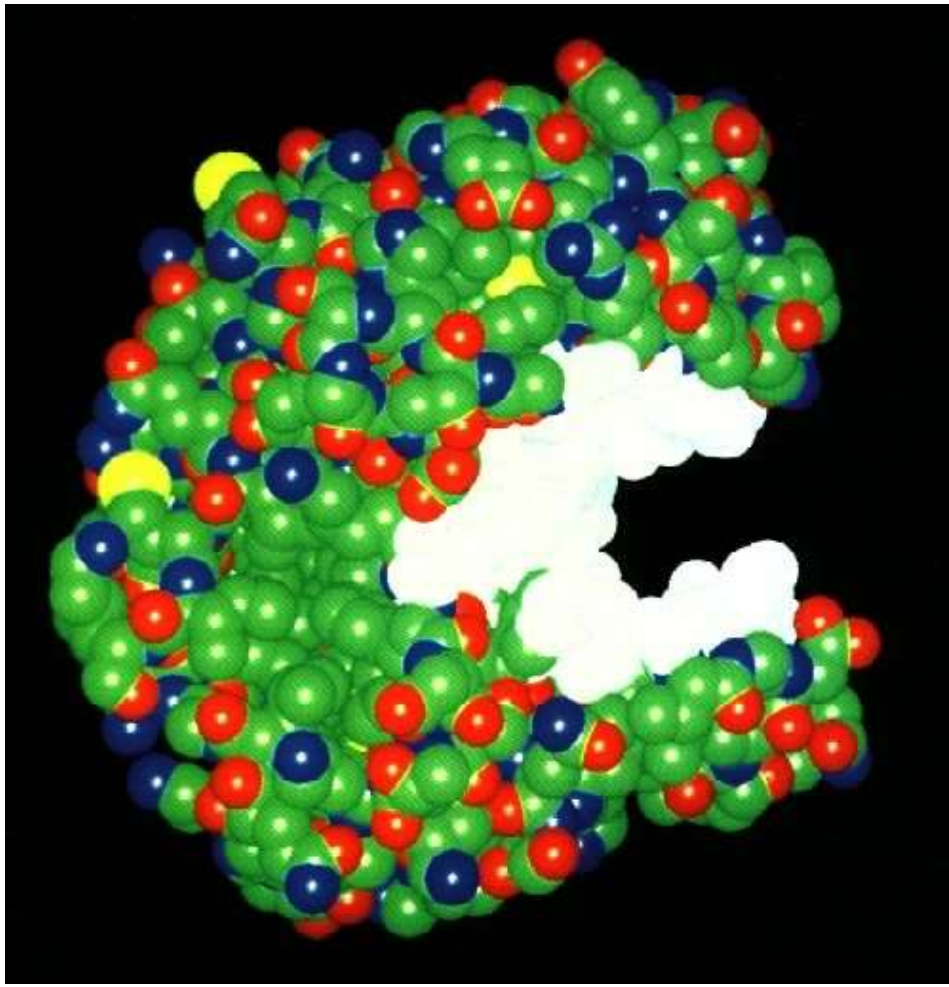
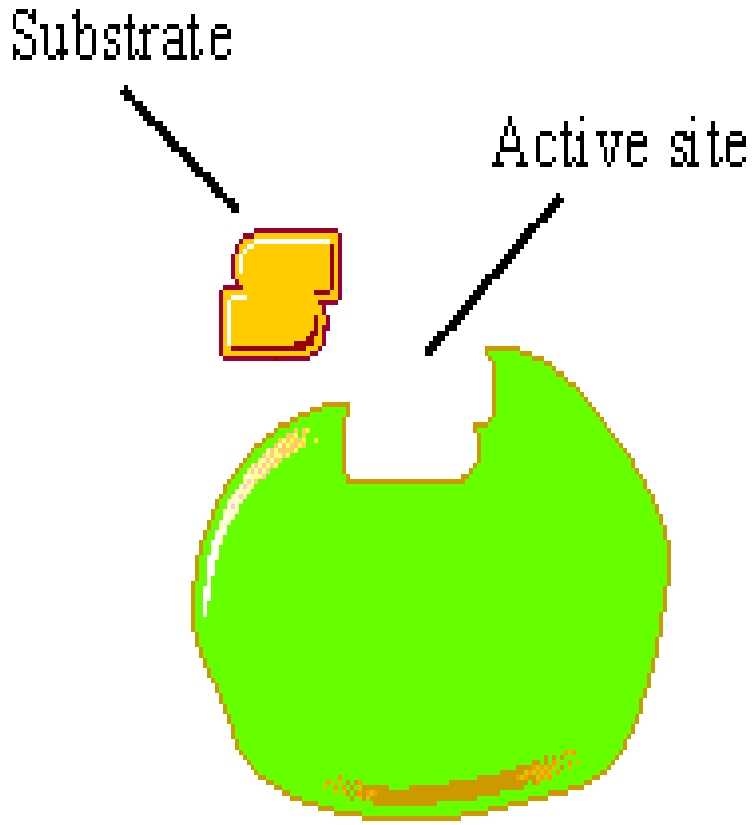
Enzymes

What Are Enzymes?

- Most enzymes are Proteins (tertiary and quaternary structures)
- Act as Catalyst to accelerates a reaction
- Not permanently changed in the process



- Are specific for what they will catalyze
- Are Reusable



Nomenclature and Classification

Enzymes are often classified by placing them in categories according to the reactions that they catalyze:

1. Oxidoreductase
2. Transferase
3. Hydrolase
4. Lyase
5. Isomerase
6. Ligase

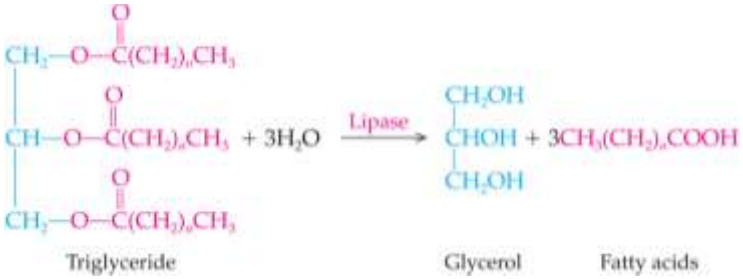
Classification of Enzymes

- Oxidoreductases catalyze redox reactions
  - Reductases
  - Oxidases

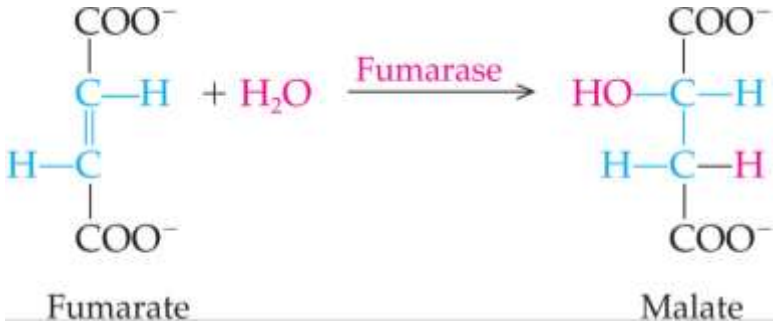
- Transferases transfer a group from one molecule to another
  - Transaminases catalyze transfer of an amino group
  - Kinases transfer a phosphate group

