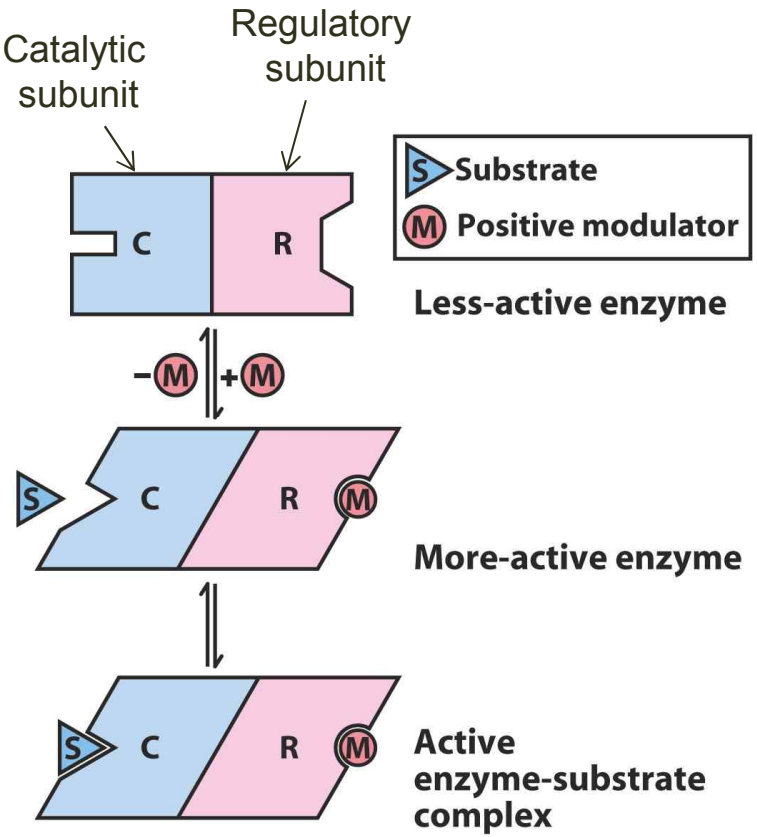
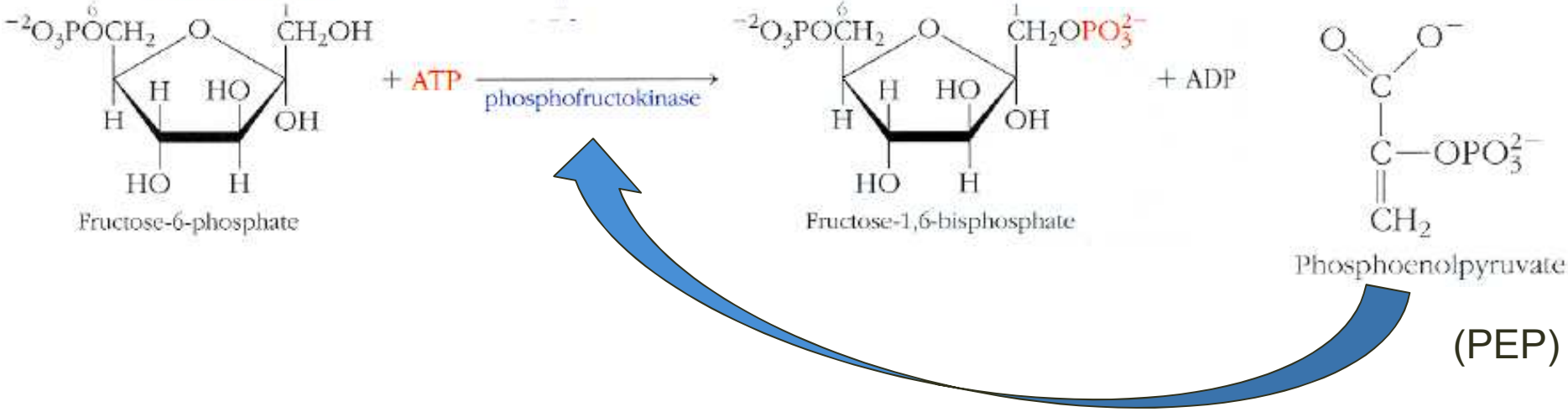
Allosteric Enzymes

