

# 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*)  
[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.

## LECTURE 5 ENZYMES CONTINUED AND COMPLICATED

- Noncompetitive inhibition example

### RECALL: INHIBITOR TYPE

*Competitive:*

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

*Noncompetitive:*

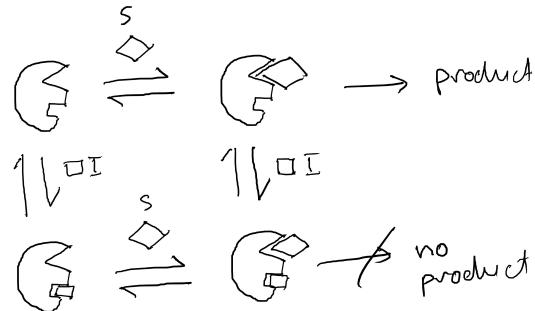
- Inhibitor can bind to either/both enzyme and complex
- Only slows product step
- Doesn't affect binding of substrate

*Uncompetitive:*

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

## ENZYMES REGULATION: NONCOMPETITIVE INHIBITION EXAMPLE

Picture



## NONCOMPETITIVE INHIBITION EXAMPLE

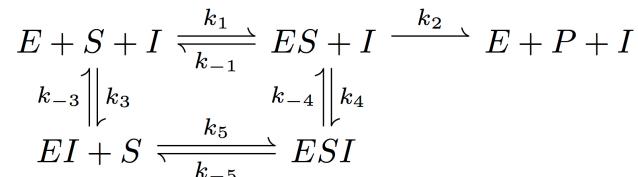
Assumptions:

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

Leads to...(see handout)

## NONCOMPETITIVE INHIBITION EXAMPLE

Reaction scheme



## NONCOMPETITIVE INHIBITION EXAMPLE

$$\begin{aligned} (E_0 - [ES] - [EI] - [ESI])[S] - K_s[ES] &= 0 \\ (E_0 - [ES] - [EI] - [ESI])[I] - K_I[EI] &= 0 \\ [EI][S] - K_s[ESI] &= 0 \\ [ES][I] - K_I[ESI] &= 0 \end{aligned}$$

which leads to...

## KEY RESULT

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

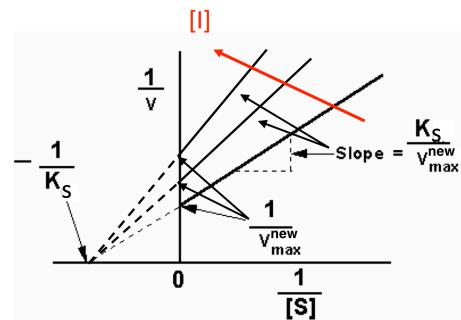
$$J_P = v = \frac{V_{\max}^{\text{new}}[S]}{K_M + [S]}$$

where here

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

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

## PLOTTING: DOUBLE-RECIPROCAL PLOT



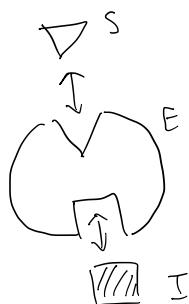
(i.e. *Lineweaver-Burk plot*)

## Biomeng 261 Lecture 5:

### Enzyme regulation cont'd

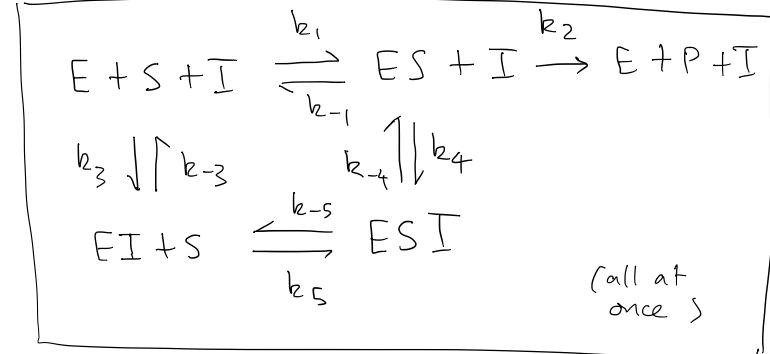
↳ non-competitive, reversible inhibition model

