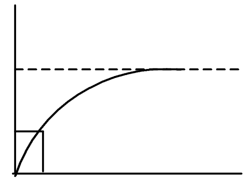
Lectures 5 and 6
Inhibition Systems and Derivation of their Rate Equations

1) $v = \frac{Vm[S]}{S + Km} =$ Vmax v



v vs. [S] - Hyperbolic

2) Lineweaver Burk $\frac{1}{v}$ vs $\frac{1}{[S]}$ **Linear Plot or Double Reciprocal Plot**

Basic Equation

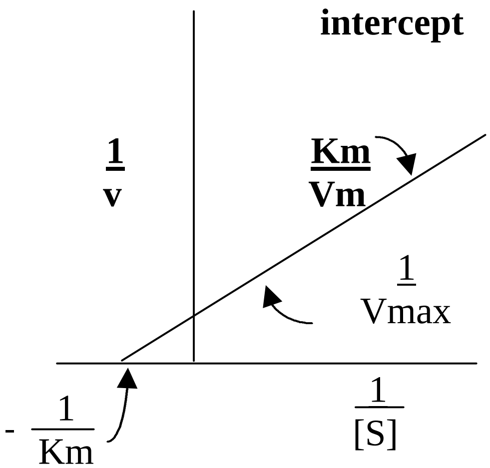
$$V = \frac{Vm[S]}{[S] + Km}$$

(TAKE RECIPROCAL)

$$\frac{1}{v} = \frac{1}{Vmax} + \frac{Km}{Vm}\,\frac{1}{[S]}$$

y = b + m X

intercept (b), **slope** (m)



@ [S] → ∞, $\frac{1}{[S]}$ → **O,** $\left(\frac{Km}{Vm} \times \frac{1}{[S]}\right) = O$

∴ **Y intercept =** $\frac{1}{Vmax}$

@ $\frac{1}{v}$ = O

$$\frac{1}{Vm} = \frac{Km}{Vm}\;\frac{1}{S}$$

Cross Multiply

$$-\frac{1}{Km} = \frac{1}{S}$$

EADIE-HOFSTEE PLOTS - v vs $\frac{v}{[S]}$ **(Gives Km & Vmax directly)**

$$V = \frac{Vm[S]}{[S]+Km}$$

$$\frac{1}{v} = \frac{1}{Vm} + \frac{Km}{Vm}\ \underline{1}$$

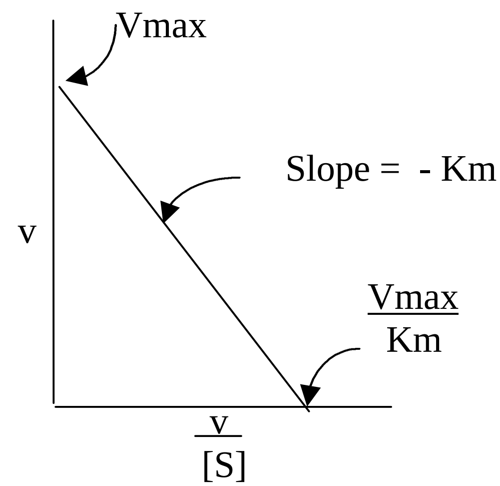
Multiply by Vmax • v

$$Vm = v + Km\ \frac{v}{s}$$

Or rearranging

$$v = Vm - (Km) \times \frac{v}{[S]}$$

Y = b m x



Rules for deriving rate laws for simple systems

1. **Write reactions involved in forming P from S**
2. **Write the conservation equation for expressing the total enzyme concentration $[E]_{total}$ among the various species**
3. **Write the velocity dependence equation, summing all the catalytic rates constants multiplied by the concentration of the respective produt - forming species.**
4. **Divide the velocity dependence equation by the conservation equation.**
5. **Express the concentration of each enzymic species in terms of free enzyme concentration & substitute**
6. **Algebra**

For a simple 1 step reaction, no inhibitor

(1) $$E + S \underset{k_2}{\overset{k_1[S]}{\rightleftarrows}} ES \xrightarrow{k_3} E + P$$

