7. Reaction Mechanisms, Pathways, Bioreactions and Bioreactors

- **Active Intermediates and Free Radicals**
  - **Pseudo-steady-state-hypothesis**
  - **Reaction pathway**
- **Enzymes**
  - **Michealis-Menten enzyme kinetics**
  - **Enzyme inhibition**
- **Bioreactors**
- **Pharmacokinetics**
- **Polymerization**

1. Active Intermediates / Free Radicals (PSSH) I

○ **Active intermediate**

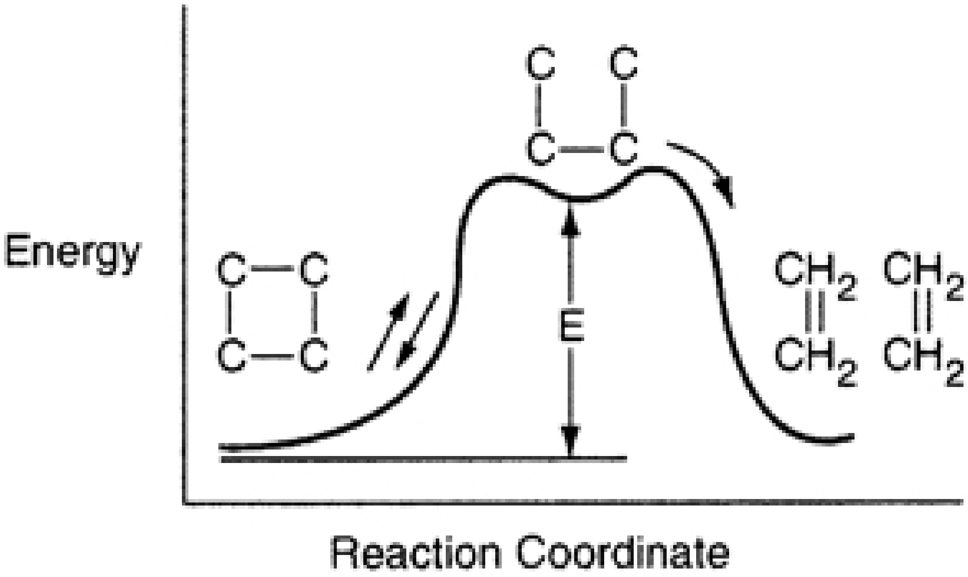
- **A molecule that is in a highly energetic and reactive state**
- **It is short lived as it disappears virtually as fast as it is formed (ca. 10^{-14}s)**
- **Very low concentrations ☞ the net rate of reaction of an active intermediate, A*, is zero**

$$r_{A^*} = \sum r_{iA} = 0$$

⇒ Pseudo Steady State Hypothesis (PSSH)

1. Active Intermediates / Free Radicals (PSSH) II

- Active intermediate 2
  - The active intermediates reside in the trough of the reaction coordinate



1. Active Intermediates / Free Radicals (PSSH) III

- Active intermediate 3 - Example

  - The rate law for the reaction A → B + C is found from experiment to be $-r_A = \dfrac{kC_A^2}{1+k'C_A}$

    Suggest a mechanism consistent with the rate law.

Sol)

- Two A molecules collide and energy is transferred from one A molecule to the other molecule making it highly reactive.

$$A + A \xrightarrow{k_1} A^* + A \qquad r_{1A^*} = -k_1 C_A^2$$

1. Active Intermediates / Free Radicals (PSSH) IV

- **Active intermediate 4 - Example**

- This activated molecule (A*) can do one of two things

- **It (A*) can collide with another molecule to become deactivated (A)**

$$A^* + A \xrightarrow{k_2} A + A \qquad r_{2A^*} = -k_2 C_{A^*} C_A$$

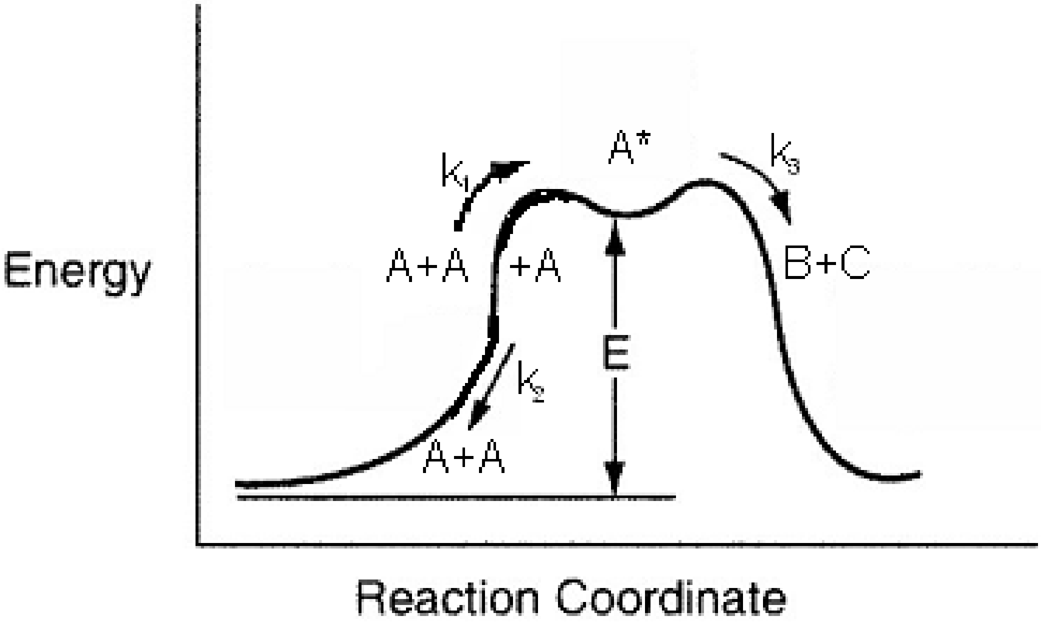
- **or (2) the activated molecule, A* can decompose to form B and C**

