Metabolic Control Analysis (MCA)
MICROCOSME: bacterial systems biology

- **MICROCOSME**: systems biology group at INRIA/Université Grenoble Alpes in Grenoble
  - Microbiologists, computer scientists, mathematicians, physicists, ...

  https://team.inria.fr/microcosme

- **Objective**: analysis, engineering, and control of the growth of bacteria
  - Specific research problems shaped by **biological questions**
  - Problems often addressed by combination of **models and experiments**
Overview

• Part 1. Systems biology and kinetic modeling
  – Introduction
  – Kinetic modeling of cellular reaction networks

• Part 2. Metabolic network modeling
  – Kinetic modeling of metabolism
  – Metabolic control analysis (MCA)
  – Flux balance analysis (FBA)
  – Practical on flux balance analysis (COBRA)

• Part 3. Gene regulatory network modeling
Biochemical reaction networks

• ODE model for growth of microbial populations:

\[ \dot{x} = N \cdot v(x) - \mu \cdot x, \]

\[ \mu = \delta \cdot \sum \alpha_i \cdot N_i \cdot v(x). \]

• Reaction rates depend on concentrations \( x \) of substrates, products, effectors
Metabolic networks

• Focus on **subsystems** that can be studied in isolation due to **modular structure** of reaction networks
  – Time-scale hierarchies
  – Connectivity structure

• **Metabolic networks**
  – Metabolites and enzymatic reactions
  – Short turn-over times of metabolite pools in comparison with enzyme pools

Metabolic networks

• Models describing dynamics of metabolism
  – Effect of growth dilution can often be ignored
  – Variables are metabolites and rates of enzyme-catalyzed reactions
  – Enzyme concentrations constant on time-scale of metabolic dynamics
    \[ \dot{x} = N v(x) \]

• Explicit introduction of dependency of model dynamics on parameters \( p \):
  – Enzyme concentrations
  – Half-saturation and catalytic constants
  – Inhibition/activation constants
    \[ \dot{x} = N v(x, p) \]
Stoichiometry matrix

- Stoichiometry matrix $N$ describes structure of reaction network

  Internal reactions and exchange reactions, reversible and irreversible

\[
N = \begin{bmatrix}
-1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\
1 & -1 & 1 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\
0 & 1 & -1 & -1 & 1 & -1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & -1 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 \\
\end{bmatrix}
\]

Stoichiometry matrix

• Stoichiometry matrix may not be full rank
  – Dependencies between rows (variables) due to conservation relations
  – Example: \([\text{ATP}] + [\text{ADP}] + [\text{AMP}] = \text{constant}\)

• Reduction of stoichiometry matrix by means of link matrix \(L\):
  \[
  N = L N^0, \quad x = L x^0
  \]

• Variables in resulting metabolic system are independent
  \[
  \dot{x}^0 = N^0 v(x^0, p)
  \]

• In what follows, we assume that \(N\) is full rank
Metabolic networks at steady state

- For many problems of interest, the metabolic system can be considered at **steady state**
  \[ N \, v(x, p) = 0 \]
  - Metabolism relaxes on short time-scale (seconds-minutes) after changes in environment
  - Difficult to measure dynamics of metabolic adaptation
- Metabolic rates at steady state: **fluxes** \( v(x^*, p) = v^*(p) \)
- Trivial steady state with zero fluxes corresponds to **thermodynamic equilibrium**
- Steady state with non-zero fluxes requires that metabolic system is **open system**
  Non-zero exchange fluxes
Stability of steady state

• Metabolism concerns almost exclusively **sustainable** processing of chemical inputs into outputs
  Biomass, energy, waste, …

• Therefore, one expects steady states to be **stable**


• **Stability criterion** given by sign of (real part of) eigenvalues of **Jacobian matrix**

\[
J(x^*) = N \left. \frac{\partial v}{\partial x} \right|_{x^*, v^*}
\]

System is stable, if real part of every eigenvalue is negative

Example of simple metabolic pathway

- Pathway of reactions converting substrate to product
  - S and P are supplied/removed (constant concentrations)
  - Reactions are reversible (Michaelis-Menten kinetics)

\[ S \xrightleftharpoons{v_1} X_1 \xrightleftharpoons{v_2} X_2 \xrightleftharpoons{v_3} P \]

