Turning metabolic networks into dynamic models

Wolfram Liebermeister
Humboldt-Universität Berlin – Theoretische Biophysik

GoFORSYS MiniSymposium, May 29, 2009
MPI of Molecular Plant Physiology, Golm
Multi-parallel data show metabolism as a dynamic system

**Metabolic flux**

Stationary fluxes in yeast during growth on glucose

**Data:** computed from Teusink et al. (2000), with additional assumptions (no lactate flux; homogeneous TCA cycle flux); no restriction to thermo-dynamic correctness

**Gene expression**

Log expression values during glucose/ethanol shift

**Data:** from DeRisi et al. (1997), normalised to mean value per ORF; for each reaction, mean over relevant ORFS

**Metabolite concentrations**

Metabolite concentrations during glucose/ethanol shift

**Data:** from D. Schomburg, normalised to minimal value for each metabolite; logarithmic values; interpolation in time
The dream of systems biology: From genomes to models...

- Genome Sequence, textbook knowledge
- Metabolic network
  - Gene annotation
  - Check topology (Iterative process)
  - Assign kinetic laws and parameters
- Stoichiometric model
  - Assign constraints (thermodynamic information?)
- Dynamic (kinetic) model
  - Transfer knowledge, Predefine steady states
- Predictions
  - Fluxes, product yields, viability of mutants
  - Dynamic behavior
  - Effect of perturbation
  - Variability of outputs
- Simulation, parameter fitting, control analysis, optimal control
Data integration in the BaSysBio project

**Shift experiment**
Glucose ↔ Malate shift in *B. subtilis*

**Data**
- Metabolome data
- Extracellular metabolites
- Proteomics (2D_gel and mass spec)
- Transcriptomics (traditional and tiling arrays)

**Modelling tasks**
- Data preprocessing + mapping
- Construction of a medium-size kinetic model
- Automatic collection of kinetic data
- Parameter estimation
- Comparison to *S. aureus* and *B. anthracis*

http://www.basysbio.eu/
Computer-assisted model building
Modelling workflow for data integration

**Large scale:**
- Stoichiometric
- Steady state fluxes,
- Constraint-based optimisation

**Small scale:**
- Hand-curated kinetics,
- Simulation,
- Control analysis
Modelling workflow for data integration

**Large scale:**
- Stoichiometric
- Steady state fluxes,
- Constraint-based optimisation

**Medium scale**
- Standard kinetics
- Data integration
- Sampling techniques

**Small scale:**
- Hand-curated kinetics,
- Simulation,
- Control analysis
Modelling workflow for data integration

- **List of reactions**
  - Kinetic constants
  - Metabolome, proteome, transcriptome, flux data

- **Network structure**
  - Building
  - Mapping

- **Collected kinetic constants**
  - Mapping
  - Balancing

- **Balanced parameters complete + feasible**
  - Mapping
  - Fitting or sampling

- **Refined parameters (prob. distribution)**

- **Kinetic model (ensemble)**

**Large scale:**
- Stoichiometric
- Steady state fluxes,
- Constraint-based optimisation

**Medium scale**
- Standard kinetics
- Data integration
- Sampling techniques

**Small scale:**
- Hand-curated kinetics,
- Simulation,
- Control analysis

**Simulation, analysis**
Problems in building large kinetic models

1. Enzymatic mechanisms are often unknown
   Use simple yet plausible standard rate laws

2. Models should obey the laws of thermodynamics
   Be aware of relevant constraints
   Use feasible parameter set in fitting, sampling, optimisation etc

3. Parameters show variation and may be uncertain
   Describe parameters by probability distributions
   Infer probabilistic statements about model outputs, dynamics etc

4. Data may not suffice to determine the parameters
   Use prior distributions and Bayesian statistics for estimation

5. Parameter estimation in large models is expensive
   Use direct sampling methods that avoid steady-state calculation
Standard kinetic laws
Standard kinetic laws; the convenience kinetics

Reaction

\[ A + B \rightleftharpoons C \]

Mass-action

\[ v = k^+ a b - k^- c \]

Linlog

\[ \frac{v}{v_0} = \frac{u}{u_0} \left( 1 + E_A \ln \frac{a}{a_0} + E_B \ln \frac{b}{b_0} + E_C \ln \frac{c}{c_0} \right) \]

