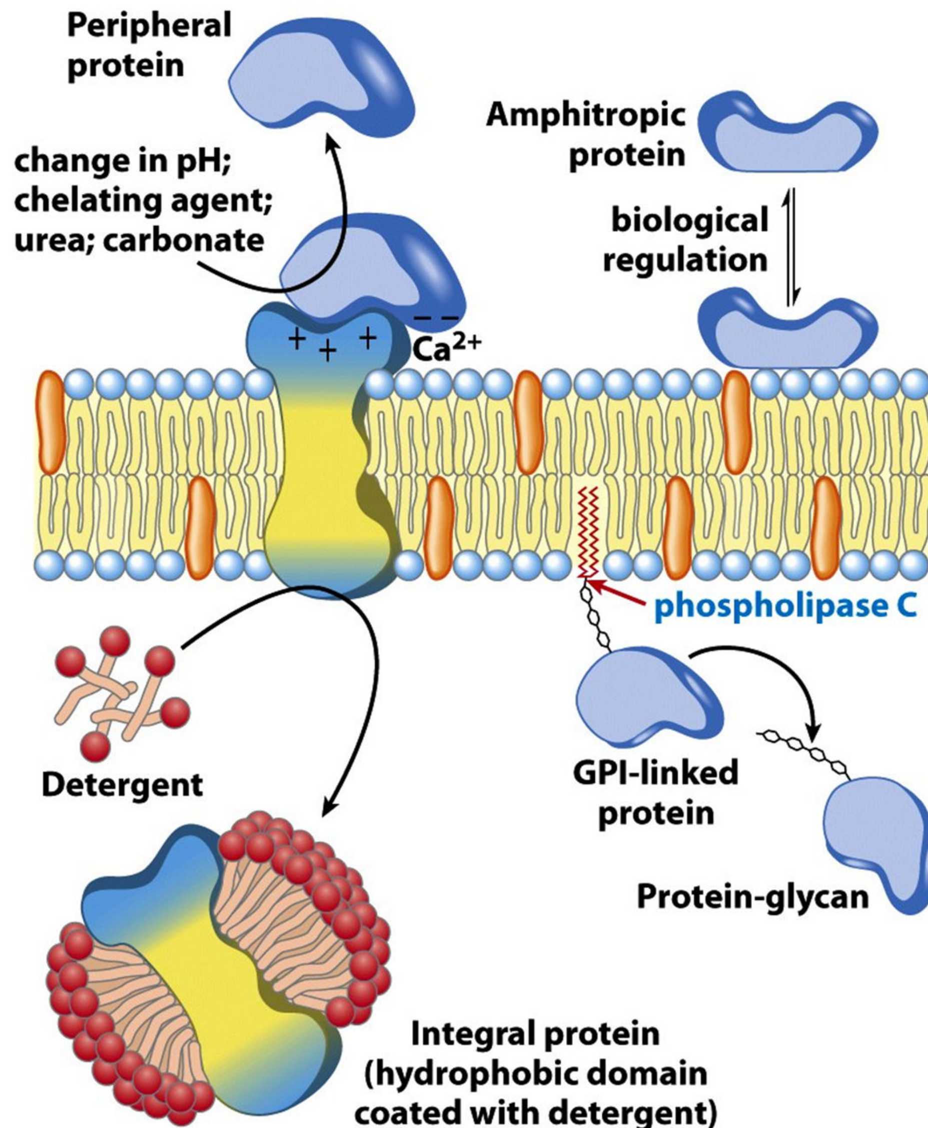
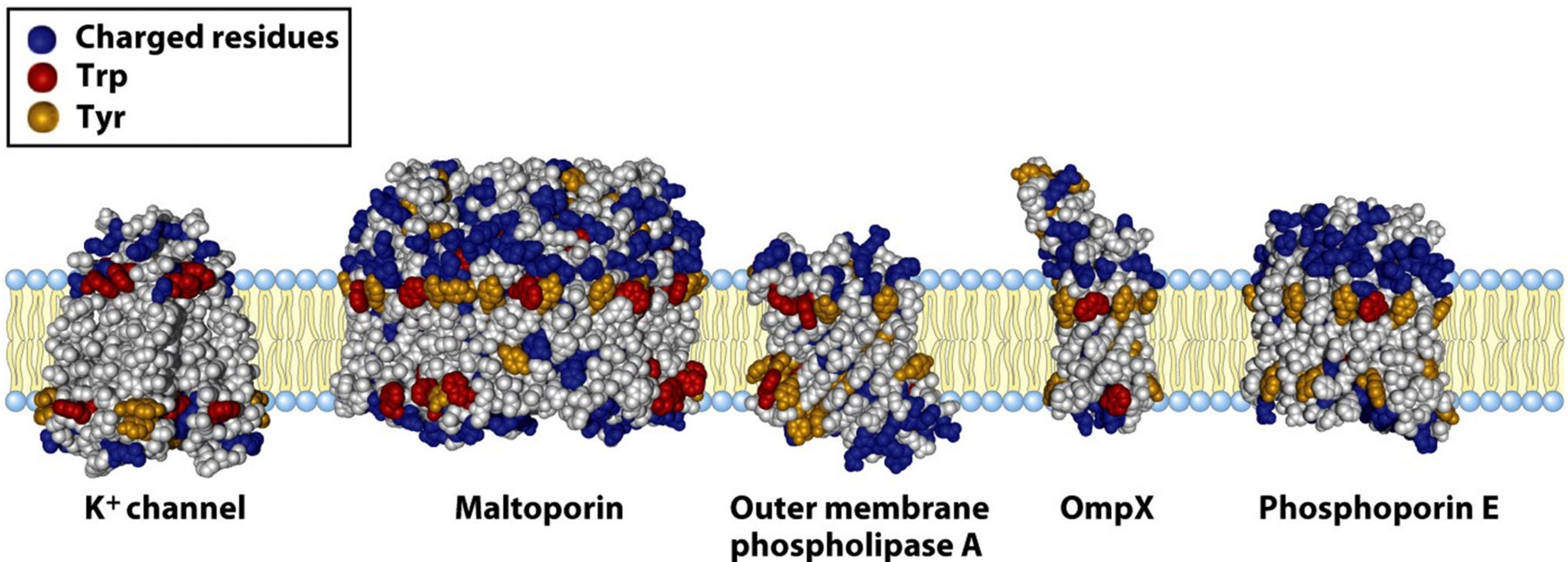
Membrane Proteins:



1. **Integral proteins:** proteins that insert into/span the membrane bilayer; or covalently linked to membrane lipids.
   (Interact with the hydrophobic part of the membrane)
2. **Peripheral proteins:** interact with integral protein through non-covalent interaction; or interact with polar head groups of the membrane lipids.
   (charge interaction is common)
3. **Amphitropic proteins:** Associate with membrane conditionally. Usually subjected to biological regulation.

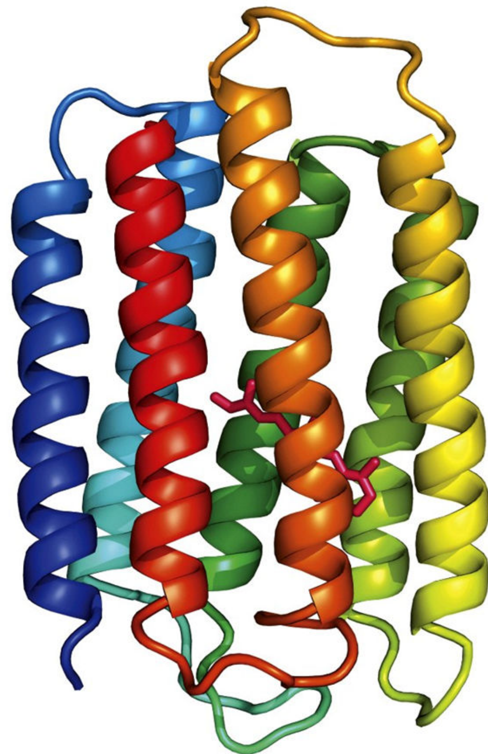
A protein's surface polarity corresponds to its environment



Tyr and Trp exhibit 'snorkeling' – pointing their polar group toward mb exterior

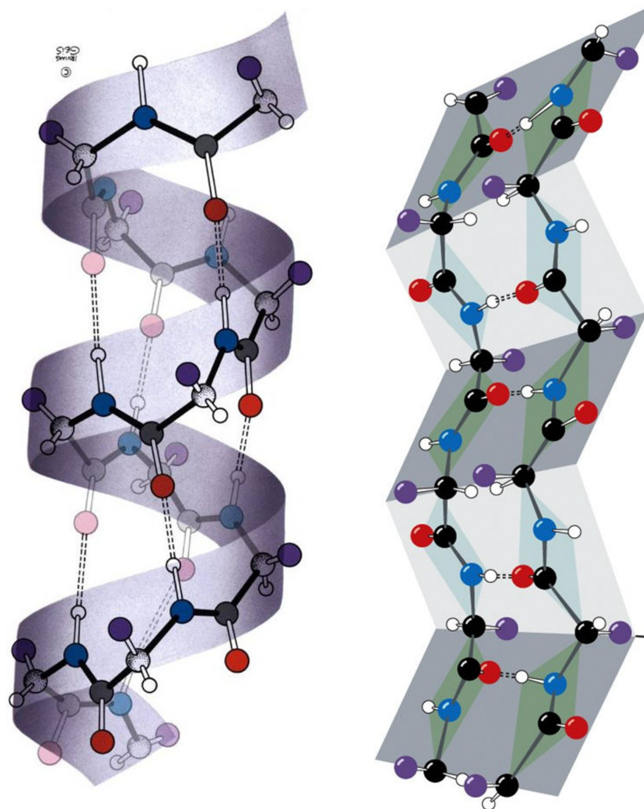
Also, often 'positive inside' – positively charged aa's facing cytoplasmic region

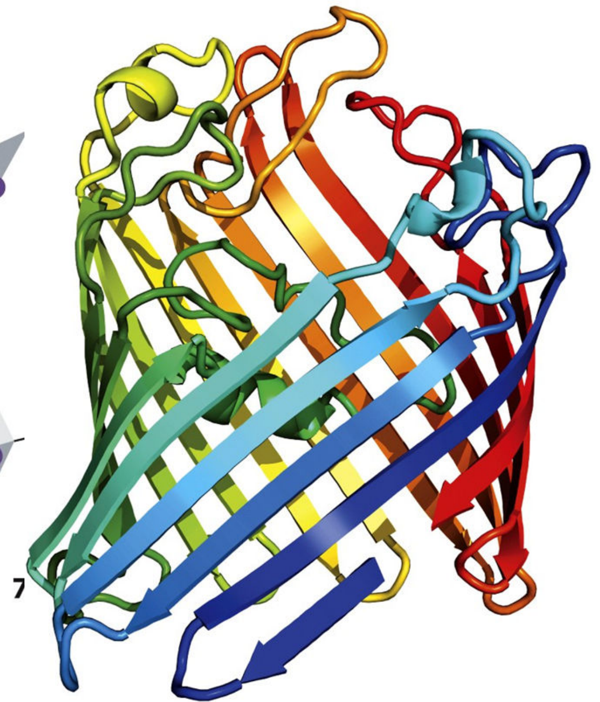
Transmembrane regions are usually α-helices or continuous β-sheets (β-barrels)



Bacteriorhodopsin:
a light-driven proton pump

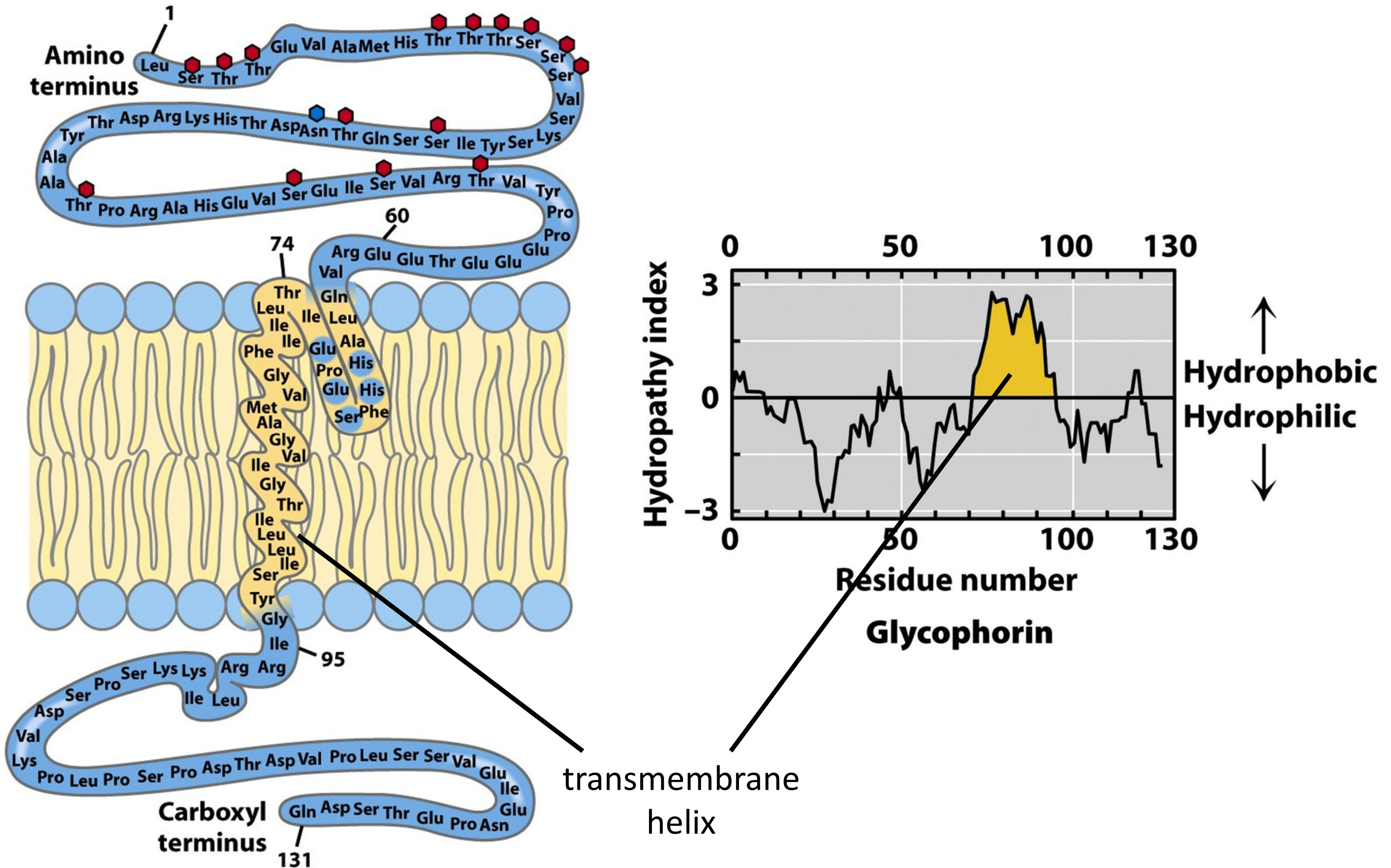
Backbone hydrogen bonds can be self-satisfied.