(2) $$[E]_T = [E] = [ES]$$

(3) $$v = k_3[ES]$$

(4) $$\frac{v}{[E]_T} = \frac{k_3[ES]}{[E] + [ES]}$$

(5) $$[ES] = k_1[S] \bullet [E] - k_2[ES] - k_3[ES]$$

$$[ES] = k_1[S] \bullet [E] - (k_2 + k_3)[ES]$$

@ steady state $\frac{d[ES]}{dt}$ **= O, ∴ rate of formation = rate of breakdown**

$$k_1 [S] \bullet [E] = (k_2 + k_3)[ES]$$

$$[ES] = \frac{k_1[S]\bullet[E]}{k_2 + k_3}$$, **Define** $Km = \left(\frac{k_2 + k_3}{k_1}\right) = \frac{[S]\bullet[E]}{Km} = [ES]$

(6) **Substitute into step (4)**

$$\frac{v}{[E_T]} = \frac{k_3 \bullet \frac{[S]}{Km} \bullet [E]}{E + \frac{[S]}{Km} \bullet [E]}$$ ÷ **Top and bottom By[E]** $= \frac{k_3 \frac{[S]}{Km}}{1 + \frac{S}{Km}} = \frac{v}{[E_T]}$

Multiply by Km

$$\frac{v}{[E_T]} = \frac{k_3 [S]}{Km + [S]}$$; **Define** $Vmax = k_3 [E_T]$; $v = \frac{Vmax [S]}{Km + [S]}$

EQUATION FOR COMPETITIVE INNIBITION • MUTUALLY EXCLUSIVE BINDING OF S AND I

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

$$\textbf{k on [I]} \downarrow\uparrow \textbf{ k off}$$

$$\mathbf{EI}$$

- Can drive <u>all</u> E to ES By increasing [S]

- Since [I] & [S] are mutually exclusive binders, [I] apparently decreases affinity for E, e.g. Km ↑

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

$$v = k_3 [ES]$$

$$\frac{v}{[E]_T} = \frac{k_3[ES]}{[E]+[ES]+[EI]}$$

$$[ES] = \frac{k_1 \bullet [S] \bullet [E]}{(k_2 + k_3)} \quad = \frac{1}{Km} \quad = \frac{[S]}{Km} \bullet [E]$$

$$[EI] = \text{k on } [E] \bullet [I] = \text{k off } [EI]$$

@ steady-state $\frac{d[EI]}{dt}$ **= O;** $\frac{\text{k on}}{\text{k off}}$ $\frac{1}{K_I}$ **i.e., receprocal dissociation constant**

$$[EI] \quad \frac{[I]}{K_I} \bullet [E]$$

$$\frac{v}{[E]_T} = \frac{k_3 \frac{[S]}{Km} \bullet [E]}{E + \frac{[S]}{Km} \bullet [E] + [I] \bullet [E]} \longrightarrow \frac{k_3 [S]}{Km + [S] + Km \frac{[I]}{K_I}}$$

Define Vmax & Collect Terms

$$\frac{Vmax\,[S]}{Km\left(1+\frac{[I]}{K_I}\right) + [S]} \; ;$$

Double Reciprocal Equation

$$\frac{1}{v} = \frac{1}{Vmax} + \frac{Km}{Vm}\,\frac{1}{[S]}\left(1+\frac{[I]}{K_I}\right)$$

b + x m

DETERMINING K_I FROM SLOPE REPLOT

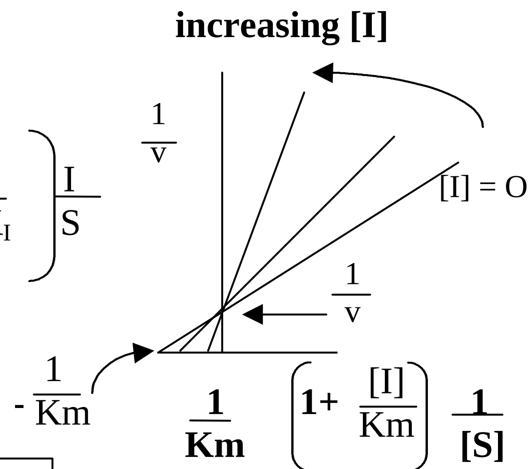
-Measure $\frac{1}{v}$ vs $\frac{1}{S}$ @ several [I]

