Metabolic Control Analysis (MCA)

So far, we have looked at metabolism from to extreme views:

- Kinetic modeling, which aims at accurate mechanistic models of enzymatic reactions. Limited to small systems in prectise
- Steady-state flux analysis, where large systems can be studied but in a limited setting where the effect of regulation is side-stepped in the modeling

Metabolic control analysis can be seen as middle ground of the two extremes: in MCA, we can model the network behaviour of the reactions and consider regulation at the same time.

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

Coefficients of control analysis

The limit can be written as

$$c_x^y = \frac{x}{y}\frac{\partial y}{\partial x} = \frac{\partial \ln y}{\partial \ln x},$$

by using the derivation rule $d/dz \ln z = 1/z$, for $z = x, y$

- The normalization factor x/y makes the coefficient independent of units, the same value will be obtained regardless of in which units y and x are expressed.
- Unnormalized coefficients $\frac{\partial y}{\partial x}$ are sometimes used as well as some mathematical derivations become easier

Types of coefficients

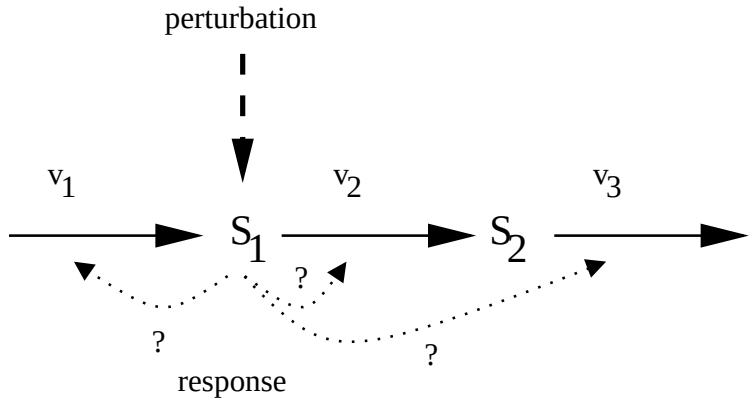
- Elasticity coefficients quantify the sensitivity of a reaction rate to the change of concentration or a parameter.
- Flux control coefficients quantify the change of a flux along a pathways in response to a change in the rate of a reaction
- Concentration control coefficients quantify the change of 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.



ϵ-elasticity coefficient

Consider a reaction catalyzed by a enzyme E, inhibited by effector I and activated by effector A

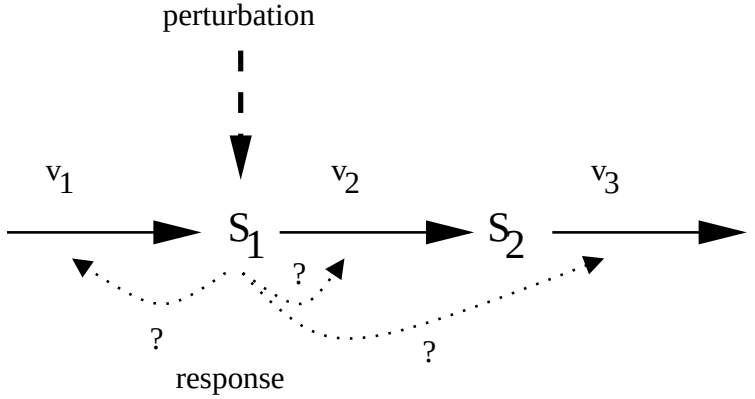
Typical values (there are exceptions) for elasticity coefficients satisfy the following:

$$\epsilon_S^v = \frac{\partial \ln v}{\partial \ln S} > 0, \epsilon_P^v = \frac{\partial \ln v}{\partial \ln P} < 0$$

i.e. i.e. the more substrate the faster the rate, the more product the slower the rate

$$\epsilon_A^v = \frac{\partial \ln v}{\partial \ln A} > 0, \epsilon_I^v = \frac{\partial \ln v}{\partial \ln I} < 0$$

i.e. the higher activator concentration the faster the rate, the higher inhibitor concentration the slower the rate



