Chapter 9: Catalytic strategies

Chapter 6: Enzyme

" 8: Enzyme concepts & Kinetics

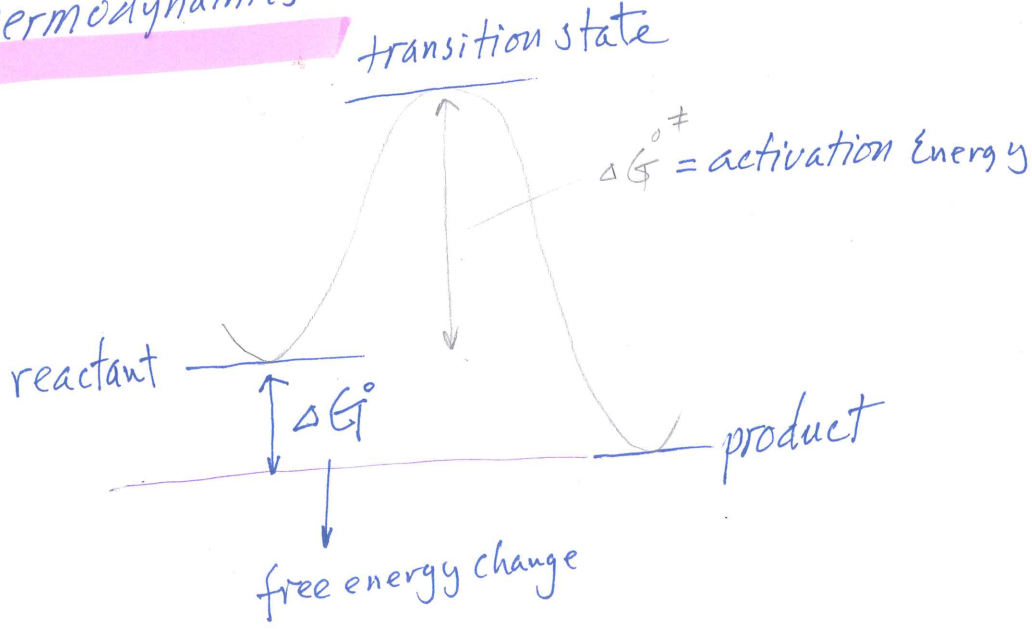
1. Enzyme: ribozyme (RNA)
   proteins

   Chemical catalyst: increase $10^2 \sim 10^4$
   Enzyme (Biological catalyst: 10^{20}

   } 若無 enzyme, 如一頓早餐要消化 50年 instead of few hours

2. catalysis: Kinetic & Thermodynamics

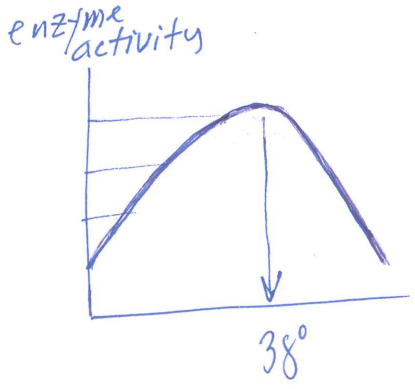
Thermodynamics



$\Delta G^{\circ} < 0$, spontaneously occurs, no rate is provide

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

Glucose + $6O_2 \rightarrow 6CO_2 + 6H_2O$ $\Delta G^{\circ} = -689$ Kcal/mole



$$2\,H_2O_2 \longrightarrow 2\,H_2O + O_2$$

	Activation Eact	Relative Rate
NO catalyst	18	1
pt	11.7	2.77×10^4
catalase	5.5	6.51×10^8

