Lecture: Computational Systems Biology
Universität des Saarlandes, SS 2012

07 Sensitivities, Metabolic Control Analysis

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Recap

- Studying the **effect of parameters on the system's behaviour**:
  - Manual changes and recalculation of quantities of interest
  - Sliders (in COPASI)
  - Parameter scanning (systematic sweep of parameter space)
  - Parameter sampling (randomly exploring parameter space)
- **Discrete events** (discrete changes in systems, described by triple [trigger condition, target, expression])
- **Rules** (describe the behaviour of additional variables in the system with algebraic expressions or ODEs)

Sensitivity analysis

Possible questions:

- How do changes of an enzyme affect the resulting steady state of the system?
- Which effector has the strongest impact on the reaction rate?
- etc.

Not easy to answer for big and highly regulated systems

Sensitivity analysis

Possible applications:

- **Biotechnology**: which enzymes should be activated to increase the rate of synthesis of a desired metabolite?
- **Medicine**: which reactions should be modified to down-regulate a metabolite which is overproduced in a metabolic disorder (without perturbing the rest of the network too much)?

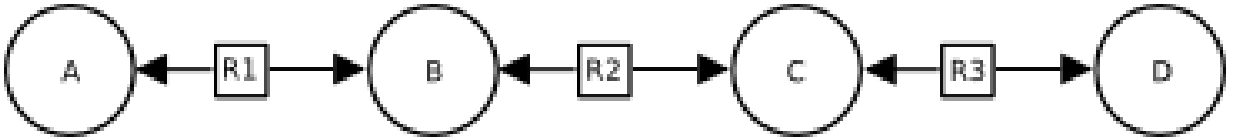
"Rate limiting step"?

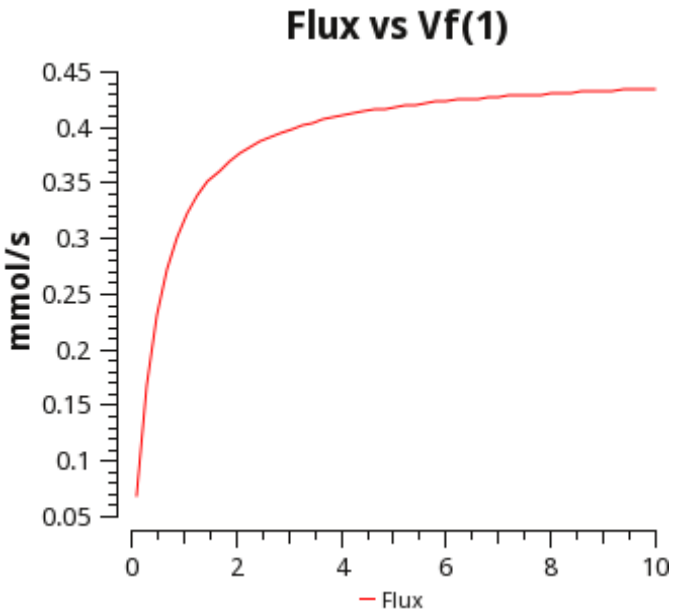
Blackman's (1905) rate-limiting principle:

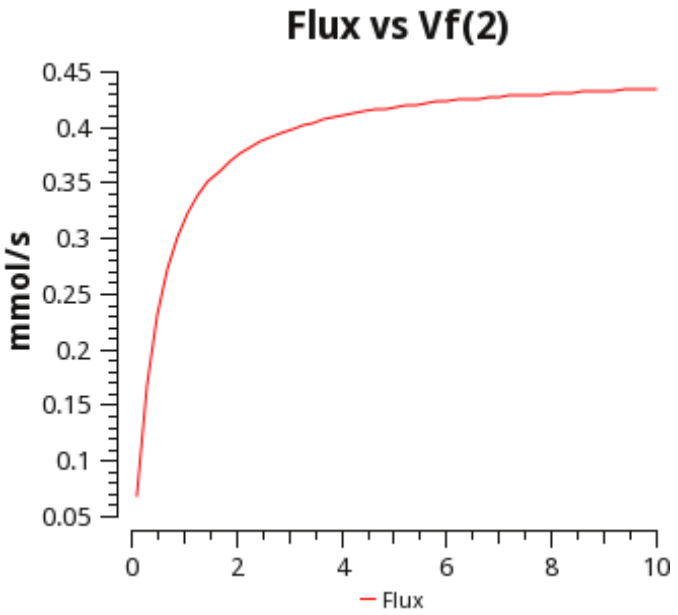
When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the 'slowest' factor