X intercept @ $\frac{1}{v} = O$

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

Cross multiply

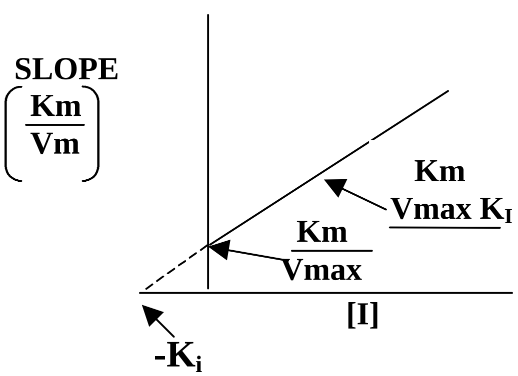
$$-\frac{1}{K_m}\left(1 + \frac{I}{K_I}\right) = \boxed{\frac{1}{[S]}}$$



Slope of $\frac{1}{s}$ vs $\frac{1}{v} = \frac{K_m}{V_m}\left(1 + \frac{[I]}{K_I}\right)$

As [I] ↑, Slope increases

PLOT SLOPE vs [I]



$$\textbf{SLOPE} = \frac{K_m}{V_{max}} + \frac{K_m[I]}{V_{max} \bullet K_I}$$

y = b + m x

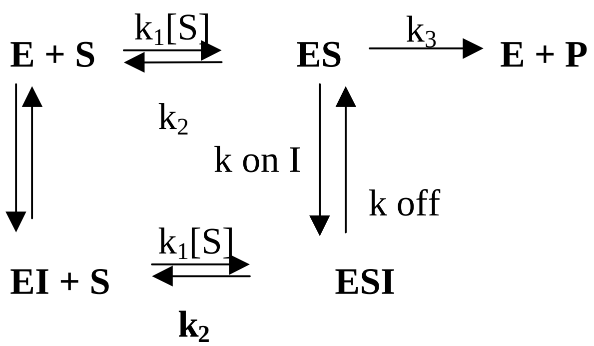
@ SLOPE O (i.e. x-intercept)

$$\frac{K_m}{V_{max}} = \frac{-K_m}{V_{max}\,K_I} \bullet I$$

Cross Multiply

$-K_I$ = [I] @ slope = O

NON COMPETITIVE INHIBITION



$$[E]_T = [E] + [ES] + [EI] + [ESI]$$

$$v = k_3 [ES]$$

$$\frac{v}{[E]T} = \frac{k_3[ES]}{[E] + [ES] + [EI] + [ESI]}$$

$$[ES] = \frac{[S]}{Km} \bullet [E] \quad ; \quad [EI] = \frac{I}{K_I} \bullet [E]$$

[ESI] = Cannot easily calculate [ESI] by steady state hypothesis

∴ Most assume equilibrium thus is valid since EI + ESI <u>are</u> in equilibrium

(i.e. k_3 = O for ESI ∴ $k_2 >>> k_3$)

THUS:

$$[ESI] = \frac{k\ on\ [ES] \bullet I}{k\ off} \quad ; \quad \frac{S}{K_m} \bullet \frac{[I]}{K_I} \bullet [E]$$

$$\frac{v}{[E]_T} = \frac{k_3 \frac{S}{Km} \bullet [E]}{E + \frac{S}{Km} \bullet E + \frac{[I]}{K_I} \bullet E + \frac{[I]}{K_I} \frac{[S]}{K_m} \bullet E}$$

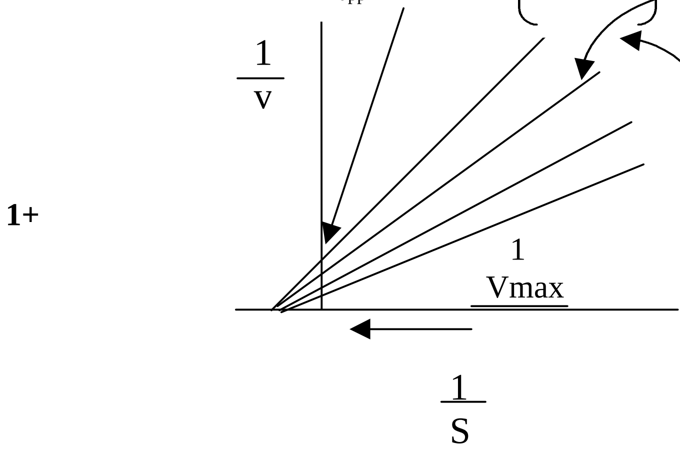
$$\frac{v}{[E]_T} = \frac{k3\ [S]}{Km + [S] + Km \frac{[I]}{K_I} + [S]}$$

