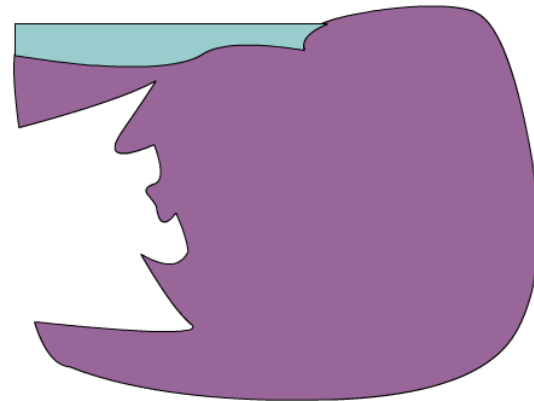
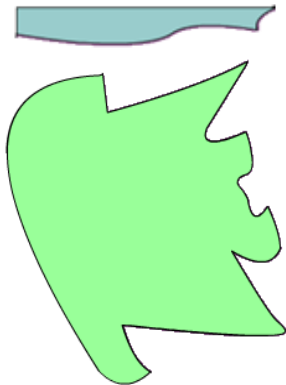


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ENZYMES

General properties of proteins

- The function of nearly all proteins depends on their ability to bind other molecules (ligands)
- Two properties of a protein characterize its interaction with ligands:
 - **Affinity:** the strength of binding between a protein and other molecule
 - **Specificity:** the ability of a protein to bind one molecule in preference to other molecules

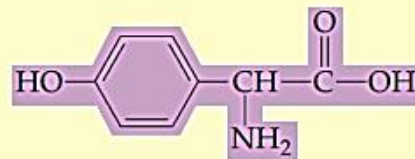


Are enzymes important?

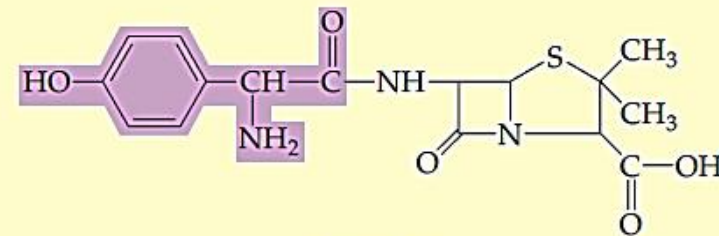
- In the human body, almost every metabolic process involve the use of enzymes



Sucrose (table sugar), yeast enzyme breaks sucrose into its two smaller sugar



p-Hydroxyphenylglycine

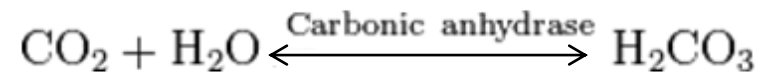
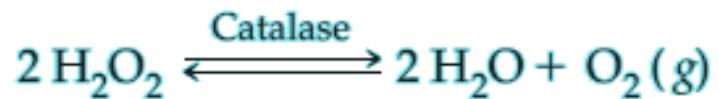


Amoxicillin

crushed leaves are exposed to the oxygen in air, a polyphenoloxidase breaks up polyphenols into tannins which impart the darker color and characteristic flavors

The Biological Catalysts; Enzymes

- What are enzymes? (specialized proteins, small amounts, acceleration, no change). Ribozymes are the exception
- Enzymes are the most efficient catalysts known
 - Usually in the range of 10^6 to 10^{14}
 - Non-enzymatic catalysts (10^2 to 10^4)
 - The actions of enzymes are fine-tuned by regulatory processes
- Examples: catalase (10^8) & carbonic anhydrase (10^7)



Reaction Conditions	Activation Free Energy		Relative Rate
	kJmol^{-1}	kcal mol^{-1}	
No catalyst	75.2	18.0	1
Platinum surface	48.9	11.7	2.77×10^4
Catalase	23.0	5.5	6.51×10^8



How to express an enzymatic reaction?

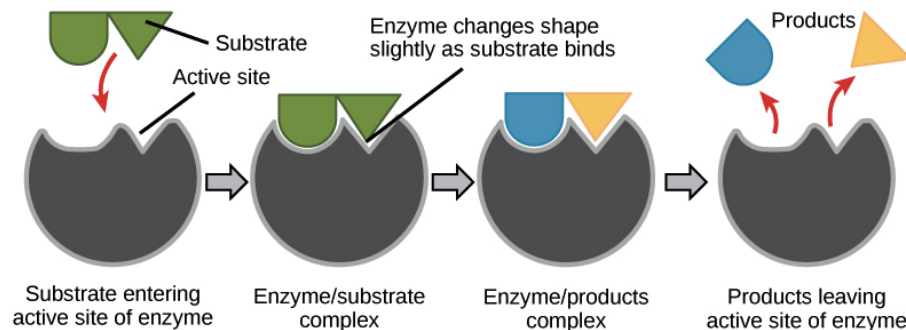
- In enzymatic reactions, reactants are known as substrates
- We can simply express an enzymatic reaction using this formula



Or

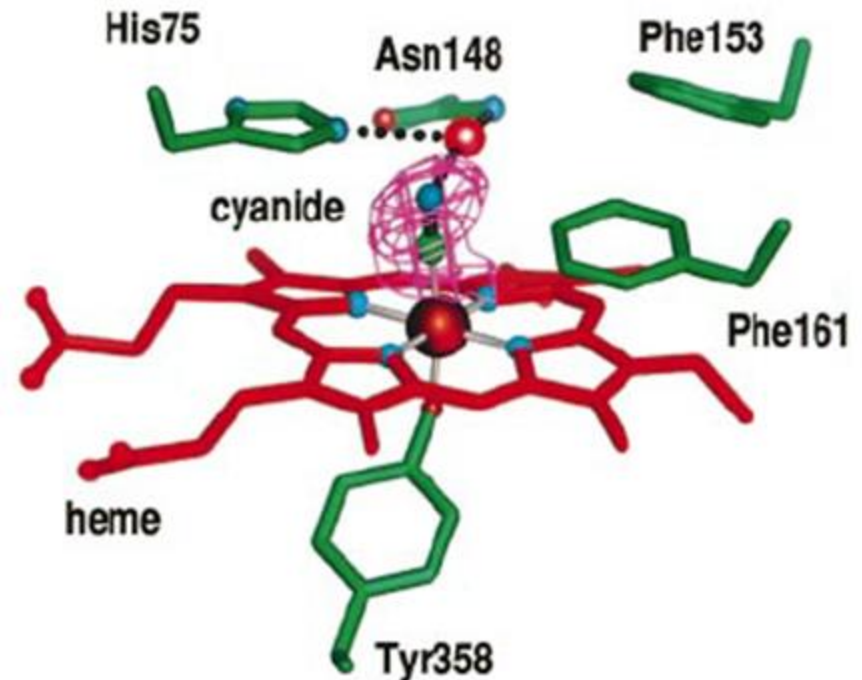
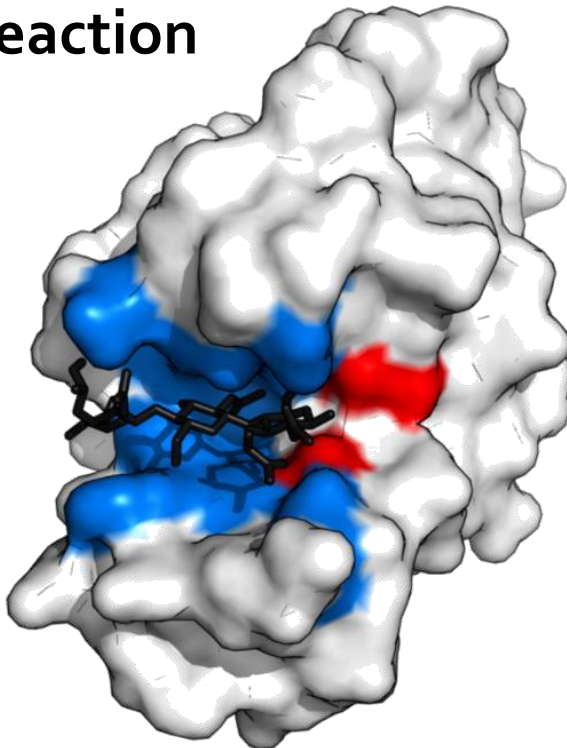


where E is the free enzyme; S is the free substrate, ES is the enzyme-substrate complex; P is the product of the reaction; and EP is the enzyme-product complex before the product is released



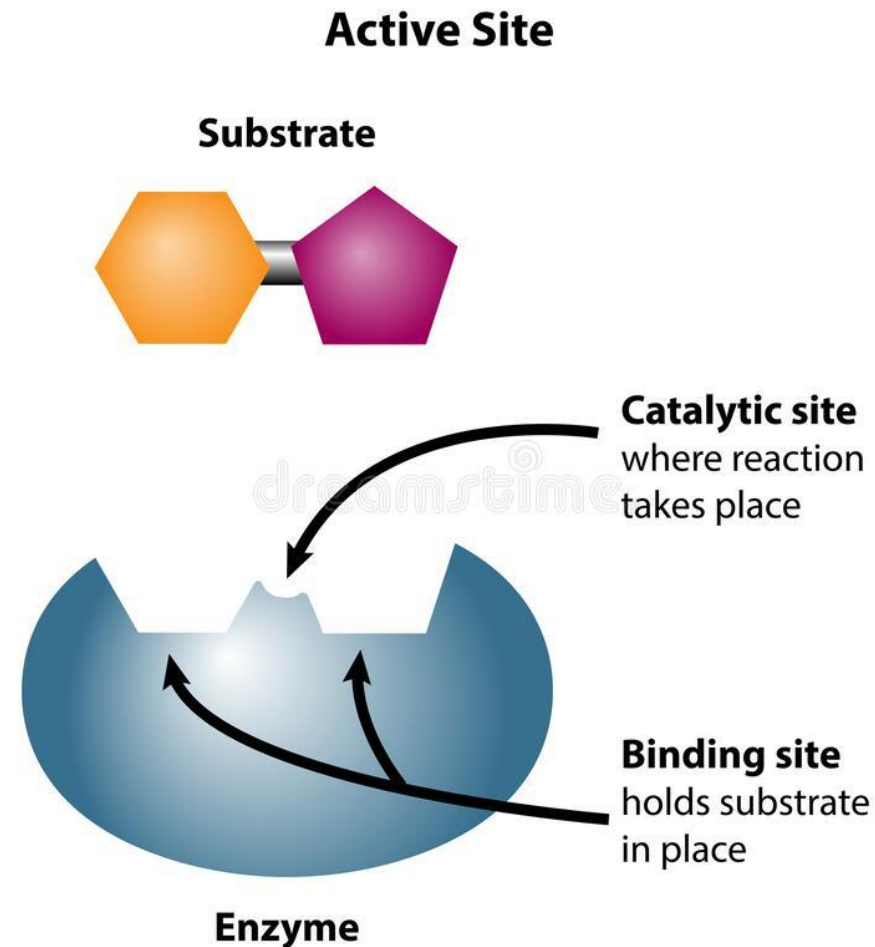
Active sites of enzymes

- A specific three-dimensional shape which includes a region where the biochemical reaction takes place
- Contains a specialized amino acid sequence that facilitates the reaction



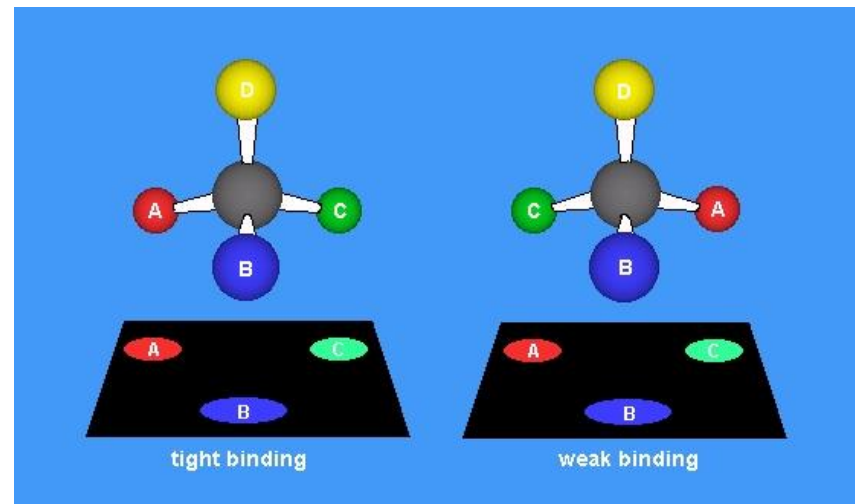
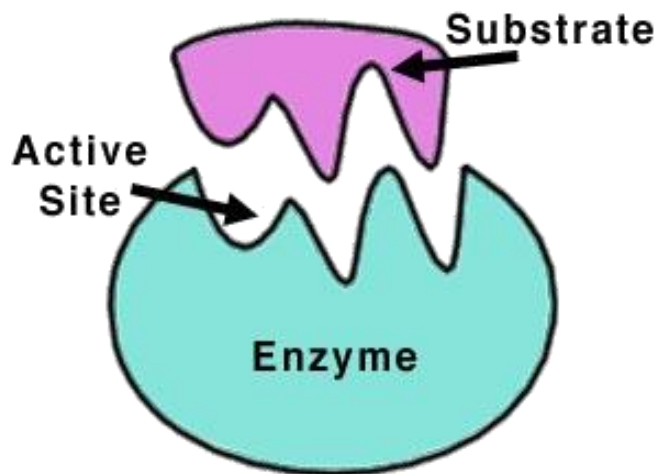
Active sites of enzymes

- Within the active site are two sub-sites, the binding site and the catalytic site. The binding & catalytic site may be the same
- Binding site: binds substrate through ionic, H-bonding or other electrostatic forces, or hydrophobic interactions
- Catalytic site: contains the catalytic groups



Features of active site

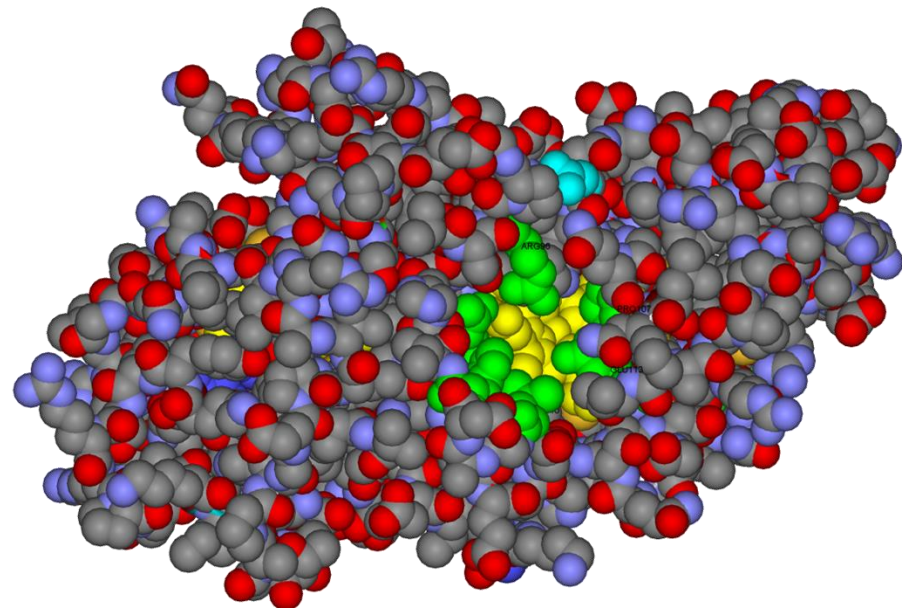
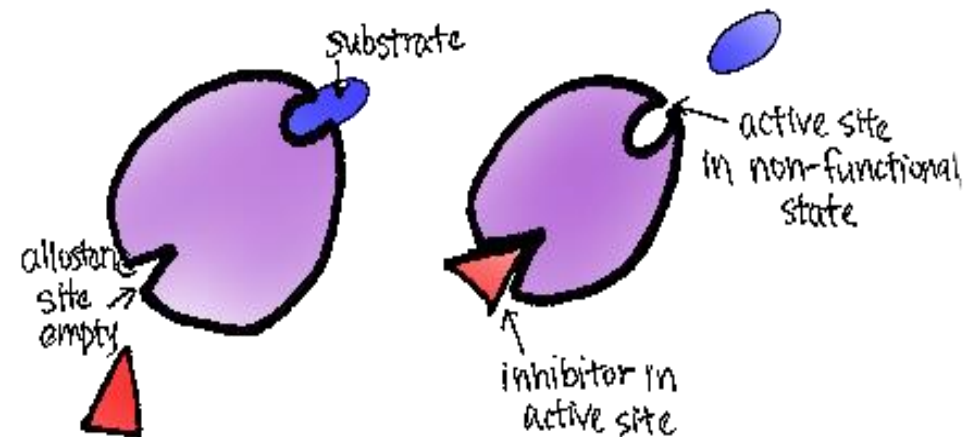
- Active sites; structures that look like canals, clefts or crevices
- Water is usually excluded after binding unless it participates in the reaction
- Substrates are bound to enzymes by multiple weak attractions (electrostatic, hydrogen, van der Waals, & hydrophobic)
- Binding occurs at least at three points (chirality)



Features of active site

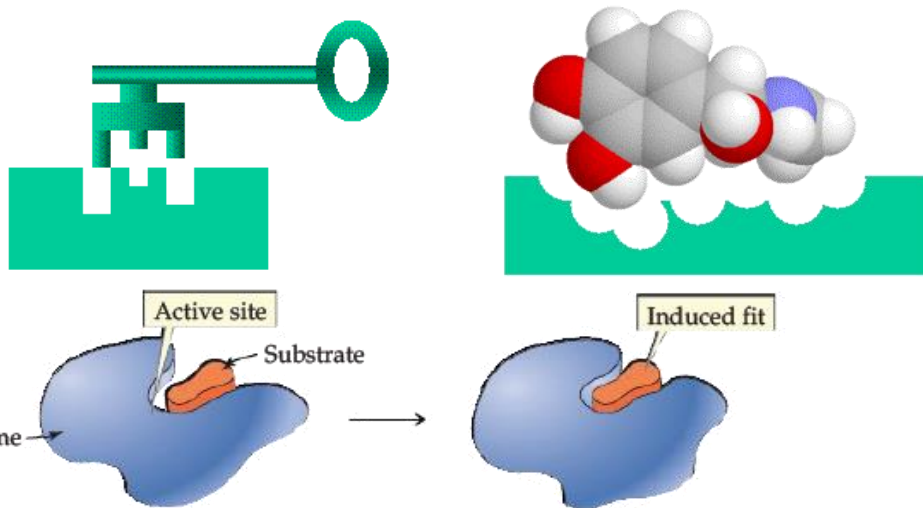
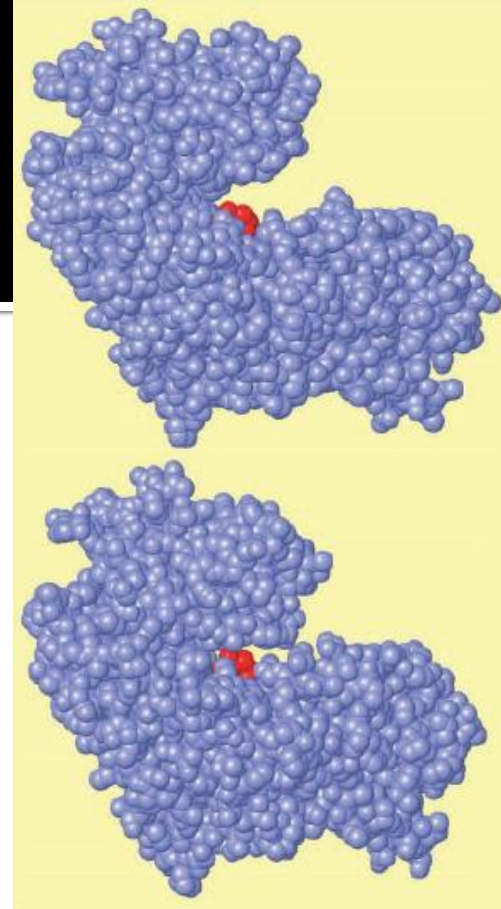
- Forms by groups from different parts of the amino acid sequence usually forming **a domain made of multiple secondary structures**
- Takes up a relatively **small part** of the total volume
- The **"extra" amino acids** help create the three-dimensional active site & in many enzymes, may create **regulatory sites**

ALLOSTERIC ENZYME

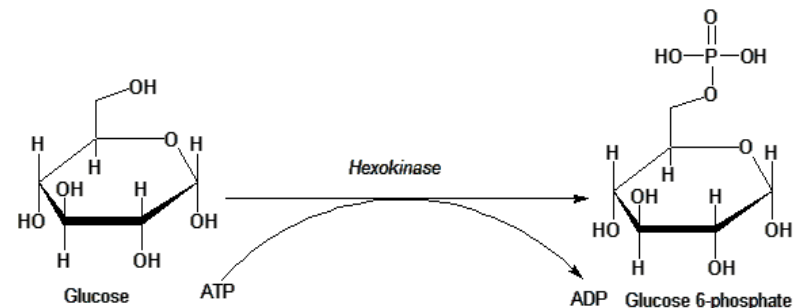


How Do Enzymes Work?

- Binding leads to formation of transition-state
- Usually, substrate binds by non-covalent interactions to the active site
- The catalyzed reaction takes place at the active site, usually in several steps
- Two models, lock-and-key vs. induced-fit model
- Glucose and hexokinase, phosphorylation

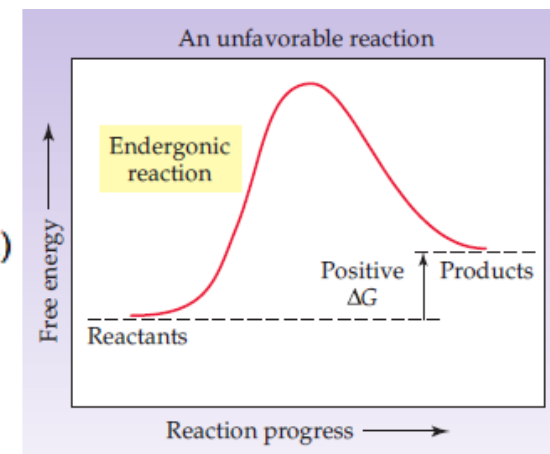
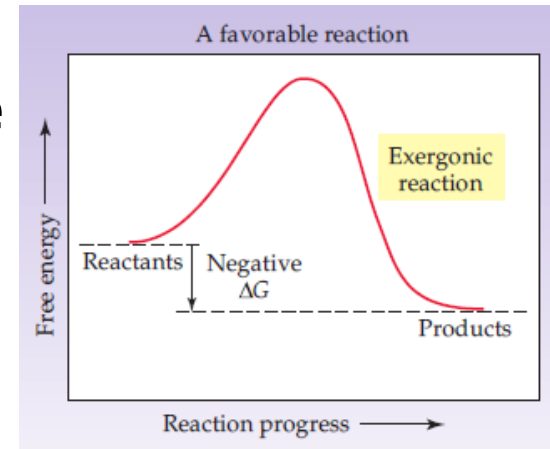
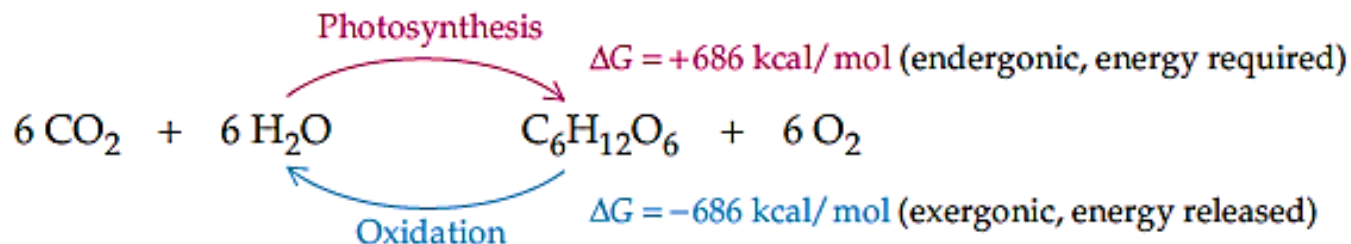


Improving the binding site for ATP
& excluding water (might interfere
with the reaction)



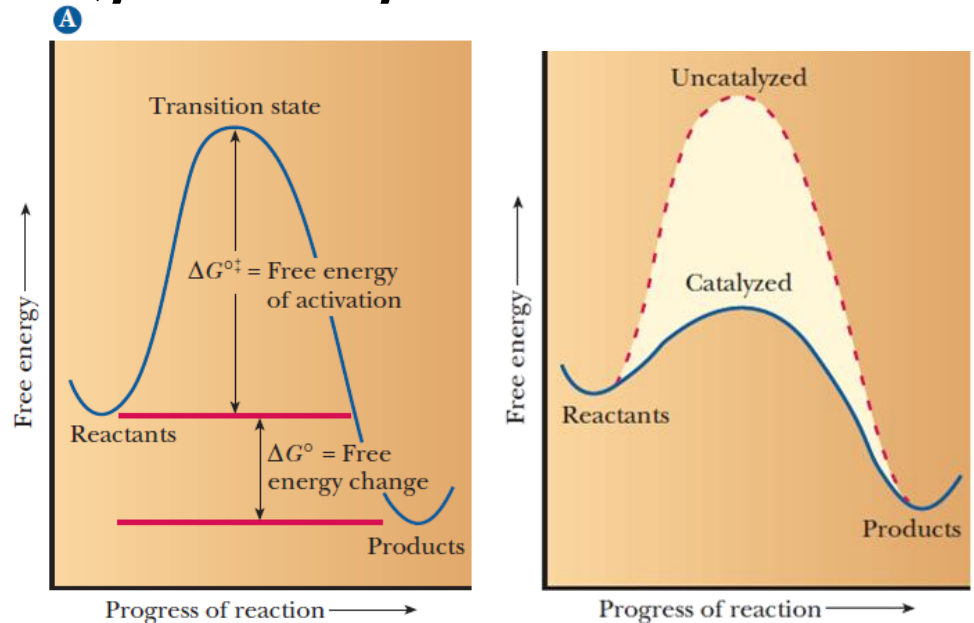
Energy & Biochemical Reactions

- $\Delta G = \Delta H - T\Delta S$
- Spontaneous vs. non-spontaneous, favorable vs. non-favorable, exergonic vs. endergonic, exothermic vs. endothermic, switch of signs
- ΔG , ΔG°
- Biochemical pathways; storage (endergonic) & release (exergonic)
- Kinetics (rate) vs. Thermodynamics (favorability)



How do enzymes work?

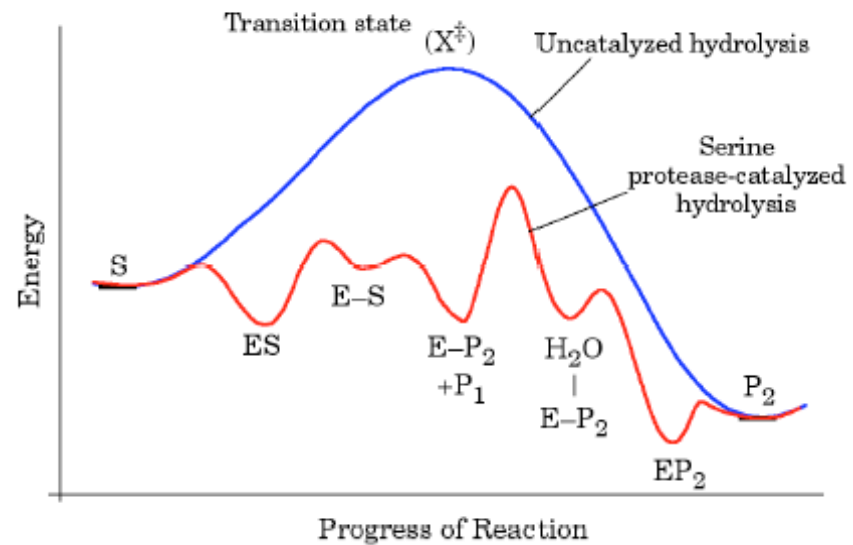
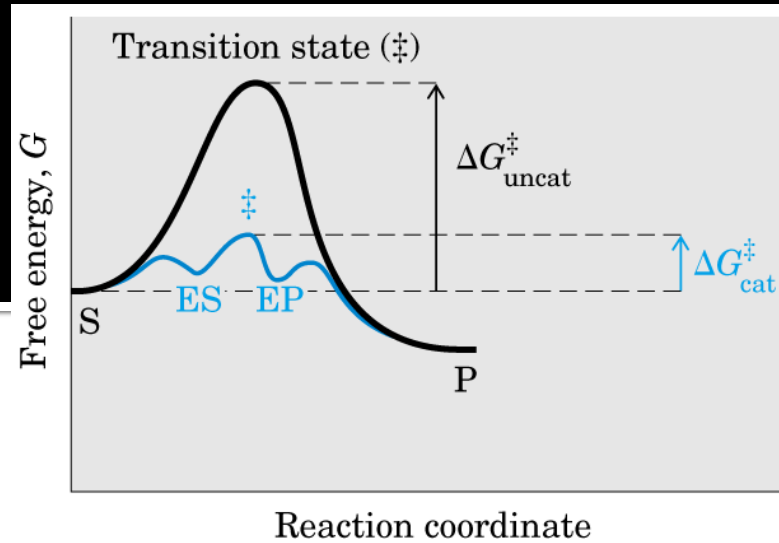
- Enzymes speed up reactions, but have no relation to equilibrium or favorability
- What is an activation energy ($\Delta G^{\ddagger}$) concept?
- Specificity varies (stereoisomers), however, there is none non-specific
- Spontaneous vs. rate!
- What is the transition state?



