Enzymes are biological catalysts; nearly every reaction that takes place in a living cell is catalyzed by an enzyme. Most enzymes are proteins, with some requiring non-protein components called coenzymes in order to function. The control of enzymatic activity plays a central role in controlling the activities and proper functioning of a living cell.
Introduction to Enzyme Catalysis

Enzymes can be amazingly proficient.

<table>
<thead>
<tr>
<th></th>
<th>Nonenzymatic rate constant ($k_n$ in s$^{-1}$)</th>
<th>Enzymatic rate constant ($k_{cat}/K_m$ in M$^{-1}$s$^{-1}$)</th>
<th>Catalytic proficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonic anhydrase</td>
<td>$10^{-1}$</td>
<td>$7 \times 10^6$</td>
<td>$7 \times 10^7$</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>$4 \times 10^{-9}$</td>
<td>$9 \times 10^7$</td>
<td>$2 \times 10^{16}$</td>
</tr>
<tr>
<td>Chorismate mutase</td>
<td>$10^{-5}$</td>
<td>$2 \times 10^6$</td>
<td>$2 \times 10^{11}$</td>
</tr>
<tr>
<td>Triose phosphate isomerase</td>
<td>$4 \times 10^{-6}$</td>
<td>$4 \times 10^8$</td>
<td>$10^{14}$</td>
</tr>
<tr>
<td>Cytidine deaminase</td>
<td>$10^{-10}$</td>
<td>$3 \times 10^6$</td>
<td>$3 \times 10^{16}$</td>
</tr>
<tr>
<td>Adenosine deaminase</td>
<td>$2 \times 10^{-10}$</td>
<td>$10^7$</td>
<td>$5 \times 10^{16}$</td>
</tr>
<tr>
<td>Mandelate racemase</td>
<td>$3 \times 10^{-13}$</td>
<td>$10^6$</td>
<td>$3 \times 10^{18}$</td>
</tr>
<tr>
<td>β-Amylase</td>
<td>$7 \times 10^{-14}$</td>
<td>$10^7$</td>
<td>$10^{20}$</td>
</tr>
<tr>
<td>Fumarase</td>
<td>$10^{-13}$</td>
<td>$10^9$</td>
<td>$10^{21}$</td>
</tr>
<tr>
<td>Arginine decarboxylase</td>
<td>$9 \times 10^{-16}$</td>
<td>$10^6$</td>
<td>$10^{21}$</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>$10^{-15}$</td>
<td>$3 \times 10^7$</td>
<td>$3 \times 10^{22}$</td>
</tr>
<tr>
<td>Orotidine 5′-phosphate decarboxylase</td>
<td>$3 \times 10^{-16}$</td>
<td>$6 \times 10^7$</td>
<td>$2 \times 10^{23}$</td>
</tr>
</tbody>
</table>
Introduction to Enzyme Catalysis

Overview

✦ Review of chemical reactions mechanisms
✦ Discussion of catalysis in general terms
✦ Examination of some major modes of enzymatic catalysis
  › acid/base catalysis
  › covalent catalysis
  › substrate binding
  › transition state stabilization
Chemical Reaction Mechanisms

A chemical mechanism lays out in detail the steps in a chemical reaction.

- With a focus on
  - the making and breaking of covalent bonds.
  - the movement of electrons at each step in a reaction.
Chemical Reaction Mechanisms

We will focus on three possible aspects to a reaction mechanism:

✦ Nucleophilic substitution
✦ Covalent bond cleavage
✦ Oxidation/Reduction

These are not necessarily independent of one another.