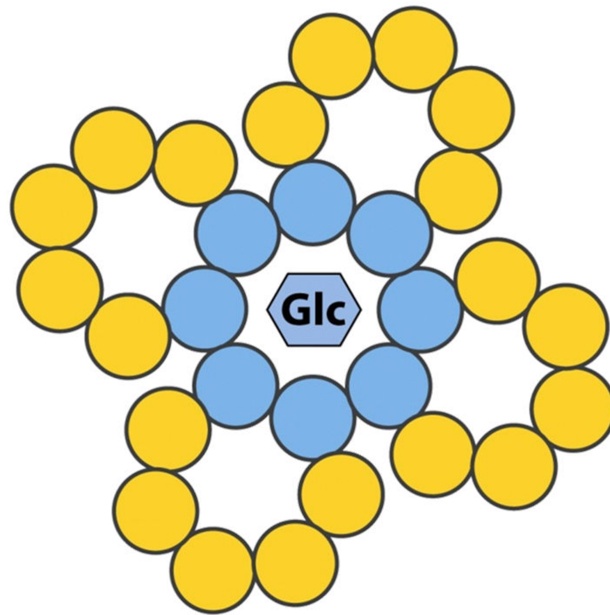
Porin:
a pore-forming protein

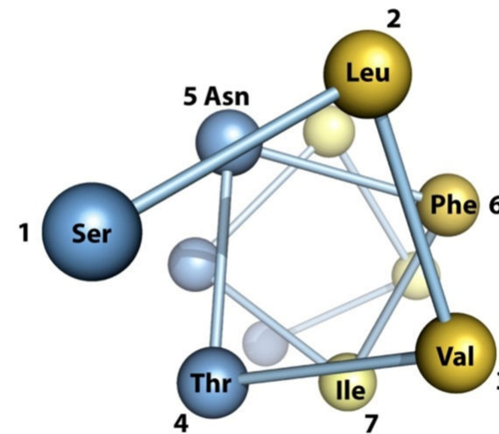
Transmembrane helices are predicted by hydrophobic stretches of 20-25 aa residues



In integral transport proteins, interiors are hydrophilic and exteriors are hydrophobic

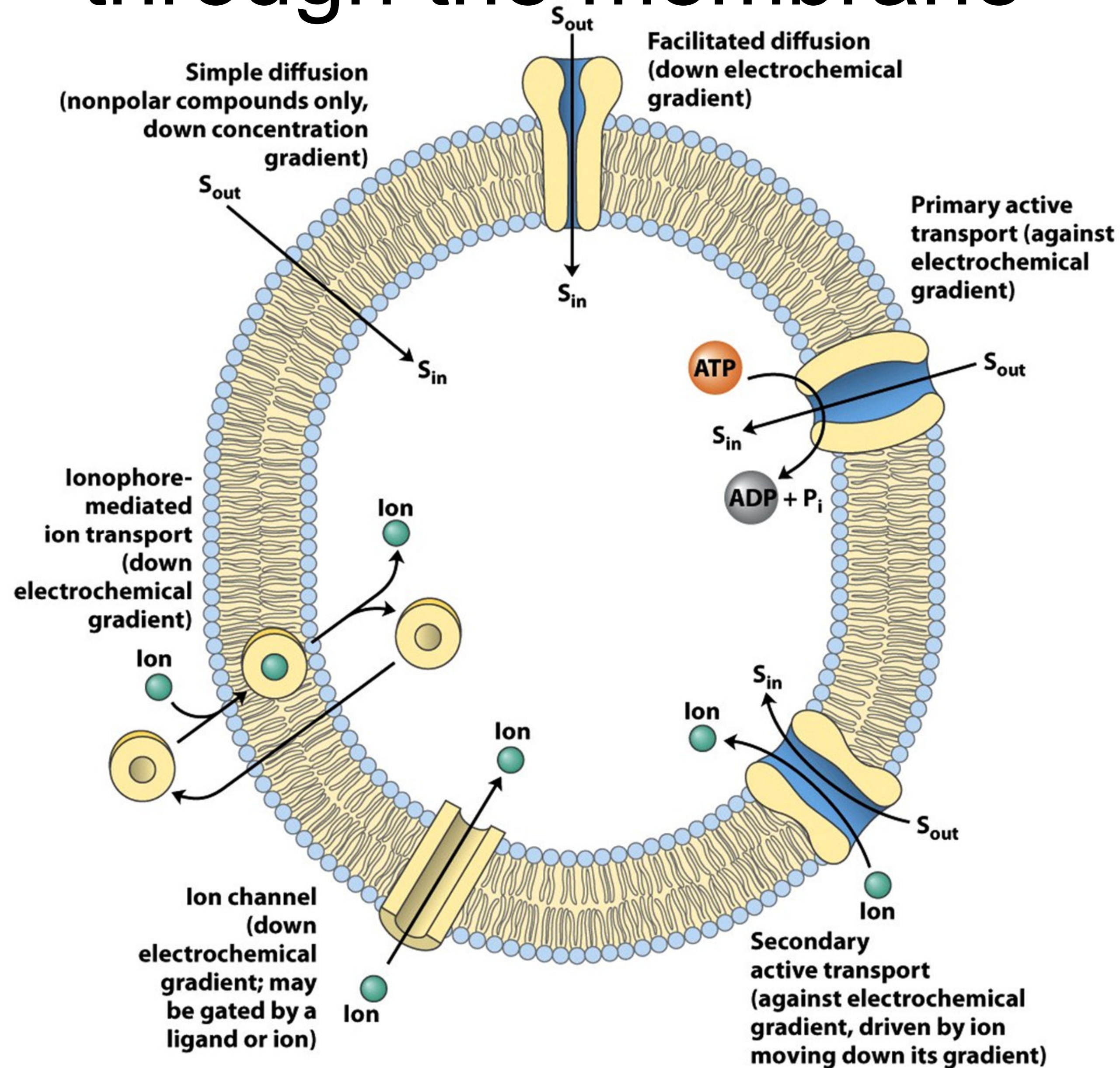
Glucose transporter

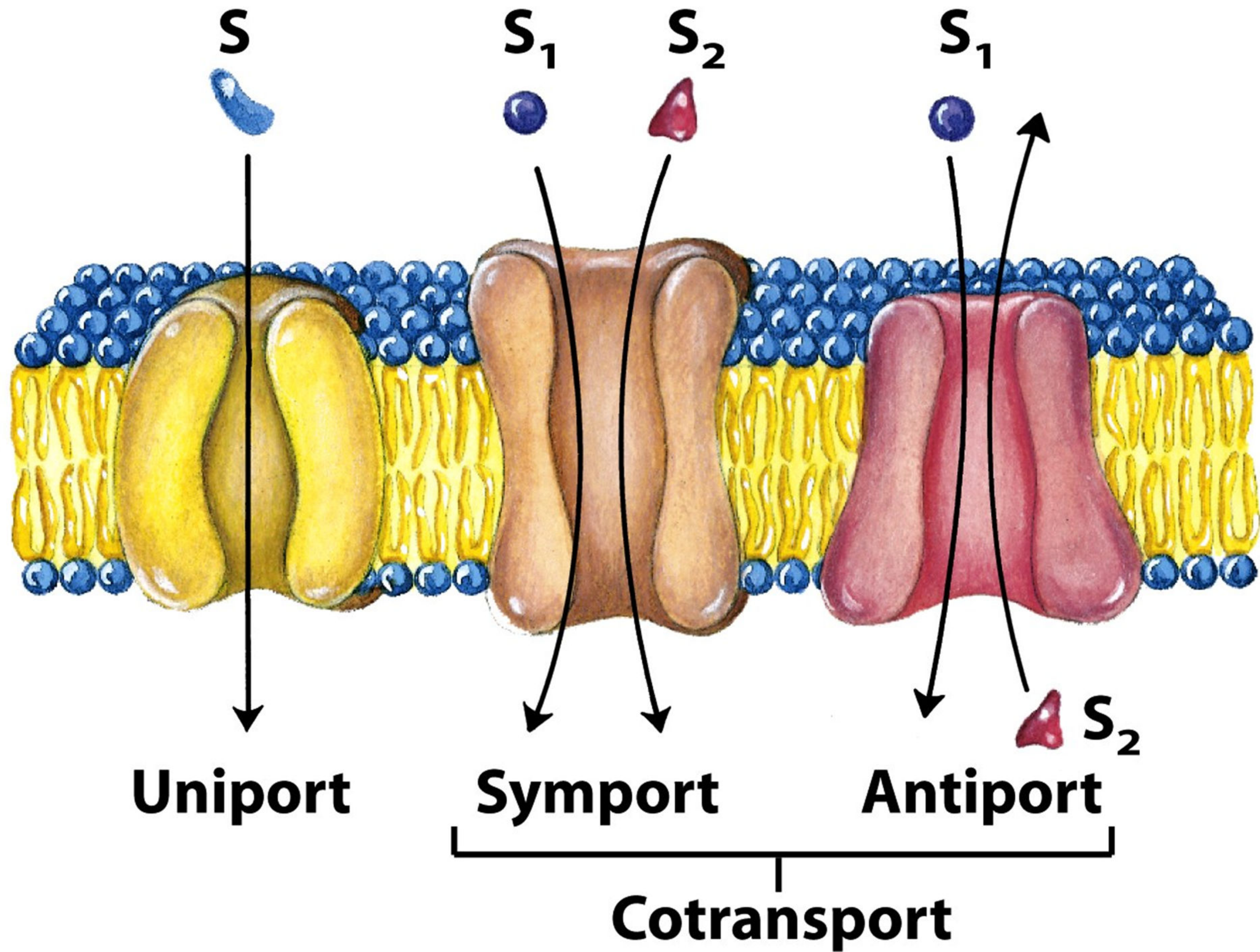




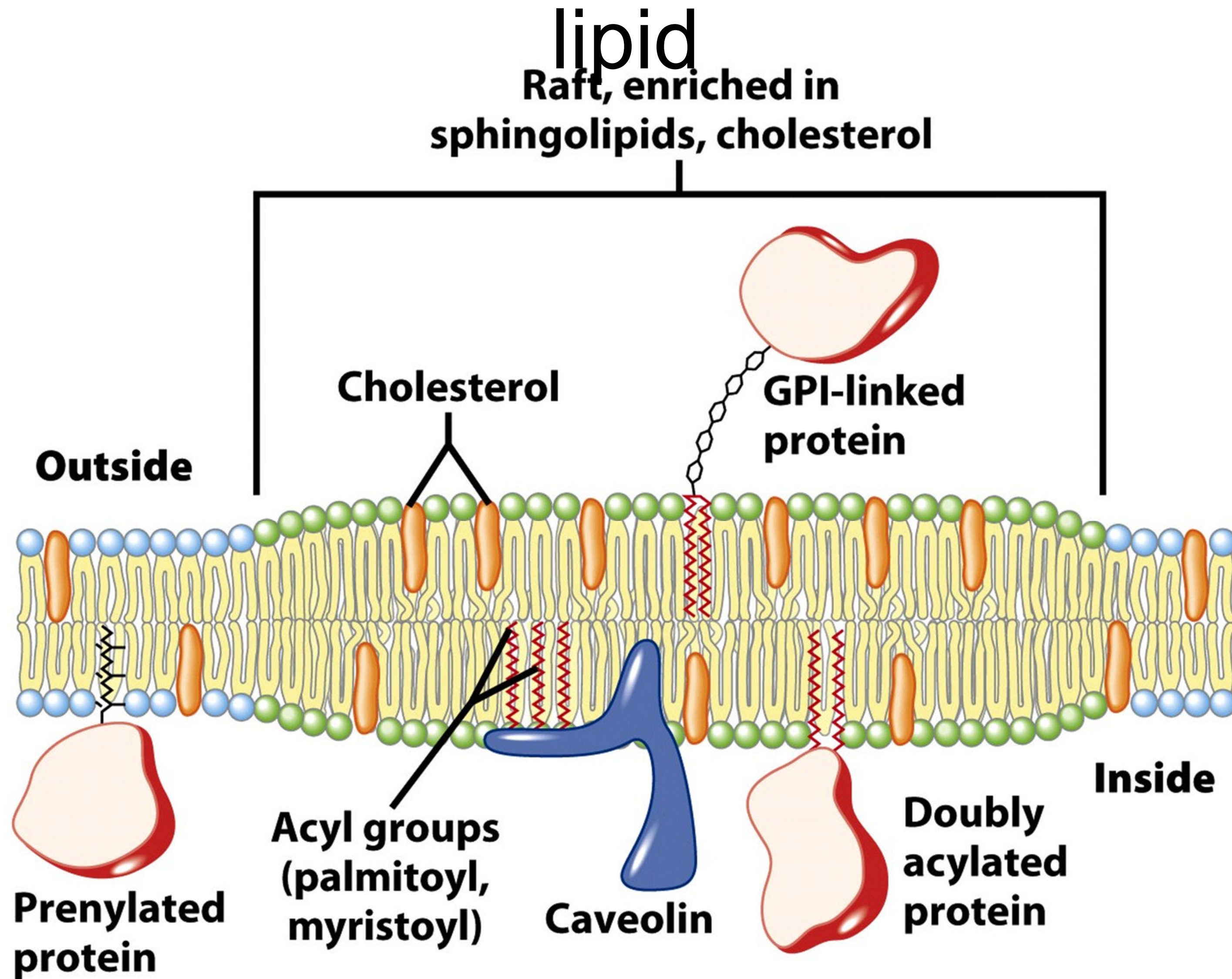
—Ser—Leu—Val—Thr —Asn—Phe—Ile—

Transporters catalyze passage through the membrane




S
S1
S2
S1
S2
Uniport
Symport
Antiport
Cotransport

Lipid-linked proteins cluster in or outside of rafts based on their linked lipid



Methods for determining protein structure

- Sequence:
  - Edman degradation: Remove one modified a.a from N-terminus at a time;
  - Mass spectrometry: Generate small fragments and measure the M/Z ratio.

