Lecture 15

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.

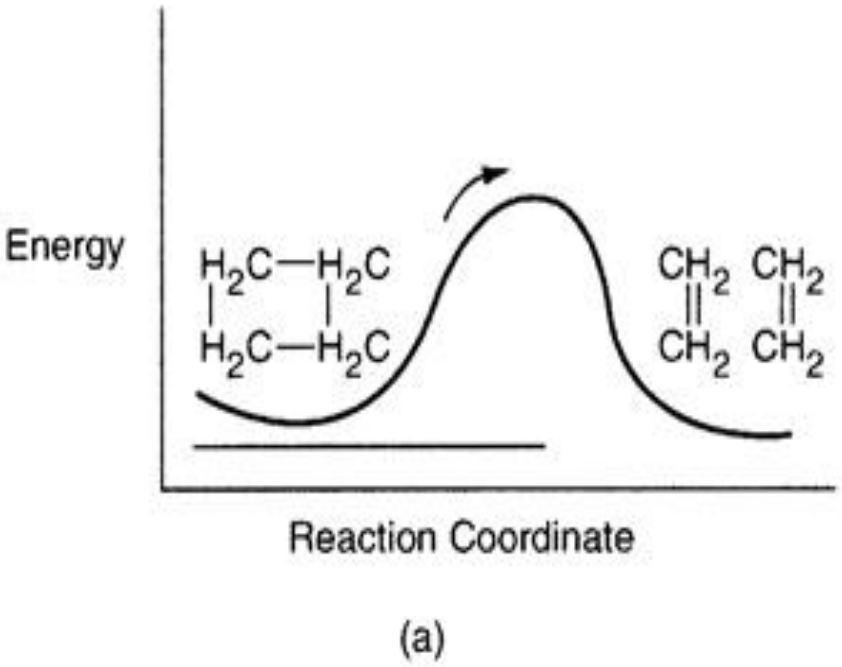
Lecture 15 – Tuesday

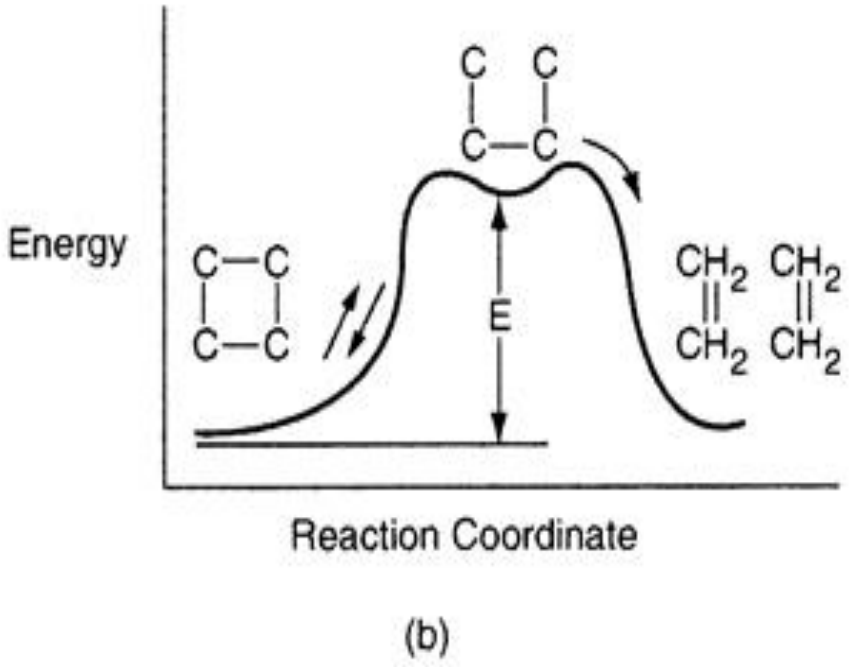
Enzymatic Reactions

- Michealis-Menten Kinetics
- Lineweaver-Burk Plot
- Enzyme Inhibition
  - Competitive
  - Uncompetitive
  - Non-Competitive

Review Last Lecture

Active Intermediates and PSSH





Reaction coordinate. Courtesy *Science News*, *156*, 247 (1999).

