

BIOCHEMISTRY (I)

LIFS2210

Enzymes and Enzyme Reactions

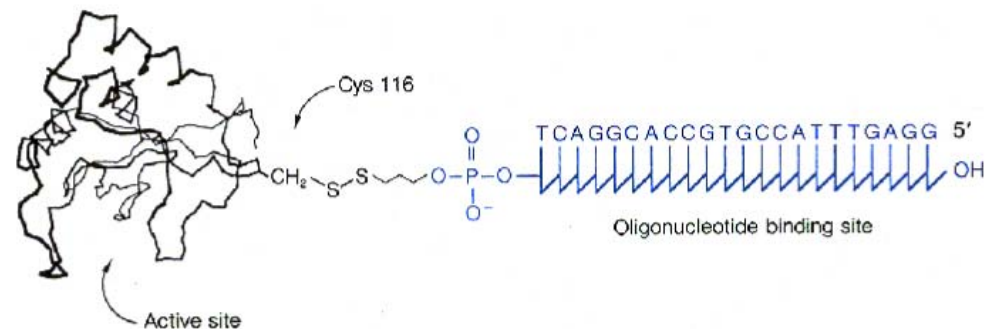
1. Enzymes: Biocatalysts

- **Catalyst**: to increase the rate or velocity of a chemical reaction without itself being changed in the overall process
- Catalyst - speeds up attainment of reaction equilibrium
- Most biological catalysts are proteins called **enzymes**

Nonprotein Biocatalysts: Ribozymes

- Some RNA molecules, called **ribozymes**, are capable of catalyzing chemical reactions.

- **A hybrid enzyme**



Properties of Enzymes

- **Enzymatic reactions** - 10^3 to 10^{17} faster than the corresponding uncatalyzed reactions
- **Substrate** - the substance acted on by an **enzyme** is called a **substrate**.

Naming Enzymes

- The name of an enzyme identifies the reacting substance
 - usually ends in *–ase*
 - For example, *sucrase* catalyzes the hydrolysis of sucrose
- The name also describes the function of the enzyme
 - For example, *oxidases* catalyze oxidation reactions
- Sometimes common names are used, particularly for the digestion enzymes such as *pepsin* and *trypsin*
- Some names describe both the substrate and the function
 - For example, *alcohol dehydrogenase* oxidizes ethanol

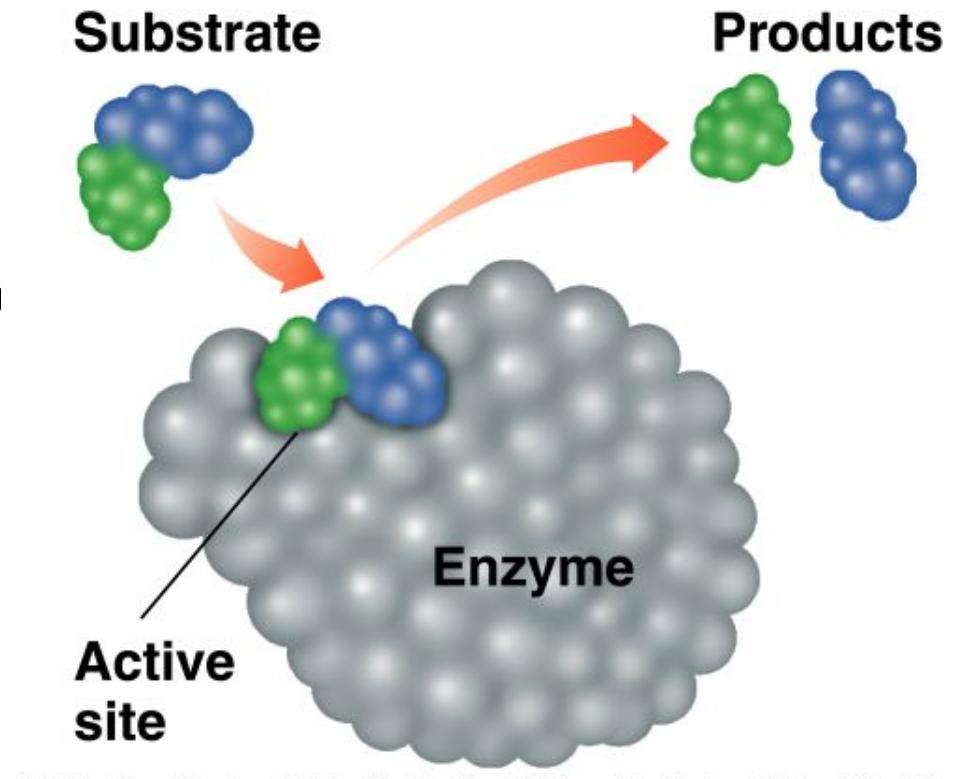
Enzyme Specificity

- Enzymes have varying degrees of **specificity** for substrates
- **Stereospecificity** - many enzymes act upon only one stereoisomer of a substrate
- Enzymes may recognize and catalyze:
 - a single substrate
 - a group of similar substrates
 - a particular type of bond

Type	Reaction Type	Example
Absolute	Catalyze one type of reaction for a single substrate	Urease catalyzes only the hydrolysis of urea
Group	Catalyze one type of reaction for similar substrates	Hexokinase adds a phosphate group to hexoses
Linkage	Catalyze one type of reaction for a specific type of bond	Chymotrypsin catalyzes the hydrolysis of peptide bonds

Active Site of an Enzyme

- The **active site** is a region within an enzyme that fits the shape of substrate molecules
- Amino acid side-chains align to bind the substrate through H-bonding, salt-bridges, hydrophobic interactions, etc.
- Products are released when the reaction is complete (they no longer fit well in the active site)

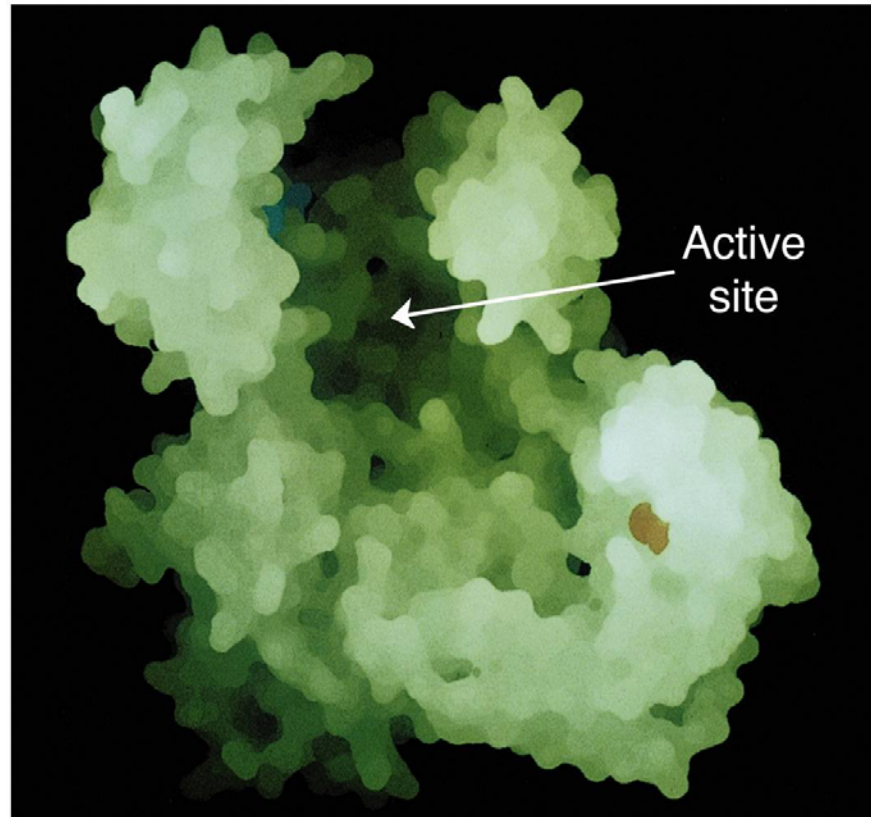


Enzyme active site

Active site:

Usually form a cleft or pocket

Substrates are bound by multiple weak interactions



2. Classification of Enzymes

- Enzymes are classified according to the type of reaction they catalyze:

<u>Class</u>	<u>Reactions catalyzed</u>
▪ Oxidoreductases	Oxidation-reduction
▪ Transferases	Transfer groups of atoms
▪ Hydrolases	Hydrolysis
▪ Lyases	Add atoms/remove atoms to/from a double bond
▪ Isomerases	Rearrange atoms
▪ Ligases	Use ATP to combine molecules

Oxidoreductases, Transferases and Hydrolases

Class	General Reactions Catalyzed	Typical Subclasses	Function
1. Oxidoreductases	Oxidation–reduction reactions	Oxidases Reductases Dehydrogenases	Oxidation Reduction Remove 2H to form double bonds
$ \begin{array}{ccccccc} \text{CH}_3\text{—CH}_2\text{—OH} & + & \text{NAD}^+ & \xrightarrow{\text{Alcohol dehydrogenase}} & \text{CH}_3\text{—}\overset{\text{O}}{\underset{\text{O}}{\text{C}}}\text{—H} & + & \text{NADH}^+ + \text{H}^+ \\ \text{Ethanol} & & \text{Coenzyme} & & \text{Acetaldehyde} & & \text{Coenzyme} \end{array} $			
2. Transferases	Transfer of functional groups	Transaminases Kinases	Transfer amino groups Transfer phosphate groups
$ \begin{array}{ccccccc} \begin{array}{c} \text{NH}_3^+ \\ \\ \text{CH}_3\text{—CH—COO}^- \end{array} & + & \begin{array}{c} \text{O} \\ \\ ^-\text{OOC—C—CH}_2\text{CH}_2\text{—COO}^- \end{array} & \xrightleftharpoons{\text{Alanine transaminase}} & \begin{array}{c} \text{O} \\ \\ \text{CH}_3\text{—C—COO}^- \end{array} & + & \begin{array}{c} \text{NH}_3^+ \\ \\ ^-\text{OOC—CH—CH}_2\text{CH}_2\text{—COO}^- \end{array} \\ \text{Alanine} & & \alpha\text{-Ketoglutarate} & & \text{Pyruvate} & & \text{Glutamate} \end{array} $			
3. Hydrolases	Hydrolysis reactions	Peptidases Lipases Amylases	Hydrolyze peptide bonds Hydrolyze ester bonds in lipids Hydrolyze 1,4-glycosidic bonds in amylose
$ \begin{array}{ccccccc} \begin{array}{c} \text{R} \quad \text{O} \quad \text{R} \\ \quad \quad \\ \text{—N—CH—C—N—CH—COO}^- \\ \quad \\ \text{H} \quad \text{H} \end{array} & + & \text{H}_2\text{O} & \xrightarrow{\text{Peptidase}} & \begin{array}{c} \text{R} \quad \text{O} \\ \quad \\ \text{—N—CH—C—O}^- \\ \\ \text{H} \end{array} & + & \begin{array}{c} \text{R} \\ \\ \text{H}_3\text{N}^+\text{—CH—COO}^- \end{array} \\ \text{Polypeptide C terminal} & & & & \text{Shorter polypeptide} & & \text{Amino acid from C terminal} \end{array} $			

Lyases, Isomerases and Ligases

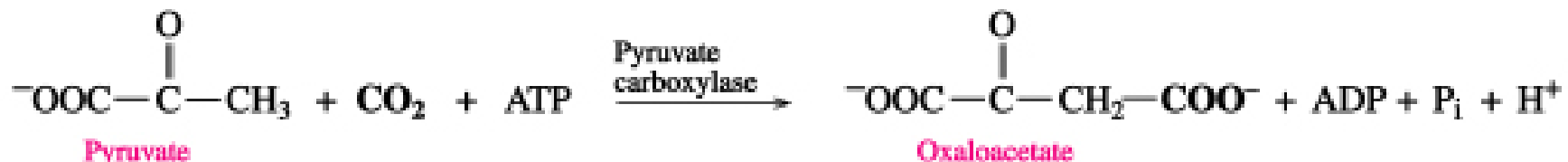
Class	General Reactions Catalyzed	Typical Subclasses	Function
4. Lyases	Addition of a group to a double bond or removal of a group from a double bond without hydrolysis or oxidation	Decarboxylases Dehydrases Deaminases	Remove CO ₂ Remove H ₂ O Remove NH ₃