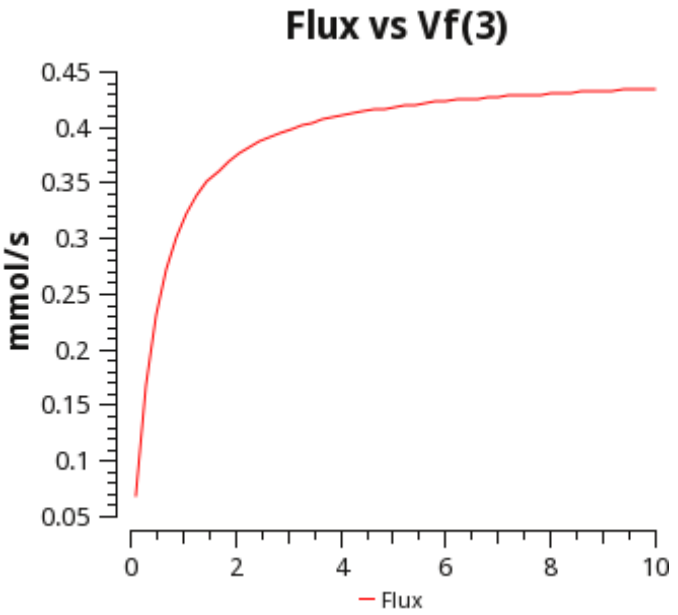
Principle is too simplistic and does not hold in many situations. E.g., obviously, in a linear chain of reactions all rates/fluxes are by definition the same in steady state. There cannot be a "slowest" one.

How to develop this fuzzy notion of rate limitation into a quantitative concept?









- No reaction is THE rate-limiting step
- All reactions can become rate-limiting if their enzyme concentration is low
- Rate-limitation has to be quantified since it is a gradual phenomenon

Quantifying control

In steady state there is no "limitation". However, what if we change one of the rates (by a very small amount). By how much does the overall steady state flux change due to this?

Distinguish between local effect and global effects!

- **local**: look at reaction in isolation. How do parameters change the flux? → derivatives of kinetic function
- **global**: local changes also affect other reactions in the network. When the system settles down to a new steady state the final change in the rate of a reaction will not be the same as if it was isolated

Remark: a "rate-limiting reaction" would be one where changes in its local rate become the same in the global rate. That would mean that all other reactions have no control at all

Metabolic control analysis (MCA)

- MCA is a special type of sensitivity analysis
- It quantifies how infinitesimal changes in a parameter influence a variable through a control coefficient
- It provides theoretical explanations for fundamental phenomena (summation theorems → shifting of control)
- MCA is local, i.e. dependent on the current state

Some facts

- Independently developed in the 70s by
  - Kacser & Burns 1973 (Edinburgh)
  - Heinrich & Rapaport 1974 (Berlin)
- Further important development by
  - Reder (1988) Metabolic control theory: a structural approach. *J. Theor. Biol.* **135**:175-201
  - and others...
- Originally intended for metabolic networks (but by now there are extensions for signalling, gene expression and other networks)

General sensitivity coefficients

Let *y(x)* be a quantity that depends, directly or indirectly, on another quantity *x*. The effect of a change *Δx* on *y* can be expressed in terms of a sensitivity coefficient

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

normalisation factor

"What percentage change in *y* results from a 1%-change *Δx* in *x*?"

Normalisation (factor *x/y*)

- **PRO** We get rid of units and can compare fluxes belonging to different branches in a network
- **CONTRA** Undefined if quantity itself is zero. If that's the case, use nonnormalised coefficients

Local ↔ global properties

Elasticities - how does an effector influence a reaction rate in isolation (local property)

these can be calculated in any state

Flux/Concentration control coefficients - how does a change in, say, an enzyme concentration change the system behaviour, i.e. a steady state (global property)

these refer to a given steady state of the system and, after a perturbation, the relaxation to a new steady state is considered

Elasticities

Elasticity coefficients quantify the sensitivity of a reaction rate to the change of a concentration or parameter in isolation (while all other arguments of the kinetic function are fixed)

ε-elasticity: sensitivity of reaction rate v_k to the change of concentration of S_i

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

π-elasticity: sensitivity of reaction rate v_k to the change of parameter p_m

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

Example: Michaelis-Menten kinetics

$$v = \frac{V_{max} S}{K_m + S}$$

$$\epsilon_S^v = \frac{S}{v} \frac{\partial}{\partial S} \left(\frac{V_{max} S}{K_m + S} \right) = \frac{S}{v} \frac{V_{max}(K_m + S) - V_{max} S}{(K_m + S)^2} = \frac{K_m}{K_m + S}$$

Remarks:

- Normalised ε-elasticity in mass action kinetics is always 1
- Elasticity is zero whenever the rate does not depend directly on a metabolite concentrations or parameter

Flux control coefficients

Refer to a stable steady state with concentrations $\boldsymbol{S}^{st} = \boldsymbol{S}^{st}(\boldsymbol{p})$ and fluxes $\boldsymbol{J} = \boldsymbol{v}(\boldsymbol{S}^{st}(\boldsymbol{p}), \boldsymbol{p})$. A small change of the rate of a reaction (by a parameter change) drives the system to a new steady state closeby

Flux control coefficient for the control of rate v_k over the flux J_j can be measured by

$$C_k^j = \frac{\partial J_j / J_j}{\partial v_k / v_k} = \frac{v_k}{J_j}\frac{\partial J_j}{\partial v_k} = \frac{\partial \log(J_j)}{\partial \log(v_k)}$$

Concentration control coefficients

Refer to a stable steady state with concentrations $\boldsymbol{S}^{st} = \boldsymbol{S}^{st}(\boldsymbol{p})$ and fluxes $\boldsymbol{J} = \boldsymbol{v}(\boldsymbol{S}^{st}(\boldsymbol{p}), \boldsymbol{p})$. A small change of the rate of a reaction (by a parameter change) drives the system to a new steady state closeby.