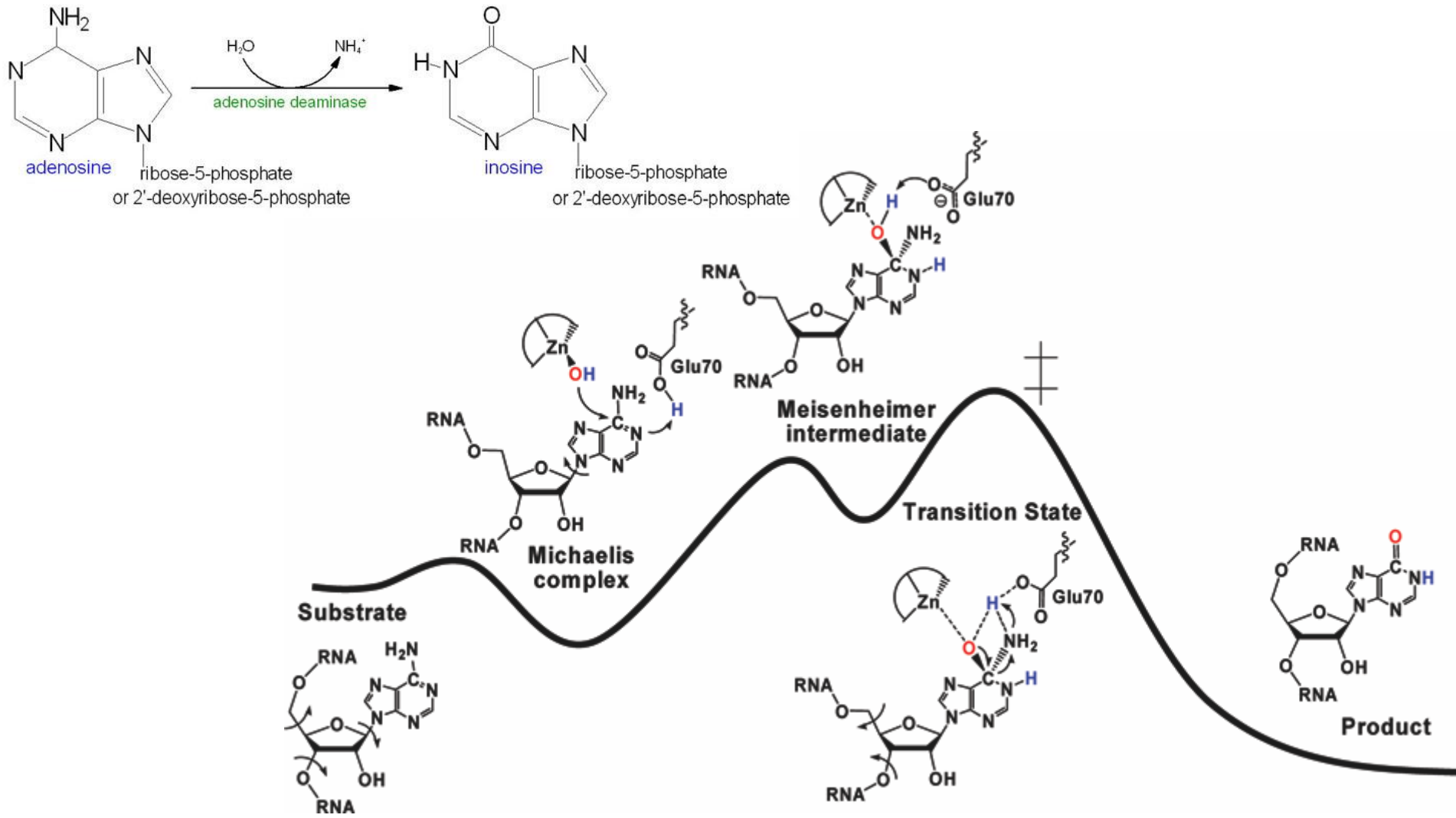
Transition-state complex binds more tightly to the enzyme compared to substrate

Alternative pathways

- Substrates of enzymatic reactions often undergo several transformations when associated with the enzyme and each form has its own free energy value
- Which one is the activation energy?
- Activation energy & final ΔG calculation

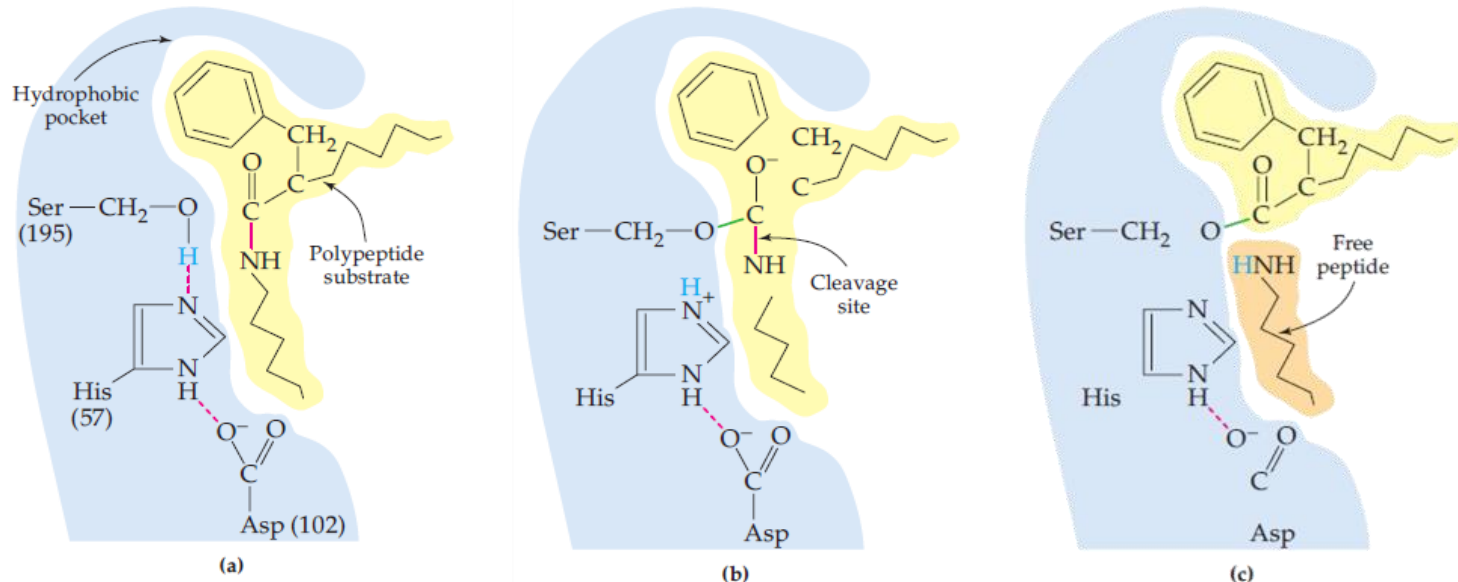


Example: Adenosine Deaminase



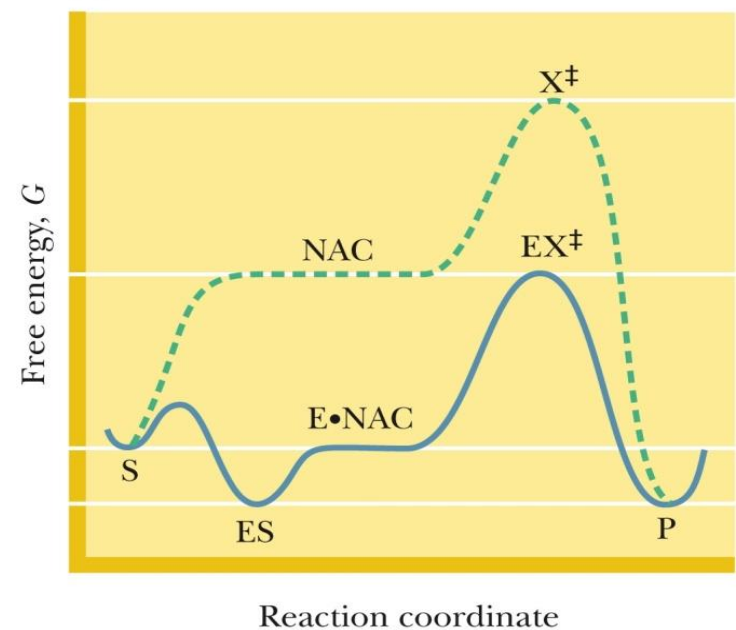
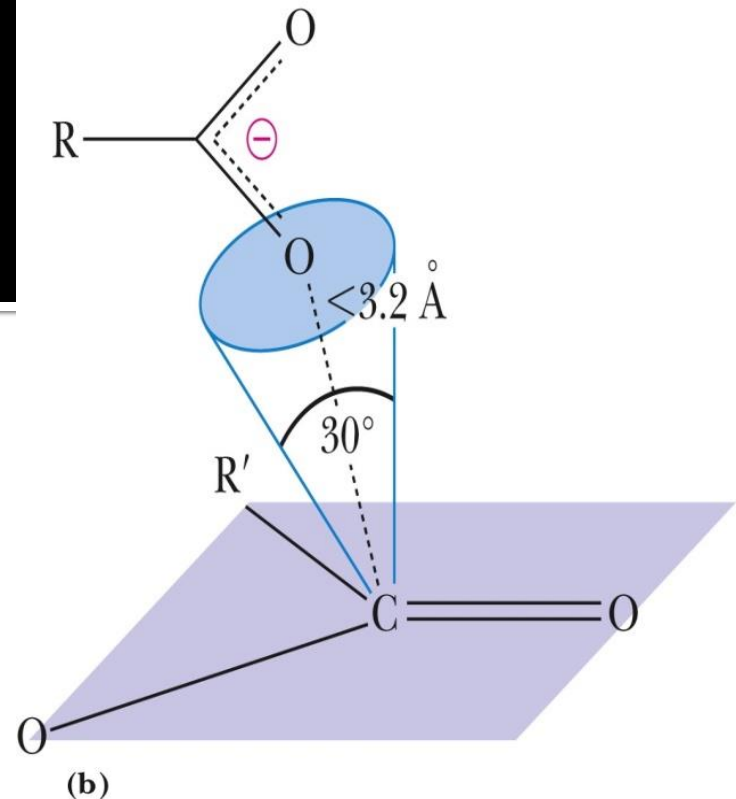
How Do Enzymes Work?

- Proximity effect: Bring substrate(s) and catalytic sites together
- Orientation effect: Hold substrate(s) at the exact distance and in the exact orientation necessary for reaction
- Catalytic effect: Provide acidic, basic, or other types of groups required for catalysis
- Energy effect: Lower the energy barrier by inducing strain in bonds in the substrate molecule



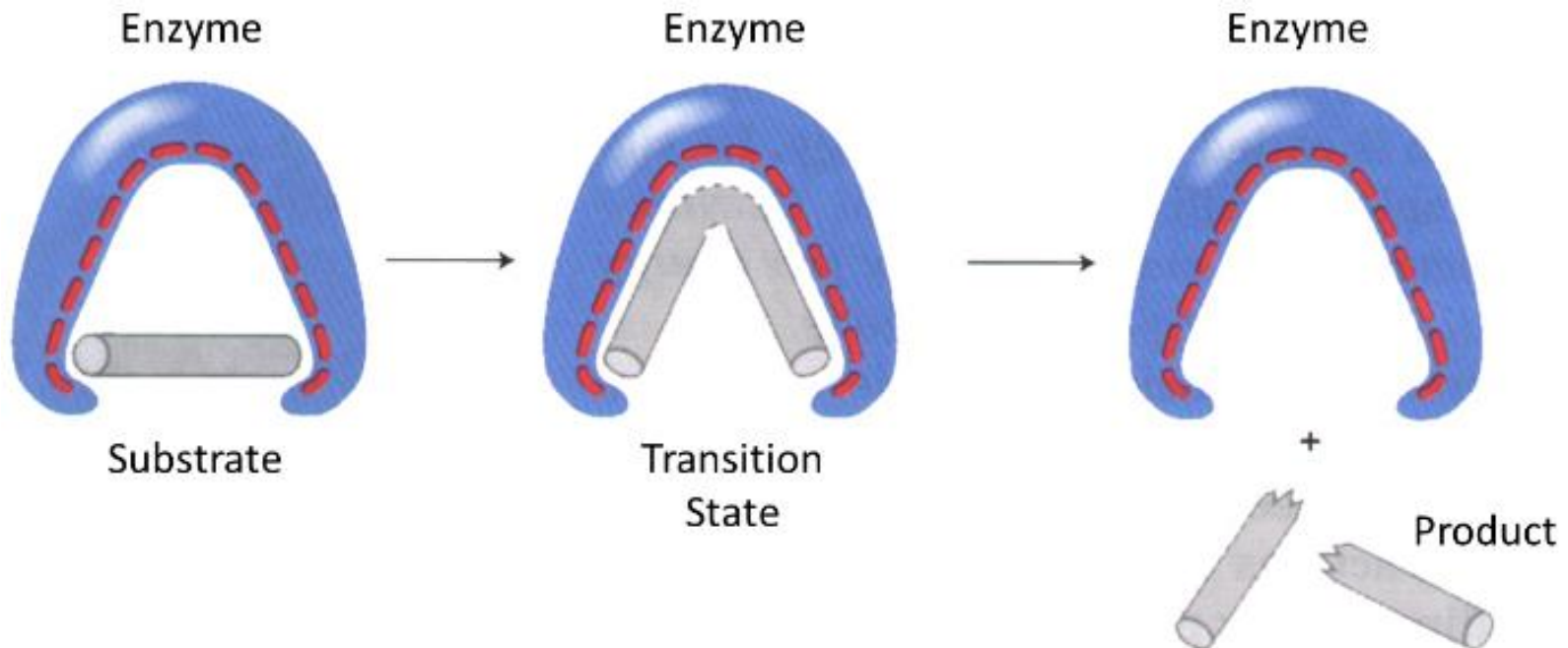
Catalysis by proximity & orientation

- Enzyme-substrate interactions orient reactive groups and bring them into proximity with one another favoring their participation in catalysis
 - Such arrangements have been termed near-attack conformations (NACs)
 - NACs are precursors to reaction transition states



Catalysis by bond strain

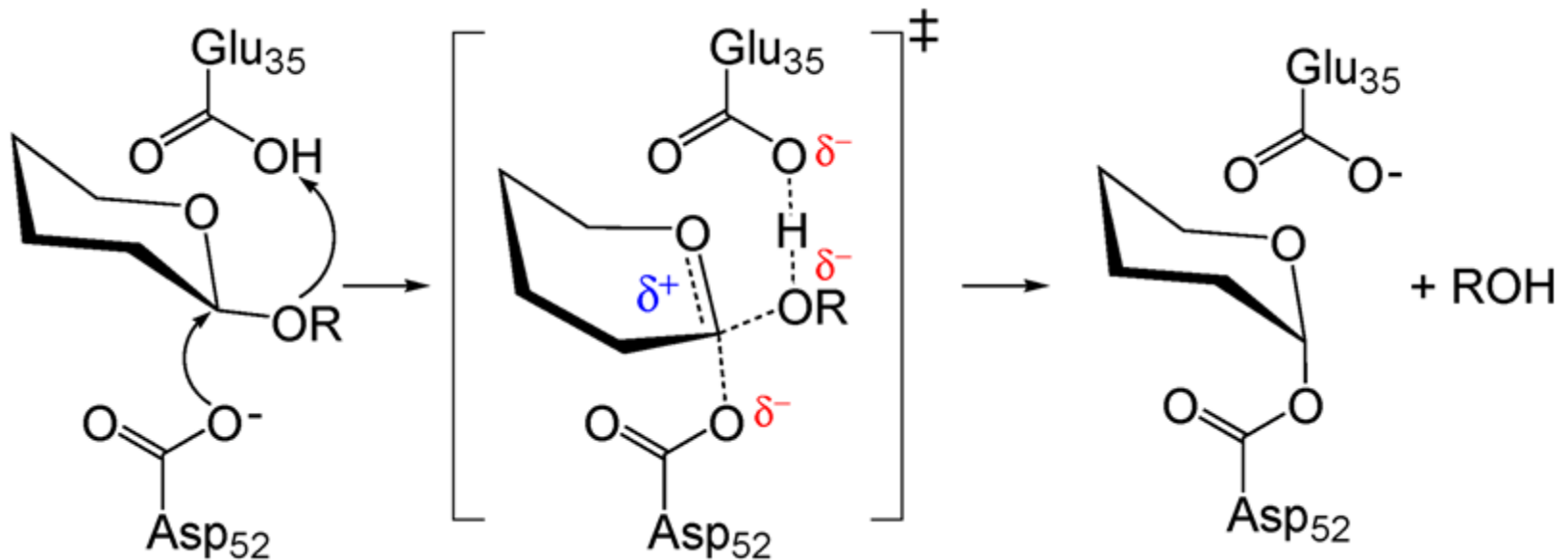
- In this form of catalysis, the induced structural rearrangements produce strained substrate bonds reducing the activation energy.



Catalysis by bond strain

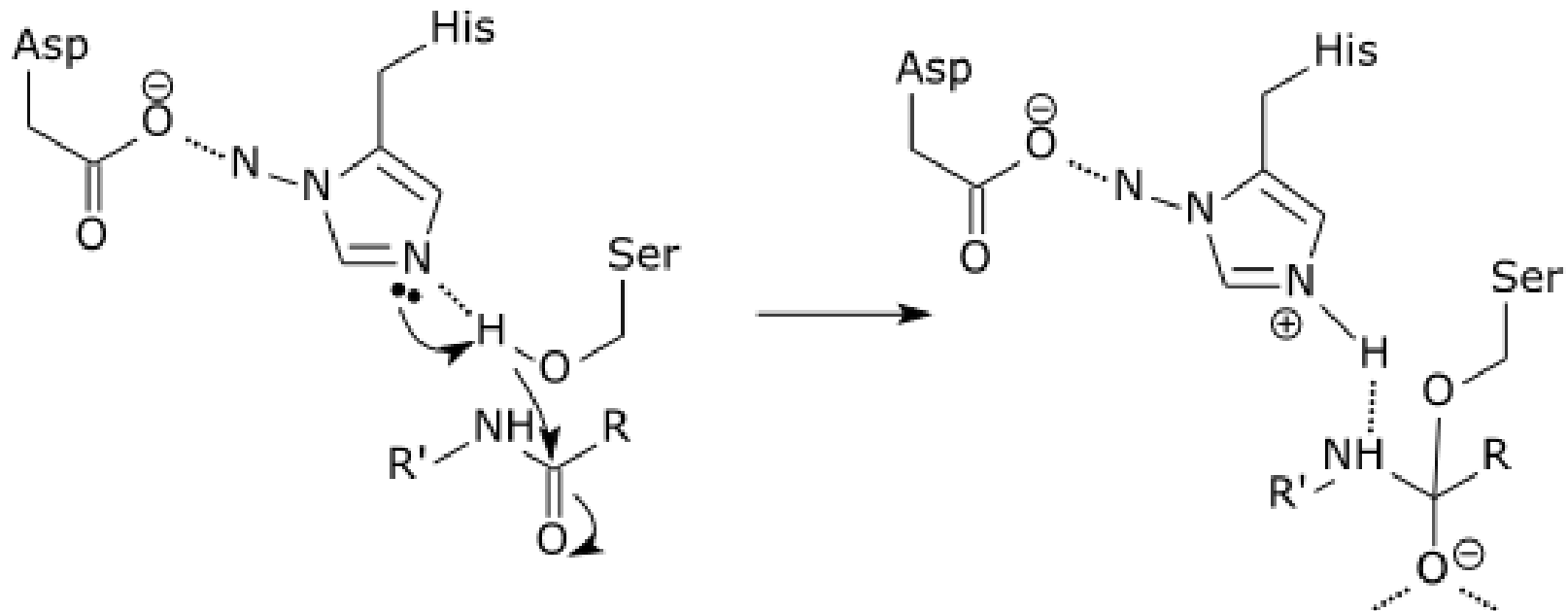
- Example: lysozyme

The substrate, on binding, is distorted from the typical 'chair' hexose ring into the 'sofa' conformation, which is similar in shape to the transition state



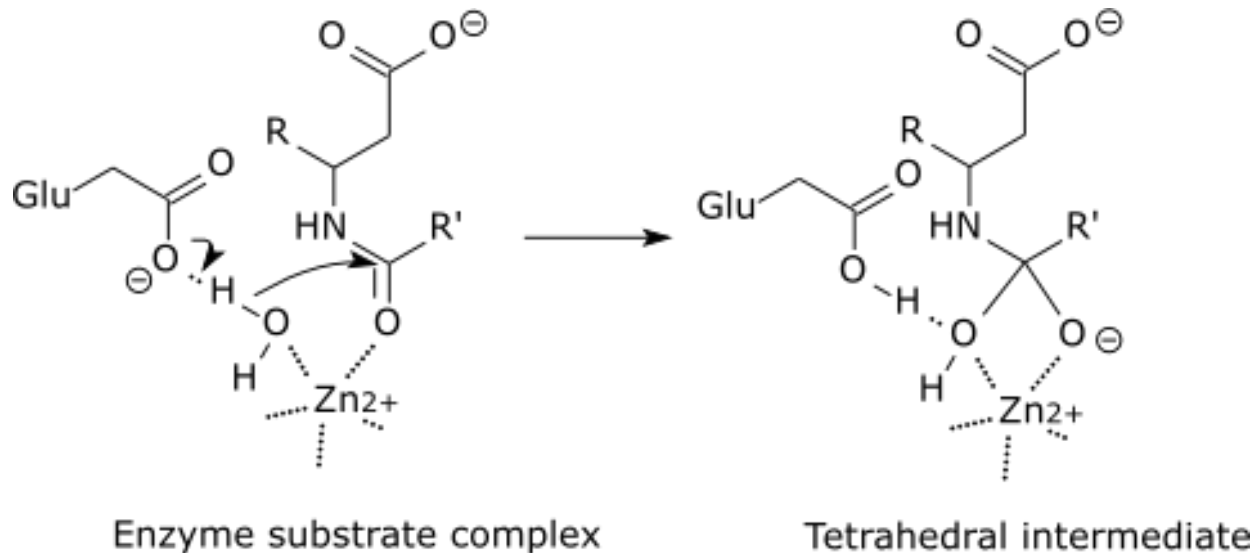
Catalysis involving proton donors (acids) & acceptors (bases)

- The R groups act as donors or acceptors of protons
 - Histidine is an excellent proton donor/acceptor at physiological pH
 - Example: serine proteases



Covalent catalysis

- A covalent intermediate forms between the enzyme or coenzyme and the substrate
 - Examples of this mechanism is proteolysis by serine proteases, which include digestive enzymes (trypsin, chymotrypsin, and elastase)



Naming of enzymes

- In general, enzymes end with the suffix (-ase)
- Most enzymes are named for their substrates and for the type of reactions they catalyze, with the suffix “ase” added
- For example; ATPase is an enzyme that breaks down ATP, whereas ATP synthase is an enzyme that synthesizes ATP
- Some enzymes have common names that provide little information about the reactions that they catalyze
- Examples include the proteolytic enzyme trypsin

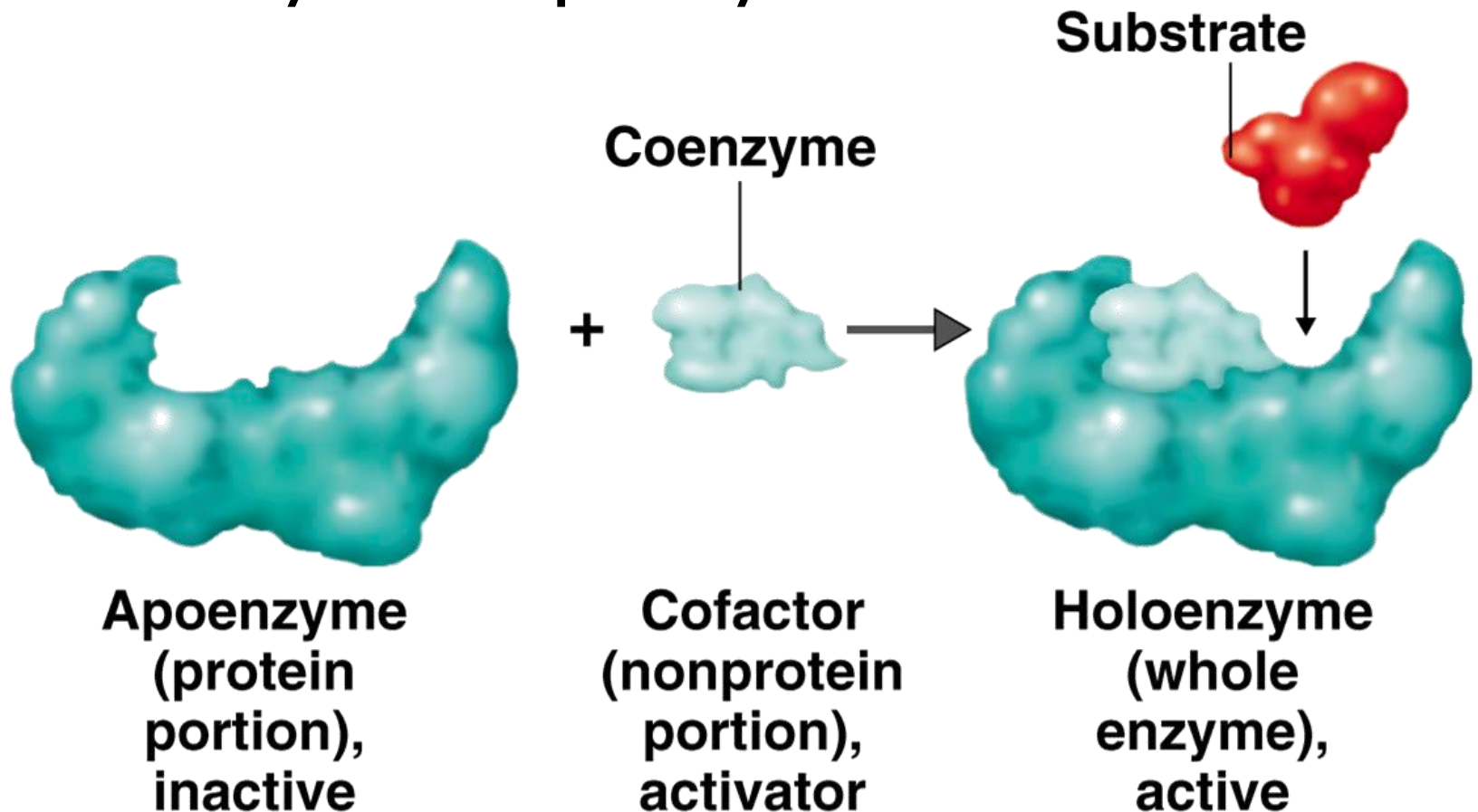
Naming of enzymes; EC numbering

Enzyme Commission number

- A numerical classification scheme for enzymes, based on the chemical reactions they catalyze
- Strictly speaking, EC numbers do not specify enzymes, but enzyme-catalyzed reactions
- Numbering format:
 - EC followed by four numbers separated by periods
 - Major class (1-7), Minor class, subclass, further sub-classification
- For example: tripeptide aminopeptidases "EC 3.4.11.4"
 - EC 3: hydrolases
 - EC 3.4: hydrolases that act on peptide bonds
 - EC 3.4.11: hydrolases that cleave off the amino-terminal of the amino acid polypeptide
 - EC 3.4.11.4: cleave off the amino-terminal end from a tripeptide

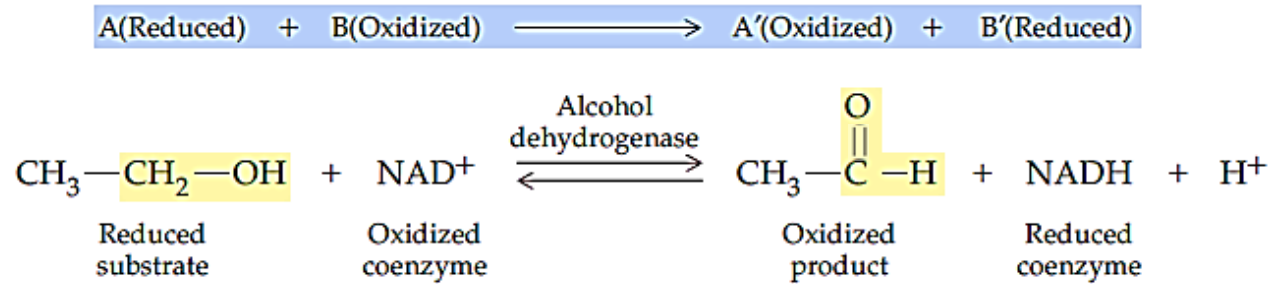
Enzyme Classification (structure)

- Simple vs. complex (conjugated)
- Holoenzyme vs. apoenzyme

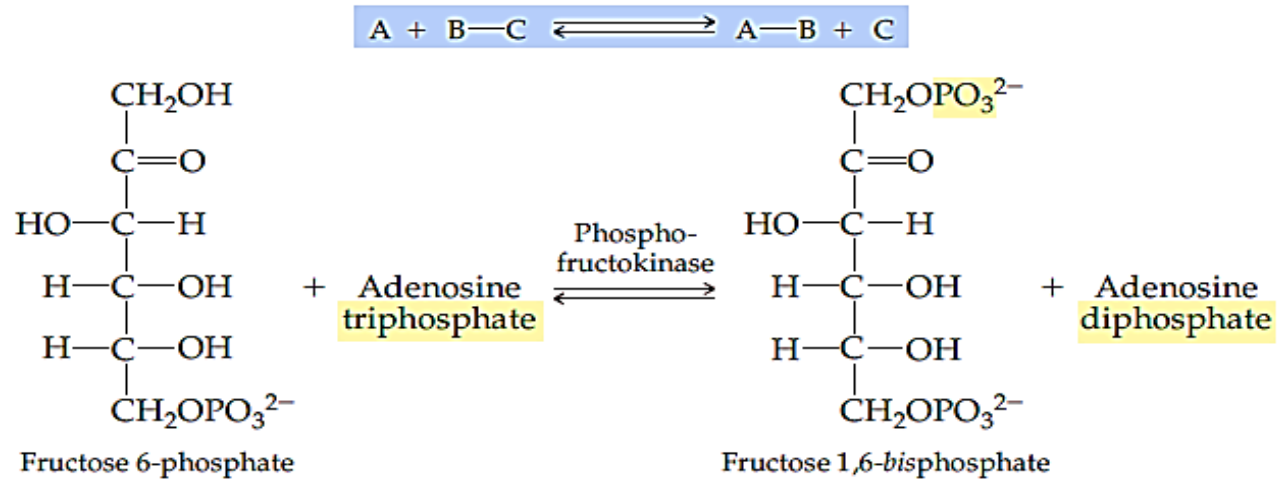


Enzyme Classification (function)

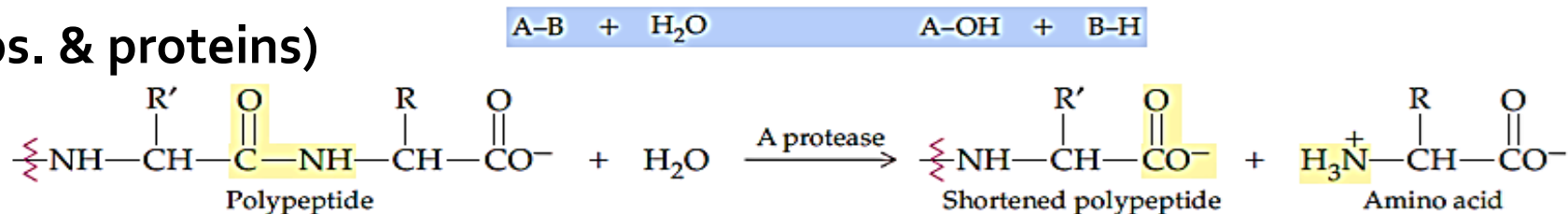
- **Oxidoreductases:**
addition or removal
of O, O₂, H. Require
coenzymes (heme)



- **Transferases:**
transfer of a group
from one molecule
to another

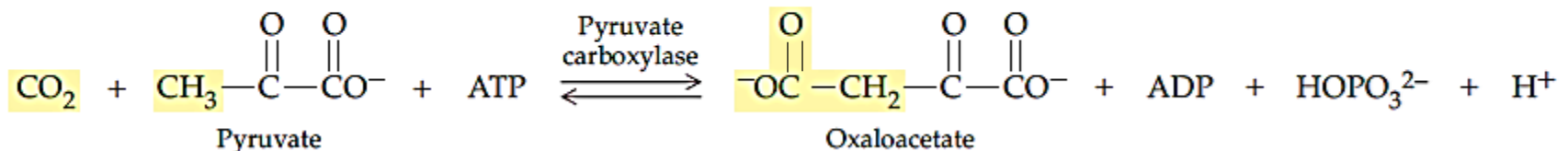
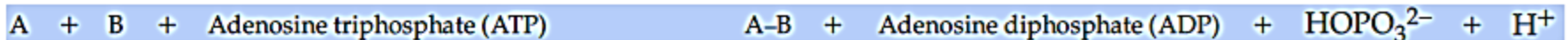
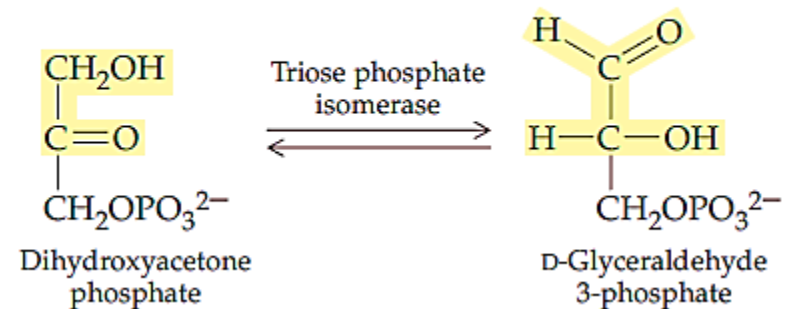
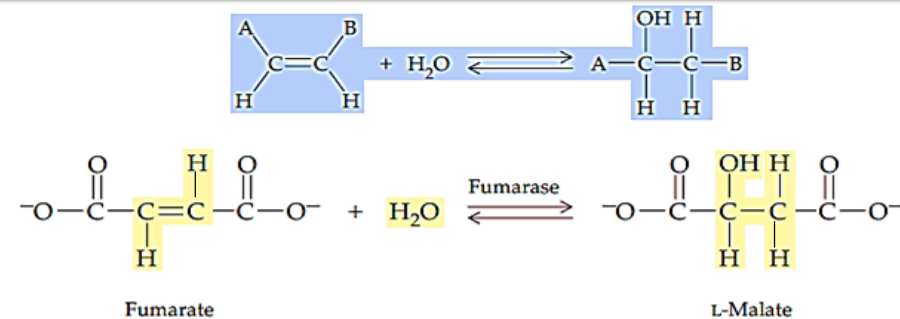


