Metabolic networks

Wolfram Liebermeister
Humboldt-Universität zu Berlin – Theoretische Biophysik
How can a living being emerge just from sugar, water, and a couple of salts?

<table>
<thead>
<tr>
<th>Minimal Medium for <em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
</tr>
<tr>
<td>KH₂PO₄</td>
</tr>
<tr>
<td>NH₄Cl</td>
</tr>
<tr>
<td>NaCl</td>
</tr>
<tr>
<td>MgSO₄</td>
</tr>
<tr>
<td>CaCl₂</td>
</tr>
</tbody>
</table>
How can a living being emerge just from sugar, water, and a couple of salts?

Minimal Medium for *E. coli*

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>5 g/l</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>6 g/l</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>3 g/l</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>1 g/l</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.5 g/l</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>0.12 g/l</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.01 g/l</td>
</tr>
</tbody>
</table>

L'essentiel est invisible pour les yeux.
Metabolic networks produce materials and energy for the cell

**Minimal Medium for *E. coli***
- Glucose: 5 g/l
- Na₂HPO₄: 6 g/l
- KH₂PO₄: 3 g/l
- NH₄Cl: 1 g/l
- NaCl: 0.5 g/l
- MgSO₄: 0.12 g/l
- CaCl₂: 0.01 g/l
Overview

What are metabolic networks and how do they work?
How can we use models to understand their dynamics?
How can we predict fluxes in large networks?
How do metabolic systems respond to perturbations?
What standards, resources, and software are available?
Metabolic networks
A genome-scale metabolic reconstruction for *Escherichia coli* K-12 MG1655 that accounts for 1260 ORFs and thermodynamic information

Adam M Feist¹, Christopher S Henry², Jennifer L Reed¹, Markus Krummenacker², Andrew R Joyce¹, Peter D Karp³, Linda J Broadbelt⁴, Vassiliy Hatzimanikatis⁷ and Bernhard Ø Palsson⁵

http://www.genome.jp/kegg/pathway/map/map01100.html
Threonine synthesis pathway
Metabolic networks have several levels of regulation.
Metabolic networks have several levels of regulation

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Reactions</th>
<th>Enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>Aspartate → ATP → ADP</td>
<td>2.7.2.4</td>
</tr>
<tr>
<td>Aspartyl-P</td>
<td>Aspartyl-P → NADPH → NADP+ → P</td>
<td>1.2.1.11</td>
</tr>
<tr>
<td>Asp semiald</td>
<td>Asp semiald → NADPH → NADP+</td>
<td>1.1.1.3</td>
</tr>
<tr>
<td>Homoserine</td>
<td>Homoserine → ATP → ADP</td>
<td>2.7.1.39</td>
</tr>
<tr>
<td>P-Homoserine</td>
<td>P-Homoserine → P</td>
<td>4.2.3.1</td>
</tr>
<tr>
<td>Threonine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Metabolic networks have several levels of regulation

Metabolites:
- Aspartate
- Aspartyl-P
- Asp semiald
- Homoserine
- P-Homoserine
- Threonine
- Lysine

Reactions:
- Aspartate → ATP
- Aspartyl-P → ADP
- Asp semiald → NADPH
- Homoserine → ATP
- P-Homoserine → P

Enzymes:
- 2.7.2.4
- 1.2.1.11
- 1.1.1.3
- 2.7.1.39
- 4.2.3.1

Metabolic regulation:
- ATP
- ADP
- NADPH
- NADP+, P
Metabolic networks have several levels of regulation

Metabolites
- Aspartate
- Aspartyl-P
- Homoserine
- P-Homoserine
- Threonine

Reactions
- Aspartate to ATP
- Aspartyl-P to ADP
- Homoserine to ATP
- P-Homoserine to P

Enzymes
- 2.7.2.4
- 1.2.1.11
- 1.1.1.3
- 2.7.1.39
- 4.2.3.1

Metabolic regulation
- Lysine

Transcriptional regulation
- asd
- thrA
- thrB
- thrC
Metabolic networks have several levels of regulation

Metabolites

Reactions

Enzymes

Metabolic regulation

Transcriptional regulation

Metabolites:
- Aspartate
- Aspartyl-P
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Enzymes:
- 2.7.2.4
- 1.2.1.11
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- 4.2.3.1

Metabolic regulation:

Transcription factors:
- asd
- thrA
- thrB
- thrC

Transcription factors
Multi-omics data show metabolism as a dynamic system

Measured uptake rates and concentrations in *B. subtilis* central metabolism after adding malate to a glucose medium.
Kinetic models
How do metabolic networks work?

