Metabolic Control Analysis (MCA)

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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

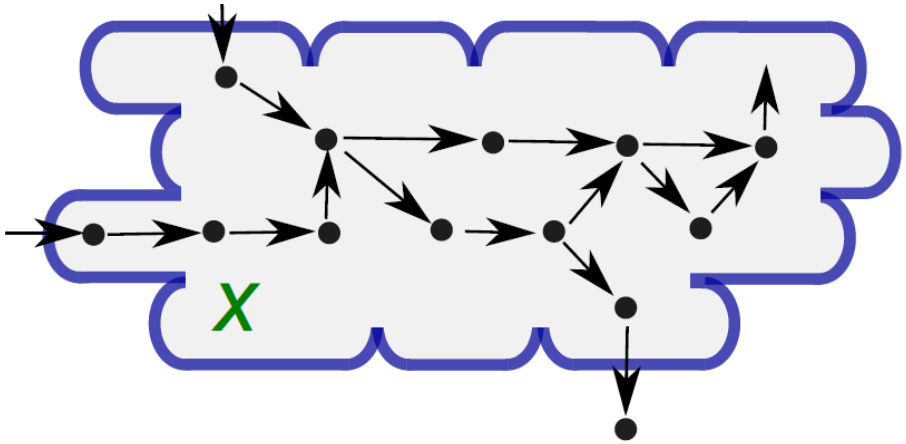
- Part 1. Systems biology and kinetic modeling
  - Introduction
  - Kinetic modeling of cellular reaction networks
- Part 2. Metabolic network modeling
  - Kinetic modeling of metabolism
  - **Metabolic control analysis (MCA)**
  - Flux balance analysis (FBA)
  - Practical on flux balance analysis (COBRA)
- Part 3. Gene regulatory network modeling

Biochemical reaction networks

- ODE model for growth of microbial populations:

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

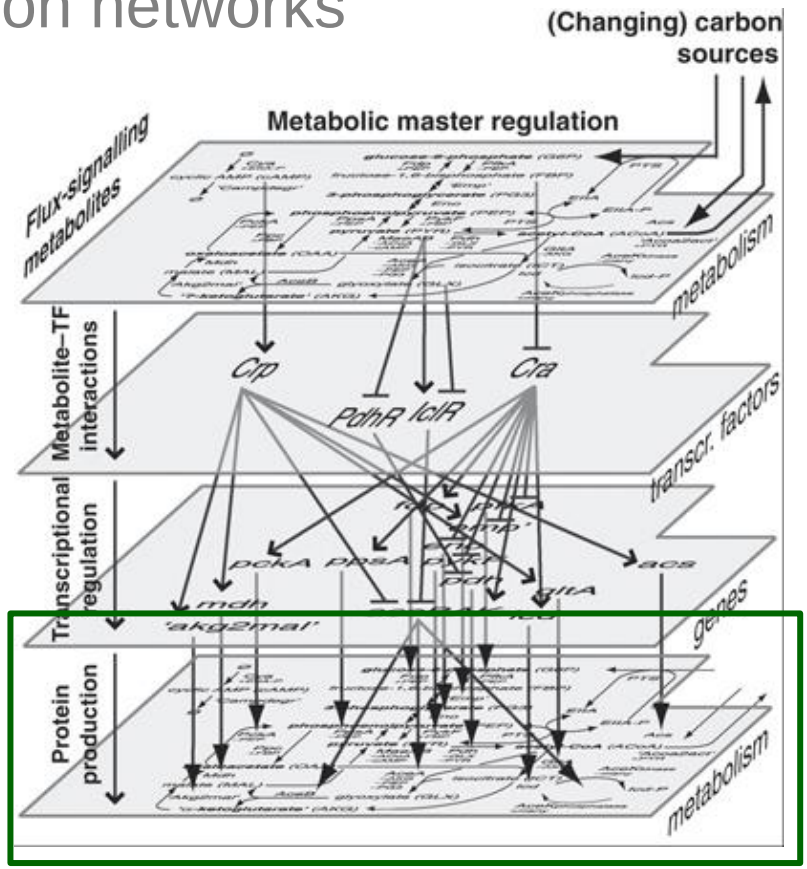
$$\mu = \delta \cdot \sum_i \alpha_i \cdot N_i \cdot v(x).$$