- Secondary structure:
  - Circular Dichroism
  - FTIR

  } Indicate the composition of secondary structures

- Tertiary, quaternary structure:
  - NMR: derived distance constraints are used to calculate likely protein conformations
  - X-ray crystallography: Electron density map allows for positioning of protein atoms, revealing structure

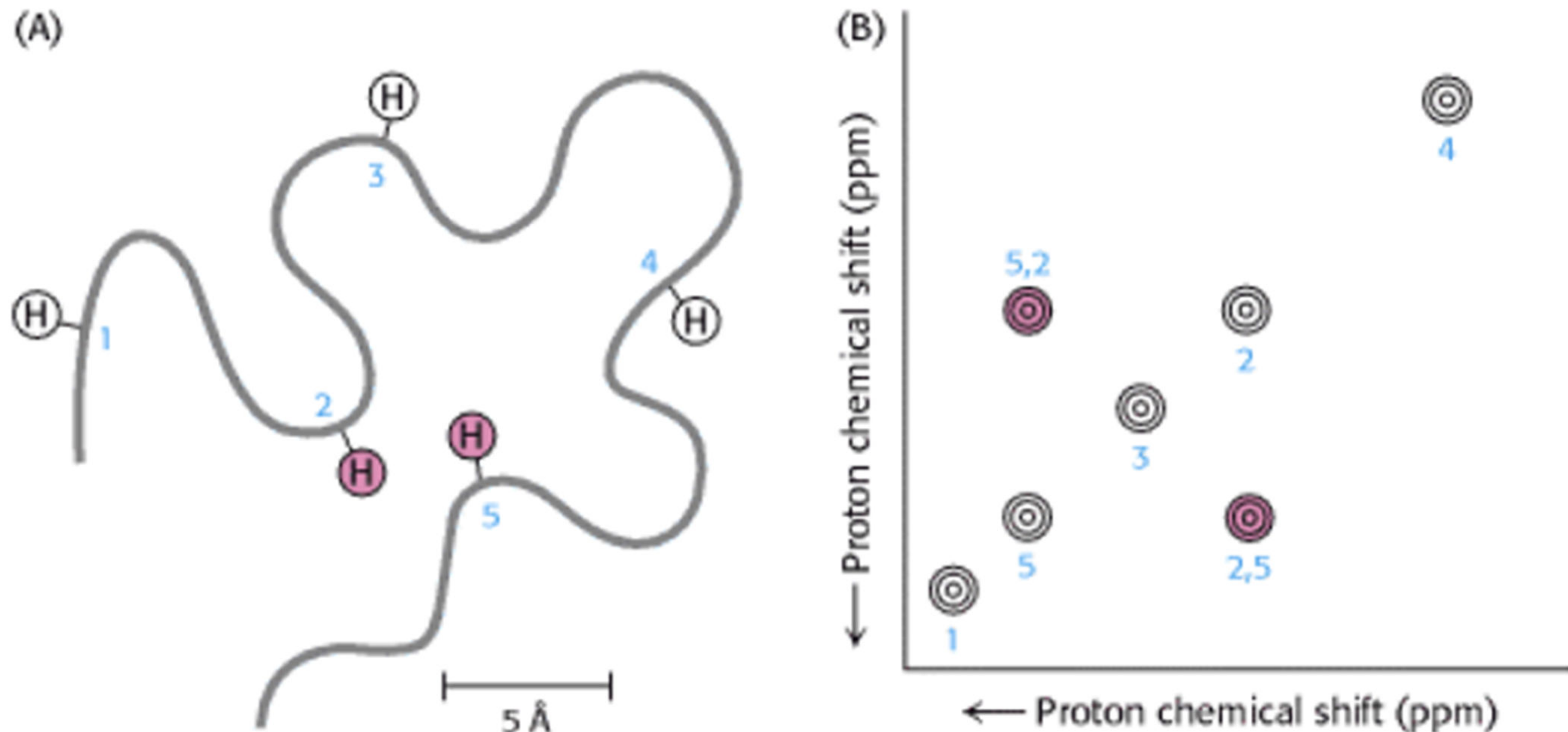
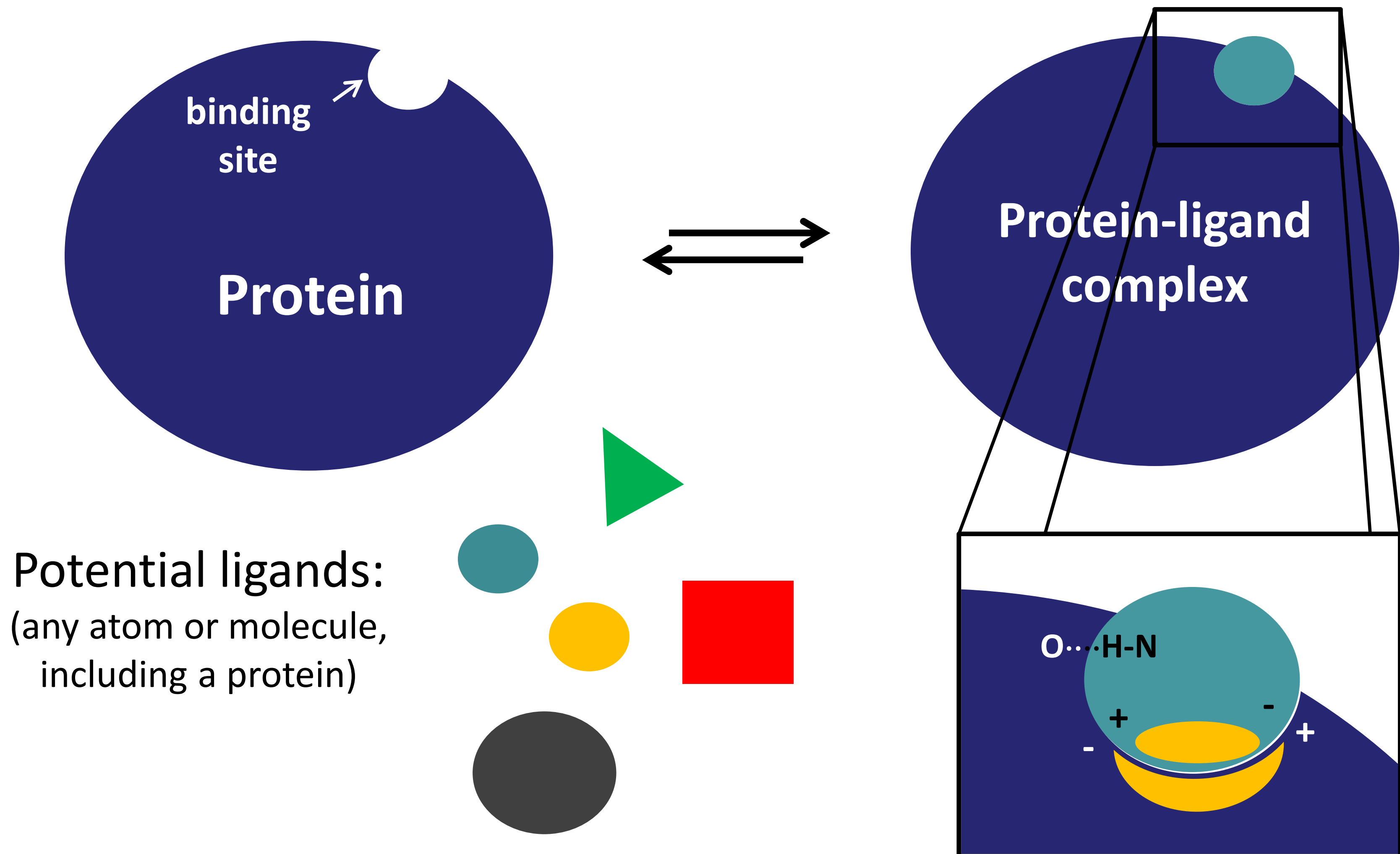
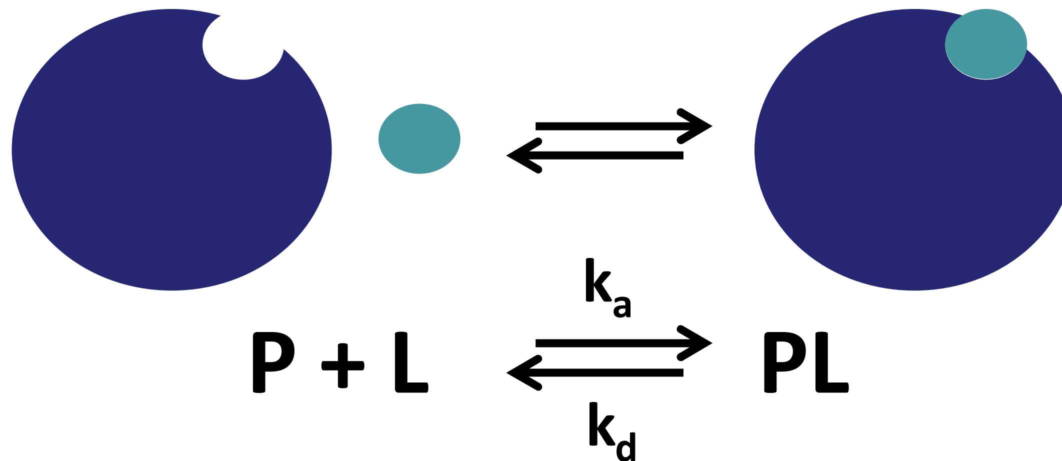


Figure 4.45. The Nuclear Overhauser Effect. The nuclear Overhauser effect (NOE) identifies pairs of protons that are in close proximity. (A) Schematic representation of a polypeptide chain highlighting five particular protons. Protons 2 and 5 are in close proximity (~4 Å apart), whereas other pairs are farther apart. (B) A highly simplified NOESY spectrum. The diagonal shows five peaks corresponding to the five protons in part A. The peaks above the diagonal and

A protein binds a ligand through a specific, reversible interaction



The association constant (K_a) and disassociation constant (K_d)provide a measure of affinity between protein & ligand

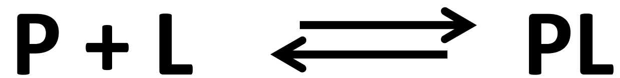


$K_a = \frac{[PL]}{[P][L]}$ = Association Constant

$$K_a = \frac{[PL]}{[P][L]} = \frac{k_a}{k_d}$$

$K_d = \frac{1}{K_a}$ = Dissociation Constant

The fraction of occupied binding sites (θ) is proportional to the ligand concentration



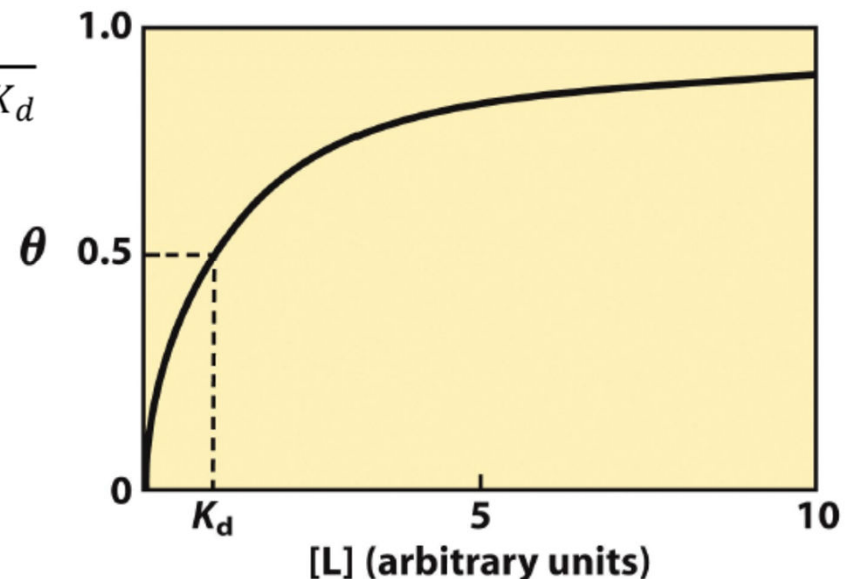
When $[L] \gg [PL] + [P]$, $[L]$ is constant (usually true for small ligands in cells)

$$\theta = \frac{binding\ sites\ occupied}{total\ binding\ sites} = \frac{[PL]}{[PL]+[P]}$$

Substitue in $[PL] = K_a[L][P]$

$$\theta = \frac{K_a[L][P]}{K_a[L][P]+[P]} = \frac{K_a[L]}{K_a[L]+1} = \frac{[L]}{[L]+\frac{1}{K_a}} = \frac{[L]}{[L]+K_d}$$

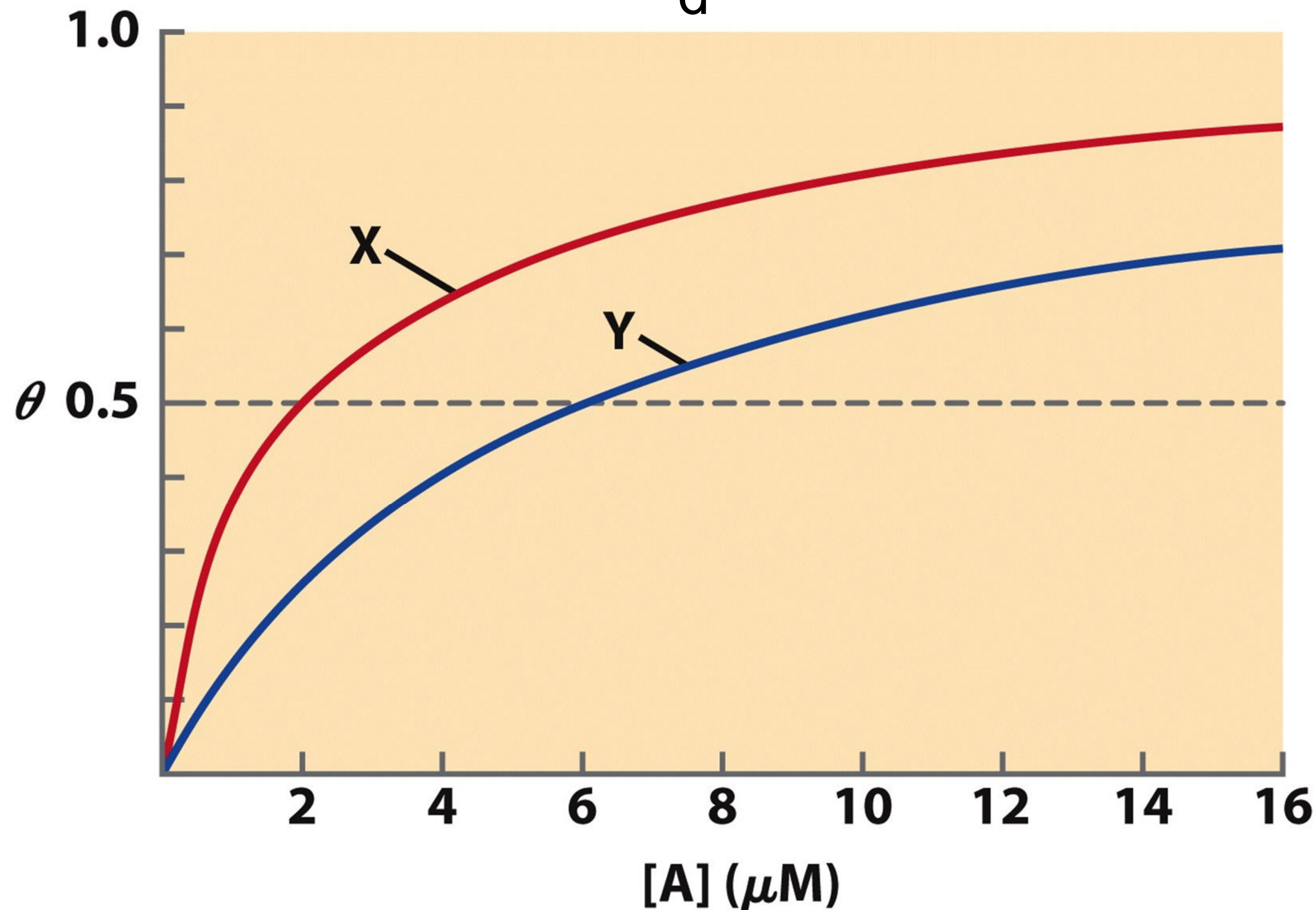
Simple binding: Hyperbolic curve



When [L] = K_d, then θ = 1/2

Lower K_d = Higher Affinity!!