- **Hydrolases:**
addition of water
(carbs. & proteins)



Enzyme Classification (function)

- **Lyases:** addition of a molecule (H_2O , CO_2 , NH_3) to a double bond or reverse
- **Isomerases:** one substrate and one product
- **Ligases:** usually not favorable, so they require a simultaneous hydrolysis reaction



Oxido-reductases

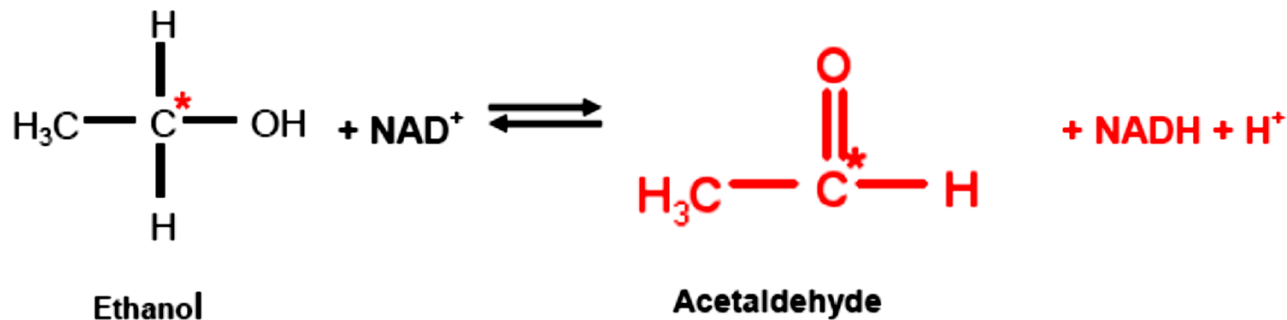
- These enzymes catalyze oxidation & reduction reactions involving the transfer of hydrogen atoms, electrons or oxygen
- This group can be further divided into 4 main classes:
 - ✓ Dehydrogenases
 - ✓ Oxidases
 - ✓ Peroxidases
 - ✓ Oxygenases

Dehydrogenases

- Dehydrogenases catalyze hydrogen transfer from the substrate to a molecule known as nicotinamide adenine dinucleotide (NAD⁺)
- Lactate dehydrogenase

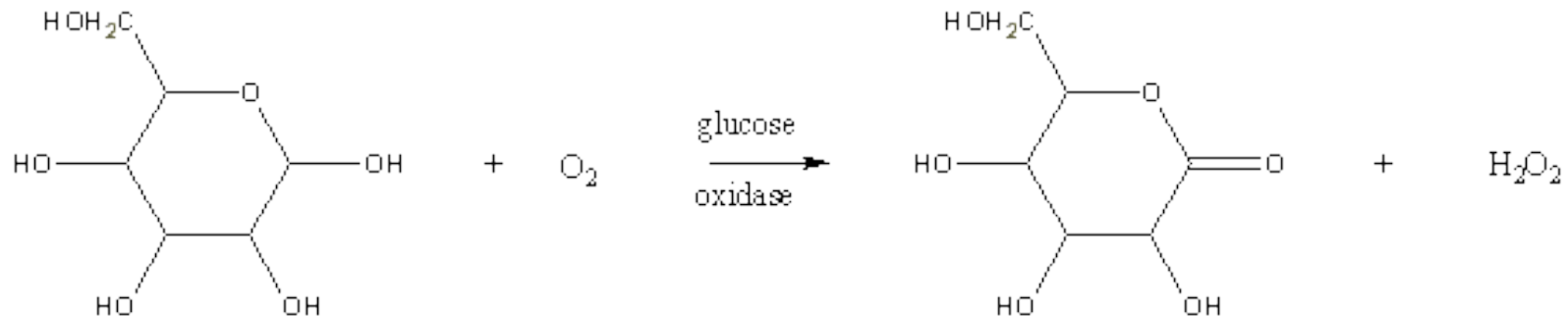


-  **Alcohol dehydrogenase**



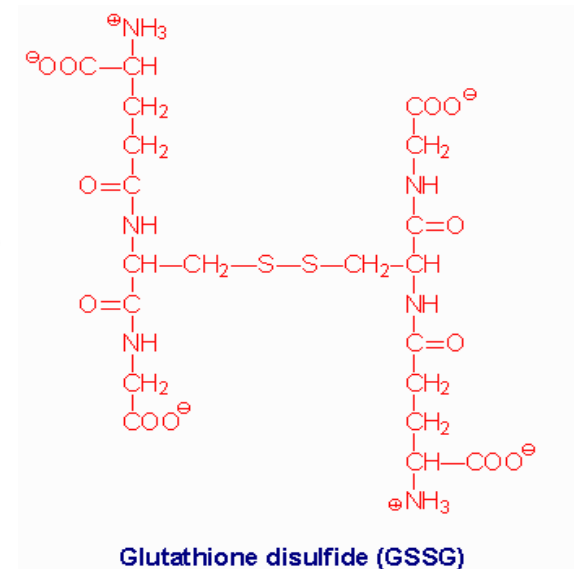
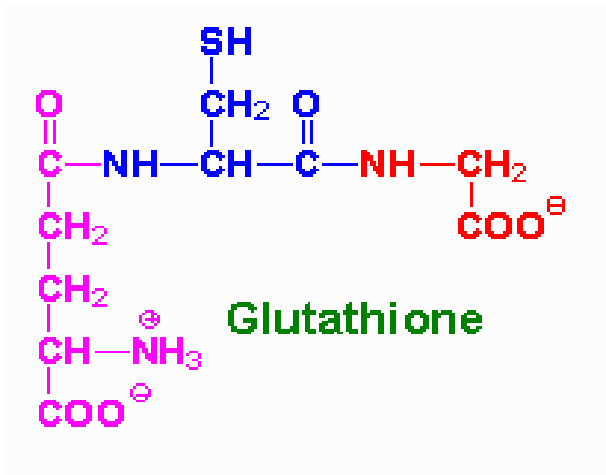
Oxidases

- Oxidases catalyze hydrogen transfer from the substrate to molecular oxygen producing hydrogen peroxide as a by-product
- Glucose oxidase



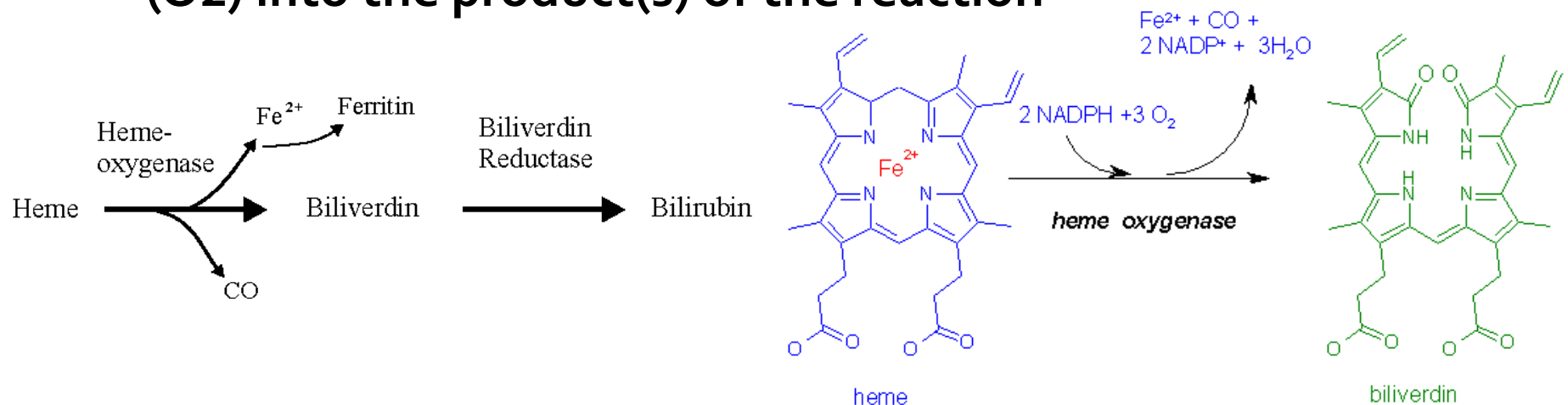
Peroxidases

- Peroxidases catalyze oxidation of a substrate by hydrogen peroxide
- Oxidation of two molecules of glutathione (GSH) in the presence of hydrogen peroxide:



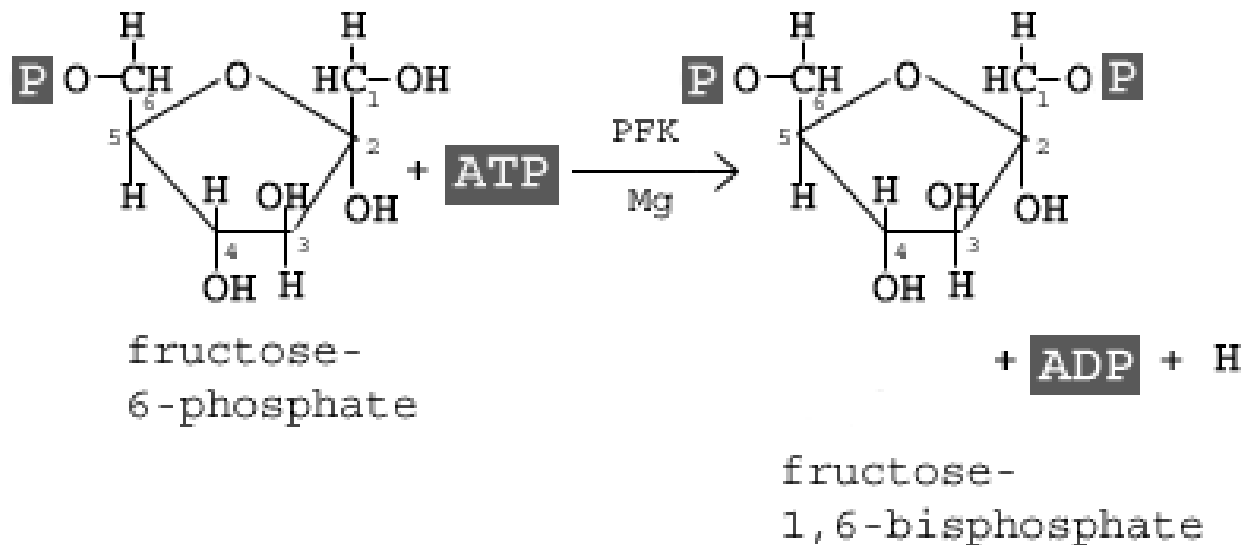
Oxygenases

- Oxygenases catalyze substrate oxidation by molecular O_2
- The reduced product of the reaction in this case is water and not hydrogen peroxide
- There are two types of oxygenases:
- Monooxygenases; transfer one oxygen atom to the substrate, and reduce the other oxygen atom to water
- Dioxygenases, incorporate both atoms of molecular oxygen (O_2) into the product(s) of the reaction



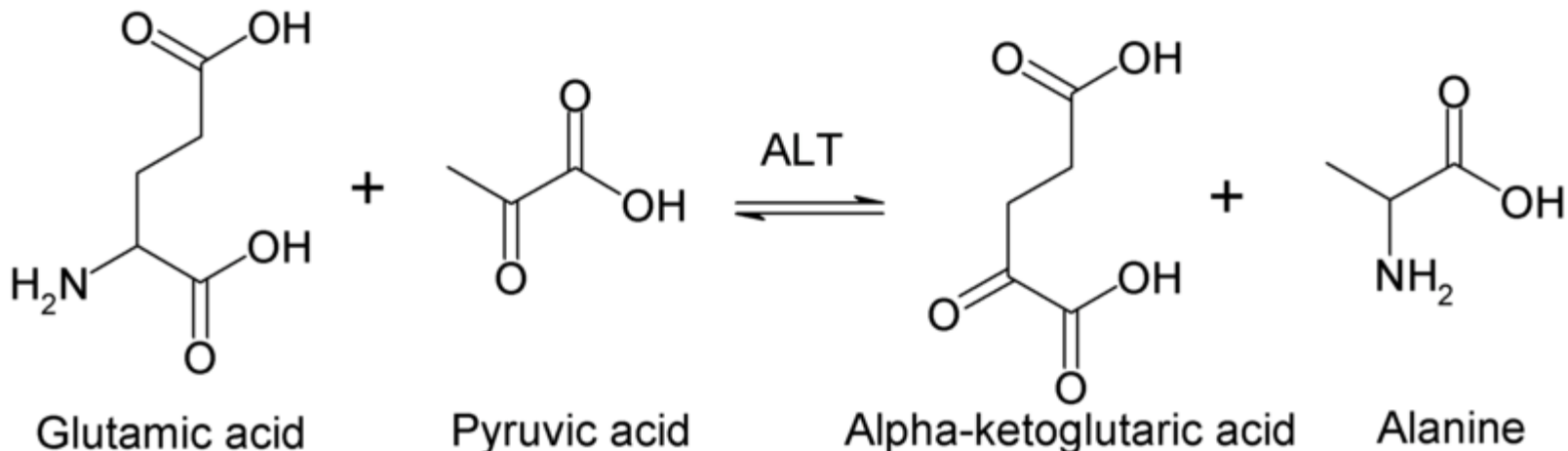
Transferases

- These enzymes transfer a functional group (C, N, P or S) from one substrate to an acceptor molecule
- Phosphofructokinase; catalyzes transfer of phosphate from ATP to fructose-6-phosphate:



Aminotransferases (Transaminases)

- A transaminase transfers an amino functional group from one amino acid to a keto acid, converting the amino acid to a keto acid and the keto acid to an amino acid
- This allows for the interconversion of certain amino acids

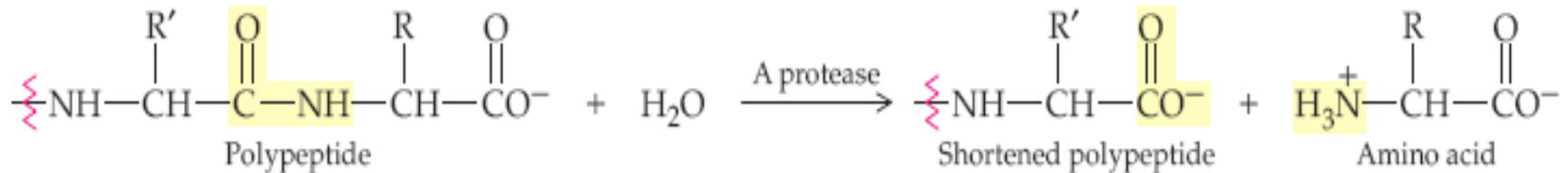


Hydrolases

- These enzymes catalyze cleavage reactions while using water across the bond being broken
- Peptidases, esterases, lipases, glycosidases, phosphatases are all examples of hydrolases named depending on the type of bond cleaved

Proteases

- These enzymes catalyze proteolysis, the hydrolysis of a peptide bond within proteins
- Proteolytic enzymes differ in their degree of substrate specificity

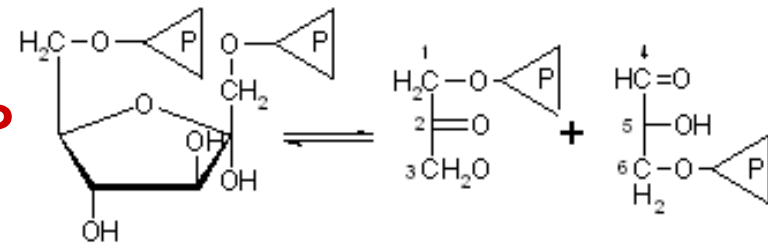


- Trypsin, is quite specific; catalyzes the splitting of peptide bonds only on the carboxyl side of lysine and arginine
- Thrombin, catalyzes the hydrolysis of Arg-Gly bonds in particular peptide sequences only

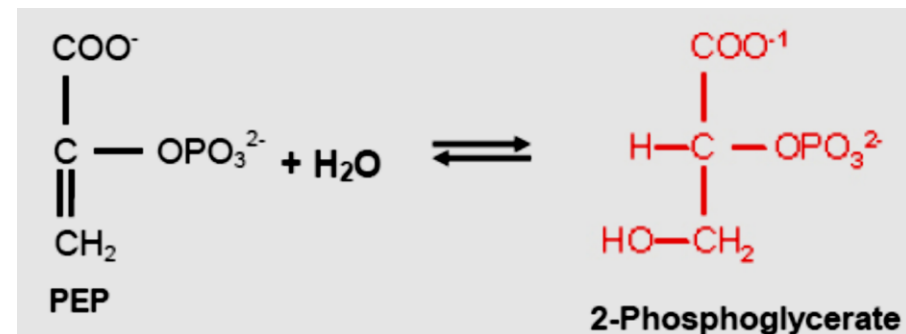
Lyases

- Catalyze the addition or removal of functional groups from their substrates with the associated formation or removal of double bonds between C-C, C-O and C-N
- Aldolase; breaks down fructose-1,6-bisphosphate into dihydroxyacetone phosphate and glyceraldehydes-3-phosphate

➤ **F 1,6 bisphosphate $\rightleftharpoons$ DHAP + GAP**

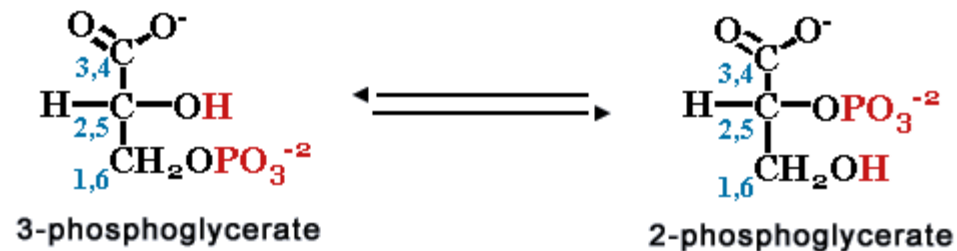
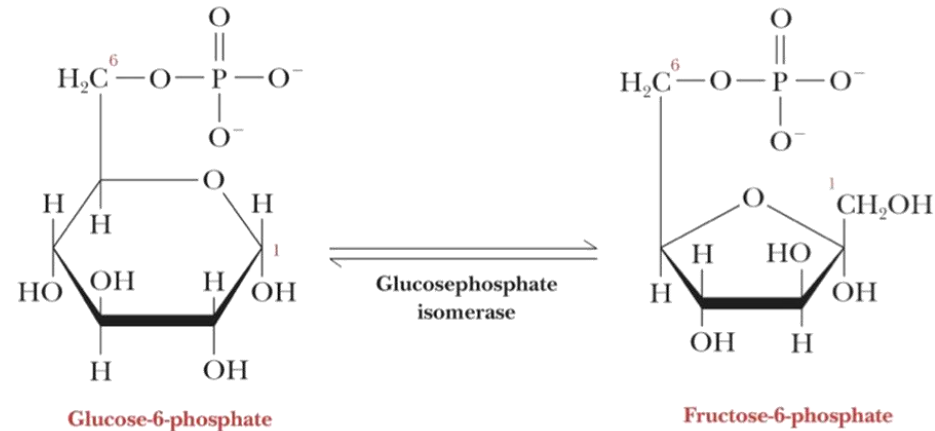


- Enolase; interconverts phosphoenolpyruvate and 2-phosphoglycerate by formation and removal of double bonds



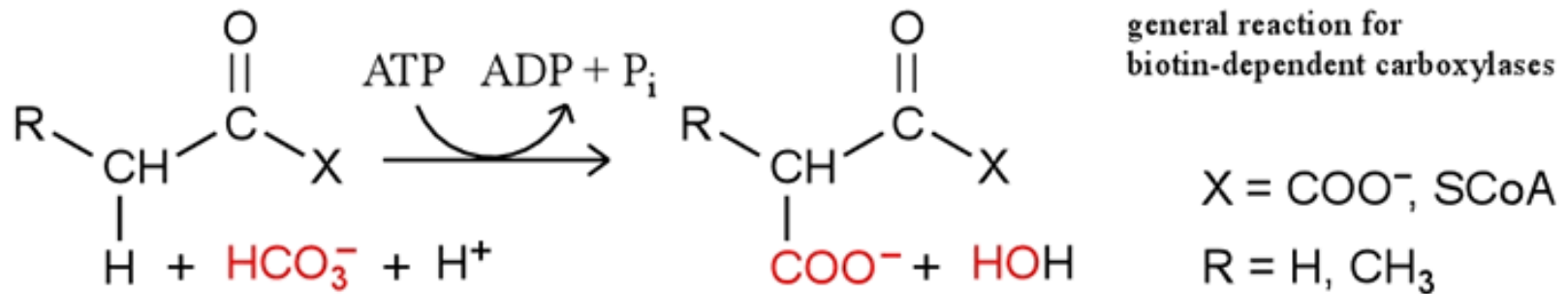
Isomerases

- Catalyze intramolecular rearrangements
- Glucose-6-phosphate isomerase; isomerizes glucose-6-phosphate to fructose-6-phosphate
- Phosphoglycerate mutase; transfers a phosphate group from carbon number 3 to carbon number 2 of phosphorylated glycerate (BPG intermediate)
- 3-P glycerate $\rightleftharpoons$ 2 P glycerate



Ligases

- Ligases join C-C, C-O, C-N, C-S and C-halogen bonds
- The reaction is usually accompanied by the consumption of a high energy compound such as ATP
- Pyruvate carboxylase
 - $\text{Pyruvate} + \text{HCO}_3^- + \text{ATP} \rightleftharpoons \text{Oxaloacetate} + \text{ADP} + \text{P}_i$



CATALYSIS

FUNCTIONAL GROUPS IN CATALYSIS

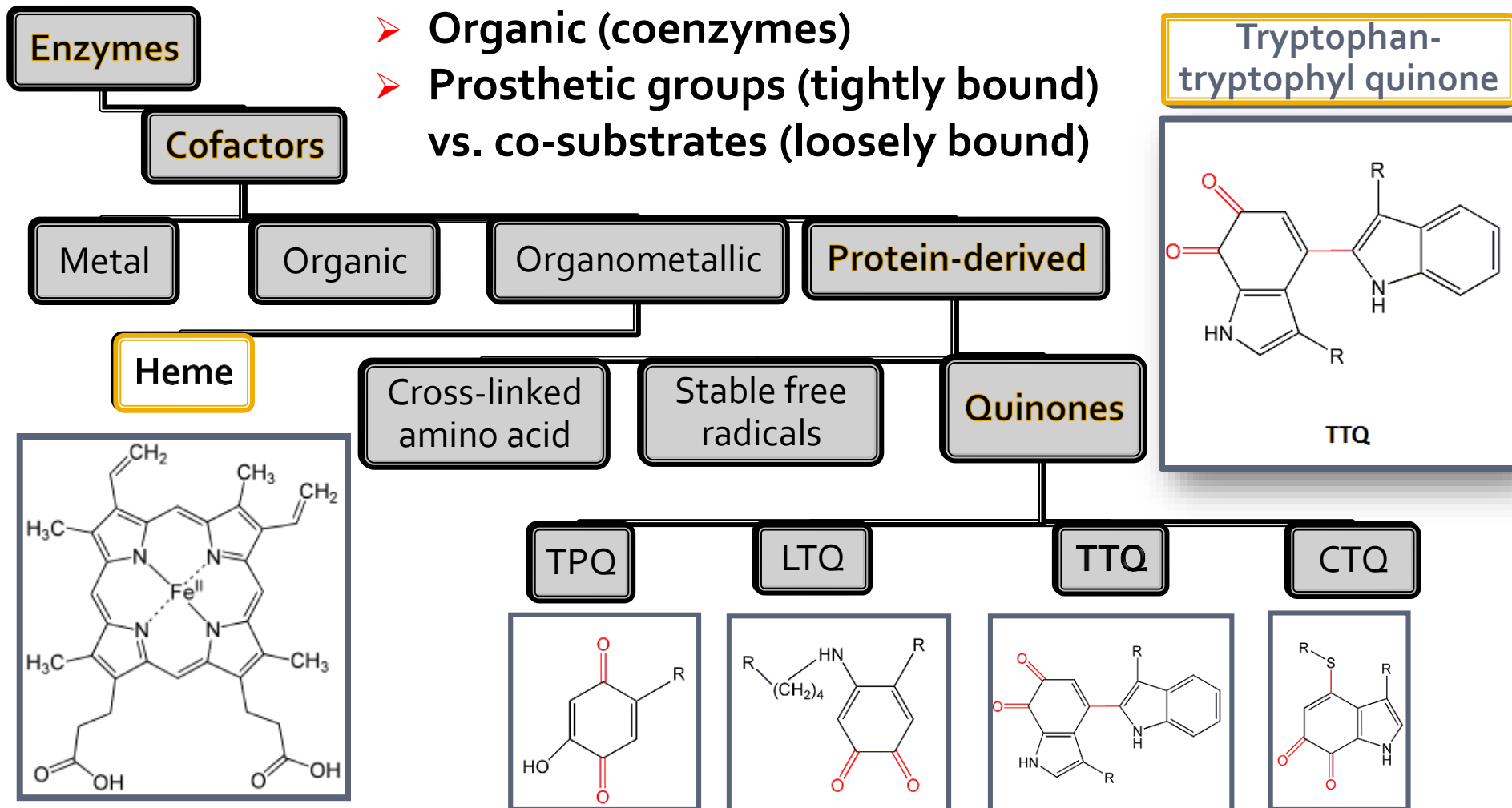
- Not all enzymes rely on their active site for catalysis (chymotrypsin vs. conjugated enzymes)
- Conjugated: coenzymes, metal ions, & metallocoenzymes

- A. Functional Groups on Amino Acid Side Chains:
 - Almost all polar amino acids (nucleophilic catalysis)
 - Ser, Cys, Lys, & His can participate in covalent catalysis
 - Histidine: pKa, physiological pH & acid–base catalysis
- B. Coenzymes in Catalysis
 - Usually (but not always) synthesized from vitamins
 - Each coenzyme is specific for a type of reaction
 - They are either:
 - * Activation-transfer coenzymes
 - * Oxidation–reduction coenzymes

Enzymes-cofactors

Enzyme cofactors

- Apoenzyme vs. holoenzyme
- Organic (coenzymes)
- Prosthetic groups (tightly bound) vs. co-substrates (loosely bound)



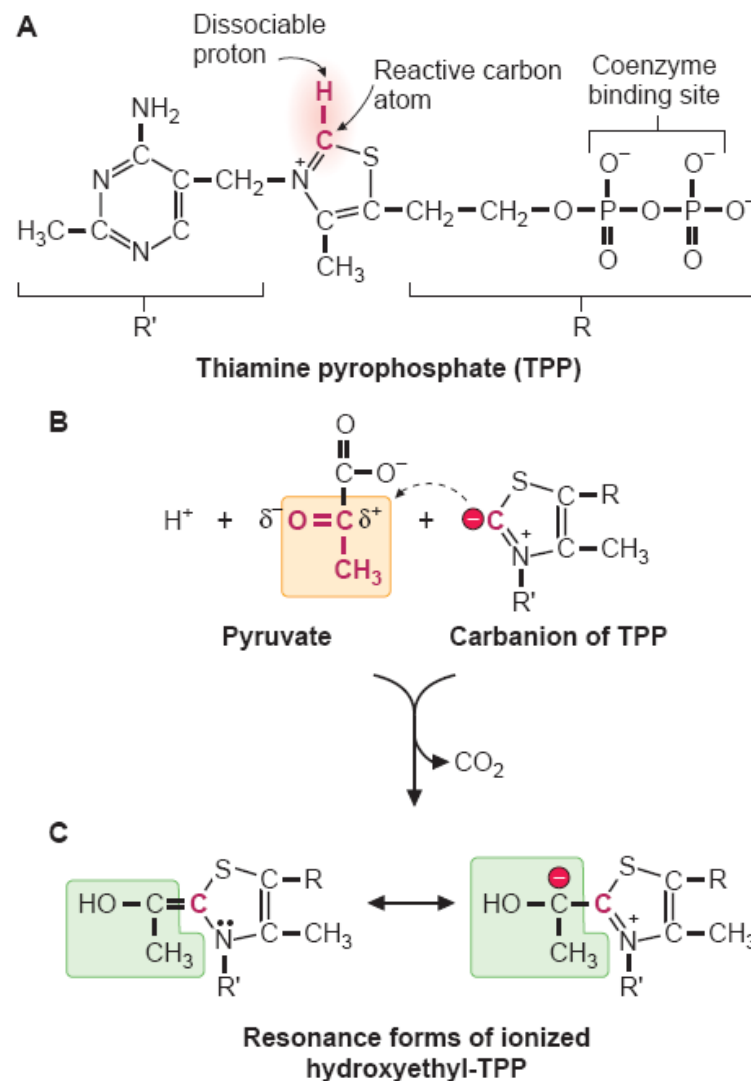
ACTIVATION-TRANSFER COENZYMES

- Usually participate directly in catalysis by forming a **covalent bond**
- Characteristics:
 - Two groups in the coenzyme:
 - Forms a covalent bond (functional group)
 - Binds tightly to the enzyme (binding group)
 - Dependence on the enzyme for additional specificity of substrate & additional catalytic power

