Bioeng 261 : L4 Enzymes cont'd & complicated

Quick recap & comments: (L1-L3)

Simplest constitutive equation
- mass action

More complicated: Enzymes
- Michaelis-Menten model
  - Each part: mass action
  - Overall : not mass action

Even more complicated
- Regulation effects
  - same shape
  - different parameter values

Today - more regulation
- cooperativity } different shape!

Recap: enzymes important for many diff. processes.

Example 1. Speed up reaction $S \rightarrow P$

$$S + E \rightleftharpoons C \rightarrow P + E$$

Example 2: Reversible phosphorylation of another protein

Kinase & Phosphatase

↑ add phosphate group

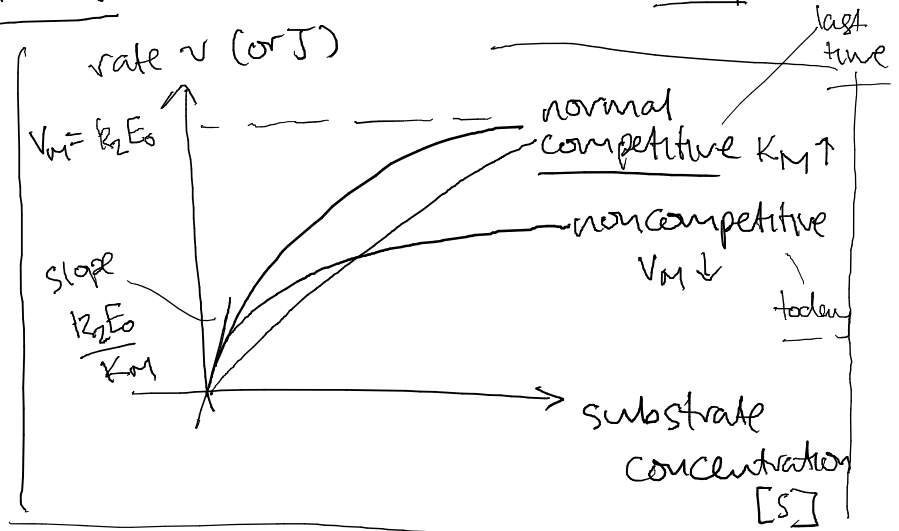
↑ remove phosphate group

(i.e. convert A to 'active' A^*)

$$A + B \rightleftharpoons C \rightarrow A^* + B$$

B 'activates' A

Recall: Michaelis-Menten Shape & Inhib.



Equation

$$J = v = \frac{\overbrace{k_2 E_0}^{V_{max}} [S]}{K_M + [S]}$$

- lump $k_1 E_0$ into V_{max}
- $K_M = \frac{k_{-1}}{k_1}$ or $\frac{k_{-1} + k_2}{k_1}$ } depending on approx

K_M empirical:

note when $K_M = [S]$

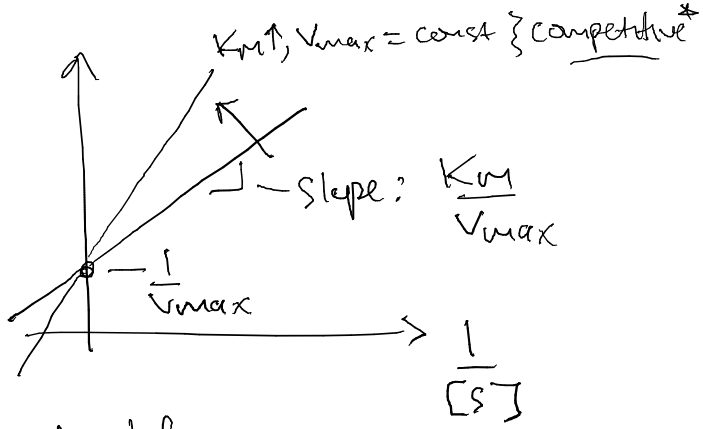
then $J = \frac{k_2 E_0}{2} = \frac{V_{max}}{2}$

$\Rightarrow$ **K_M is substrate conc. giving half-maximal reaction rate**

Recall: Lineweaver-Burk plots

→ also called 'double reciprocal' plots

$$\frac{1}{J} = \frac{1}{v}$$



can plot to identify experimental evidence for type of inhibition

* Competitive inhibition model

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1 \xrightarrow{k_2} P + E$$

$$I + E \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_2$$

Note on modelling irreversible inhibition

Example: competitive irreversible inhibition:

eg hydrogen cyanide

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1 \xrightarrow{k_2} P + E$$

$$I + E \xrightarrow{k_3} C_2$$

note!

cf $I + E \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_2$

L4 Today: - non-competitive, reversible inhibition
  - MM with diff. parameters
- cooperative effects
  - deviations from MM model.

