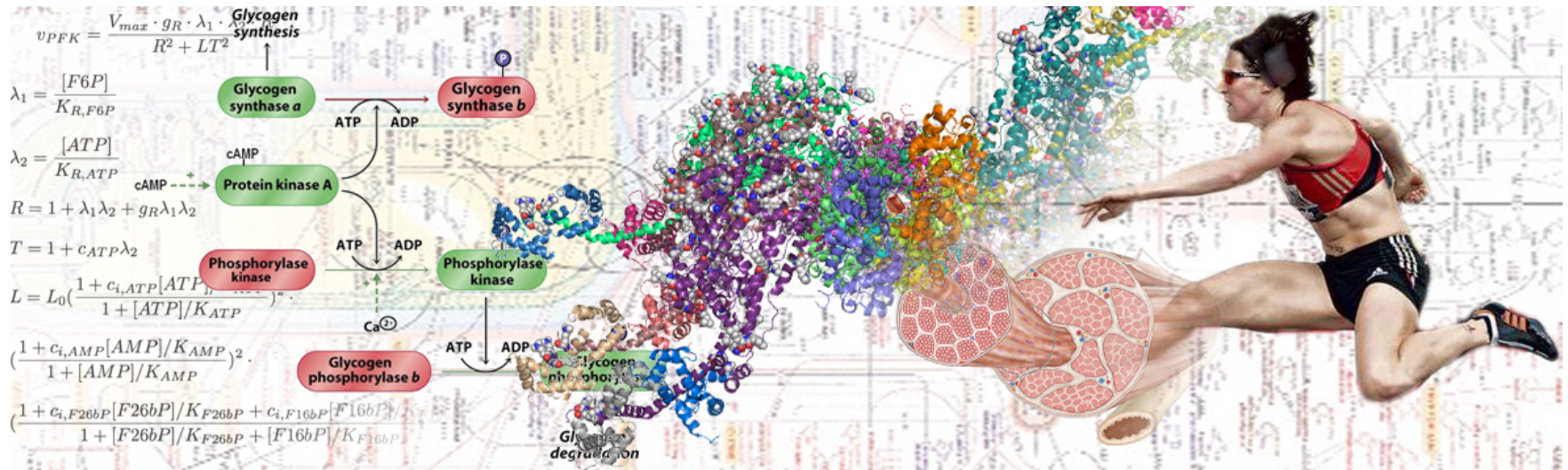


Biochemistry 714 Mini-course: Molecular Systems Biology



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

March - April 2024

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

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

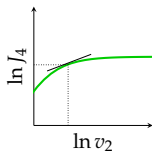
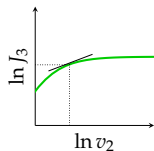
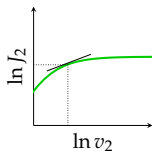
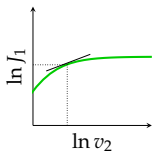
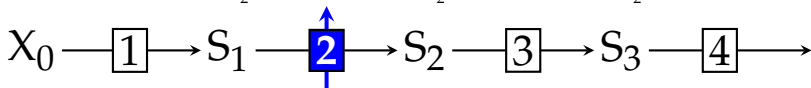
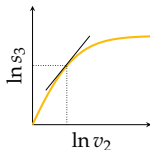
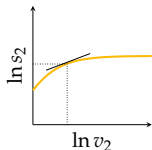
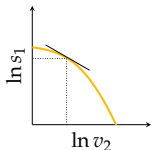
- ▶ Fixed concentrations (external)
- ▶ Thermodynamic constants (equilibrium constants)
- ▶ Kinetic constants (k_{cat} , K_M , enzyme concentrations)
- ▶ Moiety-conserved sums of concentrations

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

$$C_{v_2}^{s_1} = \frac{\partial \ln s_1}{\partial \ln v_2} < 0 \quad C_{v_2}^{s_2} = \frac{\partial \ln s_2}{\partial \ln v_2} > 0 \quad C_{v_2}^{s_3} = \frac{\partial \ln s_3}{\partial \ln v_2} > 0$$