$$A^* \xrightarrow{k_3} B + C \qquad r_{3A^*} = -k_3 C_{A^*}$$

1. Active Intermediates / Free Radicals (PSSH) V

- Active intermediate 5 - Example

  - For reactions with active intermediates, the reaction coordinated has trough in it and the active intermediate, A*, sit in this trough



1. Active Intermediates / Free Radicals (PSSH) VI

- **Active intermediate 6 - Example**

- Rate laws

Reaction (1) $r_{1A*} = k_1 C_A^2$

Reaction (2) $r_{2A*} = -k_2 C_A C_{A*}$

Reaction (3) $r_{3A*} = -k_3 C_{A*}$

- Relative rates $r_{1A} = -2r_{1A*}$, $r_{3B} = -r_{3A*}$

- Net rates = Rate of formation of product

$$r_B = r_{3B} = -r_{3A*} = k_3 C_{A*}$$

1. Active Intermediates / Free Radicals (PSSH) VII

- **Active intermediate 7 - Example**

- Use PSSH to solve for C_{A*}

$$r_{A*} = \sum r_{1A*} = r_{1A*} + r_{2A*} = r_{3A*}$$

$$= k_1 C_A^2 - k_2 C_A C_{A*} - k_3 C_{A*} = 0$$

- Solving for C_{A*}

$$C_{A*} = \frac{k_1 C_A^2}{k_3 + k_2 C_A}$$

- Substituting for C_{A*} to get the rate of formation of B

$$r_B = \frac{k_1 k_3 C_A^2}{k_3 + k_2 C_A}$$

1. Active Intermediates / Free Radicals (PSSH) VIII

- **Active intermediate 8 - Example**

- Relative rates overall

$$A \rightarrow B + C$$

$$\frac{r_B}{1} = \frac{r_A}{-1}$$

$$r_A = -r_B = \frac{-k_1 k_3 C_A^2}{k_3 + k_2 C_A}$$

- For high concentrations of A, we can neglect k_3 with regard to k_2C_A, i.e., $k_2 C_A >> k_3$

- The rate law becomes $r_A = -\frac{k_1 k_3}{k_2} C_A = -kC_A$

1. Active Intermediates / Free Radicals (PSSH) IX

- **Active intermediate 9 - Example**

- For low concentrations of A, we can neglect k_2C_A with regard to k_3, i.e., $k_3 >> k_2C_A$

- The rate law becomes

$$-r_A = -\frac{k_3k_1}{k_3}C_A^2 = -k_1C_A^2$$

- Dividing by k_3 and letting $k' = k_2/k_3$ and $k = k_1$

$$\boxed{-r_A = -\frac{kC_A^2}{1+k'C_A}}$$

Produce an active intermediate

- Collide with active intermediate

- Spontaneous decomposition

1. Active Intermediates / Free Radicals (PSSH) X

- **Active intermediate 10 - Temperature dependence**

- The reaction $2NO + O_2 \rightarrow 2NO_2$

has an elementary rate law $r_{NO_2} = kC_{NO}^2 C_{O_2}$

However... the rate law decrease with increasing temperature, why?

Sol)

- Mechanism:

$$NO + O_2 \xrightarrow{k_1} NO_3^*$$

$$NO_3^* \xrightarrow{k_2} NO + O_2$$

$$NO_3^* + NO \xrightarrow{k_3} 2NO_2$$

$$\frac{r_{NO_2}}{2} = r_{3NO_3^*} = -k_3 C_{NO_3} \cdot C_{NO} = k_3 \left[NO_3^*\right]\left[NO\right]$$

1. Active Intermediates / Free Radicals (PSSH) XI

- **Active intermediate 11 - Temperature dependence**

- Pseudo Steady State Hypothesis (PSSH)

- **net rate of species A* (in this case, NO_3^*) is zero**

$$r_{NO_3*} \cong 0 = k_1(NO)(O_2) - k_2(NO_3*) - k_3(NO_3*)(NO)$$

- All specific reaction rates are defined with respect to NO^*_3

- Assume that all reactions are <u>elementary</u> reactions

$$r_{1\,NO_3^*} = k_1[NO][O_2]$$

$$r_{2\,NO_3^*} = -k_2[NO_3^*]$$

$$r_{3\,NO_3^*} = -k_3[NO_3^*][NO]$$

1. Active Intermediates / Free Radicals (PSSH) XII

- **Active intermediate 12 - Temperature dependence**

- The net reaction rate for NO^*_3

$$r_{NO_3^\bullet} = r_{1\,NO_3^\bullet} + r_{2\,NO_3^\bullet} + r_{3\,NO_3^\bullet}$$

