Enzymes catalyze chemical reactions (thousands). Most would not occur alone at physiological conditions. Enzymes facilitate chemical reactions by increasing their rate. They act as a catalyst, i.e., the enzyme is regenerated:

They are specific: they carry out specific reactions on specific substrates.

Thermodynamics vs. Kinetics

<table>
<thead>
<tr>
<th>Thermodynamics</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔG: the change in the Gibbs free energy for the reaction</td>
</tr>
<tr>
<td>ΔG = G(product) - G(reactant)</td>
</tr>
<tr>
<td>If G(product) &gt; G(reactant), ΔG is negative.</td>
</tr>
<tr>
<td>ΔG is the driving force for the reaction.</td>
</tr>
<tr>
<td>The reaction is spontaneous and energy will be released.</td>
</tr>
<tr>
<td>ΔG is also related to the equilibrium constant (K&lt;sub&gt;eq&lt;/sub&gt;): ΔG° = -RT ln K&lt;sub&gt;eq&lt;/sub&gt;</td>
</tr>
<tr>
<td>ΔG for the reaction says nothing about how fast the reaction will go.</td>
</tr>
</tbody>
</table>
Kinetics (how fast?)

Enzymes can increase rates by $10^6$-$10^{14}$-fold.

"Active Site"

Region of the protein that:
1) binds substrate
2) catalyzes the chemical reaction

Example:
Carboxypeptidase A
-- synthesized in the pancreas
-- delivered to the small intestine lumen
-- role in digestion of protein

Removes amino acids from the C-terminus of peptides

Peptide $\rightarrow$ a COOH

A Substrate: $\text{H}_2\text{N}-\text{Gly-Tyr-COO}^-$

In water, no bond cleavage takes place.
**Active Site:** Binding of Substrate

Carboxypeptidase A
Enzyme Contains Zinc Ion

Active Site: Catalytic Mechanism
Types of Enzyme Reactions

1. Oxidoreductases
   - electrons, H or O atoms moved from one substrate to another one
     (e.g., alcohol dehydrogenase; alcohol to acetaldehyde)

2. Transferases
   - transfer of a chemical group from one molecule to another
     (e.g., a kinase that moves a phosphate from ATP to a protein)

3. Hydrolases
   - use water to make two products from a substrate
     (e.g., carboxypeptidase A described above)

4. Lyases
   - cleave –C-C-, –C-N- or C-O bonds, or sometimes make them (synthetases)

5. Isomerases
   - move a group or a double bond within the molecule

6. Ligases
   - join atoms together using energy, usually from ATP (synthetases)
Coenzymes and Cofactors

Many enzymes just use their own amino acids for catalysis. Others require nonpolypeptide coenzymes/cofactors for catalysis.

**Cosubstrate** modified during reaction and leaves the active site.

**Prosthetic group** tightly bound, regenerated after reaction.

### Enzyme Kinetics

(Rates in mathematical terms)

\[
E + S \xrightleftharpoons[k_2]{k_1} ES \xrightarrow{k_{cat}} E + P
\]

\[
E + S \xrightarrow{k_1} E + P
\]

\[
E + S \xrightarrow[k_{cat}]{k_2} E + P
\]

\(k_{cat}\) is the catalytic rate constant

(for the rate-determining step)

Add enzyme to substrate; measure substrate and product concentrations over time
Effect of [S] on Initial Velocity

Initial velocity \( v \) vs. [S]

Initial velocity is defined as the change in product over time, \( d[P]/dt \).

Michaelis-Menten Equation:

\[
\frac{\kappa_{\text{cat}} [E_0] [S]}{K_m + [S]} = v
\]

- \( [E_0] \) is total enzyme concentration
- \( \kappa_{\text{cat}} \) is the \( V_{\text{max}} \) (Maximum velocity at a given \( [E_0] \))
- \( K_m = k_1/k_2 + k_3 \)
- If \( k_3 \ll k_2 \), \( K_m \) is the **dissociation constant** (affinity)

- \( \kappa_{\text{cat}} \) and \( K_m \) are constants!!

\[
\frac{V_{\text{max}} [S]}{K_m + [S]} = v
\]

- If \( K_m = [S] \), \( v = 0.5 V_{\text{max}} \)

\( K_m \) is equivalent to the [S] that gives a velocity of one-half \( V_{\text{max}} \).
$[S] \ll K_m$  
$V = \frac{V_{max} [S]}{K_m + [S]}$  
$V = \frac{V_{max} [S]}{[S] + K_m}$  
$V = \frac{V_{max} [S]}{[E][S] + K_m}$

$V_{max} = k_{cat}[E_o]$  
Initial Velocities (Product vs. Time) for Different $[E_o]$ at high $[S]$

How Fast Does an Enzyme Go, Actually?

