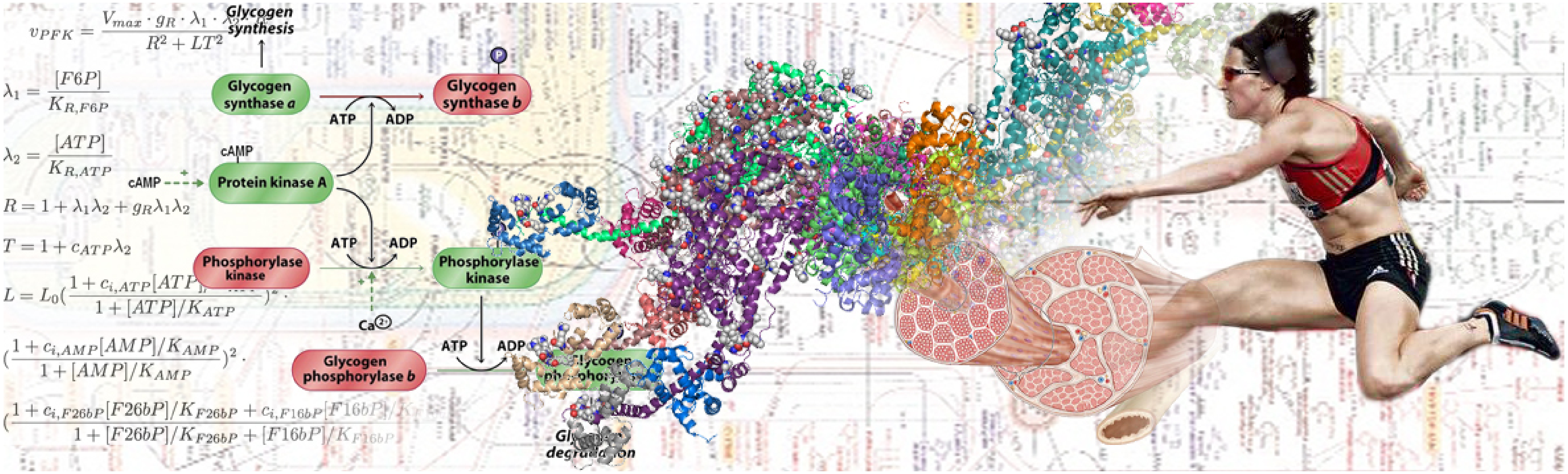
Biochemistry 714 Mini-course: Molecular Systems Biology



Prof Johann Rohwer (lectures), Dr Dawie van Niekerk (tutorials and data analysis),
Prof Jacky Snoep (tutorials and practical)

March – April 2025

Thus far

- First Lecture: Chemical kinetics
- Direction of reaction: ΔG, Γ / K_{eq}
- How far: K_{eq}, ΔG^0
- How fast: mass action kinetics
- Second Lecture: Enzyme kinetics
- Derivation of rate equations: equilibrium binding, steady-state approximation
- V_{max}, K_m, saturation, cooperativity, allostery, reversibility, product inhibition
- Third Lecture: Coupled reactions
- Parameter estimation; initial rates, progress curves
- Closed, open systems; equilibrium, steady state, rate characteristics
- Kinetic model of simple pathways in steady state
- Fourth Lecture: Structural network analysis
- N, K, L matrices
- Steady-state flux constraints, flux analysis, flux modes
- Moiety conservation relationships

This lecture:
Metabolic Control Analysis (MCA)

- Quantifies the importance of each of the enzymes in determining the steady-state system variables in terms of control coefficients
- Relates this importance for system behaviour to characteristics of the isolated components (elasticities)

Metabolic control analysis: Relationship between Response, control and elasticity coefficients

