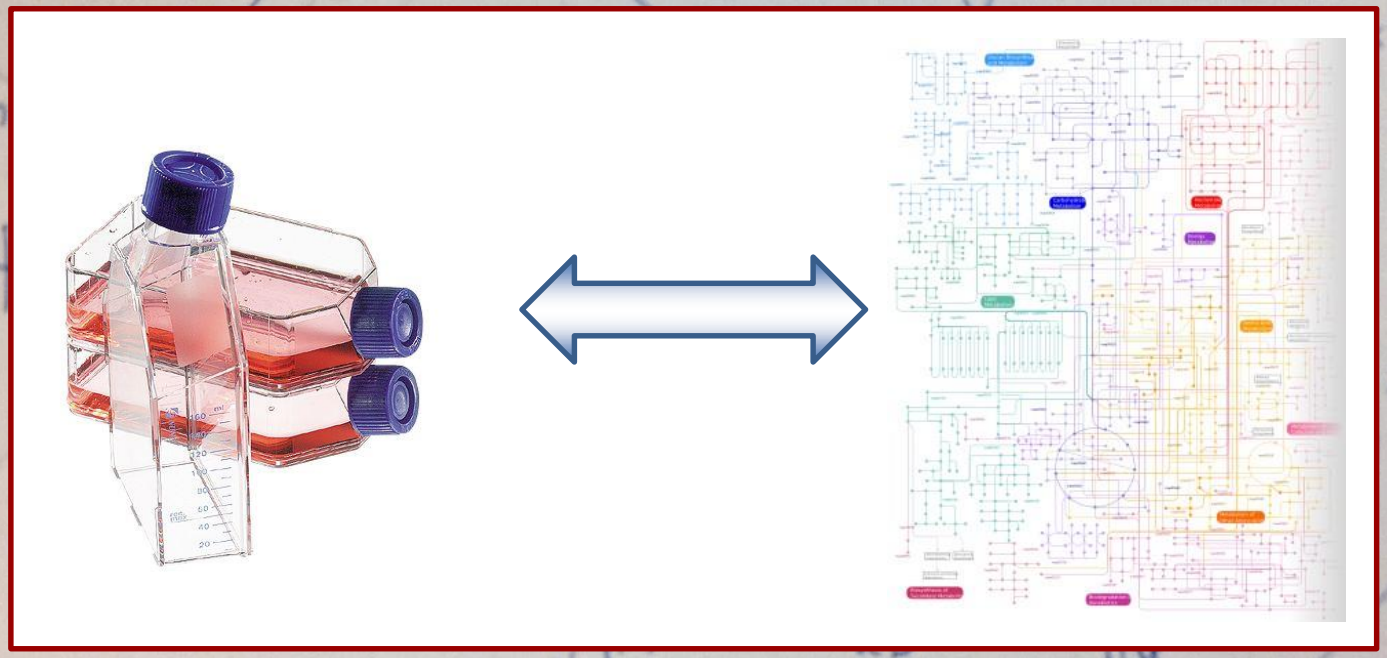
PoLiMeR

Metabolic Control Analysis



Barbara M. Bakker *University Medical Centre Groningen, University of Groningen*

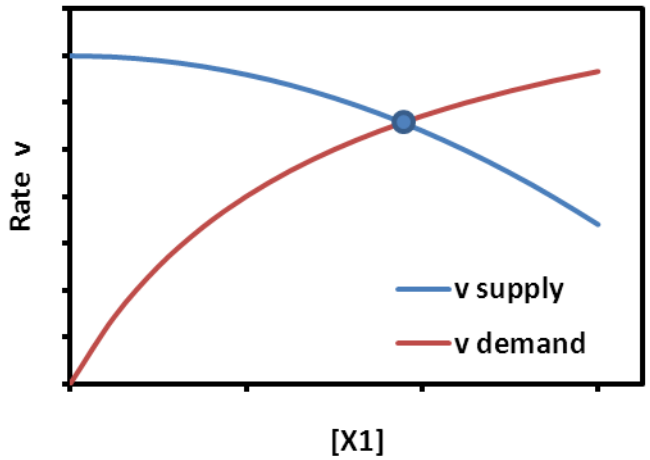
The Menu

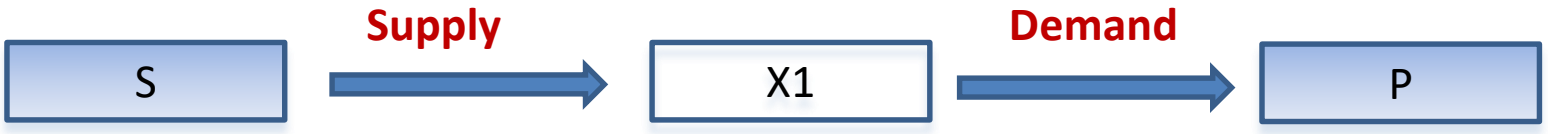
- *Flux control*
- *Relation between control and kinetics*
- *Matrix method*
- *Homeostasis*

How are metabolite fluxes controlled by metabolic processes?

Steady state

v supply = v demand, then [X1] remains constant

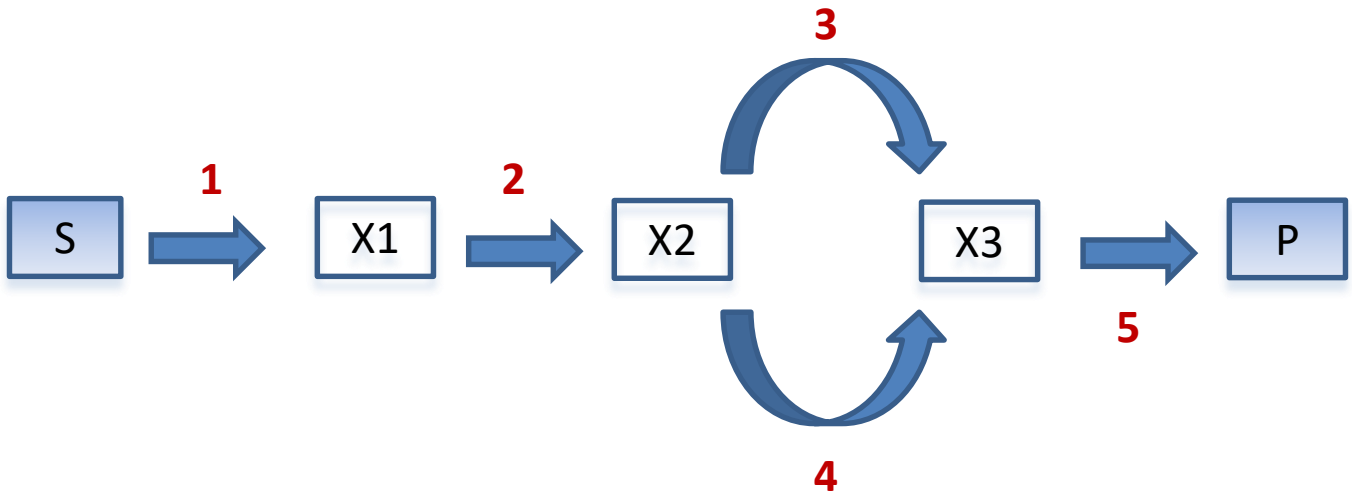




Fixed (external)

Variable(internal)

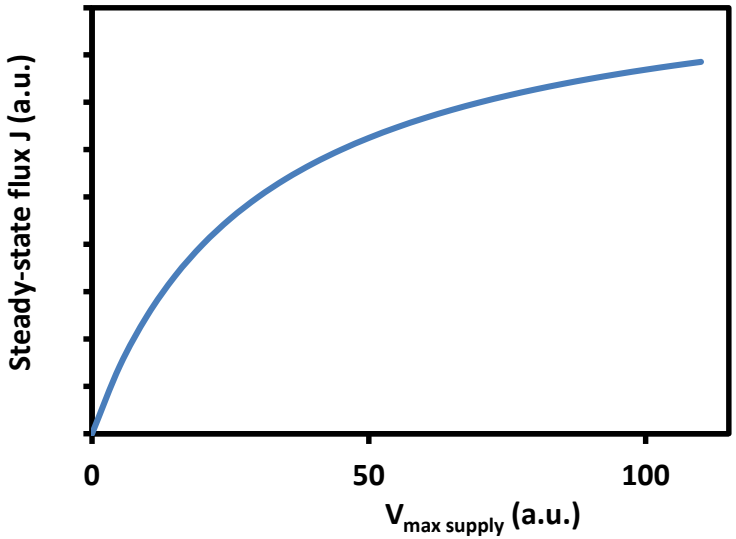
Enzymes communicate via metabolite concentrations

