

# BIOMENG 261

## TISSUE AND BIOMOLECULAR ENGINEERING

*Module I: Reaction kinetics and systems biology*

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## LECTURE 6 ENZYMES CONTINUED AND COMPLICATED...CONTINUED

- Summary of reversible inhibition types
  - Uncompetitive (briefly)
  - Double-reciprocal plots for each type
- A note on irreversible inhibition
- Cooperativity effects
  - Hill equations (non-Michaelis-Menten!)

## 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.

## 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

## COOPERATIVITY: DEFINITION

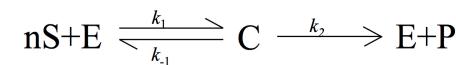
*Positive cooperativity:*

- Binding of one **substrate** molecule *increases subsequent rates* of binding of substrate molecules to *remaining active sites*

*Negative cooperativity:*

- Binding of one **substrate** molecule *decreases subsequent rates* of binding of substrate molecules to *remaining active sites*

## N BINDING SITES: THE HILL EQUATION

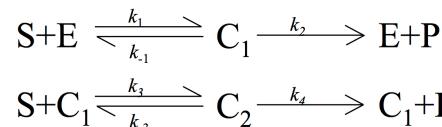


Leads to...(see handout)

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

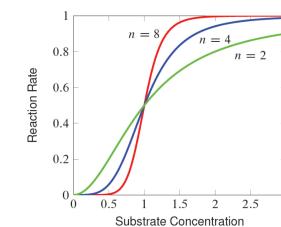
- As empirical model: *three free parameters*
- Non-hyperbolic i.e. is *sigmoidal*

## SIMPLE MODEL: TWO ACTIVE SITES



Implications: non-Michaelis-Menten, *sigmoidal* behaviour (see handout)

## EFFECT OF N AND HILL PLOTS



Convert to *Hill plot*: rewrite as

$$\ln \frac{v}{V_{\max} - v} = n \ln[S] - n \ln K_M$$

- Gives *straight line* for fitting

## Biomeng 261 Lecture 6:

### o Enzyme regulation

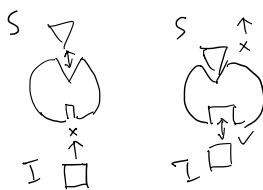
- summary of competitive, non-competitive, uncompetitive (reversible) inhibition
- note on irreversible inhibition.

### o Cooperative effects

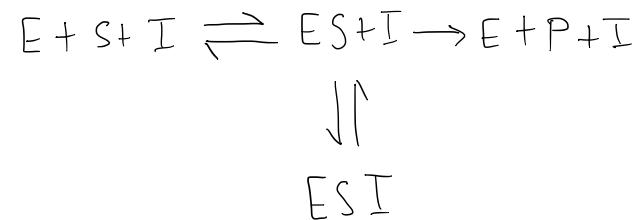
- multiple active sites for main substrate

### Uncompetitive?

- only bind to complex
- prevents subsequent unbinding & production steps



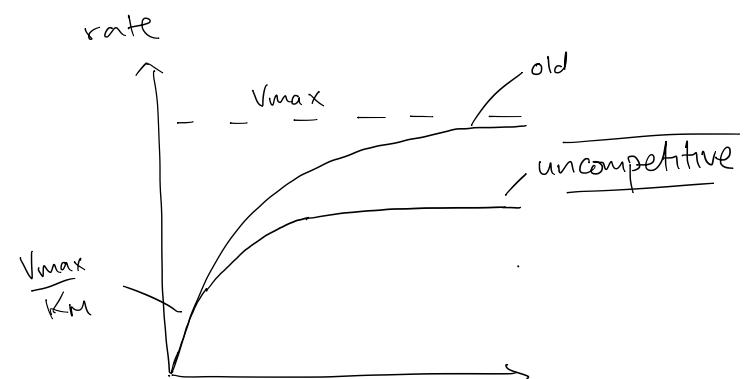
### Uncompetitive scheme:



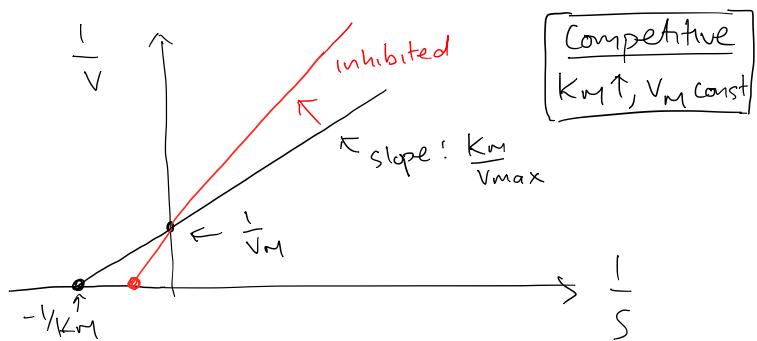
Can show (exercise?)

