Enzyme Basics

- Six Classes (IUBMB)
- Kinetics
- Michaelis-Menten Equation
- Vo, Km, Vmax, Kcat
- Lineweaver-Burk Plot

Enzymes are biological macromolecules that increase the rate of the reaction.
Seven major groups of enzymes

**Oxidoreductases:** (oxidation-reduction reactions)

**Transferases:** (group transfer reactions)

**Hydrolases:** (hydrolysis)

**Lyases:** (nonhydrolytic and nonoxidative lysis → double bond)

**Isomerases:** (isomerization)

**Ligases:** (ligation reaction requiring energy from NTP (nucleoside triphosphate)

**Translocases:** (assist in moving another molecule, usually across a cell membrane)

**YOU MUST KNOW THESE 7 GROUPS!**

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For more information on the types of enzymes see

Recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology

on the Nomenclature and Classification of Enzymes by the Reactions they Catalyse

https://www.qmul.ac.uk/sbcs/iubmb/
1. Oxidoreductases (dehydrogenases)

Catalyze oxidation-reduction reactions

\[ \text{HO} - \text{C} - \text{H} + \text{NAD}^+ \xrightarrow{\text{Lactate dehydrogenase}} \text{CO}_2 + \text{NADH} + \text{H}^+ \] (5.1)

Oxidation can be identified as:
* Addition of oxygen
* Removal of hydrogen
* Increase in valency of metal ion (e.g. Fe$^{2+}$ → Fe$^{3+}$)

See also the index and examples in your text to find more examples; e.g. pg. 380

The Oxidation States of Carbon

<table>
<thead>
<tr>
<th>Most reduced</th>
<th></th>
<th>Most oxidized</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$-CH$_3$</td>
<td>Alkane (ethane)</td>
<td>CO$_2$ Carbon dioxide</td>
</tr>
<tr>
<td>↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH$_3$-HC$_2$OH</td>
<td>Alcohol (ethanol)</td>
<td></td>
</tr>
<tr>
<td>↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH$_3$-COH</td>
<td>Aldehyde (acetaldehyde)</td>
<td></td>
</tr>
<tr>
<td>↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH$_3$-COOH</td>
<td>Carboxylic acid (acetic acid)</td>
<td></td>
</tr>
<tr>
<td>↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO$_2$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each of the arrows indicates an oxidation reaction. All except the last reaction are oxidations brought about by dehydrogenation.
2. Transferases

- Catalyze group transfer reactions

\[
\begin{align*}
\text{H}_3\text{N} - &\text{C} - \text{H} + \text{C} - \text{O} \quad \text{Alanine transaminase} \quad \text{EC} \ 2.6.1.2 \\
\text{CH}_3 \quad \text{L-Alanine} &\quad \frac{\text{COO}^-}{\text{COO}^-} \\
\alpha\text{-Ketoglutarate} &\quad \text{C} - \text{O} + \text{H}_3\text{N} - \text{C} - \text{H} \\
&\quad \text{CH}_3 \quad \text{Pyruvate} \\
&\quad \text{(CH}_2)_2 \quad \text{COO}^- \quad \text{L-Glutamate}
\end{align*}
\]

Other examples?

3. Hydrolases

- Catalyze hydrolysis reactions where water is the acceptor of the transferred group

\[
\begin{align*}
\text{PO}_{4}^- - &\text{P} - \text{O} - \text{P} - \text{O}^- + \text{H}_2\text{O} \quad \text{Pyrophosphatase} \quad \text{EC} \ 3.6.1.18 \\
\text{Pyrophosphate} &\quad 2 \text{HO} - \text{P} - \text{O}^- \\
&\quad \text{Phosphate}
\end{align*}
\]
4. Lyases