- **Allosteric Enzymes**
  - Change in enzyme activity by conformational changed induced by modulator binding
  - Mostly multisubunit proteins
- **Modulators**
  - Activators or inhibitors
  - Binding to regulatory sites



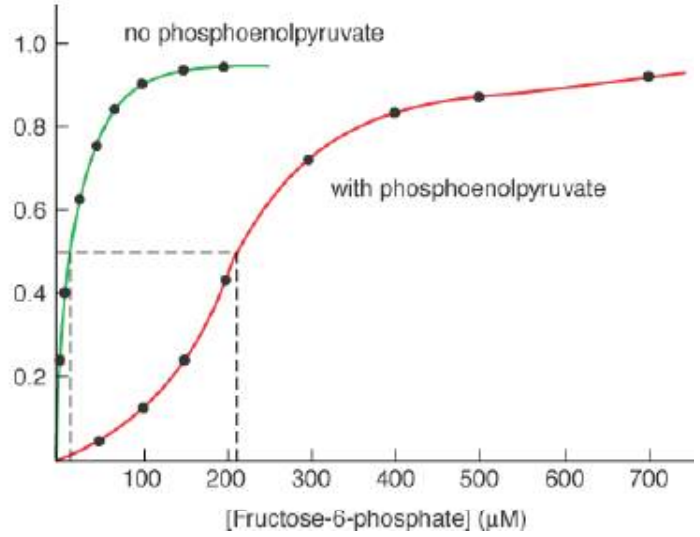
Allosteric inhibitor

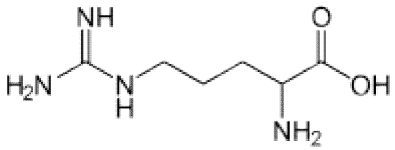
- Substrate binding to one active site may increase the activity of other sites, while inhibitor may decrease the activity of all the subunits
- Phosphofructokinase is inhibited by phosphoenolpyruvate (feedback inhibition)



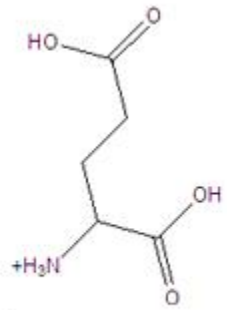
Allosteric inhibitor

- Without PEP, the enzyme shows hyperbolic kinetics with K_M of 23μM
- With 300μM of PEP, the rates become sigmoidal with K_M of 200μM
- PEP binding changes the conformation from green to red
- Arg(+) near F6P is replaced by Glu
- Glu repels the substrate

