

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*)
[11 lectures/3 tutorials/2 labs]

1. Basic principles: modelling with reaction kinetics [4 lectures]

Conservation, directional and constitutive principles. Mass action. Enzyme kinetics. Enzyme regulation. Mathematical/graphical tools for analysis and fitting.

2. Systems biology I: signalling and metabolic systems [2 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.

RECAP AND COMMENTS FOR LAST WEEK

- Mass action
- Enzymes and Michaelis-Menten model
- Enzymes and regulation effects
- Competitive inhibition model

See handout.

LECTURE 4 ENZYMES CONTINUED AND COMPLICATED

- Noncompetitive inhibition example
- Cooperativity: multiple active sites
- Cooperativity: the Hill equation

RECALL: INHIBITOR TYPE

Competitive:

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

Uncompetitive:

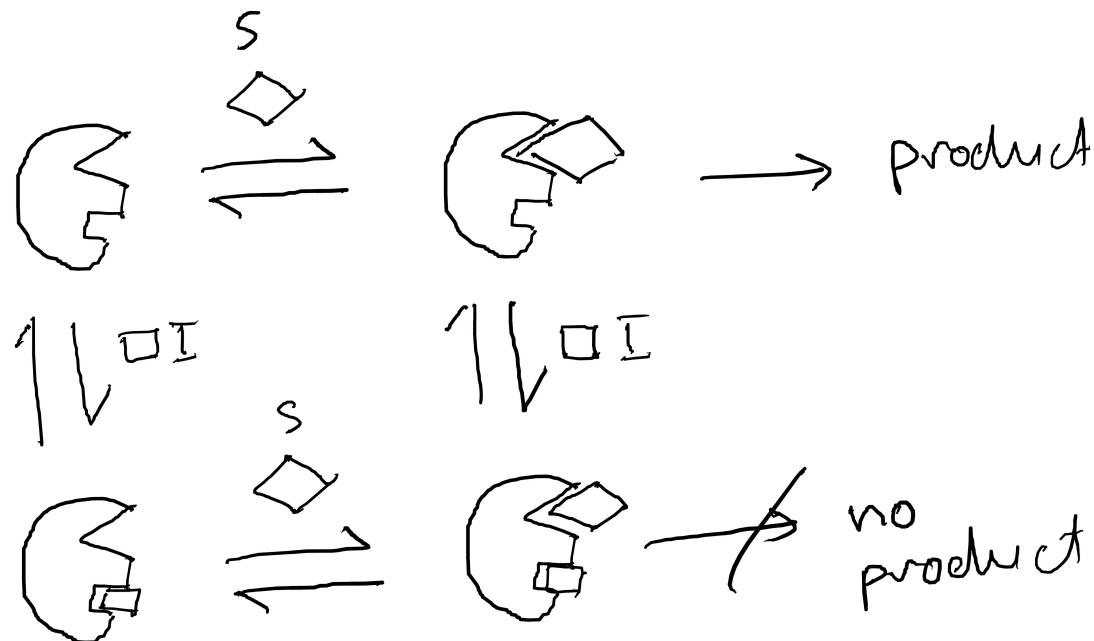
- Inhibitor can only bind to substrate-enzyme complex (not free enzyme)
- Prevents both product step and reversible unbinding step

Noncompetitive:

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

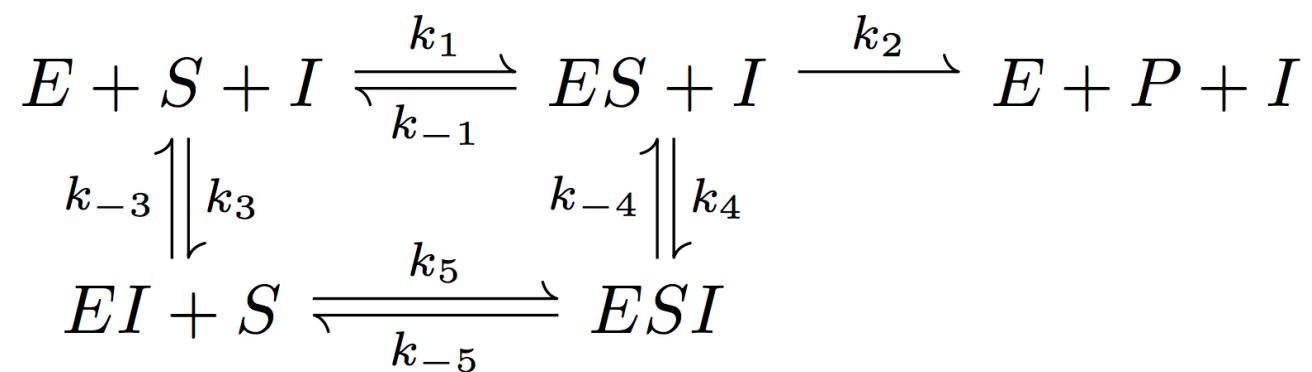
ENZYMES REGULATION: NONCOMPETITIVE INHIBITION EXAMPLE

Picture



NONCOMPETITIVE INHIBITION EXAMPLE

Reaction scheme



NONCOMPETITIVE INHIBITION EXAMPLE

Assumptions:

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

Leads to...(see handout)

NONCOMPETITIVE INHIBITION EXAMPLE

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

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

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

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

which leads to...

UPSHOT

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

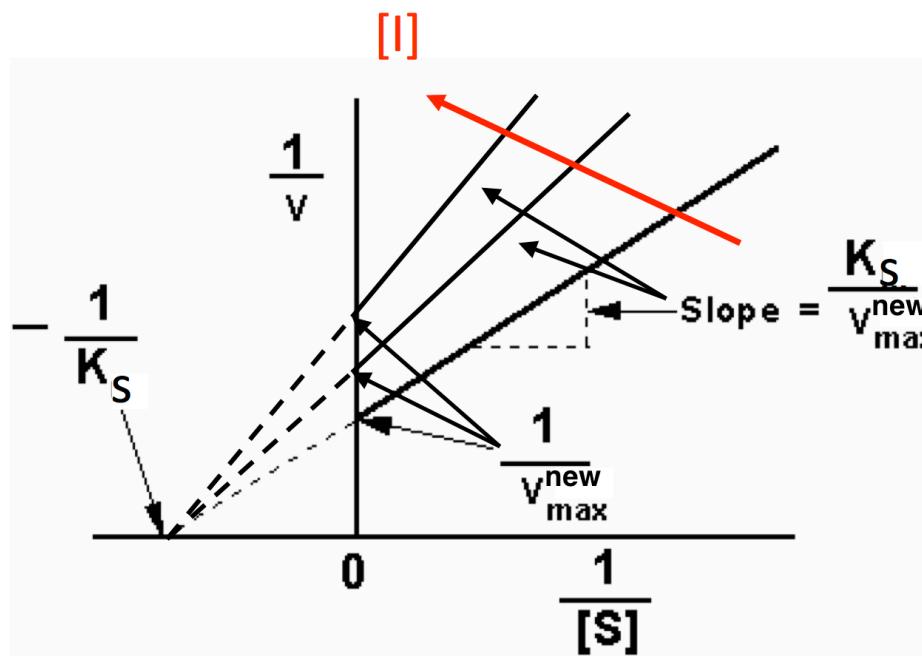
$$J_P = v = \frac{V_{\max}^{\text{new}}[S]}{K_S + [S]}$$