The steady state of an open system

Parameters - The controllers

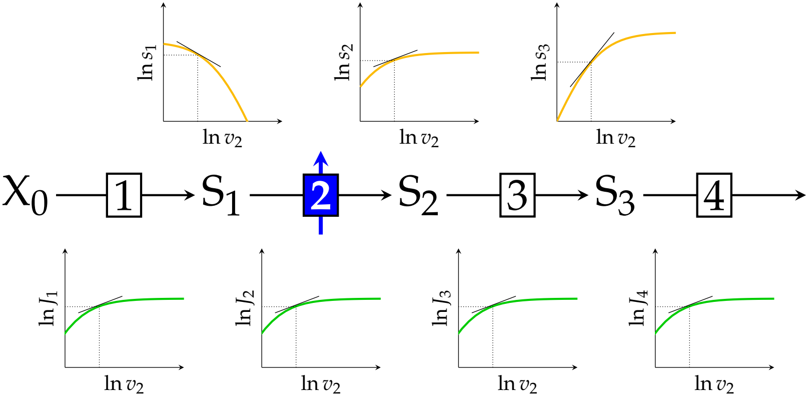
- Fixed concentrations (external)
- Thermodynamic constants (equilibrium constants)
- Kinetic constants (k_{cat}, K_{M}, enzyme concentrations)
- Moiety-conserved sums of concentrations

Variables - The controlled

- Fluxes, J (i.e., reaction rates in steady state)
- Concentrations of network intermediates
- Functions of fluxes and concentrations (ratios, potentials, etc.)

Control coefficients

$$C^{s_1}_{v_2} = \frac{\partial \ln s_1}{\partial \ln v_2} < 0 \qquad C^{s_2}_{v_2} = \frac{\partial \ln s_2}{\partial \ln v_2} > 0 \qquad C^{s_3}_{v_2} = \frac{\partial \ln s_3}{\partial \ln v_2} > 0$$



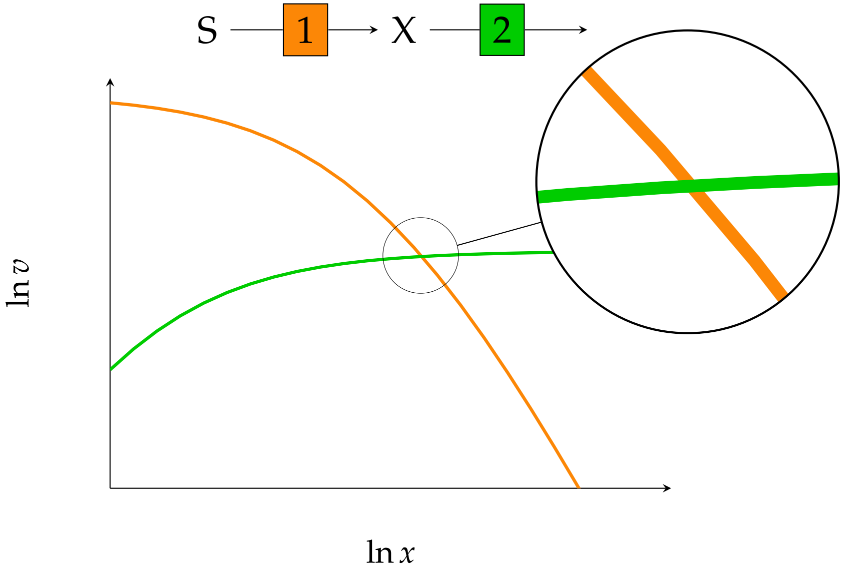
$$C^{J_1}_{v_2} = \frac{\partial \ln J_1}{\partial \ln v_2} = C^{J_2}_{v_2} = \frac{\partial \ln J_2}{\partial \ln v_2} = C^{J_3}_{v_2} = \frac{\partial \ln J_3}{\partial \ln v_2} = C^{J_4}_{v_2} = \frac{\partial \ln J_4}{\partial \ln v_2}$$

Control coefficient—Mathematical definition

For any steady-state variable y the control coefficient of reaction i is given by

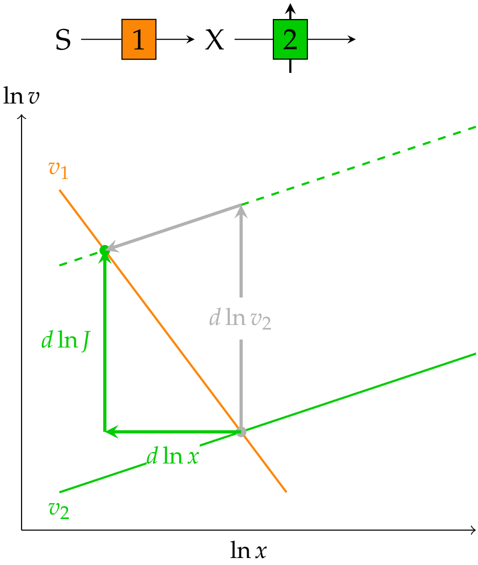
$$C^{y}_{v_i} = \frac{\partial \ln y}{\partial \ln v_i}$$

