I

FLUX CONTROL COEFFICIENTS
Greater is the number of the steps, weaker is in general the flux as compared with the vmax of the enzymes.

In other words, in a metabolic network, enzymes generally work (very) far from their maximal activity.

There is a great reserve of activity for most of the steps.

The variation of activity of an enzyme (in excess) will have in general few effect on the flux at steady-state. It is impossible to escope to such a behaviour.

Metabolic control analysis
**CONTROL COEFFICIENT**

**QUESTION** : What is the influence of a given step ($V_1$ or $V_2$) on a flux $F$?

**ANSWER** : Make a variation of the step ($\Delta V_i$ for instance) and monitor the corresponding variation of the flux $\Delta F$.

**DEFINITION** : $C_{V_1}^F = \frac{\Delta F}{\Delta V_1}$ is the control coefficient of the reaction $V_i$ on the flux $F$.

$$C_{V_1}^F = \frac{\partial F}{\partial V_1} \quad \text{or} \quad C_{V_1}^F = \frac{\Delta F/F}{\Delta V_1/V_1} = \frac{d\ln F}{d\ln V_1}$$

Idem for $C_{V_2}^F$
SUMMATION THEOREM

In a metabolic network: \[ \sum C^F_i = 1 \]

CONSEQUENCES OF THE THEOREM:

1. The control of a flux can be **shared** by several steps
2. The control distribution can **change** according to the physio(pathological) conditions
3. \[ \Delta F = \sum C^F_i \cdot \Delta V_i = C^F_1 \cdot \Delta V_1 + C^F_2 \cdot \Delta V_2 + \ldots + C^F_r \cdot \Delta V_r. \]
4. Most of the control coefficients have low values
   \( \Rightarrow \) A change in a single step will usually not affect a flux.

BIOLOGIST
The measured control coefficients sum to 1
See below the control of oxidative phosphorylation

MATHEMATICIAN
Demonstrated in general by C. Reder (see below)
Metabolic control theory: a structural approach,
APPLICATIONS / SUCCESS OF MCA

-Molecular basis of dominance: Why the fluxes in heterozygote are the same as in normal homozygote?

-The control of mitochondrial oxidative-phosphorylation. Which step(s) control the ATP synthesis according to the demand?

-Threshold effect in mitochondrial diseases and tissue specificity.

Impossible to escape to the behaviours described above
(Low control of most of the steps, thresholds etc.)

Amplification of PFK gene does not significantly increase the glycolytic flux. Amplification of several enzymes is necessary.

JP Mazat
RECENT APPLICATIONS / SUCCESS OF MCA
After Rafael Moreno-Sánchez, Emma Saavedra, Sara Rodríguez-Enríquez, and Viridiana Olin-Sandoval. J. Biomed. Biotech. (2008) (references below are from this paper)

- Glycolysis in erythrocytes [84] in which flux control distributes between HK (71%) and PFK-1 (29%);
- Carbohydrate metabolism during differentiation in Dictyostelium discoideum [127] with cellulose synthase (86%) as the main controlling step;
- Sucrose accumulation in sugar cane with HK, invertase, fructose uptake, glucose uptake, and vacuolar sucrose transporter having the most significant flux control [128];
- Glycerol synthesis in S. cerevisiae with GAPDH (85%) as the main control step [129];
- Penicillin synthesis in Penicillium chrysogenum controlled (75–98%) either by d-(a-aminoadipyl) cysteinylylvaline synthetase (short incubation times <30 hour) or isopenicillin N. synthetase (long incubation times > 100 h) [130];
- Calvin cycle [131] controlled by GAPDH (50%) and sedoheptulose-1,7-bisphosphatase (50%);
- Threonine synthesis in E. coli controlled by homoserine dehydrogenase (46%), aspartate kinase (28%), and aspartate semialdehyde dehydrogenase (25%) [111];
- Lysine production in Corynebacterium glutamicum mainly controlled by aspartate kinase and permease [132];
- Nonoxidative pentose pathway in erythrocytes mainly controlled by transketolase (74%) [133];
- EGF-induced MAPK signaling in tumor cells controlled by Ras-activation by EGF (21%), Ras dephosphorylation (43%), ERK phosphorylation by MEK (44%), and MEK phosphorylation by RAS (143%) [13].
- Aspergillus niger arabinose utilization with flux control shared by arabinose reductase (68%), arabitol dehydrogenase (17%), and xylulose reductase (14%) [134];
- Glycolysis in L. lactis in which several end products are generated (lactate, organic acids, ethanol, acetoin) [135]. Model predictions indicated that flux toward diacetyl and acetoin (important flavor compounds) was mainly controlled by LDH but not by acetolactate synthetase, the first enzyme of this branch.