- Catalyze lysis of a substrate, generating a double bond in a nonhydrolytic, nonoxidative elimination (i.e. nonhydrolytic & nonoxidative lysis that yields a double bond)

\[
\begin{align*}
\text{Pyruvate} & \quad \overset{\overset{\text{P} \text{yruvate}}{\text{d} \text{e} \text{c} \text{a} \text{r} \text{b} \text{o} \text{x} \text{y} \text{l} \text{ase}}} \quad \text{H}_2O \\
\overset{\overset{\text{E} \text{C} \text{ } 4.1.1.1}{\text{P} \text{yruvate}}}{\xrightarrow{\text{P} \text{yruvate} \text{decarboxylase}}} & \quad \overset{\overset{\text{A} \text{c} \text{e} \text{t} \text{a} \text{l} \text{d} \text{e} \text{h} \text{y} \text{d} \text{e} \text{y}}{\text{C} \text{H}_3}} \quad \overset{\overset{\text{C} \text{O} \text{O}}{\text{C} \text{O}}} \quad \text{Acetaldehyde} + \overset{\overset{\text{C} \text{O} \text{O}}{\text{C} \text{O}}} \quad \text{Carbon} \\
& \quad \text{dioxide} \quad (5.4)
\end{align*}
\]

5. Isomerases

- Catalyze isomerization reactions

\[
\begin{align*}
\overset{\overset{\text{L-}\text{A} \text{l} \text{a} \text{n} \text{i} \text{n} \text{e}}{\text{H}_3\text{N} \text{CH}_3}} & \quad \overset{\overset{\text{C} \text{O} \text{O}}{\text{C} \text{O}}} \quad \overset{\overset{\text{A} \text{l} \text{a} \text{n} \text{i} \text{n} \text{e} \text{ r} \text{a} \text{c} \text{e} \text{m} \text{a} \text{s} \text{e}}{\text{E} \text{C} \text{ } 5.1.1.1}} \quad \overset{\overset{\text{H} \text{C} \text{NH}_3}{\text{C} \text{O} \text{O}}} \quad & \quad \overset{\overset{\text{D-}\text{A} \text{l} \text{a} \text{n} \text{i} \text{n} \text{e}}{\text{H}_3\text{N} \text{CH}_3}} \\
& \quad (5.5)
\end{align*}
\]
6. Ligases (synthetases)

- Catalyze ligation, or joining of two substrates
- Require chemical energy (e.g. ATP)

\[
\text{L-Glutamate} \quad \text{H}_3\text{N} - \text{C} - \text{H} \quad (\text{CH}_2)_2 \quad + \quad \text{ATP} + \text{NH}_2\text{O}^2- \xrightarrow{\text{Glutamine synthetase}} \quad \text{H}_3\text{N} - \text{C} - \text{H} \quad (\text{CH}_2)_2 \quad + \quad \text{ADP} + \text{P}_i
\]

EC 6.3.1.2

7. Translocases:

- Assist in moving another molecule (usually across a cell)

**ABC-type polar-amino-acid transporter**

*Other name(s):* histidine permease; polar-amino-acid-transporting ATPase

*Systematic name:* ATP phosphohydrolase (ABC-type, polar-amino-acid-importing)

EC 7.4.2.1

\[
\text{ATP} + \text{H}_2\text{O} + \text{polar amino acid} \quad [\text{polar amino acid-binding protein}]_{\text{side 1}} =
\]

\[
\text{ADP} + \text{phosphate} + \text{polar amino acid} \quad [\text{side 2}] + [\text{polar amino acid-binding protein}]_{\text{side 1}}
\]

Other examples?

https://www.ocf.berkeley.edu/~eldrin/research/slides/slide03.htm
Challenge of the Weekend, Due September 10, 2019
[This is an individual assignment]

Find a minimum of three examples of enzymes, and their reactions, for each of the 7 classes of enzymes. (You should be able to find examples in your book, from the NC-IUBMB site, or from research articles, etc.)

Label an individual page for each of the 7 classes of enzymes (i.e. transferases, hydrolases, etc). On each page for that particular class of enzyme, give a list of at least 3 full reactions, including substrate(s), products and enzyme name.

A completed assignment will be six pages with a total of 21 reactions.

For credit, these must be ready to be turned in before the start of Breakout Session on Tuesday, September 10.

