Metabolic Control Analysis (MCA)

- The restriction imposed by MCA is that we only study effects of small perturbations: what will happen if we 'nudge' the metabolic system slightly of its current steady state
- Mathematically, we employ a linearized system around the steady state, thus ignoring the non-linearity of the kinetics.
- The predictions are local in nature; in general different for each steady state

Questions of interest

- How does the change of enzyme activity affect the fluxes?
- Which individual reaction steps control the flux or concentrations?
- Is there a bottle-neck or rate-limiting step in the metabolism?
- Which effector molecules (e.g. inhibitors) have the greatest effect?
- Which enzyme activities should be down-regulated to control some metabolic disorder? How to distrub the overall metabolism the least?

Coefficients of control analysis

The central concept in MCA is the *control coefficient* between two quantities (fluxes, concentations, activities, ...) y and x:

$$c_x^y = \left(\frac{x}{y} \frac{\Delta y}{\Delta x} \right)_{\Delta x \to 0}$$

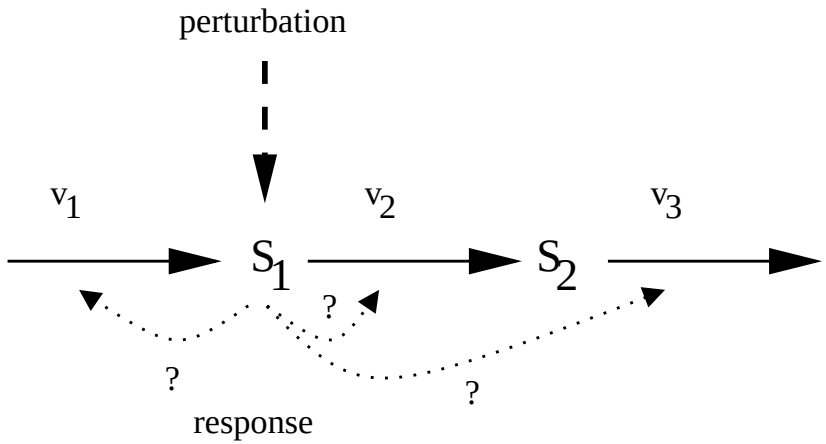
- Intuitively, c_x^y is the relative change of y in response of infinitely small change to x

ϵ-elasticity coefficient

- ϵ-elasticity coefficient

$$\epsilon_i^k = \frac{S_i}{v_k} \frac{\partial v_k}{\partial S_i}$$

quantifies the change of a reaction rate v_k in response to a change in the concentration S_i, while everything else is kept fixed.

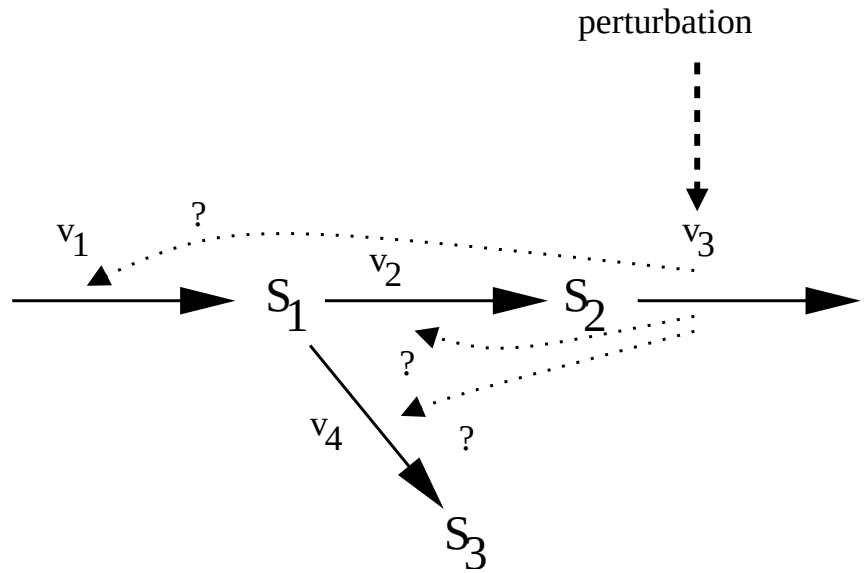


Flux control coefficients

The flux-control coefficient (FCC)

$$FCC_k^j = \frac{v_k}{J_j}\frac{\partial J_j}{\partial v_k}$$

is defined as the change of flux J_j of a given pathway, in response to a change in the reaction rate v_k.