✦ e.g. Oxidation/Reduction can also involve covalent bond cleavage.
Chemical Reaction Mechanisms

Nucleophilic substitution

✦ nucleophiles vs electrophiles
✦ Nucleophilic attack on a carbonyl group

\[ \text{SN}_2 \text{ reaction with pentacoördinate transition state} \]
Chemical Reaction Mechanisms

Covalent bond cleavage reactions

✦ Formation of carbanion and hydrogen ion

\[
\begin{align*}
R_3 \quad \text{C–H} \quad \rightarrow \quad R_3 \quad \text{C} : \quad + \quad \text{H}^{+}
\end{align*}
\]

✦ Formation of carbocation and hydride ion

\[
\begin{align*}
R_3 \quad \text{C–H} \quad \rightarrow \quad R_3 \quad \text{C}^{+} \quad + \quad \text{H}^{+}
\end{align*}
\]

✦ This mechanism is used in dehydrogenation oxidation/reduction reactions.

Both of the bonding electrons stay with one of the products.
Chemical Reaction Mechanisms

Covalent bond cleavage

- Formation of free radicals

\[ R_1O \text{-} OR_2 \rightarrow R_1O\cdot + \cdot OR_2 \]

One of the two bonding electrons stays with each of the products
Chemical Reaction Mechanisms

Oxidation/Reduction Reactions

- These reactions are used to extract energy from the foods we eat.

- Definitions of oxidation and reduction

<table>
<thead>
<tr>
<th>Oxidation</th>
<th>Reduction</th>
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</thead>
<tbody>
<tr>
<td>Gain oxygen</td>
<td>Lose oxygen</td>
</tr>
<tr>
<td>Lose electrons</td>
<td>Gain electrons</td>
</tr>
<tr>
<td>Lose hydrogen</td>
<td>Gain hydrogen</td>
</tr>
</tbody>
</table>
Chemical Reaction Mechanisms

Oxidation/Reduction

- Dehydrogenation reactions represent a large fraction of the biological oxidation/reduction reactions.
- Usually involves a cleavage reaction that forms a carbocation.
- e.g. alcohol dehydrogenase

\[
\begin{align*}
\text{CH}_3\text{-CH}_2\text{-OH} & + \text{NAD}^+ & \rightarrow & \text{CH}_3\text{-C} & \text{H} & + \text{NADH} & + & \text{H}^+ \\
(\text{ethanol}) & & & (\text{acetaldehyde}) & & & & \\
\end{align*}
\]

- In this reaction, NAD\(^+\) is the oxidizing reagent
Chemical Reaction Mechanisms

Oxidation/Reduction

- By accepting the hydride ion, NAD$^+$ is often the oxidizing reagent in dehydrogenation reactions.

- NAD stands for nicotinamide-adenosine-dinucleotide.
Chemical Reaction Mechanisms

Oxidation/Reduction

By accepting the hydride ion, NAD$^+$ is often the oxidizing reagent in dehydrogenation reactions.

NAD stands for nicotinamide-adenosine-dinucleotide.
Chemical Reaction Mechanisms

Oxidation/Reduction

- By accepting the hydride ion, NAD\textsuperscript{+} is often the oxidizing reagent in dehydrogenation reactions.

- NAD stands for \textit{nicotinamide-adenosine-dinucleotide}.
Catalysts speed up reactions by lowering the free energy of the transition state.

$\Delta G < 0$

spontaneous

$E_{\text{act}} > 0$

without catalyst

$-$

with catalyst

Chem 352, Lecture 4 - Part II, Enzyme Catalysis
Catalysts Speed Up Reactions

Intermediates are represented by valleys in the reaction profile.
Catalysts Speed Up Reactions

We will focus on two ways that enzyme catalysts do this.

✦ The enzyme provides chemical catalysts

✦ The binding of substrates and transition state intermediates lowers the entropy for the reaction and helps to stabilizes the transition states.
Catalysts Speed Up Reactions

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- **The enzyme provides chemical catalysts**
- **The binding of substrates and transition intermediates** lowers the entropy for the reaction and helps to stabilize the transition states.
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✦ The enzyme provides chemical catalysts

✦ The binding of substrates and transition state intermediates lowers the entropy for the reaction and helps to stabilizes the transition states.
Chemical Modes of Enzymatic Catalysis