Review Last Lecture

Active Intermediates and PSSH

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:

$$r_{A^*.net} = \sum_{i=1}^{m} r_{A^*i} = 0$$

2. The azomethane (AZO) decomposition mechanism is

$$2\,AZO \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AZO + AZO^*$$

$$AZO^* \xrightarrow{k_3} N_2 + ethane$$

$$\Rightarrow r_{N_2} = \frac{k(AZO)^2}{1 + k'(AZO)}$$

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

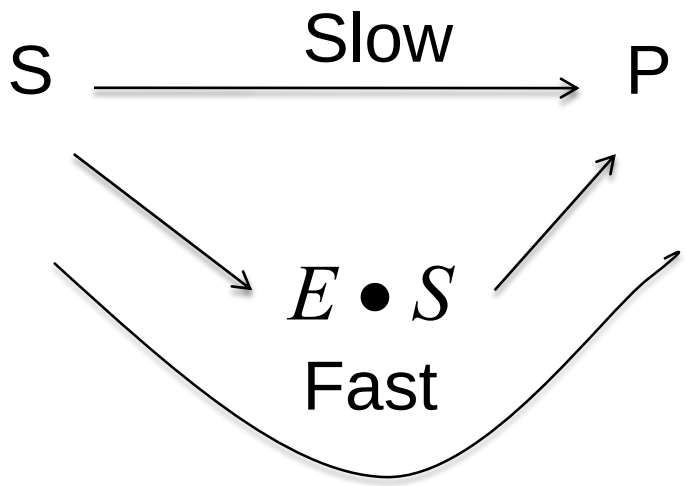
Michaelis-Menten Kinetics

Enzymes are protein-like substances with catalytic properties.

Enzyme Unease

Enzymes

Enzymes provide 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.

Enzymes - Urease

A given enzyme can only catalyze only one reaction. Urea is decomposed by the enzyme urease, as shown below.

$$NH_2CONH_2 + UREASE \xrightarrow{H_2O} 2NH_3 + CO_2 + UREASE$$

$$S + E \xrightarrow{H_2O} P + E$$

The corresponding mechanism is:

$$E + S \xrightarrow{k_1} E \bullet S$$

$$E \bullet S \xrightarrow{k_2} E + S$$

$$E \bullet S + W \xrightarrow{k_3} P + E$$

Enzymes - Michaelis-Menten Kinetics

$$r_P = k_3(E \bullet S)(W)$$

$$r_{E \bullet S} = 0 = k_1(E)(S) - k_2(E \bullet S) - k_3 W(E \bullet S)$$

$$(E \bullet S) = \frac{k_1(E)(S)}{k_2 + k_3 W}$$

$$E_t = (E) + (E \bullet S)$$

$$(E) = \frac{E_t}{1 + \left(\frac{k_1 S}{k_2 + k_3 W} \right)}$$

Enzymes - **Michaelis-Menten Kinetics**

$$r_P = k_3\left(E \bullet S\right)\left(W\right) = \frac{\overbrace{k_3 W}^{k_{cat}} E_t S}{\underbrace{\frac{k_2 + k_3 W}{k_1}}_{K_M} + S} = \frac{\overbrace{k_{cat} E_t}^{V_{\max}} S}{K_M + S}$$

