Substrate Binding and Enzyme Action

- The first step in an enzyme-catalyzed reaction is the formation of the enzyme-substrate complex. This is represented by the equation:
  \[ E + S \rightarrow ES = E + P \].
- The region of the enzyme where the substrate binds is called the active site. This consists of a substrate binding site and the catalytic site.
- The active site is usually a cleft or pocket created by the unique tertiary structure of the enzyme protein.
- Enzyme specificity is due to specificity of substrate binding driven by substrate and enzyme 3D structure.
- The ES complex is stabilized in the transition state by non-covalent interactions between substrate and the amino acid in the active site.

Factors Affecting Reaction Rates

The study of enzyme reaction rates is called enzyme kinetics. Enzyme kinetics are affected by:

- **Temp and pH:**
  - Each enzymatic reaction has an optimum pH and optimum temperature. Extreme temp or pH disrupts enzyme structure and therefore reaction rate.
- **Enzyme concentration:**
  - At saturating substrate concentration, the initial velocity is directly related to the enzyme concentration. \[ E + S \rightarrow ES = E + P \]. Thus, as long as S is not limiting, more E leads to more ES.
- **Substrate concentration:**
  - The reaction rate is \( \frac{k [P]}{[S]} \). The rate can be increased by adding more substrate, or by removing product as it is formed.
An enzyme binds a substrate in a region called the active site.
Only certain substrates can fit the active site.
Amino acid R groups in the active site help substrate bind.
Enzyme-substrate complex forms.
Substrate reacts to form product.
Product is released.

Models of Enzyme-Substrate Binding

- **Lock and key model**: This means that the enzyme’s tertiary structure consists of a unique pocket or site which is tailor-made to fit only its substrate and nothing else, just as a key fits into a lock.
- **Induced-fit model**: This updated model states that enzymes interact with substrates and in the process change their conformation such that the enzyme is snug around the substrate, sort of like a glove around a hand.

**Enzyme Action - Lock & Key Model**

- The favored model of enzyme substrate interaction is known as the induced fit model.
- This model proposes that the initial interaction between enzyme and substrate is relatively weak.
  - These weak interactions rapidly induce conformational changes in the enzyme that strengthen binding and bring catalytic sites close to substrate bonds to be altered.
- After binding takes place, one or more mechanisms of catalysis generates transition - state complexes and reaction products.
- The possible mechanisms of catalysis are four in number.
Catalysis by Bond Strain:
- In this form of catalysis, the induced structural rearrangements that take place with the binding of substrate and enzyme ultimately produce strained substrate bonds,
  • Which more easily attain the transition state.
- The new conformation often forces substrate atoms and bulky catalytic groups, such as aspartate and glutamate, into conformations that strain existing substrate bonds.

Catalysis by Proximity and Orientation:
- Enzyme-substrate interactions orient reactive groups and bring them into proximity with one another.
  • In addition to inducing strain, groups such as aspartate are frequently chemically reactive as well, and their proximity and orientation toward the substrate thus favors their participation in catalysis.

Enzyme Substrate Interactions

Enzyme Inhibitors

Enzyme Inhibitors
- Specific enzyme inhibitors regulate enzyme activity and help us understand mechanism of enzyme action. (Denaturing agents are not inhibitors)
- **Irreversible inhibitors** form covalent or very tight permanent bonds with aa at the active site of the enzyme and render it inactive. 3 classes: group specific reagents, substrate analogs, suicide inhibitors
- **Reversible inhibitors** form an EI complex that can be dissociated back to enzyme and free inhibitor. 3 groups based on their mechanism of action: competitive, non-competitive and uncompetitive.

Enzyme Inhibitors

Stickase

If enzyme just binds substrate then there will be no further reaction

Enzyme not only recognizes substrate, but also induces the formation of transition state

Enzyme Inhibitors

Enzyme Inhibitors

Specific

Denaturation

Irreversible

Reversible

Noncompetitive, Allosteric, Feedback

Competitive

Acids & Bases
Temperature
Heavy Metals
Reducing Agents

Non-specific

Competitive

Reversible

Irreversible

Denaturation

Enzyme Inhibitors

Enzyme Inhibitors

Enzyme Inhibitors

Enzyme Inhibitors

Enzyme Inhibitors

Enzyme Inhibitors
**Competitive Inhibition**

- Has a structure similar to substrate
- Occupies active site
- Competes with substrate for active site
- Has effect reversed by increasing substrate concentration

**Non-Competitive Inhibition**

- Does not have a structure like substrate
- Binds to the enzyme but not active site
- Changes the shape of enzyme and active site
- Substrate cannot fit altered active site
- No reaction occurs
- Effect is not reversed by adding substrate

**Feedback Control**

The activity of the first enzyme of the pathway is inhibited by the end product, thus controlling production of end product.

**Enzyme Inhibition (Mechanism)**

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Competitive</th>
<th>Non-competitive</th>
<th>Uncompetitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>$E + S \rightarrow ES \rightarrow E + P$</td>
<td>$E + S \rightarrow ES \rightarrow E + P$</td>
<td>$E + S \rightarrow ES \rightarrow E + P$</td>
</tr>
<tr>
<td>Inhibitor</td>
<td>$E + I \downarrow$</td>
<td>$E + I \downarrow$</td>
<td>$E + I \downarrow$</td>
</tr>
<tr>
<td>Reaction</td>
<td>$E + S \rightarrow ES \rightarrow E + P$</td>
<td>$E + S + ES \rightarrow E + P$</td>
<td>$E + S + ES \rightarrow E + P$</td>
</tr>
</tbody>
</table>

- $E$ binds to $I$ only and competes with $S$. Increasing $S$ overcomes inhibition by $I$.
- $[I]$ binds to $E$ or $ES$ complex. Increasing $S$ can not overcome $[I]$ inhibition.
- $[I]$ binds to $ES$ complex only, increasing $S$ favors the inhibition by $[I]$. 

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**Succinate Dehydrogenase**

<table>
<thead>
<tr>
<th>Product</th>
<th>Substrate</th>
<th>Competitive Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate</td>
<td>Glutarate</td>
<td>Malonate</td>
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<tr>
<td>$\text{C}-\text{OO}^-$</td>
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