

BIOMENG 261

TISSUE AND BIOMOLECULAR ENGINEERING

Module I: Reaction kinetics and systems biology

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LECTURE 6 ENZYMES CONTINUED AND COMPLICATED...CONTINUED

- Summary of reversible inhibition types
 - Uncompetitive (briefly)
 - Double-reciprocal plots for each type
- A note on irreversible inhibition
- Cooperativity effects
 - Hill equations (non-Michaelis-Menten!)

1

3

MODULE OVERVIEW

Reaction kinetics and systems biology (*Oliver Maclarens*)
[12 lectures/3 tutorials/2 labs]

1. Basic principles: modelling with reaction kinetics [6 lectures]

Physical principles: conservation, directional and constitutive. Reaction modelling. Mass action. Enzyme kinetics. Enzyme regulation. Mathematical/graphical tools for analysis and fitting.

2. Systems biology I: overview, signalling and metabolic systems [3 lectures]

Overview of systems biology. Modelling signalling systems using reaction kinetics. Introduction to parameter estimation. Modelling metabolic systems using reaction kinetics. Flux balance analysis and constraint-based methods.

3. Systems biology II: genetic systems [3 lectures]

Modelling genes and gene regulation using reaction kinetics. Gene regulatory networks, transcriptomics and analysis of microarray data.

RECALL: INHIBITOR TYPE

Competitive:

- Substrate and inhibitor can't be bound at the same time

Noncompetitive:

- Inhibitor can bind to either/both enzyme and complex
- Only slows product step
- Doesn't affect binding of substrate

Uncompetitive:

- Inhibitor can only bind to substrate-enzyme complex (not free enzyme)
- Prevents both product step and reversible unbinding step

2

4

COOPERATIVITY: DEFINITION

Positive cooperativity:

- Binding of one *substrate* molecule *increases subsequent rates* of binding of substrate molecules to *remaining active sites*

Negative cooperativity:

- Binding of one *substrate* molecule *decreases subsequent rates* of binding of substrate molecules to *remaining active sites*

5

Leads to... (see handout)

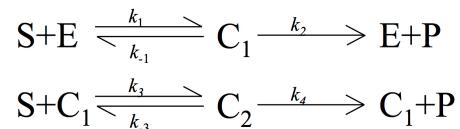
$$J_P = v = \frac{V_{\max}[S]^n}{K_M^n + [S]^n}$$

- As empirical model: *three free parameters*
- Non-hyperbolic i.e. is *sigmoidal*

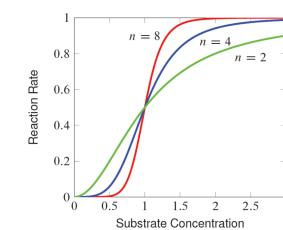
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EFFECT OF N AND HILL PLOTS

SIMPLE MODEL: TWO ACTIVE SITES



Implications: non-Michaelis-Menten, *sigmoidal* behaviour
(see handout)



Convert to *Hill plot*: rewrite as

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

- Gives *straight line* for fitting

6

8

Biomeng 261 Lecture 6:

o Enzyme regulation

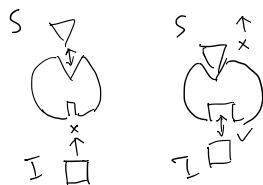
- summary of competitive, non-competitive, uncompetitive (reversible) inhibition
- note on irreversible inhibition.

o Cooperative effects

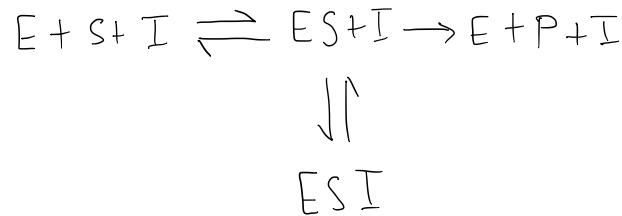
- multiple active sites for main substrate

Uncompetitive?

