بسمه تعالی

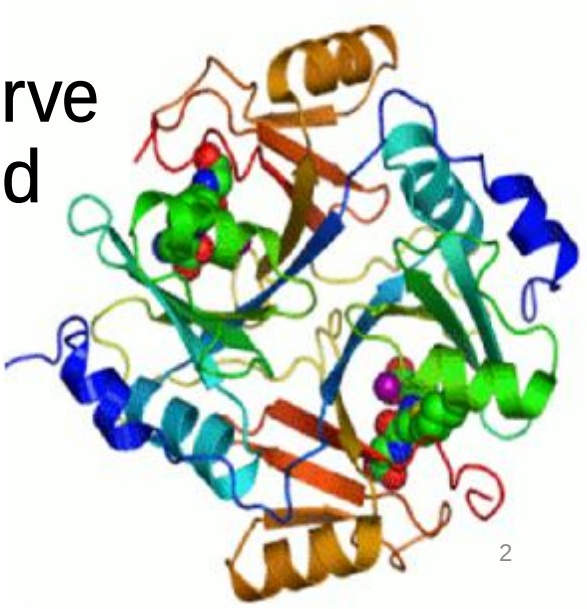
کارشناسی ارشد
بیوشیمی و بیولوژی سلول

آنزیم

ابراهیم قاسمی

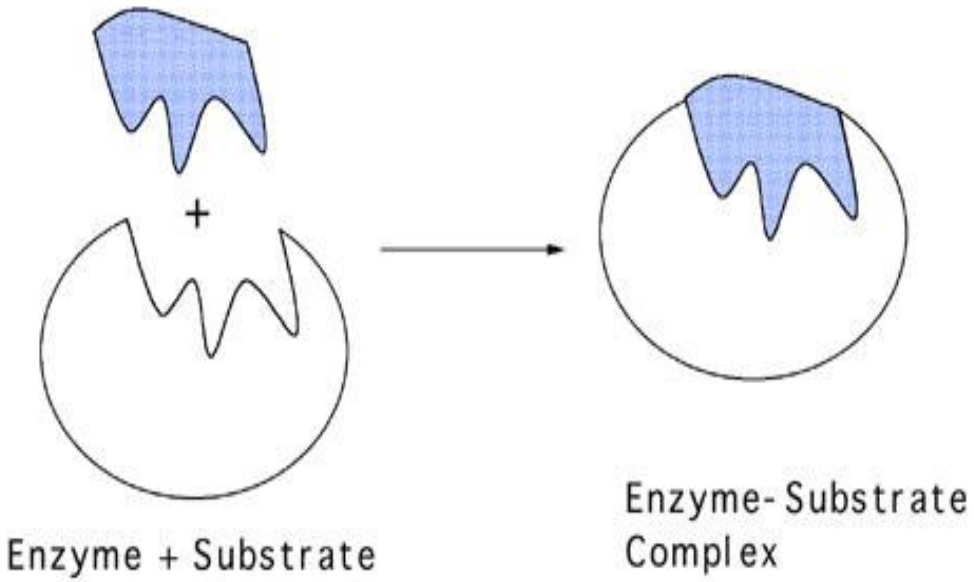
Enzyme

- Proteins with catalytic properties
- A small group of catalytic RNA molecules
- Catalyze reactions (degrade, conserve and transform chemical energy, and make biological macromolecules)



Enzyme

- The substrates of enzymes are the reactants that are activated by the enzymes

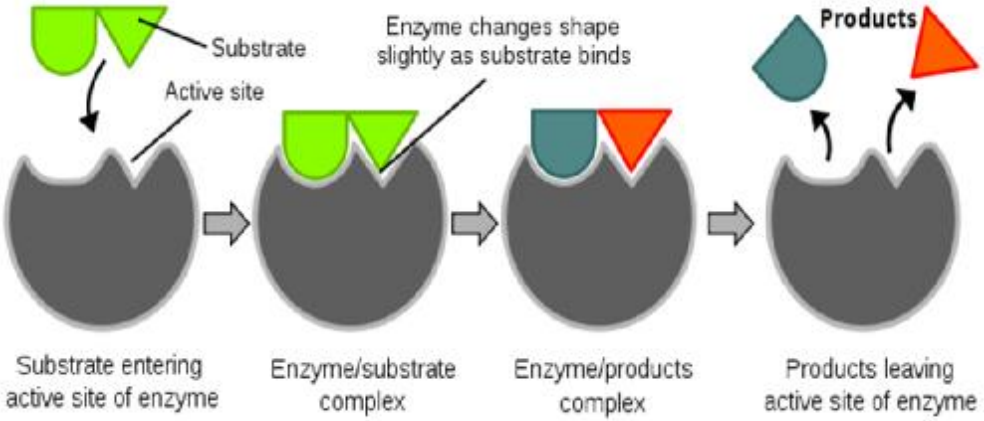


Enzyme

- Active site: The area on the enzyme where the substrate attach to is called the active site.

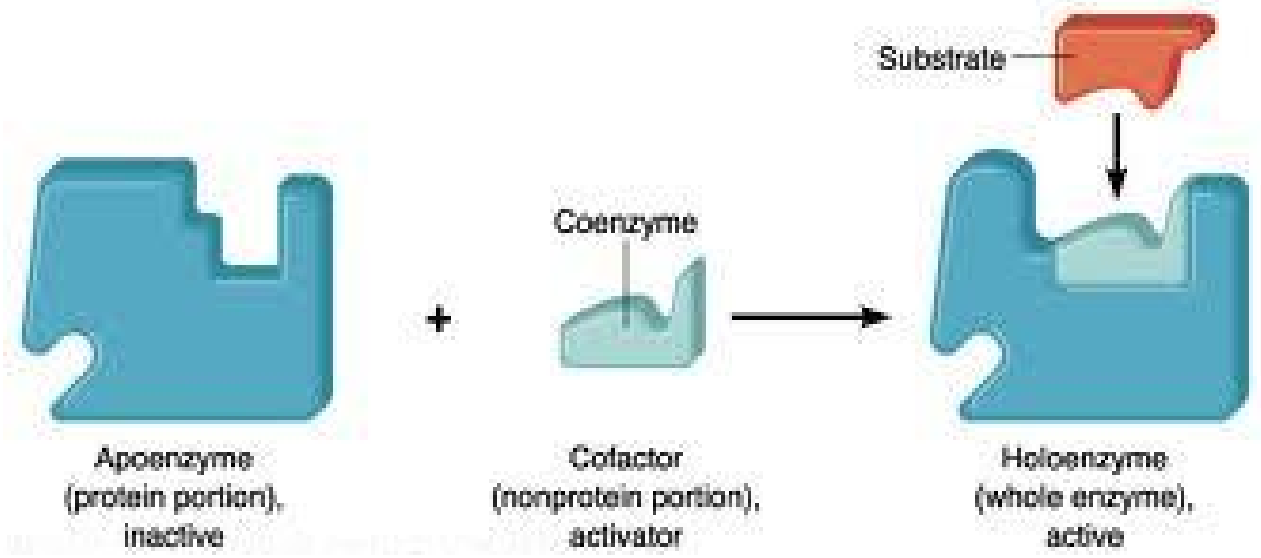
- Enzymes are specific to their substrates

- The specificity is determined by the active site.



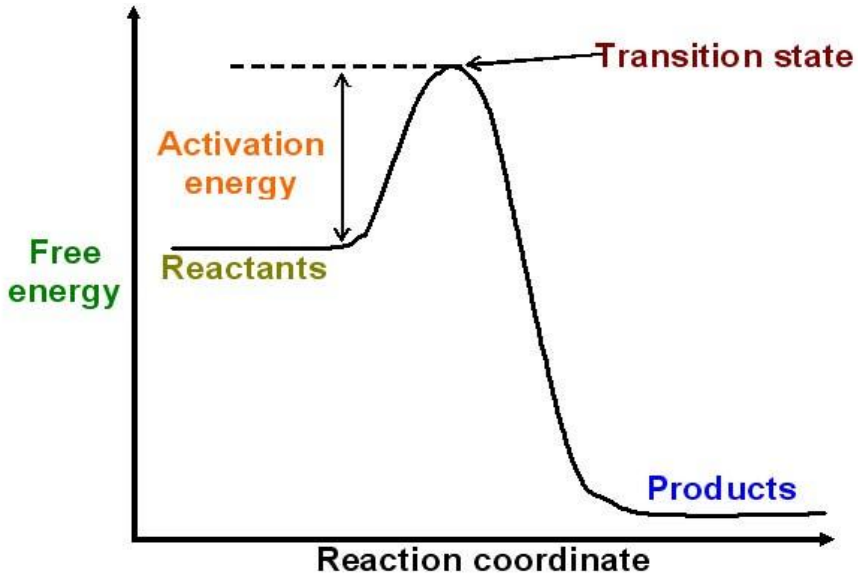
APOENZYME and HOLOENZYME

- The enzyme without its non protein moiety is termed as apoenzyme and it is inactive.
- Holoenzyme is an active enzyme with its non protein component



Why enzymes?

- A bag of sugar can remain on the shelf for years without any obvious conversion to CO_2 and H_2O.
- All chemical reactions require some amount of energy to get them started = THE ACTIVATION ENERGY
- During this part of the reaction the molecules are said to be in a transition state.



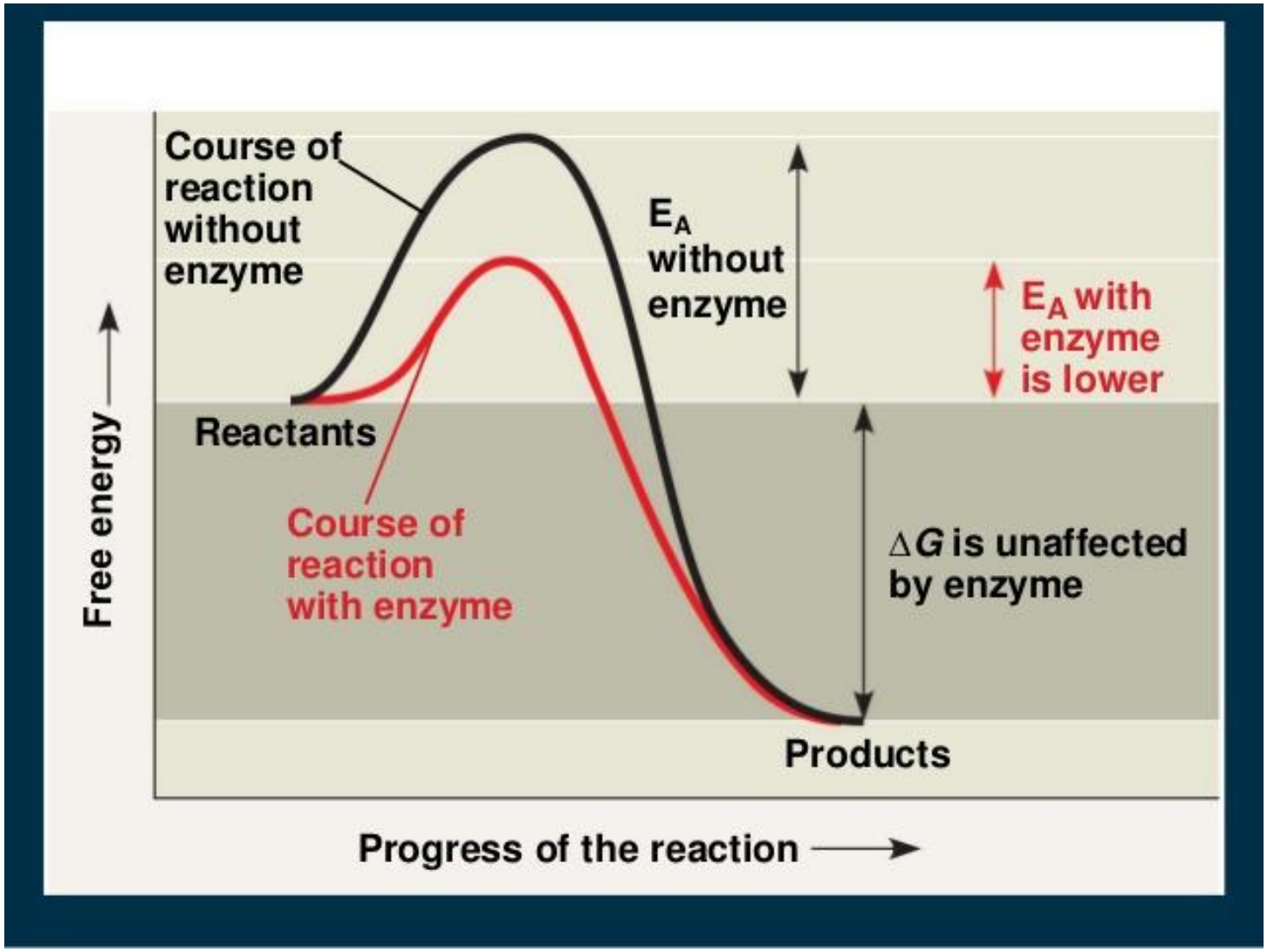
Why enzymes?

