Chapter 14. Enzyme Kinetics
Chemical kinetics

- Elementary reactions

\[ \text{Overall stoichiometry} \]

\[ A \rightarrow P \]

\[ i_1 \rightarrow i_2 \] (Intermediates)

- Rate equations

\[ aA + bB + \cdots + zZ \rightarrow P \]

\[ \text{Rate} = k[A]^a[B]^b[\cdots][Z]^z \]

\[ k: \text{rate constant} \]

- The order of the reaction (a+b+…+z): Molecularity of the reaction
  - Unimolecular (first order) reactions: \( A \rightarrow P \)
  - Bimolecular (second order) reactions: \( 2A \rightarrow P \) or \( A + B \rightarrow P \)
  - Termolecular (third order) reactions
Rates of reactions

A → P  (First-order reaction)

\[ v = \frac{d[P]}{dt} = - \frac{d[A]}{dt} = k[A] \]

2A → P  (Second-order reaction)

\[ v = \frac{d[P]}{dt} = - \frac{d[A]}{dt} = k[A]^2 \]

A + B → P  (Second-order reaction)

\[ v = \frac{d[P]}{dt} = - \frac{d[A]}{dt} = - \frac{d[B]}{dt} = k[A][B] \]
Rate constant for the first-order reaction

\[ v = -\frac{d[A]}{dt} = k[A] \]

\[ \frac{d[A]}{[A]} = -kdt \]

\[ \int_{[A]_0}^{[A]} \frac{d[A]}{[A]} = -k \int_0^t dt \]

\[ \ln[A] - \ln[A]_0 = -kt \]

\[ \ln[A] = \ln[A]_0 - kt \]

\[ [A] = [A]_0 e^{-kt} \]

The reactant concentration decreases exponentially with time.
Half-life is constant for a first-order reaction

\[ \ln[A] - \ln[A]_0 = -kt \]

\[ \ln \frac{[A]}{[A]_0} = -kt \]

\[ \ln \frac{1}{2} = -kt_{1/2} \]

\[ t_{1/2} = \frac{\ln 2}{k} = \frac{0.693}{k} \]

For the first-order reaction, half-life is independent of the initial reactant concentration
Second-order reaction with one reactant

\[2A \rightarrow P\]

\[v = -\frac{d[A]}{dt} = k[A]^2\]

\[\frac{d[A]}{[A]^2} = -k dt\]

\[\frac{1}{[A]} = \frac{1}{[A]_0} + kt\]

\[kt_{1/2} = \frac{2}{[A]_0} - \frac{1}{[A]_0} = \frac{1}{[A]_0}\]

\[t_{1/2} = \frac{1}{k[A]_0}\]

Half-life is dependent on the initial reactant concentration
Pseudo first-order reactions

\[ \nu = k[A][B] \]

When \([B] >> [A]\),

\[ \nu = k'[A] \]

where \(k' = k[B]_0\)

e.g. B is water (55.5M): \(k' = 55.5k\)
Arrhenius equation

\[ k = Ae^{-\frac{E_a}{RT}} \]
Multistep reactions have rate-determining steps.

Rate-determining step: the slow step (= the higher activation energy)
Catalysts reduce the activation energy

Rate enhancement = \( \frac{k_{\text{cat}}}{k_{\text{uncat}}} = e^{\frac{\Delta E_a}{RT}} \)

- e.g. When \( \Delta E_a = 5.7 \text{ kJ/mol} \), the reaction rate increases 10 fold
- When \( \Delta E_a = 34 \text{ kJ/mol} \), the reaction rate increases 10^6 fold
Michaelis-Menten equation

\[ v_0 = \frac{V_{\text{max}} [S]}{K_M + [S]} \]

- \( v_0 \) = the initial rate
- \( V_{\text{max}} \) = the maximum rate
- \( K_M \) = the Michaelis constant
- \([S]\) = the substrate concentration
Steady state approximation

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

\[ [E]_T = [E] + [ES] \]

\[ \frac{d[ES]}{dt} \approx 0 \]

\[ [S_0] \]

Concentration

Time

\[ [S] \quad [P] \]

\[ [E]_T \quad [ES] \quad [E] \]
Derivatization of Michaelis-Menten equation

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

\[ v = \frac{d[P]}{dt} = k_3[\text{ES}] \]

\[ \frac{d[\text{ES}]}{dt} = k_1[\text{E}][\text{S}] - k_2[\text{ES}] - k_3[\text{ES}] = 0 \] (Steady state approximation)

\[ [\text{E}]_i = [\text{E}] + [\text{ES}] \]