JP Mazat
II

THE CONTROL OXIDATIVE
PHOSPHORYLATION
RESPIRATORY CHAIN

NADH

Pyruvate
Glutamate

Complex I

FMN + 5 FeS centers

Complex II

FAD + 2 FeS centers

Succinate, Fatty Acids

Complex III

Cyt b → FeS (Rieske) → Cyt c₁ → Cyt c

Complex IV
(Cytochrome c Oxidase)

Rotenone

Antimycine

KCN

O₂
Inhibition by KCN of Cytochrome-c Oxidase and of respiration rate


Control coefficient = a/b
## CONTROL OF OXYDATIVE PHOSPHORYLATION IN MUSCLE MITOCHONDRIA

<table>
<thead>
<tr>
<th>Component</th>
<th>$V_{ATP}$</th>
<th>$V_{O2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate transport</td>
<td>0.1</td>
<td>0.09</td>
</tr>
<tr>
<td>Complex I</td>
<td>0.1</td>
<td>0.15</td>
</tr>
<tr>
<td>Complex III</td>
<td>0.26</td>
<td>0.19</td>
</tr>
<tr>
<td>Complex IV</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>ATP Synthase</td>
<td>0.11</td>
<td>0.08</td>
</tr>
<tr>
<td>Translocase</td>
<td>0.16</td>
<td>0.14</td>
</tr>
<tr>
<td>Phosphate Carrier</td>
<td>0.10</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Sum</strong></td>
<td><strong>0.91</strong></td>
<td><strong>0.87</strong></td>
</tr>
</tbody>
</table>
MISE EN ÉVIDENCE D’UN SEUIL DANS LES PATHOLOGIES MITOCHONDRIALES

Activité COX

Vitesse de respiration

(50 biopsies)

Activité COX

J.-P. Mazat Montpellier Fev 2005
Some thousands of mtDNA arranged in nucleoids

The ratio of mutated mtDNA (heteroplasmy can vary between 0% and 100%)

Fig. 4. A small minority of wild-type (WT) mtDNA can protect the mitochondrial transformants against the respiratory defect caused by the MELAS mutation.
METABOLIC THRESHOLD EFFECT in the framework of MCA

\[ C_i = \frac{\Delta F}{\Delta v_i} \]

\[ \sum_i C_i = 1 \]

\[ \Rightarrow \text{The control is shared} \]

\[ \Rightarrow \text{Most of control coefficients have a low value.} \]

\[ \Rightarrow \text{Threshold effect.} \]
RELATION BETWEEN CONTROL COEFFICIENTS AND THRESHOLD VALUES

\[ y = 90.811 - 153.42x \quad R^2 = 0.805 \]
DETERMINATION OF CONTROL COEFFICIENT
Irreversible inhibitor

\[
\frac{V}{V_{\max}} = \frac{E_t - I}{I_{\max}} = \frac{I_{\max} - I}{I_{\max}}
\]

\[
\ln V - \ln V_{\max} = \ln (I_{\max} - I) - \ln I_{\max}
\]

\[
C_{Fv}^F = \frac{d \ln F/dl}{d \ln V/dl} \bigg|_{l = 0} dl
\]

\[
d \ln F/dl = \frac{1}{F} \frac{dF}{dl}
\]

\[
d \ln V/dl = -\frac{1}{I_{\max} - I} = -\frac{1}{I_{\max}} \bigg|_{l = 0}
\]

\[
C_{Fv}^F = -\frac{dF}{dl} \frac{I_{\max}}{F}
\]
III

METABOLIC CONTROL ANALYSIS
SUBSTRATE CONTROL COEFFICIENTS
AND ELASTICITIES
QUESTION: What is the influence of a given step ($V_1$ or $V_2$) on the substrate $X_1$ concentration?

ANSWER: Make a variation of the step ($\Delta V_1$ for instance) and record the corresponding variation of the flux $\Delta X_1$.