$$r_P = k_3\left(E \bullet S\right)\left(W\right) = \frac{V_{\max} S}{K_m + S}$$

Enzymes - **Michaelis-Menten Kinetics**

$$V_{max} = k_{cat} E_t$$

Turnover Number: k_{cat}

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

For the reaction: $H_2O_2 + E \xrightarrow{k_{cat}} H_2O + O + E$

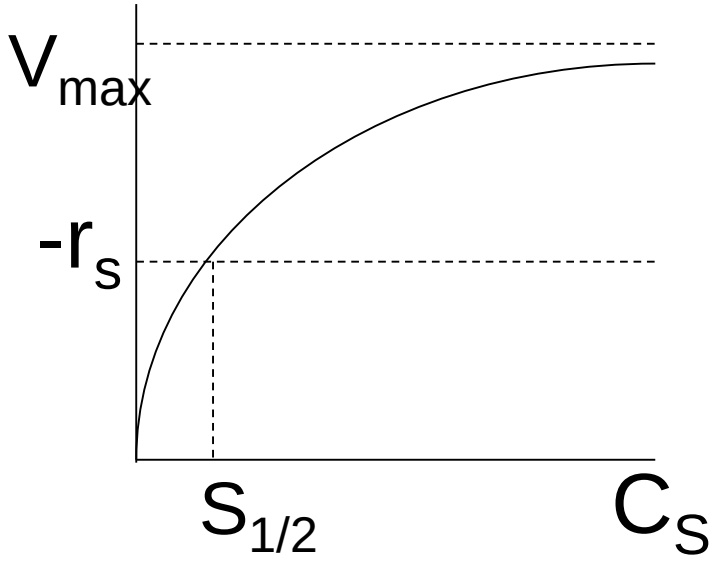
40,000,000 molecules of H_2O_2 converted to product per second on a single enzyme molecule.

Enzymes - **Michaelis-Menten Kinetics**

Michaelis-Menten Equation

$$r_P = -r_S = \frac{V_{max} S}{K_M + S}$$

(Michaelis-Menten plot)



Solving: $\frac{V_{max}}{2} = \frac{V_{max} S_{1/2}}{K_M + S_{1/2}}$

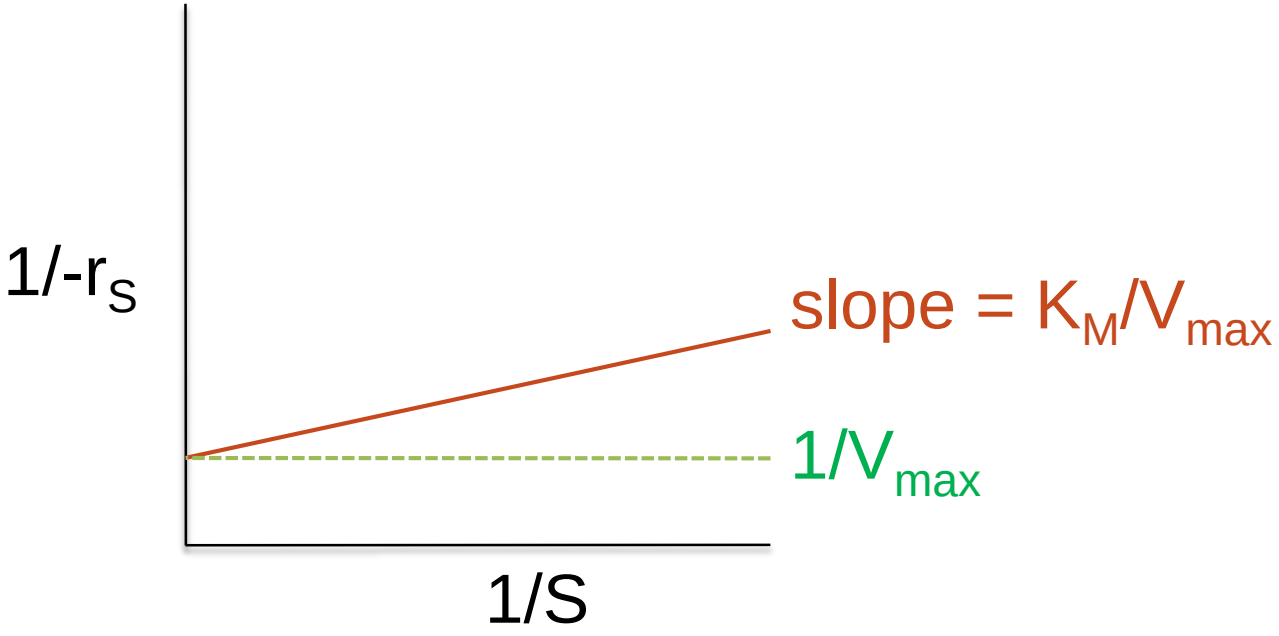
$K_M = S_{1/2}$

therefore K_M is the concentration at which the rate is half the maximum rate.