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

\[ (k_{-1} + k_2 + k_3)[\text{ES}] = k_1[\text{E}][\text{S}] \]

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

\[ [\text{ES}] = \frac{[\text{E}][\text{S}]}{k_{-1} + k_2} + [\text{S}] \]

\[ [\text{ES}] = \frac{[\text{E}][\text{S}]}{[\text{K}_u] + [\text{S}]} \quad \text{where} \quad [\text{K}_u] = \frac{k_{-1} + k_2}{k_1} \]

The initial velocity \( v_0 = k_3[\text{ES}] = \frac{k_3[\text{E}][\text{S}]}{[\text{K}_u] + [\text{S}]} \)

The maximum velocity \( (V_{\text{max}}) \) occurs at high substrate concentration when the enzyme is entirely in the [ES] form: \( V_{\text{max}} = k_3[\text{E}]_i \)

\[ v_0 = \frac{V_{\text{max}}[\text{S}]}{[\text{K}_u] + [\text{S}]} \]
Michaelis constant $K_M$

$$K_M = [S] \text{ at which } v_0 = \frac{V_{\text{max}}}{2}$$

- If an enzyme has a small value of $K_M$, it achieves maximal catalytic efficiency at low substrate concentrations
- Measure of the enzyme’s binding affinity for the substrate (The lower $K_M$, the higher affinity)
Lineweaver-Burke plot

\[ v_0 = \frac{V_{\text{max}} [S]}{K_M + [S]} \]

\[ \frac{1}{v_0} = \frac{K_M}{V_{\text{max}}} \frac{1}{[S]} + \frac{1}{V_{\text{max}}} \]

Slope = \( \frac{K_M}{V_{\text{max}}} \)
\( k_{\text{cat}} / K_M \) is a measure of catalytic efficiency

Catalytic constant or turnover number of an enzyme:

\[
k_{\text{cat}} = \frac{V_{\text{max}}}{[E]_T}
\]

When \( K_M >> [S] \),

very little ES is formed and consequently \([E] \approx [E]_T\)

\[
\nu_0 = \frac{V_{\text{max}} [S]}{K_M + [S]} \approx \frac{V_{\text{max}} [S]}{K_M} = \frac{k_{\text{cat}} [E]_T [S]}{K_M} \approx \left( \frac{k_{\text{cat}}}{K_M} \right) [E] [S]
\]
Catalytic perfection

\[
\frac{k_{\text{cat}}}{K_M} = \frac{k_2}{K_M} = \frac{k_1 k_2}{k_1 + k_2}
\]

The ratio is maximal when \( k_2 \gg k_1 \),

\[
\frac{k_{\text{cat}}}{K_M} = k_1
\]

(Diffusion-controlled limit: \( 10^8 \) to \( 10^9 \) M\(^{-1}\)s\(^{-1}\))
Inhibitors

• Substances that reduce an enzyme’s activity
  – Study of enzymatic mechanism
  – Therapeutic agents
• Reversible or irreversible inhibitors

Dihydrofolate (Dihydrofolate reductase substrate)

Methotrexate (Dihydrofolate reductase inhibitor, anticancer drug)
Modes of the reversible inhibition

- **Competitive inhibitors**
  - Binds to the substrate binding site
- **Uncompetitive inhibitors**
  - Binds to enzyme-substrate complex
- **Non-competitive inhibitors**
  - Binds to a site different from the substrate binding site
- **Mixed inhibitors**
  - Binds to the substrate-binding site and the enzyme-substrate
Competitive inhibition

\[ K_i = \frac{[E][I]}{[EI]} \]

\[ \frac{d[ES]}{dt} = k[E][S] - k_1[ES] - k_2[ES] = 0 \]  (Steady state approximation)

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

\[ [EI] = \frac{[E][I]}{K_i} = \frac{K_u[ES][I]}{[S]K_i} \]

\[ [E]_r = [E] + [EI] + [ES] \]

\[ [E]_r = \frac{K_u[ES]}{[S]} + \frac{K_u[ES][I]}{[S]K_i} + [ES] = [ES]\left( \frac{K_u}{[S]} \left( 1 + \frac{[I]}{K_i} \right) + 1 \right) \]

\[ [ES] = \frac{[E]_r[S]}{K_u \left( 1 + \frac{[I]}{K_i} \right) + [S]} \]

\[ v_0 = k_2[ES] = \frac{k_2[E][S]}{K_u \left( 1 + \frac{[I]}{K_i} \right) + [S]} \]