DEFINITION: $C_{Xj}^{Vi} = \frac{\Delta X_j}{\Delta V_i}$ is the control coefficient of the reaction $i$ on the $X_j$ concentration.

$$C_{Xj}^{Vi} = \frac{\Delta X_j}{\Delta V_i} = \frac{d X_j}{dV_i} \quad \text{or} \quad C_F^{Vi} = \frac{\Delta X_j}{\Delta V_i/V_i} = \frac{d\ln X_j}{d\ln V_i}$$

MATHEMATICIAN

- $V_i = V_i (X_j, p_i)$, $p_i$ is a specific parameter of $V_i$
- Steady-state ($N.V = 0$) $\Rightarrow X_j^° (p_i)$
- Metabolite Control Coefficient of $v_i$ on $X_j$: $C'_{Xj}^{vi} = \frac{\partial X_j}{\partial V_i} \frac{\partial p_i}{\partial p_i}$ or $C_F^{vi} = \frac{\partial \ln X_j}{\partial \ln V_i} \frac{\partial p_i}{\partial p_i}$
SUMMATION THEOREM FOR SUBSTRATES (Linear pathways)

In a linear pathway for each metabolite $X_j$:

$$\sum_i C_{X_{j}v_i} = 0$$

REMARK - ILLUSTRATION:
If all the rates are changed by the same factor all the $X_j$ remain the same and:

$$\sum_i C_{X_{j}v_i} = 0$$
QUESTION: What is the influence of the metabolite concentration $X_i$ on a given step $V_j$?

ANSWER: Make a variation of the metabolite concentration $\Delta X_i$ and look at the corresponding variation of the rate $\Delta V_j$.

DEFINITION: $\varepsilon_{Vj}^{Xj} = \frac{\Delta V_j}{\Delta X_i}$ is the elasticity of the reaction $j$ on $X_i$ concentration.

$$\varepsilon_{Vj}^{Xj} = \frac{\Delta V_j}{\Delta X_i} = \frac{dV_j}{dX_i} \quad \text{or} \quad \varepsilon_{Vj}^{Xj} = \frac{\Delta V_j}{\Delta X_i / X_i} = \frac{d\ln V_j}{d\ln X_i}$$

REMARK: Elasticity coefficients are local coefficients expressing the dependency of a rate function on a metabolite concentration, contrary to control coefficients (global coefficients).
ELASTICITY COEFFICIENTS
MICHAELIS EQUATION

\[ V = \frac{V_M \cdot X}{K_M + X} \]

Simple derivative

\[ \frac{\partial V}{\partial X} = \frac{V_M \cdot K_M}{(K_M + X)^2} \]

- \( X >> K_M \) \( \Rightarrow \) \( \frac{\partial V}{\partial X} = 0 \)
- \( X << K_M \) \( \Rightarrow \) \( \frac{\partial V}{\partial X} = \frac{V_M}{K_M} \)

Logarithmic derivative

\[ \varepsilon_{x_j} = \frac{\partial \ln V_i}{\partial \ln x_j} \]
\[ \frac{\partial \ln V}{\partial \ln X} = \frac{K_M}{K_M + X} \]

- \( X >> K_M \) \( \Rightarrow \) \( \frac{\partial \ln V}{\partial \ln X} = 0 \)
- \( X << K_M \) \( \Rightarrow \) \( \frac{\partial \ln V}{\partial \ln X} = 1 \)
### ELASTICITY COEFFICIENTS

#### GENERALIZED MICHAELIS EQUATION

\[ V = \frac{V_f \cdot S/K_S - V_r \cdot P/K_P}{1 + S/K_S + P/K_P} \]

\[ K_{eq} = \frac{P_{eq}}{S_{eq}} = \frac{V_f \cdot K_P}{V_r \cdot K_S} \]

\[ \Gamma = \frac{P}{S} \]

Generalized Michaelis equation  
Equilibrium constant and  
Haldane relationship  
At steady-state

\[ \eta_S^V = \frac{\partial \ln V}{\partial \ln S} = \frac{1}{1 - \Gamma/K_{eq}} - \frac{V_f}{V} = \frac{1}{1 - \Gamma/K_{eq}} \left[ 1 - \frac{F}{V_f} \right] \]

\[ \eta_S^P = \frac{\partial \ln V}{\partial \ln P} = \frac{\Gamma/K_{eq}}{1 - \Gamma/K_{eq}} - \frac{V_r}{V} = \frac{\Gamma/K_{eq}}{1 - \Gamma/K_{eq}} \left[ 1 + \frac{F}{V_r} \right] \]
**Example 1**

\[ \text{X}_1 \text{ is the link between reactions 1 and 2} \]

\( \implies \) Control coefficients should depend upon the way the rates change with \( \text{X}_1 \) (elasticity coefficients).