ACTIVATION-TRANSFER COENZYMES

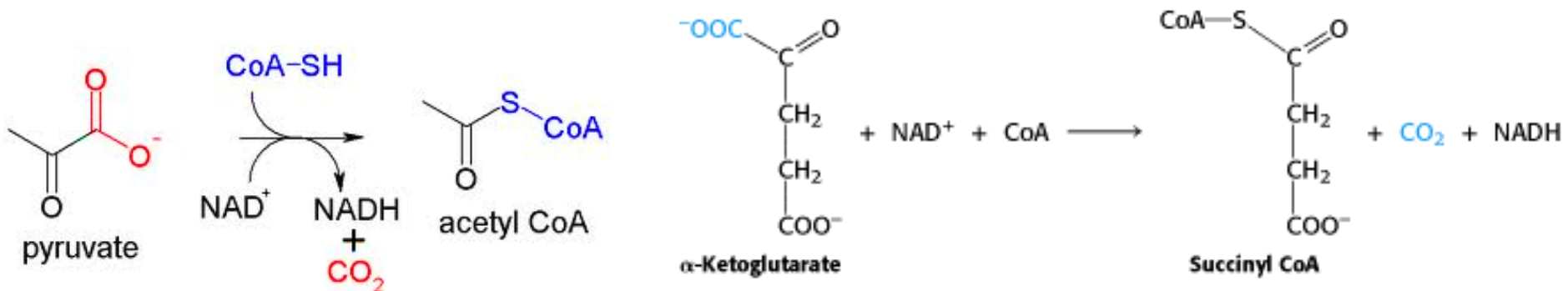
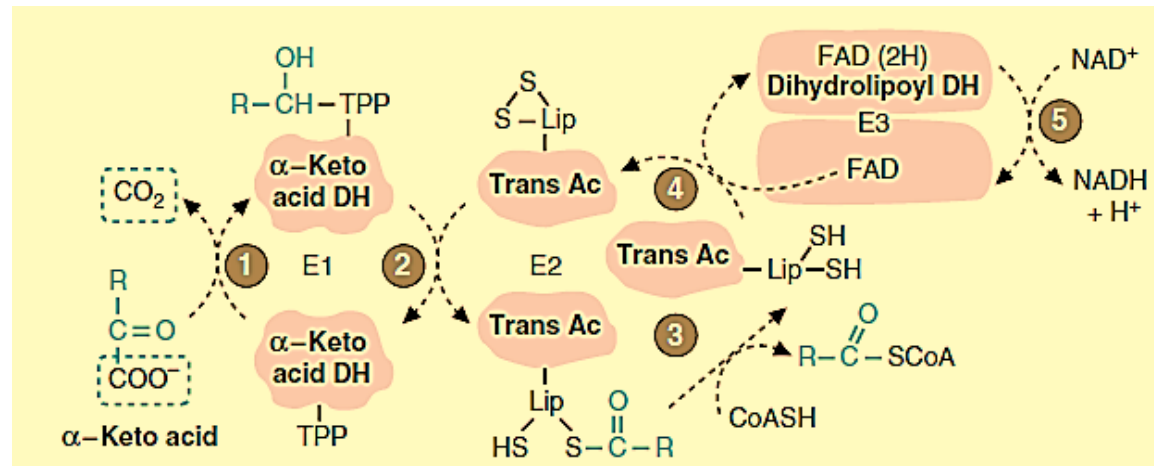
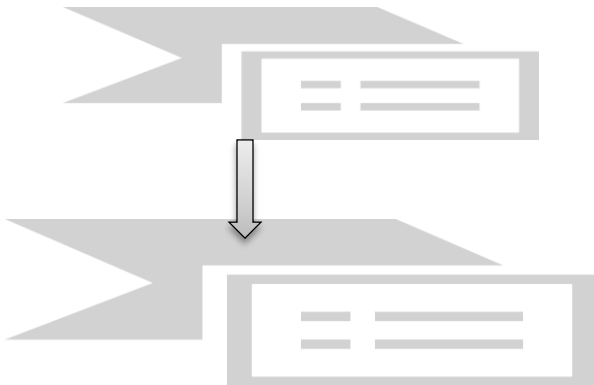
1 - TPP

- Thiamine pyrophosphate
- Source: thiamine (B₁)
- **Decarboxylation reactions**
- Pyrophosphate:
 - Provides negatively charged oxygen atoms
 - Chelate Mg²⁺ (tight binding)
- Functional group (reactive carbon atom)
- Reactive thiamine carbon forms a covalent bond with a substrate keto group while cleaving the adjacent carbon-carbon bond



Thiamin (vitamin B₁)

- Thiamin (vitamin B₁) is rapidly converted to its active form, thiamin pyrophosphate, TPP, in the brain & liver
- Required by pyruvate dehydrogenase & α-ketoglutarate dehydrogenase

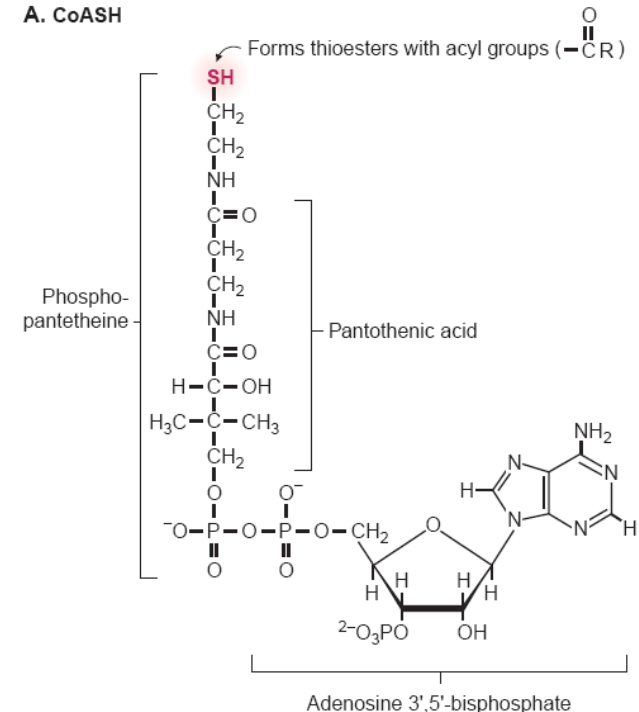


ACTIVATION-TRANSFER COENZYMES

2 - Coenzyme A (CoA)

- Source: pantothenate (B₅)
- Binding group: adenosine 3',5'-bisphosphate (tight & reversible)
- Functional group: sulfhydryl group (nucleophile)
 - Attacks carbonyl groups & forms acyl thioesters (the "A")
- How it is different from usual? (regeneration & acyl-CoA derivative)
- Like some others (NAD⁺), why do they call them coenzymes?
 - Common to so many reactions
 - The original form is regenerated by subsequent reactions
 - Synthesized from vitamins
 - The amount in the cell is nearly constant

A. CoASH



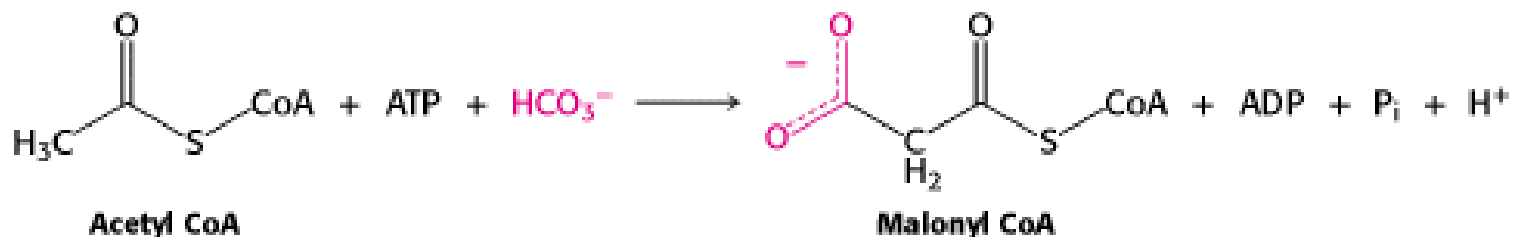
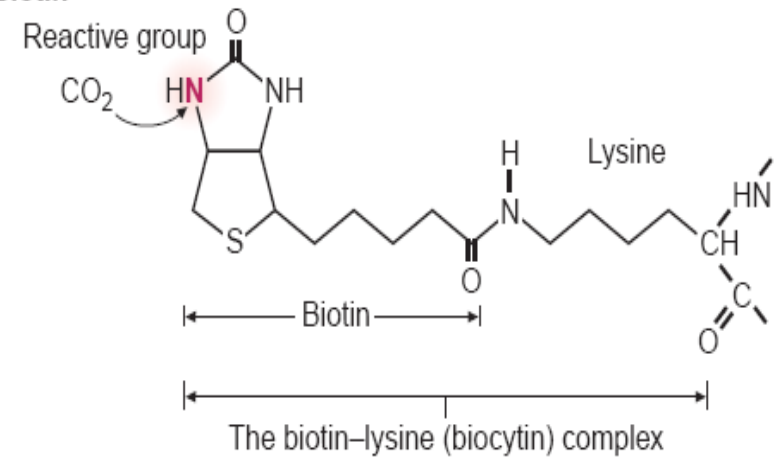
ACTIVATION-TRANSFER

COENZYMES

3 – Biotin (B7)

- Biotin is required for **carboxylation** reactions (**covalently bound to Lys**)
- **Source: food & intestinal bacteria**
- Deficiencies are generally seen
 - Long antibiotic therapies
 - **Excessive consumption of raw eggs** (egg white protein, avidin, high affinity for biotin)
- Pyruvate carboxylase
- Acetyl CoA carboxylase (fatty acid synthesis)

B. Biotin

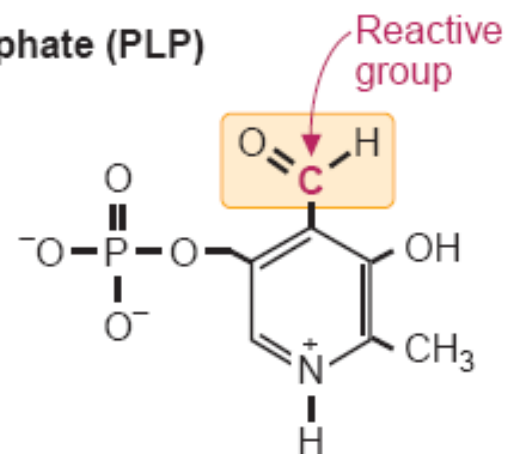


ACTIVATION-TRANSFER COENZYMES

4 - PLP

- Synthesis: Pyridoxine (B6)
- Functions in the metabolism of amino acids (**transaminases**)
- Reversible reactions

C. Pyridoxal phosphate (PLP)



- Mechanism:
 - Reactive aldehyde forms a covalent bond with the amino groups
 - Ring nitrogen withdraws electrons from bound amino acid (cleavage of bond)



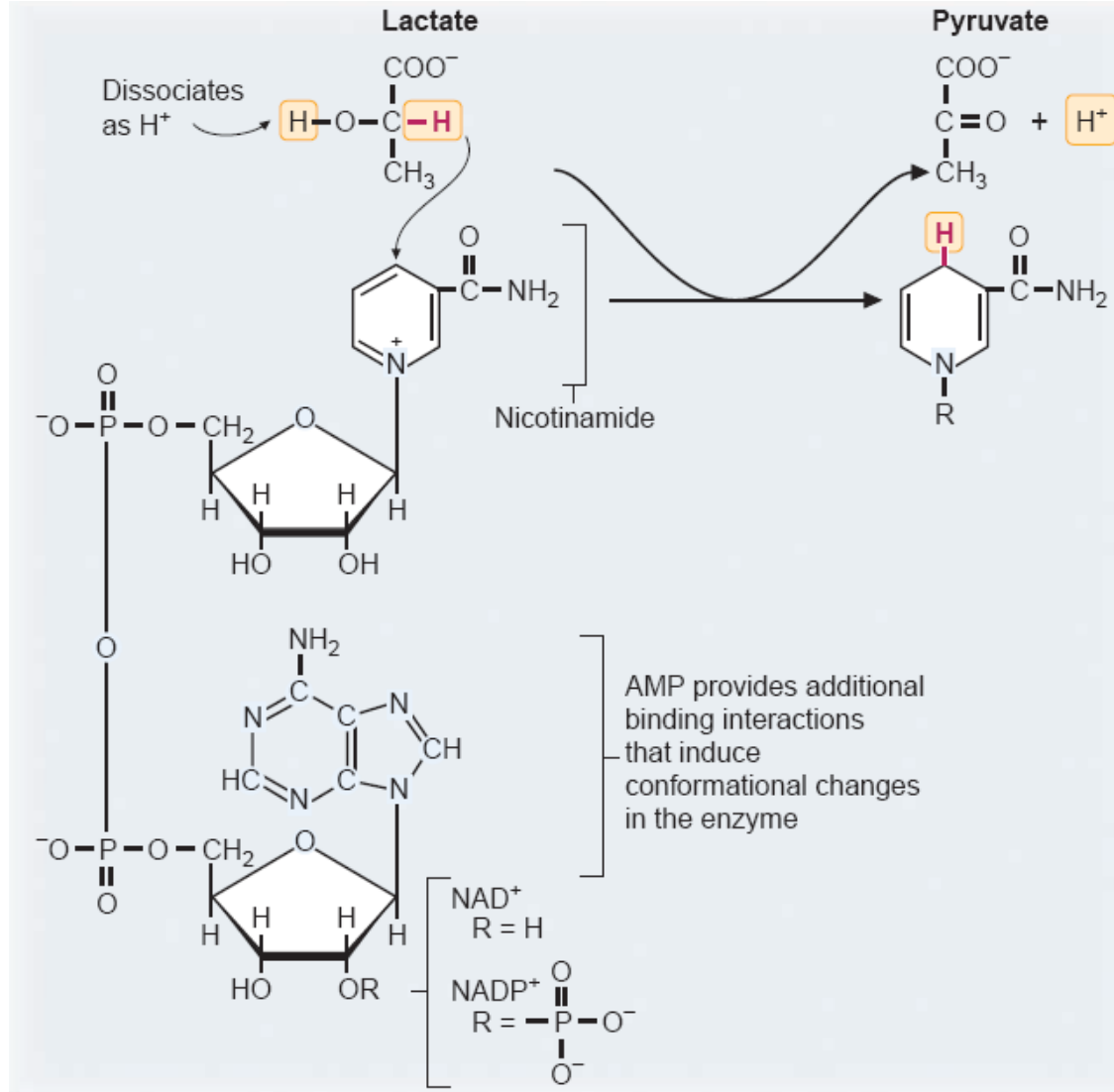
OXIDATION–REDUCTION COENZYMES

- A large number of coenzymes
- Do not form covalent bonds with the substrate
- Most common: **NAD⁺ (niacin, B₃) & FAD (riboflavin, B₂)**
- Others: work with metals to transfer single electrons to O₂ (Vitamins E & C)
- Again: Dependence on the enzyme for additional specificity of substrate & additional catalytic power

OXIDATION-REDUCTION COENZYMES

1 – NAD⁺

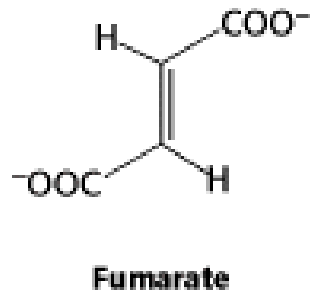
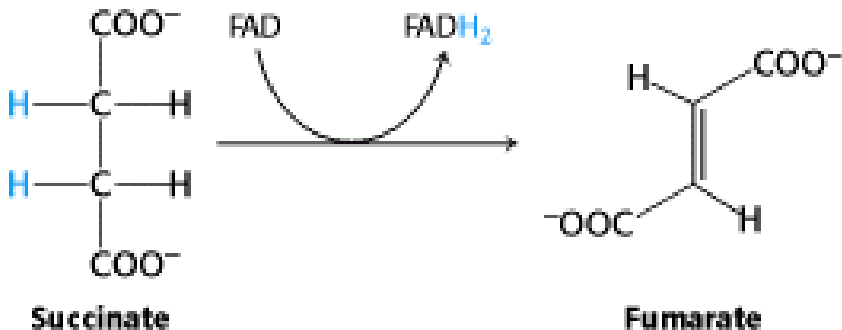
- Functional group (C opposite to N)
- Accepts a hydride ion
- The H⁺ from substrate dissociates, & a keto group (CO) is formed
- (ADP) portion of the molecule binds tightly
- The role of enzymes' Histidine



OXIDATION-REDUCTION COENZYMES

2 – FAD & FMN

- Source: Riboflavin (B₂)
- FMNH₂ and FADH₂
- Flavoproteins
- FAD and FMN are prosthetic groups (tightly bound)
- Succinate dehydrogenase
- Pyruvate dehydrogenase complex



Water-Soluble Vitamins

Name	Coenzyme or Active Form	Primary biochemical function
Thiamin	Thiamine pyrophosphate (TPP)	Aldehyde-group transfer
Riboflavin	Flavin mononucleotide (FMN) Flavin adenine dinucleotide (FAD)	Hydrogen-Atom (electron) transfer Hydrogen-Atom (electron) transfer
Nicotinic Acid	Nicotinamide adenine dinucleotide (NAD) Nicotinamide adenine dinucleotide phosphate (NADP)	Hydrogen-Atom (electron) transfer Hydrogen-Atom (electron) transfer
Pantothenic Acid	Coenzyme A (CoA)	Acyl-group transfer
Pyridoxine	Pyridoxal Phosphate	Amino-group transfer
Biotin	Biocytin	Carboxyl transfer
Folate	Tetrahydrofolate	One-Carbon group transfer
Vitamin B ₁₂	Coenzyme B ₁₂	1,2 shift hydrogen atoms
Lipoic Acid	Lipoyllysine	Hydrogen-Atom and Acyl-group transfer
Ascorbic Acid	Ascorbic acid, dehydroascorbic acid	Cofactor in hydroxylation

Catalytic Metals

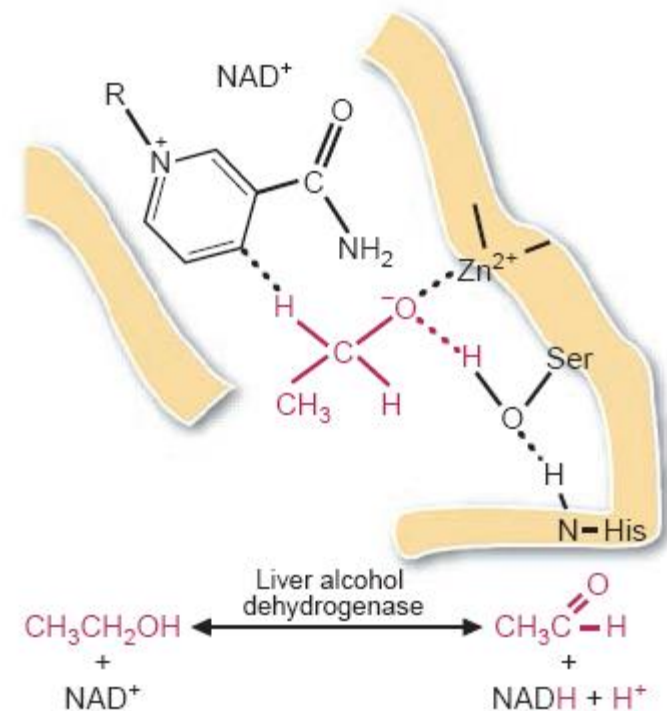
- Metals can be **tightly bound (metalloenzymes) or loosely bound (metal-activated enzymes)**
- Acting as electrophiles
- Metal-activated enzymes; the metal either required or enhances activity (Mg^{2+} , Mn^{2+} , Ca^{2+} , & K^{+})
- Phosphofructokinase & TPP; (Mg^{2+}) is required to coordinate the phosphate groups on the ATP for a successful reaction (chelation)

Metal	Enzyme
Zn^{2+}	Carbonic anhydrase
Zn^{2+}	Carboxypeptidase
Mg^{2+}	Hexokinase
Se	Glutathione peroxidase
Mn^{2+}	Superoxide dismutase

Fructose-6-phosphate + ATP → fructose-1,6-bisphosphate + ADP

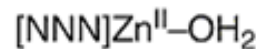
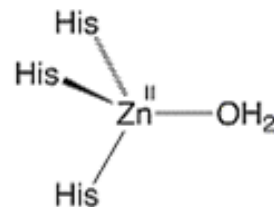
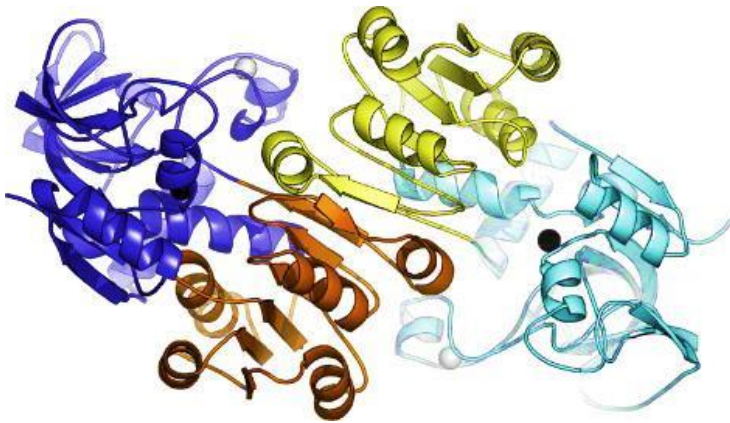
Catalytic Metals

- Alcohol dehydrogenase (ADH)
- Activated serine (pulls a proton off –OH)
- Oxyanion is stabilized by zinc
- Transfer of a hydride ion to NAD^+
- Zinc in ADH as His in lactate dehydrogenase



Metalloenzymes

- Metal ions are usually incorporated during synthesis & removal of the metal causes denaturation
- These metal ions may contribute either to the structure or the catalytic mechanism
- Liver alcohol dehydrogenase (dimer); 2 Zn^{+2} in each monomer; one for structural maintenance (joins the two subunits), the other is catalytic
- Carbonic anhydrase; A zinc atom is essentially always bound to four or more groups



Carbonic Anhydrase



Kinetics of enzymatic reactions

Kinetics

- Biochemical Kinetics: the science that studies rates of chemical reactions
- An example is the reaction ($A \rightarrow P$), The velocity, v , or rate, of the reaction $A \rightarrow P$ is the amount of P formed or the amount of A consumed per unit time, t . That is,

$$v = \frac{d[P]}{dt} \quad \text{or} \quad v = \frac{-d[A]}{dt}$$

Reaction Rate Law

- The rate is a term of change over time
- The rate will be proportional to the conc. of the reactants
- It is the mathematical relationship between reaction rate and concentration of reactant(s)
- For the reaction ($A + B \rightarrow P$), the rate law is

$$\text{Rate} = \frac{-\Delta[A]}{\Delta t} = \frac{-\Delta[B]}{\Delta t} = \frac{\Delta[P]}{\Delta t} \quad v = \frac{-d[A]}{dt} = k[A]$$

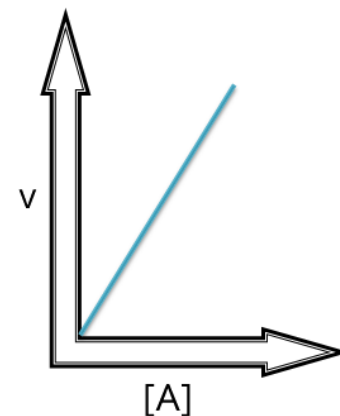
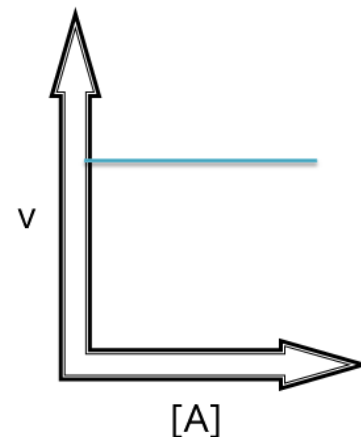
- From this expression, the rate is proportional to the concentration of A, and k is the rate constant

The order of the reaction & the rate constant (k)

- A multistep reaction can go no faster than the slowest step

$$v = k(A)^{n_1}(B)^{n_2}(C)^{n_3}$$

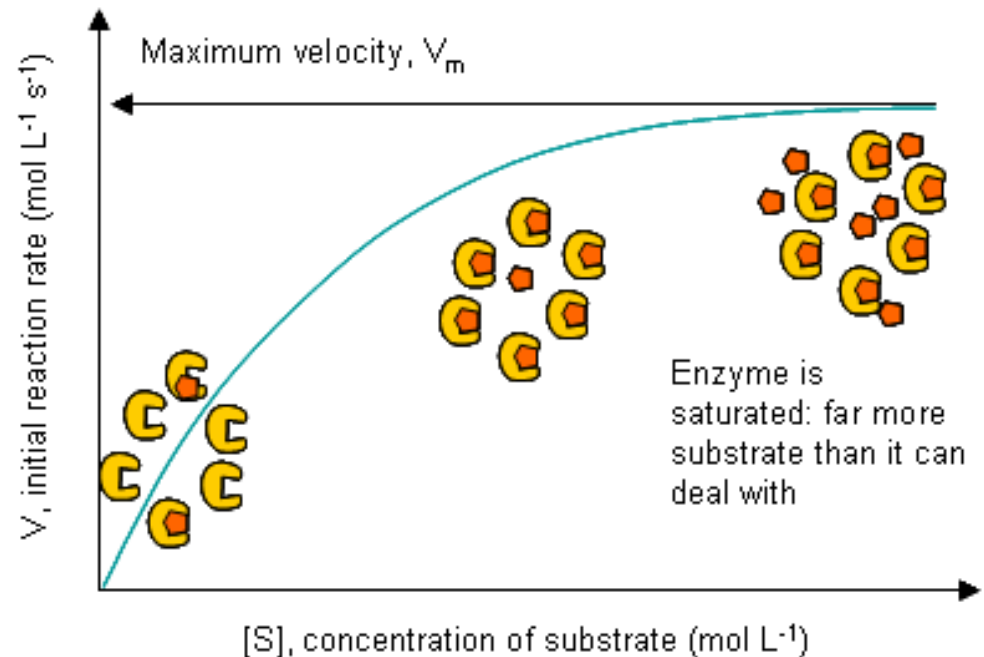
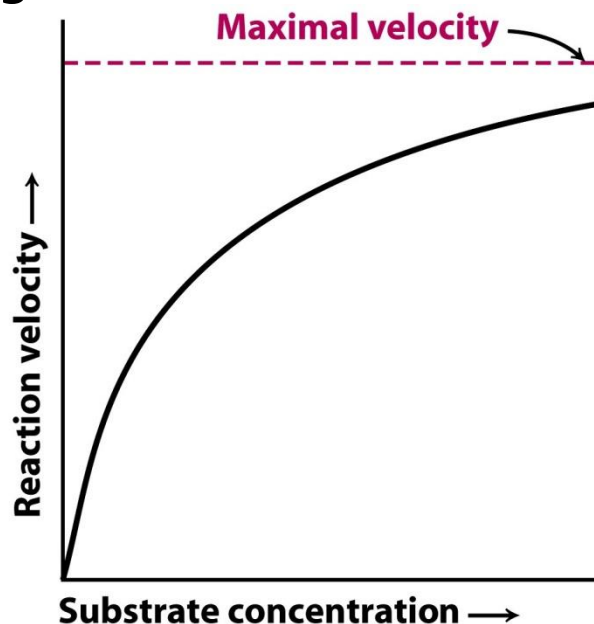
- k is the rate constant: the higher the activation energy (energy barrier), the smaller the value of k
- $(n_1+n_2+n_3)$ is the overall order of the reaction
- Dimensions of k



Overall order	$V=$	Dimensions of k
Zero	k	$(\text{conc.})(\text{time})^{-1}$
First	$k(A)$	$(\text{time})^{-1}$

Enzyme kinetics

- Enzymatic reactions may either have a simple behavior or complex (allosteric) behavior
- Simple behavior of enzymes: as the concentration of the substrate rises, the velocity rises until it reaches a limit
- Thus; enzyme-catalyzed reactions have hyperbolic (saturation) plots

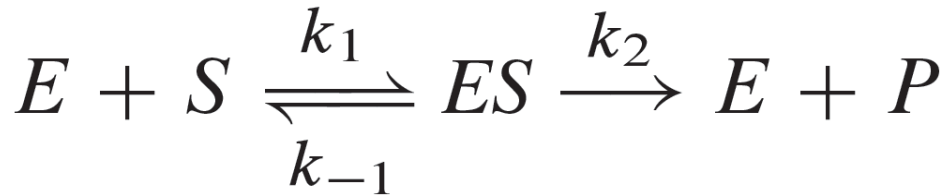
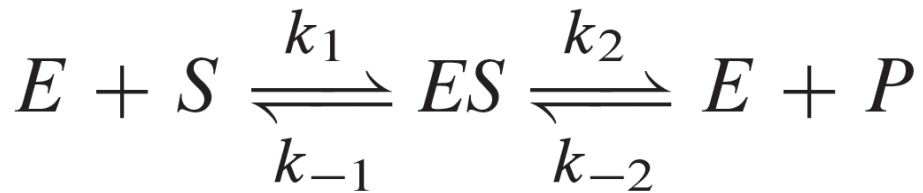


Enzyme kinetics

- The maximal rate, $V_{\max}$, is achieved when the catalytic sites on the enzyme are saturated with substrate
- $V_{\max}$ reveals the turnover number of an enzyme
 - The number of substrate molecules converted into product by an enzyme molecule in a unit of time when the enzyme is fully saturated with substrate
- At $V_{\max}$, the reaction is in zero-order rate since the substrate has no influence on the rate of the reaction

Expression of enzyme kinetic reactions

"Steady State Assumption"



$$v = k_2 ES$$

$$\frac{dES}{dt} = k_1 E \cdot S - k_{-1} ES - k_2 ES$$

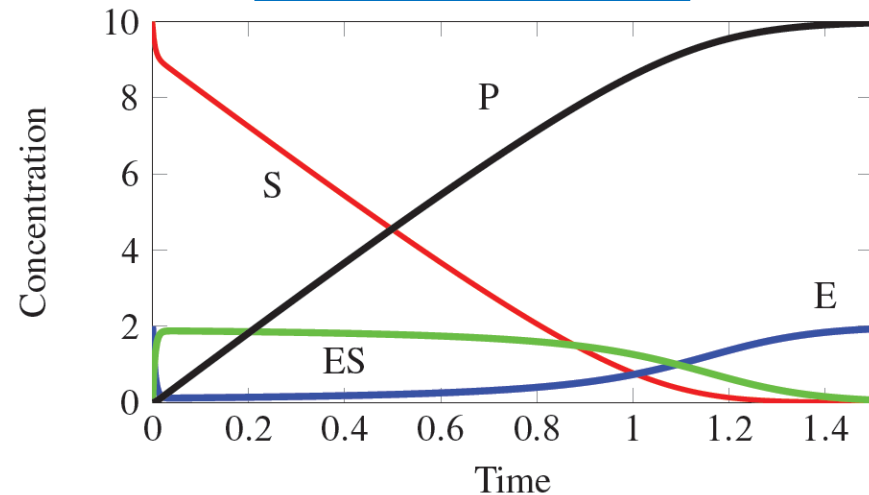
$$0 = k_1 E \cdot S - k_{-1} ES - k_2 ES$$

$$E_t = E + ES$$

$$ES = \frac{E_t \cdot S}{(k_{-1} + k_2)/k_1 + S}$$

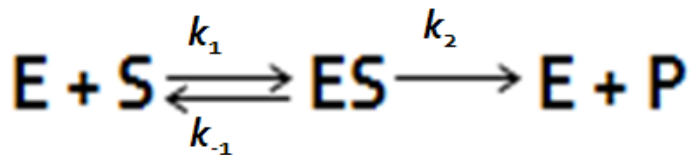
$$v = \frac{E_t k_2 S}{(k_{-1} + k_2)/k_1 + S}$$

$$v = \frac{V_{max} S}{K_m + S}$$



The Michaelis constant (K_m)

- For a reaction:



STEADY STATE APPROXIMATION

$$\frac{d[ES]}{dt} = k_1 [E] [S] - k_{-1} [ES] - k_2 [ES] = 0 \text{ (approx.)}$$

$$\frac{[E] [S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_M \quad \text{Equation 1}$$

- K_m , called the Michaelis constant is

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

- In other words, K_m is related to the rate of dissociation of substrate from the enzyme to the enzyme-substrate complex
- K_m describes the affinity of enzyme for the substrate

