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 5 ENZYMES CONTINUED AND COMPLICATED

- Noncompetitive inhibition example

RECALL: INHIBITOR TYPE

Competitive:

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

Uncompetitive:

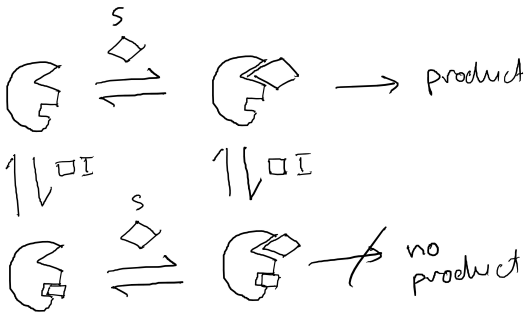
- Inhibitor can only bind to substrate-enzyme complex (not free enzyme)
- Prevents both product step and reversible unbinding step

Noncompetitive:

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

ENZYMES REGULATION: NONCOMPETITIVE INHIBITION EXAMPLE

Picture



NONCOMPETITIVE INHIBITION EXAMPLE

Reaction scheme

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

$$k_{-3} \Big\| k_3 \qquad k_{-4} \Big\| k_4$$

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

NONCOMPETITIVE INHIBITION EXAMPLE

Assumptions:

- *Noncompetitive* rates
- *Quasi-equilibrium* assumption
- *Conservation* of total enzyme

Leads to...(see handout)

NONCOMPETITIVE INHIBITION EXAMPLE

$$\left(\mathrm{E}_0 - [\mathrm{ES}] - [\mathrm{EI}] - [\mathrm{ESI}]\right)[\mathrm{S}] - K_S[\mathrm{ES}] = 0$$

$$\left(\mathrm{E}_0 - [\mathrm{ES}] - [\mathrm{EI}] - [\mathrm{ESI}]\right)[\mathrm{I}] - K_I[\mathrm{EI}] = 0$$

$$[\mathrm{EI}][\mathrm{S}] - K_S[\mathrm{ESI}] = 0$$

$$[\mathrm{ES}][\mathrm{I}] - K_I[\mathrm{ESI}] = 0$$

which leads to...

KEY RESULT

Again, *same MM form* of equation, but *modified* V_{max} constant:

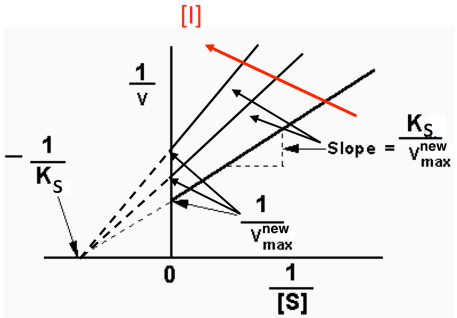
$$J_P = v = \frac{V_{max}^{new}[S]}{K_M + [S]}$$

where here

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

$$K_S = K_M = \frac{k_{-1}}{k_1} = \frac{k_{-5}}{k_5}, K_I = \frac{k_{-3}}{k_3} = \frac{k_{-4}}{k_4}$$

PLOTTING: DOUBLE-RECIPROCAL PLOT



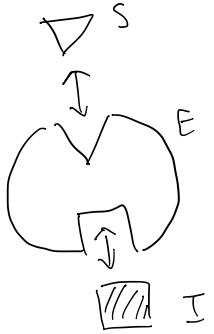
(i.e. *Lineweaver-Burk plot*)

Biomeng 261 Lecture 5:

Enzyme regulation cont'd

↳ non-competitive, reversible inhibition model

Non-competitive inhibition model



- Inhibitor I binds to either enzyme or complex at allosteric (not active) site
- stops production step
- doesn't affect binding/unbinding

General model

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

$$k_3 \downarrow\uparrow k_{-3} \qquad k_{-4} \uparrow\downarrow k_4$$

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

(all at once)

Parts:

Here just showing which quantities are used in reaction →

$$E + S \overset{1}{\rightleftharpoons} ES \xrightarrow{2} E + P$$

$$E + I \overset{3}{\rightleftharpoons} EI$$

$$ES + I \overset{4}{\rightleftharpoons} ESI$$

$$EI + S \overset{5}{\rightleftharpoons} ESI$$

9 Fluxes, including 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:

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

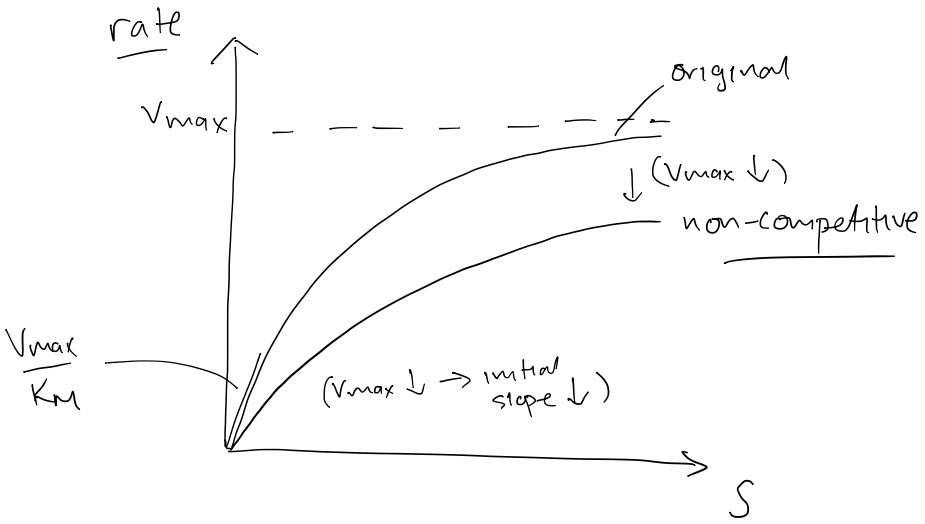
[MM form]

↓ 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 MM form as before
- New V_{max} (↓)
- Same $K_S = K_M = \frac{k_{-1}}{k_1}$ (Quasi-Eq.) as before.



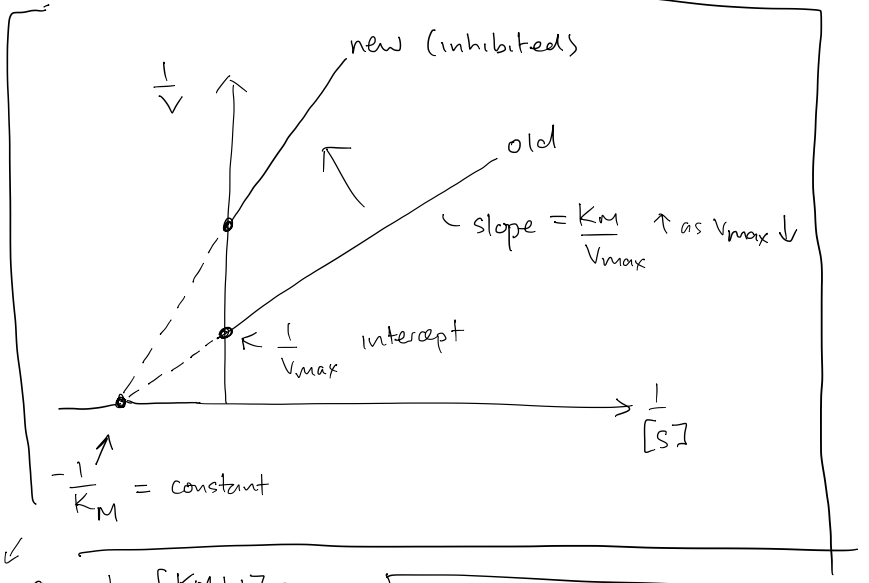
Lineweaver-Burk / Double-reciprocal plots

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

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

ie $\boxed{Y = M \cdot X + C}$

So: **Noncompetitive Inhibition**



$\frac{1}{v} = 0 \Rightarrow \frac{1}{V_{max}}\left[\frac{K_M}{[S]} + 1\right] = 0$

$\Rightarrow [S] = -K_M$

$\Rightarrow \frac{1}{[S]} = -\frac{1}{K_M}$ ✓

Exercise: do same plot for previous lecture example (competitive).