Kinetics:

$$A + B \longrightarrow P$$

$$\text{rate} = -\frac{d[A]}{dt} = -\frac{d[B]}{dt} = \frac{d[P]}{dt}$$

$$\text{rate} \propto [A]^f [B]^g = k[A]^f[B]^g$$

k: rate constant

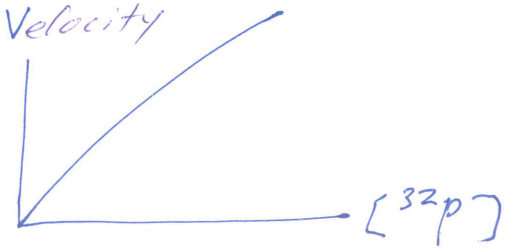
f, g: order wrt [A] or [B]

overall order: $f+g$

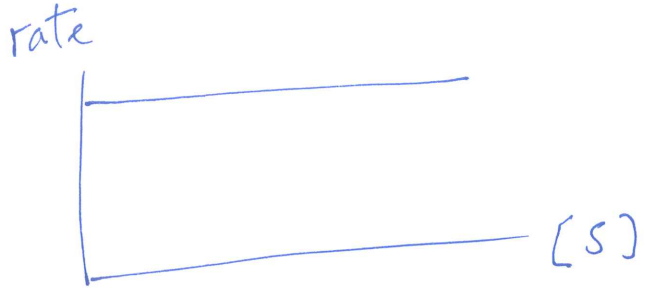
First order rxn

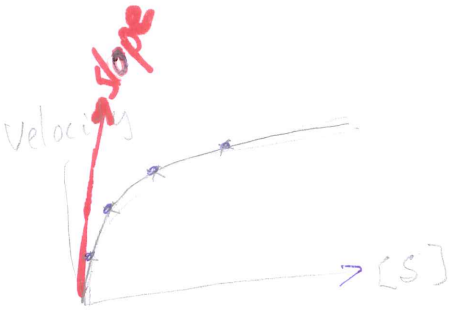
$$^{32}P \longrightarrow \text{product decay}$$

$$\text{Velocity} = \text{rate} = k[^{32}P] = \frac{-d[^{32}P]}{dt}$$

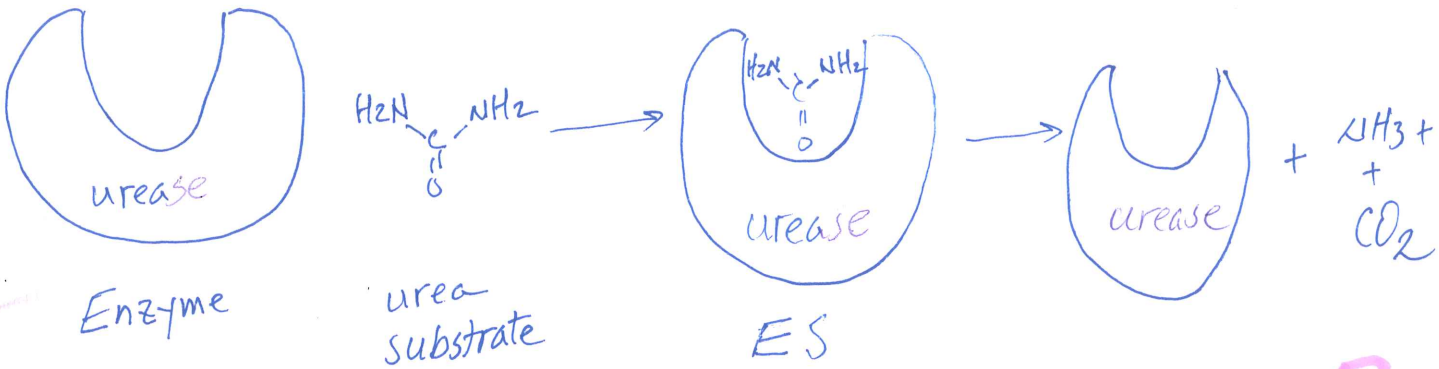


Zero order





Enzyme substrate model



Enzyme　　urea substrate　　ES

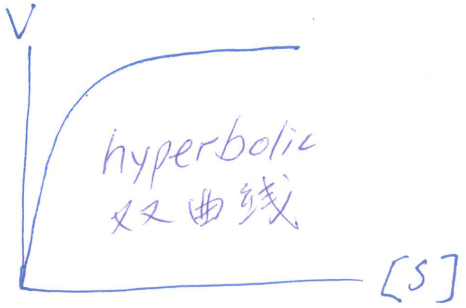
$E + S \rightarrow ES \rightarrow E + P$