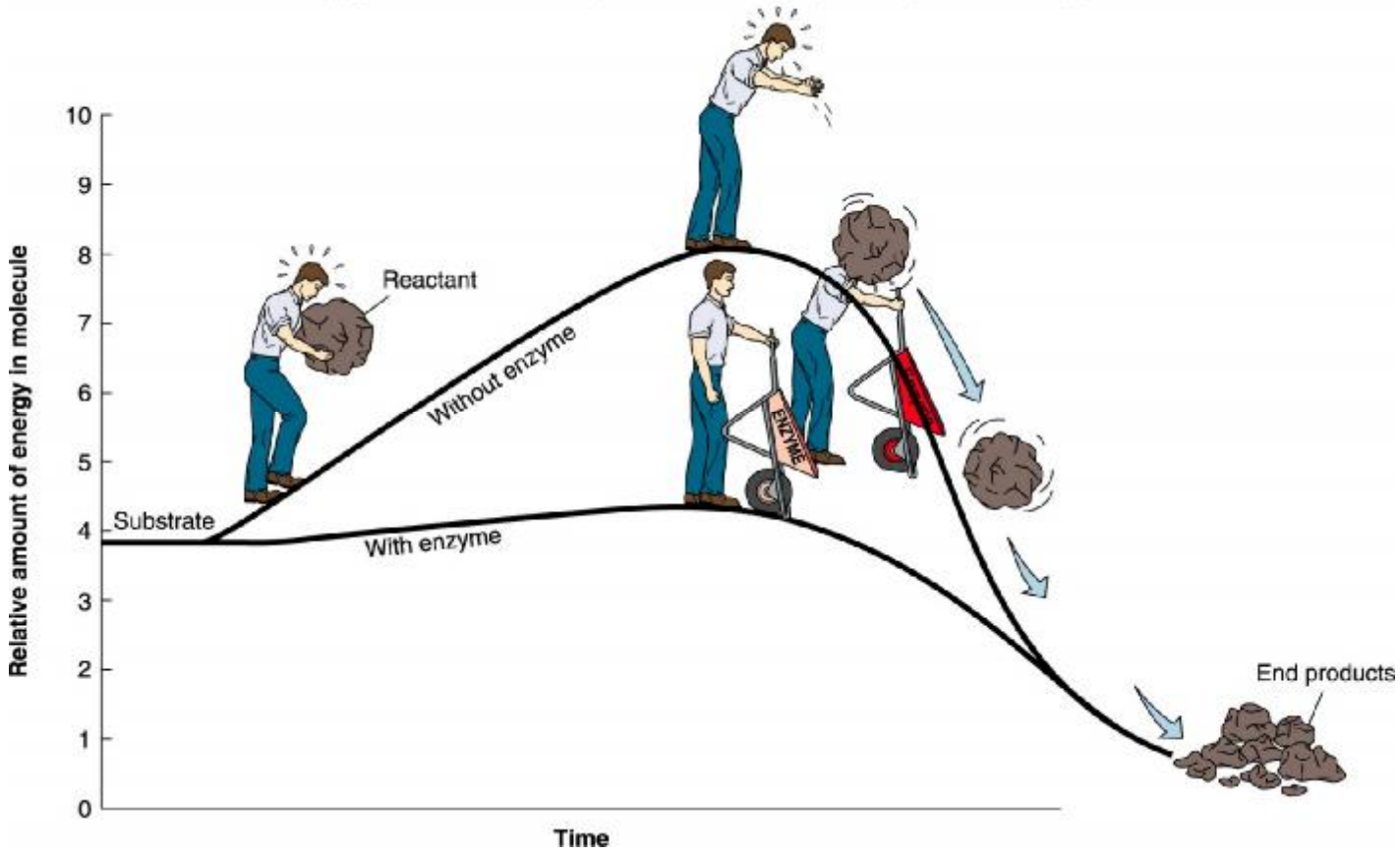
- Increasing the temperature makes molecules move faster : For chemical reactions the Q10 = 2 to 3
- Biological systems are very sensitive to temperature changes
- Enzymes can increase the rate of reactions without the need to increase the temperature
- They do this by lowering the activation energy
- They create a new reaction pathway “a short cut” .

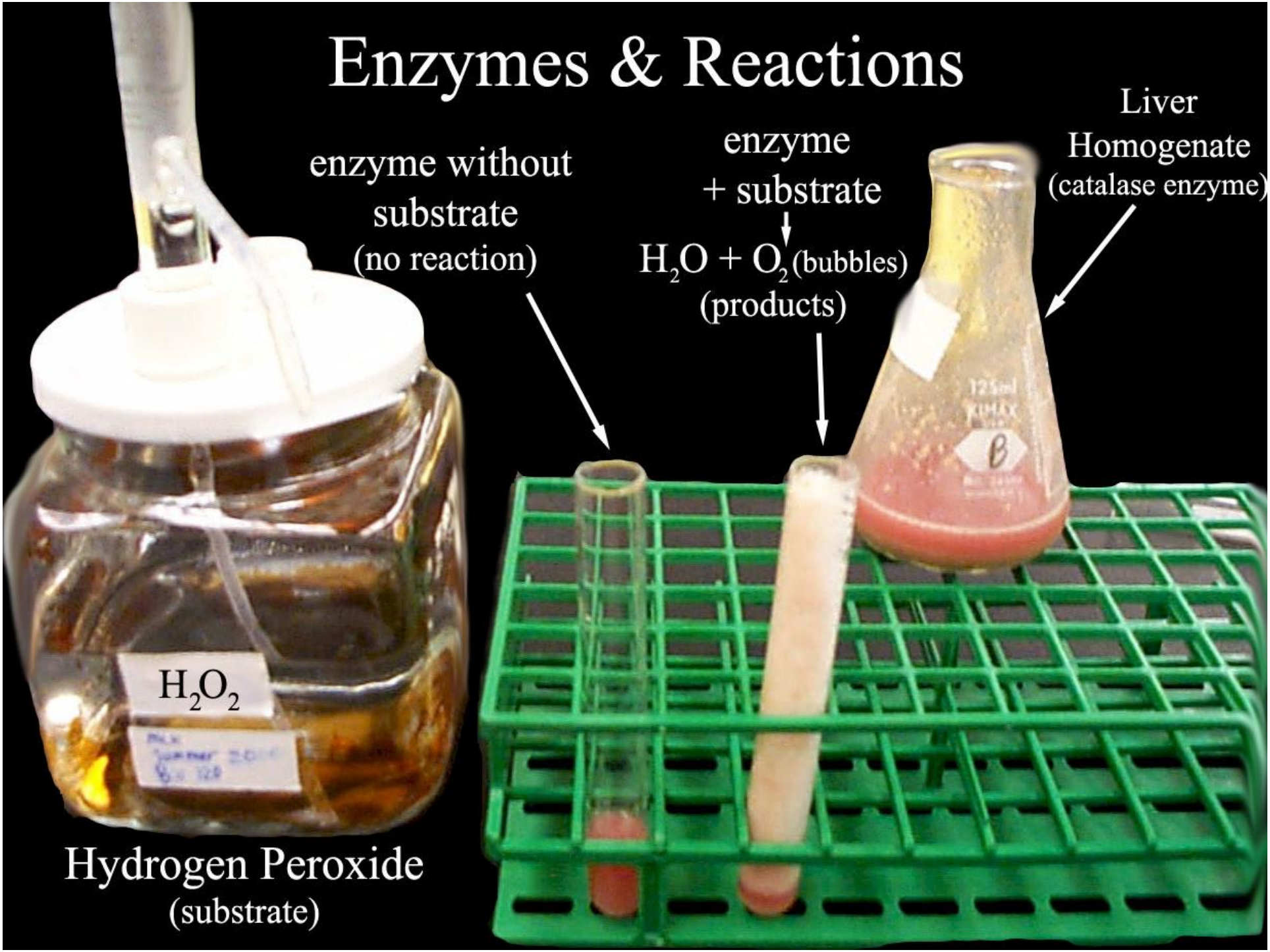
Why enzymes?

- All chemical reactions in living organisms require enzymes to work

Enzyme controlled reactions proceed 10^8 to 10^{11} times faster than corresponding non-enzymic reactions

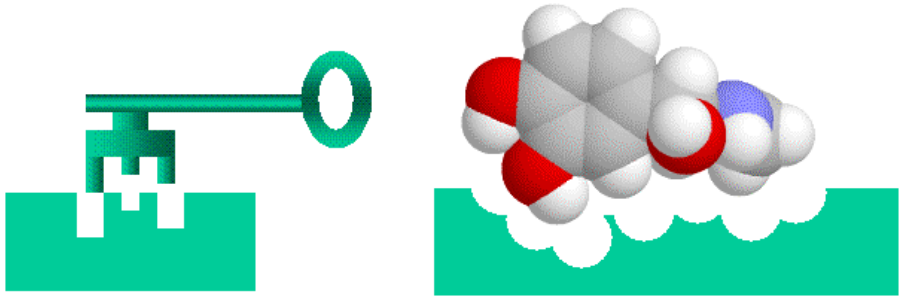




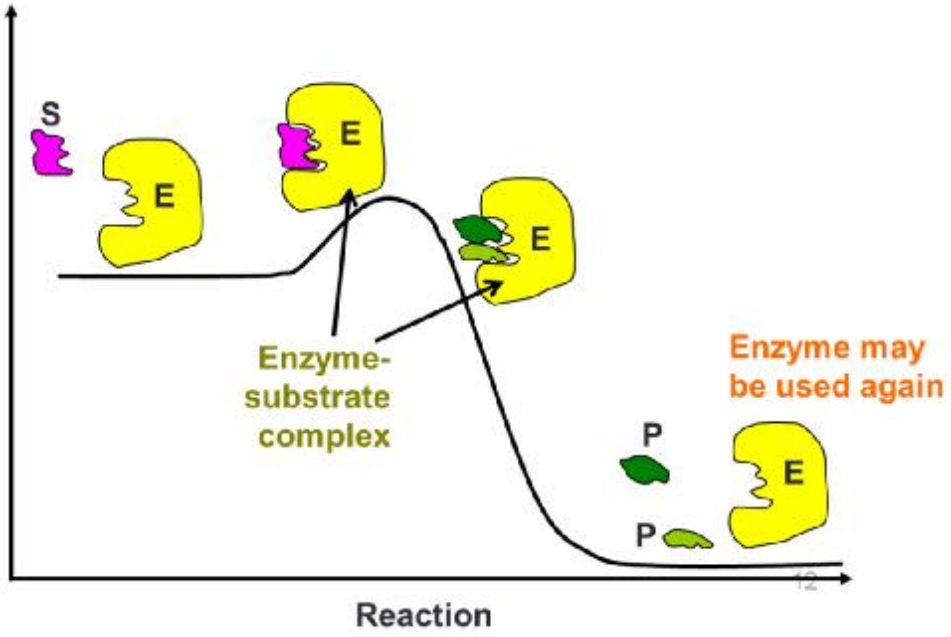

Enzymes & Reactions
enzyme without substrate (no reaction)
enzyme + substrate
$H_2O + O_2$ (bubbles)
(products)
Liver Homogenate (catalase enzyme)
H_2O_2
Hydrogen Peroxide
(substrate)

The Lock and Key Hypothesis

- Fit between the substrate and the active site of the enzyme is exact: Like a key fits into a lock very precisely

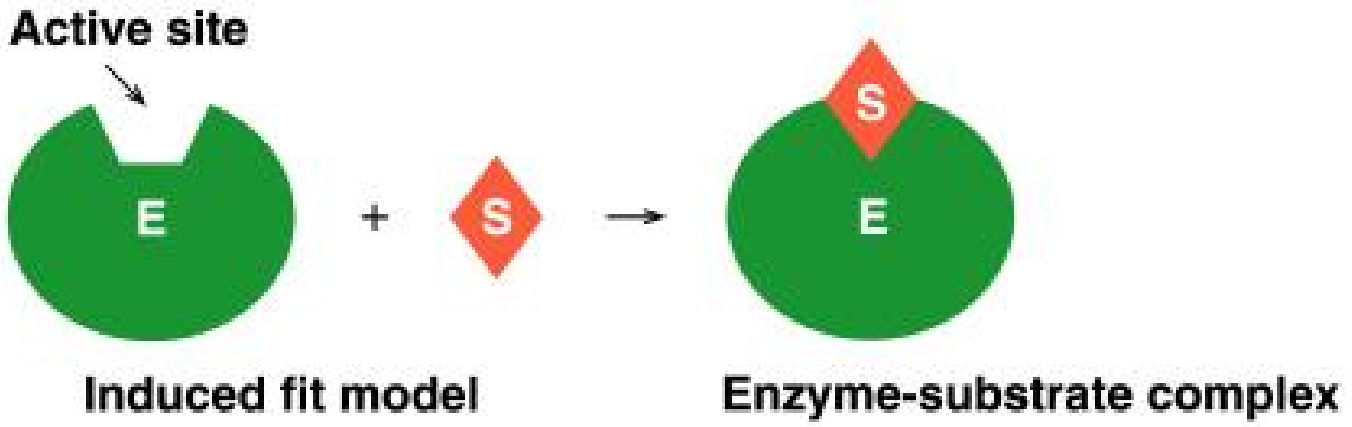


- Products have a different shape from the substrate
- This explains enzyme specificity
- This explains the loss of activity when enzymes denature

