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

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

Types of coefficients

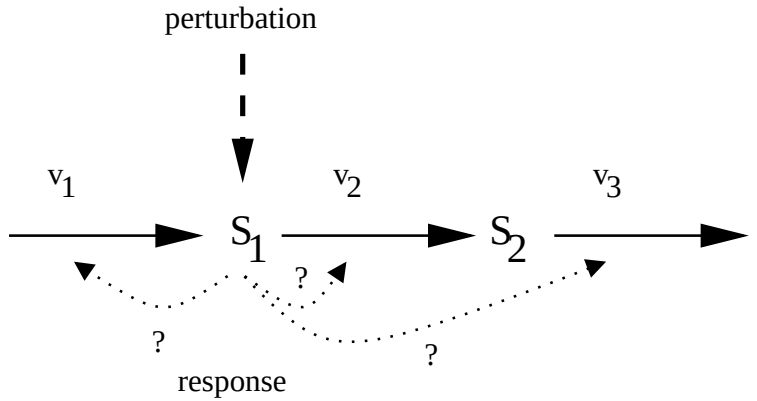
- Elasticity coefficients quantify the sensitivity of a reaction rate to the change of concentration or a parameter.
- Flux control coefficients quantify the change of a flux along a pathways in response to a change in the rate of a reaction
- Concentration control coefficients quantify the change of concentration of some metabolite S_i in response of a change in the rate of a reaction
- Response coefficients quantify the change of a flux in response to a change change in a parameter (e.g. kinetic parameters of an enzyme)

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.

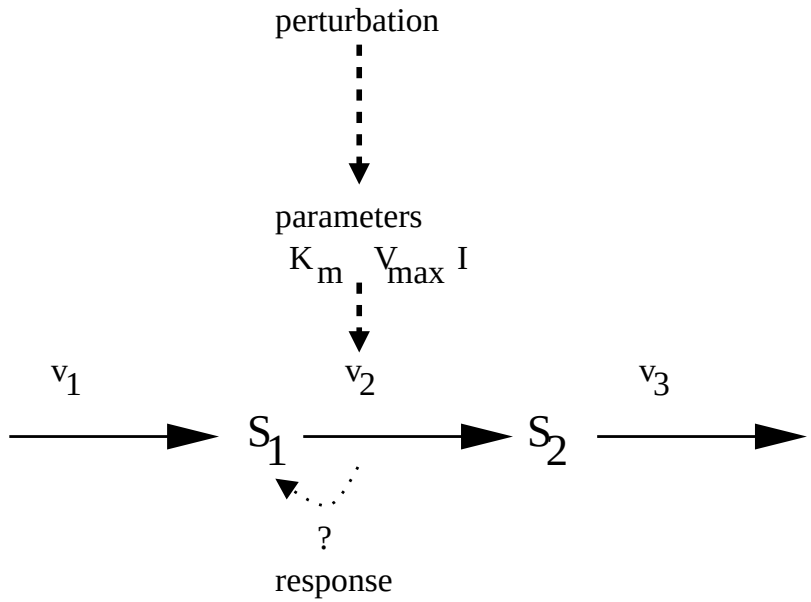


π-elasticity coefficient

π-elasticity coefficient

$$\pi_m^k = \frac{p_m}{v_k} \frac{\partial v_k}{\partial p_m}$$

is defined as the change of a reaction rate v_k in response to a change in a parameter (kinetic constant, enzyme concentration, inhibitors)

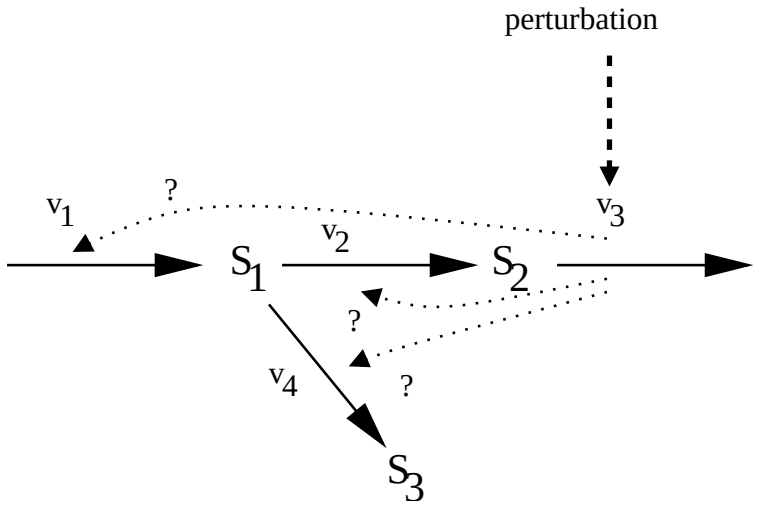


Flux control coefficients

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.

