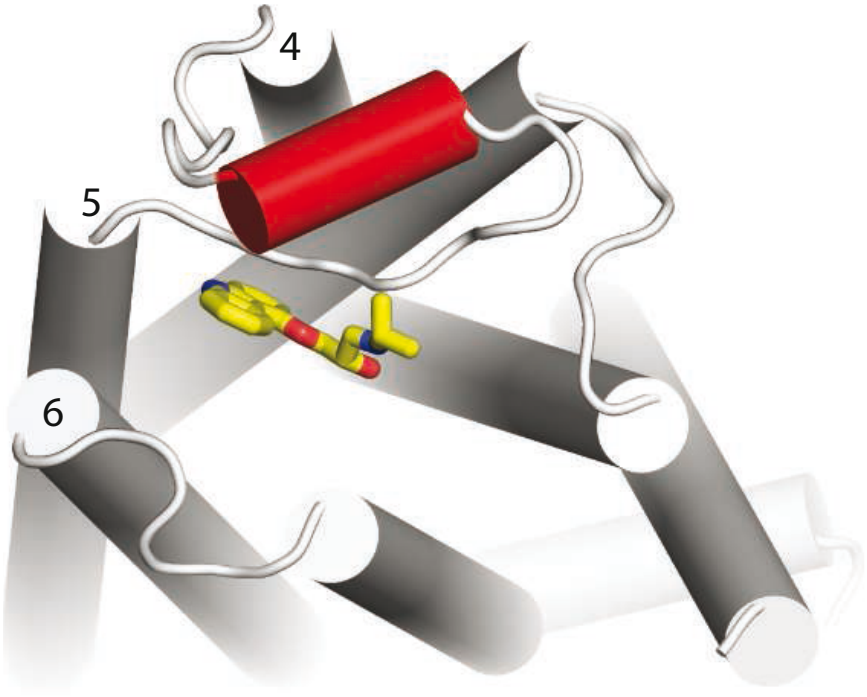
Crystal structure of Rhodopsin (2.8 Å)



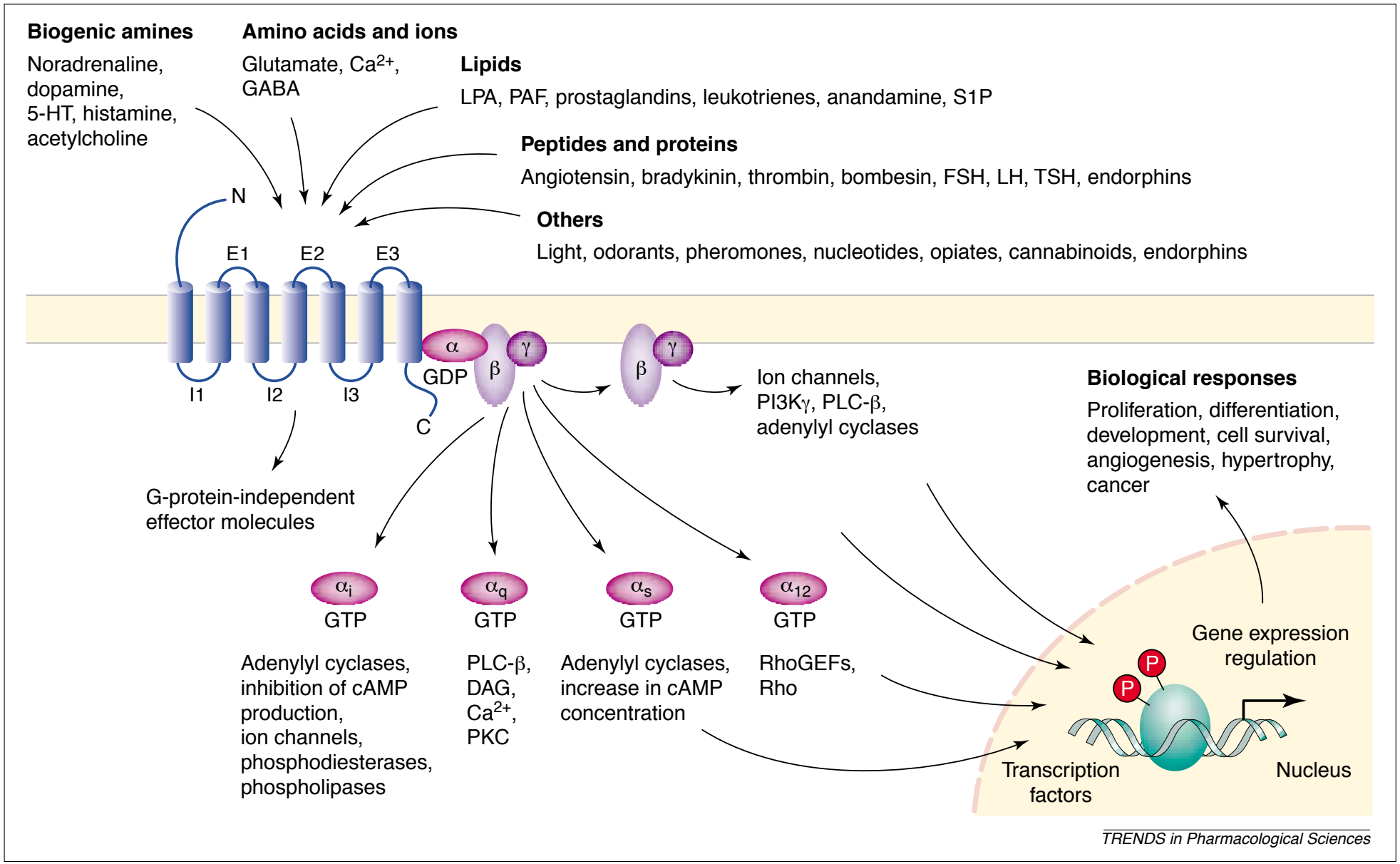
Palcewski et al.
Science (2000) 289, 739.



Rhodopsin



β-Adrenergic Receptor

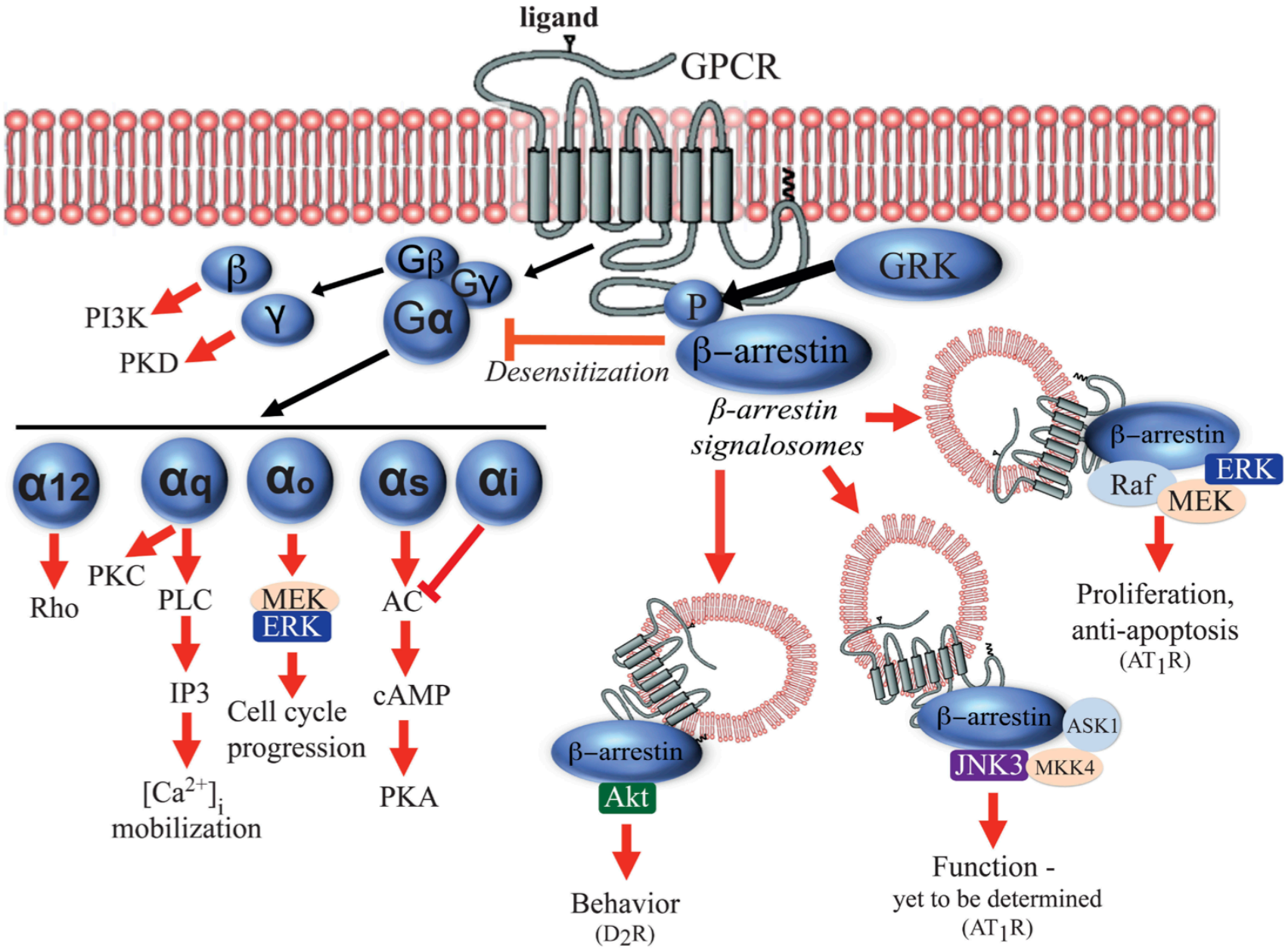


DAG, diacylglycerol; FSH, follicle-stimulating hormone; GEF, guanine nucleotide exchange factor; LH, leuteinizing hormone; LPA, lysophosphatidic acid; PAF, platelet-activating factor; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PLC, phospholipase C; S1P, sphingosine-1-phosphate; TSH, thyroid-stimulating hormone.

G α protein subtypes and signaling

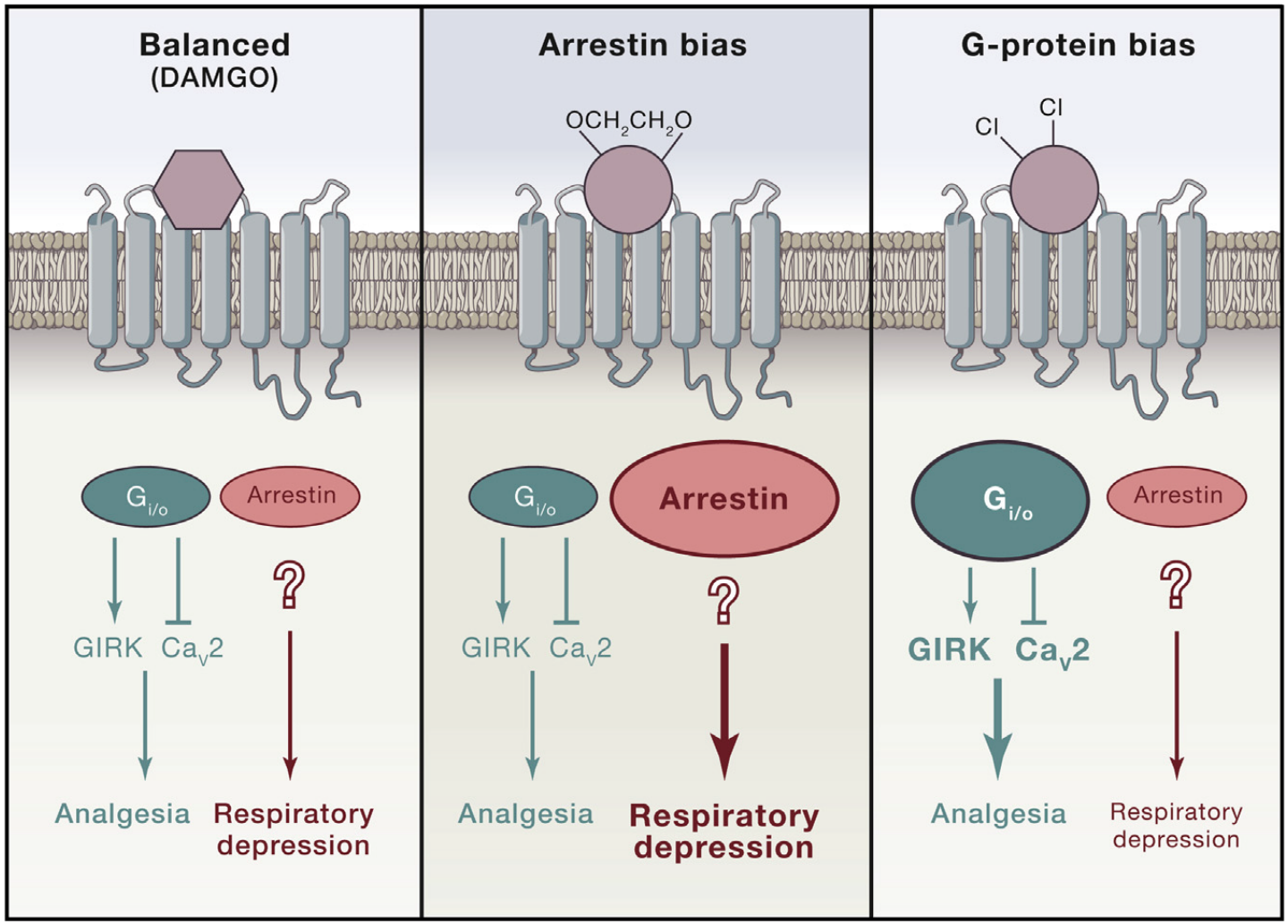
Family	Members	Actions	Function
I	G_s	a	activates adenylate cyclase, Ca^{2+} channels, cSrc tyrosine kinases
	G_{olf}	a	activates adenylate cyclase in olfactory neurons
II	G_i	a	inhibits adenylate cyclase
		βγ	activates K^+ channel
	G_o	βγ	activates K^+ channel, inactivates K+ channels
		a and βγ	activates phospholipase C-β
	G_t	a	activates cGMP phosphodiesterase in vertebrate photoreceptors
III	G_q	a	activates phospholipase C-β
IV	G_{12}	a	activates rho guanine nucleotide exchange factors

Biased Signaling



Bologna et al.,Biomol Ther. 2017;25(1):12-25

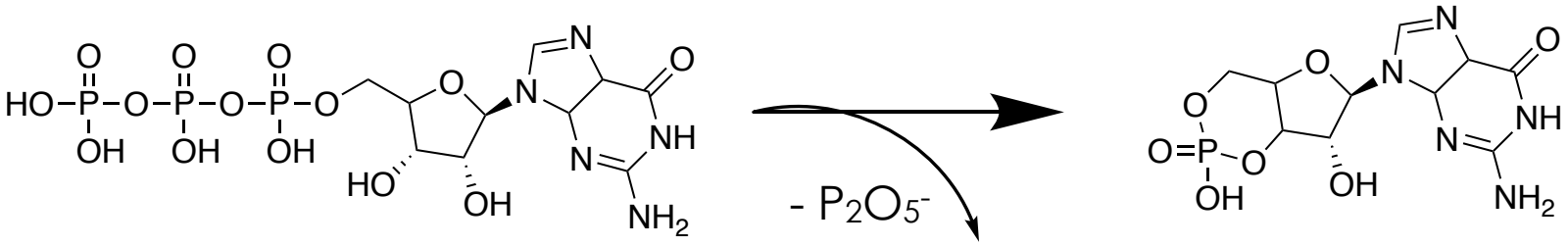
Biased Signaling



Cell *171*, November 16, 2017

Targets for G-proteins

- adenylate cyclase converts ATP to cAMP



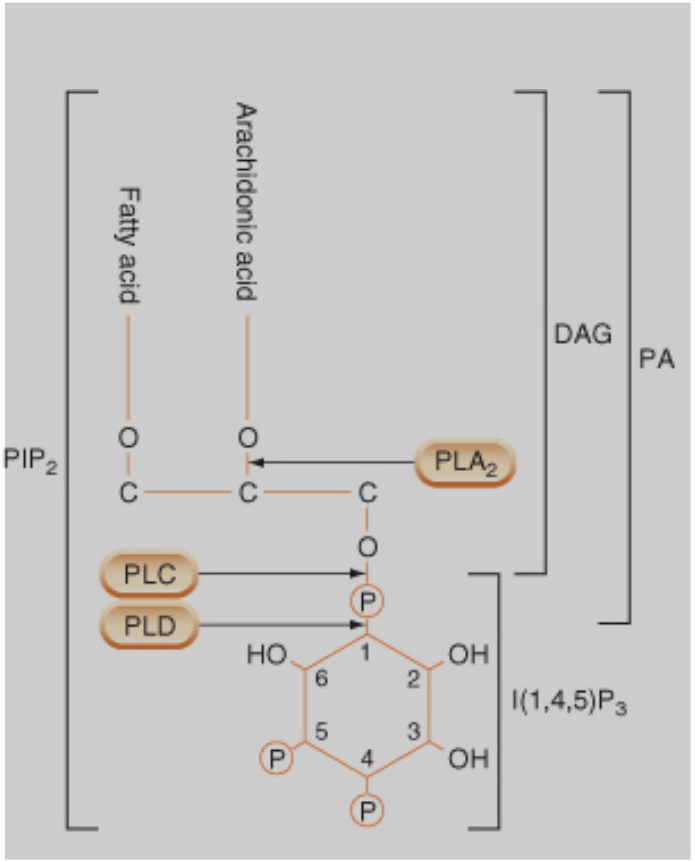
conversion of ATP to cAMP is catalysed by phosphodiesterases, which are targets of certain drugs (Viagra, caffeine)

- phospholipase C
- diacylglycerol and protein kinase C
- ion channels

Phospholipase C

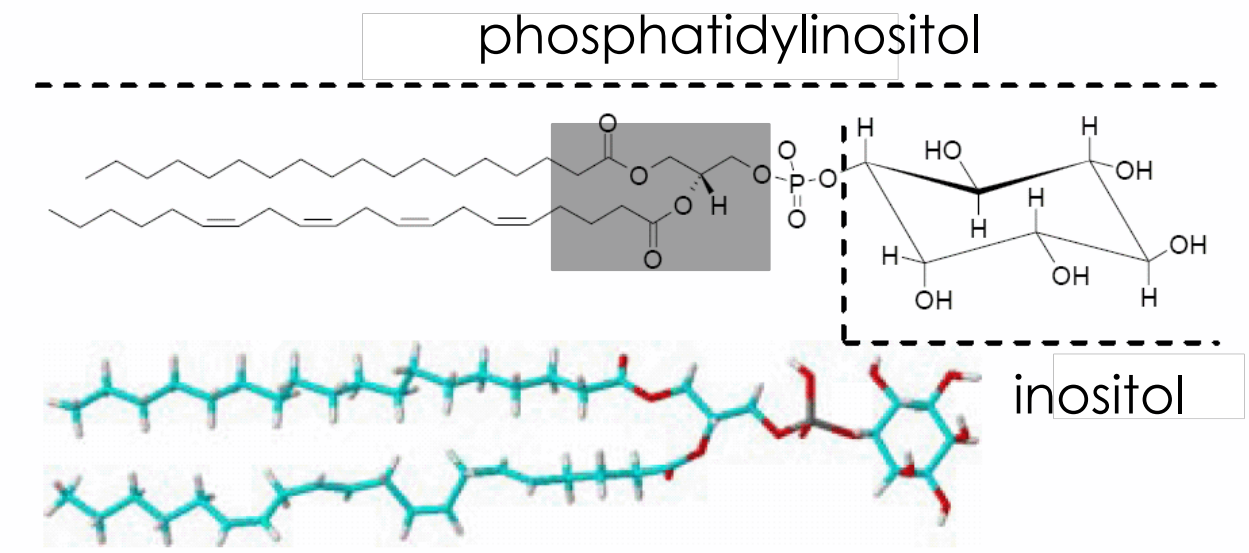
- phospholipase C catalyses the formation of IP3 and DAG from the membrane-bound phospholipid
- IP3 increases intracellular Ca2+ levels by releasing Ca2+ from intracellular storages
- increased Ca2+ levels initiate muscle contraction, secretion, enzyme activation and membrane hyperpolarization
- DAG activates protein kinase C, which acts to phosphorylate many intracellular proteins and thereby influences their function

the phospholipase/ inositol phosphate system



cleavage sites for phospholipases

phosphatidylinositol

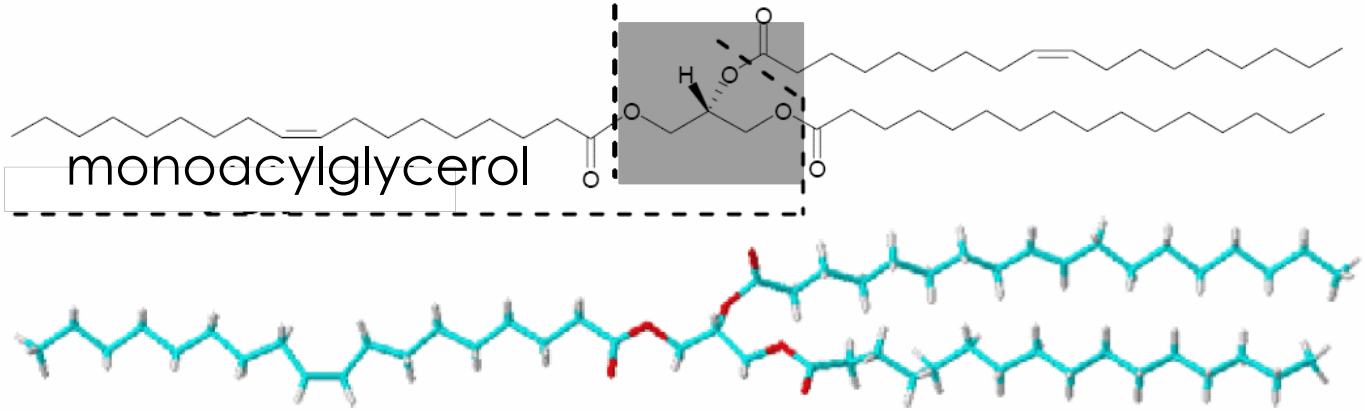


inositol

triacylglycerol

diacylglycerol

monoacylglycerol

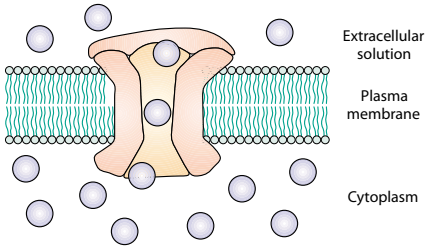


G-protein-coupled receptors		
Acetylcholine receptors	Muscarinic receptor agonists	Pilocarpine[151]
	Muscarinic receptor antagonists	Tropane derivatives[152,153]
	Muscarinic receptor M_3 antagonists	Darifenacine[154]
Adenosine receptors	Agonists	Adenosine[155]
	Adenosine A_1 receptor agonists	Lignans from valerian[156]
	Adenosine A_1 receptor antagonists	Caffeine, theophylline
	Adenosine A_{2A} receptor antagonists	Caffeine, theophylline[157]
Adrenoceptors[158,159]	Agonists	Adrenaline, noradrenaline, ephedrine
	α_1- and α_2-receptors agonists	Xylometazoline
	α_1-receptor antagonists	Ergotamine[160]
	α_2-receptor, central agonists	Methyldopa (as methylnoradrenaline)
	β-adrenoceptor antagonists	Isoprenaline
	β_1-receptor antagonists	Propranolol, atenolol
	β_2-receptor agonists	Salbutamol
	β_2-receptor antagonists	Propranolol
Angiotensin receptors	AT_1-receptors antagonists	Sartans[161]
Calcium-sensing receptor	Agonists	Strontium ions[162]
	Allosteric activators	Cinacalcet[163]
Cannabinoid receptors	CB_1- and CB_2-receptors agonists	Dronabinol[164]
Cysteinyl-leukotriene receptors	Antagonists	Montelukast[165]
Dopamine receptors[166]	Dopamine receptor subtype direct agonists	Dopamine, levodopa
	D_2, D_3 and D_4 agonists	Apomorphine
	D_2, D_3 and D_4 antagonists	Chlorpromazine, fluphenazine, haloperidol, metoclopramide, ziprasidone
Endothelin receptors (ET_A, ET_B)	Antagonists	Bosentan[167]

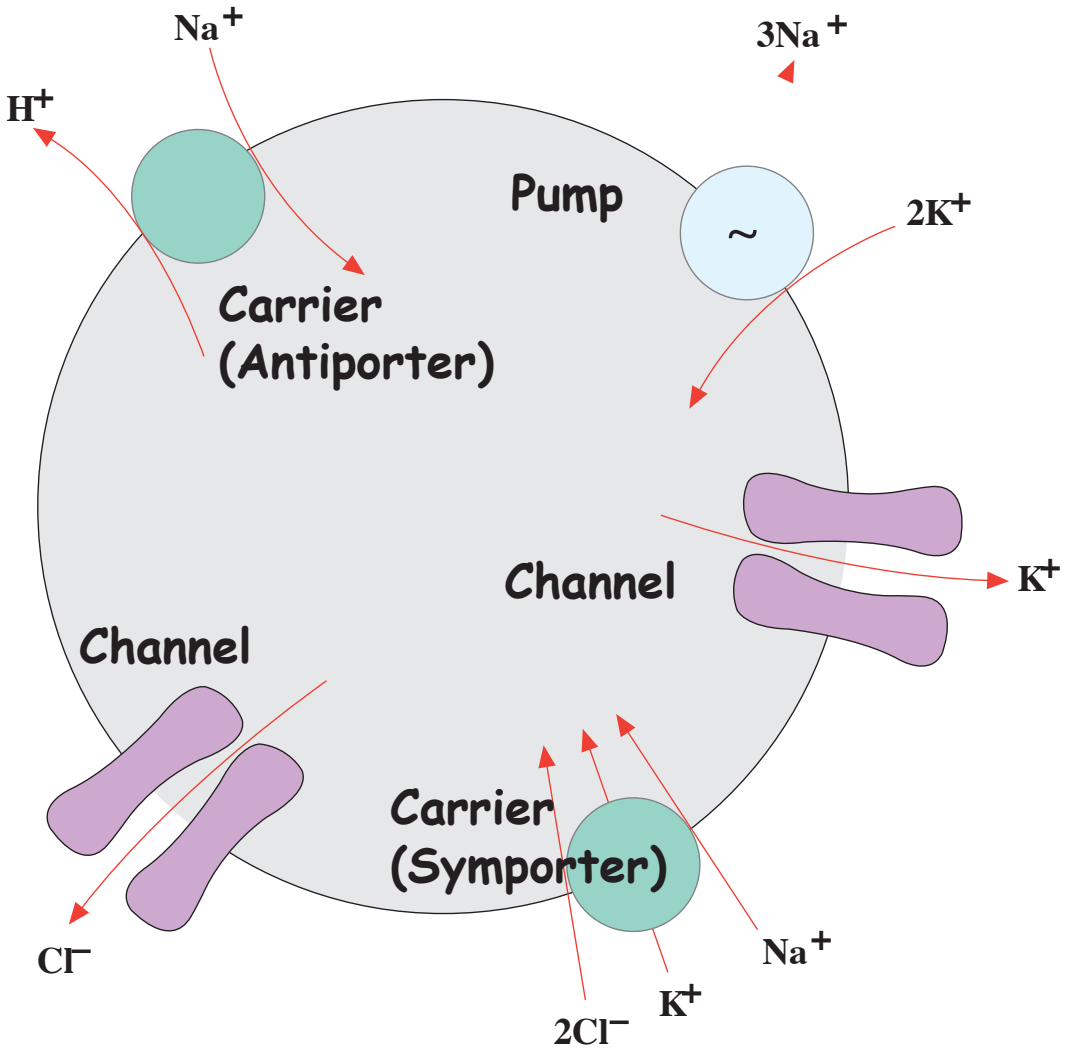
G-protein-coupled receptors (II)

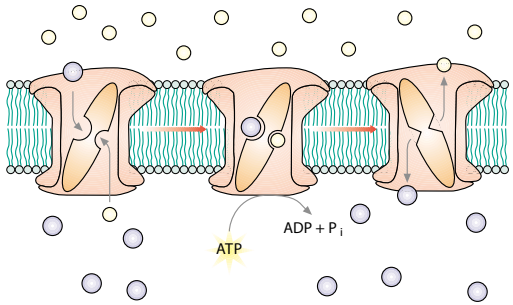
$GABA_B$ receptors	Agonists	Baclofen
Glucagon receptors	Agonists	Glucagon
Glucagon-like peptide-1 receptor	Agonists	Exenatide
Histamine receptors	H_1-antagonists	Diphenhydramine
	H_2-antagonists	Cimetidine
Opioid receptors	μ-opioid agonists	Morphine, buprenorphine
	μ-, κ- and δ-opioid antagonists	Naltrexone
	κ-opioid antagonists	Buprenorphine
Neurokinin receptors	NK_1 receptor antagonists	Aprepitant
Prostanoid receptors	Agonists	Misoprostol, sulprostone, iloprost
Prostamide receptors	Agonists	Bimatoprost
Purinergic receptors	P_2Y_{12} antagonists	Clopidogrel
Serotonin receptors	Subtype-specific (partial) agonists	Ergometrine, ergotamine
	5-HT_{1A} partial agonists	Buspirone
	5-$HT_{1B/1D}$ agonists	Triptans
	5-HT_{2A} antagonists	Quetiapine, ziprasidone
	5-HT_3antagonists	Granisetron
	5-HT_4partial agonists	Tegaserode
Vasopressin receptors	Agonists	Vasopressin
	V_1 agonists	Terlipressin
	V_2 agonists	Desmopressin
	OT agonists	Oxytocin
	OT antagonists	Atosiban

Transport of ions across the membrane

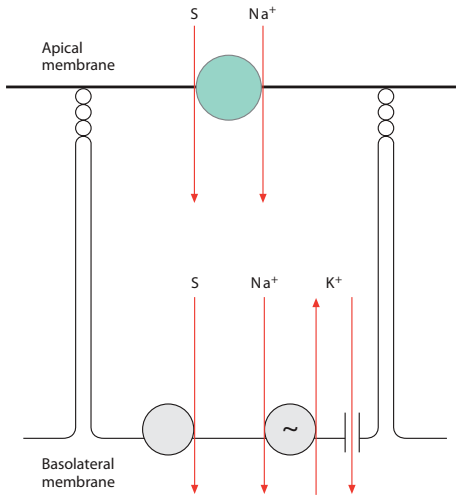


passive transport: channels





active transport: pumps



active transport: symporter/ antiporter

Type	Activity of drug	Drug examples
Cation-chloride cotransporter (CCC) family	Thiazide-sensitive NaCl symporter, human inhibitor	Thiazide diuretics
	Bumetanide-sensitive NaCl/KCl symporters, human inhibitor	Furosemide
Na^+/H^+ antiporters	Inhibitor	Amiloride, triamterene
Proton pumps	Ca^{2+}-dependent ATPase (PfATP6; Plasmodia) inhibitor	Artemisinin and derivatives
	H^+/K^+-ATPase inhibitor	Omeprazole
Na^+/K^+ ATPase	Inhibitor	Cardiac glycosides
Eukaryotic (putative) sterol transporter (EST) family	Niemann-Pick C1 like 1 (NPC1L1) protein inhibitor	Ezetimibe
Neurotransmitter/Na^+ symporter (NSS) family	Serotonin/Na^+ symporter inhibitor	Cocaine, tricyclic antidepressants, paroxetine
	Noradrenaline/Na^+ symporter inhibitor	Bupropion, venlafaxine
	Dopamine/Na^+ symporter inhibitor	Tricyclic antidepressants, cocaine, amphetamines
	Vesicular monoamine transporter inhibitor	Reserpine