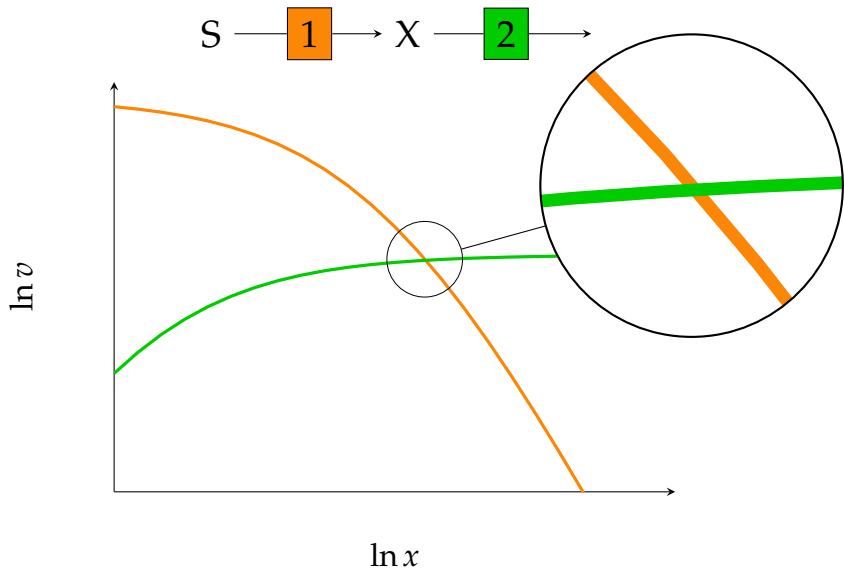
$$C_{v_2}^{J_1} = \frac{\partial \ln J_1}{\partial \ln v_2} = C_{v_2}^{J_2} = \frac{\partial \ln J_2}{\partial \ln v_2} = C_{v_2}^{J_3} = \frac{\partial \ln J_3}{\partial \ln v_2} = C_{v_2}^{J_4} = \frac{\partial \ln J_4}{\partial \ln v_2}$$

Control coefficient—Mathematical definition

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

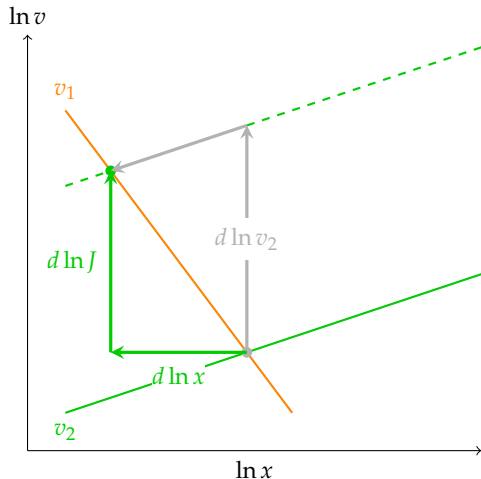
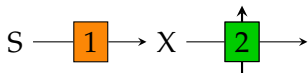
$$C_{v_i}^y = \frac{\partial \ln y}{\partial \ln v_i}$$

Rate characteristics linearised in log-log space



Control coefficients and the summation properties

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



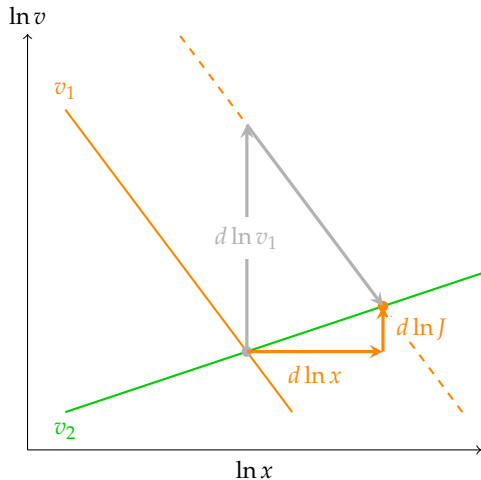
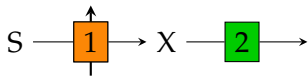
Flux-control coefficient