BCMB 3100 - Chapter 5 Lecture

- Enzymes
- Six Classes (IUBMB)
- **Kinetics**
- Michaelis-Menten Equation
- \( V_0, K_m, V_{max}, K_{cat} \)
- Lineweaver-Burk Plot
Principles of Kinetics
(for non-enzyme-catalyzed reactions) (1)

**first-order reaction**

\[ \frac{\Delta [P]}{\Delta t} = v = k[S] \quad \text{rate equation} \]

v = velocity,  
P = product,  
S = substrate,  
k = rate constant  (s\(^{-1}\))

Principles of Kinetics
(for non-enzyme-catalyzed reactions) (2)

**second-order reaction (bimolecular)**

\[ S_1 + S_2 \rightarrow P_1 + P_2 \quad v = k[S_1]_1[S_2]_1 \]

k = rate constant M\(^{-1}\) s\(^{-1}\)

**pseudo first-order reaction**

If concentration of a reactant is so high that it remains constant during reaction = zero-order

\[ v = k[S_1]_1[S_2]^0 = k[S_1]_1 \]

Important for enzyme assays
**Emil Fischer 1894**

___________ model for enzyme:substrate interactions

---

**Enzyme Kinetics**

- **Enzyme-substrate complex (ES)** - complex formed when specific substrates fit into the enzyme active site

\[
E + S \rightarrow ES \rightarrow E + P
\]

- When \([S] \gg [E]\), every enzyme binds a molecule of substrate (enzyme is saturated with substrate)

- Under these conditions the rate depends only upon \([E]\), and the reaction is **pseudo-first order**
**Enzyme kinetics:** study of the rates of enzyme-catalyzed reactions

**Why study enzyme kinetics?** - enzyme kinetics gives information about:
- enzyme catalysis
- enzyme mechanisms
- regulation of enzyme activity (i.e. inhibitors & activators)
- basis of enzyme assays

The kinetics of an enzyme are affected by:
- [enzyme]
- [substrate]
- pH & temperature
- coenzymes, activators, inhibitors

**Enzyme Kinetics**

\[ E + S \rightarrow ES \rightarrow E + P \]

Effect of enzyme concentration \([E]\) on velocity (\(v\))

- Fixed, saturating \([S]\)
- Pseudo-first order enzyme-catalyzed reaction
Progress curve for an enzyme-catalyzed reaction

\[ E + S \rightarrow ES \rightarrow E + P \]

See Fig. 6.2

• The initial velocity \( (v_o) \) is the slope of the initial \[ \text{_____} \] of the curve
• Rate of the reaction doubles when twice as much enzyme is used

Note: Assuming the reaction is set up appropriately, the leveling off of the amount of product produced over time indicates that the reaction has reached equilibrium, that is, the forward and reverse reactions are occurring at a fixed rate.

Plots of initial velocity \( (v_o) \) versus \( [S] \)

See Fig. 7.1

(a) Each \( v_o \) vs \( [S] \) point is from one kinetic run

(b) Michaelis constant \( (K_m) \) equals the concentration of substrate needed for 1/2 maximum velocity
Michaelis-Menten Kinetics apply to many enzymes

(1913) Leonor Michaelis & Maud Menten proposed simple model for enzyme kinetics  
(See pages 121-122)

\[
E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P
\]

Assumption #1: (no product reverts to S, true in initial stage of reaction, \(k_{-2} = 0\)) (initial velocity = Vo)

\[
E + S \overset{k_1}{\rightarrow} ES \overset{k_2}{\rightarrow} E + P
\]
\[ E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} E + P \]

**K\text{cat} =** catalytic constant = turnover number
number of catalytic events per second per enzyme

**Goal:** expression that relates rate of catalysis to concentration of S and E and rate constants for individual steps.

\[ v = k_2[ES] \]

[ES] can be described by [E] & [S] by considering individual rate constants

rate of formation of ES = \( k_1 [E][S] \)
rate of breakdown of ES = \( (k_{-1} + k_2) [ES] \)

**Assumption #2:** Steady state conditions (i.e. concentration of ES stays the same while concentration of E and S changes). Occurs when formation of ES = breakdown of ES.

\[ k_1 [E][S] = (k_{-1} + k_2) [ES] \]

\[ [ES] = [E][S] / (k_{-1} + k_2) / k_1 \]
[ES] = [E][S] / (k_{-1} + k_2) / k_1

Define constant \( K_m \) (Michaelis constant)

\[ K_m = \frac{(k_{-1} + k_2)}{k_1} \]

Therefore \( [ES] = [E][S] / K_m \)

Solve for \([ES] \) …….