where here

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

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

PLOTTING: DOUBLE-RECIPROCAL PLOT

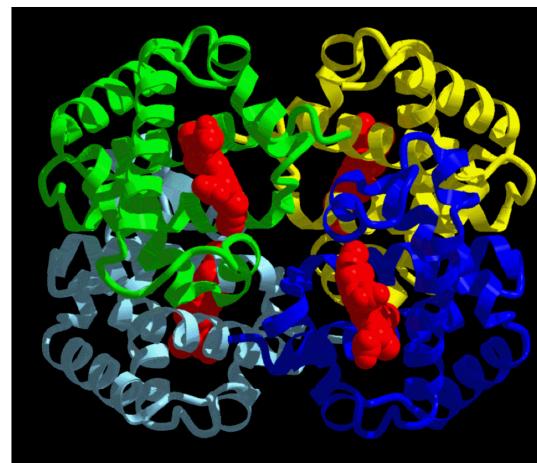
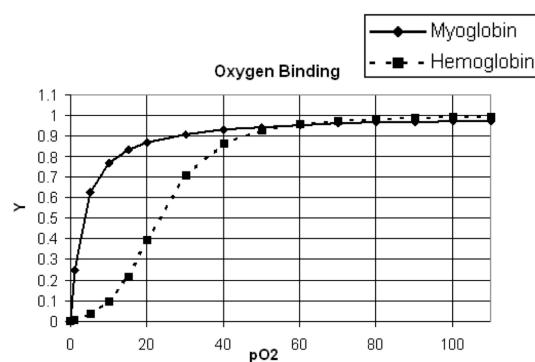


(i.e. *Lineweaver-Burk plot*)

COOPERATIVITY: SIGMOIDAL BEHAVIOUR

- We often observe non-hyperbolic, *sigmoidal kinetics*
- Can't be explained by simple Michaelis-Menten model

Classic example: *binding of oxygen to haemoglobin in red blood cells*



COOPERATIVITY: DEFINITION

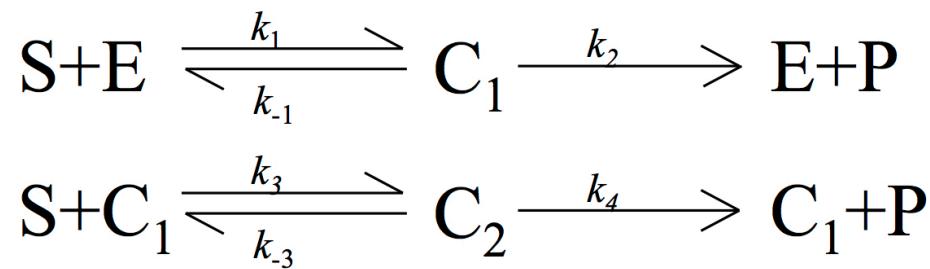
Positive cooperativity:

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

Negative cooperativity:

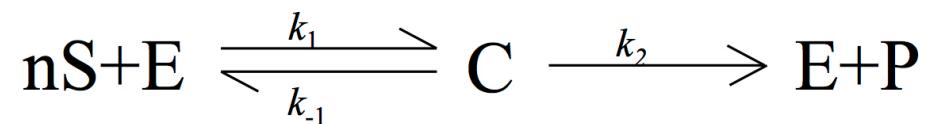
- Binding of one *substrate* molecule *decreases subsequent rates* of binding of substrate molecules to *remaining active sites*

SIMPLE MODEL: TWO ACTIVE SITES



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

N BINDING SITES: THE HILL EQUATION

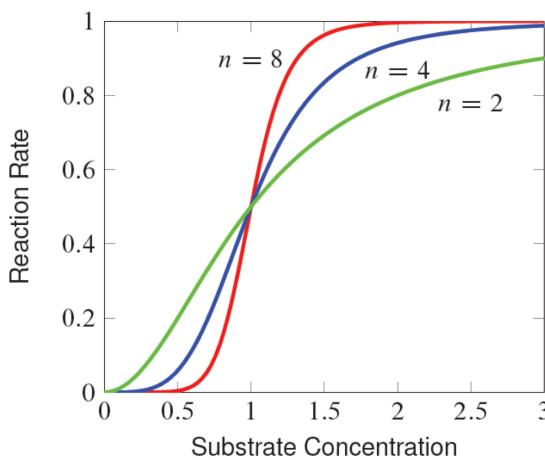


Leads to...(see handout)

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

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

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