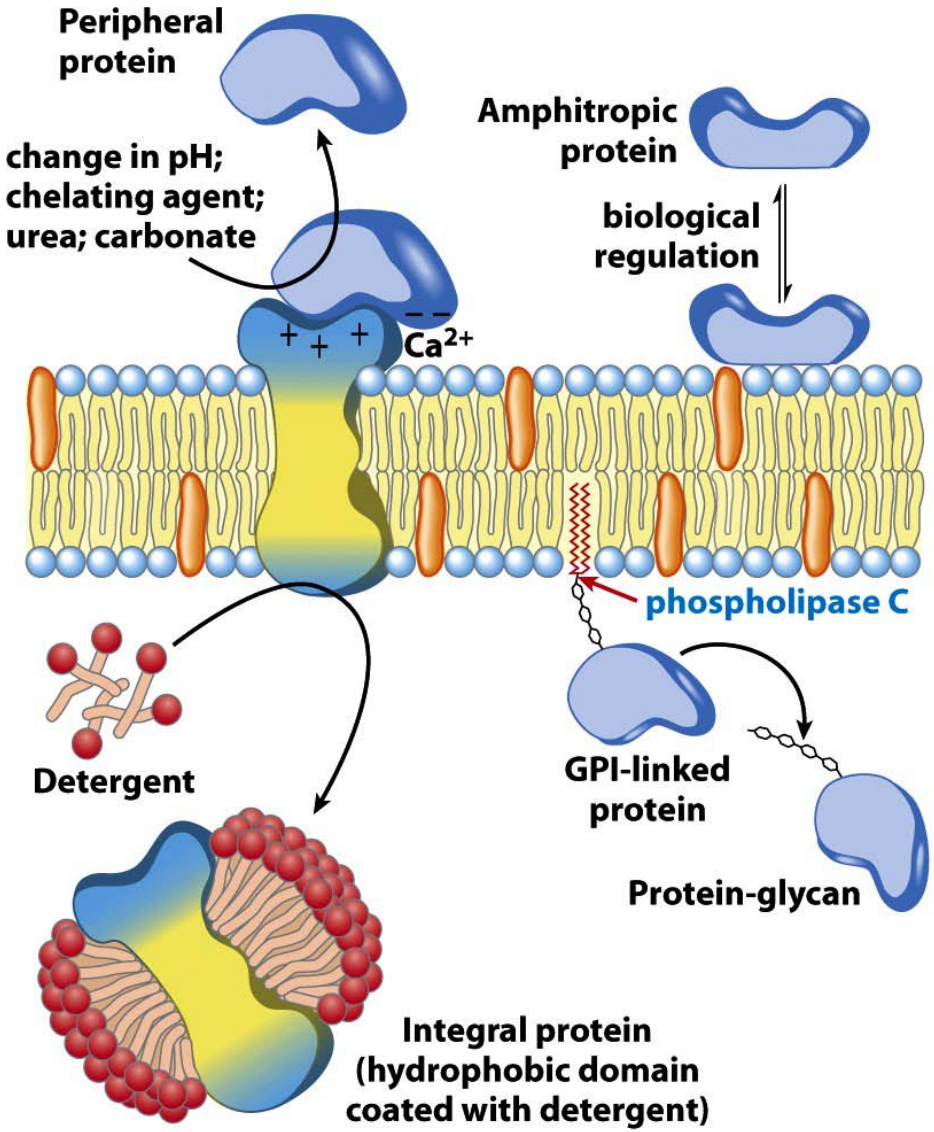
Membrane Proteins:



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

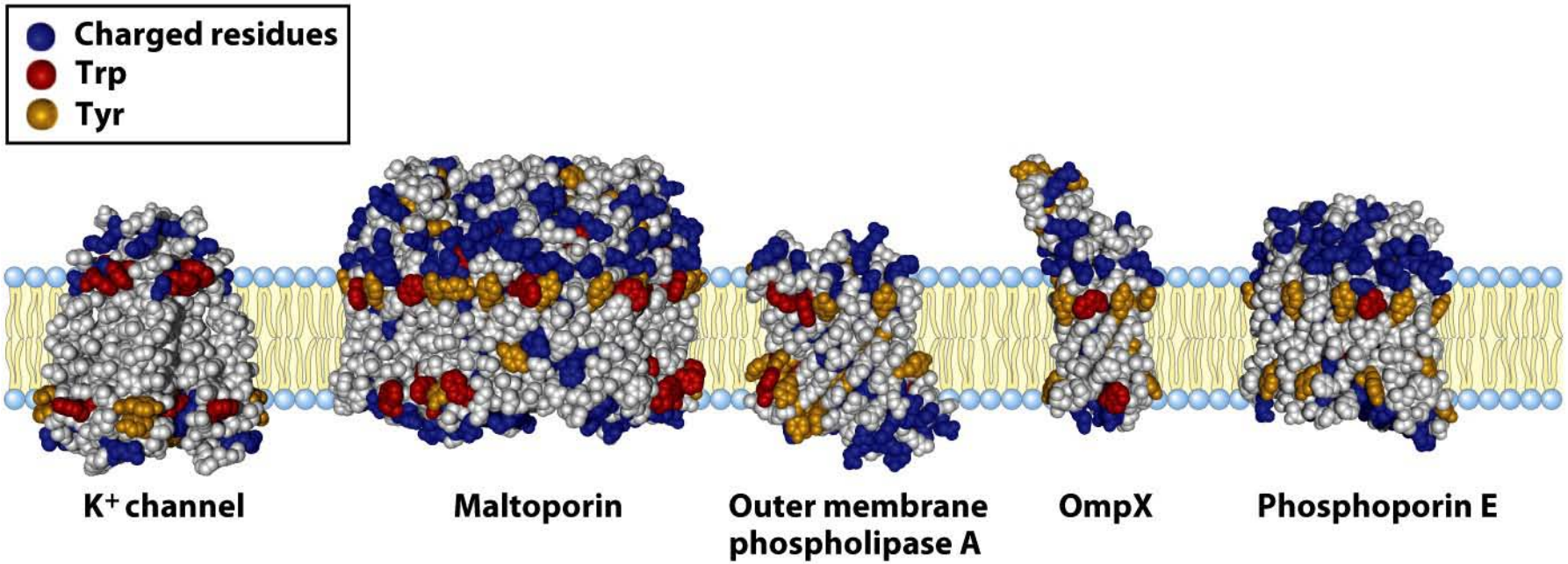


Figure 11-12
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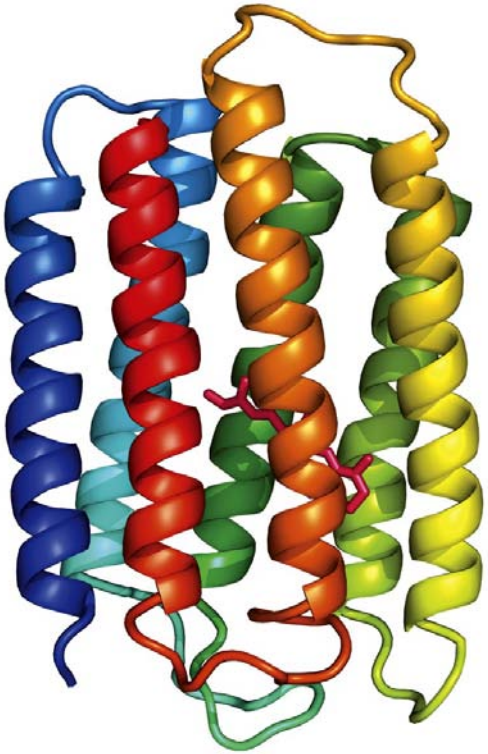
Tyr and Trp exhibit 'snorkeling' – pointing their polar group toward mb exterior

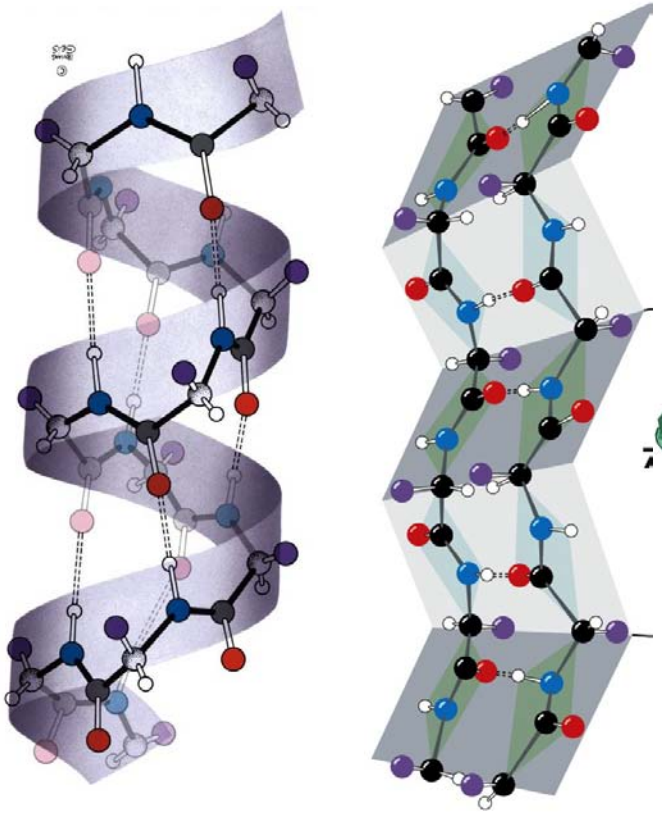
Also, often 'positive inside' – positively charged aa's facing cytoplasmic region

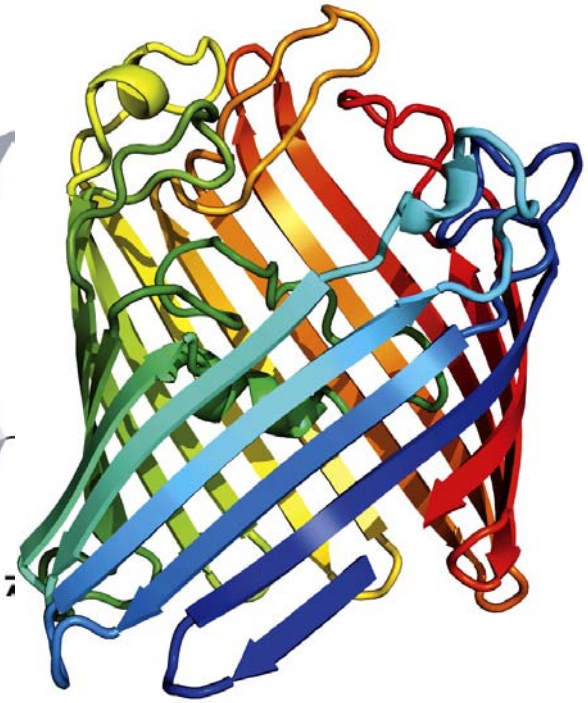
Transmembrane regions are usually α-helices or continuous β-sheets (β-barrels)



Bacteriorhodopsin:
a light-driven proton pump

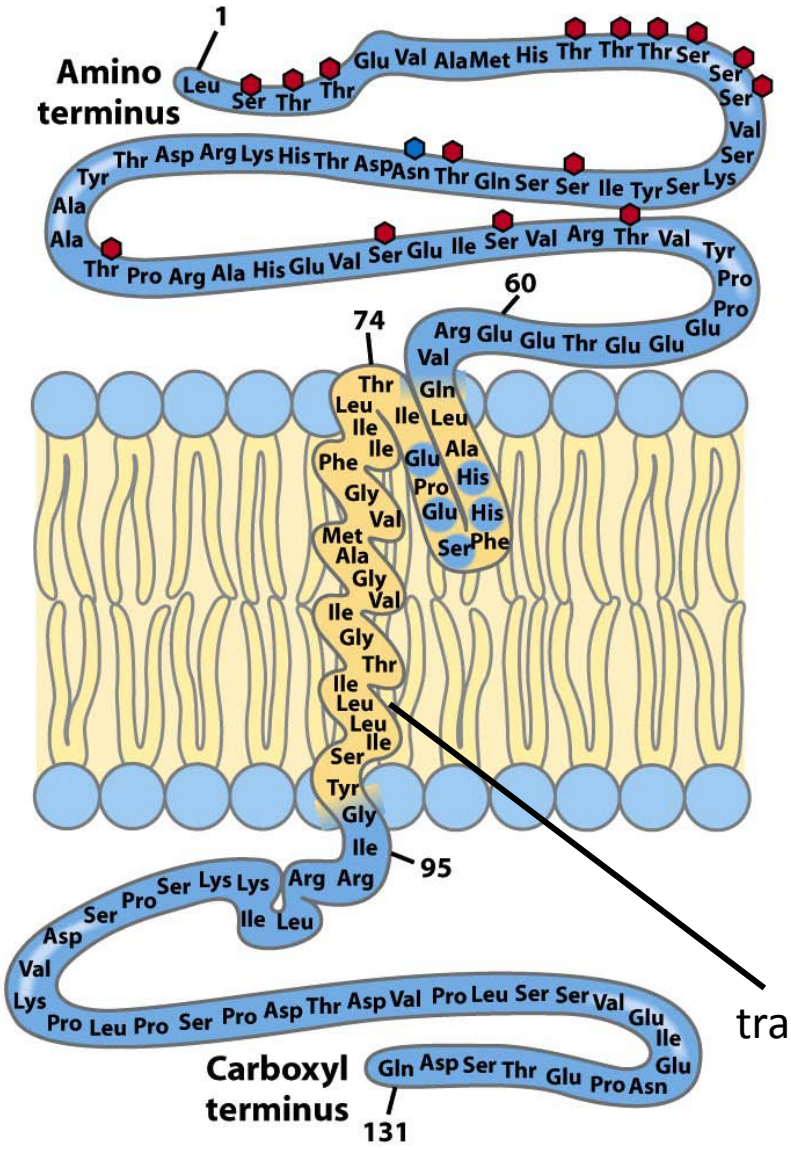
Backbone hydrogen bonds can be self-satisfied.