- 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



Kotte *et al.* (2010), *Mol. Syst. Biol.*, 6: 355

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

$$\dot{x} = N\, v(x)$$

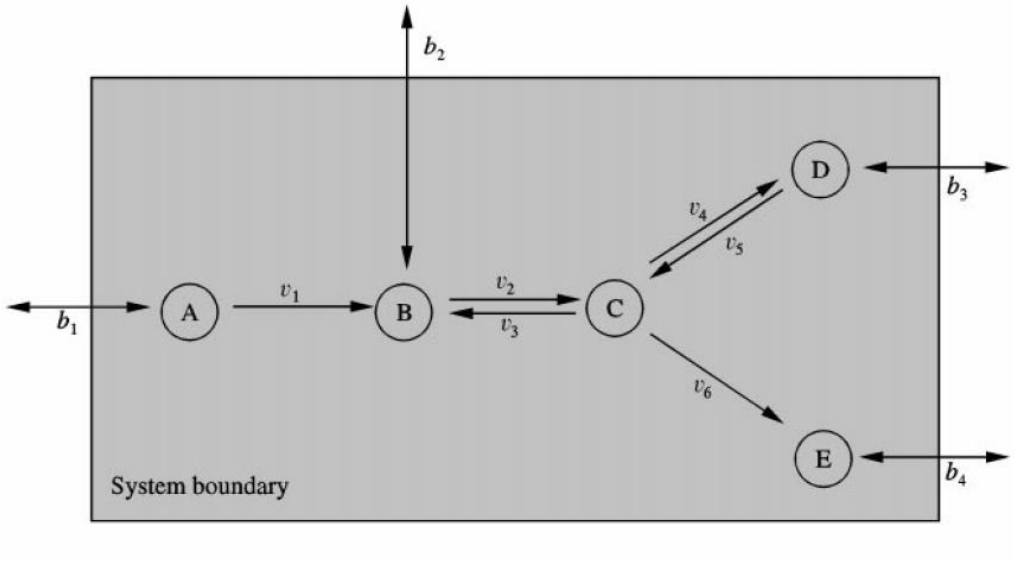
- Explicit introduction of dependency of model dynamics on parameters p :
  - Enzyme concentrations
  - Half-saturation and catalytic constants
  - Inhibition/activation constants

$$\dot{x} = N v(x, p)$$

Stoichiometry matrix

- Stoichiometry matrix N describes structure of reaction network

  Internal reactions and exchange reactions, reversible and irreversible



$$N \qquad v$$

$$\begin{bmatrix} -1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\ 1 & -1 & 1 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 1 & -1 & -1 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_6 \\ b_1 \\ b_2 \\ b_3 \\ b_4 \end{bmatrix}$$

Schilling *et al.* (2000), *J. Theor. Biol.*, 203(3):229-48

Stoichiometry matrix

- Stoichiometry matrix may not be full rank
  - Dependencies between rows (variables) due to conservation relations
  - Example: [ATP] + [ADP] + [AMP] = 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**

  Grimbs *et al.* (2007), *Mol. Syst. Biol.*, 3:146

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

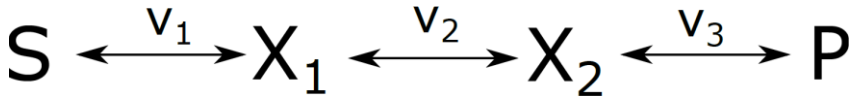
$$J(x^*) = N \left. \frac{\partial v}{\partial x} \right|_{x^*, v^*}$$

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

Kaplan and Glass (1995), *Understanding Nonlinear Dynamics*, New York

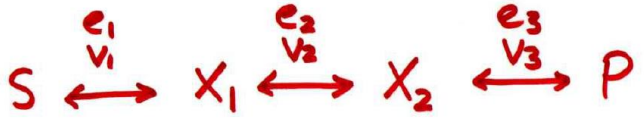
Example of simple metabolic pathway

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



- **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



$$\frac{d}{dt}\begin{bmatrix} X_1 \\ X_2 \end{bmatrix} = \underbrace{\begin{bmatrix} 1 & -1 & 0 \\ 0 & 1 & -1 \end{bmatrix}}_{N} \underbrace{\begin{bmatrix} v_1 \\ v_2 \\ v_3 \end{bmatrix}}_{v} = 0$$

$$N \cdot v = 0 \Rightarrow v_1 = v_2 \text{ et } v_2 = v_3 \Rightarrow v_1 = v_2 = v_3$$

$$J = N \cdot \frac{\partial v}{\partial x} = \begin{bmatrix} 1 & -1 & 0 \\ 0 & 1 & -1 \end{bmatrix} \cdot \begin{bmatrix} \frac{\partial v_1}{\partial x_1} & 0 \\ \frac{\partial v_2}{\partial x_1} & \frac{\partial v_2}{\partial x_2} \\ 0 & \frac{\partial v_3}{\partial x_2} \end{bmatrix} = \begin{bmatrix} \frac{\partial v_1}{\partial x_1} - \frac{\partial v_2}{\partial x_1} & -\frac{\partial v_2}{\partial x_2} \\ \frac{\partial v_2}{\partial x_1} & \frac{\partial v_2}{\partial x_2} - \frac{\partial v_3}{\partial x_2} \end{bmatrix}$$

