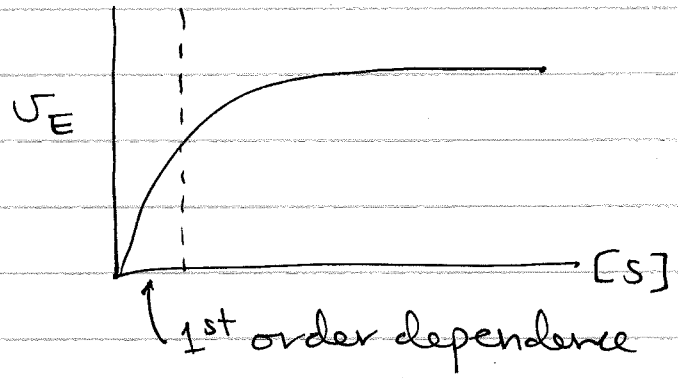
Enzymes are affected by pH & temperature.

There is a pH & temperature optimum.

Recollect:



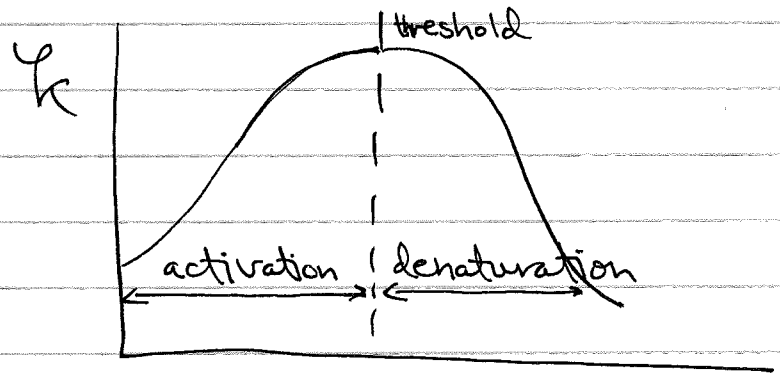
∴ when [S] is very small,

$$v_E = \frac{v_{max}[S]}{K_M + [S]} \approx k[S]$$

(1) Temp.: ∴ $v_E = k[S]$

∴ At T_1, $v_{E_1} = k_1[S]$

T_2, $v_{E_2} = k_2[S]$ & so on.



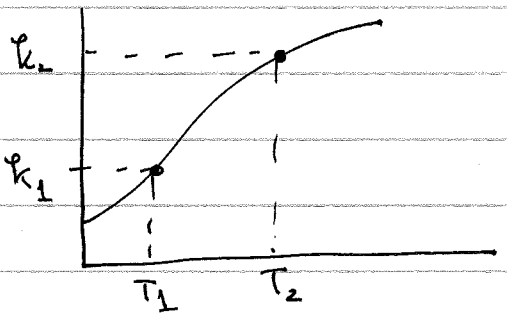
{This is what is observed}

Until the $T_{threshold}$ is reached:

$$k_1 = A \exp\left(\frac{-E_a}{RT_1}\right)$$

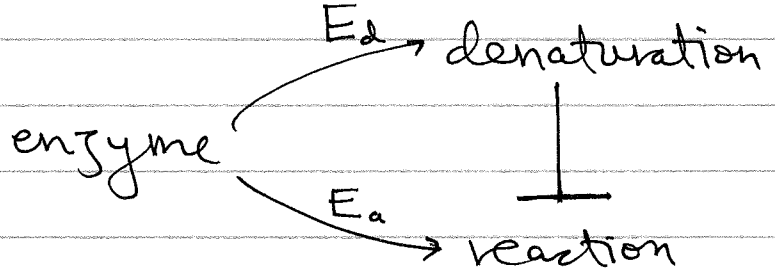
$$k_2 = A \exp\left(\frac{-E_a}{RT_2}\right)$$

i.e. $$\frac{k_1}{k_2} = \exp\left(\frac{E_a}{RT_2} - \frac{E_a}{RT_1}\right)$$



Follows the Arrhenius Law.

After $T_{threshold}$, there are two competing processes:

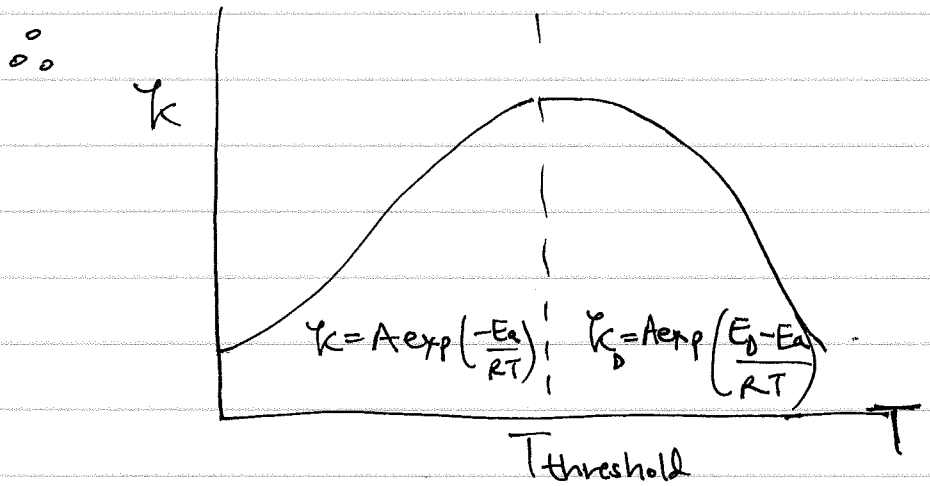


∴ The Arrhenius equation changes.