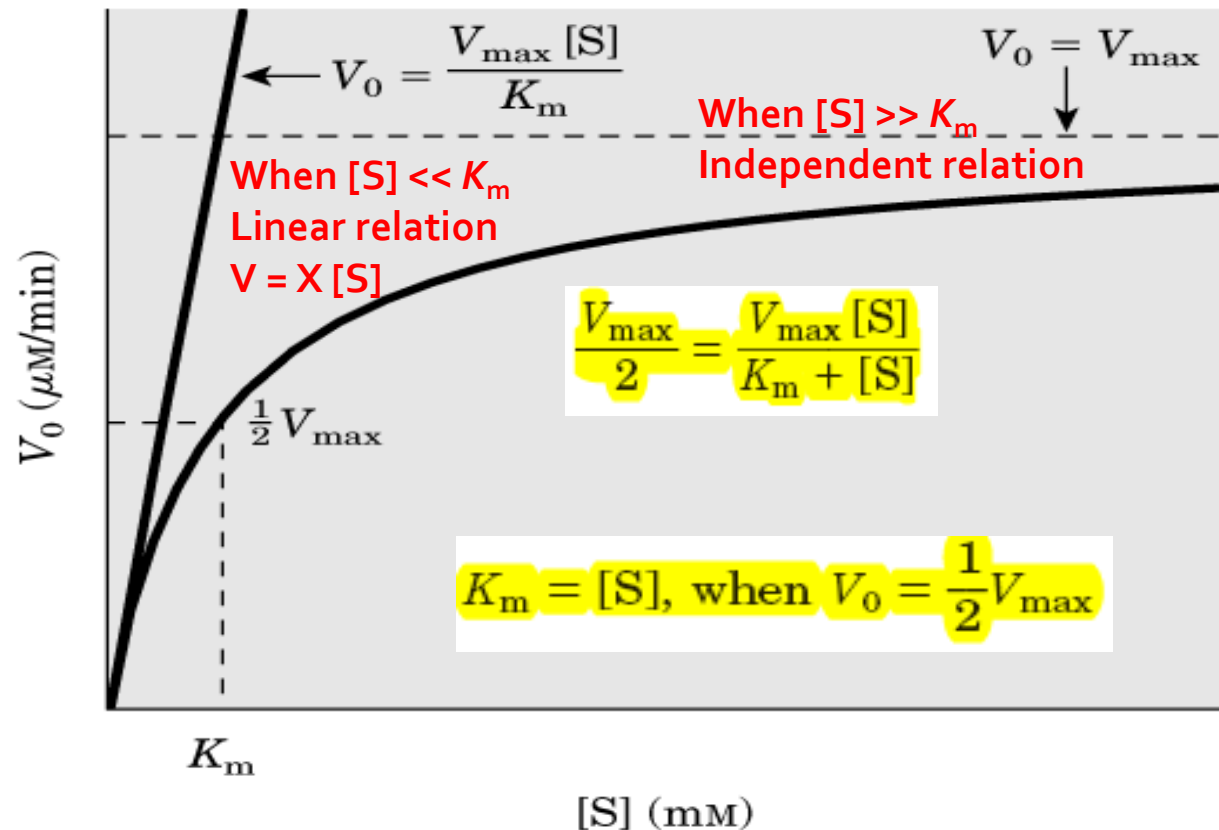
Expression of enzyme kinetic reactions

Michaelis-Menten equation

- A quantitative description of the relationship between the rate of an enzyme catalyzed reaction (V_o) & substrate concentration $[S]$
 - ✓ The rate constant (K_m) and maximal velocity (V_{max})

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

The substrate concentration at which V_o is half maximal is K_m



The Michaelis constant (K_m)

$$V_0 = V_{\max} \frac{[S]}{[S] + K_M}$$

- The lower the K_m of an enzyme towards its substrate, the higher the affinity
- When more than one substrate is involved? Each will have a unique K_m & $V_{\max}$
- K_m values have a wide range. Mostly between (10^{-1} & 10^{-7} M)

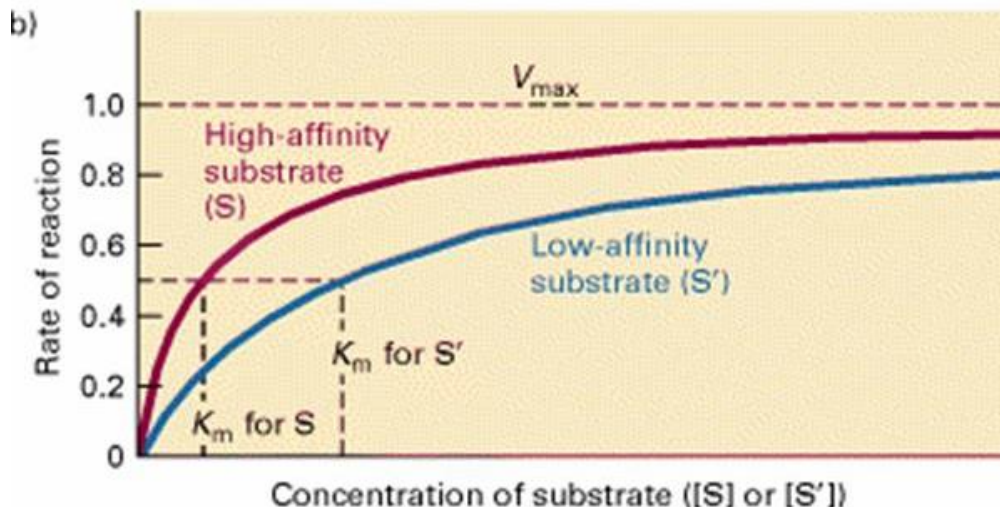


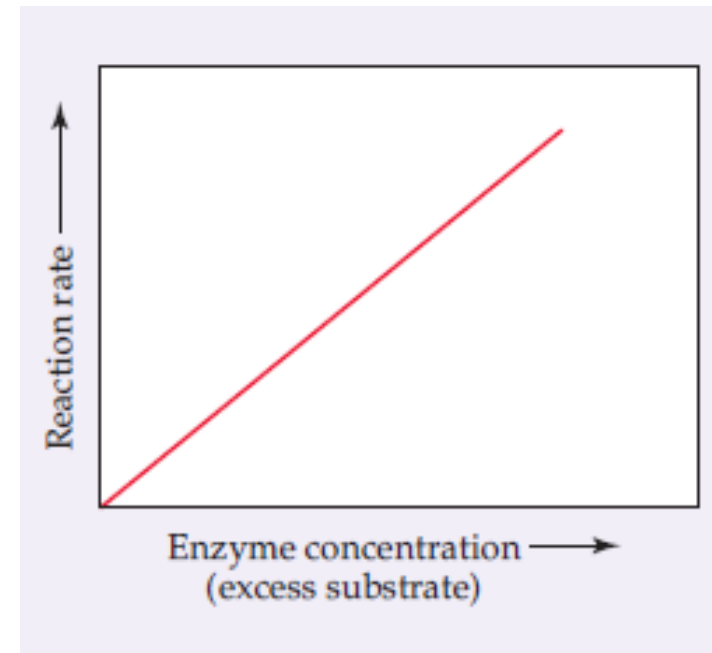
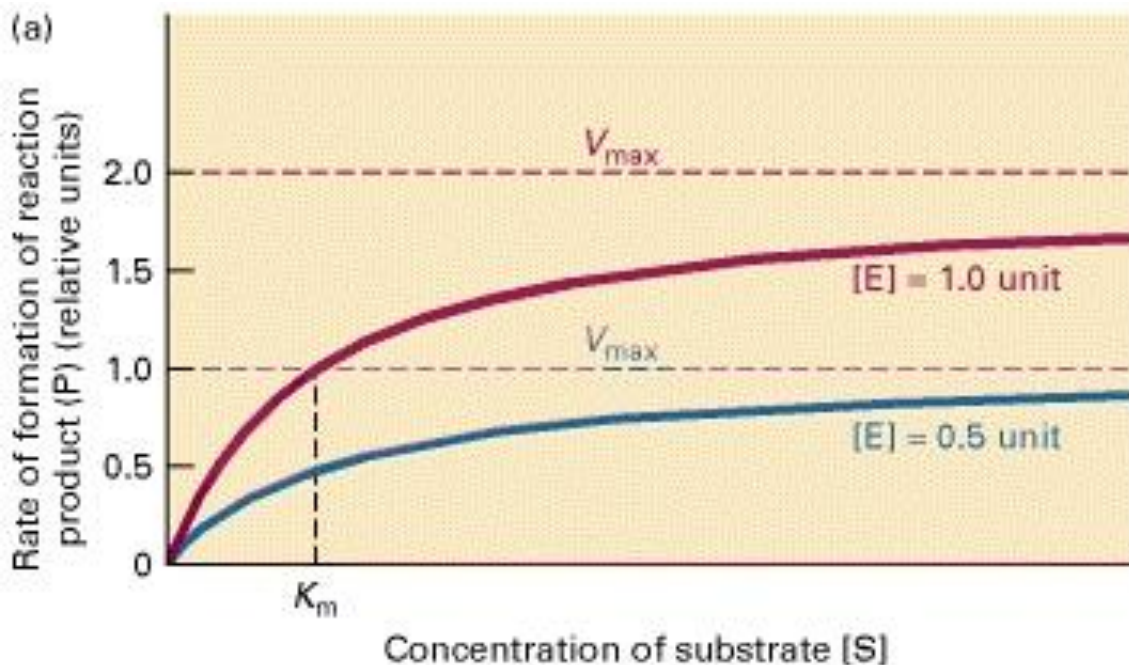
table 8-6

K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Catalase	H ₂ O ₂	25
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO ₃ ⁻	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

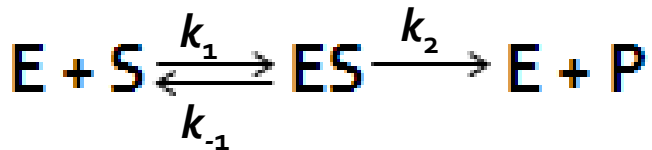
K_M & K_D [E], K_M & V_{max}

- K_D : dissociation constant, The actual measure of the affinity
 - $K_D = (k_{-1}/k_1)$
- $$E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} E + P$$
- When you increase the enzyme concentration, what will happen to V_{max} & K_m ?



$V_{\max}$ & k_{cat}

- For the enzymatic reaction



- The maximal rate, $V_{\max}$, is equal to the product of k_2 , also known as k_{cat} , and the total concentration of enzyme

$$V_{\max} = k_2 [E]_T$$

- k_{cat} , the turnover number, is the concentration (or moles) of substrate molecules converted into product per unit time per concentration (or moles) of enzyme, or when fully saturated

$$k_{\text{cat}} = V_{\max} / [E]_T$$

- In other words, the maximal rate, $V_{\max}$, reveals the turnover number of an enzyme if the total concentration of active sites $[E]_T$ is known

Turnover Numbers (k_{cat}) of Some Enzymes

Enzyme	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β -Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

Example

- a 10^{-6} M solution of carbonic anhydrase catalyzes the formation of 0.6 M H_2CO_3 per second when it is fully saturated with substrate
 - ✓ Hence, k_{cat} is $6 \times 10^5 \text{ s}^{-1}$
 - ✓ $3.6 \times 10^7 \text{ min}^{-1}$
- Each catalyzed reaction takes place in a time equal to $1/k_2$, which is $1.7 \mu\text{s}$ for carbonic anhydrase
- The turnover numbers of most enzymes with their physiological substrates fall in the range from 1 to 10^4 per second

Specificity & Efficiency

$$V = \frac{V_{\max} [S]}{K_M + [S]} = \frac{k_{\text{cat}} [E_T][S]}{K_M + [S]} \quad V = (k_{\text{cat}}/K_M) [E][S]$$

- Specificity constant (k_{cat}/K_M): determines the relative rate of the reaction at low [S]
- k_{cat}/K_M ($\text{M}^{-1} \text{min}^{-1}$) is indicative of:
 - ✓ Enzyme's substrate specificity: the higher the ratio, the higher the specificity
 - ✓ Enzyme's catalytic efficiency: the higher the ratio, the more efficient the enzyme

Table 6.2

Turnover Numbers and Km for Some Typical Enzymes

Enzyme	Function	k_{cat} = Turnover Number*	K_M **
Catalase	Conversion of H_2O_2 to H_2O and O_2	4×10^7	25
Carbonic Anhydrase	Hydration of CO_2	1×10^6	12
Acetylcholinesterase	Regenerates acetylcholine, an important substance in transmission of nerve impulses, from acetate and choline	1.4×10^4	9.5×10^{-2}
Chymotrypsin	Proteolytic enzyme	1.9×10^2	6.6×10^{-1}
Lysozyme	Degrades bacterial cell-wall polysaccharides	0.5	6×10^{-3}

k_{cat} values vary over a wide range
 K_M values also vary over a wide range
 k_{cat}/K_M , the range is only 4

Reaction rate (v); Enzyme activity; Specific activity; Turnover number

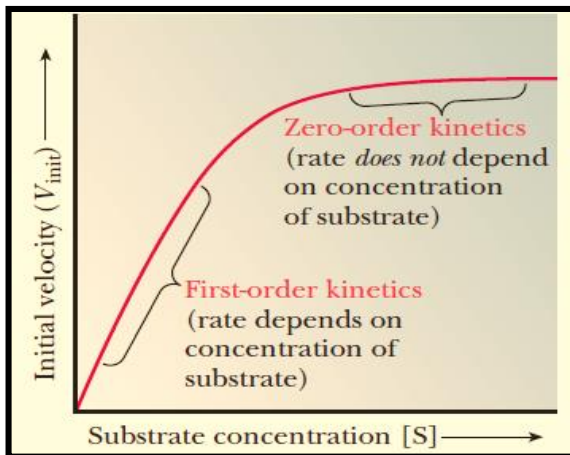
- Reaction rate; measures the concentration of substrate consumed (or product produced) per unit time ($\text{mol}/\{\text{L}\cdot\text{s}\}$ or M/s)
- Enzyme activity; measures the number of moles of substrate consumed (or product produced) per unit time (mol/s)
 - ✓ Enzyme activity = rate of reaction $\times$ reaction volume
- Specific activity; measures moles of substrate converted per unit time per unit mass of enzyme ($\text{mol}/\{\text{s}\cdot\text{g}\}$)
 - ✓ Specific activity = enzyme activity / actual mass of enzyme
 - ✓ This is useful in determining enzyme purity after purification
- Turnover number; measures moles of substrate converted per unit time per moles of enzyme (min^{-1} or s^{-1})
 - ✓ Turnover number = specific activity $\times$ molecular weight of enzyme

Sample calculations

- A solution contains initially $25 \times 10^{-4} \text{ mol L}^{-1}$ of peptide substrate and $1.5 \text{ } \mu\text{g}$ chymotrypsin in 2.5 ml volume. After 10 minutes, $18.6 \times 10^{-4} \text{ mol L}^{-1}$ of peptide substrate remain. Molar mass of chymotrypsin is $25,000 \text{ g mol}^{-1}$.
- How much is the rate of the reaction?
 - (conc./time)
- How much is the enzyme activity?
 - (mol./time)
- How much is the specific activity?
 - (enz. Act. / enz. Mass)
- How much is the turn over number?
 - (sp. Act. X enz. molar mass)

Disadvantage of Michaelis-Menten equation & Lineweaver-Burk or double-reciprocal plot

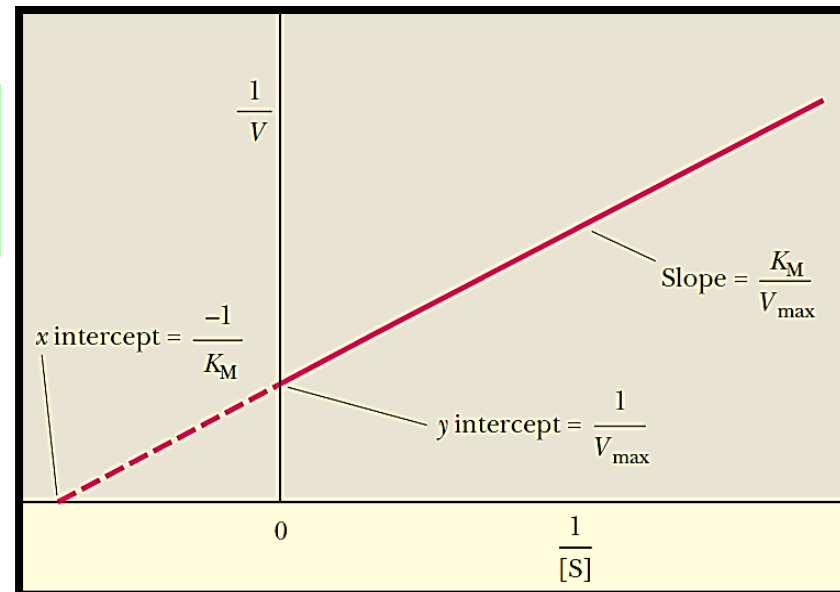
- Determining the K_m from hyperbolic plots is not accurate since a large amount of substrate is required in order to reach V_{max}
- This prevents the calculation of both V_{max} & K_m
- Lineweaver-Burk plot: A plot of $1/v_o$ versus $1/[S]$ (double-reciprocal plot), yields a straight line with an y-intercept of $1/V_{max}$ and a slope of K_M/V_{max}
- The intercept on the x-axis is $-1/K_M$



$$v = \frac{V_{max} \cdot [S]}{[S] + K_m}$$

ص = أس + ب

$$\frac{1}{v} = \left[\frac{K_m(1)}{V_{max}[S]} + \frac{1}{V_{max}} \right]$$



Example

- A biochemist obtains the following set of data for an enzyme that is known to follow Michaelis-Menten kinetics. Approximately, $V_{\max}$ of this enzyme is ... & K_m is ...?

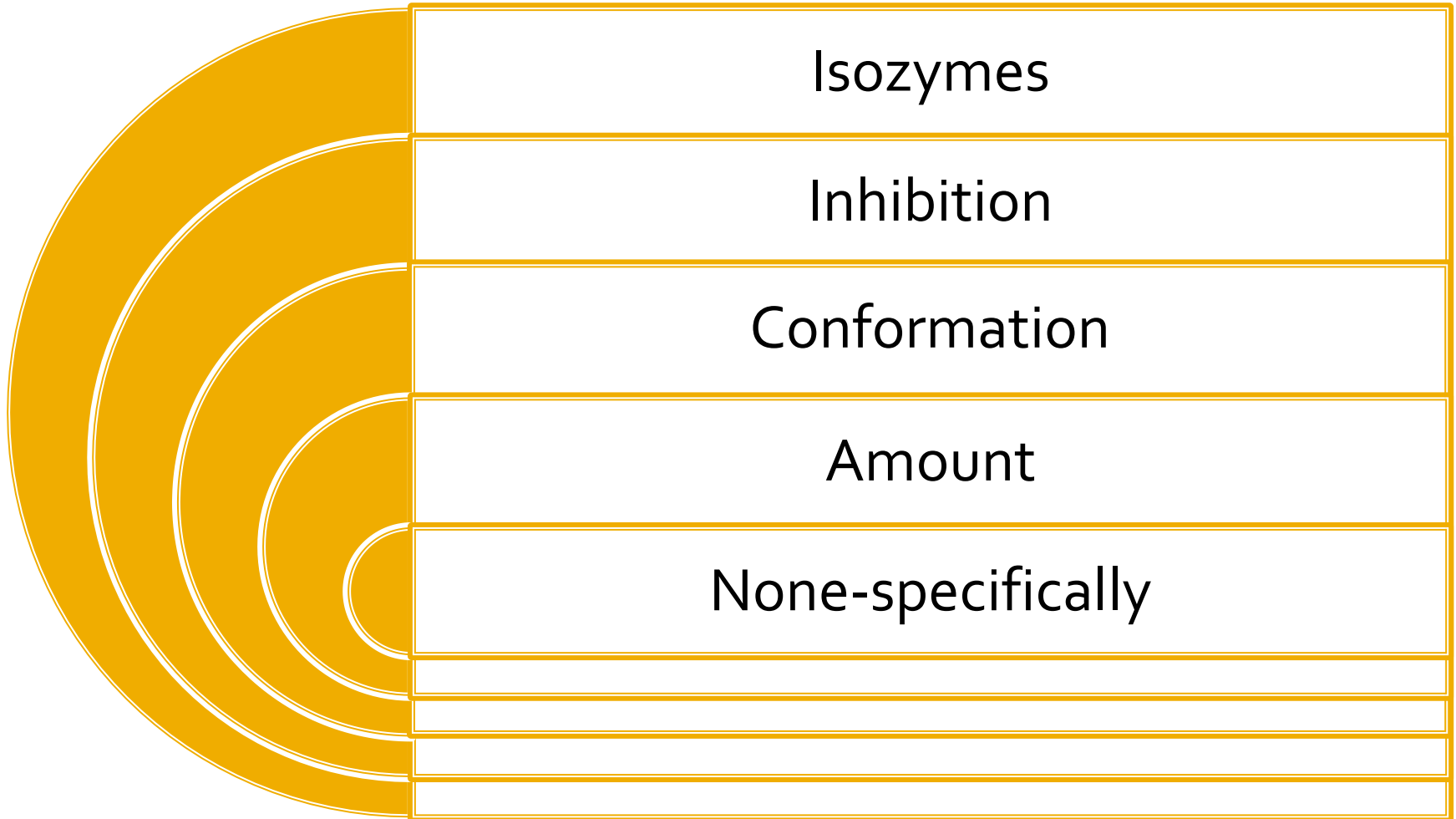
- A. 5000 & 699
- B. 699 & 5000
- C. 621 & 50
- D. 94 & 1
- E. 700 & 8

Substrate Concentration (μM)	Initial velocity ($\mu\text{mol/min}$)
1	49
2	96
8	349
50	621
100	676
1000	698
5000	699

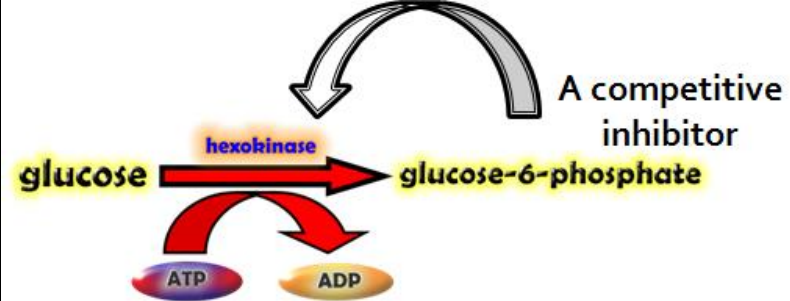
- You are working on the enzyme “Medicine” which has a molecular weight of 50,000 g/mol. You have used 10 μg of the enzyme in an experiment and the results show that the enzyme converts 9.6 μmol per min at 25°C. the turn-over number (k_{cat}) for the enzyme is:
 - A. 9.6 s^{-1}
 - B. 48 s^{-1}
 - C. 800 s^{-1}
 - D. 960 s^{-1}
 - E. 1920 s^{-1}

Enzymes Regulation

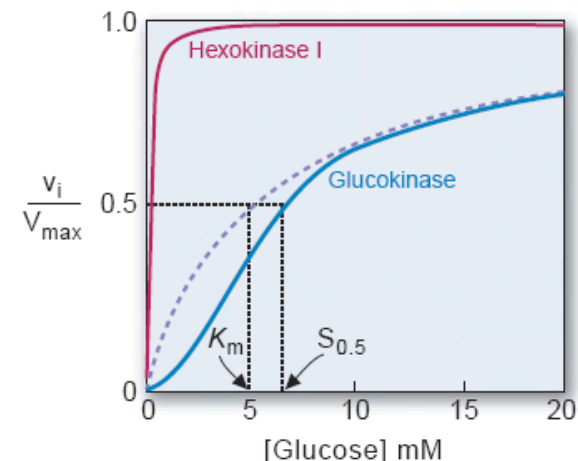
Modes of regulation



1. Isozymes (isoenzymes) The Differential K_M Value "Hexokinase"

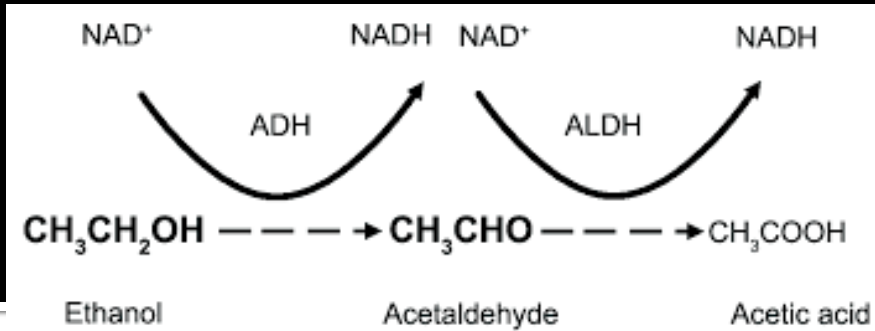


- What are isozymes? Same substrate & product, different gene, different localization, different parameters (K_m , V_{max} , k_{cat})
- Hexokinase found in RBCs & in liver
- Catalyzes the first step in glucose metabolism
- Hexokinase I (RBCs): K_M (glucose) ≈ 0.1 mM
- Hexokinase IV (glucokinase, liver, pancreas) ≈ 10 mM
- RBCs: when blood glucose falls below its normal fasting level (≈ 5 mM), RBCs could still phosphorylate glucose at rates near V_{max}
- Liver: rate of phosphorylation increases above fasting levels (after a high-carbohydrate meal)
 - High K_M of hepatic glucokinase promotes storage of glucose
- Pancreas: works as a sensor



Aldehyde dehydrogenase (ALDH)

- Oxidation of acetaldehyde to acetate.
- Four tetrameric isozymes (I-IV)
- ALDH I (low K_m ; mitochondrial) and ALDH II (higher K_m ; cytosolic)
- ~50% of Japanese & Chinese are unable to produce ALDH I (not observed in Caucasian & Negroid populations)
 - Flushing response
 - Tachycardia



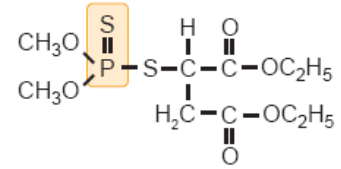
2. Inhibition

MECHANISM-BASED INHIBITORS

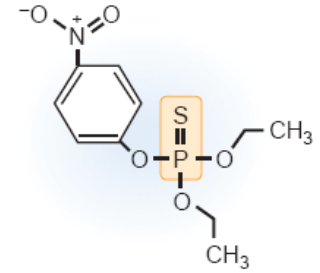
- Mechanism-based inhibitors mimic or participate in an intermediate step of the catalytic reaction
- The term includes:
 - A. Covalent inhibitors
 - B. Transition state analogs
 - C. Heavy metals
- The kinetic effect of irreversible inhibitors is to decrease the concentration of active enzyme

Covalent Inhibitors

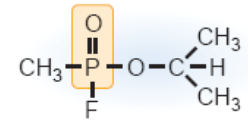
- Covalent or extremely tight bonds with active site amino acids
- Amino acids are targeted by drugs & toxins
- The lethal compound [DFP] is an **organophosphorus** compound that served as a prototype for:
 - The nerve gas sarin
 - The insecticides malathion & parathion
- DFP also inhibits other enzymes that use serine (ex. serine proteases), but the inhibition is not as lethal



Malathion

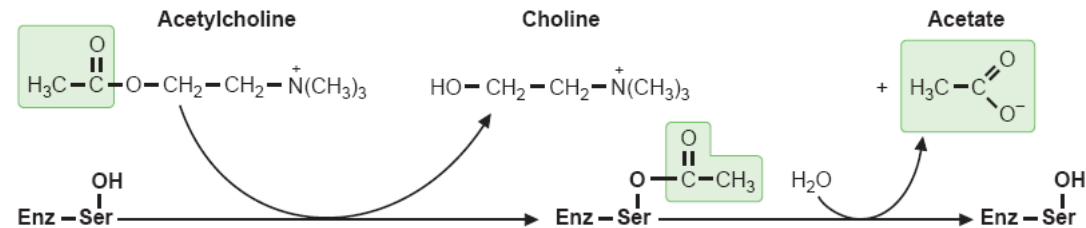


Parathion

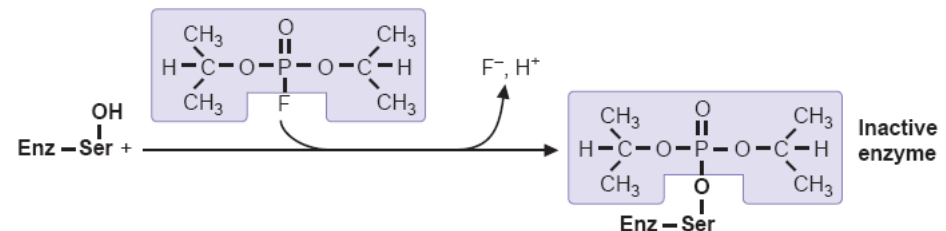


Sarin

A. Normal reaction of acetylcholinesterase

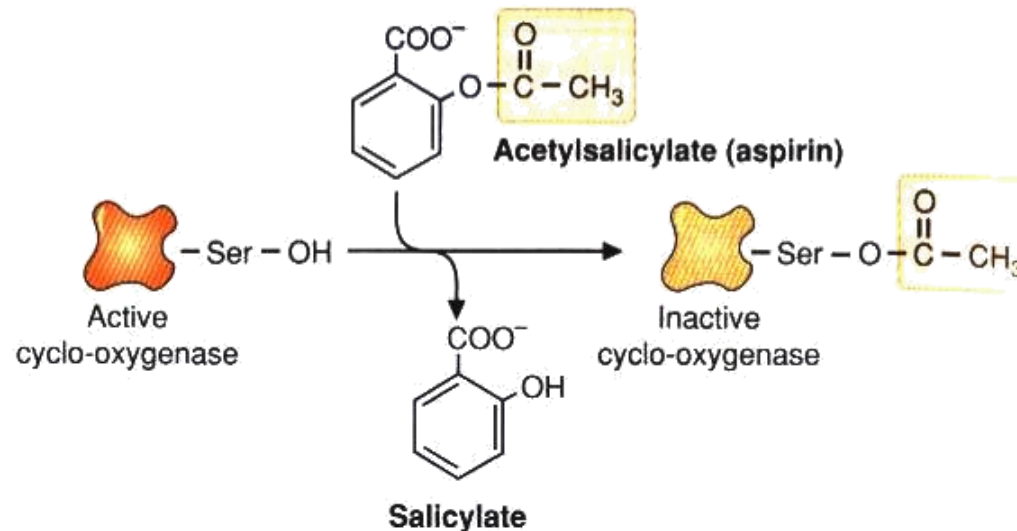


B. Reaction with organophosphorus inhibitors



Covalent Inhibitors

- Aspirin (acetylsalicylic acid): covalent acetylation of an active site serine in the enzyme prostaglandin endoperoxide synthase (cyclooxygenase)
- Aspirin resembles a portion of the prostaglandin precursor that is a physiologic substrate for the enzyme

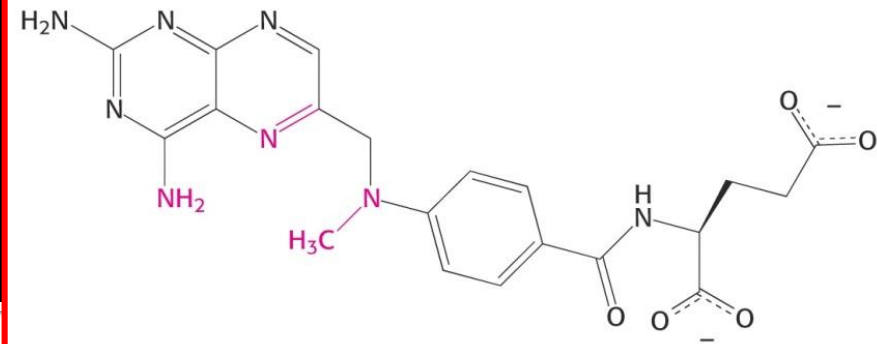


Transition-State Analogs & Compounds that Resemble Intermediate Stages of the Reaction

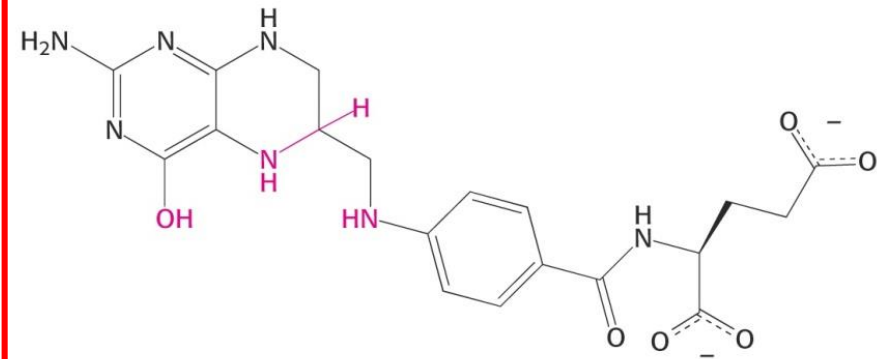
- Transition-state analogs: extremely potent inhibitors (bind more tightly)
- Drugs cannot be designed that precisely mimic the transition state! (highly unstable structure)
- Substrate analogs: bind more tightly than substrates
- Known as suicide inhibitors