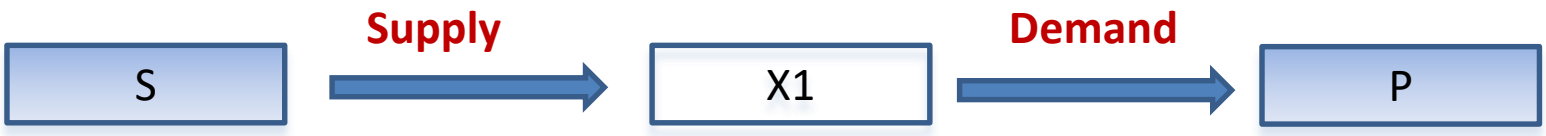


Fixed (external)

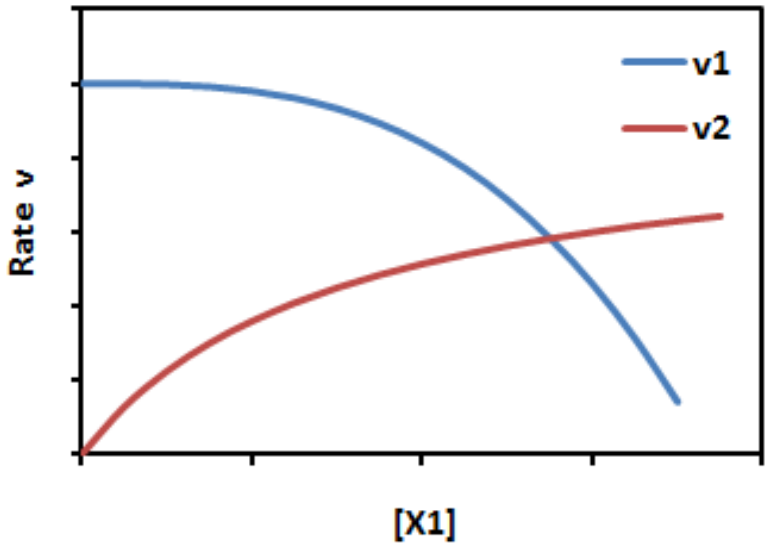
Variable(internal)

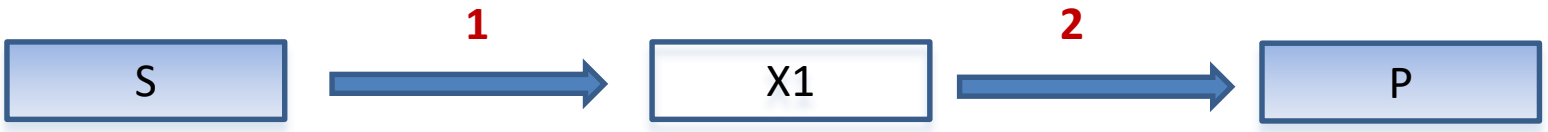
Rate-limiting steps are rare in biology





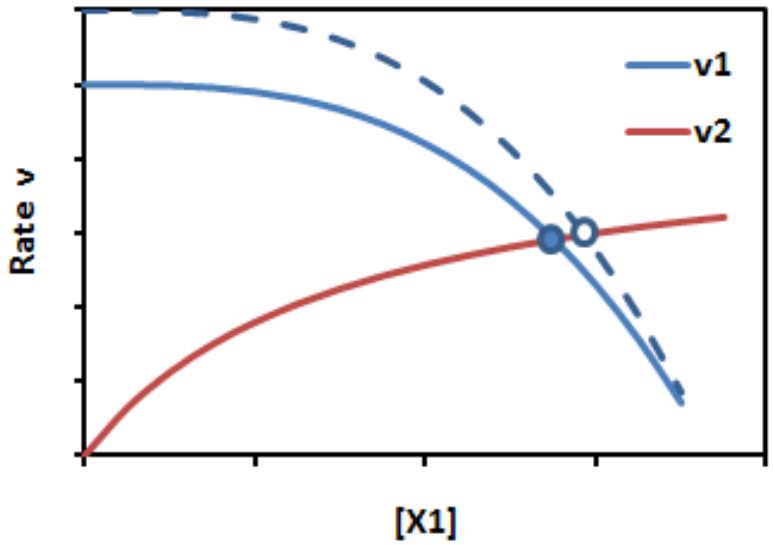
How to stimulate the flux most effectively?

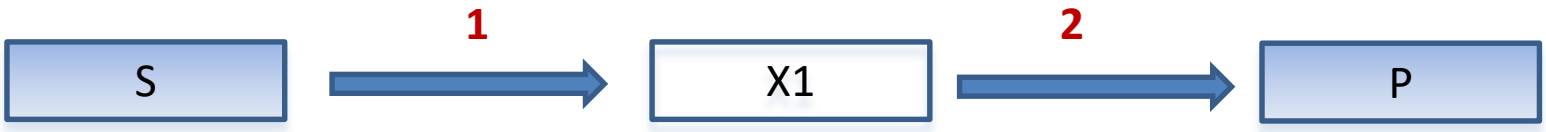




Activation of supply

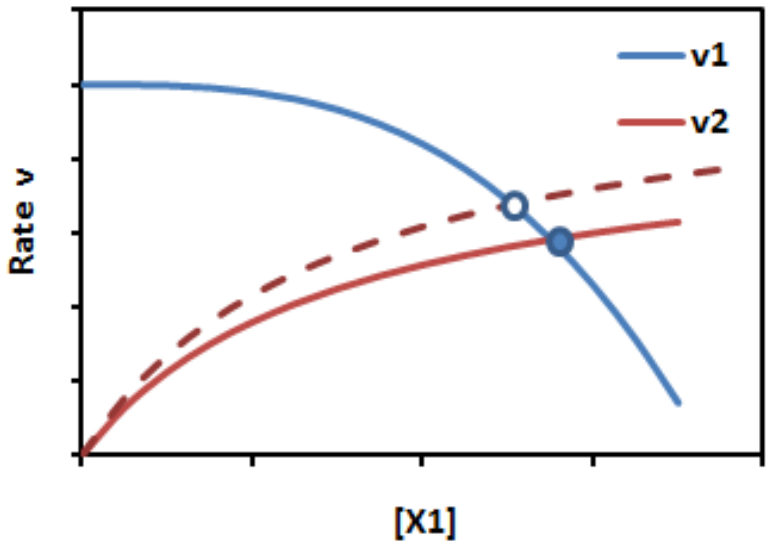
Enzyme concentration or V_{max} +20%

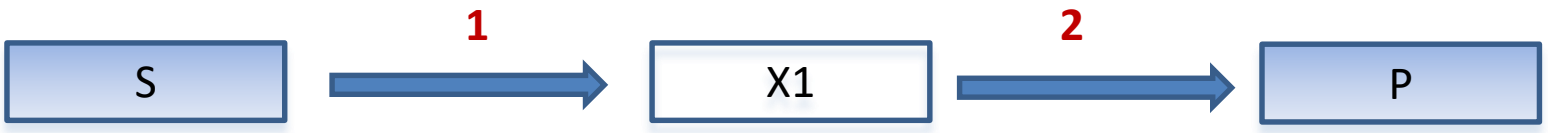




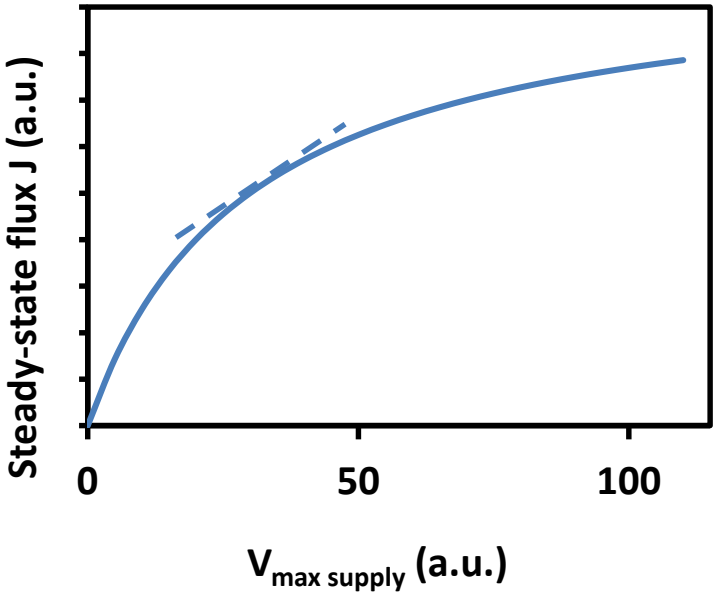
Activation of demand

Enzyme concentration or V_{max} +20%





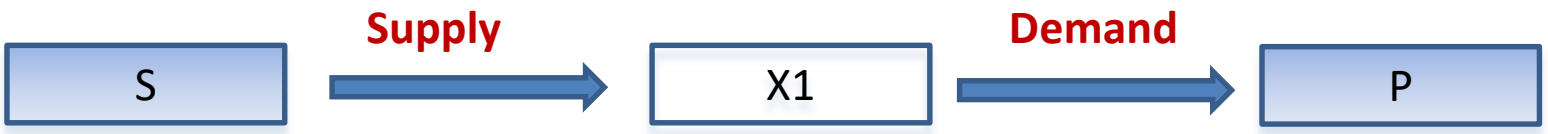
Quantification: the flux control coefficient