5. Isomerases	Rearrangement of atoms to form isomers	Isomerases Epimerases	Convert cis and trans Convert D and L isomers
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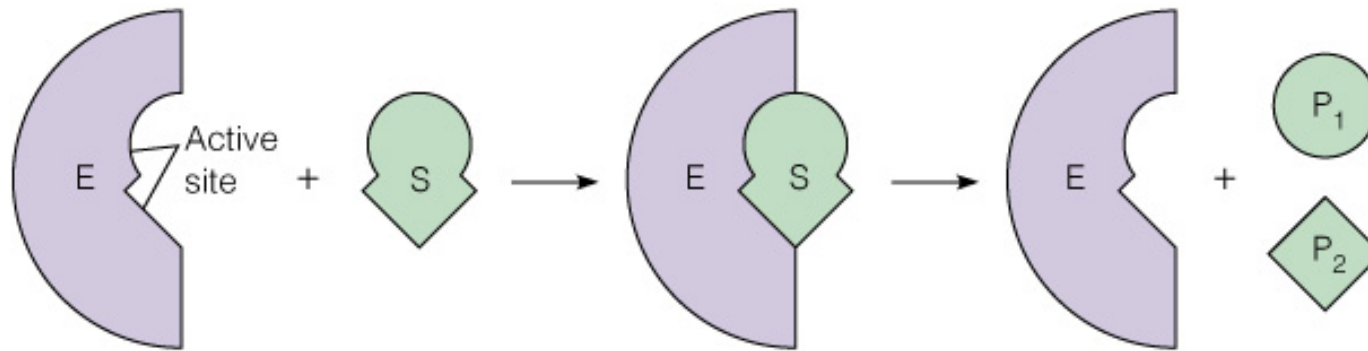
6. Ligases	Bonding of molecules using ATP energy	Synthetases Carboxylases	Combine molecules Add CO ₂
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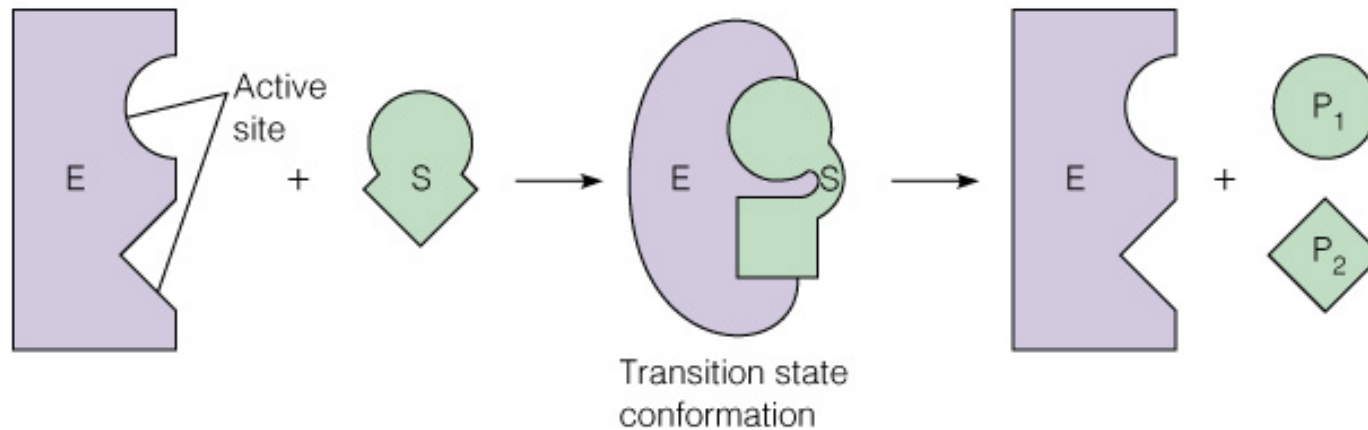
Multienzyme Complexes and Multifunctional Enzymes

- **Multienzyme complexes** - different enzymes that catalyze sequential reactions in the same pathway are bound together
- **Multifunctional enzymes** - different activities may be found on a single, multifunctional polypeptide chain