- **Exercise**: What is the stoichiometry matrix for this system?
- **Exercise**: How do the fluxes relate at steady state?
- **Exercise**: Write out the Jacobian matrix for this system
- **Exercise**: Determine the stability of the system. Hint: use the signs of the partial derivatives and the relation between eigenvalues and trace/determinant
Example of simple metabolic pathway

\[
\begin{align*}
S & \xrightarrow{v_1} X_1 \xrightarrow{v_2} X_2 \xrightarrow{v_3} P \\
\frac{d}{dt} \begin{bmatrix} x_1 \\ x_2 \end{bmatrix} &= \begin{bmatrix} 1 & -1 & 0 \\ 0 & 1 & -1 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \end{bmatrix} = 0 \\
N \cdot v &= 0 \Rightarrow v_1 = v_2 \text{ et } v_2 = v_3 \Rightarrow v_1 = v_2 = v_3
\end{align*}
\]
Example of simple metabolic pathway

Stability: trace negative (-) + (-) < 0

determinant positive

\[
J = \begin{bmatrix}
\frac{dv_1}{dx_1} - \frac{dv_2}{dx_1} \\
\frac{dv_1}{dx_1} \\
\frac{dv_2}{dx_2} - \frac{dv_3}{dx_2}
\end{bmatrix} + \begin{bmatrix}
\frac{dv_2}{dx_2} \\
\frac{dv_2}{dx_2} - \frac{dv_3}{dx_2}
\end{bmatrix}
\]

\[
= \frac{dv_1}{dx_1} \left( \frac{dv_2}{dx_2} - \frac{dv_3}{dx_2} \right) + \frac{dv_2}{dx_1} \frac{dv_3}{dx_2}
\]

> 0
Example of simple metabolic pathway

• Pathway of reactions converting substrate to product
  – S and P are supplied/removed (constant concentrations)
  – Reactions are reversible (Michaelis-Menten kinetics)

\[ S \xleftarrow{v_1} X_1 \xleftrightarrow{v_2} X_2 \xrightarrow{v_3} P \]

• Assumption: steady states are \textbf{stable}
Metabolic control analysis

• Steady state of system is **sensitive** to (local) changes in enzyme concentrations or kinetic parameters
Metabolic control analysis

- Steady state of system is **sensitive** to (local) changes in enzyme concentrations or kinetic parameters
Metabolic control analysis

• Steady state of system is sensitive to (local) changes in enzyme concentrations or kinetic parameters

• Metabolic control analysis (MCA) aims at studying this sensitivity in a systematic and rigorous manner

• MCA applies to arbitrarily complex networks

• Central questions in MCA:
  – How does the system steady state respond to changes in enzyme concentrations or kinetic parameters?
  – How does the system response depend on the network structure?
  – How constrained are sensitivities? Do they show dependencies?

Sauro (2009), Chapter 13 in Jason McDermott et al. (eds.), *Computational Systems Biology*, Humana Press, 269-309


Fell (1997), *Understanding the Control of Metabolism*, Portland Press
Elasticity coefficients

- **Elasticity coefficients** express how the rate of a reaction changes due to a change in the reaction properties
  - Change in substrate, product, enzyme, effector concentrations
  - Change in kinetic parameter

\[
\epsilon_{x_i}^{v_j} = \frac{\partial v_j}{\partial x_i} \cdot \frac{x_i}{v_j} = \frac{\partial \ln v_j}{\partial \ln x_i} = \%v_j \%x_i \quad \epsilon_{p_i}^{v_j} = \frac{\partial v_j}{\partial p_i} \cdot \frac{p_i}{v_j} = \frac{\partial \ln v_j}{\partial \ln p_i} = \%v_j \%p_i
\]

- Elasticities are **local** properties of metabolic system
- Elasticities may vary with system state for complex rate laws

- Exercise: write elasticities with respect to change in enzyme concentration for irreversible Michaelis-Menten rate law
Elasticity coefficients

\[ v = k_{cat} \cdot e \cdot \frac{s}{s + K_m} \]

\[ e_v = \frac{dv}{de} \cdot \frac{e}{v} \]

\[ = k_{cat} \cdot \frac{s}{s + K_m} \cdot e \cdot \frac{1}{v} \]

\[ = \frac{v}{v} = 1 \]
Response coefficients

- **Response coefficients** express how steady state of the system changes due to a change in reaction properties
  - Flux response coefficients
    \[ R_{p_i}^{v_j} = \left. \frac{\partial v_j}{\partial p_i} \cdot \frac{p_i}{v_j} \right|_{x^*,v^*} = \left. \frac{\partial \ln v_j}{\partial \ln p_i} \right|_{x^*,v^*} = \frac{%v_j}{%p_i} \]
  - Concentration response coefficients
    \[ R_{p_i}^{x_j} = \left. \frac{\partial x_j}{\partial p_i} \cdot \frac{p_i}{x_j} \right|_{x^*,v^*} = \left. \frac{\partial \ln x_j}{\partial \ln p_i} \right|_{x^*,v^*} = \frac{%x_j}{%p_i} \]