(You do NOT need to know how to solve for \([ES]\))

\[
\begin{align*}
E + S & \xrightarrow{k_{-1}} ES \xrightarrow{k_2} E + P \\

\text{Velocity of an enzyme-catalyzed reaction (depends upon} \\
\text{rate of conversion of ES to E + P)} \\
\nu_o &= k_2[ES] \\

\text{Recall} \quad k_2 = k_{\text{cat}} \\
\text{Vmax occurs when [S] much > than Km so} \\
\nu_{\text{max}} &= k_2[E_T]
\end{align*}
\]
The Michaelis-Menten equation

- Equation describes $v_o$ versus $[S]$ plots
- $K_m$ is the ________

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

$V_o = V_{max}[S] / Km + [S]$

At low $[S]$, $[S] <<<< Km$ & $V_o = V_{max}[S] / Km$
thus the rate is proportional to $[S]$

At high $[S]$, $[S] >>>> Km$. Thus: $V = V_{max}$
Thus rate is independent of $[S]$

When $[S] = Km$ $V = 1/2 V_{max}$

Thus $K_m = [S]$ at which reaction is half its maximal value
\[ E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} E + P \]

---

\[ \text{number of } S \text{ molecules} \]
converted into \( P \) by \( E \) per second when \( E \) is fully saturated with \( S \).

\[ k_2 = k_{\text{cat}} = s^{-1} \]

\[ \text{can be calculated from } V_{\text{max}} = k_2[ET] \]

\[ \text{of most enzymes is from 1 to } 10,000 \text{ per sec} \]

---

For most reactions \( K_m \) is a measure of the stability of the \( ES \) complex and \( K_{\text{cat}} \) is the rate constant for conversion of \( ES \) to \( P \).

For most reactions \( K_m \) is a measure of the affinity of \( E \) for \( S \). Thus, the lower the \( K_m \) value, the greater the affinity of \( E \) for \( S \).

For most cases, \( K_{\text{cat}} \) is a measure of the catalytic activity of the enzyme. Thus, the greater \( K_{\text{cat}} \), the faster the reaction.
Under physiological conditions enzymes are not normally saturated with $S$.

\[ \frac{[S]}{K_m} \text{ is usually between 0.01 and 1} \]

(Km usually $10^{-1}$ to $10^{-7} \text{M}$)

How do you characterize enzyme kinetics under these low [S]?

\[ V = [E][S] \frac{k_2}{K_m} \]

When $[S] << \lllllllllllll K_m$   

\[ [E] \approx [E_T] \text{ so} \]

\[ V = [E_T][S] \frac{k_2}{K_m} \]

Thus, $V$ depends on value of [S] & $k_2 / K_m$

$k_2 / K_m$ is limited by $k_1$ which is limited $< 10^9 \text{M}^{-1} \text{s}^{-1}$

(due to limits of diffusion). A few enzymes catalyze reactions at this upper physical rate = diffusion controlled reactions.

---

**Fig 7.3** $K_m$ and physiological substrate concentrations

• $K_m$ values for enzymes are typically just above [S], so that the enzyme rate is sensitive to small changes in [S]
Meanings of $k_{\text{cat}}$ and $k_{\text{cat}}/K_m$

Specificity constant

$[S]$

Region A: $E + S \xrightarrow{k_{\text{cat}}/K_m} E + P$
Region B: $k_{\text{cat}}[E][S]$

Turnover number

See pages 124-126

The specificity constant is a measure of catalytic efficiency

Examples of catalytic constants

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$k_{\text{cat}}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papain</td>
<td>10</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>$10^2$</td>
</tr>
<tr>
<td>Carboxypeptidase</td>
<td>$10^2$</td>
</tr>
<tr>
<td>Trypsin</td>
<td>$10^2$ (to $10^3$)</td>
</tr>
<tr>
<td>Acetylcholinesterase</td>
<td>$10^3$</td>
</tr>
<tr>
<td>Kinases</td>
<td>$10^3$</td>
</tr>
<tr>
<td>Dehydrogenases</td>
<td>$10^3$</td>
</tr>
<tr>
<td>Transaminases</td>
<td>$10^3$</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>$10^6$</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>$10^6$</td>
</tr>
<tr>
<td>Catalase</td>
<td>$10^7$</td>
</tr>
</tbody>
</table>