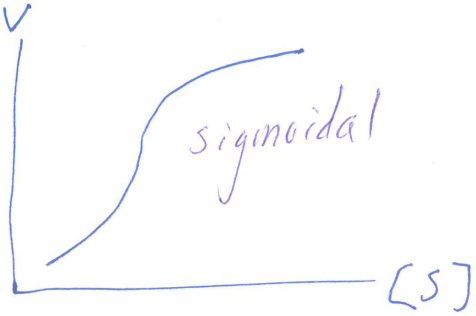
lock & key model

Induced-fit model

Two Example of Enzyme catalyzed reaction

1. Non-allosteric rxn: Chemotrypsin: hyperbolic shape
2. Allosteric rxn: ATase, Hemoglobin: sigmoidal shape





Michaelis-Menten Eq (1913)

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

1. steady state approximation of [ES]: rate of formation = rate of destruction

$$k_1[E][S] = k_2[ES] + k_{-1}[ES]$$

$$[ES] = \frac{k_1[E][S]}{k_{-1}+k_2} = \frac{[E][S]}{K_M} = \frac{([E]-[ES])[S]}{K_M}$$

2. Material Balance: $[E]_T = [E]_{free} + [ES]$

$$= [E] + [ES]$$

$$[E] = [E]_T - [ES]$$

$$[ES]K_M = [E][S] - [ES][S]$$

$$[ES] = \frac{[E][S]}{K_M + [S]}$$

$$k_1([E]_T - [ES])[S] = k_2[ES] + k_{-1}[ES] = [ES](k_2 + k_{-1})$$

$$\Longrightarrow \frac{([E]_T - [ES])[S]}{[ES]} = \frac{k_2 + k_{-1}}{k_1} = K_M \text{ (michaelis constant)}$$

$$K_M[ES] = [E]_T[S] - [ES][S]$$

$$[ES] = \frac{[E]_T[S]}{K_M + [S]}$$

$$V = \text{rate} = k_2[ES] = \frac{k_2[E]_T[S]}{K_M + [S]}$$

if $[S] \to \infty$

$\text{rate} = k_2[E]_T = k_2[ES] = V_{max}$

when substrate concentration is high $\Longrightarrow$ enzyme is complete saturated with [S]

(当 enzyme 全被 S saturated 时 $V_{max} = k_2[ES]$)

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

$[E]_T = [ES]$

rate is maximum. V_{max}

① 当 [S] is large, $V = V_{max}$ (zero order)

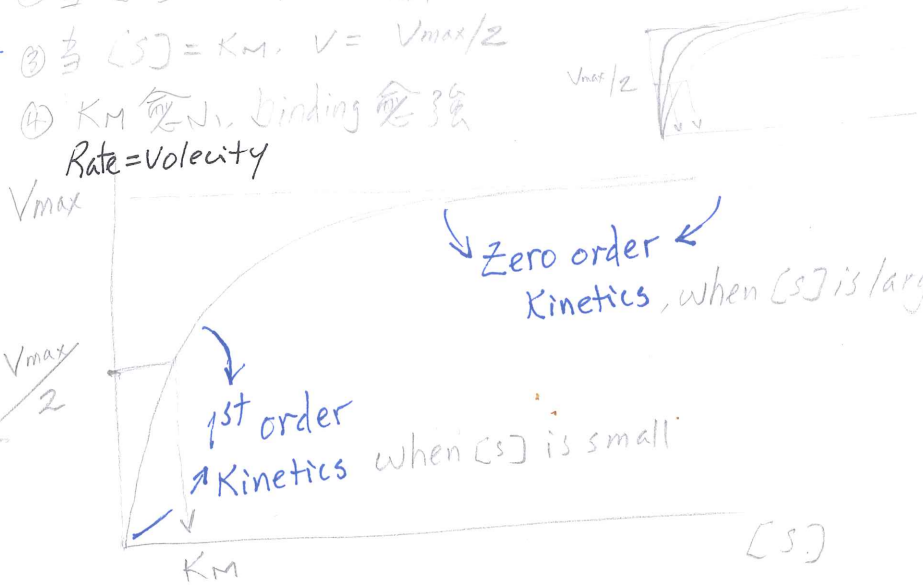
② 当 [S] is small, $V = \frac{V_{max}}{K_M}[S]$ (1st order)

