

BIOMENG 261

TISSUE AND BIOMOLECULAR ENGINEERING

Module I: Reaction kinetics and systems biology

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LECTURE 8: SYSTEMS BIOLOGY CONT'D

- Metabolic modelling
- Intro to Flux Balance Analysis (FBA)
- Null spaces (linear algebra)

MODULE OVERVIEW

Reaction kinetics and systems biology (*Oliver Maclaren*)
[12 lectures/4 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.

EXAMPLE: METABOLIC MODELS

Recall: *metabolism*

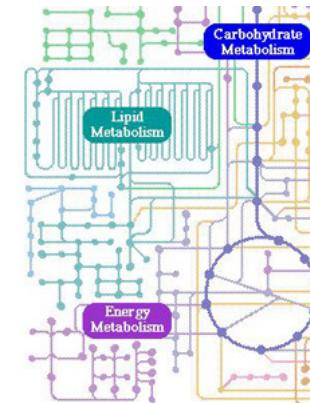
The *consumption and production of chemical substances and energy to sustain life*

- Catabolism: breakdown
- Anabolism: build up

Food → Life

EXAMPLE METABOLIC MODELS/NETWORKS

Zoomed in:

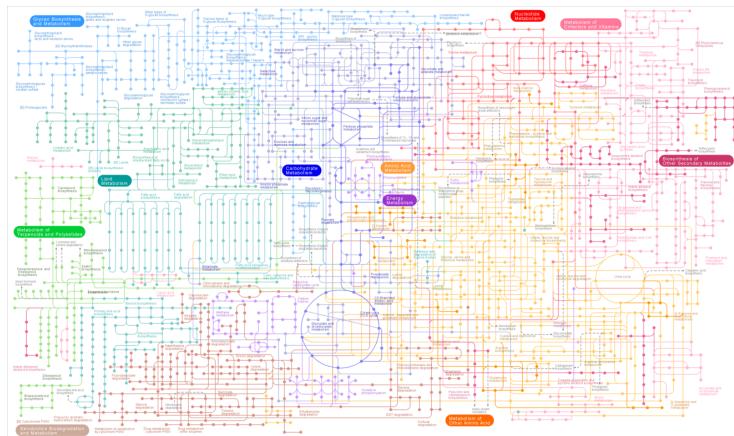


METABOLIC MODELS AS NETWORKS

Usually viewed as a *large network* of interacting pathways

Can reconstruct via *genome sequencing*

EXAMPLE METABOLIC MODELS/NETWORKS



From: http://www.genome.jp/kegg-bin/show_pathway?map01100

APPROACHES

- *Full dynamic model* and search for smaller sets of important parameters (e.g. introduce complexity trade-off)
- *Difficult to scale up* to this size! (See previous lecture).
- *Try something else!*

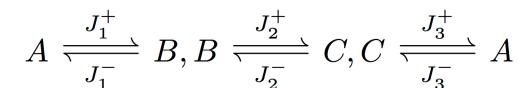
FLUX BALANCE ANALYSIS

More from Babtie and Stumpf (2017):

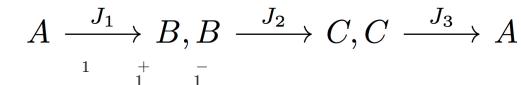
present in a system. In cell biology, for example, there are now numerous attempts at modelling aspects of metabolism, gene regulation and signalling at cellular level [17–24]. Perhaps the best established are metabolic models, where a powerful set of tools, based around *flux balance analysis* (FBA) [25], allows us to explore metabolic phenotypes *in silico* at a genomic level for an increasing range of organisms (and some individual cell types) [24,26,27]. However, such models are stoichiometric and thus give us information about biochemical reaction schemes and fluxes, but not details about the system dynamics.

MOTIVATING EXAMPLE

Consider



or, equivalently in terms of *net* fluxes:



where $J = J^+ - J^-$ etc, and the arrows represent *net* fluxes

WHAT IS FLUX-BASED ANALYSIS?