Rate characteristics linearised in log-log space



Control coefficients and the summation properties

The steady-state concentration x has changed by $d \ln x$



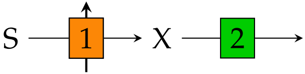
Flux-control coefficient

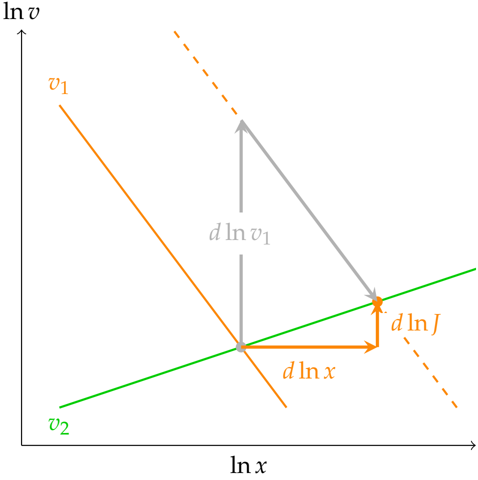
$$C^J_{v_2} = \frac{d \ln J}{d \ln v_2}$$

Concentration-control coefficient

$$C^x_{v_2} = \frac{d \ln x}{d \ln v_2}$$

The steady-state concentration x has changed by $d \ln x$





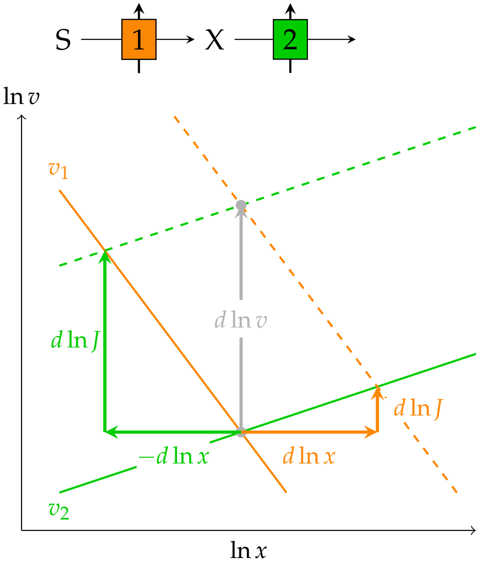
Flux-control coefficient

$$C^J_{v_1} = \frac{d \ln J}{d \ln v_1}$$

Concentration-control coefficient

$$C^x_{v_1} = \frac{d \ln x}{d \ln v_1}$$

Summation property



of flux-control coefficients

$$d \ln J + d \ln J = d \ln v$$

$$\frac{d \ln J}{d \ln v} + \frac{d \ln J}{d \ln v} = \frac{d \ln v}{d \ln v}$$

$$C^J_{v_1} + C^J_{v_2} = 1$$

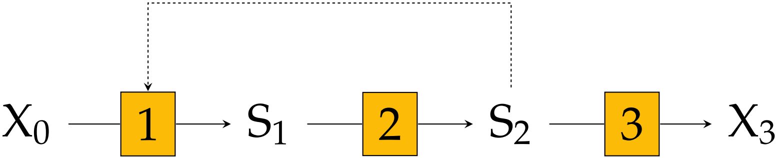
of concentration-control coefficients

$$d \ln x + (-d \ln x) = 0$$

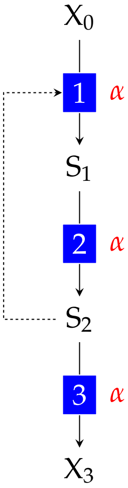
$$\frac{d \ln x}{d \ln v} + \left(\frac{-d \ln x}{d \ln v} \right) = 0$$

$$C^x_{v_1} + C^x_{v_2} = 0$$

The system



Thought experiment: Summation properties



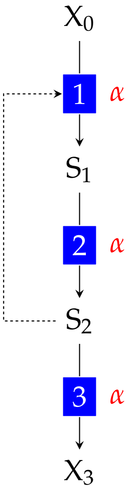
What would happen if all the reaction rates are changed simultaneously by the factor α?