Since \( V_{\text{max}} = k_2[E]_r \),

\[ v_0 = \frac{V_{\text{max}}[S]}{\alpha K_M + [S]} \]

where \( \alpha = 1 + \frac{[I]}{K_i} \)
Competitive inhibitors affect $K_M$

$$v_0 = \frac{V_{\text{max}} [S]}{\alpha K_M + [S]}$$

$[S] \to \infty; v_0 \to V_{\text{max}}$ regardless of $\alpha$
Determination of $K_i$ of the competitive inhibitor

\[
\frac{1}{V_0} = \left( \frac{\alpha K_M}{V_{\text{max}}} \right) \frac{1}{[S]} + \frac{1}{V_{\text{max}}}
\]

\[
\alpha = 1 + \frac{[I]}{K_i}
\]
Uncompetitive inhibition

\[
\begin{align*}
K' &= \frac{[ES][I]}{[ESI]} \\
[E] &= \frac{K_s[ES]}{[S]} \\
[ESI] &= \frac{[ES][I]}{K'} \\
[E]' &= [E] + [ES] + [ESI] \\
\frac{[E]'}{[ES]} &= \frac{K_s[ES]}{K_s + (1 + \frac{[I]}{K'})[S]} \\
\alpha = k_s[ES] &= \frac{k_d[ES]}{K_d + (1 + \frac{[I]}{K'})[S]} \\
\alpha' &= 1 + \frac{[I]}{K'}
\end{align*}
\]
Uncompetitive inhibitors decrease both $V_{\text{max}}$ and $K_M$

\[ v_0 = \frac{V_{\text{max}} [S]}{\alpha' K_M + [S]} \]

$[S] \rightarrow \infty; v_0 \rightarrow \frac{V_{\text{max}}}{\alpha'}$

$K_M >> [S]; v_0 \rightarrow \frac{V_{\text{max}}}{K_M} [S]$ (Effects of an uncompetitive inhibitor become negligible)

\[ \alpha' = 1 + \frac{[I]}{K'_i} \]
Determination of $K_i'$ of the uncompetitive inhibitor

$$\frac{1}{v_0} = \left( \frac{K_M}{V_{\text{max}}} \right) \frac{1}{[S]} + \frac{\alpha'}{V_{\text{max}}}$$
Mixed inhibition

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

\[
K_i = \frac{[E][I]}{[EI]} \quad K'_i = \frac{[ES][I]}{[ESI]}
\]

\[
[ESI] = \frac{[ES][I]}{K'_i}
\]

\[
[E]_r = [E] + [EI] + [ES] + [ESI]
\]

\[
[E]_r = [E] \left(1 + \frac{[I]}{K_i}\right) + [ES] \left(1 + \frac{[I]}{K'_i}\right)
\]

\[
*[E] = [E] \alpha + [ES] \alpha'
\]

\[
*[E] = \frac{K_M [ES]}{[S]} \alpha + [ES] \alpha' = [ES] \left(\frac{\alpha K_M}{[S]} + \alpha'\right)
\]

\[
*[ES] = \frac{[E]_r [S]}{\alpha K_M + \alpha' [S]}
\]

\[
v_0 = k_3 [ES] = \frac{k_3 [E]_r [S]}{\alpha K_M + \alpha' [S]}
\]

Since \( V_{\text{max}} = k_3 [E]_r \),

\[
v_0 = \frac{V_{\text{max}} [S]}{\alpha K_M + \alpha' [S]} \quad \text{where} \quad \alpha = 1 + \frac{[I]}{K_i} \quad \text{and} \quad \alpha' = 1 + \frac{[I]}{K'_i}
\]
Lineweaver-Burk plot of a mixed inhibition

\[
\frac{1}{v_0} = \left( \frac{\alpha K_M}{V_{\text{max}}} \right) \frac{1}{[S]} + \frac{\alpha'}{V_{\text{max}}}
\]
Noncompetitive inhibition

A special mixed inhibition when $K_i = K_i'$

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

\[
+ +
\]

\[
l + l
\]

\[
V_0 = \frac{V_{\max}[S]}{\alpha K_M + \alpha'[S]} \quad \text{where} \quad \alpha = 1 + \frac{[I]}{K_i} \quad \text{and} \quad \alpha' = 1 + \frac{[I]}{K_i'}
\]