$$r_{NO_3^\bullet} = k_1[NO][O_2] - k_2[NO_3^*] - \frac{1}{2}k_3[NO_3^*][NO]$$

- The PSSH assumes that the net rate of NO^*_3 is zero

$$0 = k_1[NO][O_2] - k_2[NO_3^*] - \frac{1}{2}k_3[NO_3^*][NO]$$

$$0 = k_1[NO][O_2] - [NO_3^*]\left(k_2 + k_3[NO]\right)$$

- Solving for NO_3*

$$[NO_3^*] = \frac{k_1[NO][O_2]}{k_2 + k_3[NO]}$$

1. Active Intermediates / Free Radicals (PSSH) XIII

- **Active intermediate 13 - Temperature dependence**

$$r_{NO_2} = -2r_{3NO_3^*} = 2k_3\frac{\left[k_1(NO)(O_2)\right][NO]}{k_2 + k_3(NO)}$$

$$r_{NO_2} = \frac{2k_1k_3(NO)^2(O_2)}{k_2 + k_3(NO)}$$

$$k_2 >> k_3[NO]$$

$$r_{NO_2} = \frac{k_1\,k_3}{k_2}[NO]^2[O_2] = \frac{A_1\,A_3}{A_2}e^{\frac{E_2-(E_1+E_3)}{RT}}[NO]^2[O_2]$$

$$E_2 > (E_1 + E_3)$$

1. Active Intermediates / Free Radicals (PSSH) XIV

- Gas phase chain reaction involving free radicals 1

  \- Hydrodealkylation

  - in the gas phase at high temperature and involves free radical with the sequence

Initiation : $H_2 \xrightarrow{k_1} 2H\cdot$

Propagation : $\begin{cases} H\cdot + C_6H_5CH_3 \xrightarrow{k_2} C_6H_5\cdot + CH_4 \\ C_6H_5\cdot + H_2 \xrightarrow{k_3} C_6H_6 + H\cdot \end{cases}$

Termination : $2H\cdot \xrightarrow{k_4} H_2$

  - specific reaction rates k_1 and k_4 are defined w.r.t. H_2
  - r_{C6H6} = ?

1. Active Intermediates / Free Radicals (PSSH) XV

- Gas phase chain reaction involving free radicals 2
  - For the elementary equation, the rate of formation of benzene is $r_{C_6H_6} = k_3 C_{C_6H_5\bullet} C_{H_2}$
  - We need to eliminate the concentration of the free radical $C_{C_6H_5\bullet}$ by expressing it in terms of the concentrations of toluene and hydrogen
  - Under psuedo-steady-state hypothesis, we set the rates of formation of $C_{H\bullet}$ and $C_{C_6H_5\bullet}$ equal to zero, i.e.

$$0 = r_{H\bullet} = 2k_1 C_{H_2} - k_2 C_{H\bullet} C_{C_6H_5CH_3} + k_3 C_{H_2} C_{C_6H_5\bullet} - 2k_4 \left[C_{H\bullet}\right]^2$$

$$0 = r_{C_6H_5\bullet} = k_2 C_{H\bullet} C_{C_6H_5CH_3} - k_3 C_{H_2} C_{C_6H_5\bullet}$$

1. Active Intermediates / Free Radicals (PSSH) XVI

- Gas phase chain reaction involving free radicals 3

  - Adding the above 2 equations

$$0 = r_{H\bullet} = 2k_1 C_{H_2} - 2k_4 \left[C_{H\bullet}\right]^2$$

  - Solving for $C_{H\bullet}$ yields

$$C_{H\bullet} = \sqrt{\frac{k_1 C_{H_2}}{k_4}}$$

  - Solving for $C_{C_6H_5\bullet}$ yields

$$C_{C_6H_5\bullet} = \frac{k_2 C_{C_6H_5CH_3} \sqrt{\frac{k_1 C_{H_2}}{k_4}}}{k_3 C_{H_2}}$$