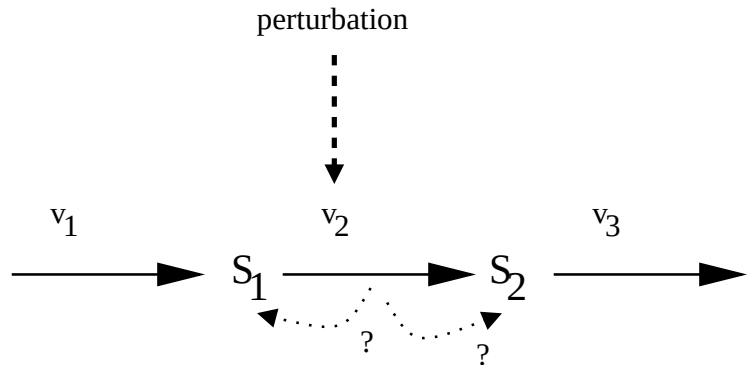


Concentration control coefficients

The concentration-control coefficient (CCC)

$$CCC_k^i = \frac{v_k}{S_i}\frac{\partial S_i}{\partial v_k}$$

is defined as the change of concentration S_i, in response to a change in the reaction rate v_k.



Theorems of MCA

- Unlike the elasticity coefficients, the control coefficients cannot be directly computed from the kinetic parameters of the reactions, even in principle.
- In order to determine the coefficients we need both some MCA theory and experimental data
- MCA theory consists of two sets of theorems:
  - Summation theorems make statements about the total control of a flux or a steady-state concentration
  - Connectivity theorems relate the control coefficients to the elasticity coefficients

Summation theorems

The first summation theorem says that for each flux J_j the flux-control coefficients must sum to unity

$$\sum_{k=1}^{r} FCC_k^j = 1$$

Thus, control of a flux is shared across all enzymatic reactions
For concentration control coefficients we have

$$\sum_{k=1}^{r} CCC_k^i = 0$$

Control of a concentration is shared across all enzymatic reactions, some exerting positive control, other exerting negative control.

Flux control connectivity theorems

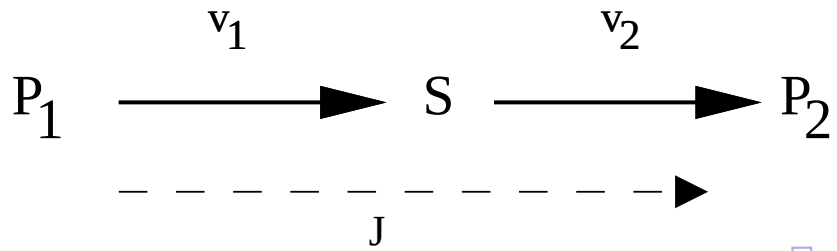
- Connectivity theorems tie elasticity coefficients $\epsilon_{S_i}^{v_k}$ and control coefficients $FCC_{v_k}^{J_j}, CCC_{v_k}^{S_i}$ together.
- Flux control connectivity is given by

$$\sum_{k=1}^{r} FCC_{v_k}^{J_j} \epsilon_{S_i}^{v_k} = 0$$

- In our example we have $FCC_1^J \epsilon_S^1 + FCC_2^J \epsilon_S^2 = 0$ giving

$$\frac{FCC_1^J}{FCC_2^J} = -\frac{\epsilon_S^2}{\epsilon_S^1}$$

which shows that, everything else remaining constant, an increase in ϵ_S^2 needs to be countered with a decrease in FCC_2^J