Example: ϵ-elasticity of a simple reaction

Consider an enzymatic reaction modelled with Michaelis-menten kinetics

$$v_k = \frac{V_{max} S_i}{K_m + S_i}$$

The elasticity with respect to the change in the substrate concentration is found to be

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

by applying the derivation rule $\frac{d}{dx} \frac{f(x)}{g(x)} = \frac{f(x)' g(x) - g(x)' f(x)}{g(x)^2}$

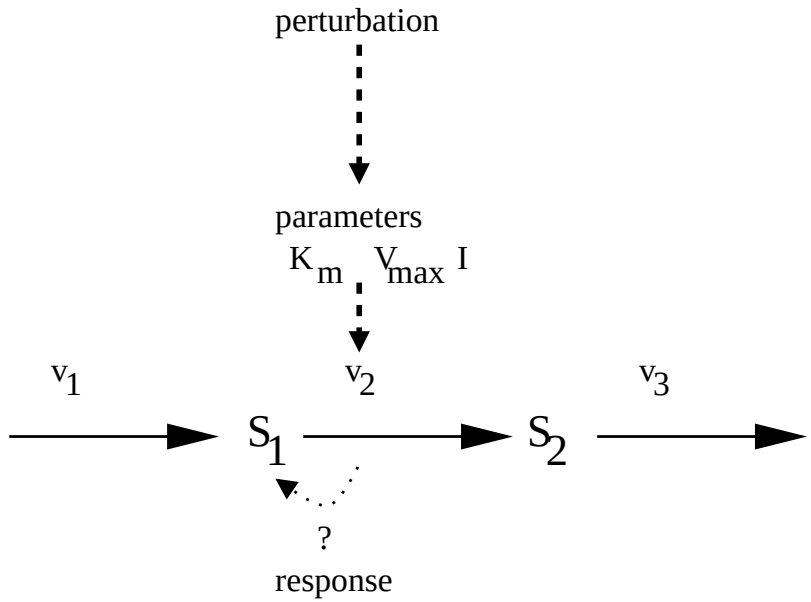
- The change of reaction rate in response to change of concentration of the substrate is the lower the higher the concentration
- The reaction rate is a concave function of the substrate concentration

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)



Example: π-elasticity of a simple reaction

Consider a reaction with the Michaelis-Menten rate equation

$$v_k = \frac{V_{max} S_i}{K_m + S_i}$$

The π-elasticity w.r.t. the K_m-parameter is given by

$$\pi^k_{K_m} = \frac{K_m}{v_k} \frac{\partial v_k}{\partial K_m} = -\frac{K_m}{K_m + S}$$

- Asymptotically tends towards -1 when S decreases i.e. for low substrate situations the change of K_m has linear effect on reaction rates
- Tends towards zero when S increases: in high substrate situations the change of K_m has little effect

Control coefficients

We consider a vector

$$\mathbf{S} = \mathbf{S}(\mathbf{p})$$

of steady state concentrations and a vector

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

of steady state fluxes, parametrized by $\mathbf{p}$, which includes kinetic parameters of enzymes and concentrations of external metabolites.

Consider a small perturbation of a reaction rate v_k via perturbation of the parameters $\mathbf{p}$.

This will cause the system to seek a new steady state in the neighborhood of of the original: $\mathbf{J} \rightarrow \mathbf{J} + \mathbf{\Delta J}$, $\mathbf{S} \rightarrow \mathbf{S} + \mathbf{\Delta S}$

Questions of interest

We wish to capture the answers to the following questions

- What is the effect of rate change of a reaction to a particular flux?
- What is the effect of rate change of a reaction to a particular concentration?