deactivation energy

$$k_D = A \exp\left(\frac{E_D - E_a}{RT}\right)$$

after $T > T_{threshold}$



(2) Effect of pH

$$E^- + H^+ \quad (K_2)$$

$$EH + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EHS \xrightarrow{k_3} EH + P$$

$$+H^+ \quad (K_1)$$

$$EH_2^+$$

K_1 & K_2 are equilibrium constants

When one writes equilibrium constants in enzymology, it is in terms of dissociation constants.

$$\therefore \frac{d[EHS]}{dt} = 0 \quad \text{.... PSSA}$$

$$\therefore [EHS] = \left(\frac{k_1}{k_2 + k_3}\right)[EH][S]$$

But $K_{Dissoc.} = \dfrac{[EH][S]}{[EHS]}$ ..... K_M is a dissociation eqb. const.!

$$\therefore [EHS] = \frac{1}{K_M}[EH][S]$$

Similarly, $[EH_2^+] = \dfrac{[EH][H^+]}{k_1}$

$$[E^-] = \frac{K_2[EH]}{[H^+]}$$

} Acid-base equilibria is PSSA too!

Also, $[E]_0 = [EH] + [EHS] + [E^-] + [EH_2^+]$

$\therefore$ After making the relevant subst., one gets:

$$[E]_o = [EH] + \frac{1}{K_M}[EH][S] + \frac{K_2[EH]}{[H^+]} + \frac{1}{K_1}[EH][H^+]$$

* We are trying to rewrite this in measurable terms

→ $[E]_o$, $[S]$ & $[H^+]$

→ $\underline{-\log_{10}[H^+] = pH}$

$$\therefore [E]_o = [EH]\left\{1 + \frac{[S]}{K_M} + \frac{K_2}{[H^+]} + \frac{[H^+]}{K_1}\right\}$$

$$\therefore [EH] = \frac{[E]_o}{1 + \frac{[S]}{K_M} + \frac{K_2}{[H^+]} + \frac{[H^+]}{K_1}}$$

$$\therefore v_E = K_3[EHS] = \frac{K_3}{K_M}[EH][S]$$

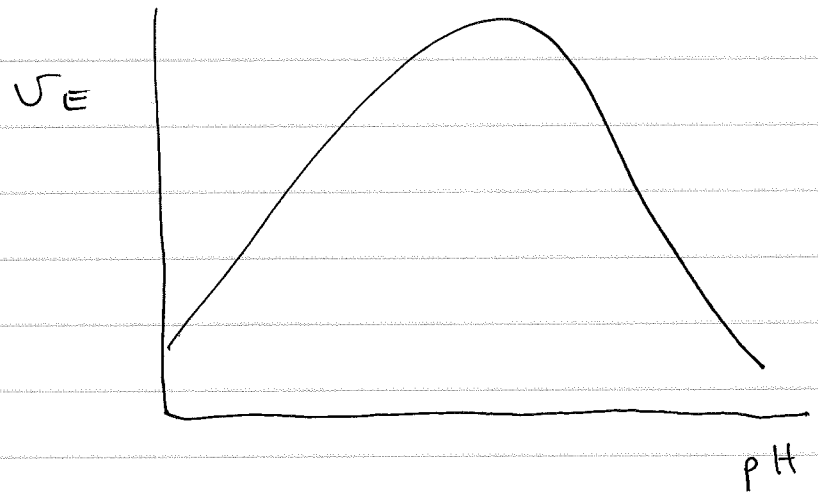
$$\therefore v_E = \frac{K_3[S]}{K_M}\left\{\frac{[E_o]}{1 + \frac{[S]}{K_M} + \frac{K_2}{[H^+]} + \frac{[H^+]}{K_1}}\right\}$$

$$\therefore v_E = \frac{K_3[E]_o[S]}{\left\{K_M + [S] + \frac{K_2 K_M}{[H^+]} + \frac{K_M[H^+]}{K_1}\right\}}$$

$$\therefore\ v_E = \frac{\overbrace{k_3[E]_o}^{v_{max}}[S]}{\underbrace{K_M\left\{1 + \frac{k_2}{[H^+]} + \frac{[H^+]}{K_1}\right\}}_{K_{M,pH}} + [S]}$$

$$\therefore\ v_E = \frac{v_{max}[S]}{K_{M,pH} + [S]}$$

→ In this form as simple Michaelis-Menten kinetics



(3) Substrate allostery:

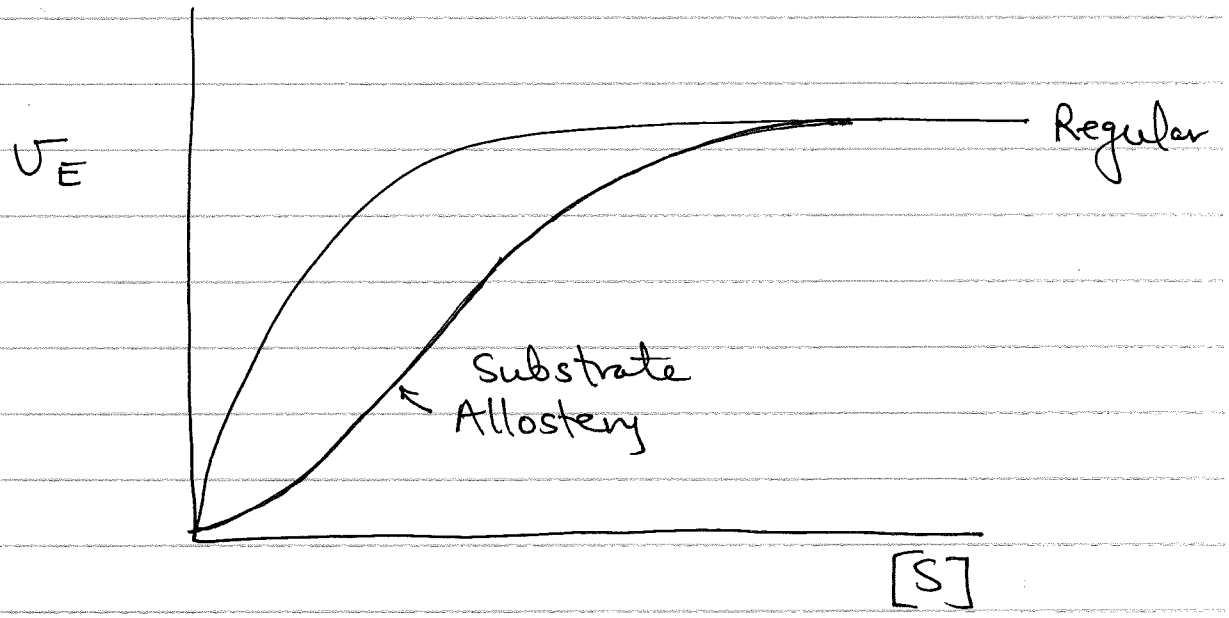
Some enzymes have more than 1 substrate binding site.

* These enzymes exhibit <u>cooperativity</u>.

What is cooperativity? Substrate binding causes acceleration / improves propensity of enzyme to bind to more substrate.

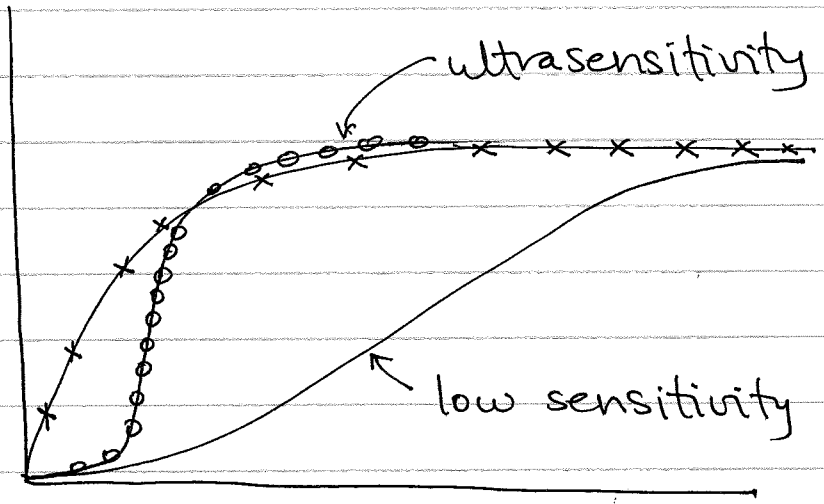
$$\therefore \quad v_E = \frac{v_{max}[S]^h}{K_M'' + [S]^h}$$

↑ expression different from regular Michaelis-Menten kinetics



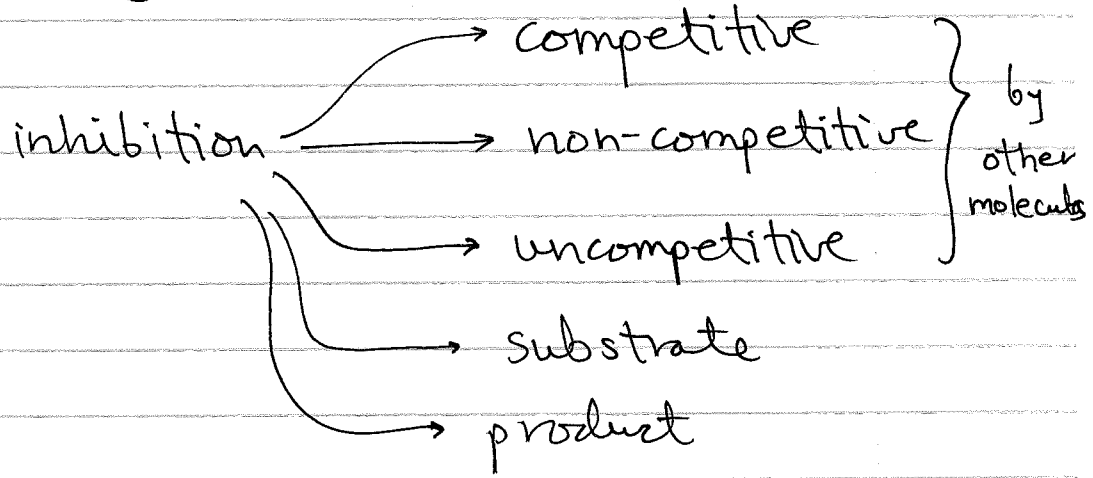
Substrate allostery = sigmoidal curve.

Where is allostery critical?



One sees such phenomena in geneswitching, action of some toxins etc.

(4) Enzyme inhibition :



General protocol in deriving v_E :

(1) write $\frac{d[P]}{dt} = v_E =$ conc. terms.

(2) make PSSA for transient / equilibrium species

eg. $[ES]$, $[EHS]$, $[E^-]$ etc.

→ Acid-base eqb. is PSSA too!

