

# BIOMENG 261

## TISSUE AND BIOMOLECULAR ENGINEERING

*Module I: Reaction kinetics and systems biology*

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### LECTURE 2 ENZYME KINETICS

- Enzymes
- The Michaelis-Menten model
- Quasi-equilibrium and quasi-steady-state analysis
- Empirical analysis

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## 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.

### ENZYMES: MOTIVATION/IDEA



might be

- *Possible* (thermodynamically favourable)  
but still
- *Too slow* (kinetic 'path' takes too long)

*Q: Can we speed it up, without changing  
the start and end points?*

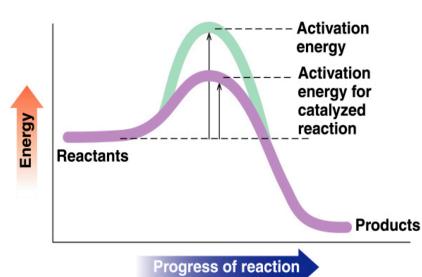
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## CATALYSTS

A **catalyst** is a substance that

- Can speed up a reaction ('help along the reaction path')
- Doesn't change the start and end points (overall free energy is the same)
- Are not used up in the reaction themselves



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## ENZYMES

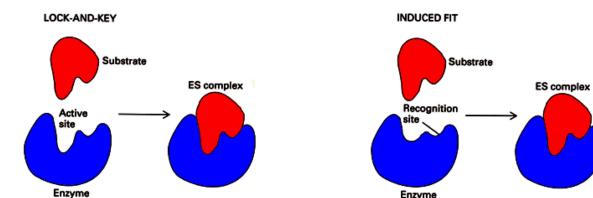
An **enzyme** is a biological **catalyst**

- Usually proteins/large macromolecules
- Think: 'helper machines'
- Usually end in '-ase'
  - permease
  - kinase
  - etcase

## CATALYSTS

## ENZYMES: MECHANISM(S)

- 'Lock and key': rigid enzyme
- 'Induced fit': same basic idea but deformable enzyme

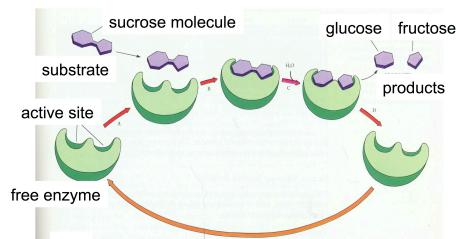


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## ENZYMES: EXAMPLE

Hydrolysis ('splitting') of sucrose into glucose and fructose



## THE MICHAELIS-MENTEN MODEL

Michaelis-Menten (1913) introduced one of the first, and simplest, mathematical models of enzyme activity

Each step of **full system** obeys mass action

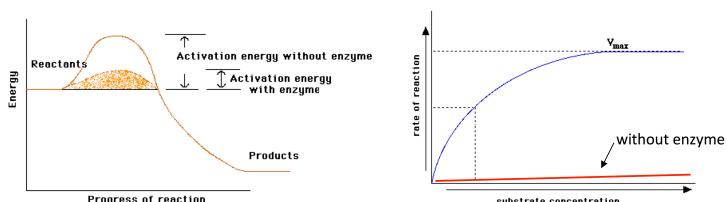
But result is

The overall, **effective**  $S \rightarrow P$  reaction does not obey mass action

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## ENZYMES: EFFECT



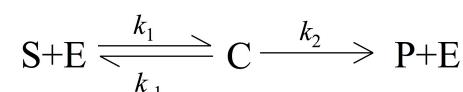
Problem: **nonlinear** in 'substrate' (reactants)

But mass action would be rate =  $k[S]$ , i.e. linear!