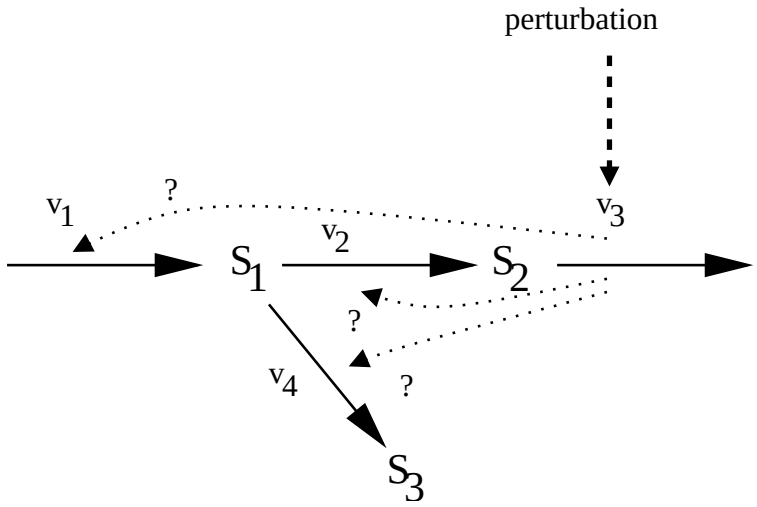
Answers to the above questions are characterized by so called control coefficients.

Flux control coefficients

The flux-control coefficient (FCC)

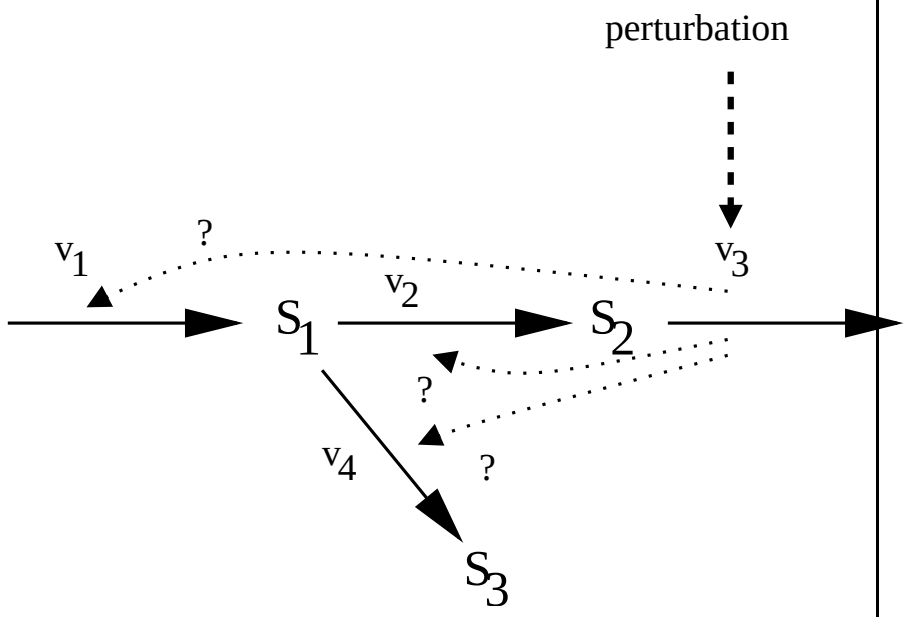
$$FCC_k^j = \frac{v_k}{J_j}\frac{\partial J_j}{\partial v_k}$$

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



Flux control coefficients

- Unlike elasticity coefficients, FCC's are global: all reaction rate have control over all fluxes, the strength of control is quantified by the FCC.
- Note that the notion of 'control' does not in general mean direct regulatory relationship e.g. FCC_3^4 denoting the control of v_3 to the flux from S_1 to S_3 will typicly be non-zero