1. Active Intermediates / Free Radicals (PSSH) XVII

- Gas phase chain reaction involving free radicals 3

  - Eliminate $C_{C_6H_5\bullet}$

$$r_{C_6H_6} = k_2 C_{C_6H_5CH_3}\sqrt{\frac{k_1 C_{H_2}}{k_4}}$$

$$r_{C_6H_6} = k\left(C_{H_2}\right)^{1/2}\left(C_T\right)$$

$$k = 10^{10.5}\exp\left[\frac{-50{,}000}{RT}\right]$$

$$C_T = \text{concentration of toluene}$$

1. Active Intermediates / Free Radicals (PSSH) XVIII

- Reaction pathway

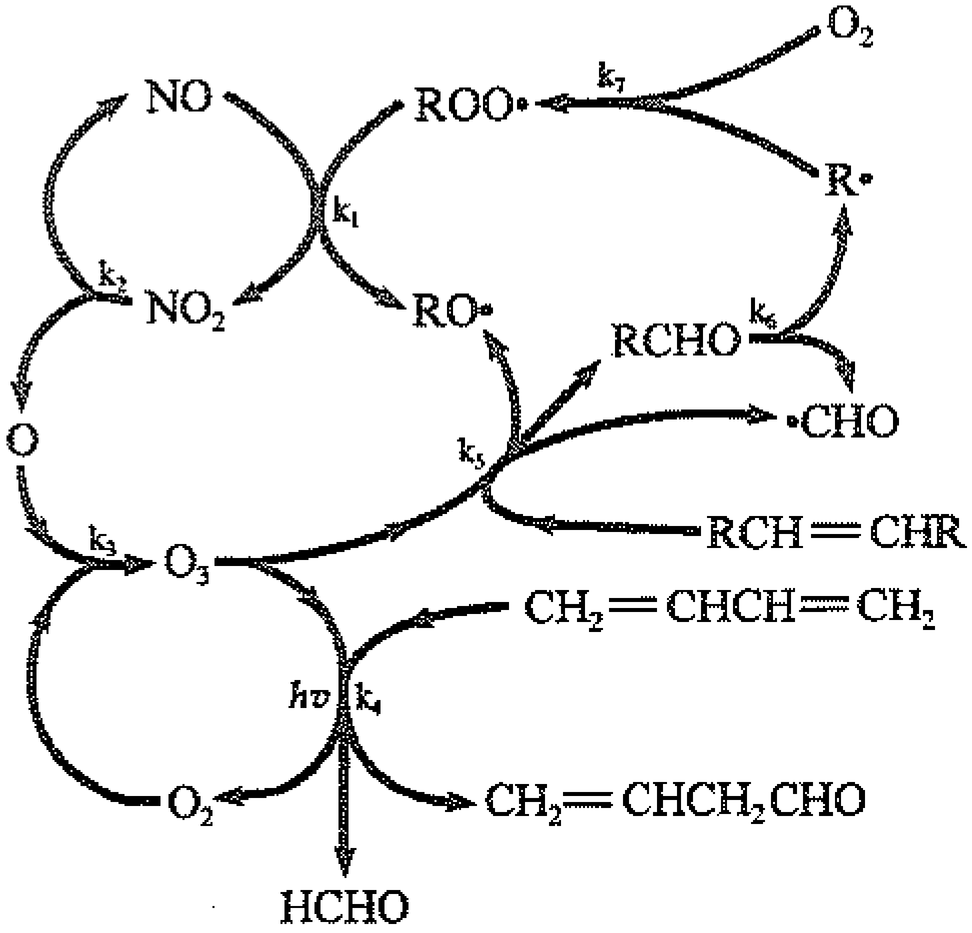
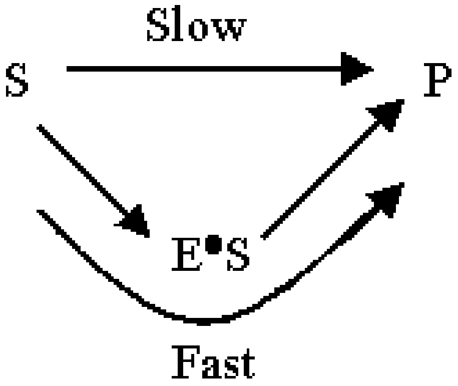


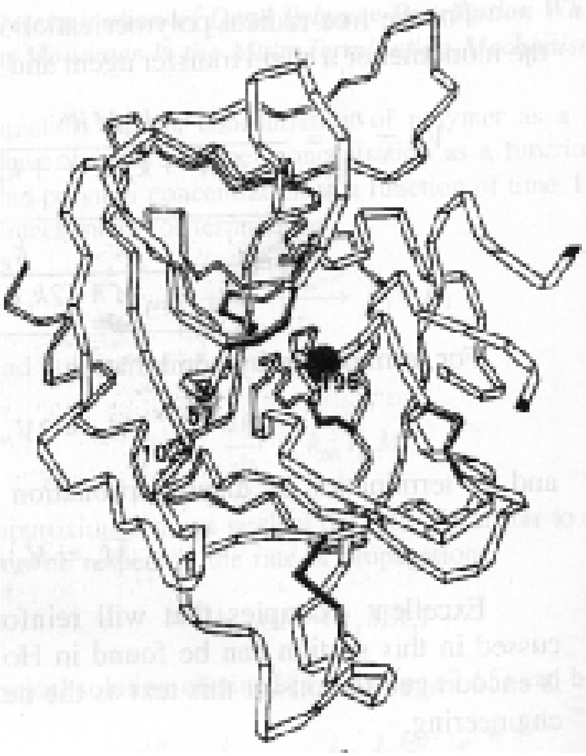
Figure 7-3 Reaction pathways in smog formation.

2. Enzymes I

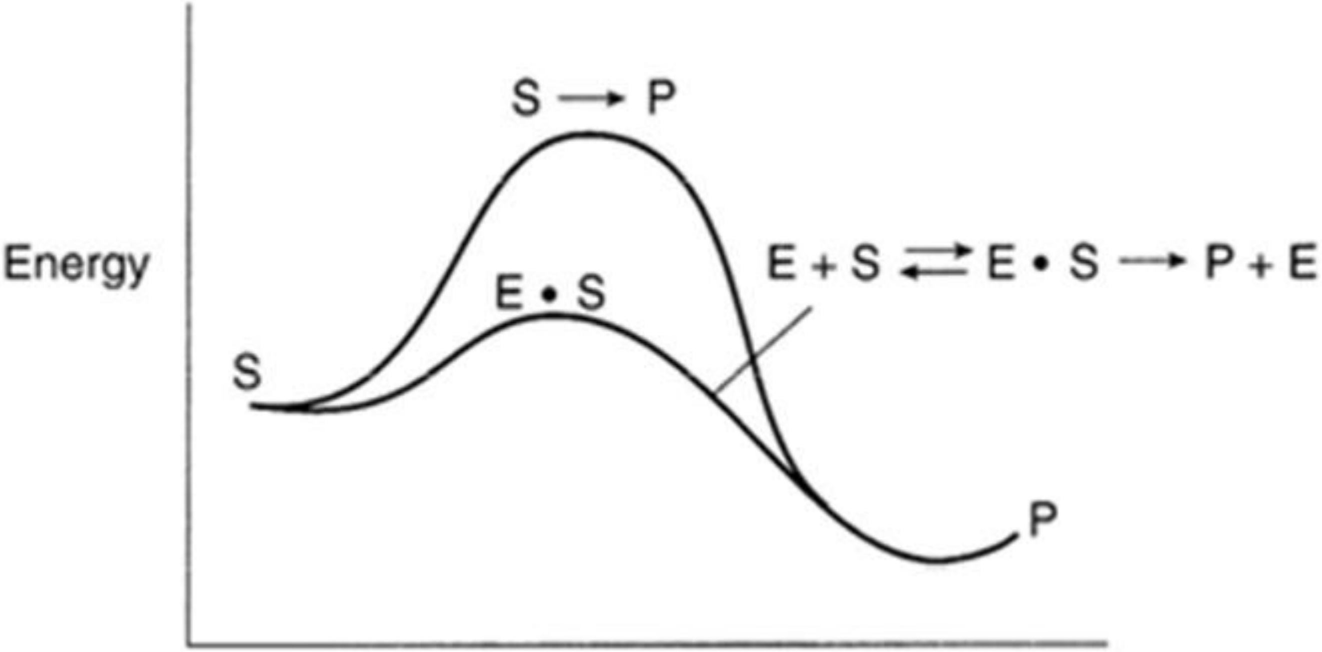
- **Enzymes are protein like substances with catalytic properties**

