Control theory of group transfer pathways

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Abstract

Relay or group-transfer pathways are important for metabolism and signal transduction. Yet, they are not addressed by standard metabolic control analysis. In this paper the control theory for this type of pathways is developed. Control coefficients are defined both with respect to modulation of enzyme concentration (enzyme control coefficient) and with respect to modulation of 'elemental' process activity (process control coefficient). Whereas the latter obeys the theorems of standard metabolic control theory, the more operational, former type of control coefficient obeys new control theorems: (i) the sum of enzyme control coefficients on the flux of group transfer equals 2 minus the control by pathway boundary substrates and products, divided by the extent to which the pathway enzymes are complexed, (ii) the sum of the controls on the concentration of any of the non-complexed pathway components is 1 (iii) the sum of the controls on the concentration of any of enzyme-enzyme complexes is 2, with the same corrections as above, (iv) the control exerted by enzyme concentrations can be calculated from the kinetic properties (elasticity coefficients).

The implications for metabolism and signal transduction of the special control properties of relay pathways are discussed.

Keywords: Metabolic control theory; Control theory; Channeling; Relay pathway; Enzyme–enzyme interaction

1. Introduction

Cell function is controlled in various ways and at various points, ranging from the catalytic activity of single enzymes to the mutually adjusted operation of biosynthetic pathways. Various theoretical treatments (e.g. [1–7]) exist which define and relate characteristics of control. Control exerted by all enzymes of a metabolic pathway on any steady-state flux through the pathway adds up to 1 whereas the control on any steady-state metabolic concentration adds up to 0 [1,2]. Along with these 'summation' theorems, so-called 'connectivity' theorems [1,8] reveal the dependence of the distribution of the control among the pathway enzymes on kinetic properties (elasticity coefficients) of the individual enzymes. Consequently, the extent to which an enzyme in a metabolic pathway controls flux or concentrations can be understood in terms of kinetic properties of all the enzymes and of pathway structure [6,9–13,32].

The initial developments of metabolic control theory focused on the control of metabolic networks. With the increasing emphasis on the roles played by regulated gene expression and signal transduction in the control of cell function, however, control theories have been extended, so as to address these aspects explicitly (e.g. [14,15]). In one of these developments it was stressed that cell function does not just consist of a single metabolic network, but rather of a number of such networks, which are connected by (allosteric) regulation or/and mass flow. A modular approach may help to reveal the essence of such regulation [15–18].

Some cellular signal transduction pathways are organized in such a modular fashion; they consist of cycles of protein phosphorylation and dephosphorylation by kinases and phosphatases which are themselves again activated by phosphorylation or dephosphorylation. The phosphate group is obtained from ATP and, within each cycle, lost as inorganic phosphate; there is no net transfer of the phosphate group along the signal transduction pathway. In other pathways, however, there is such a transfer of phosphate between enzymes. Examples are the phosphotransferase system of enterobacteria, the NtrB/NtrC system involved in the transcription regulation of the E. coli

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glutamine synthetase gene and the regulation of sporula-
tion in *B. subtilis* [19]. Other groups than phosphate are
transferred in electron-transfer chains in free-energy cou-
pling membranes (i.e., e^- or H), in channelled membrane
mediated free-energy transduction (i.e., H^+) [20–22] and
more generally whenever coenzymes are involved. In gen-
eral this type of pathway may be called a relay pathway.

In parallel to an experimental determination of the flux
control coefficients in the phosphotransferase system of *E.
coli* [23], it was recently shown that in group-transfer
pathways the sum of the flux control by the participating
enzymes can exceed the 1 that is characteristic of so-called
'ideal' metabolic pathways [24,25]. This suggested that
relay (group-transfer) pathways have their own set of
control principles, which require a separate control theory.
In this paper we develop this control theory, including the
summation and the connectivity theorems and the way to
calculate enzymes' control coefficients from properties of
the individual enzymes.

2. Results

2.1. Definitions

Fig. 1 represents the pathways we shall deal with in this
paper. A group, P, is transferred through r 'enzymes' from
a primary donor (SP) to an ultimate acceptor (W). The
rates of the reversible consecutive transfer reactions are v_i,
$\mathbf{v} = (v_1, v_2,...,v_{2r+2})$, the number ($n$) of reactions is:
$n = 2r + 2$. Steady state is achieved when all rates are
equal:

$$v_i = J \text{ (for all } i) \tag{1}$$

The concentrations (thermodynamic activities) of the
'boundary substrates' S, SP, W and WP, are taken to be
constant (to be clamped by an external bath). It is conve-
nient to designate the concentrations of these boundary
substrates as if they themselves were additional enzymes
of the pathway (E_0 and E_{r+1}, respectively):

$$E_0 = [S], \ E_0 P = [SP], \ e_0 = [S] + [SP],$$

$$E_{r+1} = [W], \ E_{r+1}P = [WP], \ e_{r+1} = [W] + [WP] \tag{2}$$

For brevity, we shall denote the concentrations of the
enzyme-enzyme complexes by Q's

$$Q_i = [E_i PE_{i+1}], \ (i = 1,...,r-1)$$

$$Q_0 = [SPE_1]; \ Q_r = [E_r PW] \tag{3}$$

There are $m = 3r + 1$ concentration variables, x_i, in the
system (Fig. 1):

$$\begin{aligned}
x_1 &= E_1, & x_{3i-2} &= E_i, & x_{3r+1} &= Q_r = E_r PW \\
x_2 &= Q_o = SPE_1, & x_{3i-1} &= Q_{i-1} & & \\
x_3 &= E_1 P,... & x_{3i} &= E_i P, & & ...
\end{aligned} \tag{4}$$

That the 'enzymes' act only catalytically in the overall
group transfer implies that they are neither consumed not
produced; they merely change from one form (e.g., E_i) to
another (e.g., $E_i P$). The sum concentration of all forms of
any enzyme i is constant, however, so that we have r
conservation constraints:

$$E_i + E_{i-1} PE_i + E_i P + E_i PE_{i+1} = e_i (i = 1,...,r) \tag{5}$$

or, in terms of x:

$$x_{3i-2} + x_{3i-1} + x_{3i} + x_{3i+2} = e_i (i = 1,...,r-1)$$

$$x_{3r-2} + x_{3r-1} + x_{3r} + x_{3r+1} = e_r \tag{6}$$

There are no further restrictions on the concentration vari-
ables x_i, so that the number of independent concentrations
is equal to $m - r = 2r + 1$.

Below it will prove convenient to indicate by coeffi-
cients γ_{ij} the concentration variables that contain a form
of enzyme i:

$$\sum_{j=1}^{3r+1} \gamma_{ij} \cdot x_j = e_i (i = 1,...,r) \tag{7}$$

γ_{ij} is equal to 1 if x contains E_i and 0 if x is not
involved. It is clear from Eqs. 6 and 7 and Fig. 1 that
enzyme i participates in concentration variables with in-
dices $3i - 2$ (E_i) $3i - 1$ ($Q_{i-1} = E_{i-1}PE_i$), $3i$ ($E_i P$) and
$3i + 2$ ($Q_i = E_i PE_{i+1}$), hence:

$$\gamma_{ij} = \delta_{3i-2,j} + \delta_{3i-1,j} + \delta_{3i,j} + \delta_{3i+2,j} (i = 1,...,r-1)$$

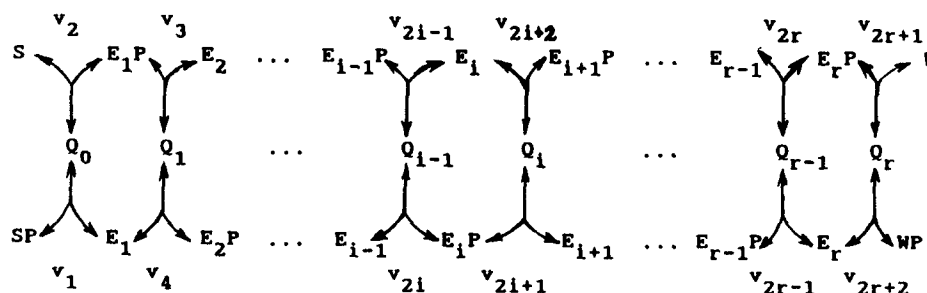$$\gamma_{rj} = \delta_{3r-2,j} + \delta_{3r-1,j} + \delta_{3r,j} + \delta_{3r+1,j}, \tag{8}$$

Fig. 1. Group-transfer (-relay) pathway. A group P is ultimately transferred from pathway substrate S to pathway product W. Enzyme-enzyme complexes
are referred to by Q, rates by v, the transferred group by P.

where the Kronecker δ is used:

$$\delta_{l,j} = 0 \text{ if } l \neq j, \ \delta_{l,l} = 1 \tag{9}$$

The overall process of group transfer from SP to W is effected by the $2r + 2$ elementary processes indicated by the arrows in Fig. 1. Any enzyme is involved in four processes, i.e., complex-formation with the phosphorylated form of the preceding enzyme, dissociation from that preceding enzyme whilst retaining the phosphoryl group, complex-formation with the subsequent enzyme and dissociation having transferred the phosphoryl group to that subsequent enzyme. Inserting i and $i + 1$ in the following equations one obtains the rates for the four corresponding processes for E_i:

$$v_{2i-1} = k_{2i-1}^{+} \cdot E_{i-1}P \cdot E_i - k_{2i-1}^{-} \cdot E_{i-1}PE_i;$$

$$v_{2i} = k_{2i}^{+} \cdot E_{i-1}PE_i - k_{2i}^{-} \cdot E_{i-1} \cdot E_iP (i = 1,...,r + 1) \tag{10}$$

The positive direction of any *odd* elemental process, v_{2i-1}, corresponds to the formation of an enzyme-enzyme complex, $E_{i-1}PE_i$ (consuming $E_{i-1}P$ and E_i). The positive direction of any *even* elemental process, v_{2i}, corresponds to the consumption (dissociation) of an enzyme-enzyme complex, $E_{i-1}PE_i$, and to the formation of free enzyme E_{i-1} and E_iP.

One of our points of interest is the extent to which the activity of any of these $2r + 2$ elemental processes determines the group-transfer flux. As in Metabolic Control Analysis, we shall wish to define this extent in terms of the ratio of the relative change in group-transfer flux and the relative change in activity of the elemental process, where the latter is taken in the limit to zero.

The activity of an elemental process may be modulated by changing the forward rate constant only or by changing the reverse rate constant only. In either case the overall Gibbs energy difference of the group-transfer reaction from SP to W will also be modulated. Since it is physically impossible to change the Gibbs energy difference of a reaction by just changing a catalyst, we prefer to modulate the activity of the elemental step such that its standard Gibbs energy difference (i.e., its equilibrium constant) is not affected; i.e., equal relative changes of the forward and reverse rate constants. To describe this type of modulation, we let the parameter ξ_s ($s = 1,...,2r + 2$) modulate the activity of the sth elemental process (equally in the forward and in the reverse direction):

$$v_s(\xi_s) = \xi_s \cdot v_s(1) \ (s = 1,...,2r + 2) \tag{11}$$

where the expressions for $v_s(1)$ are given by Eq. 10.

We define the control coefficient of the flux J with respect to any process s as:

$$C_{v_s}^J = \frac{d\ln|J|}{d\ln \xi_s} = \frac{dJ/J}{d\xi_s/\xi_s} \tag{12}$$

Although the control coefficient has been defined in terms of an infinitesimal modulation of the activity of step (s), in practice one may compare the percentage in the steady-state flux (J) to a small percentage change (e.g., 1%) in the activity of the step s. The $C_{v_i}^J$ are called elemental flux control coefficients [24,25,27,43]. The operational equivalent of this definition compares the change in flux effected by a change in a parameter p_s that affects step s only, to the change in activity of step s caused by that parameter change [[26], cf. [40]]:

$$C_{v_s}^J = \frac{(d\ln|J|/dp_s)_{sys}}{(\partial\ln|v_s|/\partial p_s)_{proc}}, \ s = 1,...,2r + 2 \tag{13}$$

Subscripts *sys* and *proc* refer to the different differentiation conditions; allowing all variables to change until the new steady state is attained (sys) versus keeping all other variables that affect processes constant (proc), respectively.

Similarly, we define quantitatively the control exerted by any elemental process on the *concentration* of any of the components in the system:

$$C_{v_s}^x = \frac{d\ln x}{d\ln \xi_s} = \frac{(d\ln x/dp_s)_{sys}}{(\partial\ln|v_s|/\partial p_s)_{proc}}, \ s = 1,...,2r + 2 \tag{14}$$

where x is any steady-state concentration. $C_{v_s}^x$ is called an elemental concentration control coefficient.

At any steady state, we shall consider flux J and the vector of concentrations $\mathbf{x}$ as functions of the total enzyme concentration vector $\mathbf{e} = (e_1,...,e_r)$, boundary substrate vector $\mathbf{B} = ([S], [SP], [W], [WP])$ and parameters $\boldsymbol{\xi} = (\xi_1, \xi_2,...,\xi_{2r+2})$, i.e.:

$$J = J(\mathbf{e}, \mathbf{B}, \boldsymbol{\xi}); \ \mathbf{x} = \mathbf{x}(\mathbf{e}, \mathbf{B}, \boldsymbol{\xi}) \tag{15}$$

Because we shall also be interested in the control exerted by the enzymes, we define coefficients for that control in terms of the system responses to an increase in total concentration of any of the enzymes:

$$C_{e_i}^J = \frac{d\ln|J|}{d\ln e_i}, \ C_{e_i}^{x_k} = \frac{d\ln x_k}{d\ln e_i} \tag{16}$$

Here, the coefficients C_{ei}^J and C_{ei}^{xk} are called the flux and concentration control coefficients, respectively, of the enzyme(concentration)(s). In a deviation from agreed terminology [41] one may prefer to call these response coefficients.