When $K_i = K_i'$, $\alpha = \alpha'$

\[
V_0 = \frac{V_{\max}[S]}{\alpha(K_M + [S])} = \frac{V_{\max}[S]}{K_M + [S]}
\]
Noncompetitive inhibitors affect not $K_M$ but $V_{\text{max}}$

$$v_0 = \frac{V_{\text{max}} [S]}{\alpha \left( \frac{K_M + [S]}{K_M} \right)}$$

where $\alpha = 1 + \frac{[I]}{K_i}$

$[S] \to \infty$; $v_0 \to \frac{V_{\text{max}}}{\alpha}$

---

**Graph:**

- $V_{\text{max}}$
- $V_{\text{max}}/2$
- $V_{\text{max}}/2$
- $V_0$
- $K_M$
- $[S]$

- **No inhibitor ($\alpha=1$)**
- **Increasing [I]**

$\alpha = 1 + \frac{[I]}{K_i}$
Determination of $K_i$ of the noncompetitive inhibitor

\[
\frac{1}{v_0} = \left( \frac{\alpha K_M}{V_{max}} \right) \frac{1}{[S]} + \frac{\alpha}{V_{max}}
\]

\[
\text{Slope} = \alpha \frac{K_M}{V_{max}}
\]

\[
\frac{\alpha}{V_{max}} = 1
\]

Increasing $[I]$
Effects of inhibitors on $V_{max}$ and $K_M$ of the Michaelis-Menten equation

\[ v_0 = \frac{V_{app}^m [S]}{K_{M app} + [S]} \]

<table>
<thead>
<tr>
<th>Type of Inhibition</th>
<th>$V_{app}^m$</th>
<th>$K_{M app}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>$V_{max}$</td>
<td>$K_M$</td>
</tr>
<tr>
<td>Competitive</td>
<td>$V_{max}$</td>
<td>$\alpha K_M$</td>
</tr>
<tr>
<td>Uncompetitive</td>
<td>$V_{max}/ \alpha'$</td>
<td>$K_M/ \alpha'$</td>
</tr>
<tr>
<td>Mixed</td>
<td>$V_{max}/ \alpha'$</td>
<td>$\alpha K_{M/ \alpha'}$</td>
</tr>
</tbody>
</table>

$^\alpha = 1 + \frac{[I]}{K_I}$ and $\alpha' = 1 + \frac{[I]}{K'_I}$. 

Effects of pH

- Binding of substrate to enzyme
- Catalytic activity of enzyme
- Ionization of substrate
- Variation of protein structure (only at extreme pHs)

\[
\begin{align*}
v_0 &= \frac{V_{\text{max}}'[S]}{K_{M}' + [S]} \\
V'_{\text{max}} &= V_{\text{max}} / f_2 \quad \text{and} \quad K_{M}' = K_M (f_1 / f_2) \\
\text{where} \\
f_1 &= \frac{[H^+]}{K_{E_1}} + 1 + \frac{K_{E_2}}{[H^+]} \\
f_2 &= \frac{[H^+]}{K_{E_1}} + 1 + \frac{K_{E_2}}{[H^+]} \\
\end{align*}
\]
Approximate identity of catalytic amino acid residues

pK_a ~4 → Catalytic Asp or Glu residue
pK_a ~6 → Catalytic His residue
pK_a ~10 → Catalytic Lys residue

Caution should be taken because pK_a of amino acid residues are environmentally sensitive
Bisubstrate reactions

\[ A + B \rightleftharpoons E \rightleftharpoons P + Q \]

- 60% of biochemical reactions involve two substrates and two products
- Transfer reactions and oxidation-reduction reactions

\[(a)\]
\[
\begin{align*}
R_1\text{C}-\text{NH}-R_2 + H_2O & \xrightarrow{\text{trypsin}} R_1\text{C}-O^- + H_3N^+ - R_2 \\
\text{Polypeptide}
\end{align*}
\]

\[(b)\]
\[
\begin{align*}
\text{CH}_3\text{C}-\text{OH} + \text{NAD}^+ & \xrightarrow{\text{alcohol dehydrogenase}} \text{CH}_3\text{C}-\text{CH} + \text{NADH} \\
\text{H} & \xrightarrow{H^+} \text{H}
\end{align*}
\]
Cleland’s nomenclature system for the enzymatic reactions

- Substrates: A, B, C, D… in the order that they add to the enzyme
- Products: P, Q, R, S… in the order that they leave the enzyme
- Inhibitors: I, J, K, L…
- Stable enzyme complexes: E, F, G, H… with E being the free enzyme
- Numbers of reactants and products: Uni (one), Bi (two), Ter (three), and Quad (four)
  e.g. Bi Bi reaction: a reaction that requires two substrates and yields two products
Types of Bi Bi Reactions