Thermodynamic-kinetic modelling

\[ v = R \left( \xi_A \xi_B - \xi_C \right) \]

\[ \xi_i = e^{\mu_i/RT} \]

Convenience kinetics

\[ v = u \frac{k^+ (a/k^M_A)(b/k^M_B) - k^- (c/k^M_C)}{(1 + a/k^M_A)(1 + b/k^M_B) + c/k^M_C} \]

Convenience kinetics

Liebermeister W. and Klipp E. (2006), Theoretical Biology and Medical Modelling 3:41
Thermodynamics and model parametrisation
Feasible kinetic constants for fitting / sampling

**Kinetic constants in convenience kinetics**

- **Substrate A**
- **Substrate B**
- **Enzyme X**
- **Product C**
- **Inhibitor I**

**Choosing the kinetic constants**

- **Full dependent set of kinetic constants**
  \[
  \ln k_r^{eq} = \sum_i n_{ir} \ln c_i^{eq} = -\frac{1}{RT} \sum_i n_{ir} G_i^{(0)}
  \]
  \[
  \ln k_r^{\pm} = \ln k_r^C + \frac{1}{2} (\ln k_r^{eq} + \sum_i n_{ir} \ln k_{ri}^M)
  \]

**Independent parameters for entire model**

Liebermeister W. and Klipp E. (2006), Theoretical Biology and Medical Modelling 3:42
Parameter balancing by Bayesian estimation
Known kinetic constants are ... ... incomplete and inconsistent

Collected equilibrium constants

Model of *B. subtilis*
central carbon metabolism

- 66 metabolites, 61 reactions
- 54 (66) Gibbs free energies
- 19 (258) Michaelis constants
- 0 (3) Activation constants
- 1 (17) Inhibition constants
- 19 (61) Equilibrium constants
- 0 (122) Turnover rates

needed in the model

found in data collection
Bayesian parameter estimation / balancing

\[ \text{Prob}(p|\text{data}) = \text{Prob}(\text{data}|p) \times \text{Prob}(p) \times \text{const.} \]

Liebermeister W. and Klipp E. (2006), Theoretical Biology and Medical Modelling 3:42
Parameter balancing for the *B. subtilis* model

Collected equilibrium constants

Balanced equilibrium constants
Thermodynamic constraints in sampling
Steady-state elasticity sampling

Instead of computing a steady state:

Predefine the steady state and sample elasticities from a random distribution

Limitation

Constraints in reversible reactions → dependencies between elasticities

→ Sampled elasticities may correspond to unfeasible parameter sets

\[
\begin{align*}
E_A &= \frac{k_+ a}{v} \\
E_B &= \frac{k_- b}{v} \\
E_A - E_B &= \frac{(k_+ a - k_- b)}{v} = 1
\end{align*}
\]
Thermodynamically feasible elasticity sampling

**A possible sampling scheme**

1. Sample feasible **fluxes** (energy balance analysis criterion)
2. Choose **concentrations** and **chemical potentials** In agreement with fluxes
3. Sample independently **all saturation constants**
4. Compute **elasticities**

**Application: interaction effects**

Second-order control coefficients
Enzyme levels → Biomass production in *S. cerevisae* model
Network component analysis
Network component analysis

The NCA model
Expression profile = linear combination of TF activity profiles
NCA: application to glucose / malate shift data

Inferred TF activity profiles

Transcription factors

Genes (mRNA species)

Expression data (BaSysBio project)

Inferred quantitative weights
Positive / negative
Kinetic models under periodic perturbations
Periodic perturbations in linearised kinetic models

Bifurcation parameter: external concentration $X$

Perturbation: chemical noise in reaction rates (small molecule numbers)

Periodic perturbations in linearised kinetic models

Bifurcation parameter: external concentration $X$

Perturbation: chemical noise in reaction rates (small molecule numbers)


A current application: Yeast cells under periodic osmotic stress

Transient behaviour
(from numerical integration)

Asymptotic periodic behaviour
(from numerical integration and Fourier synthesis of linear response modes)

Model by Zhike Zi, HU Berlin
Advertisement: Systems biology operational software live DVD

- Ubuntu Linux 8.10 booting directly from DVD
- Preinstalled software tools for SBML models: CellDesigner / COPASI / semanticSBML / ...
- Provides tools needed for: Model building / layout / simulation / fitting / annotation / merging
- Models from the BioModels.net database
- Documentation and video tutorials

Further information and download of DVD image at www.sbos.eu
Acknowledgements

HU Berlin
Edda Klipp
Jannis Uhlendorf
Falko Krause
Timo Lubitz
Simon Borger
Ivo Mainz
...