- Response coefficients are **global** properties of metabolic system
- Response coefficients generally vary with system state
- Exercise: give examples of response coefficients for simple reversible pathway and their meaning
Response coefficients

\[ R_{e_2}, R_{e_1}, R_{e_2} \]
Response coefficients

- **Response coefficients** express how steady state of the system changes due to a change in reaction properties
  - **Flux response coefficients**
    \[
    R_{p_i}^{v_j^*} = \frac{\partial v_j}{\partial p_i} \cdot \frac{p_i}{v_j} \bigg|_{x^*, v^*} = \frac{\partial \ln v_j}{\partial \ln p_i} \bigg|_{x^*, v^*} = \frac{\%v_j^*}{\%p_i}
    \]
  - **Concentration response coefficients**
    \[
    R_{p_i}^{x_j^*} = \frac{\partial x_j}{\partial p_i} \cdot \frac{p_i}{x_j} \bigg|_{x^*, v^*} = \frac{\partial \ln x_j}{\partial \ln p_i} \bigg|_{x^*, v^*} = \frac{\%x_j^*}{\%p_i}
    \]
- **Response coefficients are global** properties of metabolic system
- **Response coefficients generally vary with system state**
- **How can response coefficients be computed? How do they relate to elasticity coefficients?**
Computation of response coefficients

- Differentiation of steady-state equation w.r.t. $p$:

$$\left. N \frac{\partial v}{\partial x} \right|_{x^*,v^*} \frac{\partial x}{\partial p} + \left. N \frac{\partial v}{\partial p} \right|_{x^*,v^*} = 0$$
Computation of response coefficients

- Differentiation of steady-state equation w.r.t. $p$:

\[
N \frac{\partial v}{\partial x} \frac{\partial x}{\partial p} \bigg|_{x^*,v^*} + N \frac{\partial v}{\partial p} \bigg|_{x^*,v^*} = 0
\]

\[
\frac{\partial x}{\partial p} \bigg|_{x^*,v^*} = -(N \cdot \frac{\partial v}{\partial x})^{-1} \cdot N \cdot \frac{\partial v}{\partial p} \bigg|_{x^*,v^*}
\]

\[
\begin{align*}
\frac{\partial x}{\partial p} \bigg|_{x^*,v^*} &= -(N \cdot \frac{\partial v}{\partial x})^{-1} \cdot N \cdot \frac{\partial v}{\partial p} \cdot dg(p) \bigg|_{x^*,v^*} \\
&= -(N \cdot \frac{\partial v}{\partial x})^{-1} \cdot N \cdot \frac{\partial v}{\partial p} \cdot dg(v)^{-1} \cdot \frac{\partial v}{\partial x} \cdot dg(x) \cdot dg(x)^{-1})^{-1} \cdot N \cdot dg(v) \cdot dg(v)^{-1} \cdot \frac{\partial v}{\partial p} \cdot dg(p) \bigg|_{x^*,v^*}
\end{align*}
\]

\[
R_p^{x^*} = - (N \cdot dg(v) \cdot \epsilon^v_x)^{-1} \cdot N \cdot dg(v) \cdot \epsilon^v_p \bigg|_{x^*,v^*}
\]
Control coefficients

- Separation of reaction-specific and systemic contribution to response coefficient

\[
R_{p}^{x^*} = - \left( N \cdot dg(v) \cdot \epsilon_{x}^{v} \right)^{-1} \cdot N \cdot dg(v) \cdot \epsilon_{p}^{v} \bigg|_{x^*,v^*} \\
= C_{v}^{x^*} \cdot \epsilon_{p}^{v} \bigg|_{x^*,v^*}
\]

with **concentration control coefficients**

\[
C_{v}^{x^*} = - \left( N \cdot dg(v) \cdot \epsilon_{x}^{v} \right)^{-1} \cdot N \cdot dg(v) \bigg|_{x^*,v^*}
\]

- Concentration control coefficients describe effect of change in rate (by whatever means) on steady-state concentration

\[
R_{p_i}^{x_j^*} = \sum_{k} C_{v_k}^{x_j^*} \cdot \epsilon_{p_i}^{v_k} \bigg|_{x^*,v^*} \\
C_{v_k}^{x_j^*} = \frac{\% x_j^*}{\% v_k}
\]
Control coefficients

- Similar analysis for flux response coefficients leads to

\[ R_p^v = C_v^v \cdot \epsilon_p \bigg|_{x^*,v^*} \]

with flux control coefficients

\[ C_v^v = I + \epsilon_x^v \cdot C_x^x \bigg|_{x^*,v^*} \]