Ion channels

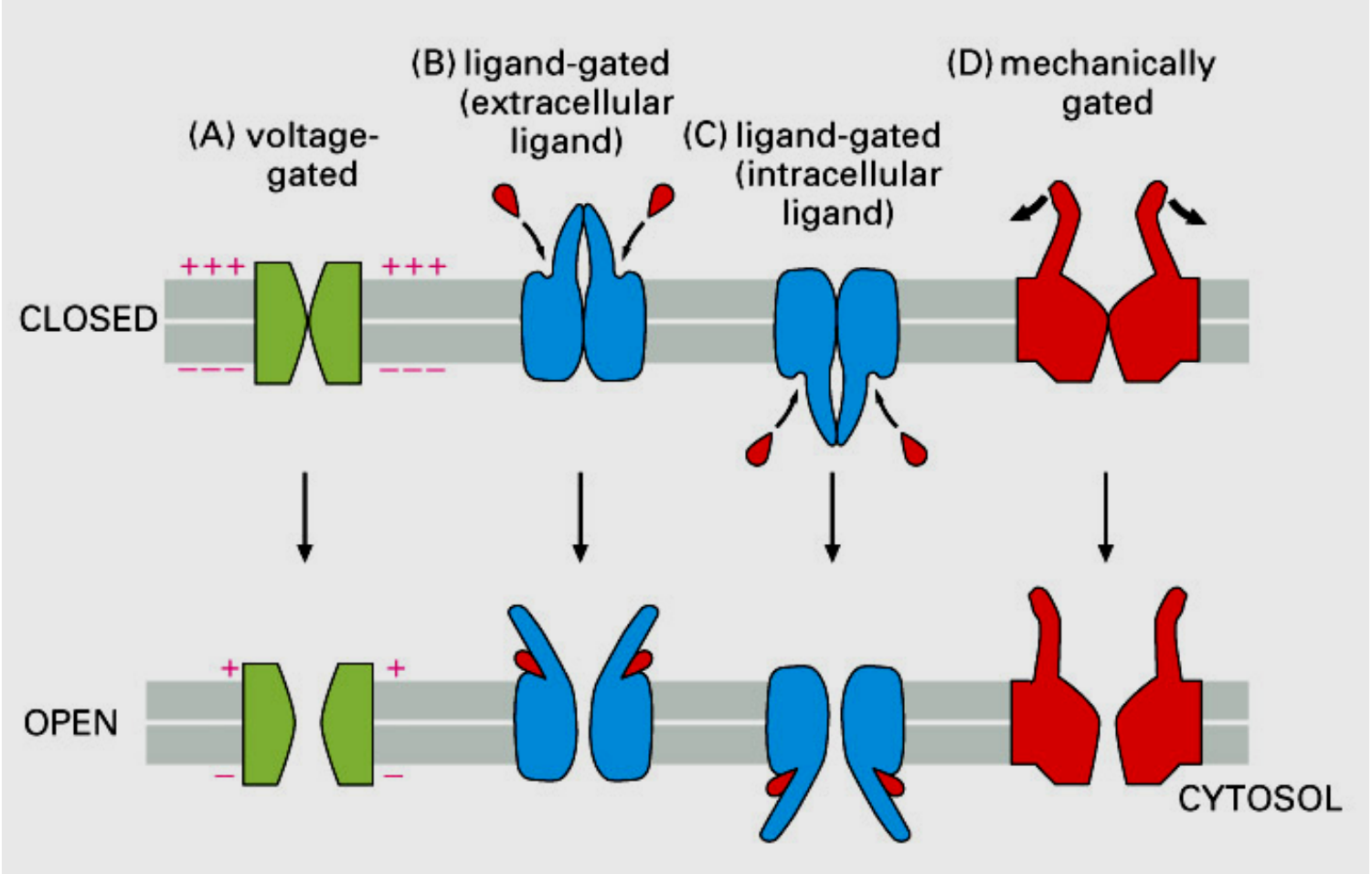
- sometimes called ionotropic receptors
- mainly involved in fast synaptic transmission
- usually pentameric assembly of subunits
- ligand-binding and gating occurs on a millisecond timescale

Types of Ion Channels

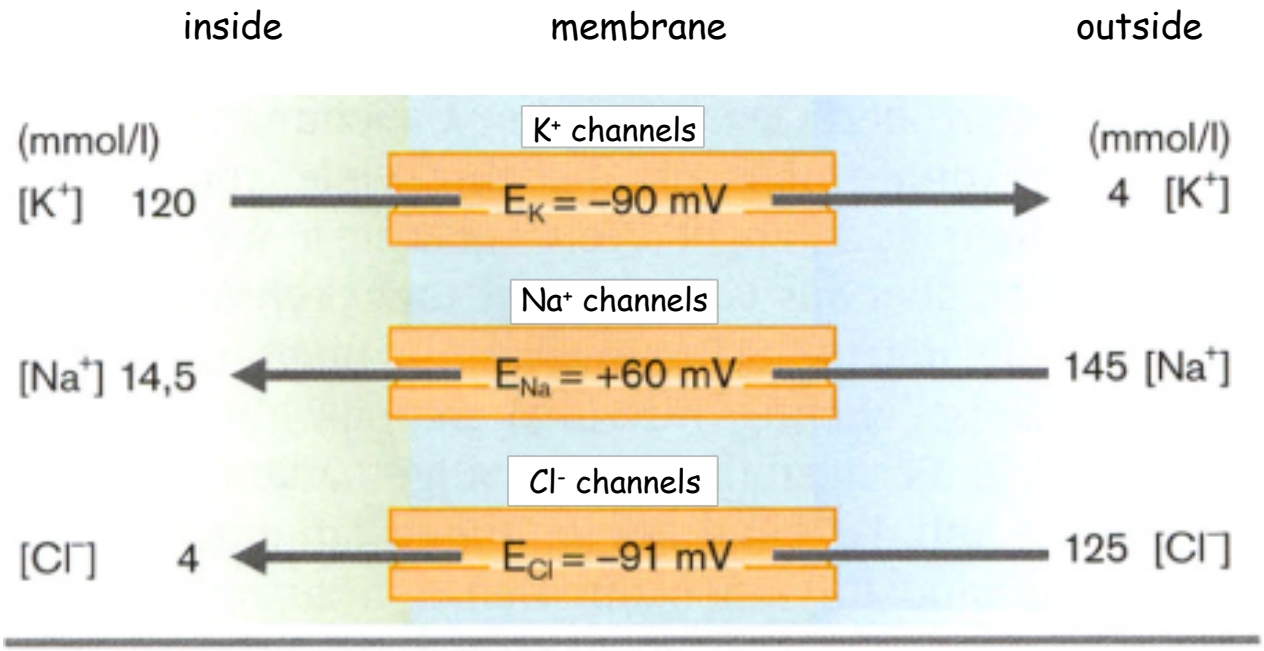
- Simple pores (GA, GAP junctions)
- Pumps (ATP-synthase, K^+,Na^+-ATPase)
- gated channels
  - Voltage-gated (K^+ channels, Na^+ channels, Ca^{2+} channels)
  - Mechanically gated (auditory hair cells)
  - Ligand-gated ion channel (pentameric structure of the pore)
    - ✓ 4-TM(nicotinic acetylcholine, GABA, serotonin (5HT3), glycine receptors
    - ✓ 3-TM (Ca^{2+}-controlled glutamate receptors)
    - ✓ 2-TM (ATP-gated)

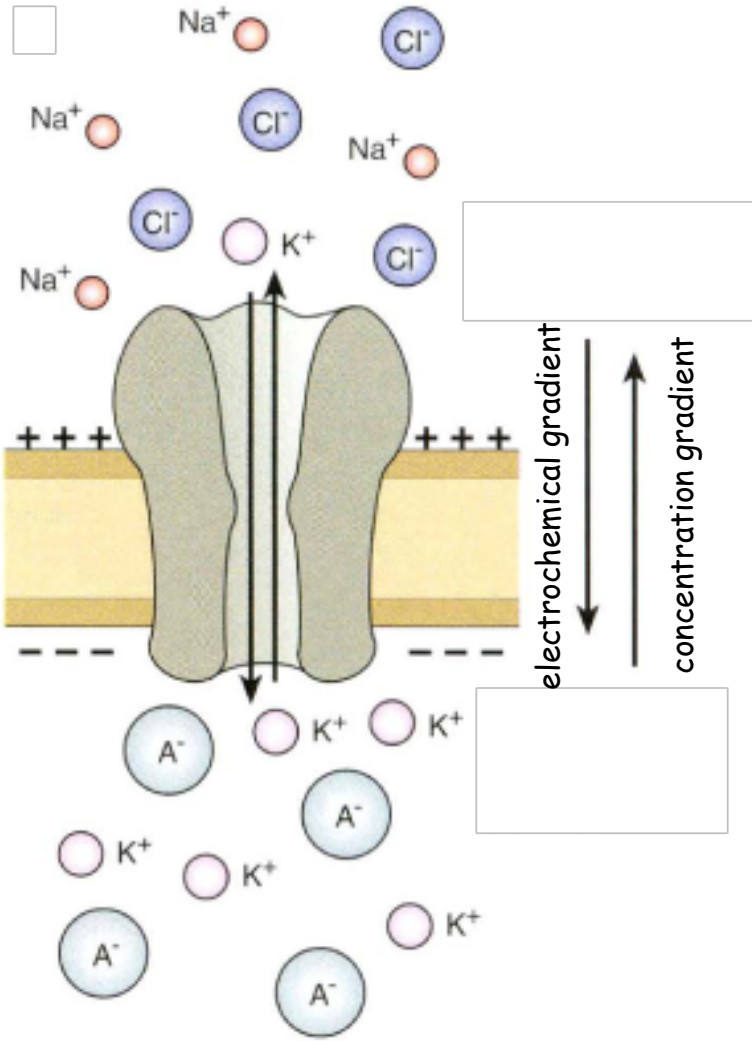
Stimulation/Inhibition

- excitatory channels
  - acetylcholine-gated cation channels
  - glutamate-gated Ca2+ channels
  - serotonin-gated cation channels
- inhibitory channels
  - GABA-gated chloride channels
  - glycine-gated chloride channels

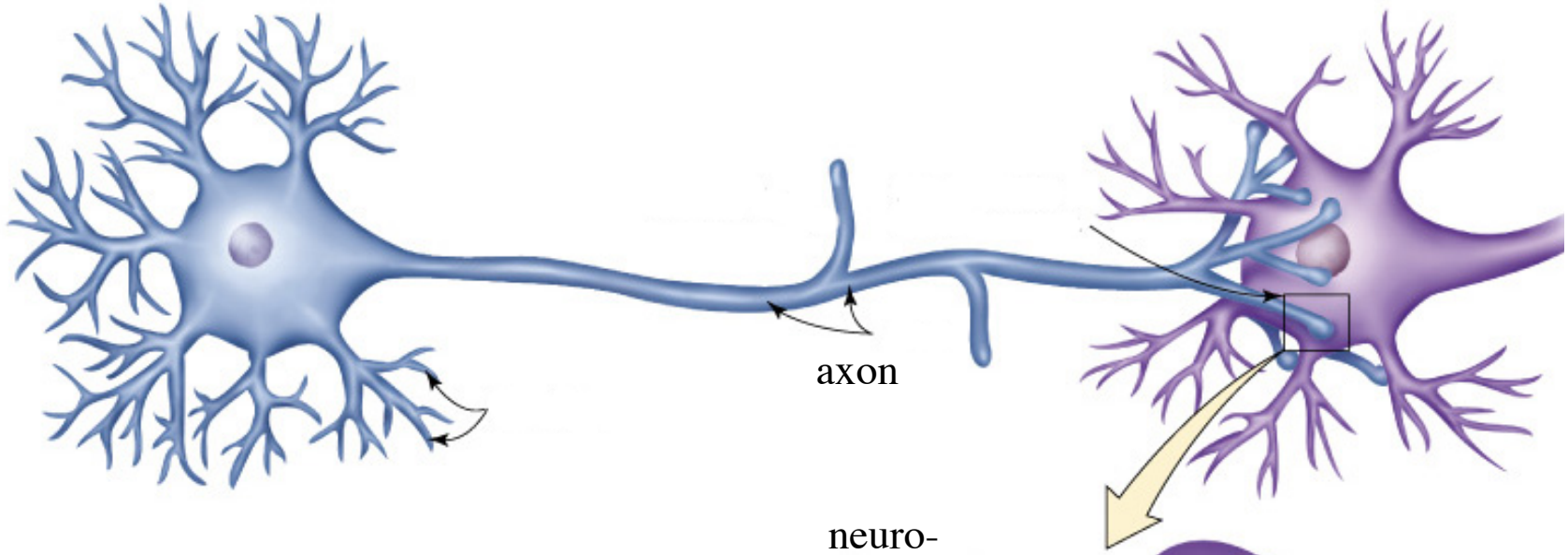

(A) voltage-gated
(B) ligand-gated (extracellular ligand)
(C) ligand-gated (intracellular ligand)
(D) mechanically gated
CLOSED
OPEN
+++
+++
– – –
– – –
+
+
–
–
CYTOSOL

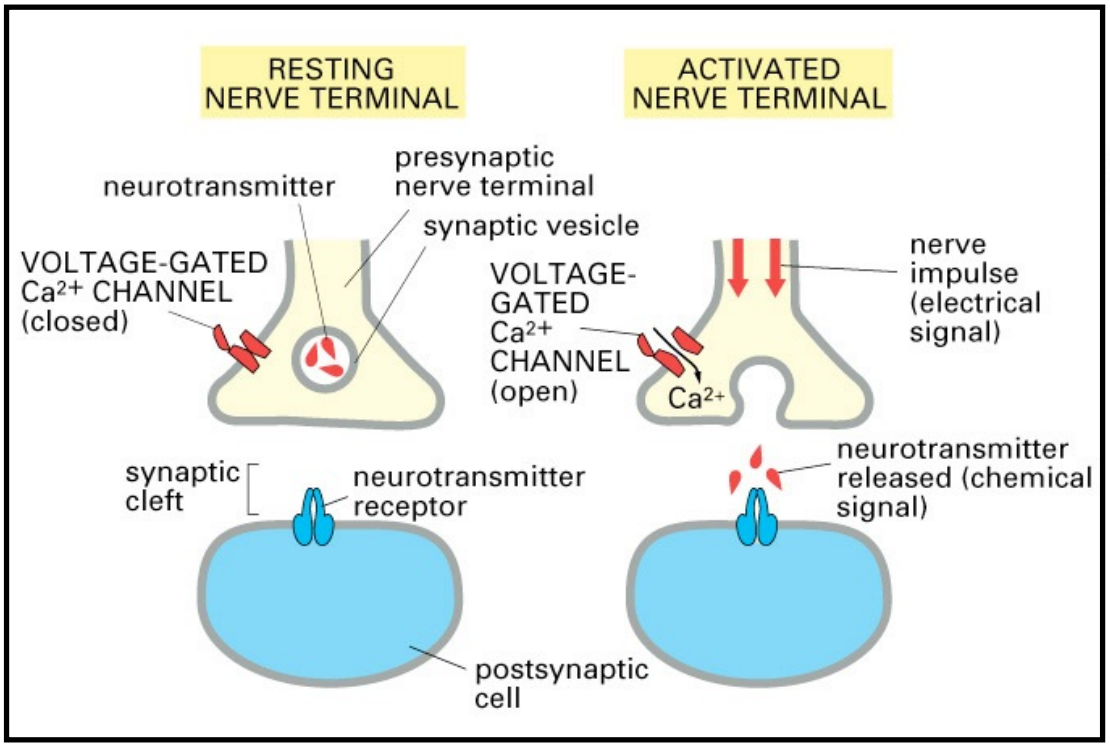
Ion channels help to maintain different ion concentrations in and outside of the cell

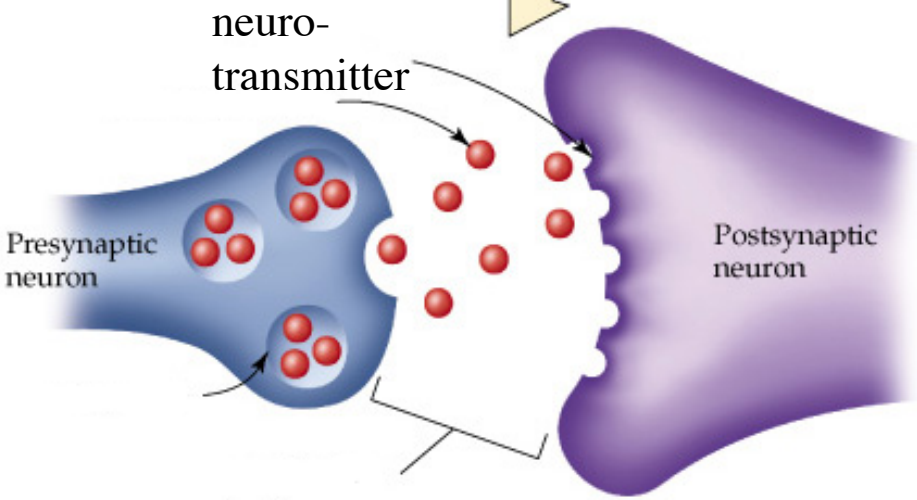




Nerve transmission







The **Action Potential**:

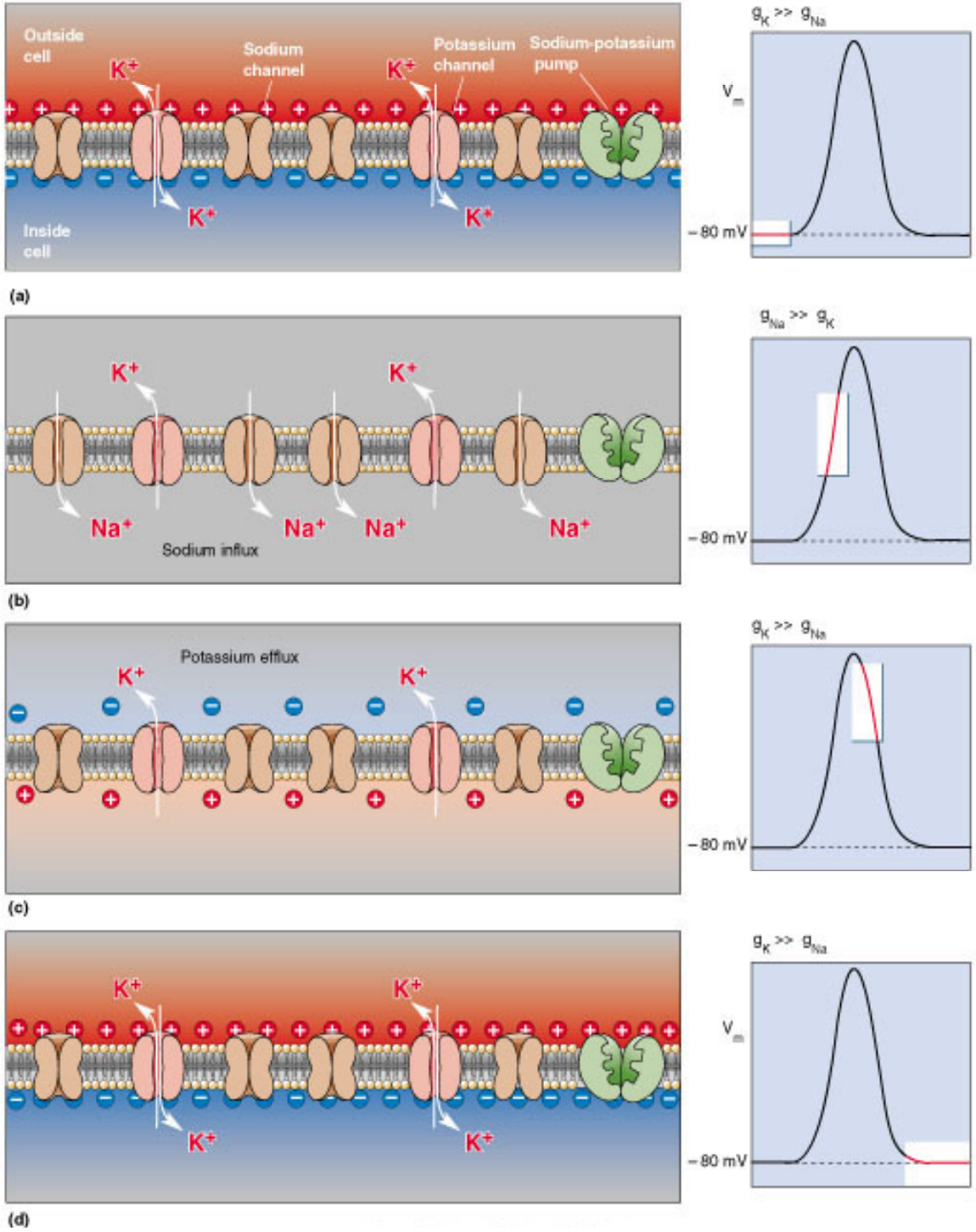
a) **Resting membrane potential** (RMP) at -70mV. Na^+ on outside and K^+ on inside of cell

b) As **depolarization** reaches threshold of -55mV, the **action potential** is triggered and Na+ rushes into cell. Membrane potential reaches +30mV on action potential

c) Propagation of the action potential at 110 m/sec (which is 225 mph)

d) **Repolarization** occurs with K^+ exiting the cell to return to -70mV RMP

e) Return of ions (Na^+ and K^+) to their extracellular and intracellular sites by the sodium potassium (Na^+K^+) pump



Rest: open K+ channels (not voltage-gated

Rise: open Na+ channels (voltage-gated

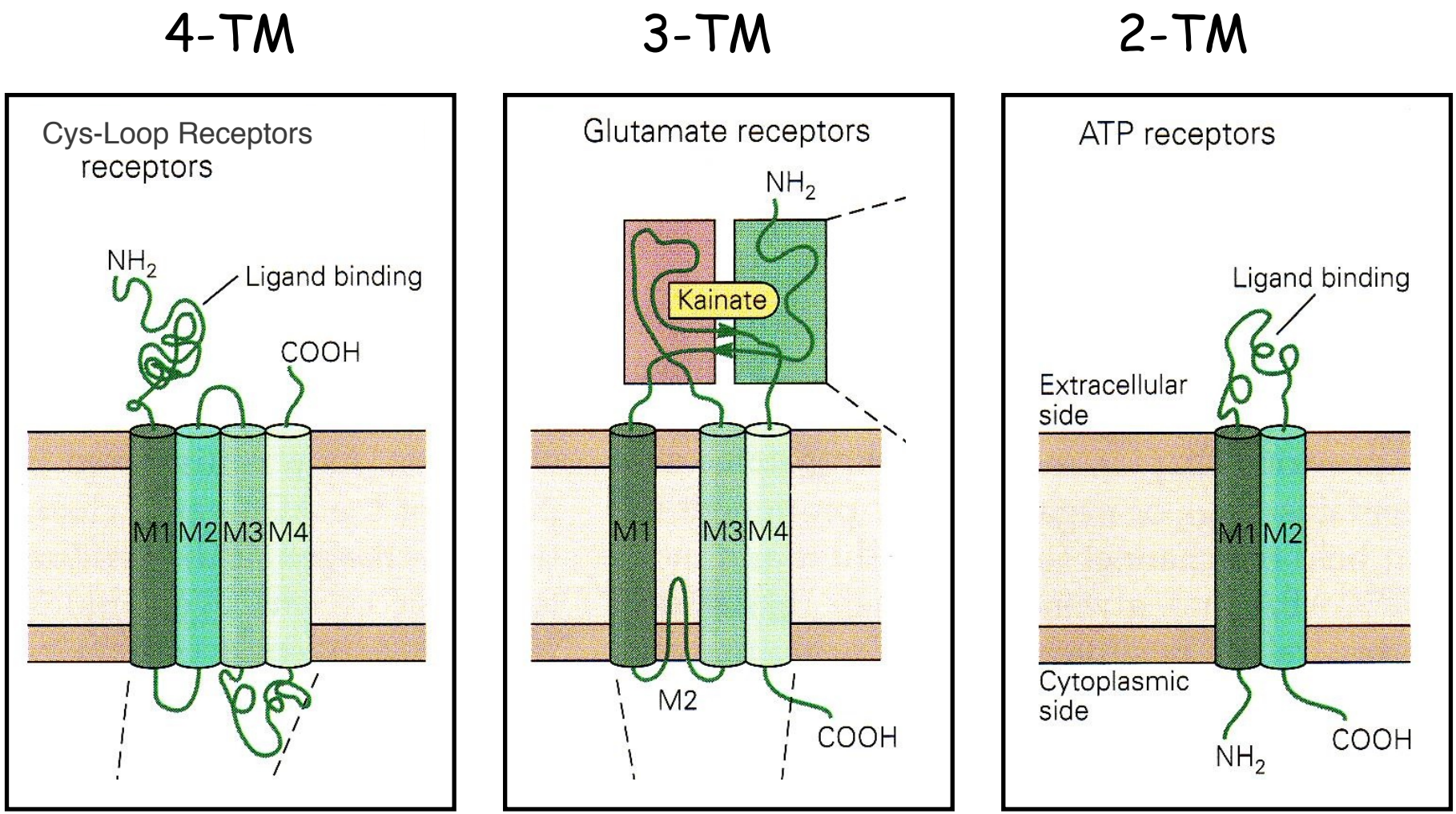
Fall: open K+ channels (voltage-gated

Rest: open K+ channels (not voltage-gated

Short Test

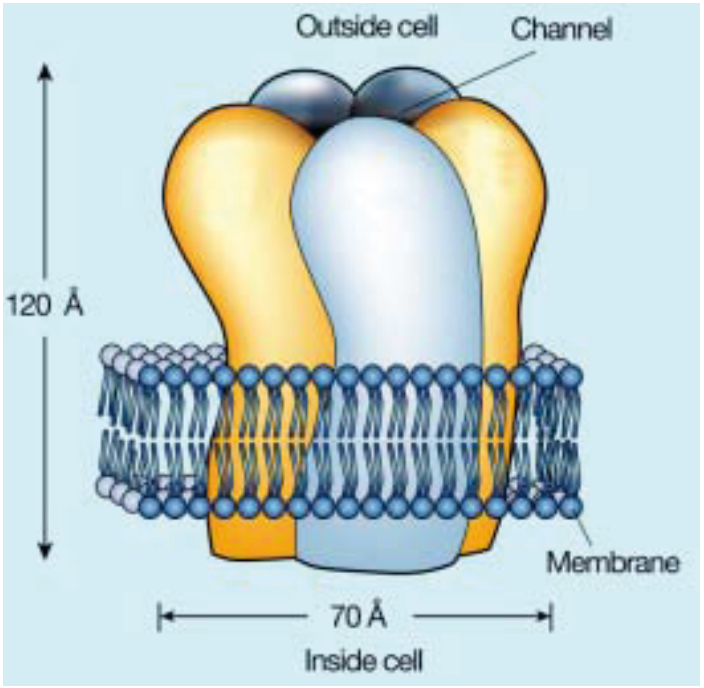
- Describe the difference between fragments and drugs
- Describe how you can analytically determine the fraction of bound drug
- Describe the path to intracellular targets for orally-uptaken drugs
- What analytical techniques for determining dissociation constants do you know for weak (1mM) and tight (10 nM) binders do you know?
- What is a prodrug? Describe prodrugs for small molecules and for proteins
- Describe SAR-by-NMR

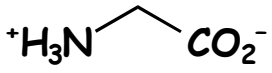
Topologies for Ligand-Gated Ion Channels



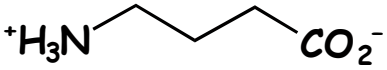
Kandel, Schwartz & Jessel, Principles of Neural Science 4th Ed. (2000)

Ion-Gated Channels



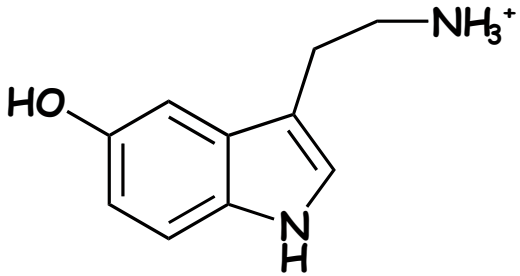


glycine



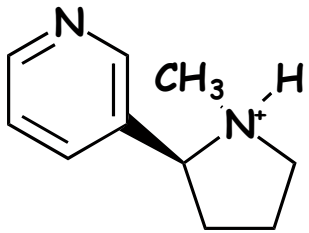
GABA

(chloride channels
regulates neuronal excitability)