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





2. Enzymes II



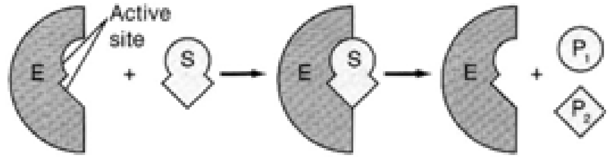
There are six classes of enzymes (E) and only six:

1.	Oxidoreductases	$AH_2 + B + E \rightarrow A + BH_2 + E$
2.	Transferases	$AB + C + E \rightarrow AC + B + E$
3.	Hydrolases	$AB + H_2O + E \rightarrow AH + BOH + E$
4.	Isomerases	$A + E \rightarrow iso - A + E$
5.	Lyases	$AB + E \rightarrow A + B + E$
6.	Ligases	$A + B + E \rightarrow AB + E$

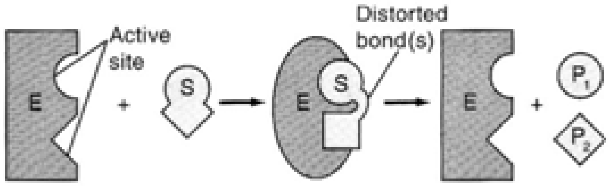
2. Enzymes III

- A given enzyme can only catalyze only one reaction

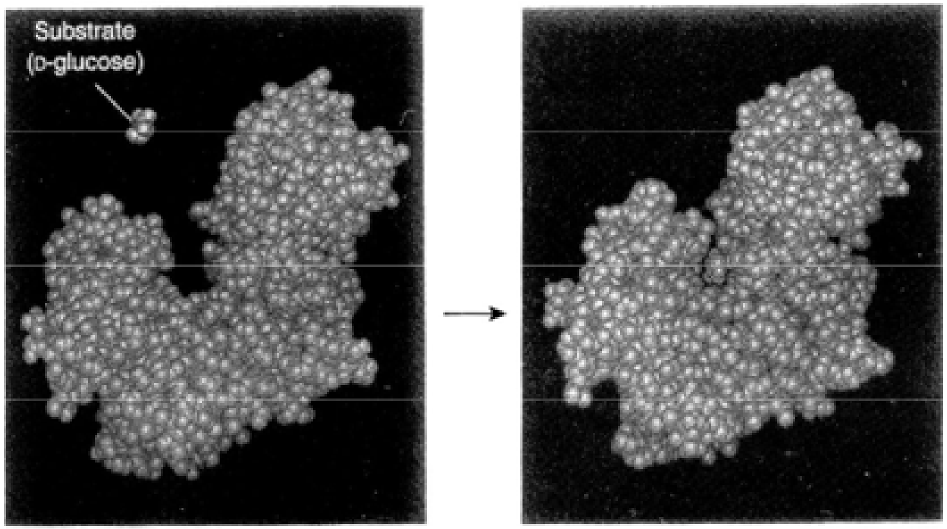
The Enzyme Substrate Complex



(a) Lock-and-key model

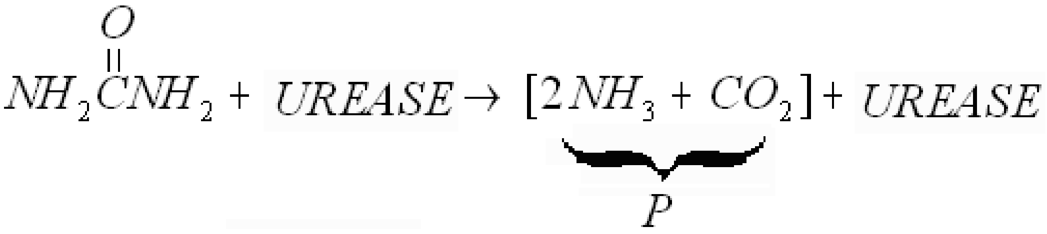


(b) Induced fit model



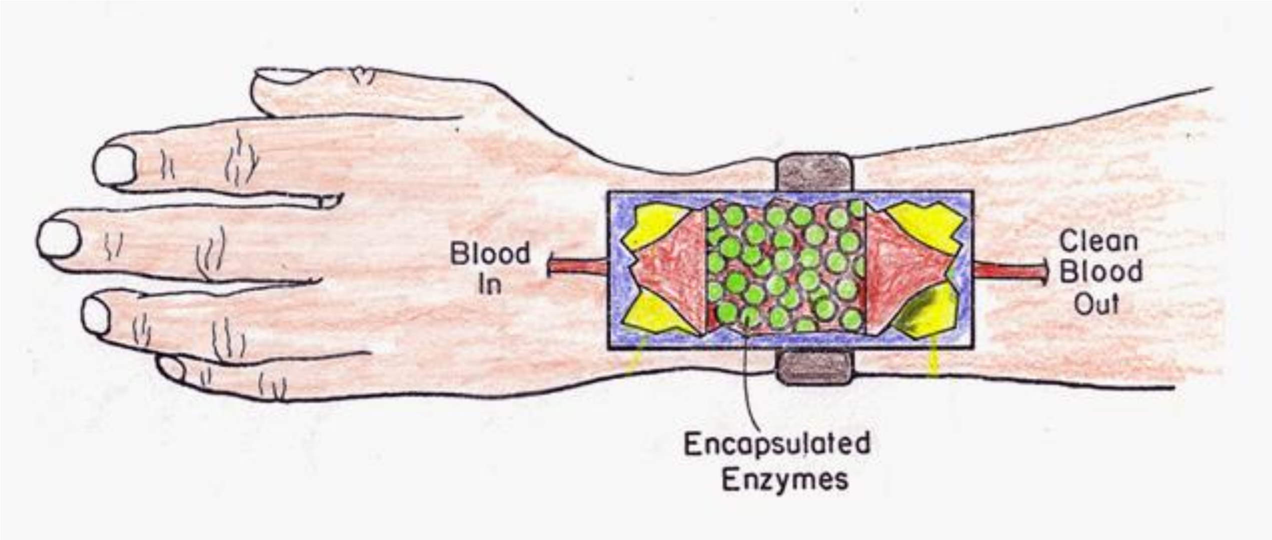
2. Enzymes IV

- **Artificial kidney**



$$NH_2\overset{\overset{O}{\|}}{C}NH_2 + UREASE \rightarrow \underbrace{[2NH_3 + CO_2]}_{P} + UREASE$$

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



2. Enzymes V

- **Artificial kidney 2**