(3) write conc. balance for $[E]_0$

(4) eliminate unmeasurable / PSSA terms in v_E & replace with measurable terms

eg. $[E]_0$, $[S]$, $[H^+]$ etc.

Competitive inhibition : very common in phamaceutical industry

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

$$+ \; I$$

$$K_I \downarrow\uparrow \; [EI]$$

When we write K, these are dissociation constants

↑ substrate analogue

* direct competition between S & I for E.

$$K_I = \frac{[E][I]}{[EI]} \quad \ldots\ldots \text{PSSA for } EI$$

Let's follow the algorithm:

$$\frac{d[P]}{dt} = k_3[ES] = v_E$$

② PSSA $\Rightarrow$ [ES] & [EI]

$$\therefore [ES] = \frac{k_1}{k_2+k_3}[E][S] = \frac{1}{K_M}[E][S]$$

③ But remember, enzyme balance:

$$[E_0] = [E] + [ES] + [EI]$$

$$\therefore [E]_0 = [E] + \frac{1}{K_M}[E][S] + \frac{1}{K_I}[E][I]$$

$$\therefore [E] = \frac{[E]_0}{1 + \frac{[S]}{K_M} + \frac{[I]}{K_I}}$$

$$\therefore \quad v_E = k_3[ES] = \frac{k_3}{K_M}[E][S]$$

(4) $$\therefore \quad v_E = \frac{k_3}{K_M}[S]\left\{\frac{[E]_o}{1+\frac{[S]}{K_M}+\frac{[I]}{K_I}}\right\}$$

$$\therefore \quad v_E = \frac{k_3[E]_o[S]}{K_M + \frac{K_M}{K_I}[I] + [S]}$$

$$\therefore \quad v_E = \frac{v_{max}[S]}{\left\{K_M + \frac{K_M}{K_I}[I]\right\} + [S]}$$

Let's make a Lineweaver-Burke plot

$$\therefore \quad \text{Let} \quad K_M + \frac{K_M}{K_I}[I] = \alpha$$

$$\Rightarrow v_E = \frac{v_{max}[S]}{\alpha + [S]}$$

$$\therefore \quad \alpha + [S] = \frac{v_{max}[S]}{v_E}$$

$$\therefore \quad \frac{\alpha + [S]}{v_{max}[S]} = \frac{1}{v_E}$$

$$\therefore \quad \frac{1}{v_E} = \frac{1}{v_{max}} + \left(\frac{\alpha}{v_{max}}\right)\frac{1}{[S]}$$

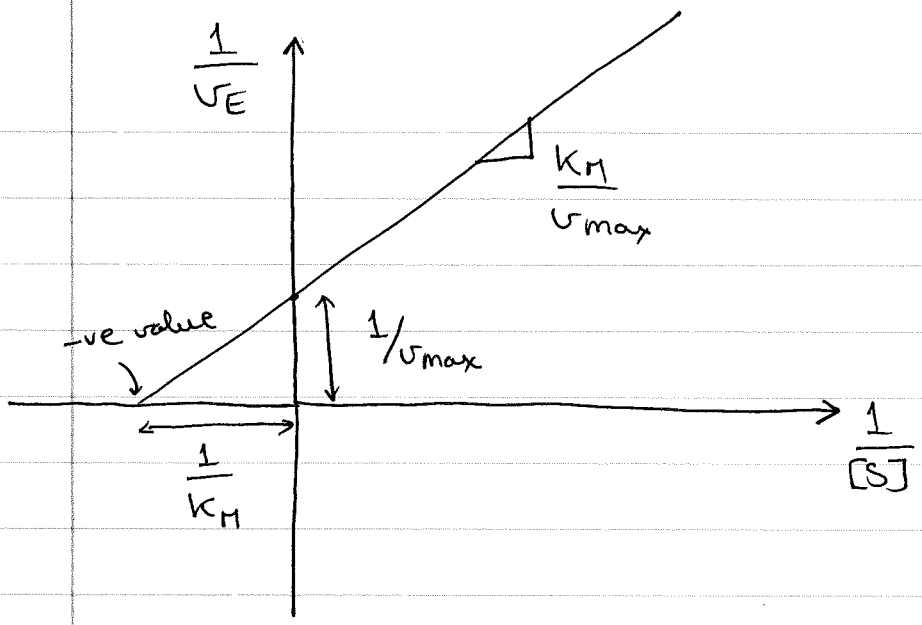
Remember: The normal Lineweaver Burke plot is:

$$\frac{1}{v_E} = \frac{1}{v_{max}} + \left(\frac{K_M}{v_{max}}\right)\frac{1}{[S]}$$

When $\underbrace{\frac{1}{v_E} = 0}_{\text{x-intercept}}$, $\quad \frac{-1}{v_{max}} = \left(\frac{K_M}{v_{max}}\right)\frac{1}{[S]}$

$$\therefore \quad \frac{1}{[S]} = \frac{-1}{K_M}$$ ← this is the x-intercept

The y-intercept is $\frac{1}{v_{max}}$



Normal Lineweaver Burke plot

For inhibition, $\frac{1}{v_E} = \frac{1}{v_{max}} + \left(\frac{\alpha}{V_{max}}\right)\frac{1}{[S]}$

slope changes

y-intercept unchanged

The slope is different.