$$C_{supply}^{J} = \frac{\%\ change\ of\ J}{\%\ change\ of\ V_{max,supply}}$$

$$= \frac{dJ}{dV_{max,supply}} \cdot \frac{V_{max,supply}}{J}$$

$$= \frac{dlnJ}{dlnV_{max,supply}}$$



Generalized definition of flux control coefficient

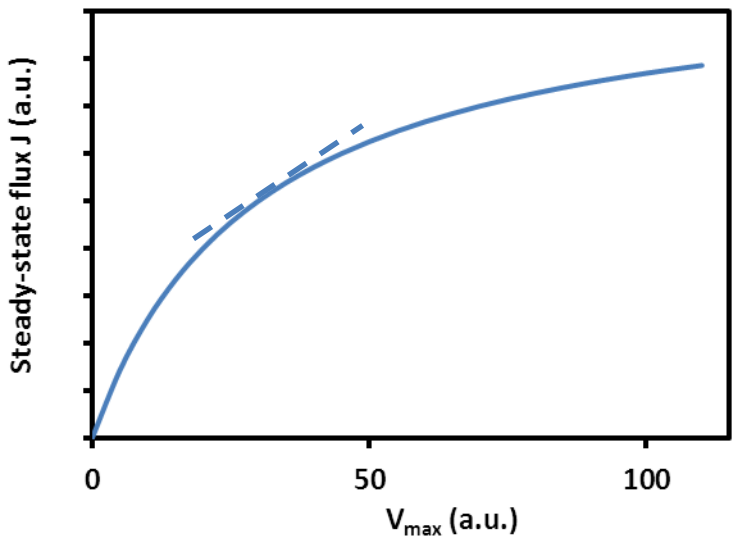
$$C_i^J = \frac{(dlnJ/dlnp)_{ss}}{\partial lnv/\partial lnp}$$

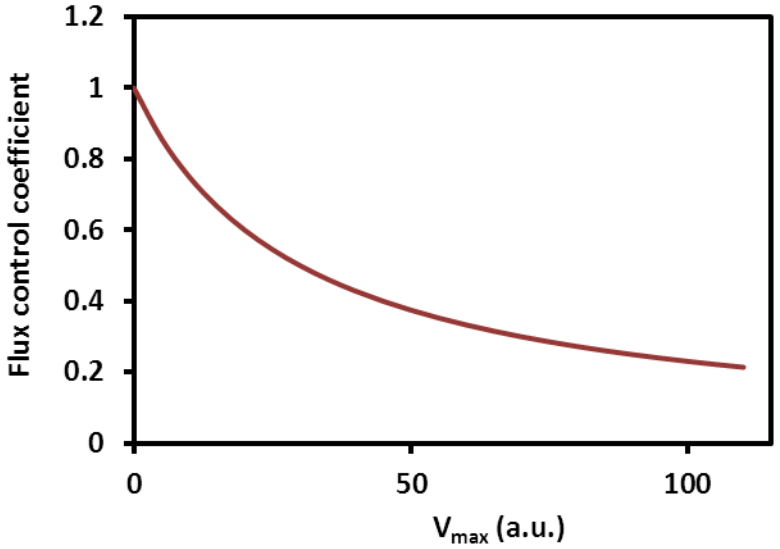
i denotes the name of the enzyme
J is the steady-state flux
v_i = the rate of enzyme i
p is any parameter that *selectively* affects v_i

Note: d denotes a total derivative (i.e. comparing two steady states), whereas ∂ is a partial derivative.

Discussion: why is this equivalent to the previous equation if $p = V_{max}$?

The flux control coefficient is not constant





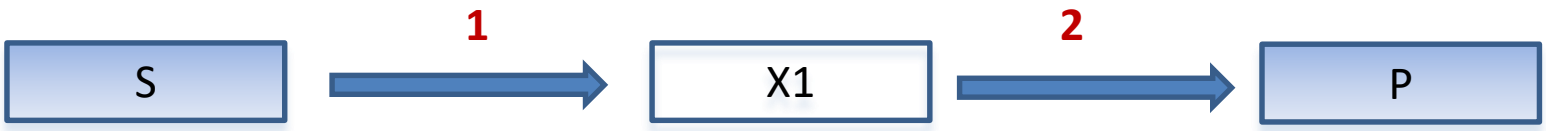
Flux control coefficient

$$C_i^J = \frac{dJ}{dV_{max,i}} \cdot \frac{V_{max,i}}{J}$$

Summation theorem

$$\sum_i C_i^J = 1$$

$$C_1^J + C_2^J = 1$$



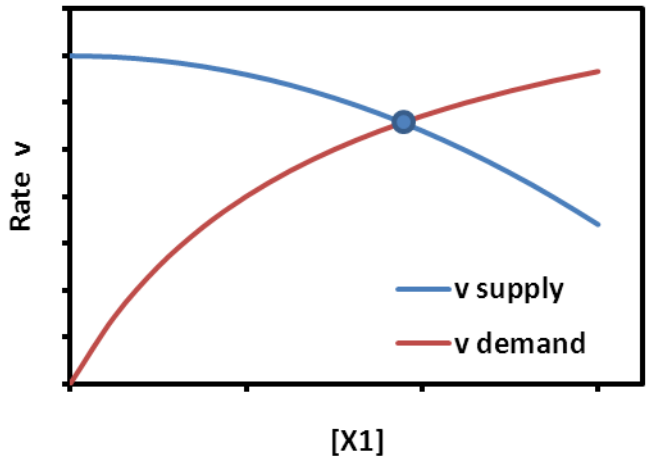
Determination of flux control coefficients

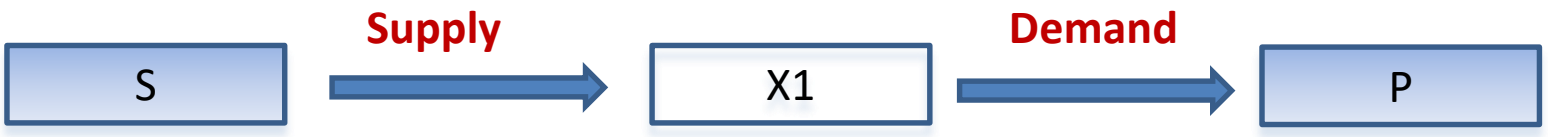
- Titration with inhibitors
  - not always specific
  - inhibitor kinetics required
  - fast
- Manipulation of gene expression
  - specific
  - slow, adaptation effects possible
- Kinetic modelling
  - kinetics of the complete system required
  - transparant and complete

Conclusion 1

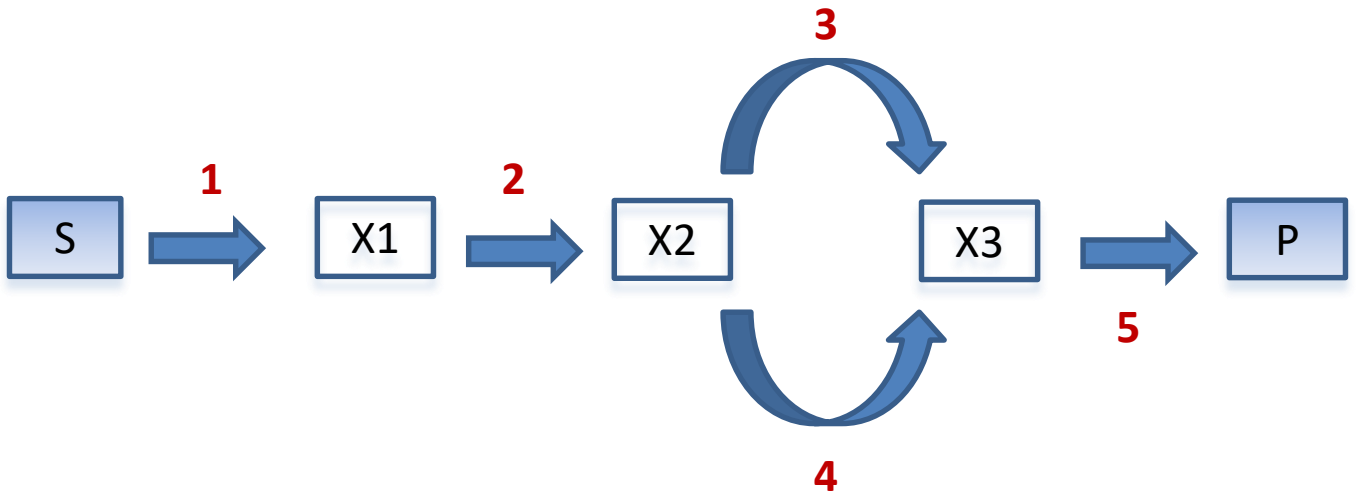
The flux control coefficient expresses the extent to which an enzyme determines the metabolic flux.