Methotrexate

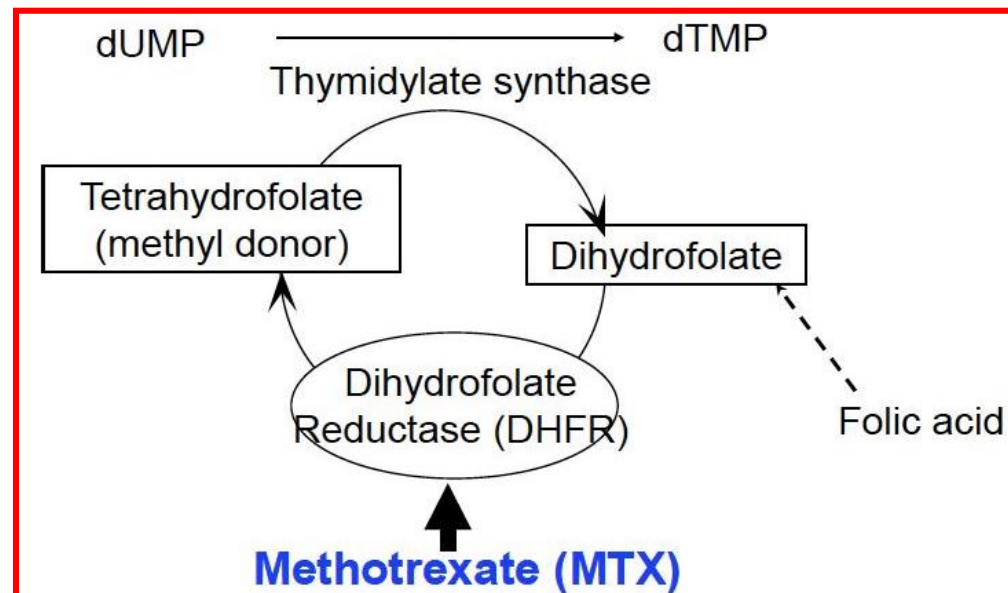
- Synthetic inhibitor
- Anticancerous
- Analog of tetrahydrofolate
- Binds to enzyme a 1000-fold more tightly
- Inhibits nucleotide base synthesis



Methotrexate

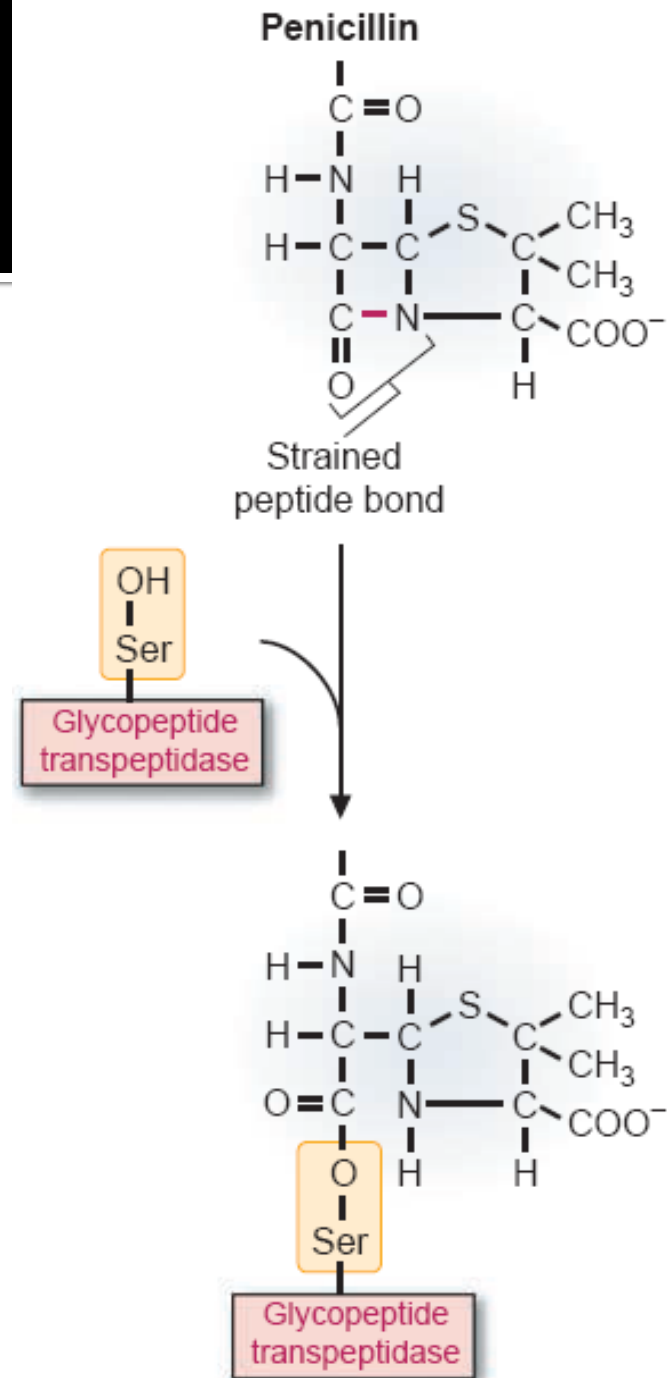


Tetrahydrofolate



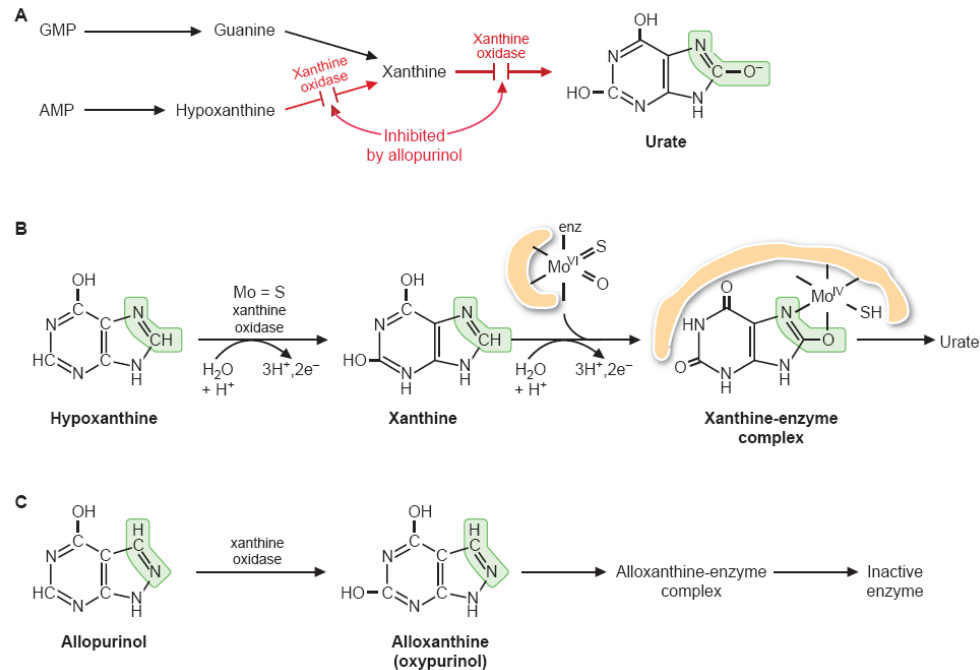
PENICILLIN

- A transition-state analog to *glycopeptidyl transferase or transpeptidase*
- Required by bacteria for synthesis of the cell wall
- The reaction is favored by the strong resemblance between the peptide bond in the β -lactam ring of penicillin & the transition-state complex of the natural transpeptidation reaction
- Inhibitors that undergo partial reaction to form irreversible inhibitors in the active site are sometimes termed *suicide inhibitors*



ALLOPURINOL

- A drug used to treat gout
- Decreases urate production by inhibiting xanthine oxidase
- **The enzyme commits suicide** by converting the drug to a transition-state analog
- **The enzyme contains a molybdenum–sulfide (Mo-S) complex** that binds the substrates and transfers the electrons required for the oxidation reactions
- **Xanthine oxidase oxidizes the drug allopurinol to oxypurinol**, a compound that binds very tightly to a molybdenum–sulfide complex in the active site



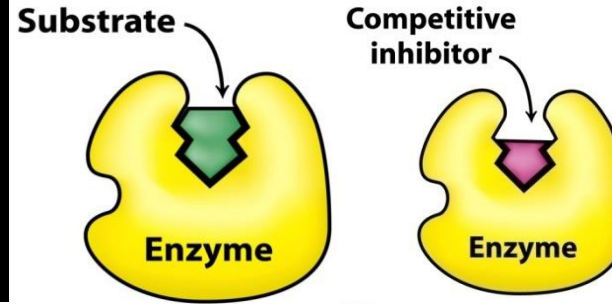
Heavy Metals

- Tight binding of a metal to a functional group in an enzyme
- Mercury (Hg), lead (Pb), aluminum (Al), or iron (Fe)
- Relatively nonspecific for the enzymes they inhibit, particularly if the metal is associated with high-dose toxicity
- **Mercury**: binds to so many enzymes, **often at reactive sulfhydryl groups** in the active site
 - It has been difficult to determine which of the inhibited enzymes is responsible for mercury toxicity
- **Lead** provides an example of a metal that inhibits through **replacing the normal functional metal in an enzyme**, such as calcium, iron, or zinc
 - Its developmental & neurologic toxicity may be caused by its ability to **replace Ca^{+2}** in several regulatory proteins that are important in the central nervous system and other tissues

Reversible Inhibitors

- Characterized by a rapid dissociation of the enzyme-inhibitor complex
- Usually these inhibitors bind through non-covalent interactions & inhibitor maintains a reversible equilibrium with the enzyme
- Reversible inhibitors can be divided into two classes: competitive & noncompetitive
- The double-reciprocal plots are highly useful for distinguishing among these inhibitors

Competitive inhibition

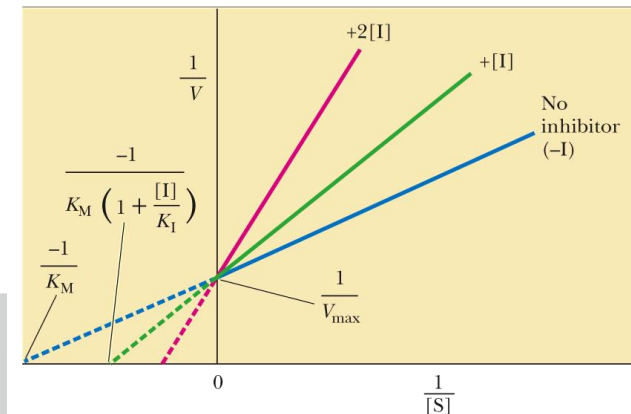
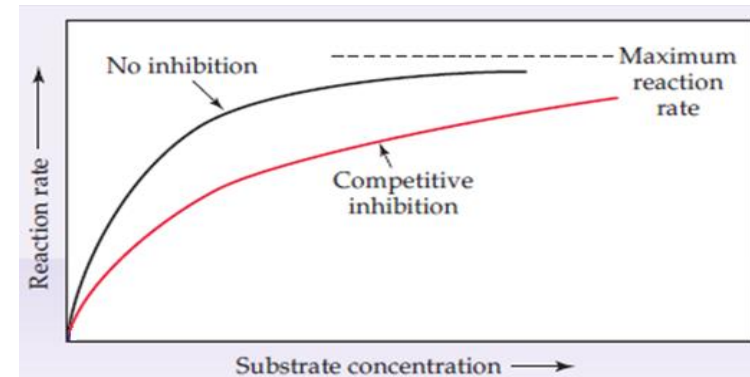
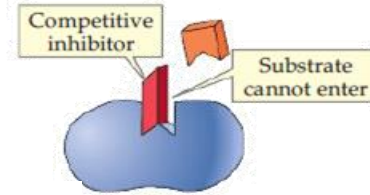
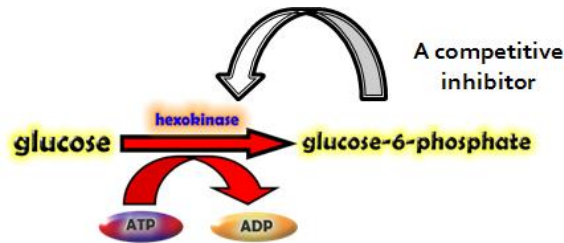


➤ The inhibitor competes with substrate

➤ Increasing $[S]$ can overcome the inhibition ($V_{\max}$)

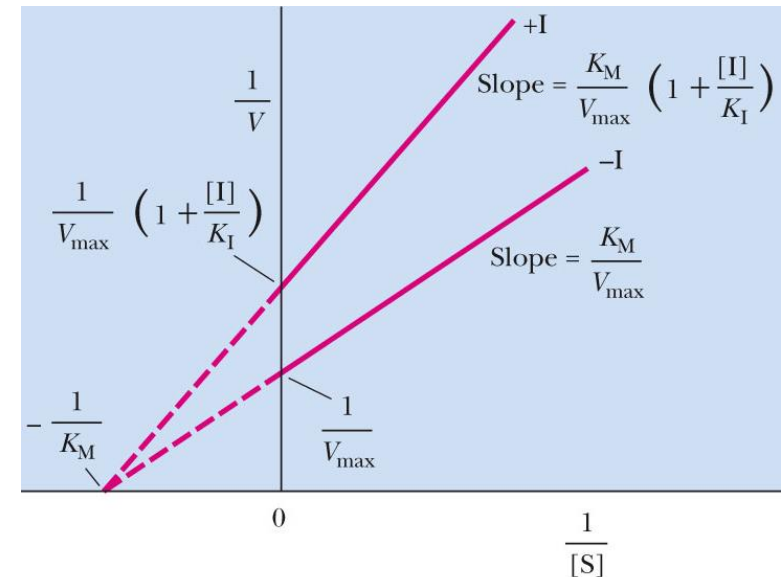
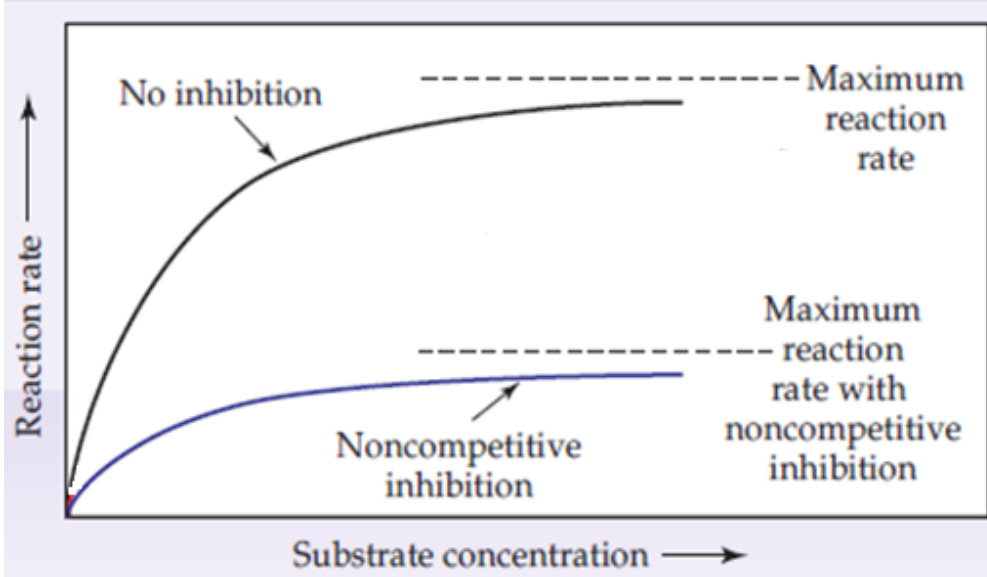
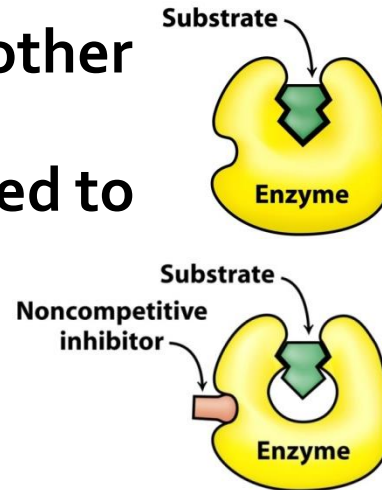
➤ Does K_M change?

➤ Significance (ex. Hexokinase)



Noncompetitive inhibition

- The inhibitor binds at a site other than the active site
- The complex does not proceed to form product or has a lower efficiency
- $V_{\max}$ vs. K_M
- Can we reach $V_{\max}$?

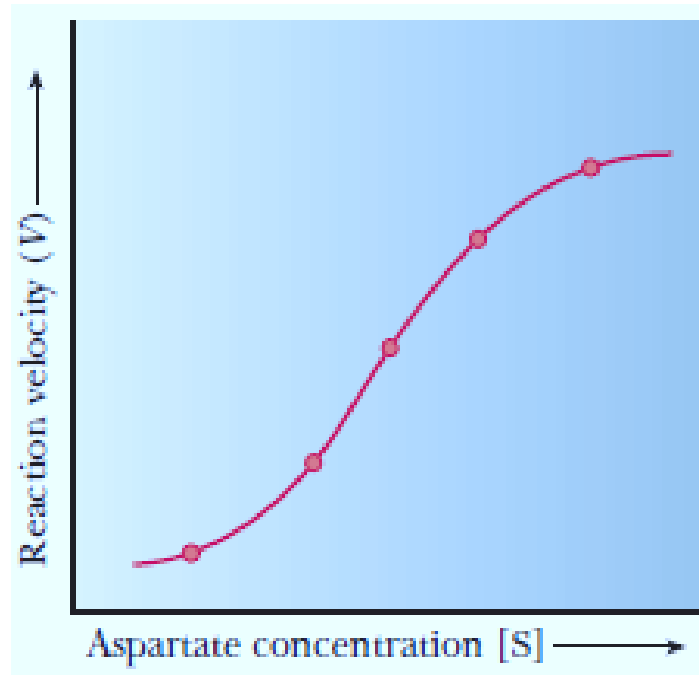
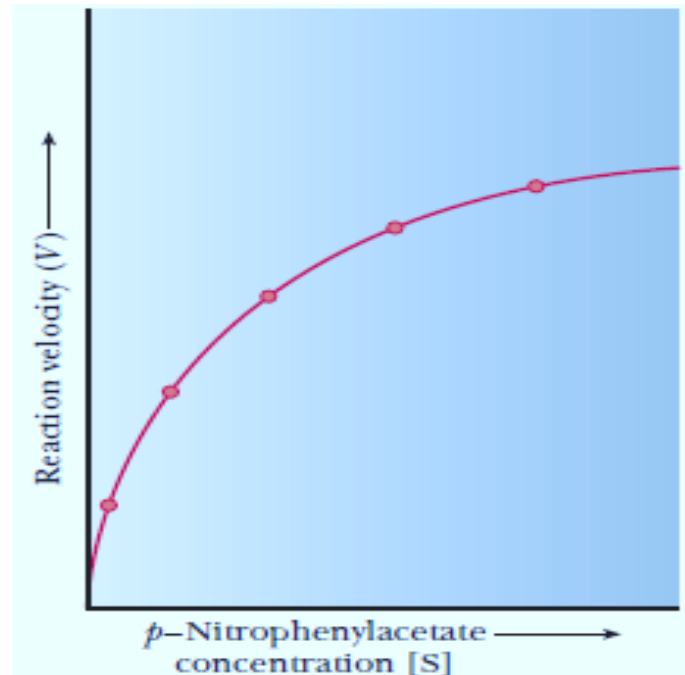
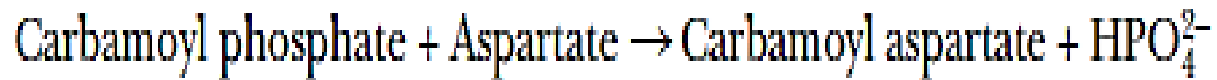


REGULATION THROUGH CONFORMATIONAL CHANGES

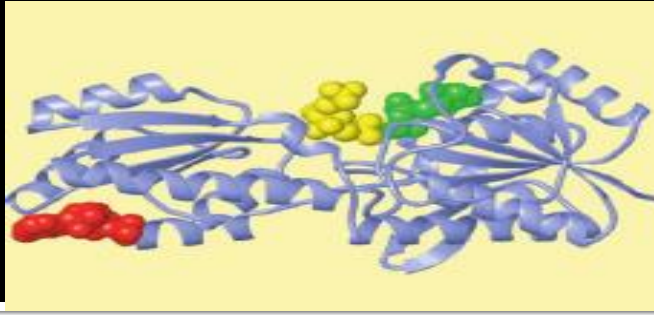
- These regulatory mechanisms include
 - A. Allosteric activation and inhibition;
 - B. Phosphorylation or other covalent modification;
 - C. Protein-protein interactions between regulatory & catalytic subunits or between two proteins;
 - D. Proteolytic cleavage
- These types of regulation can rapidly change an enzyme from an inactive form to a fully active conformation

Not all enzymes follow Michaelis-Menten equation; *Chymotrypsin vs. ATCase*

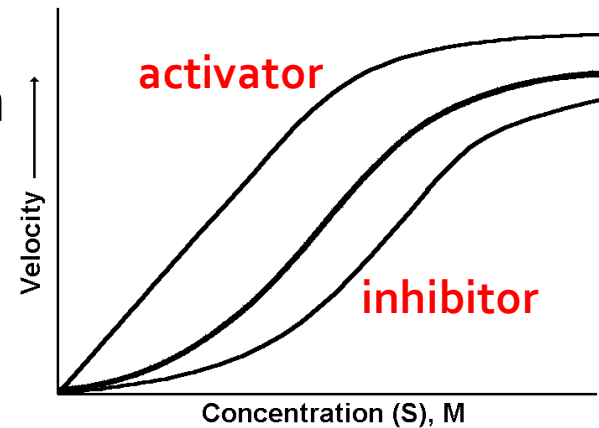
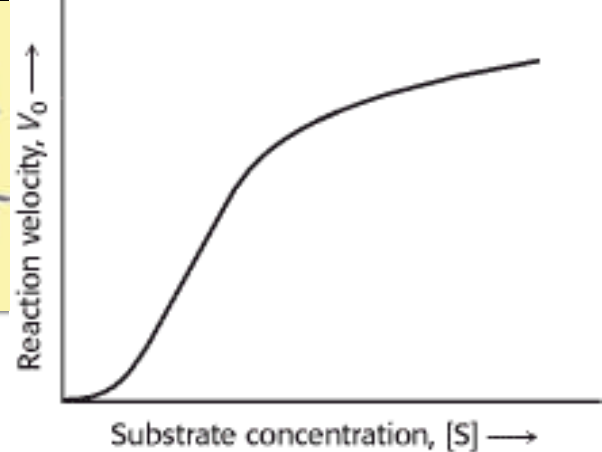
- Chymotrypsin: Specificity for aromatic residues mainly. Also, hydrolysis of ester bonds
- Aspartate transcarbamoylase (ATCase): synthesis of CTP & UTP for RNA and DNA synthesis



Allosteric regulation



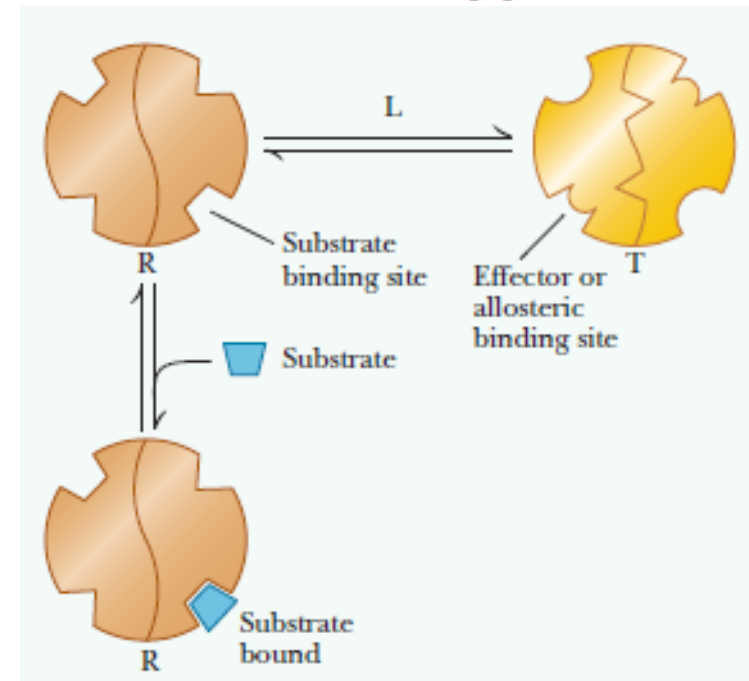
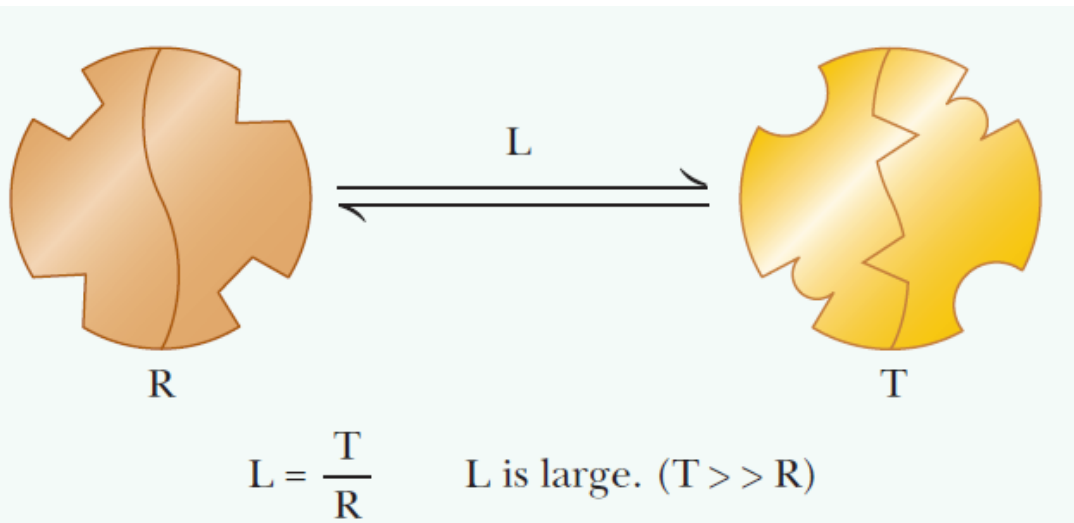
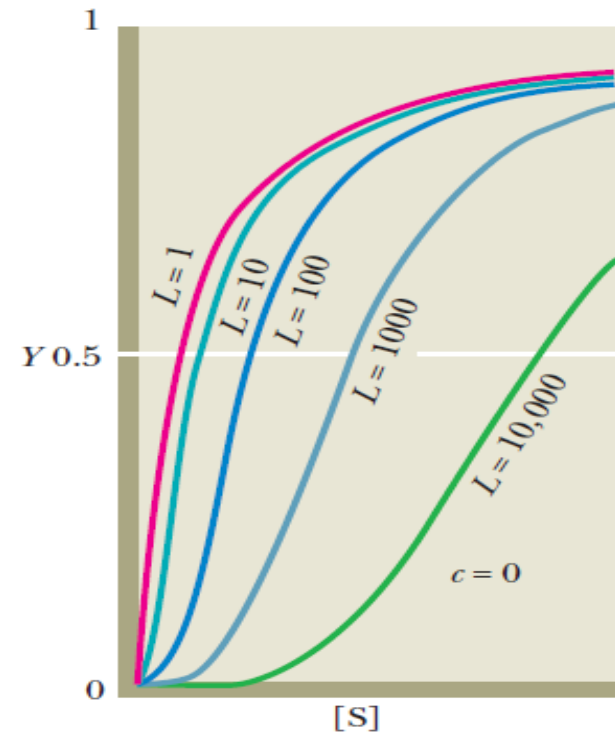
- What are allosteric enzymes? A multi-subunit enzyme with catalytic subunit(s) and regulatory subunit(s)
- Binding triggers a conformational change in the active site
- The Michaelis-Menten model can't explain the kinetic properties
- The effect of the modulators (allosteric modifiers)
- Homotropic vs. heterotropic
- The substrate concentration at half of the $V_{\max}$ is called ($K_{0.5}$)
- Allosteric inhibitors have a much stronger effect on enzyme velocity



The effect of
modifiers on
 $V_{\max}$ & $K_{0.5}$

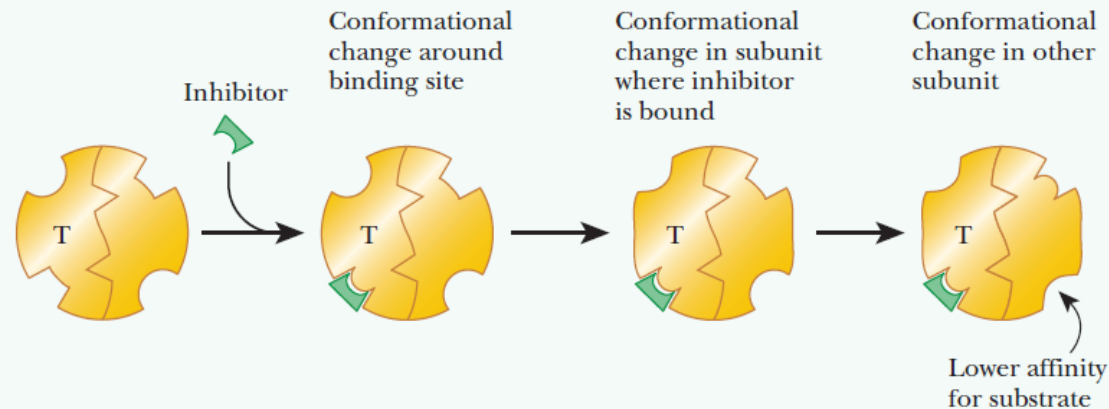
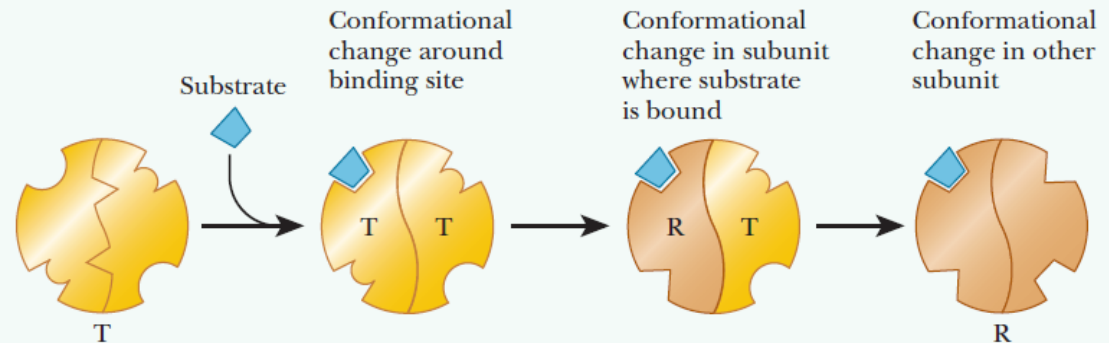
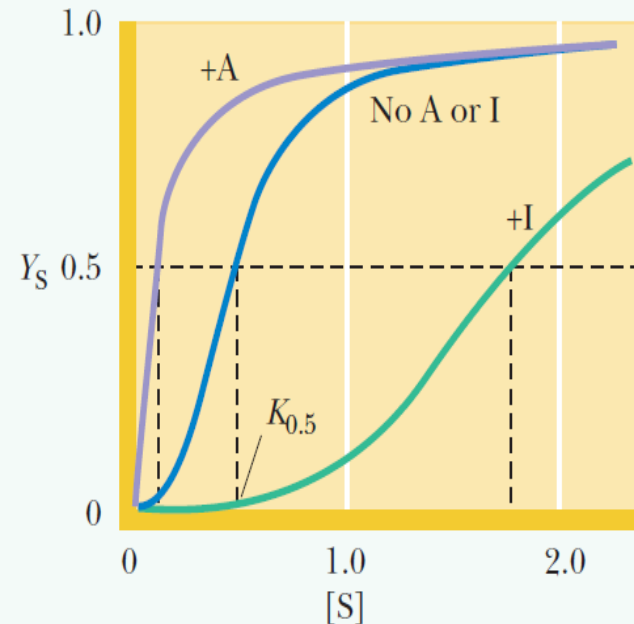
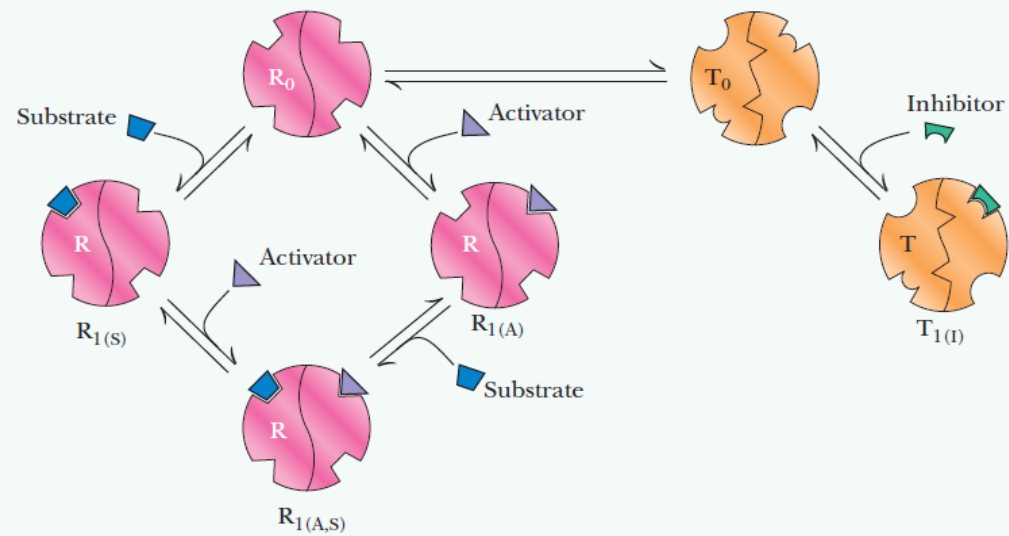
How do allosteric enzymes work?