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

$$\frac{1}{Vm_{app}} = \frac{1}{Vm}\left(1+\frac{I}{K_I}\right)$$

Slope = $\mathbf{\frac{Km}{Vm}\left(1 + \frac{[I]}{K_I}\right)}$

Increasing [I]



[I] =O $\frac{1}{Vmax_{app}}$ **=Yint =** $\frac{1}{Vmax}\left(1+\frac{I}{K_I}\right)$

$\frac{1}{V = O}$ **@Xint,** $\therefore$ $\frac{-Km}{Vmax}\left(1+\frac{I}{K_I}\right)\frac{1}{S} = \frac{1}{Vmax}\left(1+\frac{I}{K_I}\right)$

Cross Multiply

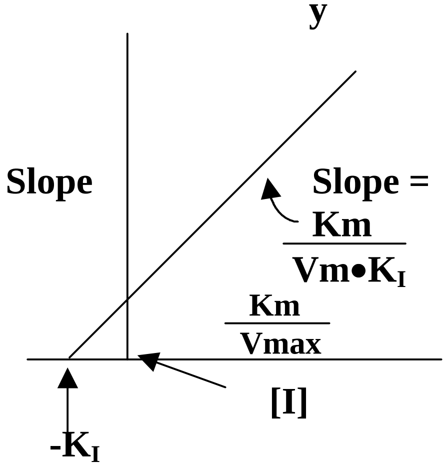
$$\mathbf{\frac{1}{[S]} = -\frac{1}{Km}}$$

Replots to determine K_I

Slope of $\frac{1}{v}, \frac{1}{S}$ **Plot =** $\frac{Km}{Vm}\left(\mathbf{1+}\ \frac{[I]}{K_I}\right)$

Slope = $\mathbf{\frac{Km}{Vm} + \frac{KM[I]}{VmK_I}}$

y b + m x



x int. of slope <u>vs</u> [I]
@ Slope = O

$\therefore\ -\frac{Km}{Vm} = \frac{Km[I]}{VmK_I}$ **[I]**

Cross Multiply

$-K_I$ **= [I] @ Slope =O**

y-
Can also do intercept replot as well

Y int = $\frac{1}{Vmaxapp} = \frac{1}{Vmax} + \frac{[I]}{Vmax \bullet K_I}$ ← x, ← m

Uncompetitive Inhibition

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

$$ES \rightleftharpoons ESI$$

I binds only to ES

There is an **obligate order of binding**
First S Then I

Like non-competitive, [S] @ ∞ **cannot** drive enzyme to ES form

$V = k_3\,[ES]$
$[E]_t = [E]+[ES]+[ESI]$

∴ I should **decrease** Km by driving reaction $E+S \rightleftharpoons ES$ towards ES formation

$$[ES] = \frac{k_1[S]}{k_2+k_3} \bullet [E], \quad \frac{k_1}{k_2+k_3} = \frac{1}{Km} \quad = \frac{[S]}{Km} \bullet [E]$$

$$ESI = \frac{Kon[I]}{Koff} \bullet [ES], \quad \frac{Kon}{Koff} = \frac{1}{K_I} \quad = \frac{I}{K_I} \bullet [ES] \quad \frac{[S] \bullet [I]}{Km \bullet K_I}$$