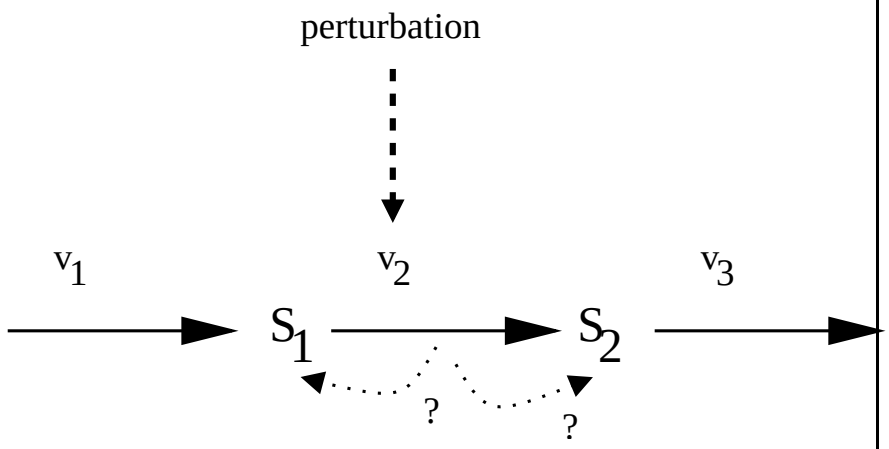


Concentration control coefficients

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.



Response coefficients

The steady state $\mathbf{S}(\mathbf{p}), \mathbf{J} = \mathbf{v}(\mathbf{S}(\mathbf{p}), \mathbf{p})$ is determined by the parameters $\mathbf{p}$ (kinetic parameters of enzymes, external metabolite concentrations, temperature, pH,...)

Response coefficients quantify the direct effect of the parameters $\mathbf{p}$ to the steady state (rather than via individual enzymatic reactions)

Given a perturbation to a parameter p_m, the response coefficient of a flux J_j is

$$R_m^j = \frac{p_m}{J_j} \frac{\partial J_j}{\partial p_m}$$

and the response coefficient of a concentration S_i is is

$$R_m^i = \frac{p_m}{S_i} \frac{\partial S_i}{\partial p_m}$$

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 theorem tie elasticity coefficients $\epsilon_{S_i}^{v_k}$ and control coefficients $FCC_{v_k}^{J_j}, CCC_{v_k}^{S_i}$ together.

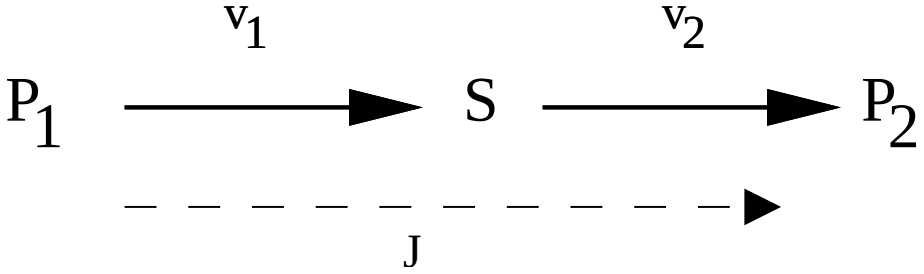
For flux control we have

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

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 FCC_2^J needs to be countered with a decrease in ϵ_S^2



Concentration control connectivity

Similar connectivity theorems hold for concentrations.