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## THE MICHAELIS-MENTEN MODEL: DERIVATION

Assumed reaction mechanism:



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## THE MICHAELIS-MENTEN MODEL: FULL MODEL

Full system of equations:

$$\begin{aligned}\frac{d[S]}{dt} &= -k_1[E][S] + k_{-1}[C] \\ \frac{d[E]}{dt} &= -k_1[E][S] + (k_{-1} + k_2)[C] \\ \frac{d[C]}{dt} &= k_1[E][S] - (k_{-1} + k_2)[C] \\ \frac{d[P]}{dt} &= k_2[C]\end{aligned}$$

Initial conditions:

$$[S](0) = S_0, \quad [E](0) = E_0, \quad [C](0) = 0, \quad [P](0) = 0$$

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## ANALYSIS METHODS

Equilibrium vs Steady-state

- *Equilibrium*: forward and backward components of a *single reaction balanced*
- *Steady state*: *concentrations constant* in time
  - multiple reactions into a particular compartment balance each other; may be unbalanced elsewhere

## THE MICHAELIS-MENTEN MODEL: REDUCED MODEL

Goal: reduction to 'effective' constitutive equation for  
 $S \rightarrow P$

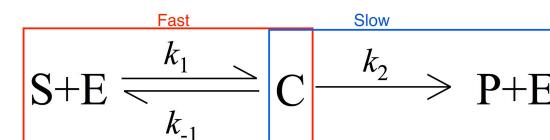
i.e.

$$J_P = \frac{d[P]}{dt} = f([S])$$

Note: people often use  $v$  instead of  $J$  in this context.

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## QUASI-EQUILIBRIUM ANALYSIS

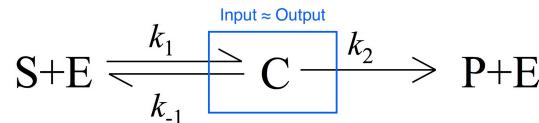


- Assume a fast reaction quickly reaches approximate (quasi-) equilibrium

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## QUASI-STEADY-STATE ANALYSIS



- Assume 'inputs and outputs' to a species or 'compartment' (e.g. to  $[C]$ ) quickly reach approximate (quasi-) balance.

## EMPIRICAL ANALYSIS: LINEWEAVER-BURK PLOTS

To fit to data we can *rewrite* the Michaelis-Menten relation

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

as

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

i.e.  $y = mx + c$  for  $y = \frac{1}{v}$  and  $x = \frac{1}{[S]}$ .

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## REGARDLESS: THE MICHAELIS-MENTEN CONSTITUTIVE EQUATION

Both result in the same *form*:

$$v([S]) := J_P([S]) = \frac{d[P]}{dt} = \frac{V_{max}[S]}{K_M + [S]}$$

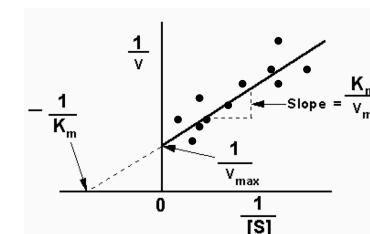
with different  $K_M$  in terms of elementary steps - often just treat *empirically*, i.e. fit  $K_M$ . Notes:

- A *nonlinear* constitutive equation for what would usually be a linear mass action reaction.

## EMPIRICAL ANALYSIS: LINEWEAVER-BURK PLOTS

Called a *Lineweaver-Burk* plot.

Also called a *double reciprocal* plot.



Can also just fit original equation for a range of initial conditions.

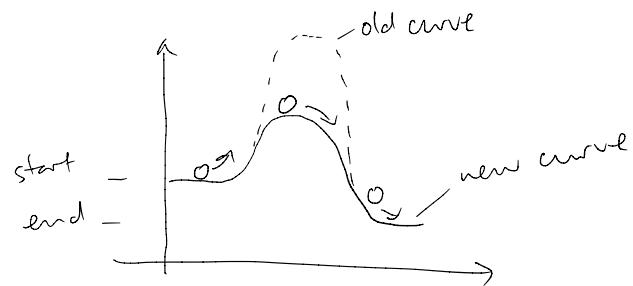
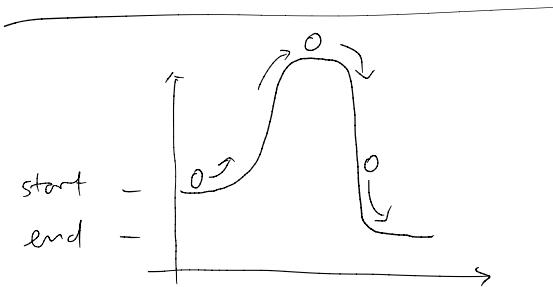
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## Biomeng 261 Lecture 02 : Enzymes

Thermodynamics : possible or not

Kinetics : how fast



- same start & end (thermo)
- different time to get there

Problem : many reactions are possible but too slow

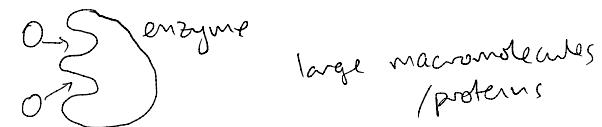
→ could 'heat up' but causes other problems!