- Flux control coefficients describe effect of change in rate (by whatever means) on fluxes at steady state

\[ R_{pi}^{v_j} = \sum_k C_{vk}^v \cdot \epsilon_{pi}^v \bigg|_{x^*,v^*} \quad C_{vk}^v = \frac{\%v_j^*}{\%v_k^*} \]

- Above analysis provides conceptual framework, but is not very practical for computational purposes
Summation and connectivity theorems

- Above analysis can be further developed into MCA summation theorems:
  \[ C_v^x \cdot \mathbf{1} = 0, \sum_k C_{vk}^x = 0 \]
  \[ C_v^v \cdot \mathbf{1} = 1, \sum_k C_{vk}^v = 1 \]

- Flux control is distributed over the system

- Idem for MCA connectivity theorems:
  \[ C_v^x \cdot \epsilon_x = -I, \sum_k C_{vk}^x \cdot \epsilon_{xi} = -\delta_{ji} \]
  \[ C_v^v \cdot \epsilon_x = 0, \sum_k C_{vk}^v \cdot \epsilon_{xi} = 0 \]

Example of simple metabolic pathway

\[ S \xleftarrow{v_1} X_1 \xrightarrow{v_2} X_2 \xleftarrow{v_3} P \]

- Exercise: write down the flux summation and connectivity theorems for the model of this pathway
- Exercise: find expressions for flux control coefficients in terms of elasticities. What can be learned from these expressions?
Example of simple metabolic pathway

\[ N.B. \quad J = v_1^* = v_2^* = v_3^* \]

\[
\begin{bmatrix}
C_v^1 & C_v^2 & C_v^3 \\
C_v^1 & C_v^2 & C_v^3 \\
C_v^1 & C_v^2 & C_v^3
\end{bmatrix}
\begin{bmatrix}
1 \\
1 \\
1
\end{bmatrix} =
\begin{bmatrix}
1 \\
1
\end{bmatrix}
\]

\[ \Rightarrow C_v^1 + C_v^2 + C_v^3 = 1 \quad (i) \]

\[
\begin{bmatrix}
C_v^1 & C_v^2 & C_v^3 \\
C_v^1 & C_v^2 & C_v^3 \\
C_v^1 & C_v^2 & C_v^3
\end{bmatrix}
\begin{bmatrix}
\epsilon_x^1 \\
\epsilon_x^2 \\
\epsilon_x^3
\end{bmatrix} =
\begin{bmatrix}
0 & 0 \\
0 & 0 \\
0 & 0
\end{bmatrix}
\]

\[ \Rightarrow C_v^1 \cdot \epsilon_x^1 + C_v^2 \cdot \epsilon_x^2 + C_v^3 \cdot \epsilon_x^3 = 0 \quad (ii) \]

\[ C_w \cdot \epsilon_x^1 + C_v^2 \cdot \epsilon_x^2 + C_v^3 \epsilon_x^3 = 0 \quad (iii) \]
Example of simple metabolic pathway

\[ \begin{align*}
C_{V_1} &= -\frac{e_{x_1}}{e_{x_2}} \cdot C_{V_2} \\
C_{V_2} &= -\frac{e_{x_3}}{e_{x_4}} \cdot C_{V_3} \\
\Rightarrow C_{V_1} &= \frac{e_{x_2}}{e_{x_1}} \cdot \frac{e_{x_3}}{e_{x_4}} \cdot C_{V_3} \\
C_{V_1} + C_{V_2} + C_{V_3} &= 1 \\
\left( \frac{e_{x_2}}{e_{x_1}} \cdot \frac{e_{x_3}}{e_{x_4}} - \frac{e_{x_1}}{e_{x_2}} + 1 \right) C_{V_3} &= 1 \\
C_{V_3} &= \frac{e_{x_2}}{e_{x_1}} \cdot \frac{e_{x_3}}{e_{x_4}} - \frac{e_{x_1}}{e_{x_2}} + 1 \\
&> 0 \\
C_{V_2} &= -\frac{e_{x_1}}{e_{x_2}} \cdot \frac{e_{x_3}}{e_{x_4}} \cdot C_{V_3} \\
C_{V_1} &= \frac{e_{x_2}}{e_{x_1}} \cdot \frac{e_{x_3}}{e_{x_4}} + \frac{e_{x_1}}{e_{x_2}} \cdot e_{x_3} \\
&> 0
\end{align*} \]
Example of simple metabolic pathway

\[ S \xrightleftharpoons{v_1} X_1 \xrightleftharpoons{v_2} X_2 \xrightleftharpoons{v_3} P \]

