

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*)

[11 lectures/3 tutorials/2 labs]

1. Basic principles: modelling with reaction kinetics [5 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 [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.

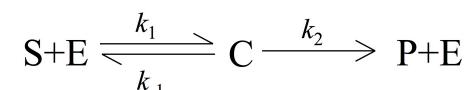
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LECTURE 3 ANALYSIS METHODS

- Quasi-steady state vs quasi-equilibrium
- Scaling and non-dimensional analysis

THE MICHAELIS-MENTEN MODEL

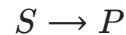
Assumed reaction mechanism:



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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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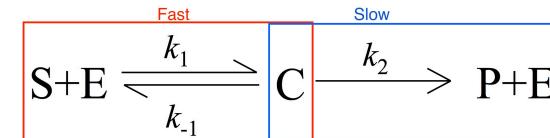
ANALYSIS METHODS

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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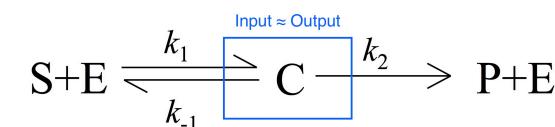
QUASI-EQUILIBRIUM ANALYSIS



- Assume a fast reaction quickly reaches equilibrium.

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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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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.

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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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Bioeng 261 Lecture 3

Bits & Pieces - Quasi-steady state analysis of MM

- conditions for QSS vs QE
- Scaling & non-dim. analysis.

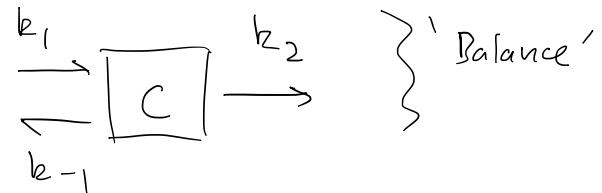
Let's look at some of the analysis methods in a little more detail!

Quasi-steady state analysis of MM

(see also L2 handouts)

(Briggs & Haldane 1925)

→ assume complex concentration doesn't change much:



i.e.:

$\frac{\text{total influx from all reactions into } [C]}{\text{total outflux from all reactions out of } [C]}$

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



$$\Rightarrow k_1 [S][E] = k_{-1}[C] + k_2[C] \quad ①$$

Also assume $[E] = E_0 - [C]$ ②

as before (total amount of enzyme, in all forms, conserved)

\Rightarrow want to eliminate $[E]$ & $[C]$ in full ODEs

\hookrightarrow use ① & ② ! (algebraic eqns)

\Rightarrow ② \rightarrow ① & rearranging gives:

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

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

cf $K_{eq} = \frac{k_1}{k_2}$

so?

let same Michaelis-Menten form:

$$\begin{aligned} v_p := J_p &= \frac{d[P]}{dt} = k_2[C] \\ \uparrow \text{reaction velocity} &= \frac{k_2 E_0 [S]}{K_{ss} + [S]} \end{aligned}$$

i.e. $v = \frac{v_{max} \cdot [S]}{K_M + [S]}$

where $v_{max} = k_2 E_0$

$$K_M = \left\{ \begin{array}{l} k_{-1}/k_2 \quad QE \\ \frac{k_{-1} + k_1}{k_2} \quad QSS \\ \text{empirical} \end{array} \right.$$



Empirical interpretation of K_m ?

$$\left(\begin{array}{l} \text{reaction} \\ \text{rate} \\ = \\ \frac{d[\text{P}]}{dt} \end{array} \right) \rightarrow v = \frac{V_{\max} [S]}{K_m + [S]}$$

set $[S] = K_m$ (note: K_m has units of conc.)

$$\Rightarrow v = \frac{V_{\max} \cdot K_m}{K_m + K_m}$$

$$= \frac{V_{\max}}{2}$$

So K_m is the concentration at which the reaction rate is half of its maximum value

Unit checks?

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

$$J, \text{ etc.} \sim \frac{\text{conc}}{\text{Time}}$$

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

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

$$\Rightarrow k_1 \sim \frac{1}{\text{conc. time}} \quad \left. \begin{array}{l} \\ \\ \end{array} \right\} \text{second order rate constant}$$

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

$$\Rightarrow k_{-1} \sim \frac{1}{\text{time}} \quad \left. \begin{array}{l} \\ \end{array} \right\} \text{first order rate const.}$$

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

$$\sim \text{conc.} \checkmark$$

Exercise: determine K_m units.

