

Introduction to Metabolic Control Theory

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Outline

1. Introduction to systemic sensitivity analysis
2. The stoichiometry matrix
 - System reduction
3. System evolution
 - System relaxation between steady-states
4. Control coefficients
5. Summation theorem
6. Response coefficients and elasticities
7. Connectivity theorem

General problem

- Let us consider an **arbitrary complex metabolic network**
- Each reaction rate responds to changes in concentrations of substrates, products and some effectors:
 - These kinetic laws are **individual molecular properties** of each enzyme in the system
- Central questions of MCT:
 - How does the **system** respond to changes in individual molecular properties (enzyme activities)?
 - How does the system's response depend on the **network structure**?
 - How constrained are systemic sensitivities?
Do they show dependencies?

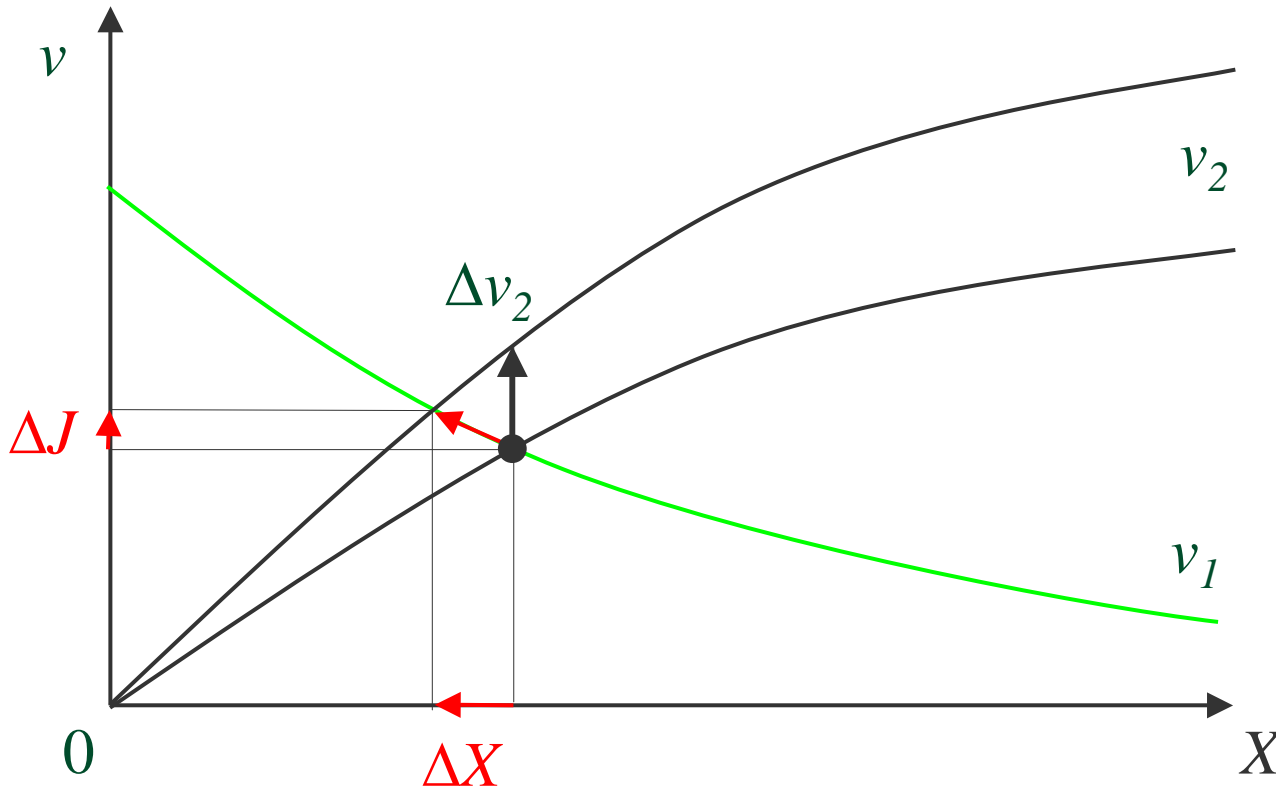
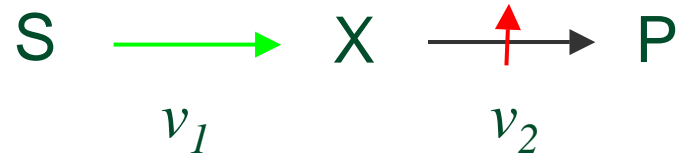
Steady-states and system definition