L4 begin.

Non-competitive inhibition model.

- Inhibitor I, binds to either enzyme or complex at allosteric (not active) site

- slows production step
- doesn't affect binding/unbinding

General model:

$$\begin{array}{ccccc} E+S+I & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & ES+I & \xrightarrow{k_2} & E+P+I \\ {\scriptstyle k_{-3}}\uparrow\downarrow{\scriptstyle k_3} & & {\scriptstyle k_{-4}}\uparrow\downarrow{\scriptstyle k_4} & & \\ EI+S & \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} & ESI & & \text{all at once.} \end{array}$$

ie here → just showing which quantities are used

$$\begin{aligned} E+S &\overset{1}{\rightleftharpoons} ES \xrightarrow{2} E+P \\ E+I &\overset{3}{\rightleftharpoons} EI \\ ES+I &\overset{4}{\rightleftharpoons} ESI \\ EI+S &\overset{5}{\rightleftharpoons} ESI \end{aligned}$$

specific steps

(9 fluxes, incl. forward/back)

(Full model)

Conservation of mass $\{E, S, I, P$ / $EI, ES, ESI\}$ complexes

> Note only include Js in an ODE if they use that quantity

$$\frac{d[E]}{dt} = -J_1 + J_{-1} + J_2 - J_3 + J_{-3}$$ eliminating total enzyme

$$\frac{d[S]}{dt} = -J_1 + J_{-1} - J_5 + J_{-5}$$

$$\frac{d[I]}{dt} = -J_3 + J_{-3} - J_4 + J_{-4}$$

$$\frac{d[P]}{dt} = J_2 = v$$ [goal] → overall reaction rate.

$$\frac{d[EI]}{dt} = +J_3 - J_{-3} - J_5 + J_{-5}$$

$$\frac{d[ES]}{dt} = +J_1 - J_{-1} - J_4 + J_{-4}$$

$$\frac{d[ESI]}{dt} = +J_4 - J_{-4} + J_5 - J_{-5}$$

want to eliminate via approx if possible

Assume mass action (constitutive model)

$$J_1 = k_1[E][S]$$
$$J_{-1} = k_{-1}[ES]$$
$$J_2 = k_2[ES]$$
$$J_3 = k_3[E][I]$$
$$J_{-3} = k_{-3}[EI]$$
$$J_4 = k_4[ES][I]$$
$$J_{-4} = k_{-4}[ESI]$$
$$J_5 = k_5[EI][S]$$
$$J_{-5} = k_{-5}[ESI]$$

> Note careful to only include 'active' participants

> Q: what can we say about k_3 vs k_4? k_1 vs k_5? A →

we could simulate etc the whole system.

→ here we want a reduced model instead

Reductions

1. Total enzyme (in all forms) is conserved

$$E = E_0 - [ES] - [EI] - [ESI]$$

2. Noncompetitive
   - assume binding/unbinding of [S]/[I] unaffected by other

gives ↓

same: substrate binding/unbinding

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

$$EI + S \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} ESI$$

set $k_1 = k_5$, $k_{-1} = k_{-5}$

define $K_S = \frac{k_1}{k_{-1}} = \frac{k_5}{k_{-5}}$

&

same: inhibitor binding/unbinding

$$E + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EI$$

$$ES + I \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} ESI$$

set $k_3 = k_4$, $k_{-3} = k_{-4}$

define $K_I = \frac{k_3}{k_{-3}} = \frac{k_4}{k_{-4}}$

Reductions: approximations

- Quasi-steady vs Quasi-equilibrium

Quasi-steady state is probably conceptually better

→ but a bit messy

⇒ Quasi-equilibrium a bit

→ easier and gives same basic result here

I will use this

Note: these approximations allow us to focus on solving system of algebraic equations

(Still good practice to write out full system!

→ & gives time soln.

So...

- Assume all enzyme binding/unbinding reactions at equilibrium
- Use conservation of total enzyme (all forms)
- Mass action with rate constants for S/I indep. of I/S binding ($\frac{k_{-1}}{k_1} = \frac{k_{-5}}{k_5} = K_S$ & $\frac{k_{-3}}{k_3} = \frac{k_{-4}}{k_4} = K_I$)

$$(E_0 - [ES] - [EI] - [ESI])[S] - K_S[ES] = 0$$

$$(E_0 - [ES] - [EI] - [ESI])[I] - K_I[EI] = 0$$

$$[EI][S] - K_S[ESI] = 0$$

$$[ES][I] - K_I[ESI] = 0$$

⇒ 4 equations but only 3 independent (note symmetry in S & I)

⇒ use to eliminate [ES, EI, ESI]