We have

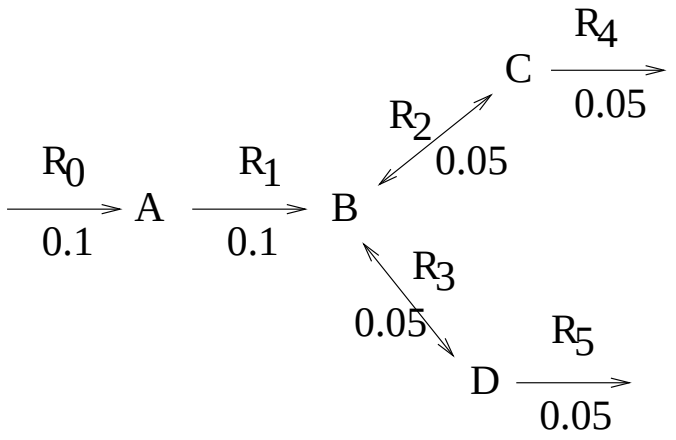
$$\sum_{k=1}^{r} CCC_{v_k}^{S_h} \epsilon_{S_i}^{v_k} = 0$$

for $h \neq i$. and

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

MCA example: simple junction

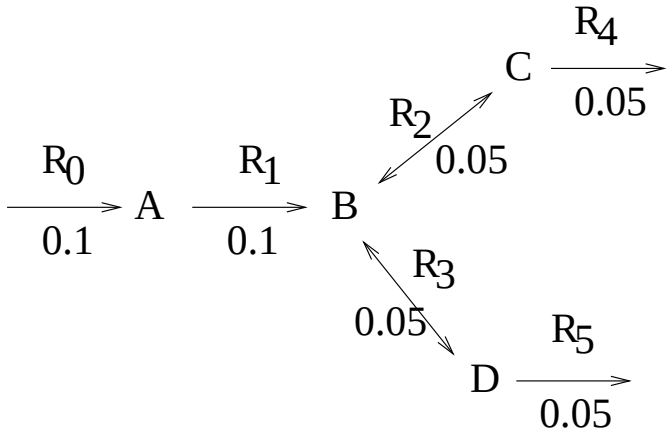
- Reaction $R0$ has constant flux $v_0 = 0.1$
- Reactions $R1, R4$ and $R5$ irreversible with mass action kinetics $v = k_+ S$
- Reactions $R2$ and $R3$ reversible with mass action kinetics $v = k_+ S - k_- P$
- All kinetic constants equal $k_+ = k_- = 0.1$
- Let us perform MCA analysis with given steady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



MCA example: simple junction

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

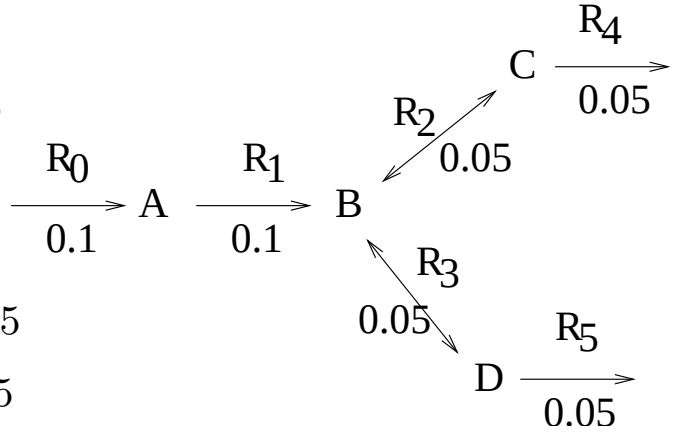
	A	B	C	D
R0	0	0	0	0
R1	1	0	0	0
R2	0	2	-1	0
R3	0	2	0	-1
R4	0	0	1	0
R5	0	0	0	1



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

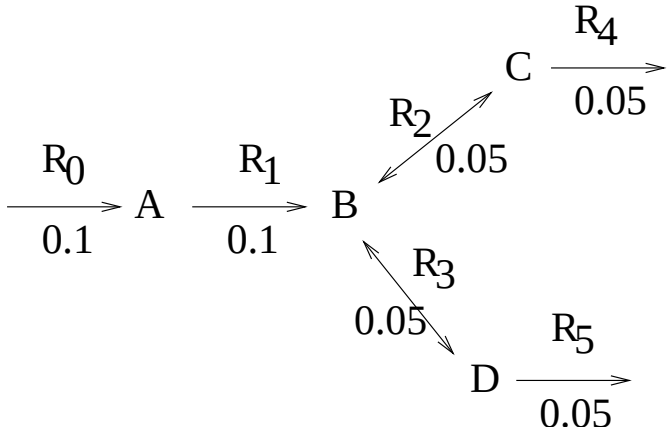


MCA example: simple junction

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: control of an unbranched pathway