- The corresponding mechanism

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

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

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

- Michaelis-Menten Equation

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

Leonor Michaelis
1875–1949

Maud Menten
1879–1960

2. Enzymes VI

- **Derive: Michaelis-Menten Kinetics**

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

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

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

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

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

$$r_p = k_3 (E \cdot S)(W) = \frac{\overbrace{k_3 W E_t}^{V_{max}} S}{\underbrace{\dfrac{k_2 + k_3 W}{k_1}}_{K_m} + S}$$

$$r_P = \frac{V_{max} S}{K_m + S}$$

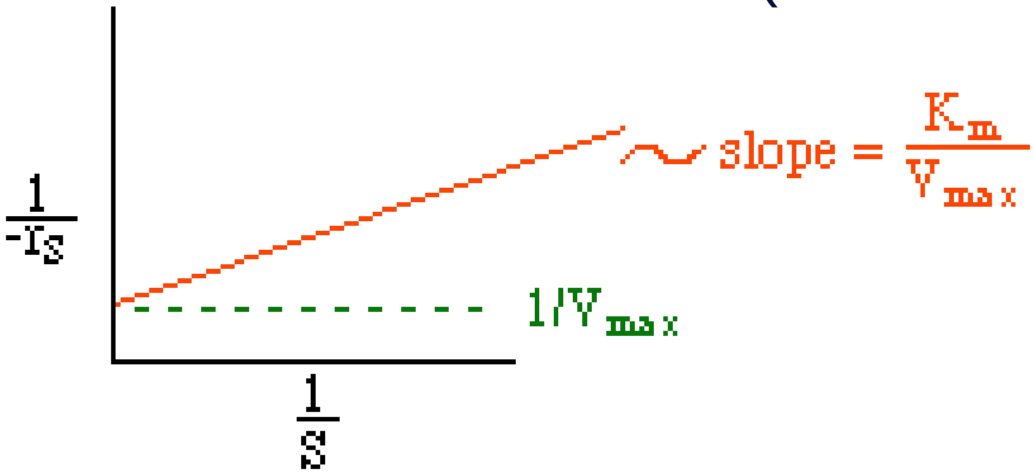
2. Enzymes VII

- **Michaelis-Menten Kinetics 2**

  - Inverting yields

$$\frac{1}{-r_s} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}}\left(\frac{1}{S}\right)$$