Solution? Enzymes

→ biological catalysts

- do help speed up
- don't change start/end
- are not themselves used up in reaction

"Helpers" - help 'get over the barrier'



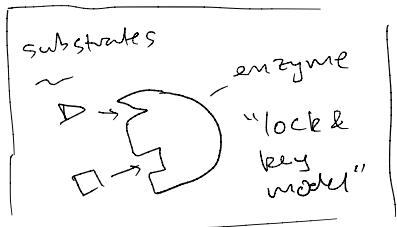
Very helpful! eg factor of  $10^7$  speed up

- eg - help break bonds
- overcome charge repulsion



enzymes are

- specific



- regulated

↳ competitive inhibition } see  
↳ non-competitive inhibition } later

- larger than reactants (substrates)  
↳ macromolecules / proteins

- don't, at first blush, obey  
mass action

Naming hint: - ase

eg permease

invertase

kinase

phosphatase

etc

Michaelis-Menten reaction

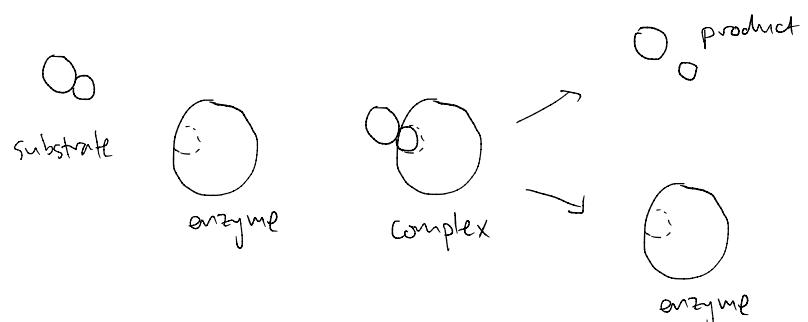
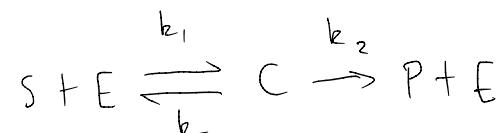
- one of first & simpliest models  
of how enzymes work (1913)

Key:

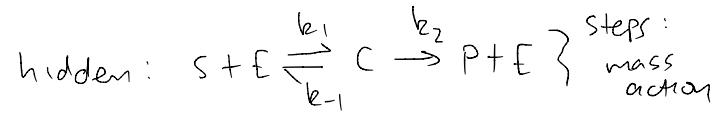
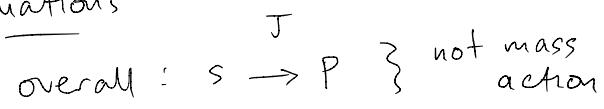
two-step reaction

↳ each step obeys mass action

↳ overall reaction doesn't



### Equations



### 1. conservation

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

$$\frac{d[E]}{dt} = -J_1 + J_{-1} + J_2$$

$$\frac{d[C]}{dt} = J_1 - J_{-1} - J_2 = -\frac{d[E]}{dt}$$

$$\frac{d[P]}{dt} = J_2$$

### 2. constitutive (mass action)

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

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

$$J_2 = k_2 [C]$$


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### Combining

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

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

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

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

### Initial conditions

$$[S](0) = S_0$$

$$[E](0) = E_0$$

$$[C](0) = 0$$

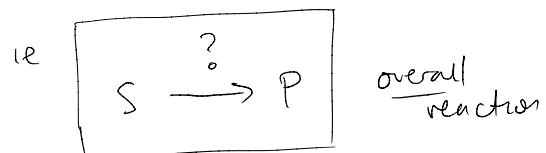
$$[P](0) = 0$$


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what now?