Classification of Enzymes

- Hydrolases cleave bonds by adding water
  - Phosphatases
  - Peptidases
  - Lipases

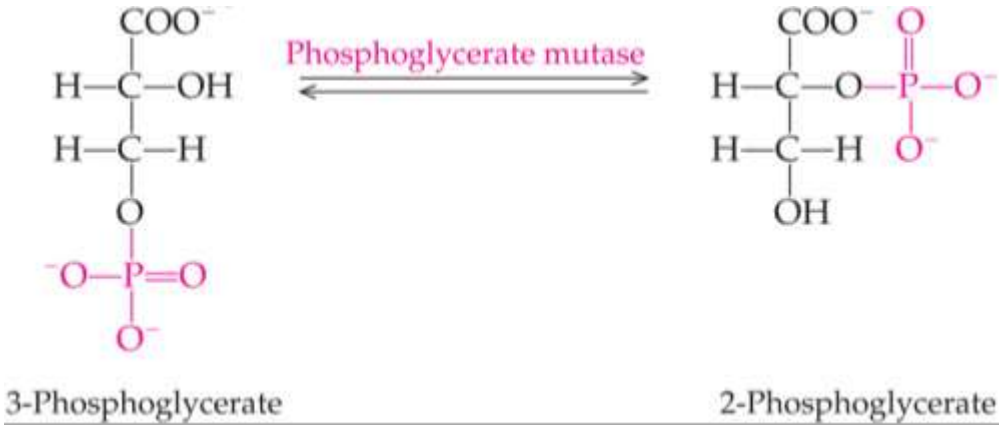


- Lyases catalyze removal of groups to form double bonds or the reverse break double bonds
  - Decarboxylases
  - Synthases

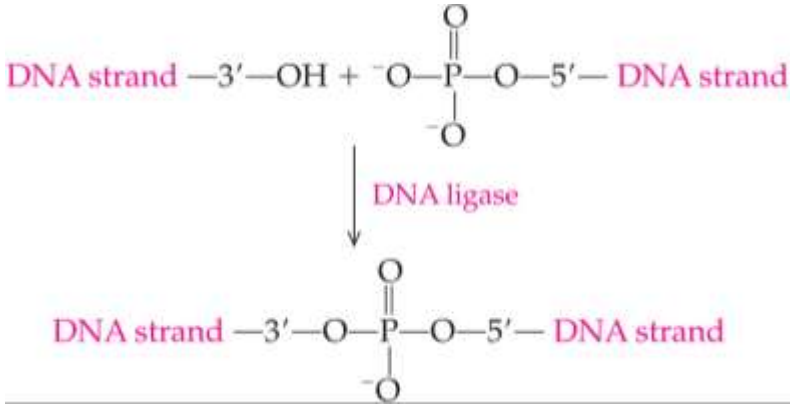


Classification of Enzymes

- Isomerases catalyze intramolecular rearrangements
  - Epimerases
  - Mutases



- Ligases catalyze a reaction in which a C-C, C-S, C-O, or C-N bond is made or broken



Nomenclature of Enzymes

- In most cases, enzyme names end in –ase
- The common name for a hydrolase is derived from the substrate
  - Urea: remove -a, replace with -ase = urease
  - Lactose: remove -ose, replace with -ase = lactase
- Other enzymes are named for the substrate and the reaction catalyzed
  - Lactate dehydrogenase
  - Pyruvate decarboxylase
- Some names are historical - no direct relationship to substrate or reaction type
  - Catalase
  - Pepsin
  - Chymotrypsin
  - Trypsin

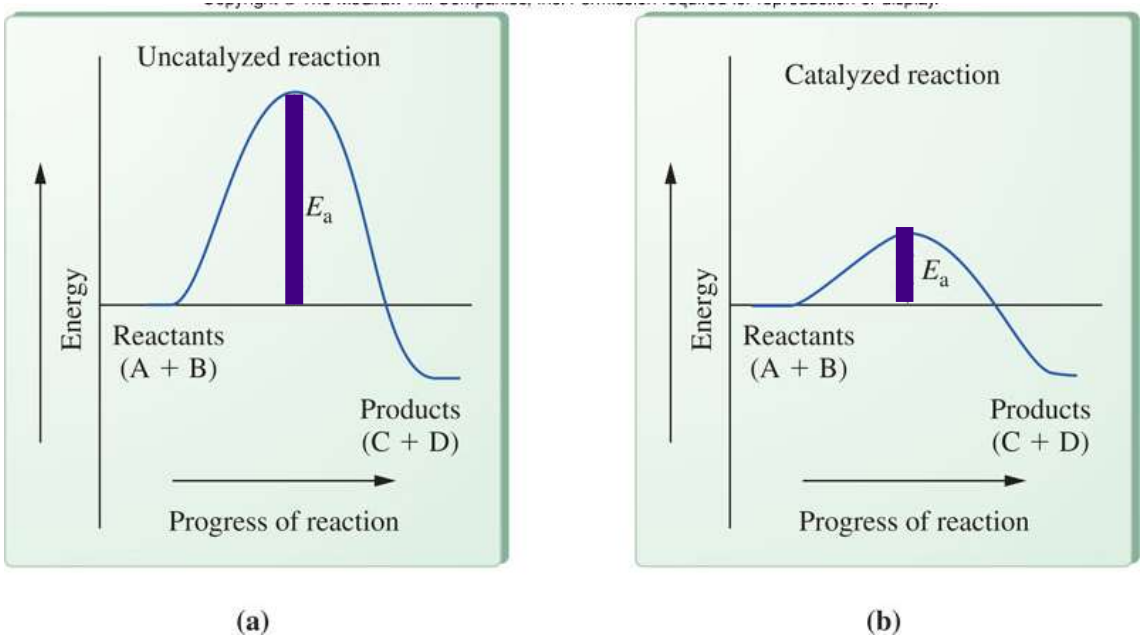
The Effect of Enzymes on the Activation Energy of a Reaction

