Chemical Reaction Engineering (CRE) is the field that studies the rates and mechanisms of chemical reactions and the design of the reactors in which they take place.
Today’s lecture

- Enzymes
  - Michealis-Menten Kinetics
  - Lineweaver-Burk Plot
  - Enzyme Inhibition
    - Competitive
    - Uncompetitive
Last lecture

1. In the PSSH, we set the rate of formation of the active intermediates equal to zero. If the active intermediate A* is involved in \( m \) different reactions, we set it to

\[
r_{A^*, \text{net}} \equiv \sum_{i=1}^{m} r_{A^*i} = 0 \quad (S7-1)
\]

This approximation is justified when the active intermediate is highly reactive and present in low concentrations.

2. The azomethane (AZO) decomposition mechanism is

\[
2\text{AZO} \xrightleftharpoons[k_2]{k_1} \text{AZO} + \text{AZO}^* \quad (S7-2)
\]

\[
\text{AZO}^* \xrightarrow[k_3]{\text{N}_2 + \text{ethane}} \quad (S7-3)
\]

By applying the PSSH to AZO*, we show the rate law, which exhibits first-order dependence with respect to AZO at high AZO concentrations and second-order dependence with respect to AZO at low AZO concentrations.
Enzymes

Michaelis-Menten Kinetics.
Enzymes are protein like substances with catalytic properties.

Enzymes

It provides a pathway for the substrate to proceed at a faster rate. The substrate, S, reacts to form a product P.

A given enzyme can only catalyze only one reaction. Example, Urea is decomposed by the enzyme urease.
(a) Lock-and-key model

(b) Induced fit model

Substrate (D-glucose)
A given enzyme can only catalyze only one reaction. Urea is decomposed by the enzyme urease, as shown below.

\[
\text{NH}_2\text{CONH}_2 + \text{UREASE} \overset{\text{H}_2\text{O}}{\longrightarrow} 2\text{NH}_3 + \text{CO}_2 + \text{UREASE}
\]

\[
\text{S + E} \overset{\text{H}_2\text{O}}{\longrightarrow} \text{P + E}
\]
The corresponding mechanism is:

\[ \begin{align*}
E + S & \xrightarrow{k_1} E \cdot S \\
E \cdot S & \xrightarrow{k_2} E + S \\
E \cdot S + W & \xrightarrow{k_3} P + E
\end{align*} \]
Michaelis-Menten Kinetics

\[ r_p = k_3 (E \cdot S)(W) \]

\[ r_{E \cdot S} = 0 = k_1 (E)(S) - k_2 (E \cdot S) - k_3 W (E \cdot S) \]

\[ (E \cdot S) = \frac{k_1 (E)(S)}{k_2 + k_3 W} \]

\[ E_t = (E) + (E \cdot S) \]

\[ (E) = \frac{E_t}{1 + \left( \frac{k_1 S}{k_2 + k_3 W} \right)} \]
Michaelis-Menten Kinetics

\[ r_p = k_3 (E \cdot S)(W) = \frac{k_{cat} k_3 WE_t S}{k_2 + k_3 W} + S = \frac{V_{max} E_t S}{k_{cat} K_m + S} \]

\[ r_p = k_3 (E \cdot S)(W) = \frac{V_{max}}{K_m} \frac{S}{S + K_m} \]
\[ V_{\text{max}} = k_{\text{cat}} \times E_t \]

**Turnover Number:** $k_{\text{cat}}$

Number of substrate molecules (moles) converted to product in a given time (s) on a single enzyme molecule

\[(\text{molecules/molecule/time})\]

For the reaction

\[ \text{H}_2\text{O}_2 + E \xrightarrow{k_{\text{cat}}} \text{H}_2\text{O} + \text{O} + E \]

40,000,000 molecules of $\text{H}_2\text{O}_2$ converted to product per second on a single enzyme molecule.
Summary

3. Enzyme Kinetics: Enzymatic reactions follow the sequence

\[
E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E \cdot S \xrightarrow{k_3} E + P
\]

Using the PSSH for \((E \cdot S)\) and a balance on the total enzyme, \(E_t\), which includes both the bound \((E \cdot S)\) and unbound enzyme \((E)\) concentrations

\[
E_t = (E) + (E \cdot S)
\]

we arrive at the Michaelis–Menten equation

\[
-r_s = \frac{V_{\text{max}}(S)}{K_M + (S)}
\]  \hspace{1cm} (S7-4)

where \(V_{\text{max}}\) is the maximum reaction rate at large substrate concentrations \((S \gg K_M)\) and \(K_M\) is the Michaelis constant. \(K_M\) is the substrate concentration at which the rate is half the maximum rate \((S_{1/2} = K_M)\).
Michaelis-Menten Equation

\[ r_P = -r_S = \frac{V_{\text{max}} S}{K_M + S} \]