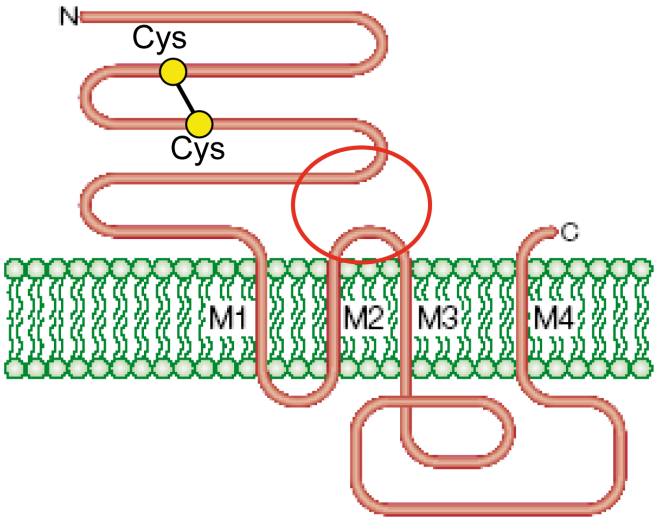


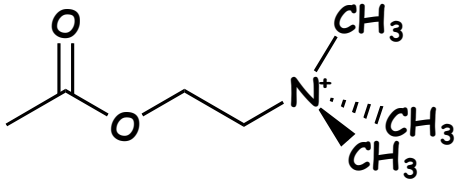
serotonin

(mood, appetite, sleep))



nicotine

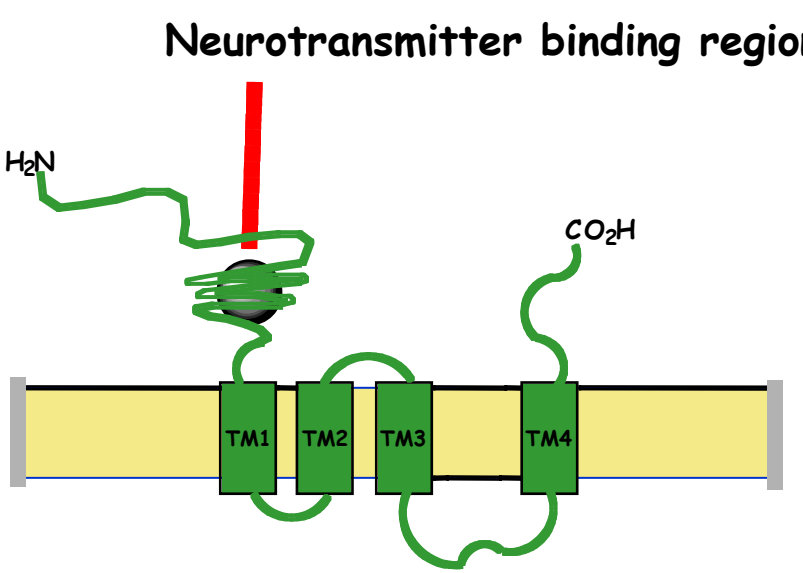


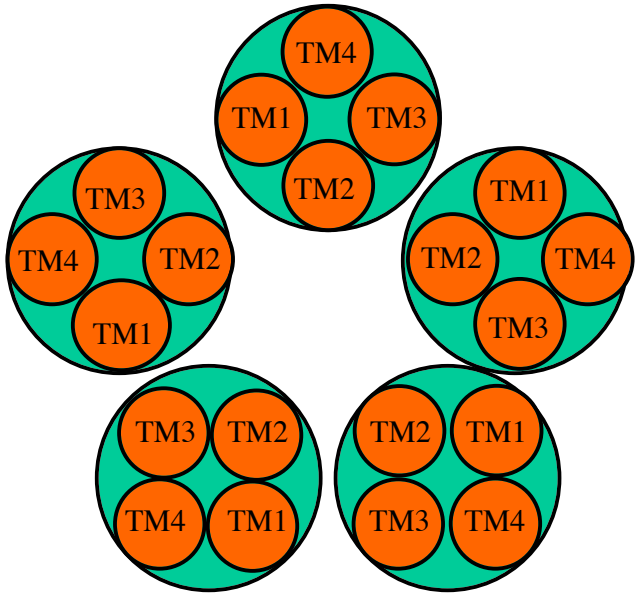


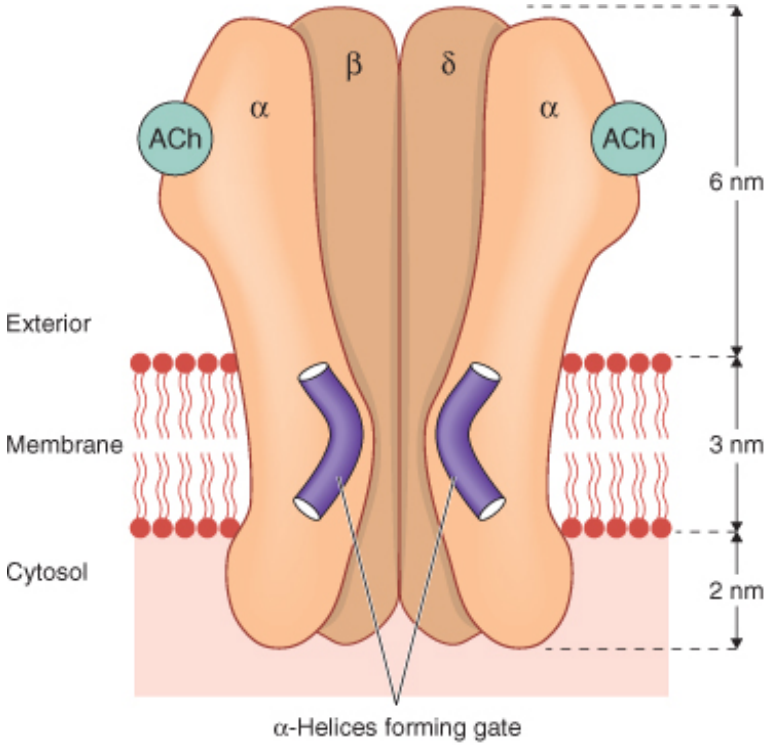
acetylcholine

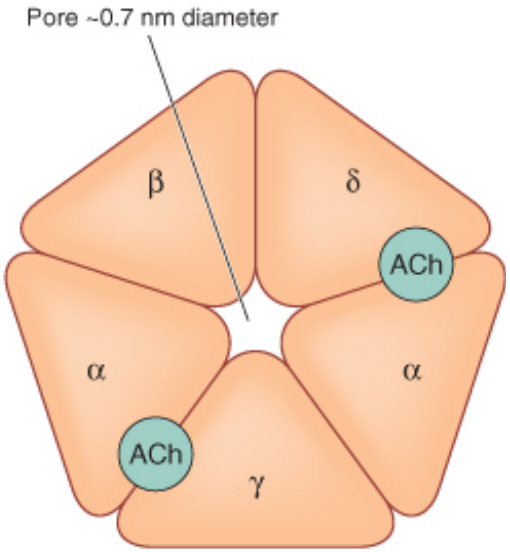
(muscles)

Structural features of ligand-gated 4TM receptors



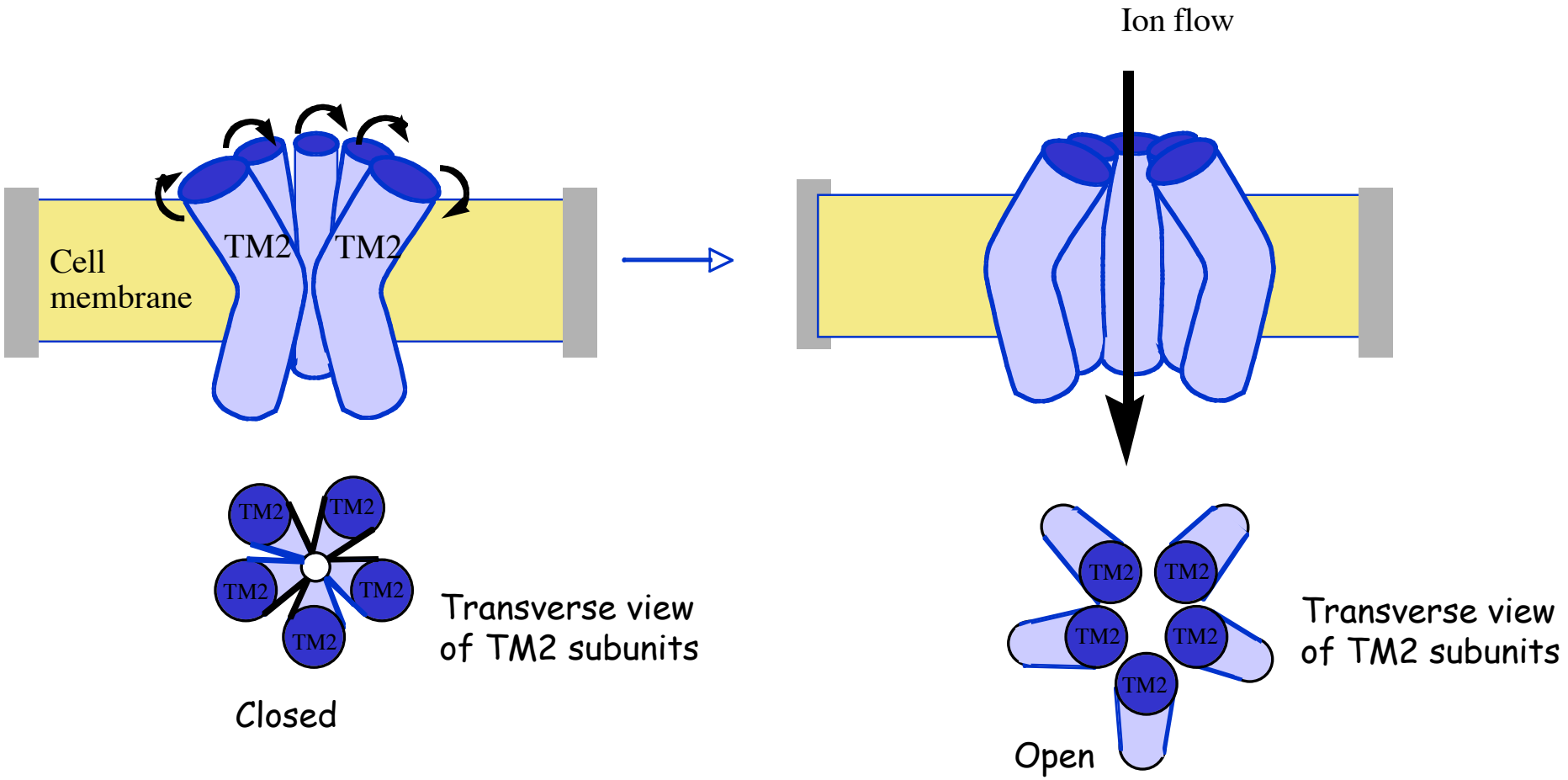




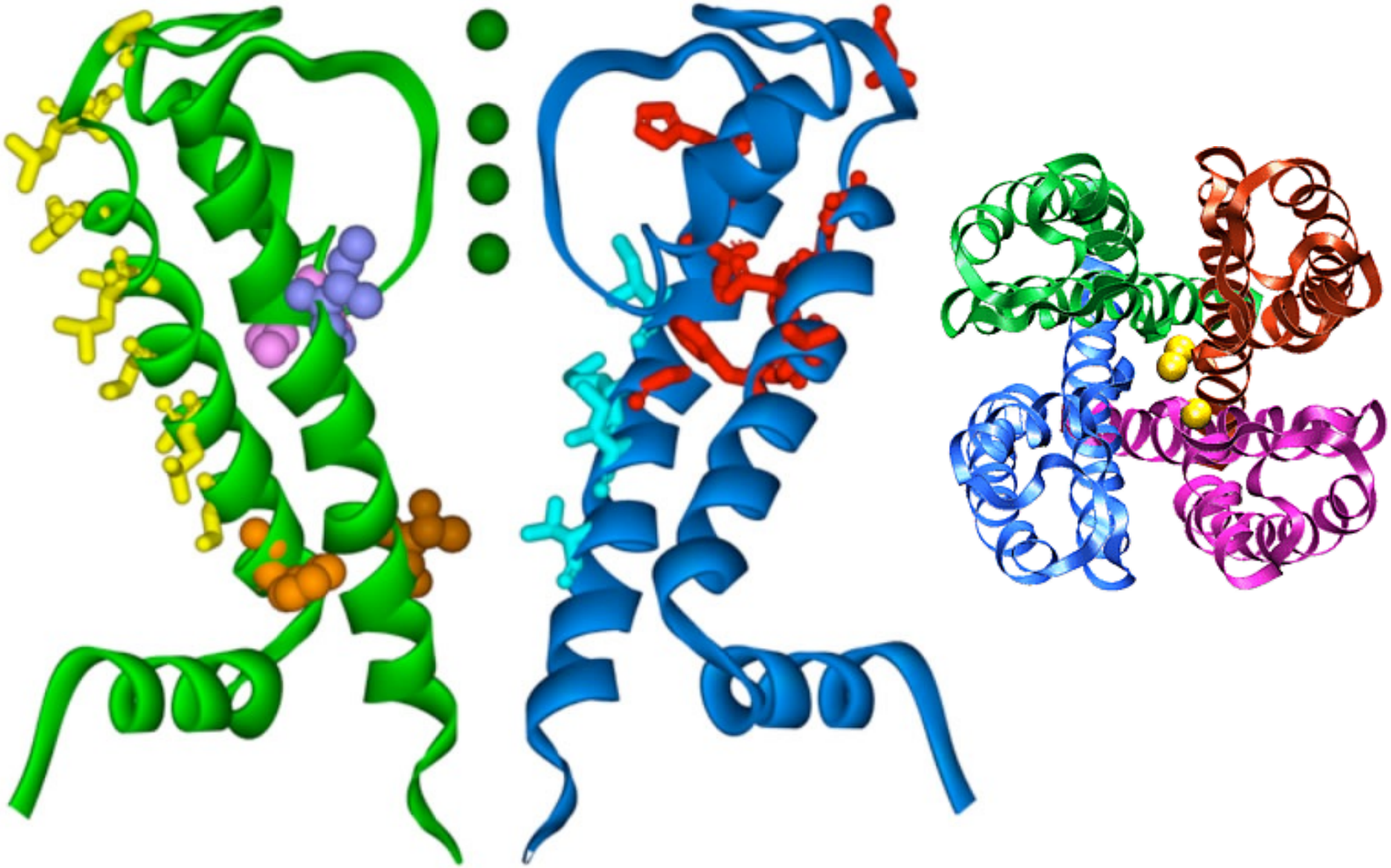


The Gating Mechanism

Neurotransmitter binds → **Induced fit at binding site** → **'Domino effect'** → **Rotation of 2TM regions of each protein subunit**

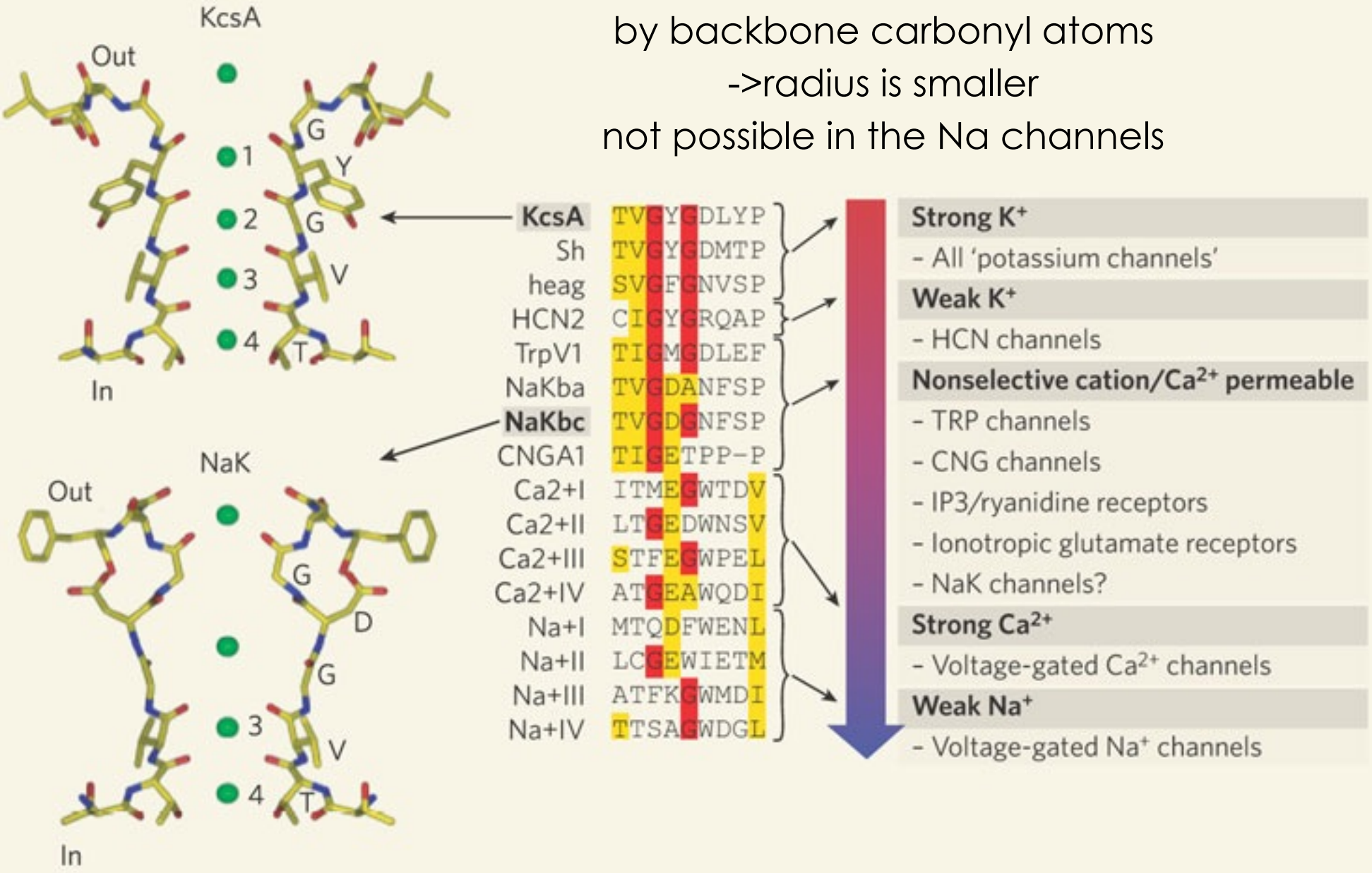


The selectivity filter



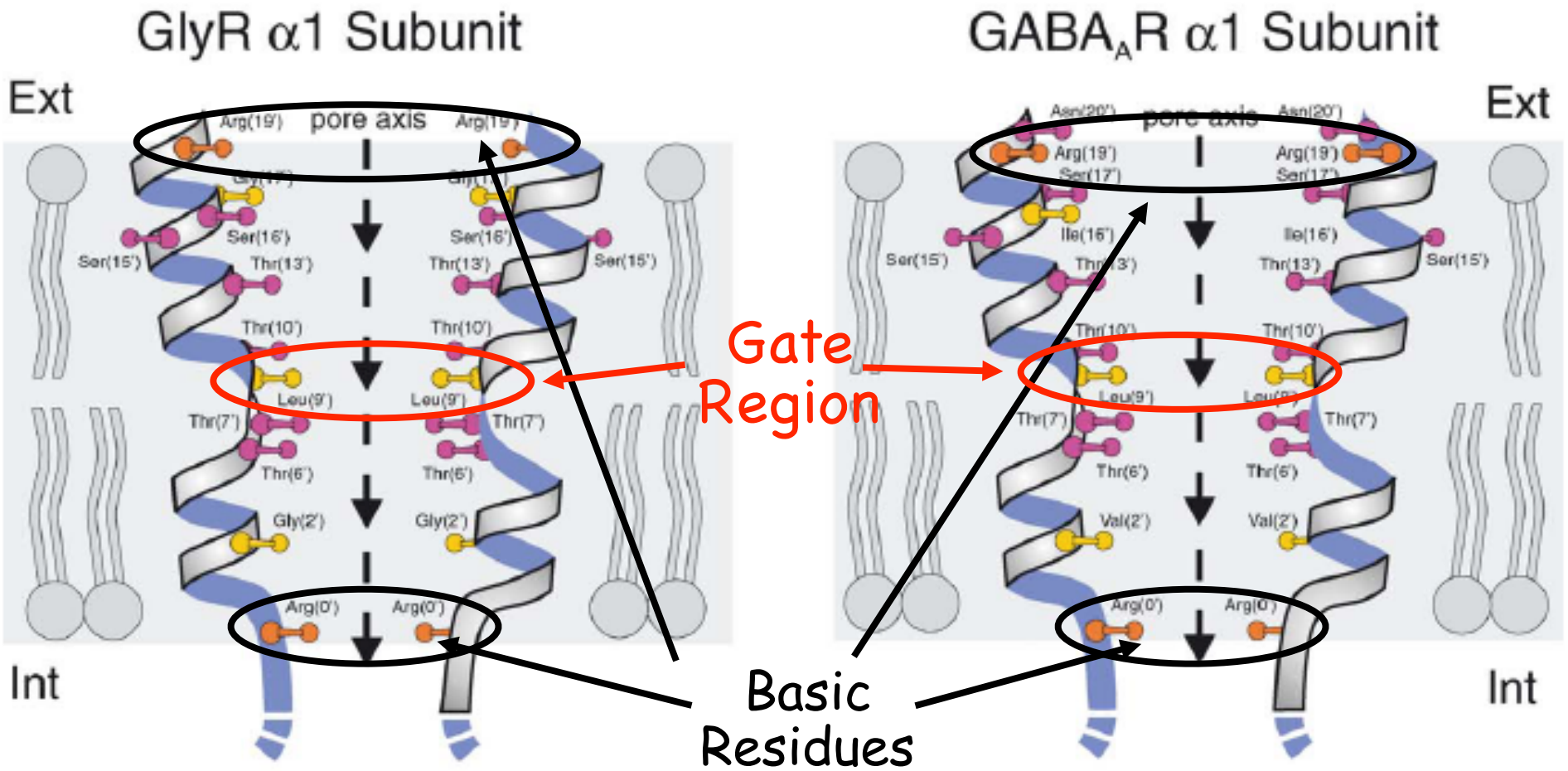
The molecular architecture of the selectivity filter

water is stripped off in the KcsA channel
by backbone carbonyl atoms
->radius is smaller
not possible in the Na channels



Rasband, M. N. (2010) Ion Channels and Excitable Cells. Nature Education 3(9):41

Ion Selection: Chloride Channels

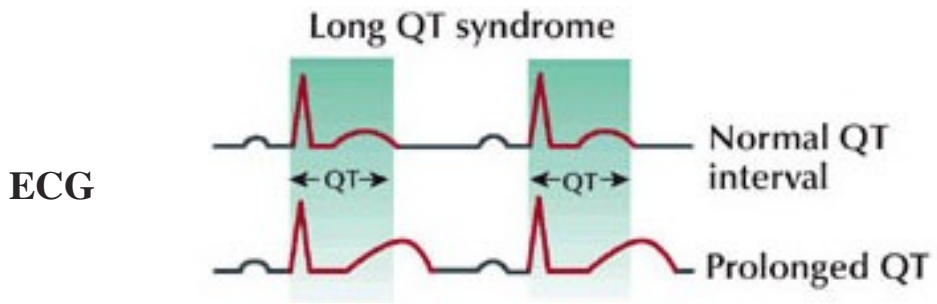


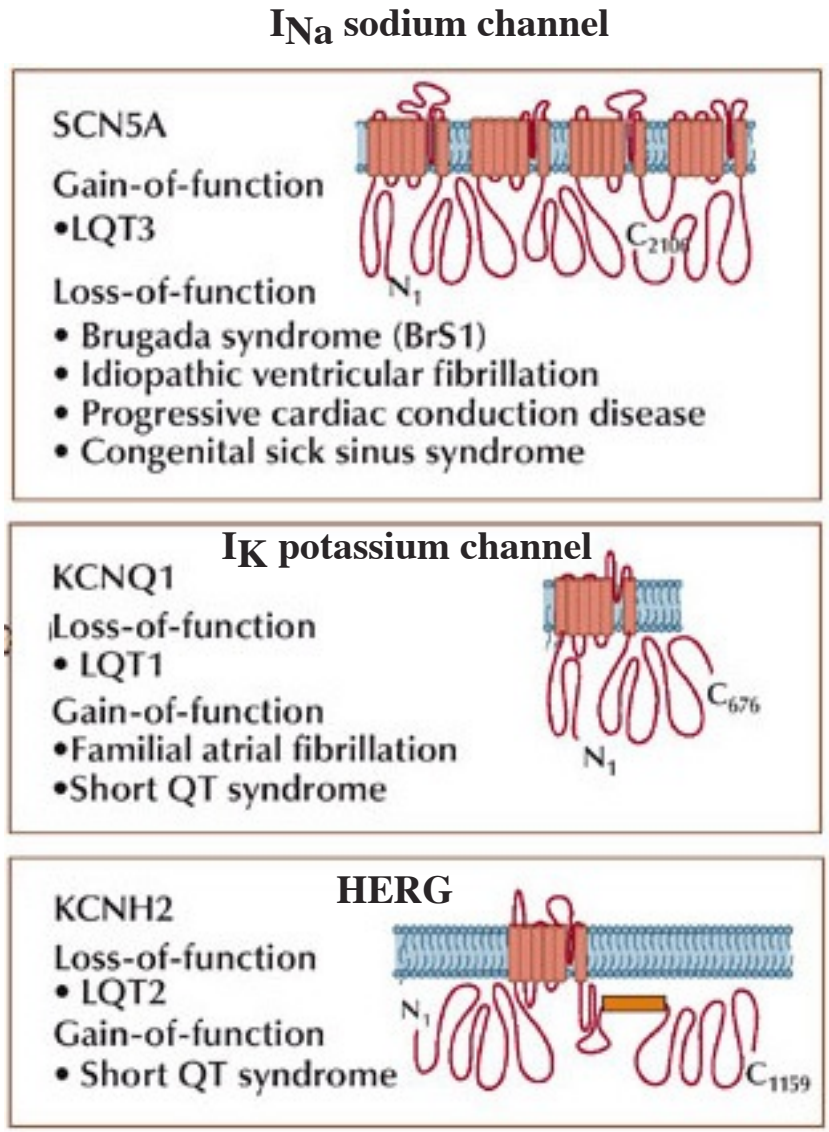
Modified from Keramidas et al., Prog. Biophys. Mol. Biol. 86: 161 (2004)

Ion channels in disease

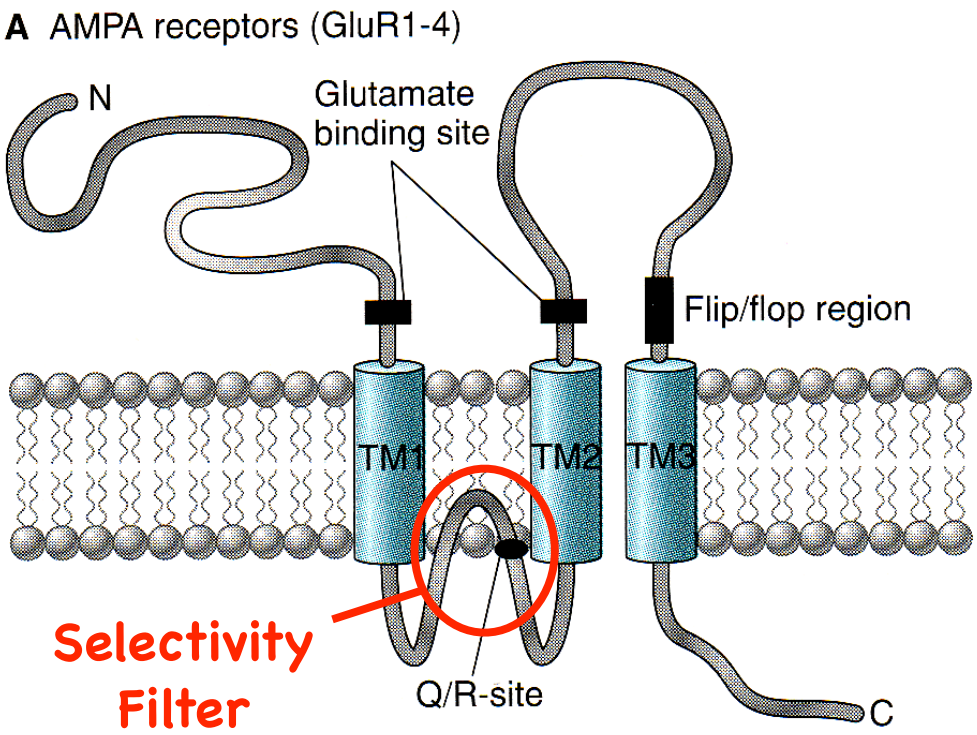
- LQTS is a genetically heterogeneous disease caused by defects in ion channels, affecting 1 in 5,000 persons

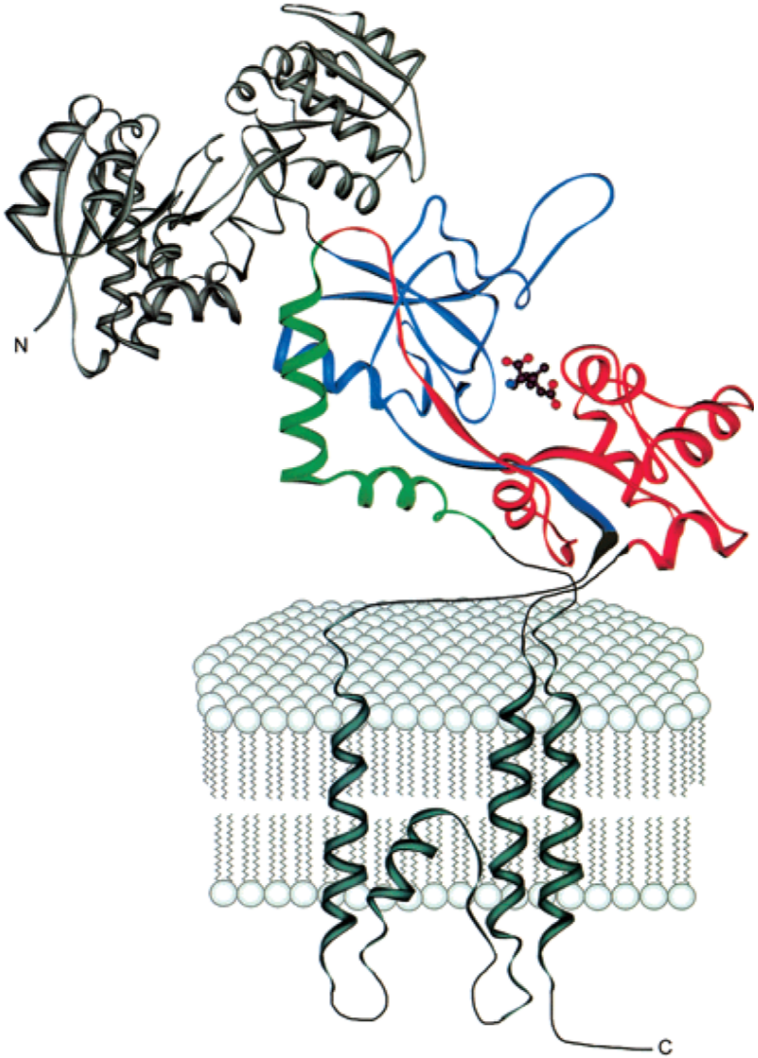
The QT represents a summation of the time required for the ventricles of the heart to electrically recharge (repolarize) in preparation for the next beat,