3. Two models for enzyme-substrate interaction

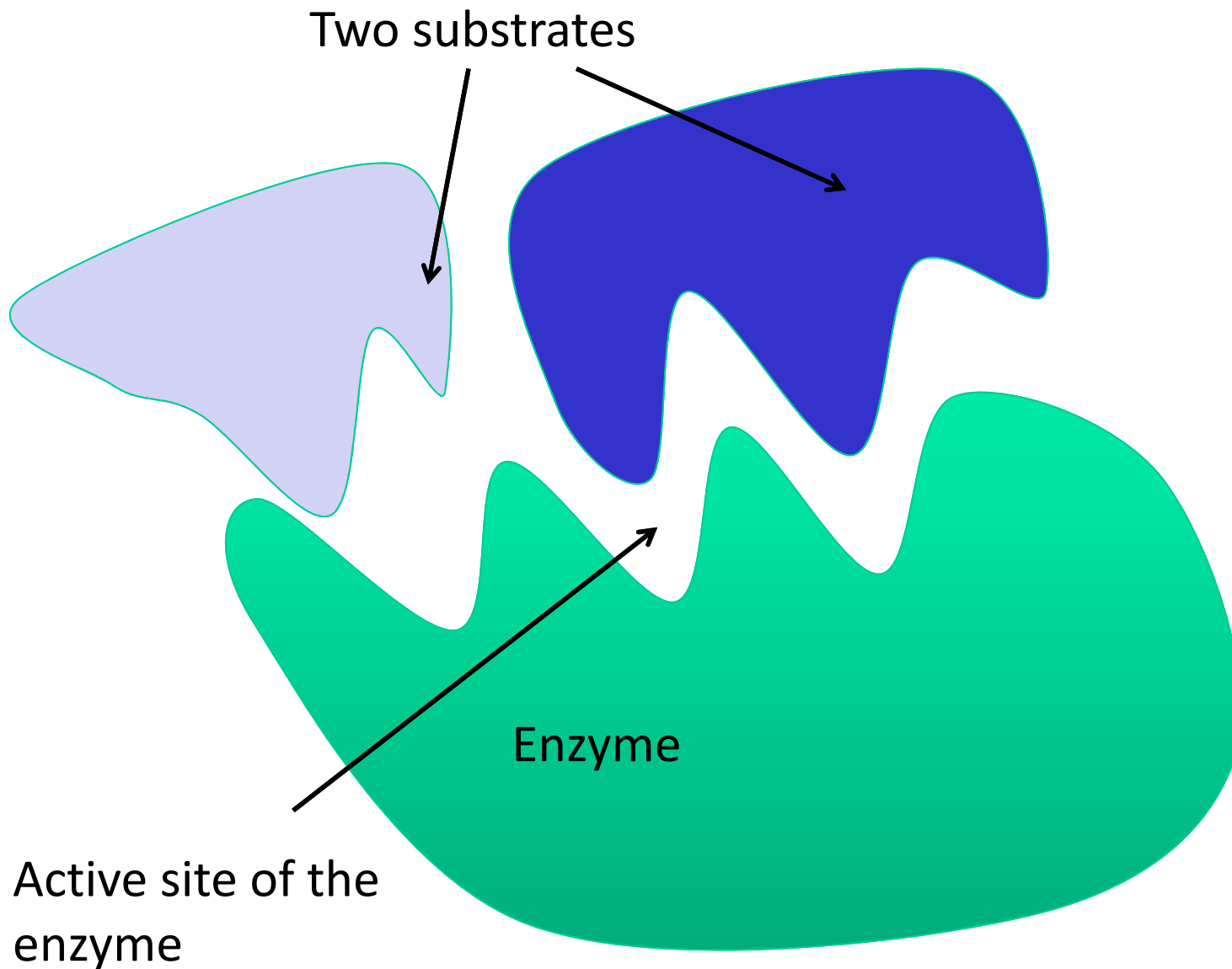


(a) Lock-and-key model



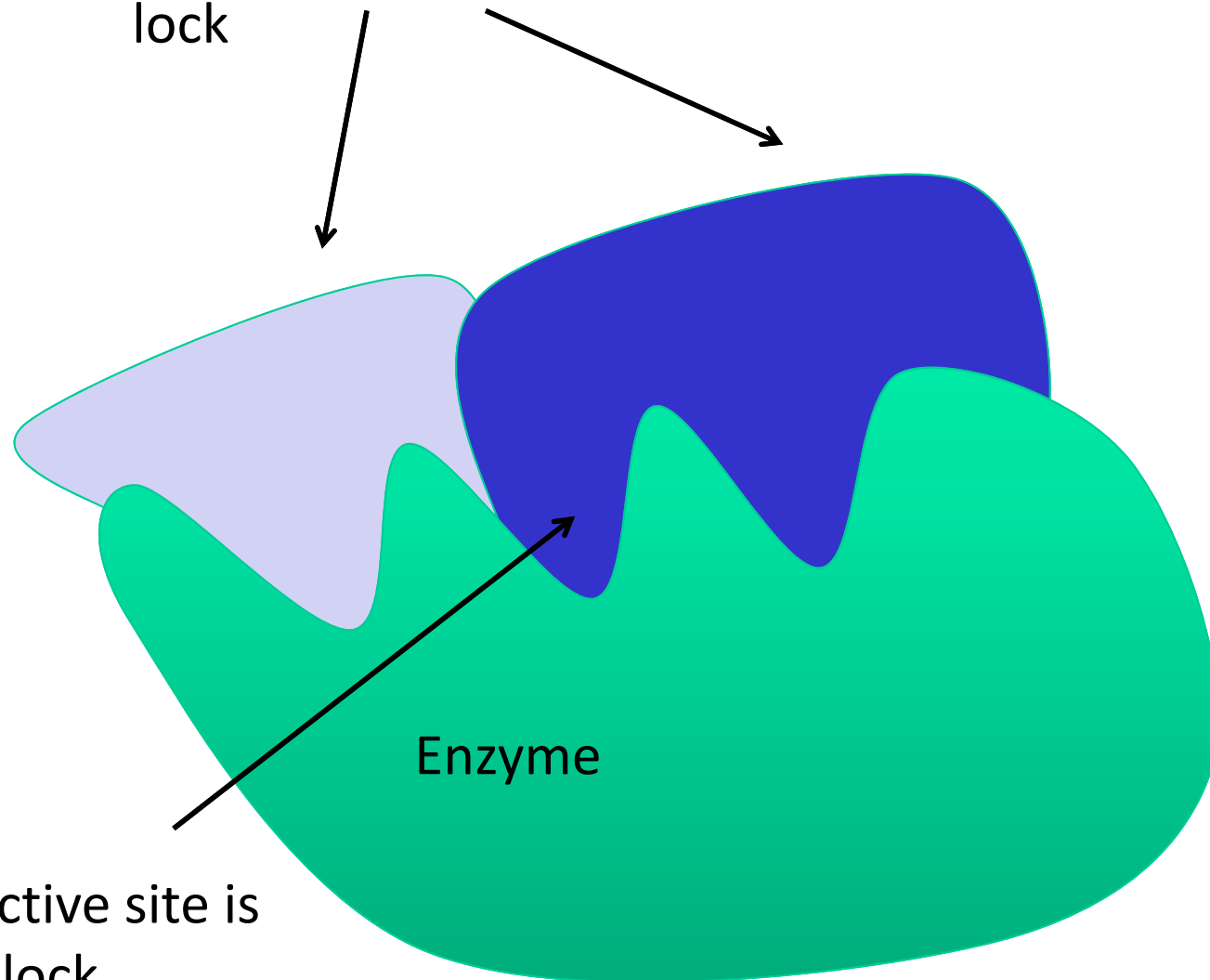
(b) Induced fit model

Lock and Key Model



Lock and Key Model

The substrates fit like a key in a lock

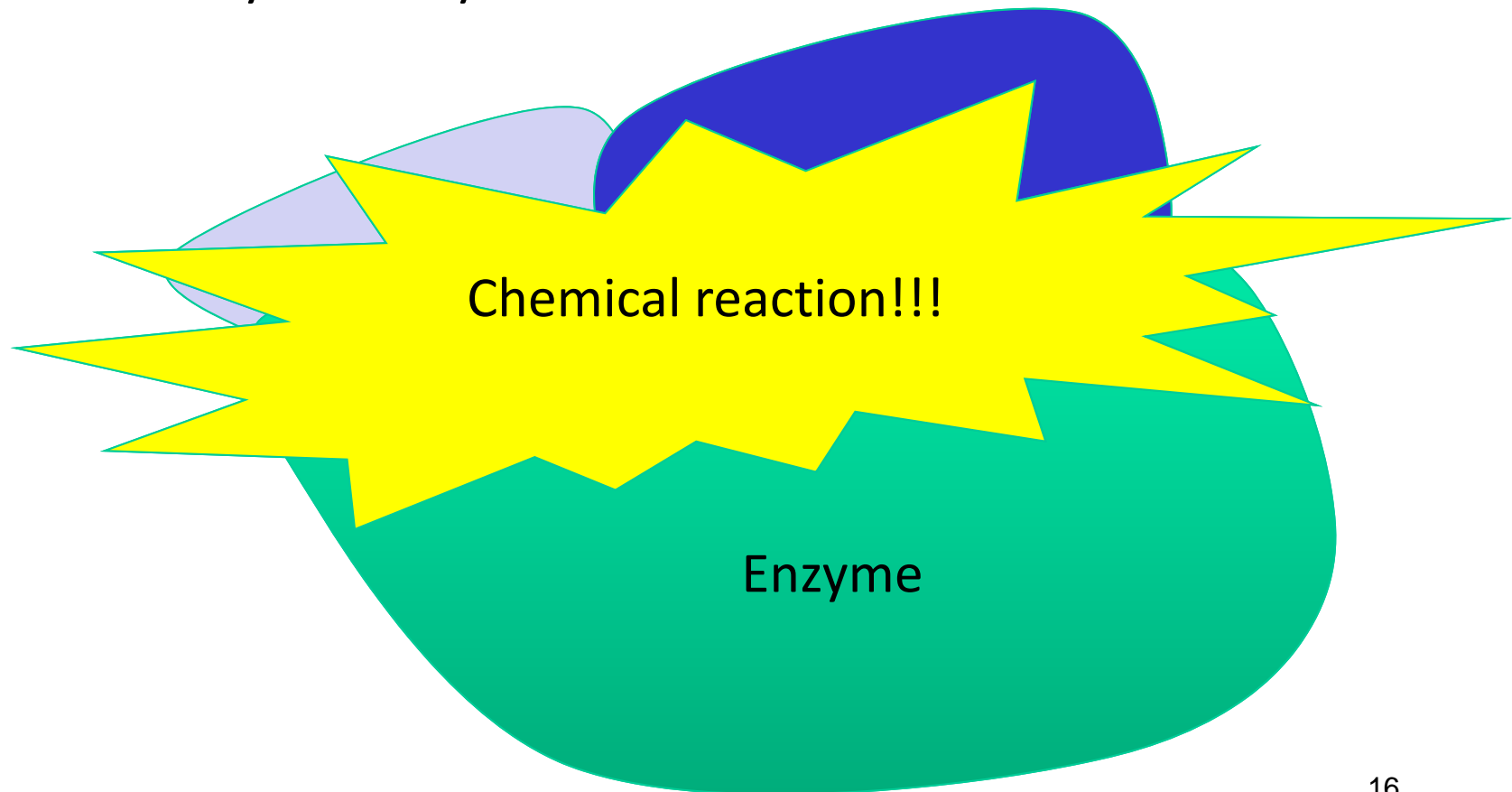


The active site is like a lock

Enzyme

Lock and Key Model

The activation energy for these substrates to bind together has been lowered by the enzyme.

