

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
- Fitting to data

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MODULE OVERVIEW

Reaction kinetics and systems biology (*Oliver Maclarens*)
[11 lectures/3 tutorials/2 labs]

1. *Basic principles: modelling with reaction kinetics* [4 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: signalling and metabolic systems* [4 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?*

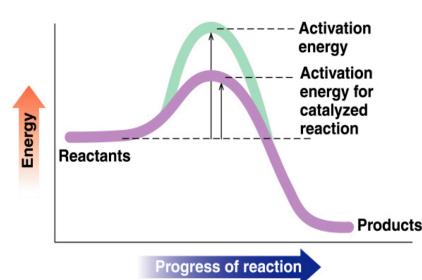
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CATALYSTS

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



ENZYMES

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

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

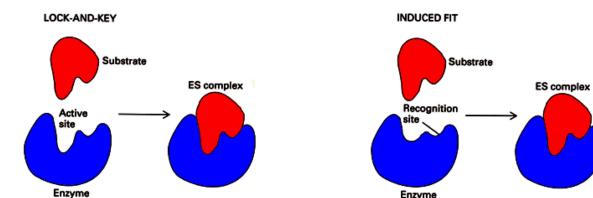
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CATALYSTS

ENZYMES: MECHANISM(S)

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

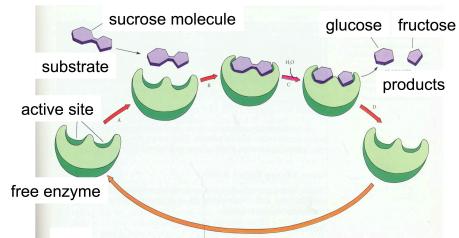


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ENZYMES: EXAMPLE

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

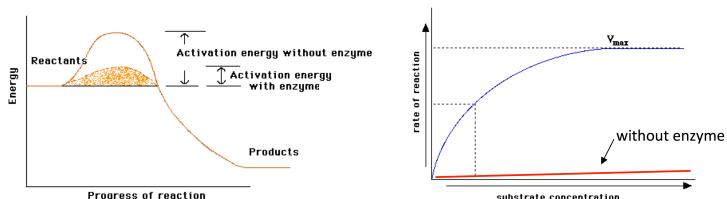
But result is

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

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ENZYMES: EFFECT



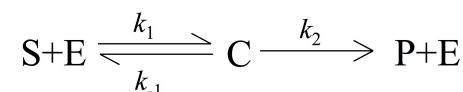
Problem: *nonlinear* in 'substrate' (reactants)

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

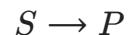
Equilibrium vs Steady-state

- *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

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THE MICHAELIS-MENTEN MODEL: REDUCED MODEL

Goal: reduction to 'effective' constitutive equation for



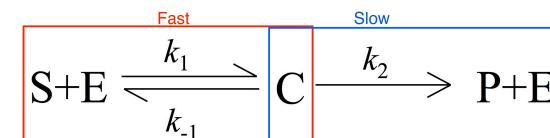
i.e.

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

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

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QUASI-EQUILIBRIUM ANALYSIS

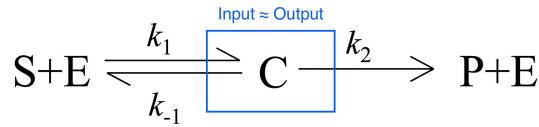


- Assume a fast reaction quickly reaches equilibrium.

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NOTE: LARGE VS SMALL?

QUASI-STEADY-STATE ANALYSIS



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

- Always compare quantities with the *same units*
- Ratio* is then independent of units i.e. *dimensionless*

e.g. quasi-equilibrium compare:

$$\frac{k_2}{k_{-1}} \ll 1?$$

quasi-steady state compare:

$$\frac{E_0}{S_0} \ll 1?$$

Justification.

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UPSHOT: 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.

USE FOR FITTING DATA: 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 $x = \frac{1}{[S]}$.