Let us consider an unbranched pathway

$$S_0 \leftrightarrow S_1 \leftrightarrow S_2 \ldots S_{r-1} \leftrightarrow S_r$$

Assume that each reaction conforms to linear kinetics:

$$v_i = k_i S_{i-1} - k_{-i} S_i, i = 1, \ldots, r$$

The reactions are in equilibrium (forward and backward flow equal) when $k_i S_{i-1} = k_{-i} S_i$ so the equilibrium constant is given by

$$K_{eq} = q_i = \frac{k_i}{k_{-i}} = \frac{S_i}{S_{i-1}}$$

MCA example: control of an unbranched pathway

The steady-state flux of the pathway can be expressed in analytical form as (proof by induction w.r.t r)

$$J = \frac{S_0 \prod_{j=1}^{r} q_j - S_r}{\sum_{l=1}^{r} 1/k_l (\prod_{m=l}^{r} q_m)}$$

and the flux control coefficients as

$$FCC_i^J = \frac{\frac{1}{k_i} \prod_{j=i}^{r} q_j}{\sum_{l=1}^{r} 1/k_l (\prod_{m=l}^{r} q_m)}$$

MCA example: control of an unbranched pathway

We consider the special case where the individual enzymes adhere to the same kinetics, i.e. $k_+ = k_i, k_- = k_{-i}$ and $q = k_+/k_- > 1$ (i.e. in equilibrium, concentration of the product is higher than the substrate which means the reactions have a tendency to happen in forward direction)

In this case the ratio of two successive flux control coefficients satisfy

$$\frac{FCC_i^J}{FCC_{i+1}^J} = \frac{(1/k_i)\prod_{j=i}^{r} q_j}{(1/k_{i+1})\prod_{j=i+1}^{r} q_j} = \frac{k_{i+1}}{k_i} q_i = q > 1$$

Thus, the reactions towards the beginning of the pathway have bigger control coefficients than the reactions towards the end.

MCA example: relaxation times

The relaxation time of an enzyme is a measure of the time it takes from the enzyme to respond to the concentration changes of the substrates and products. It is defined as

$$\tau_i = \frac{1}{k_i + k_{-i}}$$

Consider now the unbranched pathway of the previous example. We assume that the individual kinetics of the enzymes may be different, i.e. $k_i \neq k_j$ is possible, but the equilibrium constants are equal to $q = q_i = 1$ or $k_i = k_{-i}$.

This means that the equilibrium concentrations for substrates and products are all equal.

MCA example: relaxation times

As $k_i = k_{-i}$ the relaxation time simplifies to $\tau_i = 1/(k_i + k_{-i}) = 1/(2k_i)$

The flux control coefficients

$$FCC_i^J = \frac{\frac{1}{k_i}\prod_{j=i}^{r} q_j}{\sum_{l=1}^{r} 1/k_l(\prod_{m=l}^{r} q_m)}$$

simplify to the form

$$FCC_i^J = \frac{1/k_i}{\sum_{l=1}^{r} \frac{1}{k_l}} = \frac{\tau_i}{\tau_1 + \cdots + \tau_r}$$

- The control is distributed among the enzymes of the pathway, no enzyme controls the flux alone
- The higher the relaxation time of the enzyme, the more control it has over the fluxes.

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 preturbations 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: $E_1 = 3.124, E_2 = 2.209, E_3 = 1.562, E_4 = 1.105$

Determining Flux Control Coefficients

There are several ways by which the FCCs can be determined, they can broadly be classified into direct and indirect methods:

- In indirect methods, one first determines the elasticity coefficients and uses the MCA theorems to obtain the FCCs from there
- In direct methods, the FCCs are determined from flux and enzyme activity measurements following but finite activity changes

Determining elasticity coefficients

For determining elasticity coefficients several techniques exist,

- Computation from an available kinetic model for the enzymes. The limitation is that in practise we may not know the enzyme kinetics, e.g. what inhibitors and activators are relevant. (This approach was already looked at in the previous lecture)
- Double modulation experiments, where one measures the activity of two metabolites and the flux through the reaction step in three conditions (initial and two perturbed conditions), and approximates the elasticity coefficients via linear interpolation.