- An enzyme speeds a reaction by lowering the activation energy, changing the reaction pathway
  - This provides a lower energy route for conversion of substrate to product
- Every chemical reaction is characterized by an equilibrium constant, K_{eq}, which is a reflection of the difference in energy

$$K_{eq} = \frac{[B]^b}{[A]^a} = \frac{[\text{product}]^b}{[\text{reactant}]^a}$$

$$aA \rightleftharpoons bB$$

Diagram of Energy Difference Between Reactants and Products



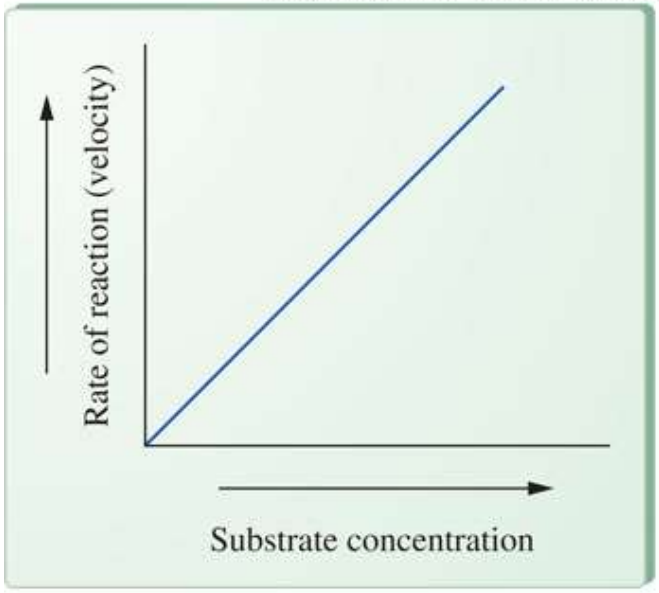
- The uncatalyzed reaction has a large activation energy, E_a, seen at left
- In the catalyzed reaction, the activation energy has been lowered significantly increasing the rate of the reaction

The Effect of Substrate Concentration on Enzyme-Catalyzed Reactions

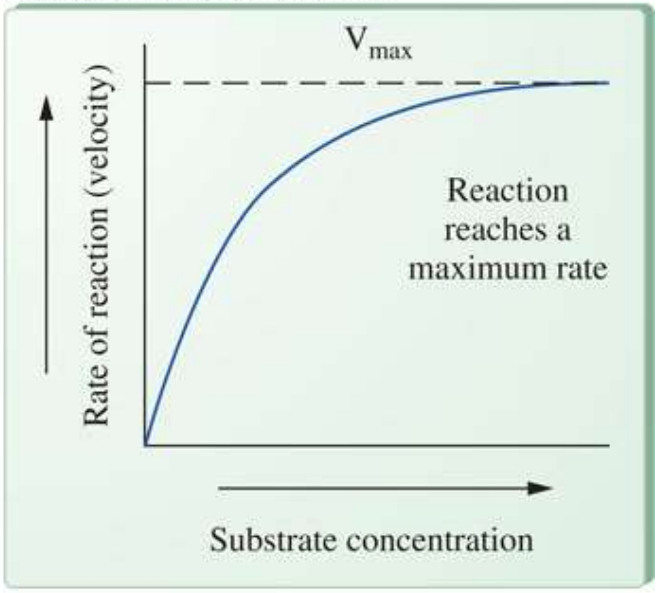
- Rates of uncatalyzed reactions increase as the substrate concentration increases
- Rates of enzyme-catalyzed reactions show two stages
  - The first stage is the formation of an enzyme-substrate complex
  - This is followed by slow conversion to product
  - Rate is limited by enzyme availability

Uncatalyzed Reaction

Enzyme-Catalyzed Reaction



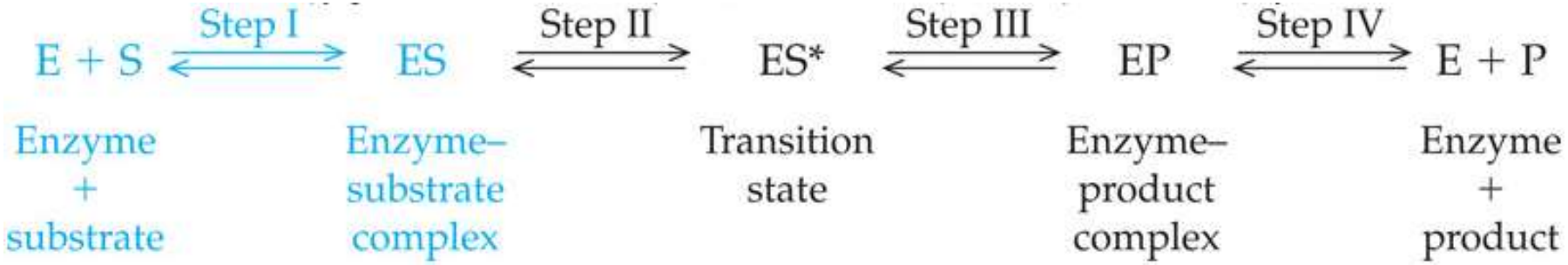
(a)



(b)

The Enzyme-Substrate Complex

- These reversible reaction steps represent the steps in an enzyme catalyzed reaction
  - The first step involves formation of an enzyme-substrate complex, E-S
  - E-S* is the transition state
  - E-P is the enzyme-product complex