Functional groups present at the active site of an enzyme can provide alternative pathways from substrate to product.
The most common catalytic groups come from the polar amino acid side chains, which are embedded in a non-polar environment of the active site.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Reactive group</th>
<th>Net charge at pH 7</th>
<th>Principal functions</th>
</tr>
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<tbody>
<tr>
<td>Aspartate</td>
<td>$\text{COO}^-$</td>
<td>$-1$</td>
<td>Cation binding; proton transfer</td>
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<td>Histidine</td>
<td>Imidazole</td>
<td>Near 0</td>
<td>Proton transfer</td>
</tr>
<tr>
<td>Cysteine</td>
<td>$\text{CH}_2\text{SH}$</td>
<td>Near 0</td>
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<td>Phenol</td>
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Chemical Modes of Enzymatic Catalysis

The most common catalytic groups come from the polar amino acid side chains, which are embedded in a non-polar environment within the active site.
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Chemical Modes of Enzymatic Catalysis

The most common catalytic groups come from the polar amino acid side chains, which are embedded in a non-polar environment of the active site.

**TABLE 6.2** Typical pKa values of ionizable groups of amino acids in proteins

<table>
<thead>
<tr>
<th>Group</th>
<th>pKa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal α-carboxyl</td>
<td>3–4</td>
</tr>
<tr>
<td>Side-chain carboxyl</td>
<td>4–5</td>
</tr>
<tr>
<td>Imidazole</td>
<td>6–7</td>
</tr>
<tr>
<td>Terminal α-amino</td>
<td>7.5–9</td>
</tr>
<tr>
<td>Thiol</td>
<td>8–9.5</td>
</tr>
<tr>
<td>Phenol</td>
<td>9.5–10</td>
</tr>
<tr>
<td>ε-Amino</td>
<td>~10</td>
</tr>
<tr>
<td>Guanidine</td>
<td>~12</td>
</tr>
<tr>
<td>Hydroxymethyl</td>
<td>~16</td>
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Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

- Example: General base catalysis can assist in the cleavage of a peptide bond.
Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

- Example: General acid catalysis can assist in a dehydration reaction.

- $\text{OH}_2^-$ makes a better leaving group than $\text{OH}^-$

$$
\begin{align*}
\text{R}^+ + \text{OH}^- & \xrightleftharpoons{\text{Slow}} \text{R-OH} \\
\text{R-OH} & \xrightleftharpoons{\text{H}^+} \text{R-OH}_2^+ \\
\text{R-OH}_2^+ & \xrightarrow{\text{Fast}} \text{R}^+ + \text{H}_2\text{O}
\end{align*}
$$
Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

- The pKa’s for acid/base groups at the active site need to be near the local pH for this to work.

- pH can affect the activity of an enzyme if there are general acid/base catalysts involved in the reaction.
Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

- The pKa's for acid/base groups at the active site need to be near the local pH for this to work.
- pH can affect the activity of an enzyme if there are general acid/base catalysts involved.

![Graph showing pH vs. relative reaction rate for Papain with peaks at pH 4.2 and pH 8.2]
Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

- The pKa's for acid/base groups at the active site need to be near the local pH for this to work.
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Papain

\[ \begin{align*}
\text{Inactive} & \xrightarrow{H^+} \text{Active} & \xrightarrow{H^+} \text{Inactive} \\
R_A & \text{H} & R_B
\end{align*} \]
Chemical Modes of Enzymatic Catalysis

**Acid/Base catalysis**

- The pKa's for acid/base groups at the active site need to be near the local pH for this to work.

- pH can affect the activity of an enzyme if there are general acid/base catalysts involved in the reaction.

**Graph**

![Graph showing relative reaction rate vs. pH for Papain](image)

- pH = 4.2
- pH = 8.2
Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

- The pKₐ's for acid/base groups at the active site need to be near the local pH for this to work.
- pH can affect the activity of an enzyme if there are general acid/base catalysts involved in the reaction.