Remember goal: production rate in terms of [S], [I] & parameters

Have: $\boxed{v = J_p = k_2[ES]}$

---- solve (by hand or computer -->

$$[ES] = \left(\frac{E_0 K_I}{K_I + [I]}\right)\left(\frac{[S]}{K_S + [S]}\right)$$

$$\Rightarrow v = J_p = k_2[ES]$$

$$= \left(\frac{k_2 E_0 K_I}{K_I + [I]}\right)\left(\frac{[S]}{K_S + [S]}\right)$$

constitutive eqn:

$$\Rightarrow \boxed{v = \frac{V_{max}^{new}[S]}{K_S + [S]}}$$

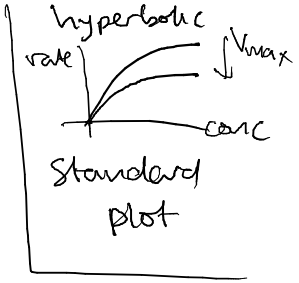
[MM form]

↓ V_{max}

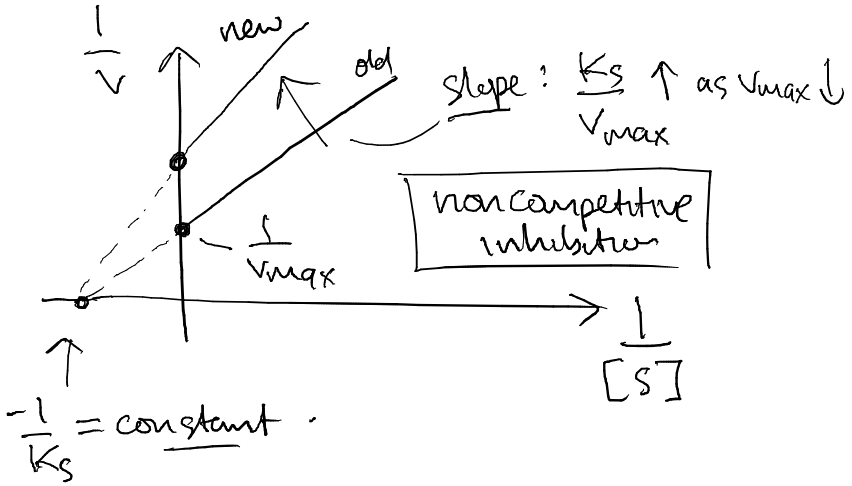
where $\boxed{V_{max}^{new} = \left(\frac{k_2 E_0 K_I}{K_I + [I]}\right) = \frac{V_{max}^{old}}{1 + [I]/K_I}}$

Plotting

- Same shape (MM) as before
- New Vmax (↓) parameter value



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



Lineweaver-Burk / Double-reciprocal plot

Cooperative effects: multiple active sites.

- So far we have considered allosteric ('other shape') site effects where the other substance (inhibitor/activator) is not a primary substrate of the reaction
  - i.e just regulates
- Many enzymes/proteins have multiple active sites & can bind multiple primary substrate molecules
- If the overall result is just a simple sum then there is no complication

→ BUT often there is an interaction

Cooperativity

Positive: binding of one substrate molecule increases the binding rate of substrate to remaining active sites

Negative: binding of one substrate molecule decreases the binding rate of substrate to remaining active sites

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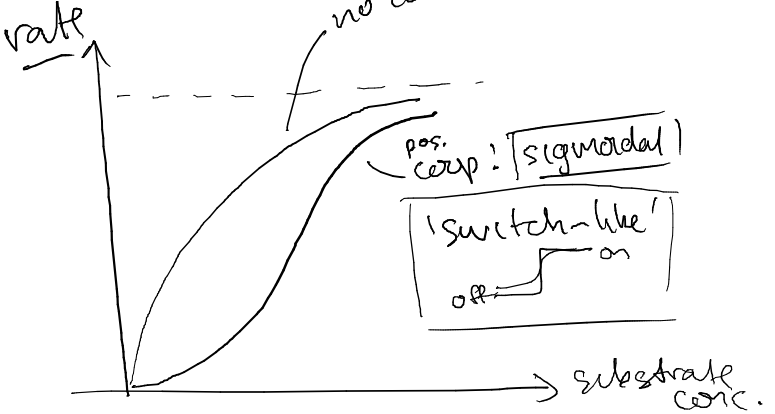
Positive: Changes curve shape → Non-MM. ∴ hyperbolic



We need a new model that can given non-hyperbolic, sigmoidal shape

Simple model: Two active sites

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1 \xrightarrow{k_2} P$$

$$S + C_1 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_2 \xrightarrow{k_4} C_1 + P$$

