

BIOMENG 261

TISSUE AND BIOMOLECULAR ENGINEERING

Module I: Reaction kinetics and systems biology

Oliver Maclarens

oliver.maclarens@auckland.ac.nz

LECTURE 5 ENZYMES CONTINUED AND COMPLICATED

- Noncompetitive inhibition example

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:

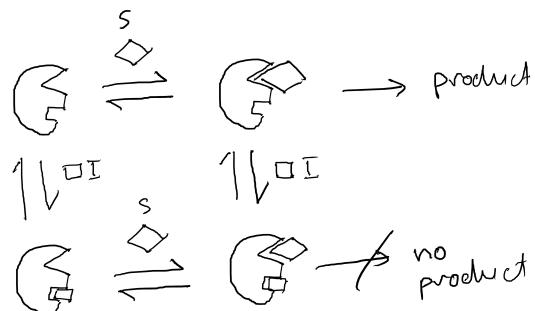
- Inhibitor can only bind to substrate-enzyme complex (not free enzyme)
- Prevents both product step and reversible unbinding step

2

4

ENZYMES REGULATION: NONCOMPETITIVE INHIBITION EXAMPLE

Picture



NONCOMPETITIVE INHIBITION EXAMPLE

Assumptions:

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

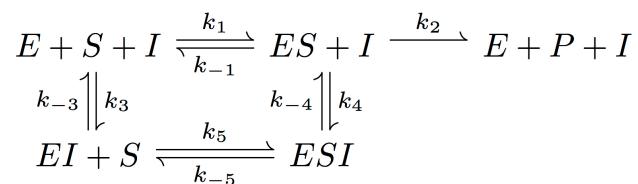
Leads to...(see handout)

5

7

NONCOMPETITIVE INHIBITION EXAMPLE

Reaction scheme



NONCOMPETITIVE INHIBITION EXAMPLE

$$(E_0 - [ES] - [EI] - [ESI])[S] - K_s[ES] = 0$$

$$(E_0 - [ES] - [EI] - [ESI])[I] - K_L [EI] = 0$$

$$[EI][S] - K_s[ESI] = 0$$

$$[ES][I] - K_s[ESI] = 0$$

which leads to...

6

8

KEY RESULT

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

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

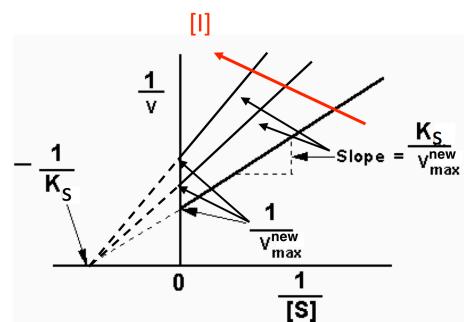
where here

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

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

9

PLOTTING: DOUBLE-RECIPROCAL PLOT



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

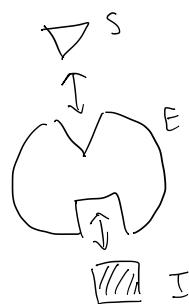
10

Biomeng 26 | Lecture 5:

Enzyme regulation cont'd

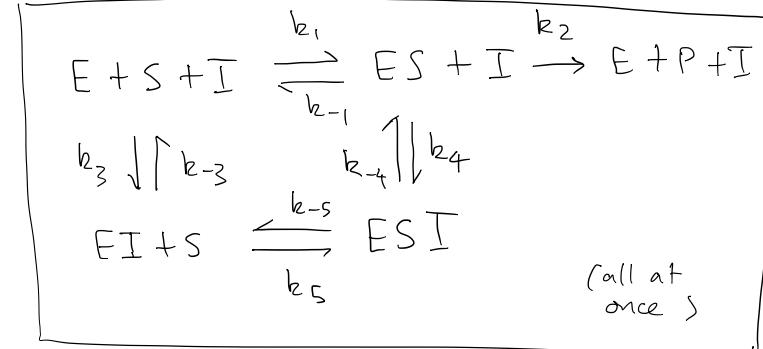
↳ non-competitive, reversible inhibition model

Non-competitive inhibition model



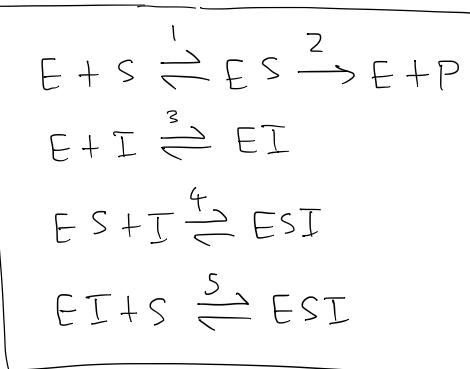
- Inhibitor I binds to either enzyme or complex at allosteric (not active) site
- stops production step
- doesn't affect binding/unbinding

General model



Parts:

Here just showing which quantities are used/made in reaction



9 Fluxes, including forward & back.

(often helpful to lump forward & back into one, especially for large systems)