Papain

\[ \text{Cys} \quad \text{His} \]

\[
\begin{align*}
\text{Inactive} & \quad \text{H}^\ominus \quad pK_\text{a} = 3.4 \\
\text{Active} & \quad \text{Cys} \quad \text{His} \\
\text{Inactive} & \quad \text{H}^\ominus \quad pK_\text{a} = 8.3
\end{align*}
\]
Chemical Modes of Enzymatic Catalysis

**Acid/Base catalysis**

- The pKa's for acid/base groups at the active site need to be near the local pH for this to work.
- pH can affect the activity of an enzyme if there are general acid/base catalysts involved.

![Graph showing the relative reaction rate of Papain vs pH]

- pH: 2 to 11
- Relative reaction rate peak at pH = 4.2
- pH = 8.2
Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

- The pKa's for acid/base groups at the active site need to be near the local pH for this to work.
- pH can affect the activity of an enzyme if there are general acid/base catalysts involved in the reaction.

Papain

![Diagram of Papain structure with pH indicated]
Chemical Modes of Enzymatic Catalysis

**Acid/Base catalysis**

- The pKa's for acid/base groups at the active site need to be near the local pH for this to work.
- pH can affect the activity of an enzyme if there are general acid/base catalysts involved in the reaction.

![Graph of Papain activity vs pH](image.png)

Relative reaction rate

- **Papain**
- **pH = 4.2**
- **pH = 8.2**

Chem 352, Lecture 4 - Part II, Enzyme Catalysis
Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

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- pH can affect the activity of an enzyme if there are general acid/base catalysts involved in the reaction.
Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

- Triose phosphate isomerase illustrates both general acid and base catalysis.
Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

Triose phosphate isomerase illustrates both general acid and base catalysis.

Glu-165

Enediol intermediate

H → C → OH
CH₂PO₄⁻²

D-Glyceraldehyde 3-phosphate (G3P)
Chemical Modes of Enzymatic Catalysis

Acid/Base catalysis

Triose phosphate isomerase illustrates both general acid and base catalysis.
Chemical Modes of Enzymatic Catalysis

TIM is Diffusion-Controlled

- Simple reactions, like that of triose phosphate isomerase (TIM), are rate limited by the binding of the substrate.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>$k_{cat}/K_m$ (M$^{-1}$ s$^{-1}$)*</th>
</tr>
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<tbody>
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</tr>
<tr>
<td>Superoxide dismutase</td>
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*The ratio $k_{cat}/K_m$ is the apparent second-order rate constant for the enzyme-catalyzed reaction $E + S \rightarrow E + P$. For these enzymes, the formation of the ES complex can be the slowest step.
TIM is Diffusion-Controlled

- Simple reactions, like that of triose phosphate isomerase (TIM), are rate limited by the binding of the substrate.
Chemical Modes of Enzymatic Catalysis

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* The ratio $k_{cat}/K_m$ is the apparent second-order rate constant for the enzyme-catalyzed reaction $E + S \rightarrow E + P$. For these enzymes, the formation of the ES complex can be the slowest step.
Covalent bond catalysis

- For some enzymes, the transition state intermediate is covalently bonded to the enzyme.

\[
\begin{align*}
A\text{–}X + E & \rightleftharpoons X\text{–}E + A \\
X\text{–}E + B & \rightleftharpoons B\text{–}X + E \\
\hline \\
A\text{–}X + B & \rightleftharpoons B\text{–}X + A
\end{align*}
\]

- We will see an example of this when we look at the details of the serine protease catalyzed reactions.
Chemical Modes of Enzymatic Catalysis

Covalent bond catalysis

- For some enzymes, the transition state intermediate is covalently bonded to the enzyme.

\[
\text{Ping-pong reaction}
\]

\[
\begin{align*}
E & \quad (EA)(FP) & F & \quad (FB)(EQ) & E \\
A & \quad \rightarrow & P & & \quad B & \quad \rightarrow & Q
\end{align*}
\]

look at the details of the serine protease catalyzed reactions
Chemical Modes of Enzymatic Catalysis

Covalent bond catalysis

For some enzymes, the transition state intermediate is covalently bonded to the enzyme.