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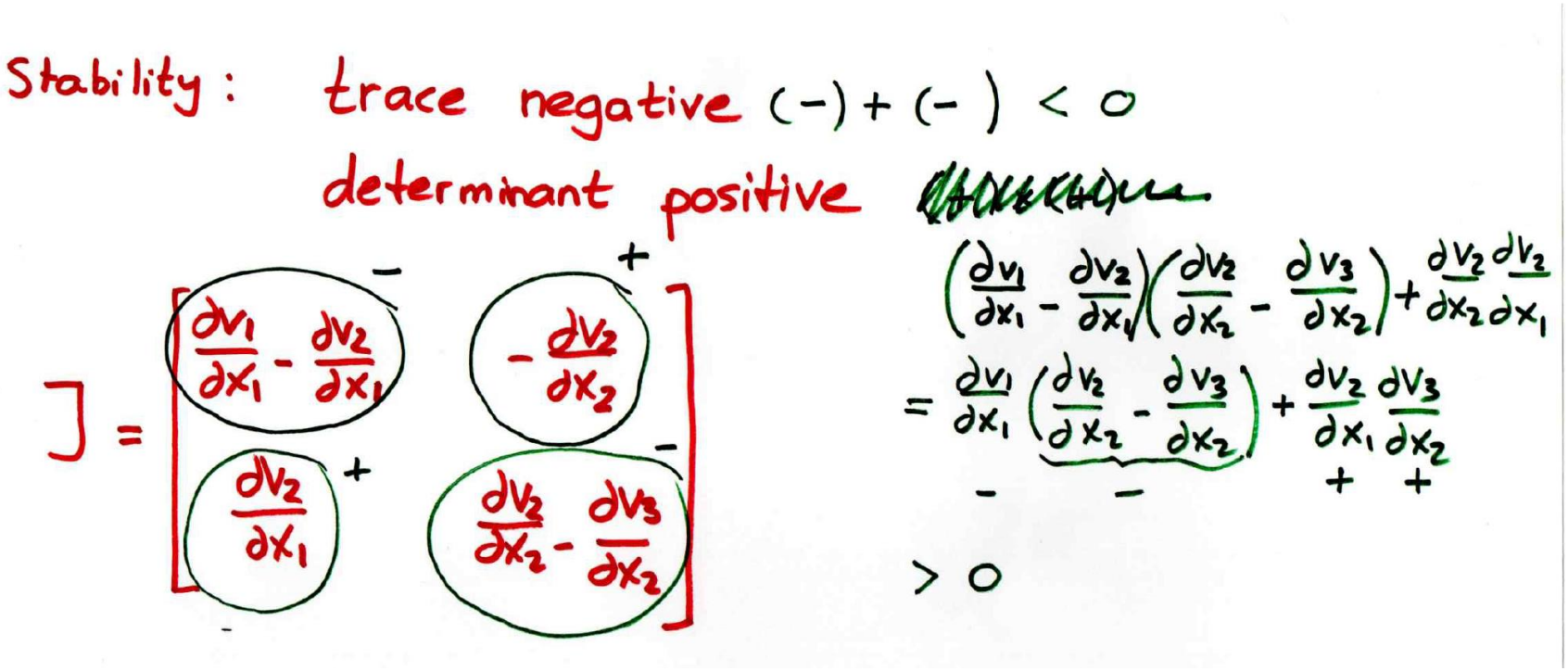
Example of simple metabolic pathway



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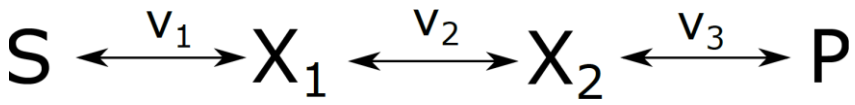
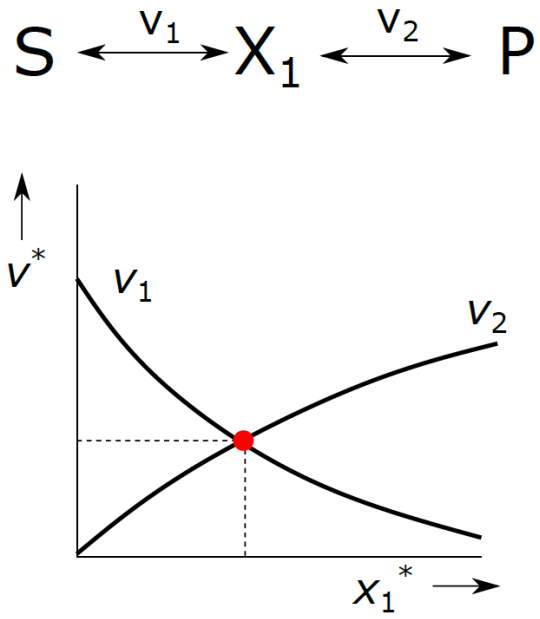


- Assumption: steady states are **stable**

Metabolic control analysis

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



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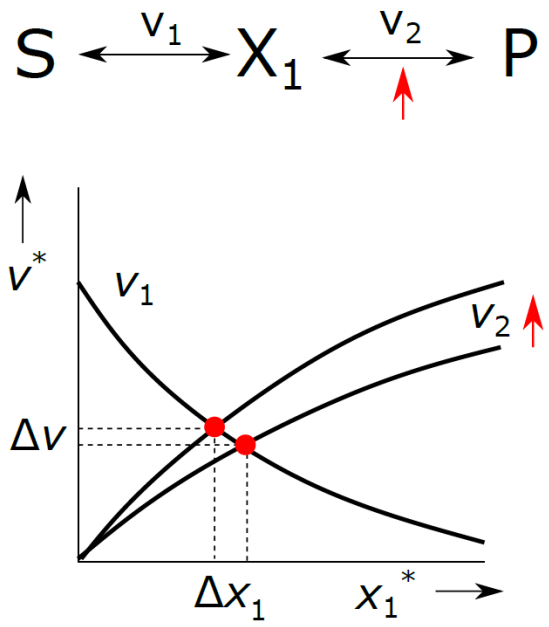