**Connection Relationship:**

\[
\begin{align*}
C_{v_1}^F \cdot \varepsilon_{x_1}^{v_1} + C_{v_2}^F \cdot \varepsilon_{x_1}^{v_2} &= 0
\end{align*}
\]

**Control Coefficient Calculation:**

\[
\begin{align*}
C_{v_1}^F &= \frac{-\varepsilon_{x_1}^{v_2}}{\varepsilon_{x_1}^{v_1} - \varepsilon_{x_1}^{v_2}} \\
C_{v_2}^F &= \frac{\varepsilon_{x_1}^{v_1}}{\varepsilon_{x_1}^{v_1} - \varepsilon_{x_1}^{v_2}}
\end{align*}
\]

\[
\begin{align*}
\frac{C_{v_1}^F}{C_{v_2}^F} &= \frac{\varepsilon_{x_1}^{v_2}}{-\varepsilon_{x_1}^{v_1}}
\end{align*}
\]
Example of example 1
Synthesis and use of ATP

\[
C_v^F = \frac{-\varepsilon_{x_1}^{v_2}}{\varepsilon_{x_1}^{v_1} - \varepsilon_{x_1}^{v_2}}
\]

\[
C_v^F = \frac{\varepsilon_{x_1}^{v_1}}{\varepsilon_{x_1}^{v_1} - \varepsilon_{x_1}^{v_2}}
\]

Elasticities

<table>
<thead>
<tr>
<th></th>
<th>ATP</th>
<th>ADP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-5</td>
<td>1</td>
</tr>
<tr>
<td>R_1</td>
<td>5</td>
<td>-1</td>
</tr>
<tr>
<td>R_2-A</td>
<td>+5</td>
<td>-1</td>
</tr>
</tbody>
</table>

Control coefficients

\[
\begin{align*}
C_{R_1} &= 0.5 \\
C_{R_2-A} &= 0.5
\end{align*}
\]

\[
\begin{align*}
C_{R_1} &= 0.14 \\
C_{R_2-A} &= 0.86
\end{align*}
\]
In general, $\varepsilon_{x_1}^{v_1} < 0$ and $\varepsilon_{x_1}^{v_2} > 0$ and the other elasticities are zero:

$$\varepsilon_{x_1}^{v_i} = 0, \; i \neq 1 \text{ or } 2.$$ 

**CONCLUSION**

$$C_{v_1}^F \cdot \varepsilon_{x_1}^{v_1} + C_{v_2}^F \cdot \varepsilon_{x_1}^{v_2} = 0$$

The ratio of control coefficients is contrary to the ratio of elasticity coefficients.
CONTROL BY AN IREVERSIBLE STEP
In a linear pathway

\[ C_{v_1}^F \cdot \varepsilon_{x_1}^{v_1} + C_{v_2}^F \cdot \varepsilon_{x_1}^{v_2} = 0 \]

- **Step 1 is irreversible:** \( \varepsilon_{x_1}^{v_1} = 0 \) \( \Rightarrow \) \( C_{v_2}^F \cdot \varepsilon_{x_1}^{v_2} = 0 \)

  In general, \( \varepsilon_{x_1}^{v_2} \neq 0 \) \( \Rightarrow \) \( C_{v_2}^F = 0 \) \( \Rightarrow \) \( C_{v_1}^F = 1 \)

- **Step 2 is irreversible:**

  In general, \( \varepsilon_{x_1}^{v_2} \neq 0 \) but also \( \varepsilon_{x_1}^{v_1} \neq 0 \) \( \Rightarrow \) *The control can be shared by step 1 and 2*
CONTROL AND REGULATION
In a linear pathway

**Remark:** $X_2$ pool is a link between steps 1, 2 and 3:

$$C_{v_1}^F \cdot \varepsilon_{x_2}^{v_1} + C_{v_2}^F \cdot \varepsilon_{x_2}^{v_2} + C_{v_3}^F \cdot \varepsilon_{x_2}^{v_3} = 0$$

For sake of simplicity, we suppose:

$$\varepsilon_{x_2}^{v_1} = 0 \Rightarrow C_{v_1}^F \cdot \varepsilon_{x_2}^{v_1} + C_{v_3}^F \cdot \varepsilon_{x_2}^{v_3} = 0 \Rightarrow \frac{C_{v_3}^F}{C_{v_1}^F} = -\frac{\varepsilon_{x_2}^{v_1}}{\varepsilon_{x_2}^{v_3}}$$

If we suppose that $V_1$ is very sensitive to $X_2$ inhibition (allosteric inhibition)

Thus $\varepsilon_{x_2}^{v_1}$ is big $\Rightarrow C_{v_4}^F$ can be much greater than $C_{v_1}^F$

**Thus the controlled (regulated step 1) is not the controlling step (step 3).**

**Explanation:** Inhibition of $V_3 \Rightarrow X_2 \Rightarrow V_3 \Rightarrow V_1 \Rightarrow V_1$ and thus $X_2 \Rightarrow V_1$ close to its previous value.