• **Sequential reactions (single displacement reactions):**
  all substrates bind before chemical event

  Ordered mechanism
  \[
  E \rightarrow EA \rightarrow EAB-EPQ \rightarrow EQ \rightarrow E
  \]

  Random mechanism
  \[
  E \rightarrow EA \rightarrow EAB-EPQ \rightarrow EQ \rightarrow E
  \]

• **Ping pong reactions (double displacement reactions):**
  chemistry occurs prior to binding of all substrates

  \[
  E \rightarrow EA-FP \rightarrow F \rightarrow FB-EQ \rightarrow E
  \]
Differentiating bisubstrate mechanisms

- Measure rates
- Change concentration of substrates and products

- Lineweaver-Burk plot
  - Intercept \((1/V_{\text{max}})\): the velocity at saturated substrate concentration → It changes when the substrate A binds to a different enzyme form with the substrate B
  - Slope \((K_M/V_{\text{max}})\): the rate at low substrate concentration → It changes when both A and B reversibly bind to an enzyme form
Ping Pong Bi Bi Mechanism

- Intercept changes because A and B bind to the different enzyme forms E and F, respectively.
- Slope remains same because the binding of A and B is irreversible due to the release of the product (P).
Sequential Bi Bi Mechanism

- Intercept changes because A and B binds to the different enzyme forms (E or EB) and (EA or E), respectively
- Slope changes because the binding of A and B is reversible
Differentiating Bi Bi mechanisms by product inhibition

Competitive inhibition → Substrate and inhibitor competitively bind to the same site of the enzyme

Ping Pong

- A vs Q: Competitive
- B vs P: Competitive
- A vs P: Noncompetitive
- B vs Q: Noncompetitive

Ordered sequential

- A vs Q: Competitive
- B vs P: Noncompetitive
- A vs P: Noncompetitive
- B vs Q: Noncompetitive

Random sequential

Under assumption of dead-end complex formation (A is similar with Q and B is similar with P)
- A vs Q: Competitive
- B vs P: Competitive
- A vs P: Noncompetitive
- B vs Q: Noncompetitive
Dead-end complexes

Dead-end complex (no chemistry)

Dead-end complex (no chemistry)

no chemistry

ATP + Creatine ⇌ ADP + Creatine-phosphate

competitive
Differentiating Bi Bi mechanisms by isotope exchange

Ping Pong Mechanism
A* → P isotope exchange is possible without B
B* → Q isotope exchange is possible without A

Sequential Mechanism
Two substrates are required for the isotope exchange
Isotope exchanges in a ping pong mechanism

Sucrose phosphorylase

\[
\text{(Sucrose)} \quad \text{Glucose-fructose} + \text{phosphate} \xleftrightarrow{E} \text{Glucose-1-phosphate} + \text{fructose}
\]

Isotope exchange experiments

\[
\text{Glucose-fructose} + \text{fructose}^* \xleftrightarrow{E} \text{Glucose-fructose}^* + \text{fructose}
\]

\[
\text{Glucose-1-phosphate} + \text{phosphate}^* \xleftrightarrow{E} \text{Glucose-1-phosphate}^* + \text{phosphate}
\]

\[
\text{Glucose-fructose} + E \xleftrightarrow{E} \text{Fructose} + \text{Glucose-E}
\]

\[
\text{Glucose-E} + \text{phosphate} \xleftrightarrow{E} \text{Glucose-1-phosphate} + E
\]

Ping Pong Mechanism (Double displacement)
Isotope exchanges in a sequential mechanism

Maltose phosphorylase

(Maltose)
Glucose-glucose + phosphate $\overset{E}{\leftrightarrow}$ Glucose-1-phosphate + glucose

Isotope exchange experiments

Glucose-glucose + glucose$^\ast$ $\overset{E}{\leftrightarrow}$ Glucose-glucose + phosphate$^\ast$  
No isotope exchange

Glucose-1-phosphate + phosphate$^\ast$ $\overset{E}{\leftrightarrow}$ Glucose-1-phosphate + glucose  
No isotope exchange

Glucose-glucose + phosphate$^\ast$ $\overset{E}{\leftrightarrow}$ Glucose-1-phosphate$^\ast$ + glucose

Glucose-1-phosphate + glucose$^\ast$ $\overset{E}{\leftrightarrow}$ Glucose-glucose + phosphate

Sequential Mechanism (Single displacement)