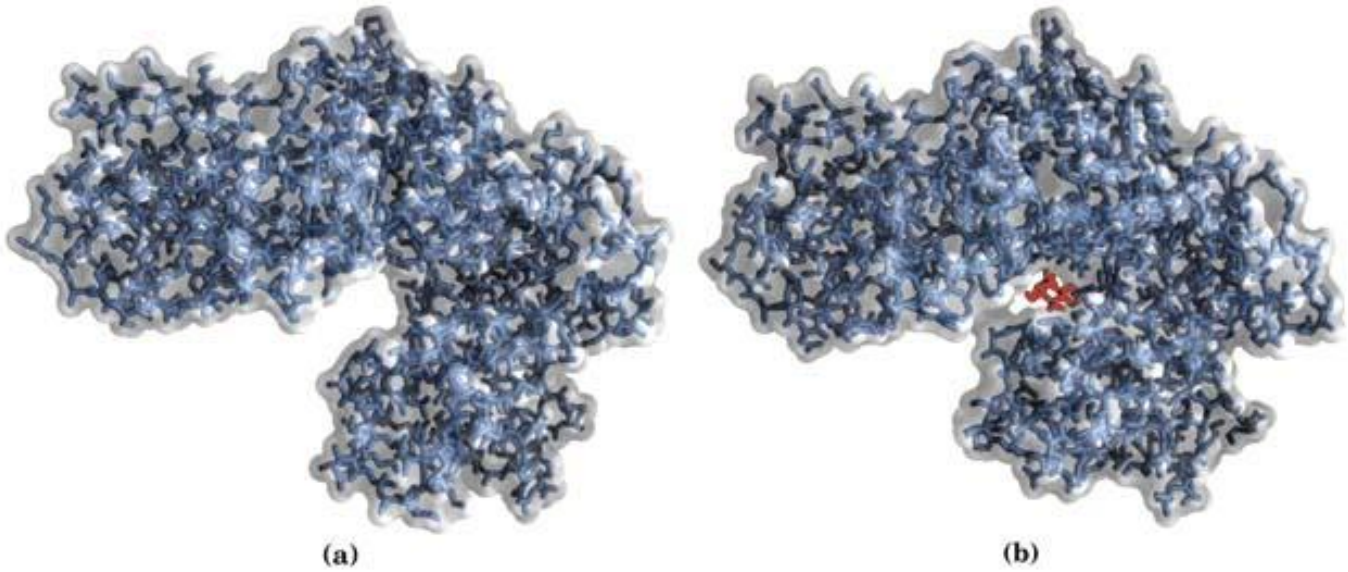


The Induced Fit Hypothesis

- The active site is flexible, not rigid and enzyme can change their shape (conformation)
- Substrate + enzyme, induces a change in the enzyme's conformation
- The chemical environment is now suitable for the reaction
- The bonds of the substrate are stretched to make reaction easier (lowers activation energy).

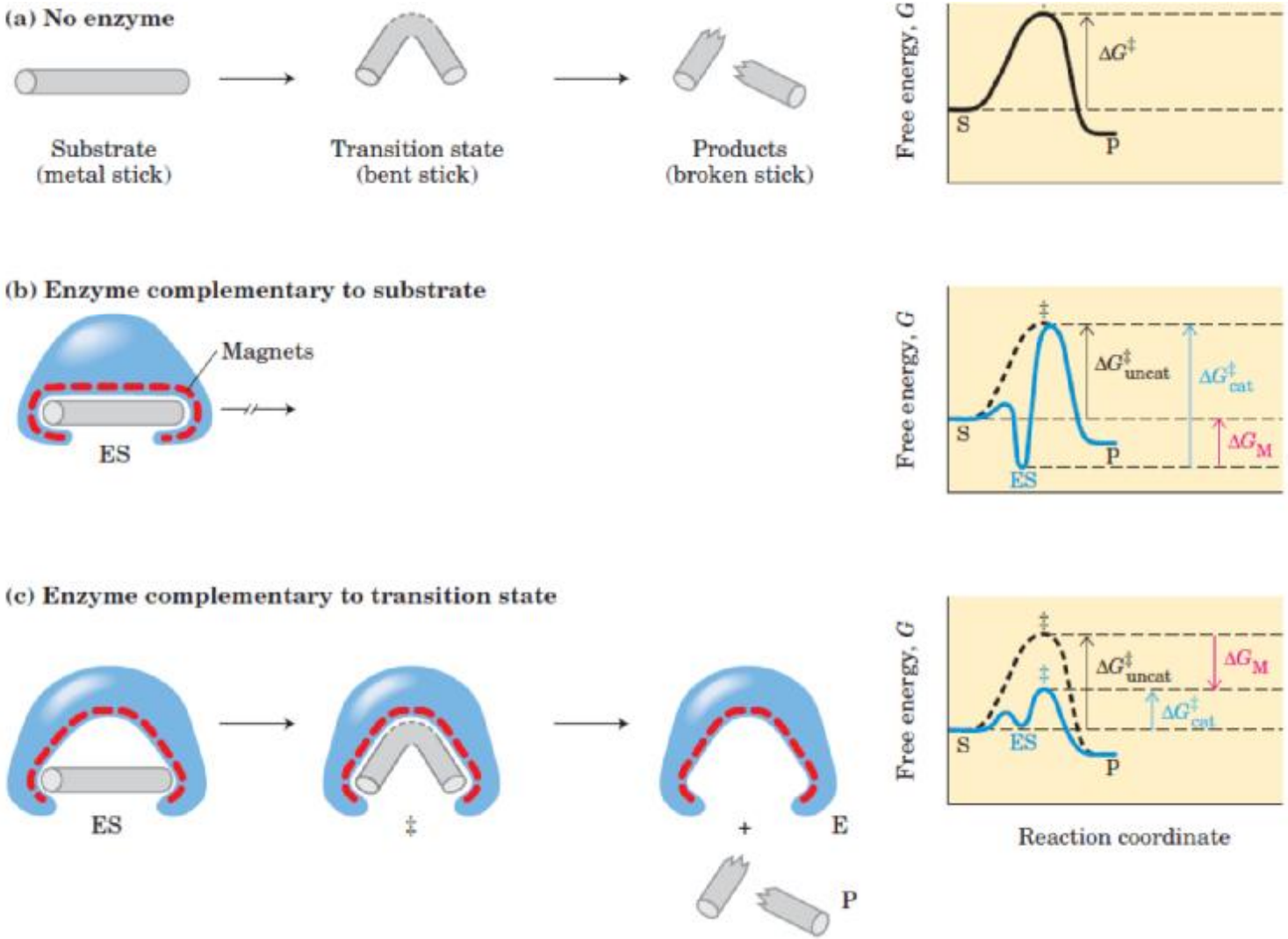


The Induced Fit Hypothesis



Hexokinase (a) without (b) with glucose substrate

- Other mechanisms: Serine protease, acid base catalysis, covalent catalysis, and transition-state stabilization

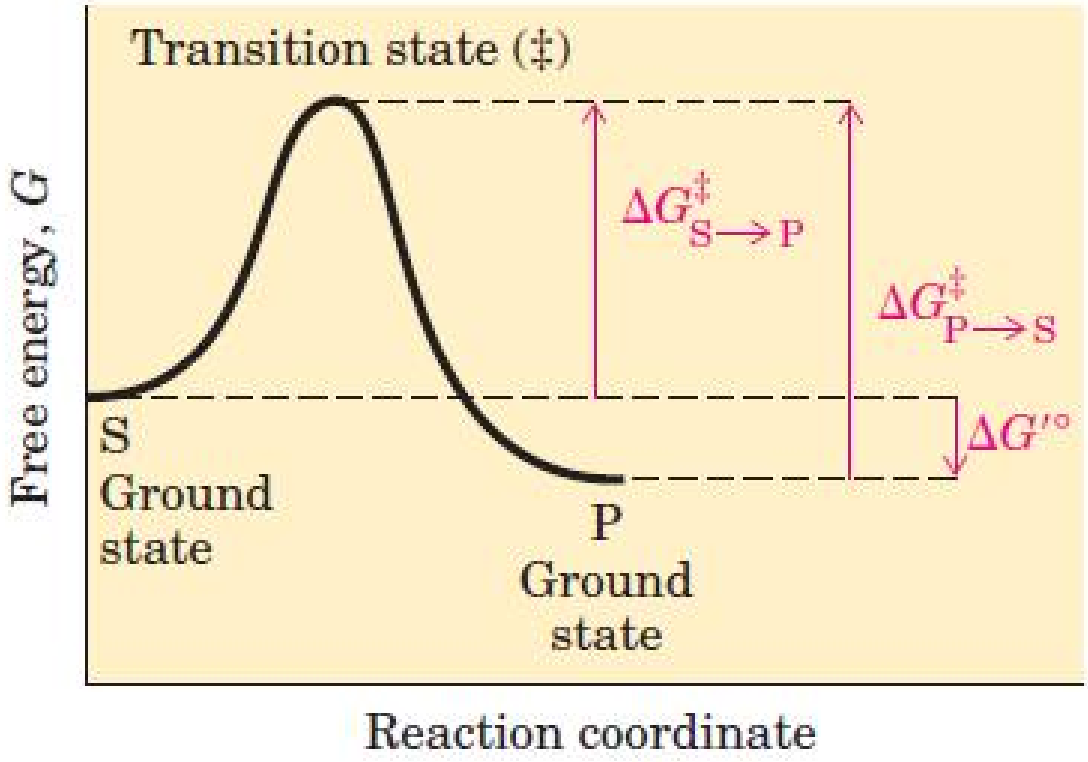

(a) No enzyme
Substrate
(metal stick)
Transition state
(bent stick)
Products
(broken stick)
Free energy, G
$\Delta G^{\ddagger}$
S
P
(b) Enzyme complementary to substrate
Magnets
ES
Free energy, G
$\Delta G^{\ddagger}_{uncat}$
$\Delta G^{\ddagger}_{cat}$
ΔG_M
S
P
ES
(c) Enzyme complementary to transition state
ES
‡
+
E
P
Free energy, G
$\Delta G^{\ddagger}_{uncat}$
ΔG_M
$\Delta G^{\ddagger}_{cat}$
S
ES
P
Reaction coordinate

Factors affecting Enzymes

- Enzyme and substrate concentration
- Environmental Conditions
  - pH, température, Ionic concentration
- Cofactors and Coenzymes
  - Inorganic substances (zinc, iron) and vitamins
- Enzyme Inhibitors

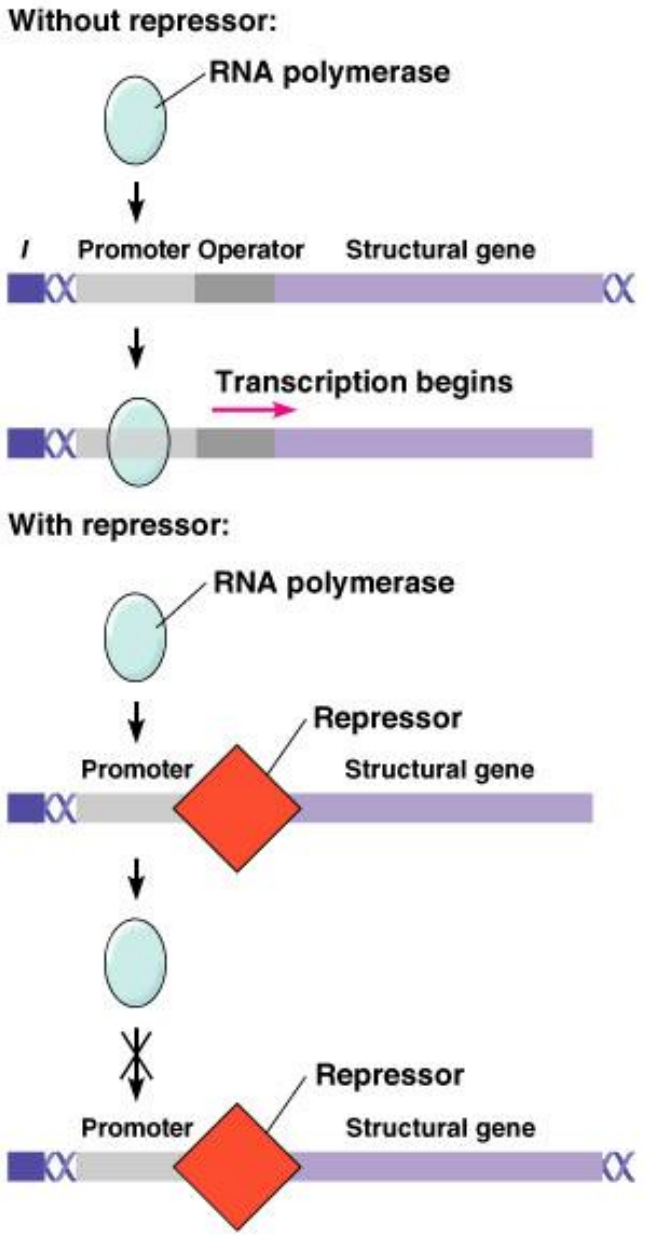
Enzymes Affect Reaction Rates, Not Equilibria