*The retroinhibition loop maintains $X_2$ and thus $F$ constant.*
CONNECTION RELATIONSHIP
Example: 3 successive reactions

\[ \begin{array}{c}
\rightarrow & V_1 & \rightarrow & X_1 & \rightarrow & V_2 & \rightarrow & X_2 & \rightarrow & V_3 & \rightarrow & F \\
\end{array} \]

- \( X_1 \) and \( X_2 \) are the links between reactions 1, 2 and 3
- 2 connection relationships for each metabolite.

**CONNECTION RELATIONSHIPS:**

\[ C_{v_1}^F \cdot \varepsilon_{x_1}^v_1 + C_{v_2}^F \cdot \varepsilon_{x_1}^v_2 + C_{v_3}^F \cdot \varepsilon_{x_1}^v_3 = 0 \]
\[ C_{v_1}^F \cdot \varepsilon_{x_2}^v_1 + C_{v_2}^F \cdot \varepsilon_{x_2}^v_2 + C_{v_3}^F \cdot \varepsilon_{x_2}^v_3 = 0 \]

**CONTROL COEFFICIENT CALCULATION:**

\[ C_{v_1}^F + C_{v_2}^F + C_{v_3}^F = 1 \]

\[ \begin{align*}
C_{v_1}^F &= \frac{\varepsilon_{x_1}^v_1 \cdot \varepsilon_{x_2}^v_3}{D} \\
C_{v_2}^F &= -\frac{\varepsilon_{x_1}^v_1 \cdot \varepsilon_{x_2}^v_3}{D} \\
C_{v_1}^F &= \frac{\varepsilon_{x_1}^v_2 \cdot \varepsilon_{x_2}^v_2}{D}
\end{align*} \]

\[ D = \varepsilon_{x_1}^v_1 \cdot \varepsilon_{x_2}^v_2 + \varepsilon_{x_1}^v_1 \cdot \varepsilon_{x_2}^v_2 - \varepsilon_{x_1}^v_1 \cdot \varepsilon_{x_2}^v_2 \]

\(( \varepsilon_{x_1}^v_3 = 0 \) and \( \varepsilon_{x_2}^v_1 = 0 \)) \((v_3 \) does not depend upon \( X_1 \) and \( v_1 \) does not depend upon \( X_2 \))
IV

GENERAL (MATHEMATICAL) THEORY OF METABOLIC CONTROL

The elasticity matrix

\[ E = \frac{\partial V}{\partial x} = \left( \begin{array}{c} \frac{\partial V_i}{\partial x_1} \\ \vdots \\ \frac{\partial V_r}{\partial x_1} \\ \vdots \\ \frac{\partial V_i}{\partial x_m} \\ \vdots \\ \frac{\partial V_r}{\partial x_m} \end{array} \right) = \left( \begin{array}{c} \varepsilon_{x_1}^{V_1} \\ \vdots \\ \varepsilon_{x_1}^{V_r} \\ \vdots \\ \varepsilon_{x_m}^{V_1} \\ \vdots \\ \varepsilon_{x_m}^{V_r} \end{array} \right) \]

- \[ \frac{\partial V_i}{\partial x_j} = \varepsilon_{x_j}^{V_i} \] is the elasticity of \( V_i \) as regards to \( x_j \) (express the local sensitivity of \( V_i \) towards \( x_j \)).

Remark-Logarithmic elasticities : \( \varepsilon_{x_j}^{V_i} = \frac{\partial \ln V_i}{\partial \ln x_j} \)

Exemple : \( V = \frac{V_M \cdot x}{K_M + x} \)

\[ \frac{\partial V}{\partial x} = \frac{V_M \cdot K_M}{(K_M + x)^2} \quad \frac{\partial \ln V}{\partial \ln x} = \frac{K_M}{K_M + x} \]

- \( x \gg K_M \Rightarrow \frac{\partial V}{\partial x} = 0 \quad \frac{\partial \ln V}{\partial \ln x} = 0 \)

- \( x \ll K_M \Rightarrow \frac{\partial V}{\partial x} = \frac{V_M}{K_M} \quad \frac{\partial \ln V}{\partial \ln x} = 1 \)
Control coefficients of substrates

