

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 Maclarens*)
[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 4 ENZYME REGULATION: INHIBITION AND ACTIVATION

- Types of enzyme regulation
- Example: competitive inhibition

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ENZYMES REGULATION: MOTIVATION/IDEA

Enzyme catalysed reactions are controlled/regulated in various ways

E.g.

- *Amount* (how many enzymes)
 - E.g. via gene expression (see later)
- *Activity* ('on' and 'off', 'up' and 'down')
 - E.g. via regulatory molecules binding to enzyme

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REVERSIBLE REGULATION TYPES

Behaviour/effect type:

REGULATION OF ENZYMES ACTIVITY

Regulatory molecules that bind to enzyme:

- *Activator*: 'turn up' enzyme
- *Inhibitor*: 'turn down' enzyme

• Competitive

• Noncompetitive

• Uncompetitive

Location of regulation interaction:

• active site (where substrate binds)

• allosteric site (not where substrate binds)

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LOCATION OF REGULATION INTERACTION

IRREVERSIBLE VS REVERSIBLE REGULATION

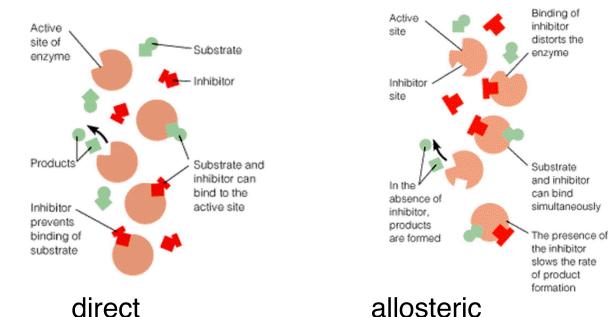
Irreversible:

- E.g. toxin that 'shuts down' enzyme permanently
- Not so good for control!
- Typically via strong (covalent) interaction

Reversible:

- *Better for control!*
- Typically via weaker (non-covalent) interaction

We'll (mainly) focus on reversible regulation and 'control'



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BEHAVIOUR/EFFECT INHIBITOR TYPE

Competitive:

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

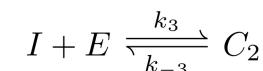
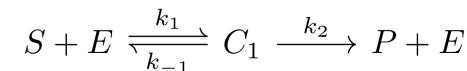
Uncompetitive:

- 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

MODEL SCHEME



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MODEL SUMMARY

MODEL

Goal here: derive model of *direct, competitive inhibition*

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$$\frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[C_1] \quad \text{and the reaction rate}$$

$$\frac{d[I]}{dt} = -k_3[E][I] + k_{-3}[C_2] \quad v = \frac{d[P]}{dt} = k_2[C_1]$$

$$\frac{d[C_1]}{dt} = k_1[E][S] - (k_{-1} + k_2)[C_1] \quad \bullet \text{ Enzyme is conserved}$$

$$\frac{d[C_2]}{dt} = k_3[E][I] - k_{-3}[C_2] \quad [E] + [C_1] + [C_2] = E_0$$

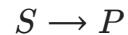
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REDUCTION: 'REMOVING' THE ENZYME

Goal is to *eliminate the enzyme terms* from the equations using:

- Conservation of total enzyme (including its complex form)
- Quasi-steady-state for C_1 and C_2

Result: as before we get an effective, MM-style constitutive equation for



but now the constants depend on I !

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KEY RESULT

Get *same MM form* of equation, but *modified* K_M constant:

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

where here

$$K_M^{\text{new}} = K_M^{\text{old}} \left(1 + \frac{[I]}{K_I}\right)$$

and

$$K_I = \frac{k_{-3}}{k_3}$$

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Biomeng 261 Lecture 4.

Enzyme regulation: inhibition & activation (mainly)

'we (ie our bodies, nature etc)

don't just want enzymes to
be 'always on'

→ they are tightly
controlled

↳ adapt to needs &
resources

How?

