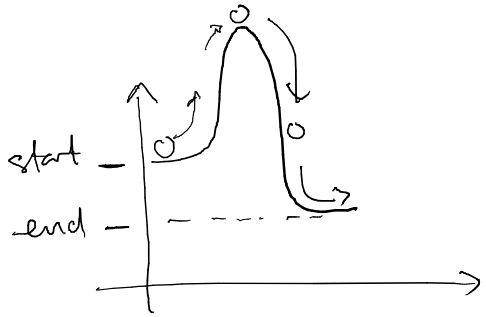
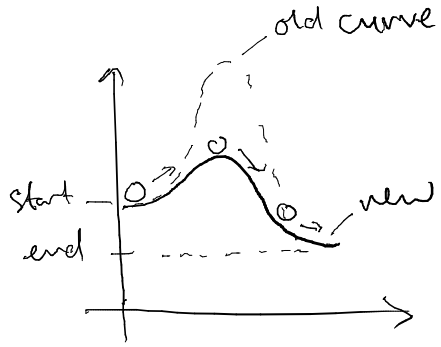
Bioeng 261 Lecture 2: Enzymes

Thermodynamics : possible or not

Kinetics : how fast



- same start & end
- different time to get there.



Problem

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

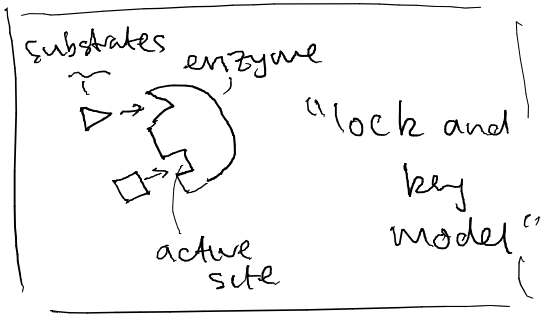
eg - help break bonds

- overcome charge repulsion

(thank: little razor)

Enzymes are

- specific:



"lock and key model"

- regulated
  - competitive inhibition
  - non-competitive inhibition

} see later

- larger than reactants (substrates)
  - macromolecules/proteins
- don't, at first blush, obey mass action

Naming hint: -ase

eg permease
invertase
kinase
phosphatase

etc

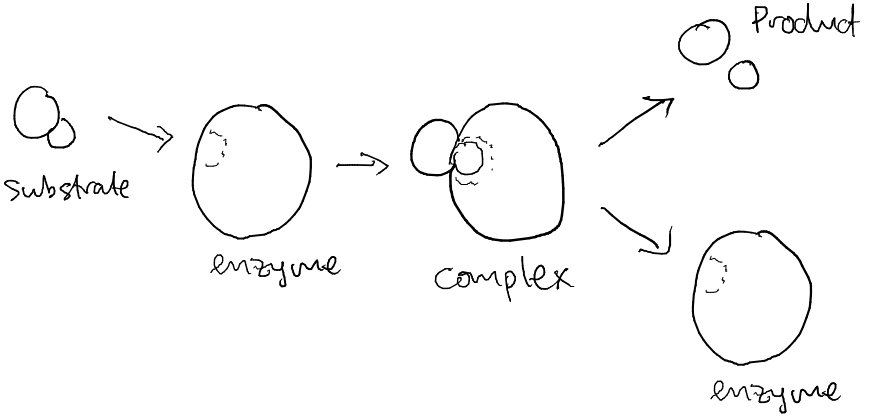
Michaelis-Menten reaction

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

Key:

two step reaction
- Each step obeys mass action
- overall reaction doesn't

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} P + E$$



Equations

overall: $S \xrightarrow{k} P$ } not mass action

hidden: $S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} P + E$ } steps: mass action

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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)

$$J_1 = k_1[S][E]$$
$$J_{-1} = k_{-1}[C]$$
$$J_2 = k_2[C]$$

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

ie

$$S \xrightarrow{?} P$$

overall reaction

(remember: doesn't sat. mass action)

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

want new constitutive eqn for J_2

Useful: (incl. in complex form)

1. Total amount of enzyme conserved

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

verify: *

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

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

$$= 0$$

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

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

$= \text{initial condition}$

$= E_0$

[* remember from before: $\frac{d[C]}{dt} = -\frac{d[E]}{dt}$]

→ use to eliminate one of [E] & [C]

↳ [E] here

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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 Mol.