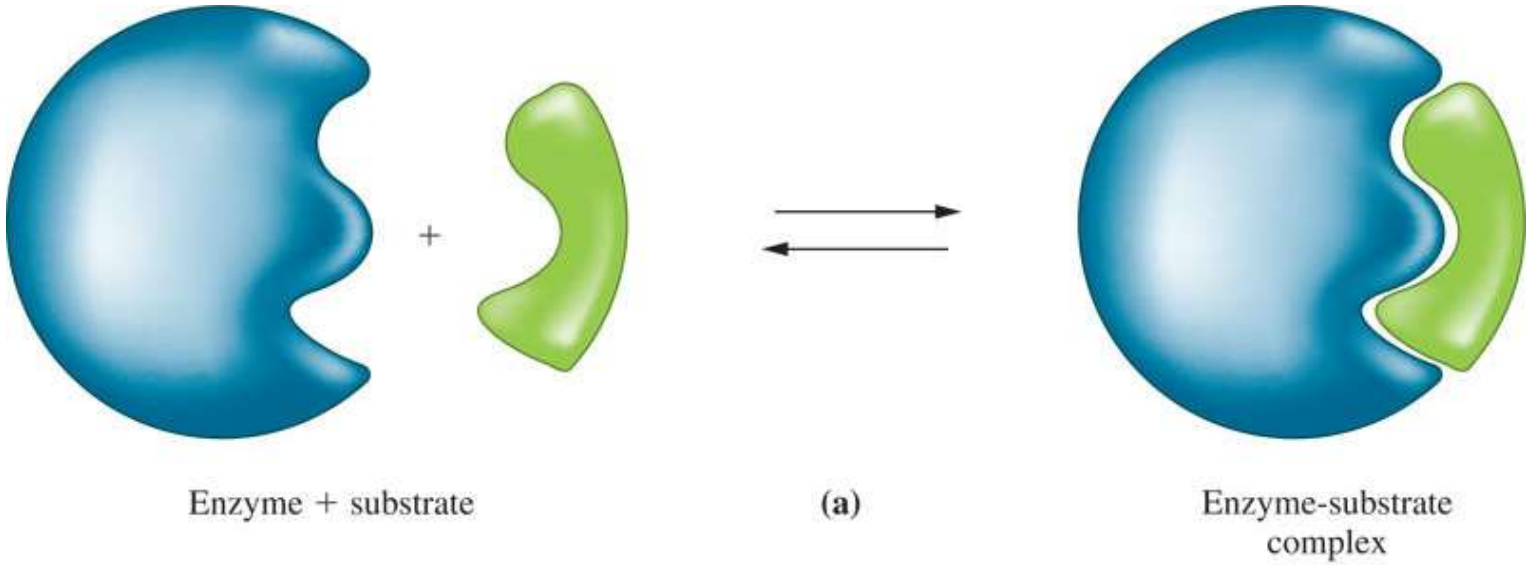


Enzyme-Substrate Complex

- The part of the enzyme combining with the substrate is the active site
- Active sites characteristics include:
  - Pockets or clefts in the surface of the enzyme
    - R groups at active site are called catalytic groups
  - Shape of active site is complimentary to the shape of the substrate
  - The enzyme attracts and holds the enzyme using weak noncovalent interactions
  - Conformation of the active site determines the specificity of the enzyme

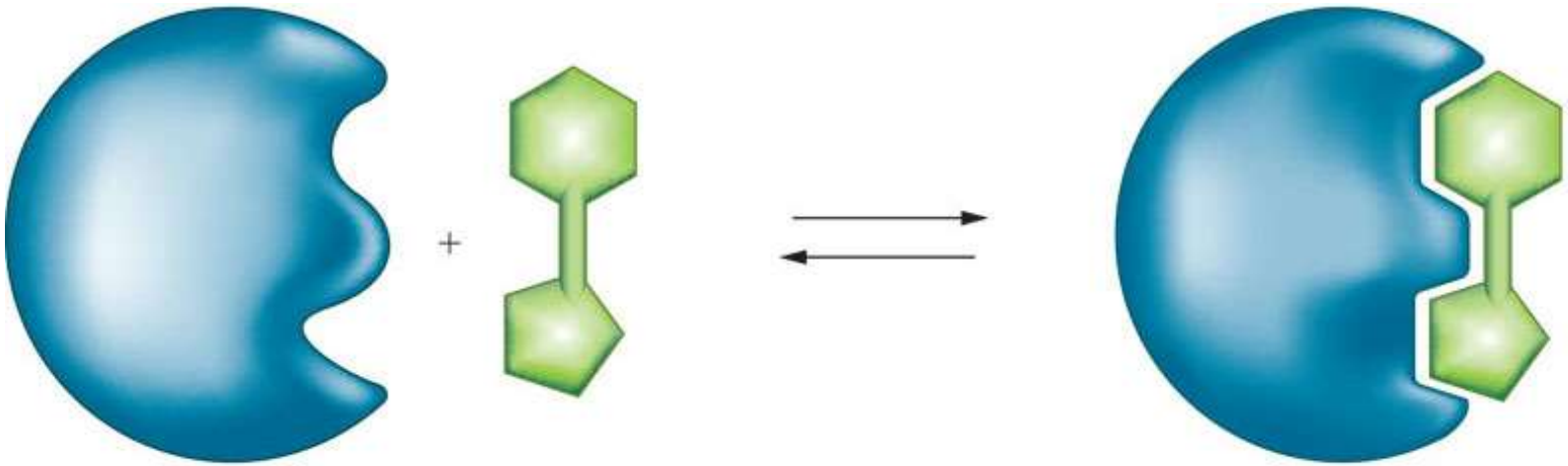
Lock and Key Enzyme Model

- In the lock-and-key model, the enzyme is assumed to be the lock and the substrate the key
  - The enzyme and substrate are made to fit exactly
  - This model fails to take into account proteins' conformational changes to accommodate a substrate molecule



Induced Fit Enzyme Model

- The induced-fit model of enzyme action assumes that the enzyme active site is more a flexible pocket whose conformation changes to accommodate the substrate molecule



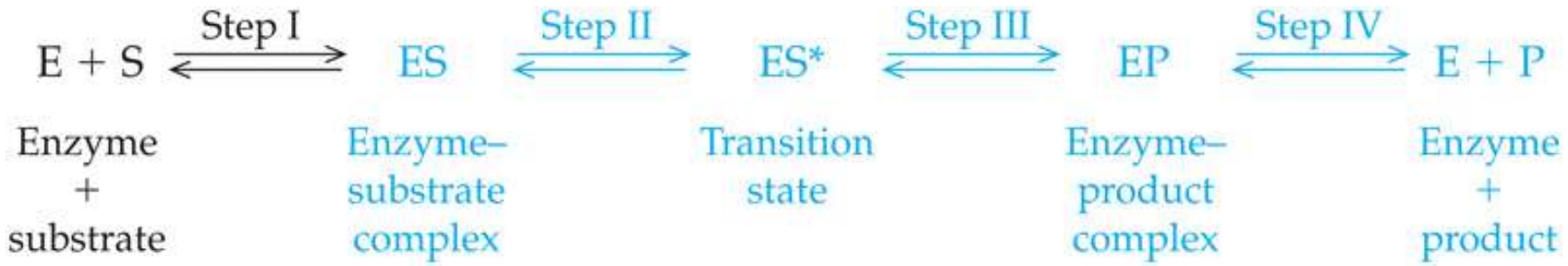
Classes of Enzyme Specificity

1. Absolute: enzyme reacts with only one substrate
2. Group: enzyme catalyzes reaction involving any molecules with the same functional group
3. Linkage: enzyme catalyzes the formation or break up of only certain category or type of bond
4. Stereochemical: enzyme recognizes only one of two enantiomers