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

or, in terms of logarithmic changes,

$$\delta \ln v_1 = \delta \ln v_2 = \delta \ln v_3 = \alpha$$

Flux summation properties



The effect on the flux, J, is the sum of all the individual rate changes:

$$\delta \ln J = C^J_{v_1} \delta \ln v_1 + C^J_{v_2} \delta \ln v_2 + C^J_{v_3} \delta \ln v_3$$

But, the flux also increases by factor α, and since

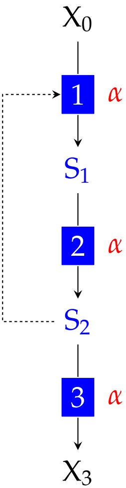
$$\delta \ln v_1 = \delta \ln v_2 = \delta \ln v_3 = \alpha$$

this simplifies to $\alpha = \alpha(C^J_{v_1} + C^J_{v_2} + C^J_{v_3})$

Therefore

$$C^J_{v_1} + C^J_{v_2} + C^J_{v_3} = 1$$

Concentration summation properties



The concentrations s_1 and s_2 remain unchanged.

Therefore, for s_1:

$$\begin{aligned} \delta \ln s_1 &= C^{s_1}_{v_1} \delta \ln v_1 + C^{s_1}_{v_2} \delta \ln v_2 + C^{s_1}_{v_3} \delta \ln v_3 \\ 0 &= \alpha \left(C^{s_1}_{v_1} + C^{s_1}_{v_2} + C^{s_1}_{v_3} \right) \end{aligned}$$

Therefore

$$C^{s_1}_{v_1} + C^{s_1}_{v_2} + C^{s_1}_{v_3} = 0$$

Similarly, for s_2:

$$C^{s_2}_{v_1} + C^{s_2}_{v_2} + C^{s_2}_{v_3} = 0$$

General summation theorems

For any steady-state flux, J_m

$$\sum_{i=1}^{n} C_{v_i}^{J_m} = 1$$

For any steady-state concentration s_j

$$\sum_{i=1}^{n} C_{v_i}^{s_j} = 0$$

where n = number of enzymes in the system.

Elasticity coefficients
and the
connectivity properties

Elasticity coefficient—Operational definition

What is the change in reaction rate v_i when a metabolite S_j that directly influences the reaction (substrate or product or effector) changes, while all other metabolites remain constant? For a small fractional change in s_j:

$$\delta \ln v_\mathrm{i} = \varepsilon^{v_i}_{s_j} \cdot \delta \ln s_j$$

Operational meaning:
If s_j changes by 1%, the rate v_i changes by $\varepsilon^{v_i}_{s}$%.

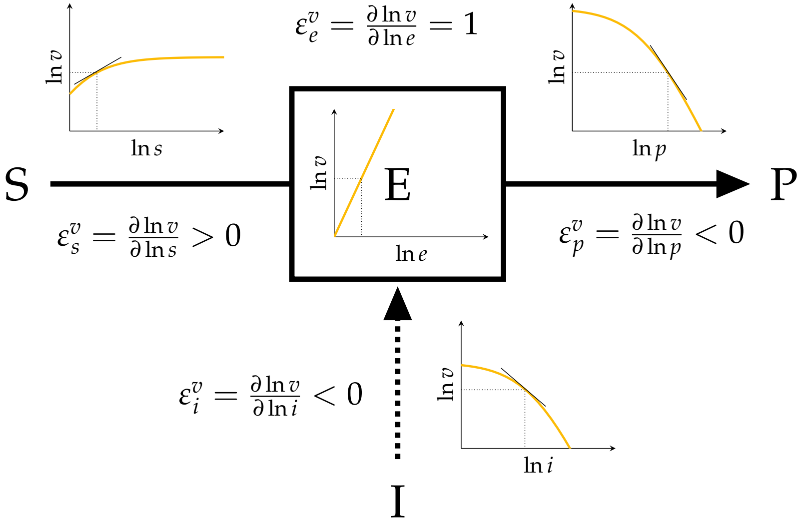
or

$\varepsilon^{v_i}_{s}$ is the % change in v_i when s_j changes by 1%.