Concentration control coefficient of concentration S_i^{st} with respect to v_k can be measured by

$$C_k^i = \frac{v_k}{S_i^{st}} \frac{\partial S_i^{st}}{\partial v_k}$$

Response coefficients

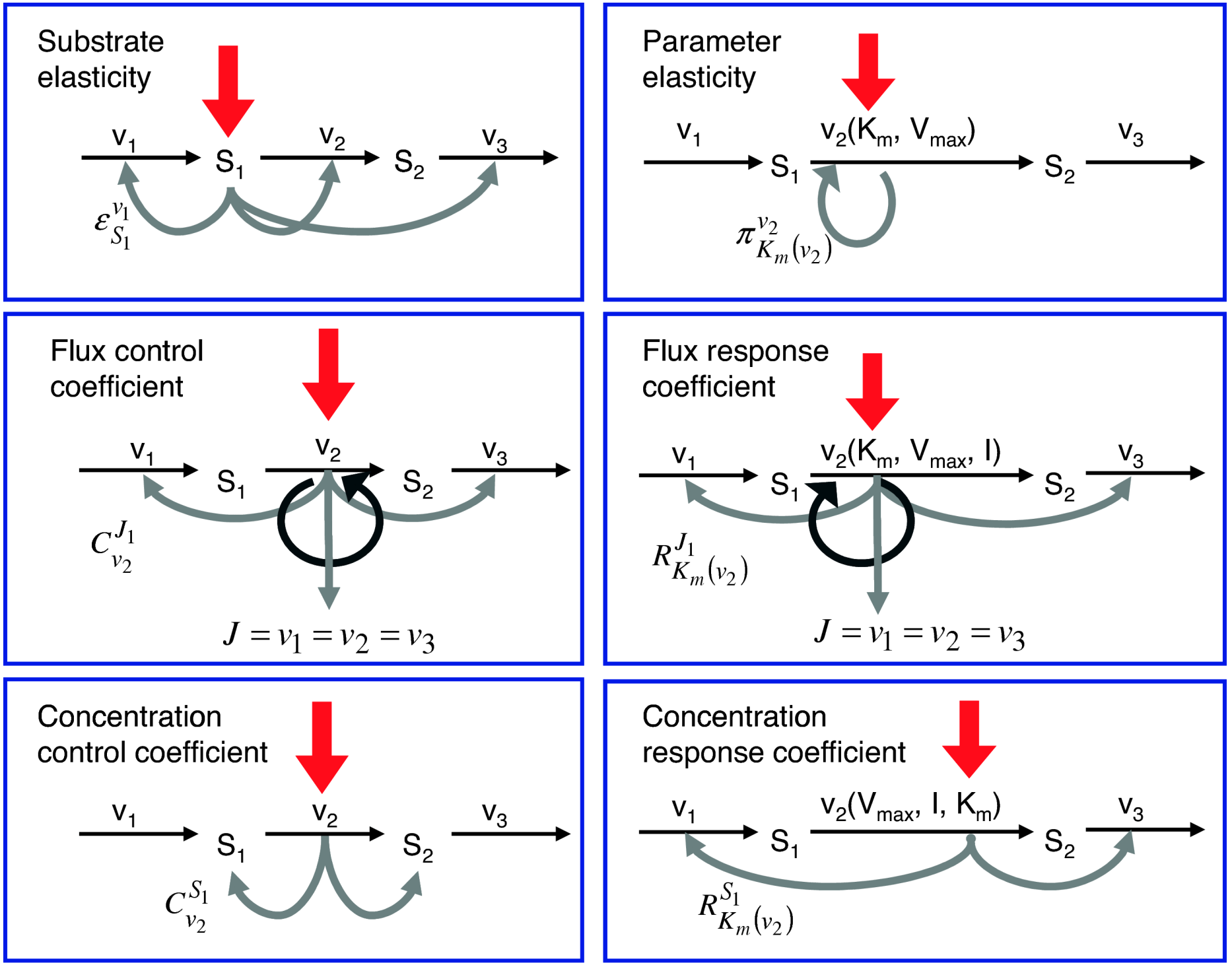
A steady state is determined by the values of the parameters. Response coefficients express this direct dependence of SS values on (external) parameters

For fluxes:

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

For concentrations:

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



Klipp - Systems Biology
ISBN: 978-3-527-31874-2 fig-02-11

Theorems of MCA

SS concentration and fluxes can usually not be expressed explicitly as function of reaction rates → Control coefficients cannot be determined by simply taking derivatives (as we did with elasticities)