Enzymes - **Michaelis-Menten Kinetics**

Inverting yields: $$\frac{1}{-r_S} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}}\left(\frac{1}{S}\right)$$

Lineweaver-Burk Plot



Types of Enzyme Inhibition

Competitive

$$E + I \Leftrightarrow I \bullet E \text{ (inactive)}$$

Uncompetitive

$$E \bullet S + I \Leftrightarrow I \bullet E \bullet S \text{ (inactive)}$$

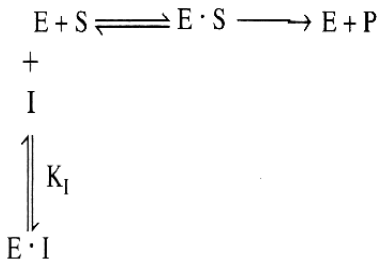
Non-competitive

$$E \bullet S + I \Leftrightarrow I \bullet E \bullet S \text{ (inactive)}$$

$$I \bullet E + S \Leftrightarrow I \bullet E \bullet S \text{ (inactive)}$$

Competitive Inhibition

Competitive inhibition pathway



Reaction Steps

(1) $E + S \xrightarrow{k_1} E \bullet S$

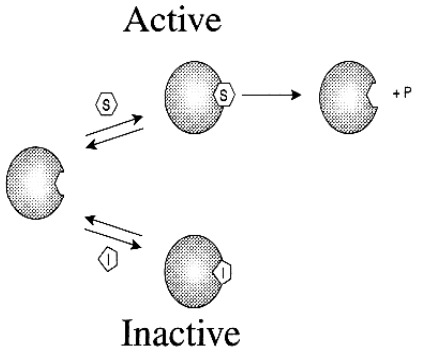
(2) $E \bullet S \xrightarrow{k_2} E + S$

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

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

(5) $E \bullet I \xrightarrow{k_5} E + I$

Competitive Inhibition Pathway



(a) Competitive inhibition. Courtesy of D. L. Nelson and M. M. Cox, *Lehninger Principles of Biochemistry*, 3rd ed. (New York: Worth Publishers, 2000), p. 266.

Competitive Inhibition

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} E \bullet S \xrightarrow{k_3} E + P$$

$$E + I \underset{k_5}{\overset{k_4}{\rightleftarrows}} E \bullet I \text{ (inactive)}$$

1) Mechanisms:

$$E + S \rightarrow E \cdot S \qquad E \cdot S \rightarrow E + S$$

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

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

$$r_P = k_3 C_{E \cdot S}$$

Competitive Inhibition

2) Rate Laws:

$$r_{E\cdot S} = 0 = k_1 C_S C_E - k_2 C_{E\cdot S} - k_3 C_{E\cdot S}$$

$$C_{E\cdot S} = \frac{k_1 C_S C_E}{k_2 + k_3} = \frac{C_S C_E}{K_m}$$

$$r_P = \frac{k_3 C_S C_E}{K_m}$$

$$r_{I\cdot E} = 0 = k_4 C_I C_E - k_5 C_{I\cdot E}$$

$$C_{I\cdot E} = \frac{C_I C_E}{K_I} \qquad K_I = \frac{k_5}{k_4}$$

Competitive Inhibition

$$C_{Etot} = C_E + C_{E\cdot S} + C_{I\cdot E}$$

$$C_E = \frac{C_{Etot}}{1 + \frac{C_S}{K_m} + \frac{C_I}{K_I}}$$

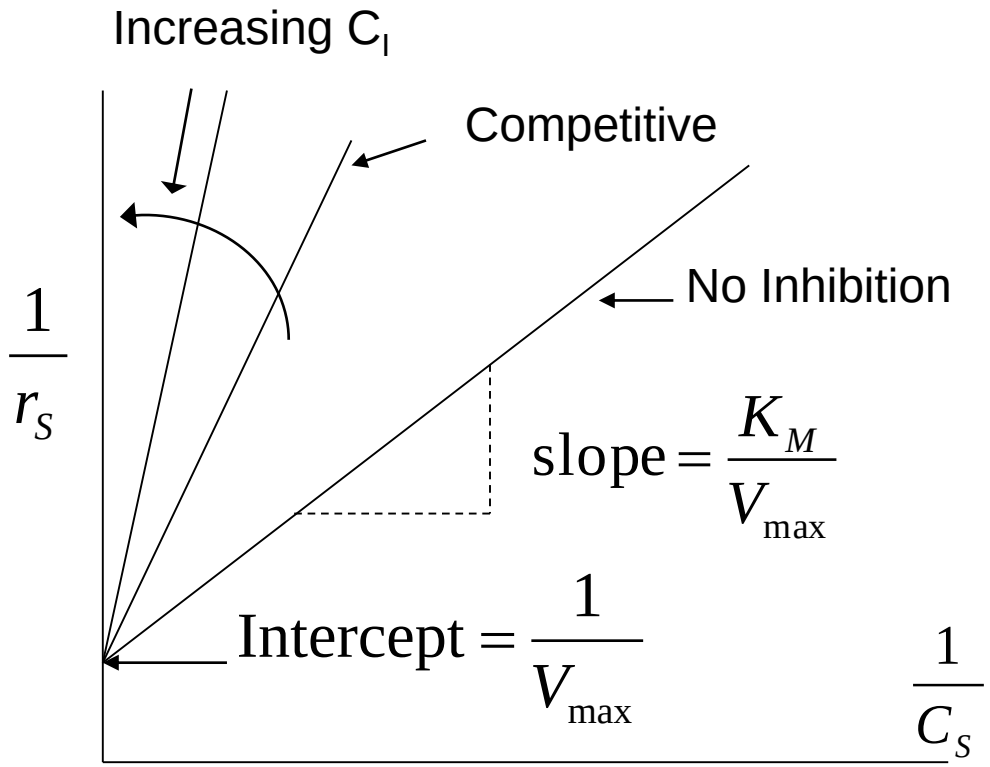
$$r_P = \frac{k_3 C_{Etot} C_S}{K_m + C_S + \frac{C_I K_m}{K_I}}$$

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

$$\frac{1}{-r_S} = \frac{1}{V_{max}} + \frac{k_m}{V_{max}}\left(1 + \frac{C_I}{K_I}\right)\frac{1}{C_S}$$

Competitive Inhibition

From before (no competition): $\frac{1}{-r_S} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}} \frac{1}{C_S}$



Competitive

$$\frac{1}{-r_S} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}} \left(1 + \frac{C_I}{K_I}\right) \frac{1}{C_S}$$

Intercept does not change, slope increases as inhibitor concentration increases

Uncompetitive Inhibition

Uncompetitive inhibition pathway

$$E + S \rightleftharpoons E \cdot S \longrightarrow E + P$$
$$+$$
$$I$$
$$\Updownarrow K_I$$
$$E \cdot S \cdot I$$

Reaction Steps

(1) $E + S \xrightarrow{k_1} E \bullet S$

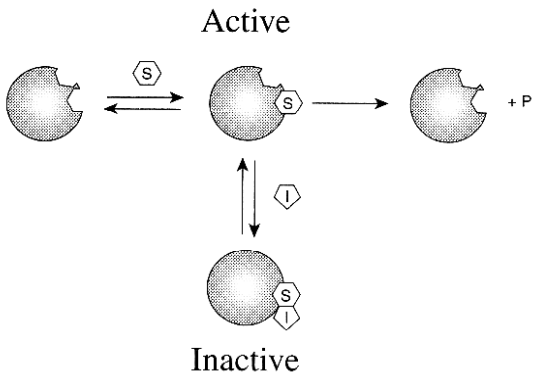
(2) $E \bullet S \xrightarrow{k_2} E + S$

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

(4) $I + E \bullet S \xrightarrow{k_4} I \bullet E \bullet S$ (inactive)

(5) $I \bullet E \bullet S \xrightarrow{k_5} I + E \bullet S$

Uncompetitive Pathway



Uncompetitive Inhibition

Inhibition only has affinity for enzyme-substrate complex

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} E \bullet S \xrightarrow{k_3} P$$

$$I + E \bullet S \underset{k_5}{\overset{k_4}{\rightleftarrows}} I \bullet E \bullet S \text{ (inactive)}$$