Solving:

\[ \frac{V_{\text{max}}}{2} = \frac{V_{\text{max}} S_{1/2}}{K_M + S_{1/2}} \]

Therefore, \( K_M = S_{1/2} \) is the concentration at which the rate is half the maximum rate.
Inverting yields

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

Lineweaver-Burk Plot

\[
1/V_{\text{max}} = \text{slope} = \frac{K_M}{V_{\text{max}}}
\]
Types of Enzyme Inhibition

Competitive
\[ E + I \Leftrightarrow I \cdot E \text{ (inactive)} \]

Uncompetitive
\[ E \cdot S + I \Leftrightarrow I \cdot E \cdot S \text{ (inactive)} \]

Non-competitive
\[ E \cdot S + I \Leftrightarrow I \cdot E \cdot S \text{ (inactive)} \]
\[ I \cdot E + S \Leftrightarrow I \cdot E \cdot S \text{ (inactive)} \]
Competitive Inhibition

Reaction Steps

(1) \( E + S \xrightarrow{k_1} E \cdot S \)

(2) \( E \cdot S \xrightarrow{k_2} E + S \)

(3) \( E \cdot S \xrightarrow{k_3} P + E \)

(4) \( I + E \xrightarrow{k_4} E \cdot I \) (inactive)

(5) \( E \cdot I \xrightarrow{k_5} E + I \)

Competitive Inhibition Pathway

Competitive Inhibition

$$E + S \xrightleftharpoons[k_2]{k_1} E \cdot S \xrightarrow{k_3} E + P$$

$$E + I \xrightleftharpoons[k_5]{k_4} E \cdot I \text{ (inactive)}$$

1) **Mechanisms:**

$$E + S \rightarrow E \cdot S \quad E \cdot S \rightarrow E + S$$

$$E \cdot S \rightarrow P + E \quad E + I \rightarrow E \cdot I$$

$$E \cdot I \rightarrow E + I$$

$$r_p = k_3 C_{E \cdot S}$$
Competitive Inhibition

2) Rates:

\[ r_{E:S} = 0 = k_1 C_S C_E - k_2 C_{E:S} - k_3 C_{E:S} \]

\[ C_{E:S} = \frac{k_1 C_S C_E}{k_2 + k_3} = \frac{C_S C_E}{K_m} \]

\[ r_P = \frac{k_3 C_S C_E}{K_m} \]

\[ r_{I:E} = 0 = k_4 C_I C_E - k_5 C_{I:E} \]

\[ C_{I:E} = \frac{C_I C_E}{K_I} \quad K_I = \frac{k_5}{k_4} \]
Competitive Inhibition

\[ C_{E_{tot}} = C_E + C_{E\cdot S} + C_{I\cdot E} \]

\[ r_p = \frac{k_3 C_{E_{tot}} C_S}{K_m + C_S + \frac{C_I K_m}{K_I}} \]

\[ C_E = \frac{C_{E_{tot}}}{1 + \frac{C_S}{K_m} + \frac{C_I}{K_I}} \]

\[ -r_S = \frac{V_{max} C_S}{C_S + K_m \left(1 + \frac{C_I}{K_I}\right)} \]

\[ \frac{1}{-r_S} = \frac{1}{V_{max}} + \frac{k_m}{V_{max}} \left(1 + \frac{C_I}{K_I}\right) \frac{1}{C_S} \]
Competitive Inhibition

From before (no competition):

Increasing $C_I$

- Intercept does not change, slope increases as inhibitor concentration increases

**Equation:**

$$\frac{1}{-r_S} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \frac{1}{C_S}$$
Uncompetitive inhibition pathway

\[ E + S \rightleftharpoons E \cdot S \rightarrow E + P \]

1. \[ E + S \xrightarrow{k_1} E \cdot S \]

2. \[ E \cdot S \xrightarrow{k_2} E + S \]

3. \[ E \cdot S \xrightarrow{k_3} P + E \]

4. \[ I + E \cdot S \xrightarrow{k_4} I \cdot E \cdot S \quad \text{(inactive)} \]

5. \[ I \cdot E \cdot S \xrightarrow{k_5} I + E \cdot S \]