Quasi vs True steady state

Quasi : $\frac{dc}{dt} \approx 0$ } often
 'dynamic' ss.

True : $\frac{dc}{dt} = 0$ } no change
 at all

Difference is subtle \rightarrow see EngSci711

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

\rightarrow rapidly reaches steady
 state

\rightarrow quickly 'readjusts' to new
 steady state if other
 variables are adjusted

\hookrightarrow fast 'slaving' to 'slow'
 variables

Often the magnitude of its changes
 are small relative to those of
 other variables over longer times

Eg $\frac{dc}{dt} / \frac{ds}{dt} \ll 1$

over longer times

$$\left. \frac{dc}{dt} \right|_{\max} \approx \frac{E_0}{T}$$

$$\left. \frac{ds}{dt} \right|_{\max} \approx \frac{S_0}{T}$$

$$\Rightarrow \left. \frac{dc}{dt} \right|_{\max} / \left. \frac{ds}{dt} \right|_{\max} \approx \underline{\frac{E_0}{S_0} \ll 1}$$

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 ✓

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

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.

Scaling & Non-dimensionalisation

Given a quantity C , introduce:

$$C = C_0 C^*$$

↑ ← ↗
 original scale, with new
 quantity dimensions dimensionless
 with dimensions quantity

i.e.

$$\boxed{\text{Variable} = \text{Constant} \times \text{Variable}}$$

Can also view as

$$\boxed{C^* = C/C_0}$$

if natural ref
≠ 0.

(sometimes use $C^* = \frac{C - C_1}{C_0}$ i.e. $C = C_1 + C_0 C^*$)

The scale factor is arbitrary so far, & can be chosen (depending on what you want to do):

→ according to scale of interest, e.g.
'1 metre'

→ according to 'natural' maximum values (& (or BC/ICs))

→ to simplify the equations

- by 'balancing' terms in equations
& assuming 'well-scaled'

↳ "method of dominant balances"

Bit of an art → see EngSci 711
etc for more theory

Example: enzyme-kinetics

$$\frac{dS}{dt} = k_{-1}C - k_1 SE$$

$$\frac{dE}{dt} = (k_{-1} + k_2)C - k_1 SE$$

$$\frac{dC}{dt} = k_1 SE - (k_2 + k_{-1})C$$

$$\frac{dP}{dt} = k_2 C$$

&

$$E + C = E_0$$

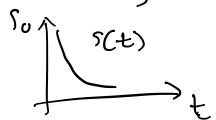
$$E(0) = E_0$$

$$C(0) = 0$$

$$S(0) = S_0$$

$$P(0) = 0$$

Note: ICs can indicate
natural max values
e.g.:



Scalings:

$$\left. \begin{aligned} S &= S_0 S^* \\ C &= E_0 C^* \\ E &= E_0 E \\ P &= S_0 P \end{aligned} \right\}$$

natural choices for max values → why?
→ see ICs!

$$\left. \begin{aligned} t &= T_0 t^* \end{aligned} \right\}$$
 arbitrary time scale
→ choose based on balances

Substitute in:

$$\frac{dS}{dt} = \frac{d(S_0 S^*)}{dT_0 t^*} = \frac{S_0}{T_0} \frac{dS^*}{dt^*} \quad (S_0, T_0 \text{ constants})$$

etc.

gives

$$\frac{S_0 \frac{dS^*}{dt^*}}{T_0} = k_{-1} E_0 C^* - (k_1 S_0 E_0) S^* E^*$$

$$\frac{E_0 \frac{dE^*}{dt^*}}{T_0} = (k_{-1} + k_2) E_0 C^* - k_1 S_0 E_0 S^* E^*$$

$$\frac{E_0 \frac{dC^*}{dt^*}}{T_0} = k_1 S_0 E_0 S^* E^* - (k_{-1} + k_2) E_0 C^*$$

$$\frac{S_0 \frac{dP^*}{dt^*}}{T_0} = k_2 E_0 C^*$$

Goal: choose T_0 to give a 'natural balance'
of interest

↳ eg simplify the 'interesting' parts



Here : product formation time scale. (of interest to us).

⇒ 'Balance':

$$\boxed{\frac{S_0 \frac{dP^*}{dt^*}}{T_0} = k_2 E_0 C^*}$$

(product equation terms
↳ usually choose two,
here only two
anyway.)