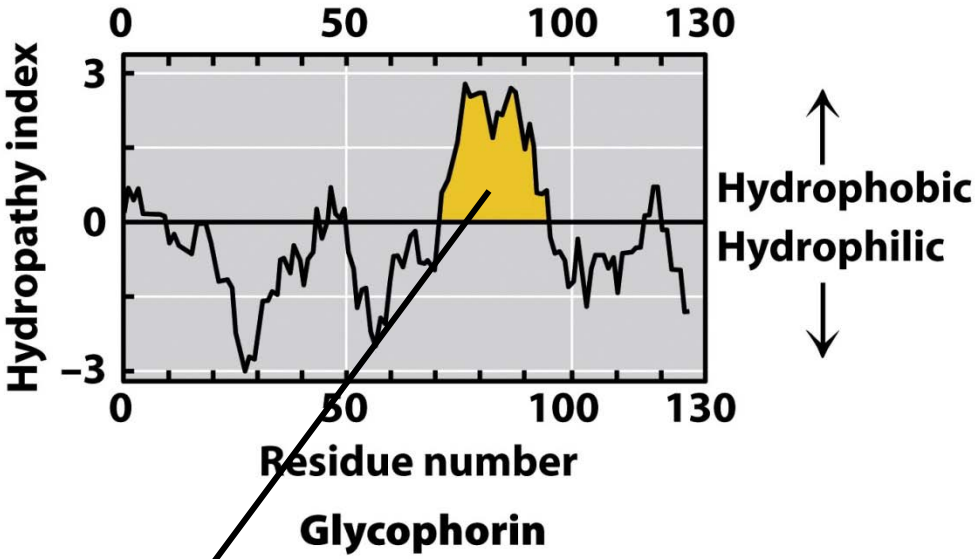




Porin:
a pore-forming protein

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

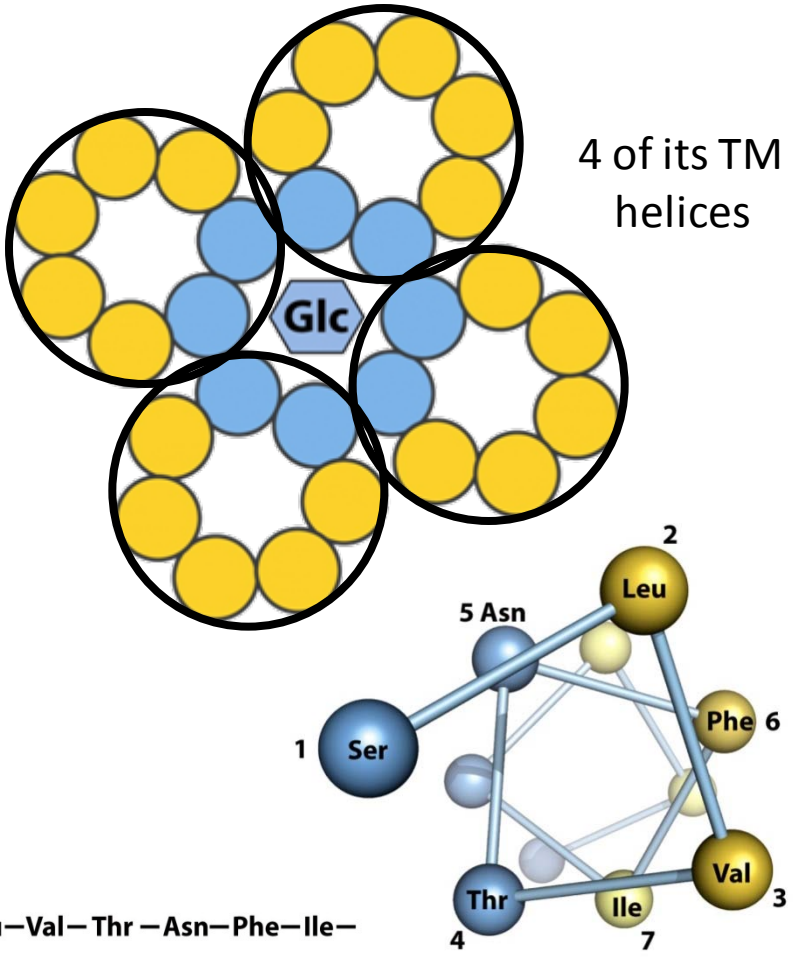




transmembrane helix

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

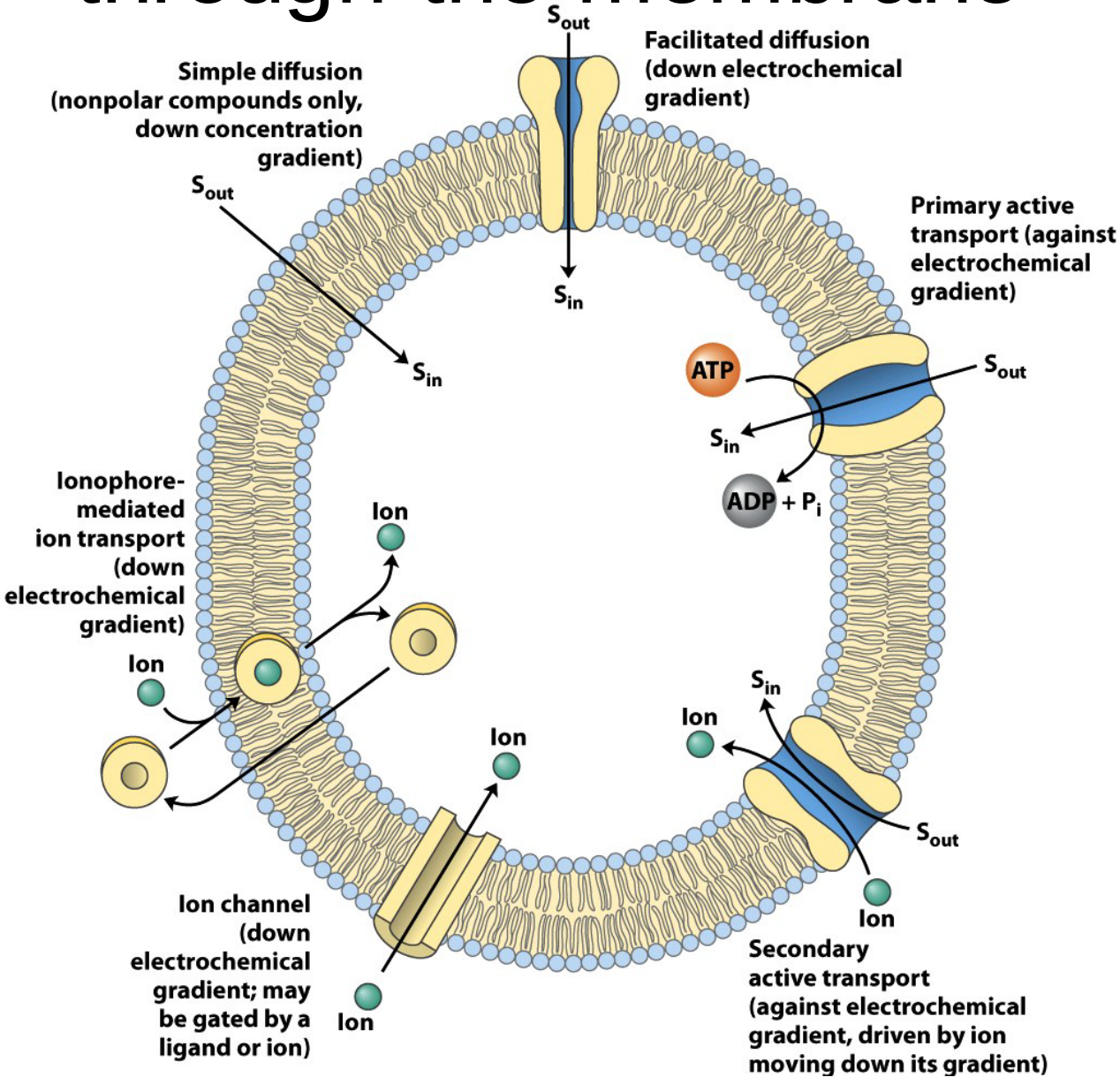
Glucose transporter

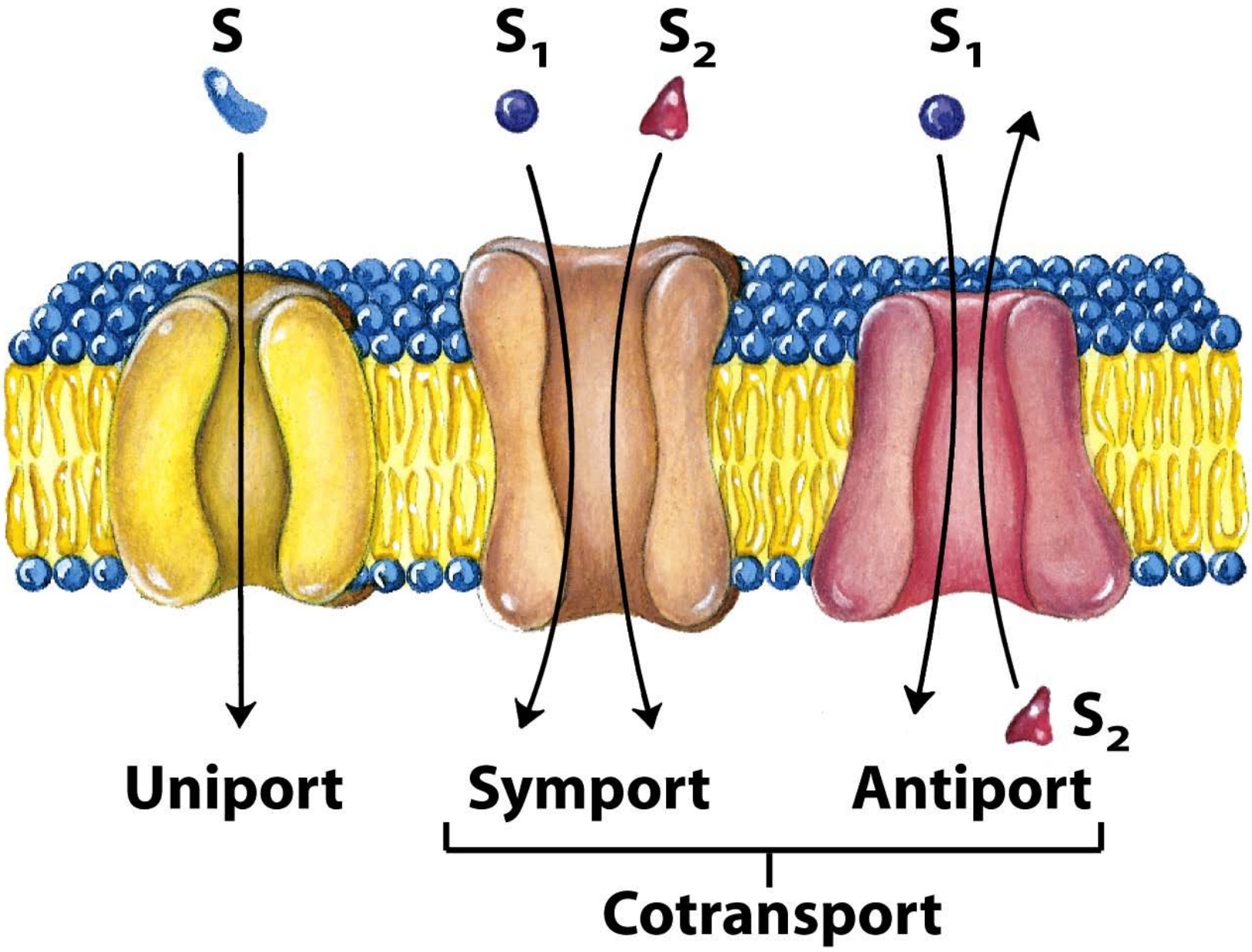


Transporter helices have 2-2 pattern: 2 hydrophobic-2 hydrophilic.
Since 3.6 a.a/turn, one side of the helix is hydrophobic, the other side is hydrophilic.

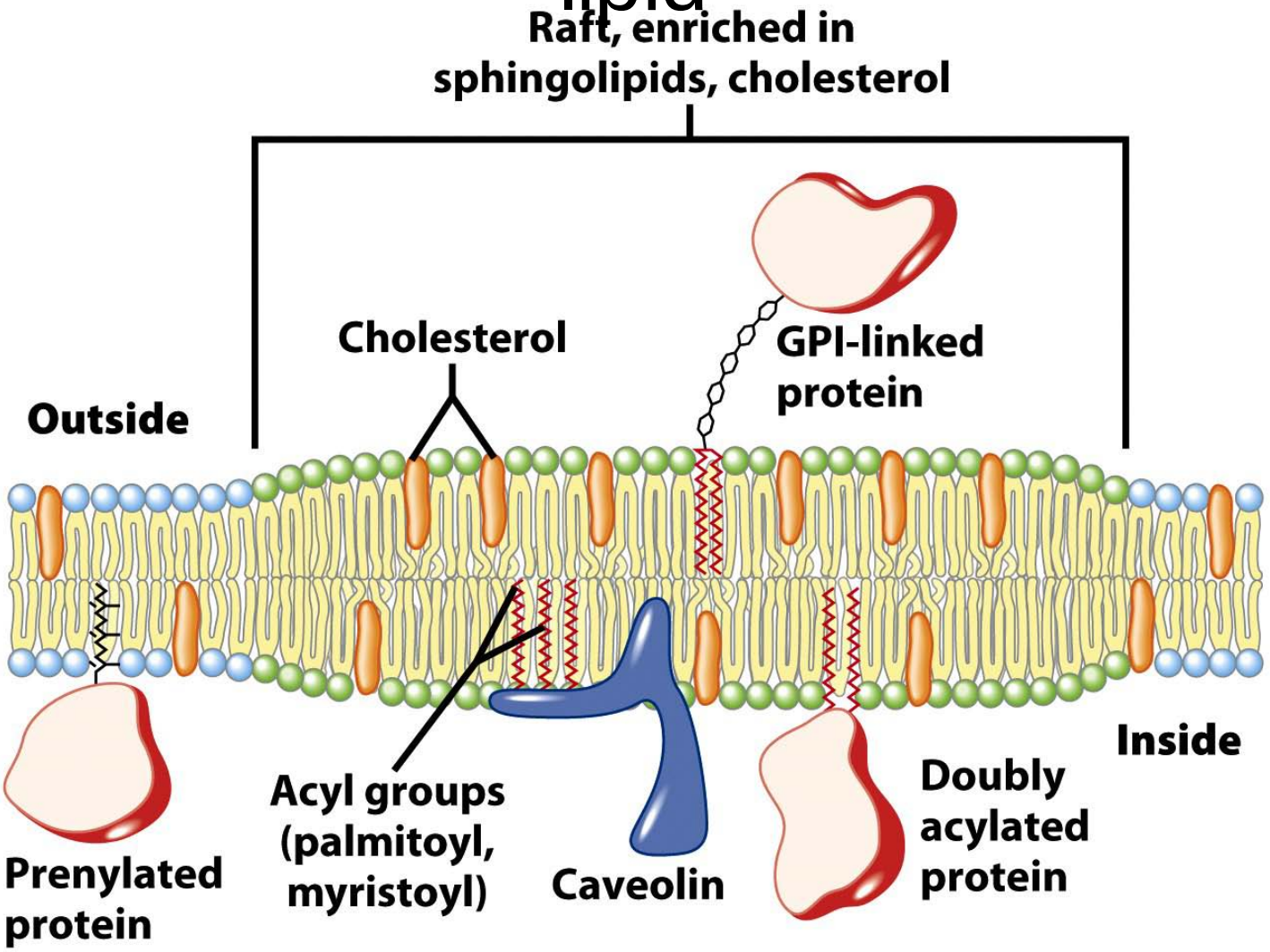
Transmembrane beta-barrels may have 1-1 pattern:
Alternating hydrophobic/hydrophilic a.a.
Since R groups of a.a. in beta-sheets facing alternating direction, one side is hydrophobic, the other side is hydrophilic.

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

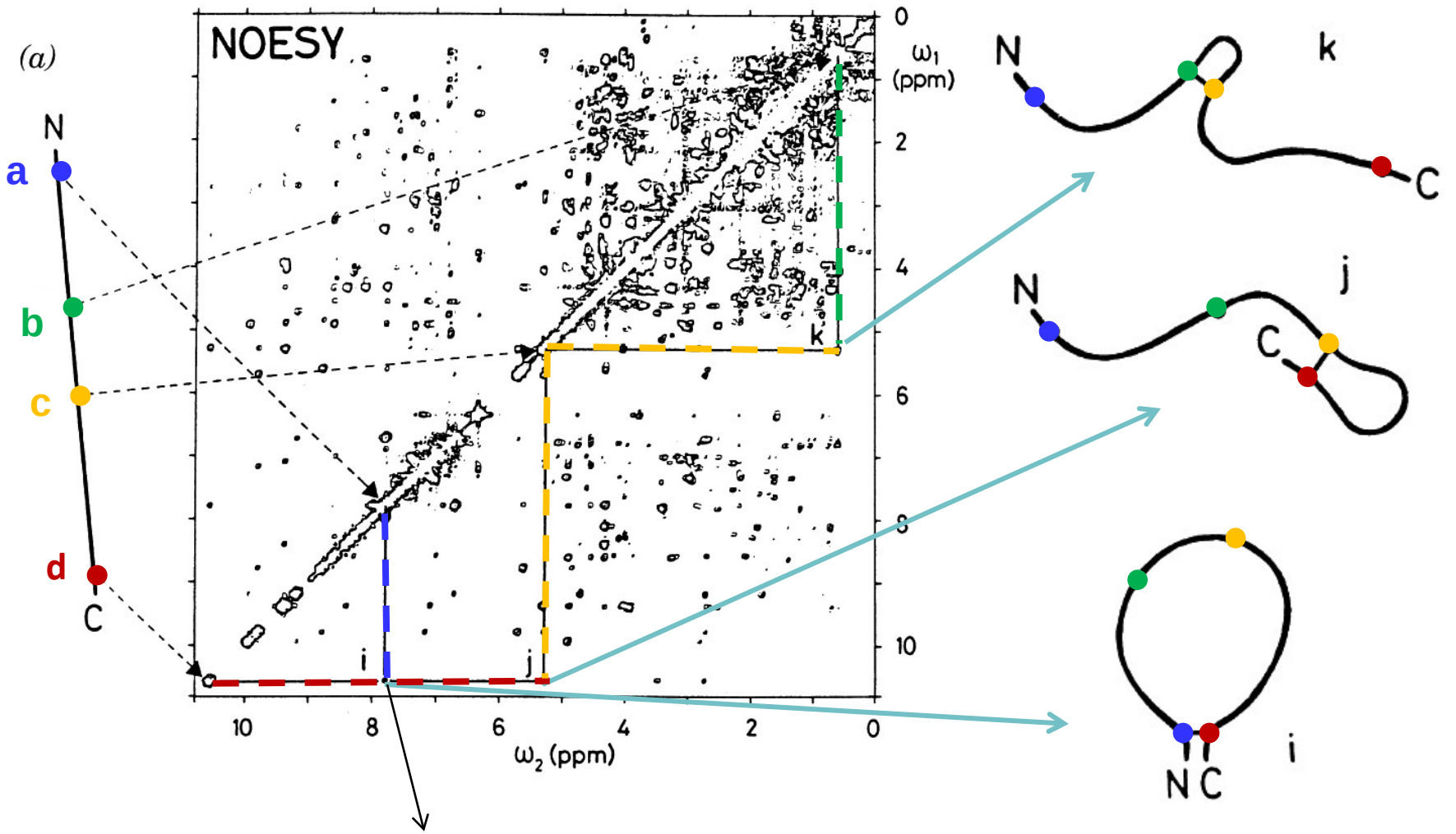
- Sequence:
  - Edman degradation: Remove one modified a.a from N-terminus at a time;
  - Mass spectrometry: Generate small fragments and measure the M/Z ratio.