- Thermodynamics as a regulatory force

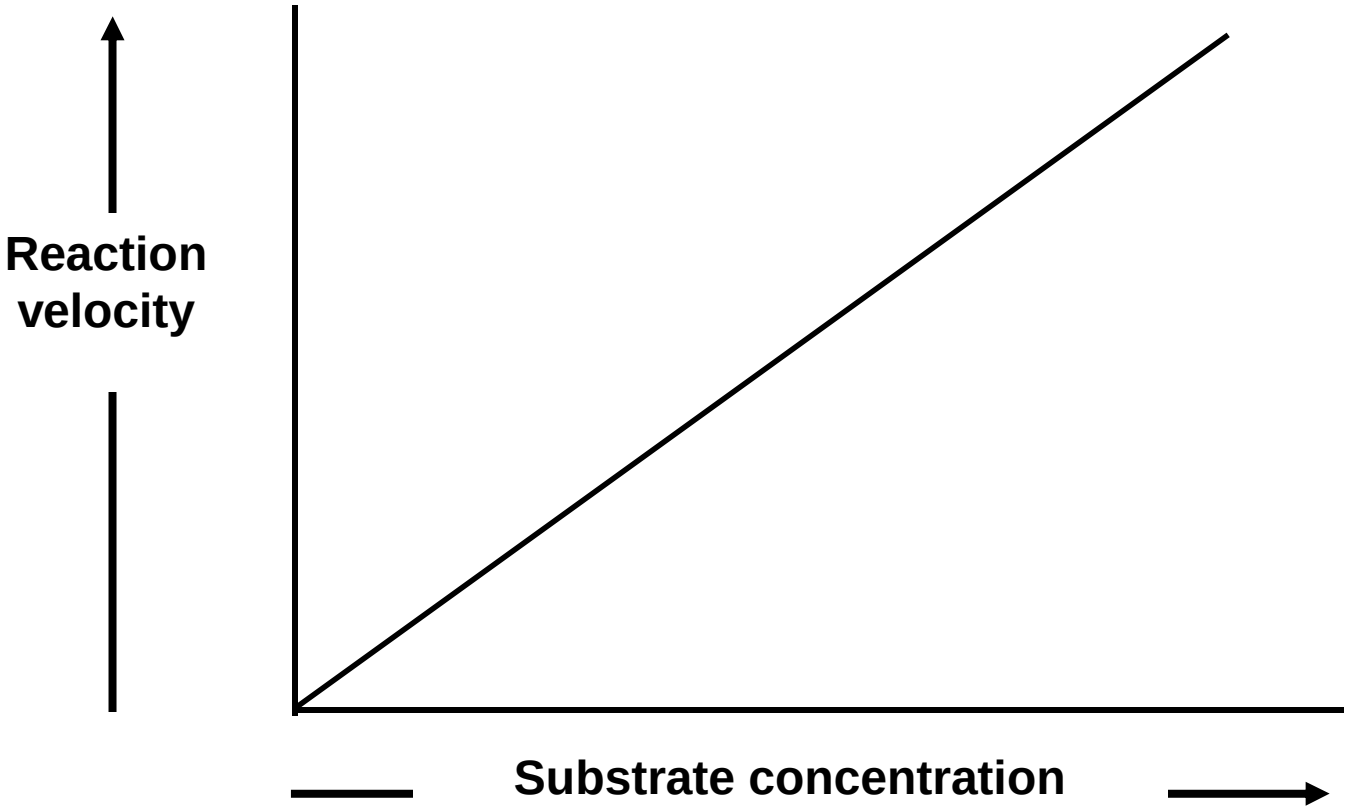


Enzyme availability

- Transcription (and translation)
- Protein processing (degradation)

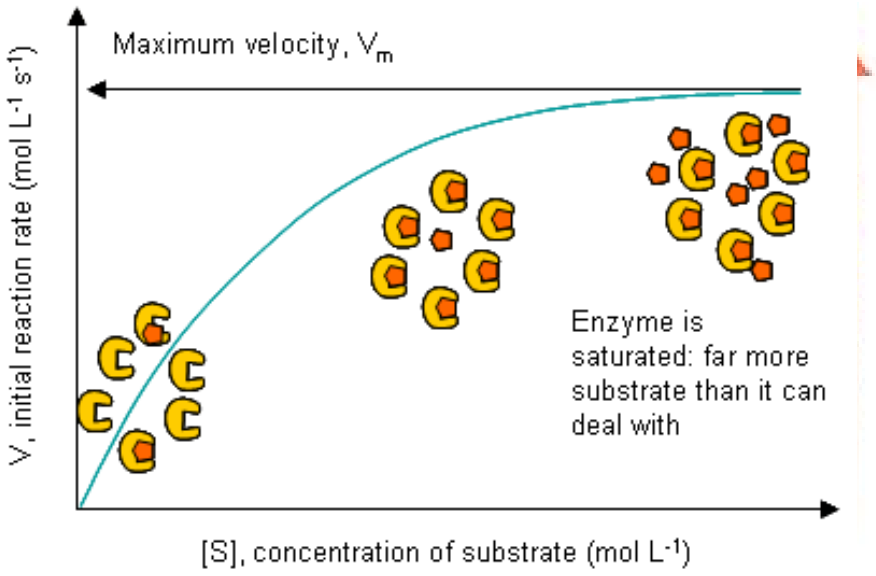


Substrate concentration: Non-enzymic reactions

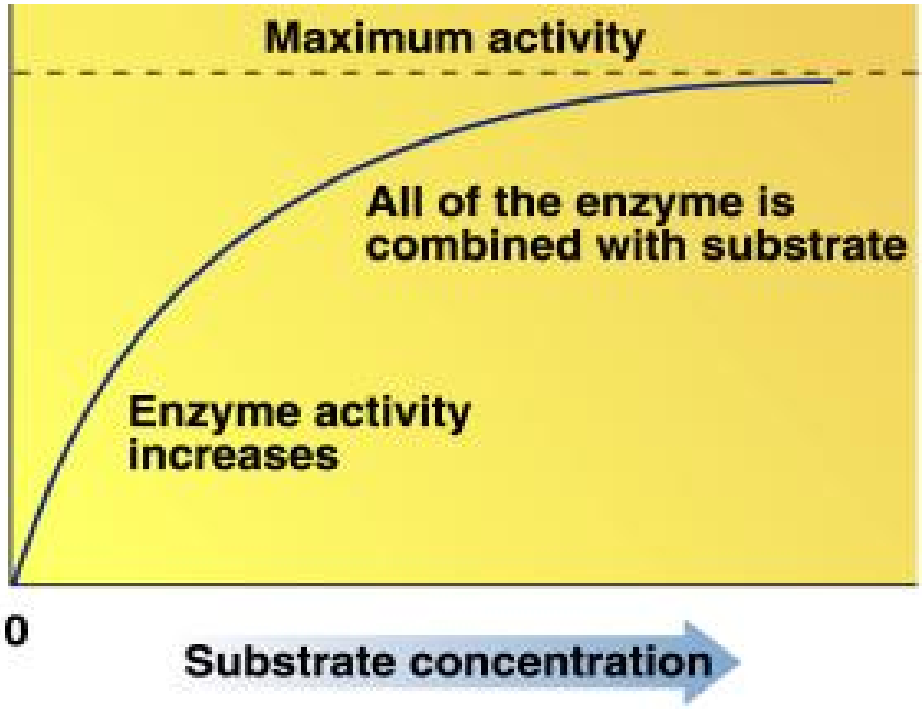


The increase in velocity is proportional to the substrate concentration.

Substrate Concentration and Reaction Rate

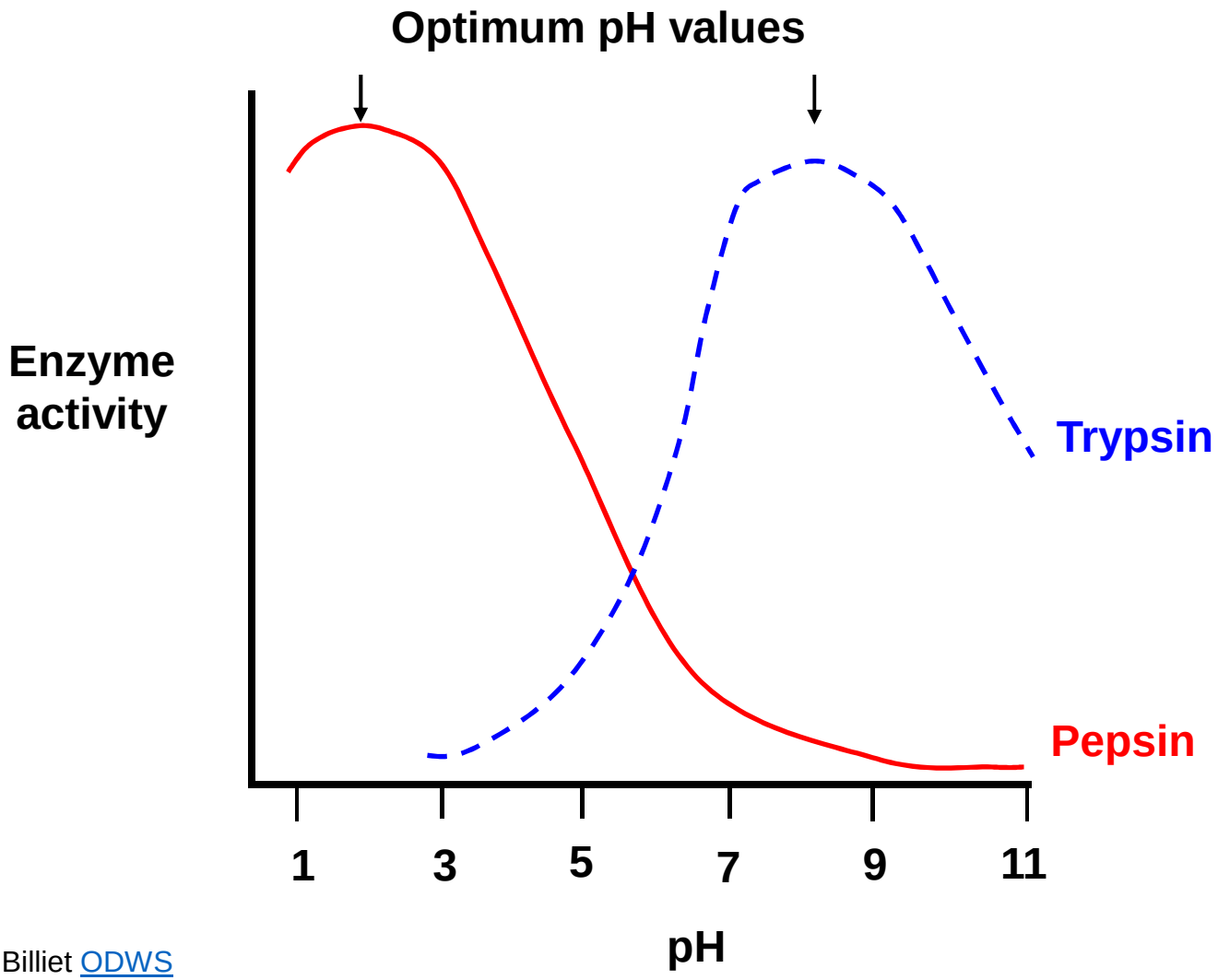


$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$



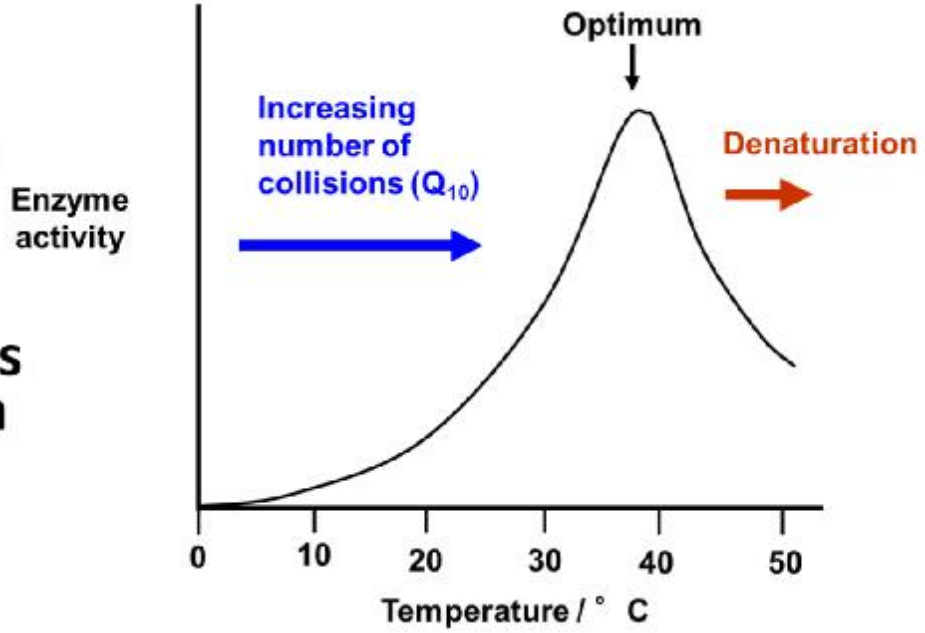
- Faster reaction but it reaches a saturation point when all the enzyme molecules are occupied
- Alter the concentration of the enzyme then V_{max} will change too.