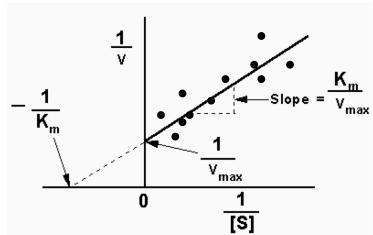
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USE FOR FITTING DATA: LINEWEAVER-BURK PLOTS

Called a *Lineweaver-Burk* plot.

Also called a *double reciprocal* plot.

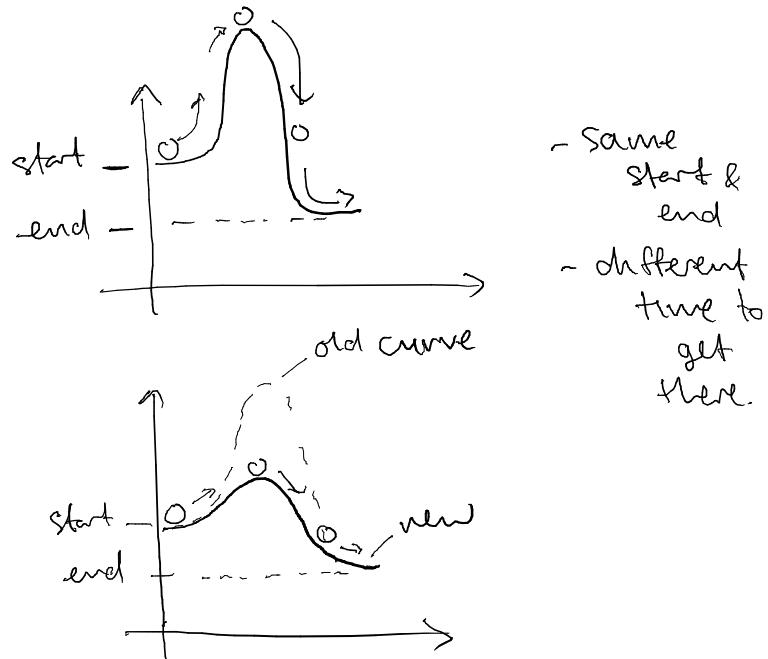


Can also just fit original equation for a range of initial conditions.

Bromeng 26 | Lecture 2: Enzymes

Thermodynamics: possible or not

Kinetics: how fast



Problems

Many reactions are possible

but too slow

→ could 'heat up' but causes other problems!

Solution? Enzymes

are biological catalysts

→ do help speed up

→ don't change start/end

→ are not themselves used up in reaction

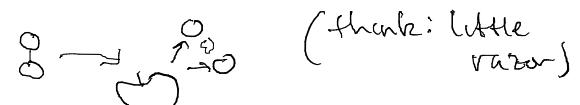
"Helpers" → help 'get over the barrier'



Very helpful - eg factor of 10^7 speed up

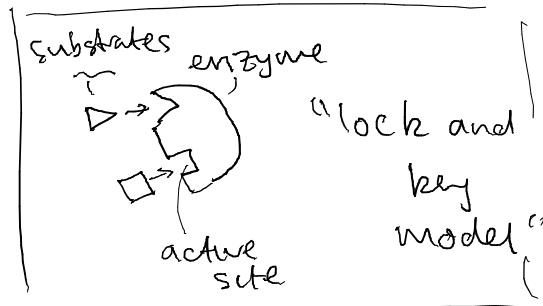
eg - help break bonds

- overcome charge repulsion



(think: little room)

Enzymes are
- specific:



- regulated

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

- larger than reactants (substrates)