Metabolism concerns almost exclusively **sustainable** processing of chemical inputs into outputs such as biomass, energy, waste, etc.: it must reach a **stable steady-state**.

Therefore:

- The system **must be open** in order to reach a thermodynamically feasible non-trivial steady-state (*i.e.*, with non-zero fluxes)
- Most reactions should be sensitive to both substrate and product concentrations, allowing for the **balancing of metabolite production and consumption rates**

Intuitively?



"Flux control"

$$\frac{\Delta J}{\Delta v_2}$$

"Concentration control"

$$\frac{\Delta X}{\Delta v_2}$$

Formally

It is possible to derive a very general treatment of metabolic control theory for metabolic systems of **arbitrary complexity**.

C. Reder (1988) *J. Theoret. Biol.* 135:175–201

General definitions:

$\mathbf{x} = \mathbf{x}(t, \mathbf{p})$ Molarity vector

$\mathbf{X} = \mathbf{X}(\mathbf{p})$ **Steady-state** molarity vector: $d\mathbf{x} / dt = 0$

$\mathbf{v} = \mathbf{v}(\mathbf{x}, \mathbf{p})$ Rate vector

$\mathbf{J} = \mathbf{J}(\mathbf{p})$ **Steady-state** flux vector

$= \mathbf{v}(\mathbf{X}(\mathbf{p}), \mathbf{p})$

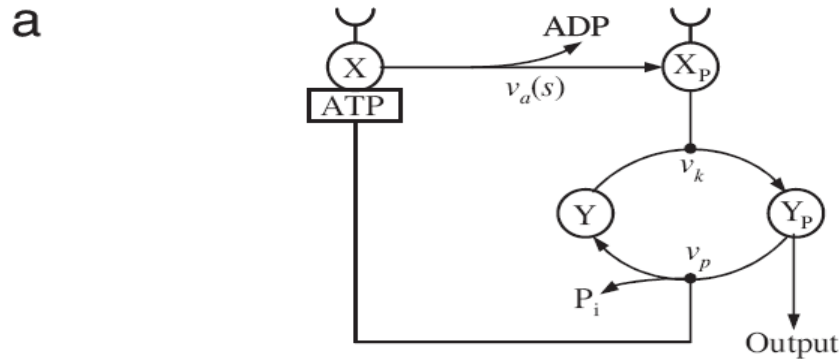
The stoichiometry matrix

- Reactions in the network are expressed in the *stoichiometry matrix* \mathbf{N} , whose columns contain the stoichiometric coefficients for each reaction
- This matrix reflects **the system's structure**
- The stoichiometry matrix \mathbf{N} is of maximal rank if and only if there is no conservation relationship constraining the different concentrations, which we will initially assume for simplicity
- Otherwise it should be reduced to a matrix \mathbf{N}^0 with maximal rank in order to deal with independent variables:

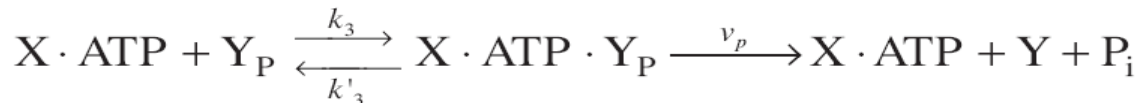
$$\mathbf{N} = \mathbf{L} \cdot \mathbf{N}^0$$

