BIOMENG 261

TISSUE AND BIOMOLECULAR ENGINEERING

Module I: Reaction kinetics and systems biology

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MODULE OVERVIEW

Reaction kinetics and systems biology (*Oliver Maclaren*)
[11-12 lectures/3 tutorials/2 labs]

1. *Basic principles: modelling with reaction kinetics* [5-6 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: 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.

LECTURE 6 ENZYMES CONTINUED AND COMPLICATED...CONTINUED

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

RECALL: INHIBITOR TYPE

Competitive:

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

Uncompetitive:

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

Noncompetitive:

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

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*

SIMPLE MODEL: TWO ACTIVE SITES

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

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

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

N BINDING SITES: THE HILL EQUATION

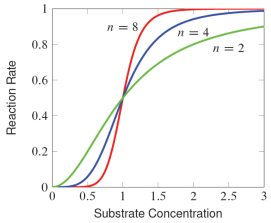
$$nS + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} E + P$$

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*

EFFECT OF N AND HILL PLOTS



Convert to *Hill plot*: rewrite as

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

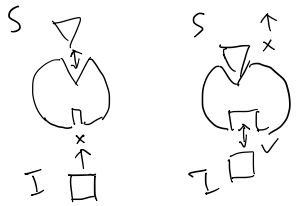
- Gives *straight line* for fitting

Biomeng 261 Lecture 6:

- Enzyme regulation
  - summary of competitive, non-competitive, uncompetitive (reversible) inhibition
  - note on irreversible inhibition.
- Cooperative effects
  - multiple active sites for main substrate

Uncompetitive?

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



Uncompetitive scheme:

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

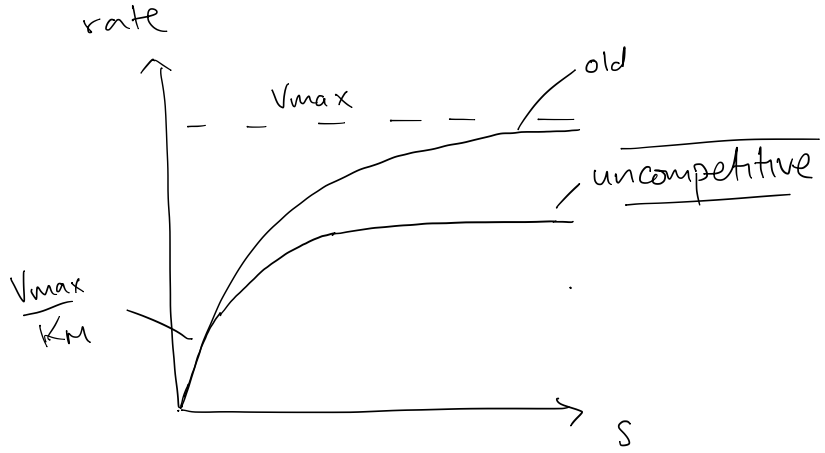
$$\downarrow\uparrow$$

$$ESI$$

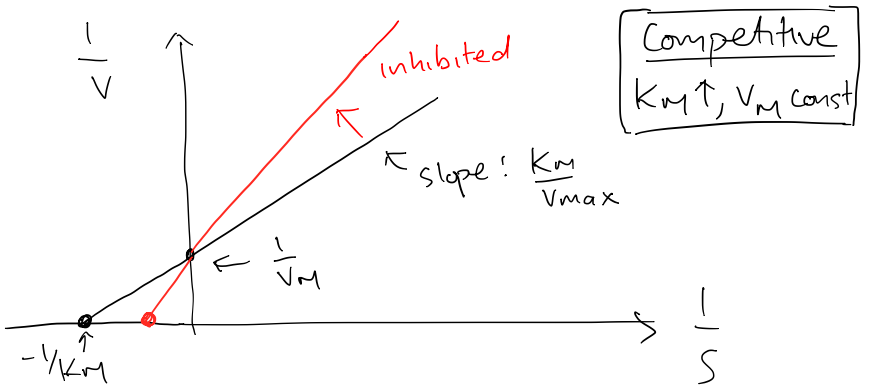
Can show (exercise?)