Elasticity coefficient—Mathematical definition

$$\begin{aligned}
\varepsilon^{v_i}_{s_j} &= \left(\frac{\partial v_i / v_i}{\partial s_j / s_j}\right)_{s_k, s_l, \ldots} \\
&= \frac{s_j}{v_i}\left(\frac{\partial v_i}{\partial s_j}\right)_{s_k, s_l, \ldots} \\
&= \left(\frac{\partial \ln v_i}{\partial \ln s_j}\right)_{s_k, s_l, \ldots}
\end{aligned}$$

Elasticity coefficients of an enzyme-catalysed reaction



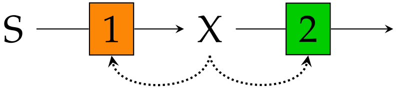
$$\varepsilon^v_e = \frac{\partial \ln v}{\partial \ln e} = 1$$

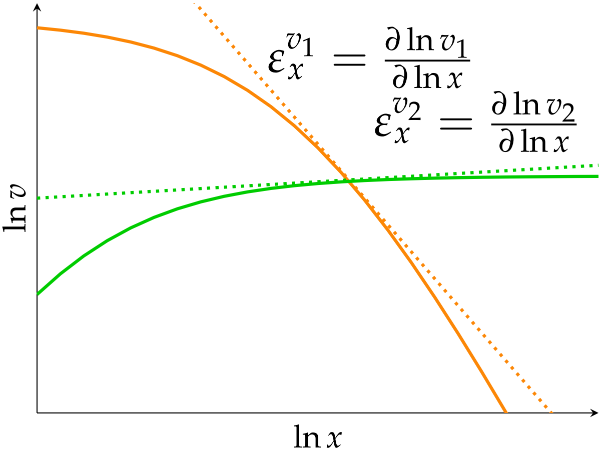
$$\varepsilon^v_s = \frac{\partial \ln v}{\partial \ln s} > 0$$

$$\varepsilon^v_p = \frac{\partial \ln v}{\partial \ln p} < 0$$

$$\varepsilon^v_i = \frac{\partial \ln v}{\partial \ln i} < 0$$

Elasticity coefficient of v_2 with respect to x





$$\varepsilon_x^{v_1} = \frac{\partial \ln v_1}{\partial \ln x}$$

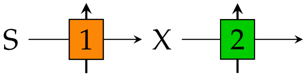
$$\varepsilon_x^{v_2} = \frac{\partial \ln v_2}{\partial \ln x}$$

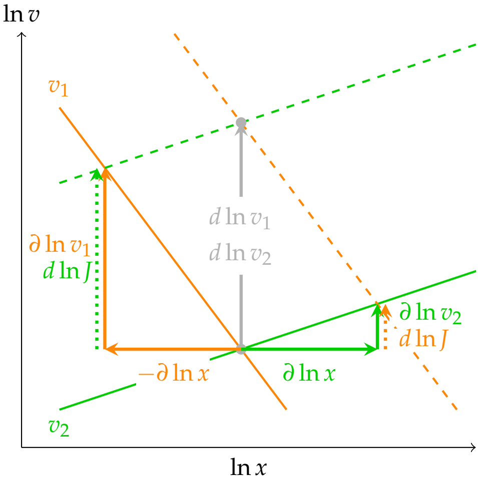
Quantitative analysis of metabolic functions

The relative values of the slopes of the tangents of the rate characteristics at steady state (the elasticity coefficients, ε) determine metabolic functions:

1. the distribution of flux control
2. the magnitude of concentration control (degree of homeostasis)