A + E ⇌ EA

EA ⇌ (EAB) ⇌ (EPQ) ⇌ EQ

E + B ⇌ EB

EB ⇌ (EBQ) ⇌ EP ⇌ P

Sequential reactions

Ordered

A
B
P
Q

Random

A
B
P
Q

Chem 352, Lecture 4 - Part II, Enzyme Catalysis
Chemical Modes of Enzymatic Catalysis

Covalent bond catalysis

- For some enzymes, the transition state intermediate is covalently bonded to the enzyme.

\[ A - Y + E \overset{\text{X}}{\longrightarrow} A - Y - E \]

Ping-pong reaction

\[ E \quad \text{(EA)(FP)} \quad F \quad \text{(FB)(EQ)} \quad E \]

Look at the details of the serine protease catalyzed reactions.
Chemical Modes of Enzymatic Catalysis

Covalent bond catalysis

- For some enzymes, the transition state intermediate is covalently bonded to the enzyme.

\[
\begin{align*}
A - X + E & \leftrightarrow X - E + A \\
X - E + B & \leftrightarrow B - X + E \\
\hline
A - X + B & \leftrightarrow B - X + A
\end{align*}
\]

- We will see an example of this when we look at the details of the serine protease catalyzed reactions.
Binding Modes of Enzymatic Catalysis

✦ Acid/Base catalysis and covalent bond catalysis can account for an approximately 10 to 100 fold increase in the reaction rates

✦ However, $10^8$ fold increases are observed
Enzymes also bind to substrates and orient them relative to one another and to catalytic groups on the enzyme.
The binding of substrates creates a high effective local concentration of substrates.

It also decreases the entropy of the substrates.
### Binding Modes of Enzymatic Reaction

<table>
<thead>
<tr>
<th></th>
<th>Reaction</th>
<th>Relative rate constants</th>
</tr>
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<tr>
<td>1.</td>
<td>( \text{H}_2\text{C} - \text{C} - \text{O} - \text{Br} ) + ( \text{H}_3\text{C} - \text{C} - \text{O} - \text{O} ) → ( \text{H}_2\text{C} - \text{C} - \text{O} ) + ( \text{H}_3\text{C} - \text{C} - \text{O} ) + ( \text{O} - \text{O} - \text{Br} )</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>( \text{H}_2\text{C} - \text{C} - \text{O} - \text{Br} ) + ( \text{H}_2\text{C} - \text{C} - \text{O} - \text{O} ) → ( \text{H}_2\text{C} - \text{C} - \text{O} ) + ( \text{H}_2\text{C} - \text{C} - \text{O} ) + ( \text{O} - \text{O} - \text{Br} )</td>
<td>( 1 \times 10^3 )</td>
</tr>
<tr>
<td>3.</td>
<td>( \text{H}_2\text{C} - \text{C} - \text{O} - \text{Br} ) + ( \text{H}_2\text{C} - \text{C} - \text{O} - \text{O} ) → ( \text{H}_2\text{C} - \text{C} - \text{O} ) + ( \text{H}_2\text{C} - \text{C} - \text{O} ) + ( \text{O} - \text{O} - \text{Br} )</td>
<td>( 2 \times 10^5 )</td>
</tr>
<tr>
<td>4.</td>
<td>( \text{O} - \text{O} - \text{C} - \text{O} - \text{Br} ) + ( \text{C} - \text{O} - \text{O} - \text{O} - \text{O} ) → ( \text{C} - \text{O} - \text{O} - \text{O} - \text{O} ) + ( \text{C} - \text{O} - \text{O} - \text{O} - \text{O} ) + ( \text{O} - \text{O} - \text{Br} )</td>
<td>( 5 \times 10^7 )</td>
</tr>
</tbody>
</table>
Binding Modes of Enzymatic

The Proximity Effect

✦ The binding of substrates creates a high effective local concentration of substrates.

✦ It also decreases the entropy of the substrates.
Binding Modes of Enzymatic Catalysis

- The favorable binding of the transition state helps to lower the activation barrier and, therefore, speed up a reaction.