- only bind to complex
- prevents subsequent unbinding & production steps



Uncompetitive scheme:

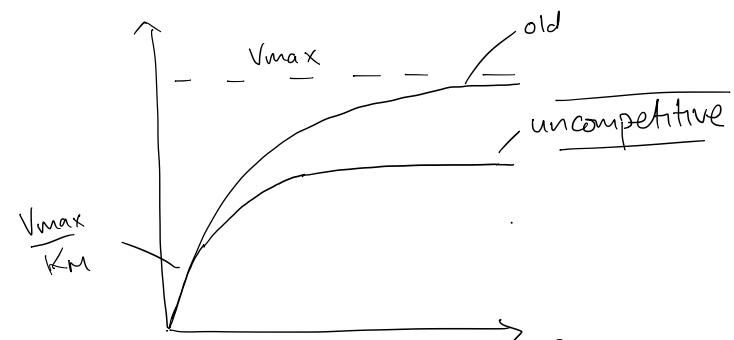


Can show (exercise?)

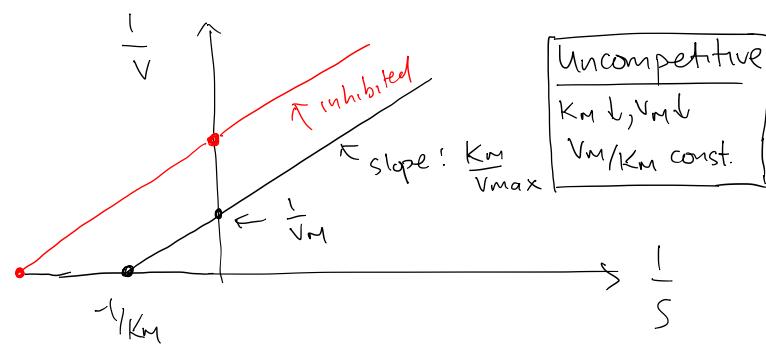
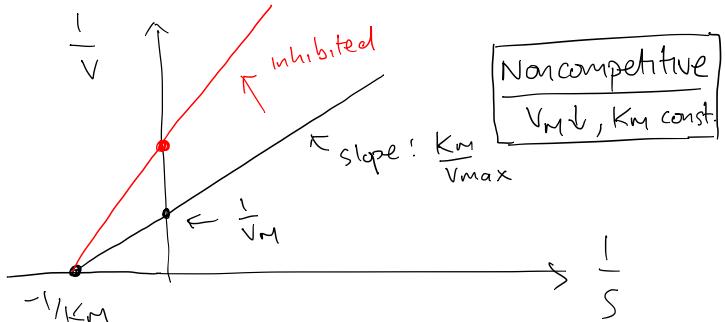
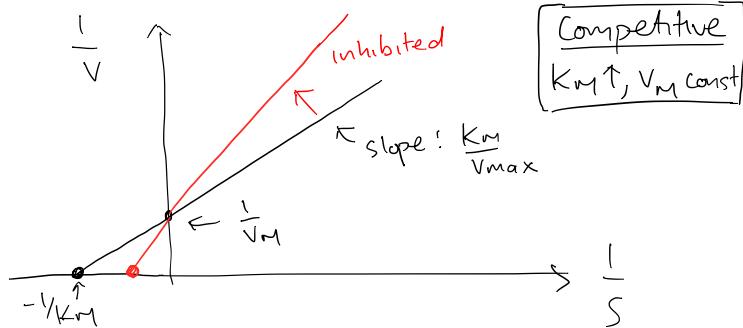
- Both V_m & K_m ↓
- But $\frac{V_m}{K_m}$ unchanged

So:

rate



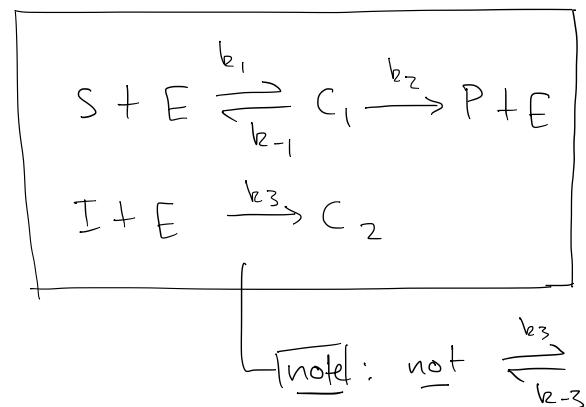
Summary : double-reciprocal plots (Key!)