Connectivity property





for the flux J

$$\frac{d\ln J}{d\ln J} = \frac{\partial \ln v_2}{\partial \ln v_1}$$

$$\frac{\frac{d\ln J}{d\ln v_1}}{\frac{d\ln J}{d\ln v_2}} = \frac{\frac{\partial \ln v_2}{\partial \ln x}}{-\left(\frac{\partial \ln v_1}{-\partial \ln x}\right)}$$

$$\frac{C^J_{v_1}}{C^J_{v_2}} = -\frac{\varepsilon^{v_2}_x}{\varepsilon^{v_1}_x}$$

$$C^J_{v_1}\varepsilon^{v_1}_x + C^J_{v_2}\varepsilon^{v_2}_x = 0$$

Connectivity property

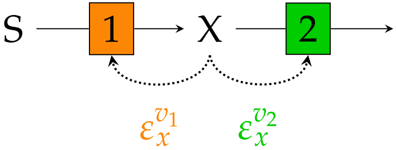
for the concentration x

$$C_{v_1}^{x}\varepsilon_{x}^{v_1} + C_{v_2}^{x}\varepsilon_{x}^{v_2} = -1$$

Derive this yourself!

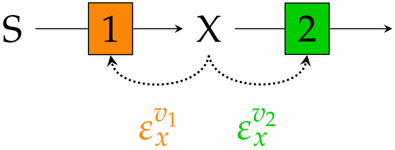
Putting it all together:
Control
in terms of
elasticities

Summary of control properties



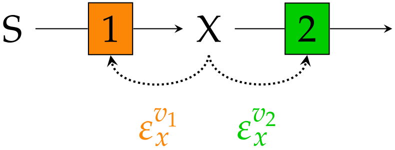
	Summation	Connectivity
Flux	$C_1^J + C_2^J = 1$	$C_1^J \varepsilon_x^{v_1} + C_2^J \varepsilon_x^{v_2} = 0$
Conc.	$C_1^x + C_2^x = 0$	$C_1^x \varepsilon_x^{v_1} + C_2^x \varepsilon_x^{v_2} = -1$

Control matrix equation



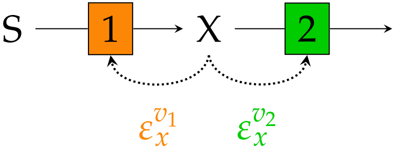
$$\begin{bmatrix} C_1^J & C_2^J \\ C_1^x & C_2^x \end{bmatrix} \begin{bmatrix} 1 & -\varepsilon_x^{v_1} \\ 1 & -\varepsilon_x^{v_2} \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}$$

Control matrix equation



$$\begin{bmatrix} C_1^J & C_2^J \\ C_1^x & C_2^x \end{bmatrix} = \begin{bmatrix} 1 & -\varepsilon_x^{v_1} \\ 1 & -\varepsilon_x^{v_2} \end{bmatrix}^{-1}$$

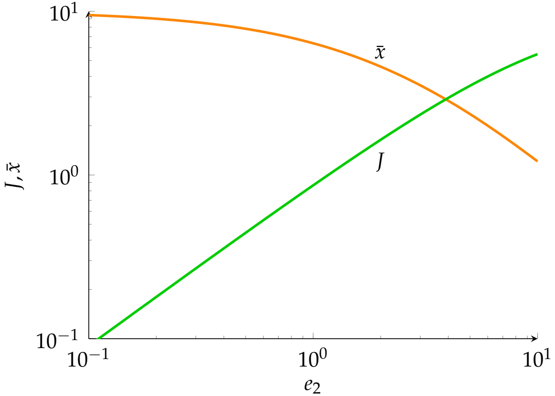
Control analytic expressions



$$C_1^J = \frac{\varepsilon_x^{v_2}}{\varepsilon_x^{v_2} - \varepsilon_x^{v_1}} \qquad C_2^J = \frac{-\varepsilon_x^{v_1}}{\varepsilon_x^{v_2} - \varepsilon_x^{v_1}}$$

$$C_1^x = \frac{1}{\varepsilon_x^{v_2} - \varepsilon_x^{v_1}} \qquad C_2^x = \frac{-1}{\varepsilon_x^{v_2} - \varepsilon_x^{v_1}}$$

The response coefficients of E_2



slopes
of lines in a
parameter portrait

$$R^J_{e_2} = \frac{d \ln J}{d \ln e_2} \qquad R^x_{e_2} = \frac{d \ln x}{d \ln e_2}$$

General definition of a response coefficient

For any steady-state variable y and any parameter p

$$R_p^y = \frac{d \ln y}{d \ln p}$$

This can be re-cast in terms of fractional changes

$$R_p^y = \frac{dy/y}{dp/p}$$

A response coefficient is therefore also a ratio of *percentage changes*.
It can be defined operationally as the %-change in y caused by a 1%-change in parameter p.

