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* oxides 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

<table>
<thead>
<tr>
<th>Type</th>
<th>Reaction Type</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute</td>
<td>Catalyze one type of reaction for a single substrate</td>
<td>Urease catalyzes only the hydrolysis of urea</td>
</tr>
<tr>
<td>Group</td>
<td>Catalyze one type of reaction for similar substrates</td>
<td>Hexokinase adds a phosphate group to hexoses</td>
</tr>
<tr>
<td>Linkage</td>
<td>Catalyze one type of reaction for a specific type of bond</td>
<td>Chymotrypsin catalyzes the hydrolysis of peptide bonds</td>
</tr>
</tbody>
</table>
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:

<table>
<thead>
<tr>
<th>Class</th>
<th>Reactions catalyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidoreductases</td>
<td>Oxidation-reduction</td>
</tr>
<tr>
<td>Transferases</td>
<td>Transfer groups of atoms</td>
</tr>
<tr>
<td>Hydrolases</td>
<td>Hydrolysis</td>
</tr>
<tr>
<td>Lyases</td>
<td>Add atoms/remove atoms to/from a double bond</td>
</tr>
<tr>
<td>Isomerases</td>
<td>Rearrange atoms</td>
</tr>
<tr>
<td>Ligases</td>
<td>Use ATP to combine molecules</td>
</tr>
</tbody>
</table>
## Oxidoreductases, Transferases and Hydrolases

<table>
<thead>
<tr>
<th>Class</th>
<th>General Reactions Catalyzed</th>
<th>Typical Subclasses</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Oxidoreductases</td>
<td>Oxidation-reduction reactions</td>
<td>Oxidases</td>
<td>Oxidation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reductases</td>
<td>Reduction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dehydrogenases</td>
<td>Remove 2H to form double bonds</td>
</tr>
<tr>
<td>CH$_3$--CH$_2$--OH + NAD$^+$ $\xrightarrow{\text{Alcohol dehydrogenase}}$ CH$_3$--C--H + NADH$^+$ + H$^+$</td>
<td>Ethanol Coenzyme</td>
<td>Acetaldehyde Coenzyme</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. Transferases</th>
<th>Transfer of functional groups</th>
<th>Transaminases</th>
<th>Kinases</th>
<th>Transfer amino groups</th>
<th>Transfer phosphate groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_3^+$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH$_3$--CH--COO$^-$ + OOC--C--CH$_2$CH$_2$--COO$^-$ $\xrightarrow{\text{Alanine transaminase}}$ CH$_3$--C--COO$^-$ + OOC--CH--CH$_2$CH$_2$--COO$^-$</td>
<td>Alanine transaminase</td>
<td>Pyruvate</td>
<td>Glutamate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3. Hydrolases</th>
<th>Hydrolysis reactions</th>
<th>Peptidases</th>
<th>Lipases</th>
<th>Amylases</th>
<th>Hydrolyze peptide bonds</th>
<th>Hydrolyze ester bonds in lipids</th>
<th>Hydrolyze 1,4-glycosidic bonds in amylose</th>
</tr>
</thead>
<tbody>
<tr>
<td>N--CH--C--NH--CH--COO$^-$ + H$<em>2$O $\xrightarrow{\text{Peptidase}}$ N--CH--C--O$^-$</em> + H$_3$N--CH--COO$^-$</td>
<td>Peptidase</td>
<td></td>
<td>Lipases</td>
<td>Amylases</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Polypeptide C terminal |                               | Shorter polypeptide | Amino acid from C terminal
## Lyases, Isomerases and Ligases

<table>
<thead>
<tr>
<th>Class</th>
<th>General Reactions Catalyzed</th>
<th>Typical Subclasses</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>4. Lyases</td>
<td>Addition of a group to a double bond or removal of a group from a double bond without hydrolysis or oxidation</td>
<td>Decarboxylases, Dehydrases, Deaminases</td>
<td>Remove CO₂, Remove H₂O, Remove NH₃</td>
</tr>
</tbody>
</table>

\[
\text{CH}_3\text{C} = \text{C} \text{COO}^- + \text{H}^+ \xrightarrow{\text{Pyruvate decarboxylase}} \text{CH}_3\text{C} = \text{C} \text{H} + \text{CO}_2
\]