The effect of pH



The effect of temperature

- Optimum temperature human enzymes 35°- 40°C (body temp = 37°C)
- Cold water fish will die at 30°C because their enzymes denature
- A few bacteria have enzymes that can withstand very high temperatures up to 100°C
- Most enzymes however are fully denatured at 70°C.



Some enzymes require cofactor or coenzyme

- Cofactor: inorganic ions

- Coenzyme: a complex organic or metalloorganic molecule

TABLE 6–1 Some Inorganic Elements That Serve as Cofactors for Enzymes

Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

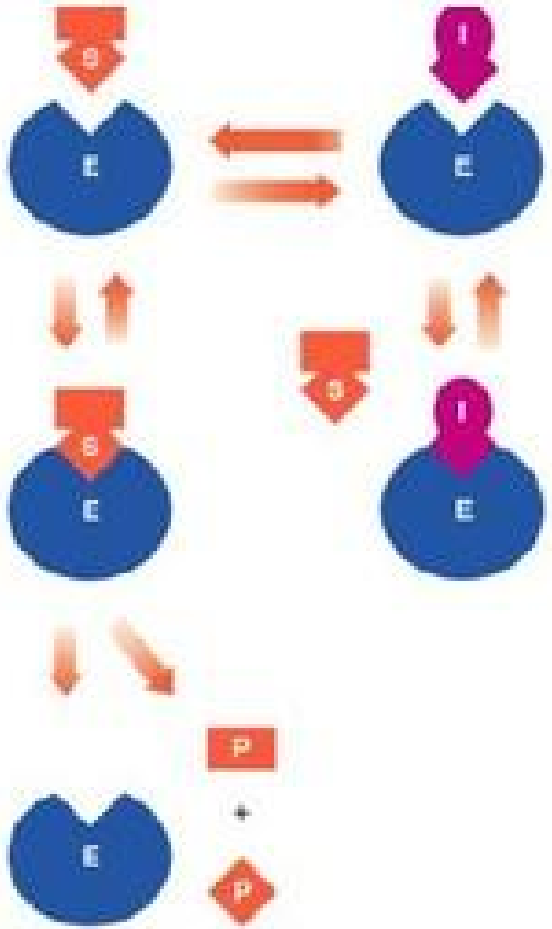
Coenzyme
Biocytin
Coenzyme A
5′-Deoxyadenosylcobalamin (coenzyme B_{12})
Flavin adenine dinucleotide
Lipoate
Nicotinamide adenine dinucleotide
Pyridoxal phosphate
Tetrahydrofolate
Thiamine pyrophosphate

Enzyme Inhibitors

Reversible Competitive Inhibition

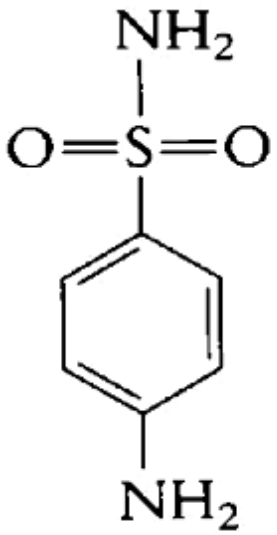
A **competitive inhibitor**:

- Has a structure like the substrate.
- Competes with the substrate for the active site.
- Has its effect reversed by increasing substrate concentration.

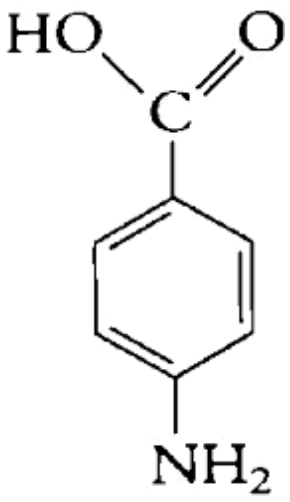


Clinically useful Competitive Inhibition

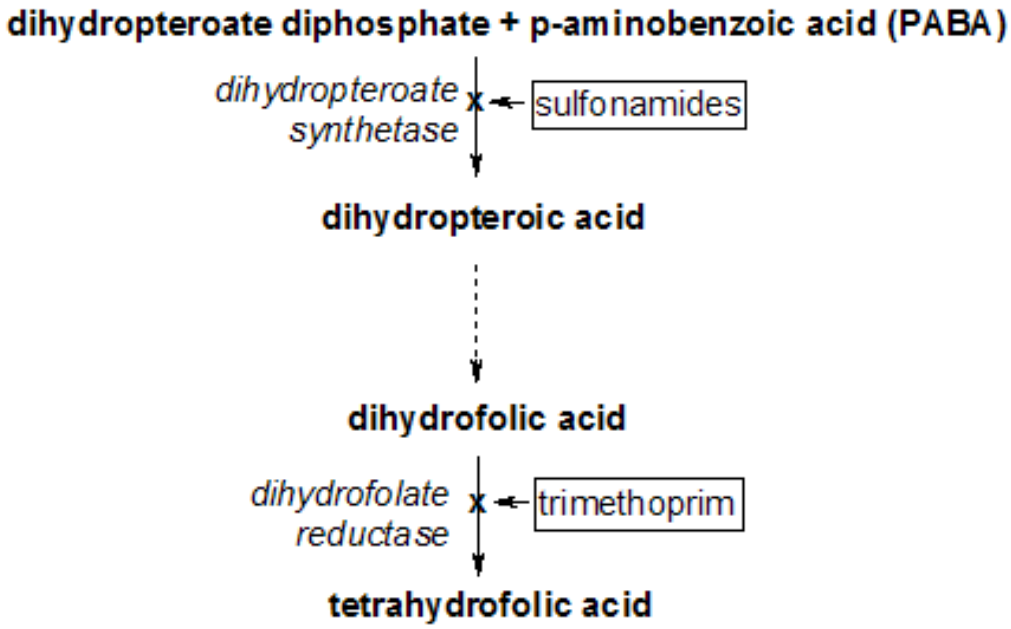
Drugs	Target Enzyme	Therapeutic Use
STATINS - Atorvastatin , simvastatin	HMG CoA reductase	Decrease plasma Cholesterol level - Antihyperlipidemic agents
Allopurinol	Xanthine oxidase	Gout
Methotrexate	Dihydrofolate reductase	Cancer
Captopril & Enalapril	Angiotensin converting enzyme	High blood pressure
Dicoumarol	Vit.K-epoxide-reductase	Anti-coagulant



Sulfanilamide



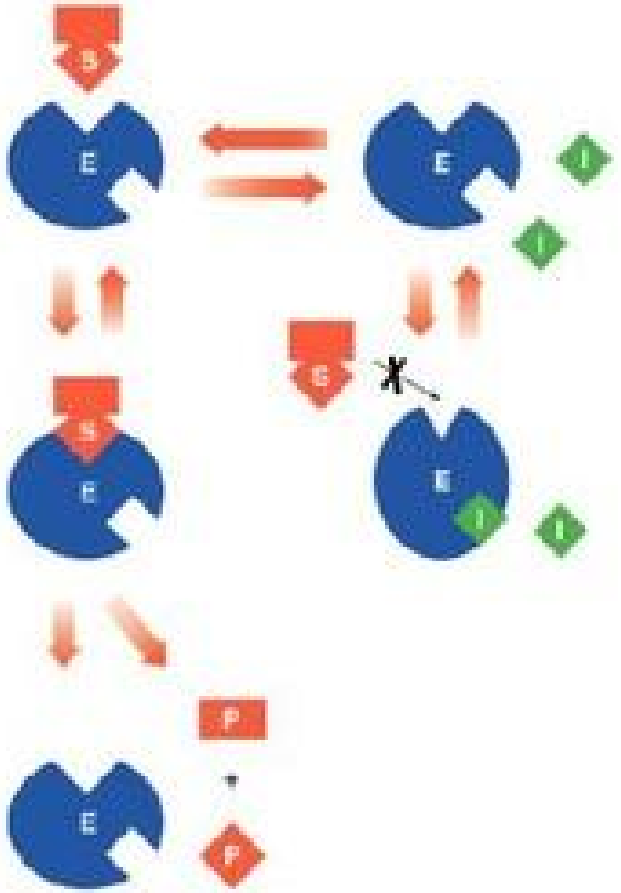
PABA



Noncompetitive Inhibition

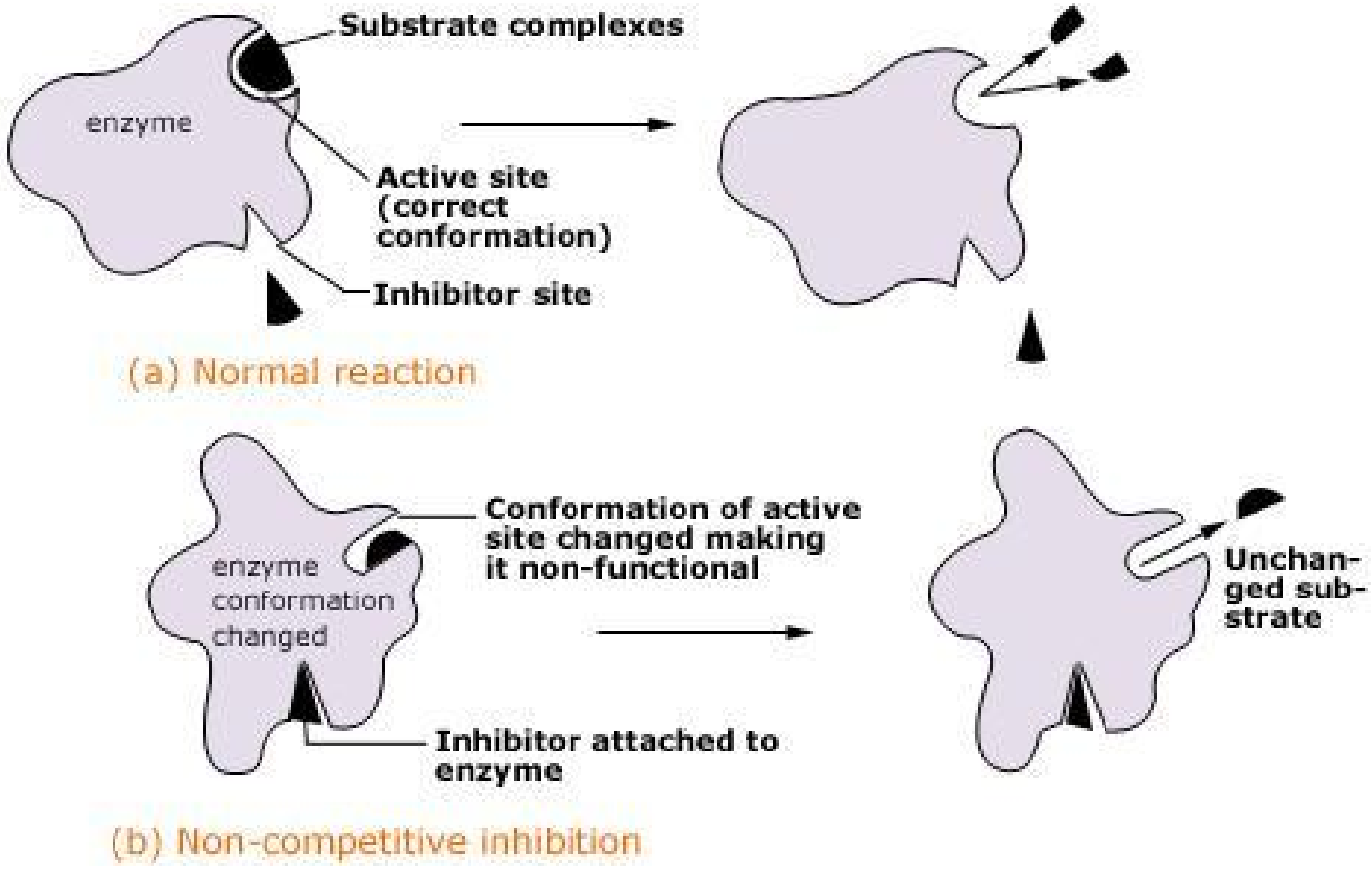
A **noncompetitive inhibitor**:

- Has a structure different than the substrate.
- Distorts the shape of the enzyme, which alters the shape of the active site.
- Prevents the binding of the substrate.
- Cannot have its effect reversed by adding more substrate.