We would like to emphasize here an important distinction between the elemental control coefficients defined by Eqs. 12–14 and the enzyme-(concentration) control coefficients. The former can be defined independently of the modulation parameter (Eq. 13), whereas the latter are always responses to a change in such a parameter as enzyme concentration, which only allow a parameterless

definition in ideal pathways (Kholodenko et al., Biophys. Chem. in press).

2.2. Relating flux control by enzymes to flux control by processes

A central point of this section is the different rôles played by the enzymes and the processes in group-transfer pathways [24], these rôles being similar in simple metabolic pathways. This difference in rôles will become apparent from the relationship expressing the control exerted by the enzymes, C_{ei}^J, in terms of control exerted by the processes, C_{ei}^J.

To obtain this relationship we shall follow a strategy developed by Kacser and Burns for metabolic pathways [1]. Fig. 2 focuses on four elemental processes in which enzyme i is involved. We now consider the following perturbation in only those concentration variables that involve enzyme i:

$$E_i(\lambda_i) = \lambda_i \cdot E_i, \quad E_iP(\lambda_i) = \lambda_i \cdot E_iP, \quad Q_{i-1}(\lambda_i)$$

$$= \lambda_i \cdot Q_{i-1};$$

$$Q_i(\lambda_i) = \lambda_i \cdot Q_i (i = 1,...,r) \tag{17}$$

where $\lambda_i = 1 + \Delta_i$, and Δ_i is sufficiently small. Note, that the concentrations of all the other enzyme forms (e.g., $E_{i+1}P$), remain unchanged and that all forms of enzyme i are amplified by the same factor. We change simultaneously, the parameters ξ_l of the rates v_l that depend on the concentrations mentioned in Eq. 17, in such a manner that the rates v_l remain the same as in the initial steady state. Appendix A derives that this requires the relative change in ξ_l to be the opposite of the perturbation in λ_i, for all processes involving enzyme i (Eq. A3). In the immediately obtained new steady state the concentrations involving enzyme i then obey Eq. 17, but the rates of all elemental processes and the flux J have remained the same as in the initial steady-state.

The above operation increases not only the total concentration (e_i) of the enzyme i but also affects the concentrations $(e_{i-1}$ and $e_{i+1})$ of the enzymes $i - 1$ and $i + 1$ with which enzyme i complexes. Because the modulations con-

sidered address both 'catalytic' activities (ξ_l) and the total enzyme concentrations (e_i), the change in flux J can be expressed through the corresponding control coefficients. Because the flux change is zero, this yields the desired relationship between control exerted by the processes (C_{vl}^J) and the control exerted by the enzymes C_{ev}^J (see Appendix A). In this manner one obtains a relationship for each of the r enzymes in the pathway plus two, slightly different equations for the boundary substrate/product couples. For the pathway internal enzymes, i.e., $i = 2,...,r - 1$ (see Appendix A):

$$C_{e_{i-1}}^J \cdot \frac{E_{i-1}PE_i}{e_{i-1}} + C_{e_i}^J + C_{e_{i+1}}^J \cdot \frac{E_iPE_{i+1}}{e_{i+1}}$$

$$= C_{v_{2i-1}}^J + C_{v_{2i}}^J + C_{v_{2i+1}}^J + C_{v_{2i+2}}^J$$

$$= \sum_{k=2i-1}^{2i+2} C_{v_k}^J (i = 2,3,...,r - 1) \tag{18}$$

Importantly, this equation affirms that there is no one-to-one relationship between control by an enzyme and control by one or two processes. Due to the involvement of two enzymes in the elemental transfer process, the relationships (Eq. 18) always involve control by several enzymes and control by several elemental processes. In addition, these relationships are moderated by the fraction of the enzymes that occur as enzyme-enzyme complexes.

For the enzyme 1 at the beginning of the pathway:

$$C_{e_1}^J + C_{e_2}^J \cdot \frac{E_1PE_2}{e_2} = C_{v_1}^J + C_{v_2}^J + C_{v_3}^J + C_{v_4}^J = \sum_{k=1}^{4} C_{v_k}^J \tag{19}$$

The interpretation of this equation holds that the control by enzyme 1, C_{e1}^J, equals the sum of the control of all four processes enzyme 1 is involved in, except for the control exerted by enzyme 2 exerted through the complex E_1PE_2. Similarly, for the enzyme r:

$$C_{e_{r-1}}^J \cdot \frac{Q_{r-1}}{e_{r-1}} + C_{e_r}^J = C_{v_{2r-1}}^J + C_{v_{2r}}^J + C_{v_{2r+1}}^J + C_{v_{2r+2}}^J$$

$$= \sum_{k=2r-1}^{2r+2} C_{v_k}^J \tag{20}$$

Eqs. 18–20 constitute r independent relations between the r enzymes' flux control coefficients. Hence, they should allow one to express the flux control coefficients of the enzymes, C_{ei}^J ($i = 1,...,r$), in terms of the control coefficients with respect to the elemental processes, C_{vi}^J and the relative fractions of the enzyme complexes (such as $(E_1PE_2)/e_2$). Because these equations are linear, the expressions are obtained most simply by the use of matrix algebra. Testifying to the independence of the r equations,
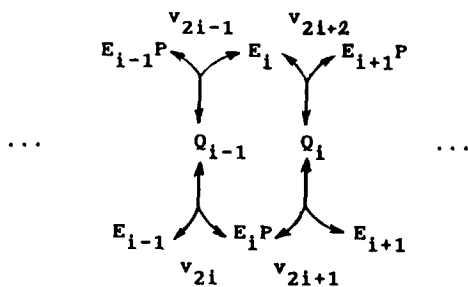

Fig. 2. An arbitrary part of a group-transfer pathway. Note the (moiety-) conservation of the total concentration of enzyme i.

the determinant of the matrix (A) of the linear equation system 18–20 is not equal to 0. This $r \times r$ matrix reads (Eq. 21):

$$A = \begin{vmatrix} 1 & \dfrac{Q_1}{\theta_2} & 0 & 0 & \cdots & 0 & 0 & 0 \\[2mm] \dfrac{Q_1}{\theta_1} & 1 & \dfrac{Q_2}{\theta_3} & 0 & \cdots & 0 & 0 & 0 \\[2mm] 0 & \dfrac{Q_2}{\theta_2} & 1 & \dfrac{Q_3}{\theta_4} & \cdots & 0 & 0 & 0 \\[2mm] \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\[2mm] 0 & 0 & 0 & 0 & \cdots & \dfrac{Q_{r-2}}{\theta_{r-2}} & 1 & \dfrac{Q_{r-1}}{\theta_r} \\[2mm] 0 & 0 & 0 & 0 & \cdots & 0 & \dfrac{Q_{r-1}}{\theta_{r-1}} & 1 \end{vmatrix}$$

In order to write Eqs. 18–20 in matrix form we define the r-dimensional vector, $\mathbf{C}_e^J$, of the flux control coefficients of enzymes (as a column-vector, T means transposed)

$$\mathbf{C}_e^J = \left(C_{e_1}^J, C_{e_2}^J, ..., C_{e_r}^J \right)^T \tag{22}$$

and the $(2r + 2)$-dimensional vector of the elemental flux control coefficients, $\mathbf{C}_v^J$,

$$C_v^J = \left(C_{v_1}^J, C_{v_2}^J, ..., C_{v_{2r+1}}^J, C_{v_{2r+2}}^J \right)^T \tag{23}$$

as well as the $r \times (2r + 2)$ rectangular matrix $\mathbf{M}$ (Eq. 24),

$$M = \begin{vmatrix} 1 & 1 & 1 & 1 & 0 & 0 & \cdots & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 1 & 1 & 1 & \cdots & 0 & 0 & 0 & 0 & 0 & 0 \\ \cdots & & & \cdots & & & & \cdots & & & \cdots & & \\ 0 & 0 & 0 & 0 & 0 & 0 & \cdots & 1 & 1 & 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \cdots & 0 & 0 & 1 & 1 & 1 & 1 \end{vmatrix}$$

With these definitions the system of linear relations Eqs. 18–20 connecting $\mathbf{C}_{e_i}^J$ and $\mathbf{C}_{v_i}^J$ can be written as:

$$A \cdot \mathbf{C}_e^J = \mathbf{M} \cdot \mathbf{C}_v^J = {}^{imp}\mathbf{C}_e^J \tag{25}$$

Due to its structure, the matrix $\mathbf{A}$ is invertible, so that:

$$\mathbf{C}_e^J = A^{-1} \cdot M \cdot C_v^J = A^{-1} \cdot {}^{imp}\mathbf{C}_e^J \tag{26}$$

The product $M.C_v^J$ has an interesting meaning: ${}^{imp}C_e^J$ is an r-dimensional vector of so-called impact control coefficients of the enzymes, ${}^{imp}C_e^J = ({}^{imp}C_{e1}^J, {}^{imp}C_{e2}^J, ..., {}^{imp}C_{er}^J)$.

The impact control coefficient of enzyme i on a flux J [27] quantifies the effect on the flux J of a simultaneous equal relative increase in the rates of all elemental processes in which enzyme i is involved:

$${}^{imp}C_{e_i}^J = \sum_{\text{all } e_i \text{ dependent reactions}} C_{v_k}^J = \sum_{k=2i-1}^{2i+2} C_{v_k}^J \tag{27}$$

In other words, this control coefficient quantifies the total impact that enzyme has on the flux.

Eq. 26 interrelates the two different modes by which enzymes in relay pathways control the flux, i.e., through their concentration $\mathbf{C}_e^J$ and through their activities in the elemental processes $\mathbf{C}_v^J$. Most strikingly, the control exerted by the concentration of an enzyme does not just depend on the control it exerts on all the reactions in which it is involved (its 'impact'), but also on the impact control coefficients of other enzymes. The persistence of ternary complexes ($E_i PE_{i+1}$) also affects the control coefficients of the enzymes (through matrix A).

We can also analyze the amount of the control exerted on the flux by the boundaries. When the ratio [SP]/[S] is clamped from the outside (leaving changes in [SP] and [S] possible), one can define for any system variable Y:

$$C_{e_0}^Y = C_{[S]+[SP]}^Y = C_{[S]}^Y + C_{[SP]}^Y, \qquad \frac{[S]}{[SP]} = \text{constant} \tag{28}$$

where the control coefficients with respect to S and SP are defined individually as:

$$C_{[S]}^Y = \frac{d\ln Y}{d\ln[S]}, \; C_{[SP]}^Y = \frac{d\ln Y}{d\ln[SP]} \tag{29}$$

For this control by the boundary substrate couple S, SP, Appendix A shows that:

$$C_{e_0}^J + C_{e_1}^J \cdot \frac{SPE_1}{e_1} = C_{v_1}^J + C_{v_2}^J \tag{30}$$

This equation specifies that the control exerted by the substrate couple on group transfer flux equals the control exerted by the two elementary steps this couple is involved in ($C_{v1}^J + C_{v2}^J$), except for the control exerted by enzyme 1 through its complex with SP ($-C_{e1}^J \cdot SPE_1/e_1$). For the control exerted by the boundary product couple W and WP we have (Appendix A):

$$C_{e_{r+1}}^J + C_{e_r}^J \cdot \frac{E_r PW}{e_r} = C_{v_{2r+1}}^J + C_{v_{2r+2}}^J \tag{31}$$

Here, under the condition of clamped ratio [W]/[WP] notation similar to that in Eqs. 28 and 29 were used:

$$C_W^Y = \frac{d\ln Y}{d\ln[W]}, \; C_{WP}^Y = \frac{\partial \ln Y}{\partial \ln[WP]}$$

$$C_{W+WP}^Y = C_{e_{r+1}}^Y = C_W^Y + C_{WP}^Y \tag{32}$$

Using Eqs. 30, 31 and 26 one can readily express the control coefficients of boundary substrates in terms of the control coefficients of elemental processe. Moreover, Eqs. 30 and 31 can be included in the matrix form of the system of linear equations connecting C_{ei}^J and C_{vi}^J. For that one should consider an extended $(r + 2)$-dimensional vector of the flux control coefficients of enzymes (cf. Eq. 22):

$$\mathbf{C_e^J} = \left(C_{e_o}^J, C_{e_1}^J, ..., C_{e_r}^J, C_{e_{r+1}}^J \right)^T \tag{33}$$

as well as an extended $(r + 2) \times (r + 2)$ matrix $\mathbf{A}$ that includes two additional rows and columns (cf. Eq. 21), corresponding to Eqs. 30, 31:

$$\mathbf{A} = \begin{vmatrix} 1 & \dfrac{Q_0}{\theta_1} & 0 & 0 & \cdots & 0 & 0 & 0 \\ 0 & 1 & \dfrac{Q_1}{\theta_2} & 0 & \cdots & 0 & 0 & 0 \\ 0 & \dfrac{Q_1}{\theta_1} & 1 & \dfrac{Q_2}{\theta_3} & \cdots & 0 & 0 & 0 \\ & & \cdot & \cdot & \cdot & & & \\ 0 & 0 & \cdots & 0 & \dfrac{Q_{r-2}}{\theta_{r-2}} & 1 & \dfrac{Q_{r-1}}{\theta_r} & 0 \\ 0 & 0 & \cdots & 0 & 0 & \dfrac{Q_{r-1}}{\theta_{r-1}} & 1 & 0 \\ 0 & 0 & \cdots & 0 & 0 & 0 & \dfrac{Q_r}{\theta_r} & 1 \end{vmatrix}$$

(34) and the extended $(r + 2) \times (2r + 2)$ rectangular matrix $\mathbf{M}$ (cf. Eq. 24):

$$\mathbf{M} = \begin{vmatrix} 1 & 1 & 0 & 0 & 0 & 0 & \cdots & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 1 & 1 & 1 & 0 & 0 & \cdots & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 1 & 1 & 1 & \cdots & 0 & 0 & 0 & 0 & 0 & 0 \\ \cdots & & & \cdots & & & & \cdots & & & \cdots & & \\ 0 & 0 & 0 & 0 & 0 & 0 & \cdots & 1 & 1 & 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \cdots & 0 & 0 & 1 & 1 & 1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & \cdots & 0 & 0 & 0 & 0 & 1 & 1 \end{vmatrix}$$

(35)

With this change in definitions, the matrix equations expressing the control coefficients of all enzymes and boundary substrates into control coefficients of the elemental processes will continue to coincide with Eq. 26.

