

BIOMENG 261

TISSUE AND BIOMOLECULAR ENGINEERING

Module I: Reaction kinetics and systems biology

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LECTURE 4 ENZYME REGULATION: INHIBITION AND ACTIVATION

- Types of enzyme regulation
- Example: competitive inhibition

MODULE OVERVIEW

Reaction kinetics and systems biology (*Oliver Maclaren*)

[12 lectures/4 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.

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

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)

LOCATION OF REGULATION INTERACTION

IRREVERSIBLE VS REVERSIBLE REGULATION

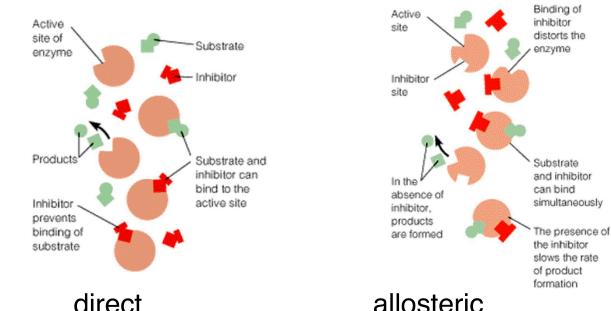
Irreversible:

- 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’



BEHAVIOUR/EFFECT INHIBITOR TYPE

Competitive:

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

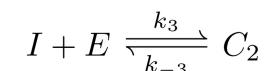
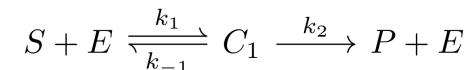
Noncompetitive:

- 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

MODEL SCHEME



MODEL

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

MODEL SUMMARY

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

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

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

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

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 !

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

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

Bioeng 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

↳ e.g. via gene expression
(see later)

- Activity

↳ Regulatory molecules

activator: turn 'up'
inhibitor: turn 'down'

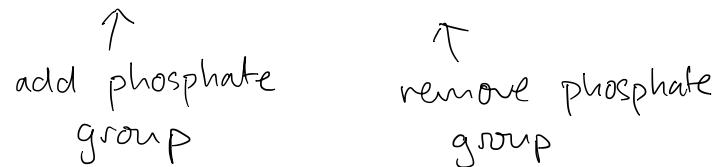
Recap: roles of enzymes - examples.

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



◦ Example: 'activate' another protein
by phosphorylating it

Kinase & Phosphatase



↳ 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 {

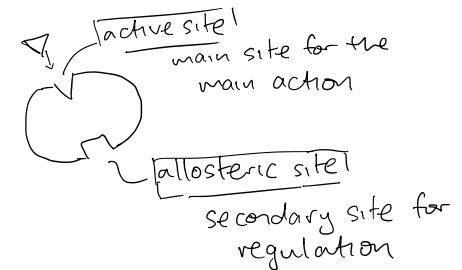
- competitive ← today
- non-competitive
- uncompetitive
- mixed

location {

- active site
- allosteric site

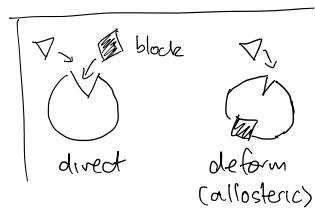
• Locations

allos: 'other'
stereos: 'solid object'



• Behaviour: competitive vs non vs un

• Competitive:

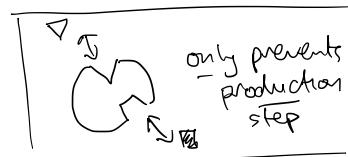


• substrate & inhibitor

can't bind enzyme at same time

↳ can be direct or allosteric mechanism/location

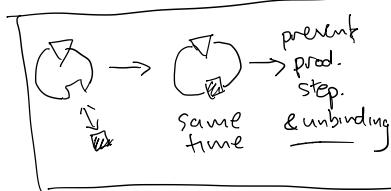
• noncompetitive:



• binds to either/both enzyme/complex

• only slows production step

• Uncompetitive:

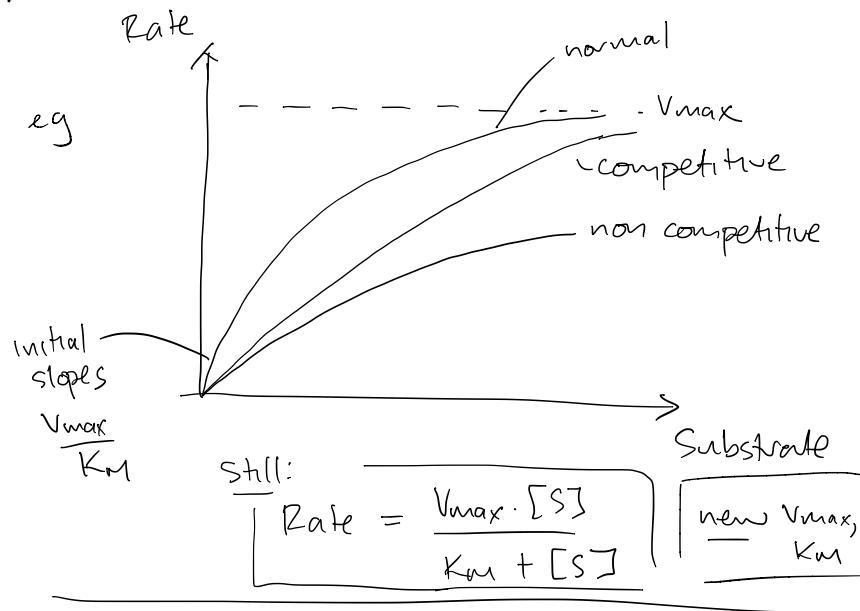


• regulator only binds to complex

• slows unbinding & production reactions

• reduces available 'active' enzyme & substrate

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



Goal: derive effects for simple
models of inhibition

1. competitive (V_{max} same, $K_m \uparrow$) ← today
2. non-competitive ($V_{max} \downarrow$, K_m same)
3. uncompetitive (both V_{max} & $K_m \downarrow$, V_{max}/K_m same)

↳ will probably just
look at result for 3.

Competitive Inhibition Model

Same idea as building MM model:

Simple (mass action) but
larger system



reduce to smaller but
complicated (non mass action)
system

Assume

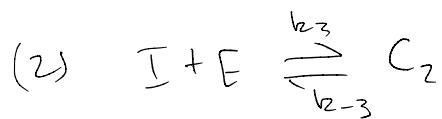
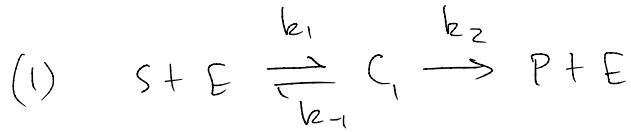


- Inhibitor I directly competes
with substrate S.

(bound to)

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

Full kinetics



Goal: derive an approximate model for $S \rightarrow P$
to understand implications of above scheme.

Competitive inhibition model

1. 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 $[E]$.

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

$$\sim := \frac{d[P]}{dt} = +J_2 \quad \boxed{\text{goal}}$$

2. Mass action

[excuse!]

gives

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

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

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

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

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

$$\sim = \frac{d[P]}{dt} = k_2 [C_1]$$

(uncoupled)

Note : using $E = f(c_1, c_2)$, have

$$1. \frac{d[S]}{dt} = f_1(c_1, c_2, S)$$

$$2. \frac{d[I]}{dt} = f_2(c_1, c_2, I)$$

$$3. \frac{d[C_1]}{dt} = f_3(c_1, c_2, S)$$

$$4. \frac{d[C_2]}{dt} = f_4(c_1, c_2, I)$$

$$5. \frac{d[P]}{dt} = f_5(c_1)$$

Goal : eliminate enzyme complex too (c_1 & c_2)

need two conditions/approx

o QSS / QE C_1
o QSS / QE C_2

$$\textcircled{1} \quad [c_1] \text{ QSS} \quad \left[\text{set } \frac{dc_1}{dt} \approx 0 \text{ relative to } \frac{ds}{dt} \right]$$