ETH Zürich
Jörg Stelling
Hans-Michael Kaltenbach
Uwe Sauer
Jörg Büscher

TU Braunschweig
Dietmar Schomburg
Kerstin Schreiber

EBI Hinxton
Nicolas LeNovere
Lukas Endler
Nick Juty

!!! The SBML community !!!
Projects planned

**Theory / methods development**
- Data integration by Bayes estimation
- Data integration by Thermodynamic sampling
- Optimal enzyme use: Value of enzymes and metabolites

**Applied projects**
- Medium-scale kinetic model energy generation / consumption / storage
- Optimal control under periodically changing conditions
- Optimal control architecture for randomly fluctuating conditions

**Implementation**
- SemanticSBML / model merging
- Data integration workflow: (Bayes + sampling in python)
Thermodynamic constraints (1): Wegscheider conditions

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

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

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

Reaction affinities
\[ K^T A = -K^T \Delta \mu = 0 \]
Thermodynamic constraints (2): Haldane relations

Example: reversible Michaelis-Menten kinetics

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

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

Equilibrium constant / Haldane relation
\[ k^{\text{eq}} = \frac{b^{\text{eq}}}{a^{\text{eq}}} = \frac{v^\text{max}_+ k_B^\text{M}}{v^\text{max}_- k_A^\text{M}} \]

Haldane relation for generalised mass-action kinetics
\[ k^\text{eq}_r = k^+_r / k^-_r \prod_i (k^\text{M}_{ri})^{n_{ir}} \]
Thermodynamic constraints (3): Flux directions

Chemical potential

\[ \mu_i := \frac{1}{V} \left( \frac{\partial G}{\partial c_i} \right)_{p,T} \]

Reaction affinity

\[ \Lambda_r := -\Delta \mu_r = -\sum_i n_{ir} \mu_i \]

Positive entropy production requires:

\[ \nu_r \neq 0 \quad \Rightarrow \quad \text{sign}(\nu_r) = \text{sign}(\Lambda_r) = -\text{sign}(\Delta \mu_r) \]

(non-zero) fluxes and reaction affinities have the same signs
Thermodynamically feasible parameter sets

Computing feasible equilibrium constants (Wegscheider condition!)

\[
\ln k_r^{eq} = \sum_i n_{ir} \ln c_i^{eq} = -\frac{1}{RT} \sum_i n_{ir} G_i^{(0)}
\]

Computation from Gibbs free energies of formation

Linear form for logarithms

Two constraints for the turnover rates:

1. Haldane relation ("mass-action" numerator)

\[
k_r^{eq} = k_r^+/k_r^- \prod_i (k_{ri}^M)^{n_{ir}}
\]

2. Fix mean turnover rate

\[
k_r^C = \sqrt{k_r^+/k_r^-}
\]

Compute turnover rates

\[
k_r^\pm = k_r^C \left( k_r^{eq} \prod_i (k_{ri}^M)^{-n_{ir}} \right)^{\pm 1/2}
\]

Linear form for logarithms

\[
\ln k_r^\pm = \ln k_r^C + \frac{1}{2} \left( \ln k_r^{eq} + \sum_i n_{ir} \ln k_{ri}^M \right)
\]

Relationships between thermodynamic quantities

**Metabolites in an ideal solution (all concentrations measured in mM)**

\[ \mu_i = \mu_i^0 + RT \ln c_i \]

**Equilibrium constants and Gibbs free energies of formation**

\[ \ln k_r^{eq} = \sum_i n_{ir} \ln c_i^{eq} = -\frac{1}{RT} \sum_i n_{ir} G_i^{(0)} \]