Metabolic control analysis

- 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

Heinrich and Schuster (1996), *The Regulation of Cellular Systems*, Chapman & Hall

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

$$\epsilon_{x_i}^{v_j} = \frac{\partial v_j}{\partial x_i} \cdot \frac{x_i}{v_j} = \frac{\partial \ln v_j}{\partial \ln x_i} = \frac{\%v_j}{\%x_i} \qquad \epsilon_{p_i}^{v_j} = \frac{\partial v_j}{\partial p_i} \cdot \frac{p_i}{v_j} = \frac{\partial \ln v_j}{\partial \ln p_i} = \frac{\%v_j}{\%p_i}$$

- 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

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

$$\varepsilon_e^v = \frac{dv}{de} \cdot \frac{e}{v}$$

$$= k_{cat} \cdot \frac{s}{s + K_m} \cdot e \,/\, v$$

$$= v / v = 1$$

Response coefficients

- **Response coefficients** express how steady state of the system changes due to a change in reaction properties
  - **Flux response coefficients**

$$R_{p_i}^{v_j^*} = \frac{\partial v_j}{\partial p_i} \cdot \frac{p_i}{v_j}\bigg|_{x^*,v^*} = \frac{\partial \ln v_j}{\partial \ln p_i}\bigg|_{x^*,v^*} = \frac{\%v_j^*}{\%p_i}$$

  - **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
- Exercise: give examples of response coefficients for simple reversible pathway and their meaning

Response coefficients

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 :

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

Computation of response coefficients

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

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

$$\left.\frac{\partial x}{\partial p}\right|_{x^*,v^*} = -\left(N \cdot \frac{\partial v}{\partial x}\right)^{-1} \cdot N \cdot \left.\frac{\partial v}{\partial p}\right|_{x^*,v^*}$$

$$\begin{aligned}
\left.\mathrm{dg}(x)^{-1} \cdot \frac{\partial x}{\partial p} \cdot \mathrm{dg}(p)\right|_{x^*,v^*} &= -\,\mathrm{dg}(x)^{-1} \cdot \left(N \cdot \frac{\partial v}{\partial x}\right)^{-1} \cdot N \cdot \left.\frac{\partial v}{\partial p} \cdot \mathrm{dg}(p)\right|_{x^*,v^*} \\
&= -\,\mathrm{dg}(x)^{-1} \cdot \left(N \cdot \mathrm{dg}(v) \cdot \mathrm{dg}(v)^{-1} \cdot \frac{\partial v}{\partial x} \cdot \mathrm{dg}(x) \cdot \mathrm{dg}(x)^{-1}\right)^{-1} \cdot \\
&\qquad \left.N \cdot \mathrm{dg}(v) \cdot \mathrm{dg}(v)^{-1} \cdot \frac{\partial v}{\partial p} \cdot \mathrm{dg}(p)\right|_{x^*,v^*}
\end{aligned}$$

$$R_p^{x^*} = -\left(N \cdot \mathrm{dg}(v) \cdot \epsilon_x^v\right)^{-1} \cdot N \cdot \mathrm{dg}(v) \cdot \left.\epsilon_p^v\right|_{x^*,v^*}$$

Control coefficients

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

$$R_p^{x^*} = -\left(N \cdot \mathrm{dg}(v) \cdot \epsilon_x^v\right)^{-1} \cdot N \cdot \mathrm{dg}(v) \cdot \epsilon_p^v \Big|_{x^*,v^*}$$
$$= C_v^{x^*} \cdot \epsilon_p^v \Big|_{x^*,v^*}$$

with **concentration control coefficients**

$$C_v^{x^*} = -\left(N \cdot \mathrm{dg}(v) \cdot \epsilon_x^v\right)^{-1} \cdot N \cdot \mathrm{dg}(v) \Big|_{x^*,v^*}$$

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

$$R_{p_i}^{x_j^*} = \sum_k C_{v_k}^{x_j^*} \cdot \epsilon_{p_i}^{v_k} \Big|_{x^*,v^*} \qquad C_{v_k}^{x_j^*} = \frac{\%x_j^*}{\%v_k}$$

Control coefficients

- Similar analysis for flux response coefficients leads to

$$R_p^{v^*} = C_v^{v^*} \cdot \epsilon_p^v \Big|_{x^*,v^*}$$

with **flux control coefficients**

$$C_v^{v^*} = I + \epsilon_x^v \cdot C_v^{x^*} \Big|_{x^*,v^*}$$

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

$$R_{p_i}^{v_j^*} = \sum_k C_{v_k}^{v_j^*} \cdot \epsilon_{p_i}^{v_k} \Big|_{x^*,v^*} \qquad C_{v_k}^{v_j^*} = \frac{\%v_j^*}{\%v_k}$$

- 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**:

$$C_v^{x^*} \cdot 1 = 0, \qquad \sum_k C_{v_k}^{x_j^*} = 0$$

$$C_v^{v^*} \cdot 1 = 1, \qquad \sum_k C_{v_k}^{v_j^*} = 1$$

- Flux control is **distributed** over the system
- Idem for MCA **connectivity theorems**:

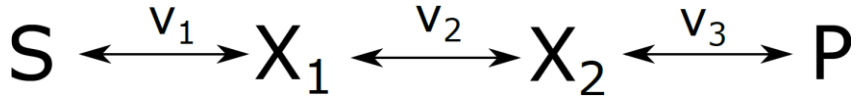
$$C_v^{x^*} \cdot \epsilon_x^v = -I, \qquad \sum_k C_{v_k}^{x_j^*} \cdot \epsilon_{x_i}^{v_k} = -\delta_{ji}$$

$$\delta_{ji} = \begin{cases} 1, & i = j, \\ 0, & i \neq j. \end{cases}$$

$$C_v^{v^*} \cdot \epsilon_x^v = 0, \qquad \sum_k C_{v_k}^{v_j^*} \cdot \epsilon_{x_i}^{v_k} = 0$$

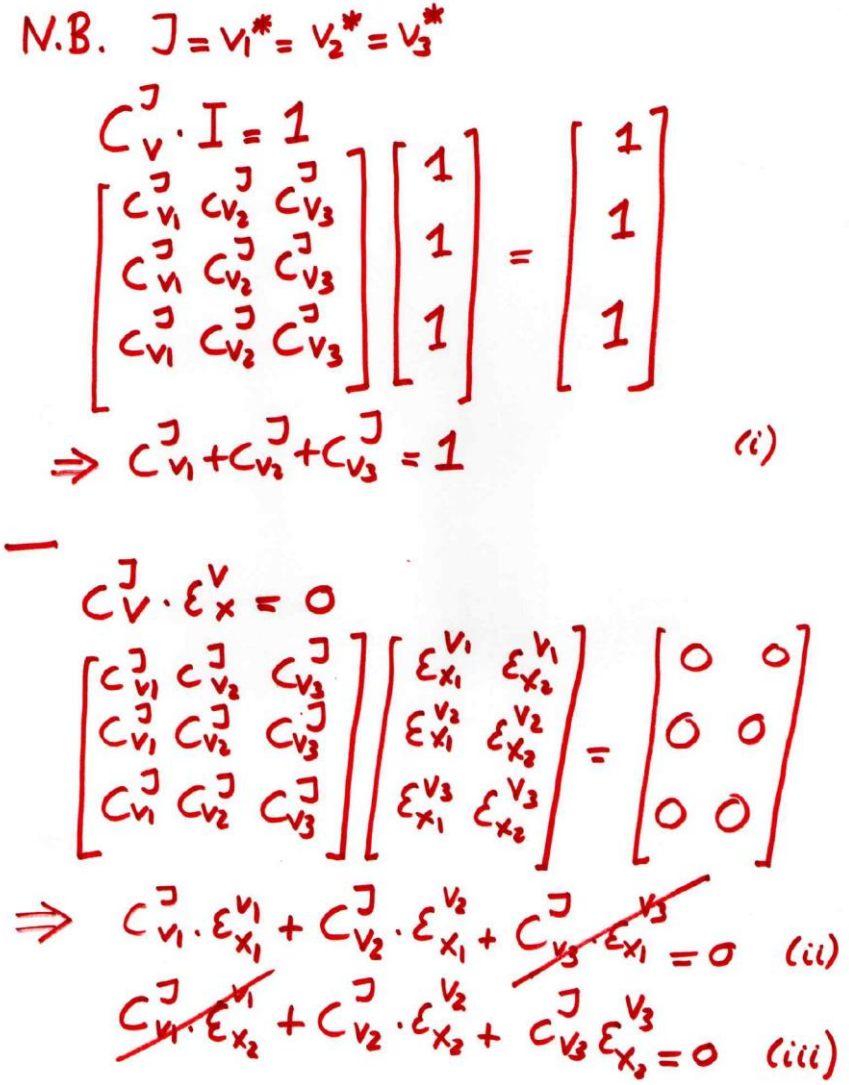
Heinrich and Schuster (1996), *The Regulation of Cellular Systems*, Chapman & Hall

Example of simple metabolic pathway



- 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



Example of simple metabolic pathway

$$C^J_{V_1} = -\frac{\varepsilon^{V_2}_{X_1}}{\varepsilon^{V_1}_{X_1}} \cdot C^J_{V_2}$$

$$C^J_{V_2} = -\frac{\varepsilon^{V_3}_{X_2}}{\varepsilon^{V_2}_{X_2}} \cdot C^J_{V_3} \Rightarrow C^J_{V_1} = \frac{\varepsilon^{V_2}_{X_1}}{\varepsilon^{V_1}_{X_1}} \cdot \frac{\varepsilon^{V_3}_{X_2}}{\varepsilon^{V_2}_{X_2}} \cdot C^J_{V_3}$$

$$C^J_{V_1} + C^J_{V_2} + C^J_{V_3} = 1$$

$$\left(\frac{\varepsilon^{V_2}_{X_1}}{\varepsilon^{V_1}_{X_1}} \cdot \frac{\varepsilon^{V_3}_{X_2}}{\varepsilon^{V_2}_{X_2}} - \frac{\varepsilon^{V_2}_{X_1}}{\varepsilon^{V_1}_{X_1}} + 1 \right) C^J_{V_3} = 1$$