$$\frac{V}{[ET]} = \frac{k_3\,[ES]}{[E]+[ES]+[ESI]}$$

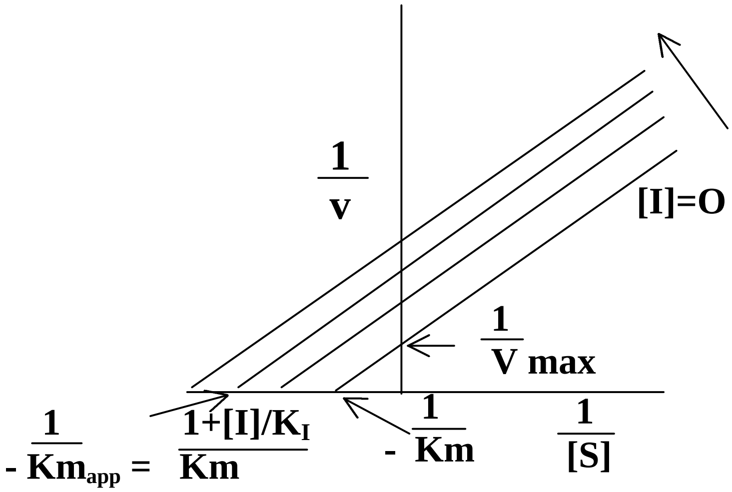
$$\frac{V}{[ET]} = \frac{k_3 \frac{S}{Km} \bullet [E]}{E + \frac{S}{Km}[E] + \frac{[S] \bullet [I]}{Km \bullet k_I}}$$

$$V = \frac{Vm\,[S]}{Km+S+\frac{[S][I]}{Km+K_I}} \xrightarrow{\text{Factor}} \frac{Vm\,[S]}{Km+S\left(1+\frac{[I]}{K_I}\right)}$$

Taking Reciprocal

$$\frac{1}{V} = \frac{Km}{Vm}\frac{1}{[S]} + \frac{1}{Vm}\left(1+\frac{[I]}{k_I}\right)$$

∴ Slope unaffected
Yint decreased by [I]



$$Y\ int = \frac{1}{Vmax}\left(1+\frac{(I)}{K_I}\right)$$

$$X\ int = \frac{1}{Y} = O;\quad -\frac{Km\ 1}{Vm[S]} = \frac{1}{Vm}\left(1+\frac{[I]}{K_I}\right)$$

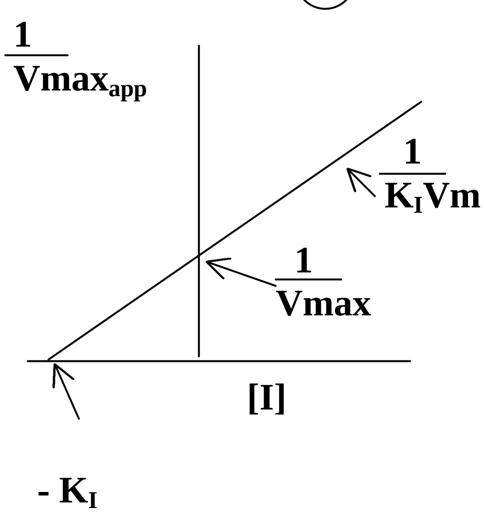
$$\therefore \frac{1}{S} \bullet \frac{1}{V} = O - \frac{1+\frac{I}{K_I}}{Km}$$

To obtain K_I, <u>Replot</u> of $\frac{1}{V}$ vs [I]

$$@\ [S] = \infty\quad \frac{1}{v} = \frac{1}{Vmax_{app}} = Y\ intercept = \frac{1}{Vmax}\left(1+\frac{[I]}{k_I}\right)$$

$$int = \frac{1}{Vmax} + \frac{[I] - X}{Vmax \bullet k_I} - m$$

(b)



$$@\ X\ int = -\frac{1}{Vmax\ app} = O$$

$$\therefore\quad \frac{1}{Vm} = -\frac{[I]}{Vmax \infty K_I}$$

$$-K_I = [I]$$

<u>COMPLEX INHIBITION</u>

$$
\begin{array}{ccccc}
E + S & \underset{k_2}{\overset{k_1}{\rightleftharpoons}} & ES & \xrightarrow{k_3} & E + P \\
+ & & & & + \\
I & & & & I \\
K_I \ \downarrow\uparrow & & & & \alpha K_s \ \downarrow\uparrow \\
EI + S & \overset{\alpha K_s}{\rightleftharpoons} & & & ESI \xrightarrow{\beta k_3} EI + P
\end{array}
$$