- could simulate whole thing
- could analyse/simplify
  - ↳ let's try!

goal: what is the rate of production of the product as a function of the substrate



remember: doesn't satisfy mass action

→ instead, want

$$\frac{d[P]}{dt} = J_2([S]) \quad \text{for some function}$$

→ want new constitutive eqn for  $J_2$

useful.

1. Total amount of enzyme conserved

$$[E] + [C] = E_0 \quad \textcircled{1}$$

verify

$$\frac{d[E]}{dt} + \frac{d[C]}{dt} = \frac{d([E] + [C])}{dt}$$

=

$$[E][S] \underbrace{(-k_1 + k_1)}_0 + [C] \underbrace{(k_{-1} + k_2 - k_{-1} - k_2)}_0$$

=

0

i.e.  $\frac{d([E] + [C])}{dt} = 0$

$$\Rightarrow [E] + [C] = \text{constant}$$

= initial amount

$$= E_0$$

→ use to eliminate one of  $[E]$  &  $[C]$

↪  $[E]$  here

get ——————

$$\frac{d[S]}{dt} = -k_1(E_0 - [C])[S] + k_{-1}[C]$$

$$\frac{d[C]}{dt} = k_1(E_0 - [C])[S] - (k_{-1} + k_2)[C]$$

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

→ 'exact' so far.

How can we eliminate  $[C]$

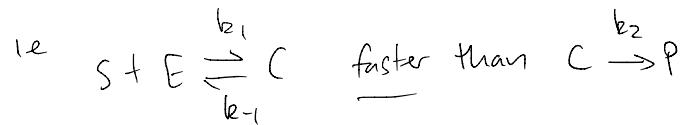
↪ need an approximation

- quasi-equilibrium } historically  
first;  
often easier

- quasi-steady state } prob. better  
'in principle'

Quasi-equilibrium (original, 1913 analysis by M&M)

- Binding/unbinding reaction  
in quasi-equilibrium



$$\underline{\text{set}} \quad k_1[S][E] = k_{-1}[C] \quad (2)$$

& use  $[E] = E_0 - [C]$  from before (1)

$$\Rightarrow k_1[S](E_0 - [C]) = k_{-1}[C]$$

$$\Rightarrow [C] = \frac{k_1[S]E_0}{k_1[S] + k_{-1}}$$

$$\Rightarrow [C] = \frac{[S]E_0}{[S] + \frac{k_{-1}}{k_1}} = \frac{[S]E_0}{[S] + K_{eq}}$$

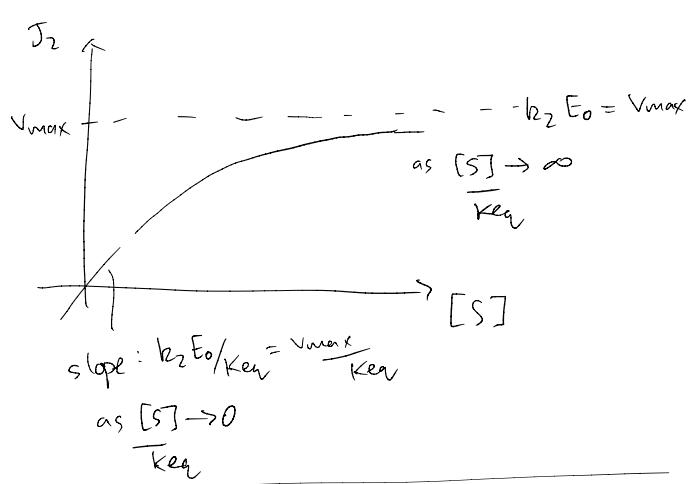
$$\text{recall: } J_2 = \frac{d[P]}{dt} = k_2[C] \rightarrow$$

so ① & ② lead to:

$$J_2 = k_2 \frac{[S] E_0}{[S] + K_{eq}} = \frac{k_2 E_0}{1 + K_{eq}/[S]}$$

where  $K_{eq} = k_1/k_2$   
 $\therefore J_2 = f([S])$

use



Note:  
 $K_{eq} \ll [S] \Rightarrow J_2 \rightarrow k_2 E_0$   
 $K_{eq} \gg [S] \Rightarrow J_2 \rightarrow \frac{k_2 E_0 [S]}{K_{eq}}$

define  $V_{max} = k_2 E_0$

Summary: Quasi-equilibrium

$$\frac{d[P]}{dt} = J_2([S]) = \frac{V_{max} [S]}{[S] + K_{eq}}$$

where  $V_{max} = k_2 E_0$

i.e.  $J_2^{QE} = \frac{V_{max} [S]}{[S] + K_{eq}}$  reaction velocity

→ an enzyme constitutive equation for  $S \rightarrow P$   
 using Michaelis-Menten mechanism