- Secondary structure:
  - Circular Dichroism
  - FTIR

  } Indicate the composition of secondary structures

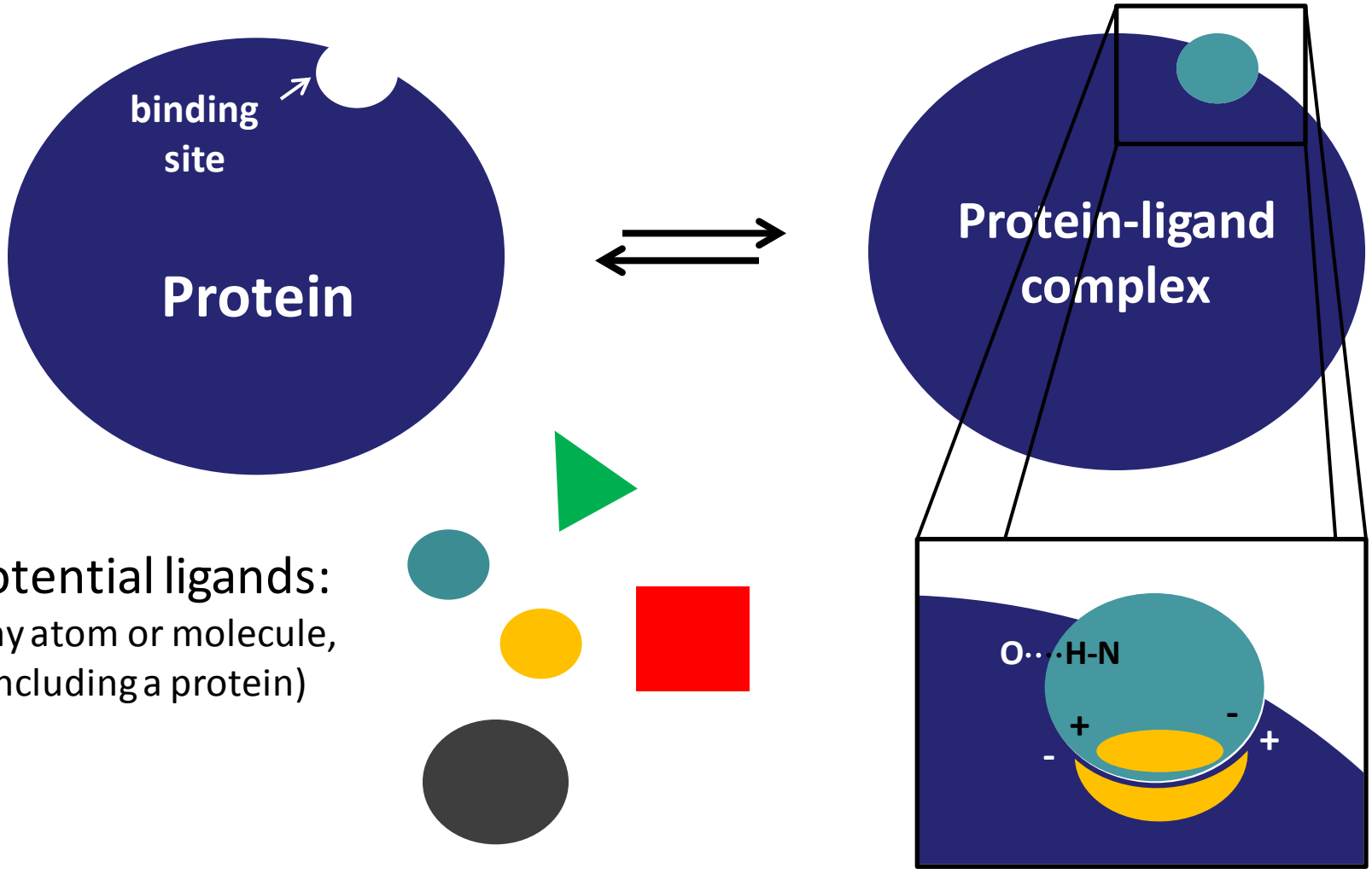
- Tertiary, quaternary structure:
  - NMR: derived distance constraints are used to calculate likely protein conformations
  - X-ray crystallography: Electron density map allows for positioning of protein atoms, revealing structure

2D NMR separates proton peaks and can reveal approximate distances between nearby atoms



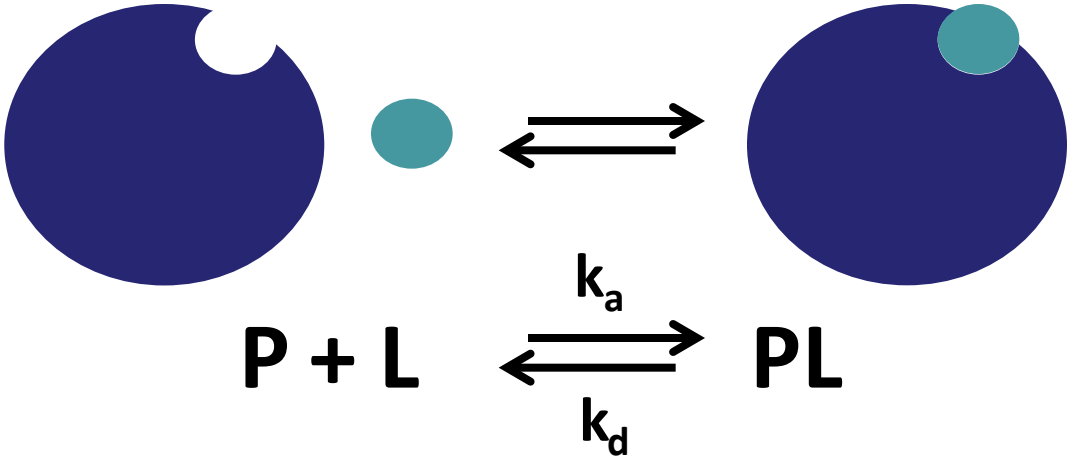
Cross-peaks indicate protons are within 5Å of each other

A protein binds a ligand through a specific, reversible interaction



Potential ligands:
(any atom or molecule, including a protein)

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

$$P + L \rightleftharpoons PL$$

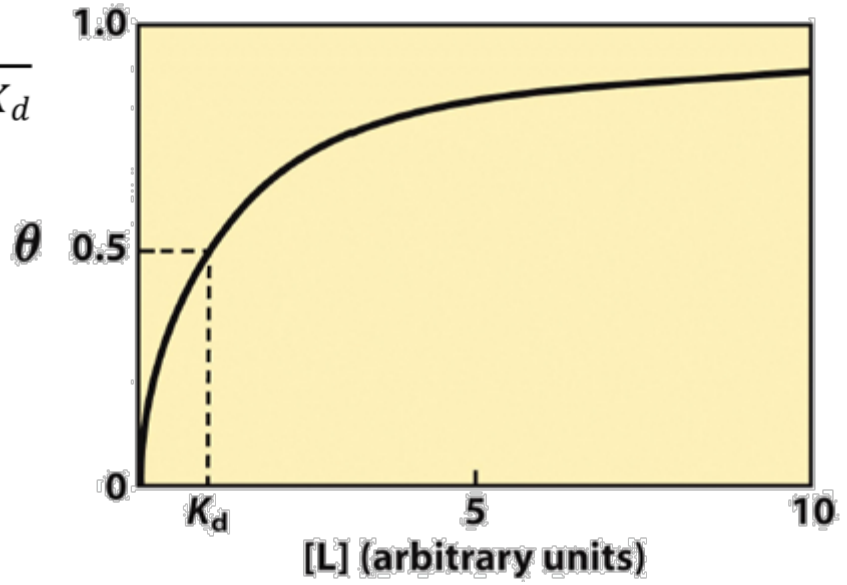
When $[L] \gg [PL] + [P]$, $[L]$ is constant (usually true for small ligands in cells)

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

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

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

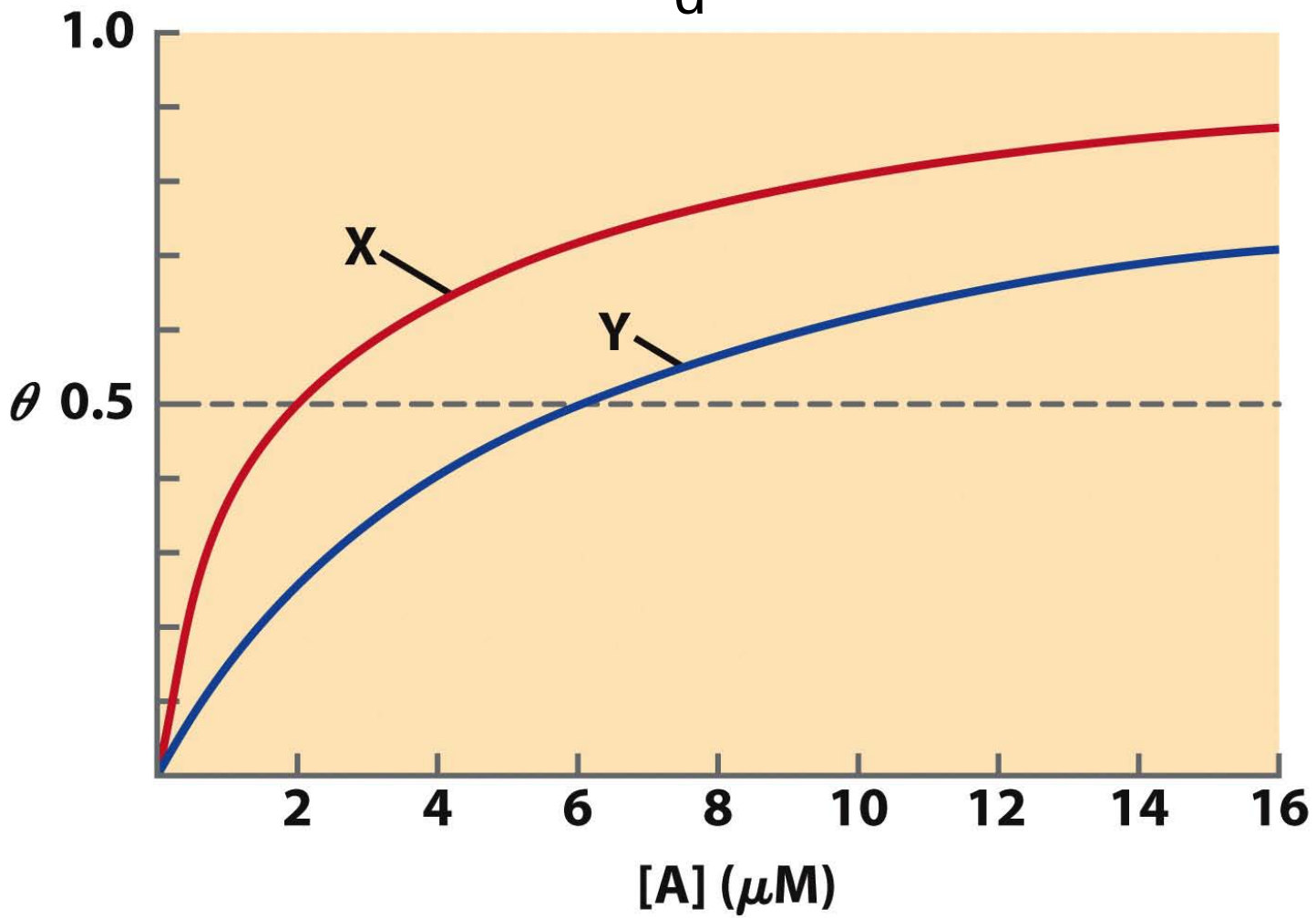
Simple binding: Hyperbolic curve



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



Unnumbered 5 p157
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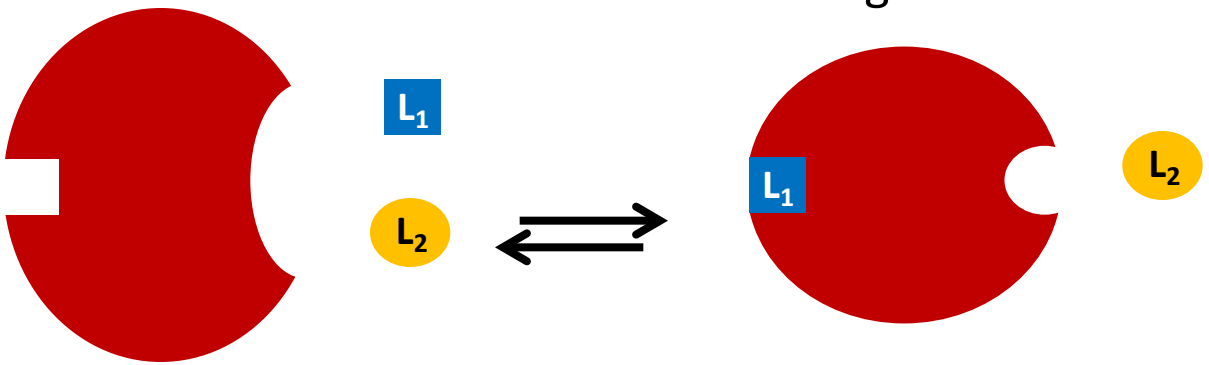
Allosteric protein

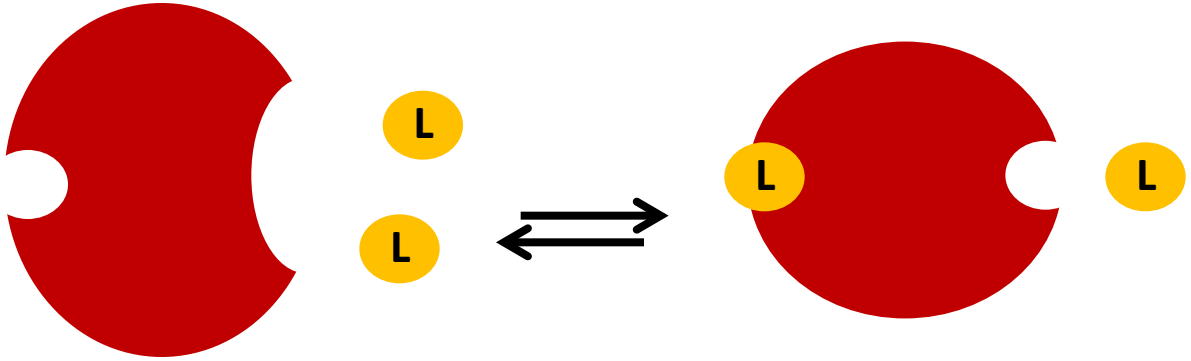
Binding of a ligand (L_1) to one site affects binding properties of ligand (L2) at another site (via a conformational change in the protein) .

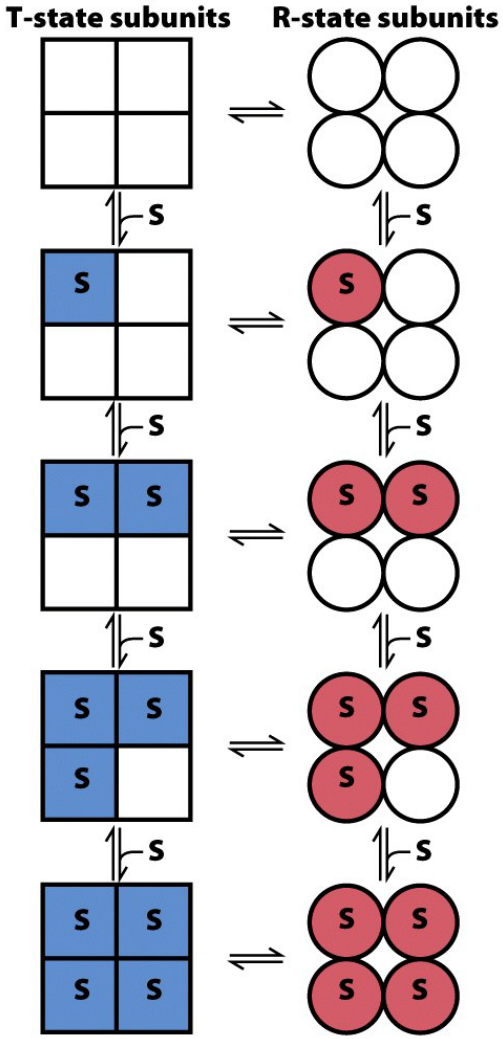
Modulator (L_1) is an 'activator' if it increases affinity at 2nd site (where L_2 binds)
Modulator (L_1) is an 'inhibitor' if it decreases affinity at 2nd site (where L_2 binds)

Heterotropic interaction: Modulator and other ligand are different



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



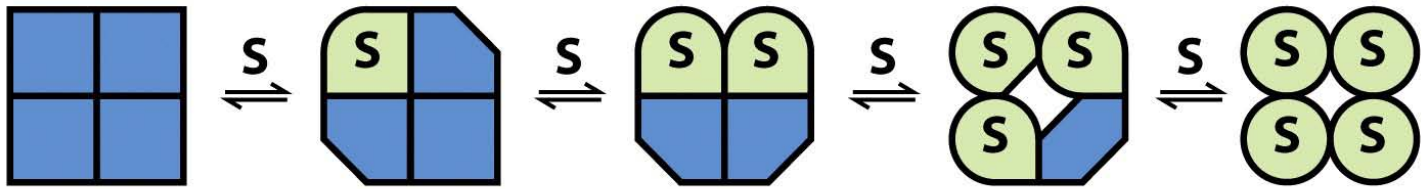


The symmetry (concerted) model of cooperativity

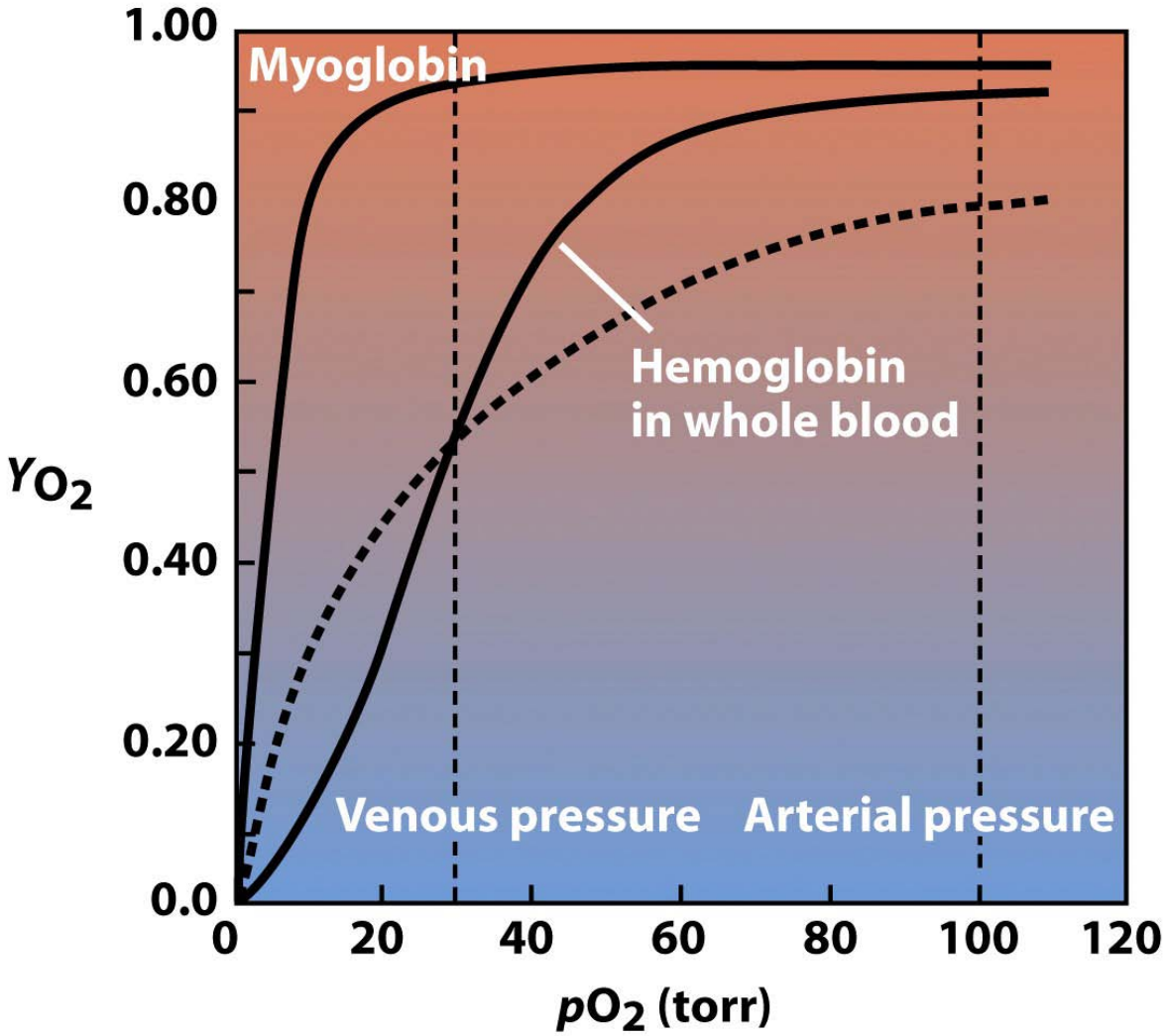
Subunits can adopt one of two possible conformations: T or R. All subunits *must* adopt the same conformation (protein is always symmetric). Equilibrium between T and R states is influenced by ligand or modulator binding.