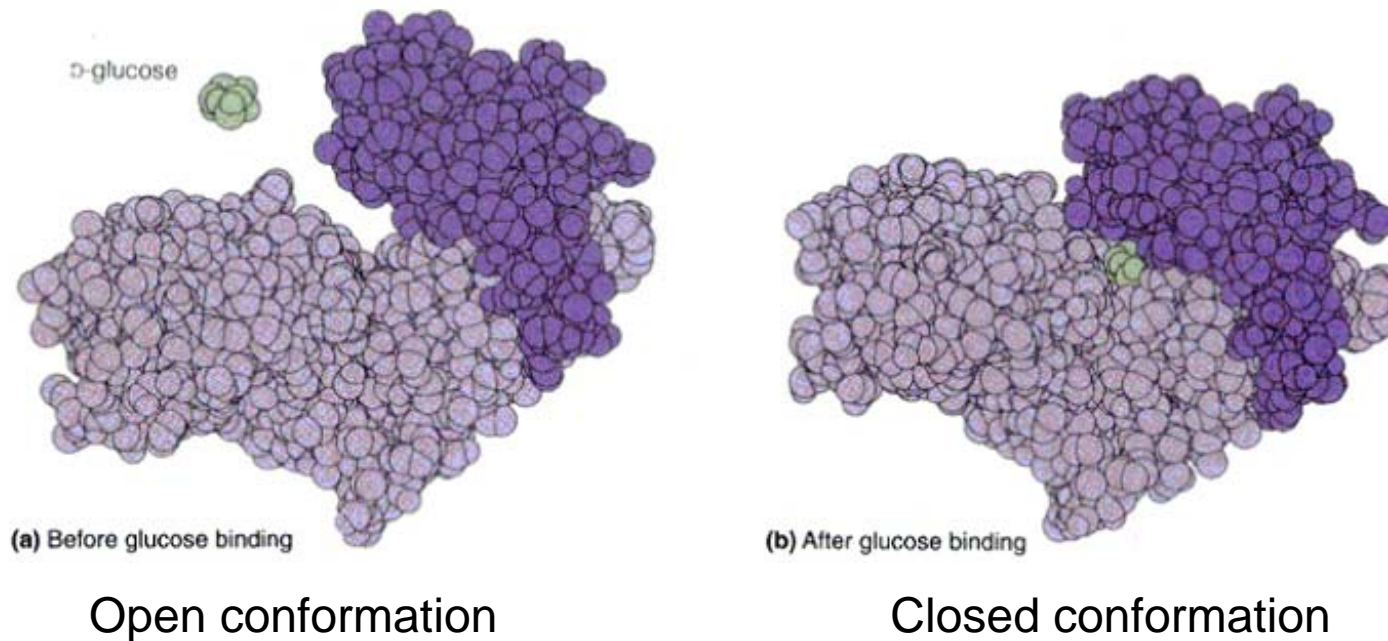


Induced Fit

- **Induced fit** activates an enzyme by substrate-initiated conformation effect
- **Induced fit** of a substrate brings chemical groups of the active site into positions that enhance their ability to catalyze the reaction
- Induced fit is a substrate specificity effect, not a catalytic mode

The induced conformational change in hexokinase

- Hexokinase mechanism requires sugar-induced closure of the active site



4. Thermodynamics

A. Enthalpy

- The **internal energy** of a system is a function of its state.
- The **enthalpy (H)** is defined as $H = E + PV$.
 - E is the internal energy
 - P is the pressure
 - V is the volume

B. Entropy

- The degree of randomness or disorder of a system is measured by a state function called the **Entropy** (**S**).
- The **entropy** of an ordered state is lower than that of a disordered state of the same system.
- The **entropy** of an isolated system will tend to increase to a maximum value.

C. Gibbs Free Energy (G)

- **Free energy (G)** is a function of state that includes both energy and entropy

$$G = H - TS$$

- **H** = enthalpy
- **S** = entropy
- **T** = absolute temperature

Free-energy Change (ΔG)

- **Free-energy change (ΔG)** is a measure of the chemical energy available from a reaction

$$\Delta G = G_{\text{products}} - G_{\text{reactants}}$$

Both entropy and enthalpy contribute to ΔG

$$\Delta G = \Delta H - T\Delta S$$

- ΔH = enthalpy change, ΔS = entropy change
 T = temp (T = degrees Kelvin)
- It measures energy change at constant temperature and pressure

Relationship between energy and entropy

Free energy change (ΔG) can predict the equilibrium concentrations and direction of a reaction

- When $\Delta G < 0$, the reaction will proceed spontaneously in the direction written
- When $\Delta G > 0$, the reaction requires energy to proceed
- When $\Delta G = 0$ the reaction is at equilibrium