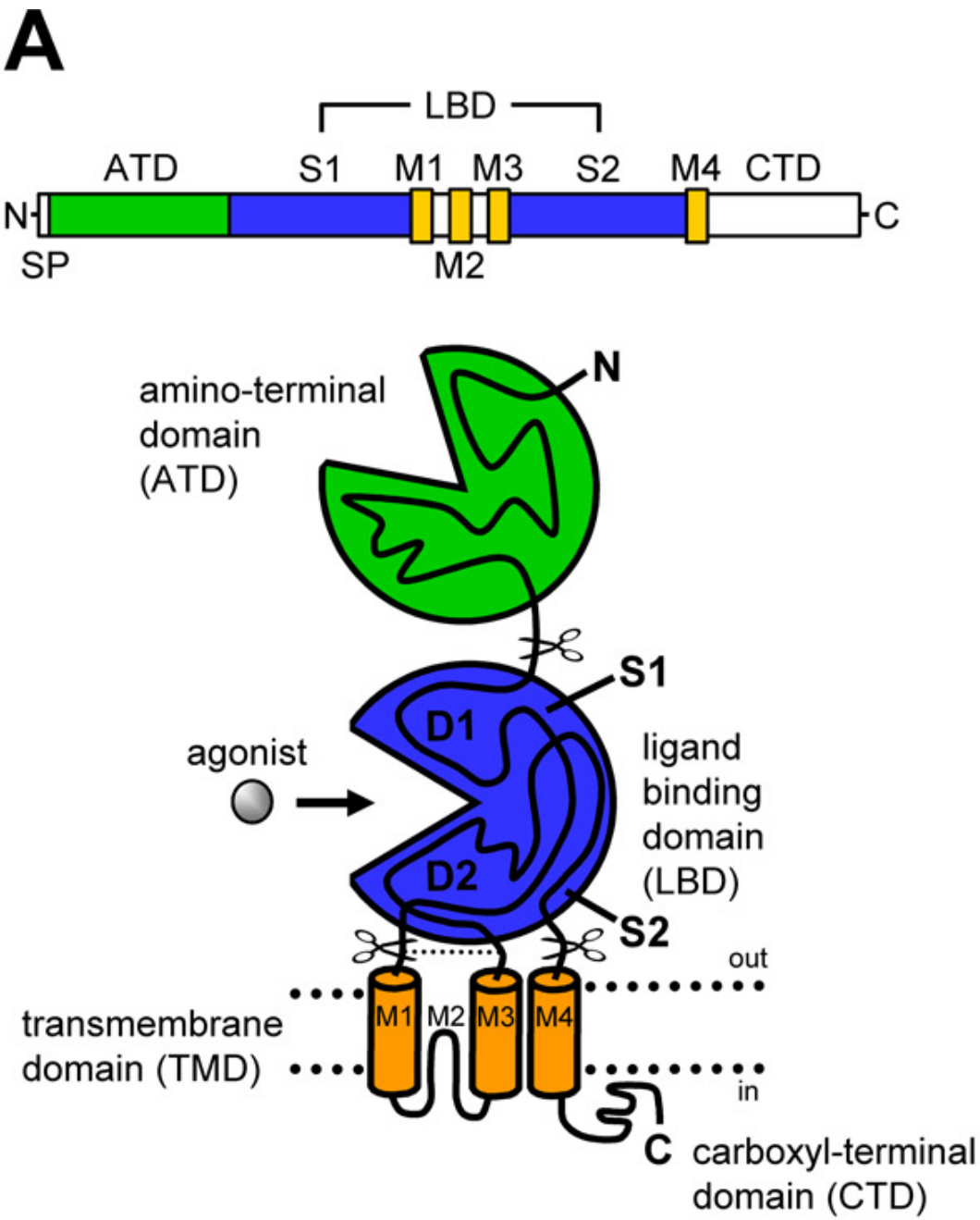
Topology of Glutamate Receptors

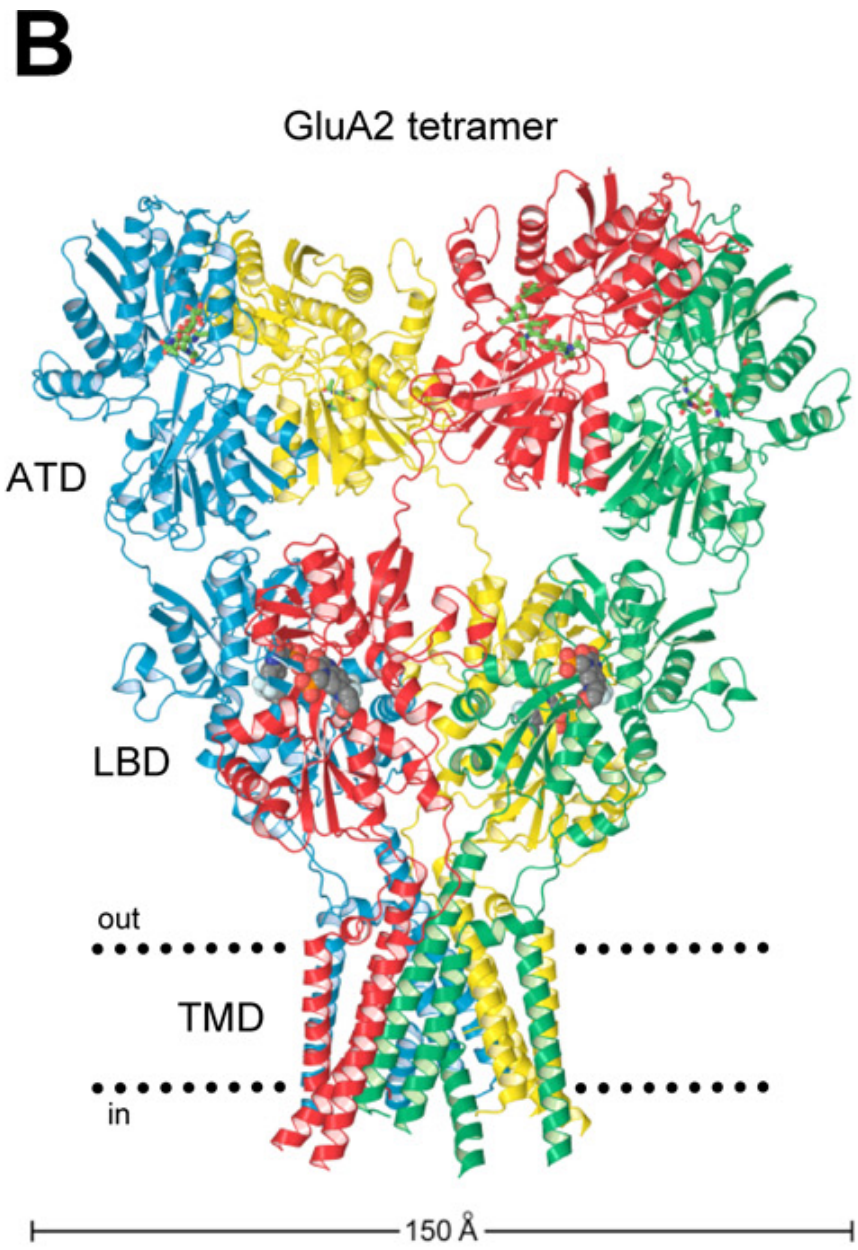




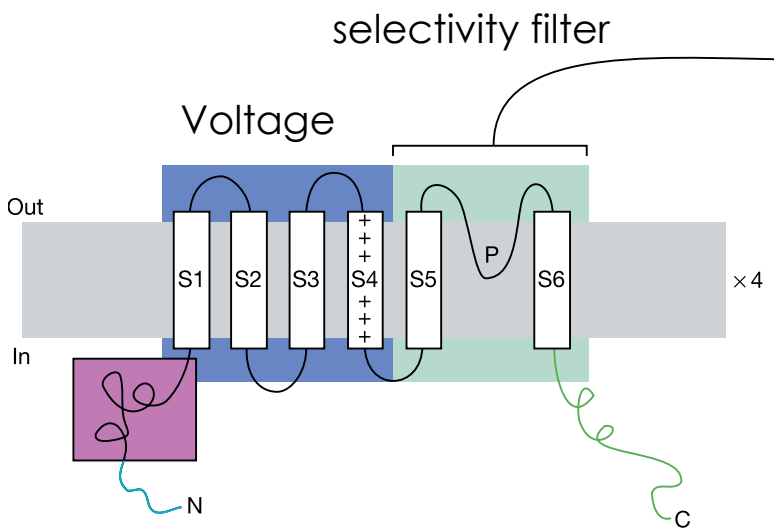
Kandel, Schwartz & Jessel,
Principles of Neural Science 4th Ed. (2000)

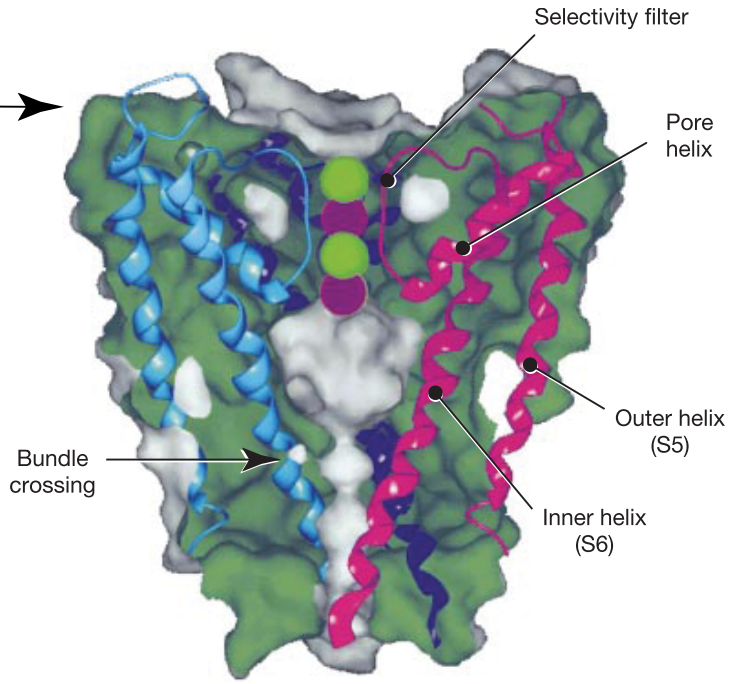
Glutamate is the **most abundant excitatory neurotransmitter** in the vertebrate nervous system. Nerve impulses trigger release of glutamate from the pre-synaptic cell. In the opposing post-synaptic cell, glutamate receptors, such as the NMDA receptor, bind glutamate and are activated. Because of its role in synaptic plasticity, glutamate is involved in cognitive functions like learning and memory in the brain.


A
LBD
ATD
S1
M1
M3
S2
M4
CTD
N
C
SP
M2
amino-terminal
domain
(ATD)
N
S1
D1
agonist
ligand
binding
domain
(LBD)
D2
S2
out
transmembrane
domain (TMD)
M1
M2
M3
M4
in
C
carboxyl-terminal
domain (CTD)


B
GluA2 tetramer
ATD
LBD
out
TMD
in
150 Å

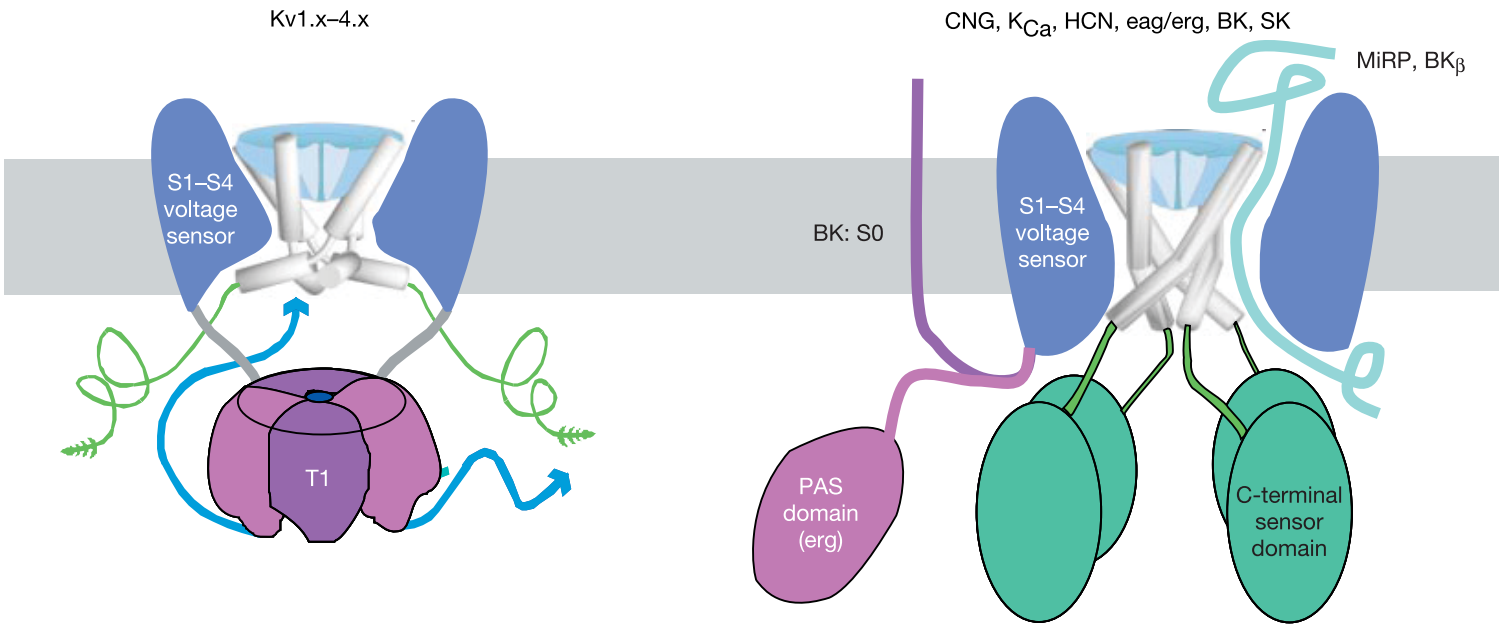
Structural Features of Voltage-Gated Ion Channels



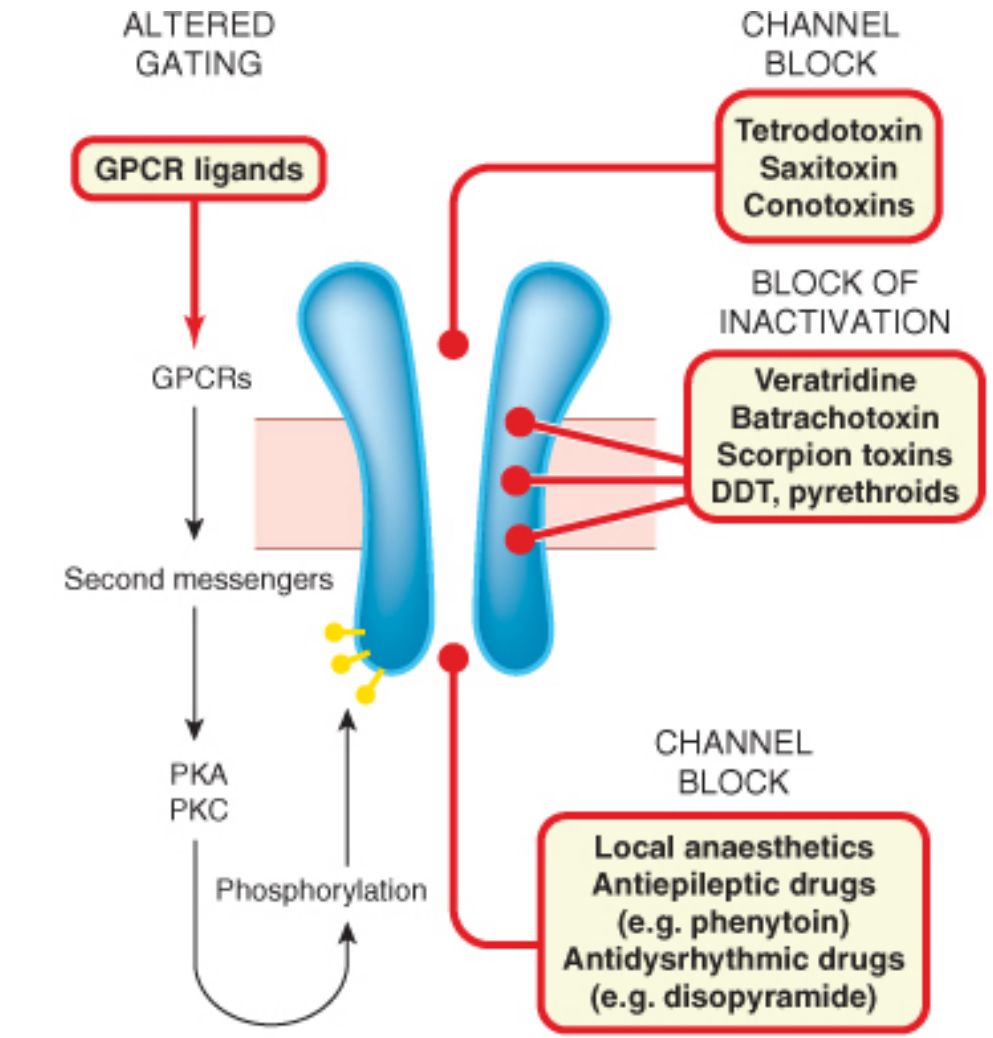


tetramerization domain

C-terminal sensor



Drugs interfering with channels

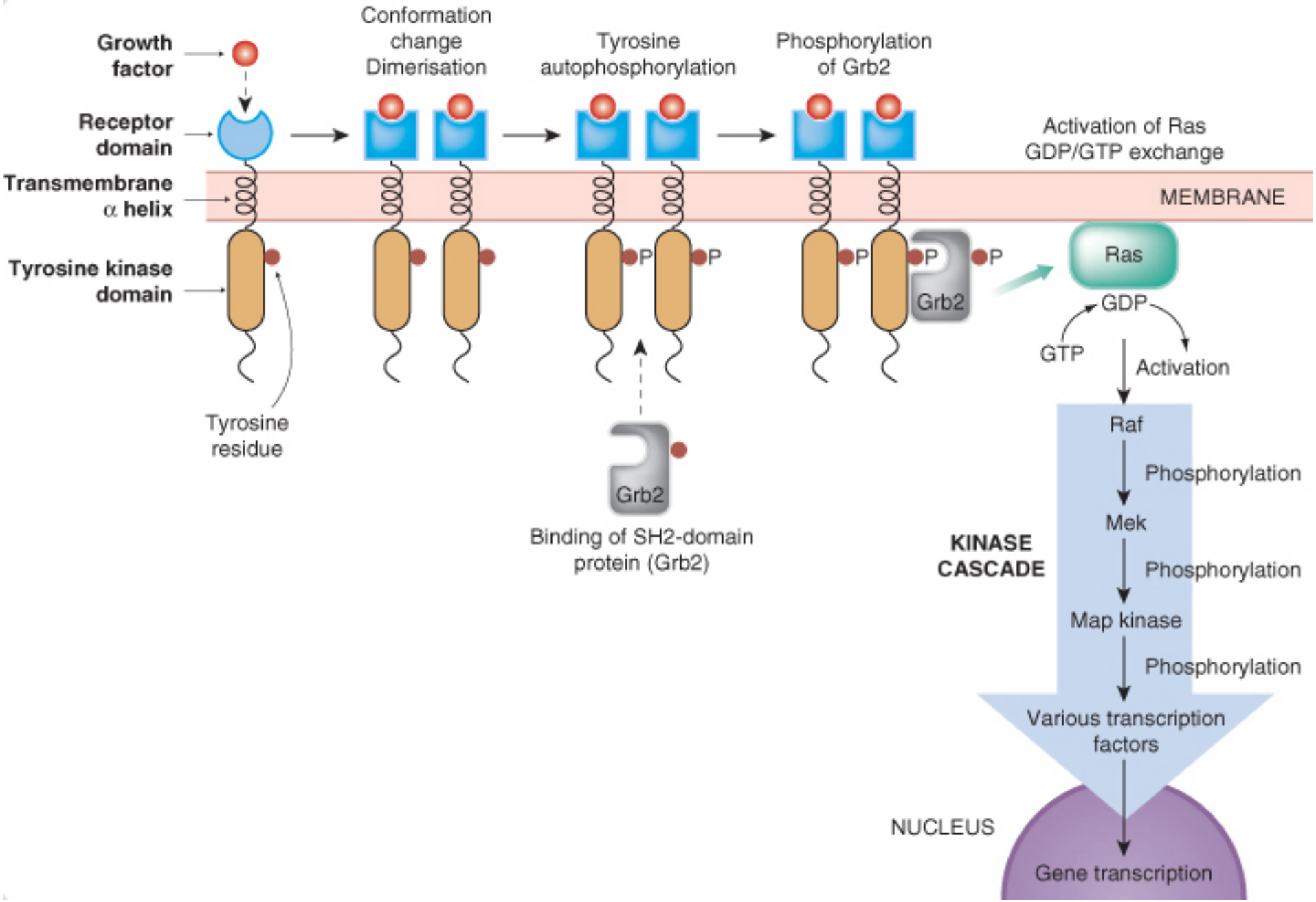


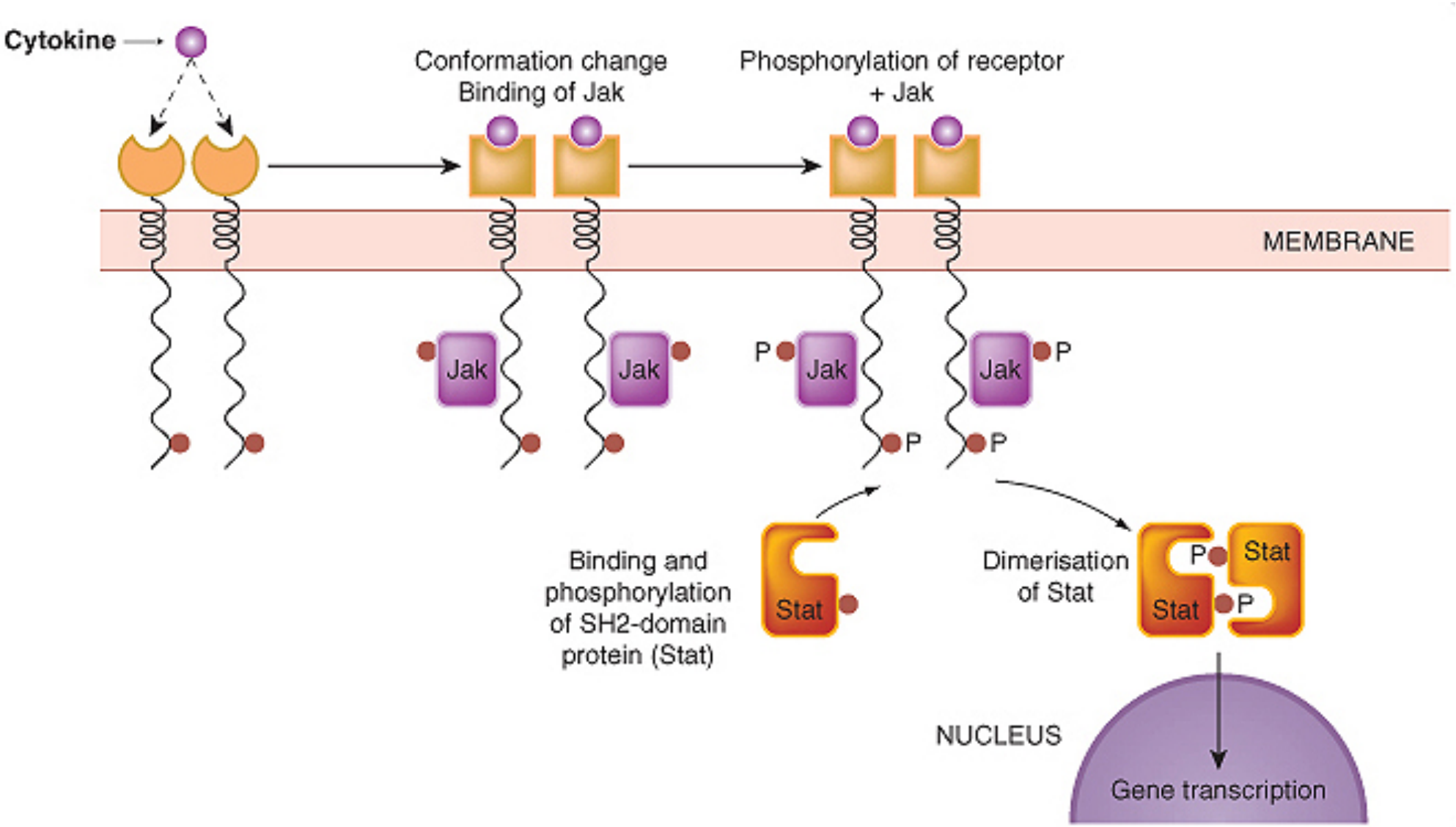
Typ	Activity of drug	Drug examples
Voltage-gated Ca^{2+} channels		
General	Inhibitor	Oxcarbazepine
In Schistosoma sp.	Inhibitor	Praziquantel
L-type channels	Inhibitor	Dihydropyridines, diltiazem, lercanidipine, pregabalin, verapamil
T-type channels	Inhibitor	Succinimides
K^+ channels		
Epithelial K^+ channels	Opener Inhibitor	Diazoxide, minoxidil Nateglinide, sulphonylureas
Voltage-gated K^+ channels	Inhibitor	Amiodarone
Na^+ channels		
Epithelial Na^+ channels (ENaC)	Inhibitor	Amiloride, bupivacaine, lidocaine, procainamide, quinidine
Voltage-gated Na^+ channels	Inhibitor	Carbamazepine, flecainide, lamotrigine, phenytoin, propafenone, topiramate, valproic acid
Ryanodine-inositol 1,4,5-triphosphate receptor Ca^{2+} channel (RIR-CaC) family		
Ryanodine receptors	Inhibitor	Dantrolene
Transient receptor potential Ca^{2+} channel (TRP-CC) family		
TRPV1 receptors	Inhibitor	Acetaminophen (as arachidonylamide)
Cl^- channels		
Cl^- channel	Inhibitor (mast cells) Opener (parasites)	Cromolyn sodium Ivermectin

Type	Activity of drug	Drug examples
Direct ligand-gated ion channel receptors		
$GABA_A$ receptors	Barbiturate binding site agonists	Barbiturate
	Benzodiazepine binding site agonists	Benzodiazepines
	Benzodiazepine binding site antagonists	Flumazenil
Acetylcholine receptors	Nicotinic receptor agonists	Pyrantel (of Angiostrongylus), levamisole
	Nicotinic receptor stabilizing antagonists	Alcuronium
	Nicotinic receptor depolarizing antagonists	Suxamethonium
	Nicotinic receptor allosteric modulators	Galantamine
Glutamate receptors (ionotropic)	NMDA subtype antagonists	Memantine
	NMDA subtype expression modulators	Acamprosate
	NMDA subtype phencyclidine binding site antagonists	Ketamine

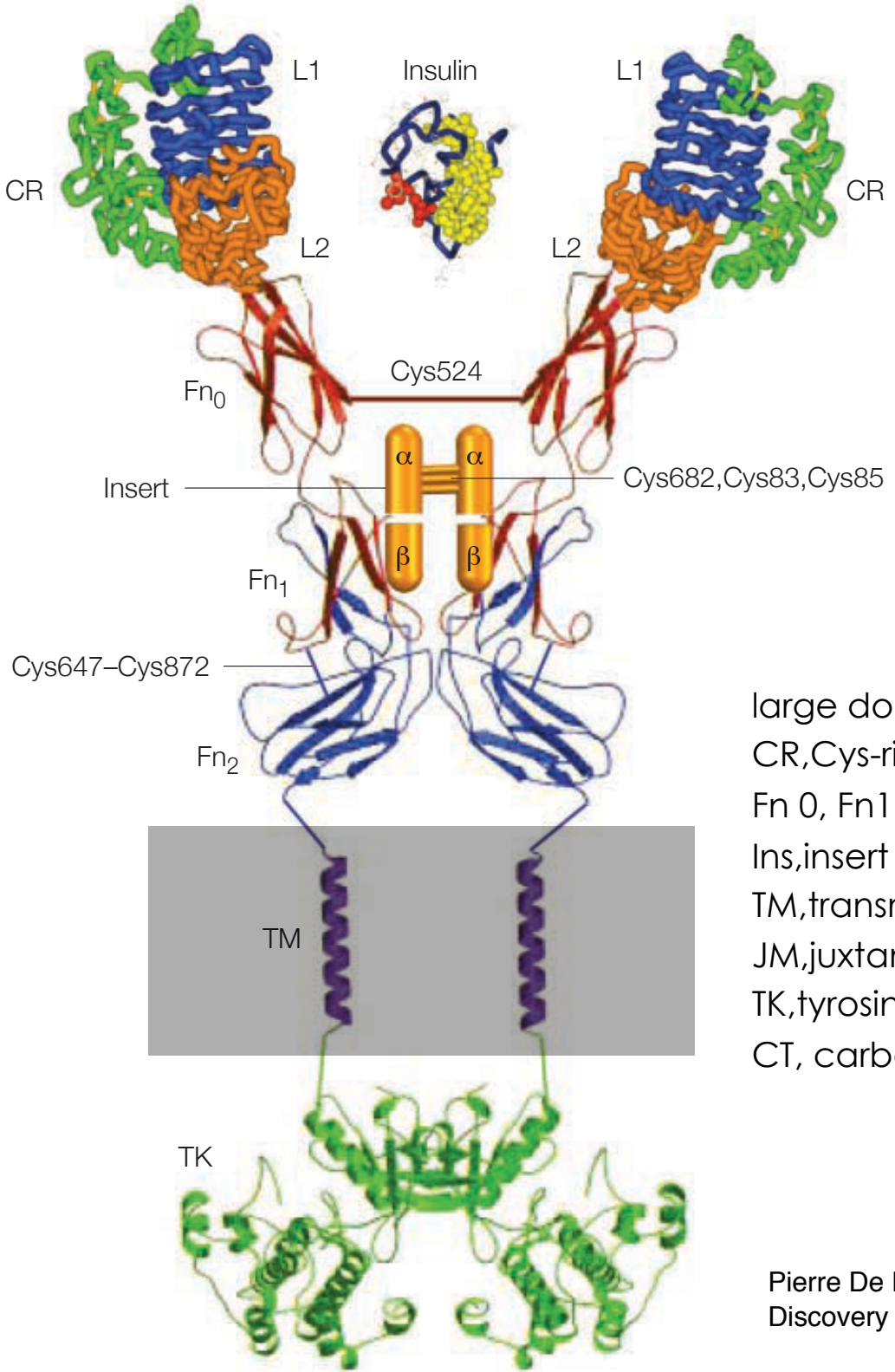
Kinase-linked receptors

- receptors for hormones and growth factors
- cytokine receptors have an intracellular domain that binds and activates cytosolic kinases when the receptor is activated
- the receptors have a large extracellular ligand-binding domain, a TM single helical domain, and a intracellular domain
- upon activation the receptor dimerizes, followed by autophosphorylation of Tyr residues.
- The P-Tyr residues bind to SH2 domains and many intracellular proteins
- 2 important pathways:
  - the RAS/Raf/MAP kinase pathway (cell division, growth and differentiation)
  - Jak/Stat pathway (stimulated by cytokines; control of synthesis and release of inflammatory mediators)


Growth factor
Receptor domain
Transmembrane α helix
Tyrosine kinase domain
Tyrosine residue
Conformation change Dimerisation
Tyrosine autophosphorylation
Phosphorylation of Grb2
Grb2
Binding of SH2-domain protein (Grb2)
Activation of Ras GDP/GTP exchange
MEMBRANE
Ras
GDP
GTP
Activation
Raf
Phosphorylation
Mek
Phosphorylation
Map kinase
Phosphorylation
KINASE CASCADE
Various transcription factors
NUCLEUS
Gene transcription



Employing **Ja**nus **k**inases (JAKs) or and **S**ignal **T**ransducers and **A**ctivators of **T**ranscription (STATs), the pathway transduces the signal carried by these extracellular polypeptides **into the cell nucleus**, where activated STAT proteins modify gene expression.