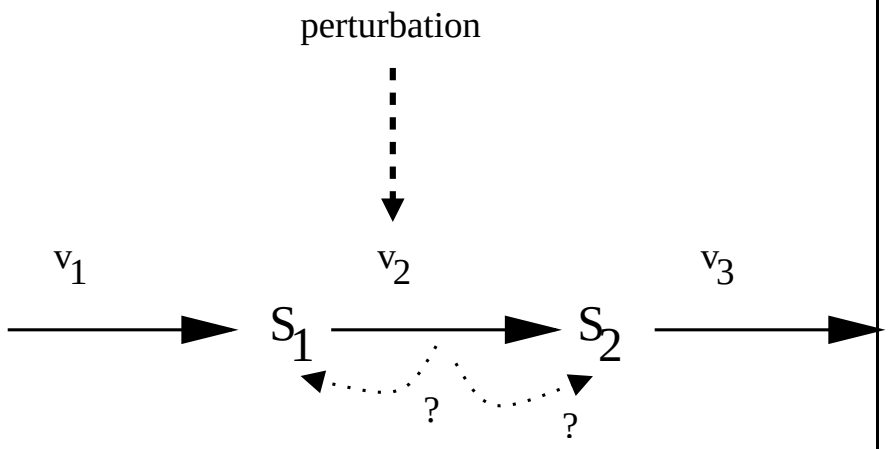


Concentration control coefficients

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

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

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

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

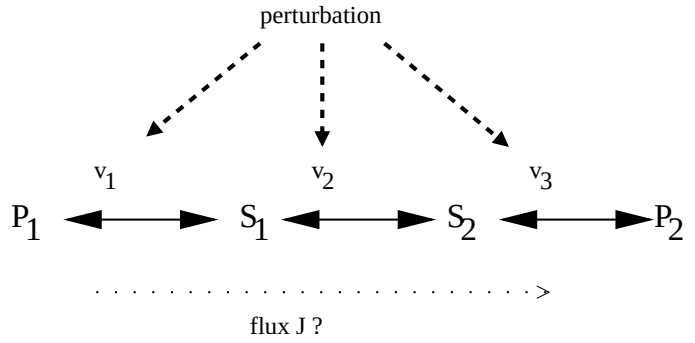
Control of a concentration is shared across all enzymatic reactions, some exerting positive control, other exerting negative control.

Flux control summation

For getting soime intuition behind flux control summation, consider the unbranched pathway on the right.

What will happen to flux J if we manipulate all three reaction rates by small fraction α, i.e

$$\frac{\delta v_1}{v_1} = \frac{\delta v_2}{v_2} = \frac{\delta v_3}{v_3} = \alpha?$$



Flux control summation

The flux through the pathway must rise by the same amount: $\frac{\delta J}{J} = \alpha$

Using the chain rule $\frac{df(x,y)}{dJ} = \frac{\partial f(x,y)}{\partial x}\frac{dx}{dJ} + \frac{\partial f(x,y)}{\partial y}\frac{dy}{dJ}$, distribute the change of flux to the individual reactions:

$$\delta J = \frac{\partial J}{\partial v_1}\delta v_1 + \frac{\partial J}{\partial v_2}\delta v_2 + \frac{\partial J}{\partial v_3}\delta v_3$$

Divide and multiply terms of the right by v_i and divide all terms by J

$$\frac{\delta J}{J} = \frac{v_1}{J}\frac{\partial J}{\partial v_1}\frac{\delta v_1}{v_1} + \frac{v_2}{J}\frac{\partial J}{\partial v_2}\frac{\delta v_2}{v_2} + \frac{v_3}{J}\frac{\partial J}{\partial v_3}\frac{\delta v_3}{v_3}$$

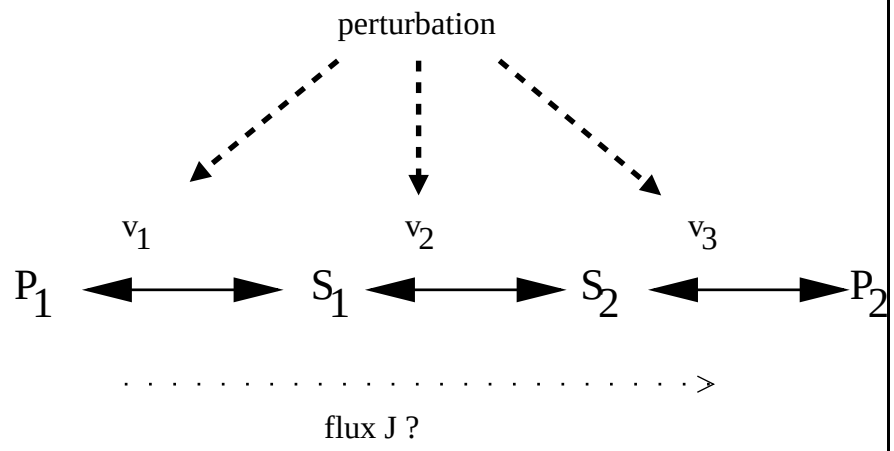
Substituting $FCC_v^J = \frac{v}{J}\frac{\partial J}{\partial v}$ obtains:

$$\frac{\delta J}{J} = \ = FCC_1^J\frac{\delta v_1}{v_1} + FCC_2^J\frac{\delta v_2}{v_2} + FCC_3^J\frac{\delta v_2}{v_3}$$