In this section we have shown how the coefficients quantifying the control exerted by the concentrations of enzymes and boundary substrates of a group-transfer pathway, may be related to the more fundamental control exerted by the elemental processes. Because the latter should obey standard control laws (see below), this will allow us to make explicit the principles governing the former control properties.

2.3. Flux control summation theorems

In ideal metabolic pathways powerful summation theorems delimit the combined control exerted by the enzymes. For control of flux, the total control by all enzymes is 1; for control on concentrations it amounts to a net total control of 0. The enzymes in group-transfer pathways are substrates for each others action. Hence, the question arises whether the summation of the enzymes' control coefficients leads to results that differ from those in ideal metabolic pathways. Summing the left-hand and right-hand sides of Eqs. 18–20, 30 and 31, one obtains:

$$\sum_{i=0}^{r+1} C_{e_i}^J + \sum_{i=2}^{r+1} C_{e_{i-1}}^J \cdot \frac{E_{i-1}PE_i}{e_{i-1}} + \sum_{i=0}^{r-1} C_{e_{i+1}}^J \cdot \frac{E_i PE_{i+1}}{e_{i+1}}$$

$$= C_{v_1}^J + C_{v_2}^J + \sum_{i=1}^{r} \sum_{l=2i-1}^{2i+2} C_{v_l}^J + C_{v_{2r+1}}^J + C_{v_{2r+2}}^J \tag{36}$$

After substituting the summation index i for $i - 1$ in the second sum in the left-hand side of this equation, and i for $i + 1$ in the third sum and rearranging, we have:

$$C_{e_0}^J + C_{e_{r+1}}^J + \sum_{i=1}^{r} C_{e_i}^J \cdot \left(1 + \frac{E_{i-1}PE_i + E_i PE_{i+1}}{e_i} \right)$$

$$= 2 \cdot \sum_{l=1}^{2r+2} C_{v_l}^J \tag{37}$$

Now we shall make use of the fact that, if the system is considered just in terms of its elemental processes, it is a special case of an ideal pathway. As a consequence, the sum of the elemental flux control coefficients, C_{vi}^J, over all the elemental processes is always equal to unity [1,24,25]:

$$\sum_{l=1}^{2r+2} C_{v_l}^J = 1 \tag{38}$$

Using Eq. 37, this yields an expression for a weighted sum of the enzyme-concentration flux-control coefficients:

$$C_{e_0}^J + \sum_{i=1}^{r} C_{e_i}^J \cdot \left(1 + \frac{E_{i-1}PE_i + E_i PE_{i+1}}{e_i} \right) + C_{e_{r+1}}^J = 2 \tag{39}$$

where $C_{e_o}^J$ and $C_{e_{r+1}}^J$ refer to control to boundary substrate/product couples, as defined by Eqs. 29 and 22. Since

$$\frac{E_{i-1}PE_i + E_i PE_{i+1}}{e_i}$$

corresponds to the fraction of enzyme i that is complexed (to either enzyme $i - 1$ or enzyme $i + 1$), Eq. 39 yields the simple result that the sum of the enzymes' and boundaries' flux control coefficients equals 2 divided by a correction factor which lies between 1 and 2. The latter correction factor is a weighted average of the fraction of the enzymes that, on average is complexed. Hence, the sum of enzyme control coefficients, ΣC_{ei}^J, that involves the control of boundary substrate/product couples, often exceeds 1 in the system of ideal group transfer ('or perfect dynamic channel') [24]. We note that, in the general case of *channelling* it can be less than 1 [28,25,27]).

The relations between C_e^J and C_v^J simplify greatly when enzymes E_i and E_{i+1} react by simple collision and do not form an enzyme-enzyme complex $E_i PE_{i+1}$ with a significant lifetime. Then we can neglect $E_i PE_{i+1}$ in Eqs. 18–20 and summation theorem, Eq. 39. If for example, the enzyme i interacts with enzymes $i - 1$ and $i + 1$ by simple collisions, that is $E_{i-2}PE_{i-1} \approx E_{i-1}PE_i = 0$, then we have:

$$C_{e_i}^J = \sum_{\text{set of } E_i\text{-dependent reactions}} C_{v_k}^J = {}^{\text{imp}}C_{e_i}^J \qquad (40)$$

That is, the total control exerted by the concentration of an enzyme becomes equal to its impact. If this is so for all enzyme combinations:

$$\sum_{i=0}^{r+1} C_{e_i}^J = 2 \qquad (41)$$

Eqs. 40 and 41 reflect that, because enzymes in group-transfer pathway are involved in two rather than one (transfer) process they exert more flux control on average than they do in ideal metabolic pathways.

2.4. Relating concentration control by enzymes to concentration control by elemental processes: c_{ei}^x and c_{vk}^x

Using much the same procedure as in section 2 one can also express the control exerted by the enzymes on any concentration x, C_{ei}^x, into those of the processes, C_{vk}^x. Again we consider the perturbation in which all forms of enzyme i are amplified by the same factor and all process activities in which enzyme i participates are reduced simultaneously. Any concentration x_j that does not correspond to one of the forms of enzyme i, remains unchanged in the new steady-state. Consequently (cf. Eq. A11),

$$\frac{d\ln x_j}{d\ln \lambda_i} = 0 =$$

$$= \sum_{l=1}^{2r+2} C_{v_l}^{x_j} \cdot \frac{d\ln \xi_l}{d\ln \lambda_i} + \sum_{v=1}^{r} C_{e_v}^{x_j} \cdot \frac{d\ln e_v}{d\ln \lambda_i},$$

$$x_j \neq (E_i, E_i P, Q_{(i-1)}, Q_i) \qquad (42)$$

When the amplified enzyme is one of the pathway internal

enzymes $(i = 2, 3, ..., r - 1)$, Eq. 42 and Eqs. A3, A5 of Appendix A require that:

$$C_{e_{i-1}}^{x_j} \cdot \frac{Q_{i-1}}{e_{i-1}} + C_{e_i}^{x_j} + C_{e_{i+1}}^{x_j} \cdot \frac{Q_i}{e_{i+1}}$$

$$= C_{v_{2i-1}}^{x_j} + C_{v_{2i}}^{x_j} + C_{v_{2i+1}}^{x_j} + C_{v_{2i+2}}^{x_j}$$

$$i = 2, 3, ..., r - 1, \quad x_j \neq (E_i, E_i P, Q_{i-1}, Q_i) \qquad (43)$$

When the amplified enzyme is the first $(i = 1)$ or the ultimate $(i = r)$ enzyme, one obtains from Eqs. 42, A3 and A7, A9:

$$C_{e_1}^{x_j} + C_{e_2}^{x_j} \cdot \frac{Q_1}{e_2} = C_{v_1}^{x_j} + C_{v_2}^{x_j} + C_{v_3}^{x_j} + C_{v_4}^{x_j}$$

$$x_j \neq (E_1, E_1 P, Q_0, Q_1) \qquad (44)$$

$$C_{e_{r-1}}^{x_j} \cdot \frac{Q_{r-1}}{e_{r-1}} + C_{e_r}^{x_j} = C_{v_{2r-1}}^{x_j} + C_{2r}^{x_j} + C_{V_{2r+1}}^{x_j} + C_{v_{2r+2}}^{x_j}$$

$$x_j \neq (E_r, E_r P, Q_{r-1}, Q_r) \qquad (45)$$

Apparently, the control exerted by the first true enzyme (e_1) in the group transfer pathway on a concentration of any form of any of the other enzymes equals the control exerted by the four elemental steps in which enzyme 1 participates minus the control exerted by enzyme 2 that forms a complex with enzyme 1.

For the metabolites (S, SP, W, WP) that serve as substrate/product couples of the pathway, we should consider perturbations given by Eqs. A12 or A13 of Appendix A and parameter changes Eq. A14 and Eq. A16, or Eq. A15 and Eq. A17, respectively. Taking into account definition Eq. 29, for $x_j \neq Q_0$, we obtain:

$$C_{e_0}^{x_j} + C_{e_1}^{x_j} \cdot \frac{Q_0}{e_1} = C_{v_1}^{x_j} + C_{v_2}^{x_j}, \qquad x_j \neq Q_0 \qquad (46)$$

Similarly, for $x_j \neq Q_r$, taking into account definition (32), we obtain:

$$C_{e_r}^{x_j} \cdot \frac{Q_r}{e_r} + C_{e_{r+1}}^{x_j} = C_{v_{2r+1}}^{x_j} + C_{v_{2r+2}}^{x_j}, \qquad x_j \neq Q_r \qquad (47)$$

When the concentrations of all molecules containing enzyme i are amplified (see Eq. 17) and the system parameters are changed correspondingly (see Eq. A2), the following variables x will be changed in the new steady state:

$$x_i = E_i, E_i P, Q_{i-1}, Q_i$$

Hence,

$$\frac{d\ln x_i}{d\ln \lambda_i} = 1 =$$

$$= \sum_{l=1}^{2r+2} C_{v_l}^{x_i} \cdot \frac{d\ln \xi_l}{d\ln \lambda_i} + \sum_{v=1}^{r} C_{e_v}^{x_i} \cdot \frac{d\ln e_v}{d\ln \lambda_i} \qquad (48)$$

Consequently, with respect to *these* concentration variables distinct summation theorems obtain. For $i = 2, 3,...,r - 1$ we obtain from Eqs. 48, A3, A5:

$$C_{e_{i-1}}^{x_i} \cdot \frac{Q_{i-1}}{e_{i-1}} + C_{e_i}^{x_i} + C_{e_{i+1}}^{x_i} \cdot \frac{Q_i}{e_{i+1}} = 1 + \sum_{k=2i-1}^{2i+2} C_{v_k}^{x_i},$$

$$x_i = (E_i, E_i P, Q_{i-1}, Q_i), \quad i = 2,3,...,r - 1 \tag{49}$$

For the perturbation in E_1- (and by analogy) E_r-containing concentrations we obtain from Eqs. 48, A3 and A7 or A9, respectively:

$$C_{e_1}^{x_i} + C_{e_2}^{x_i} \cdot \frac{Q_1}{e_2} = 1 + \sum_{k=1}^{4} C_{v_k}^{x_i},$$

$$x_i = (E_1, E_1 P, Q_0, Q_1) \tag{50}$$

$$C_{e_{r-1}}^{x_j} \cdot \frac{Q_{r-1}}{e_{r-1}} + C_{e_r}^{x_j} = 1 + \sum_{k=2r-1}^{2r+2} C_{v_k}^{x_j}$$

$$x_j = (E_r, E_r P, Q_{r-1}, Q_r) \tag{51}$$

For the perturbation corresponding to Eq. A12 or Eq. A13 in the 'boundary substrates' one obtains (cf. Eqs. 46 or 47):

$$C_{e_0}^{Q_0} + C_{e_1}^{Q_0} \cdot \frac{Q_0}{e_1} = 1 + C_{v_1}^{Q_0} + C_{v_2}^{Q_0} \tag{52}$$

$$C_{e_r}^{Q_r} \cdot \frac{Q_r}{e_r} + C_{e_{r+1}}^{Q_r} = 1 + C_{v_{2r+1}}^{Q_r} + C_{v_{2r+2}}^{Q_r} \tag{53}$$

Eqs. 43–47, 49–53 allow one to express the control of any concentration by any enzyme (C_{ei}^x) in terms of the control by the elemental processes (C_{vk}^x) and the relative fractions of enzyme-enzyme complexes $(Q_i/e_i, Q_{i-1}/e_i)$.

It must be emphasized that one may consider the control of only those $(2r + 1)$ concentrations which are chosen as independent ones, e.g. $E_i P$ $(i = 1, 2,...,r)$ and Q_i $(i = 0, 1,...,r)$, see below. For the concentrations (E_i) the control coefficients can then be also determined from the former control coefficients using relations that are obtained from the moiety conservation restrictions, see Eq. 5 [29]:

$$C_{e_l}^{E_i} \cdot \frac{E_i}{e_l} + C_{e_l}^{Q_{i-1}} \cdot \frac{Q_{i-1}}{e_l} + C_{e_l}^{E_i P} \cdot \frac{E_i P}{e_l} + C_{e_l}^{Q_i} \cdot \frac{Q_i}{e_l} = \delta_{il}$$

$$i, l = 1, 2,...,r \tag{54}$$

or in terms of the elemental control coefficients, C_{vi}^x:

$$C_{v_l}^{E_i} \cdot E_i + C_{v_l}^{Q_{i-1}} \cdot Q_{i-1} + C_{v_l}^{E_i P} \cdot E_i P + C_{v_l}^{Q_i} \cdot Q_i = 0$$

$$i = 1, 2,...,r; \ l = 1, 2,...,2r + 2 \tag{55}$$

The linear equation system of Eqs. 43–53 can most readily be solved by using matrix algebra. To this aim we write the equations as:

$$\mathbf{A} \cdot \mathbf{C}_e^x = \delta_e^x + \mathbf{M} \cdot \mathbf{C}_v^x \tag{56}$$

where C_e^x is the $(r + 2) \times (3r + 1)$ matrix of the concentration control coefficients of the enzymes, $\mathbf{C}_v^x$ is the $(2r + 2)$

$\times$ $(3r + 1)$ matrix of the concentration control coefficients of the elemental processes:

$$(\mathbf{C}_e^x)_{ij} = C_{e_i}^{x_j}, \ i = 0, 1,...,r + 1; \ j = 1, 2,...,3r + 1$$

$$(\mathbf{C}_v^x)_{ij} = C_{v_i}^{x_j}, \ i = 1, 2,...,r + 2; \ j = 1, 2,...,3r + 1 \tag{57}$$

$(r + 2) \times (3r + 1)$ matrix δ_e^x is defined by:

$$(\delta_e^x)_{ij} = 1 \ \text{if} \ x_j = E_i, E_i P, Q_{i-1} \ \text{or} \ Q_i \ \text{and} \ 0 \ \text{otherwise}$$

and matrices $\mathbf{A}$ and $\mathbf{M}$ are defined by Eqs. 34 and 35, respectively. The enzymes' concentration control coefficients can then be calculated from:

$$\mathbf{C}_e^x = \mathbf{A}^{-1} \cdot \delta_e^x + \mathbf{A}^{-1} \cdot \mathbf{M} \cdot \mathbf{C}_v^x \tag{58}$$

Note, that x in Eqs. 56 and 58 represents any concentration, i.e., not only one of the independent concentrations, since for any x_j (and any flux J) the same matrixes $\mathbf{M}$ and $\mathbf{A}^{-1}$ connect the control coefficients with respect to the enzyme concentrations to the control coefficients with respect to the activities of elemental processes.