Orth et al. (2010) in Nature Biotechnology:

What is flux balance analysis?

Jeffrey D Orth, Ines Thiele & Bernhard Ø Palsson

Flux balance analysis is a mathematical approach for analyzing the flow of metabolites through a metabolic network. This primer covers the theoretical basis of the approach, several practical examples and a software toolbox for performing the calculations.

(see Canvas)

MATRIX/VECTOR FORM

Derivation: see handout.

Result:

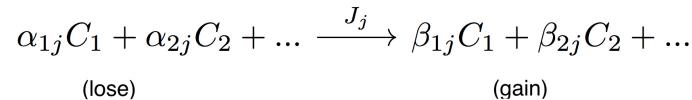
$$\frac{d\mathbf{C}}{dt} = \mathbb{S}\mathbf{J}$$

where \mathbf{C} is a *vector* of concentrations/metabolites, \mathbf{J} is a *vector* of fluxes and... \mathbb{S} is the...

MATHEMATICAL FRAMEWORK OF FLUX BALANCE ANALYSIS

STOICHIOMETRIC MATRIX \mathbb{S}

Given M species/metabolites and N reactions, each of the form



Rather than the dynamic model, we aim to solve the *steady-state* equation

$$\mathbb{S}\mathbf{J} = \mathbf{0}$$

for the vector of fluxes \mathbf{J} , *here treated as unknown*.

- No constitutive equations/no rate parameters involved here.
- We don't need to know the metabolite concentrations, just solve for fluxes

STOICHIOMETRIC MATRIX

...we write these as a *matrix*

$$\mathbb{S} = \begin{bmatrix} \beta_{11} - \alpha_{11} & \dots & \beta_{1N} - \alpha_{1N} \\ \dots & \beta_{ij} - \alpha_{ij} & \dots \\ \beta_{M1} - \alpha_{M1} & \dots & \beta_{MN} - \alpha_{MN} \end{bmatrix}$$

Note:

- Rows: species
- Columns: fluxes
- Entries: gain minus loss (stoichiometric coefficients)

WHAT'S THE CATCH?

For a given metabolic network there are *typically* (not always) *more reactions/fluxes* than species/metabolites i.e.

More columns (unknowns) than rows (equations)

The problem is *underdetermined*, i.e. there are typically *multiple solutions*.

There is a non-trivial *null space*.

NULL SPACE?

For a matrix \mathbb{A} the *null space* is just the set of solutions to the zero problem

$$\mathbb{A}\mathbf{x} = \mathbf{0}$$

i.e. here

$$N(\mathbb{S}) = \{\mathbf{J} \mid \mathbb{S}\mathbf{J} = \mathbf{0}\}$$

Example.

Biomeng 261 Lecture 8 :

Systems Biology cont'd : Metabolism

- Scaling up to much larger systems using (steady state) Flux Balance Analysis (FBA).

↳ application to metabolic modelling

What is FBA?

→ another paper!

Orth et al (2010) in Nature Biotechnology

What is flux balance analysis?

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Flux balance analysis is a mathematical approach for analyzing the flow of metabolites through a metabolic network.
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Flux Balance Analysis (Part I)

- Motivation & formulation.

→ common for metabolic models

Recall : Metabolism (cellular)

'all the chemical processes
keeping the cell alive'

→ large systems! (e.g. 100-1000s of
↳ can be genome scale! ODEs, each with many parameters)
↳ many interacting pathways'

→ naive parameter estimation doesn't work well

→ dynamic data often not available

Alternative trade-off/approach:

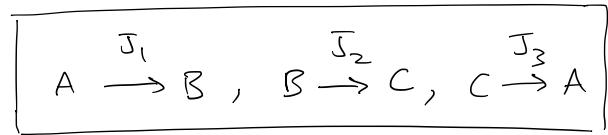
i.e. mass balance → {
but no constitutive equations
fluxes only } stoichiometric matrices
steady states (usually)
(can include some overall thermodynamic info)

FBA: simple motivating example.