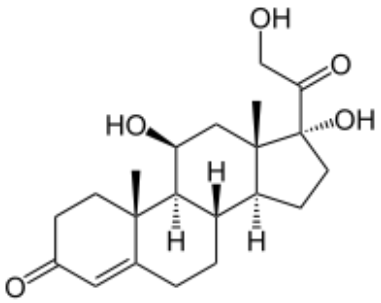


large domains L1 and L2 (leucine-rich repeats);
CR,Cys-rich domain;
Fn 0, Fn1, Fn2,fibronectin type III domains;
Ins,insert in Fn 1;
TM,transmembrane domain;
JM,juxtamembrane domain;
TK,tyrosine-kinase domain;
CT, carboxy-terminal tail.

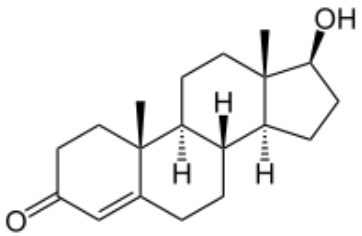
Pierre De Meyts, Jonathan Whittaker, Nature Reviews Drug Discovery 1, 769 - 783 (01 Oct 2002)

Nuclear Receptors

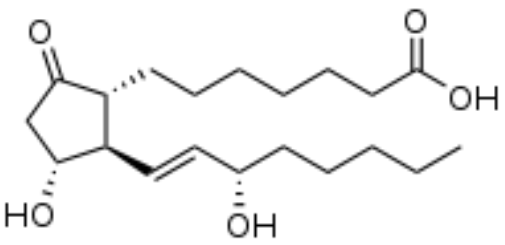
- gated by ligands such as steroids, hormones, vitamin D and retinoic acid
- act as transcription factors that regulate gene expression



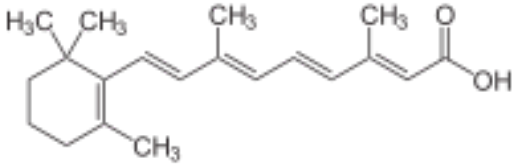
Cortisol



Testosteron

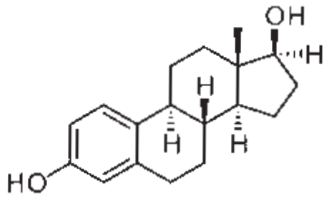


Prostaglandine

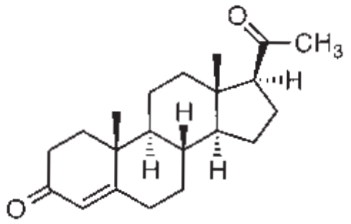


Vitamin A

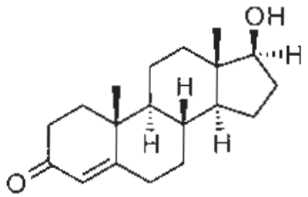
STEROIDS



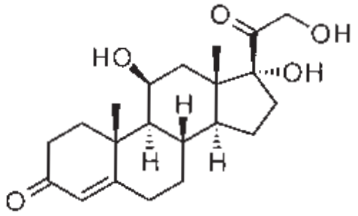
17β-Estradiol
(Estrogen Receptor)



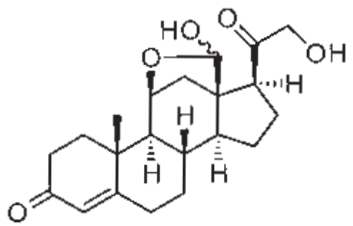
Progesterone
(Progesterone Receptor)



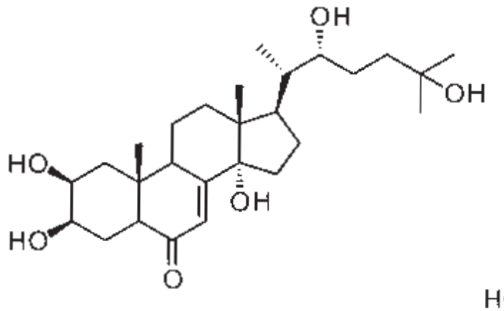
Testosterone
(Androgen Receptor)



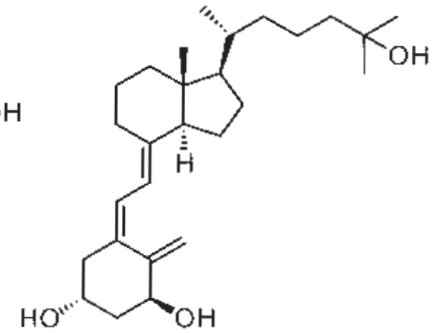
Cortisol
(Glucocorticoid Receptor)



Aldosterone
(Mineralocorticoid Receptor)

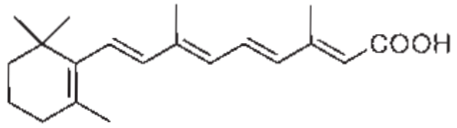


Ecdysone
(Ecdysone Receptor)

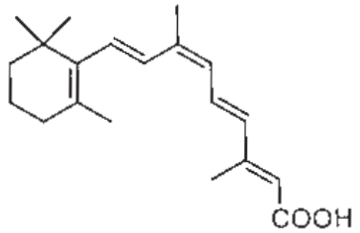


1α,25-Dihydroxyvitamin D_3
(Vitamin D Receptor)

RETINOIDS

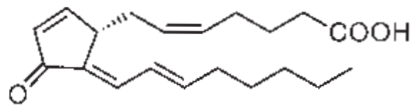


All-*trans* Retinoic Acid
(Retinoic Acid Receptor)



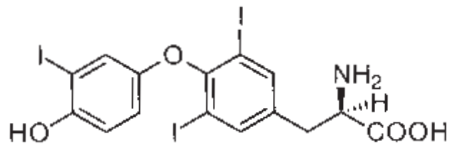
9-*cis* Retinoic Acid
(Retinoid X Receptor)

EICOSANOID



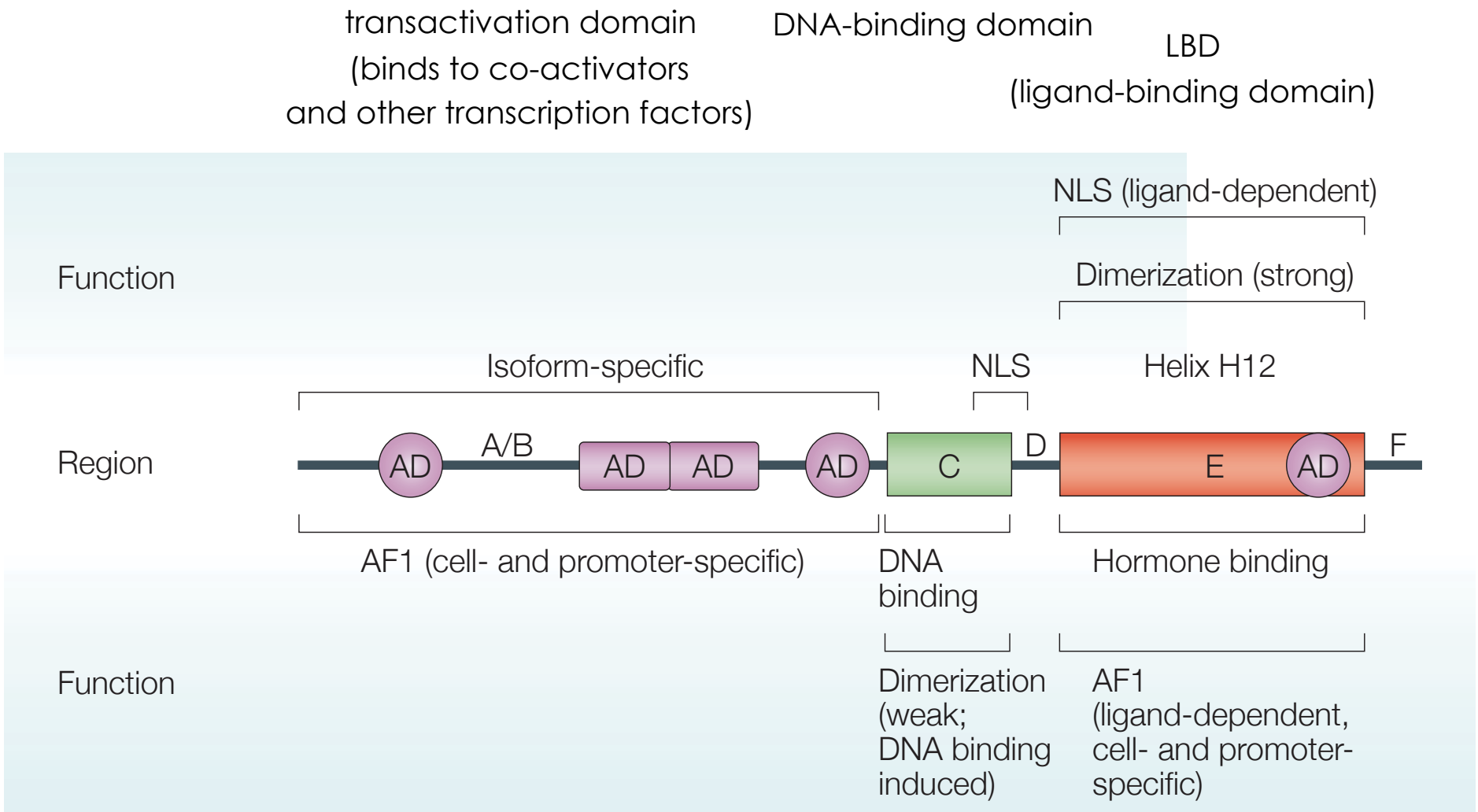
15-Deoxy-$\Delta^{12,14}$-Prostaglandin J_2
(PPAR-γ)

THYRONINE

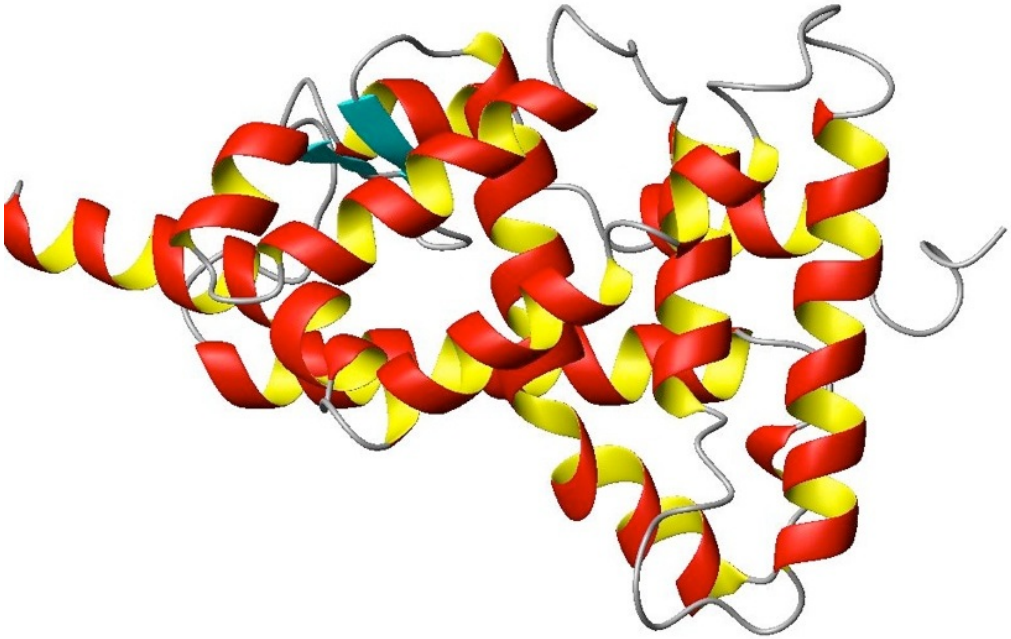


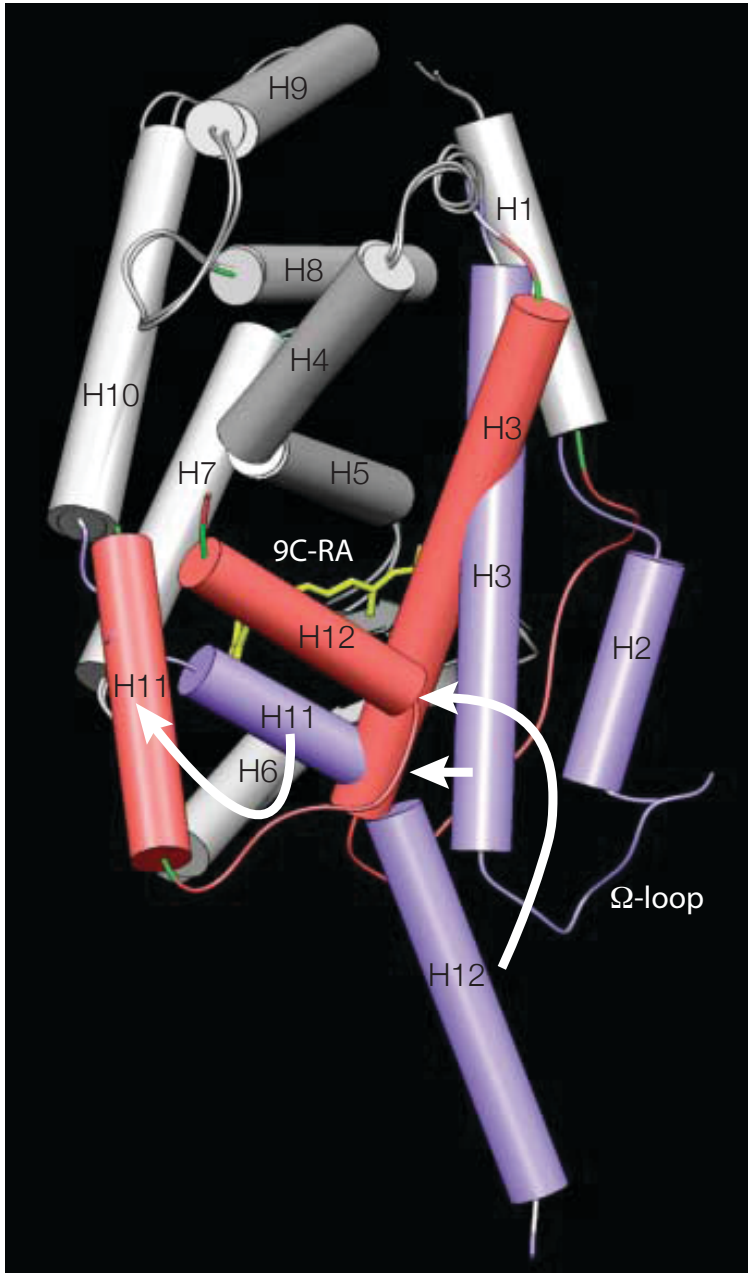
3,5,3'-L-Triiodothyronine
(Thyroid Hormone Receptor)

Structure of Nuclear Receptors



Structure of the ligand binding domain of the retinoic X receptor



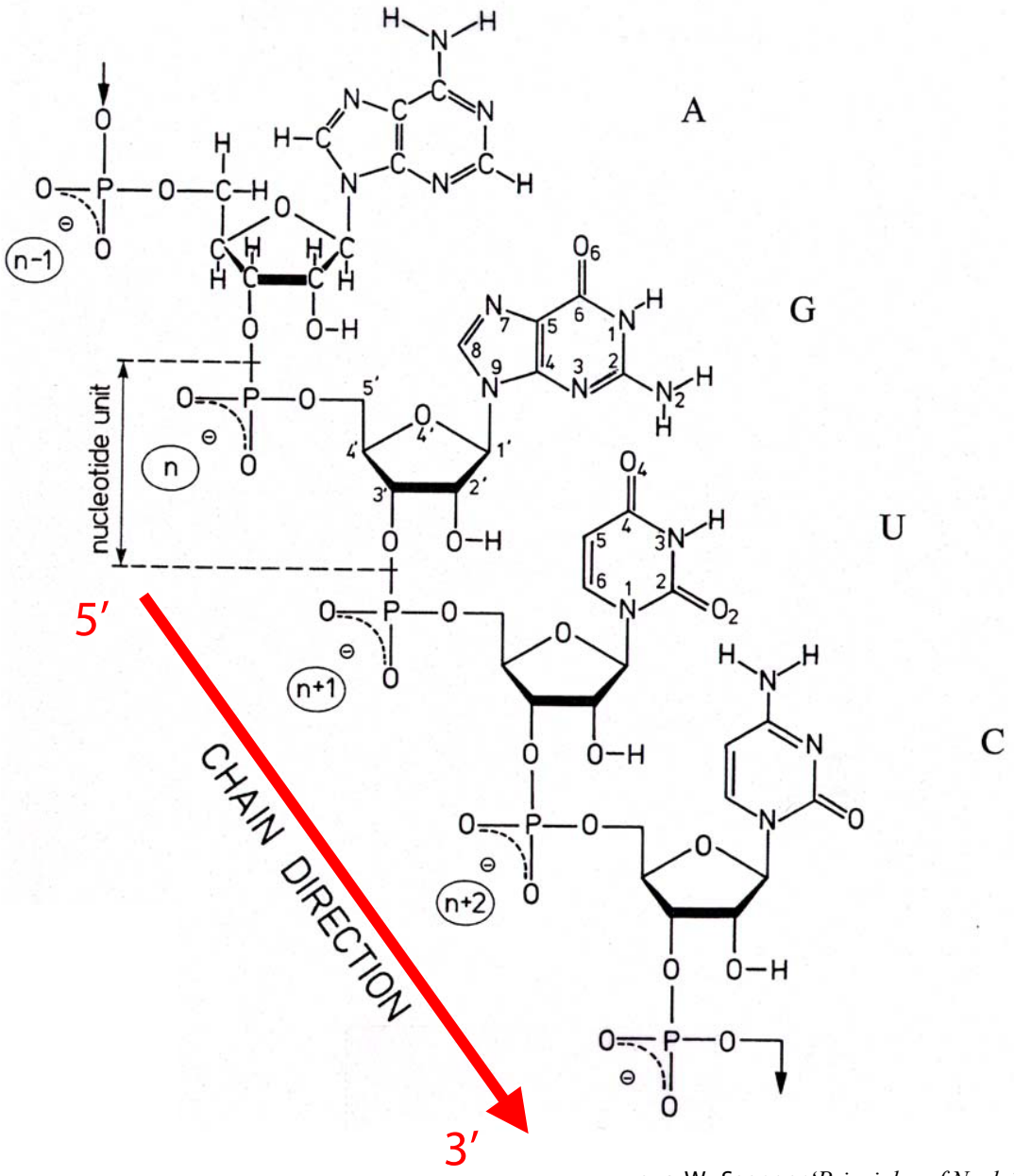


Drugs targeting nuclear receptors

Nuclear receptors (steroid hormone receptors)		
Mineralocorticoid receptor	Agonists	Aldosterone
	Antagonists	Spironolactone
Glucocorticoid receptor	Agonists	Glucocorticoids
Progesterone receptor	Agonists	Gestagens
Oestrogen receptor	Agonists	Oestrogens
	(Partial) antagonists	Clomifene
	Antagonists	Fulvestrant
	Modulators	Tamoxifen, raloxifene
Androgen receptor	Agonists	Testosterone
	Antagonists	Cyproterone acetate
Vitamin D receptor	Agonists	Retinoids
ACTH receptor agonists	Agonists	Tetracosactide (also known as cosyntropin)
Nuclear receptors (other)		
Retinoic acid receptors	RARα agonists	Isotretinoin
	RARβ agonists	Adapalene, isotretinoin
	RARγ agonists	Adapalene, isotretinoin
Peroxisome proliferator-activated receptor (PPAR)	PPARα agonists	Fibrates
	PPARγ agonists	Glitazones
Thyroid hormone receptors	Agonists	L-Thyroxine

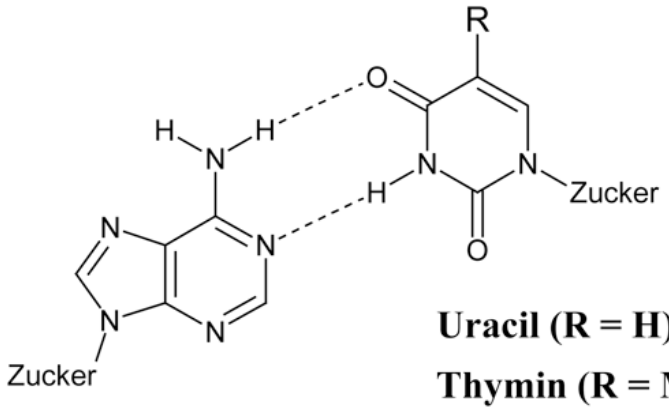
Nucleic Acids

Target	Activity of drug	Example drugs
Nucleic acids		
DNA and RNA	Alkylation	Chlorambucil, cyclophosphamide, dacarbazine
	Complexation	Cisplatin
	Intercalation	Doxorubicin
	Oxidative degradation	Bleomycin
	Strand breaks	Nitroimidazoles
RNA	Interaction with 16S-rRNA	Aminoglycoside antiinfectives
	Interaction with 23S-rRNA	Macrolide antiinfectives
	23S-rRNA/tRNA/2-polypeptide complex	Oxazolidinone antiinfectives
Spindle	Inhibition of development	Vinca alkaloids
	Inhibition of desaggregation	Taxanes
Inhibition of mitosis	—	Colchicine
Ribosome		
30S subunit (bacterial)	Inhibitors	Tetracyclines
50S subunit (bacterial)	Inhibitors	Lincosamides, quinupristin–dalfopristin



aus: W. Saenger *'Principles of Nucleic Acid Structure'* 1984, Springer

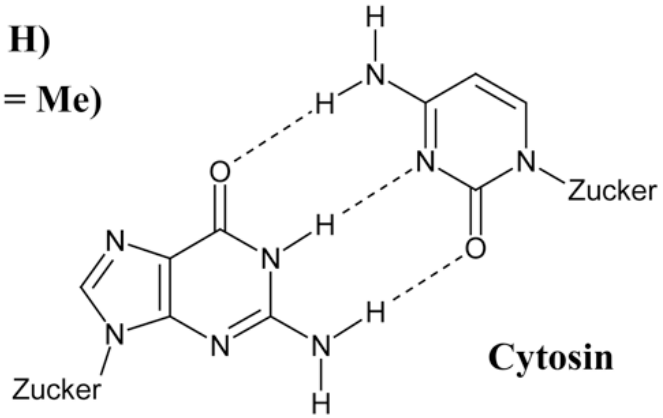
Geometry of Watson-Crick Base Pairs



Adenin

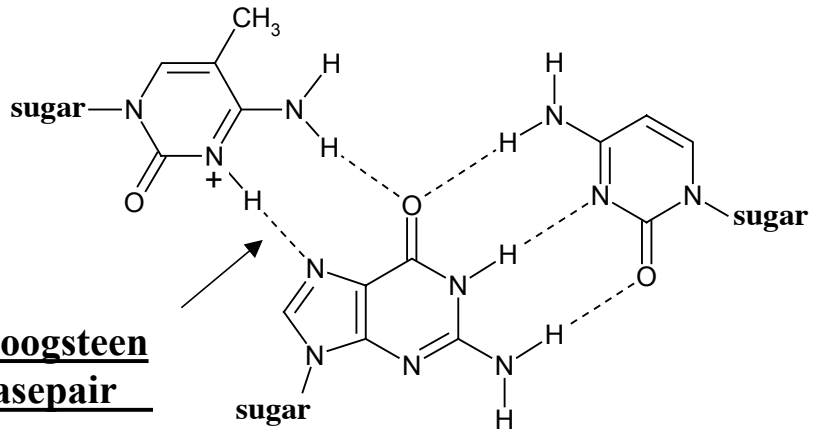
Uracil (R = H)

Thymin (R = Me)



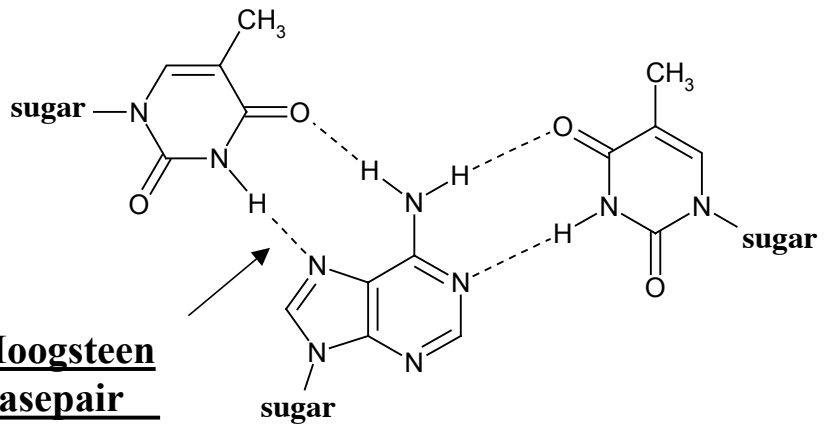
Cytosin

Guanin



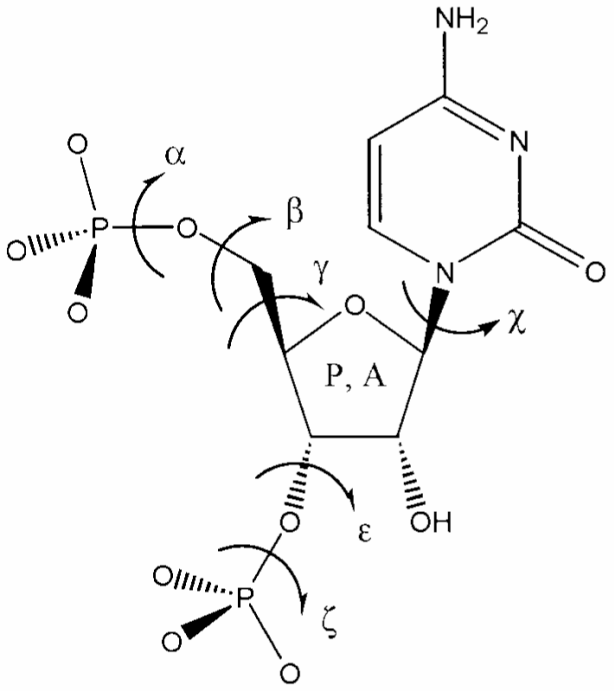
Hoogsteen basepair

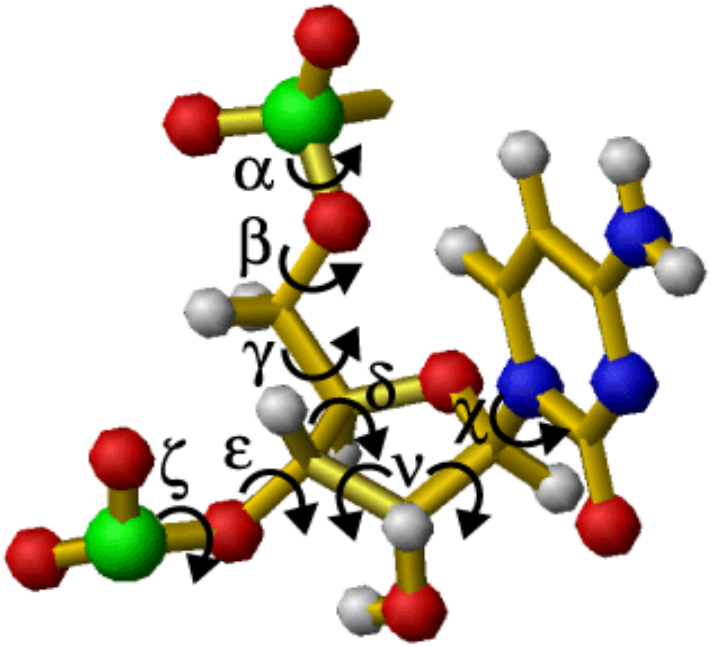
C+GC



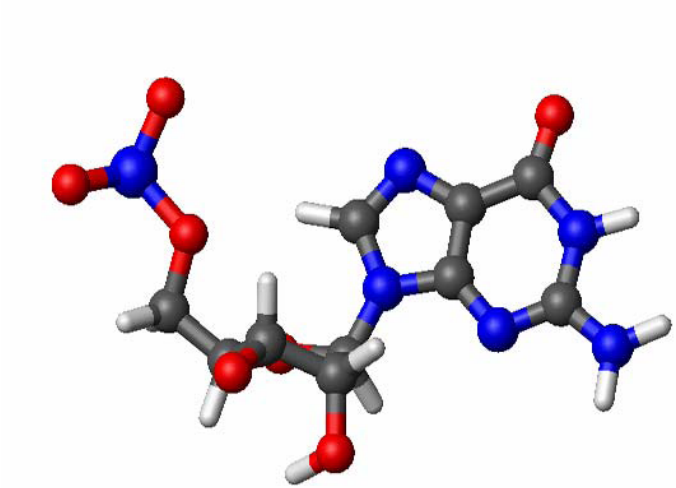
Hoogsteen basepair

TAT

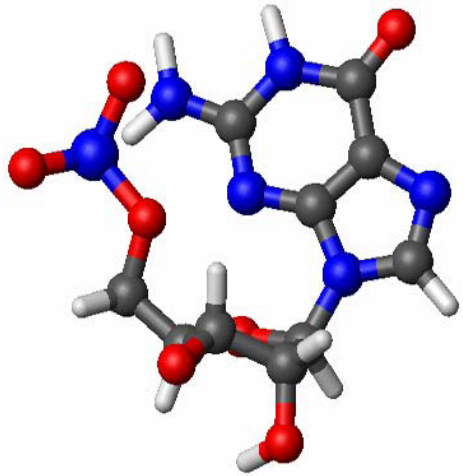




Nucleic Acid Structure involves many rotatable bonds

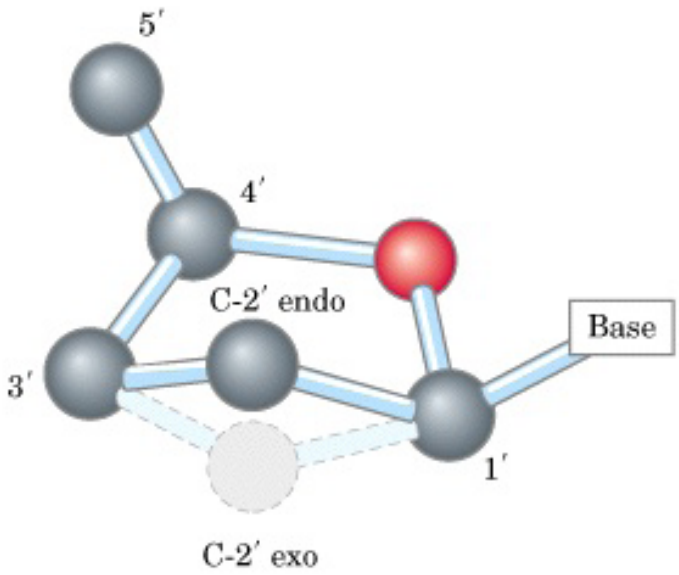


anti

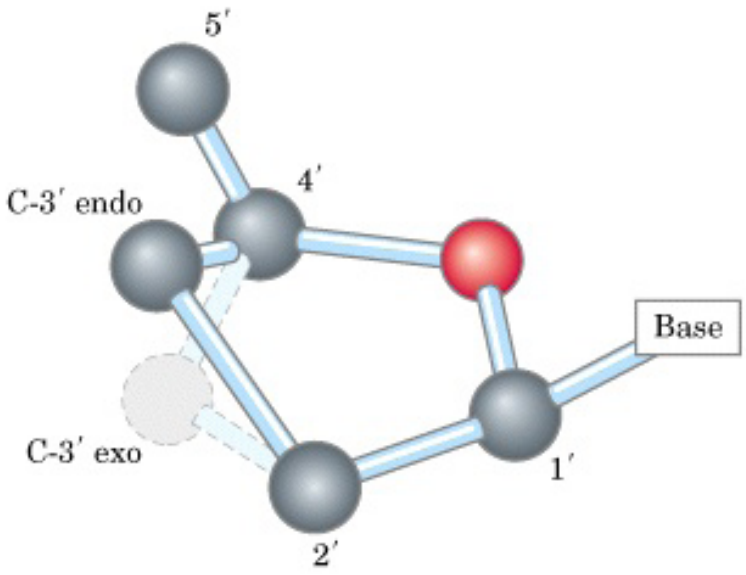


syn

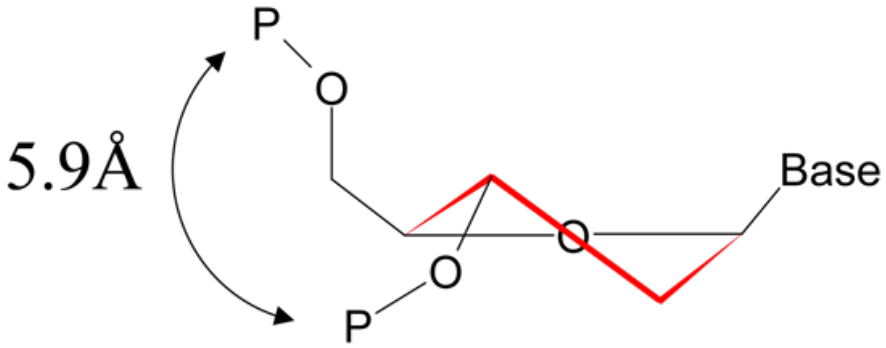
In helical oligonucleotides the glycosidic bond is usually anti