α = O or ∞ - Competitive

α = 1, β = O - <u>Non</u>-Competitive

α = 1, O < β < 1 - <u>Partial</u> Non-Competitive

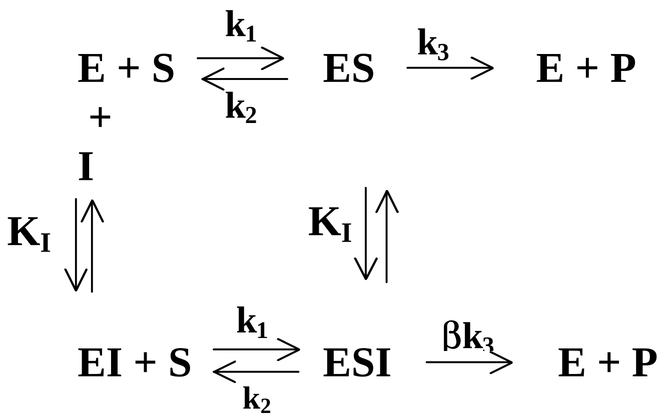
1 < α < ∞, β = 1 - <u>Partial</u> Competitive

1 < α < ∞, β = O - <u>Mixed</u> Inhibition (Type 1)

1 < α < ∞, O < β <1 - <u>Mixed Inhibition</u> (Type 2)

In <u>partial</u> inhibition, the EI or ESI complexes are <u>not</u> dead-end complexes as they are in simply inhibition schemes

Partial Non Competitive Inhibition



All forms of E (E + EI) combine equally well with S, ∴ Km does not change. Vmax is decreased because a portion of ES is ESI and ESI EI + P is slower

$$V = k_3\,[ES] + \beta k_3\,[ESI]$$

$$[E]t = [E] + [ES] + [EI] + [ESI]$$

$$[ES] = \frac{[S]}{Km}\,[E] \qquad [ESI] = \frac{I}{K_I}\,[ES] = \frac{[I]}{K_I}\,\frac{[S]}{Km}\,[E]$$

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

$$V = \frac{k_3\,\frac{[S]}{km}\,E + \beta k_3\,\frac{[I]}{K_I}\,\frac{[S]}{Km}\,E}{[F]t\;\; E + \frac{[S]}{Km} + \frac{[I]}{K_I} + \frac{[S]}{Km}\,\frac{[I]}{K_I}}$$

(÷) by E
(x) by Km
Define
$Vm = k_3\,[E]t$

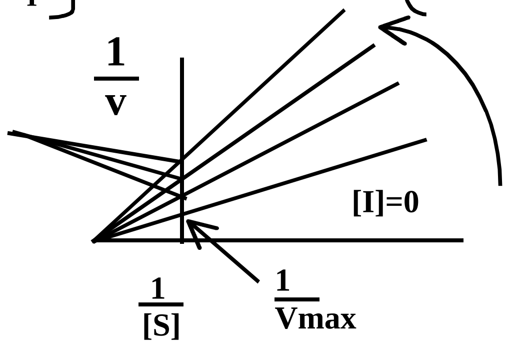
$$V = \frac{Vm\left(1 + \frac{\beta[I]}{K_I}\right)[S]}{Km\left(1 + \frac{[I]}{K_I}\right) + [S]\left(1 + \frac{[I]}{K_I}\right)}$$

$$V = \frac{Vm\,[S]}{Km\,\frac{\left(1 + \frac{[I]}{K_I}\right)}{\left(1 + \frac{\beta I}{K_I}\right)} + [S]\,\frac{\left(1 + \frac{[I]}{K_I}\right)}{\left(1 + \frac{\beta I}{K_I}\right)}}$$

Take Reciprocal

$$\frac{1}{V} = \frac{Km\left[1+\frac{[I]}{K_I}\right]}{Vm\left[1+\frac{\beta I}{K_I}\right]}\frac{1}{[S]} + \frac{1}{Vmax}\frac{\left(1+\frac{[I]}{K_I}\right)}{\left(1+\frac{\beta I}{K_I}\right)}$$

$$\frac{1}{Vmax;} = \frac{l+\frac{[I]}{K_I}}{Ymax\left(1+\frac{\beta I}{K_I}\right)}$$



Increasing [I]