Pyruvate → Acetaldehyde + Carbon dioxide

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

\[
\text{OOC} \quad \xrightarrow{\text{Maleate isomerase}} \quad \text{COO}^-  \\
\text{C} = \text{C} \quad \xrightarrow{\text{Maleate isomerase}} \quad \text{COO}^-  \\
\text{H} \quad \text{H} \quad \text{H} \quad \text{H}
\]

Maleate ↔ Fumarate

| 6. Ligases | Bonding of molecules using ATP energy | Synthetases, Carboxylases | Combine molecules, Add CO₂ |

\[
\text{OOC} = \text{C} = \text{CH}_3 + \text{CO}_2 + \text{ATP} \xrightarrow{\text{Pyruvate carboxylase}} \text{OOC} = \text{C} = \text{CH}_2\text{COO}^- + \text{ADP} + \text{P}_i + \text{H}^+
\]

Pyruvate → Oxaloacetate
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

Transition state conformation
Lock and Key Model

Two substrates

Active site of the enzyme

Enzyme
Lock and Key Model

The substrates fit like a key in a lock

The active site is like a lock

Enzyme
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

\[
\text{Glucose + ATP} \rightarrow \text{Glucose 6-phosphate + ADP}
\]
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 ($\Delta G$)

- **Free-energy change** ($\Delta G$) 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 $\Delta G$

\[
\Delta G = \Delta H - T\Delta S
\]

- $\Delta H$ = enthalpy change, $\Delta S$ = entropy change
- $T$ = temp ($T$ = degrees Kelvin)
- It measures energy change at constant temperature and pressure
Free energy change (ΔG) can predict the equilibrium concentrations and direction of a reaction

- When ΔG<0, the reaction will proceed spontaneously in the direction written
- When ΔG>0, the reaction requires energy to proceed
- When ΔG = 0, the reaction is at equilibrium
The Standard State ($\Delta G^\circ$) Conditions

• Reaction free-energy depends upon conditions

• **Standard state ($\Delta G^\circ$)** - 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

Transition state ($\ddagger$)

\[ \Delta G^\ddagger_{S \rightarrow P} \]

\[ \Delta G^\ddagger_{P \rightarrow S} \]

\[ \Delta G''^\circ \]

Free energy, $G$

S
Ground state

P
Ground state

Reaction coordinate
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

Substrate (metal stick) → Transition state (bent stick) → Products (broken stick)

Free energy, $G$

(b) Enzyme complementary to substrate

Magnets

Free energy, $G$

(c) Enzyme complementary to transition state

Free energy, $G$

Reaction coordinate
Transition-State Stabilization

• An increased interaction of the enzyme and substrate occurs in the transition-state (ES‡)

• 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

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Location</th>
<th>Substrate</th>
<th>Optimum pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepsin</td>
<td>Stomach</td>
<td>Peptide bonds</td>
<td>2</td>
</tr>
<tr>
<td>Urease</td>
<td>Liver</td>
<td>Urea</td>
<td>5</td>
</tr>
<tr>
<td>Sucrase</td>
<td>Small intestine</td>
<td>Sucrose</td>
<td>6.2</td>
</tr>
<tr>
<td>Pancreatic amylase</td>
<td>Pancreas</td>
<td>Amylose</td>
<td>7</td>
</tr>
<tr>
<td>Trypsin</td>
<td>Small intestine</td>
<td>Peptide bonds</td>
<td>8</td>
</tr>
<tr>
<td>Arginase</td>
<td>Liver</td>
<td>Arginine</td>
<td>9.7</td>
</tr>
</tbody>
</table>
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.