See also Table 7.2

ALSO NOTE: (Km usually $10^{-1}$ to $10^{-7}$M) (100 mM to 0.1 µM)
Values of $k_{\text{cat}}/K_m$

- $k_{\text{cat}}/K_m$ can approach rate of encounter of two uncharged molecules in solution ($10^8$ to $10^9\text{M}^{-1}\text{s}^{-1}$)
- $k_{\text{cat}}/K_m$ is also a measure of enzyme specificity for different substrates (_______________________)

$k_{\text{cat}}/K_m$ - This ratio is often thought of as a measure of enzyme efficiency. Either a large value of $k_{\text{cat}}$ (rapid turnover) or a small value of $K_m$ (high affinity for substrate) makes $k_{\text{cat}}/K_m$ large.

Comparison of $K_{\text{c}}/K_m$ allows direct comparison of the effectiveness of an enzyme toward different substrates (or of different enzymes for the same substrate).

The larger the $K_{\text{c}}/K_m$ the more efficient the enzyme is for a reaction.

**Table 7.3** Substrate preferences of chymotrypsin

<table>
<thead>
<tr>
<th>Amino acid in ester</th>
<th>Amino acid side chain</th>
<th>$k_{\text{cat}}/K_m$ (s$^{-1}$ M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>H</td>
<td>$1.3 \times 10^{-1}$</td>
</tr>
<tr>
<td>Valine</td>
<td>$-\text{CH}_2$</td>
<td>2.0</td>
</tr>
<tr>
<td>Norvaline</td>
<td>$-\text{CHCl}_2\text{CH}_3$</td>
<td>$3.6 \times 10^2$</td>
</tr>
<tr>
<td>Norleucine</td>
<td>$-\text{CH}_2\text{CH}_2\text{CH}_3$</td>
<td>$3.0 \times 10^3$</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>$-\text{CH}_2$</td>
<td>$1.0 \times 10^5$</td>
</tr>
</tbody>
</table>

rate acceleration = \( \frac{k_{\text{cat}}}{k_n} \)

\( k_n \) = rate constant in the absence of enzyme

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Nonenzymatic half-life</th>
<th>Uncatalyzed rate ( k_n ) ( \text{s}^{-1} )</th>
<th>Catalyzed rate ( k_{\text{cat}} ) ( \text{s}^{-1} )</th>
<th>Rate enhancement ( k_{\text{cat}}/k_n ) ( \text{s}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>OMP decarboxylase</td>
<td>78,000,000 years</td>
<td>( 2.8 \times 10^{-16} )</td>
<td>39</td>
<td>( 1.4 \times 10^{12} )</td>
</tr>
<tr>
<td>Staphylococcal nuclease</td>
<td>130,000 years</td>
<td>( 1.7 \times 10^{-13} )</td>
<td>95</td>
<td>( 5.6 \times 10^{14} )</td>
</tr>
<tr>
<td>AMP nucleosidase</td>
<td>69,000 years</td>
<td>( 1.0 \times 10^{-14} )</td>
<td>60</td>
<td>( 6.0 \times 10^{14} )</td>
</tr>
<tr>
<td>Carboxypeptidase A</td>
<td>7.3 years</td>
<td>( 3.0 \times 10^{-9} )</td>
<td>578</td>
<td>( 1.9 \times 10^{11} )</td>
</tr>
<tr>
<td>Ketosteroid isomerase</td>
<td>7 weeks</td>
<td>( 1.7 \times 10^{-7} )</td>
<td>66,000</td>
<td>( 3.9 \times 10^{15} )</td>
</tr>
<tr>
<td>Triose phosphate isomerase</td>
<td>1.9 days</td>
<td>( 4.3 \times 10^{-6} )</td>
<td>4300</td>
<td>( 1.0 \times 10^{8} )</td>
</tr>
<tr>
<td>Chorismate mutase</td>
<td>7.4 hours</td>
<td>( 2.6 \times 10^{-4} )</td>
<td>50</td>
<td>( 1.9 \times 10^{10} )</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>5 seconds</td>
<td>( 1.3 \times 10^{-1} )</td>
<td>( 1 \times 10^6 )</td>
<td>( 7.7 \times 10^6 )</td>
</tr>
</tbody>
</table>

Abbreviations: OMP, orotic acid monophosphate; AMP, adenosine monophosphate.