\Rightarrow

$$[c_1] = \frac{k_I E_0[s]}{K_m[I] + k_I[s] + K_m k_I}$$

$$\Rightarrow k_1 \underbrace{[E][s]}_{\approx} - (k_{-1} + k_2) [c_1] \approx 0$$

$$\boxed{k_1 (E_0 - [c_1] - [c_2]) [s] - (k_{-1} + k_2) [c_1] = 0}$$

$$\textcircled{2} \quad [c_2] \text{ QSS}$$

$$\Rightarrow \boxed{k_3 (E_0 - [c_1] - [c_2]) [I] - k_{-3} [c_2] = 0}$$

two eqns \Rightarrow eliminate c_1 & c_2

details: exercise!

?

$$[c_2] = \frac{K_m E_0[I]}{K_m[I] + k_I[s] + K_m k_I}$$

where $\boxed{\begin{aligned} k_I &= k_{-3}/k_3 \\ K_m &= \frac{k_{-1} + k_2}{k_1} \end{aligned}}$

\Rightarrow

$$S_0 \sim = d \frac{[P]}{dt} = k_2 [C_1]$$

$$= \frac{k_2 K_I E_0 [S]}{K_m [I] + K_I [S] + K_m K_I}$$

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

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

$$S_0 \sim = \frac{k_2 E_0 [S]}{K_m \left(1 + \frac{[I]}{K_I} \right) + [S]}$$

i.e.

$$\sim = \frac{v_{max} [S]}{K_m^{\text{new}} + [S]}$$

} MM form!

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

$$v_{max}^{\text{new}} = v_{max}^{\text{old}} = v_{max}$$

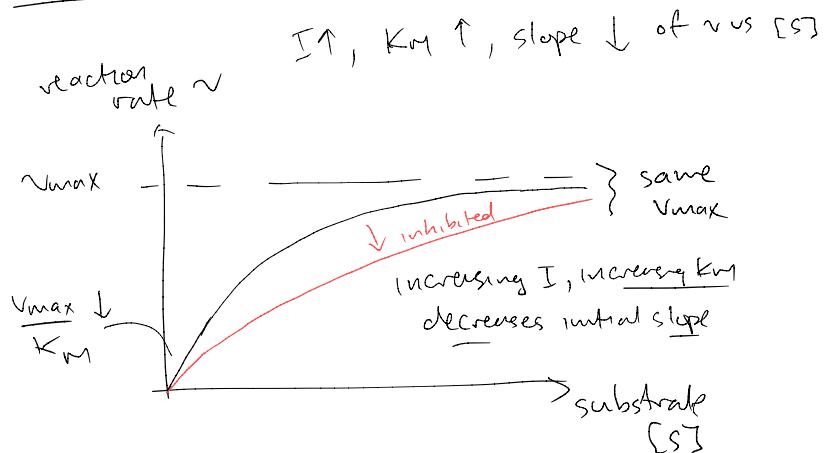
Note: as $[I] \uparrow, K_m^{\text{new}} \uparrow$

i.e. presence of inhibitor

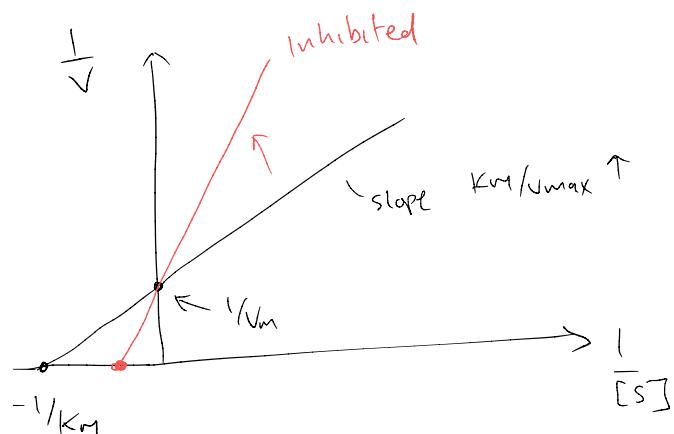
means 'effective' $\overline{K_m}$ is increased



Graphically:



Double-reciprocal:



(Note: opposite change in
slope for v or $\frac{1}{v}$!)