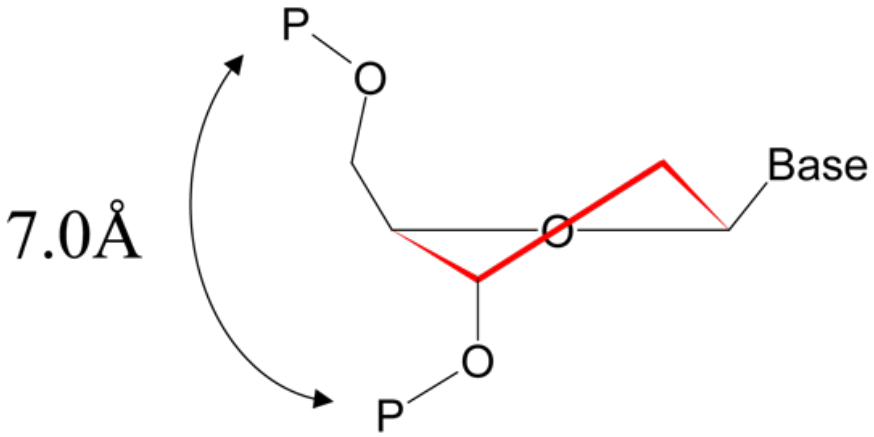
C2’-endo “South” (DNA)



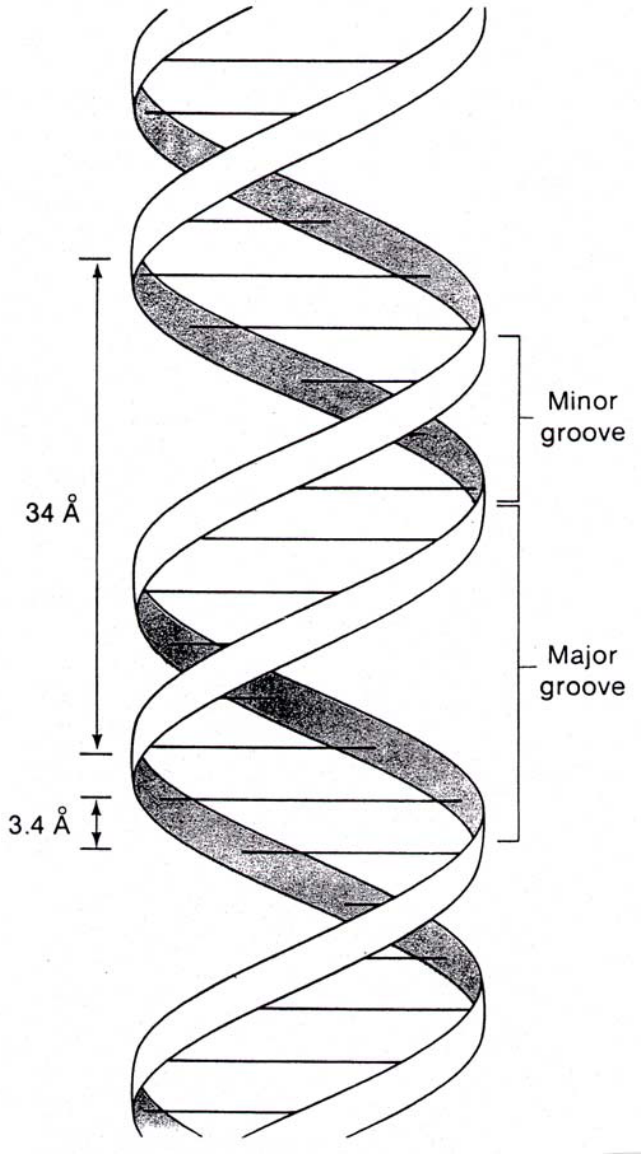
C3’-endo “North” (RNA)

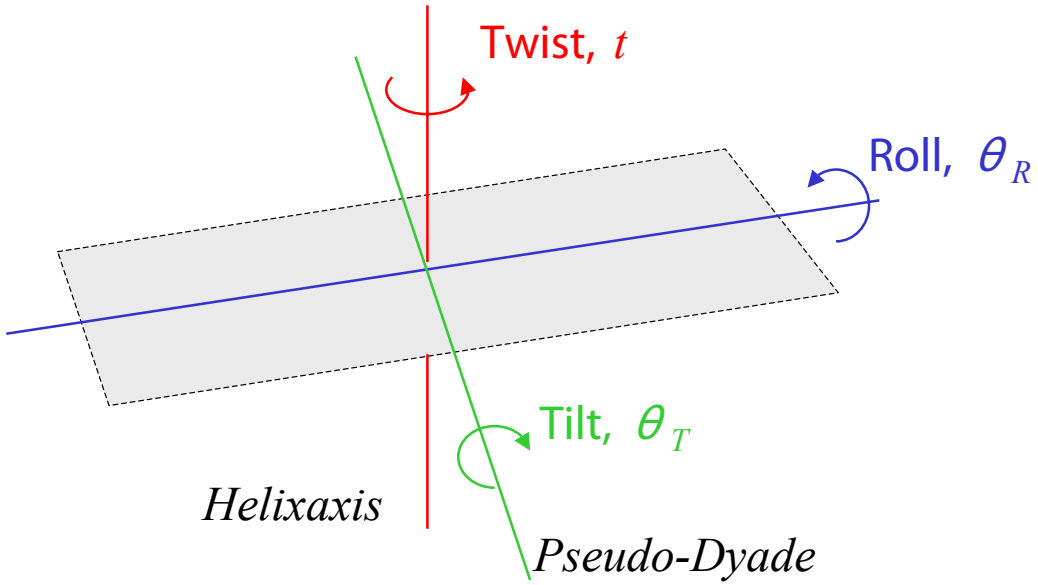


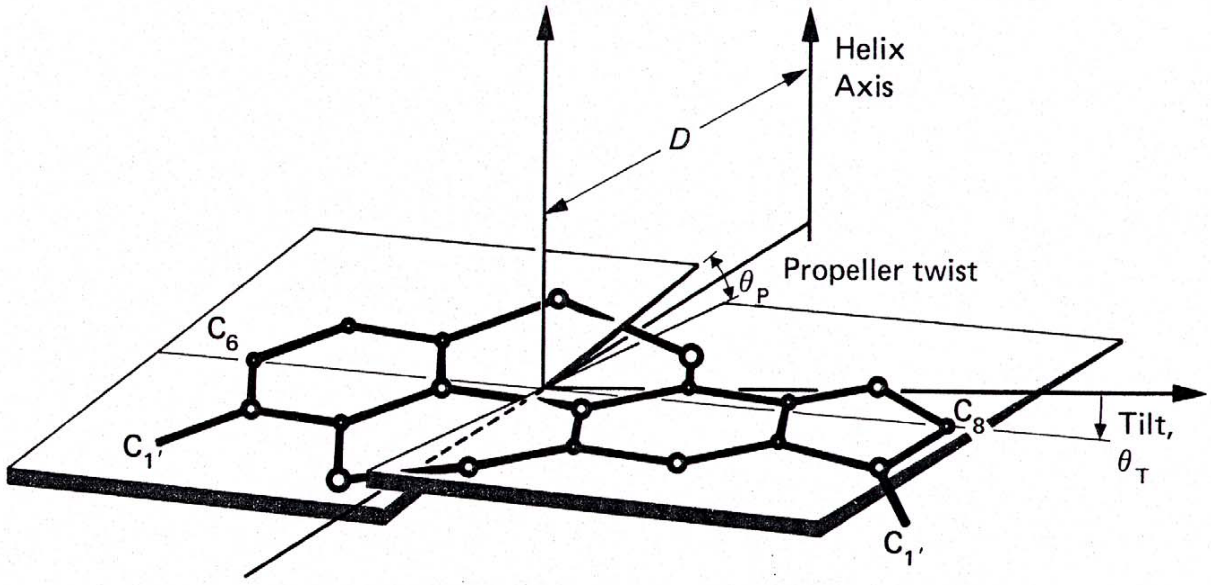
C3'-endo
(A-Konformation)



C2'-endo
(B-Konformation)

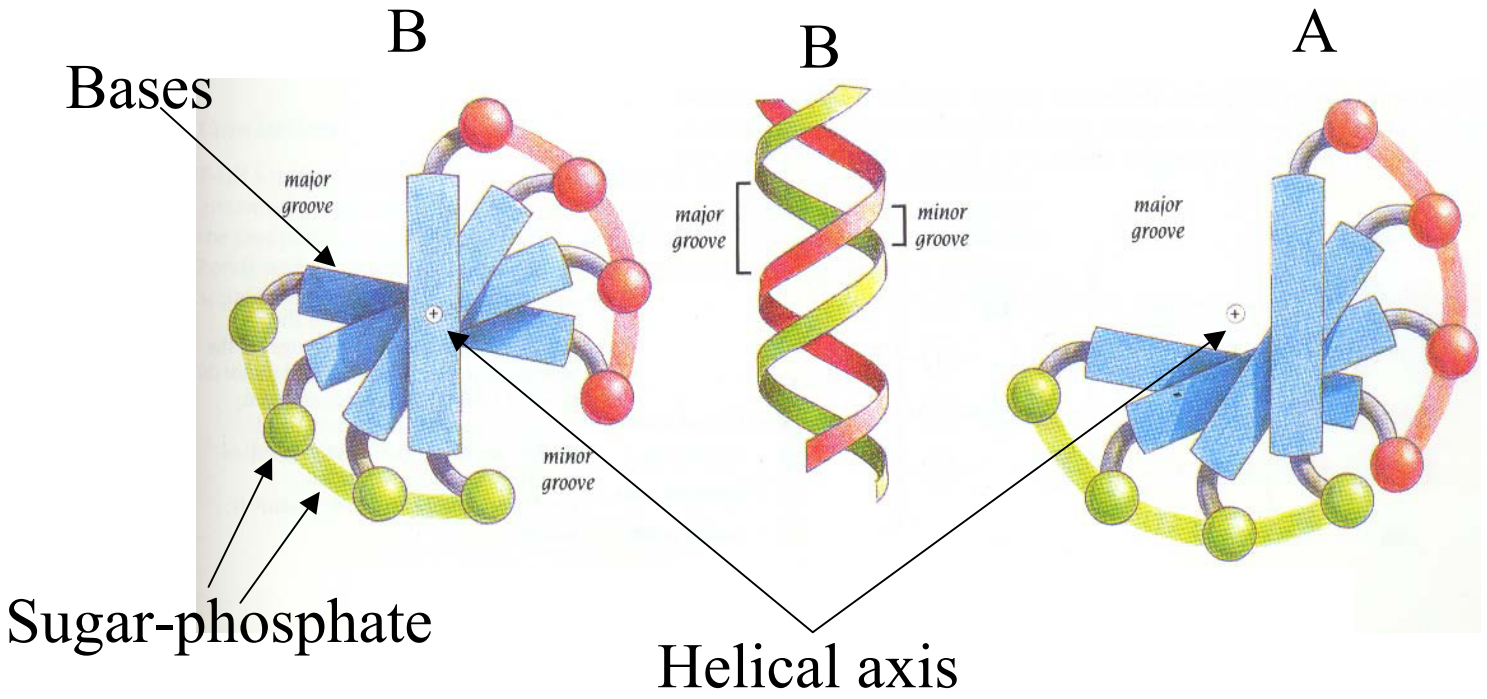






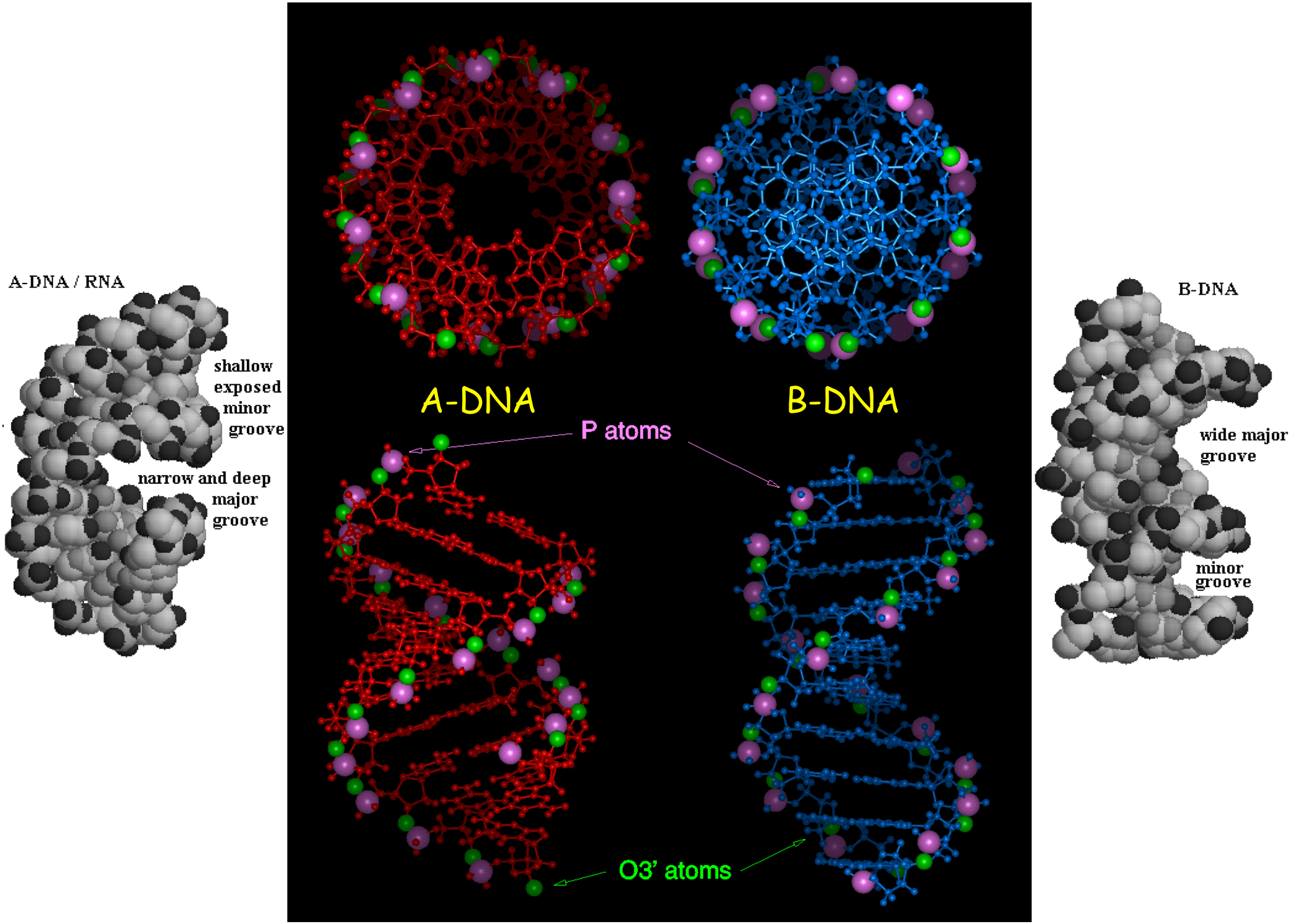
W. Saenger '*Principles of Nucleic Acid Structure*' 1984, Springer

DNA Helices

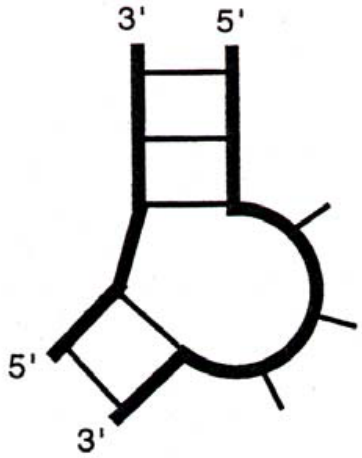


A-DNA Helical axis is shifted from the center of the bases into the major groove. Base pairs are not perpendicular to the axis. They are tilted 13° to 19°. Major groove is deep, minor groove is shallow.

B-DNA Helical axis runs through the center of each base pair which are stacked almost perpendicular to the axis. Major and minor grooves are the same depth, but major groove is wider.

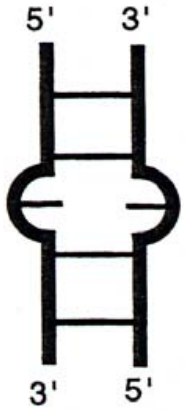

A-DNA / RNA
shallow exposed minor groove
narrow and deep major groove
A-DNA
B-DNA
P atoms
O3' atoms
B-DNA
wide major groove
minor groove

single nucleotide bulge



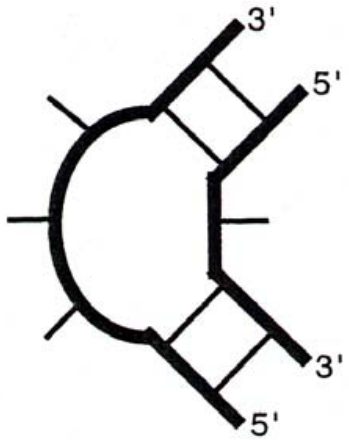
three nucleotide bulge

hairpin loop



mismatch pair
or, symmetric internal
loop of 2 nucleotides

symmetric internal loop



asymmetric internal loop

Drugs acting on DNA/RNA

- Intercalating agents
    - Topoisomerase II
    - Example – Proflavine
    - Examples – antimalarial agents
- Alkylating agents
- Chain cutters
- Antisense DNA
- siRNA
- CRISPR/CAS

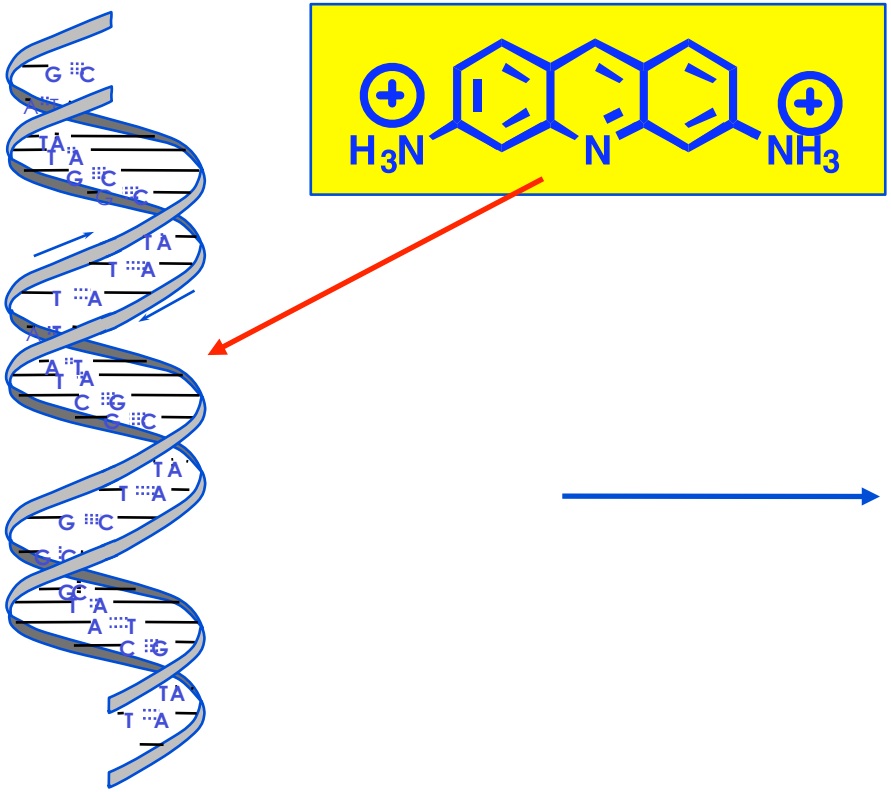
Intercalating agents

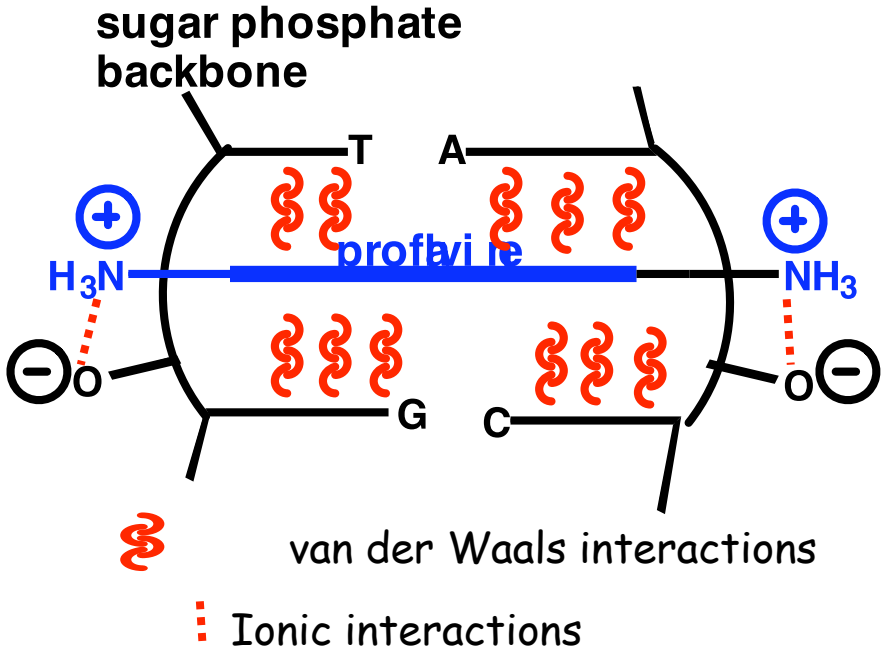
Mechanism of action

- Contain planar aromatic or heteroaromatic ring systems
- Planar systems slip between the layers of nucleic acid pairs and disrupt the shape of the helix
- Preference is often shown for the minor or major groove
- Intercalation prevents replication and transcription
- Intercalation inhibits topoisomerase II (an enzyme that relieves the strain in the DNA helix by temporarily cleaving the DNA chain and crossing an intact strand through the broken strand.

Intercalating agents

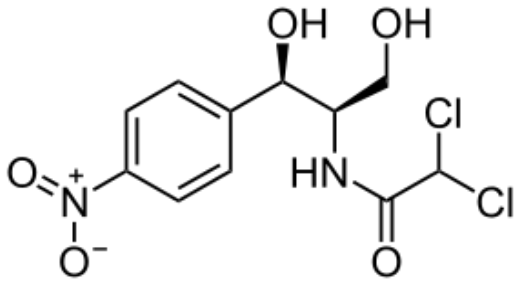
Proflavine



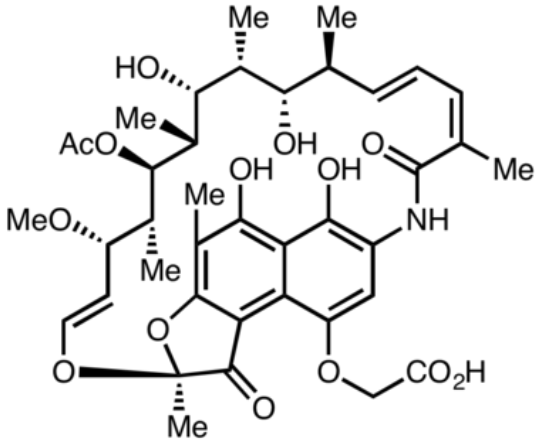


- Planar tricyclic system
- The amino substituents are protonated and charged
- Used as a topical antibacterial agent in the second world war
- Targets bacterial DNA
- Too toxic for systemic use

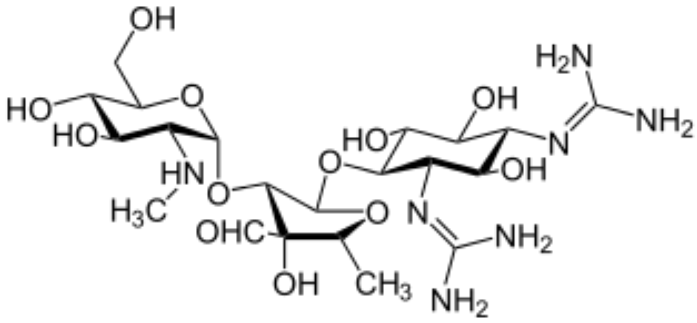
Drugs acting on rRNA: Antibiotics



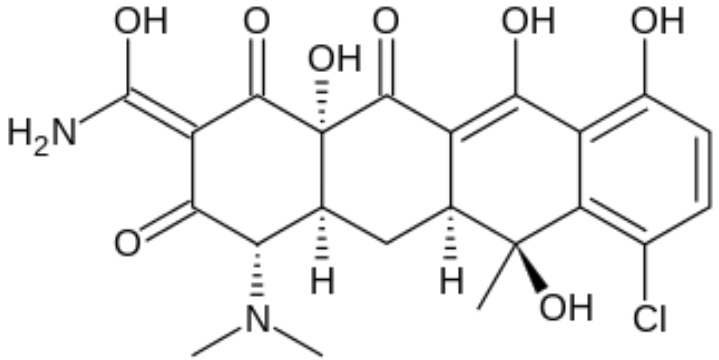
Chloramphenicol
(vs typhoid)



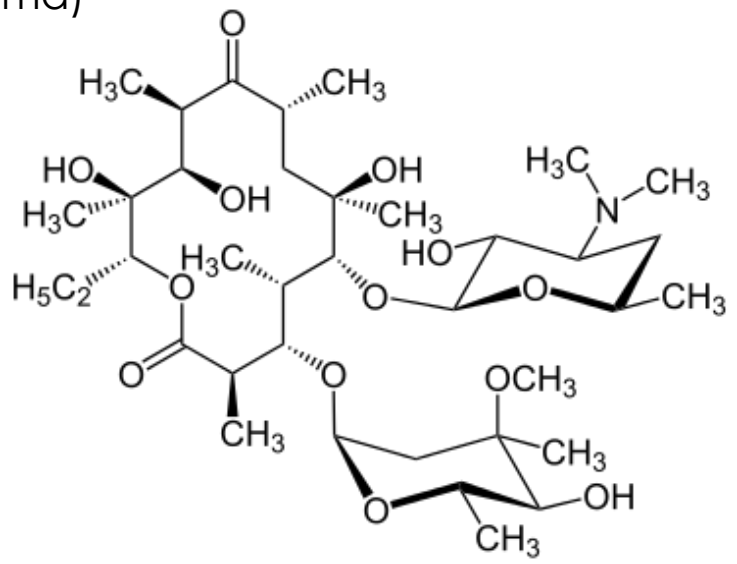
Rifamycin B
(gram-pos. bacteria, mycoplasma)



Streptomycin
(tubercolosis)



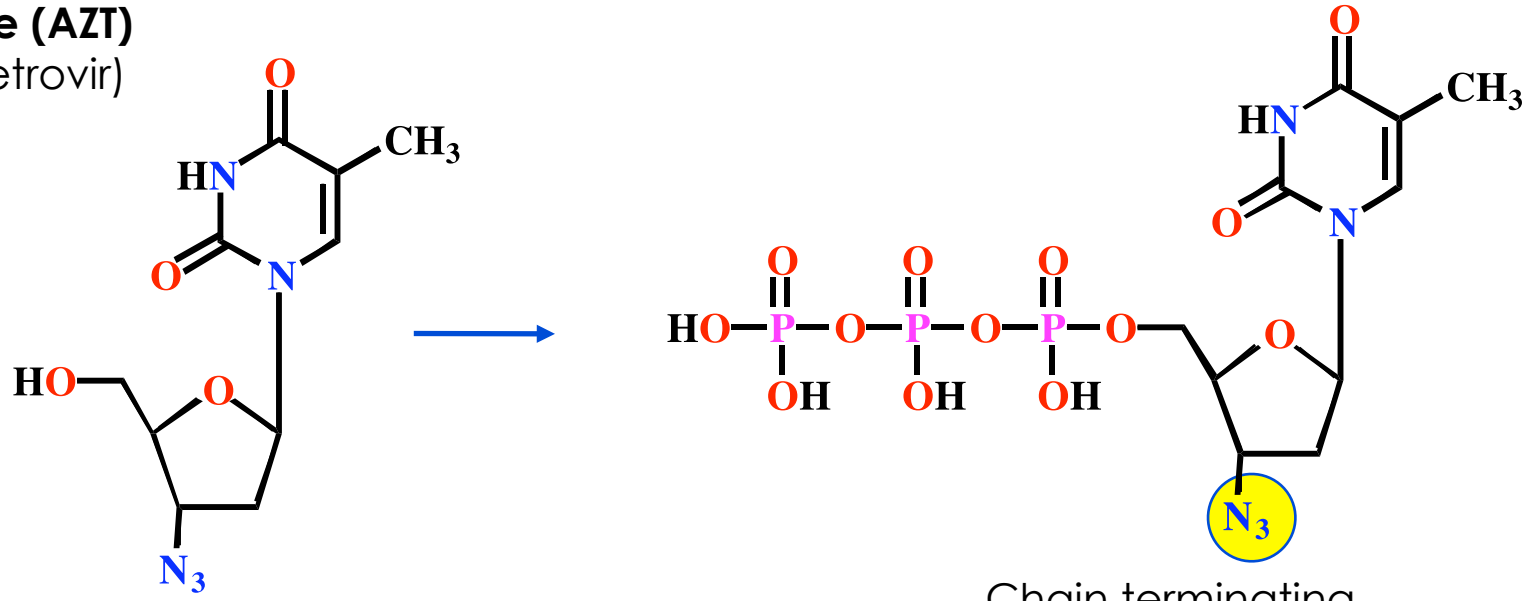
Chlortetracycline
(Aureomycin, eye infections, open wounds)