Non-competitive

- @ allosteric site



Un-competitive

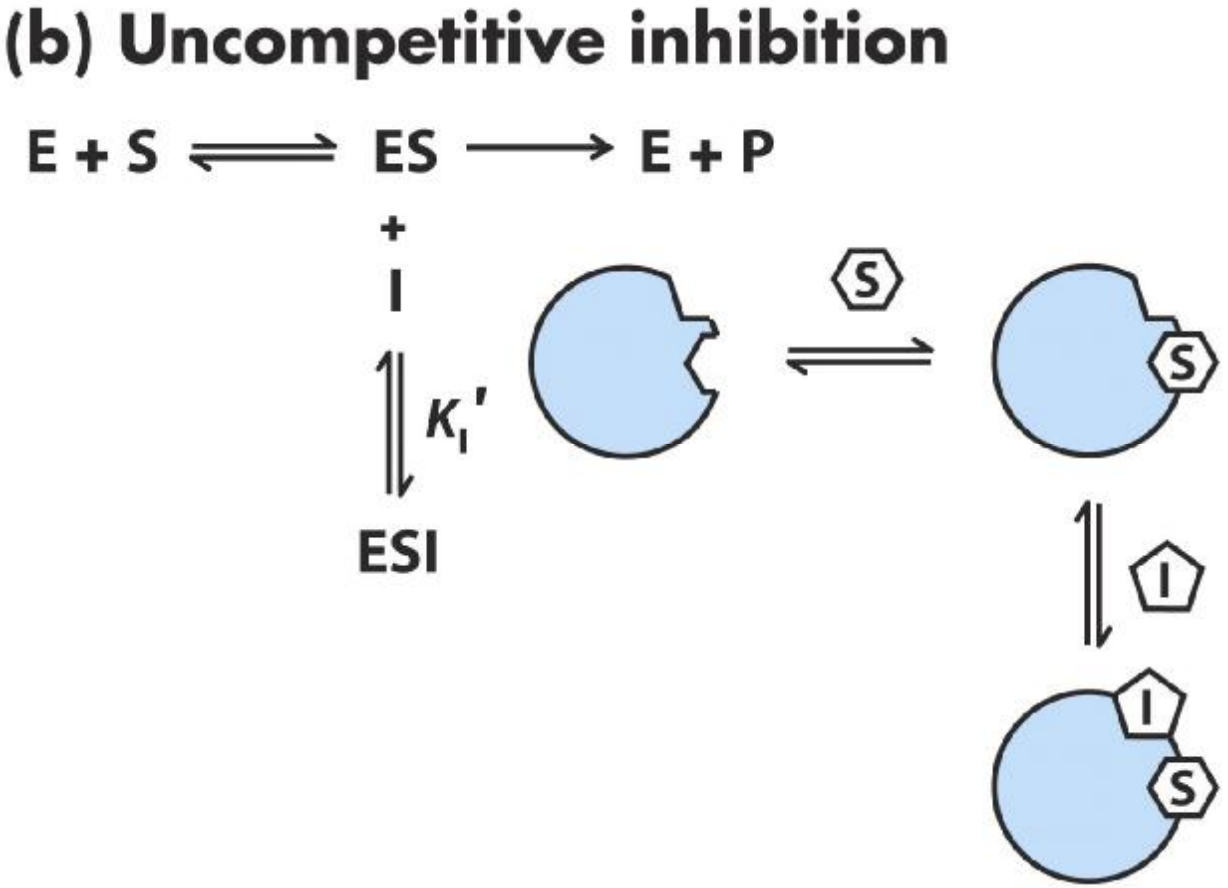
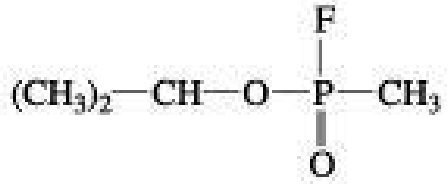
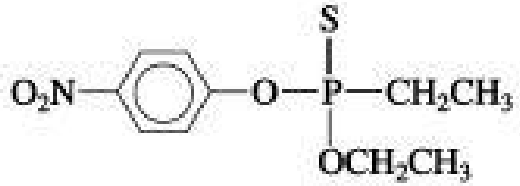
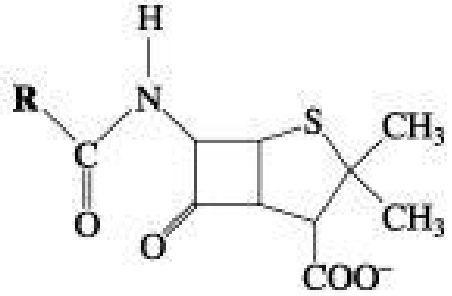
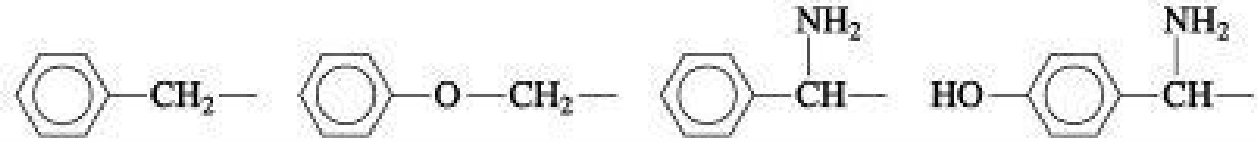


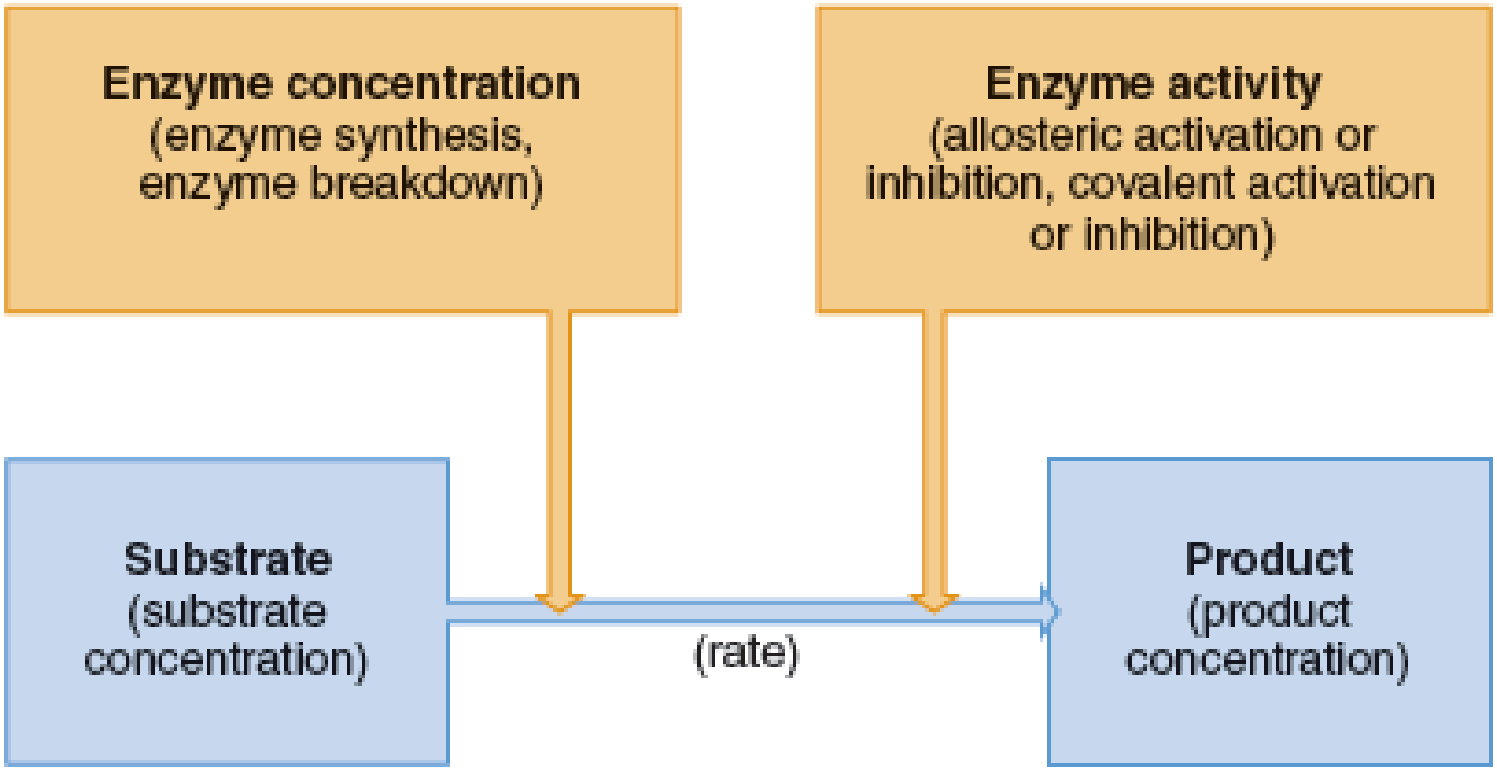
Table 21.6 Selected Irreversible Enzyme Inhibitors

Name	Structure	Natural/Synthetic Source	Inhibitory Action
Cyanide	CN^-	Bitter almonds	Bonds to metal ions in enzymes in the electron transport chain
Sarin		Nerve gas	Similar to DFP
Parathion		Insecticide	Similar to DFP
Penicillin		*Penicillium* fungus	Inhibits enzymes that build cell walls in bacteria

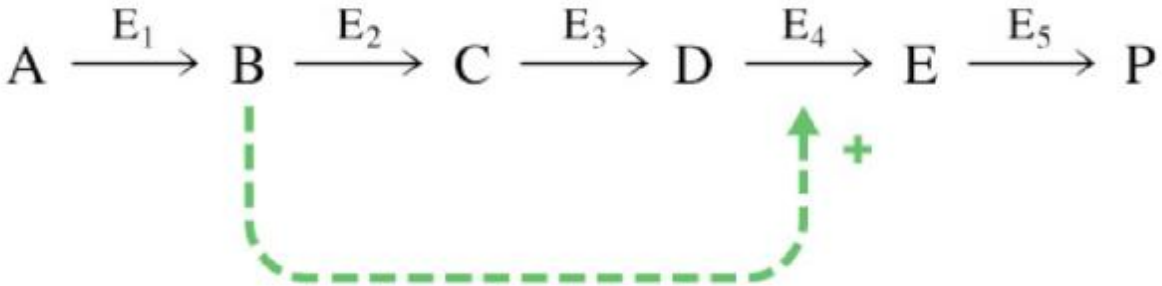
R Groups for Penicillin Derivatives



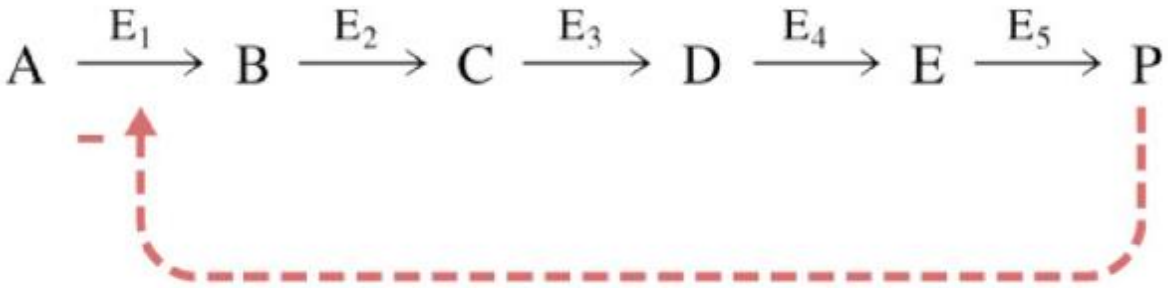
Enzyme regulation



Allosteric modification



(10.2)