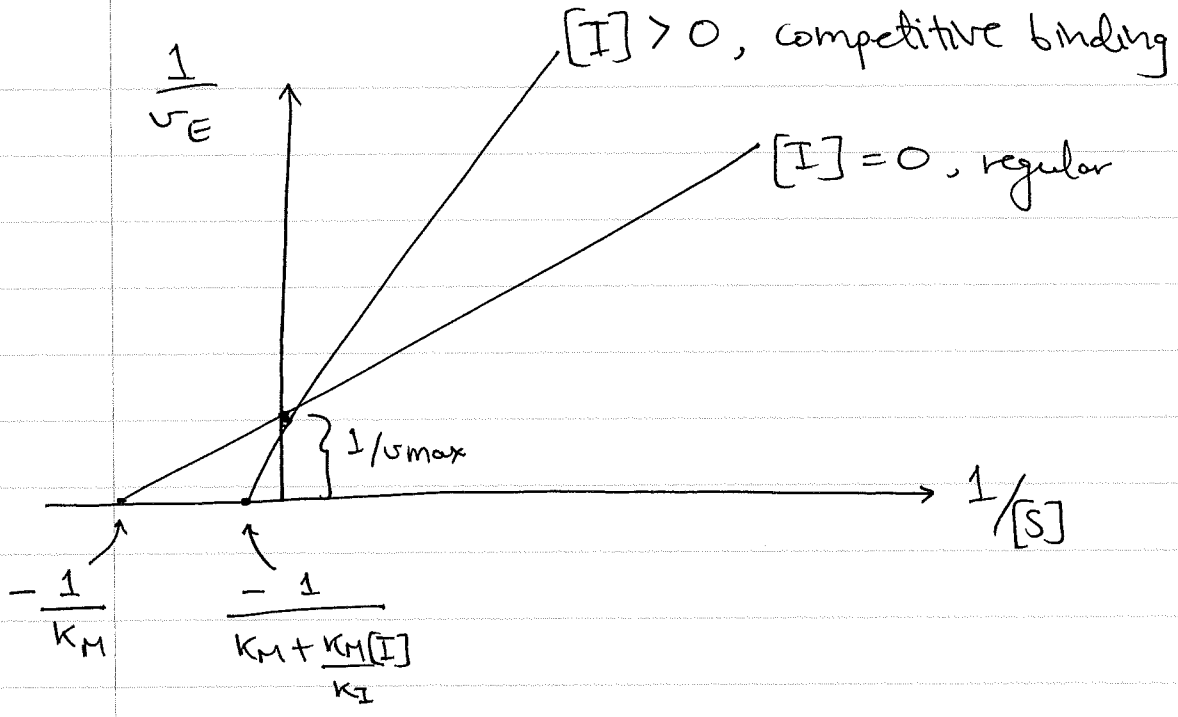
$$\text{slope} = \frac{\alpha}{v_{max}} = \frac{K_M + \frac{K_M}{K_I}(I)}{v_{max}}$$

You can see that: $\boxed{K_M + \frac{K_M}{K_I}(I) > K_M}$

The slope is greater. This also means that $\frac{1}{K_M + \frac{K_M}{K_I}(I)}$ is smaller.

x-intercept

$\therefore\ \frac{-1}{K_M + \frac{K_M}{K_I}(I)}$ is larger than $\frac{-1}{K_M}$



Case 2: non-competitive binding

$$E + S \underset{}{\overset{K_M'}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

$$\begin{array}{ccc} + & & + \\ I & & I \\ K_I \Big\updownarrow & & \Big\updownarrow K_I \\ EI + S & \rightleftharpoons & ESI \end{array}$$

K_M'

* Notice how K_I & K_M' repeat

* Non-competitive inhibition involves binding of inhibitor to enzyme but <u>not</u> in active site.

K_I represents binding between I & this distal site.

Similarly, K_M' describes binding between active site & substrate

Using equilibrium, we get:

$$K_M' = \frac{[E][S]}{[ES]} = \frac{[EI][S]}{[EIS]}$$

.... K_M' is the same as traditional MM const.

$$K_I = \frac{[E][I]}{[EI]} = \frac{[ES][I]}{[ESI]}$$

As before, $v_E = k_3[ES]$

& $[E]_0 = [E] + [ES] + [ESI] + [EI]$

When we make the relevant substitutions we get

$$\boxed{v_E = \frac{v_{max}[S]}{\left(1 + \frac{[I]}{K_I}\right)\left([S] + K_M'\right)}}$$

The Lineweaver-Burke plot becomes:

$$\left(1 + \frac{[I]}{K_I}\right)\left([S] + K_M'\right) = \frac{v_{max}[S]}{v_E}$$

$$\therefore \frac{1}{v_{max}}\left\{1 + \frac{[I]}{K_I}\right\}\left\{1 + \frac{K_M'}{[S]}\right\} = \frac{1}{v_E}$$

$$\therefore \frac{1}{v_E} = \underbrace{\frac{1}{v_{max}}\left\{1 + \frac{[I]}{K_I}\right\}}_{\text{y-intercept}} + \underbrace{\frac{K_M'}{v_{max}}\left\{1 + \frac{[I]}{K_I}\right\}}_{\text{slope}} \frac{1}{[S]}$$

What about the x-intercept?