A protein with higher affinity for a ligand has a higher binding curve and lower K_d



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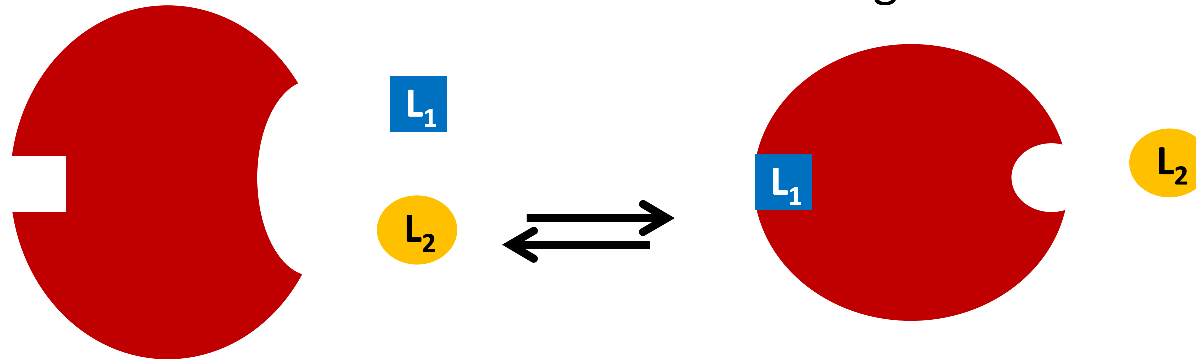
Allosteric protein

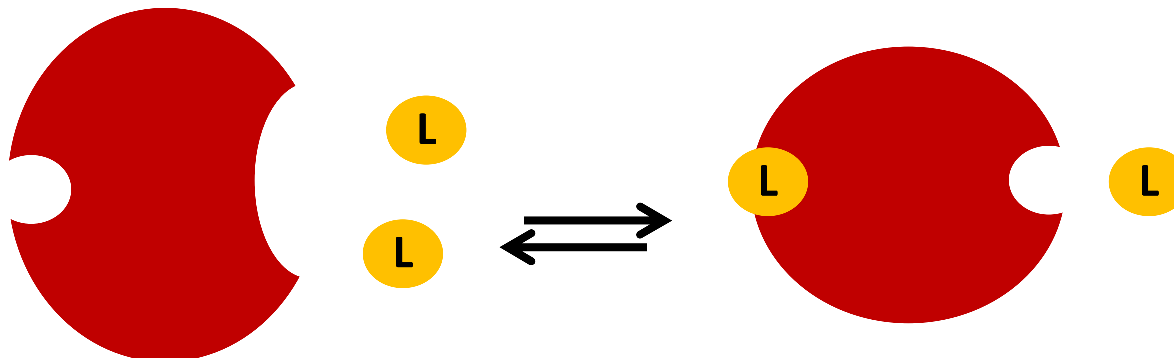
Binding of a ligand (L_1) to one site affects binding properties of ligand (L2) at another site (via a conformational change in the protein) .

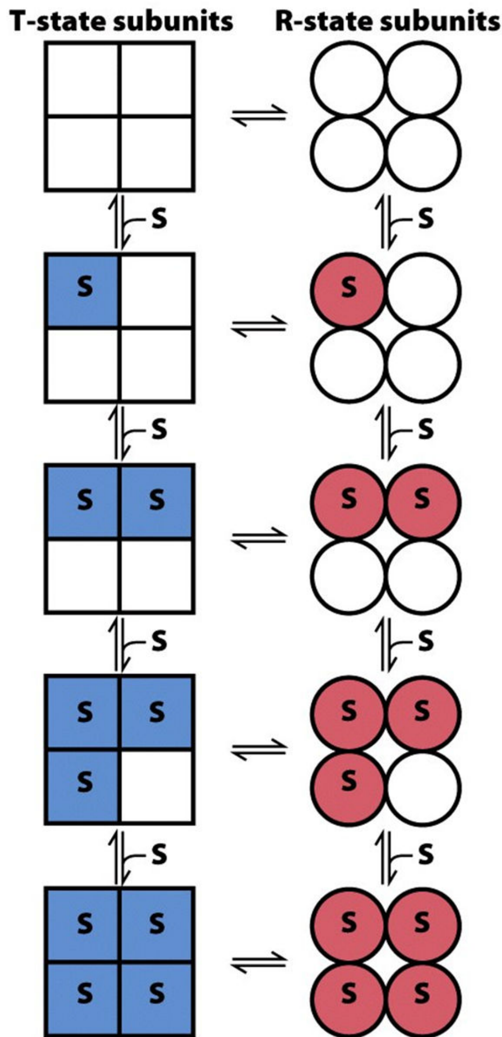
Modulator (L_1) is an 'activator' if it increases affinity at 2nd site (where L_2 binds)
Modulator (L_1) is an 'inhibitor' if it decreases affinity at 2nd site (where L_2 binds)

Heterotropic interaction: Modulator and other ligand are different



Homotropic interaction (cooperativity): Modulator and other ligand are the same





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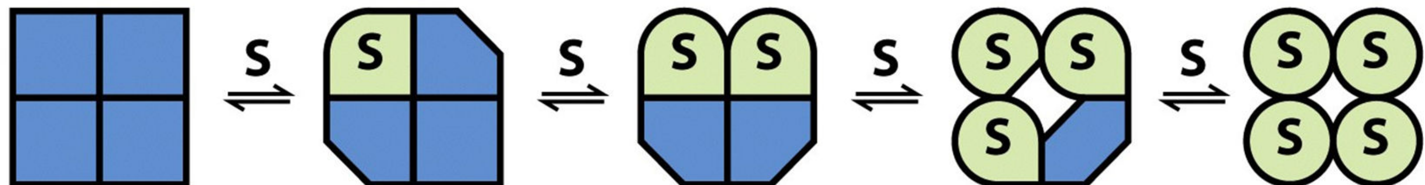
The symmetry (concerted) model of cooperativity
Subunits can adopt one of two possible conformations: T or R. All subunits *must* adopt the same conformation (protein is always symmetric). Equilibrium between T and R states is influenced by ligand or modulator binding.

The sequential (gradual) model of cooperativity
Subunits can adopt multiple conformations; Binding of ligand (S) induces conformational changes in the bound subunit and in neighboring subunits; Bound conformations may have higher or lower affinity for ligand than the free protein.



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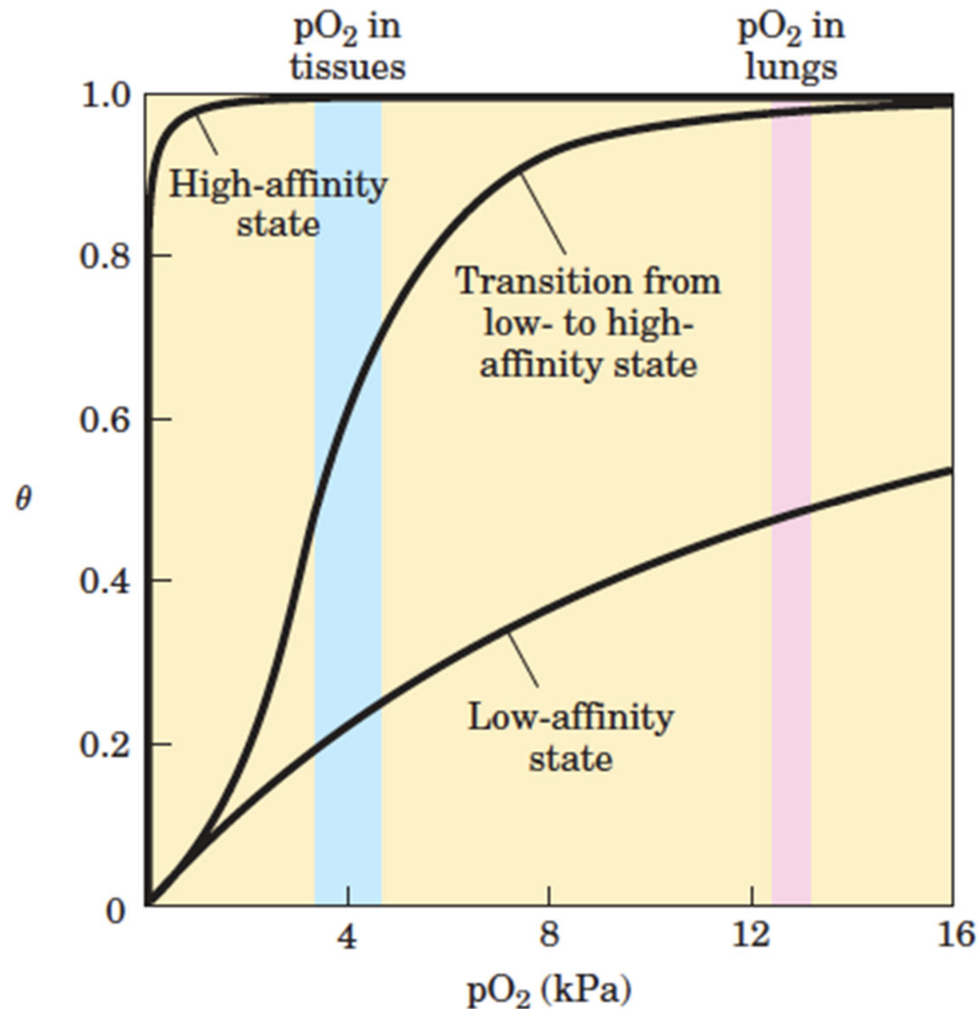
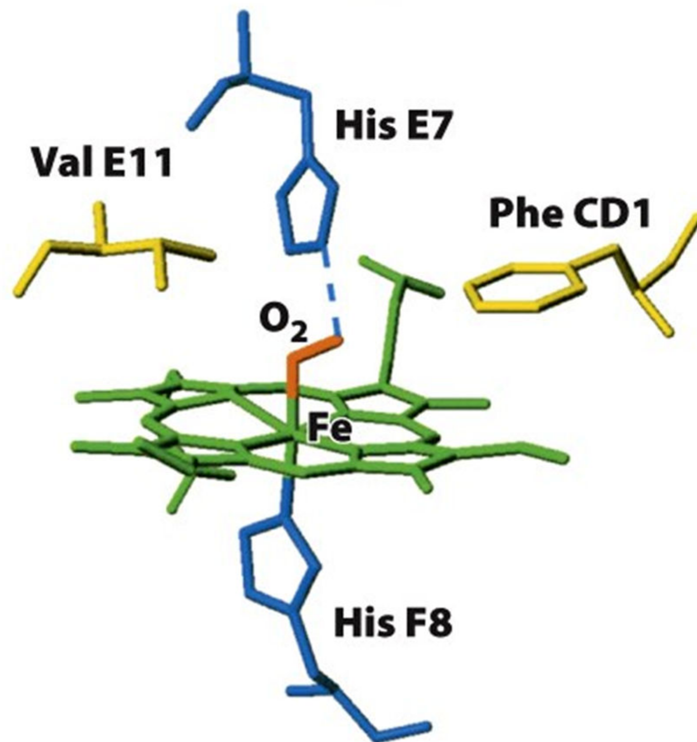
The oxygen-binding curves of Mb and Hb reflect their different functions



Myoglobin: single subunit, high affinity to oxygen, hyperbolic curve.

Hemoglobin: 4 subunits, sigmoidal curve, low affinity at tissues, high affinity at lungs. Cooperativitiy.

Heme cofactors bind O_2



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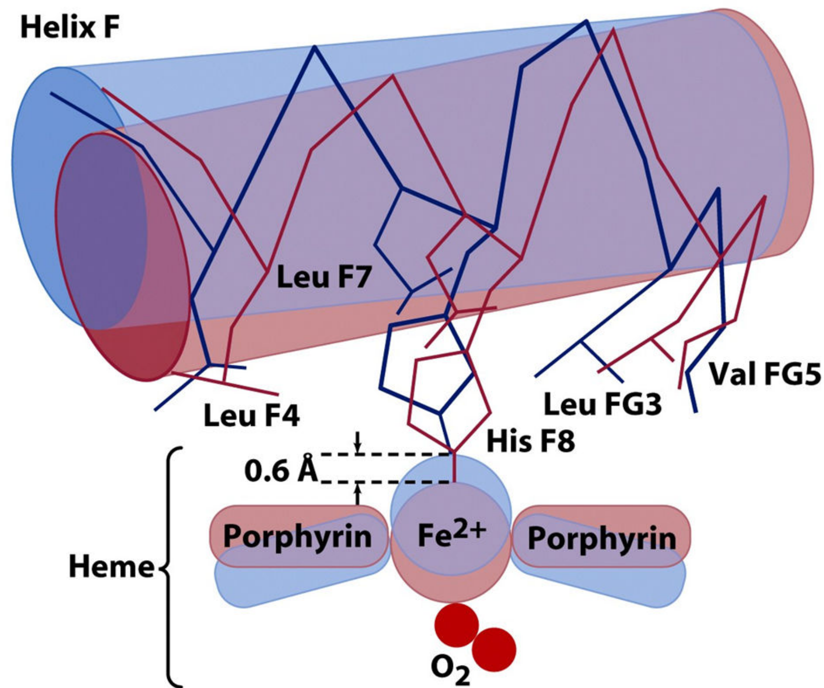
- Heme is held in place by the proximal His and by hydrophobic residues
- Proximal His of Hb covalently binds Fe of heme
- Distal His hydrogen bonds to O_2 bound to Fe. It reduces the affinity of hemoglobin to the toxic positive modulator CO by forcing CO to adopt an angle.