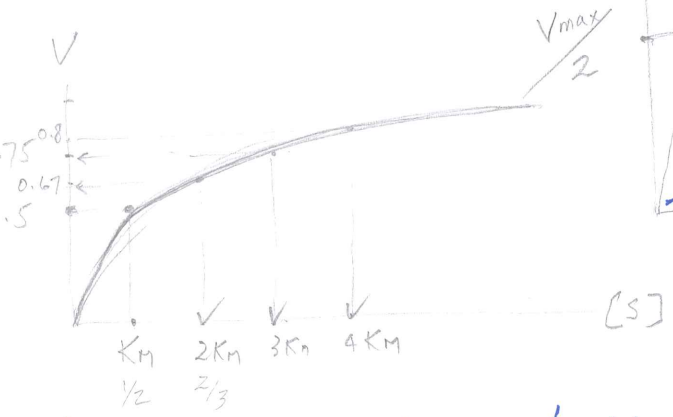
③ 当 $[S] = K_M$, $V = V_{max}/2$

④ K_M 愈小, binding 愈紧

$$V = \frac{V_{max}[S]}{K_M + [S]}$$

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





Line weaver-Burk double-reciprocal plot

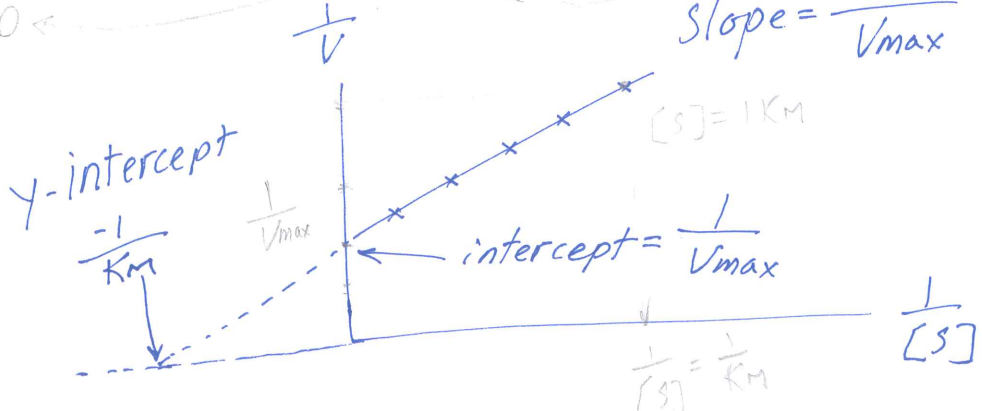
$$V = \frac{V_{max}[S]}{K_M + [S]}$$

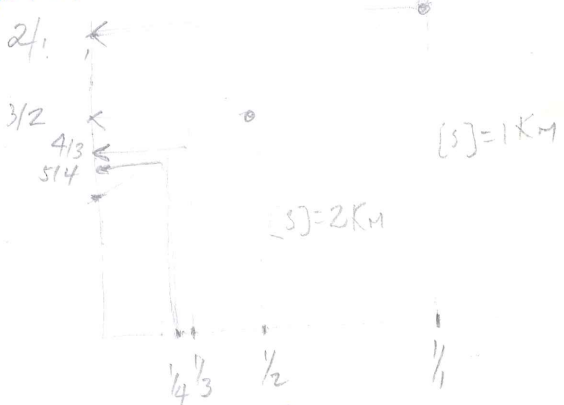
$$\frac{1}{V} = \frac{K_M}{V_{max}[S]} + \frac{1 \cancel{[S]}}{V_{max} \cancel{[S]}}$$