With Eq. 58 one can relate the control exerted by an enzyme concentration on a component of a group-transfer pathway to the more fundamental control exerted by the elemental processes. As it did for flux control, this will allow us to arrive at the principles governing the control exerted by enzymes on the concentrations of group transfer components.

2.5. Concentration control summation theorems

To formulate the summation theorems for the concentration control coefficients of the enzymes, let x be the concentration of any free enzyme or that of an enzyme carrying a group P, i.e.,

$$x = E_l \ \text{or} \ E_l P, \ (l = 1,...,r) \tag{59}$$

Obviously, one and only one of the Eqs. 49–51 corresponds to $x = E_1$ or $E_1 P$ (the 'target' equation). Summing this 'target' equation with all the Eqs. 43–47 in which x can be E_1 or $E_1 P$ and using the same rearrangement as above in section 2.3, we obtain:

$$C_{e_0}^{E_l \text{ or } E_l P} + \sum_{i=2}^{r} C_{e_i}^{E_l \text{ or } E_l P} \cdot \left(1 + \frac{Q_{i-1} + Q_i}{e_i}\right) + C_{e_{r+1}}^{E_l \text{ or } E_l P}$$

$$= 1 + 2 \cdot \sum_{k=1}^{2r+2} C_{v_k}^{E_l \text{ or } E_l P}, \ l = 1, 2,...,r \tag{60}$$

Since the sum of the elemental concentration control coefficients over all processes is equal to zero [24],

$$\sum_{k=1}^{2r+2} C_{v_k}^x = 0 \tag{61}$$

one obtains:

$$C_{e_0}^{E_l \text{ or } E_l P} + \sum_{i=1}^{r} C_{e_i}^{E_l \text{ or } E_l P} \cdot \left(1 + \frac{Q_{i-1} + Q_i}{e_i}\right) + C_{e_{r+1}}^{E_l \text{ or } E_l P}$$

$$= 1$$

$$l = 1, 2,...,r \tag{62}$$

Two and only two of the Eqs. 49–53 correspond to $x_i = Q_l$. For example, for $x = Q_0$ these 'target' equations are Eqs. 50 and 52. Note that the 'target' equations have a 1 at the right-hand side. Adding the 'target' equations to the other equations from Eqs. 43–47, in which x_j can be Q_l, one obtains:

$$C_{e_0}^{Q_l} + \sum_{i=1}^{r} C_{e_i}^{Q_l} \cdot \left(1 + \frac{Q_{i-1} + Q_i}{e_i}\right) + C_{e_{r+1}}^{Q_l} = 2,$$

$$l = 0, 1, 2, \ldots, r \tag{63}$$

Eqs. 62 and 63 imply that the sum over all enzymes of all the control coefficients with respect to any of the non-complexed pathway components ranges in between 0 and 1, and with respect to any of the enzyme-enzyme complexes lies in between 1 and 2. This result differs drastically from the case of 'nonchannelled' classical pathways, where such sums of concentration control coefficients amount to zero [3].

In principle, the different types of global control coefficient analyzed here can be measured independently by on the one hand using modifiers of enzymes (e.g., specific inhibitors) and on the other hand modulating the enzyme concentrations (e.g. by manipulating gene expression) [see the companion paper, [30]]. The differences between the values of the different control coefficients give a deeper insight into pathway regulation and mechanisms [25].

The results obtained demonstrate the interplay between the different modes by which the enzymes control fluxes and concentrations. The derived equations interrelate the different types of 'global' control properties. They do not refer to nor depend on the local enzyme properties which are commonly described in terms of so called 'elasticity' coefficients [1].

The other important aspect of analyzing pathway control structure is to understand the global control properties in terms of the local ones. The following sections are devoted to this problem.

2.6. The flux-control connectivity relations for group-transfer pathways

For any metabolic pathway with a given map the strengths of the control exerted by enzymes depends uniquely on the kinetic properties of the enzymes. The relevant part of the latter are the elasticity coefficients. The connectivity theorems relate the control coefficients to those elasticity coefficients. In this section we ask whether, in group transfer pathways, control coefficients are also uniquely related tot the kinetic properties of the enzymes, and if so, how.

2.6.a. Flux control connectivity theorems

The connectivity relations relating the flux (C_{vi}^{J}) or concentration (C_{vi}^{x}) control coefficients of the elemental processes to the elasticity coefficients of those elemental

processes $(\epsilon_{x_k}^{i})$ are also valid for group transfer pathways (by the same reasoning as for the corresponding summation theorems, see above). Together with the corresponding summation theorems, they allow one to express all the control coefficients in terms of the elasticity coefficients and the pathway stoichiometries [11,10,12,6]. Appendix B discusses this in detail.

For the elemental flux control coefficients the following connectivity relationship obtains:

$$\sum_{i=1}^{2r+2} C_{v_i}^{J} \cdot \left(\epsilon_{E_l}^{i} \cdot \frac{1}{E_l} - \epsilon_{E_lP}^{i} \cdot \frac{1}{E_lP}\right) = 0 \quad l = 1, 2, \ldots, r \tag{64}$$

This equation shows that control by elemental steps are inversely related to their local responses to relevant fluctuations of metabolic variables [cf. [5]. In other words, highly sensitive steps exert little control, also in group transfer pathways. An additional connectivity theorem is (see Appendix B):

$$\sum_{i=1}^{2r+2} C_{v_i}^{J} \cdot \left(\epsilon_{E_l}^{i} \cdot \frac{1}{E_l} - \epsilon_{Q_l}^{i} \cdot \frac{1}{Q_l} + \epsilon_{E_{l+1}}^{i} \cdot \frac{1}{E_{l+1}}\right) = 0,$$

$$l = 1, 2, \ldots, r - 1 \tag{65}$$

Two more connectivity theorems are obtained for the first and last reactions of the chain.

$$\sum_{i=1}^{2r+2} C_{v_i}^{J} \cdot \left(\epsilon_{E_1}^{i} \cdot \frac{1}{E_1} - \epsilon_{Q_0}^{i} \cdot \frac{1}{Q_0}\right) = 0 \tag{66}$$

$$\sum_{i=1}^{2r+2} C_{v_i}^{J} \cdot \left(\epsilon_{E_r}^{i} \cdot \frac{1}{E_r} - \epsilon_{Q_r}^{i} \cdot \frac{1}{Q_r}\right) = 0 \tag{67}$$

The $r + 2$ connectivity relations, Eqs. 64, 66, 67, $(r - 1)$ connectivity relations, Eqs. 65, and summation theorem Eq. 38 constitute a system of $(2r + 2)$ equations for expressing C_{vi}^{J} $(i = 1, \ldots, 2r + 2)$ in terms of the elasticity coefficients.

In the system under study the elasticities are readily expressed into the rate constants of the elemental processes:

$$\epsilon_{E_l}^{i} = 0 \quad for\ i \neq 2l - 1, 2l + 2;\ l = 1, \ldots, r$$

$$\epsilon_{E_l}^{2l-1} = \frac{E_l}{v_{2l-1}} \cdot k_{2l-1}^{+} \cdot E_{l-1}P;$$

$$\epsilon_{E_l}^{2l+2} = -\frac{E_l}{v_{2l+2}} \cdot k_{2l+2}^{-} \cdot E_{l+1}P;$$

$$\epsilon_{E_lP}^{i} = 0 \quad for\ i \neq 2l, 2l + 1;$$

$$\epsilon_{E_lP}^{2l} = -\frac{E_lP}{v_{2l}} \cdot k_{2l}^{-} \cdot E_{l-1};\ \epsilon_{E_lP}^{2l+1} = \frac{E_lP}{v_{2l+1}} \cdot k_{2l+1}^{+} \cdot E_{l+1},$$

$$for\ l = 1, \ldots, r \tag{68}$$

For the elasticities with respect to the enzyme-enzyme complexes one may have:

$$\epsilon_{Q_l}^i = 0 \ \ for \ i \neq 2l + 1, 2l + 2; \ l = 0, 1,...,r$$

$$\epsilon_{Q_l}^{2l+1} = -\frac{Q_l}{v_{2l+1}} \cdot k_{2l+1}^-, \ \ \epsilon_{Q_l}^{2l+2} = \frac{Q_l}{v_{2l+2}} \cdot k_{2l+2}^+; \quad (69)$$

Inserting Eqs. 68 into 64 and taking into account that at steady-state any $v_i = J$, we obtain for this system:

$$C_{v_{2l-1}}^J \cdot k_{2l-1}^+ \cdot E_{l-1}P + C_{v_{2l}}^J \cdot k_{2l}^- \cdot E_{l-1} - C_{v_{2l+1}}^J \cdot k_{2l+1}^+$$

$$\cdot E_{l+1} - C_{v_{2l+2}}^J \cdot k_{2l+2}^- \cdot E_{l+1}P = 0$$

$$l = 1,...,r \quad (70)$$

These equations relate elemental control coefficients to kinetic properties of the elemental steps in the group transfer pathway. Reminiscent of the control theory of metabolic pathways is their implication that, at equal concentrations, control by a step decreases with its responsiveness to changes in the concentration that affect its rate. From Eqs. 66–69 we have for the control coefficients of the first few elemental steps:

$$C_{v_1}^J \cdot (k_1^+ \cdot [SP] + k_1^-) - C_{v_2}^J \cdot k_2^+ - C_{v_4}^J \cdot k_4^- \cdot E_2 P = 0 \quad (71)$$

and for those of the ultimate elemental steps:

$$C_{v_{2r-1}}^J \cdot k_{2r-1}^+ \cdot E_{r-1}P + C_{v_{2r+1}}^J \cdot k_{2l+1}^- - C_{v_{2r+2}}^J$$

$$\cdot (k_{2r+2}^+ + k_{2r+2}^- \cdot [WP]) = 0 \quad (72)$$

and from Eqs. 65–69:

$$C_{v_{2l-1}}^J \cdot k_{2l-1}^+ \cdot E_{l-1}P + C_{v_{2l+1}}^J \cdot (k_{2l+1}^+ \cdot E_l P + k_{2l-1}^-)$$

$$- C_{v_{2l+2}}^J \cdot (k_{2l+2}^- \cdot E_{l+1}P + k_{2l+2}^+) - C_{v_{2l+4}}^J$$

$$\cdot (k_{2l+4}^- \cdot E_{l+2}P) = 0, \quad (73)$$

$$l = 1,...,r - 1$$

In all cases, several control coefficients and several kinetic properties occur in the same equation, emphasizing that there is no unique relationship between the control exerted by an elemental process and the kinetic properties of that process. Always, the kinetic properties of the rest of the pathway codetermine the control by an elemental step.

2.6.b. Concentration control connectivity theorems

Using procedures analogous to those employed in section 6a, one obtains the connectivity relations for the concentration control coefficients, C_{vi}^x, such as (see Appendix C):

$$\sum_{i=1}^{2r+2} C_{v_i}^{x_j} \cdot \left(\epsilon_{E_l}^i \cdot \frac{1}{E_l} - \epsilon_{E_lP}^i \cdot \frac{1}{E_lP} \right) = 0, \ for \ x_j \neq E_l, E_lP \quad (74)$$

$$\sum_{i=1}^{2r+2} C_{v_i}^{E_lP} \cdot \left(\epsilon_{E_l}^i \cdot \frac{1}{E_l} - \epsilon_{E_lP}^i \cdot \frac{1}{E_lP} \right) = \frac{1}{E_lP}$$

$$l = 1, 2,...,r \quad (75)$$

We here considered only the control of independent concentrations (i.e., E_lP and Q_l). For the remaining concentrations (E_l) one can obtain the connectivity relations using the conservation relations; cf., Eqs. 54, 55). For example, one can obtain from Eqs. 74, 55, 75:

$$\sum_{i=1}^{2r+2} C_{v_i}^{E_l} \cdot \left(\epsilon_{E_l}^i \cdot \frac{1}{E_l} - \epsilon_{E_lP}^i \cdot \frac{1}{E_lP} \right) = -\frac{1}{E_l}$$

$$l = 1,...,r \quad (76)$$

Alternative connectivity relations are found in Appendix C.