The Transition State and Product Formation

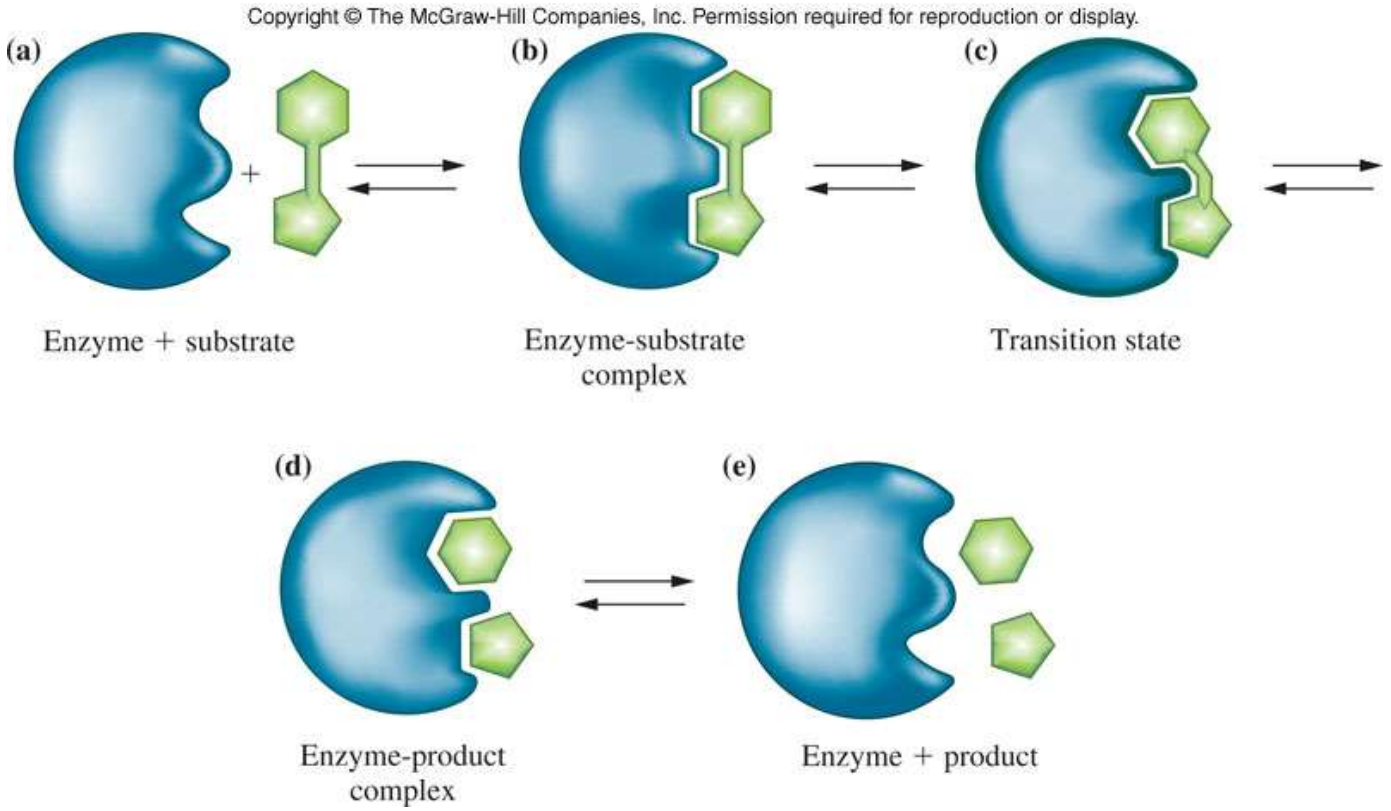
How does the enzyme promote a faster chemical reaction?

- As the substrate interacts with the enzyme, its shape changes and this new shape is less energetically stable
- This transition state has features of both substrate and product and falls apart to yield product, which dissociates from the enzyme



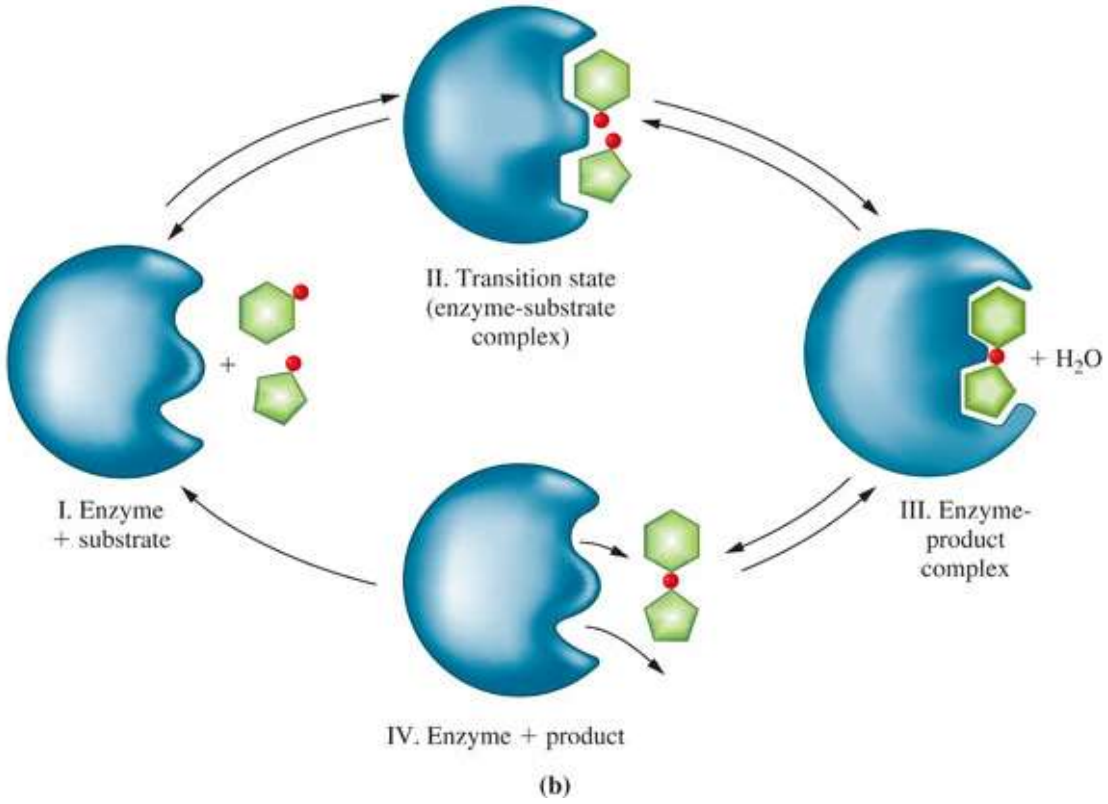
Possible Types of Transition State Changes