### Non-competitive inhibition model



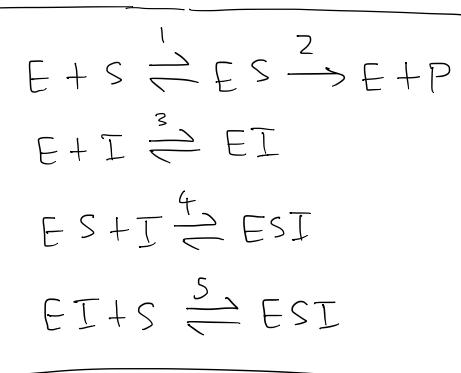
- Inhibitor I binds to either enzyme or complex at allosteric (not active) site
- stops production step
- doesn't affect binding/unbinding

### General model



### Parts:

Here just showing which quantities are used/made in reaction



↑ Fluxes, including forward & back.

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

## Full Model

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

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

$$\frac{d[E]}{dt} = -\underbrace{J_1}_{1} + \underbrace{J_{-1}}_{2} + \underbrace{J_2}_{3} - \underbrace{J_3}_{1} + \underbrace{J_{-3}}_{3}$$

will  
eliminate  
using 'total  
enzyme'  
as before

$$\frac{d[S]}{dt} = -\underbrace{J_1}_{1} + \underbrace{J_{-1}}_{5} - \underbrace{J_5}_{5} + \underbrace{J_{-5}}_{5}$$

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

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

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

$$\frac{d[ES]}{dt} = +\underbrace{J_1}_{1} - \underbrace{J_{-1}}_{4} - \underbrace{J_4}_{4} + \underbrace{J_{-4}}_{2} - \underbrace{J_2}_{2}$$

$$\frac{d[ESI]}{dt} = +\underbrace{J_4}_{4} - \underbrace{J_{-4}}_{2} + \underbrace{J_5}_{5} - \underbrace{J_{-5}}_{5}$$

## 2. Mass action constitutive model

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

$$J_{-1} = k_{-1} [ES]$$

$$J_2 = k_2 [ES]$$

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

$$J_{-3} = k_{-3} [EI]$$

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

$$J_{-4} = k_{-4} [ESI]$$

$$J_5 = k_5 [EI][S]$$

$$J_{-5} = k_{-5} [ESI]$$

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

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.

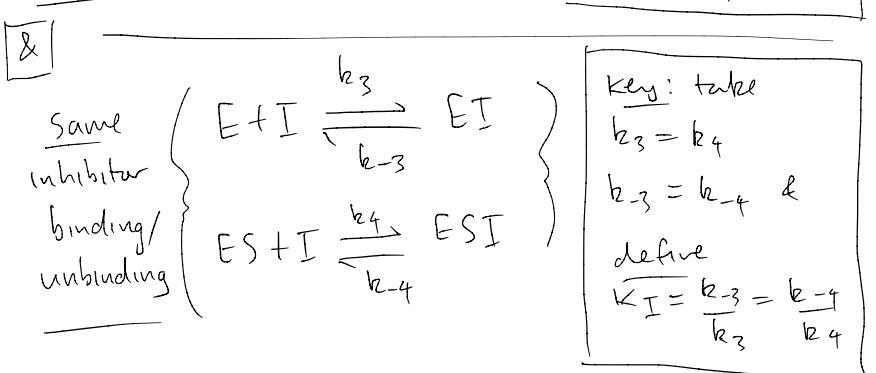
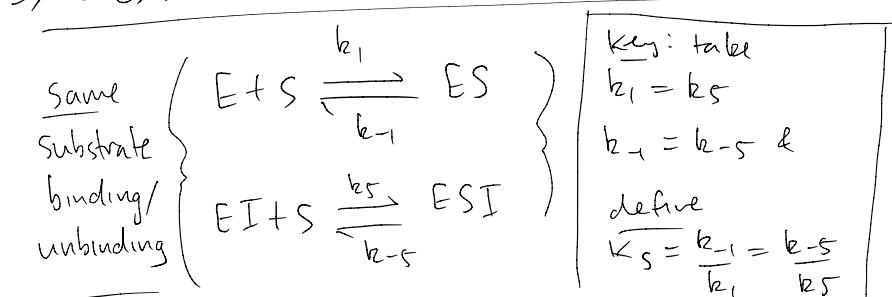
1. Total enzyme in 'all forms' is conserved:

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

2. Noncompetitive

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

⇒ gives:



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.  $\frac{k_{-1}}{k_1} = \frac{k_{-5}}{k_5} = k_S$  &  $\frac{k_{-3}}{k_3} = \frac{k_{-4}}{k_4} = k_I$

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

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

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

So ...

Again have form:

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

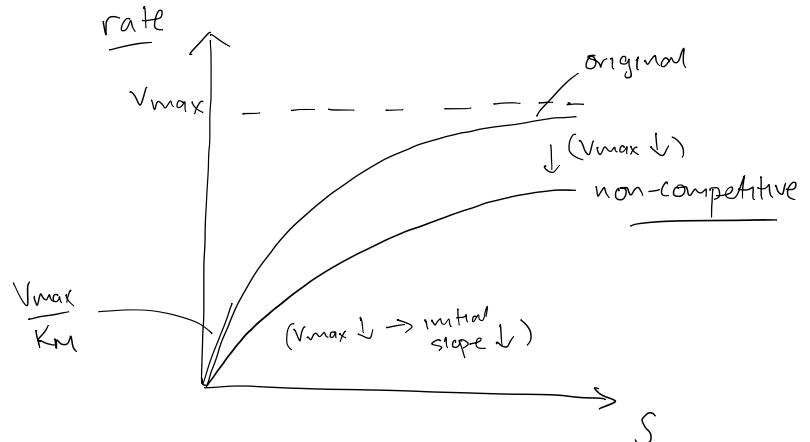
where  $\left. \begin{array}{l} V_{\max}^{\text{new}} = \left( \frac{k_2 f_0 K_I}{K_I + [I]} \right) = V_{\max}^{\text{old}} \left( \frac{1}{1 + [I]/K_I} \right) \\ K_M^{\text{new}} = K_M^{\text{old}} = K_S \end{array} \right\}$

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- $\text{Eq.}$ , no  $[I]$ ) as before.



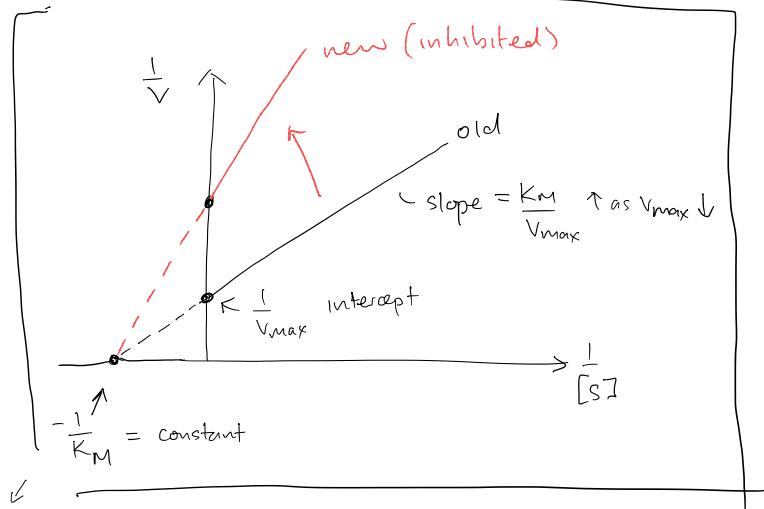
## Lineweaver-Burk / Double-reciprocal plots

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

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

i.e.  $\frac{1}{v} = m \cdot x + c$

so: | Noncompetitive inhibition |



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

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

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