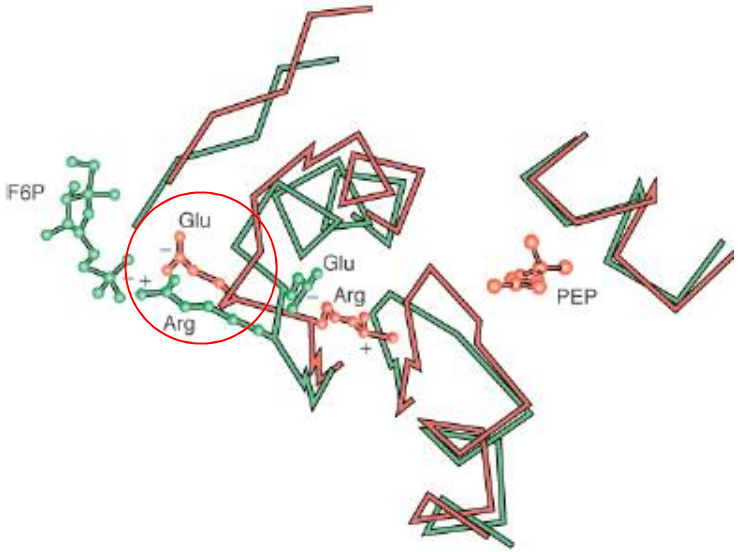




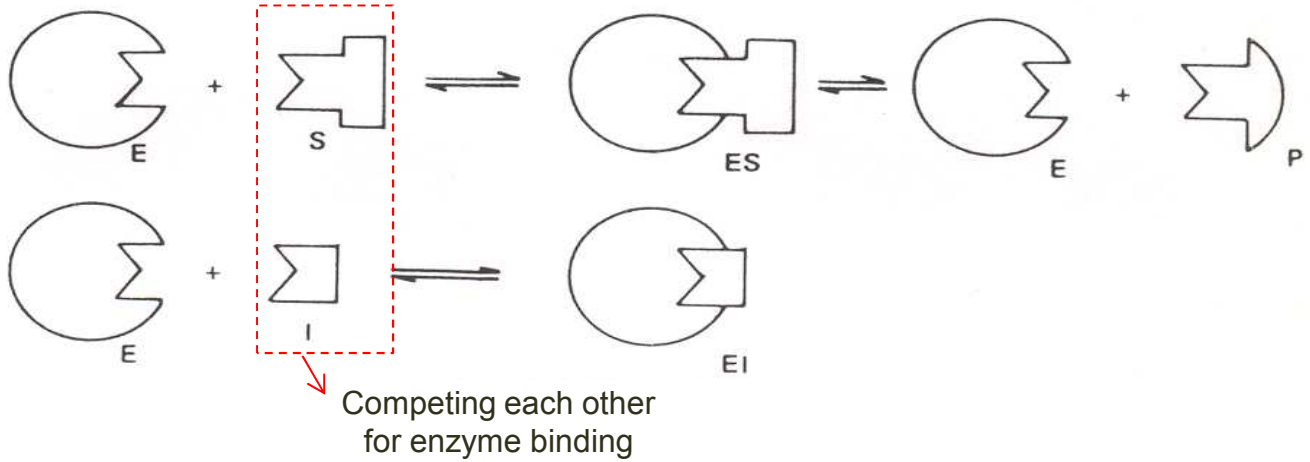
Arginine

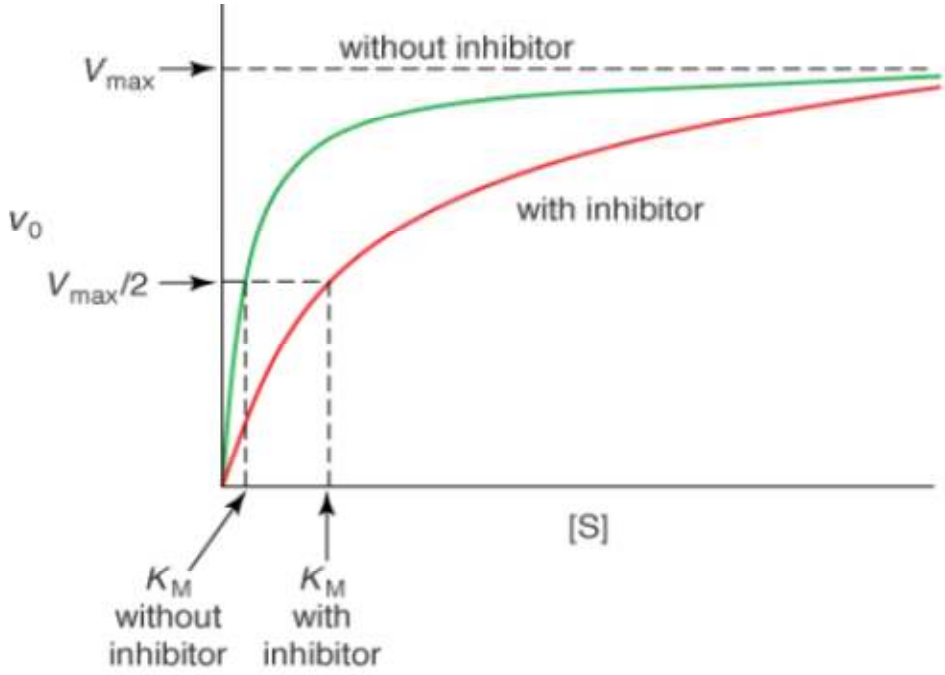


glutamic acid



Competitive inhibition





Increase in K_m
No change in V_m (why?)