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.



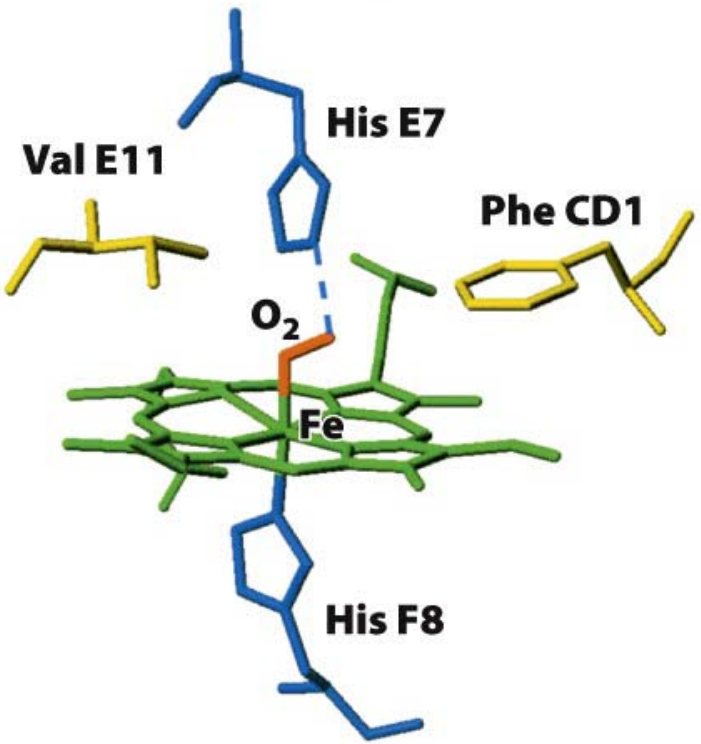
The oxygen-binding curves of Mb and Hb reflect their different functions



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

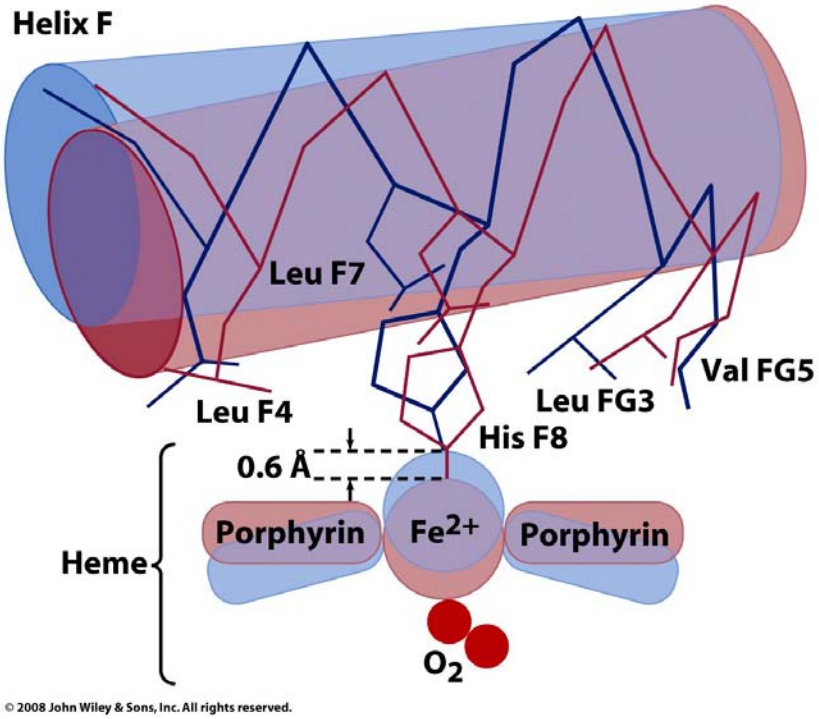
Hemoglobin: 4 subunits, sigmoidal curve, low affinity at tissues, high affinity at lungs. Cooperativitiy.

Heme cofactors bind O_2

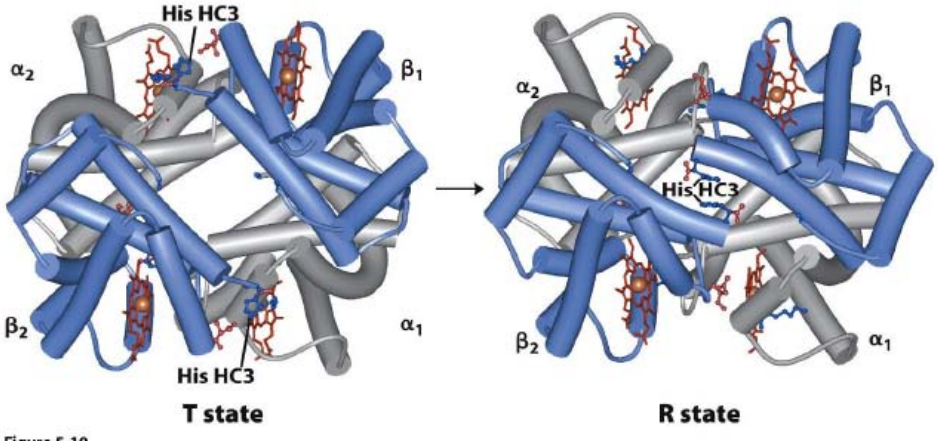


- Heme is held in place by the proximal His and by hydrophobic residues
- Proximal His of Hb covalently binds Fe of heme
- Distal His hydrogen bonds to O_2 bound to Fe. It reduces the affinity of hemoglobin to the toxic positive modulator CO by forcing CO to adopt an angle.

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



Movement of helix F shifts the entire quaternary structure of hemoglobin

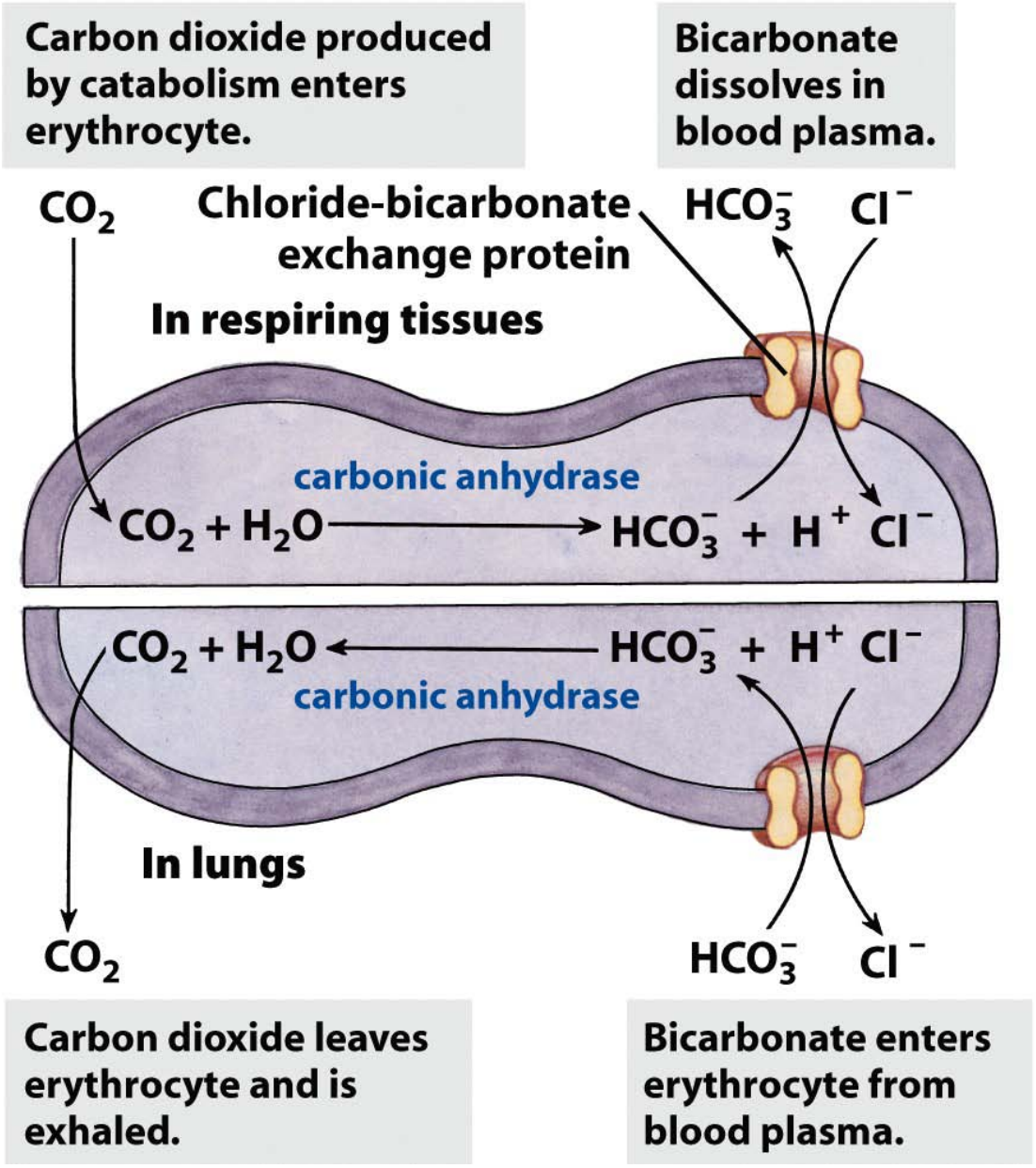


T-state = deoxygenated, low affinity
R-state = oxygenated, high affinity

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: $CO_2 + H_2O \rightarrow HCO_3^- + H^+$
  - 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

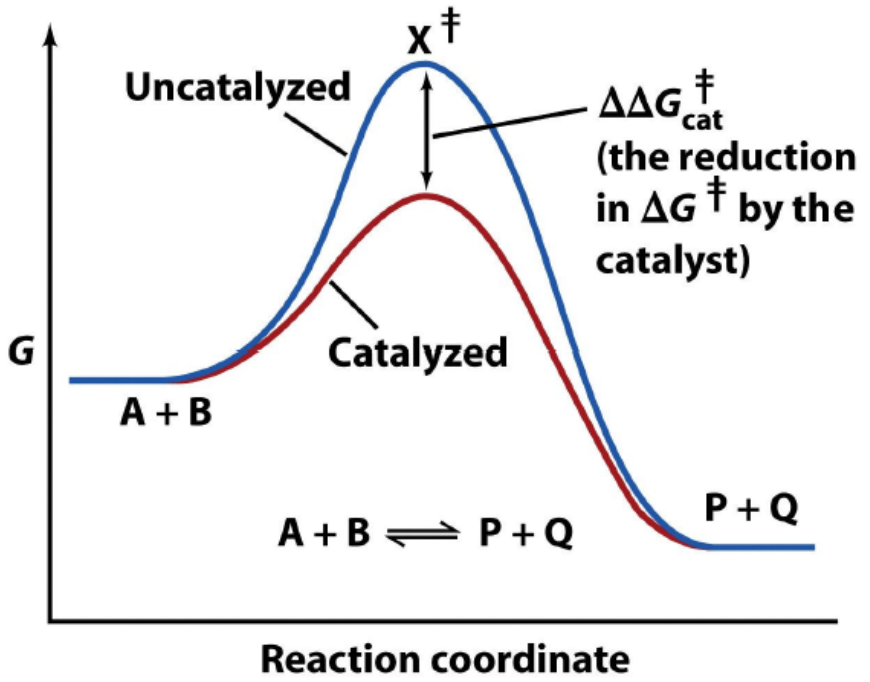


High CO2, converting to bicarbonate and proton by Carbonic anhydrase,
low pH, high Cl-
Low O2
BPG binds to stablize T-state

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)
  - **$Q = K_{eq}$ $\Delta G = 0$ @equilibrium (remember rxn quotient ,Q = [products]/[reactants])**
  - **$Q < K_{eq}$ $\Delta G < 0$ (-) spontaneous in forward direction (less products than @equil)**
  - **$Q > K_{eq}$ $\Delta G > 0$ (+) spontaneous in reverse direction (more products than @equil)**
- 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)

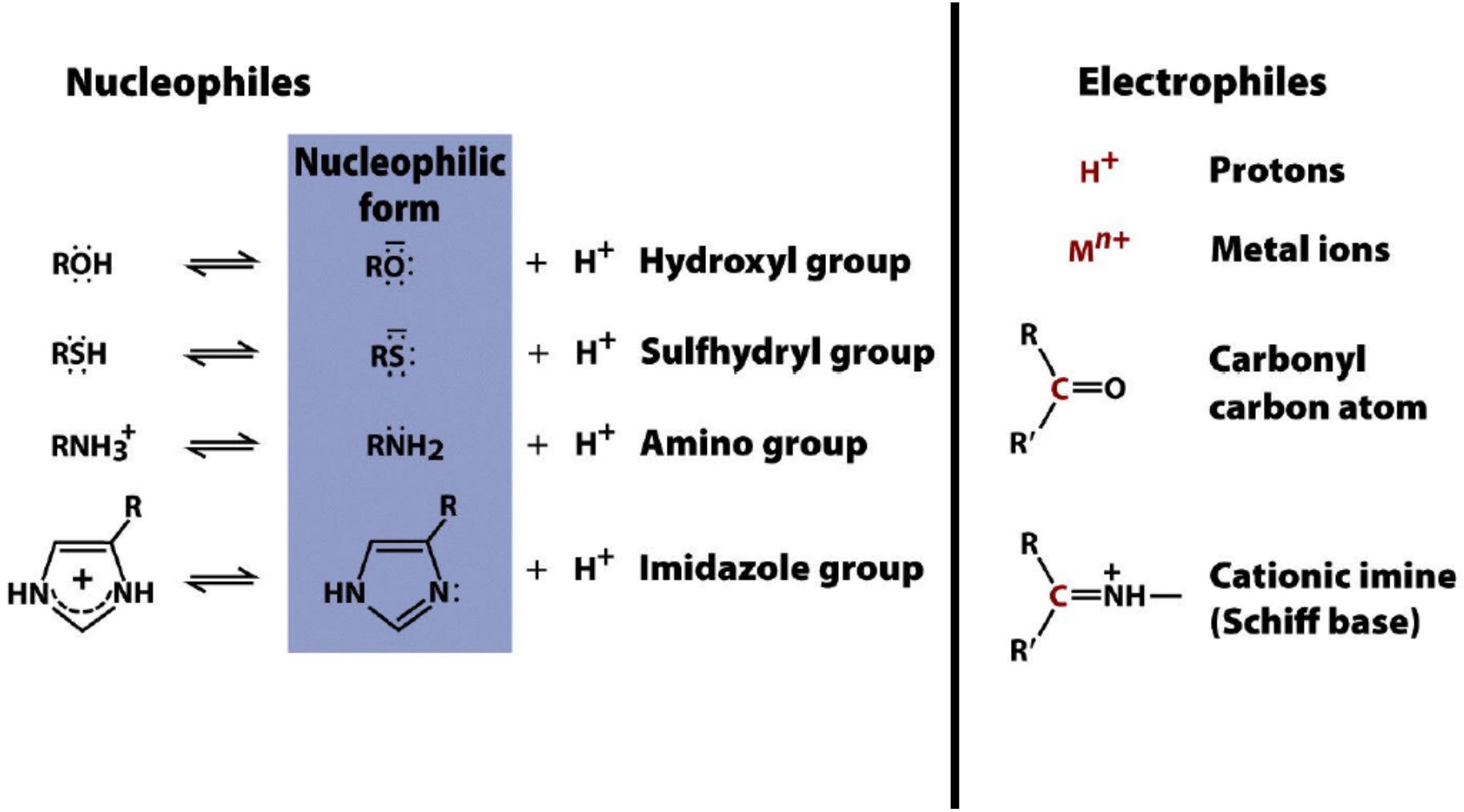
Table 11-2 Enzyme Classification According to Reaction Type