Developing the **rate law:**

$$r_P = -r_S = k_{cat}(E \bullet S)$$

$$r_{E\bullet S} = 0 = k_1(E)(S) - k_2(E \bullet S) - k_{cat}(E \bullet S) - k_4(I)(E \bullet S) + k_5(I \bullet E \bullet S) \quad (1)$$

$$r_{I\bullet E\bullet S} = 0 = k_4(I)(E \bullet S) - k_5(I \bullet E \bullet S) \quad (2)$$

Uncompetitive Inhibition

Adding (1) and (2)

$$k_1(E)(S) - k_2(E \bullet S) - k_{cat}(E \bullet S) = 0$$

$$(E \bullet S) = \frac{k_1(E)(S)}{k_2 + k_{cat}} = \frac{(E)(S)}{K_M}$$

From (2)

$$(I \bullet E \bullet S) = \frac{k_4}{k_5}(I)(E \bullet S) = \frac{(I)(E \bullet S)}{K_I} = \frac{(I)(E)(S)}{K_I K_M}$$

$$K_I = \frac{k_5}{k_4}$$

$$r_p = k_{cat}(E \bullet S) = \frac{k_{cat}(E)(S)}{K_M}$$

Uncompetitive Inhibition

Total enzyme

$$E_t = (E) + (E \bullet S) + (I \bullet E \bullet S)$$

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

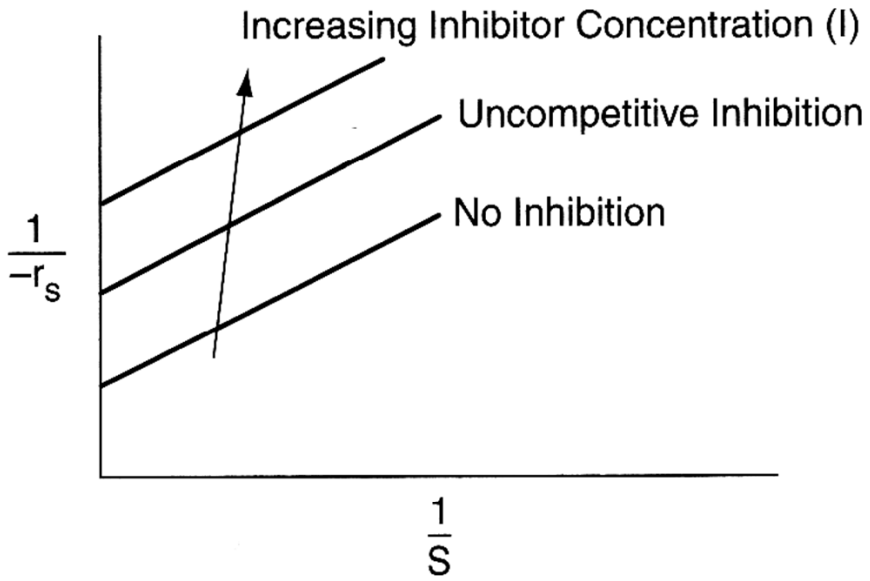
$$r_p = \frac{k_{cat} E_t (S)}{K_M \left(1 + \frac{(S)}{K_M} + \frac{(I)(S)}{K_I K_M}\right)}$$

$$-r_S = r_P = \frac{V_{\max}(S)}{K_M + (S)\left(1 + \frac{(I)}{K_I}\right)}$$

Uncompetitive Inhibition

$$\frac{1}{-r_S} = \frac{1}{V_{\max}(S)}\left(K_M + (S)\left(1 + \frac{(I)}{K_I}\right)\right)$$

$$\frac{1}{-r_S} = \frac{K_M}{V_{\max}}\left(\frac{1}{(S)}\right) + \frac{1}{V_{\max}}\left(1 + \frac{(I)}{K_I}\right)$$