Exercise: 2-component transduction

Write stoichiometry matrix for the following system:



b



Shinar *et al*, 2007, *PNAS* **104**:19931-19935

System evolution

The evolution of the system's concentration vector \mathbf{x} is a simple function of the reaction rate vector \mathbf{v} :

$$d\mathbf{x}/dt = \mathbf{N} \cdot \mathbf{v}(\mathbf{x}, \mathbf{p})$$

where \mathbf{p} is a parameter vector, and the Jacobian is :

$$\mathfrak{J} = \mathbf{N} \cdot \partial \mathbf{v} / \partial \mathbf{x}$$

$\partial v_i / \partial x_j$ are non-normalized 'elasticities'.

Shifting between steady-states

Starting from a **steady-state** \mathbf{X}_1 , what happens if we perturb the rates \mathbf{v} with a small change in parameters $\delta\mathbf{p}$?

$$\frac{d\mathbf{x}}{dt} \sim \mathfrak{J} \cdot (\mathbf{x}(t) - \mathbf{X}_2)$$

where \mathbf{X}_2 is the new steady-state.

$$\left\{ \begin{array}{l} \frac{d\mathbf{x}}{dt} = \mathbf{N} \cdot \mathbf{v}(\mathbf{x}, \mathbf{p} + \delta\mathbf{p}) \\ \frac{d\mathbf{x}}{dt}(0) = \mathbf{N} \cdot \frac{\partial \mathbf{v}}{\partial \mathbf{p}} \cdot \delta\mathbf{p} = \mathbf{N} \cdot \delta\mathbf{v} \\ \mathbf{x}(0) = \mathbf{X}_1 \end{array} \right.$$

Shifting between steady-states

which integrates into:

$$\mathbf{x}(t) = \mathbf{X}_1 - (\mathbf{I} - \exp \mathfrak{T}t) \mathfrak{T}^{-1} \cdot \mathbf{N} \cdot \delta \mathbf{v}$$

\mathfrak{T} being definitive negative for the steady-state to be stable:

$$\delta \mathbf{X} \rightarrow \mathbf{X}_2 - \mathbf{X}_1 = -\mathfrak{T}^{-1} \cdot \mathbf{N} \cdot \delta \mathbf{v}$$

$$\delta \mathbf{J} = \frac{\partial \mathbf{v}}{\partial \mathbf{x}} \delta \mathbf{X} + \frac{\partial \mathbf{v}}{\partial \mathbf{p}} \delta \mathbf{p} = \left(\mathbf{I} - \frac{\partial \mathbf{v}}{\partial \mathbf{x}} \mathfrak{T}^{-1} \cdot \mathbf{N} \right) \cdot \delta \mathbf{v}$$

These relationships express the **changes in steady-state** concentrations \mathbf{X} and fluxes \mathbf{J} in response to a change in the **enzyme rates** $\delta \mathbf{v}$

Steady-state flux constraints

- We are interested in analysing the steady-state of the system:

$$d\mathbf{x}/dt = \mathbf{N} \cdot \mathbf{v}(\mathbf{X}(\mathbf{p}), \mathbf{p}) = \mathbf{0}$$

where $\mathbf{X}(\mathbf{p})$ is the vector of steady-state concentrations

- The steady-state introduces **linear dependencies** between fluxes:

$$\mathbf{N} \cdot \mathbf{J}(\mathbf{p}) = \mathbf{0}$$

Kirchhoff's law for metabolic intermediates

- Therefore the flux vector \mathbf{J} can be expressed in a basis of $\text{Ker}(\mathbf{N})$ (often termed \mathbf{K})

Expressing systemic control

Differentiating the steady-state equation with respect to \mathbf{p} :

$$\mathbf{N} \cdot \partial \mathbf{v} / \partial \mathbf{x} \cdot \partial \mathbf{X} / \partial \mathbf{p} + \mathbf{N} \cdot \partial \mathbf{v} / \partial \mathbf{p} = \mathbf{0}$$

$$\partial \mathbf{X} / \partial \mathbf{p} = - \mathfrak{J}^{-1} \cdot \mathbf{N} \cdot \partial \mathbf{v} / \partial \mathbf{p}$$