How does an enzyme exert control?





Enzymes are connected via metabolite concentrations

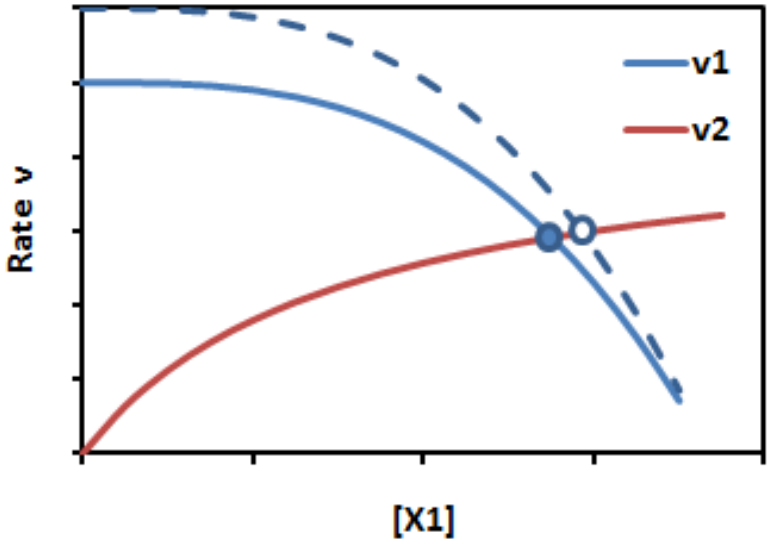


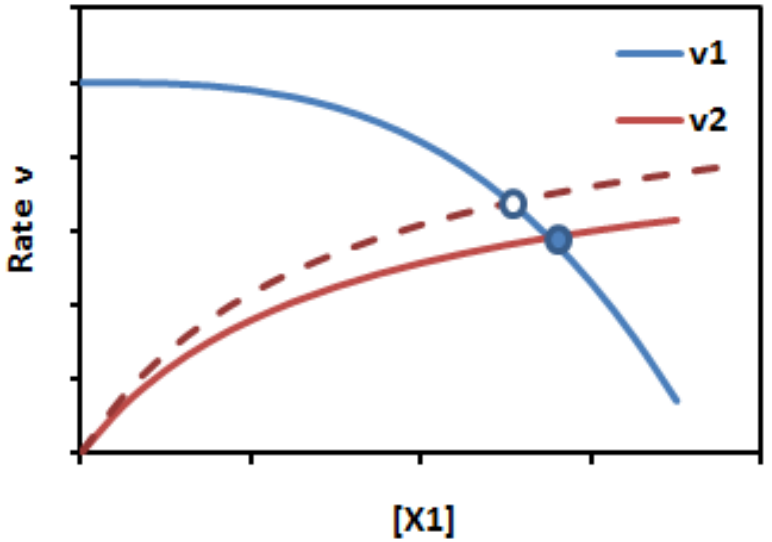
Fixed (external)

Variable(internal)

‘Inelastic’ enzymes have high flux control coefficients

Example: enzyme 1 is elastic, enzyme 2 is inelastic

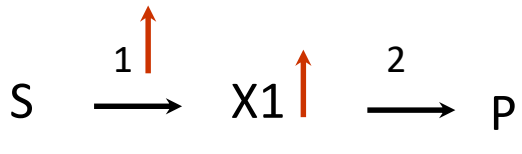


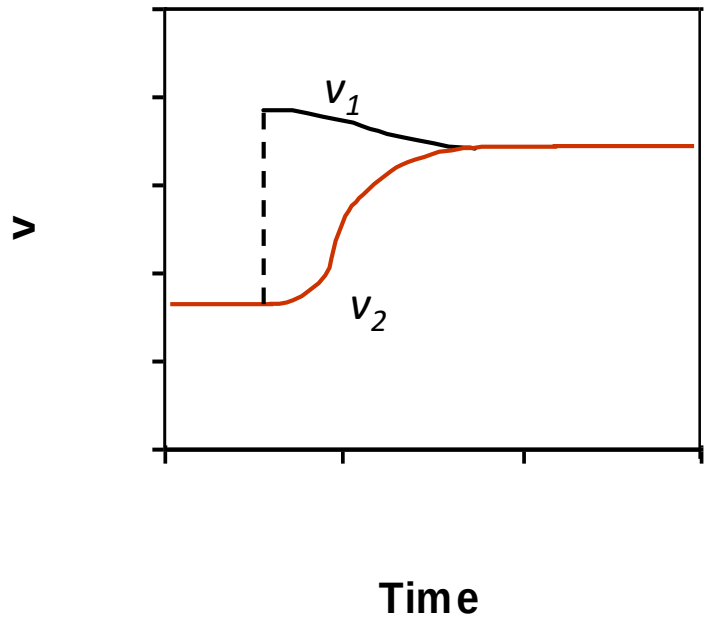


‘Inelastic’ enzymes have high flux control coefficients

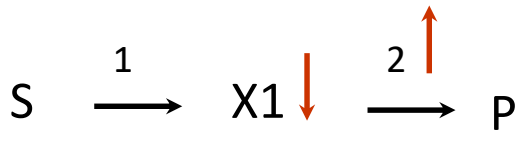
Example: v_1 is insensitive to [X1] and v_2 is rather sensitive to [X1]

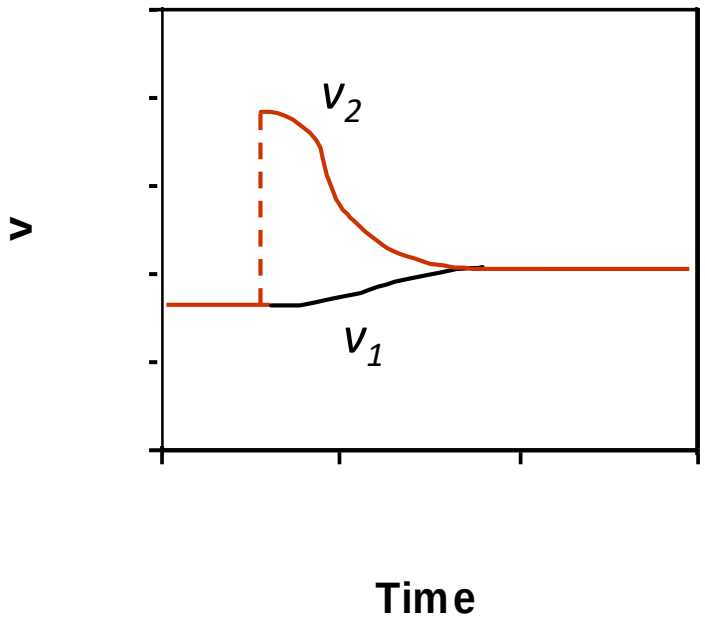
High flux control:





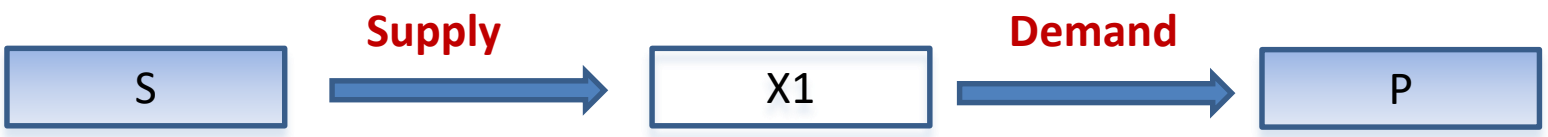
Low flux control:





Quantification: the elasticity coefficient

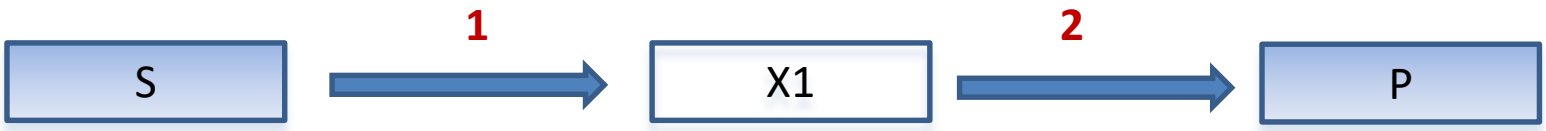
$$\varepsilon_X^{v_i} = \frac{\partial v_i}{\partial X} \cdot \frac{X}{v_i} = \frac{\partial ln v_i}{\partial ln X}$$



Connectivity theorem

$$\sum_i C_i^J \cdot \varepsilon_X^{v_i} = 0$$

$$C_1^J \cdot \varepsilon_{X_1}^{v_1} + C_2^J \cdot \varepsilon_{X_1}^{v_2} = 0$$



Implication

1. $C_1^J + C_2^J = 1$

2. $C_1^J \cdot \varepsilon_{X_1}^{v_1} + C_2^J \cdot \varepsilon_{X_1}^{v_2} = 0$

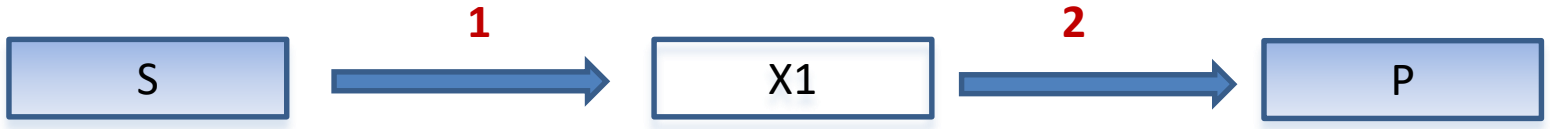
3. $C_2^J = -C_1^J \cdot \varepsilon_{X_1}^{v_1} / \varepsilon_{X_1}^{v_2}$

4. $C_1^J \left(1 - \varepsilon_{X_1}^{v_1} / \varepsilon_{X_1}^{v_2}\right) = 1$

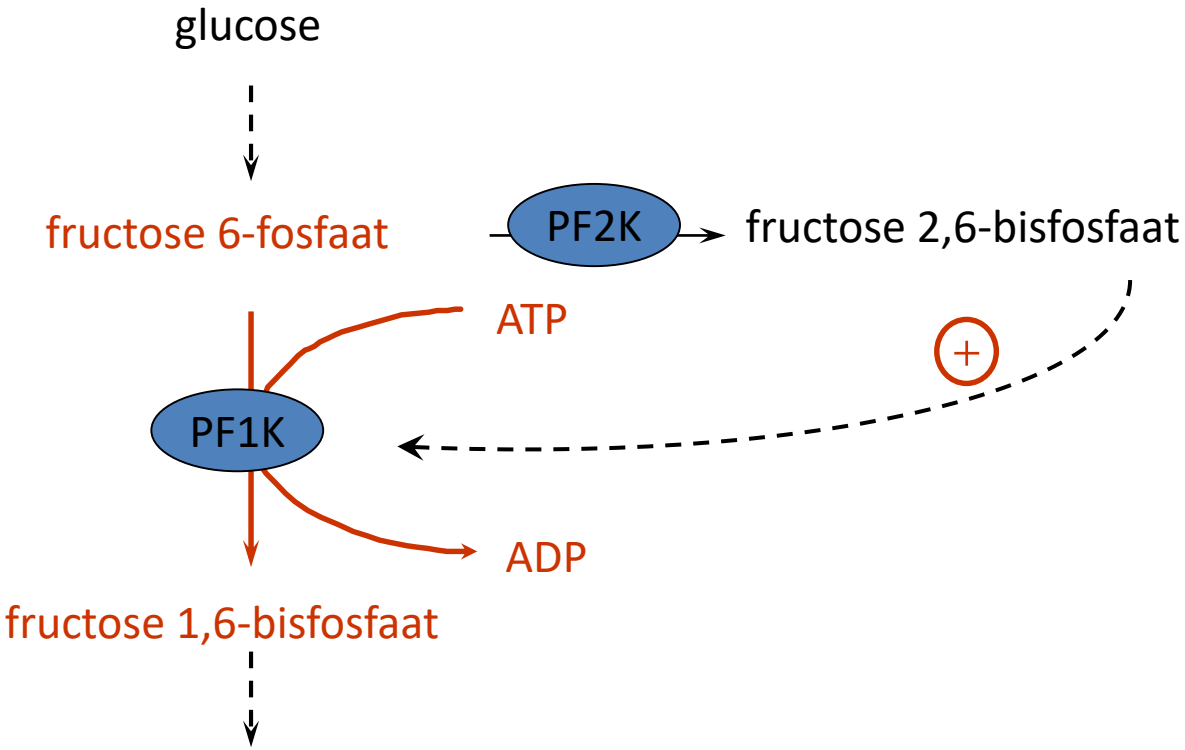
5. $C_1^J = \dfrac{\varepsilon_{X_1}^{v_2}}{\varepsilon_{X_1}^{v_2} - \varepsilon_{X_1}^{v_1}}$

6. $C_2^J = \dfrac{-\varepsilon_{X_1}^{v_1}}{\varepsilon_{X_1}^{v_2} - \varepsilon_{X_1}^{v_1}}$

7. $C_2^J / C_1^J = -\varepsilon_{X_1}^{v_1} / \varepsilon_{X_1}^{v_2}$



Example: the control by PFK on glycolysis



What will happen to the flux if phosphofructokinase (PF1K) is overexpressed?

Strong allosteric regulation → weak flux control

Anaerobic bakers'yeast

	PF1K V_{max}	$J_{glycolysis}$	PF2K V_{max}	[F26BP]
Wild-type yeast	1	1	1	1
PF1K overproducer	4.6	1.06	0.57	0.52

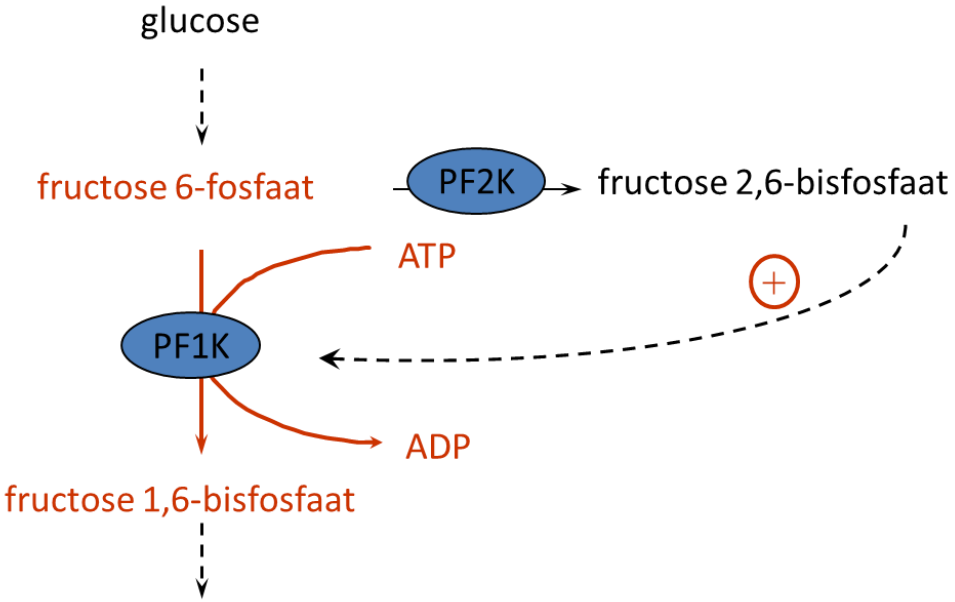
Numbers relative to wild type

The cell compensates for the overproduction of PF1K by decreasing the concentration of F26BP. Since PF1K is very sensitive to F26BP, the overproduction has little effect.