- \( N.V[X_i,p] = 0 \Rightarrow \) System of equations in \( X_i \), the solution of which if it exists \( X_i^\circ \) are the metabolites concentrations at steady-state satisfying: \( N.V[X_i^\circ(p),p] = 0 \)

- We derive this expression towards \( p \):
  
  \[
  N. \frac{\partial V}{\partial x} \cdot \frac{\partial x}{\partial p} + N. \frac{\partial V}{\partial p} = 0
  \]

  and thus
  
  \[
  \frac{\partial x}{\partial p} = - (N \cdot E)^{-1} N. \frac{\partial V}{\partial p}
  \]

  if \((N \cdot E)^{-1}\) exists

  We can write:
  
  \[
  \frac{\partial x}{\partial p} = S \cdot \frac{\partial V}{\partial p} \quad \text{with} \quad S = - (N \cdot E)^{-1} \cdot N
  \]

  - \( S \) is the matrix:
    
    \[
    S = \begin{pmatrix}
    S_{x_1}^{x_1} & \cdots & S_{x_r}^{x_1} \\
    \vdots & \ddots & \vdots \\
    S_{x_1}^{x_m} & \cdots & S_{x_r}^{x_m}
    \end{pmatrix}
    \]

  - It means that in the linear vicinity of the steady-state, one can write:
    
    \[
    \Delta X_i = S_{x_1}^{x_1} \Delta V_1 + S_{x_2}^{x_1} \Delta V_2 + \ldots + S_{x_n}^{x_i} \Delta V_n
    \]

  The variation \( \Delta X_i \) of \( X_i \) is proportionnal to the variations of the \( \Delta V_j \)weighted by the control coefficients of \( X_i \) towards \( V_j \).
Control coefficients of substrates

Example 1

\[ N = \begin{bmatrix} 1 & -1 \end{bmatrix} \]

It means that in the linear vicinity of the steady-state it can be written:

\[ S = - (N \cdot E)^{-1} \cdot N = - \begin{bmatrix} 1 \\ \varepsilon_1 - \varepsilon_2 \end{bmatrix} \begin{bmatrix} 1 & 1 \\ \varepsilon_1 - \varepsilon_2 & \varepsilon_1 - \varepsilon_2 \end{bmatrix} = \begin{bmatrix} -1 \\ \varepsilon_2 \end{bmatrix} \]

It can be checked that:

\[ S_{V_1}^{X_1} + S_{V_2}^{X_1} = 0 \]
Control coefficients of fluxes

\[ F = V[x^*(p), p] = 0 \quad (2) \]

\[ \frac{\partial F}{\partial p} = \frac{\partial V}{\partial x} \cdot \frac{\partial x}{\partial p} + \frac{\partial V}{\partial p} = E \cdot \frac{\partial x}{\partial p} + \frac{\partial V}{\partial p} \]

or \[ \frac{\partial x}{\partial p} = - (N \cdot E)^{-1} \cdot N \cdot \frac{\partial V}{\partial p} \] if \((N \cdot E)^{-1}\)

thus: \[ \frac{\partial F}{\partial p} = - E \cdot (N \cdot E)^{-1} \cdot N \cdot \frac{\partial V}{\partial p} + \frac{\partial V}{\partial p} = [- E \cdot (N \cdot E)^{-1} \cdot N + I] \cdot \frac{\partial V}{\partial p} \]

With \(C = [I - E \cdot (N \cdot E)^{-1} \cdot N]\), it can be written: \[ \frac{\partial F}{\partial p} = C \cdot \frac{\partial V}{\partial p} \]

\(C\) expresses the variation of fluxes as a function of the changes in the rates:

It means that in the linear neighborhood of the steady-state it can be written:

\[ \Delta F_i = C_{v1}^{F_i} \Delta V_1 + C_{v2}^{F_i} \Delta V_2 + \ldots + C_{vn}^{F_i} \Delta V_n \]

or: \[ \Delta F = C \Delta V \].

The variation \(\Delta F_i\) of \(F_i\) is proportionnal to the variations of \(\Delta V_j\) weighted by the control coefficients of \(F_i\) towards \(V_j\).
Properties of matrix C

- C is the projection matrix on the kernel K of N in the direction of E:
  
  It can be verified that: \( C \cdot E = 0 \) \( \Rightarrow \) Connection relationships.

  and \( C \cdot K = K \) (identity on K) \( \Rightarrow \) Summation relationships.