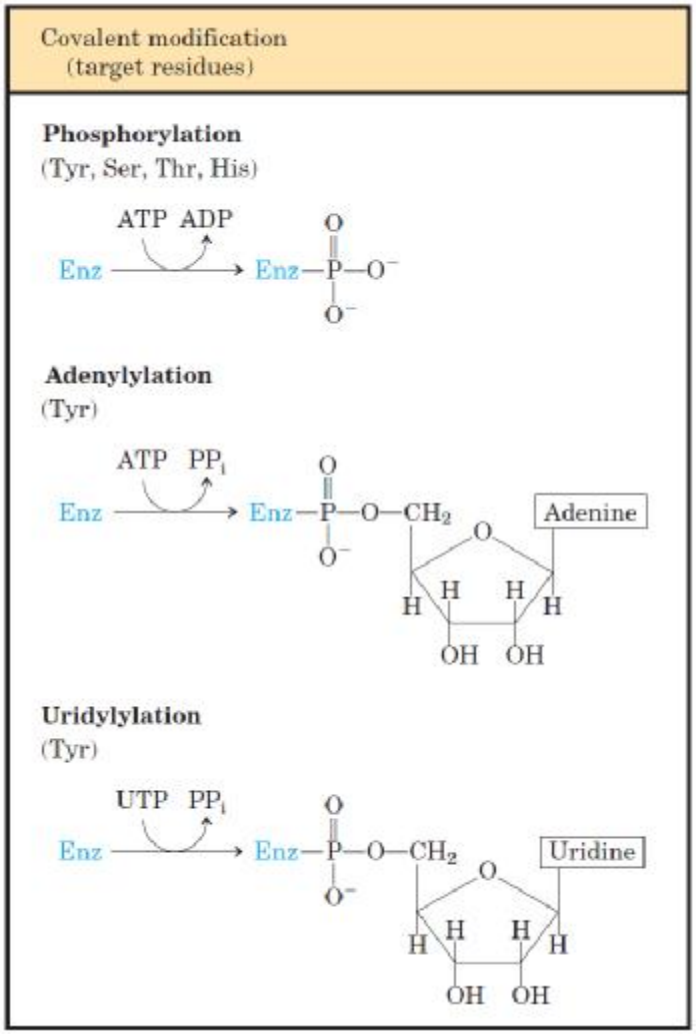


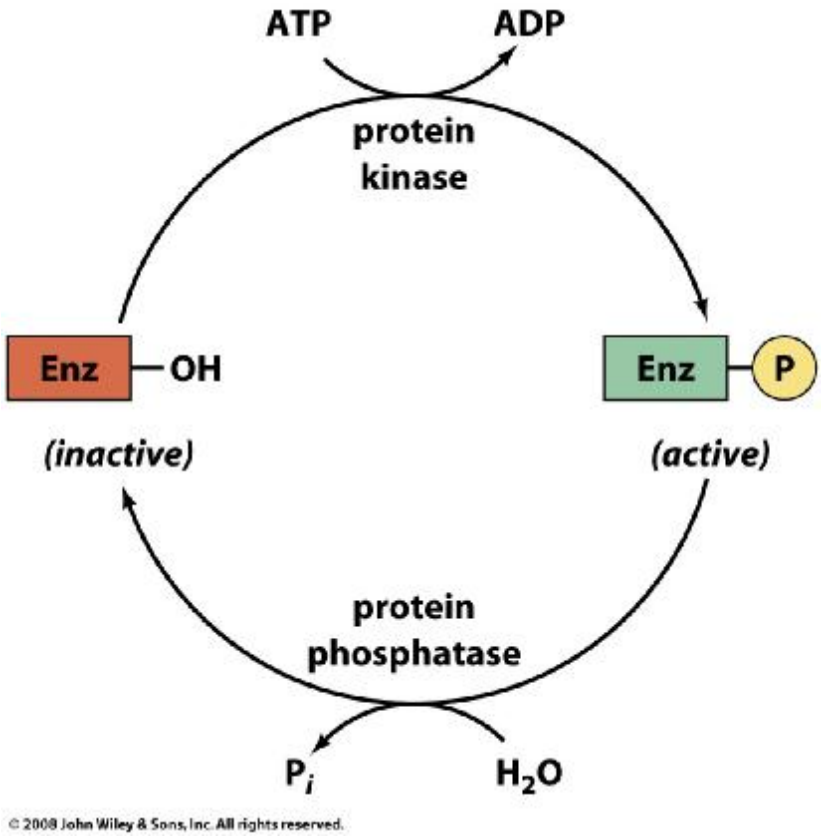
(10.1)

Covalent modification

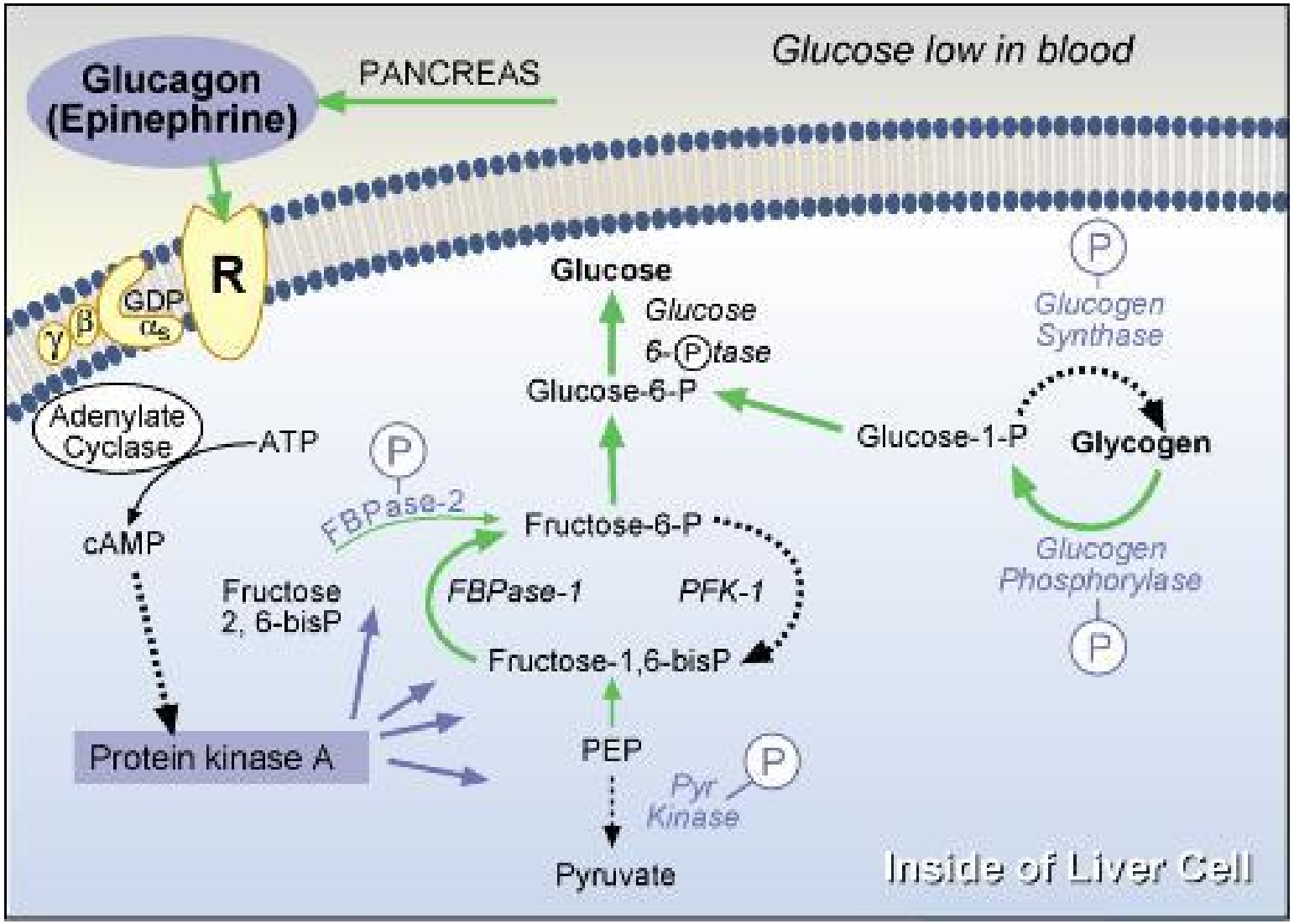
- Covalent modification of an enzyme by phosphate molecules can turn it on or off
- Usually catabolic enzymes are stimulated by phosphorylation and anabolic enzymes are turned off, but not always
- Phosphatases catalyze dephosphorylation; these have the opposite effects

Covalent modification





Covalent modification by glucagon



Naming Enzymes

- The name of an enzyme in many cases end in *–ase*, *sucrase* catalyzes the hydrolysis of sucrose

- Sometimes common names are used, particularly for the digestion enzymes such as *pepsin* and *trypsin*

- Some names describe both the substrate and the function, *alcohol dehydrogenase* oxides ethanol

EC number Classification

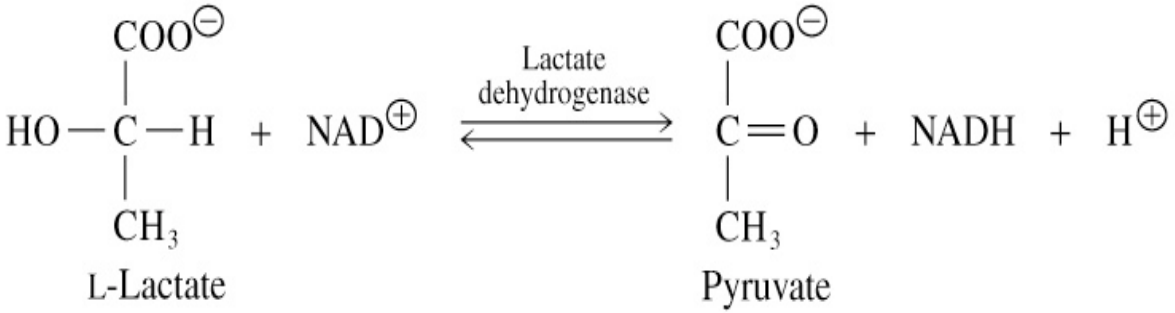
- The International Union of Biochemists (I.U.B.).

- EC 1. Oxidoreductases
- EC 2. Transferases
- EC 3. Hydrolases
- EC 4. Lyases
- EC 5. Isomerases
- EC 6. Ligases

- Each enzyme has classification number consisting of four digits:
- Example, EC: (2.7.1.1) HEXOKINASE

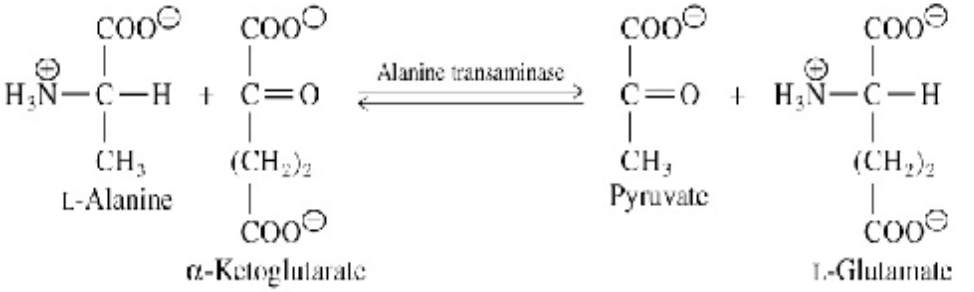
EC 1. Oxidoreductases

- Catalyze Oxidation/Reduction Reactions Act on many chemical groupings to add or remove hydrogen/oxygen atoms.
- e.g. Lactate dehydrogenase, Glucose Oxidase, Peroxidase, Catalase, Phenylalanine hydroxylase.



EC 2. Transferases

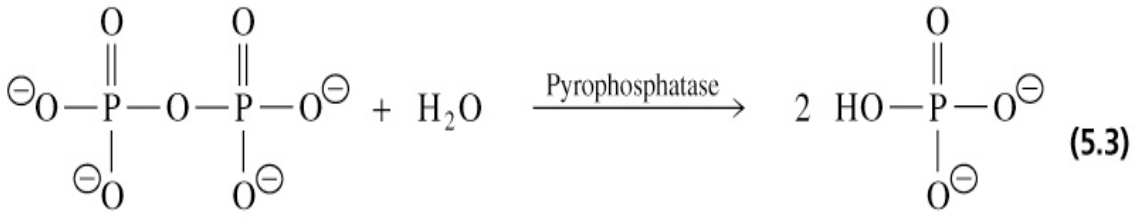
- Biochemical Activity:
  - Transfer a functional groups (e.g. methyl or phosphate) between donor and acceptor molecules.
- Examples:
  - Transaminases (ALT & AST).
  - Phosphotransferases (Kinases).
  - Transmethylases.
  - Transpeptidases.
  - Transacylases.