1. The enzyme might put "stress" on a bond facilitating bond breakage



Possible Types of Transition State Changes

2. The enzyme might bring two reactants into close proximity and maintain proper orientation



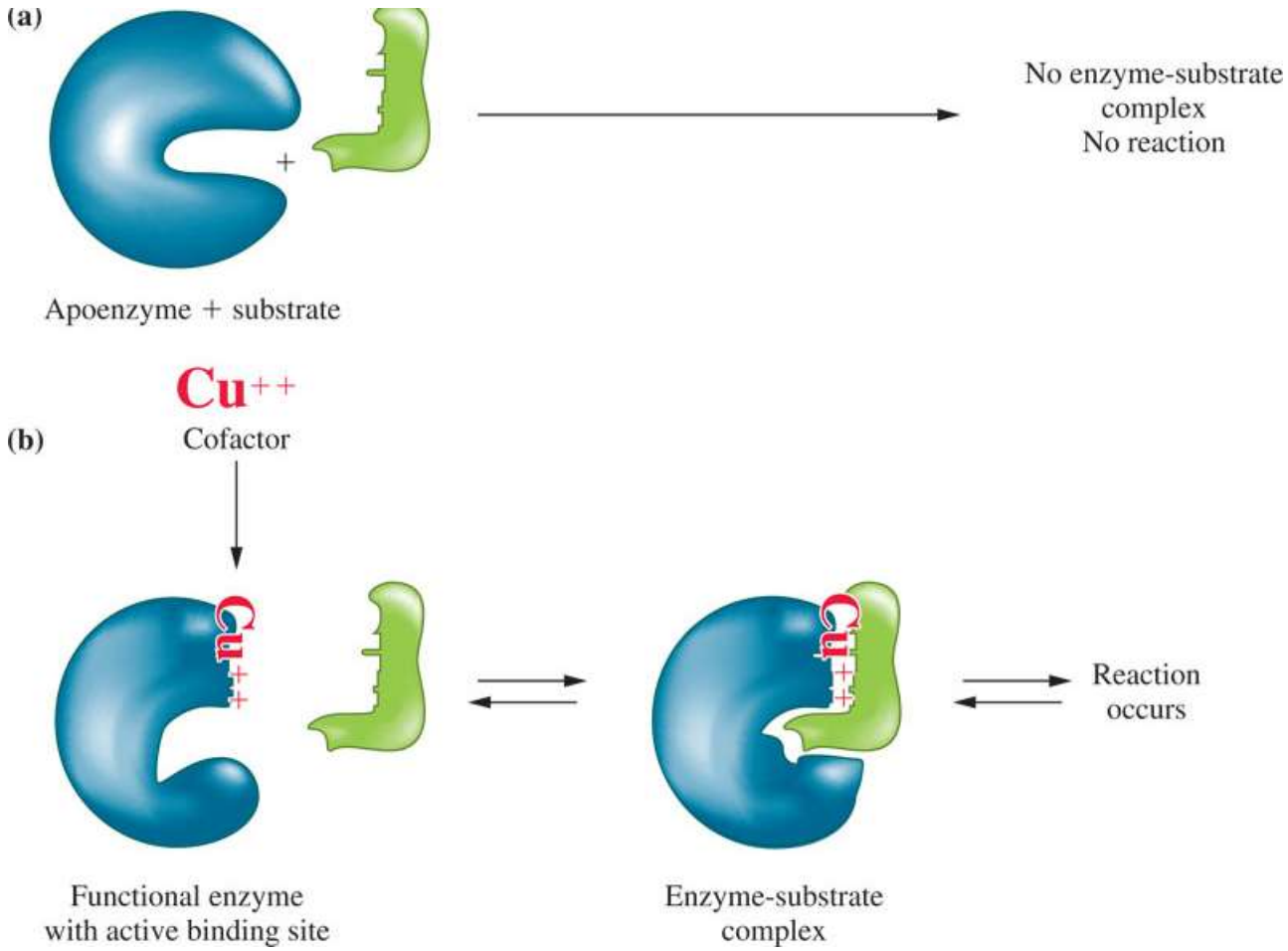
Possible Types of Transition State Changes

3. The enzyme might modify the pH of the microenvironment, donating or accepting a H^+

Cofactors and Coenzymes

- Active enzyme / Holoenzyme:
  - Polypeptide portion of enzyme (apoenzyme)
  - Nonprotein prosthetic group (cofactor)

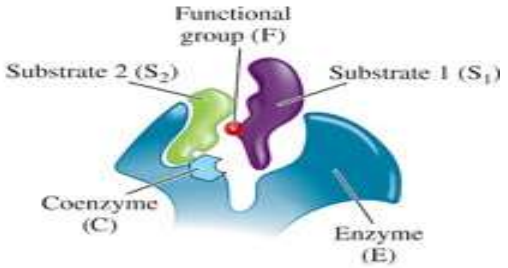
- Cofactors are bound to the enzyme for it to maintain the correct configuration of the active site
  - Metal ions
  - Organic compounds
  - Organometallic compounds


(a)
+
No enzyme-substrate complex
No reaction
Apoenzyme + substrate
Cu++
(b)
Cofactor
Cu++
Cu++
Reaction occurs
Functional enzyme with active binding site
Enzyme-substrate complex

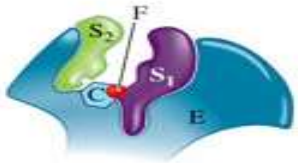
Coenzymes

- A coenzyme is required by some enzymes
  - An organic molecule bound to the enzyme by weak interactions / Hydrogen bonds
  - Most coenzymes carry electrons or small groups
  - Many have modified vitamins in their structure

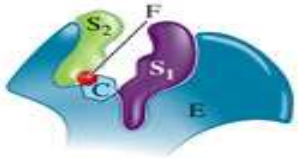
1. An enzyme with a coenzyme positioned to react with two substrates.



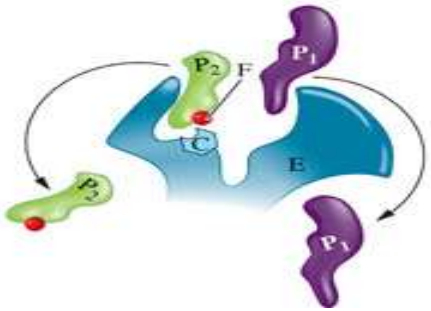
2. Coenzyme picks up a functional group from substrate 1.



3. Coenzyme transfers the functional group to substrate 2.



4. Products are released from enzyme.



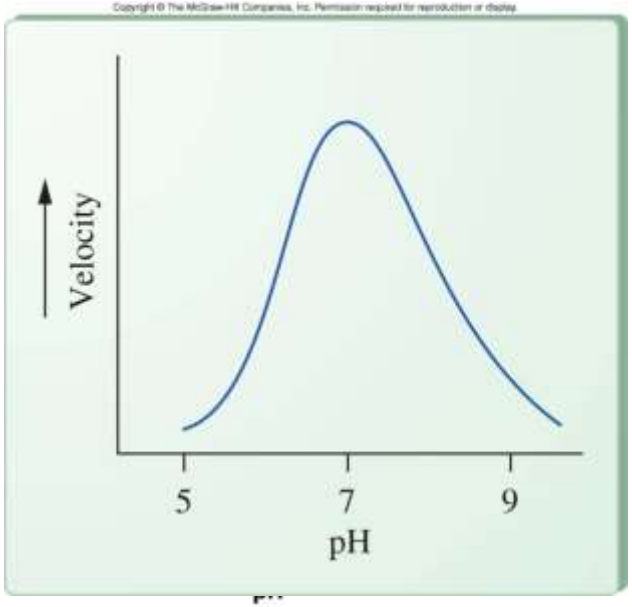
Water-Soluble Vitamins and Their Coenzymes