Erythromycin
(gram-pos. bacteria, mycoplasma)

Antiviral agents

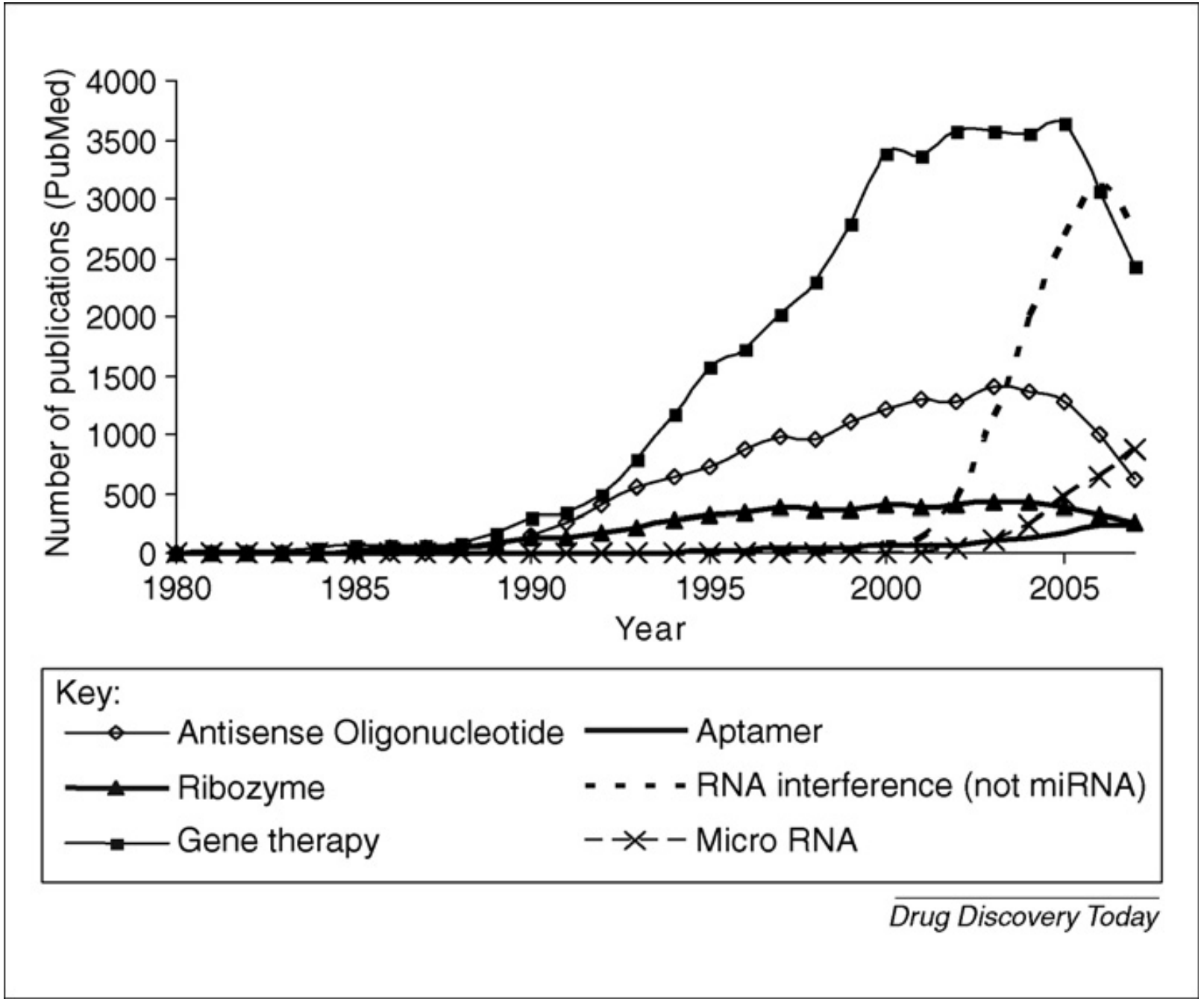
Azidothymidine (AZT)
(Zidovudine;Retrovir)



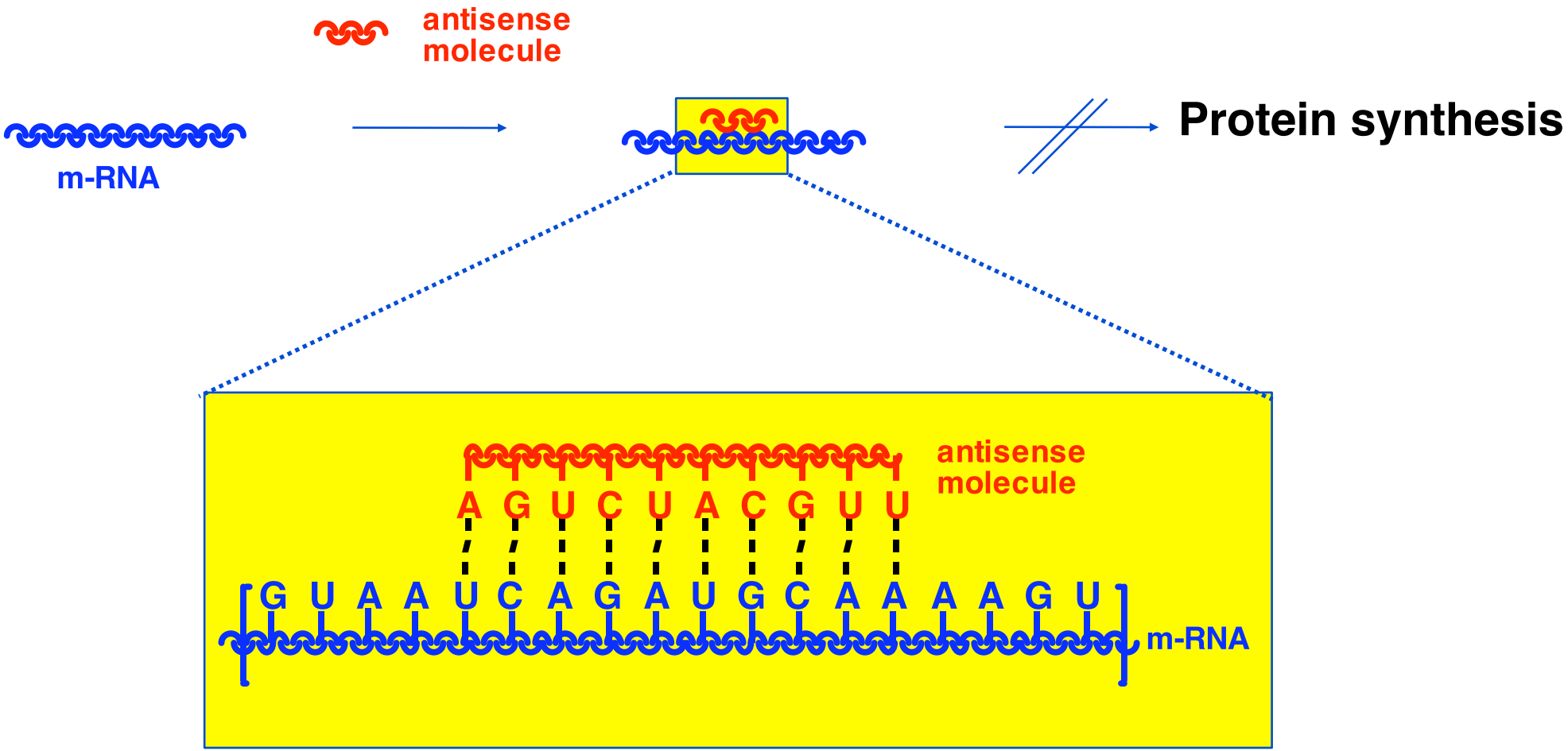
- Enzyme inhibitor
- AZT is phosphorylated to a triphosphate in the body by the viral thymidine kinase, cannot be phosphorylated in human cells
- Triphosphate has two mechanisms of action
  - inhibits a viral enzyme (reverse transcriptase)
  - added to growing DNA chain and acts as chain terminator

Gene silencing methods

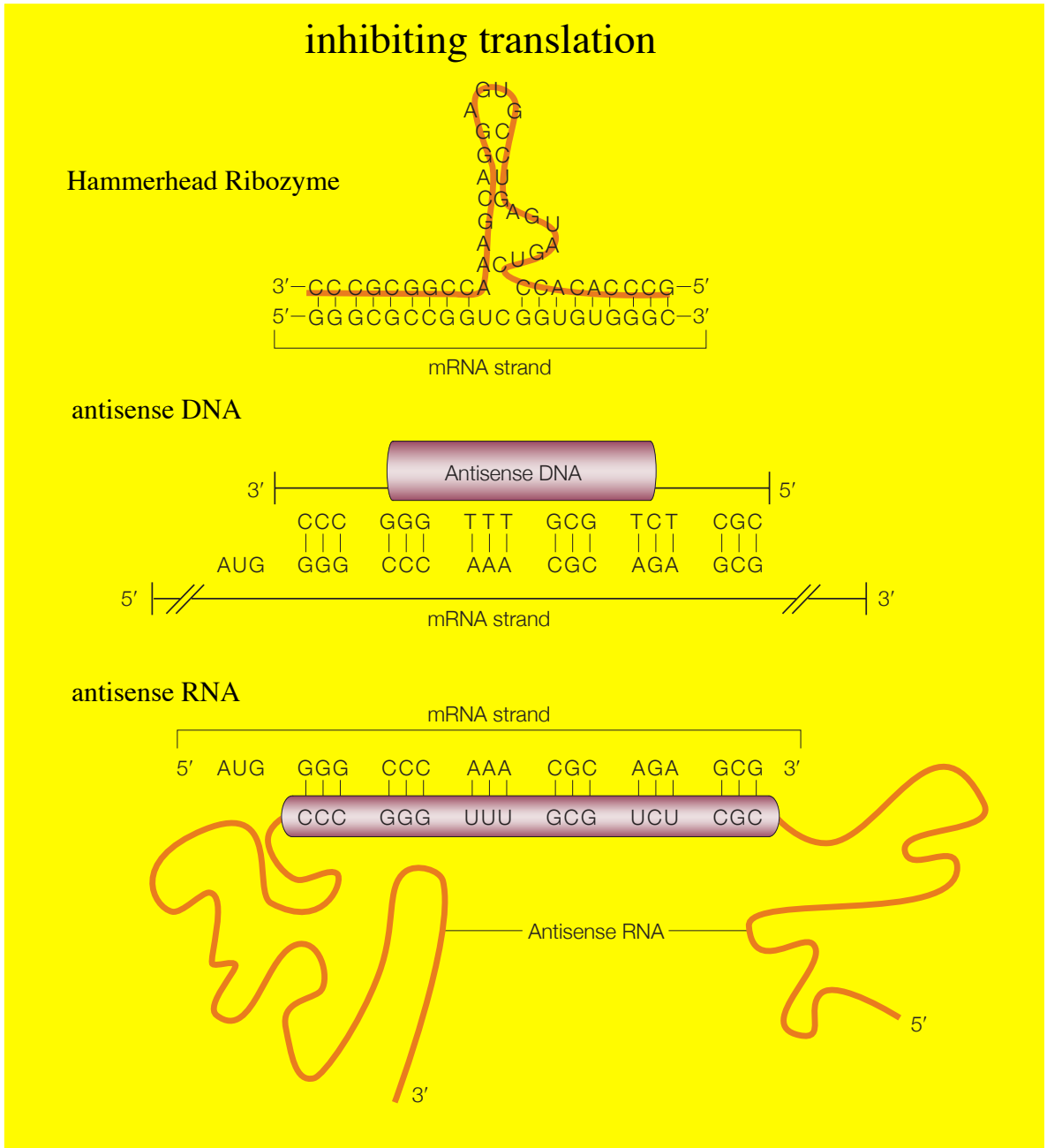
- Develop Nucleic Acid (derivatives) that bind either to DNA or RNA to **stop** transcription or translation
- Allows to rapidly develop drugs once the DNA sequence of the target is known with **high selectivity**
- Successful for all targets, even for non-druggable targets
- Rapid lead identification and optimization
- Comparably easy synthesis
- Requires modification of nucleotides to improve pharmacokinetics
- Only antagonism possible no agonism (no upregulation)


Number of publications (PubMed)
4000
3500
3000
2500
2000
1500
1000
500
0
1980
1985
1990
1995
2000
2005
Year
Key:
Antisense Oligonucleotide
Aptamer
Ribozyme
RNA interference (not miRNA)
Gene therapy
Micro RNA
Drug Discovery Today

Antisense Therapy (mRNA)



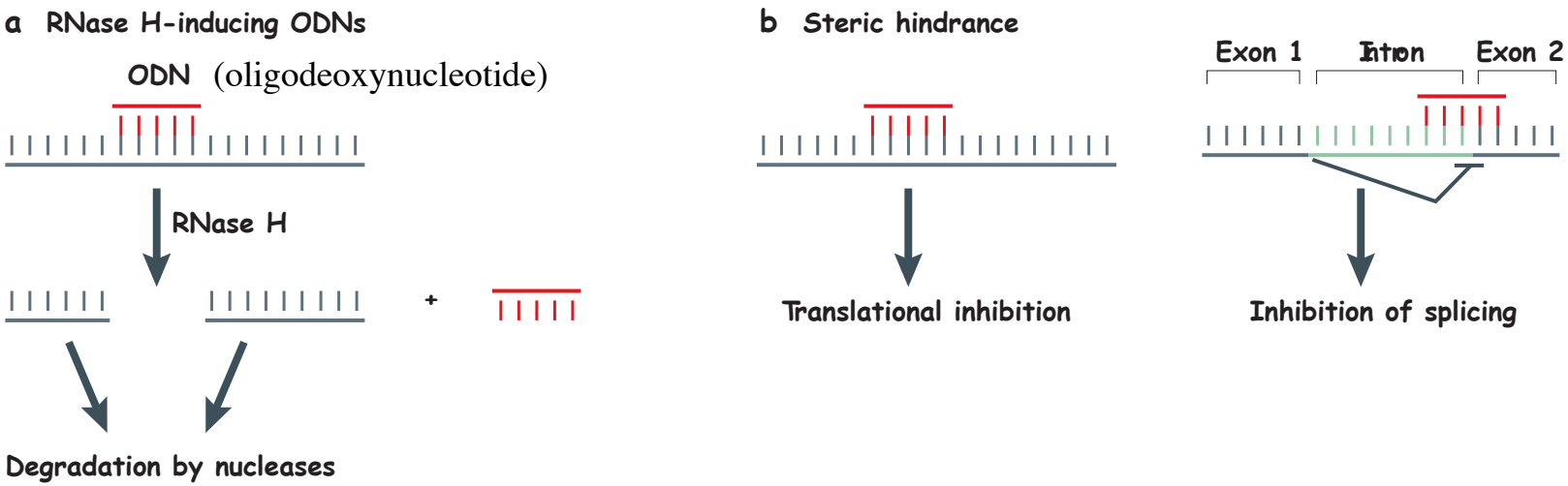
Antisense Technology



- directly targeting protein transcription or translation
- either by targeting mRNA (ribozymes or anti-sense DNA/ RNA) or by targeting DNA (not clinically successful)
- targeting DNA try to achieve homologous recombination with the DNA strand or triple-helix formation via Hoogsteen base-pairing
- mRNA is in contrast to DNA better accessible to attack

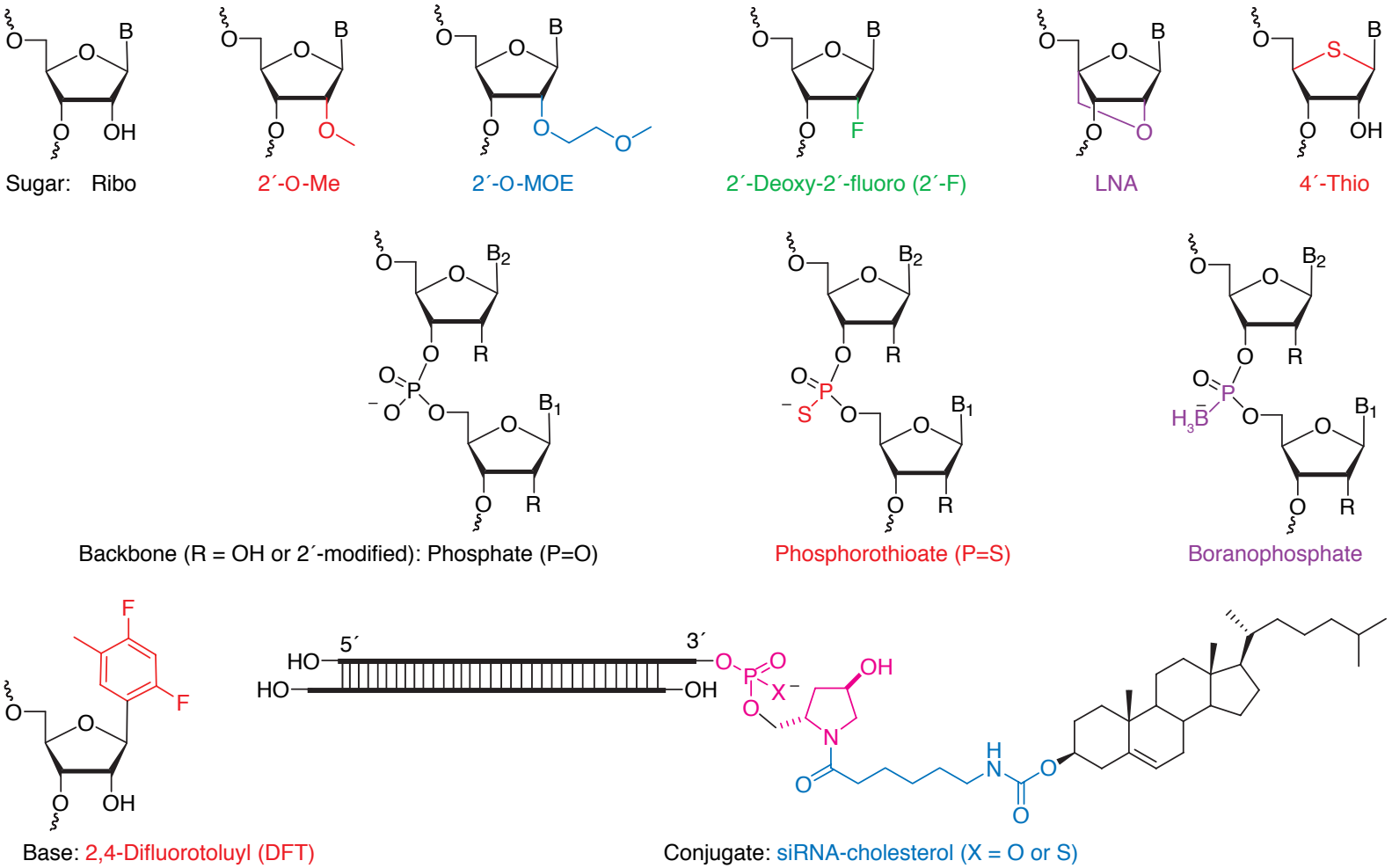
Antisense methods

- stable mRNA-antisense duplexes can
  - interfere with splicing
  - block translation
  - lead to destruction by binding to endogenous nucleases such as RNaseH



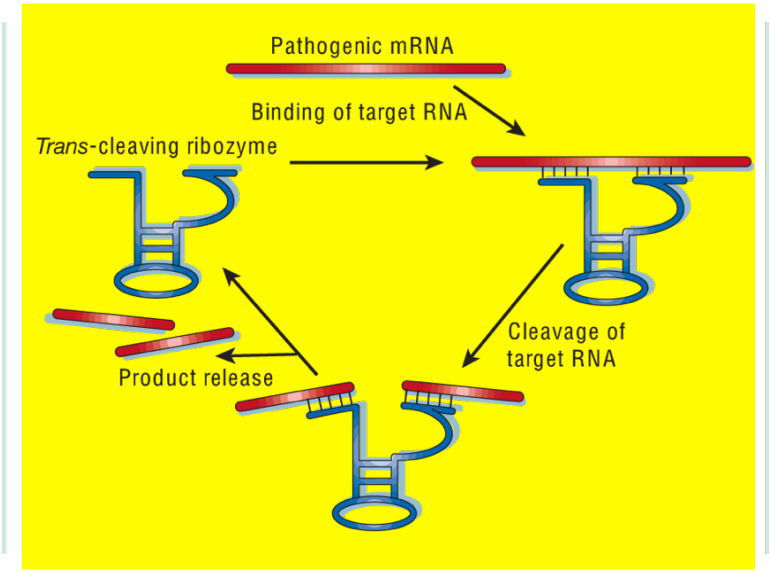
- anti-sense oligonucleotides must be chemically modified to make them more resistant against endo/ and exonucleases

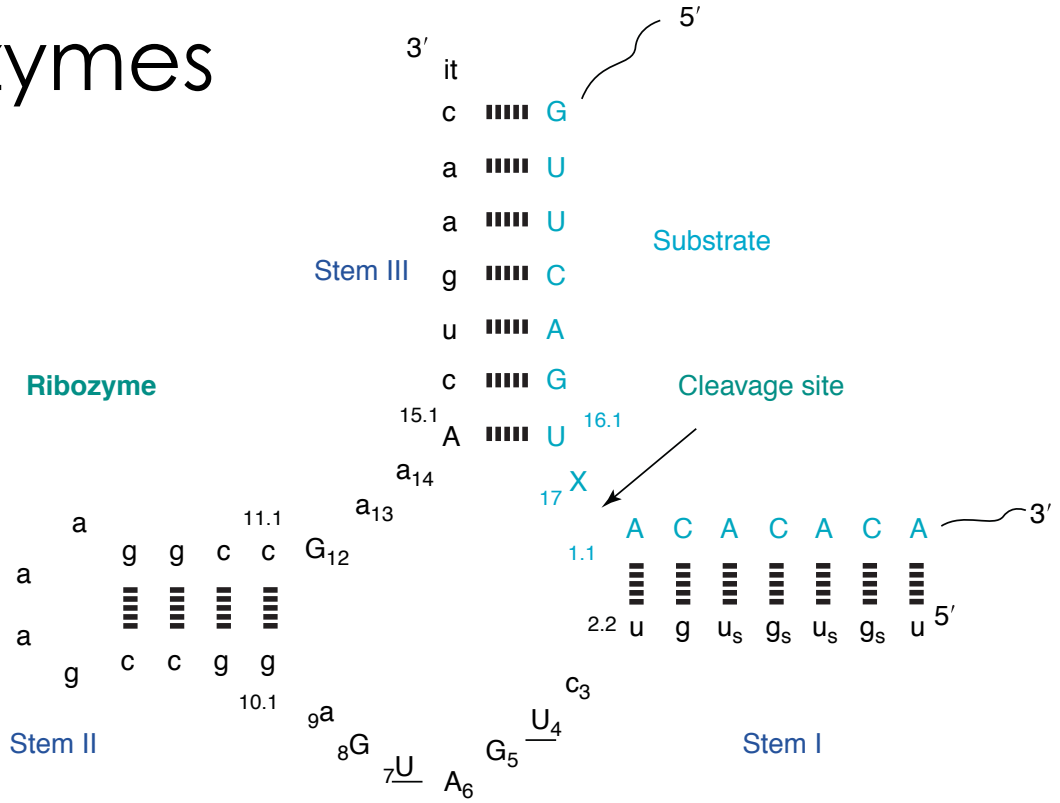
Chemical Modifications of Nucleotides to Increase Stability



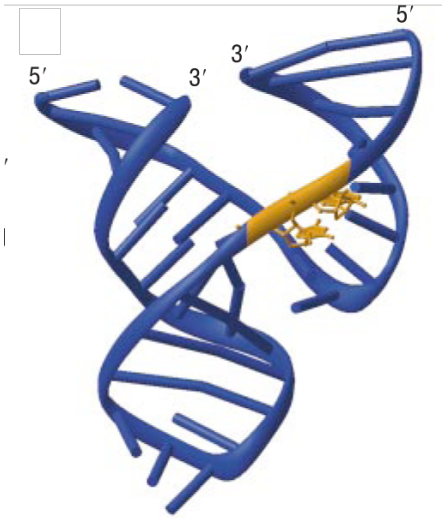
- stability against nucleases is achieved either by introducing a phosphothioate (P=S) at the 3' end for exonuclease resistance or 2' modifications for endonuclease resistance. Cholesterol conjugates improve uptake properties.

Ribozymes





- ribozymes are naturally occurring small RNA molecules with catalytic activity that cleave RNA strands (splicing, pre-mRNA into mRNA)
- can be produced from a vector intracellularly
- some ribozymes depend on the presence of divalent cations
- the catalytic motif is surrounded by a flanking sequence complementary to the target mRNA

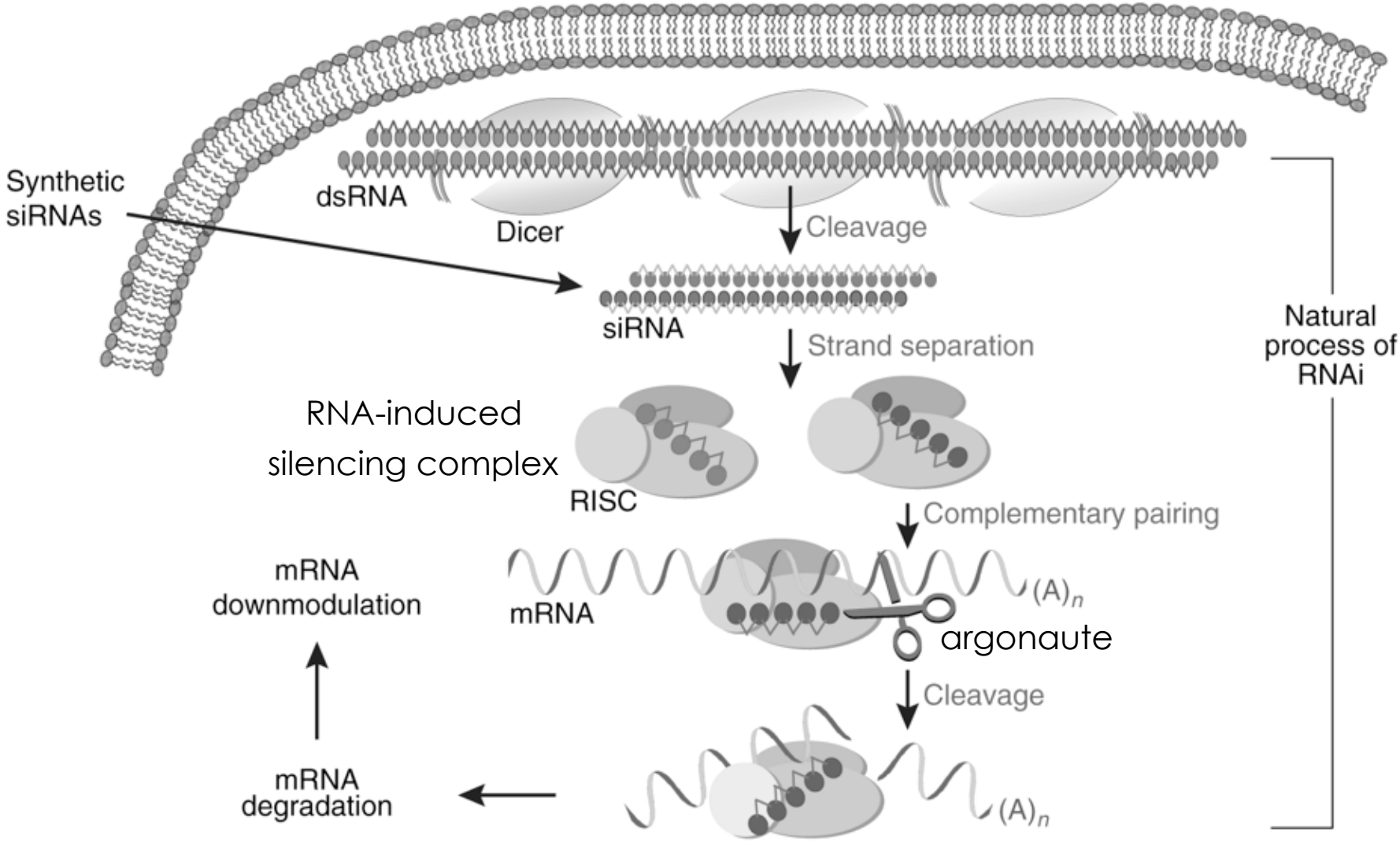


structure of the hammerhead ribozyme

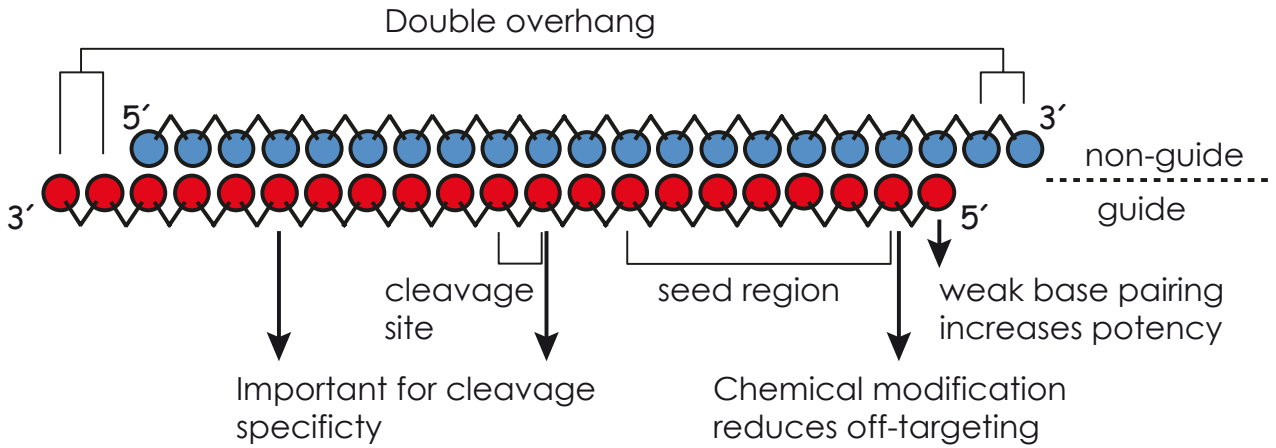
RNA interference

- RNA interference is a naturally occurring post-transcriptional gene silencing method
- By RNAi double-stranded RNA targets mRNA for destruction
- Initially, the dsRNA is cut into 21-25 bp long pieces by a member of RNA nucleases called DICER.
- The RNA fragments are then incorporated into a larger multicomponent nuclease complex (RISC, RNA-induced silencing complex)
- The RISC complex with a single-strand RNA fragment then recruits mRNA for processing and cleavage
- long RNA fragments are clinically not useful, because it triggers a immune response, that completely shuts down protein synthesis
- small interfering RNA (siRNA) do not trigger the immune response and can be used for an RNAi-based gene silencing approach.