Table 6.1
Vmax and Km can be determined by measuring the rate of the reaction at different [S] if an enzyme operates my Michaelis-Menten kinetics

Transformation of the Michaelis-Menten equation (i.e. taking the reciprocal of both sides)

\[ \frac{1}{V} = \frac{1}{V_{\text{max}}} + \left( \frac{K_m}{V_{\text{max}}} \times \frac{1}{[S]} \right) \]

Recall \( y = a + bx \) \( b = \) slope, \( a = \) y intercept

A plot of \( 1/V \) versus \( 1/[S] \) is a _________________________
(a straight line plot)
Measurement of $K_m$ and $V_{max}$

Fig 7.5 The double-reciprocal Lineweaver-Burk plot is a linear transformation of the Michaelis-Menten plot ($1/v_o$ versus $1/[S]$)

RECALL:

$$V_o = V_{max}[S] / K_m + [S]$$

Useful information regarding enzyme kinetics

$K_m$ and $V_{max}$ can be determined from the Lineweaver-Burk plot

$K_m$ depends on enzyme, pH, temperature and ionic strength of environment

The fraction of enzyme sites filled = $f_{ES}$

$$f_{ES} = V/V_{max}$$
BCMB 3100 - Chapter 5 Lecture

- Enzymes
- Six Classes (IUBMB)
- Kinetics
- Michaelis-Menten Equation
- Vo, Km, Vmax, Kcat
- Lineweaver-Burk Plot
- **Kinetics of Multisubstrate Reactions**

**Kinetics of Multisubstrate Reactions**

**Cleland notation for bi-substrate reactions**

**Kinetic Mechanisms**

- A + B → P + Q

**Fig 7.6** Notations for bisubstrate reactions

(a) Sequential (ordered or random)
**Fig. 7.6 (b) Bisubstrate reactions**

A + B → P + Q

**Ping-pong reaction**
(Double-displacement reaction)

(b) Ping-pong

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>P</th>
<th>B</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>(EA)(FP)</td>
<td>F</td>
<td>(FB)(EQ)</td>
<td>E</td>
</tr>
</tbody>
</table>

Enzyme in “F” state is temporarily modified by covalent attachment of part of original substrate to active site

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**BCMB 3100 - Chapter 5 Lecture**

- Kinetics
- Michaelis-Menten Equation
- Vo, Km, Vmax, Kcat
- Lineweaver-Burk Plot
- Kinetics of Multisubstrate Reactions
- Reversible Inhibition
Reversible Enzyme Inhibition

- Three types of reversible inhibition: **Competitive**, **Uncompetitive** and **Noncompetitive**

**Fig 8.10** Reversible enzyme inhibitors

(a) _________. S and I bind to same site on E

(b) **Nonclassical competitive.** Binding of S at active site prevents binding of I at separate site. Binding of I at separate site prevents S binding at active site.
See Fig 8.10 **Competitive inhibition.**

(a) Kinetic scheme. (b) Lineweaver-Burk plot

Apparent ______ is increased

________ stays the same.

Most common type of inhibitors

Benzamidine competes with arginine for binding to trypsin

Example of a competitive inhibitor
(c) I binds only to ES (inactivates E)

(d) I binds to either E or ES to inactivate the enzyme

See Figs 8.11 & 8.12

See Fig 8.11 Uncompetitive inhibition

Apparent ______ is decreased

_____ is decreased
See Fig 8.12  Noncompetitive inhibition

\[
\begin{align*}
E + S & \rightleftharpoons ES \rightarrow E + P \\
+ & I \\
K_i & \uparrow \quad K_i & \uparrow \\
EI + S & \rightleftharpoons ESI \\
\end{align*}
\]