- However, if the binding of substrate is too favorable, the overall reaction rate can be negatively affected.
Binding Modes of Enzymatic Catalysis

The favorable binding of the transition state helps to lower the activation barrier and, therefore, speed up a reaction. However, if the binding of substrate is too favorable, the overall reaction rate can be negatively affected.
Binding Modes of Enzymatic Catalysis

- The favorable binding of the transition state helps to lower the activation barrier and, therefore, speed up a reaction.

- However, if the binding of substrate is too favorable, the overall reaction rate can be negatively affected.
Binding Modes of Enzymatic Catalysis

“Lock and Key” model

- In the late 1880’s Emil Fischer, with his “lock and key” model, predicted what we know now to be the contribution of substrate binding to enzyme catalysis.

- In the 1960’s, Daniel Koshland proposed an alternative “induced fit” model
“Induced fit” model

- In the “induced fit” model, substrate binding induces conformational changes in the enzyme.
- Hexokinase provides a good example of “induced fit”
Binding Modes of Enzymatic Catalysis

Hexokinase, with (1BDG) and without (1HKG) bound substrate (glucose)
Binding Modes of Enzymatic Catalysis

Stabilizing the transition state

- Some of the most potent enzyme inhibitors are transition state analogues.

Adenosine deaminase

Chem 352, Lecture 4 - Part II, Enzyme Catalysis
Binding Modes of Enzymatic Catalysis

Stabilizing the transition state

- Some of the most potent enzyme inhibitors are transition state analogues.

![Chemical structures](image)
Binding Modes of Enzymatic Catalysis

Stabilizing the transition state

- Some of the most potent enzyme inhibitors are transition state analogues.

The binding affinity for the transition state analogue is $10^8$ higher than that for either the substrate or product.
Catalytic Antibodies (Abzymes)

- Transition state analogues have been used to create antibodies having catalytic activity.

\[
\begin{align*}
(b) & \quad \text{OOC}-(CH_2)_3-C-NH-CO-NH(\text{phenyl})-CO \quad \text{NO}_2 \\
& \quad \text{OH}^\ominus \\
& \quad \text{OOC}-(CH_2)_3-C-NH-CO-O^\ominus + \text{H}_2\text{N}-\text{phenyl} \quad \text{NO}_2 \\
& \quad \text{Carboxylate} \quad p-\text{Nitroaniline}
\end{align*}
\]
Catalytic Antibodies (Abzymes)

- Transition state analogues have been used to create antibodies having catalytic activity.

![Diagram showing the interaction between carboxylate and p-nitroaniline in the catalytic process.](Image)
Binding Modes of Enzymatic Catalysis

Catalytic Antibodies (Abzymes)

- Transition state analogues have been used to create antibodies having catalytic activity.

![Chemical reaction diagram](image)

(b) 

$$\Theta\text{OOC-}-(\text{CH}_2)_3-\text{C-}\text{N}$$

\[\xrightarrow{\text{OH}^-}\]

$$\Theta\text{OOC-}-(\text{CH}_2)_3-\text{C-}\text{N}$$

Carboxylate

$$+ \text{H}_2\text{N-}-(\text{CH}_2)_3-\text{C-}$$

$p$-Nitroaniline
Catalytic Antibodies (Abzymes)

- Transition state analogues have been used to create antibodies having catalytic activity.

Chem 352, Lecture 4 - Part II, Enzyme Catalysis

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Catalytic Antibodies (Abzymes)

- Transition state analogues have been used to create antibodies having catalytic activity.

Abzyme speed up reaction $10^5$ times
Serine Proteases - A Case Study

Case studies of enzyme catalyzed reactions:

✦ **Lysozyme**
  - Cleaves the polysaccharide found in bacterial cell walls.

✦ **Chymotrypsin**
  - A Serine protease that cleaves the polypeptide backbone during protein digestion.
Serine Proteases - A Case Study

Serine proteases are a group of enzymes that cleave peptide bonds.

- There are many different serine proteases
- All contain a serine side chain in their active site, along with a histidine and an aspartic acid sidechain.