Davies & Brindle (1992) Biochemistry 31, 4729

Conclusion 2

An inelastic enzyme is insensitive to metabolite concentrations and exerts strong control.
(If you don't listen to others, they can only follow or ignore you).
An elastic enzyme is sensitive to metabolites and exerts little control.
(If you listen well, others can tell you what to do)



Question: *what would happen if the elastic enzymes have insufficient capacity to follow the inelastic enzyme?*

The matrix method

$$\begin{bmatrix} C_1^J & C_2^J & \dots & C_n^J \\ C_1^{X1} & C_2^{X1} & \dots & C_n^{X1} \\ \dots & \dots & \dots & \dots \\ C_1^{Xn} & C_2^{Xn} & \dots & C_n^{Xn} \end{bmatrix} \cdot \begin{bmatrix} 1 & -\varepsilon_{X1}^{v1} & \dots & -\varepsilon_{Xn}^{v1} \\ 1 & -\varepsilon_{X1}^{v2} & \dots & -\varepsilon_{Xn}^{v2} \\ \dots & \dots & \dots & \dots \\ 1 & -\varepsilon_{Xn}^{vn} & \dots & -\varepsilon_{Xn}^{vn} \end{bmatrix} = \begin{bmatrix} 1 & 0 & \dots & 0 \\ 0 & 1 & \dots & 0 \\ \dots & \dots & \dots & \dots \\ 0 & 0 & \dots & 1 \end{bmatrix}$$

$$\mathbf{C \cdot E = I}$$

For more extensive treatment of the matrix method, including branched pathways, see:

Westerhoff (1994) Getting to the inside of cells using metabolic control analysis. Biophys Chem 50, 273-83.
Hofmeyr (2001) Metabolic Control Analysis in a nutshell. Proc 2nd Int. Conf. Syst. Biol., 291-300.

Summation theorem flux

$$\begin{bmatrix} C_1^J & C_2^J & \dots & C_n^J \\ C_1^{X1} & C_2^{X1} & \dots & C_n^{X1} \\ \dots & \dots & \dots & \dots \\ C_1^{Xn} & C_2^{Xn} & \dots & C_n^{Xn} \end{bmatrix} \cdot \begin{bmatrix} 1 & -\varepsilon_{X1}^{v1} & \dots & -\varepsilon_{Xn}^{v1} \\ 1 & -\varepsilon_{X1}^{v2} & \dots & -\varepsilon_{Xn}^{v2} \\ \dots & \dots & \dots & \dots \\ 1 & -\varepsilon_{Xn}^{vn} & \dots & -\varepsilon_{Xn}^{vn} \end{bmatrix} = \begin{bmatrix} 1 & 0 & \dots & 0 \\ 0 & 1 & \dots & 0 \\ \dots & \dots & \dots & \dots \\ 0 & 0 & \dots & 1 \end{bmatrix}$$

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Summation theorem concentrations

$$\begin{bmatrix} C_1^J & C_2^J & \dots & C_n^J \\ C_1^{X1} & C_2^{X1} & \dots & C_n^{X1} \\ \dots & \dots & \dots & \dots \\ C_1^{Xn} & C_2^{Xn} & \dots & C_n^{Xn} \end{bmatrix} \begin{bmatrix} 1 & -\varepsilon_{X1}^{v1} & \dots & -\varepsilon_{Xn}^{v1} \\ 1 & -\varepsilon_{X1}^{v2} & \dots & -\varepsilon_{Xn}^{v2} \\ \dots & \dots & \dots & \dots \\ 1 & -\varepsilon_{Xn}^{vn} & \dots & -\varepsilon_{Xn}^{vn} \end{bmatrix} = \begin{bmatrix} 1 & 0 & \dots & 0 \\ 0 & 1 & \dots & 0 \\ \dots & \dots & \dots & \dots \\ 0 & 0 & \dots & 1 \end{bmatrix}$$

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Connectivity theorems (1)

$$\begin{bmatrix} C_1^J & C_2^J & \dots & C_n^J \\ C_1^{X1} & C_2^{X1} & \dots & C_n^{X1} \\ \dots & \dots & \dots & \dots \\ C_1^{Xn} & C_2^{Xn} & \dots & C_n^{Xn} \end{bmatrix} \cdot \begin{bmatrix} 1 & -\varepsilon_{X1}^{v1} & \dots & -\varepsilon_{Xn}^{v1} \\ 1 & -\varepsilon_{X1}^{v2} & \dots & -\varepsilon_{Xn}^{v2} \\ \dots & \dots & \dots & \dots \\ 1 & -\varepsilon_{Xn}^{vn} & \dots & -\varepsilon_{Xn}^{vn} \end{bmatrix} = \begin{bmatrix} 1 & 0 & \dots & 0 \\ 0 & 1 & \dots & 0 \\ \dots & \dots & \dots & \dots \\ 0 & 0 & \dots & 1 \end{bmatrix}$$

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Connectivity theorems (2)

$$\begin{bmatrix} C_1^J & C_2^J & \dots & C_n^J \\ C_1^{X1} & C_2^{X1} & \dots & C_n^{X1} \\ \dots & \dots & \dots & \dots \\ C_1^{Xn} & C_2^{Xn} & \dots & C_n^{Xn} \end{bmatrix} \cdot \begin{bmatrix} 1 & -\varepsilon_{X1}^{v1} & \dots & -\varepsilon_{Xn}^{v1} \\ 1 & -\varepsilon_{X1}^{v2} & \dots & -\varepsilon_{Xn}^{v2} \\ \dots & \dots & \dots & \dots \\ 1 & -\varepsilon_{Xn}^{vn} & \dots & -\varepsilon_{Xn}^{vn} \end{bmatrix} = \begin{bmatrix} 1 & 0 & \dots & 0 \\ 0 & 1 & \dots & 0 \\ \dots & \dots & \dots & \dots \\ 0 & 0 & \dots & 1 \end{bmatrix}$$

$$\mathbf{C \cdot E = I}$$

For more extensive treatment of the matrix method, including branched pathways, see:

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Connectivity theorems (3)

$$\begin{bmatrix} C_1^J & C_2^J & \dots & C_n^J \\ C_1^{X1} & C_2^{X1} & \dots & C_n^{X1} \\ \dots & \dots & \dots & \dots \\ C_1^{Xn} & C_2^{Xn} & \dots & C_n^{Xn} \end{bmatrix} \cdot \begin{bmatrix} 1 & -\varepsilon_{X1}^{v1} & \dots & -\varepsilon_{Xn}^{v1} \\ 1 & -\varepsilon_{X1}^{v2} & \dots & -\varepsilon_{Xn}^{v2} \\ \dots & \dots & \dots & \dots \\ 1 & -\varepsilon_{Xn}^{vn} & \dots & -\varepsilon_{Xn}^{vn} \end{bmatrix} = \begin{bmatrix} 1 & 0 & \dots & 0 \\ 0 & 1 & \dots & 0 \\ \dots & \dots & \dots & \dots \\ 0 & 0 & \dots & 1 \end{bmatrix}$$

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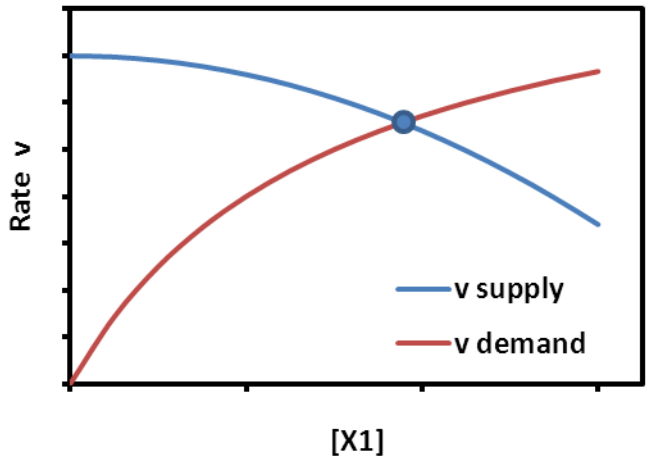
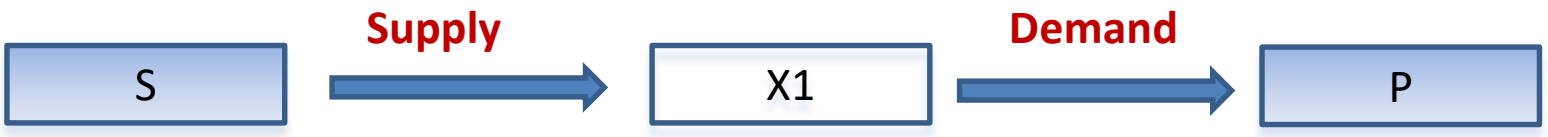
Conclusion 3