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

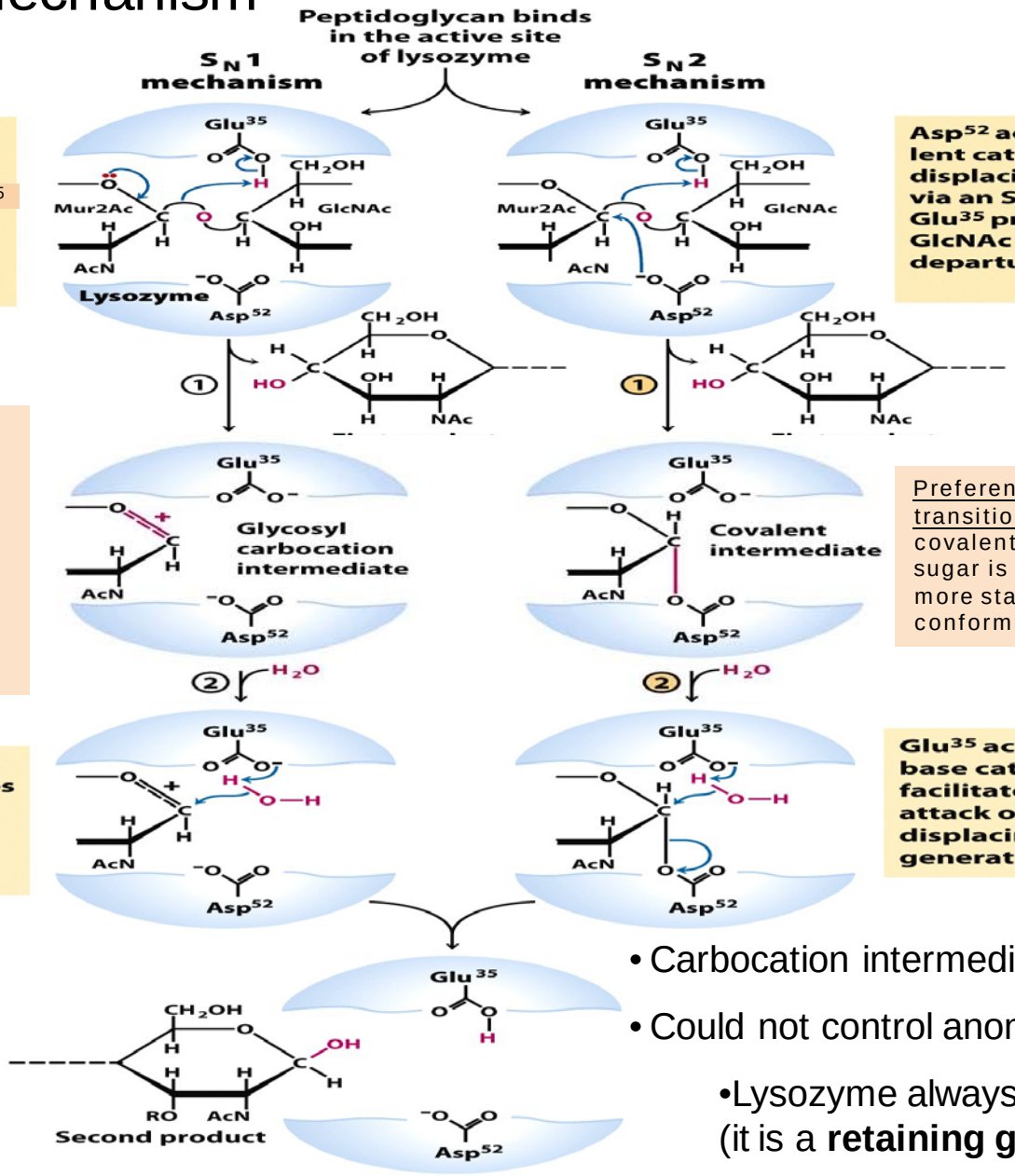
Enzymes use several catalytic mechanisms (often together) to enhance reaction rates

- 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.

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.

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

Electrostatic catalysis: Asp[52]

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

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

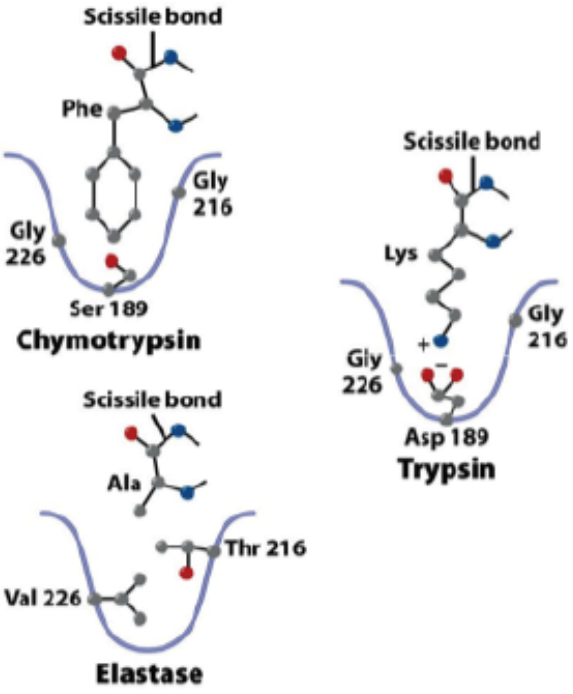
Glu[35] acts as a general base catalyst to facilitate the S_N2 attack of water, displacing Asp[52] and generating product.

Why S_N2?

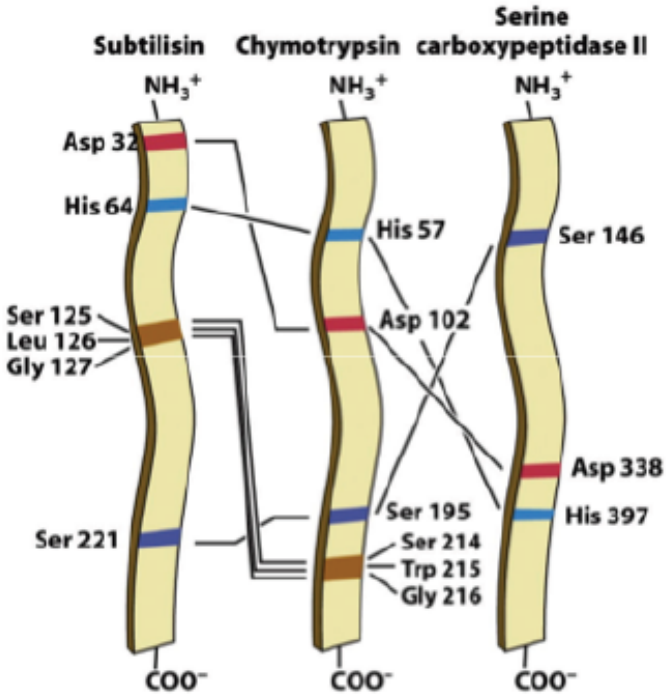
- Carbocation intermediate of S_N1 is too unstable
- Could not control anomer formed if S_N1 is used
  - Lysozyme always makes beta-anomer (it is a **retaining glycosidase**)
- Crystal structure of lysozyme with transition state analog supports covalent mechanism

Evolution of serine proteases

Divergent evolution



Convergent evolution

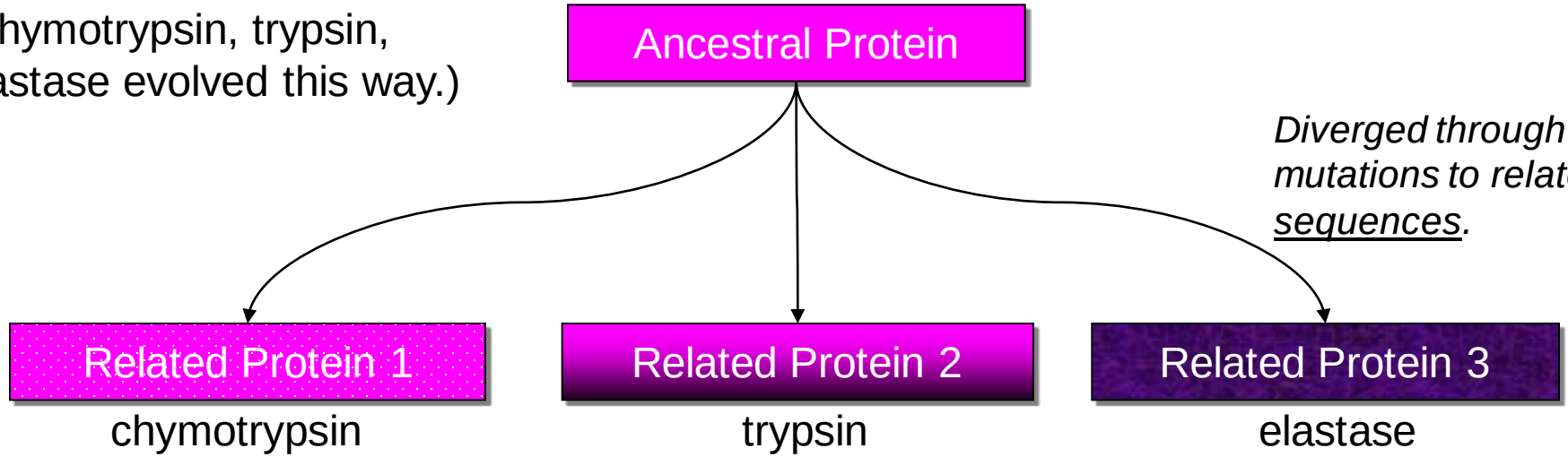


SERINE PROTEASES

(catalytic triad: Asp-His-Ser)

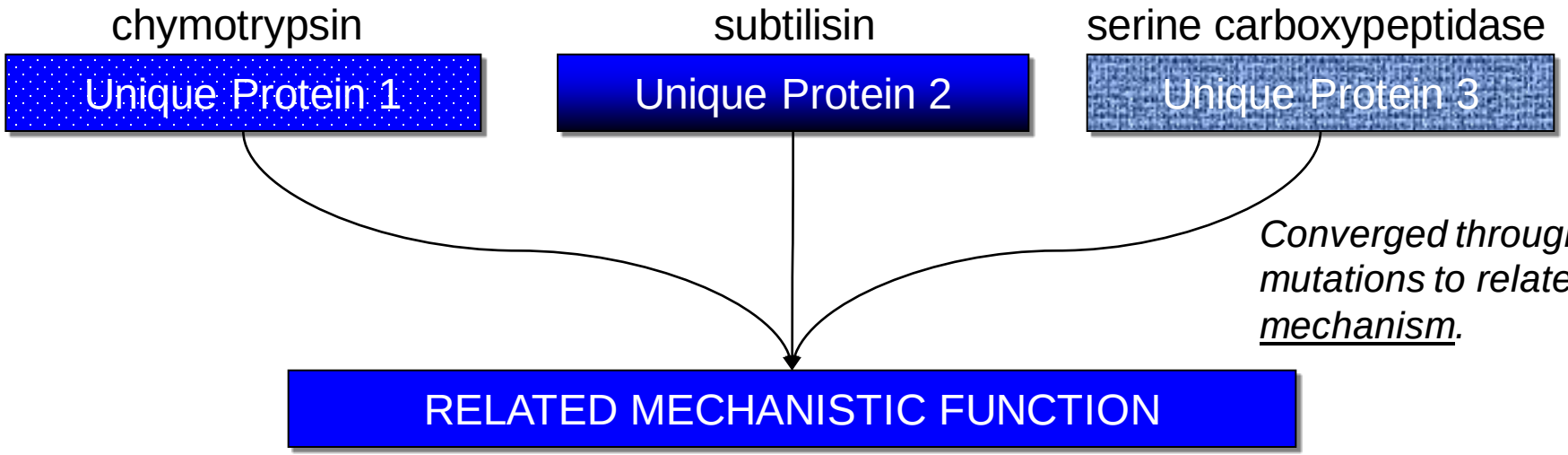
Divergent Enzyme Evolution

(Chymotrypsin, trypsin, elastase evolved this way.)



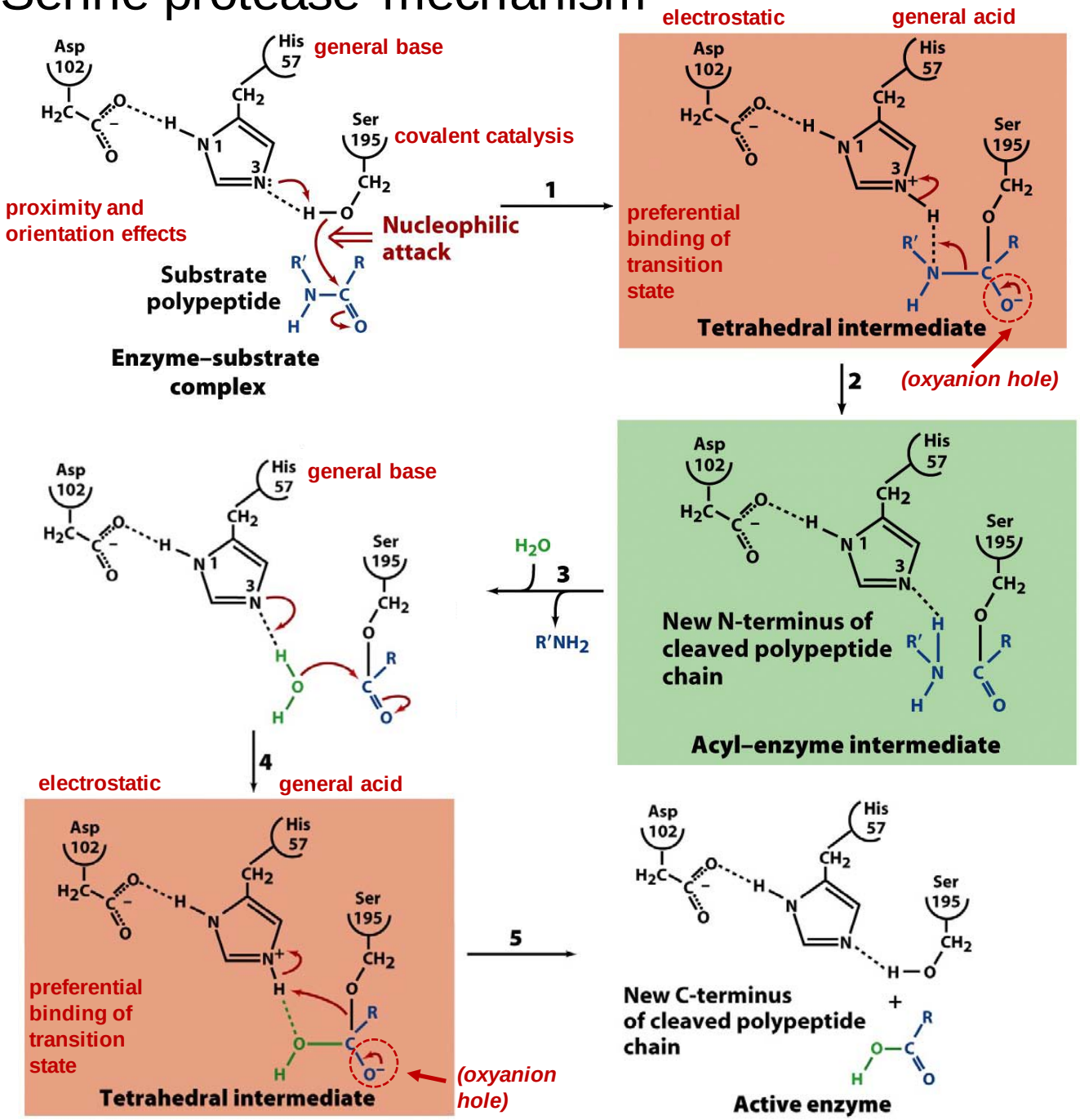
Diverged through mutations to related sequences.