- This equation relates **systemic changes** in steady-state concentrations \mathbf{X} to changes in rates \mathbf{v}
- The matrix $\mathbf{\Gamma} = - \mathfrak{J}^{-1} \cdot \mathbf{N}$ contains all **concentration control coefficients**

Flux control

- Let us calculate the resulting steady-state flux:

$$\mathbf{J} = \mathbf{v}(\mathbf{X}(\mathbf{p}), \mathbf{p})$$

and differentiate it with respect to \mathbf{p} :

$$\begin{aligned}\partial\mathbf{J}/\partial\mathbf{p} &= \partial\mathbf{v}/\partial\mathbf{x} \cdot \partial\mathbf{X}/\partial\mathbf{p} + \partial\mathbf{v}/\partial\mathbf{p} \\ &= (\partial\mathbf{v}/\partial\mathbf{x} \cdot \mathbf{\Gamma} + \mathbf{I}) \cdot \partial\mathbf{v}/\partial\mathbf{p}\end{aligned}$$

- This equation relates systemic changes in steady-state fluxes \mathbf{J} to changes in rates \mathbf{v}
- The matrix $\mathbf{\Phi} = \mathbf{I} + \partial\mathbf{v}/\partial\mathbf{x} \cdot \mathbf{\Gamma}$ contains all **flux control coefficients**

Generalisation

If the system shows **conservation relationships** such as $[ATP]+[ADP]+[AMP] = \text{constant}$ we need to reduce \mathbf{N} to a matrix \mathbf{N}^0 with maximal rank corresponding to independent metabolite molarities \mathbf{x}^0 :

$$\mathbf{N} = \mathbf{L} \cdot \mathbf{N}^0$$

$$d\mathbf{x}^0/dt = \mathbf{N}^0 \cdot \mathbf{v}(\mathbf{x}, \mathbf{p})$$

$$\mathfrak{J} = \mathbf{N}^0 \cdot \partial \mathbf{v} / \partial \mathbf{x} \cdot \mathbf{L}$$

$$\mathbf{\Gamma} = -\mathbf{L} \cdot \mathfrak{J}^{-1} \cdot \mathbf{N}^0$$

$$\mathbf{\Phi} = \mathbf{I} + \partial \mathbf{v} / \partial \mathbf{x} \cdot \mathbf{\Gamma}$$

Normalised control coefficients

It is customary to express control in terms of dimension-less **normalised** control coefficients :

Fluxes :

$$C_i^j = \frac{E_i}{J_j} \frac{\partial J_j}{\partial E_i}$$

Molarities :

$$C_i^{X_j} = \frac{E_i}{X_j} \frac{\partial X_j}{\partial E_i}$$

where the E_i parameters denote enzyme **activities**.

Scaling of fluxes with enzyme activities

The steady-state equation:

$$\mathbf{N} \cdot \mathbf{v}(\mathbf{X}, \mathbf{E}) = \mathbf{0}$$

is **invariant to an arbitrary scaling** of activities \mathbf{E} :

$$\mathbf{v}(\mathbf{X}, \alpha \mathbf{E}) = \alpha \mathbf{v}(\mathbf{X}, \mathbf{E}), \quad \forall \alpha \in \mathbb{R}^+$$

Therefore the flux vector \mathbf{J} is a 1st order homogeneous function of enzyme activities \mathbf{E} :

$$\mathbf{J}(\alpha \mathbf{E}) = \alpha \mathbf{J}(\mathbf{E}), \quad \forall \alpha \in \mathbb{R}^+$$

and concentrations \mathbf{X} are 0-order homogeneous functions:

$$\mathbf{X}(\alpha \mathbf{E}) = \mathbf{X}(\mathbf{E}), \quad \forall \alpha \in \mathbb{R}^+$$