Consider $A \xrightleftharpoons[J_1^-]{J_1^+} B, B \xrightleftharpoons[J_2^-]{J_2^+} C, C \xrightleftharpoons[J_3^-]{J_3^+} A$

In terms of net fluxes, where $J_i = J_i^+ - J_i^-$ etc

can write



where $\xrightarrow{J_i}$ represents $\xrightleftharpoons[J_i^-]{J_i^+}$ etc.

Conservation of mass is then

$$\boxed{\begin{aligned}\frac{d[A]}{dt} &= -J_1 + J_3 \\ \frac{d[B]}{dt} &= +J_1 - J_2 \\ \frac{d[C]}{dt} &= +J_2 - J_3\end{aligned}}$$

FBA: simple motivating example.

Now, we write as matrix/vector equation:

$$\bar{c} = \begin{bmatrix} [A] \\ [B] \\ [C] \end{bmatrix}, \quad \frac{d\bar{c}}{dt} = \begin{bmatrix} \frac{d[A]}{dt} \\ \frac{d[B]}{dt} \\ \frac{d[C]}{dt} \end{bmatrix}$$

$$\bar{J} = \begin{bmatrix} J_1 \\ J_2 \\ J_3 \end{bmatrix} \quad \leftarrow \text{also use } v_i \text{ & } \bar{v} \text{ instead of } J_i \text{ & } \bar{J} \text{ sometimes}$$

Gives: (overbar: vector
underbar: matrix)

$$\frac{d\bar{c}}{dt} = \underbrace{\begin{bmatrix} -1 & 0 & +1 \\ +1 & -1 & 0 \\ 0 & +1 & -1 \end{bmatrix}}_{S \text{ matrix}} \bar{J}$$

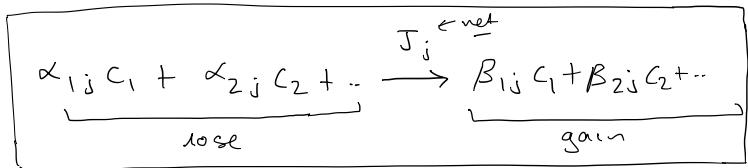
$$\text{i.e. } \boxed{\frac{d\bar{c}}{dt} = S \bar{J}}$$

Note: we are not using a constitutive equation $\bar{J} = f(\bar{c})$ so we do not have a 'closed' system of eqns

$$\frac{d\bar{c}}{dt} = F(\bar{c})$$

FBA: Stoichiometric matrices S

Given M species & N reactions, each of the form



we define the Stoichiometric matrix

$$S = \begin{matrix} & c_1 & c_2 & \cdots & c_i & \cdots & c_N \\ & \downarrow J_1 & \downarrow J_2 & \cdots & \downarrow J_i & \cdots & \downarrow J_N \\ \begin{matrix} c_1 \\ c_2 \\ \vdots \\ c_i \\ \vdots \\ c_M \end{matrix} & \left[\begin{array}{cccccc} \beta_{11} - \alpha_{11} & \cdots & \cdots & \beta_{1N} - \alpha_{1N} & & \\ \vdots & & & \vdots & & \\ & \cdots & \cdots & \cdots & & \\ & \vdots & & \vdots & & \\ & \vdots & & \vdots & & \\ & \beta_{M1} - \alpha_{M1} & \cdots & \beta_{MN} - \alpha_{MN} & & \end{array} \right] \end{matrix}$$

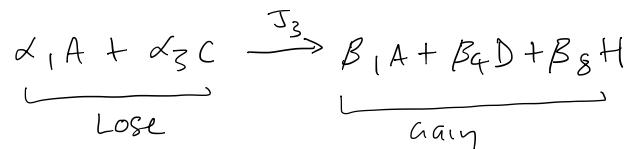
↓ just to help fill in matrix
 ↓ not part of it!

Note $+\beta$, $-\alpha$

→ sign is determined by choice of sign for net flux.