↳ macromolecules / proteins

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

Naming hint: -ase

e.g. permease

invertase

kinase

phosphatase

etc

Michaelis-Menten reaction

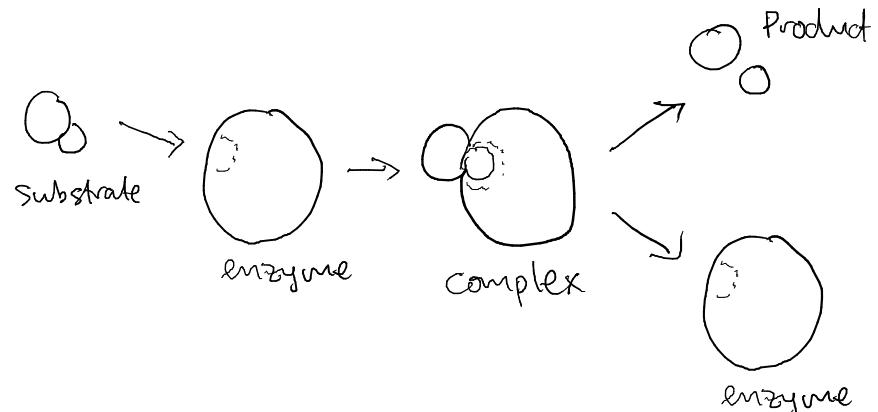
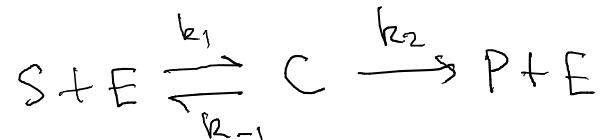
- One of first & simplest models of how enzymes work (1913)

Key:

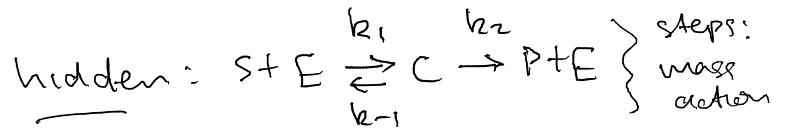
two step reaction

↳ each step obeys mass action

↳ overall reaction doesn't



Equations



1. conservation

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

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

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

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

2. constitutive (mass action)

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

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

$$J_2 = k_2 [C]$$

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

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

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

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

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

what now?

- could simulate whole thing

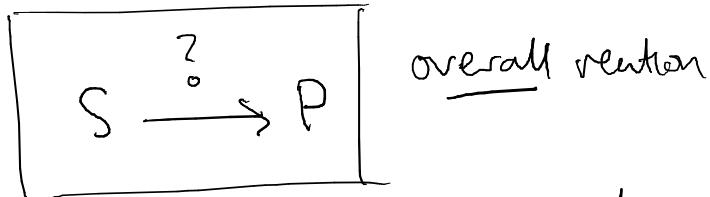
- could analyse / simplify

↳ let's try this!

Goal: what is the rate of production of the product

as a function of substrate

i.e.



(remember:
doesn't
sat.
mass action)

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

want new constitutive eqn for J_2

useful:

1. Total amount of enzyme conserved

$$[E] + [C] = E_0$$

Verify:

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

=

$$[E][S](-k_1+k_1) + [C](k_1+k_2-k_1-k_2)$$

=

0

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

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

= initial condition

$$= E_0$$

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

Let $[E]$ here

get

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

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

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

How can we eliminate $[C]$?

→ need approximation

- quasi-equilibrium } historically first
- quasi-steady state } prob. better

Quasi-equilibrium (original, 1913 analysis)
by MM.

- Binding-unbinding reaction
in quasi-equilibrium



set

$$k_1[S][E] = k_{-1}[C]$$

But $[E] = E_0 - [C]$ from before

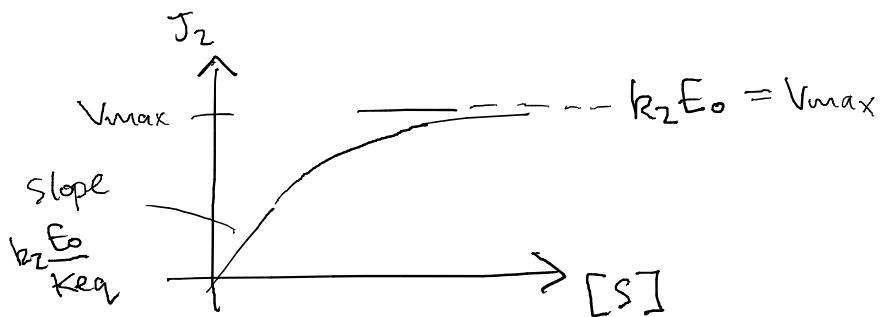
$$\Rightarrow k_1[S](E_0 - [C]) = k_{-1}[C]$$

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

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

$$\text{So } J_2 = \frac{d[P]}{dt} = k_2 \frac{[S]E_0}{[S] + K_{eq}} = \frac{k_2 E_0}{1 + \frac{K_{eq}}{[S]}}$$

$$\text{where } K_{eq} = k_1/k_2$$



$$K_{eq} \ll [S] \Rightarrow J_2 \rightarrow k_2 E_0$$

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

define —

$$\boxed{V_{max} = k_2 E_0}$$

Summary : Quasi-equilibrium

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

where $V_{max} = k_2 E_0$

i.e.