- Both  $V_m$  &  $K_m$  ↓
- But  $\frac{V_m}{K_m}$  unchanged

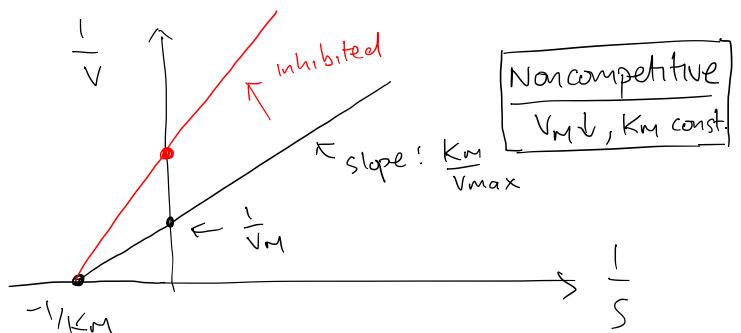
So:



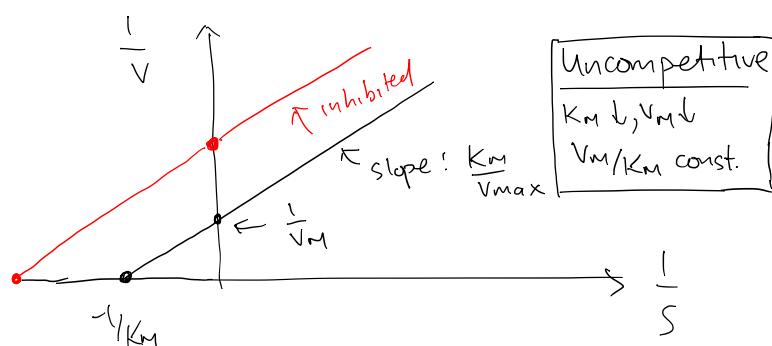
Summary: double-reciprocal plots (Key!)



Competitive  
 $K_m \uparrow, V_m \text{ const.}$



Noncompetitive  
 $V_m \downarrow, K_m \text{ const.}$

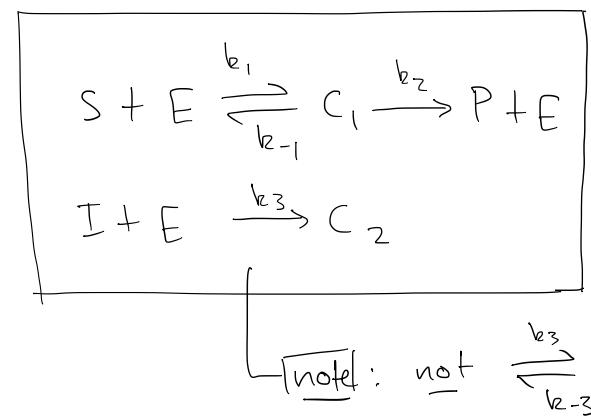


Uncompetitive  
 $K_m \downarrow, V_m \downarrow$   
 $V_m/K_m \text{ const.}$

### Note on irreversible inhibition

- We've looked at reversible inhibition
- How could we model irreversible inhibition? Same ideas!

Example: competitive irreversible inhibition [eg via hydrogen cyanide (HCN)]

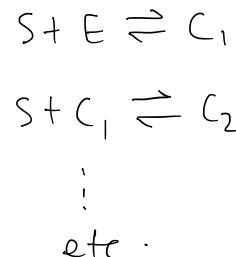
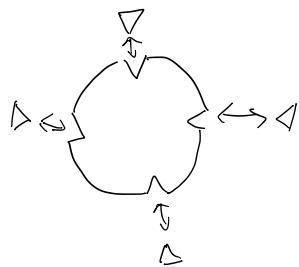


→ once in  $C_2$  form, can't get back

→ could also do  $I + E \rightarrow \emptyset$  {sink}  
(don't track)

## Cooperation?

- About how the primary substrate interacts with itself & the enzyme
- Models are based on the presence of multiple active sites (not allosteric)
- Many enzymes do in fact have multiple active sites!



## Cooperation

so:

- The activation/inhibition is due to multiple primary substrate molecules binding, not a different regulating molecule (as before)

- If the overall result is just a simple 'sum' of effects then there is no complication

BUT: often there is an interaction

what does this mean?