@ $[I] = \infty$

$$\text{Slope} = \frac{Ks}{\beta Vm}$$

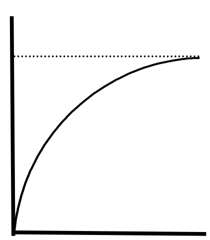
$$\text{Y int} = \frac{1}{\beta Vm}$$

$$\text{Y intercept} = \frac{\left(1+\frac{[I]}{K_I}\right)}{Vmax\left(1+\frac{\beta I}{K_I}\right)}$$

Simplifies to

$$\text{Y int} = \frac{1}{Ym_{app}} = \frac{1}{Vmax}\bullet\left(\frac{[I]+K_I}{\beta[I]+K_I}\right) \longrightarrow \text{Hyperbolic}$$

$$\text{Y int} = \frac{1}{Vmax_{app}}$$



$\longleftarrow$ Limit $= \frac{1}{\beta Vmax}$

To obtain βV<u>max</u>

Plot $Vmax_{app}$ vs $\frac{1}{[I]}$

$$Vm_{app} = \frac{Vmax\,(\beta[I]+K_I)}{[I]+K_I}$$

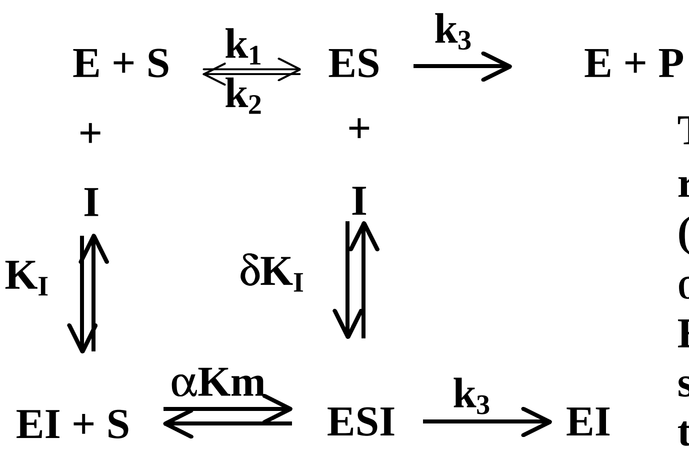
$$Vm_{app} = \beta Vmax + \frac{VmK_I}{[I]} \quad \text{multiply both terms B} \quad Vm_{app} = \frac{\beta Vm[I]}{[I]+K_I} \pm \frac{VmK_I}{[I]+K_I}$$

$\longleftarrow$

$$\frac{I+Kt}{I}$$

Partial Competitive

$$Km = \left(\frac{k_2 k_3}{k_1}\right)$$



The presence of I inhibits on rate of S, ∴ Km is increased (affinity lowered) by a factor α (kα < 0). Since ESI, EI and E are in equilibrium, and since it makes intuitive sense that S would interfere with I binding, K_I is increased by a factor 1 < α < 00.

$$\frac{V}{[E]t} = \frac{k_3\,[ES] + k_3\,[ESI]}{E + EI + ES + ESI}$$

$$[ES] = \frac{[S]}{Km} \bullet [E]$$

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

$$ESI = \frac{[I]}{\alpha K_I} \bullet [ES] = \frac{[I]}{\alpha K_I} \bullet \frac{[S]}{Km}$$

$$\frac{V}{[E]t} = \frac{k_3 \frac{S}{Km} \bullet [E] + k_3 \frac{[I]}{\alpha K_I} \bullet \frac{[S]}{Km}\,[E]}{[E] + \frac{[S]}{Km} + \frac{[I]}{K_I} \bullet [E] + \frac{[I]}{\alpha K_I}\frac{[S]}{Km}\,[E]}$$