TABLE 19.1 The Water-Soluble Vitamins and their Coenzymes

Vitamin	Coenzyme	Function
Thiamine (B_1)	Thiamine pyrophosphate	Decarboxylation reactions
Riboflavin (B_2)	Flavin mononucleotide (FMN)	Carrier of H atoms
	Flavin adenine dinucleotide (FAD)	
Niacin (B_3)	Nicotinamide adenine dinucleotide (NAD^+)	Carrier of hydride ions
	Nicotinamide adenine dinucleotide phosphate ($NADP^+$)	
Pyridoxine (B_6)	Pyridoxal phosphate	Carriers of amino and carboxyl groups
	Pyridoxamine phosphate	
Cyanocobalamin (B_{12})	Deoxyadenosyl cobalamin	Coenzyme in amino acid metabolism
Folic acid	Tetrahydrofolic acid	Coenzyme for 1-C transfer
Pantothenic acid	Coenzyme A	Acyl group carrier
Biotin	Biocytin	Coenzyme in CO_2 fixation
Ascorbic acid	Unknown	Hydroxylation of proline and lysine in collagen

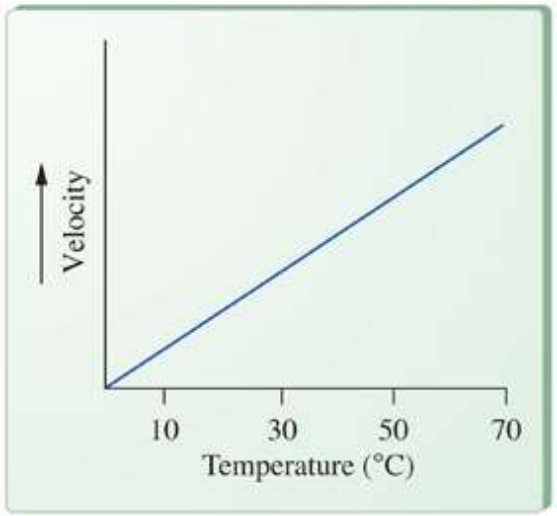
Environmental Effects

- The environment surrounding an enzyme can have a direct effect on enzyme function
- Enzymes work best within a particular range of pH
- Extreme pH changes will denature the enzyme, destroying its catalytic ability
  - Pepsin (stomach)
  - Chymotrypsin (small intestine) have different optimum pHs

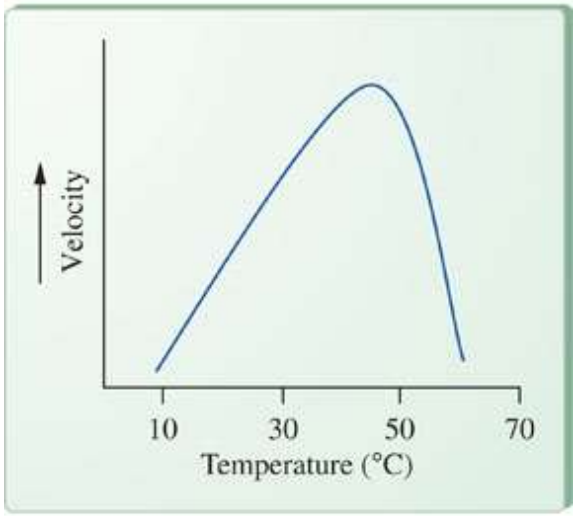


Temperature Effects

- An enzyme has an optimum temperature associated with maximal function
- The rate of an uncatalyzed reaction will increase proportionally with temperature increase
- Optimum temperature is usually close to the temperature at which the enzyme typically exists
  - 37°C for humans
- Excessive heat can denature a enzyme making it completely nonfunctional



(a)



(b)

Regulation of Enzyme Activity

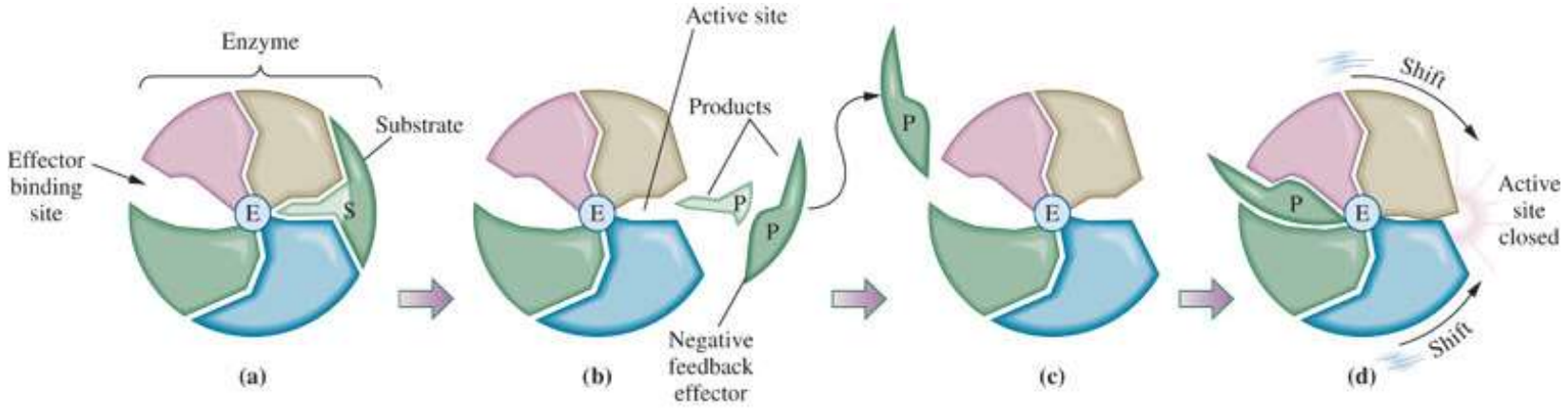
One of the major ways that enzymes differ from nonbiological catalysts is in the regulation of biological catalysts by cells

Some methods that organisms use to regulate enzyme activity are:

1. Produce the enzyme only when the substrate is present – common in bacteria
2. Allosteric enzymes
3. Feedback inhibition
4. Zymogens
5. Protein modification

Allosteric Enzymes

- Effector molecules change the activity of an enzyme by binding at a second site
  - Some effectors speed up enzyme action (positive allosterism)
  - Some effectors slow enzyme action (negative allosterism)



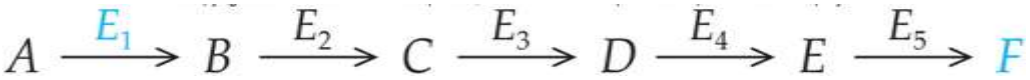
(a), (b) The allosteric enzyme has a quaternary structure with two different sites of attachment–the active site and the effector binding site. The enzyme complex normally attaches to the substrate at the active site and releases products (P).

(c) One product can function as a negative-feedback effector by fitting into the effector binding site.