Competitive inhibition

$$v = \frac{V_m [S]}{K_m \left[1 + \frac{[I]}{K_I}\right] + [S]}$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$$

\+

I → Inhibitor binds to active site on enzyme

$\updownarrow K_I$

EI → Enzyme / Inhibitor complex

- $K_{m,app}$ → *net effect of I is to increase K_m*

- *overcome inhibition by increasing [S]*

Competitive inhibition

Derive the equation for enzyme kinetics with competitive inhibition using pseudo-steady state hypothesis (PSSH).

$v = k_2[ES]$ .... (1)

$[ES] = ?$ use PSSH

$$\frac{d[\text{intermediate}]}{dt} = 0$$

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[\quad] = 0 \ (\text{PSSH})$$

$$\therefore [ES] = \frac{[E][S]}{K_m} \quad \cdots\cdots (2)$$

$$K_m = \frac{k_{-1} + k_2}{k_1}$$

(2) → (1) ;

$$v = \frac{k_2[E][S]}{K_m} \quad \cdots\cdots (3)$$

$[E] = f([S], [I], [E_0]) = ?$

$[E_0] = [E] + [ES] + [EI]$ .... (4)

(2) ?

$[EI] = ?$ use PSSH

$$\frac{d[EI]}{dt} = (\qquad) = 0$$

$$\therefore [EI] = \frac{[E][I]}{K_I} \quad \cdots\cdots (5)$$

$$K_I = \frac{k_{-3}}{k_3}$$

(2), (5) → (4) ;

$$\therefore [E] = \frac{K_I K_m [E_0]}{K_I K_m + K_I[S] + K_m[I]} \quad \cdots (6)$$

(6) → (3) ; $v = (\qquad)$

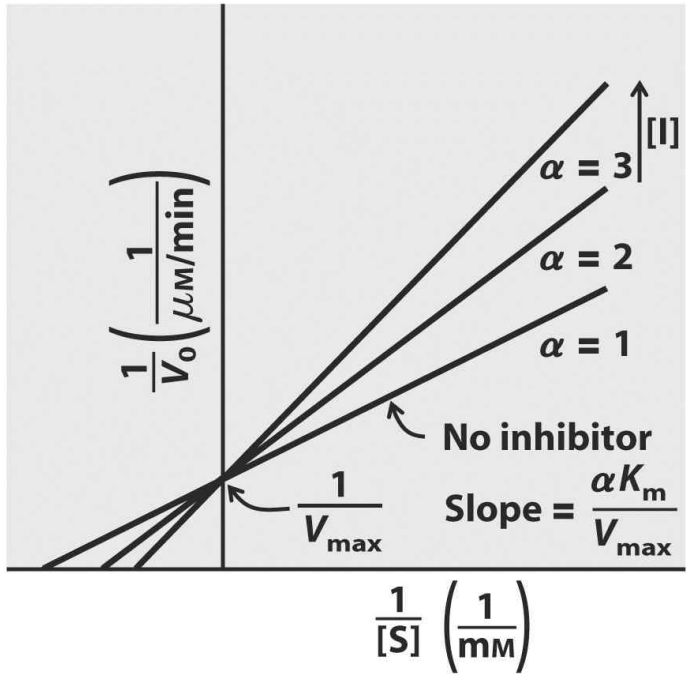
Competitive inhibition

- $V_o = V_{max}$ [S] / (αK_m + [S])
  - $\alpha = 1 + [I] / K_I$
  - $\alpha > 1$
  - $\alpha = 1$: no inhibition
- **Increase in apparent K_m by α** (decrease in substrate affinity)
- **No change in V_{max}**

Lineweaver-Burk Plot

rearrange $v = \frac{V_m[S]}{K_m + [S]}$ $\quad \boxed{\frac{1}{v} = \frac{1}{V_m} + \frac{K_m}{V_m}\frac{1}{[S]}}$