'Natural':

the corresponding non-dimensional (* terms)
quantities are ~ 1 .

⇒ Use:

$$\frac{S_0}{T_0} \times (1) = k_2 E_0 \times (1)$$

$$\text{ie } \frac{S_0}{T_0} = k_2 E_0 \Rightarrow \boxed{T_0 = \frac{S_0}{k_2 E_0}}$$

Key: natural product time scale



Using $T_0 = \frac{S_0}{k_2 E_0}$ gives:

$$1. k_2 \frac{dS^*}{dt^*} = k_{-1} C^* - (k_1 S_0) S^* E^*$$

$$\Rightarrow \boxed{\frac{dS^*}{dt^*} = \left(\frac{k_{-1}}{k_2}\right) C^* - \left(\frac{k_1 S_0}{k_2}\right) S^* E^*}$$

$$2. \frac{k_2 E_0}{S_0} \frac{dE^*}{dt^*} = (k_{-1} + k_2) C^* - k_1 S_0 S^* E^*$$

$$\Rightarrow \boxed{\frac{dE^*}{dt^*} = \left(1 + \frac{k_{-1}}{k_2}\right) \left(\frac{S_0}{E_0}\right) C^* - \left(\frac{k_1 S_0}{k_2}\right) \left(\frac{S_0}{E_0}\right) S^* E^*}$$

$$3. \boxed{\frac{dc^*}{dt^*} = -\frac{dC^*}{dt^*}} \quad \hookrightarrow \left(\frac{S_0}{E_0}\right) \left[\left(1 + \frac{k_{-1}}{k_2}\right) C^* - \frac{k_1 S_0}{k_2} S^* E^*\right]$$

$$4. \boxed{\frac{dp^*}{dt^*} = C^*} \quad \leftarrow \text{Note: Product equation simplified.}$$

AND !

$E_0 E^*(0) = E_0$	$\left\{ \begin{array}{l} E^*(0) = 1 \\ C^*(0) = 0 \\ S^*(0) = 1 \\ P^*(0) = 0 \end{array} \right.$	don't forget <u>new</u> <u>ICs</u> .
$E_0 C^*(0) = 0$		
$S_0 S^*(0) = S_0$		
$S_0 P^*(0) = 0$		

\Rightarrow

Erm... is this any simpler?

Yes!

$$\alpha \quad \beta \quad \gamma$$

\rightarrow New Parameters : $\boxed{\left\{ \frac{k_{-1}}{k_2}, \frac{k_1 S_0}{k_2}, \frac{S_0}{E_0} \right\}}$

Reduced from
5 parameters: $\{k_1, k_{-1}, k_2, S_0, E_0\}$

to 3 dimensionless parameters $\left\{ \frac{k_{-1}}{k_2}, \frac{k_1 S_0}{k_2}, \frac{S_0}{E_0} \right\}$

(drop *): $\frac{dS}{dt} = \alpha \cdot C - \beta \cdot S \cdot E$

$$\frac{dE}{dt} = (1 + \alpha) \gamma \cdot C - \beta \cdot \gamma \cdot S \cdot E$$

$$\frac{dP}{dt} = C$$

Follows from 'Buckingham π Theorem': } Key result of dimension analysis.

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

reduce to

$$P = n - k \quad \begin{matrix} \text{'free parameters'} \\ \uparrow \quad \downarrow \\ \text{number of} \\ \text{parameters} \\ \text{in problem} \end{matrix} \quad \begin{matrix} \text{dimensions} \\ (L, T, M \text{ etc}) \\ \text{in problem} \end{matrix}$$

Here 5 parameters $\{k_1, k_m, k_2, s_0, E_0\}$

$$\begin{matrix} 2 \text{ dimensions} & \begin{matrix} \text{concentration} \\ \text{time} \end{matrix} \\ \hline \Rightarrow 5 - 2 = 3 \text{ 'Independent' or remaining non-dim.} \\ \text{parameters.} \end{matrix}$$

Finally, these dim-less parameters

indicate key balances:

$$\frac{k_2}{k_1}, \frac{k_1 s_0}{k_2} \ll 1 \rightarrow QE$$

$$\frac{E_0}{s_0} \ll 1 \rightarrow QSS$$

see 7.11 for how to use do this properly.

Moral:

'Balances' are key $\left\{ \begin{matrix} \text{ratios of terms with} \\ \text{same dimensions.} \end{matrix} \right\}$

\hookrightarrow hence ratios are dim less.