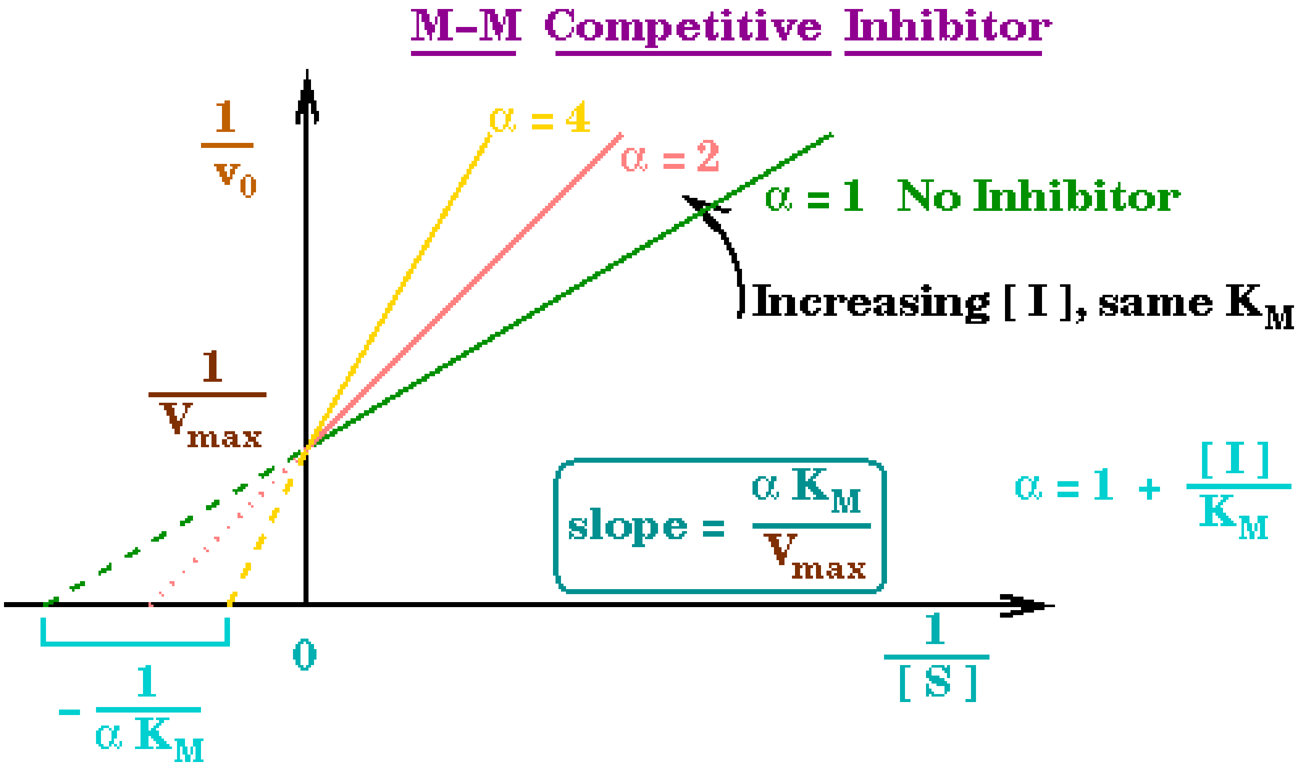
Lineweaver-Burk Plot (or double reciprocal plot)



2. Enzymes VIII

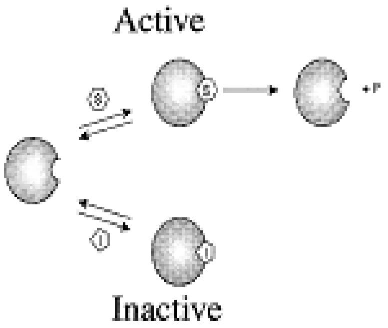
- **Michaelis-Menten Kinetics 3**

  - Lineweaver-Burk plot



Lineweaver–Burk Plot

2. Enzymes IX



○ Types of Enzyme Inhibition 1

- Competitive

Competitive
inhibition pathway

$$\begin{tikzcd}
E+S \arrow[r, shift left] & E\cdot S \arrow[l, shift left] \arrow[r] & E+P \\
+ & & \\
I \arrow[d, shift left, "K_I"] & & \\
E\cdot I \arrow[u, shift left] & &
\end{tikzcd}$$

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

2. Enzymes X

○ **Types of Enzyme Inhibition 2**

- Competitive 2 - Derive the rate

- **competitive inhibition**

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

$$E+I \underset{k_5}{\overset{k_4}{\rightleftarrows}} E \bullet I$$

- **rate laws** $r_P = -r_S = k_{cat}\left(E \bullet S\right)$

$$\text{From } r_{E \bullet S} = 0 = k_1\left(E\right)\left(S\right) - k_2\left(E \bullet S\right) - k_{cat}\left(E \bullet S\right)$$

2. Enzymes XI

- **Types of Enzyme Inhibition 3**

- Competitive 2 - Derive the rate

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

$$K_M = \frac{k_2 + k_{cat}}{k_1}$$

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

- From $r_{E \bullet I} = 0$

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

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

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

2. Enzymes XII

- **Types of Enzyme Inhibition 4**
  - **Competitive 2 - Derive the rate**
  - **Total enzyme**

