

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 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.

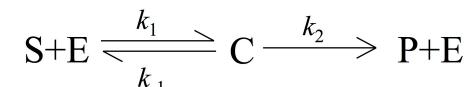
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LECTURE 3 ANALYSIS METHODS

- Quasi-steady state vs quasi-equilibrium
- Units and key dimensionless parameters

THE MICHAELIS-MENTEN MODEL

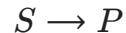
Assumed reaction mechanism:



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THE MICHAELIS-MENTEN MODEL: REDUCED MODEL

Goal: reduction to 'effective' constitutive equation for



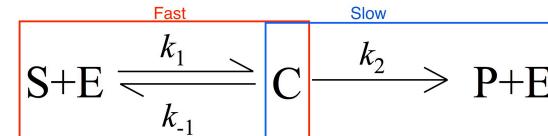
i.e.

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

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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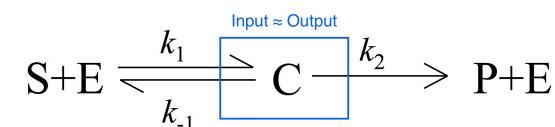
ANALYSIS METHODS

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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QUASI-STEADY-STATE ANALYSIS



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

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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.

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NOTE: LARGE VS SMALL?

- 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:

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

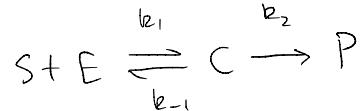
Justification.

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Biomeng 261 Lecture 03 : Enzymes cont'd

- Quasi-equilibrium vs quasi-steady
 in (a bit) more detail
- Units, key dimensionless ratios

In L2 we consider the reaction scheme for an enzyme-mediated reaction



We considered two ways of deriving an approximate $S \rightarrow P$ equation

1. Quasi-equilibrium (+ conservation of enzyme)
2. Quasi-steady state (+ conservation of enzyme)

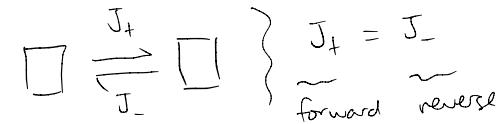
This raises the question:

which / why / when?

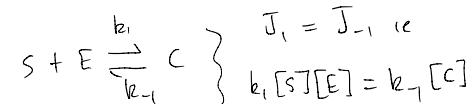
(theoretically, anyway)

Recall:

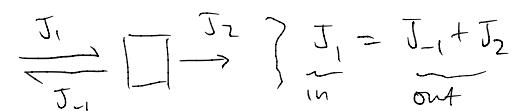
- o The quasi-equilibrium approximation sets the net result of a single reaction to zero:



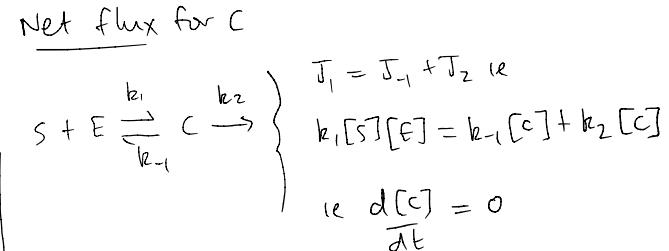
Here: binding/unbinding:



- o The quasi-steady state approximation sets the total influx for a species or 'compartment' equal to the total outflux, ie the net flux for that species to zero:



Here:



Eg QE:

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

$$\& \textcircled{2}: [E] = E_0 - [C]$$

use to eliminate $[E]$ & $[C]$ } other
in full ODEs from S & P .

$\textcircled{2} \rightarrow \textcircled{1}$ & rearrange

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

→ plug into ODEs

QSS: $\textcircled{1}': k_1 [S][E] = k_{-1} [C] + k_2 [C]$

$$\textcircled{2}: [E] = E_0 - [C]$$

same: $\textcircled{2} \rightarrow \textcircled{1}'$ & rearrange:

$$[C] = \frac{E_0 [S]}{K_{ss} + [S]}, K_{ss} = \frac{k_1 + k_2}{k_1}$$

→ plug into ODEs.