Concentration control connectivity

- Similar connectivity theorems hold for concentrations,
- The concentration control connnectivity theorem ties the elasticity of reaction v_k with respect to concentration S_i to the concentration control of v_k over the concentration S_h
- We have

$$\sum_{k=1}^{r} CCC_{v_k}^{S_h} \epsilon_{S_i}^{v_k} = 0$$

for $h \neq i$, and

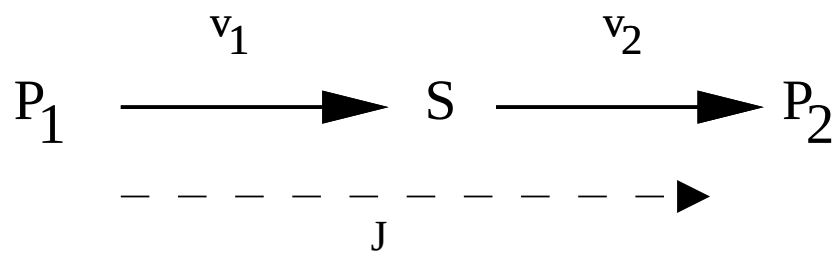
$$\sum_{k=1}^{r} CCC_{v_k}^{S_i} \epsilon_{S_i}^{v_k} = -1$$

Calculating control coefficients

- With the help of the summation and connectivity theorems and elasticities for single reactions one can determine values for the control coefficients.
- For the two step pathway below, we apply the summation theorem $FCC_1^J + FCC_2^J = 1$ and the connectivity theorem $FCC_1^J \epsilon_S^1 + FCC_2^2 \epsilon_S^2 = 0$
- We obtain

$$FCC_1^J = \frac{\epsilon_S^2}{\epsilon_S^2 - \epsilon_S^1}, FCC_2^J = \frac{-\epsilon_S^1}{\epsilon_S^2 - \epsilon_S^1}$$

where the elasticity coefficients, computed from reaction kinetics can be substituted.

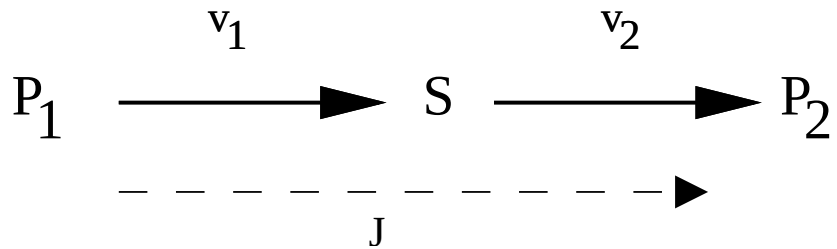


Calculating control coefficients

- Since typically we have $\epsilon_S^1 < 0$ and $\epsilon_S^2 > 0$ from

$$FCC_1^J = \frac{\epsilon_S^2}{\epsilon_S^2 - \epsilon_S^1}, FCC_2^J = \frac{-\epsilon_S^1}{\epsilon_S^2 - \epsilon_S^1}$$

we see that both reactions exert positive control over the flux of the pathway



Calculating control coefficients

- The concentration control coefficients fulfill

$$CCC^S_{v_1} + CCC^S_{v_2} = 0, CCC^S_{v_1}\epsilon^{v_1}_S + CCC^S_{v_2}\epsilon^{v_2}_S = -1$$