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

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

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

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

2. Enzymes XIII

○ Types of Enzyme Inhibition 5

- Competitive 2 - Plot

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

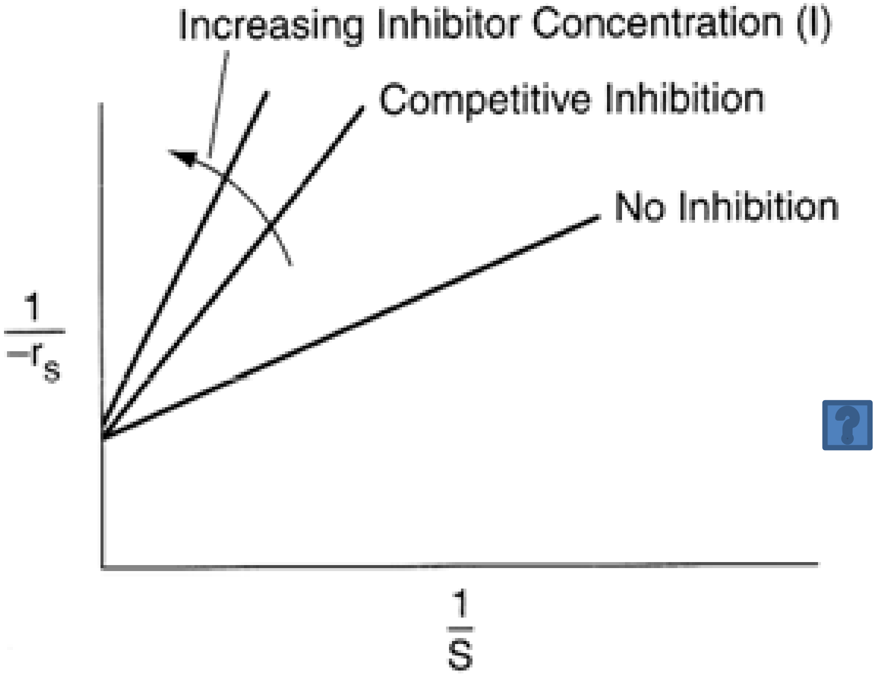
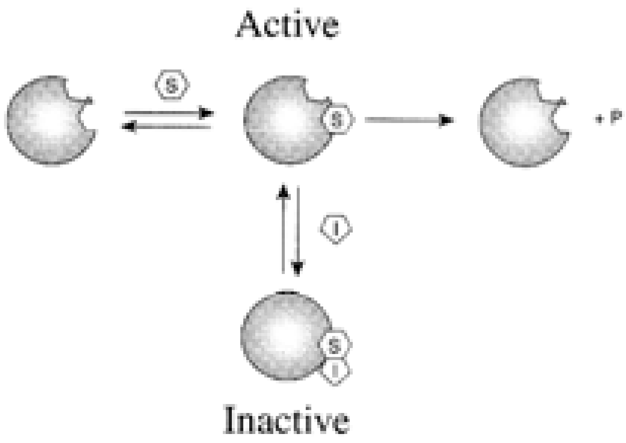
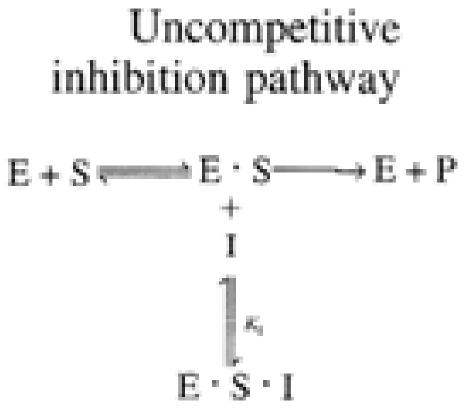


Figure 7-10 Lineweaver–Burk plot for competitive inhibition.

2. Enzymes XIV

- **Types of Enzyme Inhibition 6**
  - **Uncompetitive**





Reaction Steps

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

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

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

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

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

2. Enzymes XV

- **Types of Enzyme Inhibition 7**

  - Uncompetitive 2

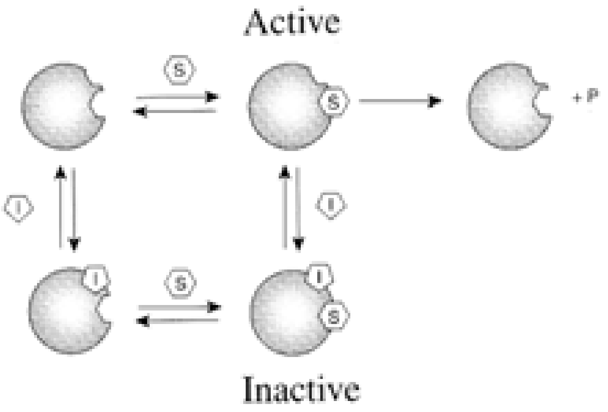
$$\boxed{-r_S = r_P = \frac{V_{max}(S)}{K_M + (S)\left(1 + \frac{(I)}{K_I}\right)}}$$

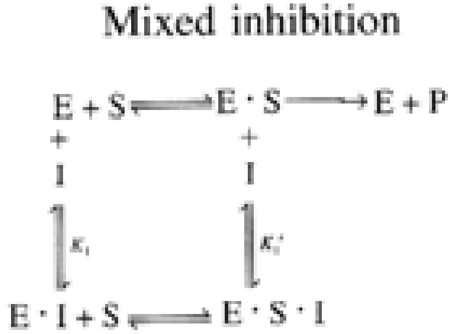
$$\frac{1}{-r_S} = \frac{1}{V_{max}(S)}\left(K_M + (S)\left(1 + \frac{(I)}{K_I}\right)\right)$$

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

2. Enzymes XVI

- **Types of Enzyme Inhibition 8**
  - **Mixed inhibition 1**




Mixed inhibition

Reaction Steps

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

$$(2)\quad E + I \rightleftharpoons I \bullet E \ \text{(inactive)}$$

$$(3)\quad I + E \bullet S \rightleftharpoons I \bullet E \bullet S \ \text{(inactive)}$$

$$(4)\quad S + I \bullet E \rightleftharpoons I \bullet E \bullet S \ \text{(inactive)}$$

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

2. Enzymes XVII

- **Types of Enzyme Inhibition 9**

  - Mixed inhibition 2

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

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

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