Summation theorems - Statement about total control over a flux or steady state-concentration

Connectivity theorems - Relate control coefficients to the elasticity coefficients

These theorems plus stoichiometric information allows the calculation of control coefficients

Summation theorems

Flux control coefficients of a metabolic network for one steady state flux sum up to one (all enzymatic reactions can share the control over this flux)

$$\sum_{k=1}^{r} C_{v_k}^{J_j} = 1$$

Concentration control coefficients of a metabolic network for one steady state concentration sum up to zero/are balanced (some reactions may exert a positive and some others a negative control)

$$\sum_{k=1}^{r} C_{v_k}^{S_i} = 0$$

Derivation

Summation theorems can be formally proved using a corollary of the Euler theorem for homogeneous functions (see corresponding literature)

A simple derivation is the following:

Consider simultaneous small relative increase *α* in all reaction rates in the system

$$\frac{\delta v_k}{v_k} = \alpha$$

For each species, relative changes in production and consumption are the same. Therefore, the concentration keeps unchanged

$$\frac{\delta S_i}{S_i} = 0$$

Derivation (cont.)

The flux in the system increases by *α* (since all reactions increased their rates by this proportion)

$$\frac{\delta J}{J} = \alpha$$

Provided that *α* is small, the total change in the flux *J* can be considered the sum of all the individual changes that would be caused by the changes in each of the reaction rates

$$\frac{\delta J}{J} = \sum_k \left(\frac{\delta J}{J} \right)_k$$

Derivation (cont.)

Using the definition of flux control coefficients

$$\frac{\left(\frac{\delta J}{J}\right)_k}{\frac{\delta v_k}{v_k}} = C_k^J$$

and therefore

$$\left(\frac{\delta J}{J}\right)_k = C_k^J \cdot \frac{\delta v_k}{v_k}$$

Derivation (cont.)

Which reduces to

$$\left(\frac{\delta J}{J}\right)_k = C_k^J \cdot \alpha$$

Summing over all reactions

$$\frac{\delta J}{J} = \sum_k \left(\frac{\delta J}{J}\right)_k = \alpha \cdot \sum_k C_k^J$$

But as

$$\frac{\delta J}{J} = \alpha$$

We arrive at the flux summation theorem

$$\sum_k C_k^J = 1$$

Derivation (cont.)

A similar reasoning can be applied to the concentration control coefficients, bearing in mind that when all reaction rates are increased by the same factor the concentration does not change. This leads to the proof of the concentration summation theorem.

Distribution of control

In summation theorems we sum over **all** reactions in the network → if one control coefficient changes this has consequences on other coefficients

- Control (coefficient) is not only dependent on the parameters of the reaction for which it is defined, but it also depends on all others
- **Control coefficients are properties of the whole system. Control is a systemic property**
- Since control over a flux sums up to one, in a large network, on average we don't expect high control coefficients. In fact, none of the reactions might have considerable control (this was also observed experimentally in Niederberger et al. (1992) *Biochemical Journal* **287**(2):473-479)

Connectivity theorems

Flux control coefficients and elasticity coefficients are related by

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

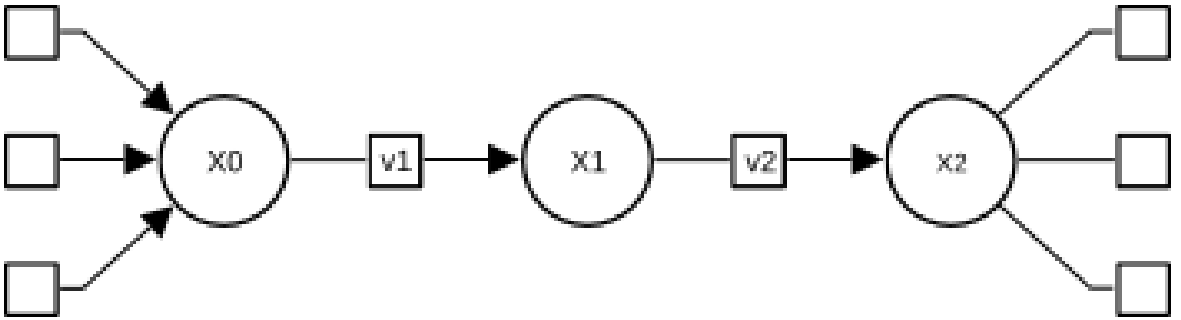
Concentration control coefficients and elasticity coefficients are related by

$$\sum_{k=1}^{r} C_{v_k}^{S_h} \epsilon_{S_i}^{v_k} = -\delta_{hi}$$

with the Kronecker symbol $\delta_{hi} = \begin{cases} 0, & if\ h \neq i \\ 1, & otherwise \end{cases}$

MCA calculations

Reactions in a biochemical network are connected by species that are products in one reaction and substrates in other reactions:



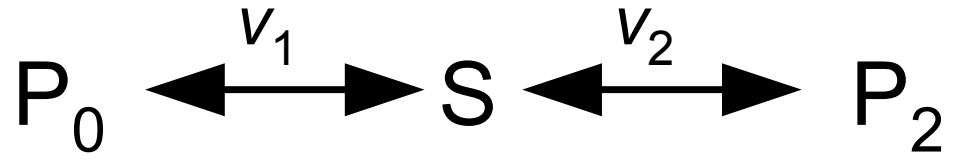
Assume simultaneous change in reaction rates v_1 and v_2 such that flux and SS concentrations of X0 and X2 do not change and only concentration of X1 changes.