$$C_{v_2}^J = \frac{d \ln J}{d \ln v_2}$$

Concentration-control coefficient

$$C_{v_2}^x = \frac{d \ln x}{d \ln v_2}$$

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



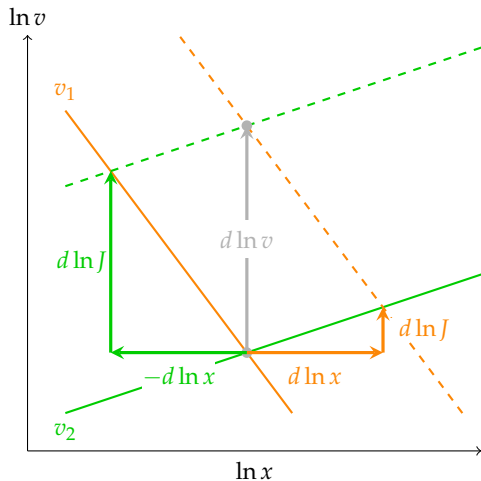
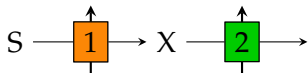
Flux-control coefficient

$$C_{v_1}^J = \frac{d \ln J}{d \ln v_1}$$

Concentration-control coefficient

$$C_{v_1}^x = \frac{d \ln x}{d \ln v_1}$$

Summation property



of flux-control coefficients

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

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

$$C_{v_1}^J + C_{v_2}^J = 1$$

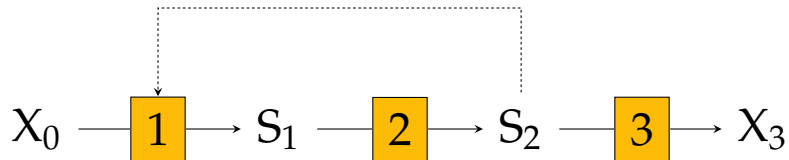
of concentration-control coefficients

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

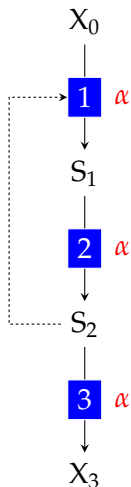
$$\frac{d \ln x}{d \ln v} + \left(\frac{-d \ln x}{d \ln v} \right) = 0$$

$$C_{v_1}^x + C_{v_2}^x = 0$$

The system



Thought experiment: Summation properties



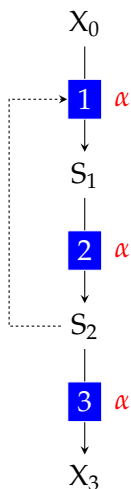
What would happen if all the **reaction rates** are changed simultaneously by the **factor α** ?

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

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:

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

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

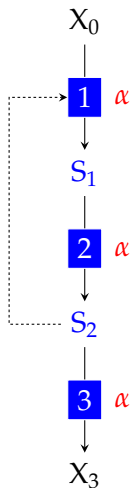
$$\delta \ln v_1 = \delta \ln v_2 = \delta \ln v_3 = \alpha$$

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

Therefore

$$C_{v_1}^J + C_{v_2}^J + C_{v_3}^J = 1$$

Concentration summation properties



The concentrations s_1 and s_2 remain unchanged.

Therefore, for s_1 :

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

Therefore

$$C_{v_1}^{s_1} + C_{v_2}^{s_1} + C_{v_3}^{s_1} = 0$$

Similarly, for s_2 :

$$C_{v_1}^{s_2} + C_{v_2}^{s_2} + C_{v_3}^{s_2} = 0$$

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 :

$$\delta \ln v_i = \varepsilon_{s_j}^{v_i} \cdot \delta \ln s_j$$

Operational meaning:

If s_j changes by 1%, the rate v_i changes by $\varepsilon_s^{v_i}$ %.