Note: not mass action  
 → saturates (approaches max)

Quasi-steady state (1925, Briggs & Haldane)

- Alternative to quasi-equilibrium

Assume complex concentration

doesn't change much

- small amount of enzyme

- fills up fast, keeps 'adjusting'



total influx from  
all reactions = total outflux  
into C    from all  
    reactions out of C

$$\frac{d[C]}{dt} \approx 0$$

new ②



i.e.  $k_1[S][E] = k_{-1}[C] + k_2[C]$  ②'

&  $[E] = E_0 - [C]$  as before ①

gives  $[C] = \frac{E_0[S]}{K_{ss} + [S]} = \frac{E_0}{1 + \frac{K_{ss}}{[S]}}$

where  $K_{ss} = \frac{k_{-1} + k_2}{k_1}$

cf:  $K_{eq} = \frac{k_{-1}}{k_1}$

Besides this, same result:

$$J_2^{ss} = \frac{V_{max} \cdot [S]}{K_{ss} + [S]}$$

where  $V_{max} = k_2 E_0$   
as before.

only K  
is different

which when?

- see L3

- OR, avoid & use empirical interp.

Empirical Interpretation: just use w/o derivation

L 'constitutive eqn' for enzyme reactions

$$\sim_p := J_p = \frac{d[P]}{dt}$$

↑  
reaction  
'velocity'

$$\sim_p = \frac{V_{max}[S]}{K_M + [S]}$$

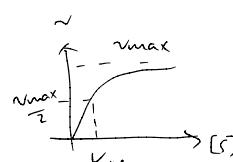
← note  $K_M$   
(not  $K_{cat}$  or  $K_{SS}$ )

Two parameters: empirical interp.  $\Rightarrow$  ie can estimate from data.

•  $V_{max}$ : max of  $\sim_p$



•  $K_M$ : "concentration at which reaction rate is half its max value"



consider  $v = \frac{V_{max}[S]}{K_M + [S]}$  ←  $K_M$  must have units (why?) of conc.

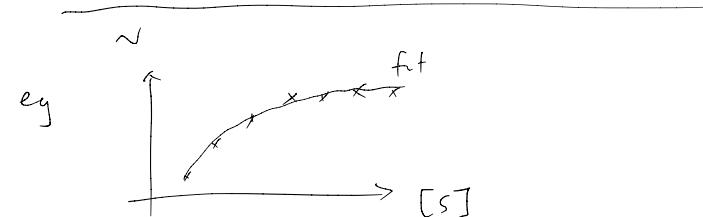
set  $[S] = K_M$  & note:

$$\Rightarrow v = \frac{V_{max} K_M}{K_M + K_M} = \frac{V_{max}}{2}$$

Experimentally:

just assume:

$$\sim([S]) = \frac{V_{max}[S]}{K_M + [S]} \quad \left. \begin{array}{l} \text{& fit to} \\ \text{data} \end{array} \right\}$$



measure initial rate for series of  $S_0$

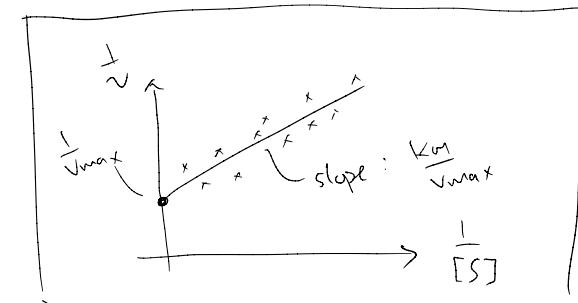
OR: rewrite as:

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

$$\downarrow \qquad \downarrow \qquad \downarrow$$

$$y = m \cdot x + c$$

ie



"Lineweaver-Burk Plot"

or "Double-reciprocal plot"