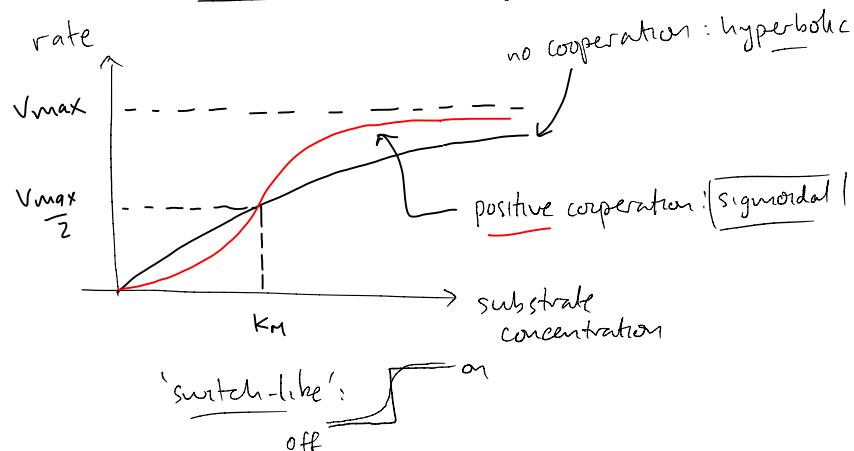


## Cooperativity

Positive: binding of one substrate molecule increases the binding rate of substrate to remaining active sites

Negative: binding of one substrate molecule decreases the binding rate of substrate to remaining active sites

Positive: changes curve 'shape' } eg hemoglobin!  
 → Not MM shape anymore }



(negative doesn't change 'shape', just slope)

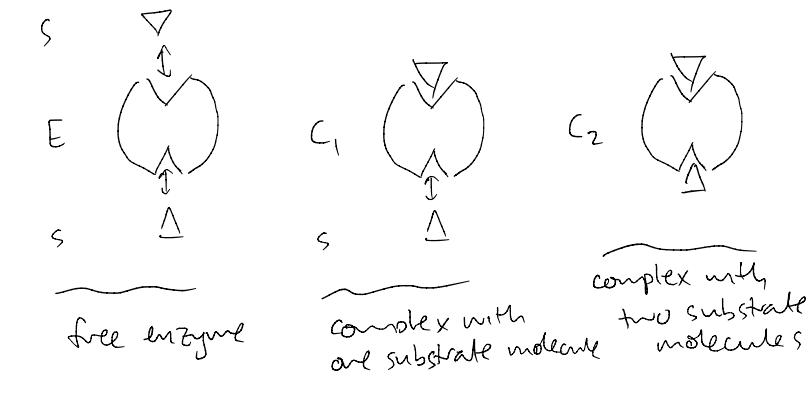


Now 'switch-like', & non-Michaelis-Menten

→ we need a new model that can give non-hyperbolic, sigmoidal shape

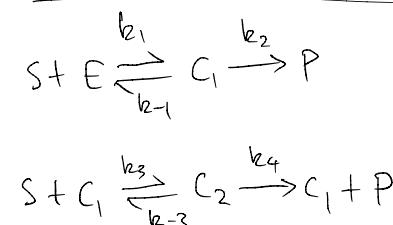
→ will again consider derivation via underlying mass action + QSS/QE approximation

First: simple two active site model



Kinetics:

&



Gives (conservation of mass + mass action):

$$\frac{d[S]}{dt} = -k_1 [S][E] + k_{-1}[c_1] - k_3 [S][c_1] + k_{-3}[c_2]$$

$$\frac{d[c_1]}{dt} = k_1 [S][E] - (k_{-1} + k_2)[c_1] - k_3 [S][c_1] + (k_3 + k_4)[c_2]$$

$$\frac{d[c_2]}{dt} = k_3 [S][c_1] - (k_{-3} + k_4)[c_2]$$

$$[E] = E_0 - [c_1] - [c_2]$$

$$\frac{d[P]}{dt} = k_2 [c_1] + k_4 [c_2] \leftarrow \text{note!}$$

Reduction?

Again: use quasi-steady state assumption (QSSA)  
to (approximately) eliminate  $c_1, c_2$

Set  $\begin{cases} \frac{d[c_1]}{dt} = 0 \\ \frac{d[c_2]}{dt} = 0 \end{cases}$

$\Rightarrow$  two algebraic equations,  
& linear in  $c_1, c_2$ .