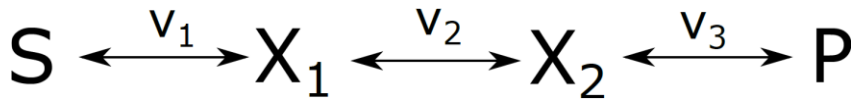
$$C^J_{V_3} = \frac{1}{\frac{\varepsilon^{V_2}_{X_1}}{\varepsilon^{V_1}_{X_1}} \cdot \frac{\varepsilon^{V_3}_{X_2}}{\varepsilon^{V_2}_{X_2}} - \frac{\varepsilon^{V_2}_{X_1}}{\varepsilon^{V_1}_{X_1}} + 1}$$

$$= \frac{\varepsilon^{V_1}_{X_1} \varepsilon^{V_2}_{X_2}}{\varepsilon^{V_2}_{X_1} \cdot \varepsilon^{V_3}_{X_2} - \varepsilon^{V_2}_{X_1} \cdot \varepsilon^{V_2}_{X_2} + \varepsilon^{V_1}_{X_1} \varepsilon^{V_2}_{X_2}} > 0$$

$$C^J_{V_2} = \frac{-\varepsilon^{V_1}_{X_1} \varepsilon^{V_3}_{X_2}}{\varepsilon^{V_2}_{X_1} \cdot \varepsilon^{V_3}_{X_2} - \varepsilon^{V_2}_{X_1} \varepsilon^{V_2}_{X_2} + \varepsilon^{V_1}_{X_1} \varepsilon^{V_2}_{X_2}} > 0$$

$$C^J_{V_1} = \frac{\cdots + \varepsilon^{V_2}_{X_1} \varepsilon^{V_3}_{X_2}}{\varepsilon^{V_2}_{X_1} \cdot \varepsilon^{V_3}_{X_2} - \varepsilon^{V_2}_{X_1} \varepsilon^{V_2}_{X_2} + \varepsilon^{V_1}_{X_1} \varepsilon^{V_2}_{X_2}} > 0$$

Example of simple metabolic pathway

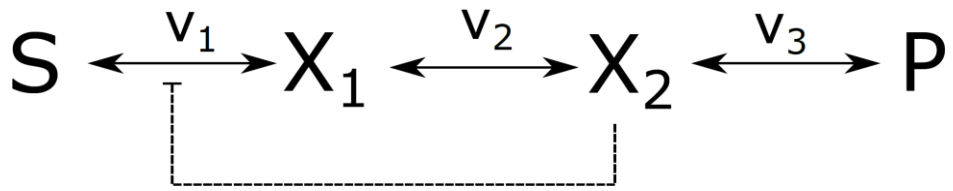


- Distributed control of enzymes over pathway flux
  - Contrary to idea of rate-limiting step

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

Example of simple pathway with feedback

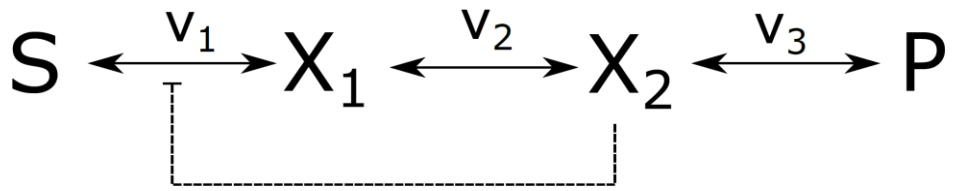
- Pathway with negative feedback on level of enzyme activity



- 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

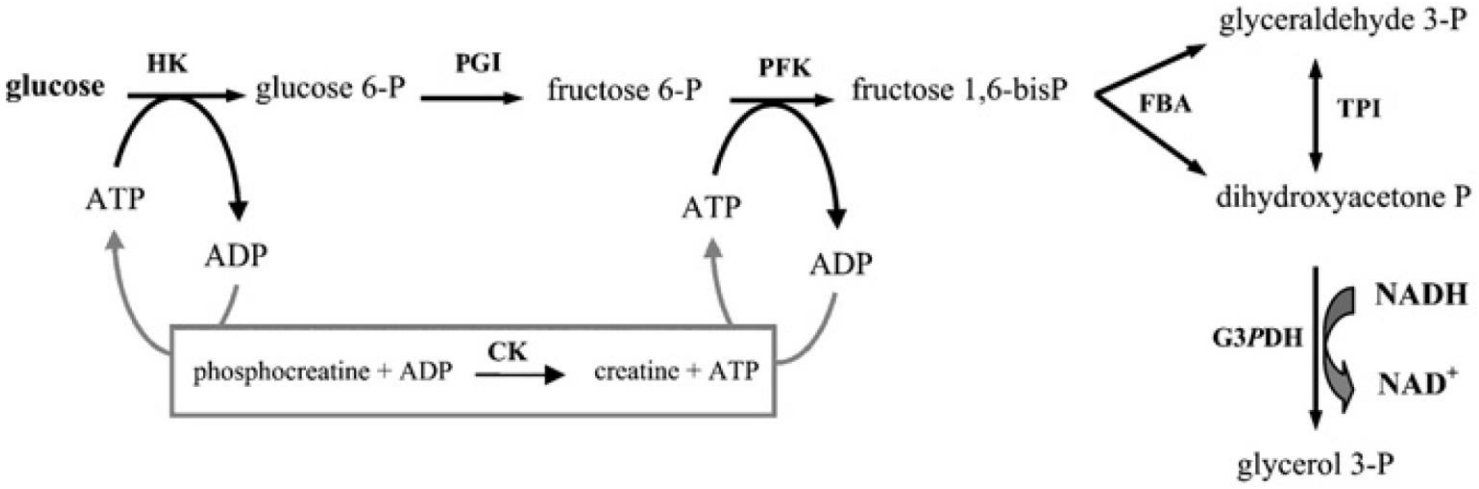


- Question: in case of strong feedback, if we would like to increase production of P, which reaction should we target?
- Answer: reaction 3 (counter-intuitive)