With connectivity theorem $\sum_{k=1}^{r} C_{v_k}^{J_j} \epsilon_{S_i}^{v_k} = 0$

we have $C_{v_1}^{J} \cdot \epsilon_{X1}^{v_1} + C_{v_2}^{J} \cdot \epsilon_{X1}^{v_2} = 0$

Example

Consider a simple example system



The flux control coefficients obey the summation and connectivity theorems, respectively

$$C_1^J + C_2^J = 1 \qquad C_1^J \epsilon_S^1 + C_2^J \epsilon_S^2 = 0$$

This can be solved for the control coefficients to yield

$$C_1^J = \frac{\epsilon_S^2}{\epsilon_S^2 - \epsilon_S^1} \qquad C_2^J = \frac{-\epsilon_S^1}{\epsilon_S^2 - \epsilon_S^1}$$

MCA calculations

Connectivity theorems describe how changes in the concentration of intermediate species' concentrations propagate through the network.
(Local) kinetic properties of each reaction propagate the perturbation to and from its immediate neighbours.

In an unbranched pathway the collection of connectivity theorems and flux summation theorems form a system of *n* (with *n* the number of internal metabolites) linear equations in *n* variables which allow us to **calculate the flux control coefficients from the elasticity coefficients**.

MCA calculations

Calculation of control coefficients in metabolic networks are complicated by:

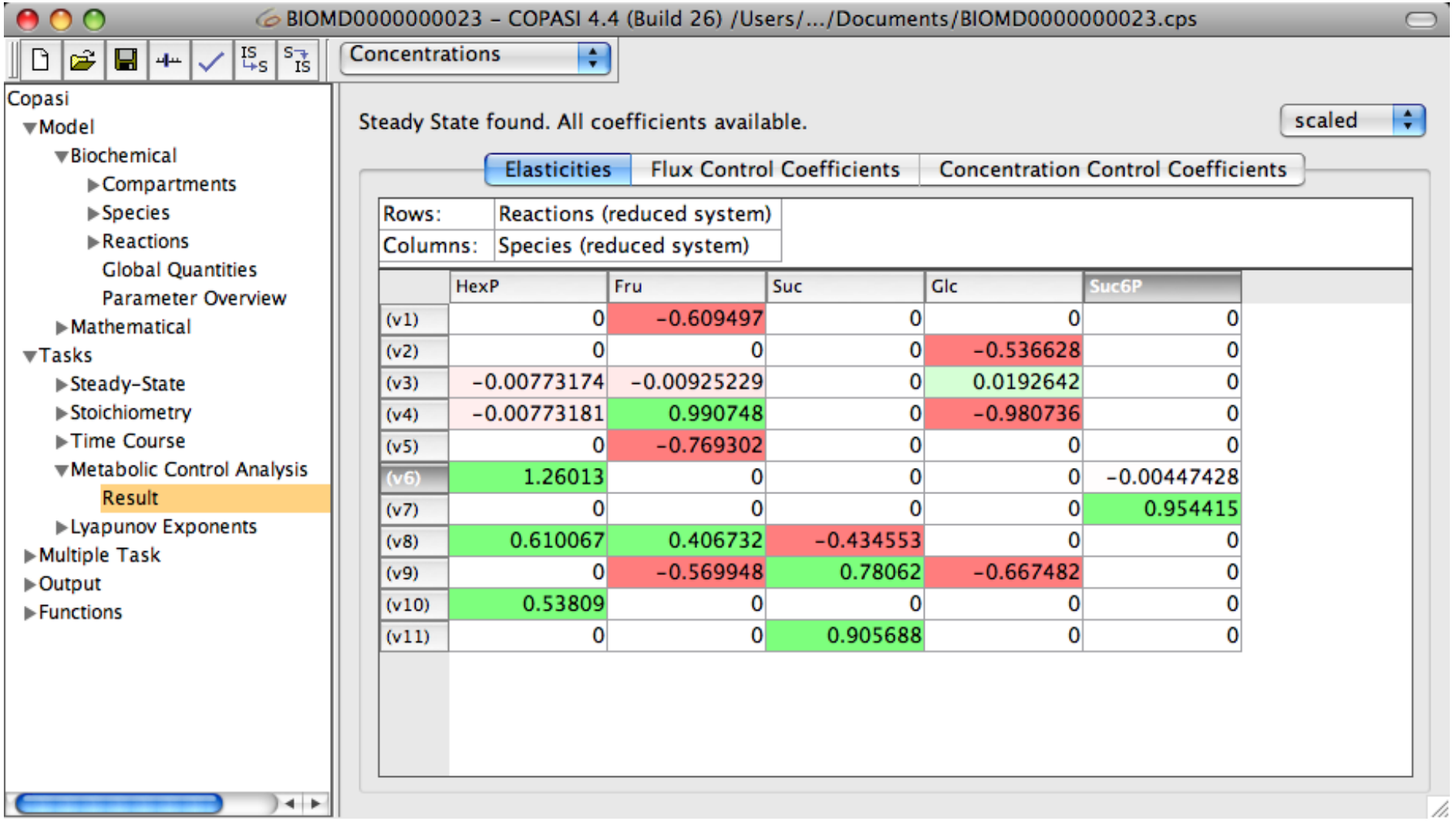
- branches, and
- mass conservations

Branches make the system of equations undetermined. Additional equations are required and can be provided by so-called branch-point theorems

If mass conservations are present the dependent species have to be eliminated before the calculations

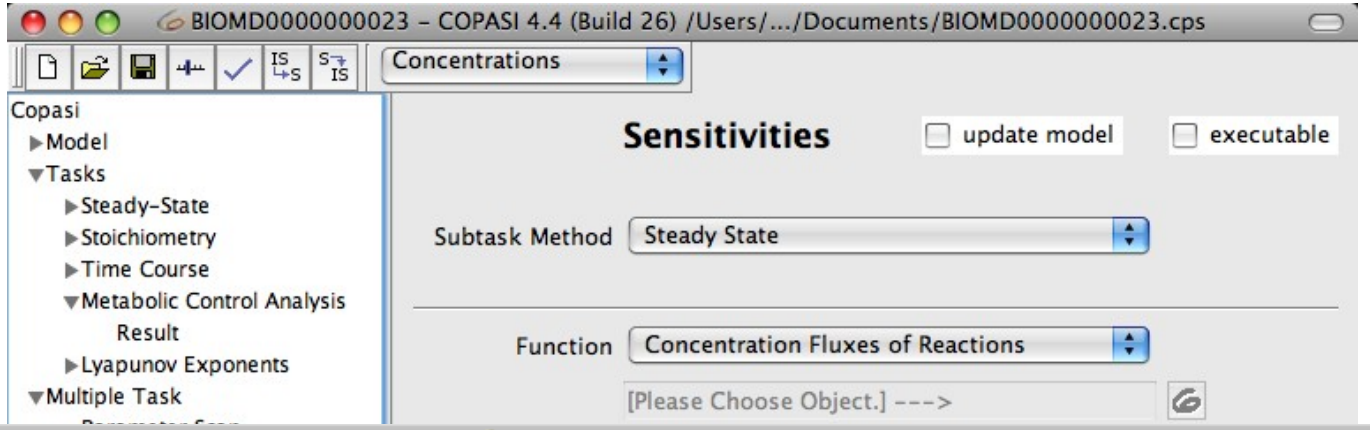
For details of the (matrix) calculation involved in MCA consult the textbook Klipp et al. (section 2.3.2.9, pp. 59)