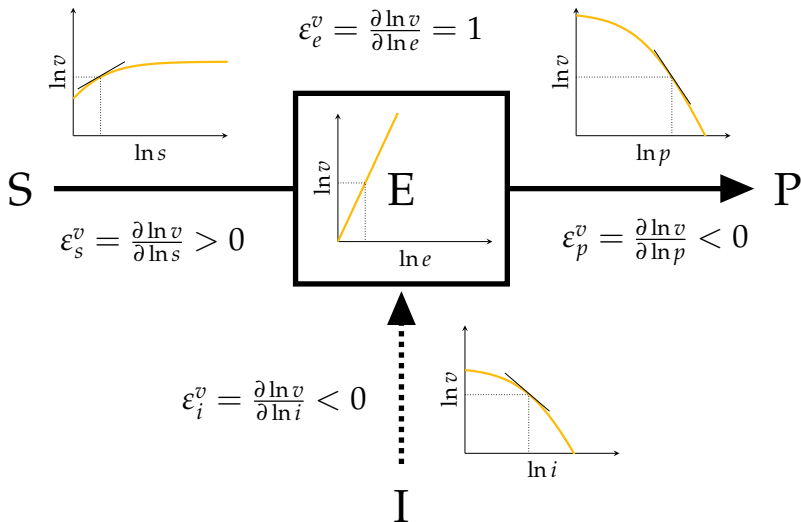
or

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

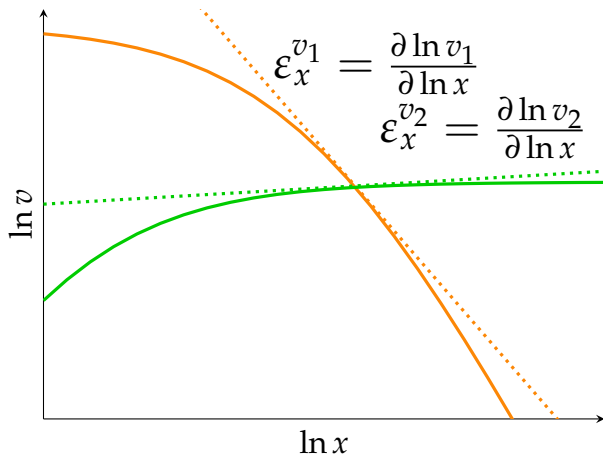
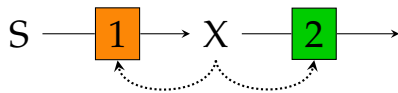
Elasticity coefficient—Mathematical definition

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

Elasticity coefficients of an enzyme-catalysed reaction



Elasticity coefficient of v_2 with respect to x

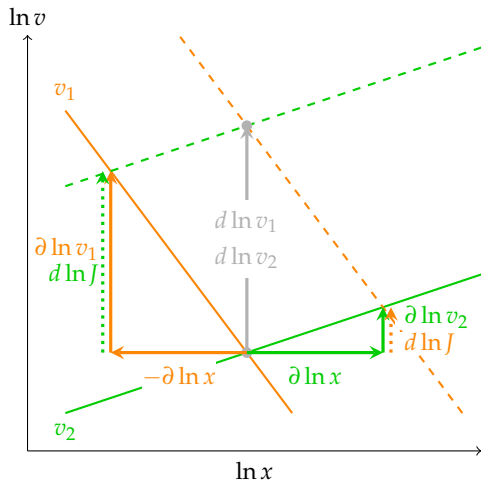
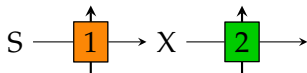


Quantitative analysis of metabolic functions

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

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

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

$$\frac{C_{v_1}^J}{C_{v_2}^J} = -\frac{\varepsilon_x^{v_2}}{\varepsilon_x^{v_1}}$$

$$C_{v_1}^J \varepsilon_x^{v_1} + C_{v_2}^J \varepsilon_x^{v_2} = 0$$

Connectivity property

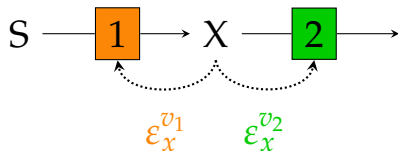
for the concentration x

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

Derive this yourself!

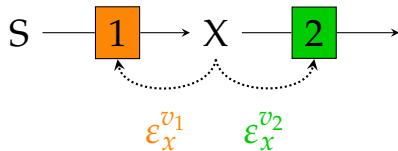
Putting it all together:
Control
in terms of
elasticities

Summary of control properties



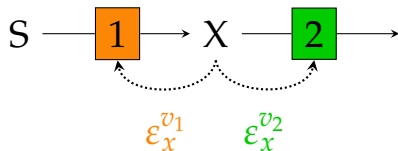
	Summation	Connectivity
Flux	$C_1^J + C_2^J = 1$	$C_1^J \epsilon_x^{v_1} + C_2^J \epsilon_x^{v_2} = 0$
Conc.	$C_1^x + C_2^x = 0$	$C_1^x \epsilon_x^{v_1} + C_2^x \epsilon_x^{v_2} = -1$