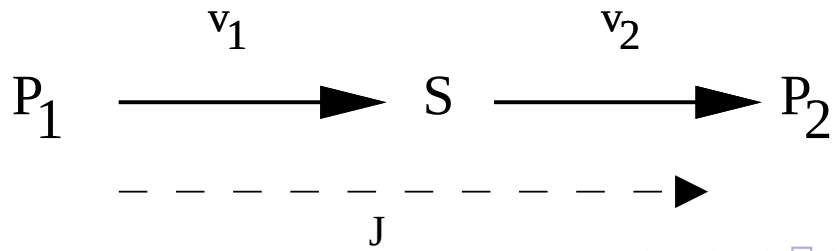
which yields

$$CCC^S_1 = \frac{1}{\epsilon^{v_2}_S - \epsilon^{v_1}_S}$$

and

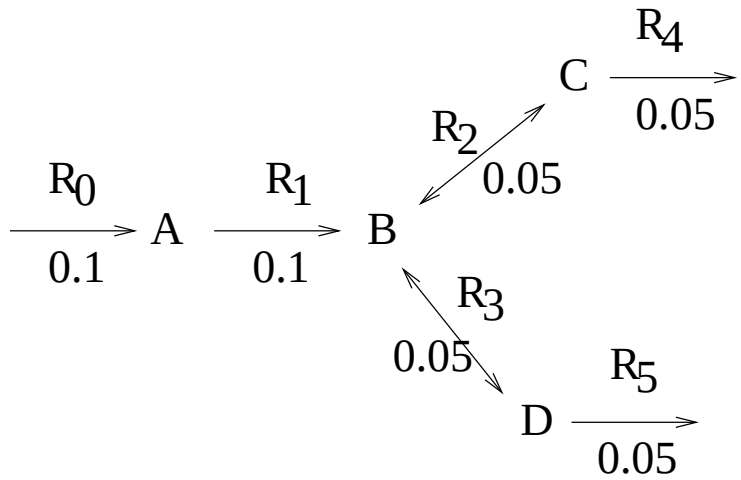
$$CCC^S_2 = \frac{-1}{\epsilon^{v_2}_S - \epsilon^{v_1}_S}$$

- With $\epsilon^1_S < 0$ and $\epsilon^2_S > 0$ we get $CCC^S_{v_1} > 0$ and $CCC^S_{v_2} < 0$, that is the rise of first reaction rate rises the concentration of S while rise of the second reaction rate lowers the concentration of S



MCA example: simple junction

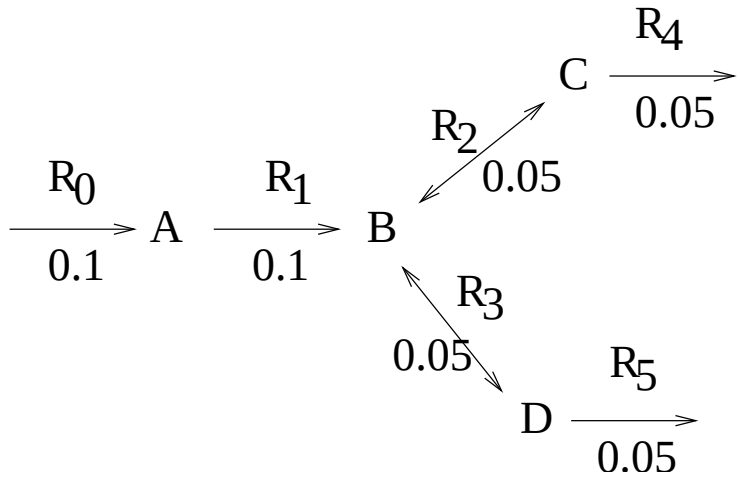
- Reaction $R0$ has constant flux $v_0 = 0.1$
- Reactions $R1, R4$ and $R5$ irreversible with mass action kinetics $v = k_+ S$
- Reactions $R2$ and $R3$ reversible with mass action kinetics $v = k_+ S - k_- P$
- All kinetic constants equal $k_+ = k_- = 0.1$
- Let us perform MCA analysis with given steady state
- Results computed with the COPASI simulator (www.copasi.org)



MCA example: simple junction

- Elasticities $\epsilon_i^k = \frac{S_i}{v_k} \frac{\partial v_k}{\partial S_i}$

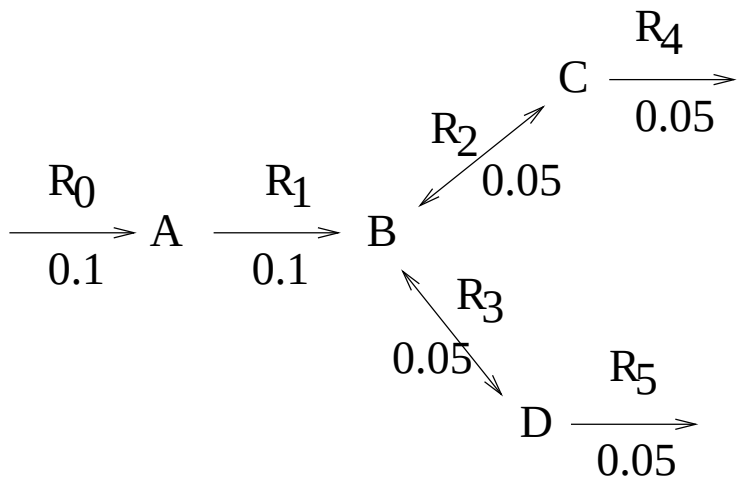
	A	B	C	D
R0	0	0	0	0
R1	1	0	0	0
R2	0	2	-1	0
R3	0	2	0	-1
R4	0	0	1	0
R5	0	0	0	1