- Summation theorem:
  For any metabolic network, the sum of flux control coefficients (logarithmic definition) is equal to 1.

  \[ C_{v1}^{Fi} + C_{v2}^{Fi} + \ldots + C_{vn}^{Fi} = 1 \]

  With: \( C_{vj}^{Fi} = \frac{\partial \ln F_i}{\partial \ln V_j} \cdot \frac{\partial p}{\partial p} \)

- Consequences of summation theorem:
  - The control can be shared: it is rare to observe a unique limiting step.
  - The control distribution change when the steady-state is changed.
  - Experimentally the control coefficient values appear low
    
    \( \Rightarrow \) Fluxes are weakly sensitive towards the enzyme activity.

- It can also be checked that: \( N \cdot C = 0 \)
Properties of matrix S

- Matix S satisfies:
  - $S \cdot K = 0 \Rightarrow$ Summation relationship.
  - $S \cdot E = -I \Rightarrow$ Connection relationship.
Determination of control coefficients from summation and connection relationships

\[
\begin{pmatrix}
C \\
S
\end{pmatrix}
\cdot
\left[
\begin{array}{cc}
K & 0 \\
0 & E
\end{array}
\right]
=
\begin{pmatrix}
K & 0 \\
0 & -1
\end{pmatrix}
\]

If the matrix \( [K; E] \) is invertible, i.e. \( [K; E]^{-1} \) exists,

\[
\begin{pmatrix}
C \\
S
\end{pmatrix}
=
\begin{pmatrix}
K & 0 \\
0 & -1
\end{pmatrix}
\cdot
[K; E]^{-1}
\]


Flux control coefficients

Example 1

\[
N = \begin{bmatrix} 1 & -1 \end{bmatrix} \quad C = I - E(N \cdot E)N = I + E.S
\]

\[
E.S = \begin{pmatrix} \varepsilon_1 \\ \varepsilon_2 \end{pmatrix} \left( \begin{array}{cc} 1 & 1 \\ \frac{1}{\varepsilon_1 - \varepsilon_2} & \frac{1}{\varepsilon_1 - \varepsilon_2} \end{array} \right) = \begin{pmatrix} -\varepsilon_1 & \varepsilon_1 \\ \frac{\varepsilon_2}{\varepsilon_1 - \varepsilon_2} & \frac{\varepsilon_2}{\varepsilon_1 - \varepsilon_2} \end{pmatrix}
\]

\[
C = \begin{pmatrix} \frac{-\varepsilon_2}{\varepsilon_1 - \varepsilon_2} & \frac{\varepsilon_1}{\varepsilon_1 - \varepsilon_2} \\ \frac{\varepsilon_2}{\varepsilon_1 - \varepsilon_2} & \frac{\varepsilon_2}{\varepsilon_1 - \varepsilon_2} \end{pmatrix}
\]

\[
\varepsilon_1 = \varepsilon_{x_1}^{V_1} \quad \varepsilon_2 = \varepsilon_{x_1}^{V_2}
\]

It is checked: \( C_{V_1}^{F_1} + C_{V_2}^{F_1} = 1 \)

And also: \( C_{V_1}^{F_1} \cdot \varepsilon_1 + C_{V_2}^{F_1} \cdot \varepsilon_2 = 0 \) and thus:

\[
\frac{C_{V_1}^{F_1}}{C_{V_2}^{F_1}} = -\frac{\varepsilon_2}{\varepsilon_1}
\]

J.-P. Mazat
EXAMPLE 1-1

\[ V_1 = \frac{k_1}{K_i + [X_1]} \]
\[ V_2 = \frac{k_2 [X_1]}{K_M + [X_1]} \]

\[ k_1 = 10 \]
\[ K_i = 1 \]
\[ k_2 = 10 \]
\[ K_M = 1 \]

\[ \Delta F = \frac{1}{2} \Delta V_i \Rightarrow C_{V_1}^F = C_{V_2}^F = 0,5 \]

We notice: \[ C_{V_1}^F + C_{V_2}^F = 1 \]
EXAMPLE 1-1

\[ V_1 = \frac{k_1}{K_I + [X_1]} \]

\[ V_2 = \frac{k_2 [X_1]}{K_M + [X_1]} \]

\[ k_1 = 10 \]
\[ K_I = 1 \]
\[ k_2 = 10 \]
\[ K_M = 1 \]

\[ \Delta F = \frac{1}{2} \Delta V_i \Rightarrow C^F_{V_1} = C^F_{V_2} = 0.5 \]