Control matrix equation



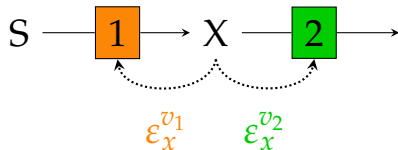
$$\begin{bmatrix} C_1^J & C_2^J \\ C_1^x & C_2^x \end{bmatrix} \begin{bmatrix} 1 & -\epsilon_x^{v_1} \\ 1 & -\epsilon_x^{v_2} \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}$$

Control matrix equation



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

Control analytic expressions



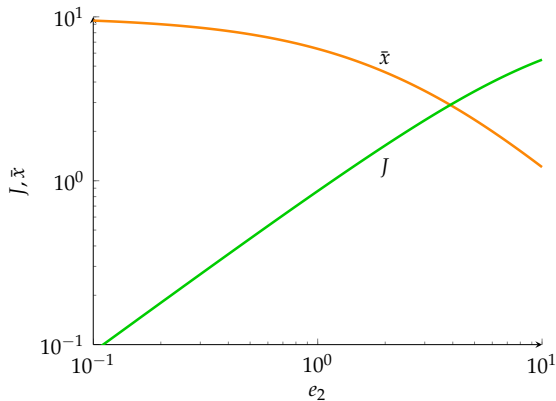
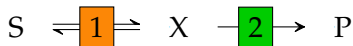
$$C_1^J = \frac{\epsilon_x^{v2}}{\epsilon_x^{v2} - \epsilon_x^{v1}}$$

$$C_1^x = \frac{1}{\epsilon_x^{v2} - \epsilon_x^{v1}}$$

$$C_2^J = \frac{-\epsilon_x^{v1}}{\epsilon_x^{v2} - \epsilon_x^{v1}}$$

$$C_2^x = \frac{-1}{\epsilon_x^{v2} - \epsilon_x^{v1}}$$

The response coefficients of E_2



slopes
of lines in a
parameter portrait

$$R_{e_2}^J = \frac{d \ln J}{d \ln e_2} \quad R_{e_2}^x = \frac{d \ln x}{d \ln e_2}$$

General definition of a response coefficient

For any *steady-state variable* y and any *parameter* p

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

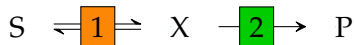
This can be re-cast in terms of *fractional changes*

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

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,

$$R_{e_2}^J = \epsilon_{e_2}^{v_2} C_{v_2}^J$$

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 $C_{v_i}^y$).

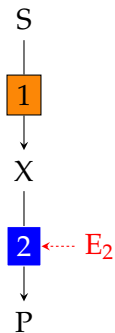
If p affects only reaction i :

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

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_e^y and C_e^y are usually equivalent



$$R_{e_2}^J = C_{v_2}^J \varepsilon_{e_2}^{v_2}$$

$$R_{e_2}^x = C_{v_2}^x \varepsilon_{e_2}^{v_2}$$

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

$$R_{e_2}^J = C_{v_2}^J$$

$$R_{e_2}^x = C_{v_2}^x$$

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).

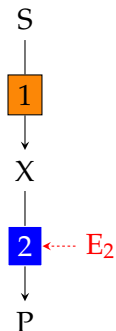
A response coefficient is the **property of a parameter**.

$$C_{v_i}^y = \frac{R_p^y}{\varepsilon_p^{v_i}} = \frac{d \ln y / d \ln p}{d \ln v_i / d \ln p} = \frac{d \ln y}{d \ln v_i}$$

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

$$C_{v_i}^y = \frac{R_{e_i}^y}{\varepsilon_{e_i}^{v_i}} = R_{e_i}^y$$

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

$$\delta \ln v_2 = \varepsilon_{e_2}^{v_2} \cdot \delta \ln e_2$$

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 :

$$\delta \ln J = C_{v_2}^J \cdot \delta \ln v_2$$

Thought experiment: $R = C\varepsilon$

Linking the **local effect** to the subsequent **systemic effect** gives:

$$\delta \ln J = C_{v_2}^J \varepsilon_{e_2}^{v_2} \delta \ln e_2 \quad \text{or} \quad \frac{\delta \ln J}{\delta \ln e_2} = C_{v_2}^J \varepsilon_{e_2}^{v_2}$$

which can be written as

$$R_{e_2}^J = C_{v_2}^J \varepsilon_{e_2}^{v_2}$$

Similarly, for the steady-state concentration X:

$$R_{e_2}^X = C_{v_2}^X \varepsilon_{e_2}^{v_2}$$