- What compounds can the cell produce?
- On which nutrient media can the cell survive?
- What do the metabolic fluxes look like?
- How do they respond to varying conditions?
- How is all this regulated?
- What conclusions can we draw from limited data?
Modelling approaches for different complexity

Topological Analysis

Flux Balance Analysis

Kinetic modeling

\[ \frac{dS}{dt} = N \cdot v = 0 \]

\[ v_1 + v_2 = v_3 \]

\[ S_0 \rightarrow S_1 \rightarrow S_2 \]

\[ \frac{dS}{dt} = N \cdot v(S, p) \]
Kinetic models describe the dynamics of biochemical reactions

\[ \text{Homoserine} \xrightarrow{v_1} \text{Phospho-homoserine} \xrightarrow{v_2} \text{Threonine} \]
Kinetic models describe the dynamics of biochemical reactions

How often does the reaction occur per time?

\[ v = k_+ a - k_- b \]

reaction rate, concentration, kinetic constant
Kinetic models describe the dynamics of biochemical reactions

Reaction rate ("kinetic equations")
How often does the reaction occur per time?

\[ v = k_+ a - k_- b \]

System equations
How do the concentrations change over time?

\[
\begin{align*}
\frac{da}{dt} &= -v_1 \\
\frac{db}{dt} &= v_1 - v_2 \\
\frac{dc}{dt} &= v_2
\end{align*}
\]

A → B → C

Homoserine → Phospho-homoserine → Threonine
Kinetic models describe the dynamics of biochemical reactions

Homoserine \(\xrightarrow{v_1} B\) Phosphohomoserine \(\xrightarrow{v_2} C\) Threonine

Reaction rate ("kinetic equations")
How often does the reaction occur per time?

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\end{align*}
\]
System equations – an example

\[ \mathbf{v} = \begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \end{pmatrix}, \quad \mathbf{S} = \begin{pmatrix} S_1 \\ S_2 \\ S_3 \\ S_4 \end{pmatrix}, \quad \mathbf{N} = \begin{pmatrix} 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & -1 & 1 & 0 \end{pmatrix} \]
# System equations – an example

<table>
<thead>
<tr>
<th>Metabolite Concentrations</th>
<th>Reaction rates</th>
<th>Stoichiometric Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_1$</td>
<td>$v_1$</td>
<td>$v_1$</td>
</tr>
<tr>
<td>$S_2$</td>
<td>$v_2$</td>
<td>$v_2$</td>
</tr>
<tr>
<td>$S_3$</td>
<td>$v_3$</td>
<td>$v_3$</td>
</tr>
<tr>
<td>$S_4$</td>
<td>$v_4$</td>
<td>$v_4$</td>
</tr>
<tr>
<td>$S_5$</td>
<td>$v_5$</td>
<td>$v_5$</td>
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The big problem in kinetic modelling: each enzyme is different !!

Mass-action kinetics (non-enzymatic reactions)
\[ v = k_+ a - k_- b \]

Michaelis-Menten kinetics (simple enzymatic law)
\[ v = \frac{v_{+}^{\text{max}} (a/k_A^M) - v_{-}^{\text{max}} (b/k_B^M)}{1 + (a/k_A^M) + (b/k_B^M)} \]
The big problem in kinetic modelling: each enzyme is different!!

Mass-action kinetics (non-enzymatic reactions)

\[ v = k_+ a - k_- b \]

Michaelis-Menten kinetics (simple enzymatic law)

\[ v = \frac{v_{+\max} (a/k_A^M) - v_{-\max} (b/k_B^M)}{1 + (a/k_A^M) + (b/k_B^M)} \]

Thermodynamics helps to reduce unknown parameters

Chemical equilibrium

\[ 0 = v(a^{eq}, b^{eq}) = v_{+\max} \frac{a^{eq}}{k_A^M} - v_{-\max} \frac{b^{eq}}{k_B^M} \]

Haldane relation

\[ k^{eq} = b^{eq} a^{eq} = \frac{v_{+\max} k_B^M}{v_{-\max} k_A^M} \]
**Constraint-based models predict metabolic fluxes in large networks**

**External metabolites (e.g. extracellular or buffered)**
Treated as fixed parameters

**Intracellular metabolites (dynamic)**
Concentration changes due to chemical reactions

**Stationary (=steady) state**
A state in which all variables remain constant in time

**Stationarity condition in kinetic models**

\[
\frac{dc}{dt} = Nv = 0
\]

Condition on the flux vector
Kinetic rate laws do not play a role!
Constraint-based models predict metabolic fluxes in large networks

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Stationarity condition in kinetic models
\[ \frac{dc}{dt} = Nv = 0 \]
Condition on the flux vector
Kinetic rate laws do not play a role!