Flux control summation

As $\alpha = \frac{\delta J}{J} = \frac{\delta v_1}{v_1} = \frac{\delta v_2}{v_2} = \frac{\delta v_3}{v_3}$ we get

$$\alpha = \frac{\delta J}{J} =$$
$$= FCC_1^J \frac{\delta v_1}{v_1} + FCC_2^J \frac{\delta v_2}{v_2} + FCC_3^J \frac{\delta v_2}{v_3}$$
$$= \alpha(FCC_1 + FCC_2 + FCC_3)$$



This can be seen to adhere to the summation theorem

$$\sum_j FCC_j = 1$$

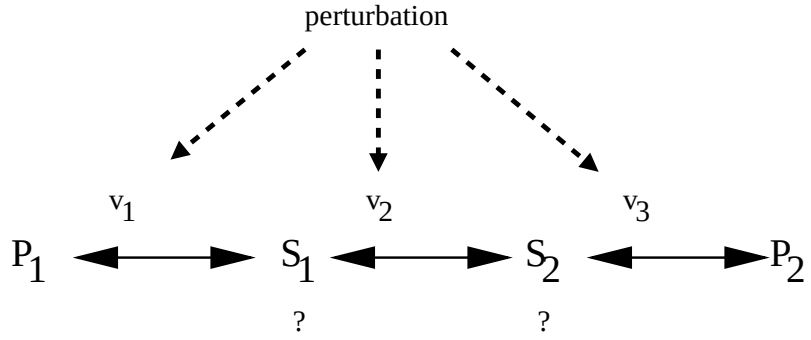
Concentration control summation

By similar argument we find that the distributed effect on the concentrations satisfies

$$\frac{\delta S_1}{S_1} = CCC_1^{S_1}\frac{\delta v_1}{v_1} + CCC_2^{S_1}\frac{\delta v_2}{v_2} + CCC_3^{S_1}\frac{\delta v_3}{v_3}$$

Because of the steady state, for concentrations S_1 and S_2 we have

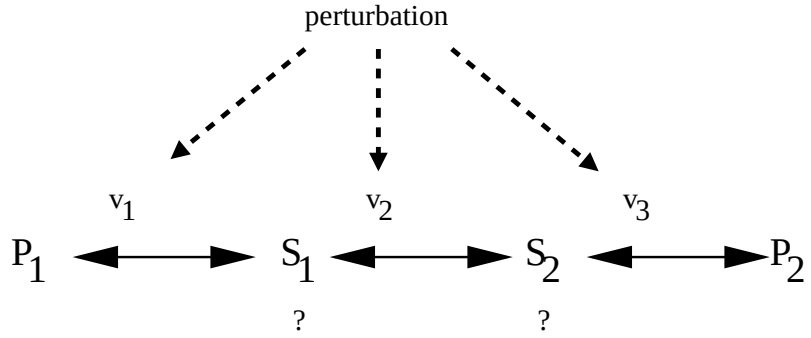
$$\frac{\delta S_1}{S_1} = \frac{\delta S_2}{S_2} = 0$$