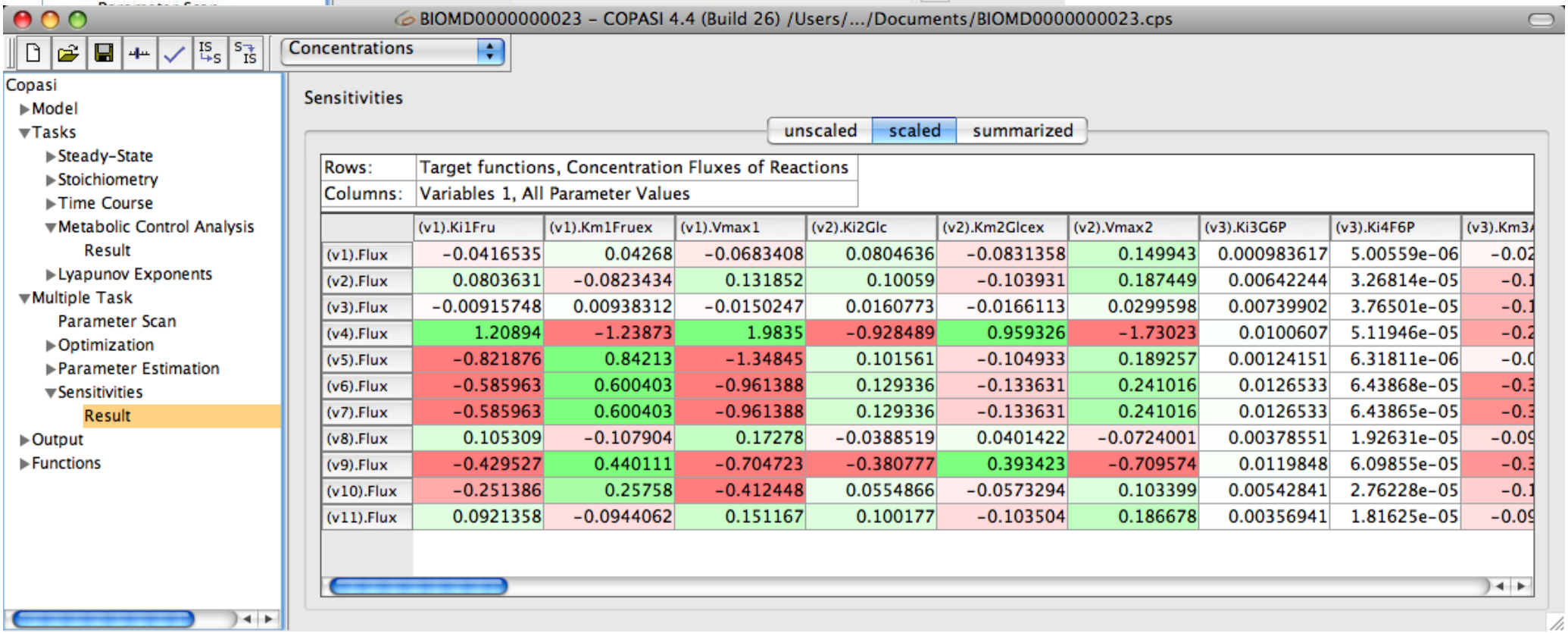
MCA in COPASI



	HexP	Fru	Suc	Glc	Suc6P
(v1)	0	-0.609497	0	0	0
(v2)	0	0	0	-0.536628	0
(v3)	-0.00773174	-0.00925229	0	0.0192642	0
(v4)	-0.00773181	0.990748	0	-0.980736	0
(v5)	0	-0.769302	0	0	0
(v6)	1.26013	0	0	0	-0.00447428
(v7)	0	0	0	0	0.954415
(v8)	0.610067	0.406732	-0.434553	0	0
(v9)	0	-0.569948	0.78062	-0.667482	0
(v10)	0.53809	0	0	0	0
(v11)	0	0	0.905688	0	0

Sensitivity Analysis (general) in COPASI





	(v1).Ki1Fru	(v1).Km1Fruex	(v1).Vmax1	(v2).Ki2Glc	(v2).Km2Glcex	(v2).Vmax2	(v3).Ki3G6P	(v3).Ki4F6P	(v3).Km3A
(v1).Flux	-0.0416535	0.04268	-0.0683408	0.0804636	-0.0831358	0.149943	0.000983617	5.00559e-06	-0.02
(v2).Flux	0.0803631	-0.0823434	0.131852	0.10059	-0.103931	0.187449	0.00642244	3.26814e-05	-0.1
(v3).Flux	-0.00915748	0.00938312	-0.0150247	0.0160773	-0.0166113	0.0299598	0.00739902	3.76501e-05	-0.1
(v4).Flux	1.20894	-1.23873	1.9835	-0.928489	0.959326	-1.73023	0.0100607	5.11946e-05	-0.2
(v5).Flux	-0.821876	0.84213	-1.34845	0.101561	-0.104933	0.189257	0.00124151	6.31811e-06	-0.0
(v6).Flux	-0.585963	0.600403	-0.961388	0.129336	-0.133631	0.241016	0.0126533	6.43868e-05	-0.3
(v7).Flux	-0.585963	0.600403	-0.961388	0.129336	-0.133631	0.241016	0.0126533	6.43865e-05	-0.3
(v8).Flux	0.105309	-0.107904	0.17278	-0.0388519	0.0401422	-0.0724001	0.00378551	1.92631e-05	-0.09
(v9).Flux	-0.429527	0.440111	-0.704723	-0.380777	0.393423	-0.709574	0.0119848	6.09855e-05	-0.3
(v10).Flux	-0.251386	0.25758	-0.412448	0.0554866	-0.0573294	0.103399	0.00542841	2.76228e-05	-0.1
(v11).Flux	0.0921358	-0.0944062	0.151167	0.100177	-0.103504	0.186678	0.00356941	1.81625e-05	-0.09