These connectivity relations can be reformulated in terms of rate constants rather than elasticities (cf. Eqs. 70–73). Moreover, together with summation theorems, they allow the calculation of the concentration control coefficients of the elemental processes, C_{vi}^{xj} $(i = 1, 2,...,2r + 2)$ from the elasticity coefficients.

2.7. Expressing control coefficients into enzyme properties (elasticity coefficients)

Above we have derived sufficient summation and connectivity relations to allow one to express flux control and concentration control coefficients into elasticity coefficients. The calculations involve the solution of a large number of linear equations. Consequently, it pays to apply matrix algebra. Appendices D and E elaborate this in terms of the non-normalized control and elasticity coefficients. Here we transform the results into the matrix equations for normalized coefficients. To this purpose, we define the following diagonal matrices $\mathbf{x_d}$ and $\mathbf{v_d}$,

$$(x_d)_{2l-1,2l-1} = E_lP, \ (l = 1,...,r)$$

$$(x_d)_{2l,2l} = Q_l$$

$$(x_d)_{2r+1,2r+1} = Q_0$$

$$(x_d)_{2r+2,2r+2} = J \quad (77)$$

$$(v_d)_{l,l} = v_l, \ l = 1,...,2r + 2 \quad (78)$$

We define the $(2r + 2) \times (2r + 2)$ matrices $\mathbf{E}$ and $\mathbf{C}_v$ by:

$$\mathbf{E} = \mathbf{x_d} \cdot \mathbf{D} \cdot (\mathbf{v_d})^{-1} \quad (79)$$

$$\mathbf{C_v} = \mathbf{v_d} \cdot \boldsymbol{\Gamma} \cdot (\mathbf{x_d})^{-1} \quad (80)$$

Matrices $\mathbf{D}$ and $\boldsymbol{\Gamma}$ are defined in Appendix E (see Eqs. E1, E7 and E9). Matrices $\mathbf{E}$ and $\mathbf{C}_v$ contain normalized elasticity coefficients and control coefficients. In a more explicit form the matrix $\mathbf{E}$ reads:

$$
E = \begin{pmatrix}
\frac{E_1P}{E_1}\epsilon_{E_1}^1 & -\epsilon_{E_1P}^2 & -\epsilon_{E_1P}^3 & \frac{E_1P}{E_1}\epsilon_{E_1}^4 & 0 & 0 & \cdots & 0 & 0 & 0 & 0 \\[2mm]
\frac{Q_1}{E_1}\epsilon_{E_1}^1 & 0 & \frac{Q_1}{E_2}\epsilon_{E_2}^3-\epsilon_{Q_1}^3 & \frac{Q_1}{E_1}\epsilon_{E_1}^4-\epsilon_{Q_1}^4 & 0 & \frac{Q_1}{E_2}\epsilon_{E_2}^6 & \cdots & 0 & 0 & 0 & 0 \\[2mm]
0 & 0 & \frac{E_2P}{E_2}\epsilon_{E_2}^3 & -\epsilon_{E_2P}^4 & -\epsilon_{E_2P}^5 & \frac{E_2P}{E_2}\epsilon_{E_2}^6 & \cdots & 0 & 0 & 0 & 0 \\[2mm]
\cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\[2mm]
0 & 0 & 0 & 0 & 0 & 0 & \cdots & \frac{E_rP}{E_r}\epsilon_{E_r}^{2r-1} & -\epsilon_{E_rP}^{2r} & -\epsilon_{E_rP}^{2r+1} & \frac{E_rP}{E_r}\epsilon_{E_r}^{2r+2} \\[2mm]
0 & 0 & 0 & 0 & 0 & 0 & \cdots & \frac{Q_r}{E_r}\epsilon_{E_r}^{2r-1} & 0 & -\epsilon_{Q_r}^{2r+1} & \frac{Q_r}{E_r}\epsilon_{E_r}^{2r+2}-\epsilon_{Q_r}^{2r+2} \\[2mm]
\frac{Q_0}{E_1}\epsilon_{E_1}^1-\epsilon_{Q_0}^1 & -\epsilon_{Q_0}^2 & -\epsilon_{Q_0}^3 & \frac{Q_0}{E_1}\epsilon_{E_1}^4 & 0 & 0 & \cdots & 0 & 0 & 0 & 0 \\[2mm]
1 & 1 & 1 & 1 & 1 & 1 & \cdots & 1 & 1 & 1 & 1
\end{pmatrix}
$$

(81) Then Eq. E10 of Appendix E can be written as:

$$
E \cdot C_v = I_{2r+2} = x_d \cdot D \cdot (v_d)^{-1} \cdot v_d \cdot \Gamma \cdot (x_d)^{-1} \tag{82}
$$

From which it follows (whenever E is non-singular):

$$
C_v = E^{-1} \tag{83}
$$

i.e., the elemental control coefficients can be calculated from the elasticity coefficients by matrix inversion. Eqs. 25 and 57 may be combined as:

$$
A \cdot C_e = \delta + M \cdot C_v \tag{84}
$$

where δ is a $(2r + 2) \times (2r + 2)$ diagonal matrix defined by having δ_e^x as its $(2r + 1) \times (2r + 1)$ upper left submatrix, and otherwise zeroes.

The combination of Eqs. 83 and 84 allows one to express all enzymes control coefficients into all elasticity coefficients by:

$$
C_e = A^{-1} \cdot \delta + A^{-1} \cdot M \cdot E^{-1} \tag{85}
$$

This equation shows that also in a group transfer pathway the control exerted by enzyme concentrations on pathway flux or concentrations is completely determined by the elasticity coefficients of the participating enzymes (in E), i.e., by the kinetic properties of the interactions between these enzymes.

3. Discussion

In this paper the control theory for group-transfer or 'relay' pathways has been developed. Consequently, the control exerted by participating enzymes on the relay flux and on the concentration of pathway components can now be understood in terms of kinetic properties (the elasticity coefficients) of the protein components of the pathway. In the Results section and in Appendix F we elaborate two complementary ways of doing this. For some pathways many kinetic characteristics are known (e.g., [33]) and the method may soon be applicable in detail.

In the dawn of such a detailed application, the developed theory already reveals properties that are of interest beyond any particular system. One of these is that the sum of all flux control coefficients over all enzymes in the pathway equals 2 if the pathway substrates and products exert no control (are present far above and below their respective K'_m, K'_i, repectively) and if the complexes between the enzymes live briefly. In cases where the complexes are longlived, such that the enzymes hardly occur in their uncomplexed forms, the sum of the flux control reduces to 1. Because the average extent of complex formation between the enzymes will tend to increase with overall enzyme concentration, this finding suggests that the control of group transfer flux by the participating enzymes varies with the average protein concentration and for that reason alone may differ between vivum and vitrum. This stresses that measurement of control coefficients should be performed in vivo.

Measurement of the sum of the enzymes' flux control coefficients and in particular its deviation from 1 and 2 may serve as a measure of the extent of complexation of the proteins in the pathway. This may be one of the few methods by which such complex formation can be demonstrated in cases where cell disruption leads to dissociation of such complexes [34].

In many relay pathways, it is not the flux, but the concentration of one of the components that functions as a signal for other parts of cell physiology. for instance, the factor III^{Glc} (recently renamed to IIA^{Glc}) of the phosphotransferase system of E. coli is involved in the catabolic repression by glucose [35]. Glucose causes factor III^{Glc} ∼ P to be dephosphorylated and III^{Glc} inhibits the activity of

transporters of alternative growth substrates. Adenylate cyclase may be affected by the ratio $III^{Glc} \sim P$ to III^{Glc}. For these cases, the results of the present study may be relevant. Non-critical application of existing metabolic control theory might have suggested that the control exerted by all PTS enzymes on III^{Glc} be zero. The present study shows that control should lie between zero and 1, depending on the same extra conditions as does the sum of the flux control coefficients. That is, if pathway substrates and products lack control and if enzyme complexation is minor, that sum is 1 and regulated expression of the genes encoding PTS enzymes may have a strong impact on signalling, contrary to what might have been expected.

It is of interest that many pathways that might have been considered to be of the straightforward 'metabolic' type, are actual relay pathways. An important example is that of the electron transfer chain in free-energy coupling membranes. In particular, the present study suggests that in the case of a dynamic organization of the components of these chains (as proposed by Hackenbrock [36]), the sum of the control coefficients on the flux of reducing equivalents should equal 2, whereas in the case of a static organization (cf. as proposed by Ferguson-Miller et al., [37]) it should equal 1. Existing experimental data [38] lack sufficient resolution to decide between the two alternatives.

In the light of the latter study, it should be noted that if the relay pathway is part of a larger network, then the result on the sum of control coefficients should be adjusted so as to state that the sum of the control coefficients over the components of the relay chain is double that otherwise expected. This can be understood in the light of modular metabolic control theory [18].

It may be noted that the group-transfer pathway is analogous to metabolic pathways where the metabolites are channelled between subsequent enzymes. For a long time (but see [39,27]), control theory for channelled pathways has been lacking, except for cases where there was channelling between enzymes in static complexes [28]. The present theory applies to cases where the complexes are dynamic and transfer of metabolite and association/dissociation fo the enzymes alternate. As such it is yet another part of a comprehensive control theory for metabolic channelling.

A most important conclusion arrived at in this paper is that enzymes participating in relay pathways control the relay flux in more that one manner. In a sense, this derives from the phenomenon that in such pathways the enzymes play both the role of catalyst and the role of metabolite. Or, to put it differently, the enzymes are involved in more than one elemental reaction and control can be attributed to each of these activities. In a parallel paper we shall demonstrate that these various modalites of control can be defined operationally and measured experimentally using a combination of inhibitor-titration and gene-expression-modulation methods [30].

Acknowledgements

Appendix 1

Appendix A: Relating enzyme control to process control (equation numbers also refer to main text)

Let us consider the perturbation in only those concentration variables that involve enzyme i, as given by Eq. 17 of the main text. Let us change simultaneously, the parameters ξ_l of the rates v_l that depend on the concentrations mentioned in Eq. 17, in such a manner that the rates v_l remain the same as in the initial steady state. In the new steady state immediately obtained, the concentrations involving enzyme i obey Eq. 17, but the rates of all elemental processes have remained the same as in the initial steady-state. That is, let $\mathbf{x}'$ be the vector of concentrations in the new (perturbed) steady state and $\mathbf{x}°$ be the vector of concentrations in the initial steady state. According to Eq. 17, $\mathbf{x}' = \mathbf{x}(\lambda_i)$ and the perturbed rates read:

$$v_{2i-1}(\mathbf{x}') = \xi_{2i-1} \cdot (k_{2i-1}^+ \cdot E_i(\lambda_i)$$
$$\cdot E_{i-1}P - k_{2i-1}^- \cdot Q_{i-1}(\lambda_i)) =$$
$$= \xi_{2i-1} \cdot \lambda_i \cdot v_{2i-1}(\mathbf{x}°),$$

and, similarly,

$$v_{2i}(\mathbf{x}') = \xi_{2i} \cdot \lambda_i \cdot v_{2i}(\mathbf{x}°)$$
$$v_{2i+1}(\mathbf{x}') = \xi_{2i+1} \cdot \lambda_i \cdot v_{2i+1}(\mathbf{x}°)$$
$$v_{2i+2}(\mathbf{x}') = \xi_{2i+2} \cdot \lambda_i \cdot v_{2i+2}(\mathbf{x}°) \tag{A1}$$

The rates in the new steady state will coincide with the rates in the initial one, if:

$$\xi_{2i-i} \cdot \lambda_i = \xi_{2i} \cdot \lambda_i = \xi_{2i+1} \cdot \lambda_i = \xi_{2i+2} \cdot \lambda_i = 1, \tag{A2}$$

or since λ_i may have an arbitrary magnitude: the changes in the rate of process l should obey:

$$\frac{d\ln \xi_l}{d\ln \lambda_i} = -1 \ (l = 2i - 1, 2i, 2i + 1, 2i + 2;$$

for $i = 1, 2, ..., r)$ \tag{A3}

The operation characterized by λ_i increases the total concentration, e_i, of the enzyme i and the enzymes with which enzyme i complexes, i.e., enzymes $i - 1$ and $i + 1$:

$$e_i(\lambda_i) = E_i(\lambda_i) + E_iP(\lambda_i) + Q_{i-1}(\lambda_i) + Q_i(\lambda_i)$$
$$= \lambda_i \cdot e_i,$$

$$e_{i-1}(\lambda_i) = E_{i-1} + E_{i-1}P + Q_{i-2} + \lambda_i \cdot Q_{i-1}, \tag{A4}$$
$$e_{i+1}(\lambda_i) = E_{i+1} + E_{i+1}P + \lambda_i \cdot Q_i + Q_{i+1},$$

$i = 2, 3, ..., r - 1)$

From Eq. A4 it follows that:

$$\left[\frac{d\ln e_i}{d\ln \lambda_i}\right]_{\lambda_i=1} = 1, \left[\frac{d\ln e_{i-1}}{d\ln \lambda_i}\right]_{\lambda_i=1} = \frac{Q_{i-1}}{e_{i-1}} = \frac{E_{i-1}PE_i}{e_{i-1}},$$

$$\left[\frac{d\ln e_{i+1}}{d\ln \lambda_i}\right]_{\lambda_i=1} = \frac{Q_i}{e_{i+1}} = \frac{E_i PE_{i+1}}{e_{i+1}}, (i = 2,3,...,r-1)$$

(A5)

When the perturbation affects enzyme 1, only e_1 and e_2 change, since there is no conservation of S-containing forms:

$$e_1(\lambda_1) = E_1(\lambda_1) + E_1 P(\lambda_1) + Q_0(\lambda_1) + Q_1(\lambda_1) = \lambda_1 \cdot e_1$$

$$e_2(\lambda_1) = E_2 + E_2 P + \lambda_1 \cdot Q_1 + Q_2 \qquad (A6)$$

Hence,

$$\left[\frac{d\ln e_1}{d\ln \lambda_1}\right]_{\lambda_1=1} = 1, \left[\frac{d\ln e_2(\lambda_1)}{d\ln \lambda_1}\right]_{\lambda_1=1} = \frac{Q_1}{e_2} \qquad (A7)$$