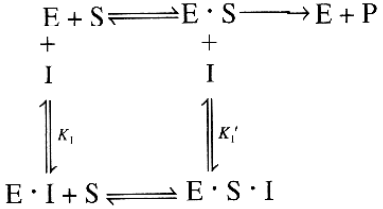


Slope remains the same but intercept changes as inhibitor concentration is increased

Lineweaver-Burk Plot for uncompetitive inhibition

Non-competitive Inhibition

Mixed inhibition



Reaction Steps

(1) $E + S \rightleftharpoons E \bullet S$

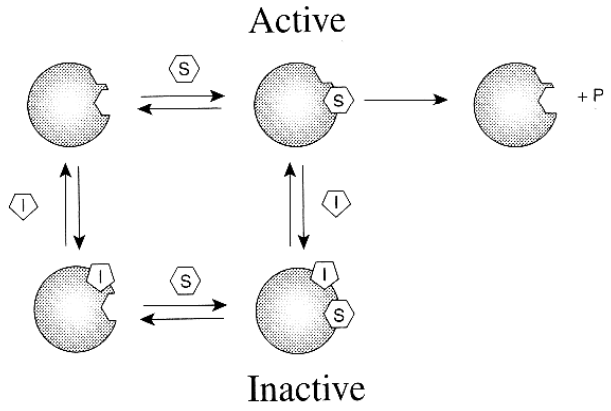
(2) $E + I \rightleftharpoons I \bullet E$ (inactive)

(3) $I + E \bullet S \rightleftharpoons I \bullet E \bullet S$ (inactive)

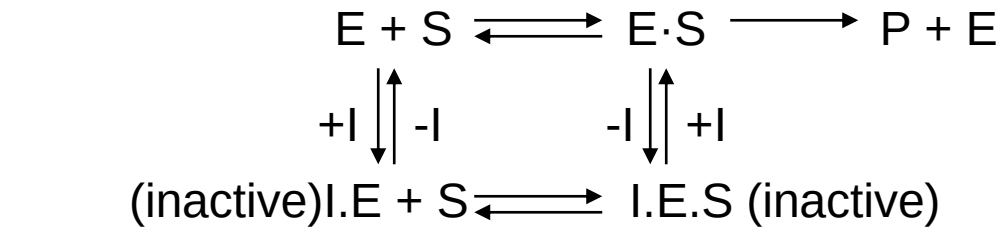
(4) $S + I \bullet E \rightleftharpoons I \bullet E \bullet S$ (inactive)