Solving gives

$$[c_1] = \frac{E_0 K_2 [S]}{K_1 K_2 + K_2 [S] + [S]^2}$$

$$[c_2] = \frac{E_0 [S]^2}{K_1 K_2 + K_2 [S] + [S]^2}$$

where  $K_1 = \frac{k_{-1} + k_2}{k_1} = K_M^{(1)}$

$$K_2 = \frac{k_{-3} + k_4}{k_3} = K_M^{(2)}$$

so  $\dot{T} = v = \frac{d[P]}{dt} = k_2 [c_1] + k_4 [c_2]$

becomes

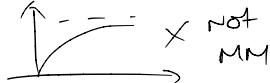
$$v = \frac{(k_2 K_2 + k_4 [S]) E_0 [S]}{K_1 K_2 + K_2 [S] + [S]^2}$$

new type of 'constitutive equation'  
for cooperative effects  $\rightarrow$

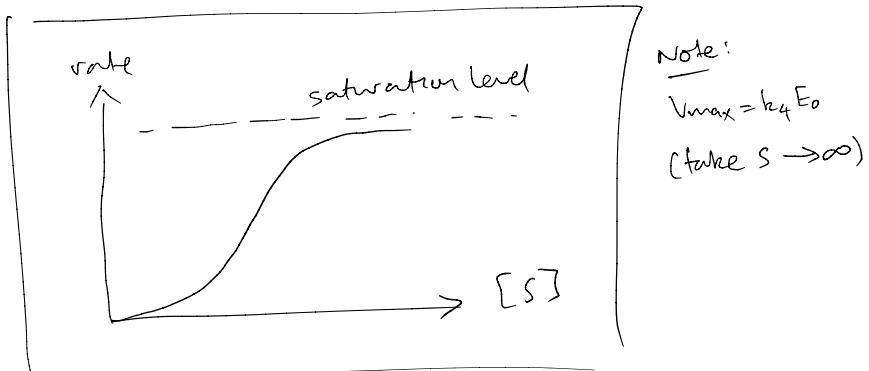
Understanding?

$$v = \frac{(k_2 K_2 + k_4 [S]) E_0 [S]}{K_1 K_2 + K_2 [S] + [S]^2}$$

Plot: not hyperbolic



is sigmoidal / ie looks like:

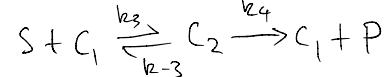
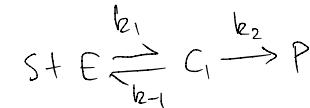


Qs: - can we simplify further?

- can we generalise to n binding sites?

Additional simplification of two-site case

Recall:



} what can we say about  $k_2, k_3$ ?

Consider two possible cases.

o Case 1: Independent & identical binding sites

o  $k_1 = 2k_3 = 2k_f$ , where  $k_f$  is individual site rate

forward: { two free sites to bind to make  $C_1$ , one free site to bind to make  $C_2$

o  $k_{-3} = 2k_{-1} = 2k_r$ ,  $k_r$  is individual site rate

reverse: {  $C_2$  has two bound molecules,  $C_1$  has one bound molecule

o  $k_4 = 2k_2$

forward: {  $C_2$  has two bound molecules,  $C_1$  has one bound molecule

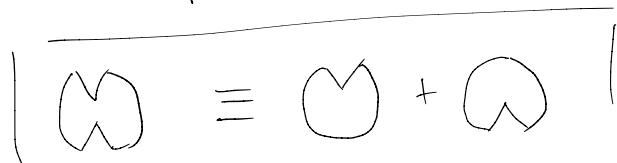
Case 1 assumptions lead to ...

$$v = 2 \left( \frac{k_2 E_0 [S]}{K_m + [S]} \right)$$

Boring!

$$\text{where } K_m = \frac{k_- + k_2}{k_+} \quad \left. \begin{array}{l} \text{individual} \\ \text{site } K_m \end{array} \right\}$$

→ just double reaction rate as expected



→ no cooperativity

Case 2. Proper interaction effects (coop.)

• Limiting case (simplification!)

→ 'second binding much faster'

Math: (details a bit beyond scope?)

use  $\left[ \frac{k_3}{k_1} \gg 1 \right] \quad \text{ie} \quad \left[ \frac{k_3 \rightarrow \infty}{k_1 \rightarrow 0} \right]$

while  $\left[ \frac{k_1, k_3 = \text{constant}}{} \right]$

gives  $\frac{K_1}{K_2} = \left( \frac{k_- + k_2}{k_1} \right) \times \left( \frac{k_3}{k_4 + k_3} \right) \gg 1$

ie take  $K_1 \rightarrow \infty, K_2 \rightarrow 0$

while  $K_1 K_2 = \left( \frac{k_- + k_2}{k_1} \right) \left( \frac{k_4 + k_3}{k_3} \right)$

ie take  $K_1 K_2 = \text{const.}$

Result:  $v = \frac{k_4 E_0 [S]^2}{K_m^2 + [S]^2}$

not  $[S]^2$   
not  $[S]$ .