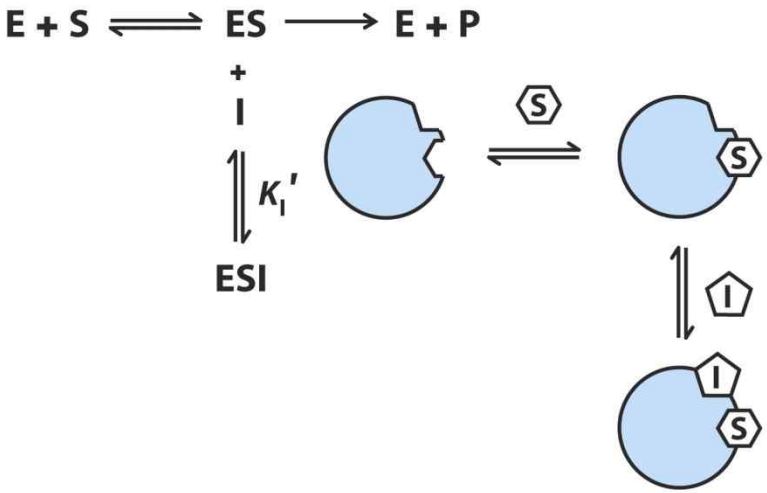
$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

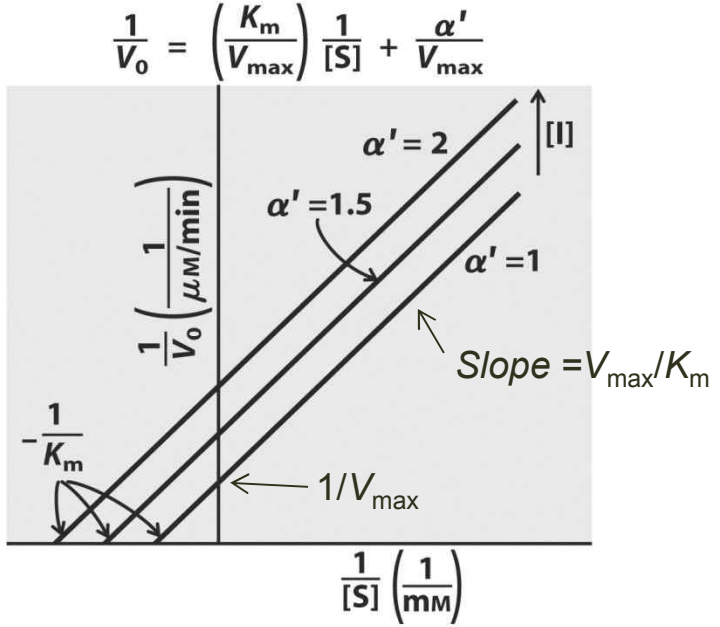


Uncompetitive inhibition

- Inhibitor binds only to ES complex at a site distinct from the active site
- Decrease in V_{max} ($\because V_{max} \rightarrow V_{max}/\alpha'$)
- Decrease in apparent K_m ($\because K_m \rightarrow K_m/\alpha'$)

$$V = \frac{V_{max}[S]}{K_m + \alpha'[S]} \qquad \alpha' = 1 + \frac{[I]}{K_I'} \qquad K_I' = \frac{[ES][I]}{[ESI]}$$





Uncompetitive inhibition

Derive the equation for enzyme kinetics with uncompetitive inhibition using equilibrium constants.

$$K_m' = \frac{[E][S]}{[ES]} \rightarrow [ES] = \frac{[E][S]}{K_m'} \quad \cdots (1)$$

$$K_I = \frac{[ES][I]}{[ESI]} \rightarrow [ESI] = \frac{[ES][I]}{K_I} \quad \cdots (2)$$

$$[E_o] = [E] + [ES] + [ESI]$$

$$= [E]\left(\qquad \cdot \qquad \right)$$

$$\therefore [E] = \frac{[E_o]}{1 + \frac{[S]}{K_m'} + \frac{[S][I]}{K_m' K_I}} \quad \cdots (3)$$