$J_2^{QE} = \frac{V_{max} [S]}{[S] + K_{eq}}$	$\frac{J}{S}$
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an enzyme constitutive equation for



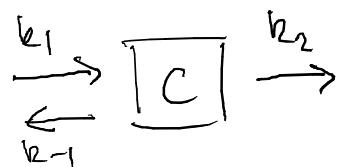
using Michaelis-Menten mechanism.

Note : not mass action!

\rightarrow saturates

Quasi-steady state (1925, Briggs & Haldane)

assume that the complex concentration doesn't change much



- small amount of enzymes
- fill up fast
- overall change in

$$\begin{aligned} \text{total influx} \\ \text{from all} \\ \text{reactions} \\ \text{into } [C] \end{aligned} = \begin{aligned} \text{total outflux} \\ \text{from all} \\ \text{reactions} \\ \text{out of } [C] \end{aligned}$$

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

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

$$\& [E] = E_0 - [C] \text{ from before}$$

gives

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

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

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

Besides this, same result:

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

$$\text{where } V_{max} = k_2 E_0$$

When? Compare scales (here: roughly may return to)

Quasi-equilibrium:



eg compare $\left| \frac{k_1}{k_2} \gg 1 \right\} \text{ie } \frac{J_1}{J_2} \gg 1 \right\}$

Quasi-steady state } separation of scales.

- small number of enzymes } $\left[S_0 \gg E_0 \right]$
- large number of substrate }

$$\left. \frac{d[C]}{dt} \right|_{\max} \approx \frac{E_0}{T}$$

$$\left. \frac{d[S]}{dt} \right|_{\max} \approx \frac{S_0}{T}$$

$$\left| \frac{\frac{d[C]}{dt}}{\frac{d[S]}{dt}} \approx \frac{E_0}{S_0} \ll 1 \right\|$$

Short note on dimensional analysis & scaling

Note: 'big' or 'small' are relative

⇒ compare two quantities with same units

$k_1 \text{ vs } k_2 \text{ is OK}$ } why?
 $k_1 \text{ vs } k_2 \text{ is not}$

Example

$$\frac{1m}{10m} = 0.1 \text{ no units}$$

→ always same ✓

vs $\frac{1m}{10s} = 0.1 \text{ m/s}$

But now measure same thing in different units

$$1m = 10cm$$

$$\Rightarrow \frac{1m}{10s} = \frac{10cm}{10s} = 1 \text{ cm/s} \quad X$$

↑
number changes with units.

→ here can't say is 'big' or 'small'
since changes even though same thing is measured.

Experimentally

(careful!)

- assume Michaelis-Menten

& fit

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

(v = reaction rate = $\frac{\Delta}{t}$)
 K_m = experimentally det.

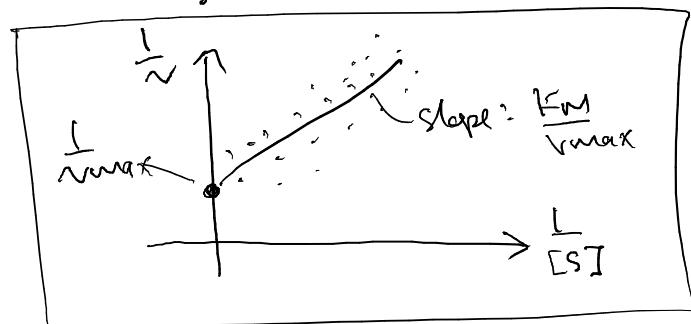
Fit - e.g. measure instant rate for a range of $[S]$ values

OR

- rewrite as

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

i.e. $y = m \cdot x + c$



'Lineweaver-Burk plot'