Full Model

1. Conservation of mass $\{[E], [S], [I], [P]$
 $[EI], [ES], [ESI]\}$ complex

Note: only include Js in an ODE if they directly involve that quantity

$$\frac{d[E]}{dt} = -\underbrace{J_1}_{1} + \underbrace{J_2}_{2} - \underbrace{J_3}_{3} + J_4 \quad \left. \begin{array}{l} \text{will} \\ \text{eliminate} \\ \text{using 'total} \\ \text{enzyme' } \\ \text{as before} \end{array} \right\}$$

$$\frac{d[S]}{dt} = -\underbrace{J_1}_{1} + \underbrace{J_2}_{5} - \underbrace{J_3}_{5} + J_4$$

$$\frac{d[I]}{dt} = -\underbrace{J_3}_{3} + \underbrace{J_4}_{4} - J_5 + J_6$$

$$\frac{d[P]}{dt} = J_2 = \sim \quad \left. \begin{array}{l} \text{goal} \\ \rightarrow \text{overall reaction} \\ \text{rate.} \end{array} \right\}$$

$$\frac{d[EI]}{dt} = +\underbrace{J_3}_{3} - \underbrace{J_4}_{5} - \underbrace{J_5}_{5} + J_6$$

$$\frac{d[ES]}{dt} = +\underbrace{J_1}_{1} - \underbrace{J_2}_{4} - \underbrace{J_3}_{4} + J_4 - J_5$$

$$\frac{d[ESI]}{dt} = +\underbrace{J_4}_{4} - J_5 + \underbrace{J_5}_{5} - J_6$$

2. Mass action constitutive model

$$J_1 = k_1 [E][S]$$

$$J_2 = k_2 [ES]$$

$$J_3 = k_3 [EI][I]$$

$$J_4 = k_4 [ES][I]$$

$$J_5 = k_5 [ESI]$$

$$J_6 = k_6 [EI][S]$$

$$J_7 = k_7 [ESI]$$

Note: $[ES] \neq [E][S]$

also: only include 'active' participants

Q: can we say anything about k_3 vs k_4 , k_2 vs k_5 , etc?

A: ... →

We could simulate whole system
 → again want a reduced model for understanding, interpretation, simplicity etc..

Reduction: 'exact' parts.

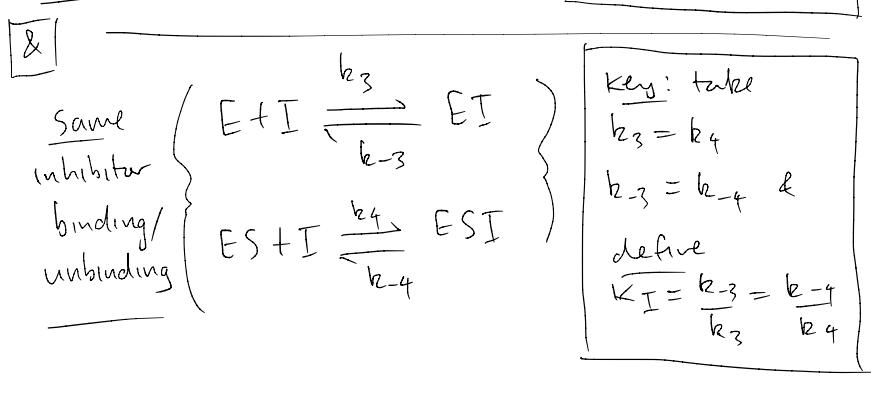
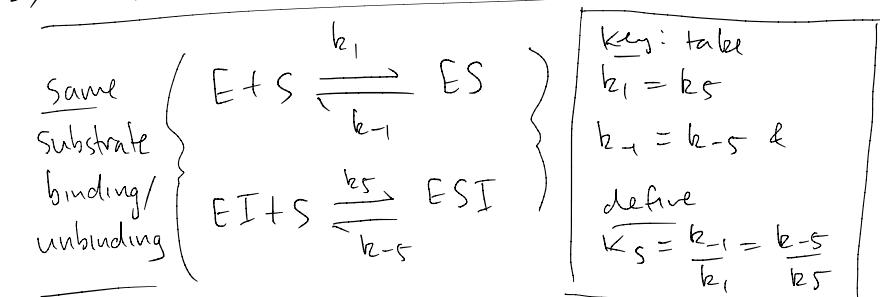
1. Total enzyme in 'all forms' is conserved:

$$[E = E_0 - [ES] - [EI] - [ESI]]$$

2. Noncompetitive

- assume binding/unbinding of
[S] &/or [I] unaffected by
other

⇒ lives:



Reduction: approximations

o Quasi-steady state vs Quasi-equilibrium

- QSS: probably conceptually better
 → but a bit messy

- QE: a bit easier & gives same basic result (here)

↳ will use this!

⇒ These approximations allow us to focus on solving a system of algebraic equations

(still good practice to write out full system → this also gives full time-dep. soln).

So ...