The proximal His links flattening of the heme to shifting of helix F in the T→ R transition.



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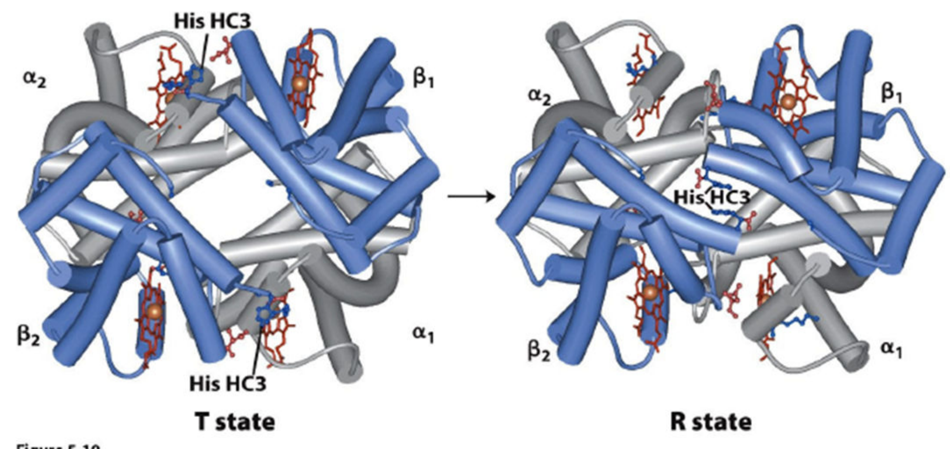
Movement of helix F shifts the entire quaternary structure of hemoglobin

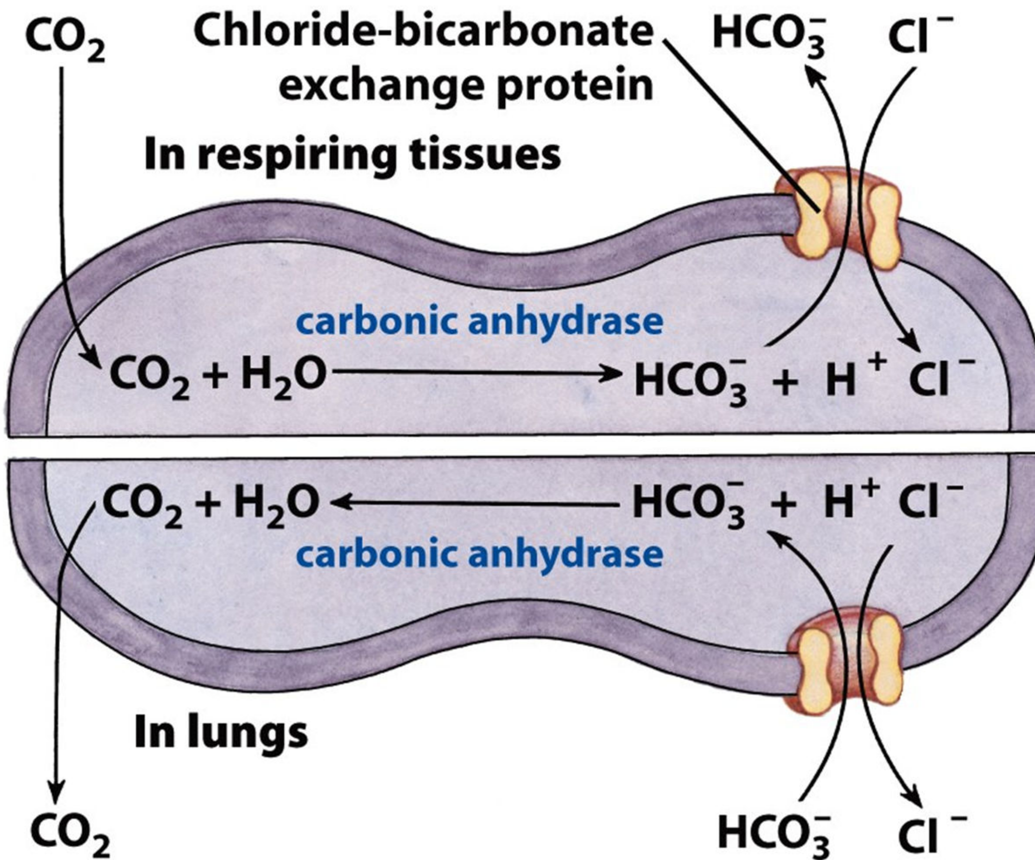


T-state = deoxygenated, low affinity
R-state = oxygenated, high affinity

There are also several ion pairs in the T-state that are broken upon transition to the R-state.

Modulators/Effectors of O_2 binding

- **Positive (stabilize R-state)**
  - O_2
  - CO (competitive inhibitor, P_{50} = 200x lower than O_2 (would be 20,000x lower if distal His were not there)
  - NO
  - H_2S
- **Negative (stabilize T-state)**
  - 2,3 BPG
    - Very negatively charged. Makes ionic interactions with Lys, Arg, His, N-terminus in center of tetramer. Keeps Hb in T-state
    - In R-state, conformation change closes up this central cavity and BPG cannot bind
    - At high altitudes, BPG helps transfer more oxygen to the tissues
  - H^+ "Bohr Effect"
    - Protons help salt bridges of T-state to form
    - Protons from reaction of carbonic anhydrase: $CO_2 + H_2O$ □ $HCO_3^- + H^+$
  - CO_2
    - Can add to N-terminal residues of Hb subunits to make carbamate (stabilizes T-state)
    - Enhances reaction of carbonic anhydrase to make H^+
  - Cl^-
    - Stabilizing "bridging ion" for ion pairs of T-state

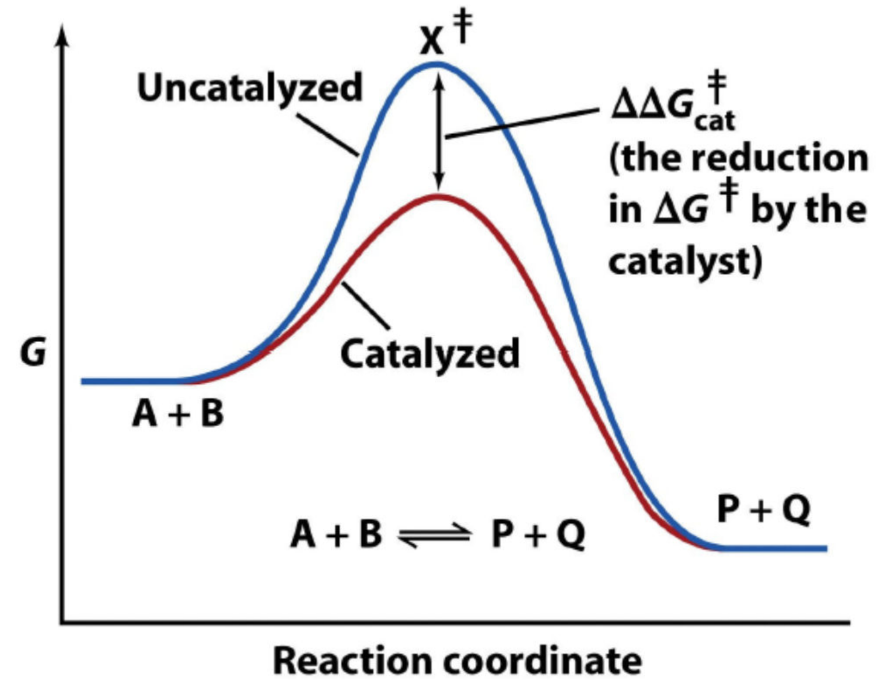


High CO2, converting to bicarbonate and proton by Carbonic anhydrase,
low pH, high Cl-
Low O2
BPG binds to stablize T-state

Low CO2,
bicarbonate and proton being converted to CO2 by Carbonic anhydrase,
high pH, low Cl-
High O2
BPG cannot bind to R-state

Enzymes

catalyze a reaction by lowering the activation energy (E_A)



- Do not change the equilibrium state of reactants or products (do not change thermodynamics)
- Enhance the rate (kinetics) of a reaction
- Kinetics are determined by the state with the highest E_A
- Enzymes act by PREFERENTIALLY BINDING the transition state – this lowers the E_A

Enzyme Classification (see handout also)

Table 11-2 Enzyme Classification According to Reaction Type

Classification	Type of Reaction Catalyzed
1. Oxidoreductases	Oxidation–reduction reactions
2. Transferases	Transfer of functional groups
3. Hydrolases	Hydrolysis reactions
4. Lyases	Group elimination to form double bonds
5. Isomerases	Isomerization
6. Ligases	Bond formation coupled with ATP hydrolysis

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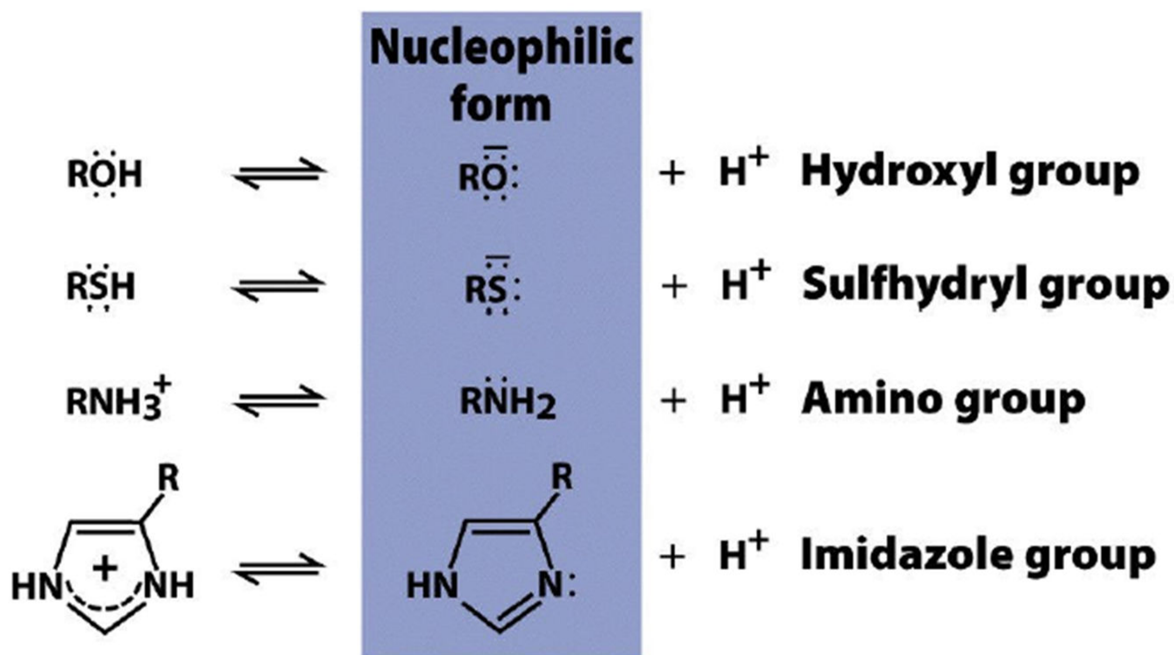
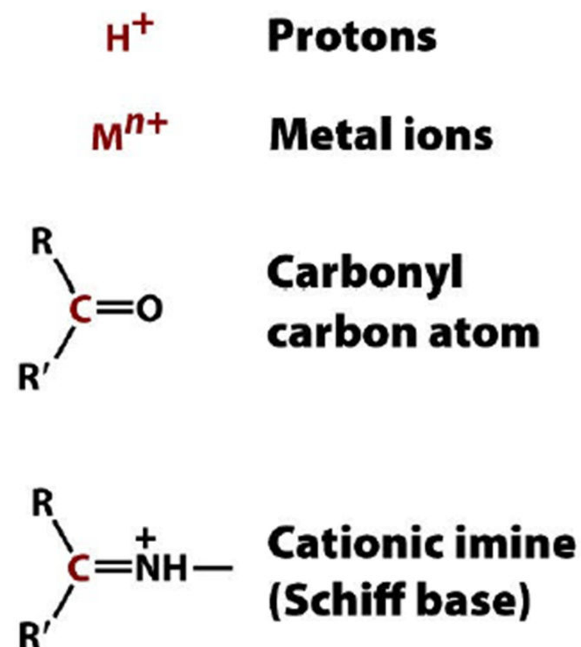
Enzymes use several catalytic mechanisms (often together) to enhance reaction rates

- Proximity and orientation effects: the enzyme positions substrates (with respect to each other and to enzyme functional groups) to maximize reactivity
- Electrostatic catalysis: the enzyme uses charge-charge interactions in catalysis
- Preferential binding of transition state: binding interactions between the enzyme and TS are maximized; they are greater than those in the enzyme-substrate or enzyme-product complexes
- General acid and general base catalysis: functional groups of the enzyme donate &/or accept protons
- Covalent catalysis: the enzyme forms a covalent bond with the substrate
- Metal-ion catalysis: the enzyme uses a metal ion to aid catalysis