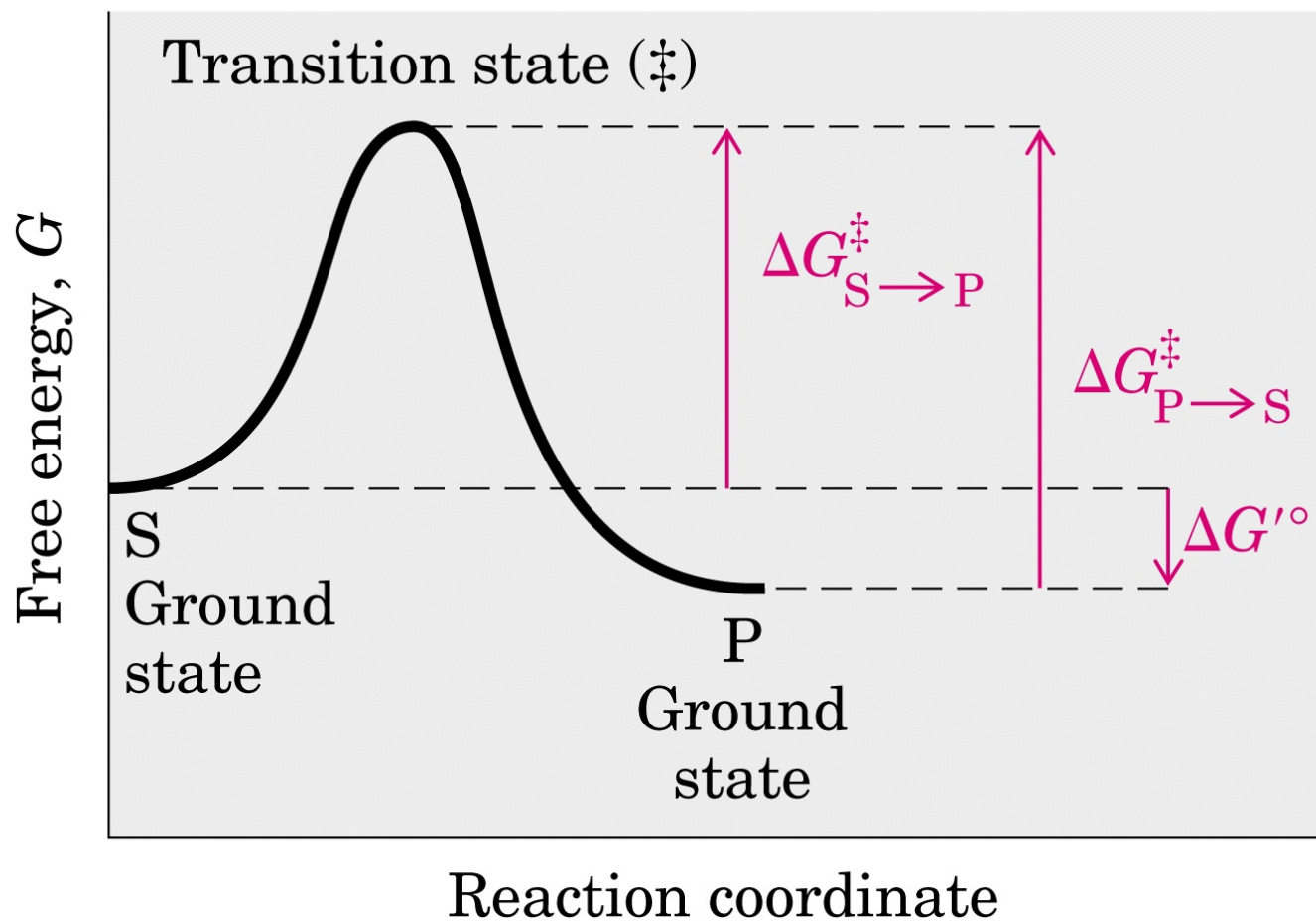
The Standard State (ΔG°) Conditions

- Reaction free-energy depends upon conditions
- **Standard state (ΔG°)** - defined reference conditions
 - Standard Temperature = 298K (25°C)
 - Standard Pressure = 1 atmosphere
 - Standard Solute Concentration = 1.0 M
- **Biological standard state = $\Delta G^{\circ'}$**
 - Standard H^+ concentration = 10^{-7} (pH = 7.0) rather than 1.0 M (pH = 0)

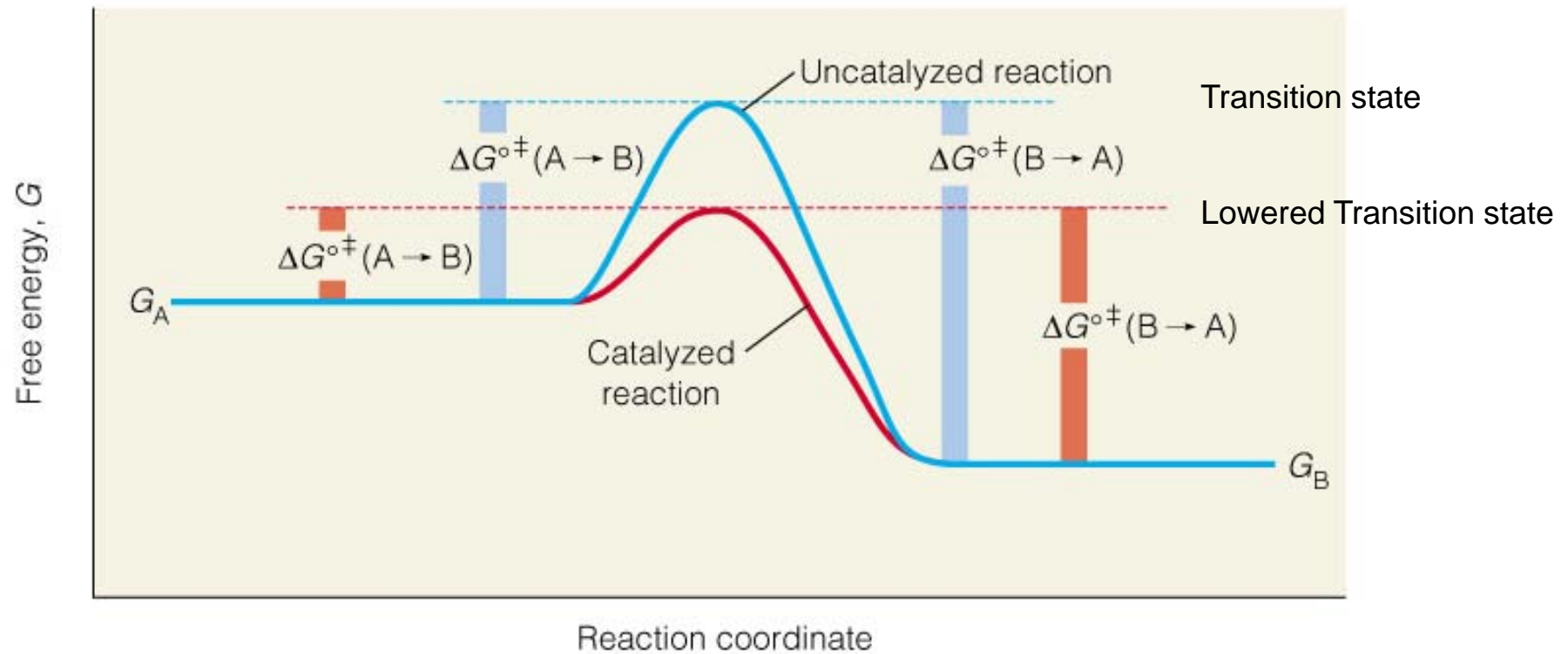
5. Mechanisms of Enzymes

- Mechanisms - the molecular details of catalyzed reactions
- Enzyme mechanisms deduced from:
 - Kinetic experiments
 - Protein structural studies
 - Studies of nonenzymatic model systems

Energy diagram for a single-step reaction



Effect of a catalyst on activation energy



Enzymes lower the activation energy of a reaction

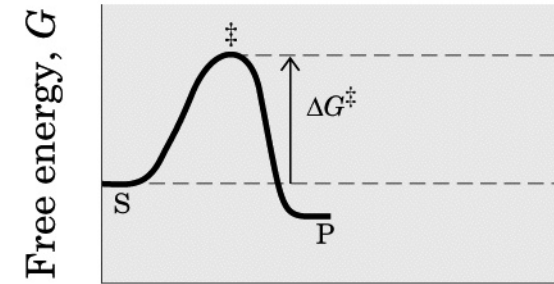
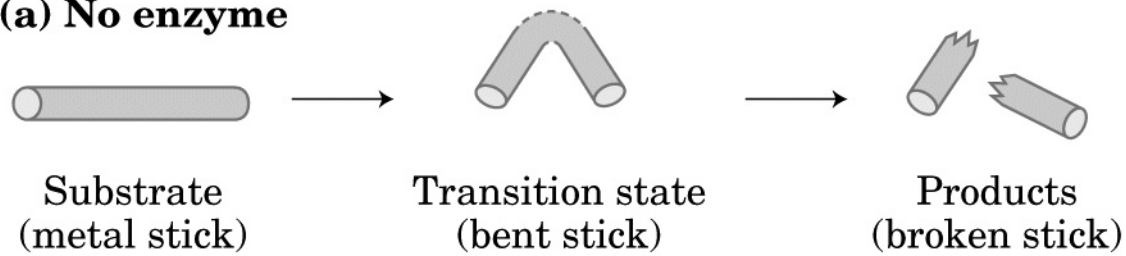
(1) Substrate binding