Reaction Steps

Uncompetitive Pathway

Active

Inactive

- [Diagram of active and inactive states]
Uncompetitive Inhibition

Inhibition only has affinity for enzyme-substrate complex

\[
\begin{align*}
E + S & \xrightarrow{k_1} E \cdot S \xrightarrow{k_3} P \\
& \xleftarrow{k_2} \\
I + E \cdot S & \xrightarrow{k_4} I \cdot E \cdot S \text{ (inactive)} \\
& \xleftarrow{k_5}
\end{align*}
\]

Developing the rate law

\[
\begin{align*}
r_p &= -r_S = k_{cat} (E \cdot S) \\
r_{E \cdot S} &= 0 = k_1 (E)(S) - k_2 (E \cdot S) - k_{cat} (E \cdot S) - k_4 (I)(E \cdot S) + k_5 (I \cdot E \cdot S) \quad (1) \\
r_{I \cdot E \cdot S} &= 0 = k_4 (I)(E \cdot S) - k_5 (I \cdot E \cdot S) \quad (2)
\end{align*}
\]
Adding (1) and (2)

\[ k_1(E)(S) - k_2(E \cdot S) - k_{\text{cat}}(E \cdot S) = 0 \]

\[ (E \cdot S) = \frac{k_1(E)(S)}{k_2 + k_{\text{cat}}} = \frac{(E)(S)}{K_M} \]

From (2)

\[ (I \cdot E \cdot S) = \frac{k_4(I)(E \cdot S)}{k_5} = \frac{(I)(E \cdot S)}{K_I} = \frac{(I)(E)(S)}{K_I K_M} \]

\[ K_I = \frac{k_5}{k_4} \]

\[ r_p = k_{\text{cat}}(E \cdot S) = \frac{k_{\text{cat}}(E)(S)}{K_M} \]
Total enzyme

\[ E_t = (E) + (E \cdot S) + (I \cdot E \cdot S) \]

\[ = (E) \left( 1 + \frac{(S)}{K_M} + \frac{(I)(S)}{K_I K_M} \right) \]

\[ r_p = \frac{k_{cat} E_t (S)}{K_M \left(1 + \frac{(S)}{K_M} + \frac{(I)(S)}{K_I K_M} \right)} \]

\[ -r_S = r_p = \frac{V_{max} (S)}{K_M + (S) \left(1 + \frac{(I)}{K_I} \right)} \]
Slope remains the same but intercept changes as inhibitor concentration is increased

Lineweaver-Burk Plot for uncompetitive inhibition

\[
\begin{align*}
\frac{1}{-r_s} &= \frac{1}{V_{\text{max}}(S)} \left( K_M + (S) \left( 1 + \frac{(I)}{K_I} \right) \right) \\
\frac{1}{-r_s} &= \frac{K_M}{V_{\text{max}}(S)} + \frac{1}{V_{\text{max}}} \left( 1 + \frac{(I)}{K_I} \right)
\end{align*}
\]
Mixed inhibition

\[ E + S \leftrightarrow E \cdot S \rightarrow E + P \]

\[ I \quad I \]

\[ E \cdot I + S \leftarrow E \cdot S \cdot I \]

**Reaction Steps**

1. \( E + S \leftrightarrow E \cdot S \)
2. \( E \cdot I \leftrightarrow I \cdot E \) (inactive)
3. \( I + E \cdot S \leftrightarrow I \cdot E \cdot S \) (inactive)
4. \( S + I \cdot E \leftrightarrow I \cdot E \cdot S \) (inactive)
5. \( E \cdot S \rightarrow P + E \)

**Noncompetitive Pathway**

**Active**

**Inactive**
Noncompetitive Inhibition (Mixed)

\[
\begin{align*}
E + S & \underset{+I}{\overset{-I}{\rightleftharpoons}} E \cdot S \underset{-I}{\overset{+I}{\rightarrow}} P + E \\
(\text{inactive})I \cdot E + S & \underset{-I}{\overset{+I}{\rightarrow}} I \cdot E \cdot S \text{ (inactive)}
\end{align*}
\]

Increasing I

No Inhibition

Both slope and intercept changes

\[
\begin{align*}
- \frac{1}{r_s} &= \frac{V_{\text{max}} C_S}{(k_m + C_S)(1 + \frac{C_I}{k_I})} \\
\frac{1}{-r_s} &= \frac{1}{V_{\text{max}}} \left(1 + \frac{C_I}{k_I}\right) + \frac{k_m}{V_{\text{max}}} \left(\frac{1}{C_S}\right) \left(1 + \frac{C_I}{k_I}\right)
\end{align*}
\]
4. The three different types of inhibition—competitive, uncompetitive, and non-competitive (mixed) inhibition—are shown on the Lineweaver–Burk plot:
End of Lecture 15