- Amount

Leg via gene expression
(see later)

- Activity

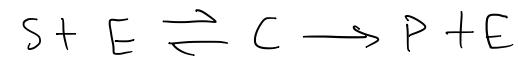
↳ Regulatory molecules

activator: turn 'up'

inhibitor: turn 'down'

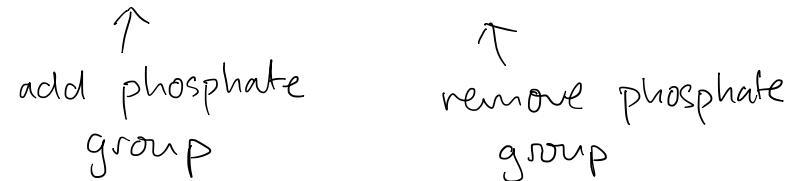
Recap: roles of enzymes - examples.

- Example: speed-up reaction $S \rightarrow P$



- Example: 'activate' another protein by phosphorylating it

Kinase & Phosphatase



i.e. convert $A \rightarrow A^*$ 'active' form



B 'activates' A

Regulation of activity: types

- irreversible (eg toxins)

↳ not usually for control

↳ typically covalent (strong)

— main focus { — reversible

- ↳ good for control
- ↳ typically non-covalent (weaker)

Reversible regulation: more terminology

behaviour {

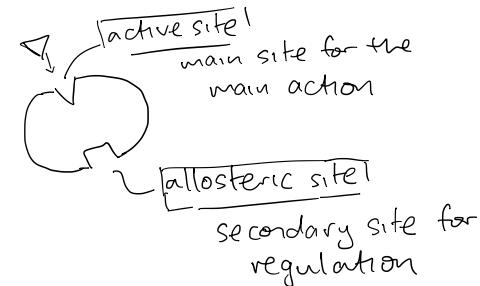
- competitive ← today
- non-competitive
- uncompetitive
- mixed

location {

- active site
- allosteric site

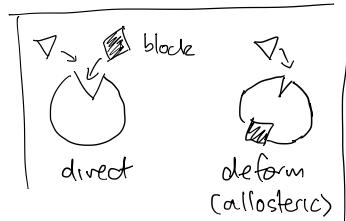
Locations

allos: 'other'
stereos: 'solid object'



Behaviour: competitive vs un vs non

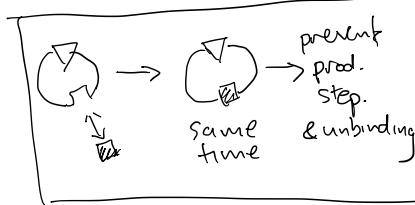
• competitive:



◦ substrate & inhibitor
[can't bind] enzyme
at same time

↳ can be direct or allosteric
mechanism/location

• uncompetitive:

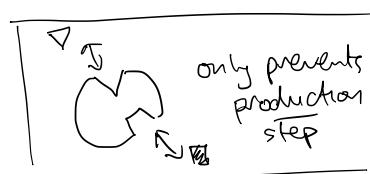


◦ regulator only binds
to complex

◦ slows unbinding &
production reactions

◦ reduces available
'active' enzyme &
substrate

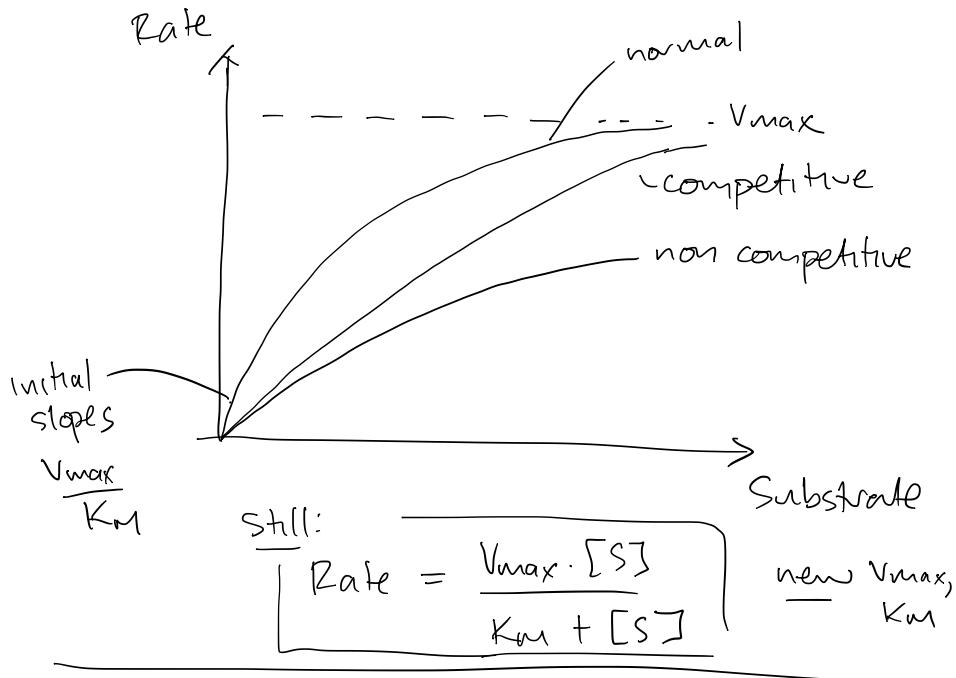
• non-competitive:



◦ binds to either/both
enzyme/complex

◦ only slows production
step

Will show we get same final form
 for equation, but modified
constants, i.e.



Goal: derive effects for simple
 — models of inhibition

- 1. competitive (V_{max} same, $K_m \uparrow$)
- 2. non-competitive ($V_{\text{max}} \downarrow$, K_m same)

(uncompetitive: both V_{max} & K_m affected)

Competitive inhibition model

Same idea as building MM model

Simple but larger systems
 ↓
 reduce to smaller but
modified system

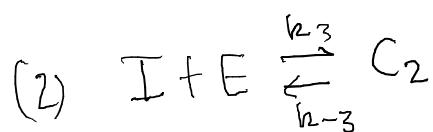
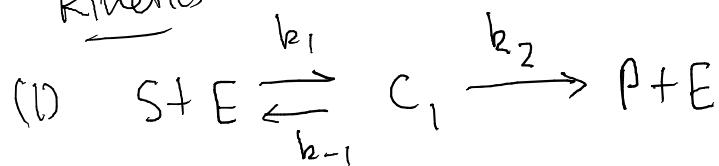


Assume

- Inhibitor I directly competes with substrate S.

⇒ Two types of complex $\begin{cases} C_1 = E:S \\ C_2 = E:I \end{cases}$

Kinetics:



Competitive inhibition model cont'd

i. conservation $[S], [I], [C_1], [C_2], [E], [P]$

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

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

$$\frac{d[C_1]}{dt} = +J_1 - J_{-1} - J_2$$

$$\frac{d[C_2]}{dt} = +J_3 - J_{-3}$$

can always eliminate $[E]$

$$\begin{aligned} \frac{d[E]}{dt} &= (-J_1 + J_{-1} + J_2) + (-J_3 + J_{-3}) \\ &= -\frac{d[C_1]}{dt} - \frac{d[C_2]}{dt} \\ \Rightarrow \boxed{[E] + [C_1] + [C_2] &= E_0} \end{aligned}$$

$$\approx \frac{d[P]}{dt} = +J_2 \quad \boxed{\text{Goal}}$$

2. Using mass action

[exercise!]

gives:

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

$$\frac{d[I]}{dt} = -k_3[E][I] + k_{-3}[C_2]$$

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

$$\frac{d[C_2]}{dt} = k_3[E][I] - k_{-3}[C_2]$$

$$\& \quad [E] = E_0 - [C_1] - [C_2]$$

$$v = \frac{d[P]}{dt} = k_2[C_1] \quad (\text{uncoupled})$$

Note :