Given by $k_{cat}$ in units of sec$^{-1}$:

$$k_{cat} = \frac{V_{max}}{[E_o]}$$

Also known as the turnover number: the number of substrate molecules converted to product per enzyme molecule per second

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Turnover Number ($k_{cat}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellobiase</td>
<td>989.937</td>
</tr>
<tr>
<td>Acetobacterium</td>
<td>24.875</td>
</tr>
<tr>
<td>Trehalase</td>
<td>6.480</td>
</tr>
<tr>
<td>Bgl</td>
<td>0.28</td>
</tr>
<tr>
<td>Cerevisiae</td>
<td>1.76</td>
</tr>
<tr>
<td>Diphtheria</td>
<td>11</td>
</tr>
<tr>
<td>Rubisco</td>
<td>0.5</td>
</tr>
<tr>
<td>Nucleo</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Values are based on a standard alanine substrate and pH 7.8. This list is subject to change based on conditions (temperature, pH, etc.)*
Transform Data to a Lineweaver-Burk Plot:

\[ \frac{V}{V_{\text{max}}} = \frac{[S]}{K_m + [S]} \]

Lineweaver-Burk Plot

Enzyme Inhibition
Enzyme Inhibition

**Competitive inhibition:**

--- inhibitor binds to part or all of the active site
--- blocks substrate binding.

Michaelis-Menten: Competitive Inhibition

- apparent $K_m$ higher: $S$ must compete with $I$ to fill $50\%$ of active sites
- $V_{max}$ not affected: $S$ can outcompete $I$ at high concentrations

Competitive inhibitor
Noncompetitive Inhibition:

- binds to an allosteric site
- affects the active site
- enzyme can’t catalyze the reaction
  (ESi complex nonproductive; lowers ES decreases $V_{\text{max}}$)
- substrate still binds; $K_m$ not changed

Michaelis-Menten: Noncompetitive Inhibition

- $K_m$ not affected: $S$ does not compete with $I$ at the active site
- $V_{\text{max}}$ decreases to $V_{\text{max}}$ (app)
Michaelis-Menten Equations for Inhibition

**Competitive:**

\[ v = \frac{V_{\text{max}} [S]}{K_m (1 + \frac{[I]}{K_i})} \]  

**Noncompetitive:**

\[ v = \frac{V_{\text{max}}}{K_m (1 + \frac{[S]}{V_{\text{max}}})} \]

\( K_i \) is the EI dissociation constant.

---

### Enzyme Inhibitors in Medicine: Examples

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target Enzyme</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avadex-21®</td>
<td>Thymidylate 2</td>
<td>Cancer chemotherapy</td>
</tr>
<tr>
<td>Aceram®</td>
<td>Aldicholine elevating</td>
<td>Anti-cancer</td>
</tr>
<tr>
<td>Cygnex®</td>
<td>Angiotensin-converting enzyme</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Citoren®</td>
<td>Cytosinease 2</td>
<td>Anti-cancer</td>
</tr>
<tr>
<td>Dipace®</td>
<td>NC-512 Exchanging</td>
<td>Anti-parkinsonism</td>
</tr>
<tr>
<td>Miacide®</td>
<td>Thymidylase 5</td>
<td>Cancer chemotherapy</td>
</tr>
<tr>
<td>Stripl®</td>
<td>3-Hydroxy-3-methylglutaryl CoA</td>
<td>High cholesterol</td>
</tr>
<tr>
<td>Vira®</td>
<td>Prophosphatase 5</td>
<td>Esophageal cancer</td>
</tr>
</tbody>
</table>