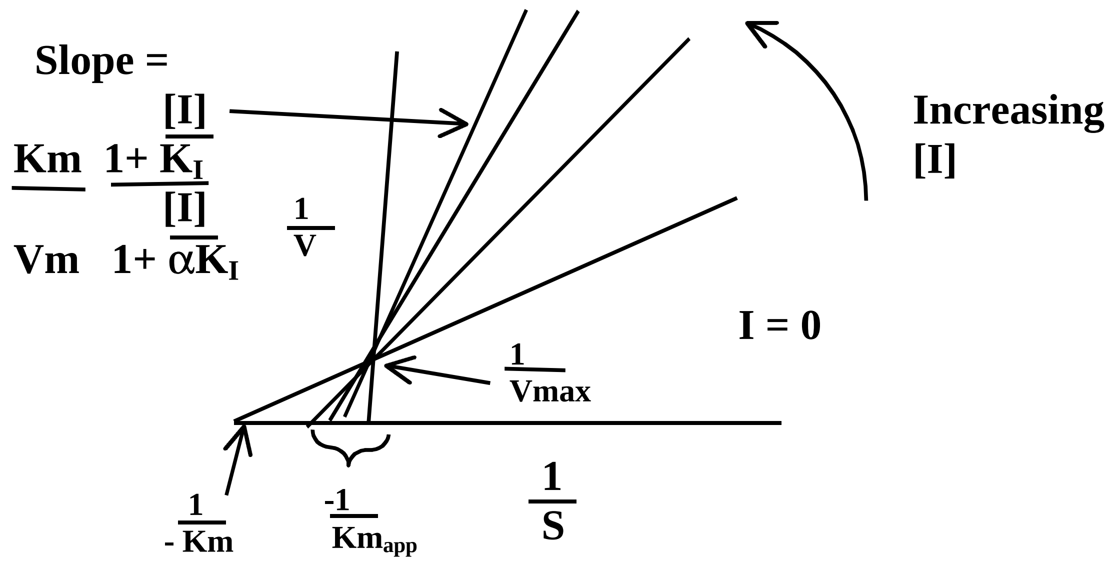
÷ by [E]
x Km
assume
$Vm = k_3\,[E]t$

$$\frac{Vm\,[S] + \frac{[I]\,[S]}{\alpha K_I}}{Km + [S] + \frac{Km\,[I]}{K_I} + \frac{[I]\,[S]}{\alpha K_I \bullet Km}} \xrightarrow{\text{Factor}} \frac{Vm\left(1 + \frac{[I]}{\alpha K_I}\right) S}{Km\left(1 + \frac{[I]}{K_I}\right) + S\left(1 + \frac{[I]}{\alpha K_I}\right)}$$

$$\div \text{ by } \frac{I}{1 + \alpha K_I} = \frac{Vmax\,[S]}{Km\,\frac{1 + \frac{[I]}{K_I}}{1 + \frac{[I]}{\alpha K_I}} + [S]} \quad \text{0r} \quad \frac{Vmax[S]}{Km\,App + [S]}$$

Take Reciprocal

$$\frac{1}{V} = \frac{Km}{Vmax}\left(\frac{1+\frac{[I]}{K_I}}{1+\frac{[I]}{\alpha K_I}}\right)\frac{1}{[S]} + \frac{1}{Vmax}$$

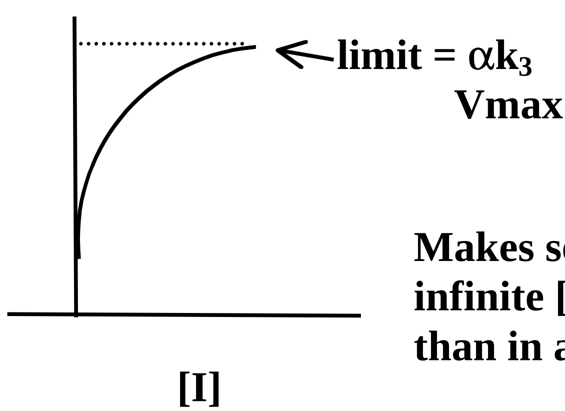


$$\text{Slope} = \frac{Km}{Vmax}\left(\frac{1+\frac{[I]}{K_I}}{1+\frac{[I]}{\alpha K_I}}\right) = \frac{Km}{Vmax} + \frac{Km\ \frac{I}{K_I}}{Vm\ \frac{I}{\alpha K_I}}$$

Simplified to:

$$\text{Slope} = \frac{\alpha Km}{Vm}\left(\frac{I+K_I}{I+\alpha K_I}\right) =$$

Hyperbolic Plot



Makes sense in that in presence of infinite [I] can still bind S, albeit weaker than in absence of I