FBA: Stoichiometric matrices

Example : 3rd reaction in some system is



Then:

$$S = \begin{bmatrix} A & J_1 & J_2 & J_3 & \cdots \\ B & & & \beta_1 - \alpha_1 & \\ C & & & 0 & \\ D & & & -\alpha_3 & \\ \vdots & & & +\beta_4 & \\ H & & & 0 & \\ & & & \vdots & \\ & & & +\beta_8 & \end{bmatrix}$$

↑
3rd reaction : 3rd column

As mentioned, we are working in terms of pure fluxes J (ie conservation of mass)

- no constitutive eq's
- no rate parameters
- no 'closure' eg $\frac{dc}{dt} = F(c)$.

FBA: Steady states

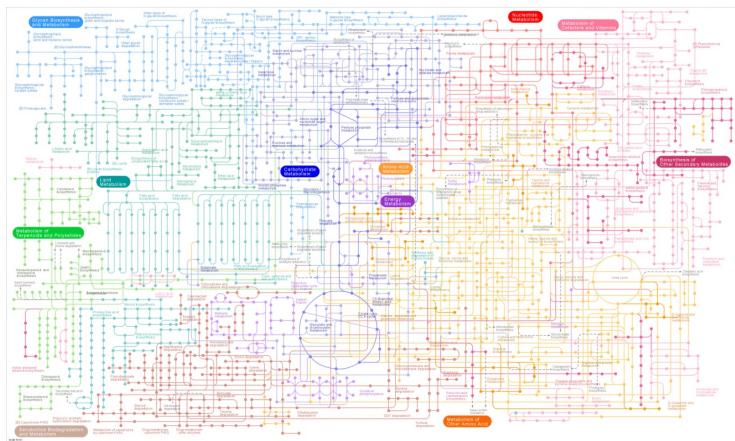
To avoid needing constitutive equations &/or needing dynamic data, in FBA we usually

- just consider steady states
- treat fluxes as unknowns to determine.

→ makes sense when thinking about eg overall metabolism & homeostasis
 ↳ hence popular in this area

→ often have eg 'metabolic network'
 maps available to 'help' build models:

Eg



FBA: Steady states

Idea:

- Take a system of 'fluxes'
 J_1, J_2, \dots etc

◦ Could write as $\frac{dC}{dt} = S\bar{J}$

FBA

- Instead, just construct S
- Solve $S\bar{J} = \bar{0}$ for fluxes \bar{J} as unknown.
 ↳ no constitutive eq's.

So: [New goal]

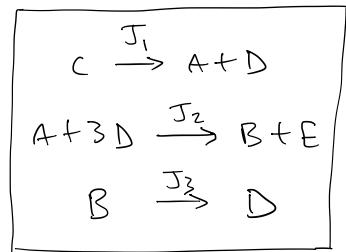
solve $S\bar{J} = \bar{0}$

steady state only.

for fluxes \bar{J}
 ↑
 not rate constants.

FBA: Examples

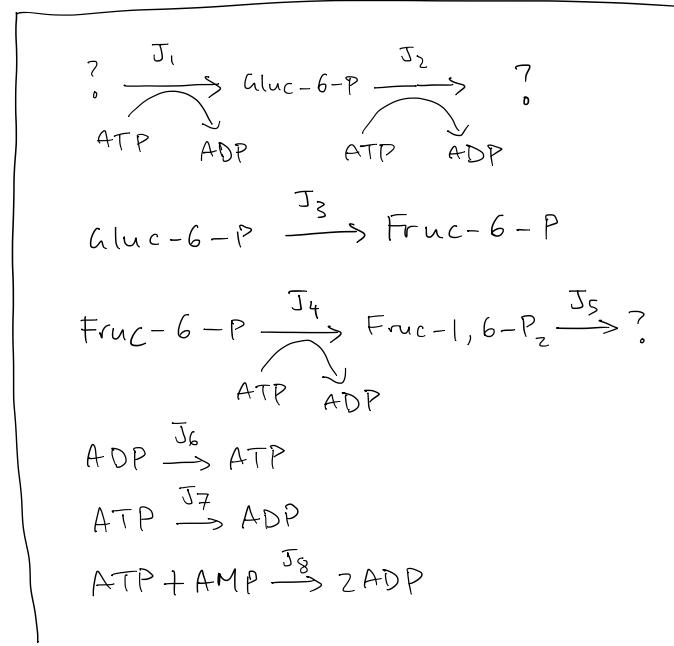
A. Determine S for:



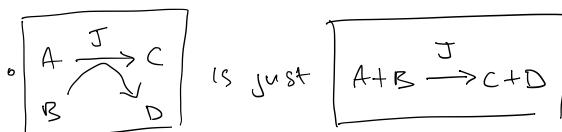
Answer:

$$S = \begin{matrix} & J_1 & J_2 & J_3 \\ \begin{matrix} A \\ B \\ C \\ D \\ E \end{matrix} & \left[\begin{matrix} +1 & -1 & 0 \\ 0 & +1 & -1 \\ -1 & 0 & 0 \\ +1 & -3 & +1 \\ 0 & +1 & 0 \end{matrix} \right] \end{matrix}$$

Examples: B. Determine S for the system:



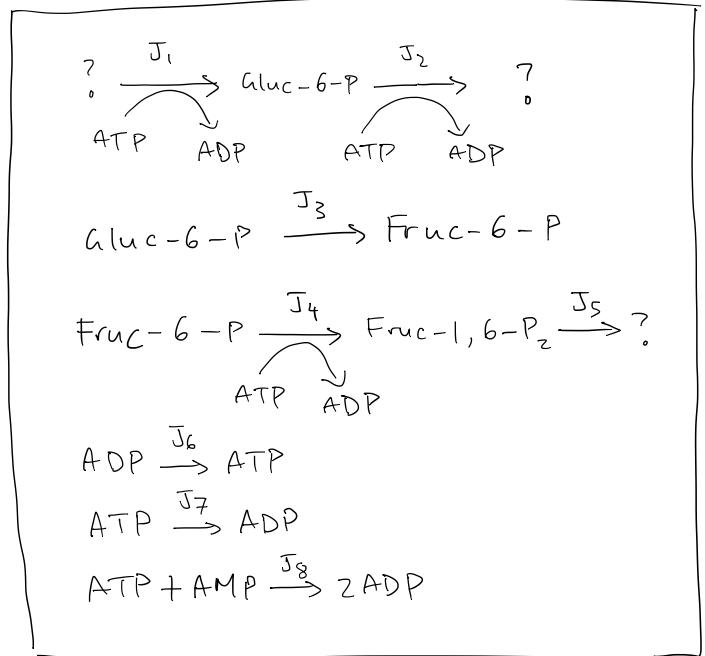
Notes:



- we allow 'unknown' metabolites via '?'
 \rightarrow no row in S , but still include reaction col!



Answers: for



we have:

$$\leq = \begin{pmatrix} J_1 & J_2 & J_3 & J_4 & J_5 & J_6 & J_7 & J_8 \\ 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 \\ -1 & -1 & 0 & -1 & 0 & 1 & -1 & -1 \\ 1 & 1 & 0 & 1 & 0 & -1 & 1 & 2 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \end{pmatrix} \begin{array}{l} \leftarrow \text{Gluc-6-P} \\ \leftarrow \text{Fruc-6-P} \\ \leftarrow \text{Fruc-1,6-P}_2 \\ \leftarrow \text{ATP} \\ \leftarrow \text{ADP} \\ \leftarrow \text{AMP} \end{array}$$

What's the catch? $\sum \bar{J} = \bar{0}$ soln?