$$\frac{1}{V} = \frac{K_M}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

$y = mx + b$

Slope $= \frac{K_M}{V_{max}}$





$[S] = K_M$ $\quad \frac{1}{V} = \frac{K_M}{V_{max}} \quad \frac{1}{K_M} + \frac{1}{V_{max}} = \frac{2}{V_{max}}$

$[S] = 2K_M$ $\quad \frac{1}{V} = \frac{K_M}{V_{max}} \cdot \frac{1}{2K_M} + \frac{1}{V_{max}} = \frac{3}{2V_{max}}$

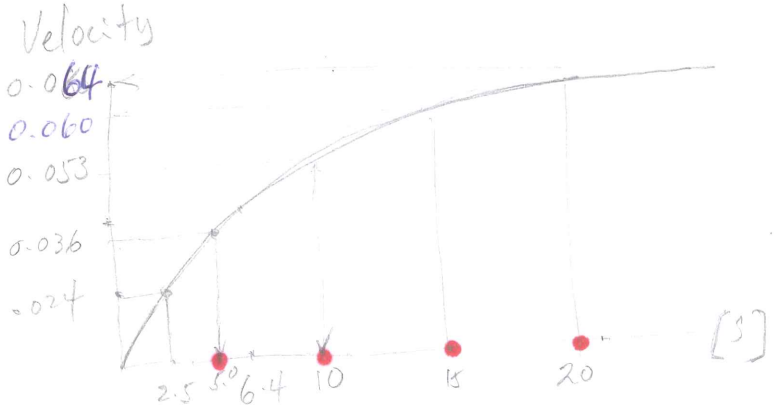
$[S] = 3K_M$ $\quad \frac{1}{V} = \frac{4}{3V_{max}}$

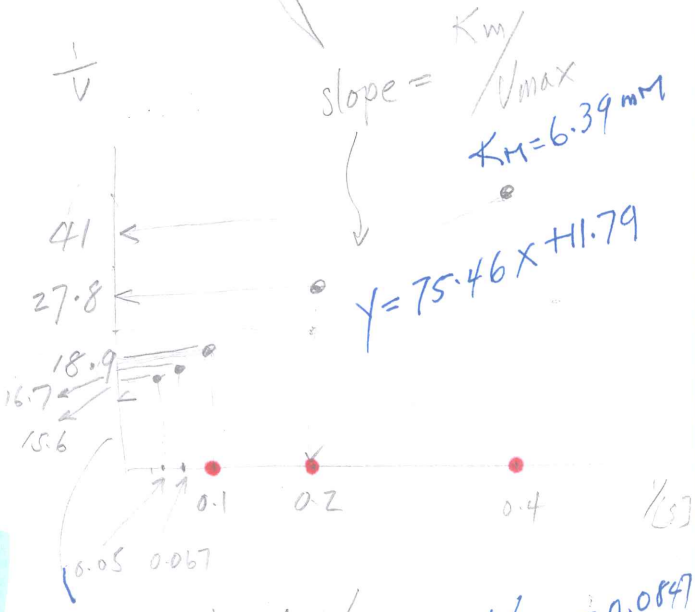
$[S] = 4K_M$ $\quad \frac{1}{V} = \frac{5}{4V_{max}}$