The partitioned response

Consider a perturbation in a parameter that only affects v_2, e.g. the enzyme concentration e_2.

The effect on a steady-state variable such as J must be propagated through a local change in v_2.

$$\frac{d\ln J}{d\ln e_2} = \frac{\partial \ln v_2}{\partial \ln e_2} \cdot \frac{d\ln J}{d\ln v_2}$$

or in terms of coefficient symbols,

$$R^J_{e_2} = \varepsilon^{v_2}_{e_2} C^J_{v_2}$$

The generalised partitioned response

A partitioned response equation exists for any parameter p and any steady-state variable y (flux or concentration).

The effect of a change δp on the variable y can be partitioned into a local rate effect, δv_i, (quantified by the elasticity coefficient, $\varepsilon_p^{v_i}$) and the subsequent system-wide effect of δv_i on y (quantified by the control coefficient $C_{v_i}^y$).

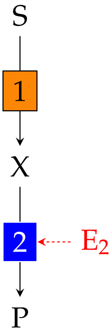
If p affects only reaction i:

$$R_p^y = C_{v_i}^y \varepsilon_p^{v_i}$$

If p affects more than one step in a network of n reactions:

$$R_p^y = \sum_{i=1}^{n} C_{v_i}^y \varepsilon_p^{v_i}$$

R^y_e and C^y_e are usually equivalent



$$R^J_{e_2} = C^J_{v_2}\varepsilon^{v_2}_{e_2}$$

$$R^x_{e_2} = C^x_{v_2}\varepsilon^{v_2}_{e_2}$$

Reaction rate is usually proportional to enzyme concentration, i.e. $\varepsilon^{v_2}_{e_2} = 1$. Therefore,

$$R^J_{e_2} = C^J_{v_2}$$

$$R^x_{e_2} = C^x_{v_2}$$

This is not normally true if the parameter is something different from an enzyme concentration (e.g. K_M).

Control *vs.* response coefficients

A control coefficient is the property of a step (i.e. parameter-independent).

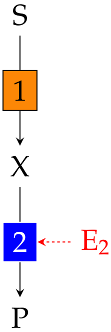
A response coefficient is the property of a parameter.

$$C^y_{v_i} = \frac{R^y_p}{\varepsilon^{v_i}_p} = \frac{d\ln y / d\ln p}{d\ln v_i / d\ln p} = \frac{d\ln y}{d\ln v_i}$$

If the parameter is an enzyme concentration, then, because $\varepsilon^{v_i}_{e_i} = 1$,

$$C^y_{v_i} = \frac{R^y_{e_i}}{\varepsilon^{v_i}_{e_i}} = R^y_{e_i}$$

Thought experiment: Partitioned response



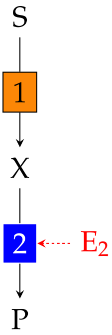
Consider a small change in the concentration of a parameter, say δe_2. The immediate localised effect is to change the rate v_2 by the amount

$$\delta \ln v_2 = \varepsilon^{v_2}_{e_2} \cdot \delta \ln e_2$$

The change in v_2 is then propagated through the system to cause a change in the steady state. For example, for the flux J:

$$\delta \ln J = C^J_{v_2} \cdot \delta \ln v_2$$

Thought experiment: $R = C\varepsilon$



Linking the local effect to the subsequent systemic effect gives:

$$\delta \ln J = C^J_{v_2} \varepsilon^{v_2}_{e_2} \delta \ln e_2 \quad \text{or} \quad \frac{\delta \ln J}{\delta \ln e_2} = C^J_{v_2} \varepsilon^{v_2}_{e_2}$$

which can be written as

$$R^J_{e_2} = C^J_{v_2} \varepsilon^{v_2}_{e_2}$$

Similarly, for the steady-state concentration X:

$$R^x_{e_2} = C^x_{v_2} \varepsilon^{v_2}_{e_2}$$