Convergent Enzyme Evolution



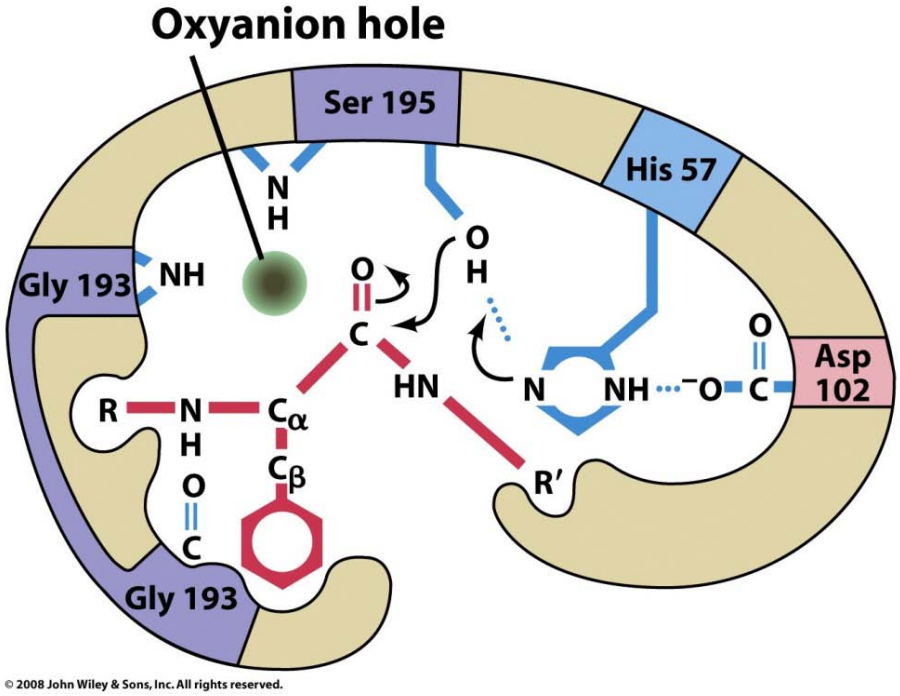
Converged through mutations to related mechanism.

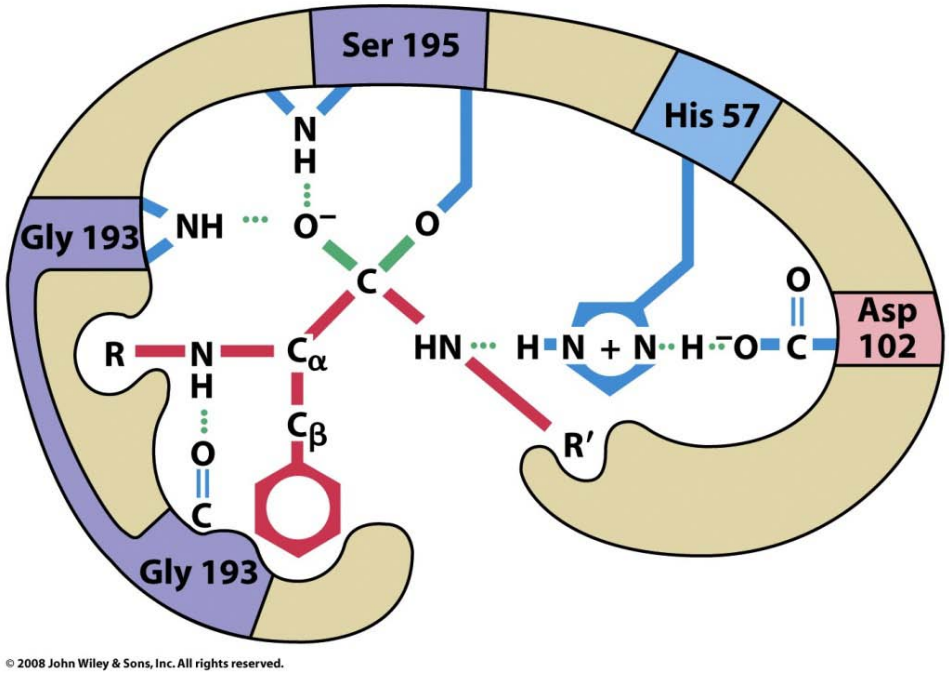
Serine protease mechanism



- 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





Michaelis-Menten kinetics of enzymes

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

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

Binding (fast) **Catalysis (slow)**

- 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:

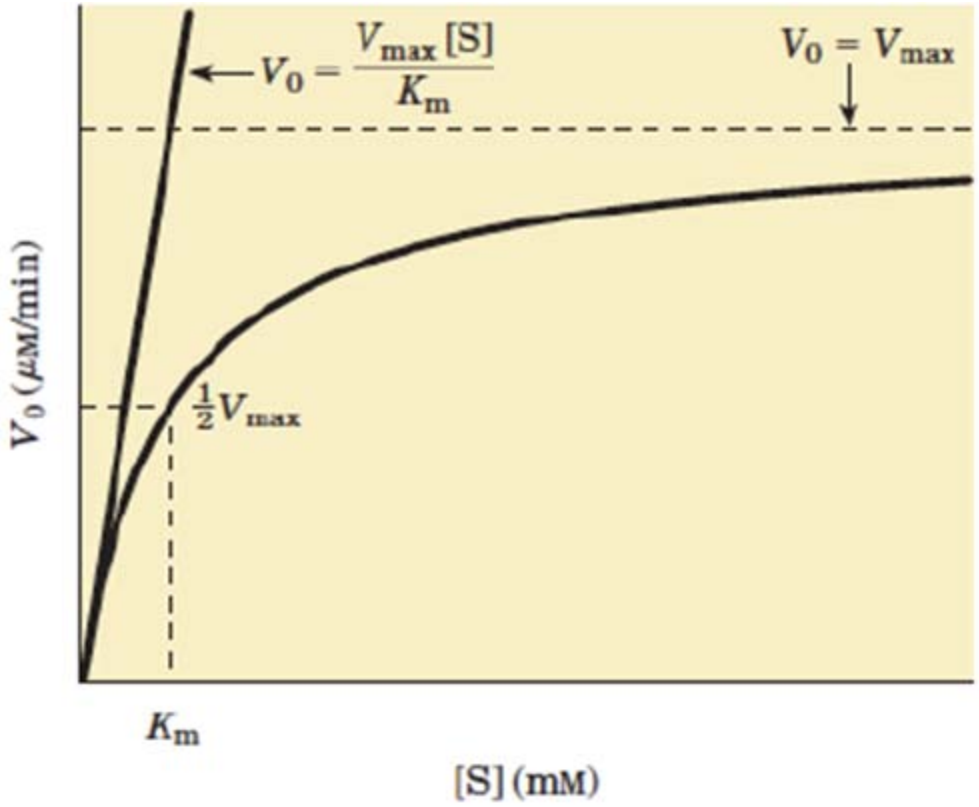
- @ time = 0, [P] ≈ 0
- [ES] is constant
- @ high [S], $v_0 = V_{max}$ and $[ES] = [E_T]$

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:

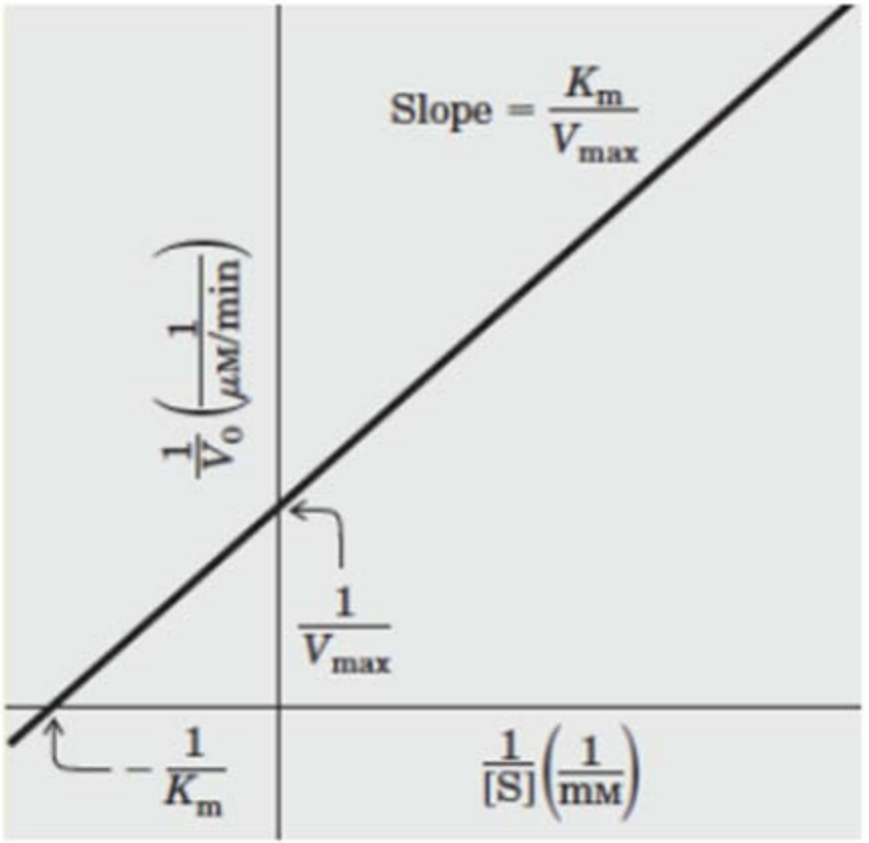
$$\frac{1}{v_0} = \frac{K_M}{V_{max}[S]} + \frac{1}{V_{max}}$$

•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}



Enzyme Inhibition – General overview

Described by the following equation:

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

- αK_M (also denoted K_M'), apparent K_M, describes in a way the binding affinity of inhibitor to enzyme
- K_M^{app} is the K_M observed in the presence of inhibitor

where

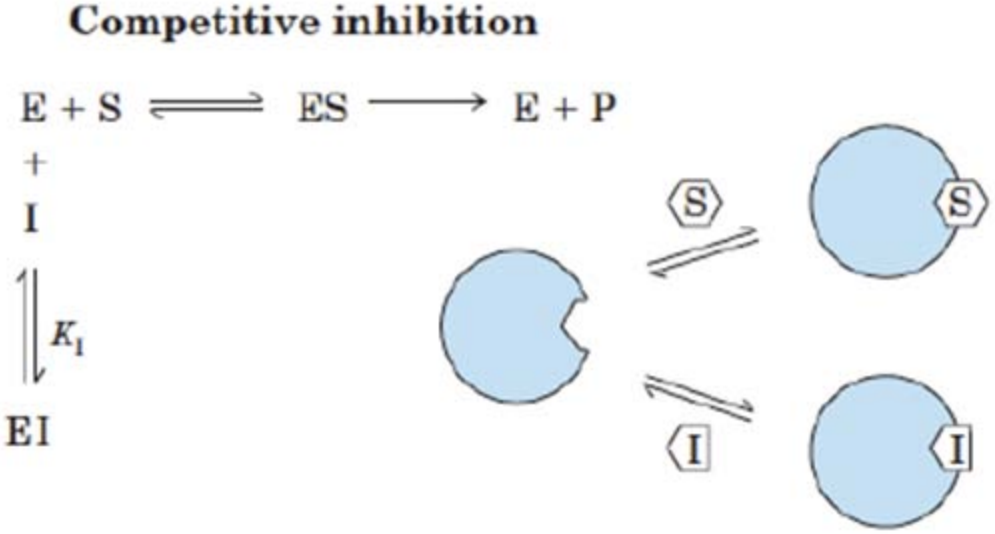
$$\alpha = 1 + \frac{[I]}{K_i} \quad \text{and} \quad K_i = \frac{[E][I]}{[EI]}$$

$$\alpha' = 1 + \frac{[I]}{K'_i} \quad \text{and} \quad K_i' = \frac{[ES][I]}{[ESI]}$$

For competitive inhibition: $\alpha' = 1$

For uncompetitive inhibition: $\alpha = 1$

Reversible Inhibitors - Competitive



$$K_i = \frac{[E][I]}{[EI]} \qquad \alpha = 1 + \frac{[I]}{K_i}$$

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

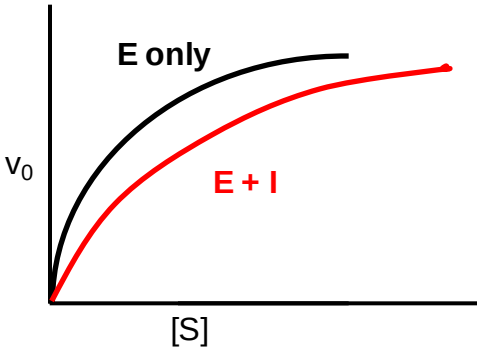
$$K_M^{apparent} = \alpha K_M$$

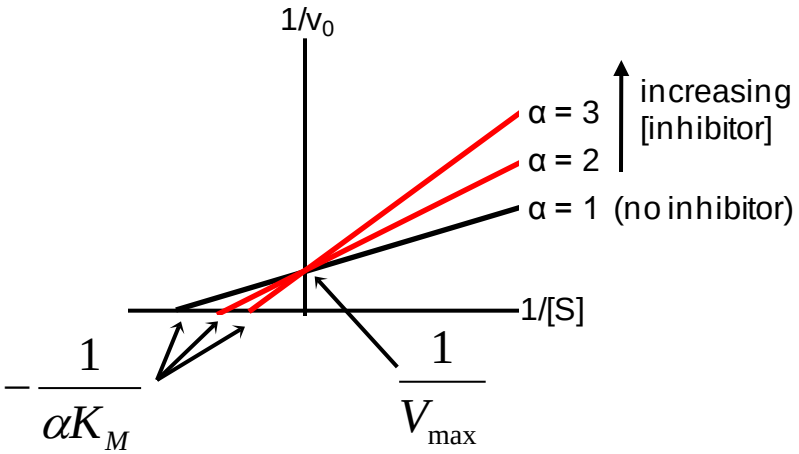
$$V_{max}^{apparent} = V_{max}$$

- Competes with substrate for the active site
- Can reverse inhibition by adding more substrate

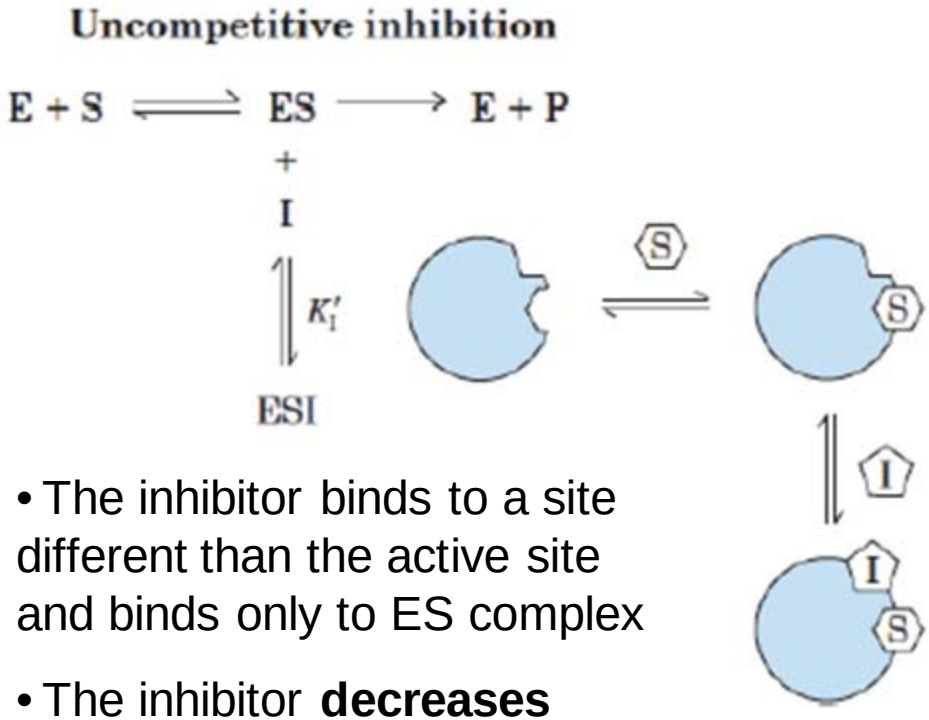
V_{max} is **unchanged** and **K_M increases** with [I]

$$\frac{1}{v_0} = \left(\frac{\alpha K_M}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$





Reversible Inhibitors - Uncompetitive



$$K_i' = \frac{[ES][I]}{[ESI]} \qquad \alpha' = 1 + \frac{[I]}{K_i'}$$

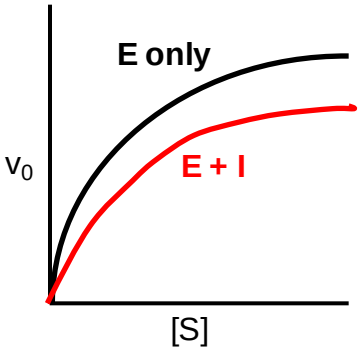
$$v_0 = \frac{V_{max}[S]}{K_M + \alpha'[S]}$$

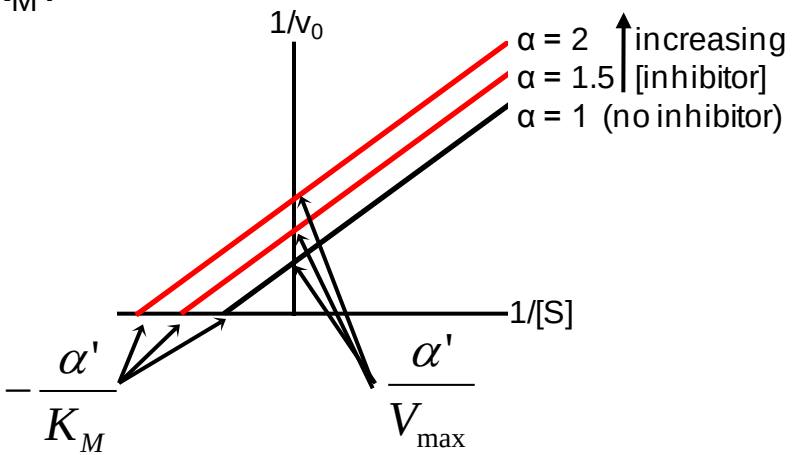
$$K_M^{apparent} = \frac{K_M}{\alpha'}$$

$$V_{max}^{apparent} = \frac{V_{max}}{\alpha'}$$

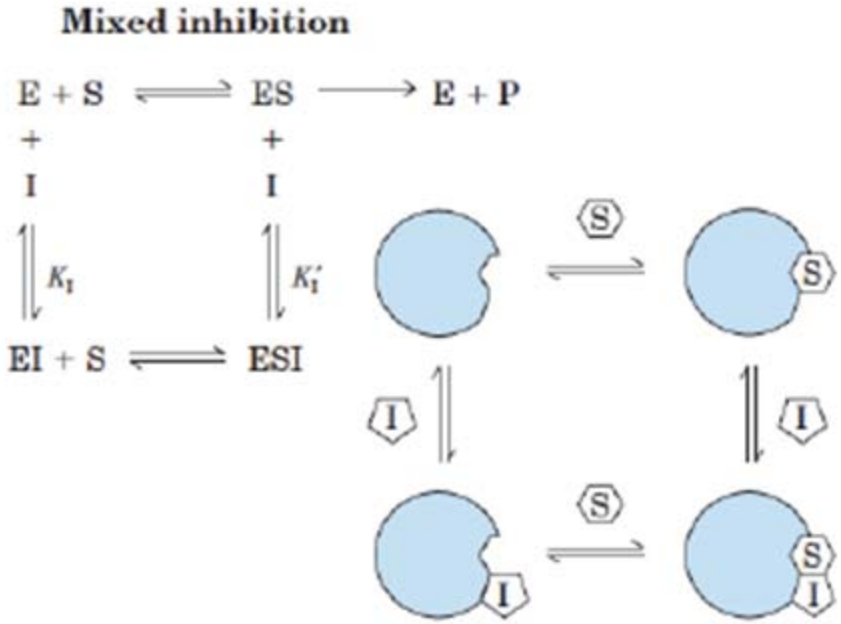
$$\frac{1}{v_0} = \left(\frac{K_M}{V_{max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$

- The inhibitor binds to a site different than the active site and binds only to ES complex
- The inhibitor **decreases both V_{max}** and **K_M**
  - Why does it lower K_M?





