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Determination of Flux Control Coefficients from transient metabolite concentrations

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Flux Control Coefficients have been used in the analysis of metabolic regulation for quantifying the effect of an enzyme on the overall steady-state flux. However, the experimental determination of these coefficients is very time-consuming, involving either determining the individual enzyme kinetics or perturbing the enzyme activity by genetic or other means. We developed a methodology that enables the determination of the Flux Control Coefficients from transient metabolite concentrations without knowing kinetic parameters. The transient states can be generated by changing the incubation conditions or adding the initial substrate. This approach is suitable for investigating metabolic regulation *in vivo* or multiple enzyme systems *in vitro*. It is particularly helpful if used in conjunction with n.m.r. measurements. The approach is based on a relationship between transient metabolite concentrations and the Flux Control Coefficients. The methodology has been improved from our previous results, and it is illustrated by three examples with simple pathway topologies.

INTRODUCTION

The quantitative analysis of metabolic regulation requires simultaneously considering most, if not all, of the variables involved in the system. In this regard, the Flux Control Coefficients (Kacser & Burns, 1973; Heinrich & Rapoport, 1974) provide a useful basis for quantifying metabolic regulations: the higher the Flux Control Coefficient, the more controlling the enzyme is to the steady-state flux. Although the Control Coefficients reveal only the effect of infinitesimal changes in parameters (such as enzyme concentrations or $V_{max.}$) on the overall fluxes, they are conceptually useful in biochemical research. If all the enzyme kinetics or the Elasticity Coefficients are available, one can calculate the Flux Control Coefficients without much difficulty (e.g. Groen *et al.*, 1986; Fell & Snell, 1988). However, in most cases, enzyme kinetics are not available, and determining individual enzyme kinetics is very time-consuming.

On the other hand, direct experimental determination of Flux Control Coefficients involves altering enzymic activity or kinetic parameters by genetic means or by the use of inhibitors and measuring the change in the steady-state flux. Several examples of the experimental determination of the Flux Control Coefficients have been reported (e.g. Flint *et al.*, 1981; Groen *et al.*, 1982; Mazat *et al.*, 1986; Salter *et al.*, 1986; Torres *et al.*, 1986, 1989; Brand *et al.*, 1988; Kruckeberg *et al.*, 1989). However, these approaches are not generally applicable to all the systems of interest. As an alternative, we proposed using the transient metabolite concentrations to calculate the Control Coefficients (Delgado & Liao, 1991). This methodology provides a useful tool to extract information from transient metabolic data, and can simplify the experiments required to determine the Control Coefficients. As measurement techniques such as n.m.r. *in vivo* and h.p.l.c. become more accurate and user-friendly, it is possible that the measurement of transient metabolite concentrations will eventually become a routine task. Therefore the approach based on the transient metabolic data is promising.

In this paper we present a refined version of the methodology, which greatly improves the accuracy of the estimated Flux Control Coefficients. For simplicity, we discuss only the linear and branched pathways without substrate cycles or conserved metabolites, and we assume that the metabolites are homogeneously distributed in the system. Extension to more complex systems will need to be discussed elsewhere. The derivations of the theory are presented in Appendixes, as they are not critical for understanding the essence of the approach.

BACKGROUND

For definition purposes, let us consider the pathway depicted in Fig. 1, where X_1 and X_{n+1} are extracellular substrate and product respectively. Although several representations have been used, the Flux Control Coefficient has been defined as (Burns *et al.*, 1985):

$$C^J_{e_i} = \left[\frac{e_i}{J}\left(\frac{\partial J}{\partial e_i}\right)\right]_{ss} \quad (1)$$

where $C^J_{e_i}$ is the steady-state Flux Control Coefficient of enzyme i, J is the steady-state flux through the pathway and e_i is the concentration of enzyme i. If one is interested in the effect of activity change in enzyme i then e_i can be defined as $V_{max.}$. The subscript 'ss' denotes that the coefficient is evaluated at the steady state. This definition quantifies the fractional change in the steady-state flux per unit fractional change in enzyme concentration, if the latter change is infinitesimal. If the change is large, it does not necessarily provide precise information because of the non-linear nature of the kinetics.