Flux balance analysis predicts flux distributions for large networks

Stationarity + Upper and lower bounds on fluxes
→ Convex set in flux space

Linear optimisation (e.g. maximal product yield)
→ Linear programming problem
Fluxes have to satisfy thermodynamic constraints

1. Wegscheider conditions

\[ f = -\nabla \Phi \quad \Rightarrow \quad \oint f(s) \cdot ds = 0 \]

\[ \Delta x = N^T x \quad \Rightarrow \quad K^T \Delta x = 0 \quad \text{(where } N K = 0) \]

Equilibrium constants

\[ K^T \ln k^{\text{eq}} = 0 \]

Mass-action ratios

\[ K^T \ln q^{\text{ma}} = 0 \]

Reaction affinities

\[ K^T A = -K^T \Delta \mu = 0 \]
Fluxes have to satisfy thermodynamic constraints

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Reaction affinities

\[ K^T A = -K^T \Delta \mu = 0 \]

2. Flux directions and affinities (positive entropy production !)

\[ \nu_r \neq 0 \implies \text{sign}(\nu_r) = \text{sign}(A_r) = -\text{sign}(\Delta \mu_r) \]
Metabolic control analysis traces the global effects of local changes.
Metabolic control analysis traces the global effects of local changes.

1. **Stationary concentrations** $s(p)$
   
   Solution of $0 = N v(s(p), p)$

2. **Response coefficients**

   **Systemic effect:** flux or concentration
   
   **Local cause:** e.g., single enzyme level

   $\Delta s_i \approx R_{p_m}^{s_i} \Delta p_m$
Summary: Modelling formalisms for biochemical systems

**Kinetic models**

\[
\frac{dx_i}{dt} = \sum_l n_{il} v_l(x, p)
\]
Summary: Modelling formalisms for biochemical systems

**Kinetic models**

\[
\frac{dx_i}{dt} = \sum_{l} n_{il} v_l(x, p)
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**Constraint-based models** (e.g., flux balance analysis)

\[
\frac{dx}{dt} = 0 \quad \Rightarrow \quad Nv = 0
\]
Summary: Modelling formalisms for biochemical systems

**Kinetic models**

\[
\frac{dx_i}{dt} = \sum_l n_{il} \, v_l(x, p)
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**Constraint-based models** (e.g., flux balance analysis)

\[
\frac{dx}{dt} = 0 \quad \Rightarrow \quad N \, v = 0
\]

**Metabolic control theory**

Systemic effect: flux or concentration

Local cause: e.g., single enzyme level

Slope at standard state = “control coefficient”
Summary: Modelling formalisms for biochemical systems

**Kinetic models**

\[
\frac{dx_i}{dt} = \sum_l n_{il} v_l(x, p)
\]

- enzyme
- concentration
- stoichiometry
- reaction rate
- parameters

**Constraint-based models** (e.g., flux balance analysis)

\[
\frac{dx}{dt} = 0 \Rightarrow Nv = 0
\]

**Metabolic control theory**

- Systemic effect: flux or concentration
- Local cause: e.g., single enzyme level
- Slope at standard state = "control coefficient"

**Thermodynamic analysis**
Technical resources for modelling
Playing with biochemical models?
Playing with biochemical models?
“Most of the published quantitative models in biology are lost for the community because they are either not made available or they are insufficiently characterized to allow them to be reused.”

Le Novere et al, (2005)
Systems Biology Markup Language (SBML)
Systems Biology Markup Language (SBML)

One exchange format - about 170 tools that understand each other

<?xml version="1.0" encoding="UTF-8"?>
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      <compartment id="ext" name="ext" size="1"/>
    </listOfCompartments>
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      ...
      ...
      <species id="C00008_c" name="product" compartment="c"> </species>
    </listOfSpecies>
    <listOfReactions>
      <reaction id="reaction_8">
        <listOfReactants>
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          ...
          <speciesReference species="O2_c" stoichiometry="0.01"/>
        </listOfReactants>
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          <speciesReference species="C00008_c" stoichiometry="0.81"/>
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        </listOfModifiers>
      </reaction>
    </listOfReactions>
  </model>
</sbml>

SBML main site http://sbml.org/
Systems Biology Graphical Notation (SBGN)

Process description diagram

http://sbgn.org/
Data, modelling software, and models are available on the web

**Network reconstructions**

- Egg
- BioCyc

**Databases for biological numbers**

- BRENDAX
- NIST

**Model repositories**

- BIOMODELS.NET: Database of curated annotated models
  - http://biomodels.org/

- JWS online: database of curated models
  - http://jjj.biochem.sun.ac.za/

**Modelling software**

- SB.OS - Live DVD with free modelling software
  - http://sbml.org/

**Model repositories**

- SB.MML
  - http://sbml.org/
Systems Biology

A Textbook

Edda Klipp, Wolfram Liebermeister, Christoph Wierling, Axel Kowal, Hans Lehrach, and Ralf Herwig
Thank you!