Introduction to Metabolic Control Theory

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Outline

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?

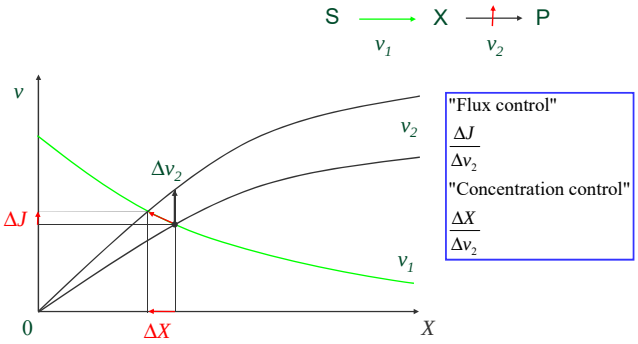
Steady-states and system definition

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?



Quantitatively

Let E_2 be the activity of enzyme 2.

At steady-state:

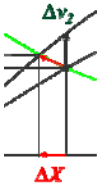
$$v_1(X, E_1) = v_2(X, E_2)$$

$$\frac{\partial v_1}{\partial x}\Delta X \simeq \frac{\partial v_2}{\partial x}\Delta X + \frac{\partial v_2}{\partial E_2}\Delta E_2$$

and at the limit

$$\frac{\partial X}{\partial E_2} = \frac{-1}{\frac{\partial v_2}{\partial x} - \frac{\partial v_1}{\partial x}}$$

if v_2 and E_2 are expressed in the same units



Concentration control

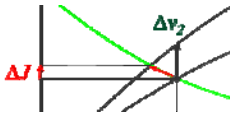
Quantitatively

$$J = v_2(X, E_2)$$

$$\Delta J \simeq \frac{\partial v_2}{\partial x}\Delta X + \frac{\partial v_2}{\partial E_2}\Delta E_2$$

and at the limit

$$\frac{\partial J}{\partial E_2} = \frac{-\frac{\partial v_1}{\partial x}}{\frac{\partial v_2}{\partial x} - \frac{\partial v_1}{\partial x}}$$



Flux control

Quantitatively

If now we modulate E_1 we get similarly:

$$\frac{\partial X}{\partial E_1} = \frac{1}{\frac{\partial v_2}{\partial x} - \frac{\partial v_1}{\partial x}} \qquad \frac{\partial J}{\partial E_1} = \frac{\frac{\partial v_2}{\partial x}}{\frac{\partial v_2}{\partial x} - \frac{\partial v_1}{\partial x}}$$

- Flux control by supply reaction 1 is proportional to sensitivity of demand reaction 2 to intermediate metabolite

Quantitatively

and we obtain the following remarkable *summation relationships*:

$$\frac{\partial X}{\partial E_1} + \frac{\partial X}{\partial E_2} = 0$$

$$\frac{\partial J}{\partial E_1} + \frac{\partial J}{\partial E_2} = 1$$

- Concentration control by supply and demand of opposite signs
- Flux control by supply and demand add up to 1

More generally

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

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

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, including enzyme activities.
The Jacobian is :

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

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

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 $\mathrm{Ker}(\mathbf{N})$ (often termed $\mathbf{K}$)

Expressing systemic control

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

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

$$\boxed{\partial\mathbf{X}/\partial\mathbf{p} = -(\mathbf{N} \cdot \partial\mathbf{v}/\partial\mathbf{x})^{-1} \cdot \mathbf{N} \cdot \partial\mathbf{v}/\partial\mathbf{p}}$$

- This equation relates systemic changes in steady-state concentrations $\mathbf{X}$ to changes in rates $\mathbf{v}$
- The matrix $\boldsymbol{\Gamma} = -(\mathbf{N} \cdot \partial\mathbf{v}/\partial\mathbf{x})^{-1} \cdot \mathbf{N}$ contains all concentration control coefficients

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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 $\mathbf{p}$:

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

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

Generalisation?

If the system shows conservation relationships such as $[ATP]+[ADP]+[AMP] = \text{constant}$

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 $\mathbf{x}^0$:

$$\mathbf{N} = \mathbf{L} \cdot \mathbf{N}^0$$
$$d\mathbf{x}^0/dt = \mathbf{N}^0 \cdot \mathbf{v}(\mathbf{x},\mathbf{p})$$
$$\boldsymbol{\mathfrak{J}} = \mathbf{N}^0 \cdot \partial\mathbf{v}/\partial\mathbf{x} \cdot \mathbf{L}$$
$$\boldsymbol{\Gamma} = -\mathbf{L} \cdot \boldsymbol{\mathfrak{J}}^{-1} \cdot \mathbf{N}^0$$
$$\boldsymbol{\Phi} = \mathbf{I} + \partial\mathbf{v}/\partial\mathbf{x} \cdot \boldsymbol{\Gamma}$$

Normalised control coefficients

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

Flux control $$C_i^j := \frac{E_i}{J_j}\frac{\partial J_j}{\partial E_i} = \frac{\partial \ln J_j}{\partial \ln E_i}$$

Concentration control $$C_i^{X_j} := \frac{E_i}{X_j}\frac{\partial X_j}{\partial E_i} = \frac{\partial \ln X_j}{\partial \ln E_i}$$

where the E_i parameters denote enzyme activities, usually expressed in the same units as J_i $(M.s^{-1})$.

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

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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:

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

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

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

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

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

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

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: $\delta_{ij} = \begin{cases} 1 \text{ if } i = j \\ 0 \text{ if } i \neq j \end{cases}$

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