(use $E = f(C_1, C_2)$)

$$1. \frac{d[S]}{dt} = f_1(\underline{C_1}, \underline{C_2}, S)$$

$$2. \frac{d[I]}{dt} = f_2(\underline{C_1}, \underline{C_2}, I)$$

$$3. \frac{d[C_1]}{dt} = f_3(\underline{C_1}, \underline{C_2}, S)$$

$$4. \frac{d[C_2]}{dt} = f_4(\underline{C_1}, \underline{C_2}, I)$$

$$5. \frac{d[P]}{dt} = f_5(\underline{C_1})$$

Goal: eliminate enzyme complex
too (C_1 & C_2)

\Rightarrow need two conditions/approx.

• QSS C_1
• QSS C_2

① $[C_1]$ QSS ie

$$\boxed{\text{set } \frac{d[C_1]}{dt} \approx 0 \text{ relative to } \frac{d[S]}{dt} \text{ (see also later)}}$$

$$\Rightarrow k_1 [E] [S] - (k_1 + k_2) [C_1] \approx 0$$

$$\Rightarrow \boxed{k_1 (E_0 - [C_1] - [C_2]) [S] - (k_1 + k_2) [C_1] = 0}$$

② $[C_2]$ QSS

$$\Rightarrow \boxed{k_3 (E_0 - [C_1] - [C_2]) [I] - k_{-3} [C_2] = 0}$$

two equations \Rightarrow eliminate two variables

C_1, C_2 .

! !
details:
(exercise!) } tutorial Q
! !

$$\Rightarrow \boxed{[C_1] = \frac{K_I E_0 [S]}{K_M [I] + K_I [S] + K_M K_I}}$$
$$[C_2] = \frac{K_M E_0 [I]}{K_M [I] + K_I [S] + K_M K_I}$$

where

$$\boxed{K_I = \frac{k_{-3}}{k_3}}$$
$$\boxed{K_M = \frac{k_1 + k_2}{k_1}}$$

so

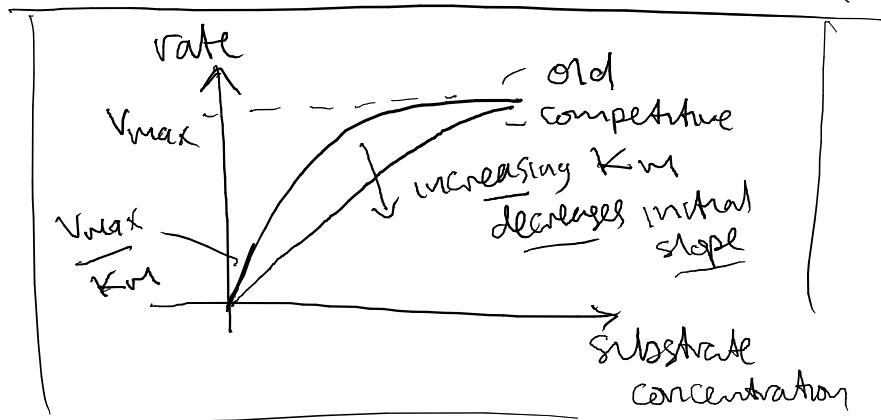
$$\begin{aligned} \sim &= \frac{d[P]}{dt} = k_2 [C_1] \\ &= k_2 K_I E_0 [S] \\ &= \frac{k_2 K_I E_0 [S]}{K_M [I] + K_I [S] + K_M K_I} \\ &= \frac{K_I (R_2 E_0 [S])}{K_I (K_M (1 + \frac{[I]}{K_I}) + [S])} \end{aligned}$$

$$\Rightarrow v = \frac{k_2 E_0 [S]}{K_m \left(1 + \frac{[I]}{K_I} \right) + [S]}$$

i.e.

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

$$\Rightarrow \begin{array}{l} \text{same } V_{max} \\ \underline{\text{new }} K_M \quad (K_M^{\text{new}} > K_M^{\text{old}}) \end{array}$$



Next time: noncompetitive inhibitors
(& other complications)