(d) Binding of the effector in the effector binding site causes a conformational shift of the enzyme that closes the active site and inactivates the enzyme.

Feedback Inhibition

- Allosteric enzymes are the basis for feedback inhibition
- With feedback inhibition, a product late in a series of enzyme-catalyzed reactions serves as an inhibitor for a previous allosteric enzyme earlier in the series



- In this example, product F serves to inhibit the activity of enzyme E_1
  - Product F acts as a negative allosteric effector on one of the early enzymes in the pathway

Proenzymes

- A proenzyme, an enzyme made in an inactive form
- It is converted to its active form
  - By proteolysis (hydrolysis of the enzyme)
  - When needed at the active site in the cell
    - Pepsinogen is synthesized and transported to the stomach where it is converted to pepsin

Protein Modification

- In protein modification a chemical group is covalently added to or removed from the protein
  - Covalent modification either activates or turns off the enzyme
- The most common form of protein modification is addition or removal of a phosphate group
  - This group is located at the R group (with a free –OH) of:
    - Serine
    - Threonine
    - Tyrosine

Inhibition of Enzyme Activity

- Chemicals can bind to enzymes and eliminate or drastically reduce catalytic activity
- Classify enzyme inhibitors on the basis of reversibility and competition
  - Irreversible inhibitors bind tightly to the enzyme and thereby prevent formation of the E-S complex
  - Reversible competitive inhibitors often structurally resemble the substrate and bind at the normal active site
  - Reversible noncompetitive inhibitors usually bind at someplace other than the active site
    - Binding is weak and thus, inhibition is reversible

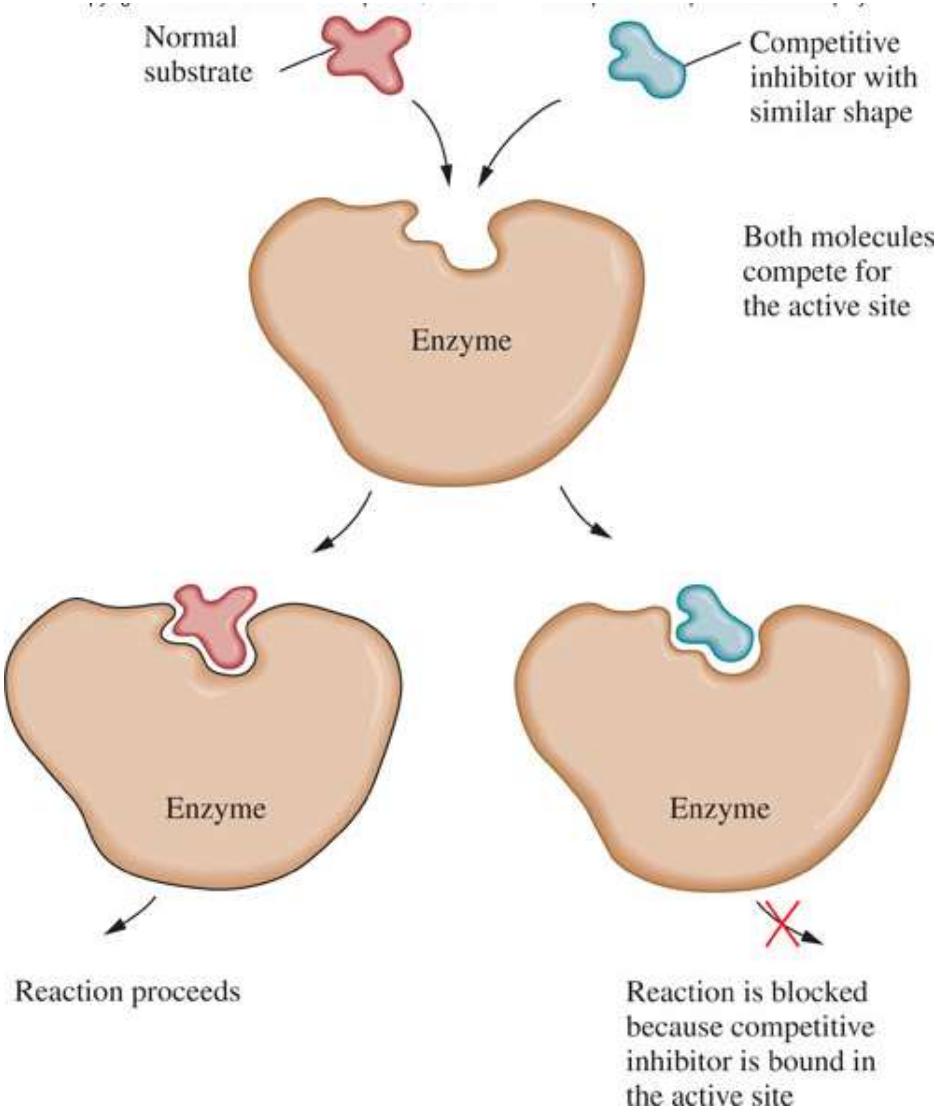
Irreversible Inhibitors

- Irreversible enzyme inhibitors bind very tightly to the enzyme
  - Binding of the inhibitor to one of the R groups of a amino acid in the active site
    - This binding may block the active site binding groups so that the enzyme-substrate complex cannot form
    - Alternatively, an inhibitor may interfere with the catalytic group of the active site eliminating catalysis
  - Irreversible inhibitors include:
    - Arsenic
    - Snake venom
    - Nerve gas

Reversible, Competitive Inhibitors

- Reversible, competitive enzyme inhibitors are also called *structural analogs*
  - Molecules that resemble the structure and charge distribution of a natural substance for an enzyme
  - Resemblance permits the inhibitor to occupy the enzyme active site
  - Once inhibitor is at the active site, no reaction can occur and the enzyme activity is inhibited
- Inhibition is competitive because the inhibitor and the substrate compete for binding to the active site
  - Degree of inhibition depends on the relative concentrations of enzyme and inhibitor

Reversible, Competitive Inhibitors



Reversible, Noncompetitive Inhibitors

- Reversible, noncompetitive enzyme inhibitors bind to R groups of amino acids or to the metal ion cofactors
  - This binding is weak
  - Enzyme activity is restored when the inhibitor dissociates from the enzyme-inhibitor complex
  - **These inhibitors:**
    - Do not bind to the active site
    - Do modify the shape of the active site once bound elsewhere in the structure

Uses of Enzymes in Medicine

- Diagnostic – enzyme levels altered with disease
  - Heart attack:
    - Lactate dehydrogenase
    - Creatine phosphate
    - Serum glutamate-oxaloacetate transaminase (SGOT)
  - Pancreatitis:
    - Amylase
    - Lipase
- Analytical reagents – enzyme used to measure another substance
  - Urea converted to NH_3 via urease
  - Blood urea nitrogen (BUN) measured
- Replacement therapy
  - Administer genetically engineered β-glucocerebrosidase for Gaucher's disease