Common nucleophiles and electrophiles in biochemistry





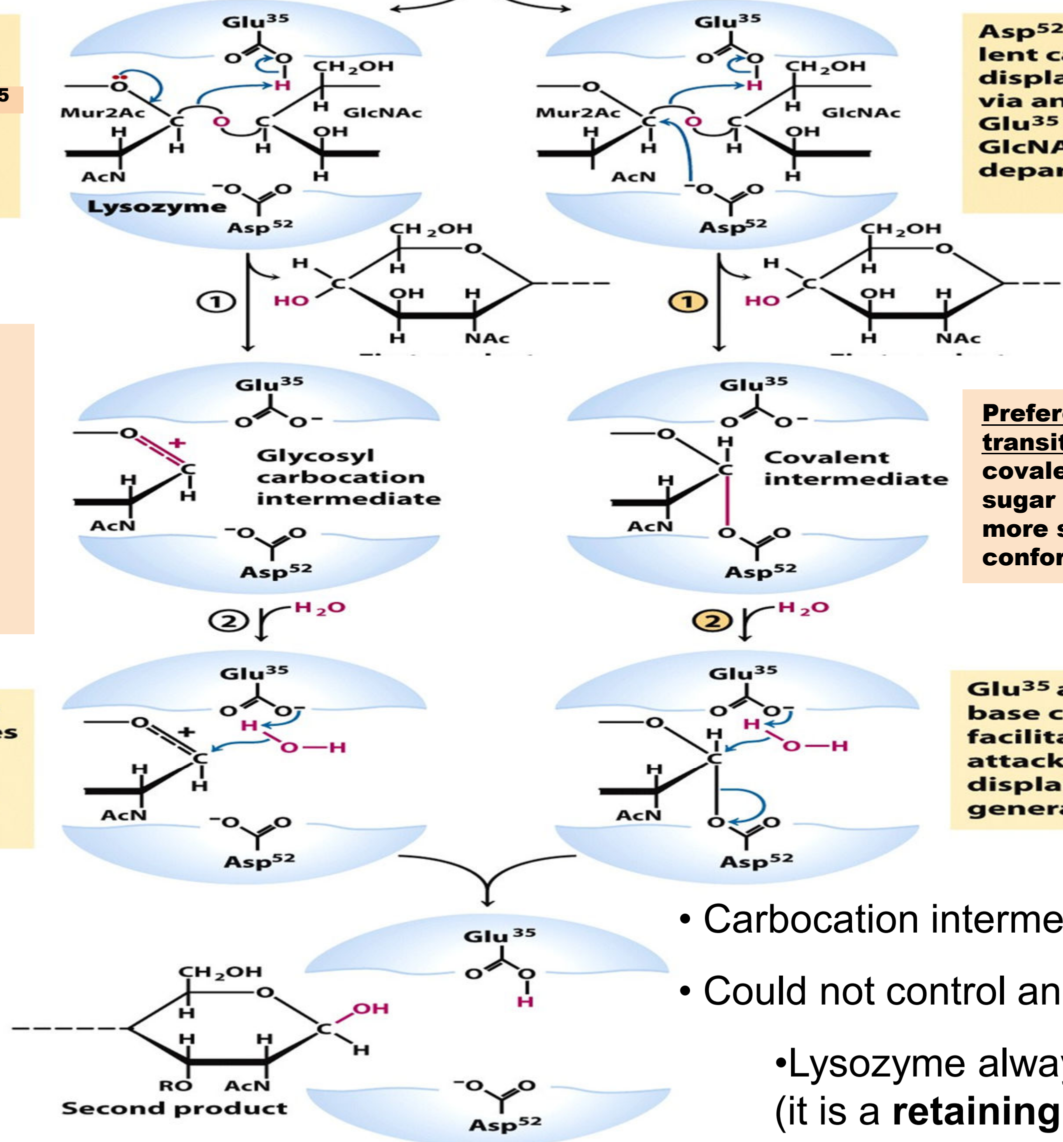
Lysozyme mechanism

A rearrangement produces a glycosyl carbocation. General acid catalysis by Glu[35] protonates the displaced GlcNAc oxygen and facilitates its departure.

<u>Preferential binding of transition state</u>: carbocation D-sugar is more stable than the original substrate D-sugar in half-chair conformation

<u>Electrostatic catalysis</u>: Asp[52]

General base catalysis by Glu[35] facilitates the attack of water on the glycosyl carbocation to form product.



Asp[52] acts as a covalent catalyst, directly displacing the GlcNAc via an S_N2 mechanism. Glu[35] protonates the GlcNAc to facilitate its departure.

<u>Preferential binding of transition state</u>: covalently bound D-sugar is forced into more stable chair conformation

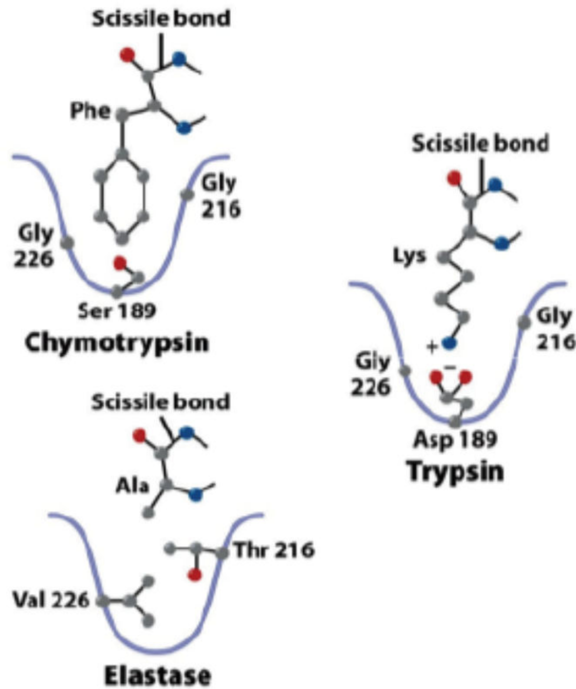
Glu[35] acts as a general base catalyst to facilitate the S_N2 attack of water, displacing Asp[52] and generating product.

Why S_N2?

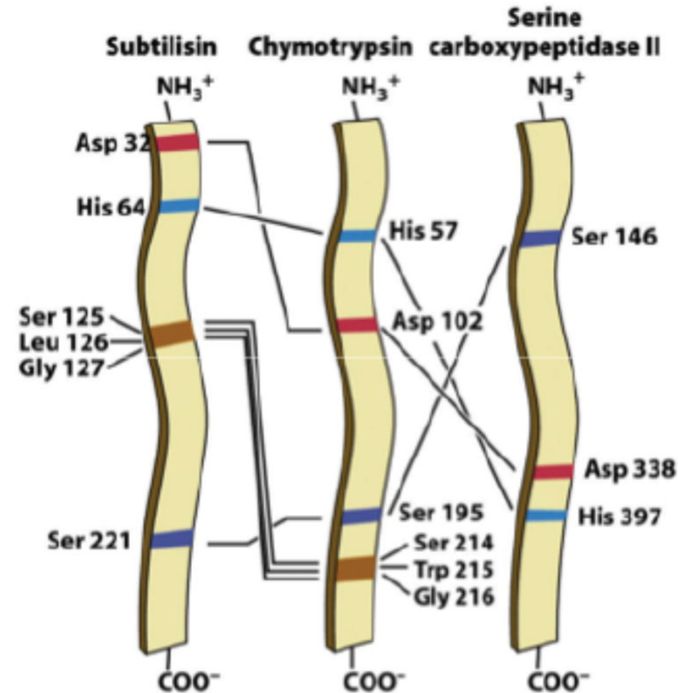
- Carbocation intermediate of S_N1 is too unstable
- Could not control anomer formed if S_N1 is used
  - Lysozyme always makes beta-anomer (it is a **retaining glycosidase**)
- Crystal structure of lysozyme with transition state analog supports covalent mechanism

Evolution of serine proteases

Divergent evolution



Convergent evolution

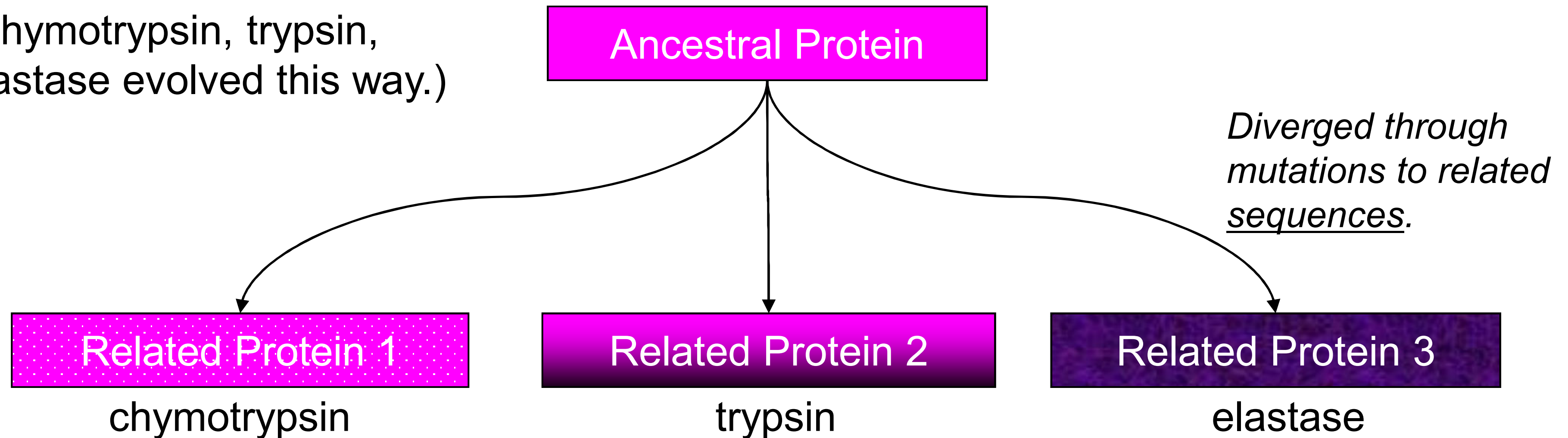


SERINE PROTEASES

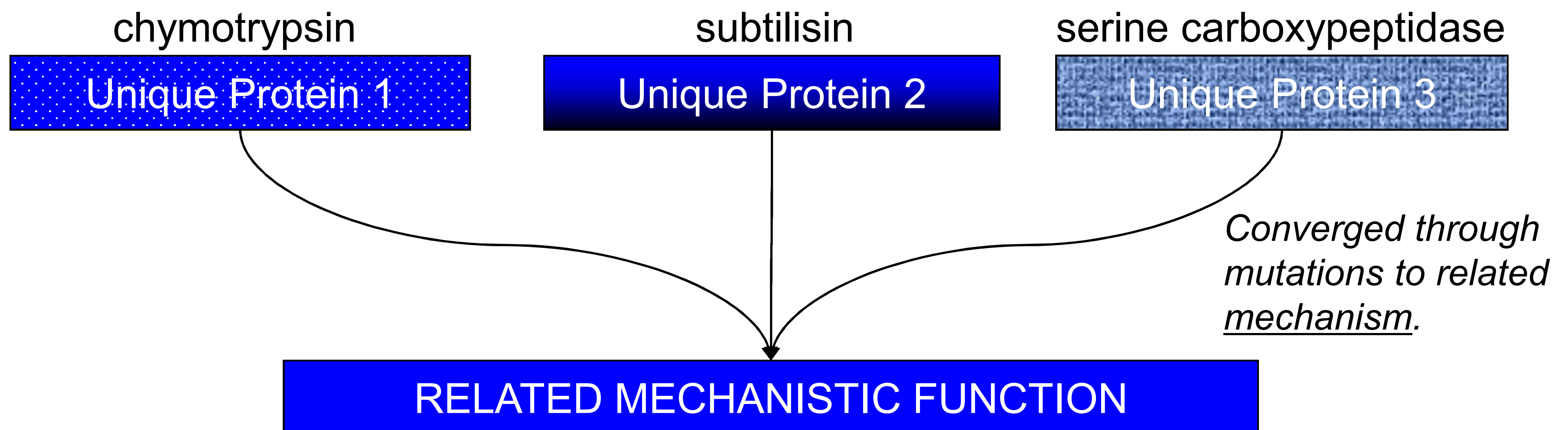
(catalytic triad: Asp-His-Ser)

Divergent Enzyme Evolution

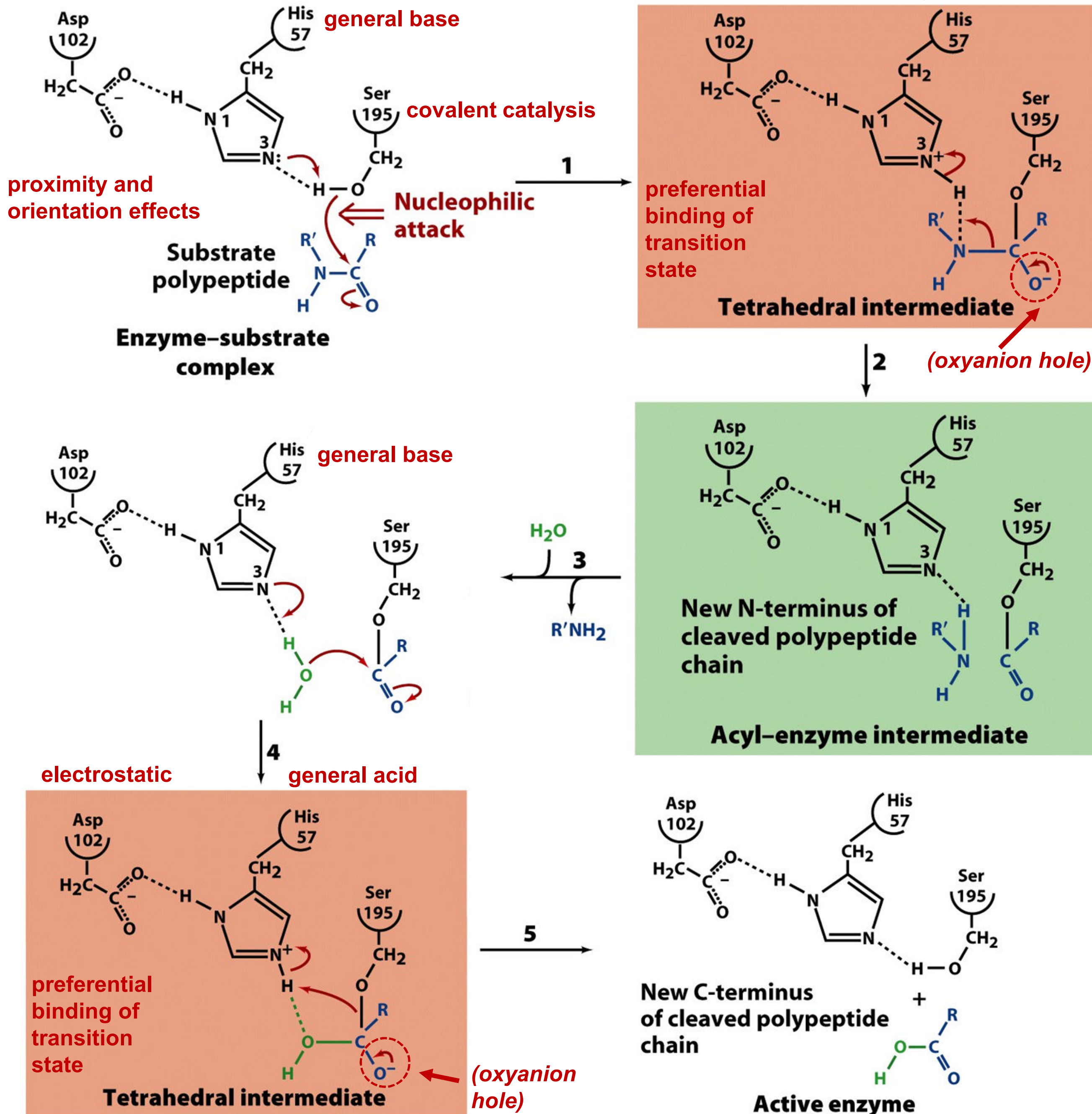
(Chymotrypsin, trypsin, elastase evolved this way.)