Apparent _______ stays the same

_______ is decreased

BCMB 3100 - Chapter 5 Lecture

- Michaelis-Menten Equation
- Vo, Km, Vmax, Kcat
- Lineweaver-Burk Plot
- Kinetics of Multisubstrate Reactions
- Reversible Inhibition
- Irreversible Inhibition
**Irreversible Enzyme Inhibition**

- Irreversible inhibitors form **stable covalent bonds** with the enzyme.
- Irreversible inhibitors can be used to identify the amino acid residues at enzyme active sites.
- Incubation of inhibitor with enzyme results in loss of activity.

**Covalent complex with lysine residues**

- Reduction of a Schiff base forms a stable substituted enzyme.

![Chemical reactions](image)
**Example of irreversible inhibition**

Inhibition of acetylcholine esterase (enzyme that hydrolyses the neurotransmitter acetylcholine & restores polarization of a nerve).

Nerve gases are organic phosphate compounds (e.g. DFP) that form stable phosphoryl-enzymes complexes by reacting with the OH of the active-site Ser. They kill by causing respiratory paralysis.

- Reaction of DFP with Ser-195 of chymotrypsin

**DFP = diisopropyl fluorophosphate**
**Affinity labels for studying enzyme active sites**

- are active-site directed reagents
- They are irreversible inhibitors
- Affinity labels resemble substrates, but contain reactive groups to interact covalently with the enzyme

**Site-Directed Mutagenesis Modifies Enzymes**

- (SDM) can be used to test the functions of individual amino acid side chains
- One amino acid is replaced by another using molecular biology techniques
- Example: Subtilisin protease (used in detergents) was made more resistant to chemical oxidation by replacing Met-222 with Ala-222
General Features of Regulatory Enzymes (1)

1. Regulatory compounds (_______________) bind regulatory sites separate from catalytic sites. They can be **activators** or **inhibitors**.

2. **Allosteric modulators** bind noncovalently, may alter Km or Vmax, and are NOT chemically altered by the enzyme they regulate

3. Catalytic and regulatory sites are different
General Features of Regulatory Enzymes (2)

4. $v_0$ vs $[S]$ curve for regulatory enzymes is often sigmoidal, suggesting cooperativity of $S$ binding

5. Most regulatory enzymes have 4º structure. Subunits may be identical or different

Levels of enzyme regulation

1. allosteric $R \rightarrow T$ transition (fastest)
2. covalent modification of enzyme (e.g. phosphorylation)
3. regulation of enzyme synthesis or degradation (slowest)

Some enzymes do NOT obey Michael-Menten kinetics

Example: Allosteric enzymes often have a sigmoidal rather than hyperbolic $V$ vs $[S]$ plot

For enzymes that do not obey Michaelis-Menten kinetics, measurement of kinetics at different $[S]$ can distinguish competitive from noncompetitive inhibition.

_________________: $V_{max}$ is NOT altered, but apparent $K_m$ is increased

_________________: $V_{max}$ is decreased but $K_m$ is not affected

_________________: $V_{max}$ and $K_m$ are decreased
Enzymes can also be regulated by positive allosteric regulators

Plots of initial velocity versus [F6P] for PFK-1 (phosphofructokinase-1)
[Important regulatory enzyme in glycolysis]

Fructose 6-phosphate + ATP → Fructose 1,6-bisphosphate + ADP

- ADP is an allosteric activator of PFK-1 and lowers the apparent $K_m$ without affecting $V_{max}$
- For a given F6P concentration the $v_o$ is larger in the presence of ADP

Regulatory enzymes usually occur at the first committed step of a pathway

Regulatory enzymes are bound by allosteric effectors at the regulatory site

Role of cooperativity of binding in regulation

- Addition of modulators alters enzyme activity
- Activators can lower $K_m$, inhibitors can raise $K_m$

See pg 129
Pyruvate dehydrogenase regulation

- **Phosphorylation** stabilizes the inactive state (red)
- **Dephosphorylation** stabilizes the active state (green)