Note on irreversible inhibition

- We've looked at reversible inhibition
- How could we model irreversible inhibition? Same ideas!

Example: competitive irreversible inhibition [eg via hydrogen cyanide (HCN)]

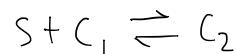
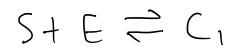
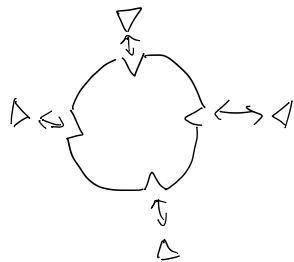


→ once in C_2 form, can't get back

→ could also do $I + E \rightarrow \emptyset \quad \{ \text{sink} \}$
 (don't track)

Cooperation?

- About how the primary substrate interacts with itself & the enzyme
- Models are based on the presence of multiple active sites (not allosteric)
- Many enzymes do in fact have multiple active sites!



⋮
etc.

Cooperation

so:

- The activation/inhibition is due to multiple primary substrate molecules binding, not a different regulating molecule (as before)

- If the overall result is just a simple 'sum' of effects then there is no complication

BUT: often there is an interaction

what does this mean?

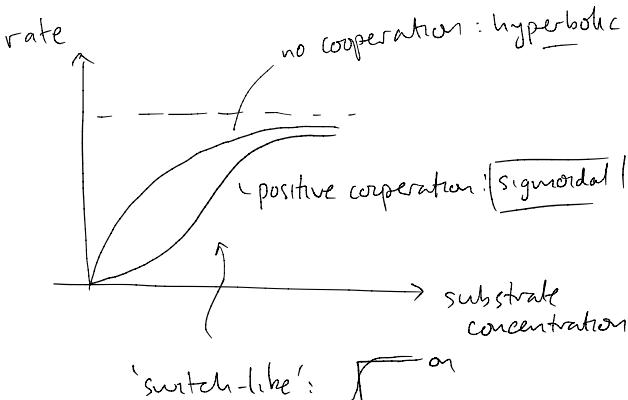


Cooperativity

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

Positive: changes curve 'shape' } ^{eg} hemoglobin!
→ Not MM shape anymore



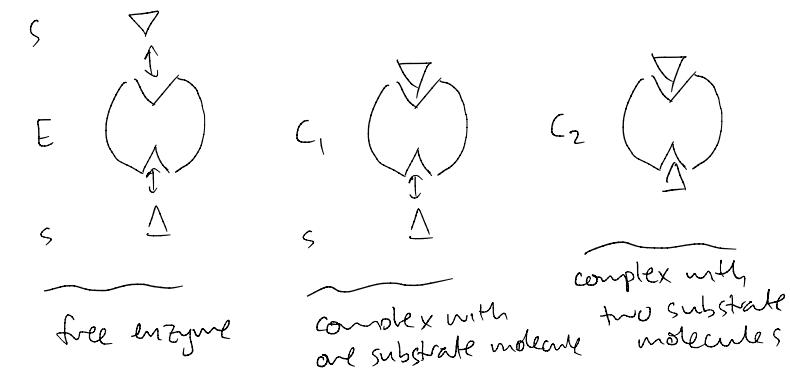
(negative doesn't change 'shape', just slope)

Now 'switch-like', & non-Michaelis-Menten

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

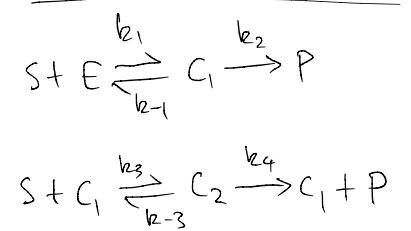
→ will again consider derivation via underlying mass action + QSS/QE approximation

First: simple two active site model



Kinetics:

&



Gives (conservation of mass + mass action):

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

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

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

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

$$\frac{d[P]}{dt} = k_2[C_1] + k_4[C_2] \leftarrow \text{note!}$$

Reduction?