Note that in order for the definition of Flux Control Coefficients to be meaningful, the internal metabolite concentrations have to be able to reach a unique non-trivial stable steady or quasi-steady state. Therefore it is commonly assumed that X_1

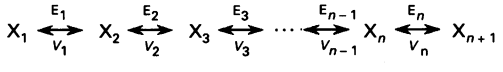

Fig. 1. Metabolic pathway for definition purposes

X_1 and X_{n+1} are extracellular substrate and product respectively; v_i is the flux through reaction i catalysed by enzyme E_i.

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and X_{n+1} are sufficiently buffered or that they do not affect the intracellular enzyme kinetics. This assumption can be relaxed as long as there is a non-trivial quasi-steady state for the internal metabolite concentrations.

If the enzyme kinetics around the steady state are known, one can calculate the Elasticity Coefficients, which are defined as:

$$\epsilon_k^i = \left[\frac{x_k}{v_i}\left(\frac{\partial v_i}{\partial x_k}\right)\right]_{ss} \quad (2)$$

where x_k is the concentration of metabolite k and v_i is the flux through enzyme i. The Flux Control Coefficients can then be obtained by solving a set of linear algebraic equations, known as the Summation and Connectivity Theorems (e.g. Fell & Sauro, 1985; Westerhoff & Kell, 1987).

In most cases, however, the enzyme kinetics or the Elasticity Coefficients are not readily available, or not accurate enough. This situation is one of the major difficulties in determining Flux Control Coefficients in practical systems.

THEORY

We proposed a methodology using the transient metabolite concentrations to calculate the Flux Control Coefficients without using the Elasticity Coefficients (Delgado & Liao, 1991). The methodology requires measuring the metabolite concentrations in a transient state, and it is derived with the use of four assumptions: (1) as in other metabolic control analyses, the external (pool) metabolites do not affect the pathway kinetics or these metabolites are buffered; (2) the kinetic rate laws are sufficiently linear around the steady state of interest; (3) the calculation of the transient fluxes from the measurement of metabolite concentrations for every reaction in the pathway must be theoretically possible; (4) the metabolites are homogeneously distributed in the system. The first assumption makes the definition of the Control Coefficients mathematically meaningful. These conditions enable the internal metabolite concentrations to reach a unique non-trivial stable steady or quasi-steady state. The second assumption is in effect equivalent to the use of Elasticity Coefficients evaluated at the steady state. Although most of the enzyme kinetics are non-linear, the approximation by the above equation is satisfactory for practical purposes. The third assumption is necessary because the methodology implicitly converts the transient metabolite concentrations into the transient flux through each enzyme. Such conversion will not be possible if the number of metabolites is less than the number of enzymes. The fourth assumption is necessary because the cases of enzyme–enzyme complex and substrate channelling are not investigated in the present paper. If there are any enzyme–enzyme complexes, each of them is treated as a single step.

Using these assumptions, we derived a relationship between the Flux Control Coefficients and the transient flux through each enzyme (Delgado & Laio, 1991):

$$\sum_{i=1}^{n} C_{e_i}^J \left(\frac{v_i(t)}{J_i}\right) = 1 \quad (3)$$

where $v_i(t)$ and J_i are the transient and steady-state fluxes through enzyme i respectively. Although the original derivation of eqn. (3) was based on simple pathways, it is applicable to any pathway topology as long as the assumptions stated above are valid. A derivation based on the approach developed by Reder (1988) for an arbitrary pathway configuration is presented in Appendix A.