Example

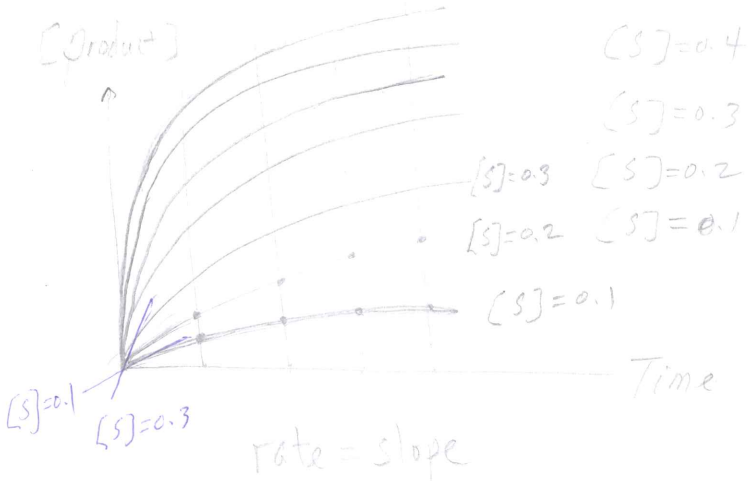
Carbobenzoxyglycyl-Tryptophan + H_2O —Chemotrypsin→ carbobenzoglycine + tryptophan

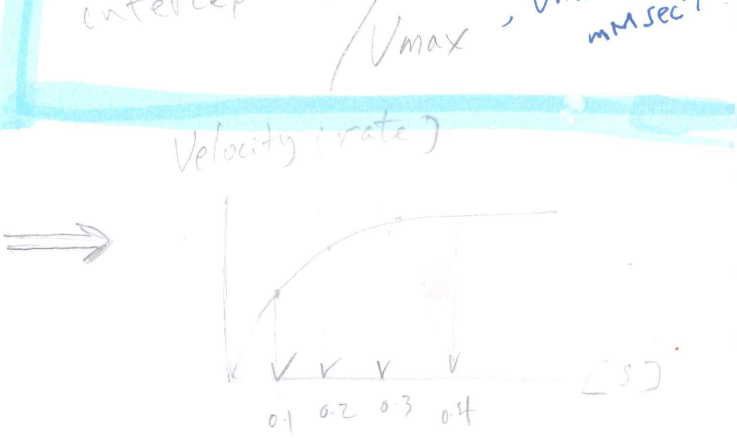
1/[S]	substrate [mM]	velocity	1/v
0.400	2.5	0.024	41.7
0.200	5.0	0.036	27.8
0.100	10.0	0.053	18.9
0.067	15.0	0.060	16.7
0.050	20.0	0.064	15.6





E + S → ES → Product





Significance of K_M & V_{max}

① K_M = Substrate concentration at which 50% of Enzyme active site are occupied by substrate

② $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$

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

when $k_{-1} \gg k_2$ (dissociation rate (k_{-1}) is greater than product formation, k_2)

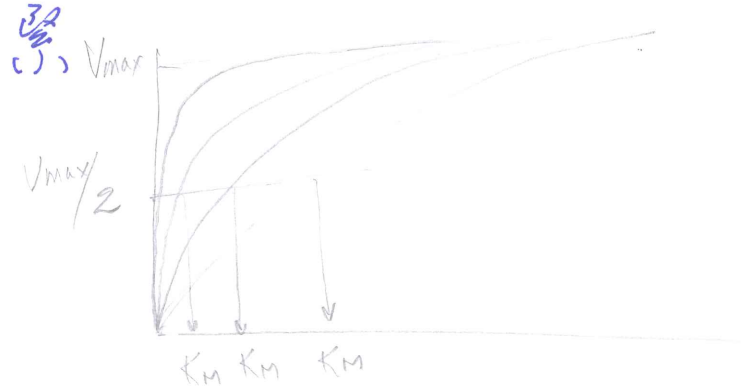
$$K_M = \frac{k_{-1}}{k_1} = \text{dissociation of ES complex}$$

$$\left(E + S \rightleftharpoons\right) ES \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} E + S$$