Again: use quasi-steady state assumption (QSSA)
— to (approximately) eliminate C_1, C_2

set $\begin{cases} \frac{d[C_1]}{dt} = 0 \\ \frac{d[C_2]}{dt} = 0 \end{cases}$

\Rightarrow two algebraic equations,
& linear in C_1, C_2 .

Solving gives

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

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

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

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

so $J = v = \frac{d[P]}{dt} = k_2[C_1] + k_4[C_2]$

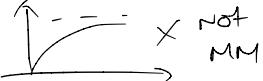
becomes

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

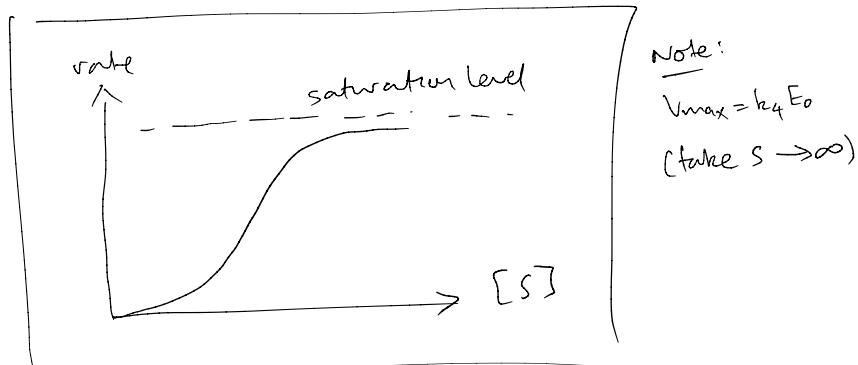
new type of 'constitutive equation'
for cooperative effects \rightarrow

Understanding?

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

Plot: not hyperbolic 

is sigmoidal ie looks like:

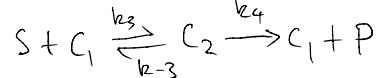
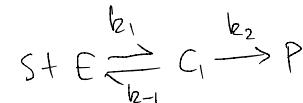


Qs! - can we simplify further?

- can we generalise to n binding sites?

Additional simplification of two-site case

Recall:



} what can we say about k_2 ?

consider two possible cases.

o Case 1: Independent & Identical binding sites

o $k_1 = 2k_3 = 2k_f$, where k_f is individual site rate

forward: { two free sites to bind to make C_1 , one free site to bind to make C_2

o $k_{-3} = 2k_{-1} = 2k_r$, k_r is individual site rate

reverse: { C_2 has two bound molecules, C_1 has one bound molecule

o $k_4 = 2k_2$

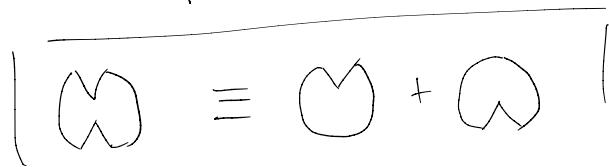
forward: { C_2 has two bound molecules, C_1 has one bound molecule

Case 1 assumptions lead to ...

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

$$\text{where } K_m = \frac{k_f + k_2}{k_f} \quad \left. \begin{array}{l} \text{individual} \\ \text{site } K_m \end{array} \right\}$$

→ just double reaction rate as expected



→ no cooperativity

Case 2. Proper interaction effects (coop.)

• Limiting case (simplification!)

→ 'second binding much faster'

Math: (details a bit beyond scope?)

use $\frac{k_3}{k_2} \gg 1 \quad \text{i.e. } \frac{k_3 \rightarrow \infty}{k_2 \rightarrow 0}$

while $\frac{k_1 k_3}{k_2} = \text{constant}$

then $\frac{K_1}{K_2} = \left(\frac{k_1 + k_2}{k_1} \right) \times \left(\frac{k_3}{k_4 + k_3} \right) \gg 1$

i.e. take $K_1 \rightarrow \infty, K_2 \rightarrow 0$

while $K_1 K_2 = \left(\frac{k_1 + k_2}{k_1} \right) \left(\frac{k_4 + k_3}{k_3} \right)$

i.e. take $K_1 K_2 = \text{const.}$

Result: $v = \frac{k_f E_0 [S]^2}{K_m^2 + [S]^2}$

note $[S]^2$
not $[S]$.