Gives

$$\frac{d[S]}{dt} = -k_1[S][E] + k_{-1}[C_1] - k_3[S][C_1] + k_{-3}[C_2]$$

$$\frac{d[C_1]}{dt} = k_1[S][E] - (k_{-1}+k_2)[C_1] - k_3[S][C_1] + (k_4+k_{-3})[C_2]$$

$$\frac{d[C_2]}{dt} = k_3[S][C_1] - (k_4+k_{-3})[C_2]$$

$$[E] = E_0 - [C_1] - [C_2]$$

conservation + mass action

Quasi-steady state assumption (QSSA)

$$\frac{d[C_1]}{dt} = \frac{d[C_2]}{dt} = 0$$ (eliminate complexes)

Solve algebraic equations

$$\Rightarrow [C_1] = \frac{E_0 K_2 [S]}{K_1 K_2 + K_2 [S] + [S]^2}$$

$$[C_2] = \frac{E_0 [S]^2}{K_1 K_2 + K_2 [S] + [S]^2}$$

where $K_1 = \frac{k_{-1} + k_2}{k_1} = K_M^{(1)}$

$$K_2 = \frac{k_{-3} + k_4}{k_3} = K_M^{(2)}$$

$\Rightarrow$

So...

$$J = v = k_2 [C_1] + k_4 [C_2]$$

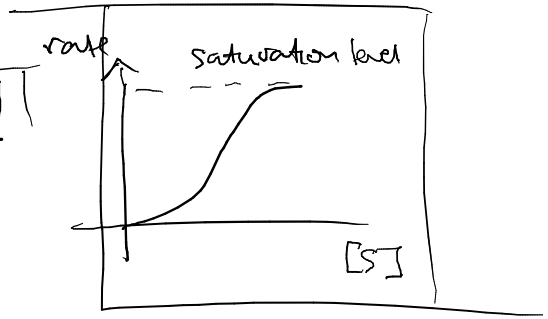
i.e.

$$v = \frac{(k_2 K_2 + k_4 [S]) E_0 [S]}{K_1 K_2 + K_2 [S] + [S]^2}$$

Constit. eqn for cooper. effects

Not hyperbolic ✗ NOT MM

eq is sigmoidal



Qs: - can we simplify further?

- can we generalise to n binding sites?

(More) Simplification of two-site case

Case 1: Independent & identical binding sites

- $k_1 = 2k_3 = 2k_+$, where k_+ is indiv. site rate
  - forward: { two free sites to bind to on C_1; one free site " " " " C_2
- $k_{-3} = 2k_{-1} = 2k_-$, (k_- is indiv.)
  - reverse: { C_2 has two bound molecules; C_1 has one " "
- $k_4 = 2k_3$
  - forward: { C_2 has two bound molecules. C_1 has one

Gives $$v = 2\left(\frac{k_2 E_0 [S]}{K_M + [S]}\right)$$ Boring!

just double reaction rate as expected

where $K_M = \frac{k_- + k_2}{k_+}$ (individual site K_M)

No cooperativity

Case 2: Proper interaction effects (coop.).

- Limiting case (simplification)

use $\frac{k_3}{k_1} >> 1$ i.e. $k_3 \to \infty$, $k_1 \to 0$

while $k_1 k_3 = \text{const}$

$\Rightarrow$ second binding much faster

Gives $$\frac{K_1}{K_2} = \frac{k_{-1} + k_2}{k_1} \times \frac{k_3}{k_4 + k_{-3}} >> 1$$

i.e. $k_1 \to \infty, k_2 \to 0$

while $K_1 \cdot K_2 = \frac{(k_{-1} + k_2)(k_4 + k_{-3})}{k_1 k_3}$

$= \text{const.}$

Result: $$v = \frac{k_4 E_0 [S]^2}{K_M^2 + [S]^2} = \frac{V_{max}[S]^2}{K_M + [S]^2}$$

$V_{max} = k_4 E_0$

$K_M^2 = K_1 K_2$

Note: $[S]^2$ not $[S]$!

Sigmoidal

Hill equation for n cooperative sites.

General case

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

- use n quasi-equilibrium eq's

- $K_n / K_1 >> 1$ & $K_1 K_n = \text{constant}$

Leads to 'Hill equation'

$$v = \frac{V_{max}[S]^n}{K_M^n + [S]^n}$$

where $K_M = \prod_{i=1}^{n} K_i$

Hill coeff. & Hill plot

We typically treat the Hill Eq^n as an empirical model for cooperativity & fit

"Hill coefficient" → n

V_{max}, K_M, n — 3 free parameters

Taking logs & rearranging gives

$$\ln\left(\frac{v}{V_{max} - v}\right) = n \ln[S] - n \ln K_M$$

ie $Y = mx + c$

Notes:

- in real systems we let n be non-integer

- v is also interpreted as (prop. to) the 'saturation fraction', ie total proportion of occupied binding sites (eg $[C]/E_0$)