MCA example: simple junction

- Flux control coefficients $FCC_J^k = \frac{v_k}{J}\frac{\partial J}{\partial v_k}$

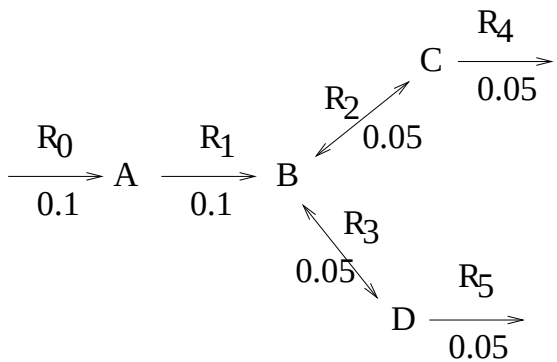
	R0	R1	R2	R3	R4	R5
R0	1	0	0	0	0	0
R1	1	0	0	0	0	0
R2	1	0	0.25	-0.25	0.25	-0.25
R3	1	0	-0.25	0.25	-0.25	0.25
R4	1	0	0.25	-0.25	0.25	-0.25
R5	1	0	-0.25	0.25	-0.25	0.25



MCA example: simple junction

- Concentration control coefficients $CCC_i^k = \frac{v_k}{S_i}\frac{\partial S_i}{\partial v_k}$

	R0	R1	R2	R3	R4	R5
A	1	-1	0	0	0	0
B	1	0	-0.25	-0.25	-0.25	-0.25
C	1	0	0.25	-0.25	-0.75	-0.25
D	1	0	-0.25	0.25	-0.25	-0.75



MCA example: predicting the results of perturbation

- Let us consider optimization of the flux over a linear pathway of four reactions by modulating enzyme concentrations.
- Assume the following kinetics $v_i = E_i(k_i S_{i-1} - k_{-i} S_i)$, initial enzyme concentrations $E_i = 1$ and rate constants $k_i = 2, k_{-i} = 1$ and concentrations of external substrates $S_0 = S_5 = 1$
- The steady state flux $J = 1$ and the flux control coefficients $FCC_1^J = 0.533, FCC_2^J = 0.267, FCC_3^J = 0.133, FCC_4^J = 0.067$ can be solved from the above equations.

MCA example: predicting the results of perturbation

- According to MCA, increasing the concentration of a single enzyme E_i by $p\%$ will increase the flux approximately by $\Delta_i = FCC_i^J(p/100)$, giving
  $\Delta_1 = 0.00533, \Delta_2 = 0.00267, \Delta_3 = 0.00133, \Delta_4 = 0.00067.$
- On the other hand, the underlying 'true' kinetic model would predict
  $\tilde{\Delta}_1 = 0.00531, \tilde{\Delta}_2 = 0.00265, \tilde{\Delta}_3 = 0.00132, \tilde{\Delta}_4 = 0.00066.$
- Thus MCA predicts fairly accurately the results of a small preturbation.

MCA example: predicting the results of perturbation

- Large perturbations would not be equally accurately predicted by MCA.
- Assume we can double the total enzyme concentration $\sum E_i = 4 \mapsto 8$. How should the enzyme be allocated for best results?
- $E_1 \mapsto 5E_1$: MCA predicts $\Delta_1 = 0.533 \cdot 5 = 2.665$, kinetic model gives $\tilde{\Delta}_1 = 0.7441$
- $E_4 \mapsto 5E_4$: MCA predicts $\Delta_4 = 0.067 \cdot 5 = 0.335$, kinetic model 0.0563
- The maximal increase of 1.2871 for the flux is obtained by modifying all the enzyme concentrations: $E_1 = 3.124, E_2 = 2.209, E_3 = 1.562, E_4 = 1.105$

The End

Course Exam

- Wednesday 29.4.2009 9am-12pm, in A111
- Examined contents: lecture slides and exercises
- Exam will consist of five questions, each worth 8 points
- Types of questions: defining concepts, essays as well as technical questions asking for analysis of a given metabolic model