Similarly, for perturbation of the ultimate enzyme (r) in the pathway only e_{r-1} and e_r change:

$$e_r(\lambda_r) = E_r(\lambda_r) + E_r P(\lambda_r) + Q_{r-1}(\lambda_r) + Q_r(\lambda_r)$$

$$= \lambda_r \cdot e_r$$

$$e_{r-1}(\lambda_r) = E_{r-1} + E_{r-1}P + Q_{r-2} + \lambda_r \cdot Q_{r-1} \qquad (A8)$$

Also

$$\left[\frac{d\ln e_r}{d\ln \lambda_r}\right]_{\lambda_r=1} = 1, \left[\frac{d\ln e_{r-1}(\lambda_r)}{d\ln \lambda_r}\right]_{\lambda_r=1} = \frac{Q_{r-1}}{e_{r-1}} \qquad (A9)$$

Since the flux J remains unchanged:

$$\left[\frac{d\ln|J|}{d\ln \lambda_i}\right]_{\lambda_i=1} = 0 \qquad (A10)$$

Because the modulations considered address both the step activities (ξ_i) and the total enzyme concentrations (e_i), the value of $d\ln|J|/d\ln \lambda_i$ can be expressed through the corresponding control coefficients. Using that $(d\ln|J|)/(d\ln \xi_i) = C_{v1}^J$, see Eq. 12), this leads to:

$$0 = \left[\frac{d\ln|J|}{d\ln \lambda_i}\right] = \sum_{i=1}^{2r+2} C_{v_i}^J \cdot \frac{d\ln \xi_l}{d\ln \lambda_i} + \sum_{v=1}^{r} c_{e_v}^J \cdot \frac{d\ln e_v}{d\ln \lambda_i}$$

$$(i = 1,...,r) \qquad (A11)$$

Eq. A11 gives one equation for each of the r enzymes in the pathway. These equations differ somewhat between pathway-internal enzymes, and enzymes at the beginning and end of the pathway. For the pathway internal enzymes, i.e., $i = 2,...,r-1$, we have from Eqs. A3–A5 and A11, to obtain Eq. 18 of the main text.

For the perturbation in E_1-containing concentrations we obtain Eq. 19 of the main text from Eqs. A11 and A3, A7. From Eqs. A11, A3, A9 for the perturbation in E_r-containing concentrations we obtain Eq. 20 of the main text.

We can also analyze the amount of control exerted on the flux by the boundaries. For these 'boundary substrates' S, SP and W, WP, one may consider the following perturbations of concentrations and parameters:

$$S\lambda_0 = \lambda_0 \cdot S; SP\lambda_0 = \lambda_0 \cdot SP; Q_0 \lambda_0$$

$$= \lambda_0 \cdot Q_0 \qquad (A12)$$

$$W(\lambda_{r+1}) = \lambda_{r+1} \cdot W; WP(\lambda_{r+1}) = \lambda_{r+1} \cdot WP;$$

$$Q_r(\lambda_{r+1}) = \lambda_{r+1} \cdot Q_r \qquad (A13)$$

Simultaneously we change the parameters ξ_1 and ξ_2 for the perturbation Eq. A12 and ξ_{2r+1}, ξ_{2r+2} for the perturbation Eq. A13, respectively such that the rates v_1 and v_2 or, correspondingly, v_{2r+1} and v_{2r+2} remain the same as in the initial steady-state. It follows that (cf. Eqs. A2, A3):

$$\xi_1 \cdot \lambda_0 = \xi_2 \cdot \lambda_0 = 1, \text{ hence } \frac{d\ln \xi_1}{d\ln \lambda_1} = \frac{d\ln \xi_2}{d\ln \lambda_1} = -1$$

(A14)

$$\xi_{2r+1} \cdot \lambda_{r+1} = \xi_{2r+2} \cdot \lambda_{r+1} = 1,$$

$$\text{hence } \frac{d\ln \xi_{2r+1}}{d\ln \lambda_{r+1}} = \frac{d\ln \xi_{2r+2}}{d\ln \lambda_{r+1}} = -1 \qquad (A15)$$

In the new steady-states we have the following values of the parameters e_1 and e_r for the perturbations defined by Eqs. A12 and A13, respectively:

$$e_1(\lambda_0) = E_1 + \lambda_0 \cdot Q_0 + E_1 P + Q_1, \left[\frac{d\ln e_1(\lambda_0)}{d\ln \lambda_o}\right]_{\lambda_0=1}$$

$$= \frac{Q_0}{e_1} \qquad (A16)$$

$$e_r(\lambda_{r+1}) = E_r + Q_{r-1} + E_r P + \lambda_{r+1} \cdot Q_r,$$

$$\left[\frac{d\ln e_r(\lambda_{r+1})}{d\ln \lambda_{r+1}}\right]_{\tau=1} = \frac{Q_r}{e_r} \qquad (A17)$$

Since J remains unchanged in the new steady state, we have for the perturbation defined by Eq. A12:

$$0 = \frac{d\ln J}{d\ln[\lambda_0]} = \frac{d\ln|J|}{d\ln[S]} \cdot \frac{d\ln[S]}{d\ln \lambda_0} + \frac{d\ln|J|}{d\ln[SP]} \cdot \frac{d\ln[SP]}{d\ln \lambda_0}$$

$$+ C_{e_1}^J \cdot \frac{d\ln e_1}{d\ln \lambda_0} + C_{v_1}^J \cdot \frac{d\ln \xi_1}{d\ln \lambda_0} + C_{v_2}^J \cdot \frac{d\ln \xi_2}{d\ln \lambda_0}, \qquad (A18)$$

and, using Eqs. A12, A14, A16 we obtain:

$$\frac{d\ln J}{d\ln[S]} + \frac{d\ln J}{d\ln[SP]} + C_{e_1}^J \cdot \frac{Q_0}{e_1} = C_{v_1}^J + C_{v_2}^J \qquad (A19)$$

From Eq. A19 we obtain Eq. 30 of the main text.

Similarly, for, the perturbation specified Eq. A13 we obtain:

$$\frac{d\ln J}{d\ln[W]} + \frac{d\ln J}{d\ln[WP]} + C_{e_r}^J \cdot \frac{Q_r}{e_r} = C_{v_{2r+1}}^J + C_{v_{2r+2}}^J$$

(A20)

From Eq. A20, Eq. 31 of the main text follows.

Appendix B. Connectivity theorems

Here we shall follow the approach by Kholodenko [6,31]. In order to obtain all the connectivity relations we choose $2r + 1 = \text{rank}$ (**N**) linearly independent vectors $\boldsymbol{\lambda}^{(v)} = (\lambda_1^{(v)}, \lambda_2^{(v)},..., \lambda_{3r+1}^{(v)})$, $(v = 1,...,2r + 1)$, such that the following perturbation in concentrations x:

$$x_j(\alpha) = x_j^o + \alpha \cdot \lambda_j^{(v)} = x_j^o \cdot \left(1 + \alpha \cdot \frac{\lambda_j^{(v)}}{x_j^o}\right),$$

$$j = 1,...,3r + 1 \tag{B1}$$

(where α has an arbitrary, but sufficiently small, value) does not change the moiety-conserved sums (Eq. 6). In other words, vectors $\boldsymbol{\lambda}^{(v)}$ should be orthogonal to all vectors $(\boldsymbol{\gamma}_i)$ of the stoichiometric coefficients of the metabolites in the moiety-conservation sums (see Eqs 6):

$$\sum_{j=1}^{3r+1} \gamma_{ij} \cdot \lambda_j^{(v)} = 0, \; i = 1,...,r$$

$$v = 1, 2,...,2r + 1 \tag{B2}$$

For the flux control coefficients, C_{vi}^J, the connectivity relations read [6,31]:

$$\sum_{i=1}^{2r+2} C_{v_i}^J \cdot \sum_{j=1}^{3r+1} \epsilon_{x_j}^i \cdot \frac{\lambda_j^{(v)}}{x_j} = 0 \tag{B3}$$

where $\epsilon_{x_j}^i = \partial \ln v_i / \partial \ln x_j$ are the elasticity coefficients, and the $2r + 1$ linearly independent vectors $\boldsymbol{\lambda}^{(v)}$ satisfy Eqs. B2.

Since the concentrations E_l and E_lP, where $l = 1,2,...,r$, enter only one conservation sum (i.e., that for e_l), we may choose the vector $\boldsymbol{\lambda}^{(l)}$ as:

$\lambda_j^{(l)} = 0$ if $x_j \neq E_l$ and $x_j \neq E_lP$,

$\lambda_j^{(l)} = 1$ if $x_j = E_l$, $\tag{B4}$

$\lambda_j^{(l)} = -1$ if $x_j = E_lP$, $l = 1, 2,...,r$

i.e. the changes corresponding to the vector $\boldsymbol{\lambda}^{(l)}$ only increase the concentration E_l at the expense of E_lP and do not change the total concentration of the enzyme l. Inserting such a vector $\boldsymbol{\lambda}^{(l)}$ into Eq. B3, one obtains Eq. 64 of the main text. This equation defines r connectivity theorems.

Two more connectivity theorems are obtained considering the first and last reactions of the chain. Taking into account that each of the complexes Q_0 and Q_r enters one conserved sum, i.e., e_1, or e_r, respectively, we choose:

$\lambda_j^{(0)} = 0$ if $x_j \neq E_1$ and $x_j \neq Q_o$,

$\lambda_j^{(0)} = 1$ if $x_j = E_1$, $\lambda_j^{(0)} = -1$ if $x_j = Q_o$ $\tag{B5}$

and

$\lambda_j^{(r+1)} = 0$ if $x_j \neq E_r$ and $x_j \neq Q_r$,

$\lambda_j^{(r+1)} = 1$ if $x_j = E_r$, $\lambda_j^{(r+1)} = -1$ if $x_j = Q_r$ $\tag{B6}$

Applying Eqs. B5 and B6 to Eq. B3 one arrives at Eqs. 66 and 69 of the main text.

The second choice for the vectors $\boldsymbol{\lambda}^{(l)}$ touches upon complexes Q_l $(l = 1,2,...,r - 1)$ that enter two moiety-conserved sums $(e_l$ and $e_{l+1})$ simultaneously. For any $l = 1,2,...,r - 1$ we choose:

$\lambda_j^{(l)} = 0$ if $x_j \neq E_l, Q_l, E_{l+1}$;

$\lambda_j^{(l)} = 1$ if $x_j = E_l$, $\lambda_j^{(l)} = -1$ if

$x_j = Q_l$, $\lambda_j^l = 1$ if $x_j = E_{l+1}$,

$l = 1,...,r - 1 \tag{B7}$

i.e., we do not change the total concentration of either of the enzymes l and $l + 1$. From Eq. B7 one obtains the connectivity theorem, Eq. 65 of the main text.

Appendix C. Concentration control connectivity

Using procedures analogous to those employed in section 2.6.a, one obtains the connectivity relations for the concentration control coefficients, C_{vi}^x. Following Kholodenko [6,31]:

$$\sum_{i=1}^{2r+2} C_{v_i}^{x_j} \cdot \sum_{s=1}^{3r+1} \epsilon_{x_s}^i \cdot \frac{\lambda_s^{(v)}}{x_s} = -\frac{\lambda_j^{(v)}}{x_j}, \; j = 1,2,...,3r + 1 \tag{C1}$$

where $(2r + 1)$ linearly independent vectors $\boldsymbol{\lambda}^{(v)}$ should satisfy Eqs. B2. Choosing the vectors $\boldsymbol{\lambda}^{(l)}$ $(l = 1,...,r)$ as in Eqs. B4 of Appendix B, one arrives at Eqs. 74 and 75 of the main text.

Choosing two vectors $\boldsymbol{\lambda}^{(0)}$ and $\boldsymbol{\lambda}^{(r+1)}$ as in Eqs. B5 and B6, one obtains from Eq. C1, respectively:

$$\sum_{i=1}^{2r+2} C_{v_i}^{x_j} \cdot \left(\epsilon_{E_1}^i \cdot \frac{1}{E_1} - \epsilon_{Q_0}^i \cdot \frac{1}{Q_0}\right) = 0, \; for \; x_j \neq E_1, Q_0 \tag{C2}$$

$$\sum_{i=1}^{2r+2} C_{v_i}^{Q_0} \cdot \left(\epsilon_{E_1}^i \cdot \frac{1}{E_1} - \epsilon_{Q_0}^i \cdot \frac{1}{Q_0}\right) = \frac{1}{Q_0} \tag{C3}$$

and

$$\sum_{i=1}^{2r+2} C_{v_i}^{x_j} \cdot \left(\epsilon_{E_r}^i \cdot \frac{1}{E_r} - \epsilon_{Q_r}^i \cdot \frac{1}{Q_r}\right) = 0, \; for \; x_j \neq E_r, Q_r \tag{C4}$$

$$\sum_{i=1}^{2r+2} C_{v_i}^{Q_r} \cdot \left(\epsilon_{E_r}^i \cdot \frac{1}{E_r} - \epsilon_{Q_r}^i \cdot \frac{1}{Q_r}\right) = \frac{1}{Q_r} \tag{C5}$$

Choosing the vectors $\boldsymbol{\lambda}^{(l)}$ $(l = 1,...,r - 1)$ as in Eq. B7 and using Eq. C1, one finds:

$$\sum_{i=1}^{2r+2} C_{v_i}^{x_j} \cdot \left(\epsilon_{E_l}^i \cdot \frac{1}{E_l} - \epsilon_{Q_l}^i \cdot \frac{1}{Q_l} + \epsilon_{E_{l+1}}^i \cdot \frac{1}{E_{l+1}}\right) = 0,$$

$$if \; x_j \neq E_l, Q_l, E_{l+1}, \; l = 1,...,r - 1 \tag{C6}$$

$$\sum_{i=1}^{2r+2} C_{v_i}^{Q_l} \cdot \left(\epsilon_{E_l}^i \cdot \frac{1}{E_l} - \epsilon_{Q_l}^i \cdot \frac{1}{Q_l} + \epsilon_{E_{l+1}}^i \cdot \frac{1}{E_{l+1}}\right) = \frac{1}{Q_l},$$

$$l = 1,...,r - 1 \tag{C7}$$

Taking into account the expressions Eqs. 68 and 69 of the main text for the elasticity coefficients, Eqs. 74–76, C2–C7 can be transformed to equations corresponding to Eqs. 70–73. Moreover, for any concentration, x_j, the r corresponding equations chosen from Eqs. 74–76, two additional equations (one from Eqs. C2, C3 and one from Eqs. C4, C5), the $(r - 1)$ equations from C6, C7 and the summation theorem, Eq. 61, constitute a complete system of $2r + 2$ equations. These allow the calculation of the concentration control coefficients of the elemental processes, C_{vi}^{xj} ($i = 1, 2,...,2r + 2$) from the elasticity coefficients.