**Reaction affinities and concentrations**

\[ A_r = RT \ln k_r^{eq} / q_r^{ma} \]

**“Generalised mass-action” form of many rate laws**

\[ \sim (k^+ a \cdot b \cdot ... - k^- c \cdot d \cdot ...) \]

**Ratio of forward and backward velocities**

\[ \zeta_r := v_r^+ / v_r^- \]

\[ \zeta_r = e^{A_r/RT} = k_r^{eq} / q_r^{ma} \]

**Lower bound of reaction affinities**

\[ v_r^+ / v_r \leq \rho \]

\[ v_r \neq 0 \quad \Rightarrow \quad |A_r| \geq RT / \rho \]
Oscillations and noise in linearised kinetic models

Pulse-response function
(response to a peak-like perturbation)

\[ K^S(t - t') = L \ e^{N^R E_s L (t - t')} \ N^R \ E_p \ \Theta(t - t') \]

Spectral response coefficients:

\[ \tilde{R}^S(\omega) = -L(N^R E_s L - i \omega I)^{-1} N^R \ E_p \]
\[ \tilde{R}^I(\omega) = E_s R^S(\omega) + E_p \]

Spectral density
(Fourier transform of correlation function)

\[ \Phi_y = \tilde{R}^Y(\omega) \Phi_p \tilde{R}^Y(\omega) \]

Analogous to (static parameters):

\[ \text{cov}(\ln y) = R^Y \ \text{cov}(\ln p) R^{Y^T} \]

Joint distributions of model parameters

Use logarithmic values!

Multiplicative relationship between parameters
→ Linear relationships between logarithms
Log-normal distribution
→ Gaussian distribution for logarithms

Linear form for log. turnover rates

$$k_r^\pm = k^C_r \left( k_r^{eq} \prod_i (k_r^M)^{-n_{ir}} \right)^{\pm 1/2}$$

$$\ln k_r^\pm = \ln k_r^{eq} + \frac{1}{2} \left( \ln k_r^{eq} + \sum_i n_{ir} \ln k_r^M \right)$$
Multiplicative saturable kinetics & its elasticities

Reaction
\[ A + B \Leftrightarrow C \]

MS kinetics
\[
v(a, b, c, u) = u \frac{k^+ \left(\frac{a}{k^M_A}\right) \left(\frac{b}{k^M_B}\right) - k^- \left(\frac{c}{k^M_C}\right)}{(1 + a/k^M_A)(1 + b/k^M_B)(1 + c/k^M_C)}
\]

MS kinetics (compact form)
\[
v_r = u_r \left[ k^+_r \beta^M_{rA} \beta^M_{rB} \alpha^M_{rC} - k^-_r \alpha^M_{rA} \alpha^M_{rB} \beta^M_{rC} \right]
\]
with saturation values
\[
\alpha^X_{ri} := \frac{1}{1 + c_i/k^X_{ri}} \quad \beta^X_{ri} := \frac{c_i/k^X_{ri}}{1 + c_i/k^X_{ri}} \quad \gamma^X_{ri} := \alpha^X_{ri} \beta^X_{ri}
\]

Elasticity coefficients (scaled derivatives)
\[
E^v_{ci} = w^+_r \alpha^A_{ri} - w^-_r \beta^I_{ri} + m^+_r \alpha^M_{ri} - m^-_r \beta^M_{ri} + \frac{m^+_r - m^-_r}{\zeta_r}
\]
\[
E^v_{cij} = -\delta_{ij} \left[ w^+_r \gamma^A_{rj} + w^-_r \gamma^I_{rj} + m^+_r \gamma^M_{rj} + m^-_r \gamma^M_{rj} \right]
\]

Can be chosen independently

Computable from chemical potentials
Merging of SBML models
SemanticSBML: a tool for annotation and merging of SBML models

Stand-alone version

Online version

http://www.semanticsbml.org
Playing with biochemical models?
Playing with biochemical models?
Matching elements between models


Systems biology markup language (SBML)