(5.2)

EC 3. Hydrolases

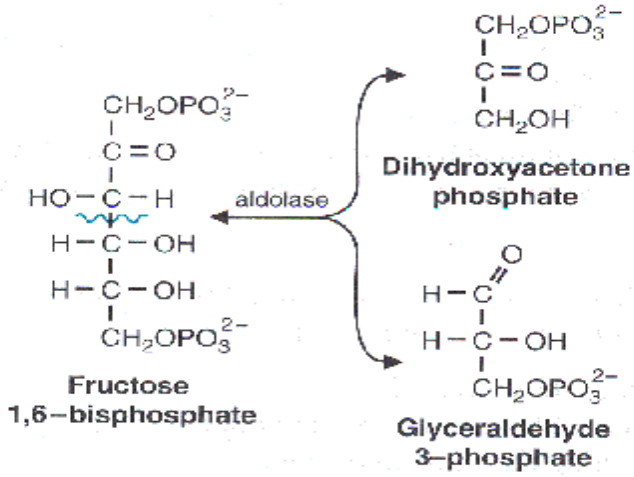
- Biochemical Activity:
  - Catalyse the hydrolysis of various bonds Add water across a bond.
- Examples:
  - Protein hydrolyzing enzymes (Peptidases).
  - Carbohydrases (Amylase, Maltase, Lactase).
  - Lipid hydrolyzing enzymes (Lipase).
  - Deaminases.
  - Phosphatases.



(5.3)

EC 4. Lyases

- Biochemical Activity:
  - Cleave various bonds by means other than hydrolysis and oxidation.
  - Add Water, Ammonia or Carbon dioxide across double bonds, or remove these elements to produce double bonds.
- Examples:
  - Fumarase.
  - Carbonic anhydrase.

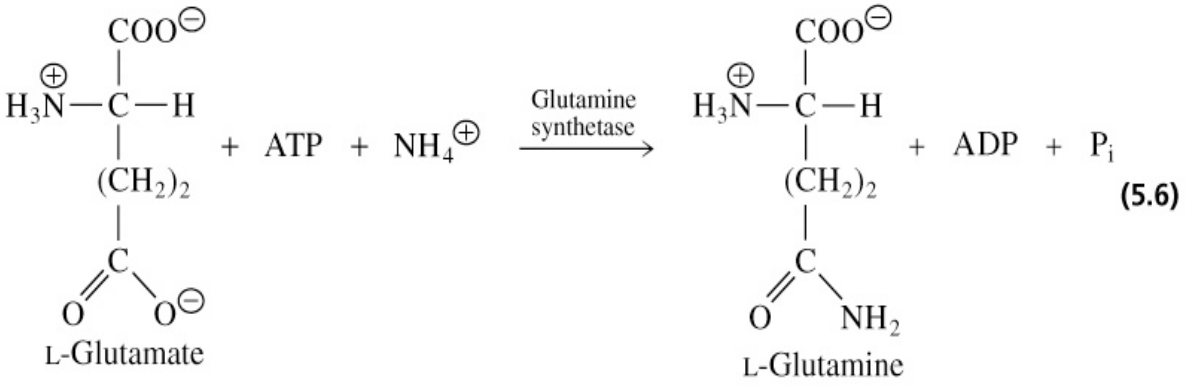


EC 5. Isomerases

- Biochemical Activity:
  - Catalyse isomerization changes within a single molecule.
  - Carry out many kinds of isomerization:
    - L to D isomerizations.
    - Mutase reactions (Shifts of chemical groups).
- Examples:
  - Isomerase.
  - Mutase.

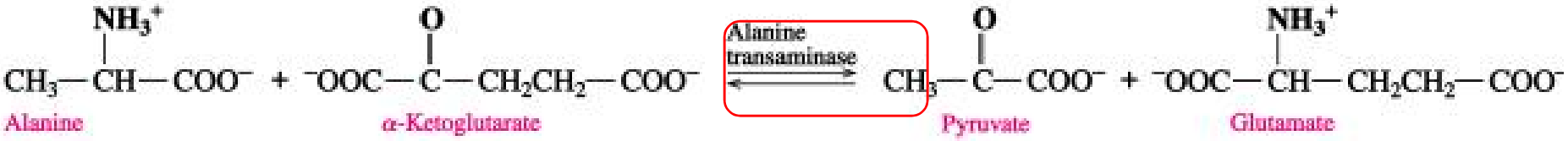
EC 6. Ligases

- Biochemical Activity:
  - Join two molecules with covalent bonds Catalyse reactions in which two chemical groups are joined (or ligated) with the use of energy from ATP.
- Examples:
  - Acetyl~CoA Carboxylase.
  - Glutamine synthetase

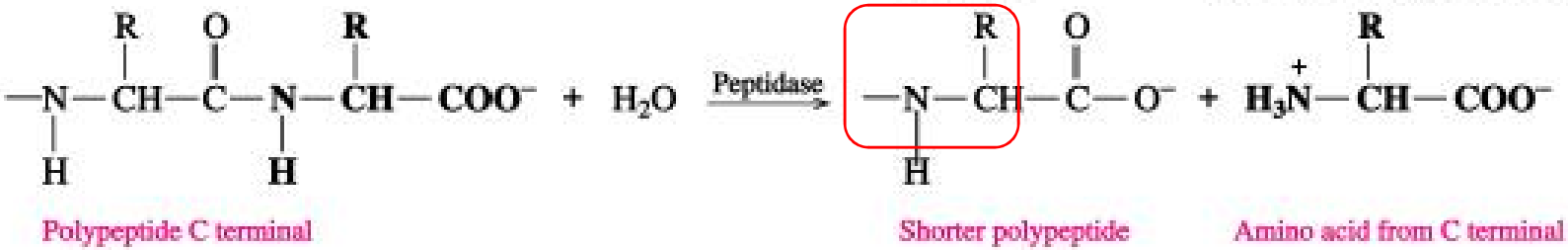


Class	General Reactions Catalyzed	Typical Subclasses	Function
1. Oxidoreductases	Oxidation–reduction reactions	Oxidases Reductases Dehydrogenases	Oxidation Reduction Remove 2H to form double bonds

2. Transferases	Transfer of functional groups	Transaminases Kinases	Transfer amino groups Transfer phosphate groups



3. Hydrolases	Hydrolysis reactions	Peptidases Lipases Amylases	Hydrolyze peptide bonds Hydrolyze ester bonds in lipids Hydrolyze 1,4-glycosidic bonds in amylose



Class	General Reactions Catalyzed	Typical Subclasses	Function
4. Lyases	Addition of a group to a double bond or removal of a group from a double bond without hydrolysis or oxidation	Decarboxylases Dehydrases Deaminases	Remove CO_2 Remove H_2O Remove NH_3

5. Isomerases	Rearrangement of atoms to form isomers	Isomerases Epimerases	Convert cis and trans Convert D and L isomers

6. Ligases	Bonding of molecules using ATP energy	Synthetases Carboxylases	Combine molecules Add CO_2

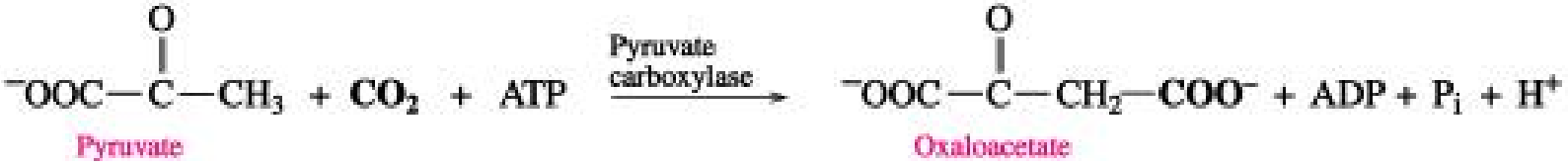


TABLE 12-1 CLASSIFICATION OF FREQUENTLY QUANTITATED ENZYMES

CLASS	RECOMMENDED NAME	COMMON ABBREVIATION	STANDARD ABBREVIATION	EC CODE NO.	SYSTEMATIC NAME
Oxidoreductases	Lactate dehydrogenase	LDH	LDH	1.1.1.27	L-Lactate:NAD^+ oxidoreductase
	Glucose-6-phosphate dehydrogenase	G-6-PDH	G-6-PD	1.1.1.49	D-Glucose-6-phosphate:$NADP^+$ 1-oxidoreductase
	Glutamate dehydrogenase	GLD	GLD	1.4.1.3	L-glutamate:NAD(P) oxidoreductase, deaminase
Transferases	Aspartate amino-transferase	GOT (glutamate oxaloacetate transaminase)	AST	2.6.1.1	L-Aspartate:2-oxaloglutarate aminotransferase
	Alanine amino-transferase	GPT (glutamate transaminase)	ALT	2.6.1.2	L-Alanine:2-oxaloglutarate aminotransferase
	Creatine kinase	CPK (creatine phosphokinase)	CK	2.7.3.2	ATP:creatine *N*-phosphotransferase
	γ-Glutamyl-transferase	GGTP	GGT	2.3.2.2	(5-Glutamyl)peptide: amino acid-5-glutamyltransferase
	Glutathione-*S*-transferase	α-GST	GST	2.5.1.18	Glutathione transferase
	Glycogen phosphorylase	GP	GP	2.4.1.1	1,4-α-D-Glucan: orthophosphate α-D-glucosyltransferase
	Pyruvate kinase	PK	PK	2.7.1.40	Pyruvate kinase
Hydrolases	Alkaline phosphatase	ALP	ALP	3.1.3.1	Orthophosphoric monoester phosphohydrolase (alkaline optimum)
	Acid phosphatase	ACP	ACP	3.1.3.2	Orthophosphoric monoester phosphohydrolase (acid optimum)
	α-Amylase	AMY	AMS	3.2.1.1	1,4-D-Glucan glucanohydrolase
	Cholinesterase	PCHE	CHE	3.1.1.8	Acylcholine acylhydrolase
	Chymotrypsin	CHY	CHY	3.4.21.1	Chymotrypsin
	Elastase-1	E1	E1	3.4.21.36	Elastase
	5-Nucleotidase	NTP	NTP	3.1.3.5	5′-Ribonucleotide phosphohydrolase
	Triacylglycerol lipase		LPS	3.1.1.3	Triacylglycerol acylhydrolase
	Trypsin	TRY	TRY	3.4.21.4	Trypsin
Lyases	Aldolase	ALD	ALD	4.1.2.13	D-D-Fructose-1, 6-bisdiphosphate D-glyceraldehyde-3-phosphate-lyase
Isomerases	Triosephosphate isomerase	TPI	TPI	5.3.1.1	Triose-phosphate isomerase
Ligase	Glutathione Synthetase	GSH-S	GSH-S	6.3.2.3	Glutathione synthase

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Regulatory Enzymes

- The activities of metabolic pathways in cells are regulated by control of the activities of certain enzymes
- The activity of allosteric enzymes is adjusted by reversible binding of a specific modulator to a regulatory site. Modulators may be the substrate itself or some other metabolite, and the effect of the modulator may be inhibitory or stimulatory. The kinetic behavior of allosteric enzymes reflects cooperative interactions among enzyme subunits.
- Other regulatory enzymes are modulated by covalent modification of a specific functional group necessary for activity. The phosphorylation of specific amino acid residues is a particularly common way to regulate enzyme activity

Enzyme Regulation

- **Allosteric enzymes** exist in either an active or inactive state.

  - possess an **allosteric site** where molecules other than the substrate bind

  - **allosteric inhibitors** bind to the allosteric site to inactivate the enzyme

  - **allosteric activators** bind to the allosteric site to activate the enzyme

The study of enzymes

- In some diseases, especially inheritable genetic disorders, there may be a deficiency or even a total absence of one or more enzymes

- Level of enzyme in blood are of diagnostic importance e.g. it is a good indicator in disease such as myocardial infarction.

- The quantity or concentration of an enzyme can be expressed in molar amounts, as with any other chemical, or in terms of activity in enzyme units.

Enzyme activity

- The SI unit is the katal, 1 katal = 1 mol s^{-1}
- This is an excessively large unit
- enzyme unit (U) = 1 μmol min^{-1}.
- 1 Unit = amount of enzyme that will convert one μmole of substrate to product in one minute at a given pH (optimum value) and temperature (usually 25°C or 37°C).
- 1 U corresponds to 16.67 nanokatals

"Specific Activity"

- Units of enzyme activity per mg protein
- Specific activity gives a measurement of enzyme purity in the mixture
- Moles of product formed by an enzyme in a given amount of time (minutes) under given conditions per milligram of total proteins