- Both V_M & K_M ↓
- But $\frac{V_M}{K_M}$ unchanged

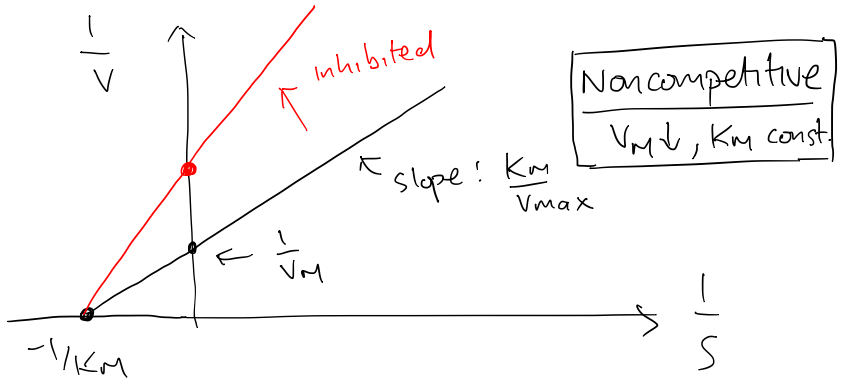
So:



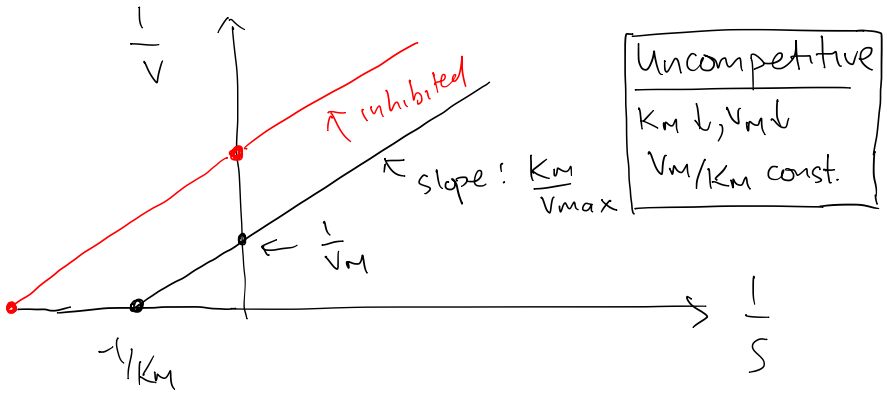
Summary: double-reciprocal plots (key!)



Competitive
K_M ↑, V_M const.



Noncompetitive
V_M ↓, K_M const.



Uncompetitive
K_M ↓, V_M ↓
V_M/K_M const.

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)]

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

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

note: not $\underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}}$

→ once in C_2 form, can't get back

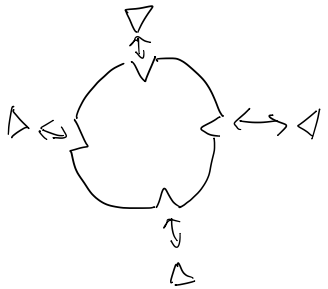
→ could also do $I + E \rightarrow \emptyset$ } 'sink' (don't track)

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!



$S + E \rightleftharpoons C_1$

$S + C_1 \rightleftharpoons C_2$

⋮

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

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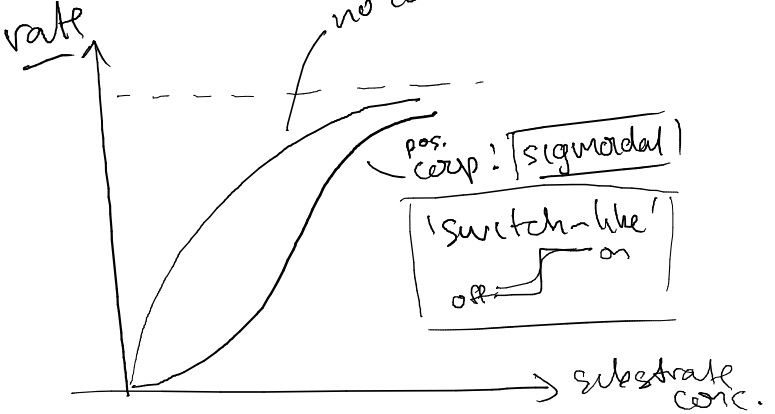
Positive: Changes curve shape → Non-MM. ∴ hyperbolic



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...

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

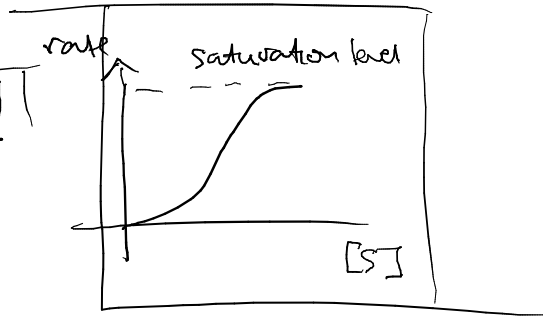
i.e.

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

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 $[C]/E_0$)