- Assume all enzyme binding/unbinding reactions are (approx) at equilibrium
- Use conservation of total enzyme
(in all forms)
- Use mass action kinetics with
rate constants for S/I that
are independent of I/S binding
i.e. $\frac{k_{-1}}{k_1} = \frac{k_{-5}}{k_2} = k_s$ & $\frac{k_{-3}}{k_3} = \frac{k_{-4}}{k_4} = k_I$

Gives:

- $(E_0 - [ES] - [EI] - [ESI])[S] - k_s [ES] = 0$
- $(E_0 - [ES] - [EI] - [ESI])[I] - k_I [EI] = 0$
- $[EI][S] - k_s [ESI] = 0$
- $[ES][I] - k_I [ESI] = 0$

\Rightarrow 4 equations, but only 3 independent
(note symmetry in S & I)

\Rightarrow use to eliminate $\boxed{[ES], [EI], [ESI]}$

Remember goal: production rate in terms
of $[S]$, $[I]$ & parameters

Have

$$\boxed{v = J_p = k_2 [ES]}$$

... solve (by hand or computer...) ...

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

$$\Rightarrow v = J_p = k_2 [ES]$$

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

Note $\begin{cases} K_s = K_M \text{ (eq.) for no } [I], \text{ or } K_M^{\text{old}} \\ \text{& } V_{\text{Max}}^{\text{old}} = k_2 E_0 \end{cases}$

So ...

Again have form:

$$v = \frac{V_{\max}^{\text{new}} [S]}{K_M + [S]} \quad \left. \begin{array}{l} \text{again} \\ \text{MM form} \end{array} \right\}$$

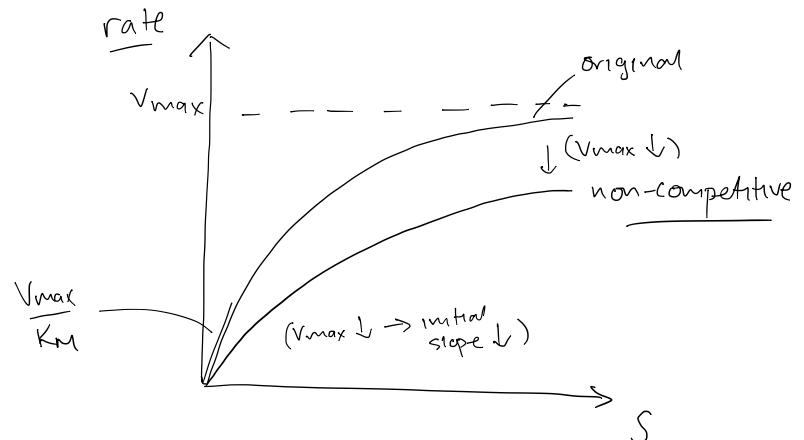
where $\left. \begin{array}{l} V_{\max}^{\text{new}} = \left(\frac{k_2 E_0 K_I}{K_I + [I]} \right) = V_{\max}^{\text{old}} \left(\frac{1}{1 + [I]/K_I} \right) \\ K_M^{\text{new}} = K_M^{\text{old}} = K_S \end{array} \right\}$

Note $\left\{ \begin{array}{l} \text{as } [I] \uparrow, \frac{1}{1 + [I]/K_I} \downarrow \\ \text{so } \boxed{V_{\max}^{\text{new}}} \downarrow \text{ with inhibition} \end{array} \right.$

& $K_M = \text{same}$.

Plotting

- Same MM form as before
- New V_{\max} (\downarrow)
- Same $K_S = K_M = \frac{k_{-1}}{k_1}$ (Quasi-eq., no $[I]$) as before.



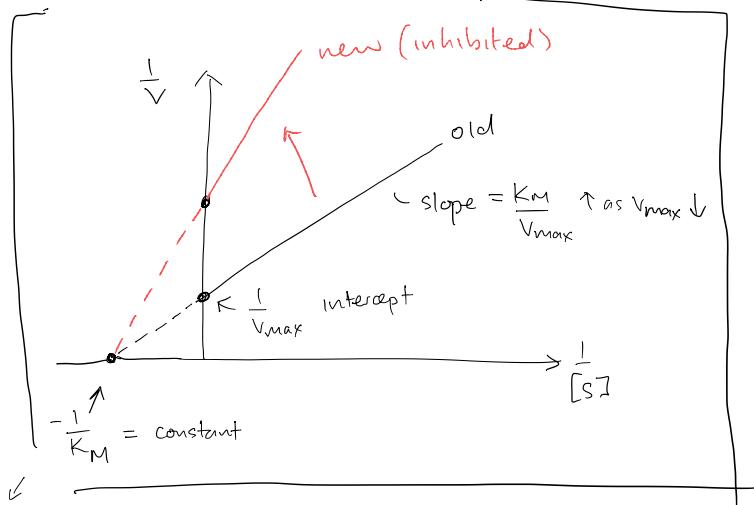
Lineweaver-Burk / Double-reciprocal plots

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

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

i.e. $\frac{1}{v} = M \cdot \frac{1}{[S]} + C$

So : |Noncompetitive inhibition|



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

$$\Rightarrow [S] = -K_m$$

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