We notice: \[ C^F_{V_1} + C^F_{V_2} = 1 \]
EXAMPLE 1-2

\[ V_1 = \frac{k_1}{K_l + [X_1]} \]

\[ V_2 = \frac{k_2 [X_1]}{K_M + [X_1]} \]

\[ k_1 = 10 \]
\[ K_l = 10 \]

\[ k_2 = 10 \]
\[ K_M = 1 \]
How a variation of a rate modulates the flux

\[ \Delta F = 0 \Rightarrow CF_1 = \frac{\Delta F}{\Delta V_1} = 0 \]

\[ \Delta F \approx \Delta V_2 \Rightarrow CF_2 = \frac{\Delta F}{\Delta V_2} = 1 \]

\[ \text{We still notice: } CF_{V_1} + CF_{V_2} = 1 \]
HOW A VARIATION OF A RATE MODULATES THE FLUX?

\[ \text{Amplification Factor} \]

\[ F \]

\[ V_1 \rightarrow X_1 \rightarrow V_2 \rightarrow F \]

Graph showing the relationship between Flux and Amplification Factor.
EXAMPLE 1-2

\[ V_1 = \frac{k_1}{K + [X]^4} \]
\[ V_2 = \frac{k_2 [X]}{K_M + [X]} \]

\( k_1 = 10 \)
\( K = 10 \)
\( k_2 = 0.1 \)
\( K_M = 0.2 \)
CONTROL COEFFICIENT IN EXAMPLE 1-2

Steady-state unchanged $\Rightarrow \Delta F \approx 0$

$\Delta V_1 \neq 0 \Rightarrow C^F_{V_1} = 0$

Steady-state x 5 $\Rightarrow \Delta F \approx 4F$

$\Delta V_2 = 4V_2 \Rightarrow C^F_{V_2} = 4F/4V_2 \approx 1$

It can be noticed that $C^F_{V_1} + C^F_{V_2} = 1$
L’État stationnaire

\[ S \rightarrow X_1 \rightarrow P \]

A l’état stationnaire,

\[ V_1 = V_2 \Rightarrow X_1^\circ \text{ et } F^\circ = V_1(X_1^\circ) = V_2(X_1^\circ) \]
L’ÉTAT STATIONNAIRE ET LE NOMBRE D ’ÉTAPES

\[ S \rightarrow X_1 \rightarrow X_2 \rightarrow X_3 \rightarrow P \]

Plus on a d ’étapes, plus le flux à l ’état stationnaire s’éloigne de la vitesse maximale de chaque étape.
Un exemple simple (exemple 1)

\[ V_1 = k_1 (a - x_1) \quad ... \quad V_i = k_i (x_{i-1} - x_i) \quad ... \quad V_n = k_n x_{n-1} \]

Etat stationnaire \( V_1 = V_2 = \ldots = V_n \)

- \( k_1 = k_2 = k_3 = \ldots = 1 \) \( \Rightarrow \) \( F_1 = a/n \) \( x^o_1 = (n-1)a/n \); \( x^o_2 = (n-2)a/n \); \ldots \( x^o_{n-1} = a/n \)

On fixe toutes les vitesses \( k_i = 1 \) sauf \( v_2 \) (\( k_2 \) variable)

On fait varier \( v_2 \) et on regarde comment varie le flux \( F \) à l'état stationnaire

\[ F = \frac{a \cdot k_2}{1 + (n-1) \cdot k_2} \]

\[ V_2 = k_2 (x_1 - x_2) = a / n \]

\[ \Delta F / \Delta v_2 = \frac{\partial F / \partial k_2}{\partial v_2 / \partial k_2} = \frac{1}{n} \quad (k_2 = 1) \]

La variation d'un flux n'est pas égale à la variation de la vitesse

Plus il y a d'étapes, moins, en général, le flux est sensible à une étape particulière
Pour 5 étapes $F = 20\%$ de l'activité maximale de chaque étape

⇒ Il ya en général pour chaque étape une grande réserve d’activité

**Prédiction :** la diminution d'une étape (5 fois en excès) aura peu d'effet sur le flux.
• Coefficient de contrôle

\[ C_{F_{v2}}^F = \frac{\Delta F}{\Delta v_2} \]

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• Théorie générale développée par Christine Reder : \( \sum C_{F_{v2}}^F = 1 \)

• Théorie appliquée avec succès au réseau des oxydations phosphorylantes mitochondriales et aux pathologies mitochondriales (TL)
COURBES D’EFFET DE SEUIL MÉTABOLIQUE Complex IV, ATP synt., Pi carrier, Pyr. Carrier, ANT


J.-P. Mazat Montpellier Fev 2005
METABOLIC THRESHOLD CURVES
Complex I and III

ROSSIGNOL, R., MALGAT, M., MAZAT, J.-P and LETELLIER, T., . . (1999)