... Both give same $\frac{d[P]}{dt} = k_2 [C] = v$ form if

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

"Michaelis
Menten"
equation.

where $V_{max} = k_2 E_0$

$$K_m = \begin{cases} k_{-1}/k_1 = K_{eq} \\ \frac{k_{-1} + k_2}{k_1} = K_{ss} \end{cases}$$

theoretical

or $v_{max} = \dots \text{max of } v$

$$K_m = \frac{\text{concentration}^*}{\text{which } v = v_{max}/2}$$

empirical

QSS probably makes more 'physical' sense
-'life' is more like a 'non-equilibrium
steady state' if anything

* unit check? →

Unit checks? Keq

$$\frac{d[C]}{dt} \text{ etc.} \sim \frac{\text{conc}}{\text{Time}}$$

(~ means
units/dim
of 'ie
[=])

$$J_i \text{ etc.} \sim \frac{\text{conc}}{\text{Time}}$$

So $k_1 \cdot [S][E] \sim \frac{\text{conc}}{\text{Time}}$

where $[S][E] \sim \text{conc}^2$

$$\Rightarrow k_1 \sim \frac{1}{\text{conc. time}}$$

} second
order
rate
constant

Similarly $k_{-1} \cdot [C] \sim \frac{\text{conc}}{\text{time}}$

$$\Rightarrow k_{-1} \sim \frac{1}{\text{time}}$$

} first order
rate const.

$$\Rightarrow K_{eq} = \frac{k_{-1}}{k_1} \sim \frac{1}{\text{time}} \cdot \frac{\text{conc. time}}{\text{conc.}}$$

✓

Exercise: determine K_{ss} units.

Both QE/QSS are approximations, ie we do not expect them to hold exactly

Under what conditions can we expect them to be 'OK' approximations?

- expect something is 'big' or 'small'!
- Note though: 'big' or 'small' are relative

compare two quantities with same units
to give dimensionless/unit-less number

Example: $\frac{1m}{10m} = \underline{0.1}$ no units ✓ OK comparison
 ↳ always same regardless of how measure.

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

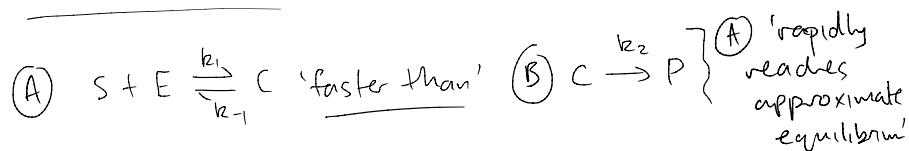
→ now measure same thing
different units:

$$\frac{1m}{10s} = \frac{100cm}{10s} = \underline{10 \text{ cm/s}}$$

↳ different numbers

⇒ can't say 'big' or 'small'

Quasi-equilibrium



Consider:

$$k_{-1} [=] \frac{1}{T} \quad (\text{first order reaction})$$

$$k_2 [=] \frac{1}{T} \quad (\text{first order})$$

$$k_1 [=] \frac{1}{\text{conc}} \cdot \frac{1}{T} \quad (\text{second order})$$

can compare eg

$$\left\{ \begin{array}{l} \frac{k_{-1}}{k_2} = J_{-1}/J_2 \\ \text{or} \\ \frac{k_1 E_0}{k_2} = \frac{V_{\max}}{k_2} \end{array} \right.$$

Usually look at:

$$\left[\frac{k_{-1}}{k_2} \gg 1 \text{ for QE} \right]$$

More sophisticated: dimensional analysis
scaling
perturbation theory
centre-manifolds

} See e.g.
EngSci.
711

Quasi-steady state

we used the approximation

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

but in reality only have

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

Difference is subtle → see e.g. EngSci711 } multiple timescales etc.

To start:

- in QSS $[C]$ can be changing in time, but magnitude of changes are small

↳ relative to something else!

↳ what?

First cut:

Compare $\frac{d[C]}{dt}$ to $\frac{d[S]}{dt}$ over 'some' time interval of interest T

\Rightarrow QSS when: $\left| \frac{\frac{d[C]}{dt}}{\frac{d[S]}{dt}} \right| \ll 1$ over 'long' times T

Relate to problem parameters $k_1, k_{-1}, E_0, S_0 \dots$ etc

note: $\left| \frac{d[C]}{dt} \right|_{\max} \sim \frac{E_0}{T}$ } $T \approx$ timescale for which all enzyme becomes complex

& $\left| \frac{d[S]}{dt} \right|_{\max} \sim \frac{S_0}{T}$ } $T \approx$ timescale for which all substrate becomes product

(compare)

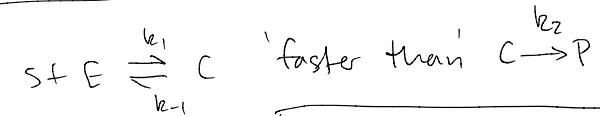
$$\Rightarrow \left| \frac{\frac{d[C]}{dt}}{\frac{d[S]}{dt}} \right|_{\max} \sim \frac{E_0}{S_0}$$