Concentration control summation

Combining we get:

$$0 = \frac{\delta S_1}{S_1} = \alpha(CCC_1^{S_1} + CCC_2^{S_1} + CCC_3^{S_1})$$



which adheres to the summation theorem

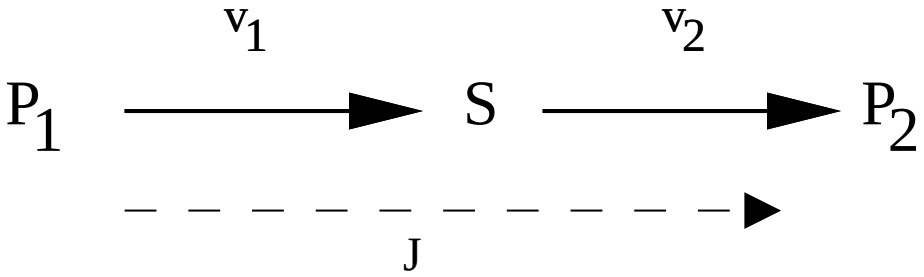
$$\sum_i CCC_1^{S_1} = 0$$

Connecting elasticity and control coefficients

Flux control coefficients and elasticities are typically coupled: given a high elasticity $\epsilon_i^k = \frac{S_i}{v_k}\frac{\partial v_k}{\partial S_i}$ the flux control coefficient $FCC_k^j = \frac{v_k}{J_j}\frac{\partial J_j}{\partial v_k}$ will typically be low, and vice versa

In the example below, if the activity of enzyme catalyzing reaction v_2 is dropped, the concentration of S will rise.

If the reaction has high elasticity it will compensate the reduced activity thus keeping the overall flux J_j close to the original.



Flux control connectivity theorems

This property is captured by so called connectivity theorems that tie elasticity coefficients $\epsilon_{S_i}^{v_k}$ and control coefficients $FCC_{v_k}^{J_j}, CCC_{v_k}^{S_i}$ together.

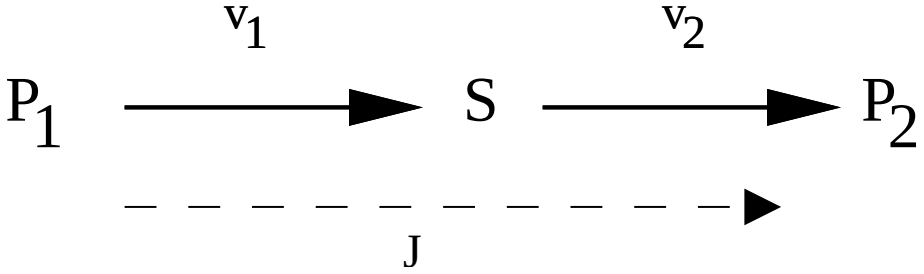
For flux control we have

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

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

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

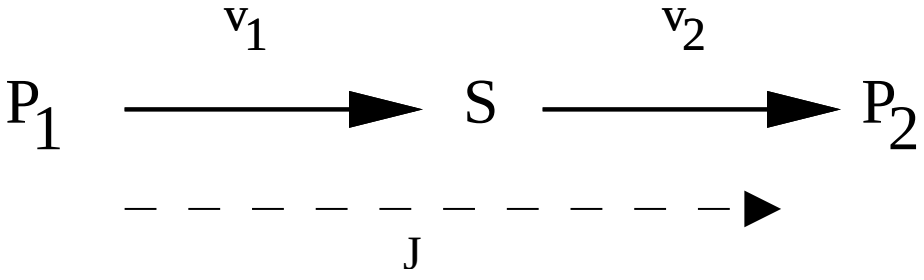
Calculating control coefficients

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 $FCC_1^J \epsilon_S^1 + FCC_2^2 \epsilon_S^2 = 0$ to solve

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

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

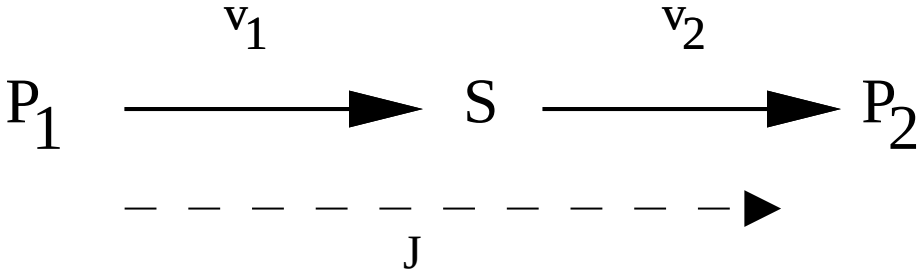


Calculating control coefficients

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