- Two conformations: more active (R) & less active or inactive (T),
- The equilibrium ratio (T/R) is called **L** and assumed to be high
- As **L** (T/R) increases, the shape becomes more sigmoidal



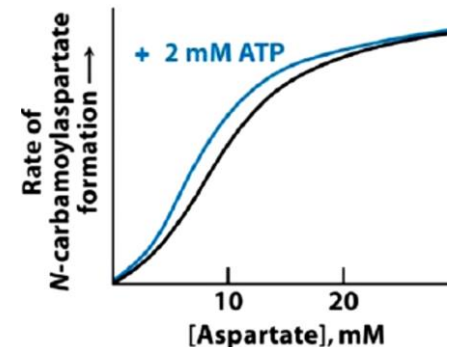
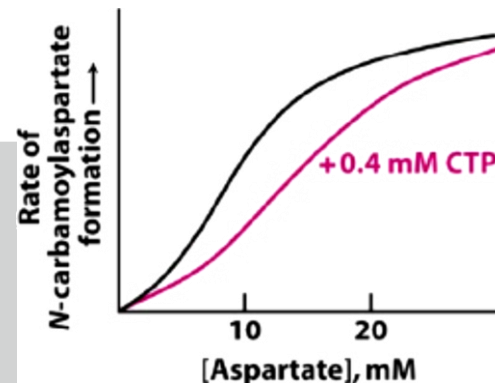
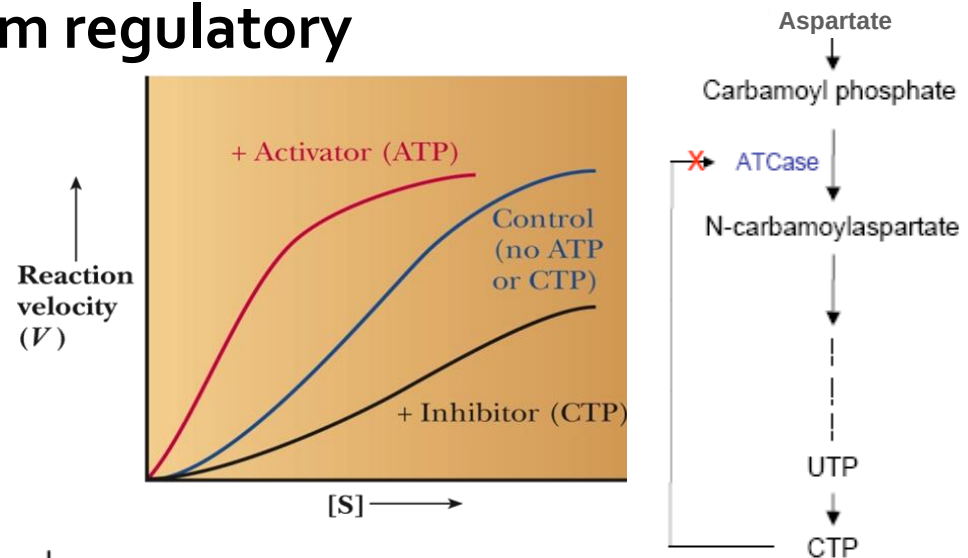
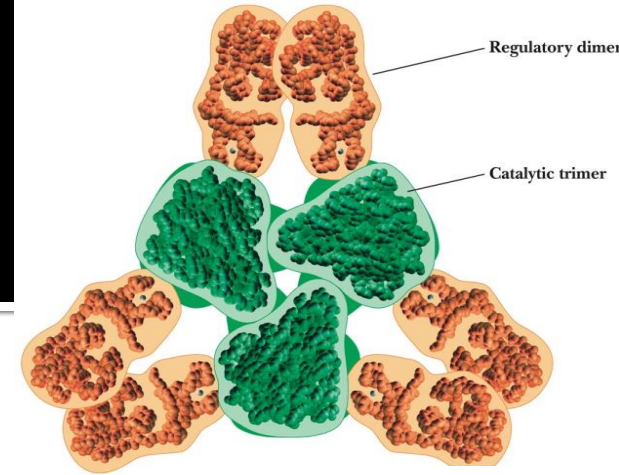
Concerted or sequential?

- Either substrate or activator must be increased to overcome the effects of the allosteric inhibitor
- Conformational change

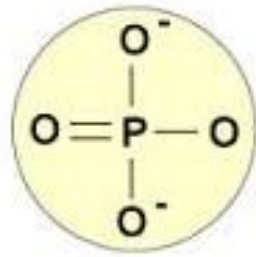


Allosteric regulation – ATCase "synthesis of pyrimidine nucleotides"

- ATCase and Hb are allosteric proteins (**cooperative behavior**)
- Catalytic can be separated from regulatory (**hyperbolic**)
- Cooperativity in relation to substrate
- **CTP is an inhibitor** of ATCase (feedback inhibition), **ATP is an activator**



Conformational Changes from Covalent Modification - 1. PHOSPHORYLATION



Phosphate
group

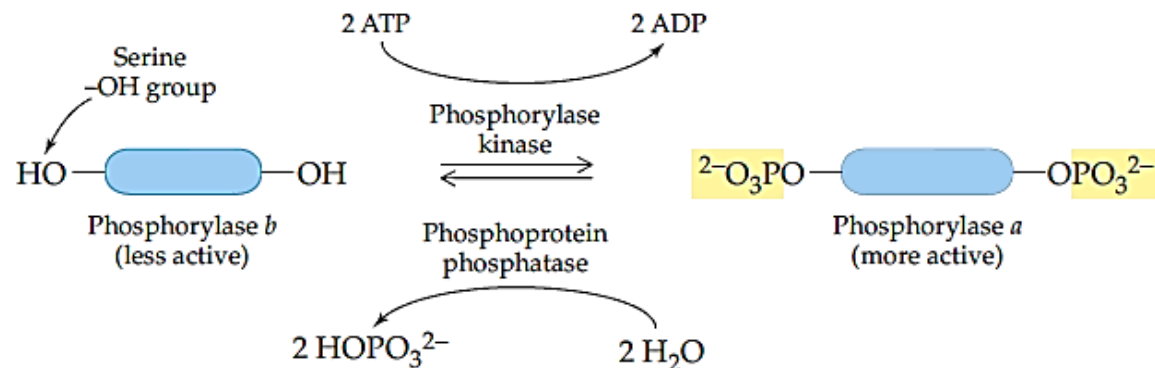
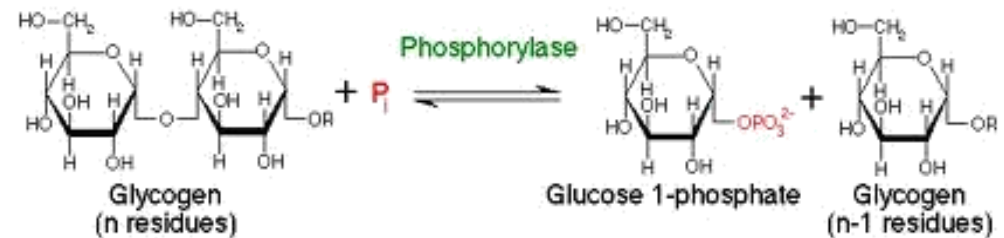
- **Why is it effective?**
- Adds two negative charges: new electrostatic interactions and accordingly conformation
- Can form three or more hydrogen bonds: specific interactions with hydrogen-bond donors
- Can take place in less than a second or over a span of hours
- Often causes highly amplified effects

Conformational Changes from Covalent Modification - 1. PHOSPHORYLATION

- Rapid and transient regulation of enzyme activity - **REVERSIBLE**
- Phosphorylation: (**Ser, Thr, & Tyr**)
 - ✓ Mostly, ATP is the donor
 - ✓ **Kinases vs. phosphatases**

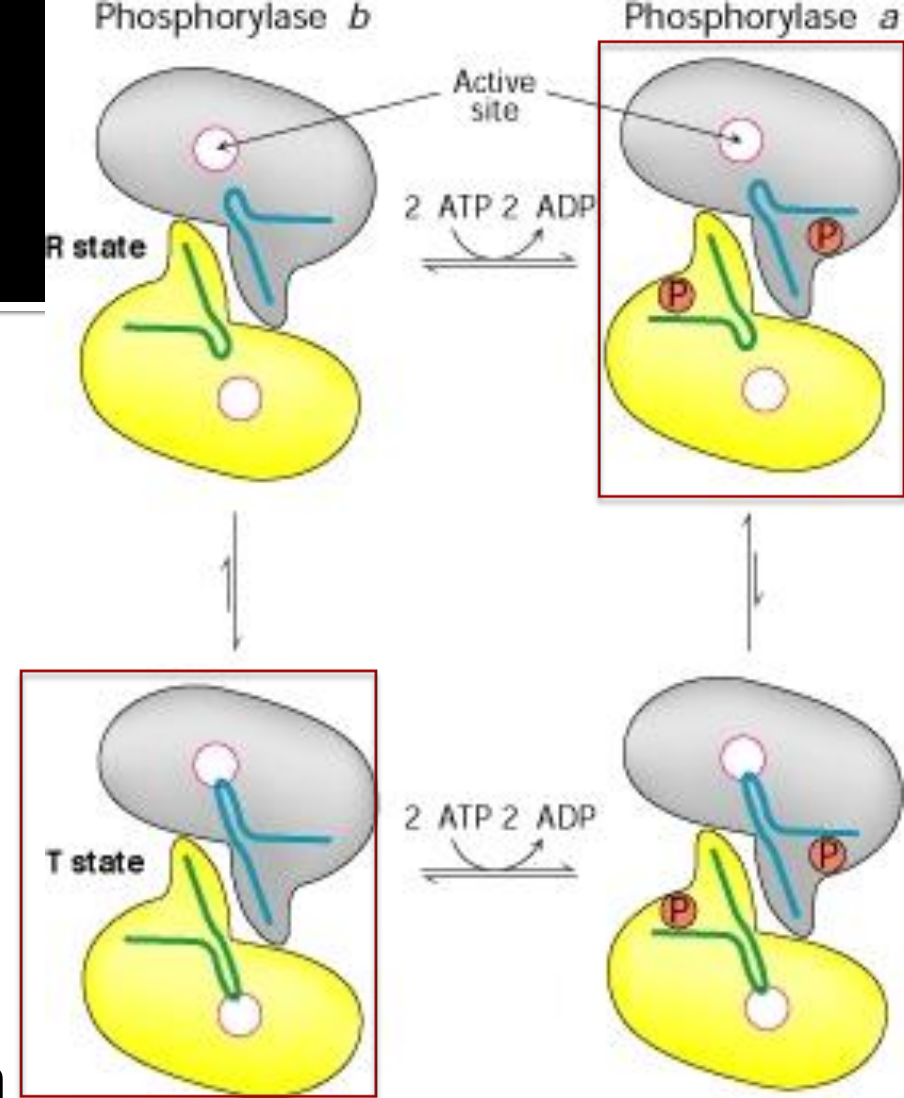
✓ Phosphorylation does not lead always to activation of enzymes

✓ **Glycogen phosphorylase-reaction** (two forms; a & b). Ser is away from the active site



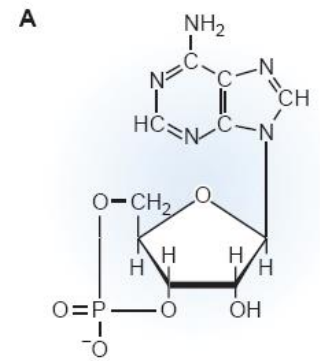
The two forms of the enzyme

- Both phosphorylase *b* and phosphorylase *a* exist as equilibria between an active R state and a less-active T state
- Phosphorylase *b* is usually inactive because the equilibrium favors the T state
- Phosphorylase *a* is usually active because the equilibrium favors the R state

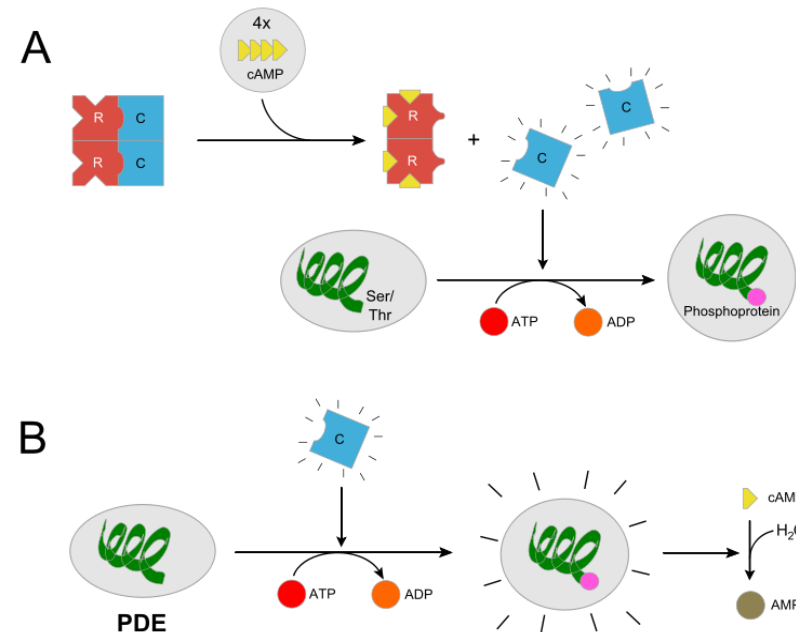


The transition of phosphorylase *b* between the T and the R state is controlled by the energy charge of the muscle cell.

Protein kinase A (PKA)



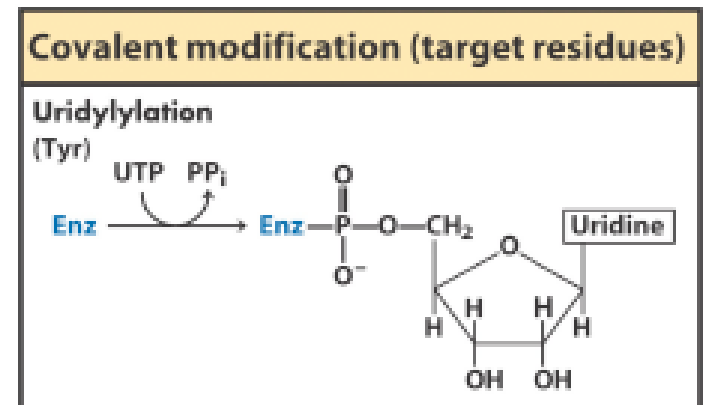
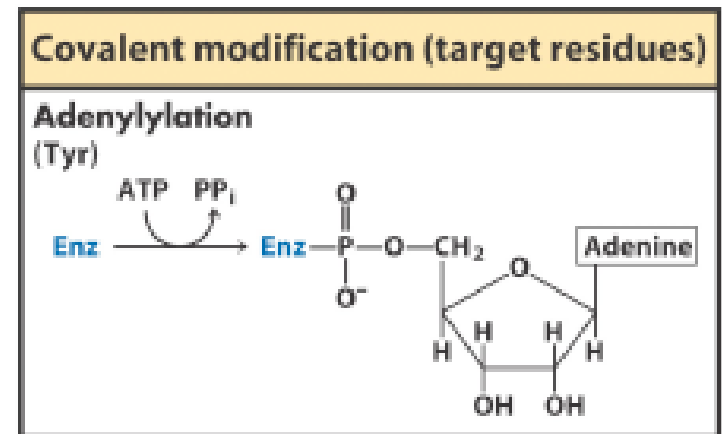
- Protein kinase A (PKA): refers to a family of enzymes whose activity is dependent on cellular levels of cyclic AMP (cAMP)
- cAMP: referred to as a hormonal 2nd messenger
- Either dedicated or not
- Has several functions in the cell, including regulation of glycogen, sugar, & lipid metabolism



- Adrenaline (epinephrine) → ↑cAMP → activates protein kinase A → phosphorylates & activates glycogen phosphorylase kinase → phosphorylates & activates glycogen phosphorylase
- Phosphorylation cascade

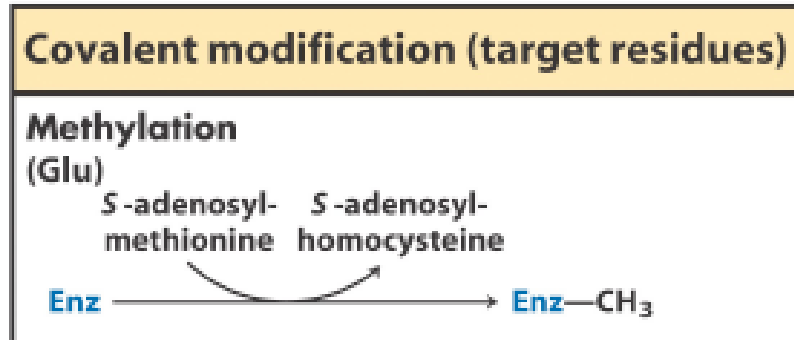
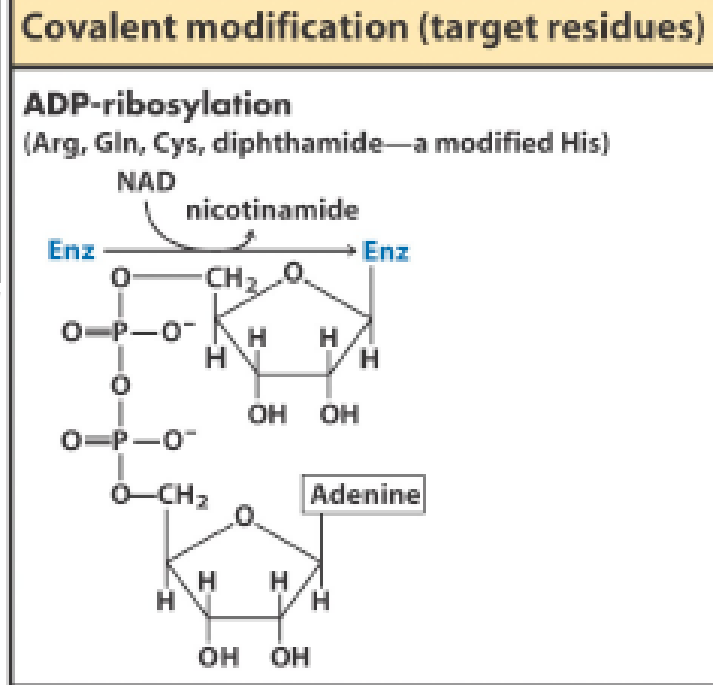
Other covalent modifiers

- Adenylylation (addition of adenylyl group). AMP (from ATP) is transferred to a Tyr hydroxyl by a phosphodiester linkage. The addition of bulky AMP inhibits certain cytosolic enzymes.
- Uridylylation (addition of uridylyl group).



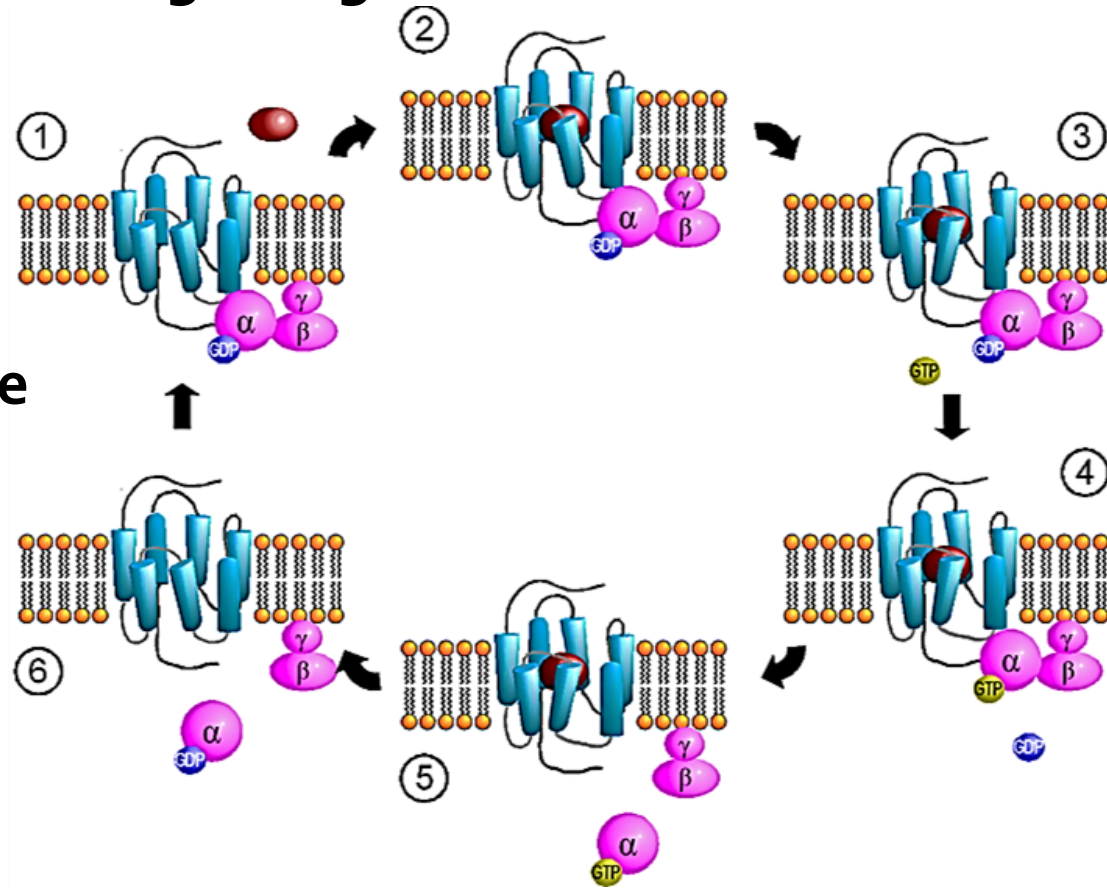
Other covalent modifiers

- ADP-ribosylation: inactivates key cellular enzymes
- Methylation: masks a negative charge & add hydrophobicity on carboxylate side chains
- Acetylation: masks positive charges when added to lysine residues



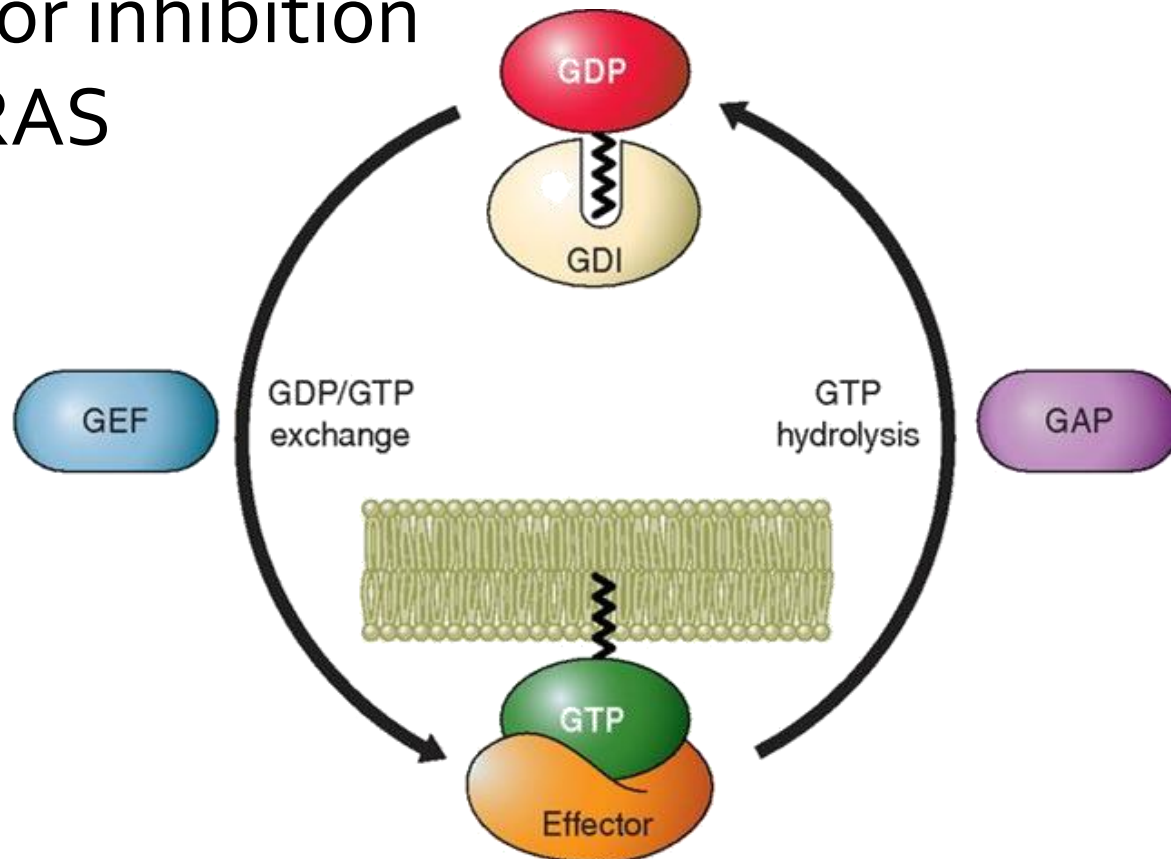
Conformational Changes from Protein-Protein Interactions

- G protein: a family of trans-membrane proteins causing changes inside the cell. They communicate signals from hormones, neurotransmitters, and other signaling factors
- When they bind guanosine triphosphate (GTP), they are 'on', and, when they bind guanosine diphosphate (GDP), they are 'off'
- α -Subunit can be stimulatory or inhibitory



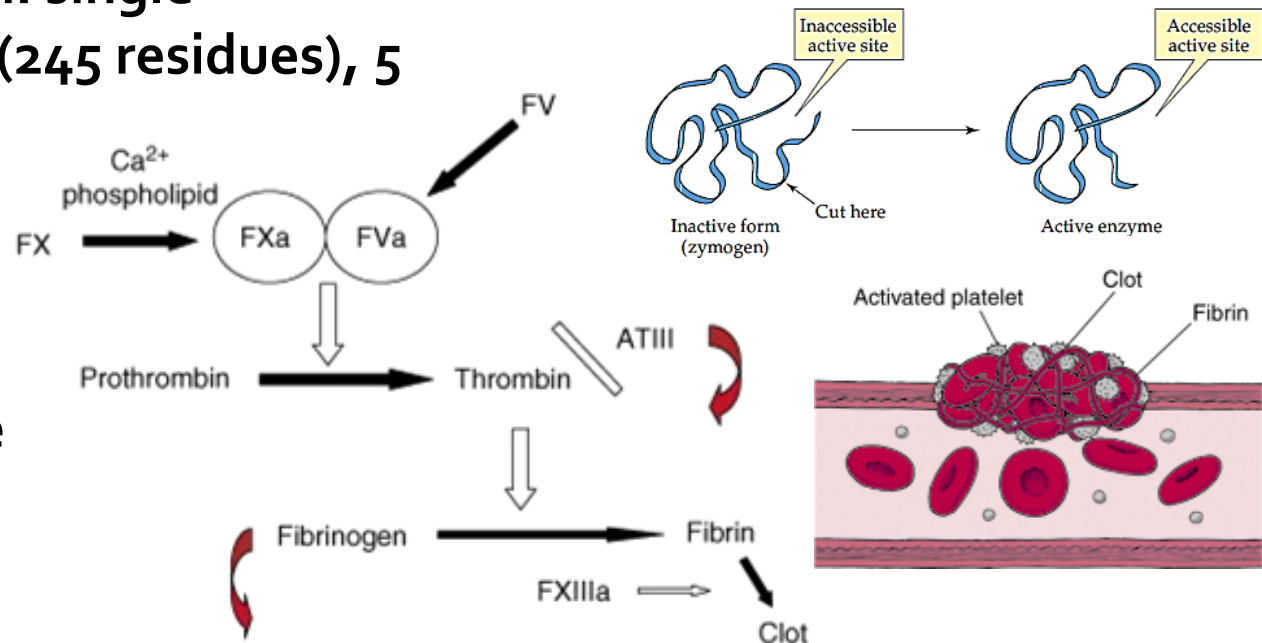
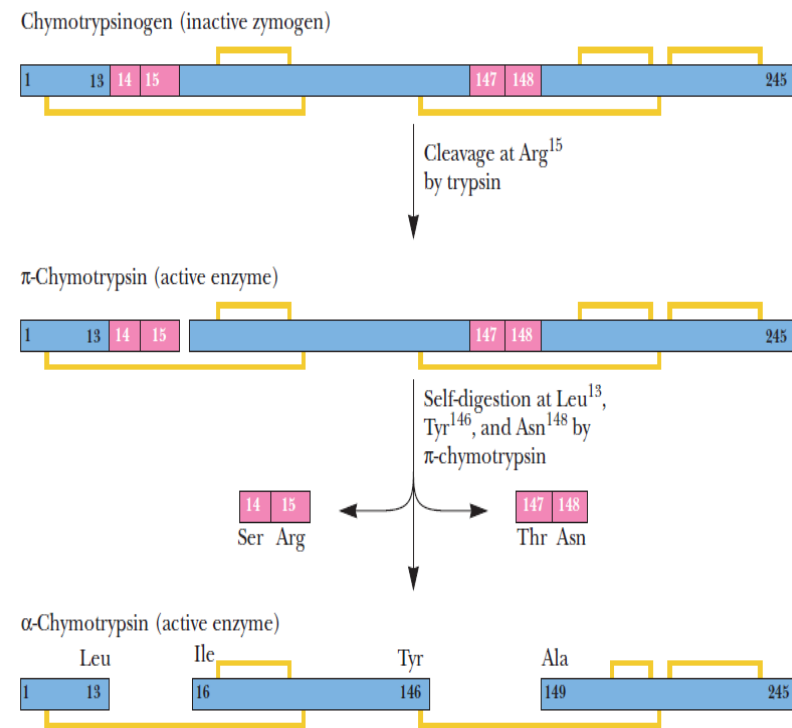
Monomeric "G" proteins