- Binding-unbinding reaction in quasi-equilibrium

ie $S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C$ faster than $C \xrightarrow{k_2} P$

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

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

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

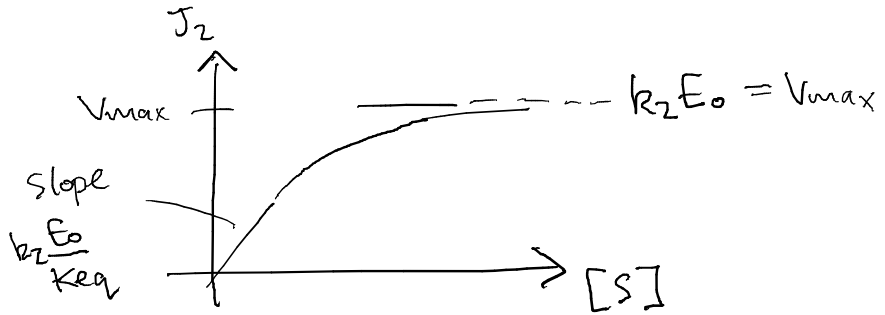
$\Rightarrow [C] = \frac{k_1[S]E_0}{k_1[S] + k_{-1}}$

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

so

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

where $K_{eq} = k_{-1}/k_1$



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

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

define

$$V_{max} = k_2 E_0$$

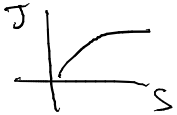
Summary: Quasi-equilibrium

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

where $V_{max} = k_2 E_0$

if

$$J_2^{QE} = \frac{V_{max}[S]}{[S]+K_{eq}}$$



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an enzyme constitutive equation for

$S \to P$

using Michaelis-Menten mechanism.

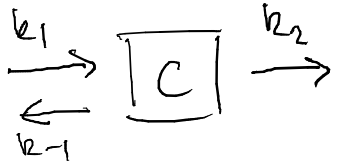
Note: not mass action!

→ 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 is small



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

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

ie $k_1[S][E] = k_{-1}[C] + k_2[C]$

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

→

gives

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

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

→ cf $K_{eq} = \frac{k_{-1}}{k_1}$

Besides this, same result:

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

where $V_{max} = k_2 E_0$

when? Compare scales (here: rough, may return to)

- Quasi-equilibrium:

$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C$ faster than $C \xrightarrow{k_2} P$

eg compare $\boxed{\frac{k_{-1}}{k_2} \gg 1}$ ie $\frac{J_{-1}}{J_2} \gg 1$

- Quasi-steady state } separation of scales.
  - small number of enzymes
  - large number of substrate } $\boxed{S_0 \gg E_0}$

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

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

$$\boxed{\frac{d[C]}{dt} \Big/ \frac{d[S]}{dt} \simeq \frac{E_0}{S_0} \ll 1}$$

Short note on dimensional analysis & scaling

Note: 'big' or 'small' are relative

⇒ compare two quantities with same units

k_{-1} vs k_2 is OK
k_1 vs k_2 is not } why?

Example

$\frac{1m}{10m} = 0.1$ no units → always same ✓

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

But now measure same thing in different units

$1m = 10cm$

$\Rightarrow \frac{1m}{10s} = \frac{10cm}{10s} = 1$ cm/s ✗

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 = $\frac{1}{T}$
K_M = experimentally det.)

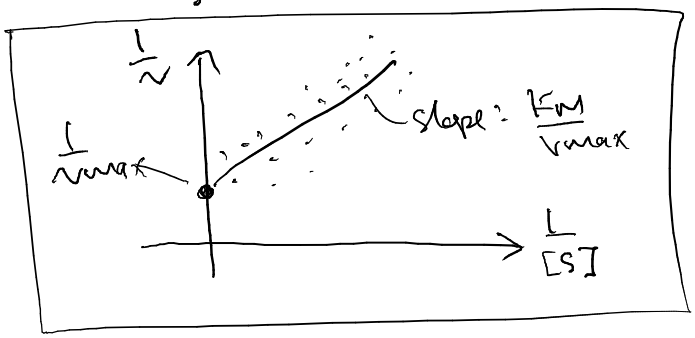
Fit - eg measure initial rate for a range of S_0 values 

OR

- rewrite as

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

ie $y = m \cdot x + c$



'Lineweaver-Burk plot'