RNA interference



siRNA



- potency largely depends on the **efficiency with which the strand is incorporated into the RISC complex**
- the RISC machinery incorporates preferably the strand whose 5' end binds less tightly with the other strand
- hence **siRNAs are designed to less strongly interact at the 5' end**
- modification at a single nucleotide position is sufficient to suppress the majority of off-targeting
- far more potent than anti-sense nucleotide approaches, because secondary structure formation of the mRNA is less of a problem, and hence much lower concentrations can be used (less off-targeting)
- **mostly injected**

The CRISPR/CAS Technology

Biology

Cell lines
HEK293
U2OS
K562

Model organisms
Mice
Rats
Fruit flies
Nematodes
Arabidopsis
Salamanders
Frogs
Monkeys

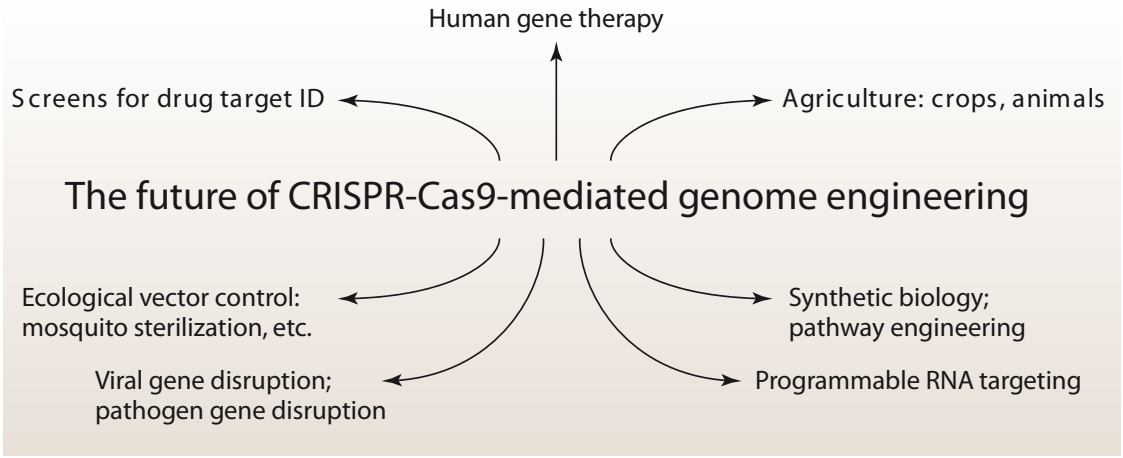
Biotechnology

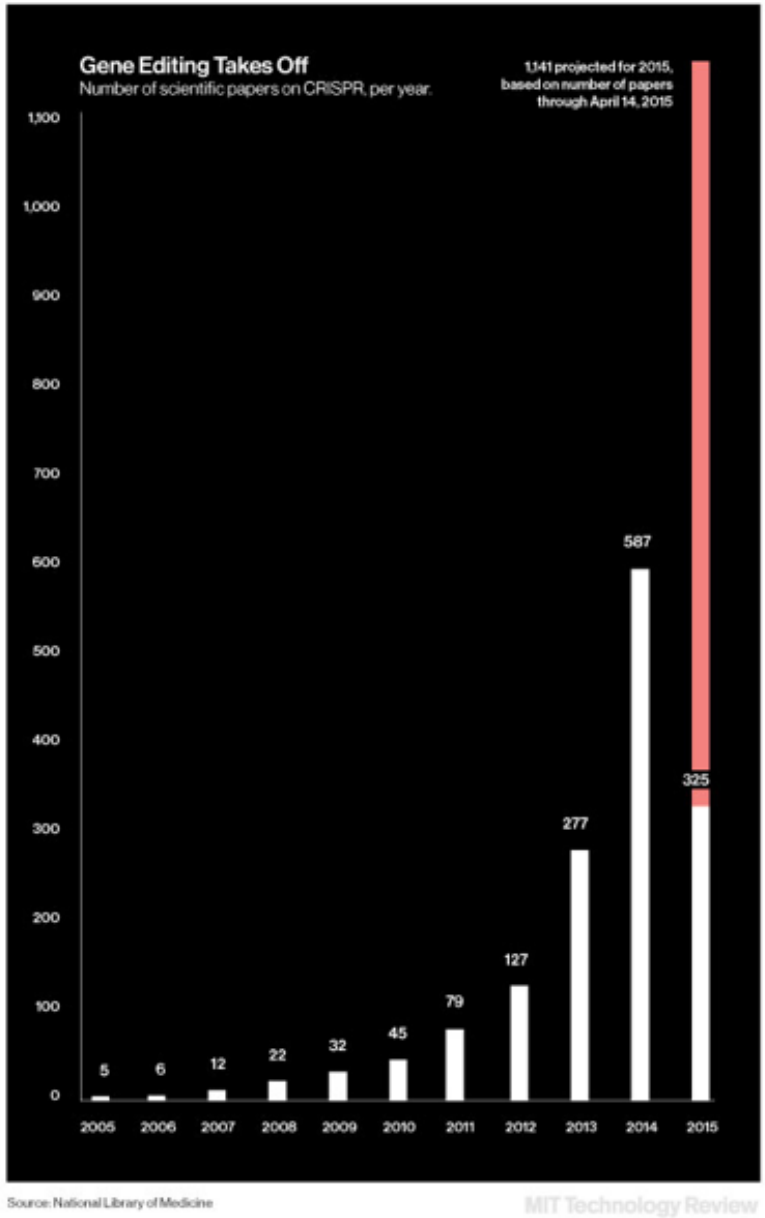
Crop plants
Rice
Wheat
Sorghum
Tobacco

Fungi
Kluyveromyces
Chlamydomonas

Biomedicine

Organoids
hESCs
iPSCs





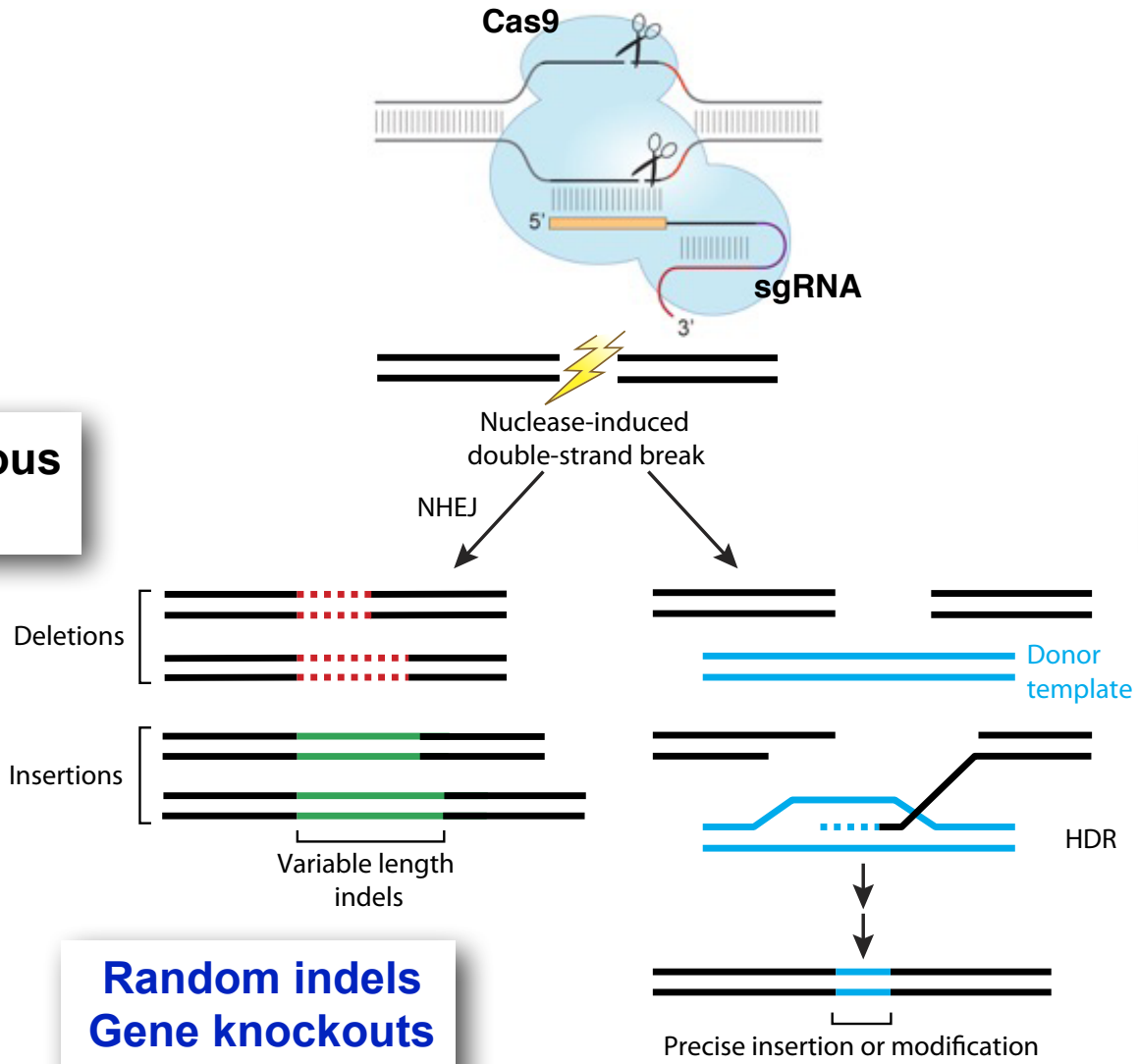
slides:courtesy of Martin Jinek

Doudna and Charpentier, *Science* (2014)
Regalado, *MIT Technology Review* (2015)

Gene Therapy

- In gene therapy DNA is inserted, deleted or replaced in the genome of a living organism using engineered nucleases, or "**molecular scissors**". Thereby genetic diseases might be completely cured.
- These nucleases create site-specific double-strand breaks (DSBs) at desired locations in the genome.
- The induced double-strand breaks are repaired through **nonhomologous end-joining** (NHEJ) or **homologous recombination** (HR), resulting in targeted mutations
- The following nucleases are used presently: meganucleases, **zinc finger nucleases** (ZFNs), transcription activator-like effector-based nucleases (**TALEN**), and the **c**lustered **r**egularly **i**nterspaced **s**hort **p**alindromic **r**epeats CRISPR-Cas system.

RNA-guided genome editing based on Cas9



Non-homologous end joining

Homology-directed repair

Random indels
Gene knockouts
Deletions

Specific insertions
Correction of point mutations
"Gene surgery"

Sander and Joung, *Nat Biotechnol* (2014)

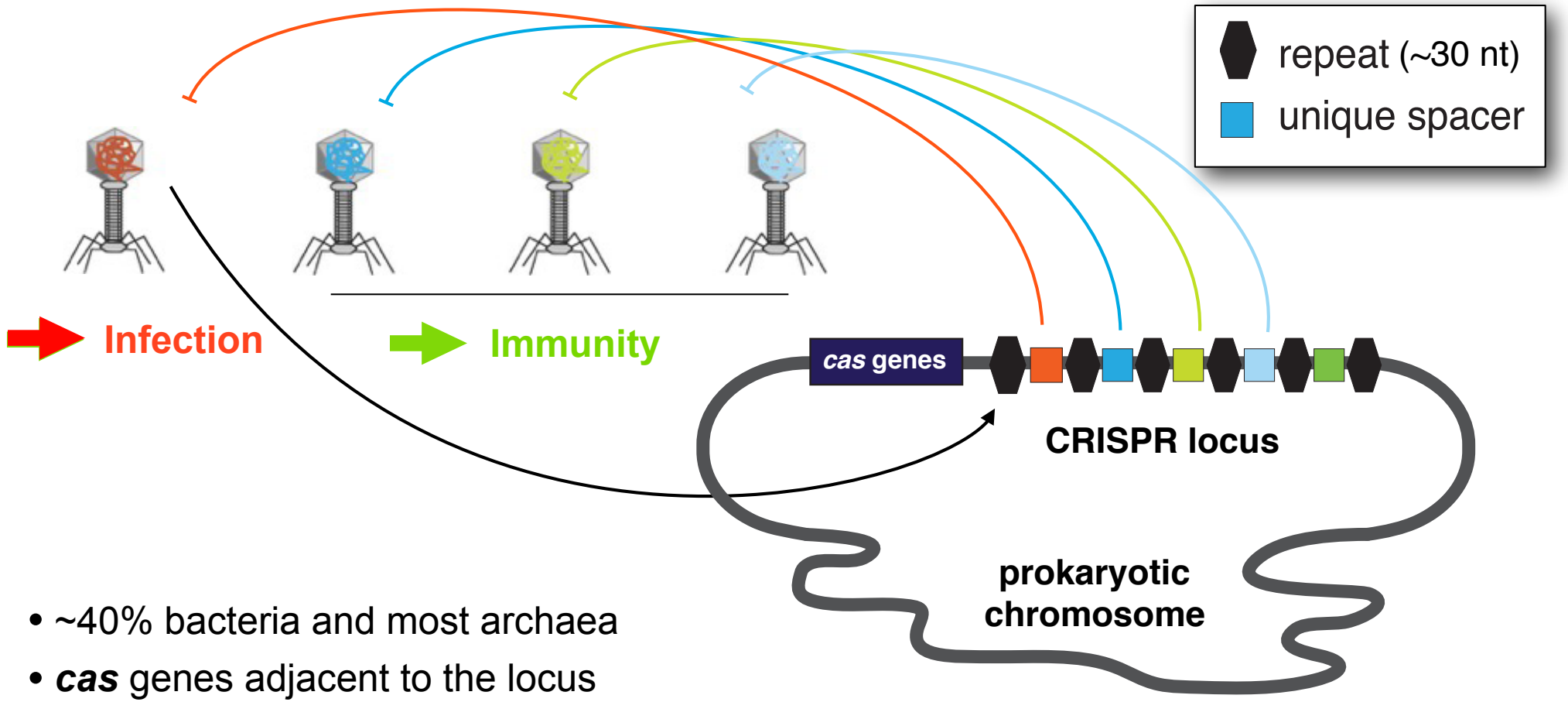
The history of CRISPR/CAS Technology

- Clustered repeats were first described in 1987 for the bacterium Escherichia coli by Yoshizumi Ishino. It was co-recognized by Francisco Mojica from the University of Alicante in 1989.
- In 2000, similar repeats were identified in other bacteria and archaea, and were termed Short Regularly Spaced Repeats (SRSR). SRSR were renamed CRISPR in 2002.
- In 2005, three independent research groups showed that some CRISPR spacers are derived from phage DNA and extrachromosomal DNA such as plasmids
- Doudna and Charpentier jointly studied a simpler CRISPR system that relies on a protein called Cas9.
- Jinek combined tracrRNA and spacer RNA into a "single-guide RNA" molecule that, mixed with Cas9, could find and cut the correct DNA targets. Jinek et al proposed that such synthetic guide RNAs could be used for gene editing
- CRISPR was first shown to work as a genome engineering/editing tool in human cell culture by 2012

source: Wikipedia

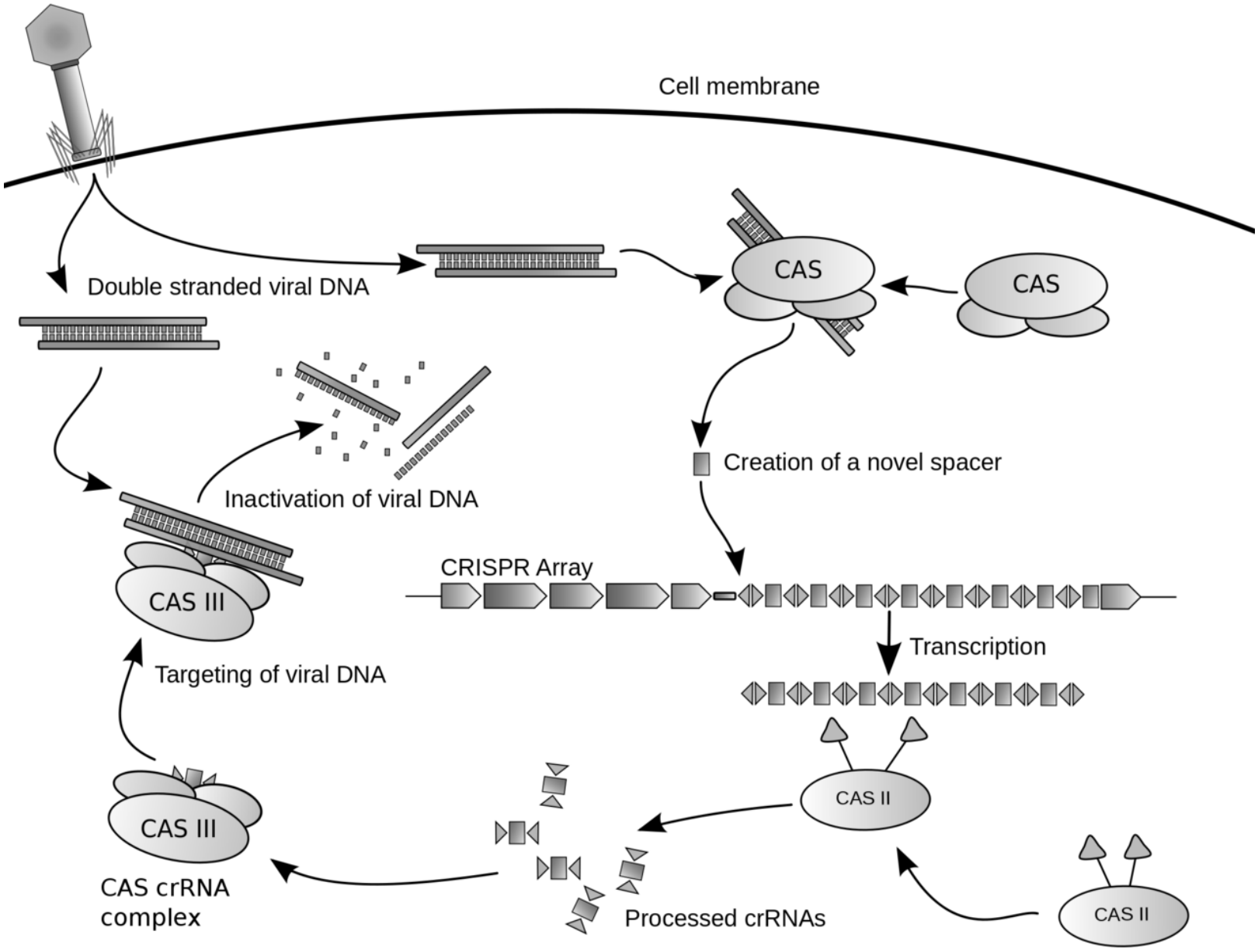
CRISPRs provide molecular memory of genetic invaders

Clustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats



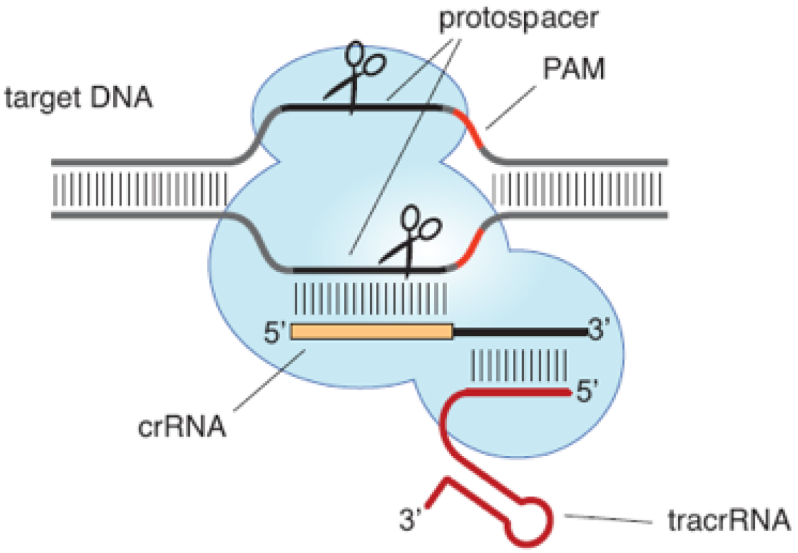
- ~40% bacteria and most archaea
- ***cas*** genes adjacent to the locus
- Spacers matching sequences in mobile genetic elements provide immunity
- Acquisition of new spacers at the leader end of the locus

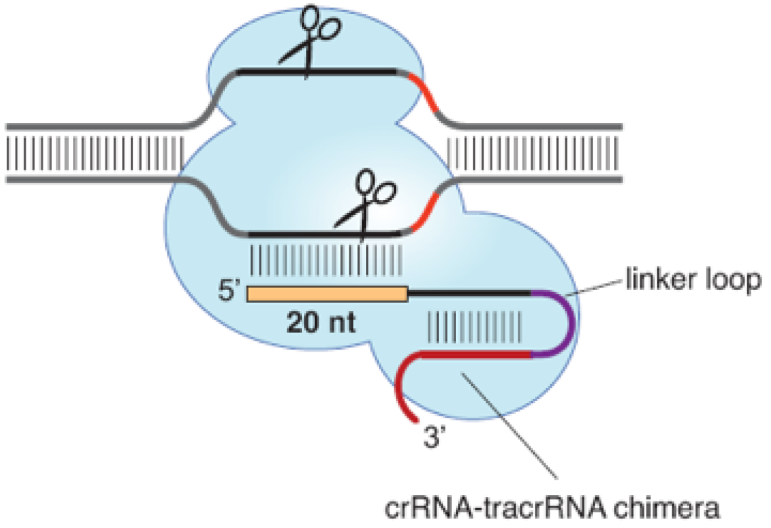
RNA-guided targeting by CRISPR-Cas



source: Wikipedia

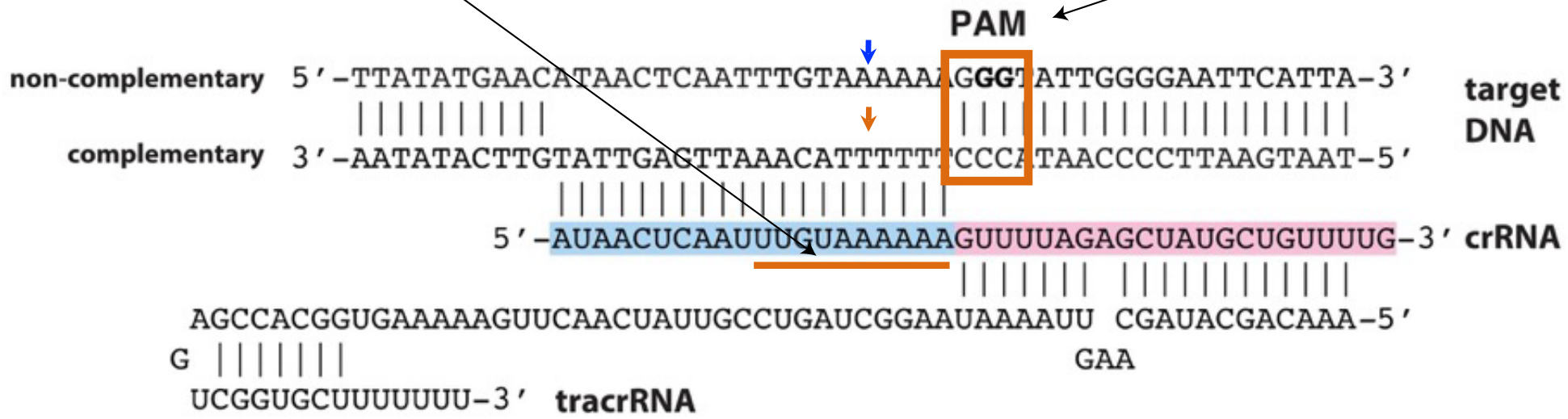
Dual RNA-guided DNA cleavage by Cas9

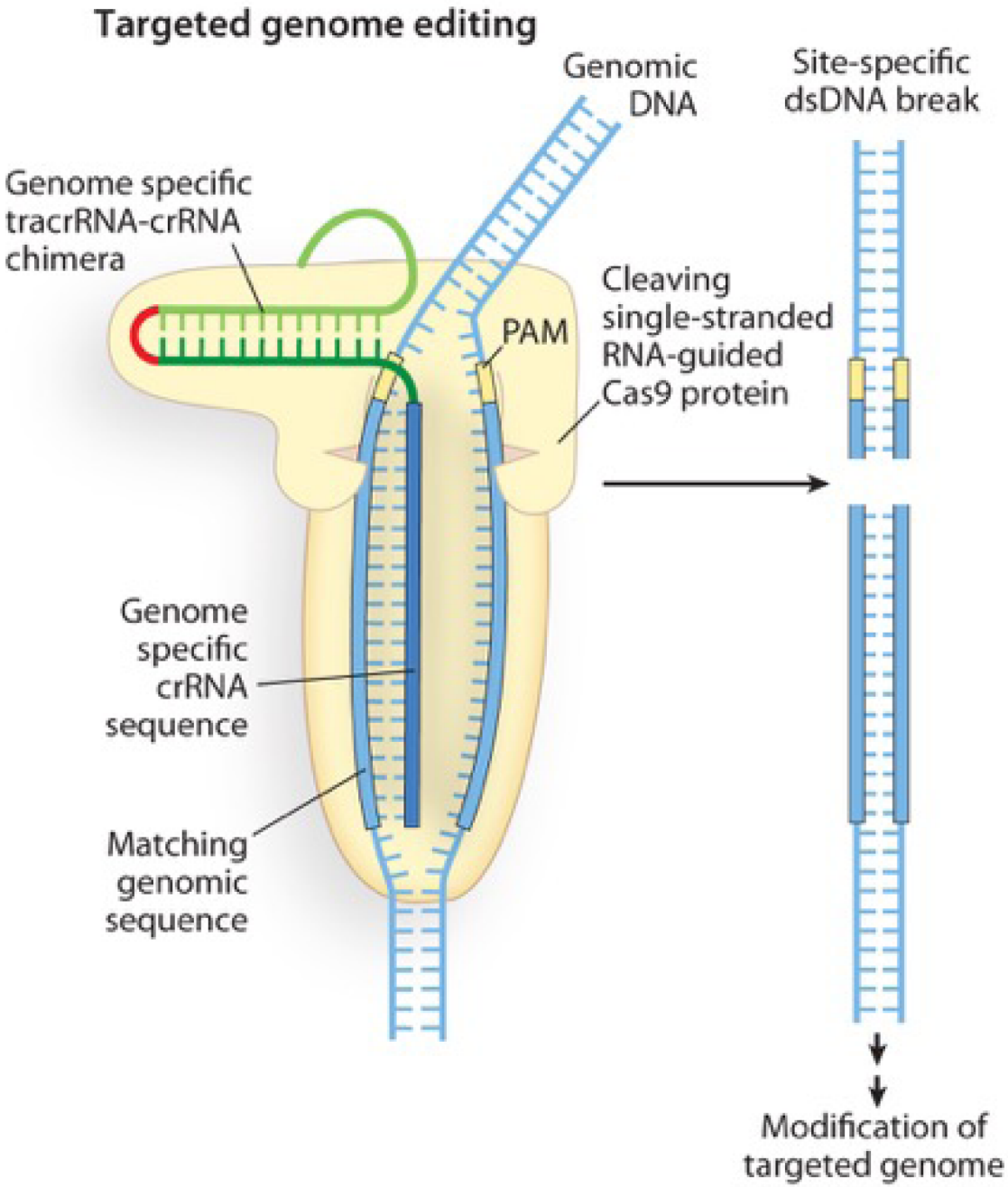




8-12 nt "Seed" sequence in crRNA

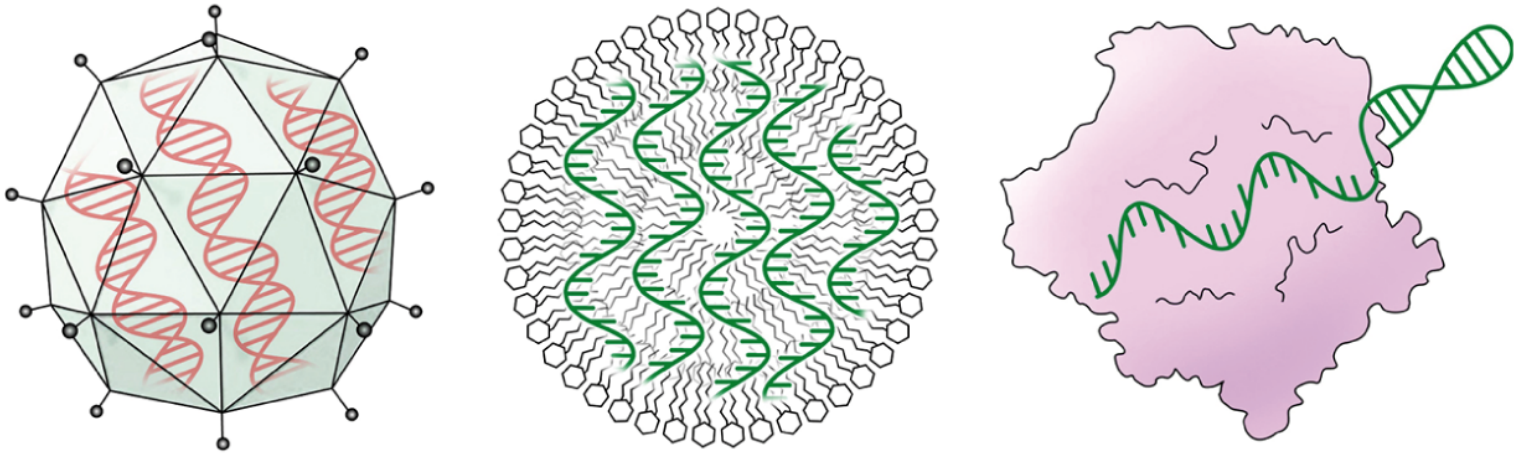
PAM motif in target DNA
(N**GG** for *S. pyogenes* Cas9)





- many different DNA targets but one generic protein (CAS9)
- can down-regulate genes but also up-regulate genes !
- works with many organisms from simple to mammalian ones
- patent situation unclear presently
- first applications in gene therapy appeared

Vehicles to transport the CAS/CRISPR components into the cell



Approach	Virus	Nanoparticle	Enzyme complex
Example	Adeno-associated virus (AAV) packaged with DNA encoding Cas9 & sgRNA	Liposomes encapsulating mRNA & sgRNA	Ribonucleoprotein (RNP) complex of Cas9 protein and sgRNA
Size	20 nm	50-500 nm	12 nm
Advantages	Extremely effective; prior use with classic gene therapy	Straightforward to prepare; low immunogenicity	Short lifetime and lower risk of off-target cutting
Disadvantages	Risk of increased off-target cutting & genomic integrations; can be immunogenic; capacity for DNA storage can be limiting	Toxicity not fully characterized	Unknown immunogenicity profile; requires additional engineering, transduction reagents, or electroporation to enter cells
Tissue Specificity	Inherent tropism inherent to various strains; additional tropism can be engineered	Tends to accumulate in the liver; deliberate targeting is being developed	No inherent cell-penetrating properties, so specificity can be engineered precisely

Wilson et al., ACS Chem Biol. 2017