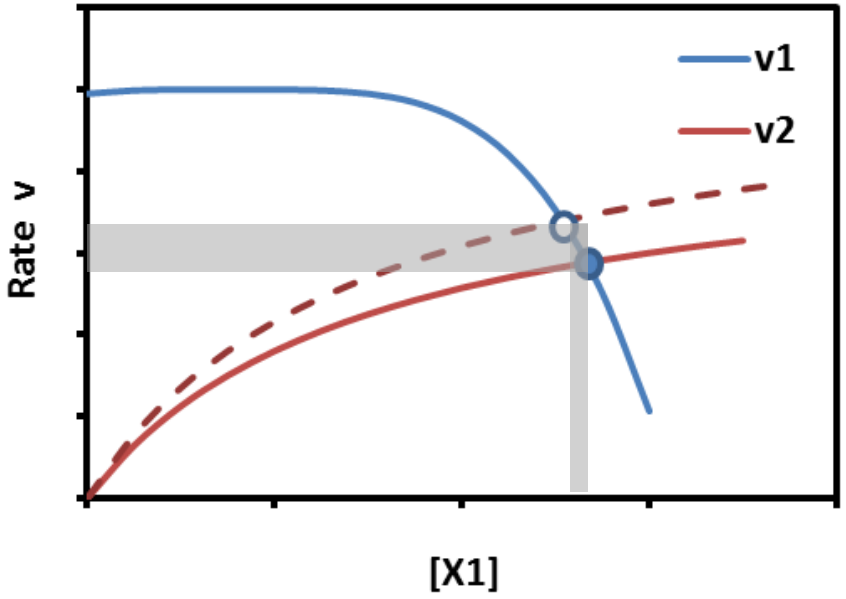
- The matrix method for Metabolic Control Analysis gived a complete and systematic overview of all control properties.
- The matrix method allows to compute control coefficients as a function of elasticities and vice versa.
- The matrix method is a valuable tool to check accuracy of calculated control coefficients.

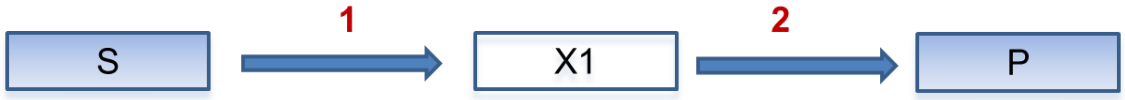
How to maintain metabolite homeostasis and alter the flux?





Control in demand:
Metabolite homeostasis by high elasticity



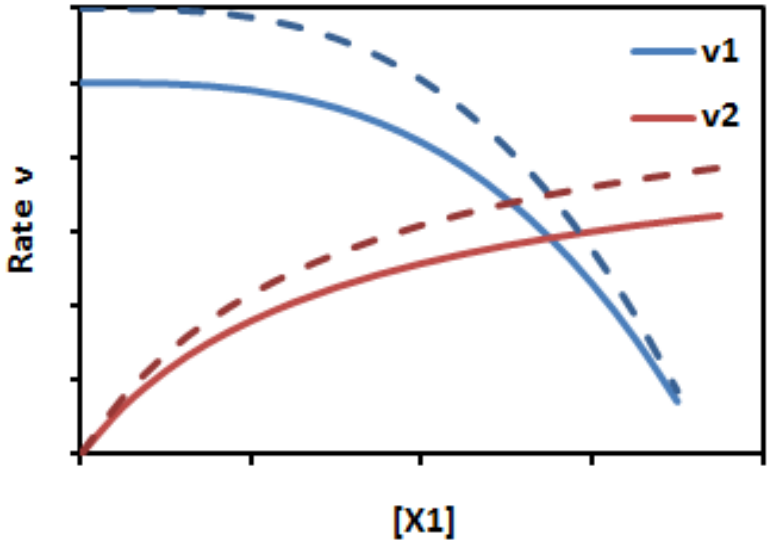


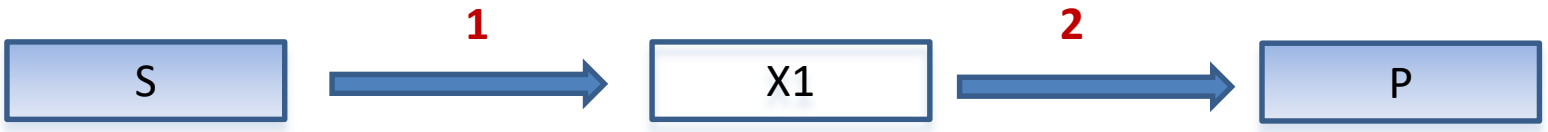
To work out for yourself what happens if the demand becomes more elastic (steeper) to: 1. flux control; 2. concentration control

See also: Hofmeyr & Rohwer (2011) In: Meth. Enzymol 500, pp 533-

Multisite modulation

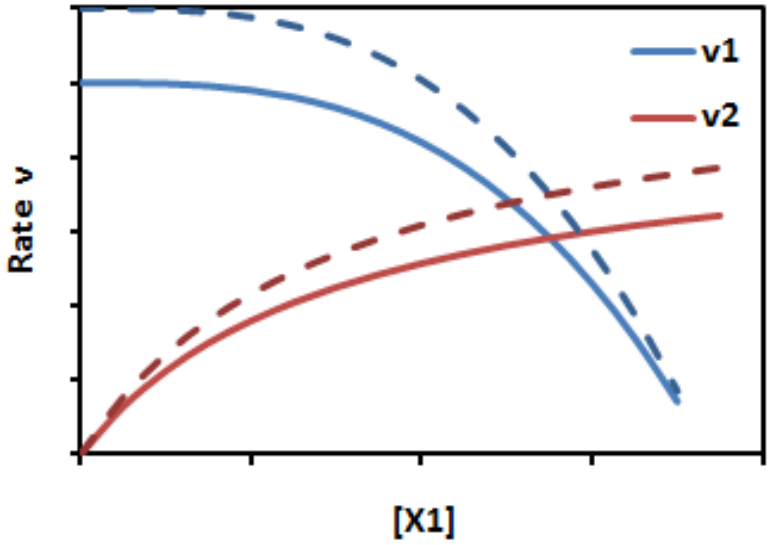
Enzyme concentration or V_{max} +20%

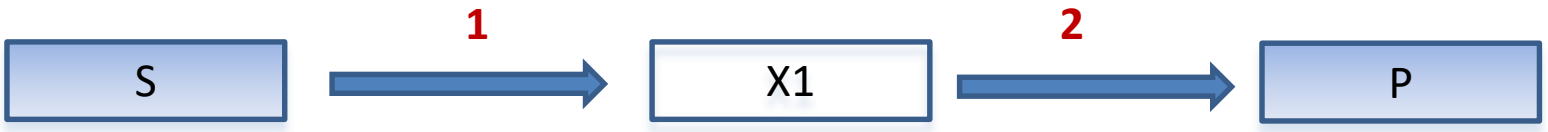




Multisite modulation

Enzyme concentration or V_{max} +20%





Protein phosphorylation can regulate metabolite concentrations rather than control flux: The example of glycogen synthase