Appendix D. Connectivity theorems in terms of the non-normalized control and elasticity coefficients

For the pathway under study the connectivity theorems for flux and concentrations simplify when they are formulated in terms of the non-normalized control and elasticity coefficients (cf. [12,15]). The latter will be designated by D_x^i,

$$D_x^i = \frac{\partial v_i}{\partial x} = \frac{\partial \ln|v_i|}{\partial \ln x} \cdot \frac{v_i}{x} = \epsilon_x^i \cdot \frac{v_i}{x} \qquad (D1)$$

Since at steady state in the relay pathway all the rates equal J (Eq. 1), the non-normalized flux control coefficients of the *elemental processes* (Γ_{vi}^J) coincide with the normalized ones (C_{vi}^J) (see Eqs. 1 and 13):

$$\Gamma_{vi}^J = \frac{(dJ/dp_i)_{sys}}{(\partial v_i/\partial p_i)_{proc}} = \frac{J}{v_i} \cdot C_{vi}^J = C_{vi}^J \qquad (D2)$$

The non-normalized concentration control coefficients (Γ_i) are defined by (cf. Eq. 14):

$$\Gamma_{vi}^x = \frac{x}{v_i} \cdot C_{vi}^x \qquad (D3)$$

In non-normalized form, the flux control connectivity theorems read (see Eqs. 64–67 of the main text):

$$\sum_{i=1}^{2r+2} \Gamma_{vi}^J \cdot \left(D_{E_l}^i - D_{E_lP}^i \right) = 0, \ l = 1,...,r \qquad (D4)$$

$$\sum_{i=1}^{2r+2} \Gamma_{vi}^J \cdot \left(D_{E_l}^i - D_{Q_l}^i + D_{E_{l+1}}^i \right) = 0, \ l = 1,2,...,r - 1 \qquad (D5)$$

$$\sum_{i=1}^{2r+2} \Gamma_{vi}^J \cdot \left(D_{E_r}^i - D_{Q_r}^i \right) = 0 \qquad (D6)$$

$$\sum_{i=1}^{2r+2} \Gamma_{vi}^J \cdot \left(D_{E_1}^i - D_{Q_0}^i \right) = 0 \qquad (D7)$$

The corresponding concentration connectivity theorems are (see Eqs. 74–76 and C3–C7):

$$\sum_{i=1}^{2r+2} \Gamma_{vi}^{E_lP} \cdot \left(D_{E_l}^i - D_{E_lP}^i \right) = 1 \qquad (D8)$$

$$\sum_{i=1}^{2r+2} \Gamma_{vi}^{xj} \cdot \left(D_{E_l}^i - D_{E_lP}^i \right) = 0, \ for \ x_j \neq E_l, E_lP \qquad (D9)$$

$$\sum_{i=1}^{2r+2} \Gamma_{vi}^{Q_l} \cdot \left(D_{E_l}^i - D_{Q_l}^i + D_{E_{l+1}}^i \right) = 1 \qquad (D10)$$

$$\sum_{i=1}^{2r+1} \Gamma_{vi}^{xj} \cdot \left(D_{E_l}^i - D_{Q_l}^i + D_{E_{l+1}}^i \right) = 0, \ for \ x_j \neq E_l, Q_l, E_{l+1} \qquad (D11)$$

$$\sum_{i=1}^{2r+2} \Gamma_{vi}^{Q_0} \cdot \left(D_{E_1}^i - D_{Q_0}^i \right) = 1 \qquad (D12)$$

$$\sum_{i=1}^{2r+2} \Gamma_{vi}^{xj} \cdot \left(D_{E_1}^i - D_{Q_0}^i \right) = 0, \ for \ x_j \neq E_1, Q_0 \qquad (D13)$$

$$\sum_{i=1}^{2r+2} \Gamma_{vi}^{Q_r} \cdot \left(D_{E_r}^i - D_{Q_r}^i \right) = 1 \qquad (D14)$$

$$\sum_{i=1}^{2r+2} \Gamma_{vi}^{xj} \cdot \left(D_{E_r}^i - D_{Q_r}^i \right) = 0, \ x_j \neq E_r, Q_r \qquad (D15)$$

Appendix E. Expressing control into enzyme properties; matrix equations for non-normalized coefficients

Let us define the $(2r + 2)$-dimensional vector $\mathbf{T}^J$, containing 0 and 1, i.e., $(T^J)_i = \delta_{i,2r+2}$ ($i = 1,2,...,2r + 2$) and the square $(2r + 2) \times (2r + 2)$ matrix $\mathbf{D}$ (which depends on the non-normalized elasticities D),

$$D_{2l-1,j} = D_{E_l}^j - D_{E_lP}^j, \ l = 1,2,...,r;$$

$$D_{2l,j} = D_{E_l}^j - D_{Q_l}^j + D_{E_{l+1}}^j, \ l = 1,2,...,r - 1;$$

$$D_{2r,j} = D_{E_r}^j - D_{Q_r}^j,$$

$$D_{2r+1,j} = D_{E_1}^j - D_{Q_0}^j,$$

$$D_{2r+2,j} = 1,$$

where $j = 1,2,...,2r + 2$ \hspace{2em} (E1)

Then the system of equations 64–67, 38 for calculating C_{vi}^J via elasticities can be written as

$$\mathbf{D} \cdot \mathbf{C}_v^J = \mathbf{T}^J \qquad (E2)$$

where $\mathbf{C}_v^J$ is the $(2r + 2)$-dimensional vector of flux control coefficients with respect to elemental processes (see Eq. 23). Matrix $\mathbf{D}$ can be written more explicitly if we take into account that most of its elements are equal to 0. From Eqs. 68, 69 we have:

For odd rows ($i = 2l - 1$, $l = 1, 2,...,r$):

$$(D)_{ij} = 0 \ for \ j \neq 2l - 1, 2l, 2l + 1, 2l + 2 \qquad (E3)$$

For even rows ($i = 2l$, $l = 1, 2,...,r - 1$):

$$(D)_{ij} = 0 \ for \ j \neq 2l - 1, 2l + 1, 2l + 2, 2l + 4 \qquad (E4)$$

For row $2r$ ($i = 2r$):

$$(D)_{2rj} = 0, \ for \ j \neq 2r - 1, 2r + 1, 2r + 2, \qquad (E5)$$

For row $(2r + 1)$ ($i = 2r + 1$):

$$(D)_{2r+1,j} = 0 \ for \ j \neq 1, 2, 4 \qquad (E6)$$

That is, in every row of $\mathbf{D}$ there are not more than 4 non-zero elements,

$$
\mathbf{D} = \begin{vmatrix}
D^1_{E_1} & -D^2_{E_1P} & -D^3_{E_1P} & D^4_{E_1} & 0 & 0 & \cdots & 0 & 0 & 0 & 0 \\
D^1_{E_1} & 0 & D^3_{E_2}-D^3_{Q_1} & D^4_{E_1}-D^4_{Q_1} & 0 & D^6_{E_2} & \cdots & 0 & 0 & 0 & 0 \\
0 & 0 & D^3_{E_2} & -D^4_{E_2P} & -D^5_{E_2P} & D^6_{E_2} & \cdots & 0 & 0 & 0 & 0 \\
\cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\
0 & 0 & 0 & 0 & 0 & 0 & \cdots & D^{2r-1}_{E_r} & -D^{2r}_{E_rP} & -D^{2r+1}_{E_rP} & D^{2r+2}_{E_r} \\
0 & 0 & 0 & 0 & 0 & 0 & \cdots & D^{2r-1}_{E_r} & 0 & -D^{2r+1}_{Q_r} & D^{2r+2}_{E_r}-D^{2r+2}_{Q_r} \\
D^1_{E_1}-D^1_{Q_0} & -D^2_{Q_0} & 0 & D^4_{E_1} & 0 & 0 & \cdots & 0 & 0 & 0 & O \\
1 & 1 & 1 & 1 & 1 & 1 & \cdots & 1 & 1 & 1 & 1
\end{vmatrix}
$$

Now from Eq. E2:

$$
\mathbf{D} \cdot \begin{vmatrix} C^J_{v_1} \\ C^J_{v_2} \\ \cdots \\ C^J_{v_{2r+2}} \end{vmatrix} = \begin{vmatrix} 0 \\ 0 \\ \cdots \\ 1 \end{vmatrix}
$$

Eq. E7 shows that for non-pathological cases the matrix $\mathbf{D}$ is non-singular. As a consequence, Eq. E8 allows one to calculate the flux control coefficients by all $2r + 2$ processes as the last column of the inverse of $\mathbf{D}$.

The concentration control coefficients may be calculated in a similar manner; the control coefficients with respect to the enzyme-enzyme complexes (Q_l) and the phosphorylated enzyme forms (E_lP) correspond to the other columns of $\mathbf{D}^{-1}$, as will be shown subsequently. The control coefficients with respect to free enzyme (E_l) may be calculated subsequently through Eqs. C7 of Appendix C).

The $(2r + 2) \times (2r + 2)$ control matrix $\boldsymbol{\Gamma}$ for this system be defined by:

$$(\boldsymbol{\Gamma})_{i,2l-1} = \Gamma^{E_lP}_{v_i} \quad (i = 1,2,...,2r + 2, l = 1,2,...,r)$$

$$(\boldsymbol{\Gamma})_{i,2l} = \Gamma^{Q_l}_{v_i}$$

$$(\boldsymbol{\Gamma})_{i,2r+1} = \Gamma^{Q_0}_{v_i}$$

$$(\boldsymbol{\Gamma})_{i,2r+2} = C^J_{v_i} = \Gamma^J_{v_i} \quad \text{(E9)}$$

All summation and connectivity theorems obtained above are then summarized by:

$$\mathbf{D} \cdot \boldsymbol{\Gamma} = \mathbf{I}_{2r+2} \quad \text{(E10)}$$

where $\mathbf{I}_{2r+2}$ is the $(2r + 2) \times (2r + 2)$ identity matrix. $\mathbf{D}$ is usually invertible, hence:

$$\boldsymbol{\Gamma} = \mathbf{D}^{-1} \quad \text{(E11)}$$

Not only does this equation express all the control coefficients into the properties (elasticity coefficients) of the enzymes, it also does this in a rather meaningful way, demonstrating that the former are the (matrix) inverse of the latter. For metabolic pathways this has also been shown, be it in terms of the normalized control and elasticity coefficients [11,10,32,13,42].

Appendix F. An alternative approach to relate control by enzyme concentration to control by elemental processes

The flux control coefficients

This appendix focuses on a more direct method of calculating the enzyme control coefficients, C^J_{ei}, C^{xj}_{ei} than that of section 2.2. In the systems under study these coefficients can be considered as the response coefficients towards a change in the moiety-conserved sums, e_i:

$$C^Y_{e_i} = \frac{d\ln Y}{d\ln e_i} = R^Y_{e_i}, \text{ where } Y = (J, x_j) \quad \text{(F1)}$$

We will use the formulas for the response coefficients, obtained by Kholodenko [6,31]. For the flux response coefficient, C^J_{el}, we have:

$$C^J_{e_l} = e_l \cdot \sum_{i=1}^{2r+2} C^J_{v_i} \cdot \left(\sum_{j=1}^{3r+1} \varepsilon^i_{x_j} \cdot \frac{\lambda^{(r,l)}_j}{x_j} \right), \, l = 1,...,r \quad \text{(F2)}$$

where the vectors $\boldsymbol{\lambda}^{(v,l)}$ are chosen in such a way that the

perturbation Eq. B1 in concentrations, x_j, changes only the moiety-conserved sum e_l, i.e.,

$$\sum_{j=1}^{3r+1} \gamma_{ij} \cdot \lambda_j^{(v,l)} = \delta_{il}, \; i = 1, 2, \ldots, r \quad \text{(F3)}$$

where $\delta_{il} = 0$, $i \neq l$, $\delta_{ll} = 1$.