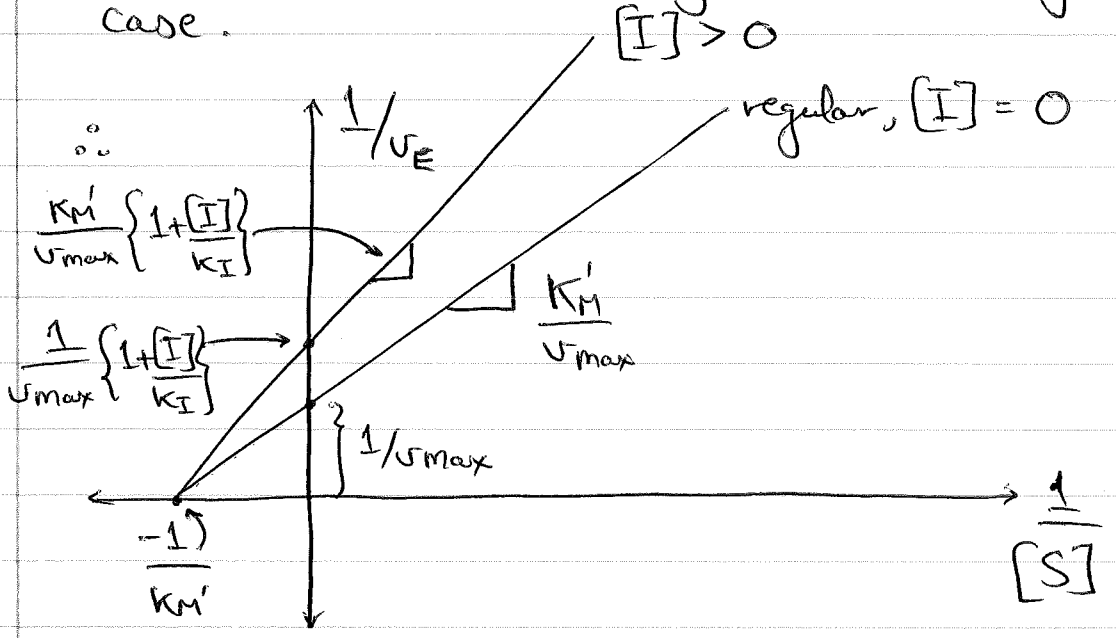
Setting $\frac{1}{v_E} = 0$, we get:

$$\left(\frac{1}{[S]}\right)\left[\frac{K_M'}{v_{max}}\left\{1 + \frac{[I]}{K_I}\right\}\right] = -\frac{1}{v_{max}}\left\{1 + \frac{[I]}{K_I}\right\}$$

$$\therefore \frac{1}{[S]} = -\frac{1}{K_M'} \quad \text{.... same as regular case}$$

$$\therefore \text{x-intercept} = -\frac{1}{K_M'}$$

y-intercept & slope are greater than regular case.



Case 3: Uncompetitive inhibition

$$E + S \underset{}{\overset{K_M'}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

$$ES + I \rightleftharpoons ESI \quad (K_I)$$

Here, substrate binding might induce a conformation change in the enzyme that exposes a previously concealed distal site for inhibitor binding.

$$K_M' = \frac{[E][S]}{[ES]} \quad \& \quad K_I = \frac{[ES][I]}{[ESI]}$$

Also, $[E]_0 = [E] + [ES] + [ESI]$

$$v_E = k_3 [ES]$$

Following our 4-step algorithm, we get:

$$\boxed{v_E = \frac{v_{max}[S]}{K_M' + [S]\left\{1 + \frac{[I]}{K_I}\right\}}}$$

The Lineweaver-Burke plot is:

$$\frac{1}{v_{max}[S]}\left\{K_M' + [S] + \frac{[S][I]}{K_I}\right\} = \frac{1}{v_E}$$

$$\therefore \quad \frac{1}{v_E} = \frac{K_M'}{v_{max}}\left(\frac{1}{[S]}\right) + \frac{1}{v_{max}} + \frac{[I]}{v_{max} K_I}$$

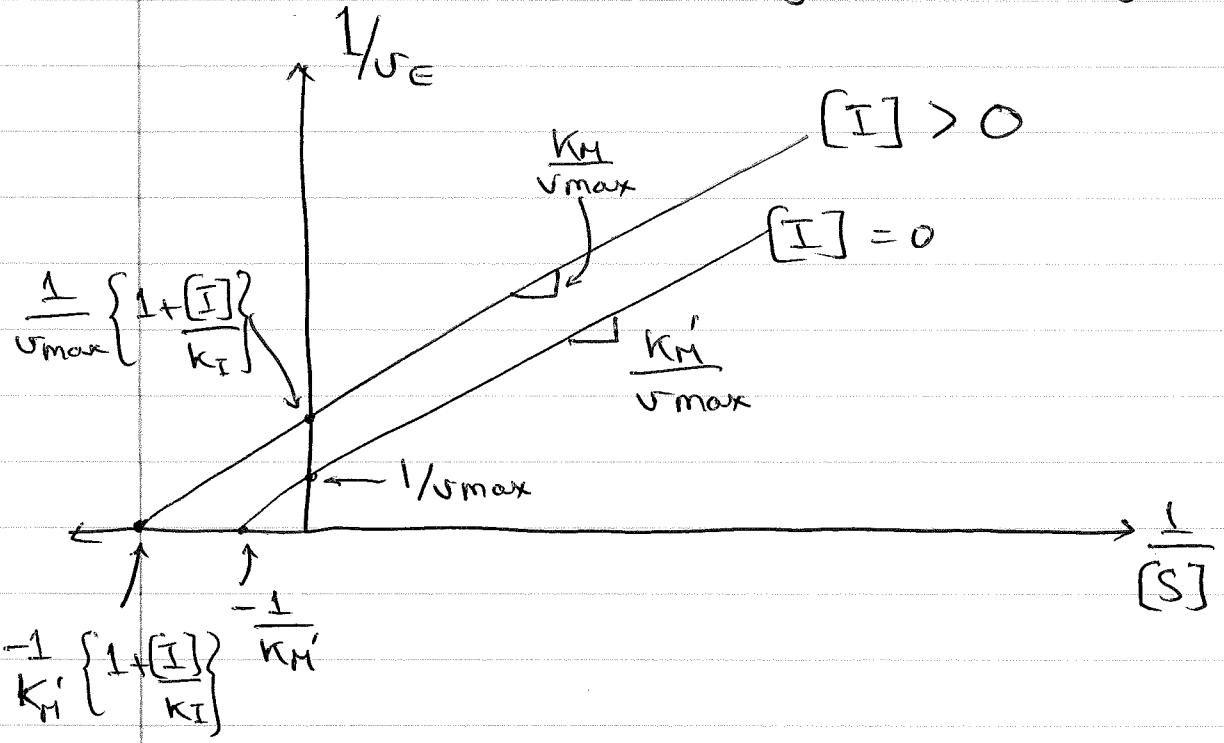
$$\therefore \frac{1}{v_E} = \frac{K_M'}{v_{max}}\left(\frac{1}{[S]}\right) + \underbrace{\frac{1}{v_{max}}\left\{1 + \frac{[I]}{K_I}\right\}}_{\text{y-intercept}}$$