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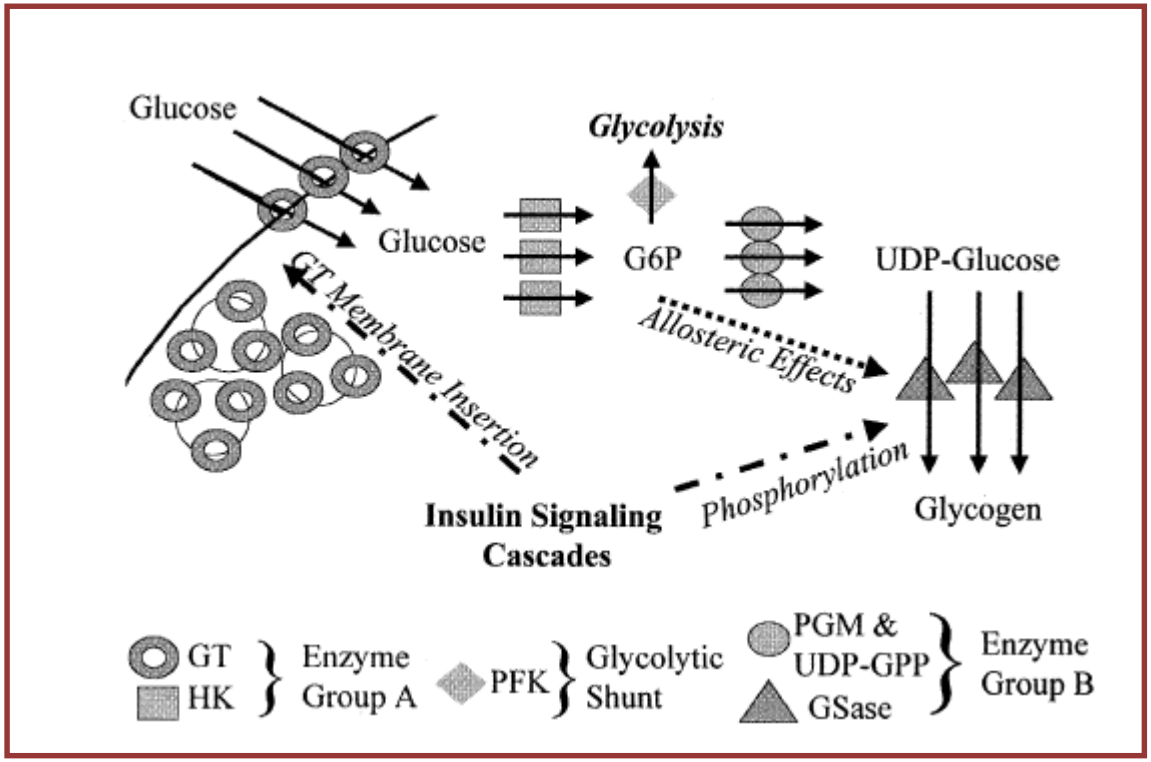
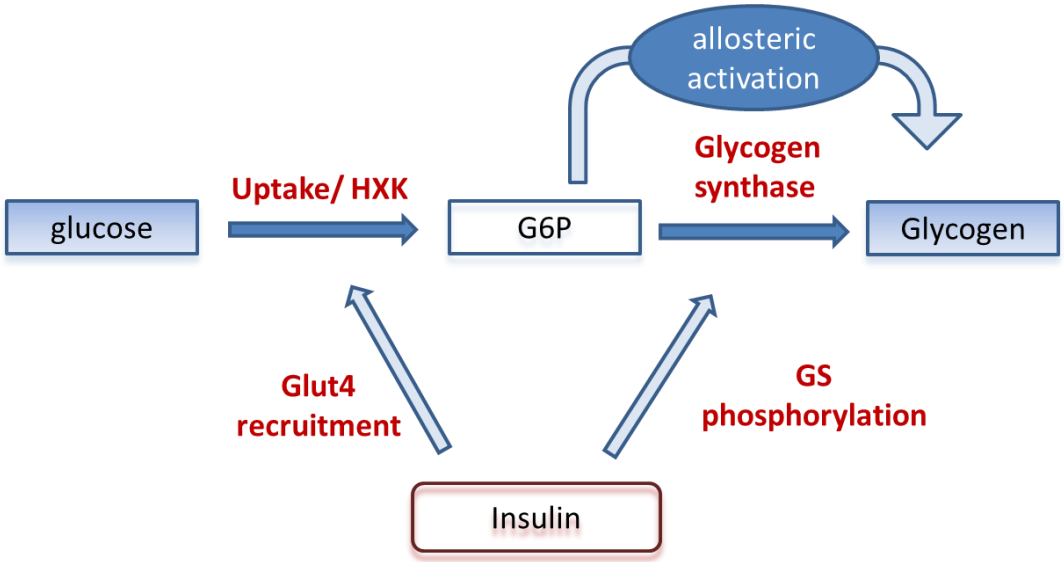
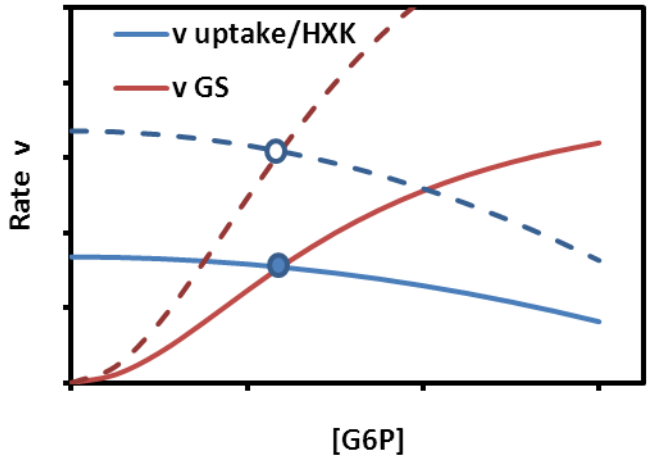


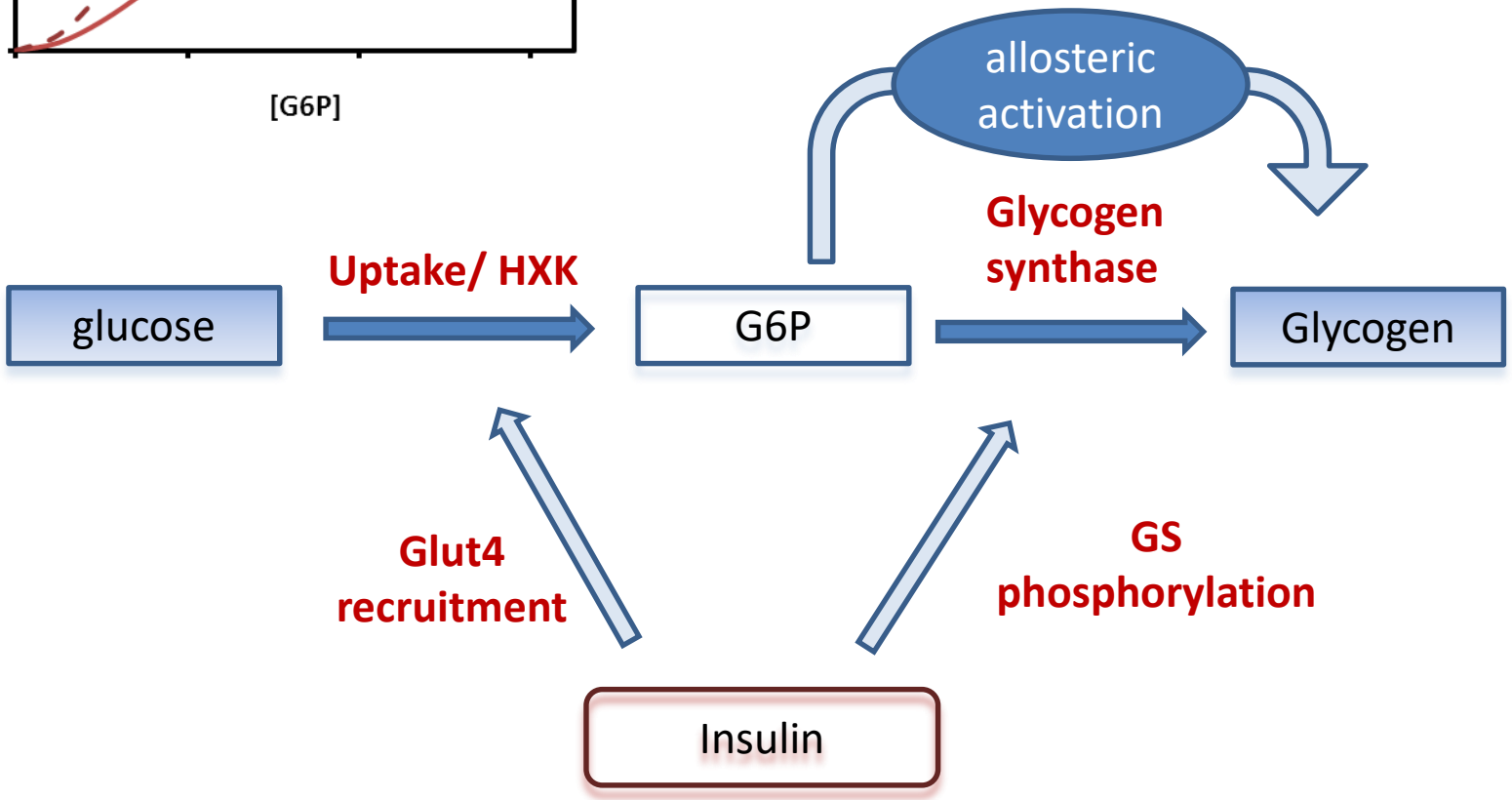


Given: Glycogen synthesis flux is controlled by glucose uptake + HXK (i.e. glucose uptake and HXK have a high flux control coefficient)

Question: What is the function of simultaneous activation of glucose uptake and glycogen synthase by insulin?

Multisite-modulation → metabolite homeostasis





Conclusion 4

To affect a flux with little effect on concentrations (homeostasis):

1. The other enzyme(s) need to be very sensitive to metabolite concentrations (high elasticity), or:

2. Both enzymes need to be up/down regulated together (multisite modulation)