Convergent Enzyme Evolution

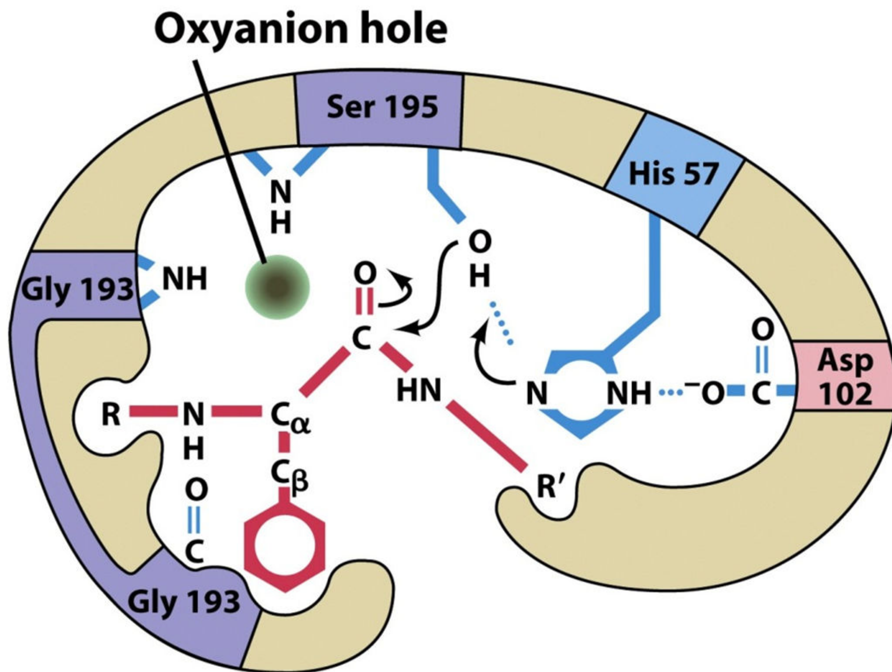


Serine protease mechanism



- Hydrolyze peptide bonds using active site Ser, which gets deprotonated by His and nucleophilically attacks the carbonyl carbon
- Later on His donates this proton to break the peptide bond (acid catalysis) – C-terminal half of polypeptide leaves with a newly created N-terminus
- His acts as a base and deprotonates H_2O so that water can attack carbonyl and make carboxyl group
- This proton finally gets transferred back to Ser to release the C-terminus and **regenerate the enzyme active site**

Chymotrypsin preferentially binds the tetrahedral intermediate



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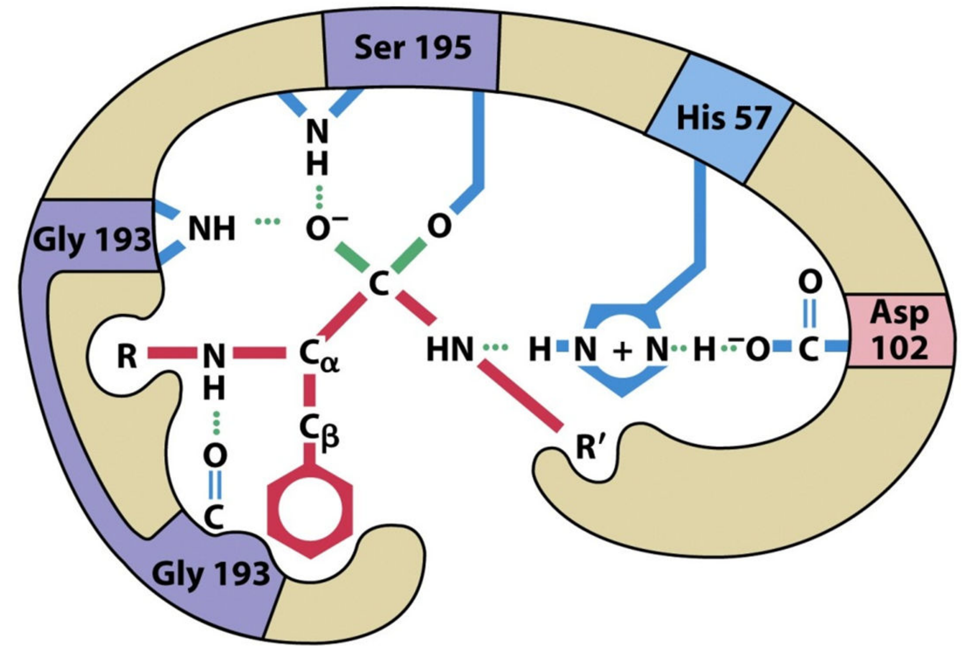

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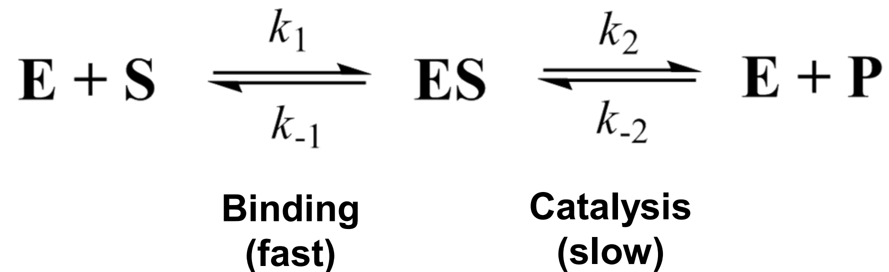
Michaelis-Menten kinetics of enzymes

$$v_0 = \frac{V_{\max}[S]}{K_M + [S]}$$



- v_0 is the velocity of the reaction
  - Units of (concentration of product / time)

$$K_M = \frac{k_2 + k_{-1}}{k_1}$$

$K_M = K_D$ when $k_2 <<< k_{-1}$

$$V_{\max} = k_2[E_T] = k_{cat}[E_T]$$

- In a reaction with many steps, k_{cat} is the rate constant for the rate-limiting step
- k_{cat} is also known as the turnover number and it represents the maximum # of substrate molecules converted to products per active site per unit time

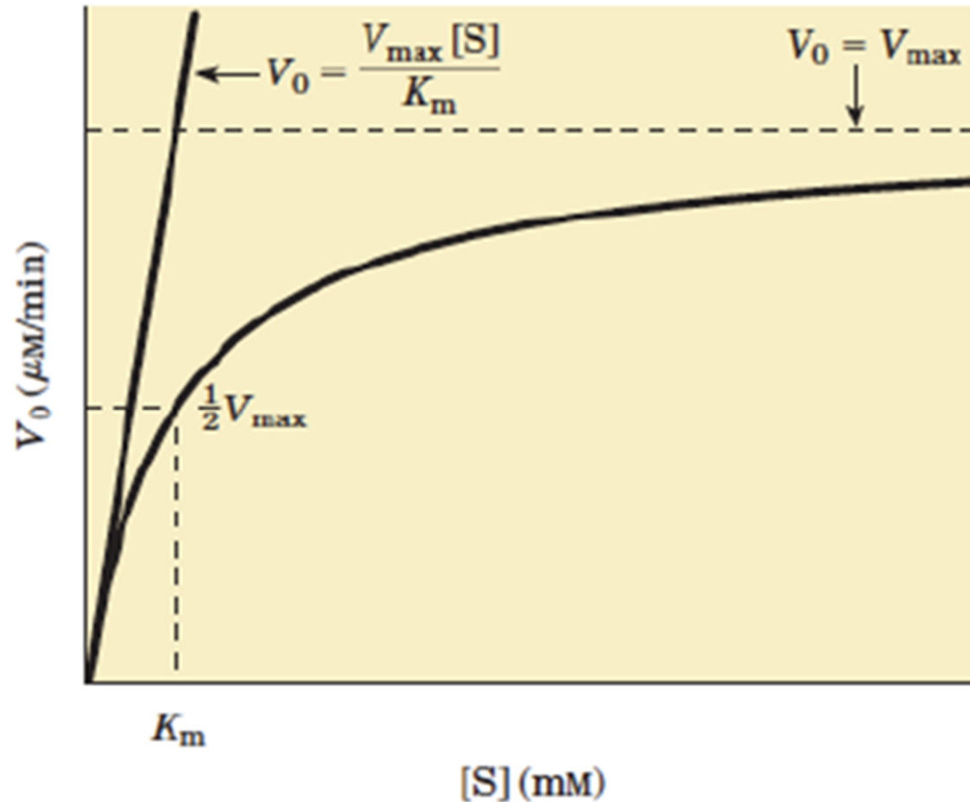
Some of the important assumptions for derivation:

- @ time = 0, [P] ≈ 0
- [ES] is constant
- @ high [S], $v_0 = V_{max}$ and [ES] = $[E_T]$

Catalytic perfection

- $\frac{k_{cat}}{K_M}$ is known as the catalytic efficiency and the upper limit is the rate at which E and S can diffuse together in aqueous solution.
  - Diffusion-controlled limit is 10^8-10^9 $M^{-1}s^{-1}$. Enzymes in this range have achieved catalytic perfection. When can an enzyme exceed this limit?

More on K_M



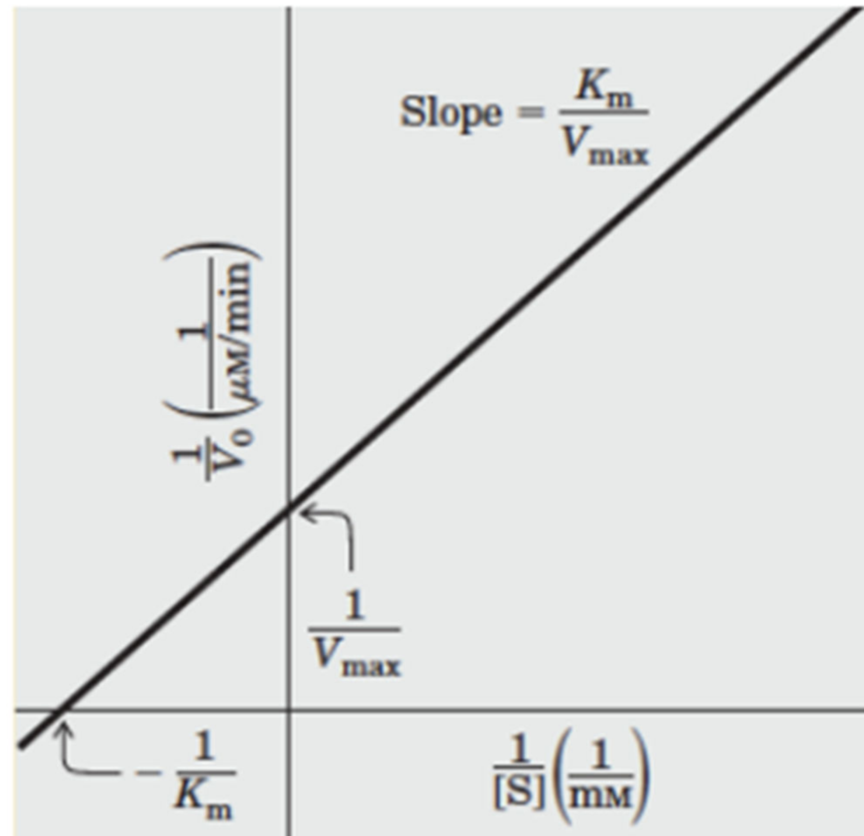
- Difficult to determine V_{max} reliably here

- K_M = [S] when $v_0 = 0.5V_{max}$
  - K_M represents the [S] at which half of the enzyme active sites are filled by substrate molecules

Lineweaver-Burk plot

• Take the reciprocal of the M-M equation which will yield:

$$\frac{1}{v_0} = \frac{K_M}{V_{max}[S]} + \frac{1}{V_{max}}$$



•Since this is a linear plot, it relies on the equation for a line, y = mx + b

•Where if x=0, then the y-intercept is $1/V_{max}$

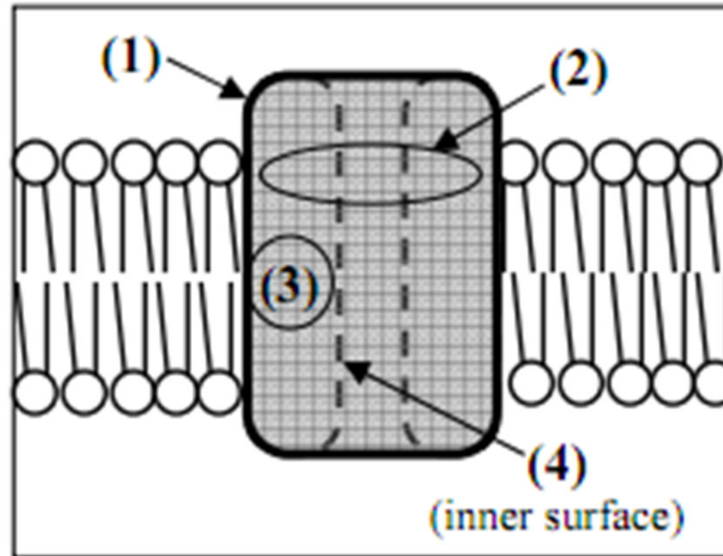
•If y=0, then the x-intercept is $-1/K_M$

•The slope is K_M/V_{max}

Questions to review

(4) Given the following diagram of a pore-forming integral membrane protein (shown in grey), match each amino acid to the *surface* location most likely to contain it.

a. Leu
b. Pro
c. Tyr
d. Asn



1) b;
2) c;
3) a;
4) d.