- Same as $G\alpha$
- Hydrolysis vs. exchange
- Activation or inhibition
- Example: RAS



Proteolytic Cleavage Zymogens (Pro- or -gen)

- Irreversible cut usually at the N-terminus
- Trypsin, chymotrypsin, pepsin (trypsinogen, pepsinogen, chymotrypsinogen)
- ✓ Chymotrypsinogen: single polypeptide chain (245 residues), 5 (S—S) bonds
- Blood clotting
- ✓ The soluble protein fibrinogen is converted to the insoluble protein fibrin



Non-specific regulators

REGULATION THROUGH CHANGES IN AMOUNT OF ENZYME

A. Regulated Enzyme Synthesis

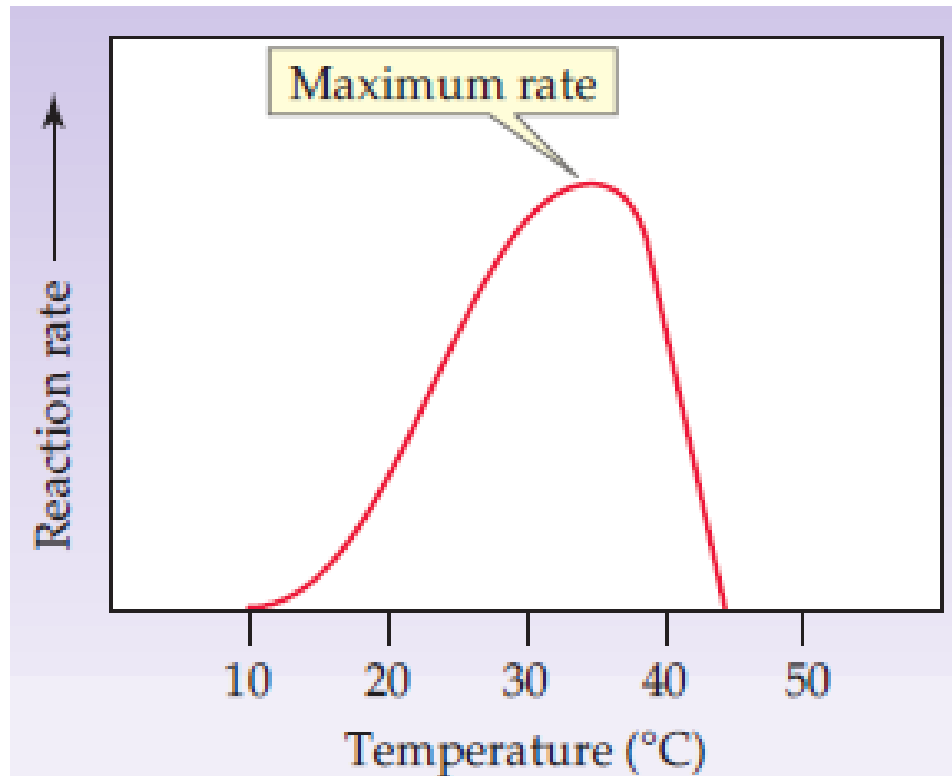
- Regulated by increasing or decreasing the rate of gene transcription (induction & repression)
 - Usually slow in humans (hours to days)
- Sometimes through stabilization of the messenger RNA

B. Regulated Protein Degradation

- Can be degraded with a characteristic half-life within lysosomes
 - During fasting or infective stress: gluconeogenesis increase & synthesis of antibodies (protein degradation increases)
 - Increased synthesis of ubiquitin

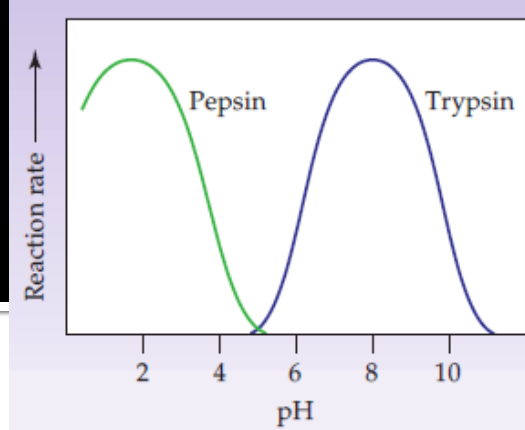
Effect of Temperature

- Increase in T° increases the rate until reaches a max ($\approx 50^\circ$): the optimal temperature of each enzyme is its' denaturation
- Autoclave steam heating
- Hypothermia, metabolic reactions, cardiac surgery



Effect of pH

- Usually a well defined optimum point
- Most enzymes have their max. activity between (5-9)
- Extremes of pH denatures protein
- pH can alter binding of substrate to enzyme (K_M) by altering the protonation state of the substrate, or altering the conformation of the enzyme



The effect of pH is enzyme-dependent

Extremozymes



Taq
polymerase
and PCR

Thermophiles (heat lovers)

Psychrophiles (cold lovers)



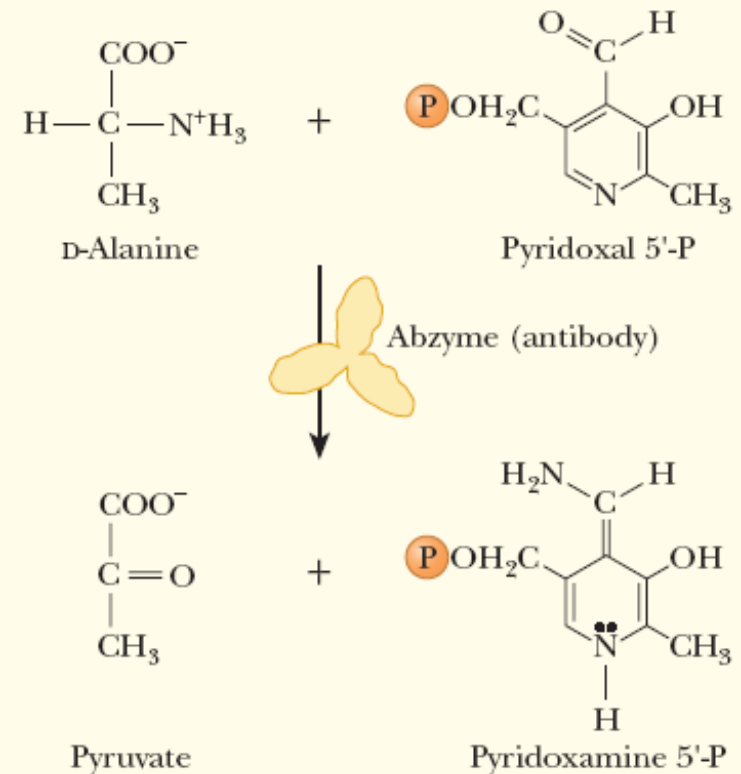
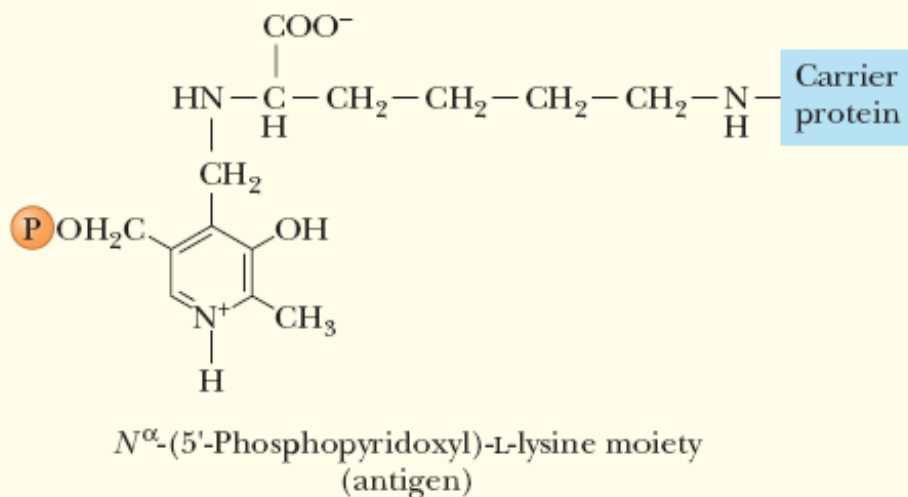
Biobleaching of paper
pulp using heat-stable
xylanases



lipases and proteases

Abzymes – cutting edge science

- An antibody that is produced against a transition-state analog (active)
- An abzyme is created in animals



An exception to protein enzymes

Ribozymes

- RNA molecules
- Examples: telomerase & RNase P
- Catalyze splicing reactions and are involved in protein synthesis
- The catalytic efficiency of catalytic RNAs is less than that of protein enzymes, but can greatly be enhanced by the presence of protein subunits

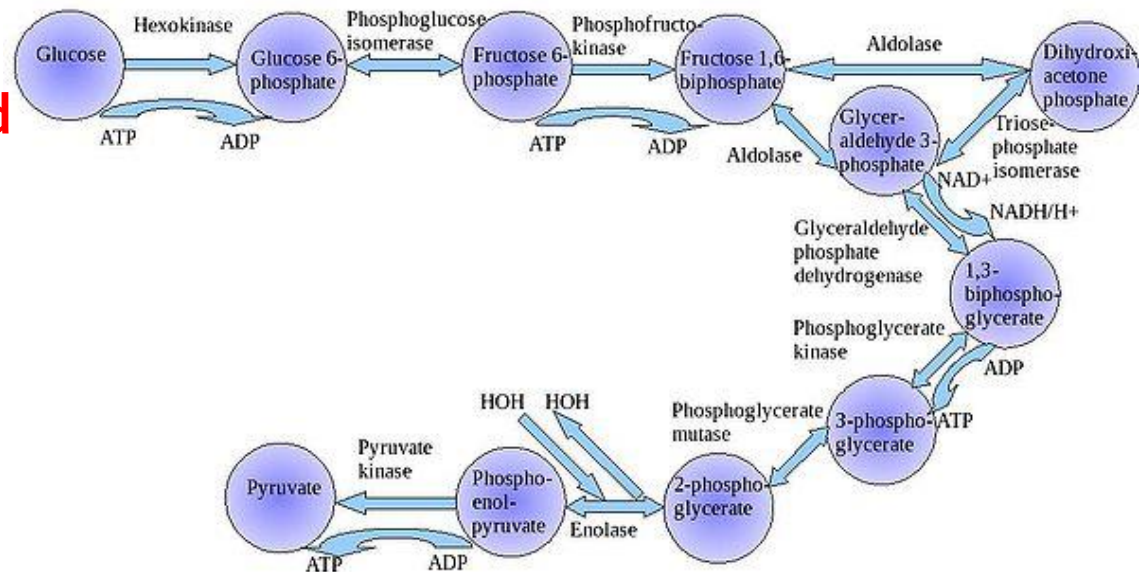


REGULATION OF METABOLIC PATHWAYS

Principles of Pathway Regulation

- **1. COUNTERREGULATION OF OPPOSING PATHWAYS**
- Synthesis vs. degradation (a different regulatory enzyme)
- **2. TISSUE ISOZYMES OF REGULATORY PROTEINS**
- **3. REGULATION AT THE RATE-LIMITING STEP**
- Pathways are principally regulated at their rate-limiting step
- The slowest step & is usually not readily reversible
 - Changes in this step can influence flux through the rest of the pathway

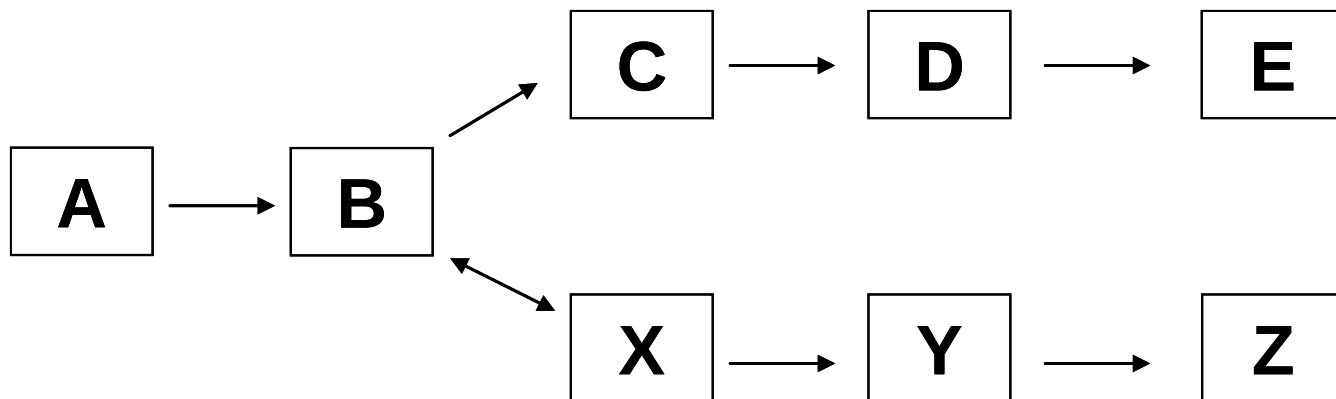
- **Usually the first committed step in a pathway**
- **Requirement for high amount of energy**
- **High K_M values of enzyme towards its substrate**



Principles of Pathway Regulation

■ 4. *The committed step*

- A committed step in a metabolic pathway is the first irreversible reaction that is unique to a pathway and that, once occurs, leads to the formation of the final substrate with no point of return
- Committed steps are exergonic reaction
- For example, the committed step for making product E is ($B \rightarrow C$), not ($A \rightarrow B$)

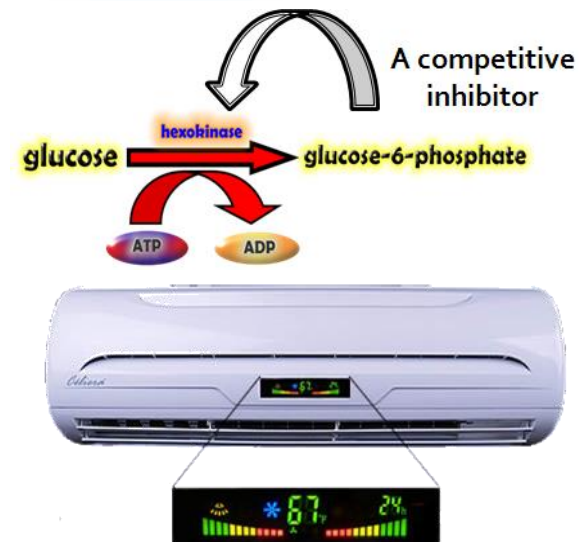
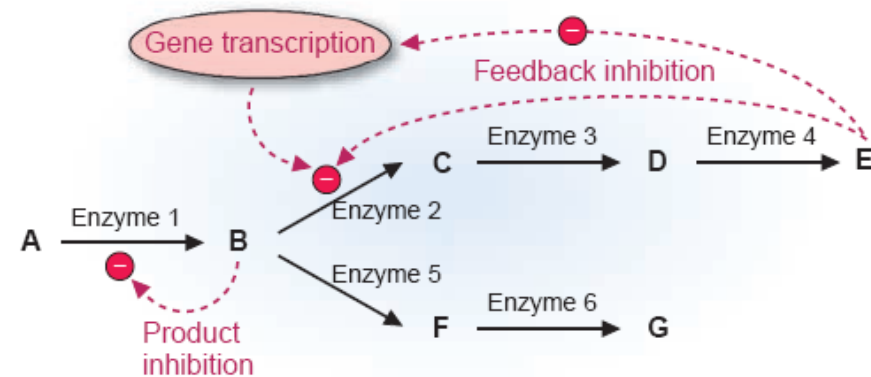
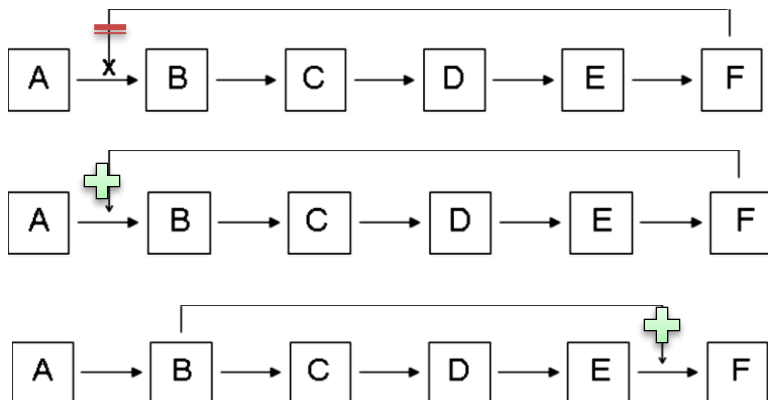


Principles of Pathway Regulation

■ 5. FEEDBACK REGULATION

- This type of regulation is much slower to respond to changing conditions than allosteric regulation

- Negative feedback regulation (feedback inhibition)
- Positive feedback regulation
- Feed-forward regulation
- Disposal of toxic compounds

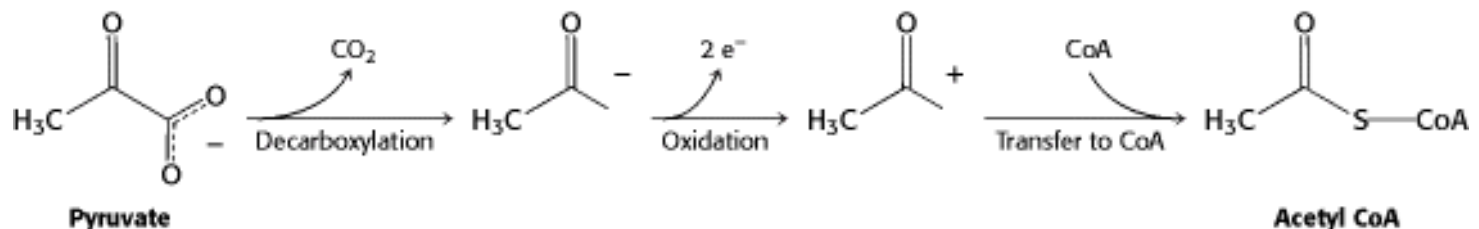
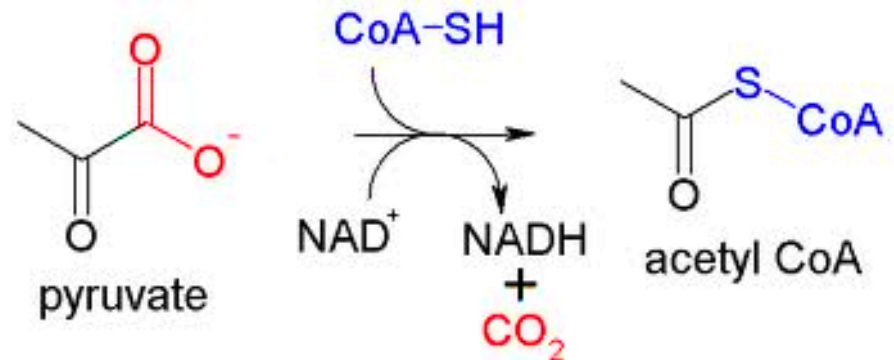
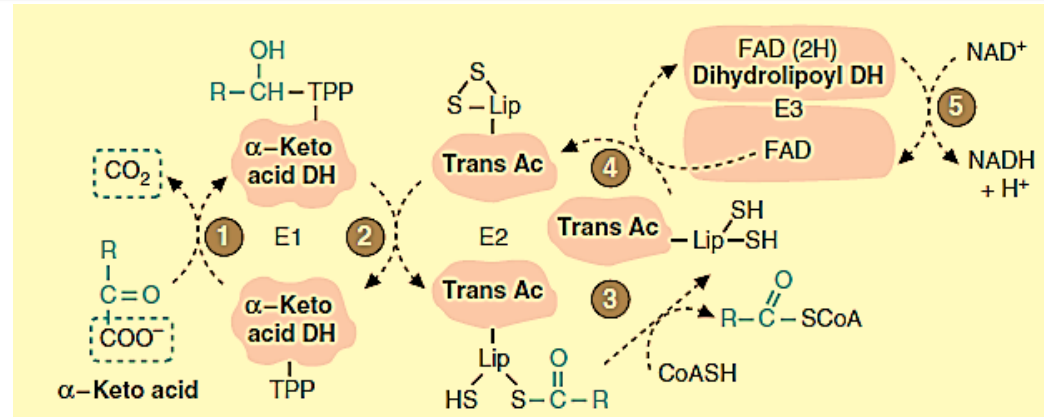


Principles of Pathway Regulation

- *6. Enzyme compartmentalization*
- Both enzymes and their substrates are present in relatively small amount in a cell
- A mechanism by which rate of reactions become faster is their compartmentalization; reducing area of diffusion
- In this way, enzymes are sequestered inside compartments where access to their substrates is limited
- Lysosomes; proteins get transported to lysozymes
- Mitochondria; energy metabolic pathways
- Metabolism of fatty acids; synthesis (cytosol) vs. degradation (mitochondria)

Principles of Pathway Regulation

- **7. Enzyme complexing**
(A multienzyme complex)
- Complexing various enzymes that share one process
- Product of enzyme A pass directly to enzyme B
- Pyruvate dehydrogenase (mitochondria) 3 enzymes: decarboxylation, oxidation, & transfer of the resultant acyl group to CoA



Enzymes in Medical Diagnosis

Diagnostic Enzymes & Liver Disease

- Concept
- Examples: ALT, AST, LDH, CK (CPK)
- Liver disease: ALT & AST
 - ALT is the most specific
 - Ratio can also be diagnostic (ALT/AST)
 - In liver disease or damage (not of viral origin):
 - ratio is less than 1
 - With viral hepatitis:
 - ratio will be greater than 1

Protein profile in myocardial infarction

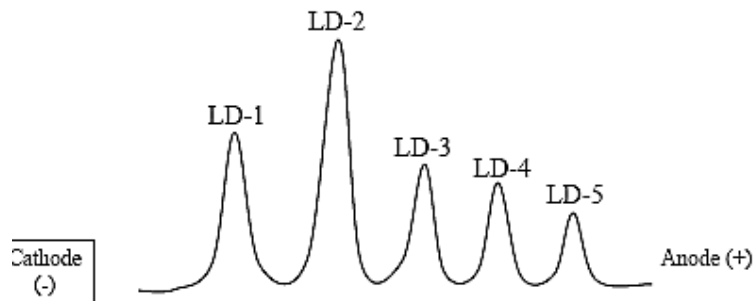


LDH

- LDH-1/LDH-2 ratio is diagnostic for myocardial infarction (heart attacks)
- Normally, this ratio is less than 1
- Following an acute myocardial infarct, the LDH ratio will be more than 1

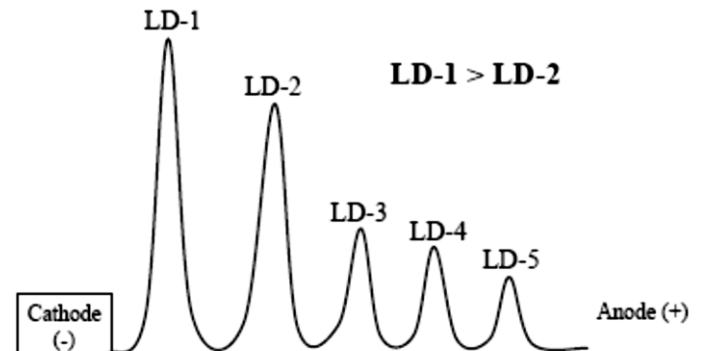
LD isoenzyme electrophoresis
(normal)

$LD-2 > LD-1 > LD-3 > LD-4 > LD-5$



LD isoenzyme electrophoresis
(abnormal)

$LD-1 > LD-2$



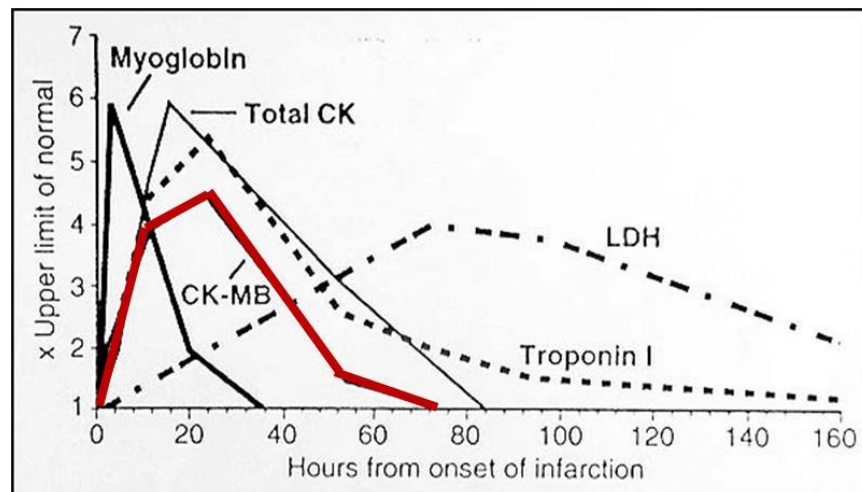
CPK

- Heart, skeletal muscles, & brain
- Like LDH, there are tissue-specific isozymes of CPK:
 - CPK₃ (CPK-MM): the predominant isozyme in muscle
 - CPK₂ (CPK-MB): accounts for ≈35% of CPK activity in cardiac muscle, but less than 5% in skeletal muscle
 - CPK₁ (CPK-BB) is the characteristic isozyme in brain and is in significant amounts in smooth muscle

Serum	Skeletal Muscle	Cardiac Muscle	Brain
0 trace BB <6% MB >94% MM	0 trace BB 1% MB 99% MM	0% BB 20% MB 80% MM	97% BB 3% MB 0%MM

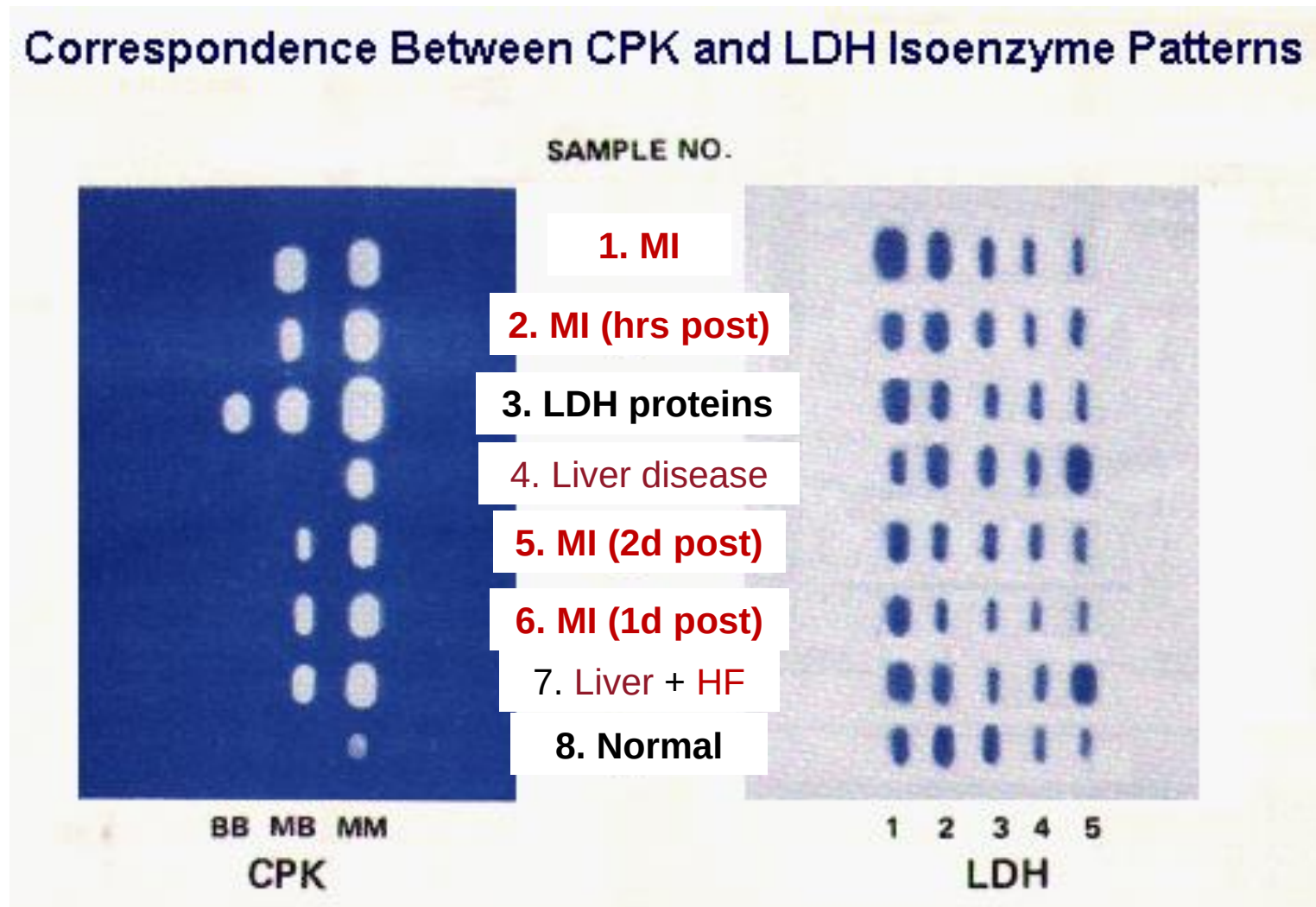
CPK and myocardial infarction

- Most of released CPK after MI is CPK-MB
- Increased ratio of CPK-MB/total CPK may diagnose acute infarction, but an increase of total CPK in itself may not
- The CPK-MB is also useful for diagnosis of reinfarction because it begins to fall after a day and disappears in 1 to 3 days, so subsequent elevations are indicative of another event



Example

Correspondence Between CPK and LDH Isoenzyme Patterns



Interpretation

- Sample #3 represents results for a control.
- Sample #8 results are from a normal specimen.
- Sample# 1 MI patient. The specimen was collected at a time when the activity of both LDH and CK were elevated. Note the LDH flip and the high relative activity of the MB isoenzyme.
- Sample# 2 MI patient who experienced chest pain only several hours previously. Total CK is significantly elevated with a high relative MB isoenzyme activity.
- Sample# 6 MI patient (the 1st day post MI); CK activity is definitely elevated with a high relative MB isoenzyme activity and the LDH flip is evident.
- Sample# 5 MI patient (2 days post MI) so that CK has almost returned to normal activity and the LDH flip is definite.
- Sample# 7 MI patient with complications of heart failure and passive liver congestion or the patient was involved in an accident as a consequence of the MI, and suffered a crushing muscle injury.
- Sample# 4 a patient with liver disease. Although the LDH isoenzyme pattern is indistinguishable from muscle disease or injury, the absence of at least a trace of CK-MB isoenzyme is inconsistent with the muscle CPK isoenzyme distribution as is the apparently normal total activity.

Troponins in MI

- Like all cardiac markers, troponins have a unique diagnostic window
- Troponin levels rise within four to six hours after the beginning of chest pain or heart damage, and stay elevated for at least one week.
- This long elevation allows detection of a myocardial infarction that occurred days earlier, but prevents detection of a second infarction if it occurred only days after the first