(5) $E \bullet S \longrightarrow P + E$

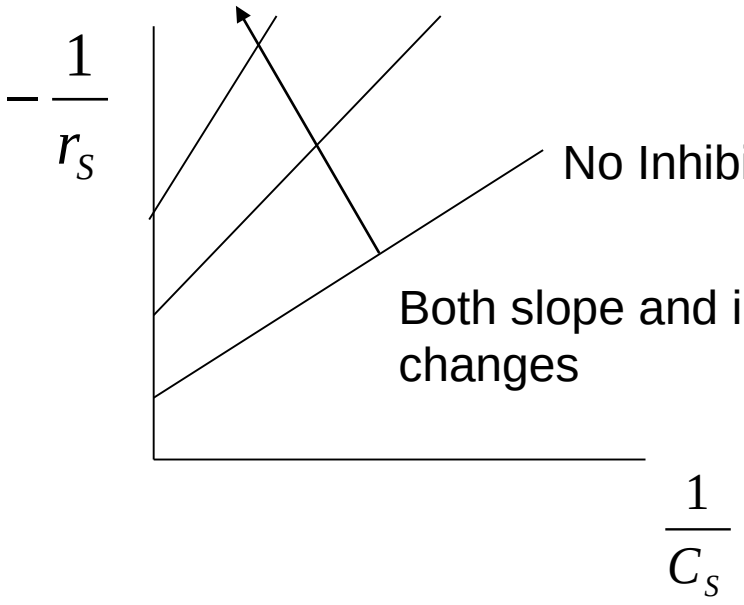
Noncompetitive Pathway



Non-competitive Inhibition



Increasing I

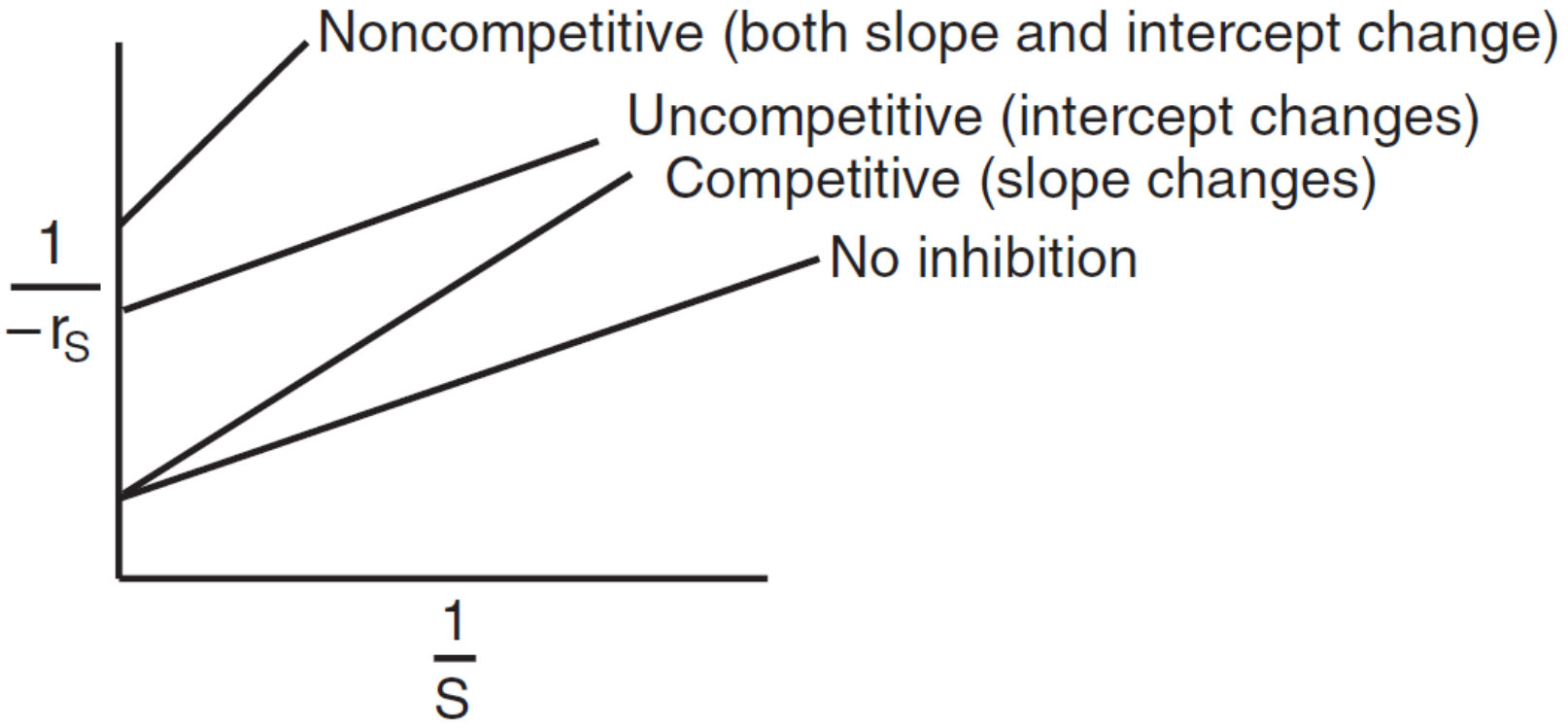


$$-r_S = \frac{V_{\max} C_S}{\left(k_M + C_S\right)\left(1 + \frac{C_I}{k_I}\right)}$$

$$\frac{1}{-r_S} = \frac{1}{V_{\max}}\left(1 + \frac{C_I}{k_I}\right) + \frac{k_M}{V_{\max}}\left(\frac{1}{C_S}\right)\left(1 + \frac{C_I}{k_I}\right)$$

Summary: Types of Enzyme Inhibition

Lineweaver–Burk plots for three types of enzyme inhibition.



End of Lecture 15