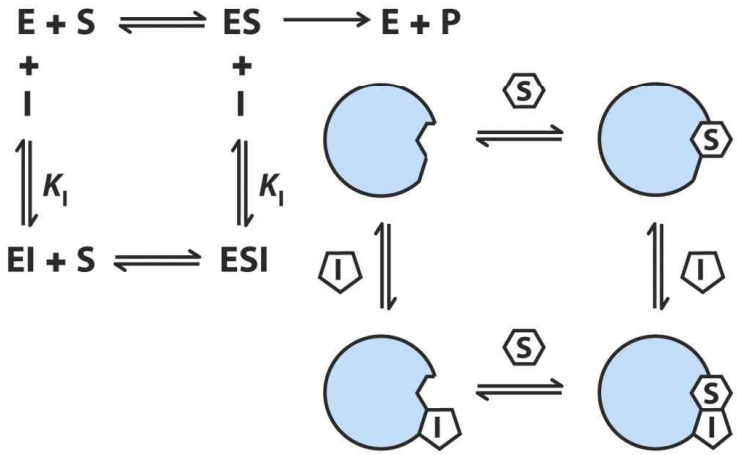
$$v = k_2[ES] \leftarrow (1)$$

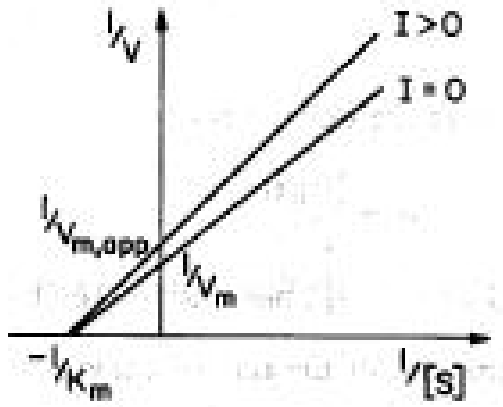
$$= \frac{k_2}{K_m'}[S][E] \leftarrow (3)$$

$$= \left(\qquad \right)$$

Noncompetitive inhibition

- Inhibitor binds to either E or ES at a site distinct from the active site
- Decrease in V_{max} by α
- $\alpha = 1 + [I] / K_I$
- No change in K_m : Inhibitor binding does not affect affinity of substrate to active site





$$v = \frac{V_m [S]}{\left[1 + \frac{[I]}{K_I}\right](K_m + [S])}$$

$$V_{m,app} = \frac{V_m}{\left[1 + \frac{[I]}{K_I}\right]}$$

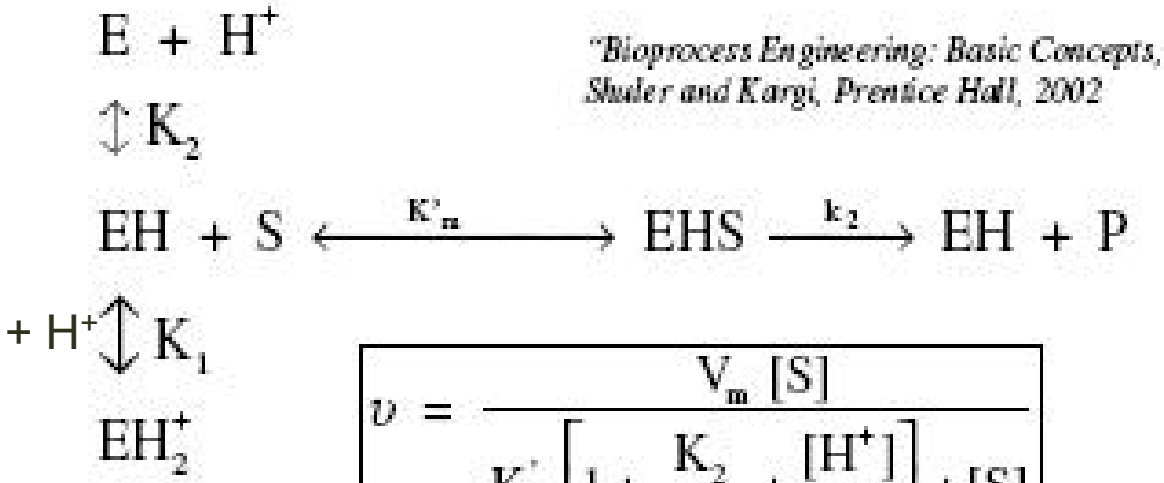
Noncompetitive inhibition

H.W.

Derive the equation for enzyme kinetics with noncompetitive inhibition using equilibrium constants.