$$K_d = \frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_1} = K_M$$

K_M 表 How tightly[a] substrate binds to Enzyme

K_M 愈小，bind 得愈緊



Vmax: turnover number

$$\frac{V_{max}}{[E]_T} = \text{turnover number} = k_{cat} = k_2$$

# of mole of substrate converted to product per mole of enzyme per second

		$k_{cat}(k_2)$	K_M	k_{cat}/K_M
catalase	$H_2O_2 \rightarrow H_2O + O_2$	10^7	25	
carbonic Anhydrase	$CO_2 + H_2O \rightarrow H_2CO_3$	10^6	12	
~~Acetylcholinesterase~~	~~regenerate Ach~~	~~10^4~~	~~9.5×10^{-2}~~	
chymotrypsin	cleave peptide	10^2	6.6×10^{-1}	
Lysozyme	Degrade polysaccharide	0.5	6×10^{-3}	

Inhibition of Enzymatic RxN

Competitive inhibition

Vmax unchanged

K_M changed (變大)

Michaelis-Menten

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

$$E + I \rightleftharpoons EI$$

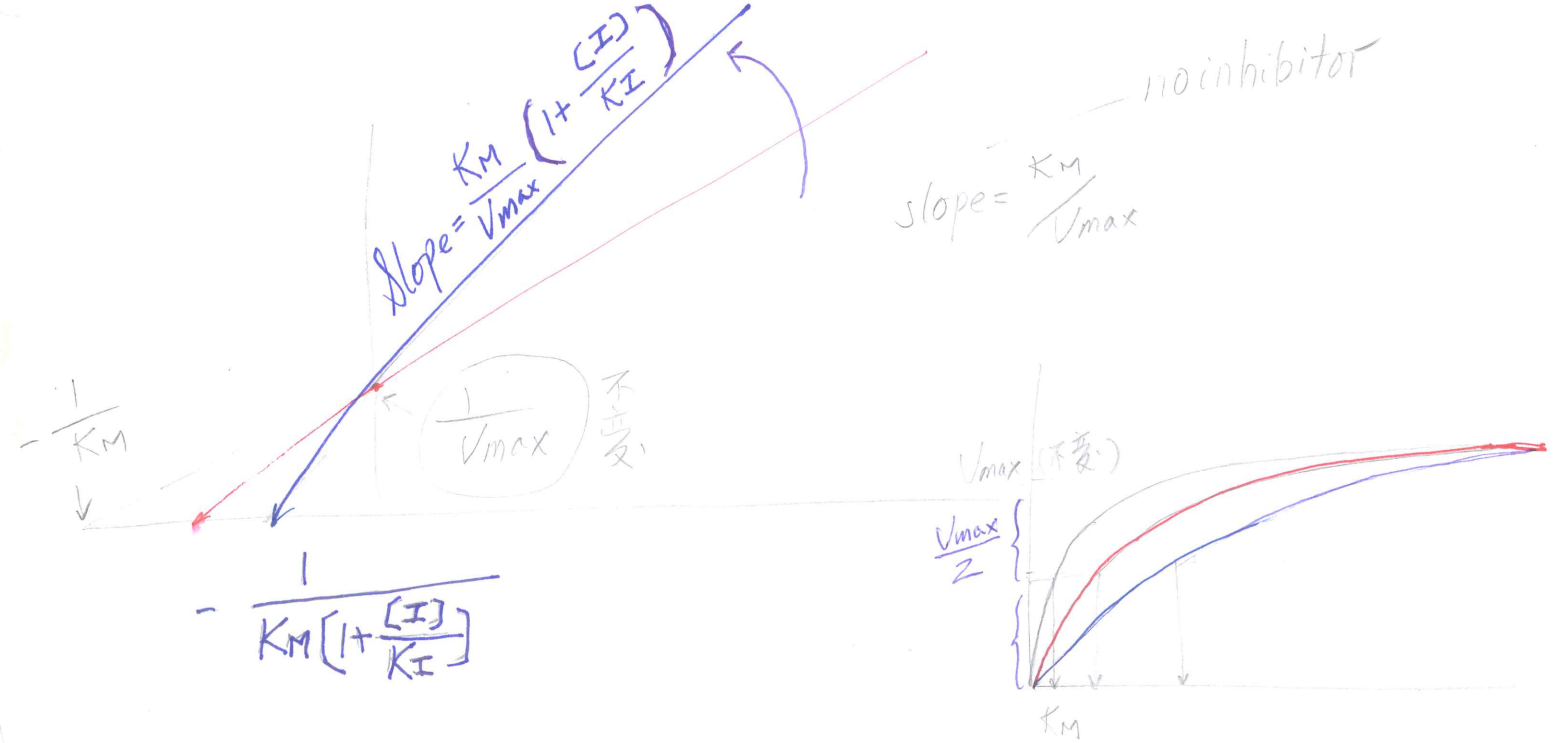
$$V = \frac{V_{max}[S]}{K_M + [S]} \qquad K_I = \frac{[E][I]}{[EI]}$$