Reversible Inhibitors - Mixed



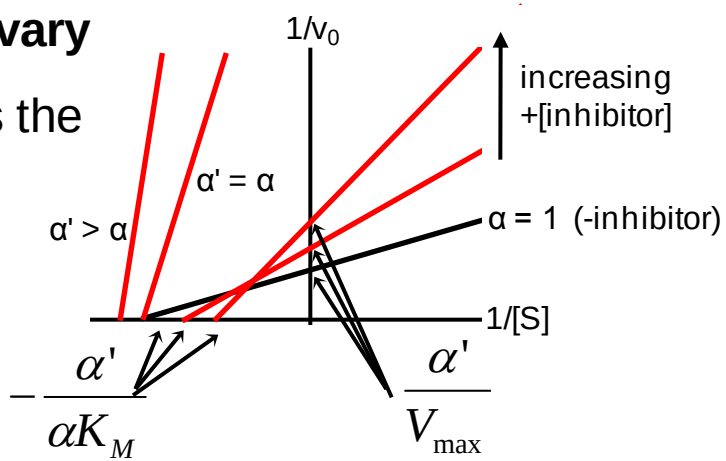
$$v_0 = \frac{V_{max}[S]}{\alpha K_M + \alpha'[S]}$$

$$K_M^{apparent} = \frac{\alpha K_M}{\alpha'}$$

$$V_{max}^{apparent} = \frac{V_{max}}{\alpha'}$$

$$\frac{1}{v_0} = \left(\frac{\alpha K_M}{V_{max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$

- The inhibitor binds to a site other than the active site but can bind to either E or ES
- **V_{max} decreases** and **K_M** can **vary**
- **Noncompetitive inhibition** is the special case in which $\alpha = \alpha'$ and K_M is unchanged (rarely encountered experimentally)



α' < α +inhibitor and -inhibitor lines cross
K_M^{app} increases

α' > α lines do not cross
K_M^{app} decreases

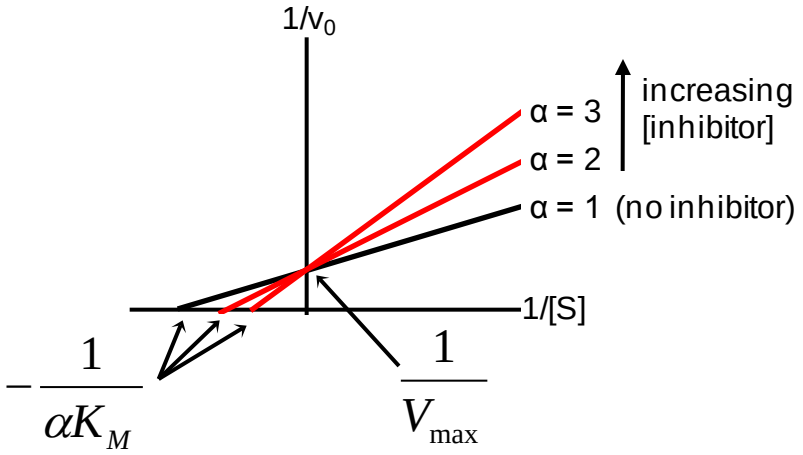
α' = α lines intersect at x-int
K_M^{app} no change

Summary of kinetic effects of enzyme inhibition

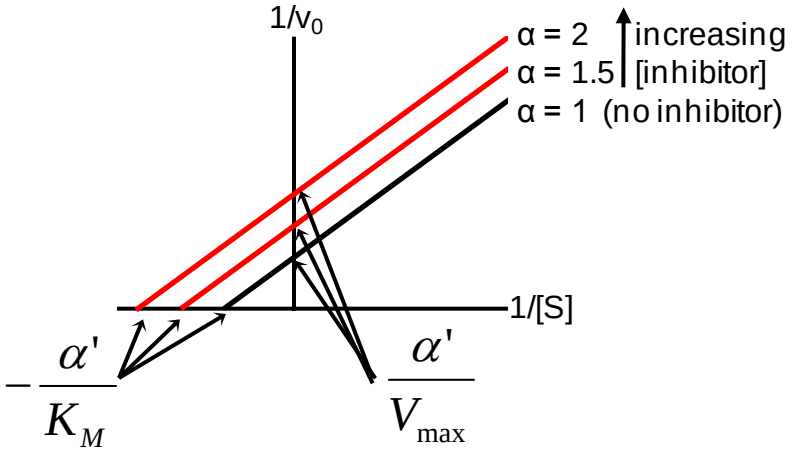
Inhibition Type	$V_{\max}^{apparent}$	$K_M^{apparent}$	**Michaelis-Menten Equation**	**Lineweaver-Burk Equation**
None	$V_{\max}$	K_M	$v_0 = \frac{V_{\max}[S]}{K_M + [S]}$	$\frac{1}{v_0} = \left(\frac{K_M}{V_{\max}}\right)\frac{1}{[S]} + \frac{1}{V_{\max}}$
Compeititve	$V_{\max}$	αK_M	$v_0 = \frac{V_{\max}[S]}{\alpha K_M + [S]}$	$\frac{1}{v_0} = \left(\frac{\alpha K_M}{V_{\max}}\right)\frac{1}{[S]} + \frac{1}{V_{\max}}$
Uncompetitive	$\frac{V_{\max}}{\alpha'}$	$\frac{K_M}{\alpha'}$	$v_0 = \frac{V_{\max}[S]}{K_M + \alpha'[S]}$	$\frac{1}{v_0} = \left(\frac{K_M}{V_{\max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{\max}}$
Mixed	$\frac{V_{\max}}{\alpha'}$	$\frac{\alpha K_M}{\alpha'}$	$v_0 = \frac{V_{\max}[S]}{\alpha K_M + \alpha'[S]}$	$\frac{1}{v_0} = \left(\frac{\alpha K_M}{V_{\max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{\max}}$

Summary of kinetic effects of enzyme inhibition: Lineweaver-Burk plots

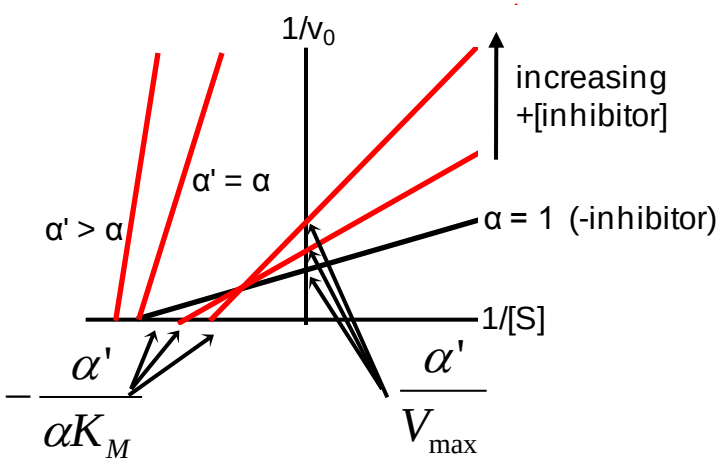
Competitive inhibition



Uncompetitive inhibition



Mixed inhibition



α' < α +inhibitor and -inhibitor lines cross
K_M^{app} increases

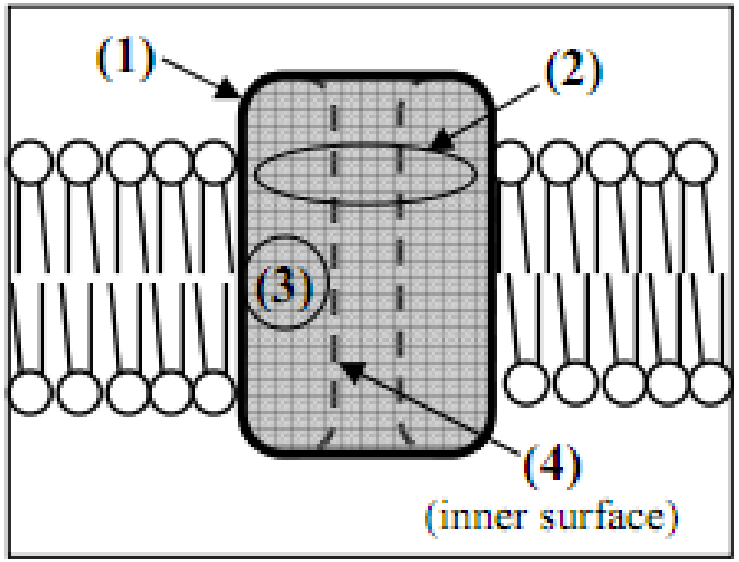
α' > α lines do not cross
K_M^{app} decreases

α' = α lines intersect at x-int
K_M^{app} no change

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

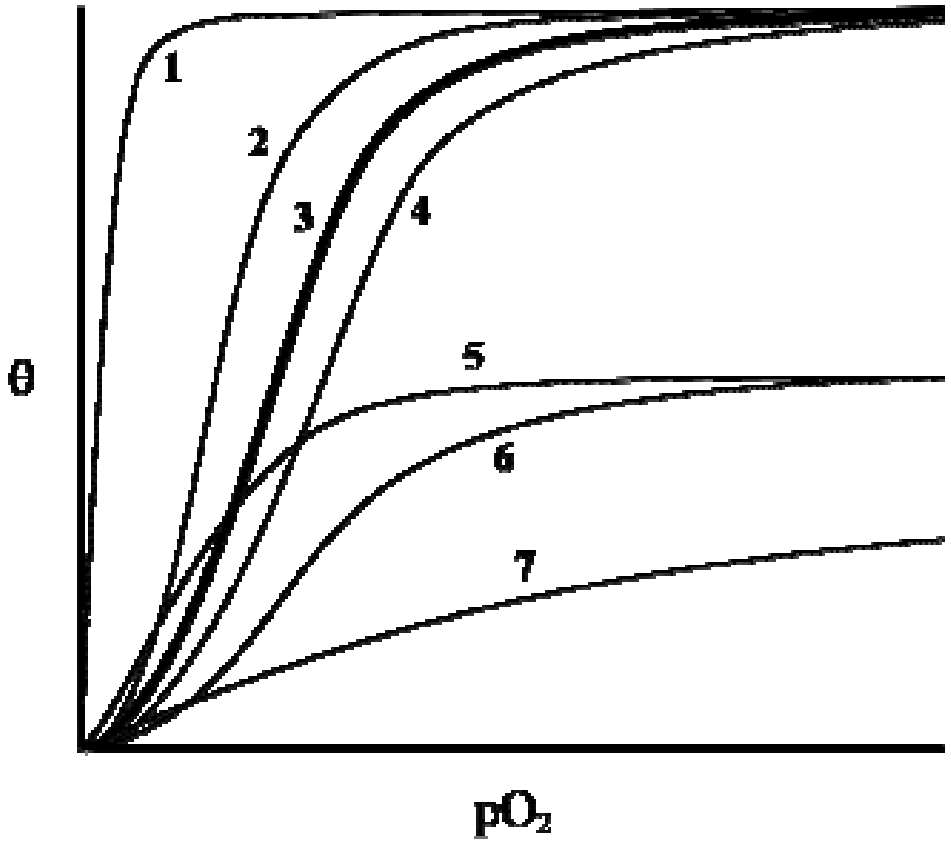
(12 pts) Shown below are several O_2 binding curves. The curve in bold (#3) represents O_2 binding by red blood cells of an average person living at sea level. Which curve below best represents O_2 binding:

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:

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

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.

Solution

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 c the enzyme-substrate complex?

$K_M = \frac{k_2 + k_{-1}}{k_1}$ if k_2 rate-limiting, $k_2 \ll\ll k_{-1}$ 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 $K_M = 1 \times 10^{-5}\ M$

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

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

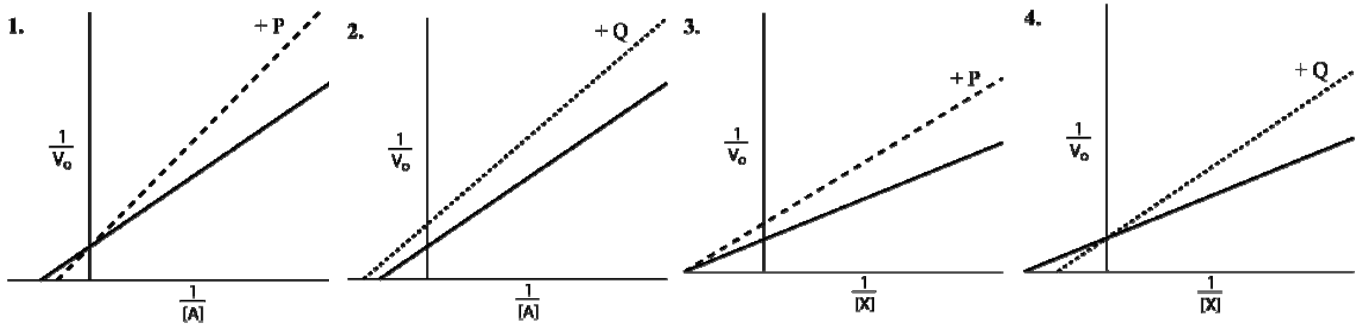
b. What would the catalytic efficiency be?

$\frac{k_{cat}}{K_M} = \frac{23.3\ s^{-1}}{1 \times 10^{-5}\ M} = 2.3 \times 10^6\ M^{-1} s^{-1}$

9. (28 pts) The enzyme 'convertase' catalyzes the following reaction:

$$A + X \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B + Y$$

You have discovered two molecules, P and Q, that inhibit catalysis by this enzyme. You examine the effects of each inhibitor on convertase and produce the following plots. (Solid lines show the enzyme action in the absence of inhibitor; dotted lines show the enzyme action in the presence of the indicated inhibitor. In plots 1 and 2, X is present at saturating concentrations; in plots 3 and 4, A is present at saturating concentrations.)



a. What type of inhibitor is P with respect to substrate A (plot 1)?

b. What type of inhibitor is Q with respect to substrate A (plot 2)?

c. What type of inhibitor is P with respect to substrate X (plot 3)?

d. What type of inhibitor is Q with respect to substrate X (plot 4)?

e. What effect(s) would increasing the concentration of *convertase* have on the dotted line in plot 1? (Circle your selections on the answer sheet.)
   A. The slope would: (increase/decrease/not change)
   B. The x-intercept would: (increase/decrease/not change)
   C. The y-intercept would: (increase/decrease/not change)

f. What effect(s) would increasing the concentration of Q have on the dotted line in plot 2? (Circle your selections on the answer sheet.)
   A. The slope would: (increase/decrease/not change)
   B. The x-intercept would: (increase/decrease/not change)
   C. The y-intercept would: (increase/decrease/not change)

g. Which of the following are true for the dotted line in plot 1? List all that are correct:

A. $\alpha < 1$	D. $\alpha' < 1$	G. $\alpha < \alpha'$
B. $\alpha = 1$	E. $\alpha' = 1$	H. $\alpha = \alpha'$
C. $\alpha > 1$	F. $\alpha' > 1$	I. $\alpha > \alpha'$

h. Which of the following are true for the dotted line in plot 2? List all that are correct:

A. $\alpha < 1$	D. $\alpha' < 1$	G. $\alpha < \alpha'$
B. $\alpha = 1$	E. $\alpha' = 1$	H. $\alpha = \alpha'$
C. $\alpha > 1$	F. $\alpha' > 1$	I. $\alpha > \alpha'$

i. Based on all four plots, would you expect P and Q to be able to bind convertase simultaneously? Explain your reasoning in 40 words or fewer.

