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?

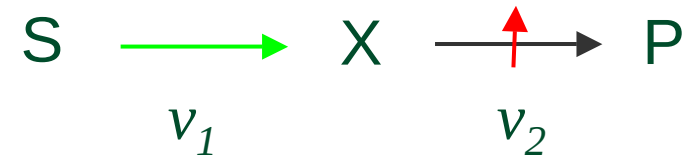
Steady-states and system definition

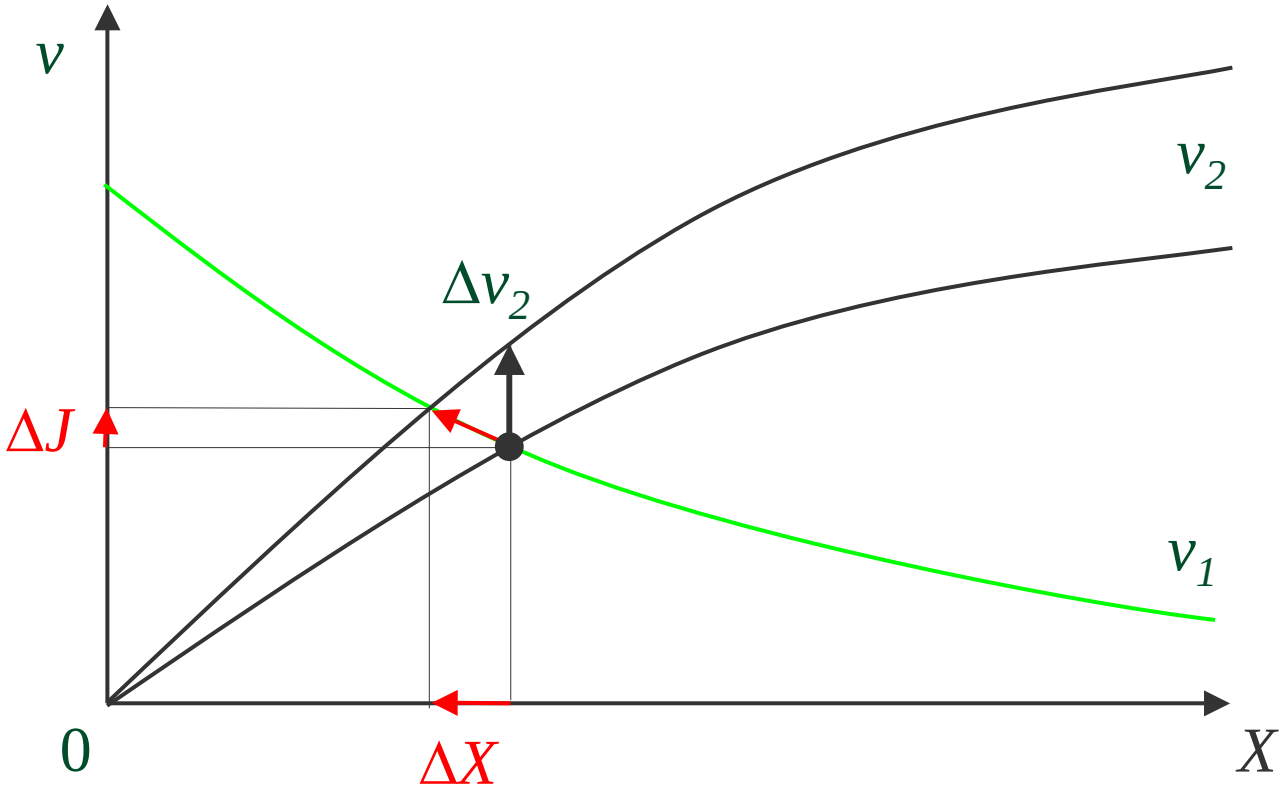
Metabolism concerns almost exclusively sustainable processing of chemical inputs into outputs such as biomass, energy, waste, etc.: it must reach a stable steady-state.

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 assume here for simplicity
- Otherwise it should be reduced to a matrix $\mathbf{N}^0$ with maximal rank in order to deal with independent variables:

$$\mathbf{N} = \mathbf{L} \,.\, \mathbf{N}^0$$

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 $\delta\mathbf{p}$?

$$\frac{d\mathrm{x}}{dt} \sim \mathfrak{J}.\left(\mathrm{x}(t) - \mathrm{X}_2\right)$$

where X_2 is the new steady-state.

$$\begin{cases} \dfrac{d\mathrm{x}}{dt} = \mathrm{N}.\mathrm{v}(\mathrm{x}, \mathrm{p} + \delta\mathrm{p}) \\ \dfrac{d\mathrm{x}}{dt}(0) = \mathrm{N}.\dfrac{\partial \mathrm{v}}{\partial \mathrm{p}}.\delta\mathrm{p} = \mathrm{N}.\delta\mathrm{v} \\ \mathrm{x}(0) = \mathrm{X}_1 \end{cases}$$

Shifting between steady-states

which integrates into:

$$\mathrm{x}(t) = \mathrm{X}_1 - \left(\mathrm{I} - \exp \Im t\right) \Im^{-1}.\mathrm{N}.\delta\mathrm{v}$$

$\Im$ being definitive negative for the steady-state to be stable:

$$\delta\mathrm{X} \to \mathrm{X}_2 - \mathrm{X}_1 = -\Im^{-1}.\mathrm{N}.\delta\mathrm{v}$$

$$\delta\mathrm{J} = \frac{\partial \mathrm{v}}{\partial \mathrm{x}}\delta\mathrm{X} + \frac{\partial \mathrm{v}}{\partial \mathrm{p}}\delta\mathrm{p} = (\mathrm{I} - \frac{\partial \mathrm{v}}{\partial \mathrm{x}}\Im^{-1}.\mathrm{N}).\delta\mathrm{v}$$

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:

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

  where $\mathbf{X}$ 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 $\mathrm{Ker}(\mathbf{N})$ (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 $\Gamma = - \mathfrak{J}^{-1} \cdot \mathbf{N}$ contains all concentration control coefficients

Flux control

- Let us calculate the resulting steady-state flux:

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

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

$$\partial\mathbf{J}/\partial\mathbf{p} = \partial\mathbf{v}/\partial\mathbf{x} \cdot \partial\mathbf{X}/\partial\mathbf{p} + \partial\mathbf{v}/\partial\mathbf{p}$$
$$= (\partial\mathbf{v}/\partial\mathbf{x} \cdot \Gamma + \mathbf{I}\,) \cdot \partial\mathbf{v}/\partial\mathbf{p}$$

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

Generalisation

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

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

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

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

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

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

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 $\mathbf{E}$:

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

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

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

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

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

Summation relationships

Summation theorems follow directly by derivation with respect to α:

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

Flux control is distributed across the system

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

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:

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

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

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

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

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

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

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