- Enzymes properly position substrates for reaction (makes the formation of the transition state more frequent and lowers the energy of activation)

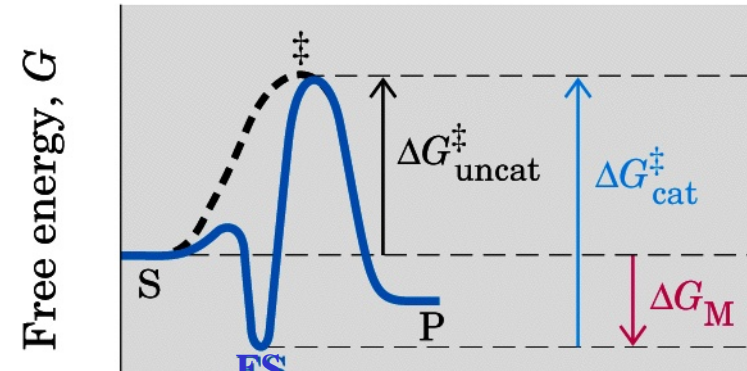
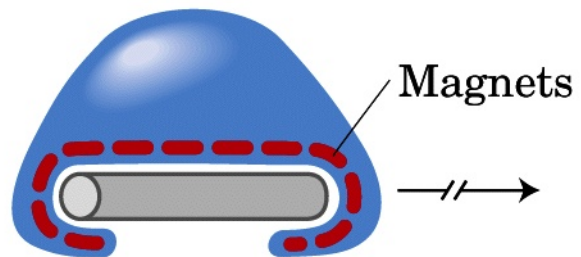
(2) Transition state binding

- Transition states are bound more tightly than substrates (this also lowers the activation energy)

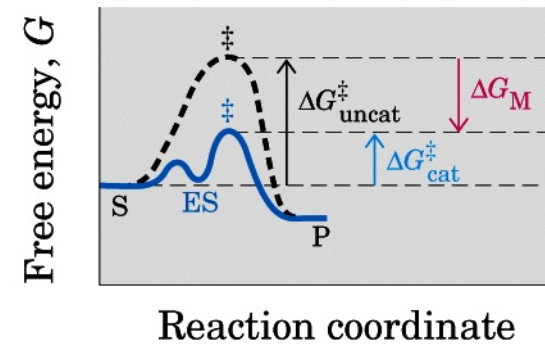
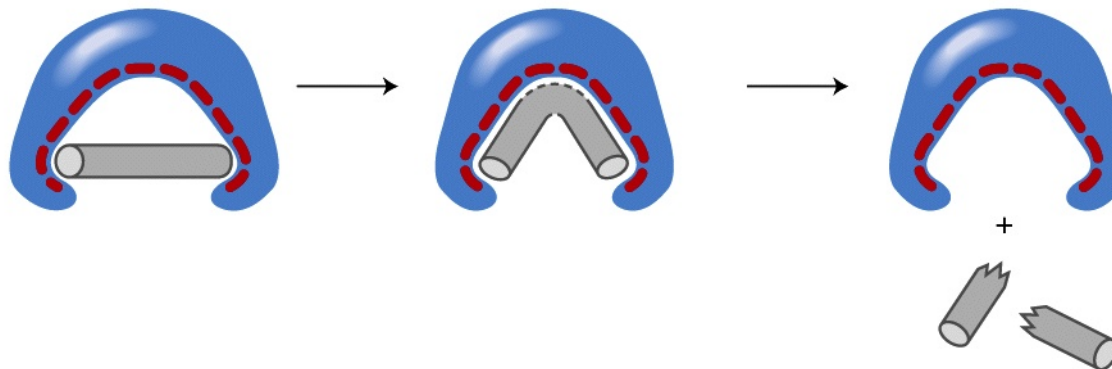
(a) No enzyme



(b) Enzyme complementary to substrate



(c) Enzyme complementary to transition state

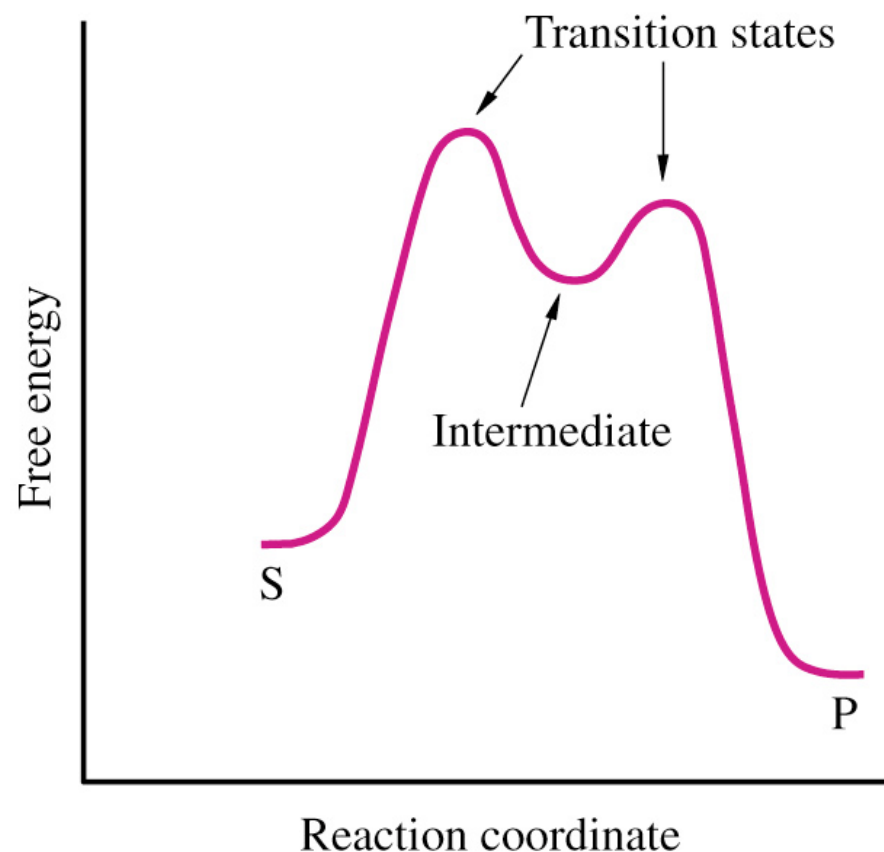


Transition-State Stabilization

- An increased interaction of the enzyme and substrate occurs in the transition-state (ES^\ddagger)
- The enzyme distorts the substrate, forcing it toward the transition state
- An enzyme must be complementary to the transition-state in shape and chemical character
- Enzymes may bind their transition states 10^{10} to 10^{15} times more tightly than their substrates

Energy diagram for reaction with intermediate

- Intermediate occurs in the trough between the two transition states
- Rate determining step in the forward direction is formation of the first transition state
- It may lead to multiple intermediate states that bypass the transition state.