Summation relationships

Summation theorems follow directly by derivation with respect to α

For fluxes :
$$\sum_i E_i \frac{\partial J_j}{\partial E_i} = J_j \Rightarrow \sum_i C_i^j = 1$$

Flux control is **distributed** across the system

For molarities :
$$\sum_i C_i^{X_j} = 0$$

Response coefficients

The linearised response of the system to a change in any parameter p_i can be expressed from control coefficients and elasticity coefficients:

$$R_i^j = \frac{p_i}{J_j} \frac{\partial J_j}{\partial p_i} = \frac{p_i}{J_j} \sum_k \frac{\partial J_j}{\partial E_k} \frac{\partial v_k}{\partial p_i} = \sum_k C_k^j \varepsilon_i^k$$

where $\varepsilon_i^k = \frac{p_i}{v_k} \frac{\partial v_k}{\partial p_i}$

are normalised elasticity coefficients expressing the sensitivities of rates to parameter changes.

The R_i^j are called **response coefficients**

Response coefficients

$$R_i^j = \sum_k C_k^j \varepsilon_i^k$$

The response of the network depends on two factors:

- the sensitivities of enzymes to parameter p_i (a molecular property)
- the control exerted by these enzymes on the flux (a systemic property)

One can similarly define response coefficients for metabolite concentrations:

$$R_i^{X_j} = \sum_k C_k^{X_j} \varepsilon_i^k$$

Connectivity relationships

$$\Gamma = -\mathbf{L} \cdot \mathfrak{J}^{-1} \cdot \mathbf{N}^0$$

$$\Rightarrow \Gamma \cdot \partial \mathbf{v} / \partial \mathbf{x} \cdot \mathbf{L} = -\mathbf{L}$$

$$\Phi = \mathbf{I} + \partial \mathbf{v} / \partial \mathbf{x} \cdot \Gamma$$

$$\Rightarrow \Phi \cdot \partial \mathbf{v} / \partial \mathbf{x} \cdot \mathbf{L} = \mathbf{0}$$

Connectivity relationships

When using normalised elasticities, connectivity relationships must be expressed with respect to **independent variables** x_i^0 :

$$\varepsilon_i^k = \frac{x_i^0}{v_k} \frac{\partial v_k}{\partial x_i^0}$$

$$\sum_k C_k^{X_j} \varepsilon_i^k = -\delta_{ij}$$

$$\sum_k C_k^j \varepsilon_i^k = 0$$

where δ_{ij} is Kronecker's symbol.

Connectivity relationships

$$\sum_k C_k^{X_j} \varepsilon_i^k = -\delta_{ij}$$

$$\sum_k C_k^j \varepsilon_i^k = 0$$

These relationships can be interpreted in terms of the **internal system's response** to perturbations of x_i^0

They are necessary for the **system's stability**:

The system counteracts fluctuations of x_i^0

The rest of the system is insensitive to these fluctuations at 1st order approximation

Summary

- The system's response depends on both enzyme properties and network structure
- **Fluxes are constrained** to a low-dimension subspace because of metabolite pool balancing at steady-state
- Control of flux is generally **distributed** across the system (no 'bottleneck')
 - This is important for biotechnology and pharmacology!
- The system's behaviour can be thought of under a general **action-reaction** principle:
 - It usually buffers changes imposed externally
 - It counteracts internal fluctuations

Further reading

- Part 1 to 3.2 of Sauro (2004) *Network dynamics in Computational Systems Biology, Methods in Molecular Biology* vol. 541, pp. 269-290, Humana Press
- *Understanding the Control of Metabolism*, by David Fell Portland Press, London, 1997

For the practical course

- Familiarize yourself with the COPASI modeling environment
<http://www.copasi.org>
 - COPASI handbook
- Be prepared to use your favourite mathematical package such as Scilab, Maple, R or Matlab