$S_0 :$

$$\boxed{\frac{E_0}{S_0} \ll 1 \text{ for QSS}}$$

Summary

• [Quasi-equilibrium] } relative reaction rates



& quantify via $\boxed{\frac{k_{-1}}{k_2} \gg 1 \text{ for QE}}$

• [Quasi-steady state] } relative rates of change

$$\left| \frac{d[C]}{dt} \right|_{\max} \text{'smaller than' } \left| \frac{d[S]}{dt} \right|$$

... over time scale of interest

quantify via

$$\left| \frac{d[C]}{dt} \right|_{\max} \approx \boxed{\frac{E_0}{S_0} \ll 1 \text{ for QSS}}$$

$$\left| \frac{d[S]}{dt} \right|_{\max}$$

Variable 'slaving' & fast & slow timescales

Even more confusingly, for QSS esp.) these variables often quickly adjust to new steady states over short times when other variables are changed! There are two timescales involved:

- fast transients (rapidly reaches QSS)
- slow longer term evolution (approx. QSS)

Intuition: usually a variable can be set to QSS if it

- rapidly reaches steady state
- quickly 'readjusts' to new steady state if other variables are adjusted
- ↳ fast 'slaving' to 'slow' variables

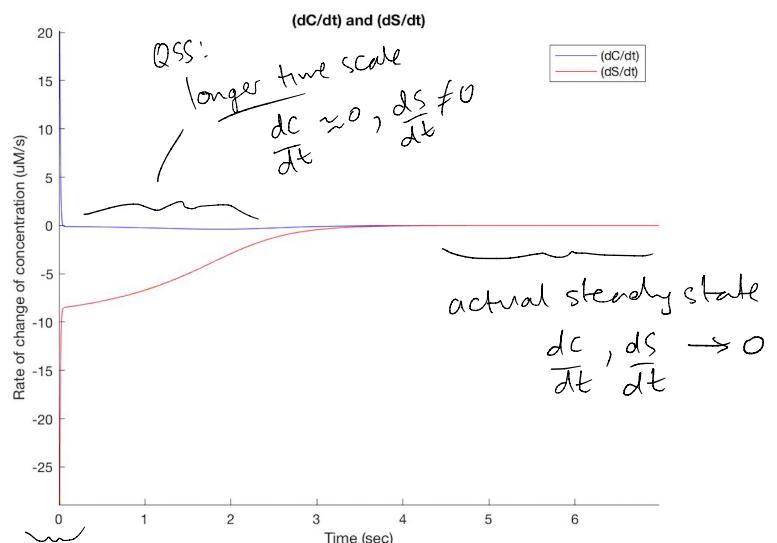
Consider $E_0 / S_0 \ll 1$: small amount of enzyme
: lots of substrate.

fast { → enzyme rapidly 'fills up' with substrate
slow { → stops changing after : bottleneck
years → overall change is small

Example

→ you will look at in computer (lab)

sneak peak:



initially /

$\frac{dc}{dt}$ & $\frac{ds}{dt}$ both large

Verbal.

Q: Describe QSS approximation

- assume c rapidly reaches steady state, after which we take $\frac{dc}{dt} \approx 0$
- occurs when $E_0/S_0 \ll 1$ ie much less enzyme than substrate
- since there is much less enzyme than substrate, initially it rapidly forms complex & hence rapidly reaches steady state, where the complex concentration is approximately constant in time.
- any time product is formed & free enzyme appears, the enzyme is rapidly 're-filled', keeping the complex concentration \approx constant

Extra [not examinable]

How many non-dimensional parameters determine behaviour of problem?

→ 'Buckingham Pi Theorem'

Short version:

Any problem with n parameters & k independent physical dimensions can be re-written in terms of $n-k$ dimensionless parameters

ie reduce to
$$P = n - k$$

\uparrow \rightarrow
 number of parameters number of physical dimensions
 in problem (L, T, M etc)
 in problem

Here 5 parameters $\{k_1, k_2, k_3, S_0, E_0\}$

2 dimensions \leq concentration
 \leq time

$\Rightarrow 5 - 2 = 3$ 'independent' or remaining non-dim. parameters.

Extra [not examinable]

These dim-less parameters indicate key balances:

$$\left. \begin{array}{l} \frac{k_2}{k_1}, \frac{k_1 S_0}{k_2} \ll 1 \rightarrow Q_E \\ \frac{E_0}{S_0} \ll 1 \rightarrow Q_{SS} \end{array} \right\}$$

see e.g.
7.1 for
how to
use do
this
properly.

Moral:

'Balances' are key \Leftrightarrow ratios of terms with
same dimensions.

\hookrightarrow hence ratios
are dim less.