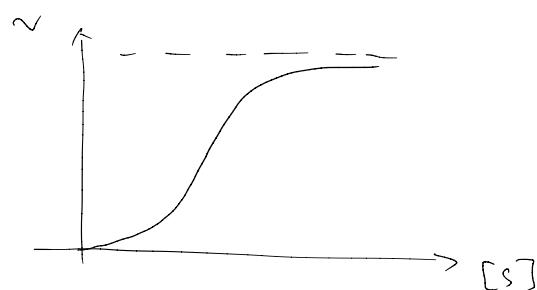
Case 2 result:

$$v = \frac{k_4 E_0 [S]^2}{K_M^2 + [S]^2} = \frac{v_{\max} [S]^2}{K_M^2 + [S]^2}$$

where:  $\underset{\text{def'n}}{\{} v_{\max} = k_4 E_0 \}$   
 $K_M^2 = K_1 K_2 \}$

→ almost MM, but...

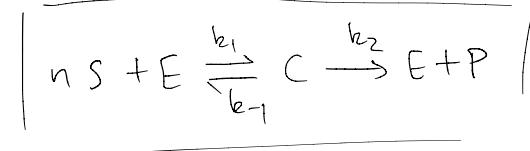
Plot:



Sigmoidal !

General case: Hill equation for n cooperative sites

write schematically as



- use • n quasi-equilibrium eq's
- $k_1/k_n \gg 1$  &  $K_1 K_n = \text{const.}$

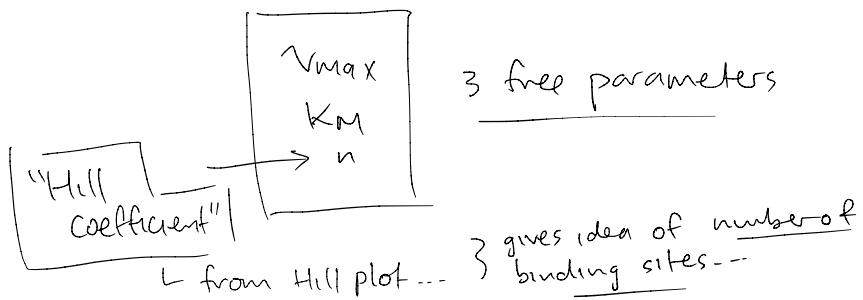
leads to the 'Hill equation'

$$v = \frac{v_{\max} [S]^n}{K_M^n + [S]^n}$$

where  $\left\{ \begin{array}{l} K_M^n = \prod_{i=1}^n K_i \\ v_{\max} = k_2 E_0 \end{array} \right.$

## Hill coeff. & Hill Plot

we typically treat the Hill eqn as  
an empirical constitutive equation/  
model for cooperativity & fit



### Hill Plot

Taking reciprocals & then taking logs &  
then rearranging -- gives

$$\ln\left(\frac{v}{V_{max}-v}\right) = n \ln[S] - n \ln K_m$$

↑ slope

ie  $y = m \cdot x + c$

(straight line)

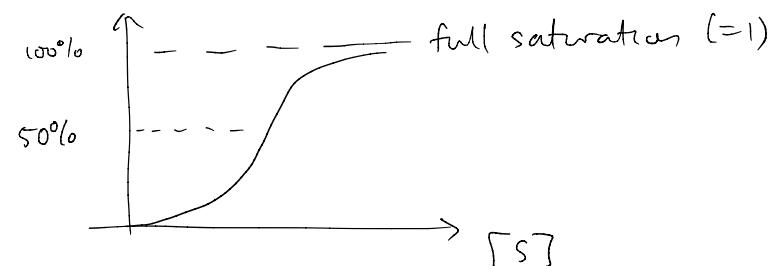
### Notes:

- in real systems we usually  
let  $n$  be non-integer

- $\begin{cases} n > 1 & \text{positive coop.} \\ n = 1 & \text{no coop.} \\ n < 1 & \text{negative coop.} \end{cases}$

-  $v$  can also be interpreted as approx.  
(proportional to) the 'saturation fraction',  
ie the total proportion of occupied  
binding sites to available binding sites:

$$v/v_{max}$$



For more, see e.g.:

Or