- Distributed control of enzymes over pathway flux
  Contrary to idea of rate-limiting step

Sauro (2009), Chapter 13 in Jason McDermott et al. (eds.), *Computational Systems Biology*, Humana Press, 269-309
Example of simple pathway with feedback

- Pathway with negative feedback on level of enzyme activity

\[
\begin{array}{c}
S \xlongequal{v_1} X_1 \xlongequal{v_2} X_2 \xlongequal{v_3} P \\
& \Downarrow & \Downarrow & \Downarrow \\
\end{array}
\]

- Question: in case of strong feedback, if we would like to increase production of P, which reaction should we target?
Example of simple pathway with feedback

- Pathway with negative feedback on level of enzyme activity

\[
S \xrightarrow{v_1} X_1 \xrightarrow{v_2} X_2 \xrightarrow{v_3} P
\]

- Question: in case of strong feedback, if we would like to increase production of P, which reaction should we target?

- Answer: reaction 3 (counter-intuitive)

Sauro (2009), Chapter 13 in Jason McDermott et al. (eds.), *Computational Systems Biology*, Humana Press, 269-309
In-vitro reconstruction of glycolysis

• Upper part of glycolysis pathway has been reconstructed in vitro and quantitatively modeled

Fiévet et al. (2006), Biochem. J., 396:317–26
In-vitro reconstruction of glycolysis

- Upper part of glycolysis pathway has been reconstructed in vitro and quantitatively modeled
- How does flux respond to change in enzyme concentration?

- Positive flux control coefficients for all enzymes, as expected from theoretical analysis

Fiévet et al. (2006), Biochem. J., 396:317–26
**In-vitro reconstruction of glycolysis**

- Upper part of glycolysis pathway has been reconstructed *in vitro* and quantitatively modeled.
- How does flux respond to change in enzyme concentration?
- How can flux be optimized for given total enzyme concentration?

- Maximum attained for intermediate enzyme concentrations

In-vivo control of glycolytic flux

- How is flux through glycolysis controlled in bacteria? What is role of ATP demand?
  ATP produced by glycolysis and consumed by other cellular processes
- Approach: augment intracellular ATP consumption
  Inducible (uncoupled) ATPase activity

Koebmann et al. (2002), J. Bacteriol., 184(14):3909-16
**In-vivo control of glycolytic flux**

- How is flux through glycolysis controlled in bacteria? What is role of ATP demand?

  ATP produced by glycolysis and consumed by other cellular processes

- Approach: augment intracellular ATP consumption

- Metabolic control analysis of simplified system

  \[
  \text{substrate} \xrightarrow{e_1} \Delta G_p \xrightarrow{e_2} \text{growth}
  \]

- ATPase overexpression decreases growth rate and increases glycolytic fluxes

![Graph showing the relationship between [ATP]/[ADP] ratio and relative flux, with separate plots for Glyc. flux and Growth rate.](image)
**In-vivo control of glycolytic flux**

- How is flux through glycolysis controlled in bacteria? What is role of ATP demand?
  - ATP produced by glycolysis and consumed by other cellular processes
- Approach: augment intracellular ATP consumption
- Metabolic control analysis of simplified system
  
  \[ \text{substrate} \xrightarrow{e_1} \Delta G_p \xrightarrow{e_2} \text{growth} \]

  \[ C_{e_2} = \frac{-\epsilon_{e_1}^p}{\epsilon_{e_2}^p - \epsilon_{e_1}^p} \]

- Experimental determination of elasticities
**In-vivo control of glycolytic flux**

- How is flux through glycolysis controlled in bacteria? What is the role of ATP demand?
  
  ATP produced by glycolysis and consumed by other cellular processes.

- Approach: augment intracellular ATP consumption.

- Metabolic control analysis of simplified system:

  \[
  \text{substrate} \xrightarrow{e_1} \Delta G_p \xrightarrow{e_2} \text{growth}
  \]

  \[
  C_{e_2} = \frac{-\varepsilon_{e_1}}{\varepsilon_{e_2} - \varepsilon_{e_1}}
  \]

- Experimental determination of elasticities.

- In wild-type cells at least 75% of glycolytic control exerted by ATP demand.
Conclusions

- Metabolic systems often analyzed at (stable) steady state
- Metabolic flux analysis (MCA) quantifies sensitivity of fluxes and concentrations to changes in parameters and inputs
- Well-established and powerful mathematical framework
- Dedicated computer tools supporting the analysis
- Many applications demonstrating its practical use in systems biology and synthetic biology/metabolic engineering
Thanks!

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