Which of the following are reasons why mass spectrometry is a useful tool in protein sequencing?(choose all that apply)

a. Sequence identification can be automated;
b. It is possible to identify modifications to amino acids;
c. A peptide’s sequence can be unambiguously determinded;
d. Mass spectrometric sequencing is faster than Edman sequencing;
e. The masses of all amino acids are unique.

a, b, d

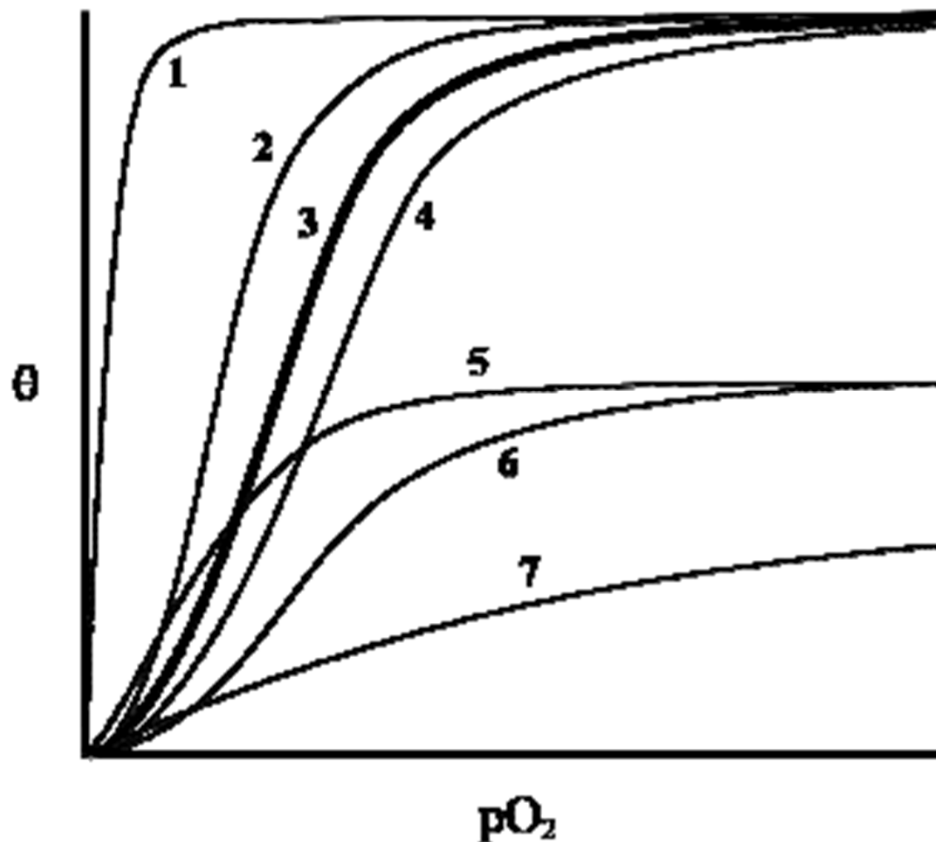
(12 pts) Shown below are several O_2 binding curves. The curve in bold (#3) represents O_2 binding by red blood cells of an average person living at sea level. Which curve below best represents O_2 binding:

a. For a resident of Tibet, who has a higher-than-average BPG concentration. 4

b. For an elite athlete, who has a higher-than-average red blood cell count. 3

c. For an altered hemoglobin that can only adopt the R state. 1

d. For hemoglobin with half of its binding sites bound by carbon monoxide. 5



(12) The P_{50} for oxygen binding to myoglobin is 2.8 torr. Binding experiments with an altered myoglobin show that it is 90% saturated at an oxygen partial pressure of 44 torr.

a. Calculate the P_{50} for oxygen binding to the altered myoglobin. Show your work.
b. Draw the binding curves for normal (N) and altered (A) myoglobin. Label the axes with names, units, and number values, and indicate which curve is which.
c. Does the altered myoglobin have higher or lower oxygen-binding affinity than normal myoglobin?
d. Additional experiments show that the normal and altered myoglobins bind oxygen *equally quickly*. Briefly explain how this is possible (in 35 words or fewer).

a. (3) $\theta = \frac{pO_2}{P_{50}+pO_2}$

$\theta(P_{50} + pO_2) = pO_2$

$\theta \cdot P_{50} + \theta \cdot pO_2 = pO_2$

$\theta \cdot P_{50} = pO_2 - \theta \cdot pO_2$

$\theta \cdot P_{50} = pO_2(1 - \theta)$

$$P_{50} = pO_2\frac{(1-\theta)}{\theta} = 44\text{ torr} \cdot \frac{0.1}{0.9} = 4.9\text{ torr}$$

b. (4) x-axis: pO_2 (torr); y-axis: θ, with values 0 to 1.0; 'N' curve: hyperbolic, passing through (2.8, 0.5) and approaching 1.0 in y; 'A' curve (right of 'N' curve): hyperbolic, passing through (4.9, 0.5) and (44, 0.9)

c. (2) lower

d. (3) Affinity depends on the rate constants for binding and unbinding. To have a lower affinity, O_2 would unbind (dissociate) faster from the altered myoglobin.

Given the enzyme catalyzed reaction:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

a. What assumption must be made about this reaction in order for K_m to approach the K_d of the enzyme-substrate complex.

b. Briefly define 'first-order' as it applies to rate constants (15 words or less).

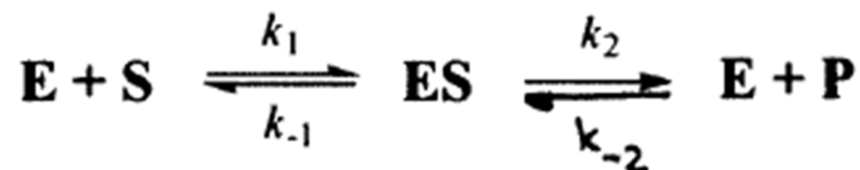
c. Of the rate-constants above, which are first-order?

d. Write two different expressions for the K_d of the enzyme substrate complex.

e. Under what condition is ES at steady state? Write an expression using concentrations and rate constants.

Given the enzyme catalyzed reaction:

Solution



$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

a. What assumption must be made about this reaction in order for K_m to approach the K_d c the enzyme-substrate complex?

$K_M = \frac{k_2 + k_{-1}}{k_1}$ if k_2 rate-limiting, $k_2 <<< k_{-1}$ and $K_M = \frac{k_{-1}}{k_1} = K_d$

b. Briefly define 'first-order' as it applies to rate constants (15 words or less).

First order refers to unimolecular reactions where only one reactant is going to product(s).

c. Of the rate-constants above, which are first-order?

k_2 and k_{-1}

d. Write two different expressions for the K_d of the enzyme substrate complex.

$$K_d = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]}$$

e. Under what condition is ES at steady state? Write an expression using concentrations and rate constants.

rate of formation of ES = rate of breakdown of ES

$$k_1[E][S] + k_{-2}[E][P] = k_{-1}[ES] + k_2[ES]$$

1. Estimate the V_{max} and K_m from the following data:

2.If 0.1µM enzyme was used in each of the above reactions, determine the k_{cat}

3.What would the catalytic efficiency be?

[S] (M)	V_o (µM/min)	[S] (M)	V_o (µM/min)
2.5×10^{-6}	28	4×10^{-5}	112
4.0×10^{-6}	40	1×10^{-4}	128
1×10^{-5}	70	2×10^{-3}	139
2×10^{-5}	95	1×10^{-2}	140

Solution

3. Estimate the V_{max} and K_m from the following data:

[S] (M)	V_o (µM/min)	[S] (M)	V_o (µM/min)
2.5×10^{-6}	28	4×10^{-5}	112
4.0×10^{-6}	40	1×10^{-4}	128
1×10^{-5}	70	2×10^{-3}	139
2×10^{-5}	95	1×10^{-2}	140

$V_{max} = 140\ \mu M/min$

$K_M = [S]$ when $v_o = \frac{V_{max}}{2}$

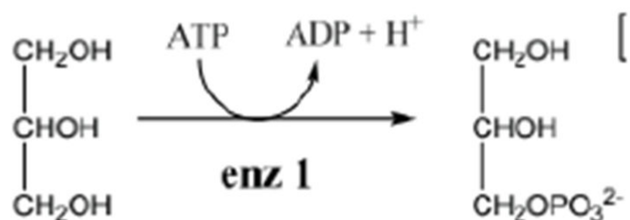
$\frac{V_{max}}{2} = 70$, so $K_M = 1\times10^{-5} M$

a. If 0.1µM enzyme was used in each of the above reactions, determine the k_{cat}

$$k_{cat} = \text{catalytic turnover} = \frac{V_{max}}{[E_T]} = \frac{140\ \mu M/min}{0.1\ \mu M} = 1400\ min^{-1} = 23.3\ s^{-1}$$

b. What would the catalytic efficiency be?

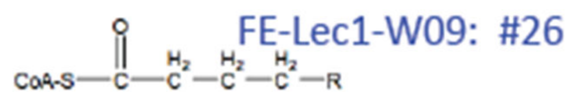
$$\frac{k_{cat}}{K_M} = \frac{23.3\ s^{-1}}{1\times10^{-5}\ M} = 2.3\times10^{6}\ M^{-1}\ s^{-1}$$

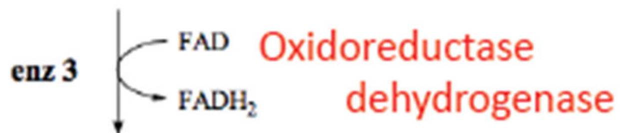


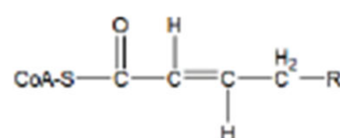
c. Name the class and subclass of enzyme 1.

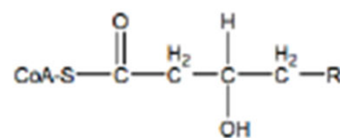
- Transferase
    - Kinase

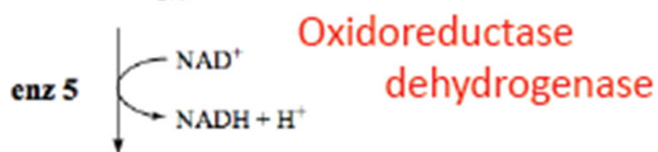
ENZYMES

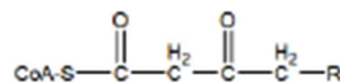


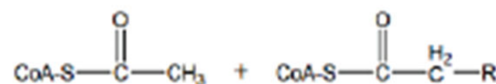












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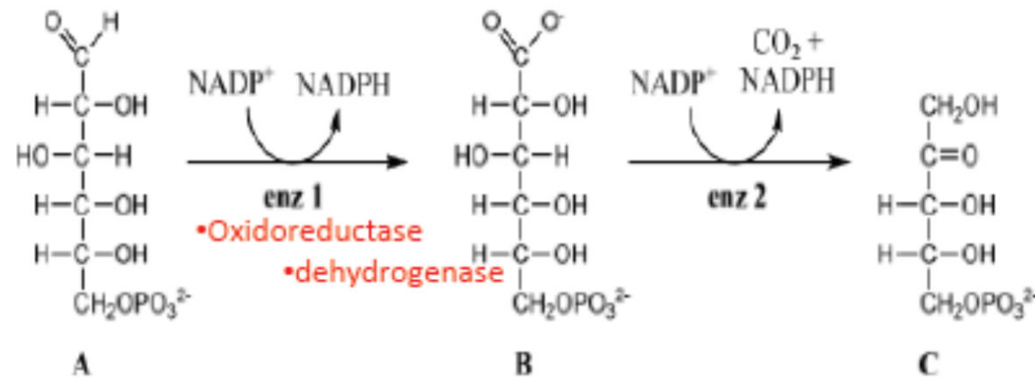
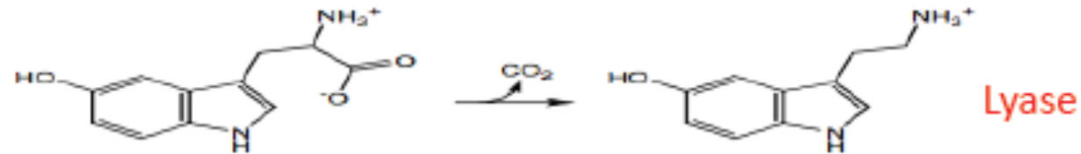
FE-Lec2-W09: #23



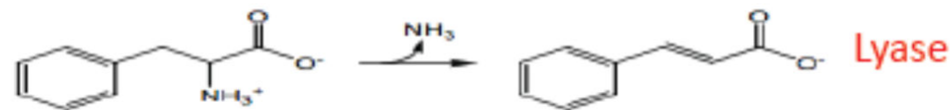
7. (6 pts) Name the *class* of enzyme that catalyzes each of the following reactions:

MTE#2-S09: #7

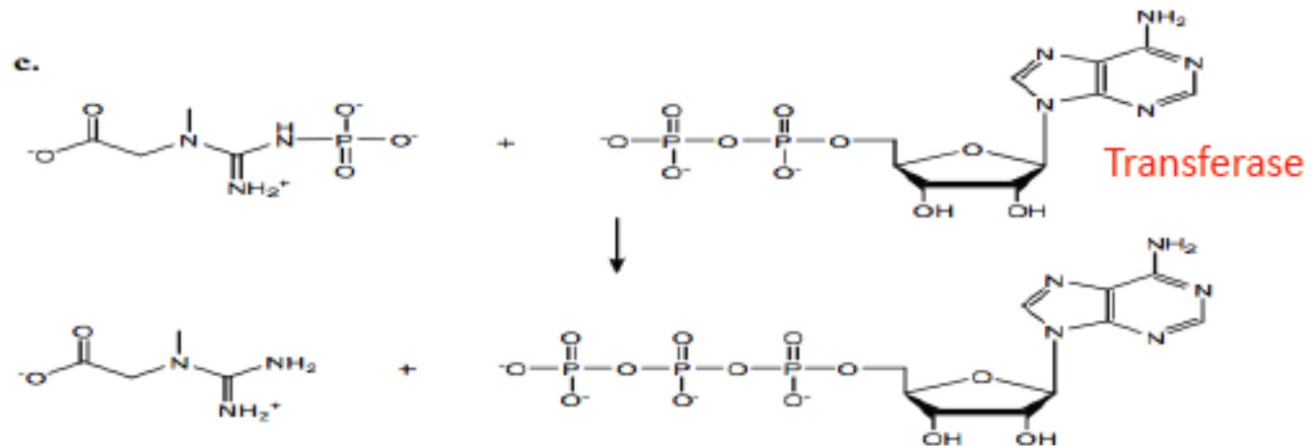
a.



b.



c.



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