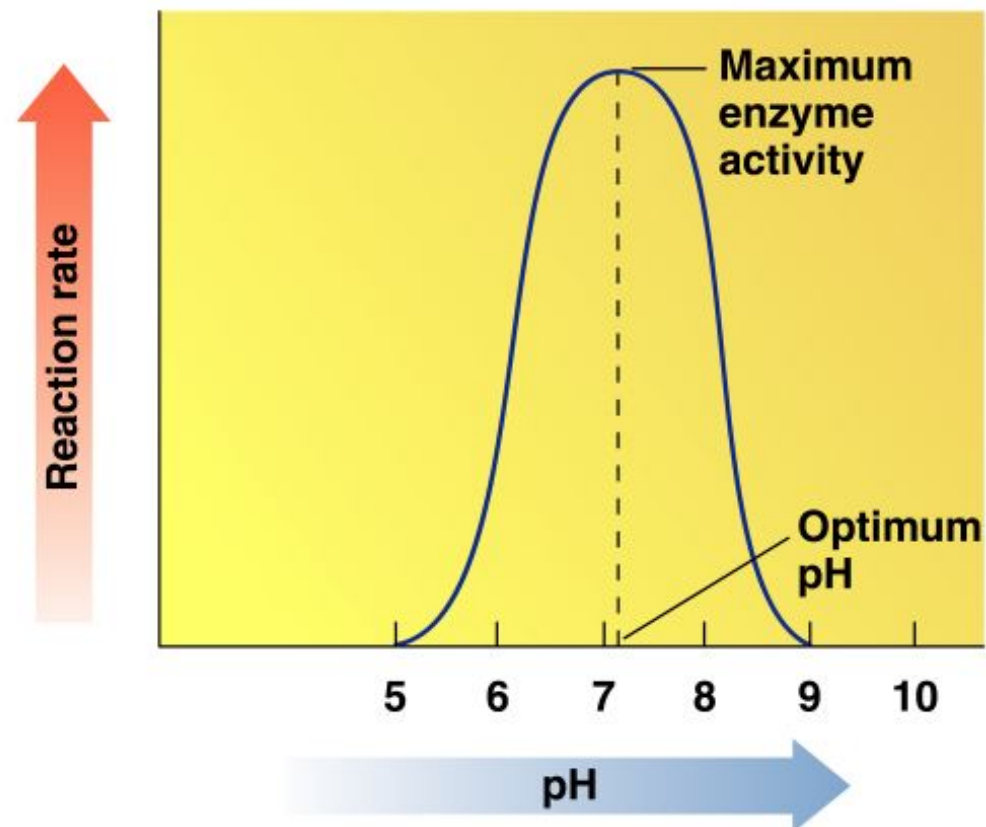


6. Factors That Affect Enzyme Activity

- Enzyme reactions are affected by reaction conditions such as
 - pH
 - Temperature
 - Substrate concentration
 - The presence of inhibitors

pH and Enzyme Activity

- Enzymes are most active at optimum pH
- Amino acids with acidic or basic side-chains have the proper charges when the pH is optimum
- Activity is lost at low or high pH as tertiary structure is disrupted



Optimum pH for Selected Enzymes

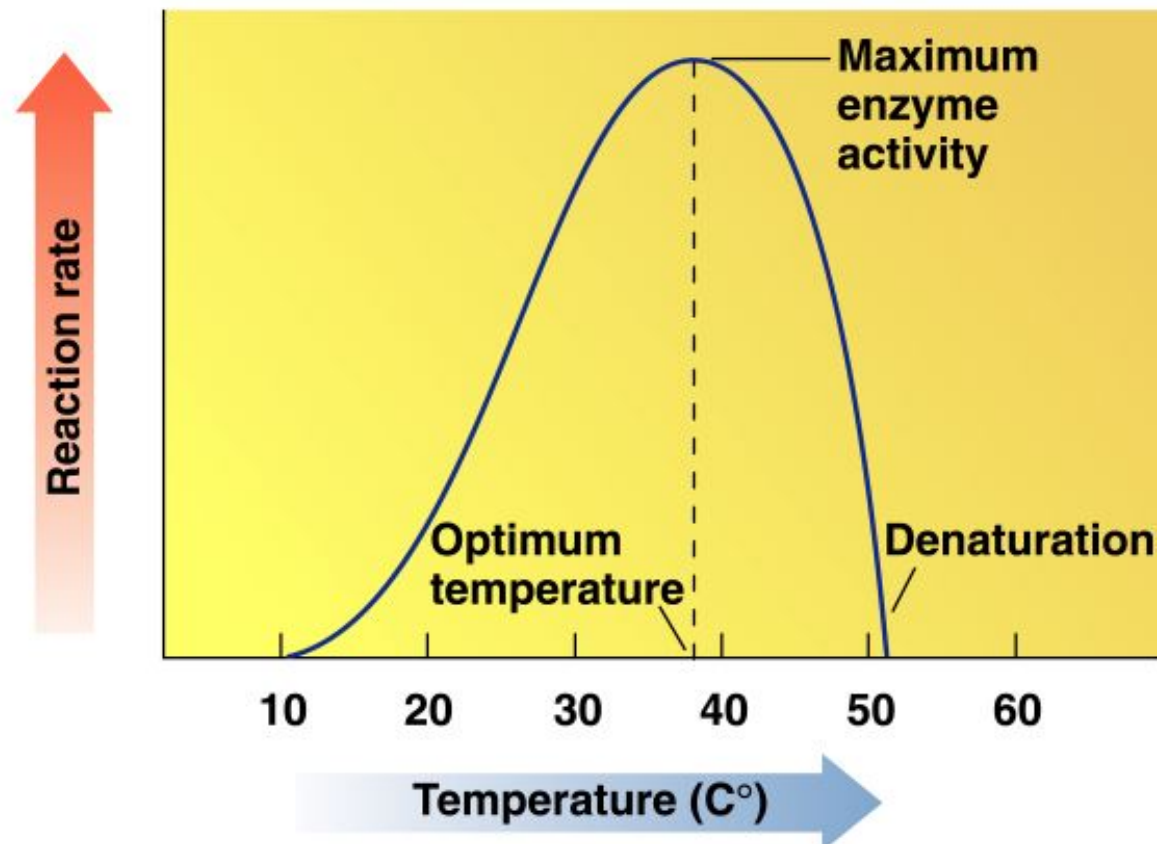
- Most enzymes of the body have an optimum pH of about 7.4
- However, in certain organs, enzymes operate at lower and higher optimum pH values

Optimum pH for Selected Enzymes

Enzyme	Location	Substrate	Optimum pH
Pepsin	Stomach	Peptide bonds	2
Urease	Liver	Urea	5
Sucrase	Small intestine	Sucrose	6.2
Pancreatic amylase	Pancreas	Amylose	7
Trypsin	Small intestine	Peptide bonds	8
Arginase	Liver	Arginine	9.7

Temperature and Enzyme Activity

- Enzymes are most active at an optimum temperature (usually 37°C in humans)
- They show little activity at low temperatures
- Activity is lost at high temperatures as denaturation occurs



Temperature and Enzyme Activity

- At low temperatures, enzyme activity is low due to a lack of energy for the reaction to occur.
- Food is stored in a refrigerator or freezer to slow spoilage brought on by enzymes.
- Boiling contaminated water will destroy enzymes in bacteria that are present in the water.