For every C_{el}^J $(1, \ldots, r)$ one can choose $(2r + 2)$ linearly independent vectors $\lambda^{(v,l)}$ $(v = 1, 2, \ldots, r + 2)$ satisfying Eq. F3. We first choose 3 such vectors for every l. The remaining independent vectors $\lambda^{(v,l)}$ will correspond to the connectivity relations for given l. Obviously, for any e_l $(l = 1, 2, \ldots, r)$ we can choose such vectors $\lambda^{(v,l)}$ in several ways and, hence, we can obtain several (different) expression for any C_{el}^Y, which, of course, give the same value. For example, concentrations E_l and $E_l P$ enter only one moiety-conserved cycle, e_l, and choosing:

$$\lambda_j^{(l)} = 0, \; \text{for } x_j \neq E_l,$$

$$\lambda_j^{(l)} = 1, \; \text{for } x_j = E_l \quad \text{(F4)}$$

one obtains from Eq. F2 and Eqs. 68, 69 of the main text, expressing the elasticities:

$$C_{e_l}^J = \frac{e_l}{E_l} \cdot \sum_{i=1}^{2r+2} C_{v_i}^J \cdot \varepsilon_{E_l}^i$$

$$= \frac{e_l}{J} \cdot \left(C_{v_{2l-1}}^J \cdot k_{2l-1}^+ \cdot E_{l-1} P - C_{v_{2l+2}}^J \cdot k_{2l+2}^- \cdot E_{l+1} P \right)$$

$$(l = 1, 2, \ldots, r) \quad \text{(F5)}$$

Choosing,

$$\lambda_j^{(l)} = 0, \; \text{for } x_j \neq E_l P,$$

$$\lambda_j^{(l)} = 1, \; \text{for } x_j = E_l P \quad \text{(F6)}$$

a different expression for C_l is obtained:

$$C_{e_l}^J = \frac{e_l}{E_l P} \cdot \sum_{i=1}^{2r+2} C_{v_i}^J \cdot \varepsilon_{E_l P}^i$$

$$= \frac{e_l}{J} \cdot \left(C_{v_{2l+1}}^J \cdot k_{2l+1}^+ \cdot E_{l+1} - C_{v_{2l}}^J \cdot k_{2l}^- \cdot E_{l-1} \right)$$

$$(l = 1, \ldots, r) \quad \text{(F7)}$$

Eqs. F5 and F7 express the flux control by enzyme concentration C_{el}^J into the flux control by processes C_{vj}^J. As such they parallel to Eq. 26. A difference is that in Eqs. F5 and F7 the elasticities mediate the relationship, whereas Eq. 26 is formulated in terms of the fractions of the enzymes that are complexed (the Q's).

Other choices for the vectors λ involve the complex Q_l in the perturbation. Since Q_0 and Q_r enter only a single moiety-conserved cycle, e_1 or e_r, one may select:

$$\lambda_j^{(0)} = 0, \; \text{for } x_j \neq Q_0;$$

$$\lambda_j^{(0)} = 1, \; \text{for } x_j = Q_0 \quad \text{(F8)}$$

and

$$\lambda_j^{(r+1)} = 0, \; \text{for } x_j \neq Q_r;$$

$$\lambda_j^{(r+1)} = 1, \; \text{for } x_j = Q_r \quad \text{(F9)}$$

Combining Eqs. F8 and F9 with Eq. F2 and Eq. 69 of the main text one finds:

$$C_{e_1}^J = \frac{e_1}{Q_0} \cdot \sum_{i=1}^{2r+2} C_{v_i}^J \cdot \varepsilon_{Q_0}^i = \frac{e_1}{J} \cdot \left(C_{v_2}^J \cdot k_2^+ - C_{v_1}^J \cdot k_1^- \right)$$

$$\text{(F10)}$$

and,

$$C_{e_r}^J = \frac{e_r}{Q_r} \cdot \sum_{i=1}^{2r+2} C_{v_i}^J \cdot \varepsilon_{Q_r}^i$$

$$= \frac{e_r}{J} \cdot \left(C_{v_{2r+2}}^J \cdot k_{2r+2}^+ - C_{v_{2r+1}}^J \cdot k_{2r+1}^- \right) \quad \text{(F11)}$$

The other complexes Q_l $(l = 1, 2, \ldots, r - 1)$ enter two moiety-conserved sums, i.e., e_l and e_{l+1}. The perturbation can be confined to moiety-conserved sum e_l by choosing $\lambda_j^{(i)}$ in the following way:

$$\lambda_j^{(l)} = 0, \; \text{for } x_j \neq E_{l+1}, Q_l;$$

$$\lambda_j^{(l)} = -1, \; \text{for } x_j = E_{l+1}$$

$$\lambda_j^{(l)} = 1, \; \text{for } x_j = Q_l$$

$$(l = 1, 2, \ldots, r - 1) \quad \text{(F12)}$$

and,

$$C_{e_l}^J = \frac{e_l}{Q_l} \cdot \sum_{i=1}^{2r+2} C_{v_i}^J \cdot \left(\varepsilon_{Q_l}^i - \varepsilon_{E_{l+1}}^i \cdot \frac{Q_l}{E_{l+1}} \right) =$$

$$\frac{e_l}{J} \cdot \left(C_{v_{2l+1}}^J \cdot (-k_{2l+1}^- - k_{2l+1}^+ \cdot E_l P) + C_{v_{2l+2}}^J \cdot k_{2l+2}^+ \right.$$

$$\left. + C_{v_{2l+4}}^J \cdot k_{2l+4}^- \cdot E_{l+2} P \right),$$

$$(l = 1, 2, \ldots, r - 1) \quad \text{(F13)}$$

Here we have obtained additional expressions for the flux control coefficients of the enzymes $e_1, e_2, \ldots, e_r$ in terms of the elemental flux control coefficients (i.e., those of the processes) and elasticities (Eqs. F5, F7, F10, F11, F13). Some of these expressions appear to be simple, e.g., Eqs. F10, F11, in that they do not implicate information on steady-state concentration values.

All the connectivity theorems for the flux control coefficients (see section 6 of the main text) can be readily derived from these expressions for C_{ei}^J. For example, subtracting Eq. F7 from Eq. F5 one obtains the connectivity relations Eqs. 64 and 70. The relations Eq. 66 and Eq. 67 can be obtained if we subtract Eq. F10 from Eq. F5 at $l = 1$ and Eq. F11 from Eq. F5 at $l = r$. Substraction of Eq. F13 from Eq. F7 yields the connectivity relations Eqs. 65.

From the equations obtained for C_e^J, additional summation equations can be derived. Summing Eqs. F5 for $l = 1,...,r$,

$$\sum_{i=1}^{r} C_{e_i}^J \cdot \frac{E_i}{e_i} = \sum_{i=1}^{r} \left(C_{v_{2i-1}}^J \cdot \varepsilon_{e_i}^{2i-1} + C_{v_{2i+2}}^J \cdot \varepsilon_{e_i}^{2i+2} \right),$$

$$(F14)$$

or in terms of elemental rate constants:

$$\sum_{i=1}^{r} C_{e_i}^J \cdot \frac{J}{e_i}$$

$$= \sum_{i=1}^{r} \left(C_{v_{2i-1}}^J \cdot k_{2i-1}^+ \cdot E_{i-1}P - C_{v_{2i+2}}^J \cdot k_{2i+2}^- \cdot E_{i+1}P \right)$$

$$(F15)$$

Note that the sum on the left-hand side of Eq. F15 represents the sum of non-normalized control coefficients. Similarly, from Eq. F7 one can obtain:

$$\sum_{i=1}^{r} C_{e_i}^J \cdot \frac{E_i P}{e_i} = \sum_{i=1}^{r} \left(C_{v_{2i}}^J \cdot \varepsilon_{E_i P}^{2i} + C_{v_{2i+1}}^J \cdot \varepsilon_{E_i P}^{2i+1} \right) \quad (F16)$$

or,

$$\sum_{i=1}^{r} C_{e_i}^J \cdot \frac{J}{e_i} = \sum_{i=1}^{r} \left(C_{v_{2i+1}}^J \cdot k_{2i+1}^+ \cdot E_{i+1} - C_{v_{2i}}^J \cdot k_{2i}^- \cdot E_{i-1} \right)$$

$$(F17)$$

The concentration control coefficients

For the concentration control coefficients we have [6,31]:

$$C_{e_l}^{x_j} = e_l \cdot \left[\frac{\lambda_j}{x_j} + \sum_{i=1}^{2r+2} C_{v_i}^{x_j} \cdot \left(\sum_{s=1}^{3r+1} \varepsilon_{x_s}^{v_i} \cdot \frac{\lambda_s^{(v,l)}}{x_s} \right) \right]$$

$$j = 1,2,...,3r+1; \ l = 1,...,r \qquad (F18)$$

where the vectors $\boldsymbol{\lambda}^{v,l} = (\lambda_1^{v,l}, \lambda_2^{v,l},...,\lambda_{3r+1}^{v,l})$ satisfy the system of linear equations F3.

Choosing the vectors $\boldsymbol{\lambda}^{(l)}$ ($l = 1, 2,...,r$) as in Eq. F4, one obtains:

$$C_{e_l}^{x_j} = \frac{e_l}{E_l} \cdot \sum_{i=1}^{2r+2} C_{v_i}^{x_j} \cdot \varepsilon_{E_l}^i,$$

$$if \ x_j \neq E_l; \ l = 1,2,...,r \qquad (F19)$$

These equations refer to the control of independent concentrations (e.g., $E_l P$ and Q_l). The control coefficient of 'dependent' concentration, $C_{e_l}^{E_l}$ can be readily obtained from Eqs. F19, 54, 56,

$$C_{e_l}^{E_l} = \frac{e_l}{E_l} \left(1 + \sum_{i=1}^{2r+2} C_{v_i}^{E_l} \cdot \varepsilon_{E_l}^i \right) \qquad (F20)$$

and if $\boldsymbol{\lambda}^{(l)}$ is chosen as in Eq. F6, we have:

$$C_{e_l}^{x_j} = \frac{e_l}{E_l P} \cdot \sum_{i=1}^{2r+2} C_{v_i}^{x_j} \cdot \varepsilon_{E_l P}^i, \ if \ x_j \neq E_l P$$

$$C_{e_l}^{E_l P} = \frac{e_l}{E_l P} \cdot \left(1 + \sum_{i=1}^{2r+2} C_{v_i}^{E_l P} \cdot \varepsilon_{E_l P}^i \right), \ l = 1,2,...,r \quad (F21)$$

Choosing the vectors $\boldsymbol{\lambda}$ as in Eqs. F8, F9 (i.e., $\boldsymbol{\lambda}^{(0)}$ and $\boldsymbol{\lambda}^{(r+1)}$), we obtain:

$$C_{e_1}^{x_j} = \frac{e_1}{Q_0} \cdot \sum_{i=1}^{2r+2} C_{v_i}^{x_j} \cdot \varepsilon_{Q_0}^i, \ \text{(for} \ x_j \neq Q_0\text{)}$$

$$C_{e_1}^{Q_0} = \frac{e_1}{Q_0} \left(1 + \sum_{i=1}^{2r+2} C_{v_i}^{Q_0} \cdot \varepsilon_{Q_0}^i \right), \qquad (F22)$$

and

$$C_{e_r}^{x_j} = \frac{e_r}{Q_r} \cdot \sum_{i=1}^{2r+2} C_{v_i}^{x_j} \cdot \varepsilon_{Q_r}^i, \ \text{(for} \ x_j \neq Q_r\text{)}$$

$$C_{e_r}^{Q_r} = \frac{e_r}{Q_r} \left(1 + \sum_{i=1}^{2r+2} C_{v_i}^{Q_r} \cdot \varepsilon_{Q_r}^i \right). \qquad (F23)$$

Substituting the expressions for λ of Eq. F12 into Eq. F18 one finds:

$$C_{e_l}^{x_j} = e_l \cdot \sum_{i=1}^{2r+2} C_{v_i}^{x_j} \cdot \left(\varepsilon_{Q_l}^i \cdot \frac{1}{Q_l} - \varepsilon_{E_{l+1}}^i \cdot \frac{1}{E_{l+1}} \right)$$

$$\left(for \ x_j \neq E_{l+1}, Q_l \right)$$

$$C_{e_l}^{Q_l} = e_l \cdot \left(\frac{1}{Q_l} + \sum_{i=1}^{2r+2} C_{v_i}^{Q_l} \cdot \left(\varepsilon_{Q_l}^i \cdot \frac{1}{Q_l} - \varepsilon_{E_{l+1}}^i \cdot \frac{1}{E_{l+1}} \right) \right),$$

$$(l = 1,2,...,r-1) \qquad (F24)$$

All connectivity relations for the concentration control coefficients can be derived from Eqs. F19–F24. Indeed, subtracting Eqs. F21 from Eqs. F19 one finds Eqs. 74–76 of the main text. Subtracting Eqs. F22 from Eqs. F19 in which $l = 1$, one retrieves Eqs. C2, C3 of Appendix C, and subtracting Eqs. F23 from Eqs. F19 at $l = r$, one obtains Eqs. C4, C5. Similarly, the connectivity relations C6, C7 are obtained from Eqs. F19, F24.

One can use the expressions Eqs. 68 and 69 for the elasticity coefficients to simplify the obtained formulas Eqs. F19–F24. For example, from Eqs. F19 and 68 one obtains:

$$C_{e_l}^{x_j} = \frac{e_l}{J} \cdot \left(C_{v_{2l-1}}^{x_j} \cdot k_{2l-1}^+ \cdot E_{l-1}P - C_{v_{2l+2}}^{x_j} \cdot k_{2l+2}^- \cdot E_{l+1}P \right)$$

$$\left(for \ x_j \neq E_l \right),$$

$$C_{e_l}^{E_l} = \frac{e_l}{E_l} \cdot \left(1 + \frac{E_l}{J} \right.$$

$$\left. \cdot \left(C_{v_{2l-1}}^{E_l} \cdot k_{2l-1}^+ \cdot E_{l-1}P - C_{v_{2l+2}}^{E_l} \cdot k_{2l+2}^- \cdot E_{l+1}P \right) \right),$$

$$l = 1,2,...,r \qquad (F25)$$

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