Solution

a. What type of inhibitor is P with respect to substrate A (plot 1)? competitive
b. What type of inhibitor is Q with respect to substrate A (plot 2)? mixed
c. What type of inhibitor is P with respect to substrate X (plot 3)? non-competitive (mixed)
d. What type of inhibitor is Q with respect to substrate X (plot 4)? competitive

* V_{max} decreased but K_M same
$\alpha = \alpha'$

e. What effect(s) would increasing the concentration of *convertase* have on the dotted line in plot 1? (Circle your selections on the answer sheet.)
A. The slope would: (increase/decrease/not change)
B. The x-intercept would: (increase/decrease/not change)
C. The y-intercept would: (increase/decrease/not change)

slope = K_M/V_{max} more enzyme ↑ V_{max}
x-int. = $-\frac{1}{K_M}$ no Δ in K_M
y-int. = $\frac{1}{V_{max}}$ more enzyme ↑ V_{max}

f. What effect(s) would increasing the concentration of Q have on the dotted line in plot 2? (Circle your selections on the answer sheet.)
A. The slope would: (increase/decrease/not change)
B. The x-intercept would: (increase/decrease/not change)
C. The y-intercept would: (increase/decrease/not change)

mixed inhibitor slope = $\left(\frac{\alpha K_M}{\alpha'}\right)\Big/\left(\frac{V_{max}}{\alpha'}\right) = \frac{\alpha K_M}{V_{max}}$ more inhibitor ↑ slope
$\alpha' > \alpha \rightarrow K_i' < K_i$
so more inhibitor ↑ α' faster than α,
V_{max} is lower

g. Which of the following are true for the dotted line in plot 1? List all that are correct:

A. $\alpha < 1$
B. $\alpha = 1$
C. $\alpha > 1$
D. $\alpha' < 1$
E. $\alpha' = 1$ (competitive)
F. $\alpha' > 1$
G. $\alpha < \alpha'$
H. $\alpha = \alpha'$
I. $\alpha > \alpha'$

so $K_M^{apparent}$ becomes smaller and x-intercept is more negative
$K_M^{app} = \frac{\alpha K_M}{\alpha'}$

Solution

h. Which of the following are true for the dotted line in plot 2? List all that are correct:

A. $\alpha < 1$
B. $\alpha = 1$
C. $\alpha > 1$
D. $\alpha' < 1$
E. $\alpha' = 1$
F. $\alpha' > 1$
G. $\alpha < \alpha'$
H. $\alpha = \alpha'$
I. $\alpha > \alpha'$

(mixed)

i. Based on all four plots, would you expect P and Q to be able to bind convertase simultaneously? Explain your reasoning in 40 words or fewer.

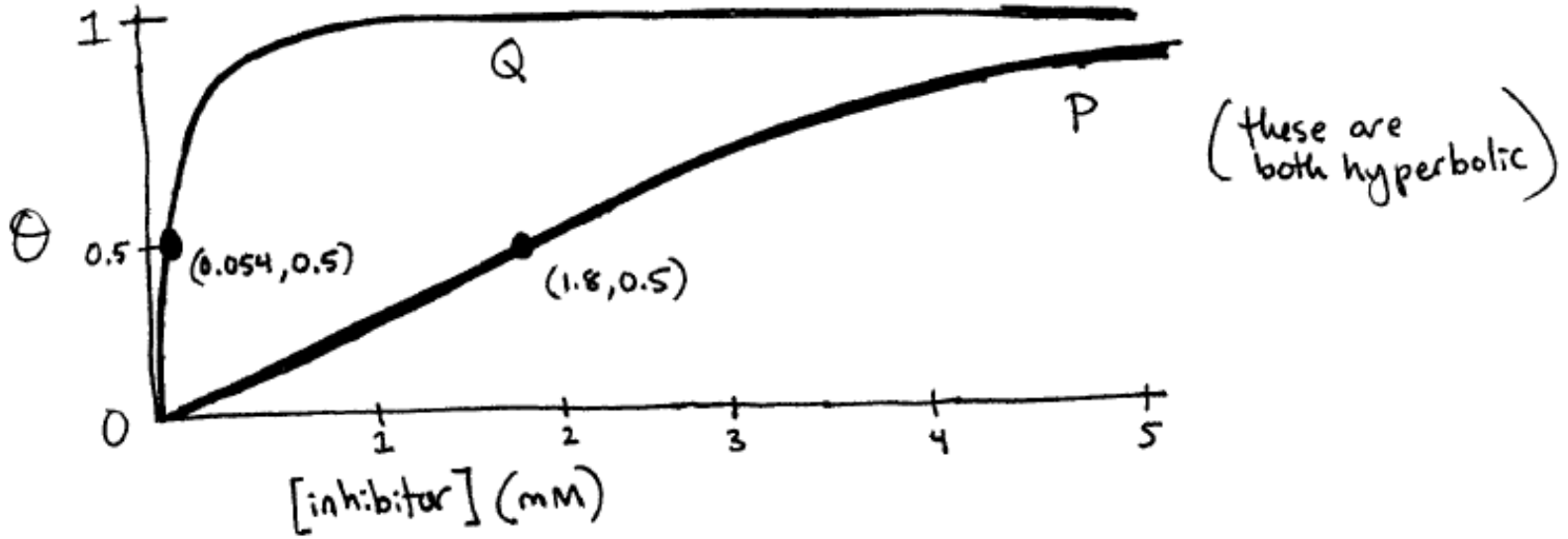
Yes, P is a competitive inhibitor for A and Q is a competitive inhibitor for X, but P is not competitive for X and Q is not competitive for A. Neither inhibitor interferes with the binding of the other.

You determine that the KI for P binding to convertase is 1.8 mM, and the KI for Q binding to convertase is 54 μM.

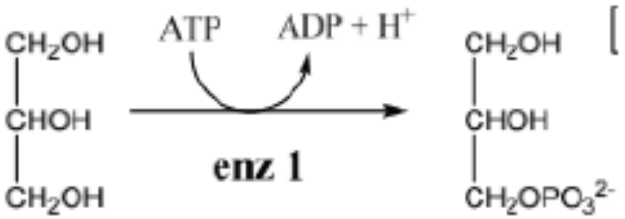
j. Does P or Q have a higher affinity for convertase? Briefly explain your answer (10 words or fewer).

Q because its K_i is lower (higher affinity)

k. Draw the binding curves for P and Q (independently) binding to convertase. Label your curves (P or Q), and label your axes with labels (axis names), units, and number values.



(these are both hyperbolic)

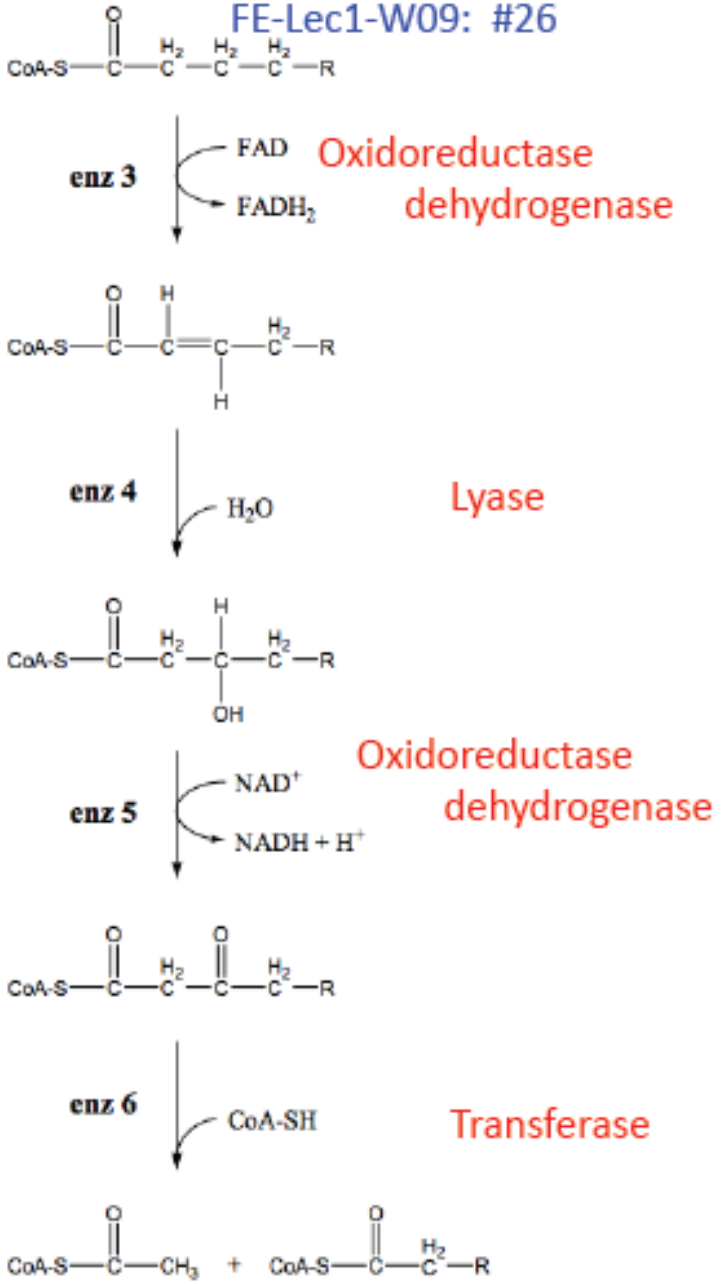


c. Name the class and subclass of enzyme 1.

- Transferase
- Kinase

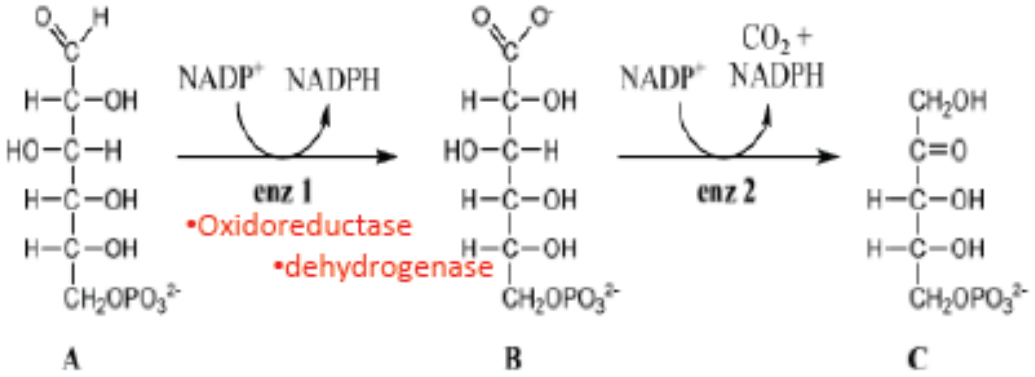
ENZYMES

FE-Lec1-W09: #26



ENZYMES

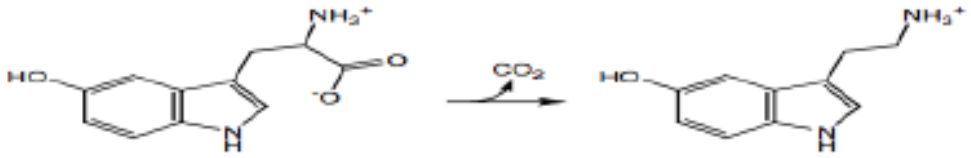
FE-Lec2-W09: #23



MTE#2-S09: #7

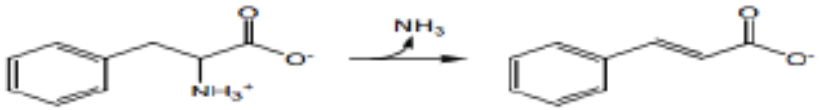
7. (6 pts) Name the *class* of enzyme that catalyzes each of the following reactions:

a.



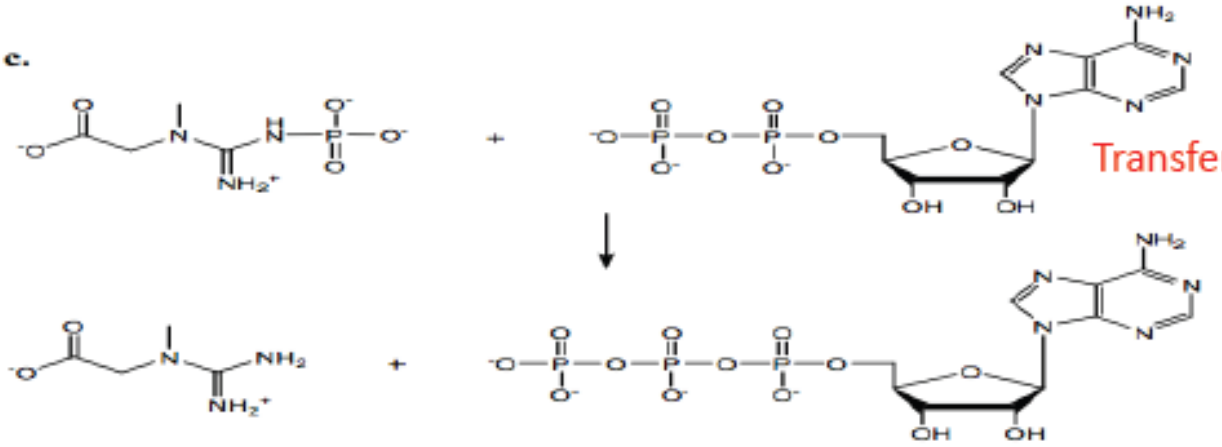
Lyase

b.



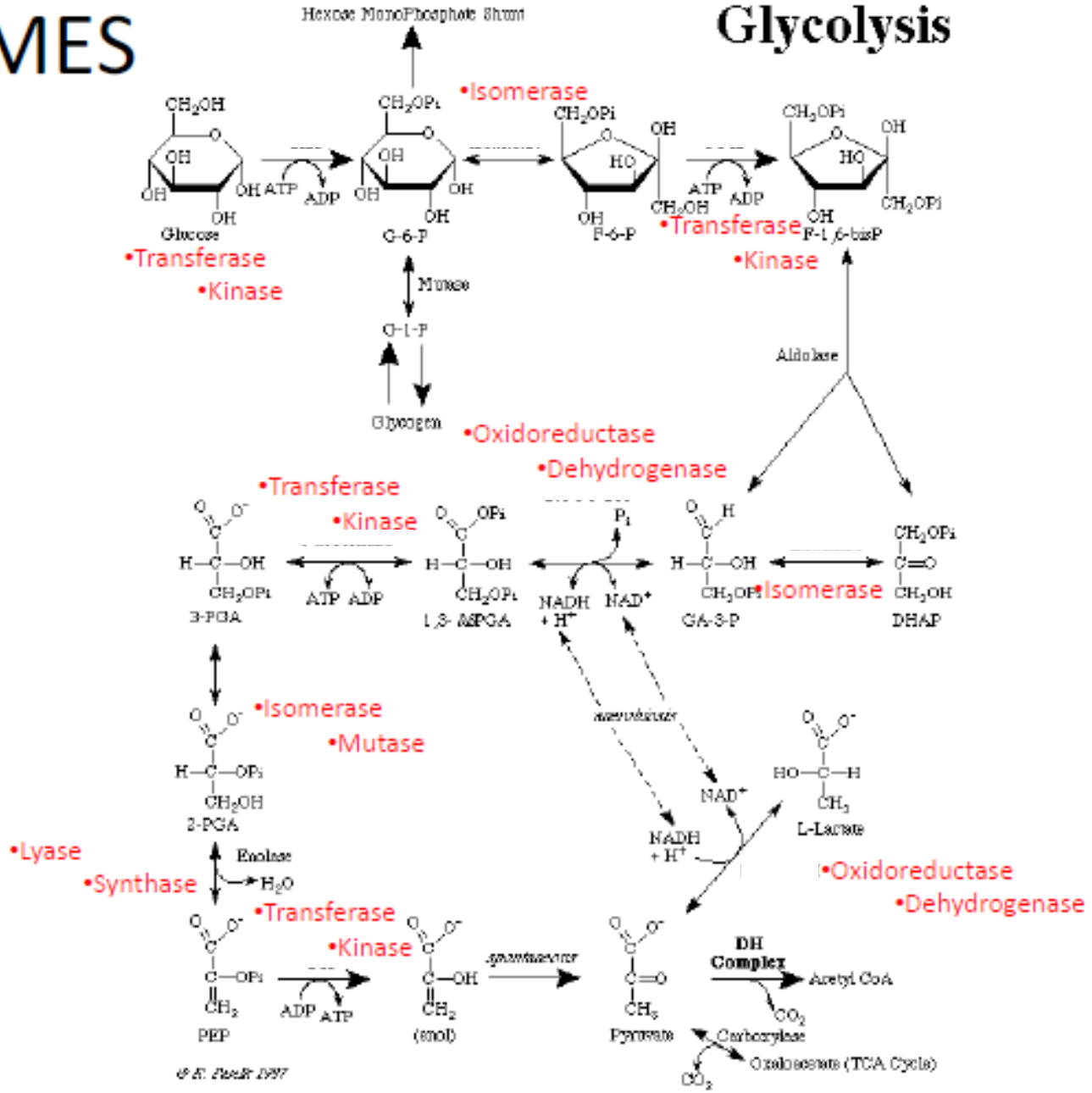
Lyase

c.



Transferase

ENZYMES


Glycolysis
Hexose MonoPhosphate Shunt
Glucose
G-6-P
F-6-P
F-1,6-bisP
G-1-P
Glycogen
Aldolase
3-PGA
1,3- BPGA
GA-3-P
DHAP
2-PGA
PEP
(enol)
Pyruvate
L-Lactate
Acetyl CoA
Oxaloacetate (TCA Cycle)
ATP
ADP
NADH + H+
NAD+
H2O
CO2
•Isomerase
•Transferase
•Kinase
•Oxidoreductase
•Dehydrogenase
•Mutase
•Lyase
•Synthase