(larger than regular MM)

* slope is unchanged (parallel lines)

* x-intercept: $[S] = \frac{-1}{K_M'}\left\{1 + \frac{[I]}{K_I}\right\}$

more negative than regular case



Case 4: Substrate inhibition

$$E + S \underset{}{\overset{K_M'}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

$$+S \Big\uparrow\downarrow K_{SI}$$

$$ES_2$$

$$K_M' = \frac{[E][S]}{[ES]} \quad , \quad K_{SI} = \frac{[ES][S]}{[ES_2]}$$

& $[E]_0 = [E] + [ES] + [ES_2]$

$$\therefore \boxed{v_E = \frac{v_{max}[S]}{K_M' + [S] + \frac{[S]^2}{K_{SI}}}}$$

$\Rightarrow$ When $[S]$ is very small:

$$v_E \approx \frac{v_{max}}{K_M'}[S]$$

$$\therefore \frac{1}{v_E} \approx \frac{K_M'}{v_{max}}\left(\frac{1}{[S]}\right)$$

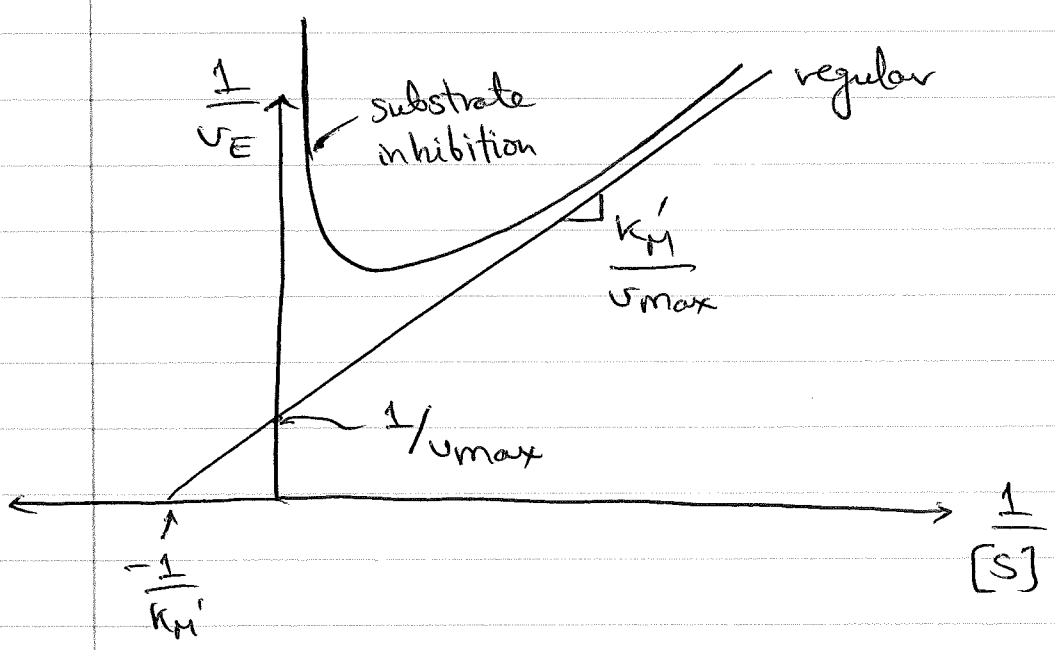
$\Rightarrow$ when [S] is very large:

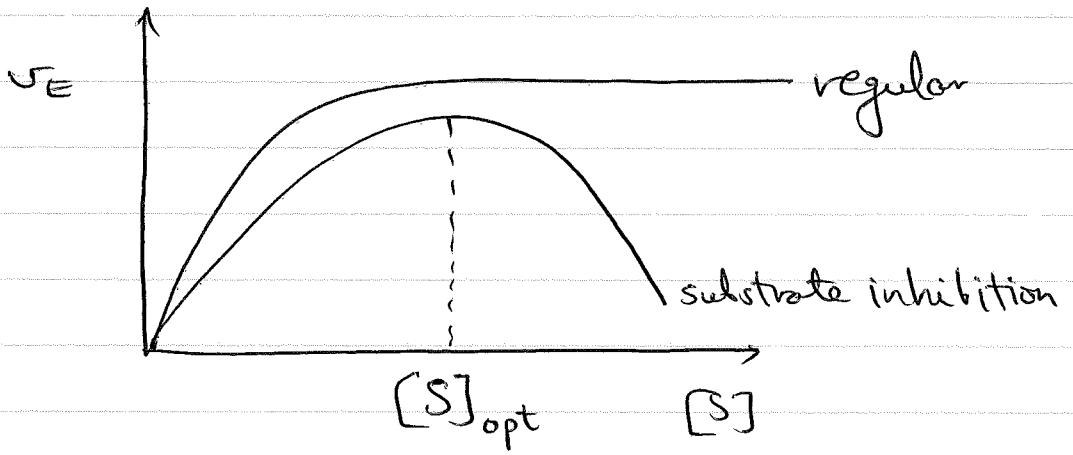
$$v_E \approx \frac{v_{max}[S]}{[S]^2/K_{SI}}$$