Here:

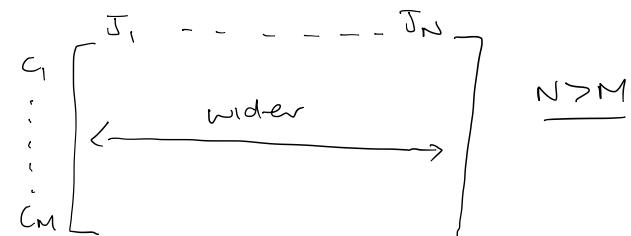
6 rows \leftarrow equations/constraints
8 columns \leftarrow unknowns (J)

unknowns > eqns

In general (in real problems)

- more reactions/fluxes (unknowns) than metabolites/concentrations (equations)
- often don't know all metabolites involved (lose a row)

shape:



$\rightarrow \sum \bar{J} = \bar{0}$ is usually underdetermined

\rightarrow ie multiple solutions

\rightarrow makes sense since only using conservation of mass.

To understand this mathematically / geometrically
we need some linear algebra

$$\hookrightarrow \{ \begin{matrix} \text{today} \\ \text{tomorrow} \end{matrix} \}$$

Null spaces (of \underline{S} say)

- The nullspace of a matrix \underline{A} is the set of all solutions to $\underline{A}\bar{x} = \bar{0}$
- zero vector is always in nullspace $\underline{A}\bar{0} = \bar{0}$
- A non-trivial null-space is when we have non-zero solutions in the nullspace
- dimension of null-space is #vars - #indp. constraints

here:
 $\bar{x} = \bar{J}$
 ie fluxes &
 $\underline{A} = \underline{S}$

Example

$$\underline{S} \bar{J} = \bar{0} : \begin{bmatrix} 1 & -1 & 0 & 0 \\ 0 & -1 & 1 & -1 \end{bmatrix} \begin{bmatrix} J_1 \\ J_2 \\ J_3 \\ J_4 \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \end{bmatrix}$$

' $\underline{A}\bar{x} = \bar{0}$ '

4 unknowns (J_1, \dots, J_4)

2 eqns (indep.).

expect $4-2 = 2$ free vars
 \Rightarrow two indep. sol's.

① $J_1 - J_2 = 0$

② $-J_2 + J_3 - J_4 = 0$

choose J_1 free (say)

① \rightarrow gives J_2

② & above: can choose one of J_3 or J_4

$\rightarrow J_3 \rightarrow$ determines J_4

so J_1, J_3 free $\rightarrow J_2, J_4$ dependent / det.

where: $J_2 = J_1$

$$J_4 = -J_2 + J_3 = -J_1 + J_3$$

$$\overbrace{J_2, J_4}^{\sim}$$

$$\overbrace{J_1, J_3}^{\sim}$$



Mathematically : vectors
 \downarrow
 $N(\underline{A}) = \{ \bar{x} \mid \underline{A}\bar{x} = \bar{0} \}$

↑
 null space of \underline{A}
 set of such that

Independent vector sol's?

All sol's have form

$$\begin{pmatrix} J_1 \\ J_1 \\ J_3 \\ J_3 - J_1 \end{pmatrix}$$

Note:
entries J_1, J_2, \dots
& vectors: $\bar{J}^{(1)}$

Can generate two independent vectors (= free vars)

by setting $\begin{cases} J_1 = 1, J_3 = 0 \\ J_1 = 0, J_3 = 1 \end{cases}$ in turn

i.e. get two vectors:

$$\bar{J}^{(1)} = \begin{pmatrix} 1 \\ 1 \\ 0 \\ -1 \end{pmatrix}$$

procedure ensures
neither can
be written
as linear
combo of
other(s) i.e. they
are linearly indep.

$$\& \quad \bar{J}^{(2)} = \begin{pmatrix} 0 \\ 0 \\ 1 \\ 1 \end{pmatrix}$$

Can show:

→ all other sol's can be written
as $\bar{J} = a\bar{J}^{(1)} + b\bar{J}^{(2)}$

for some a, b

$\{\bar{J}^{(1)}, \bar{J}^{(2)}\}$
form 'basis'
for null
space.

↳ see tomorrow!

Exercise:

- write down a (metabolic) reaction system that could have given rise to the previous stoichiometric matrix.