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

In-vitro reconstruction of glycolysis

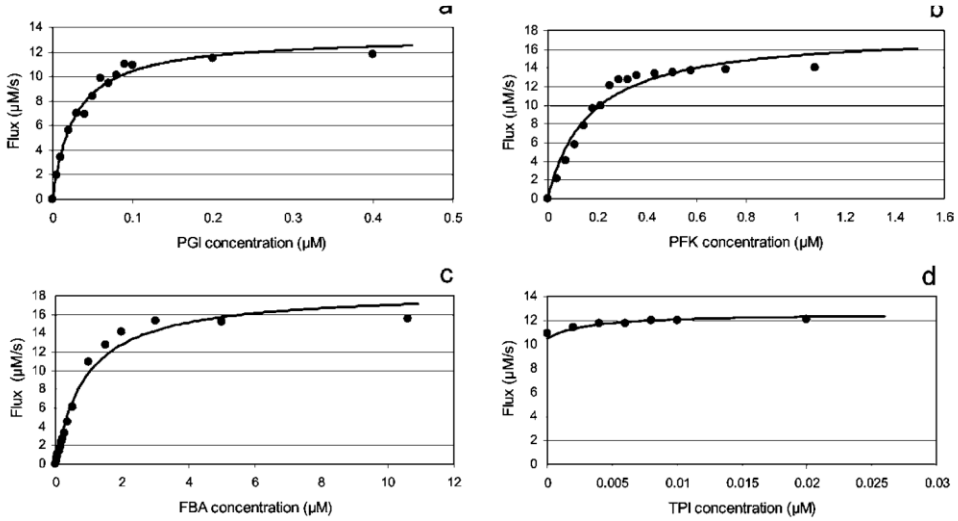
- Upper part of glycolysis pathway has been reconstructed *in vitro* and quantitatively modeled



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?



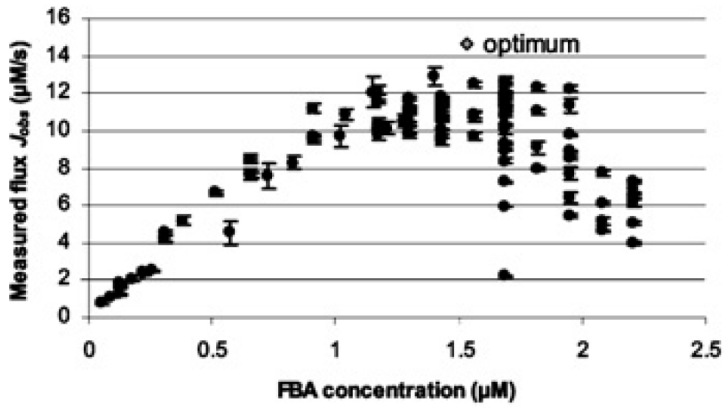
- Positive flux control coefficients for **all** enzymes, as expected from theoretical analysis

Fiévet *et al.* (2006), *Biochem. J.*, 396:317–26

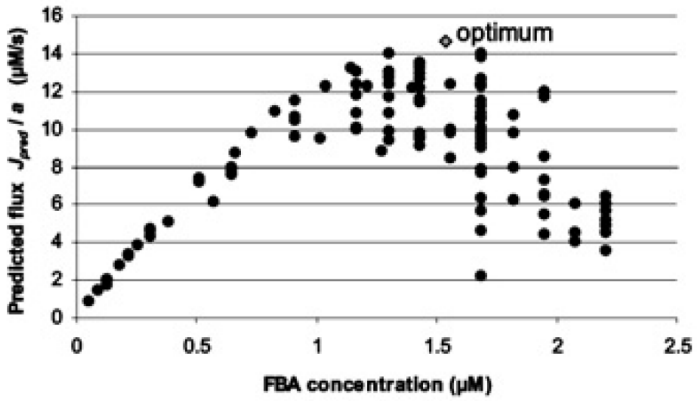
In-vitro reconstruction of glycolysis

- 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?