Enzyme Activity Depends on pH

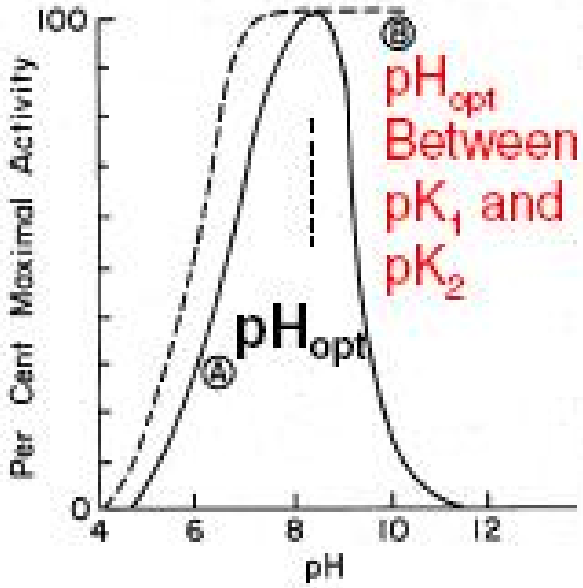
- **pH affects enzyme activity**
  - Charge of active site functional group is pH-dependent
  - Tertiary structure of enzyme is pH-dependent



"Bioprocess Engineering: Basic Concepts, Shuler and Kargi, Prentice Hall, 2002

$$v = \frac{V_m [S]}{K_m' \left[1 + \frac{K_2}{[H^+]} + \frac{[H^+]}{K_1}\right] + [S]}$$

$K_{m,app}' \rightarrow \infty$ at high and low $[H^+]$



pH = -log[H+]

How to derive this equation? Refer to textbook, page 75~76

Enzyme Activity Depends on Temperature

The rate of enzyme conversion of substrate will increase with temperature up to an optimum. Above this temperature, enzyme activity will decrease as enzyme denatures (Tertiary structure lost). Figure 3.15 shows a typical response.

"Bioprocess Engineering: Basic Concepts, Shuler and Kargi, Prentice Hall, 2002

Temperature activation

$V_m = k_2 [E_o]$, $T < T_{opt}$

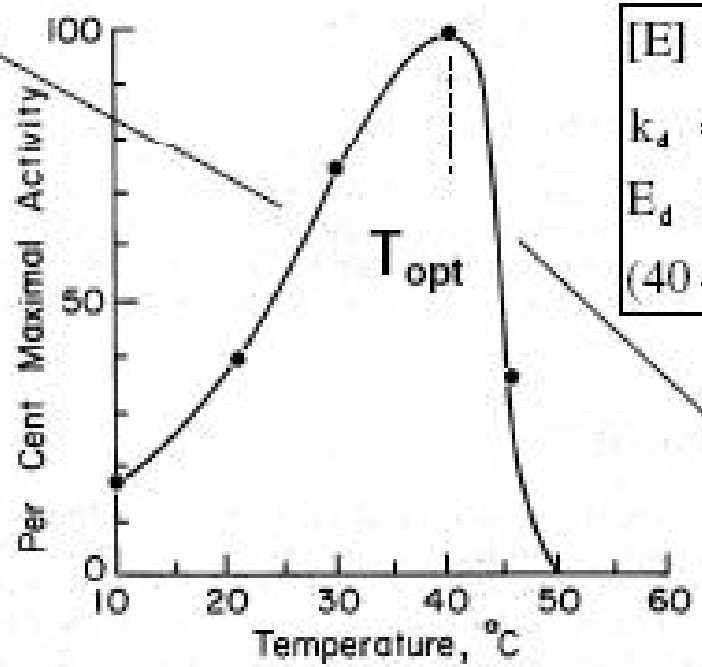
$= k_2 [E]$, $T > T_{opt}$

[E] is concentration of active enzyme

$k_2 = A\, e^{-E_a/RT}$

E_a = activation energy

(4 - 20 kcal / mole)



$[E] = [E_o]\, e^{-k_d t}$

$k_d = A_d\, e^{-E_d/RT}$

E_d = deactivation energy

(40 - 130 kcal / mole)

Temperature deactivation