without inhibitor, Lineweaver-Burk Eq

$$\frac{1}{V} = \frac{K_M}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

with inhibitor, $K_M^I \xrightarrow[\text{a factor}]{\text{increase}} \left(1 + \frac{[I]}{K_I}\right) K_M$ (代入)

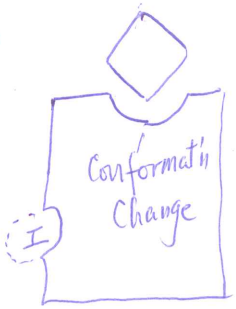
$$\frac{1}{V} = \frac{K_M}{V_{max}} \left(1 + \frac{[I]}{K_I}\right) \frac{1}{[S]} + \frac{1}{V_{max}}$$

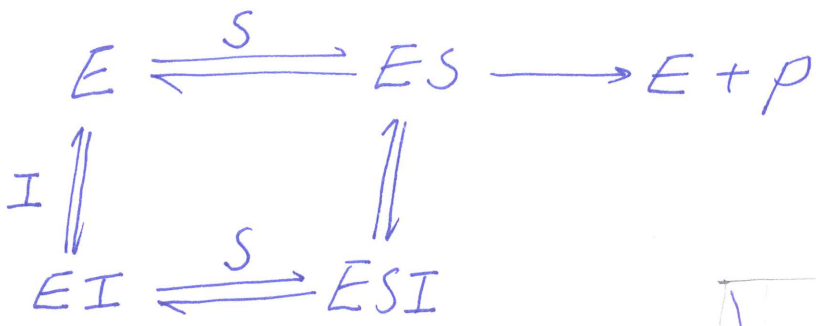


Non-competitive inhibitor

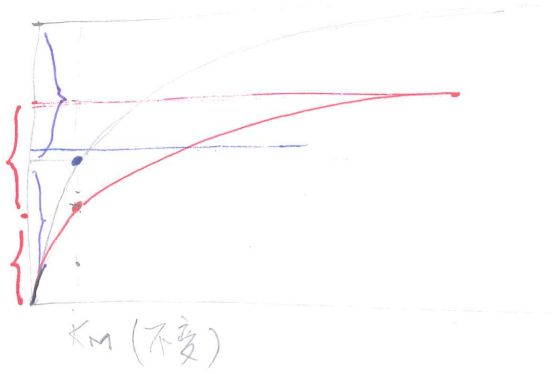
ti

V_{max} 变小
K_M 不变





$$V_{max}^{I} = \frac{V_{max}}{1 + \frac{[I]}{K_I}}$$



No non-competitive inhibitor

$$\frac{1}{V} = \frac{K_M}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

with non-competitive inhibitor

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

Lineweaver-Burk Equation: $\frac{1}{V}$