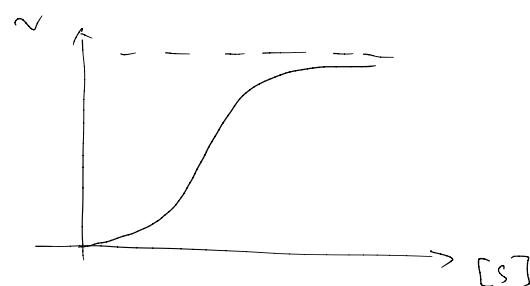
Case 2 result:

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

where: $\begin{cases} v_{\max} = k_4 E_0 \\ K_M^2 = K_1 K_2 \end{cases}$

→ almost MM, but...

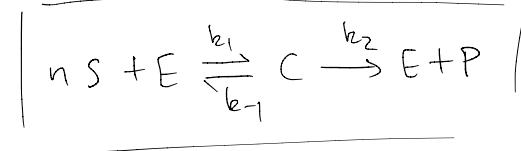
Plot:



Sigmoidal !

General case: Hill equation for n cooperative sites

write schematically as



- use • n quasi-equilibrium eqns
- $K_1/K_n \gg 1$ & $K_1 K_n = \text{const.}$

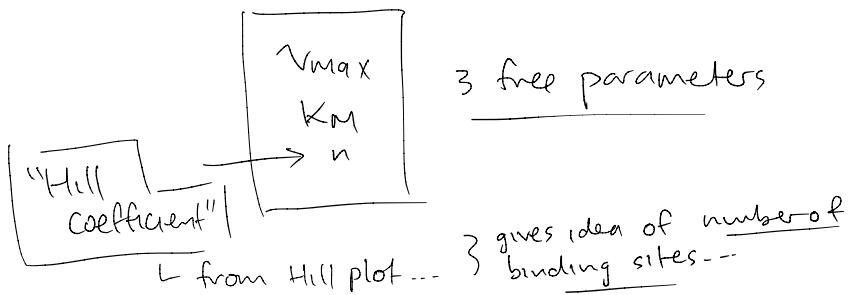
leads to the 'Hill equation'

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

where $\begin{cases} K_M^n = \prod_{i=1}^n K_i \\ v_{\max} = k_2 E_0 \end{cases}$

Hill coeff. & Hill Plot

we typically treat the Hill eqn as
an empirical constitutive equation/
model for cooperativity & fit



Hill Plot

Taking reciprocals & then taking logs &
then rearranging -- gives

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

↑ slope

$$\text{ie } Y = m \cdot x + c$$

(straight line)

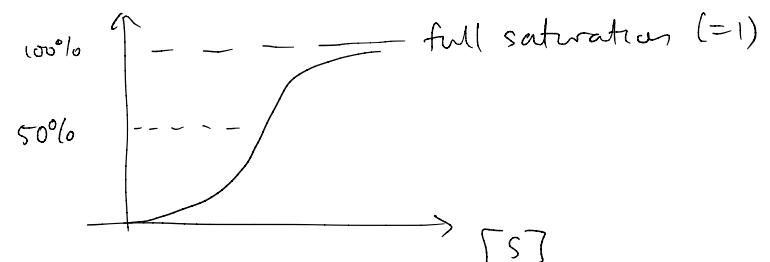
Notes:

- in real systems we usually
let n be non-integer

- $\begin{cases} n > 1 & \text{positive coop.} \\ n = 1 & \text{no coop.} \\ n < 1 & \text{negative coop.} \end{cases}$

- n can also be interpreted as approx.
(proportional to) the 'saturation fraction',
ie the total proportion of occupied
binding sites to available binding sites:

$$N/N_{\max}$$



For more, see e.g.:

Or