Double modulation experiment

Consider a single reaction $S \rightarrow P$ and let J denote the flux trough it.

The total derivative of the flux J satisfies

$$dJ = \frac{\partial v}{\partial S} dS + \frac{\partial v}{\partial P} dP$$

Scale this by the steady state flux $v = J$ to obtain

$$\frac{1}{J} dJ = \frac{\partial v}{\partial S} \frac{1}{v} dS + \frac{\partial v}{\partial P} \frac{1}{v} dP$$

Substituting the equation for elasticity coefficients $\epsilon_S^v = \frac{\partial v}{\partial S} \frac{S}{v}$ and the derivative $d \ln S = \frac{1}{S} dS$ we finally get an expression

$$d \ln J = \epsilon_S^v d \ln S + \epsilon_P^v d \ln P$$

Double modulation experiment

Assume measurements J^0, J^1, J^2 of the flux J and the concentrations S^0, S^1, S^2 of substrate S and P^0, P^1, P^2 of the product P, in three conditions (initial = 0, perturbed = 1,2).

We make the linear approximations $\Delta_i \ln J = \ln J^i - \ln J^0$, $\Delta_i \ln S = \ln S^i - \ln S^0$ and $\Delta_i \ln P = \ln P^i - \ln P^0$

And substitute them to the above derived equation

$$\Delta_1 \ln J = \epsilon_S^v \Delta_1 \ln S + \epsilon_P^v \Delta_1 \ln P$$

$$\Delta_2 \ln J = \epsilon_S^v \Delta_2 \ln S + \epsilon_P^v \Delta_2 \ln P$$

From there the elasticity coefficients can be solved, if the equations are linearly independent.

Limitations of double modulation

The double modulation experiment suffers from some problems:

- The difficulty of obtaining perturbations giving equations that are truly linearly independent: if the perturbations cause similar responses, we have

$$\frac{\Delta_1 \ln J}{\Delta_1 \ln S} \approx \frac{\Delta_2 \ln J}{\Delta_2 \ln S}$$

  and the linear system is ill-conditioned and thus prone to experimental errors

- The reactions often are dependent on more than two parameters, so instead of double modulation one needs to do an experimental plan k-fold modulation, which may be costly.

Direct methods for FCC determination

There are also direct experimental methods for FCC determination:

- Genetic alteration of expressed enzyme activity. This has the benefit that the effect can be studied in vivo (enzyme in the context of the living cell) rather than in vitro (enzyme isolated in a test tube). The limitation is that the perturbations generated by the approach will in general not be small as required by the MCA theory. Also the genetic engineering work is substantial.
- Adding purified enzyme to a cell-free extract. This is an approach that is prone to experimental errors. This is a in vitro method, so the coefficients will also not be the same as in a living cell.
- Adding the cell culture with specific inhibitors (in vivo). One measures the change in flux as a function of the concentration of the inhibitor. Requires knowing the enzymes response to the inhibitor (e.g. elasticity). Also, the inhibitor needs to be truly specific so that it does not interact with anything else but the enzyme in question.

Generalizations and variants of MCA

MCA has been extended and generalized many ways

- Large perturbations: by assuming simple linearized kinetics,

$$v = e(S_i - S_i/K)$$

  it possible to consider large pertubations rather than the infinitesimally small as required by standard MCA. If the simple kinetics is not very far from the truth, the predictions of this variant under large preturbations will typically be better than standard MCA

- Control analysis of other variables than fluxes and concentrations. Such variables include the transition time, free energy differences, growth rate, ...

Generalizations and variants of MCA

- Time-dependent Control Coefficients. It is possible to perform MCA to other than steady state systems. There one needs to define control operators $FCC(t)$, $CCC(t)$ rather than single coefficients.
- Spatial heterogeneity instead of the standard 'well-mized bag-of-enzymes' model.
- Hierarchical control analysis considers the change of enzyme activity due to translation, proteolysis, binding to other proteins, and covalent modification. It is also possible to consider mRNA concentrations explicitly, which are also variables due to transcription and degradation.

The End

- Tue 24.4. Recap lecture
- Fri 27.4. Exercise session
- Wed 2.5. Course exam, 9.00am-12.00pm, room B123