SBML model exchange format
http://sbml.org/

<?xml version="1.0" encoding="UTF-8"?>
<sbml xmlns="http://www.sbml.org/sbml/level2/version3" level="2" version="3">
  <model id="model" name="model">
    <listOfCompartments>
      <compartment id="c" name="c" size="1"/>
      <compartment id="ext" name="ext" size="1"/>
    </listOfCompartments>
    <listOfSpecies>
      <species id="C00022_c" name="Pyruvate" compartment="c"/>
    </listOfSpecies>
    <reaction id="reaction_8">
      <listOfReactants>
        <speciesReference species="C00022_c" stoichiometry="0.03"/>
        <speciesReference species="O2_c" stoichiometry="0.01"/>
      </listOfReactants>
      <listOfProducts>
        <speciesReference species="C00008_c" stoichiometry="0.81"/>
      </listOfProducts>
      <listOfModifiers>
        <modifierSpeciesReference species="enzyme_reaction_8_c"/>
      </listOfModifiers>
    </reaction>
  </model>
</sbml>

Database of curated annotated models
http://biomodels.org/

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

Systems biology ontology
http://www.ebi.ac.uk/sbo/
## Biological annotations in SBML

<table>
<thead>
<tr>
<th>SBO term</th>
<th>Species called “enzyme_R00001” represents an enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="http://www.ebi.ac.uk/sbo/">www.ebi.ac.uk/sbo/</a></td>
<td>&lt;species id=&quot;enzyme_R00001&quot; sboTerm=&quot;SBO:0000014&quot;/&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MIRIAM annotation</th>
<th>Species called “ATP” represents KEGG C06262 (ATP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;species metaid=&quot;..&quot; id=&quot;ATP&quot; name=&quot;ATP concentration&quot; compartment=&quot;cytosol&quot;&gt;</td>
</tr>
<tr>
<td></td>
<td>&lt;annotation&gt;</td>
</tr>
<tr>
<td></td>
<td>&lt;rdf:RDF xmlns:rdf=&quot;<a href="http://www.w3.org/1999/02/22-rdf-syntax-ns#">http://www.w3.org/1999/02/22-rdf-syntax-ns#</a>&quot;</td>
</tr>
<tr>
<td></td>
<td>xmlns:bqbiol=&quot;<a href="http://biomodels.net/biology-qualifiers/">http://biomodels.net/biology-qualifiers/</a>&quot;</td>
</tr>
<tr>
<td></td>
<td>xmlns:bqmodel=&quot;<a href="http://biomodels.net/model-qualifiers/%22%3E">http://biomodels.net/model-qualifiers/&quot;&gt;</a></td>
</tr>
<tr>
<td></td>
<td>&lt;rdf:Description rdf:about=&quot;#metaid_0000076&quot;&gt;</td>
</tr>
<tr>
<td></td>
<td><a href="">bqbiol:is</a></td>
</tr>
<tr>
<td></td>
<td><a href="">rdf:Bag</a></td>
</tr>
<tr>
<td></td>
<td>&lt;rdf:li rdf:resource=&quot;urn:miriam:obo.chebi:CHEBI%3A15422&quot;/&gt;</td>
</tr>
<tr>
<td></td>
<td>&lt;rdf:li rdf:resource=&quot;urn:miriam:kegg.compound:C00002&quot;/&gt;</td>
</tr>
<tr>
<td></td>
<td>&lt;/rdf:Bag&gt;</td>
</tr>
<tr>
<td></td>
<td>&lt;/bqbiol:is&gt;</td>
</tr>
<tr>
<td></td>
<td>&lt;/rdf:Description&gt;</td>
</tr>
<tr>
<td></td>
<td>&lt;/rdf:RDF&gt;</td>
</tr>
<tr>
<td></td>
<td>&lt;/annotation&gt;</td>
</tr>
<tr>
<td></td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>&lt;/species&gt;</td>
</tr>
</tbody>
</table>

- Species
- Qualifier
- Database
- Identifier

"A simple scheme for annotating SBML with references to controlled vocabularies and database entries" Le Novere and Finney, 2005
Matching elements between models


Can yeast glycolysis be understood in terms of in vitro kinetics
Matching elements between models

**Metabolic Control Analysis of Glycerol Synthesis in**
*Saccharomyces cerevisiae*  Garth R. Cronwright et al (2002)

**Can yeast glycolysis be understood in terms of in vitro kinetics**