Data



Model



- Maximum attained for intermediate enzyme concentrations

Fiévet *et al.* (2006), *Biochem. J.*, 396:317–26

In-vivo control of glycolytic flux

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

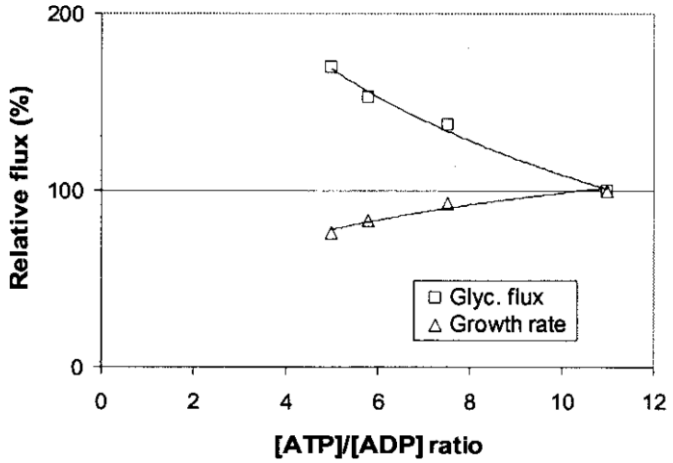
Koebmann *et al.* (2002), *J. Bacteriol.*, 184(14):3909-16

In-vivo control of glycolytic flux

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



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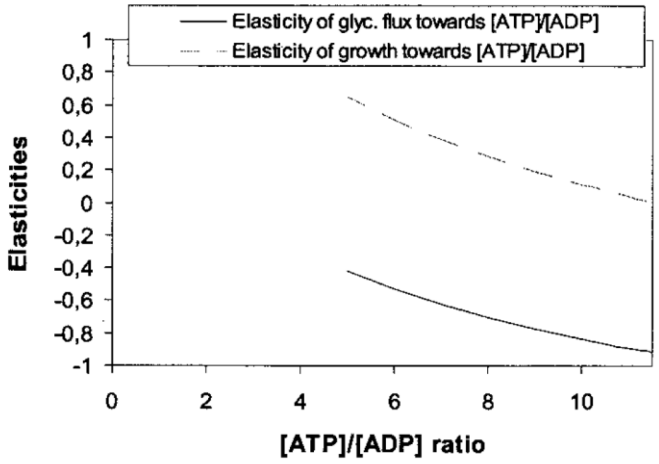
- Approach: augment intracellular ATP consumption
- Metabolic control analysis of simplified system

$$\text{substrate} \xrightarrow{e_1} \Delta G_p \xrightarrow{e_2} \text{growth}$$

$$C^J_{e_2} = \frac{-\varepsilon^{e_1}_p}{\varepsilon^{e_2}_p - \varepsilon^{e_1}_p}$$

- Experimental determination of elasticities



In-vivo control of glycolytic flux

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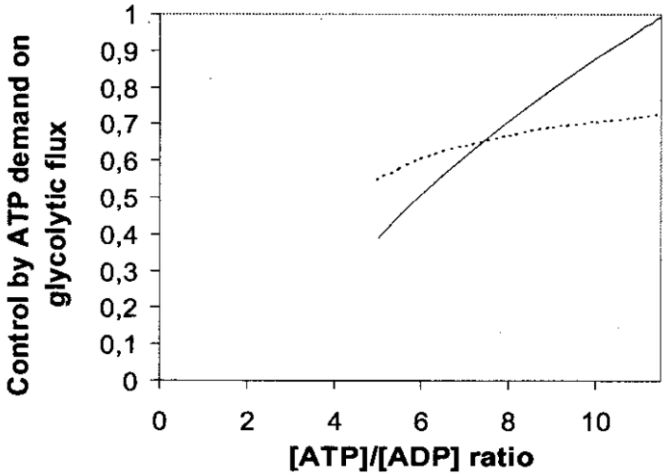
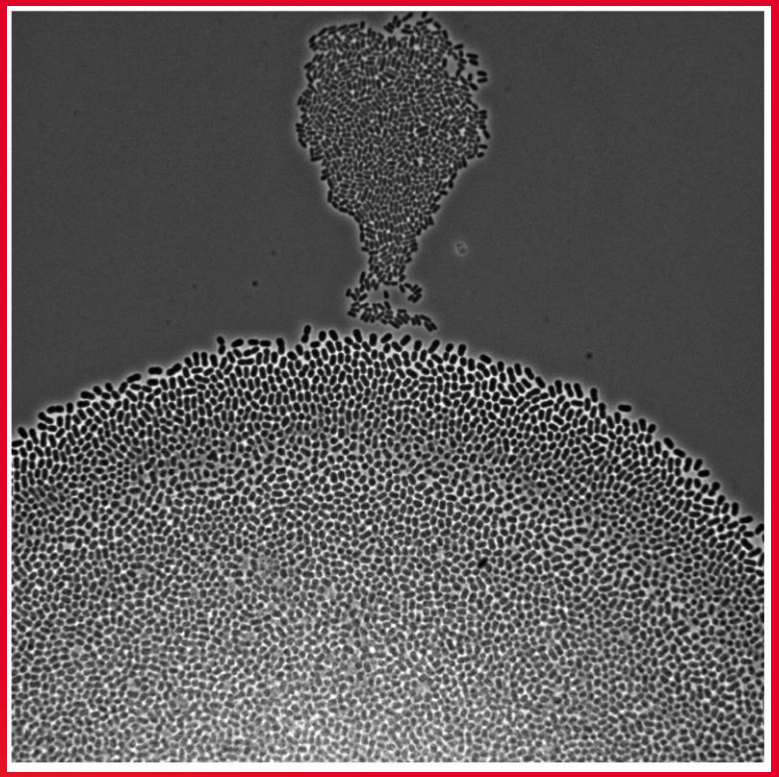
  - Experimental determination of elasticities
  - In wild-type cells at least 75% of glycolytic control exerted by ATP demand



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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