![Catalytic Triad Diagram]

The catalytic triad
Serine Proteases - A Case Study

Serine proteases nicely illustrate many of the tricks that can be used to speed up chemical reactions

- Catalytic modes of enzymatic catalysis
  - Acid/base catalysis
  - Covalent catalysis
- Binding modes of enzymatic catalysis
  - Proximity effect
  - Transition state stabilization
Serine Proteases - A Case Study

They also illustrate:

- Importance of protein folding in creating a functional protein
- Substrate specificity
- Activation through irreversible covalent modifications
Serine Proteases - A Case Study

They also illustrate

- Importance of folding to creating a functional protein
- Substrate specificity
- Activation through irreversible covalent modifications
Serine Proteases - A Case Study

They also illustrate:

✦ Importance of folding to creating a functional protein
✦ Substrate specificity
✦ Activation through irreversible covalent modifications
Serine Proteases - A Case Study

They also illustrate:

✦ Importance of folding to creating a functional protein
✦ Substrate specificity
✦ Activation through irreversible covalent modifications
✦ Additional mechanisms involving active sites
Serine Proteases - A Case Study

They also illustrate

✦ Importance of folding to creating a functional protein
✦ Substrate specificity
✦ Activation through irreversible covalent modifications
Serine Proteases – A Case Study

They also illustrate

- Importance of folding to creating a functional protein
- Substrate specificity
- Activation through irreversible covalent modifications

(a) chymotrypsin (5CHA)  (b) trypsin (1TLD)  (c) elastase (3EST)
Serine Proteases - A Case Study

They also illustrate

- Importance of folding to creating a functional protein
- Substrate specificity
- Activation through irreversible covalent modifications

(a) Chymotrypsin
(b) Trypsin
(c) Elastase

(a) chymotrypsin (5CHA)  
(b) trypsin (1TLD)  
(c) elastase (3EST)
Serine Proteases - A Case Study

They also illustrate

✦ Importance of folding to creating a functional protein
✦ Substrate specificity
✦ Activation through irreversible covalent modifications
Serine Proteases - A Case Study

They also illustrate:

- **Zymogen** (inactive precursor) synthesized in the pancreas
- **Active Enzyme** activated in the small intestine
Serine Proteases - A Case Study

They also illustrate:

✦ Importance of folding to creating a functional protein
✦ Substrate specificity
✦ Activation through irreversible covalent modifications

![Diagram of Serine Proteases]

- Trypsinogen
  - Converted to Trypsin by Enteropeptidase
  - Converts Chymotrypsinogen to Chymotrypsin
  - Converts Proelastase to Elastase

Chem 352, Lecture 4 - Part II, Enzyme Catalysis
Serine Proteases - A Case Study

They also illustrate

✦ Importance of folding to creating a functional protein
✦ Substrate specificity
✦ Activation through irreversible covalent modifications
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle

Proximity Effect
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle

Acid/Base Catalysis
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle

Covalent Bond Catalysis

Transition State Stabilization
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle

Diagram of serine protease catalytic cycle with labeled amino acids and reaction steps.
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle
Serine Proteases - A Case Study

Step-by-Step through the catalytic cycle
Serine Proteases - A Case Study

Protein Structure - Chymotrypsin

- Select model: cartoon, ball & stick, spacefill
- Select Secondary Structures: α-helix, β-sheet, other
- Highlight the Catalytic Triad: Asp 102, His 57, Ser 195
- Highlight the N-terminal Half of Peptide Product Covalently Bound to the Enzyme: On/Off
- Solvent Accessible Surface: On/Off, Translucent
- Highlight the Peptide-Enzyme Backbone Interactions: On/Off
- Highlight the Activating Interaction: Asp 194, Ile 18 and Leu 10
Next Up

✦ At this time we will skip over Chapter 7 (Cofactors and Vitamins)
  ‣ We will discuss cofactors and vitamins as we encounter them throughout the rest of the semester.

✦ Carbohydrates (Chapter 8)