$$\therefore \quad v_E \approx \frac{K_{SI} v_{max}}{[S]} \quad \cdots\cdots \quad v_E \to 0$$

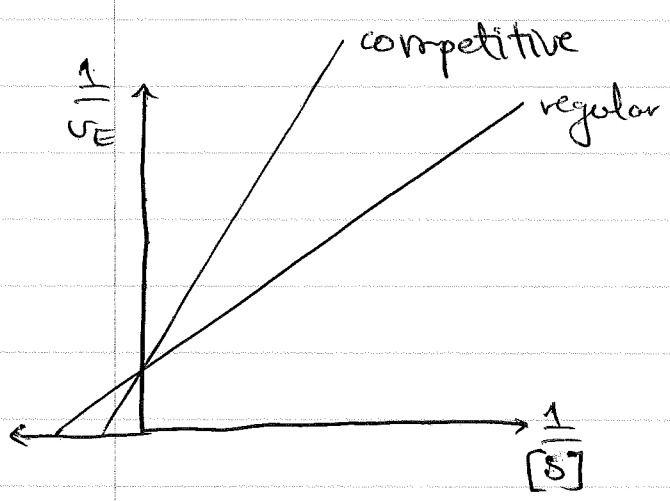
$$\therefore \quad \frac{1}{v_E} \approx \left\{\frac{K_{SI} v_{max}}{[S]}\right\}^{-1}$$

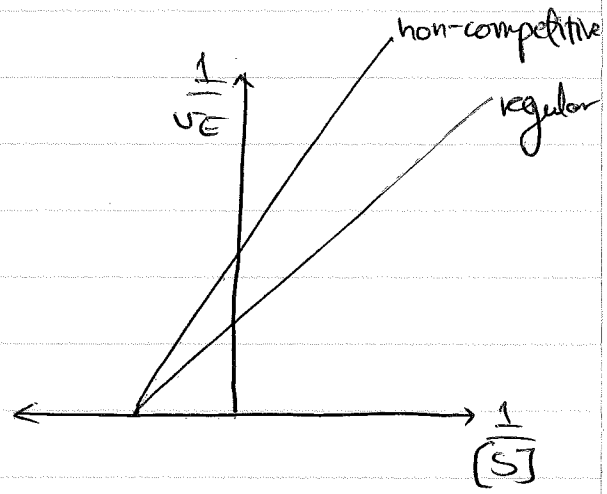
$$\therefore \quad \frac{1}{v_E} \approx \frac{[S]}{K_{SI} v_{max}} \quad \cdots\cdots \quad \frac{1}{v_E} \to \infty$$

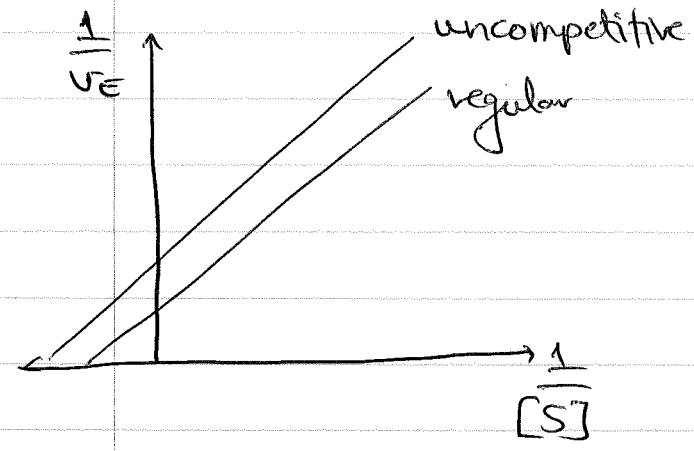


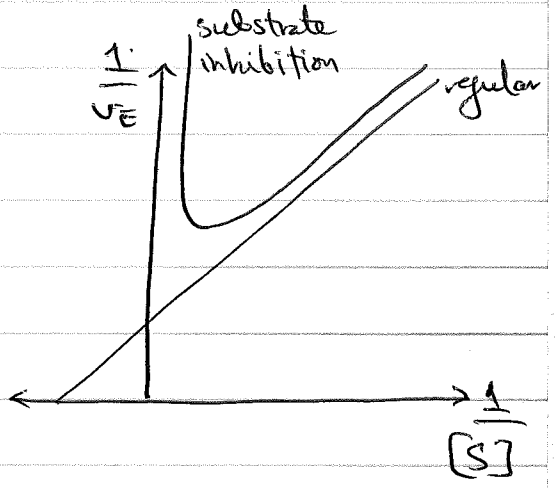


Summary: Inhibition plots









Mass transfer

* also arises in enzyme kinetics
* most common in enzyme immobilization