MCA

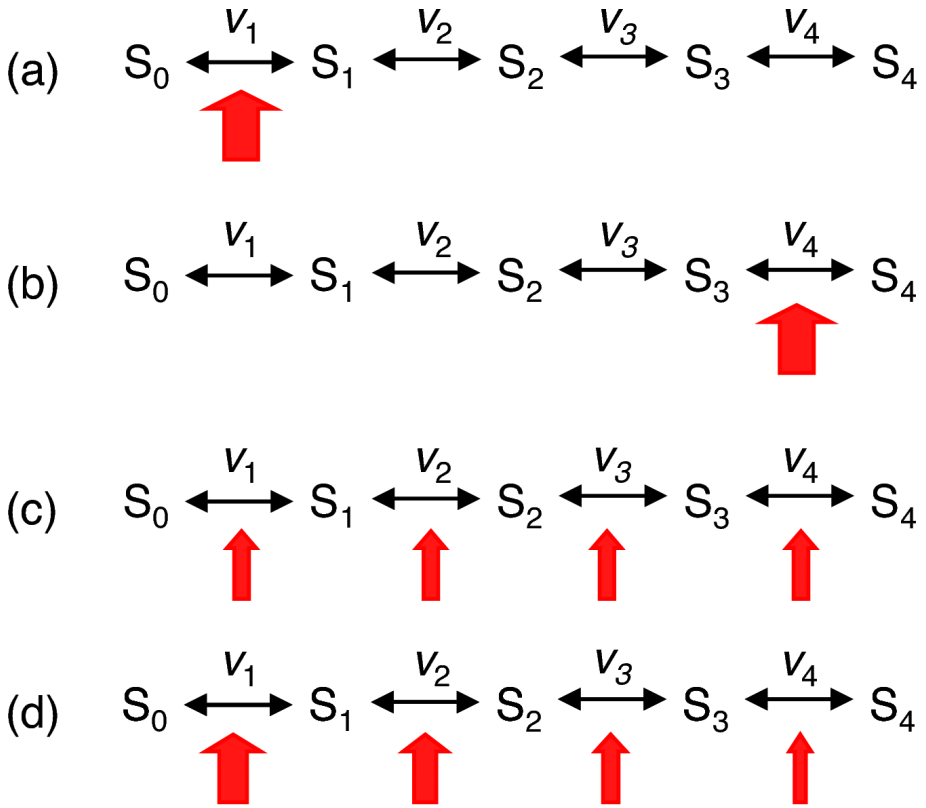
The main coefficients of MCA (elasticity, flux and concentration control coefficients) are useful quantities to characterise systems:

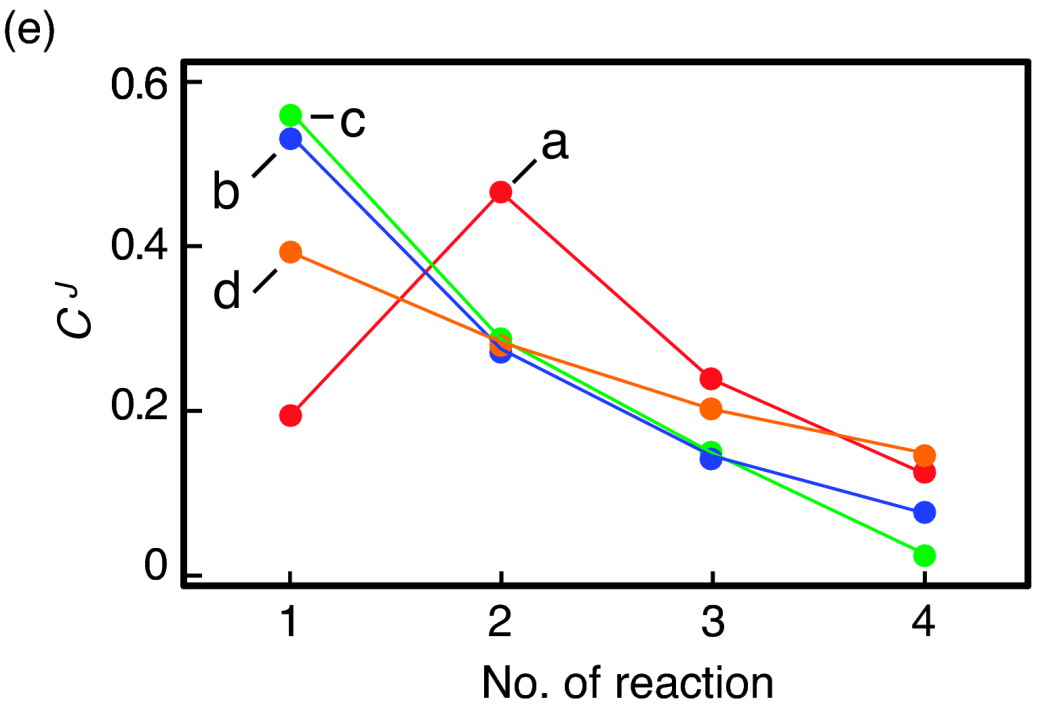
- Experiments:
  - Elasticities can be determined from enzyme kinetic experiments
  - Control coefficients can be determined by changing the V_{max} through a change in the enzyme concentration
- Steady state-models:
  - Elasticities can be determined by partial derivation of the kinetic functions
  - Control coefficients can be calculated numerically using finite differences or using MCA matrix calculations using summation and connectivity theorems

Remark

Keep in mind that, basically, MCA is restricted to (infinitesimally) small parameter changes. Mathematically, the system is linearised around a steady state

→ The change in the real system upon finite changes, e.g. in enzyme concentrations, might be different from what the MCA predicts!





Changing the concentration of the first enzyme to five times its original value in a) leads to a considerable drop of its control over the flux in the pathway with respect to the reference state in c)

Administrative stuff

- **No** exercise on Thursday, 14th, of June
  (this will give you more than a week to work on the questions...)
- New exercise sheet will appear online on Thursday latest