Note that, although the Flux Control Coefficient has been defined for the steady state, transient reaction rates or fluxes are used in the above equation. This equation states that the ratios between the transient and the steady-state fluxes through each reaction are related to each other by the Flux Control Coefficients. Therefore the reactions with larger Flux Control Coefficients have more significant effects on the other reactions, even in the transient state. The reactions with small Flux Control Coefficients can fluctuate significantly during the transient state without affecting others, whereas reactions with large Flux Control Coefficients must be held relatively constant. This can be seen in the computer simulation of human erythrocyte glycolysis (Schauer *et al.*, 1981): the fluxes through controlling enzymes, hexokinase, phosphofructokinase and pyruvate kinase, stay relatively constant, whereas the fluxes through the 'fast-equilibrium' enzymes can adjust very rapidly (within a few seconds) to dissipate the free energy.

The direct application of eqn. (3) has been illustrated (Delgado & Liao, 1991). However, the calculation of the transient fluxes involves differentiation of the transient metabolite concentrations, and is highly sensitive to experimental error. Here we present an integral form of eqn. (3), where transient metabolite concentrations are used directly to calculate the Flux Control Coefficients without differentiation. The derivation is shown in Appendix B. The reader who is more interested in the applications does not have to attend to the detailed derivations. Rather, the results in the following should be emphasized.

The integral form of eqn. (3) is:

$$\sum_{i=1}^{n+1} \alpha_i[x_i(t) - x_i(0)] = t \quad (4)$$

where α_i are coefficients to be determined. Note that both internal and external metabolites are considered in eqn. (4). This equation states that the transient metabolite concentrations are not completely independent: they are related by a linear constraint. The coefficients in the constraint are related to the Flux Control Coefficients, steady-state fluxes and the stoichiometric coefficients [Appendix B, eqn. (B-9)]. Note that the stoichiometric coefficients of the pathway are usually known and that the steady-state fluxes can be measured experimentally. In practice, we first determine α_i from the transient metabolite concentrations, and then calculate the Flux Control Coefficients as discussed below.

It is interesting to note that, even if one deletes some of the metabolite concentrations, there still exists a constraint in the form of eqn. (4). This is because metabolite concentrations are used implicitly to calculate the flux through each enzyme. In pathways without direct substrate cycling, such calculation is possible if the number of measured metabolites is greater than or equal to the number of enzymes. Therefore, for pathways without cycling, $n+1$ metabolites can be deleted if there are n branch points. We take advantage of this result in the examples.

Linear pathways

To determine the Flux Control Coefficients experimentally, one first measures the metabolite concentrations $x_i(t)$ in a transient state. The transient state can be generated by shifting incubation conditions for the whole cell, or adding initial substrate to the pathway reconstituted *in vitro*. These transient metabolite data are then regressed to obtain the coefficients α_i. Knowing α_i, the stoichiometric coefficients and the steady-state fluxes, one can then calculate the Flux Control Coefficients with:

$$[C_{e_1}^J C_{e_2}^J \ldots C_{e_n}^J] = [\alpha_1 \alpha_2 \ldots \alpha_{n+1}]\mathbf{A}J \quad (5)$$

where J is the steady-state flux through the pathway and $\mathbf{A}$ is the stoichiometric matrix of the pathway. The element of $\mathbf{A}$ in the ith row and jth column is the stoichiometric coefficient of metabolite

i in reaction j. It is defined such that the stoichiometric coefficient is negative for reactants and positive for products. Of course, if metabolite i is not present in reaction j, its stoichiometric coefficient is zero. Illustrations of the stoichiometric matrix are shown in the examples. Note that the methodology may appear to be mathematically complicated, but linear regression and matrix operations are suitable for computerization.

The major difficulty, however, residues in the estimation of α_i by linear regression, because the system has more than one constraint (multicollinearity). For example, the mass-balance equation:

$$\sum_{i=1}^{n+1} [x_i(t)-x_i(0)] = 0 \qquad (6)$$

holds for any accurate data point. The regression results may be any linear combination of eqns. (4) and (6). Fortunately, the mass-balance equation presents no serious problem because it will be automatically filtered out by eqn. (5) (see Appendix C for proof). Similarly, other stoichiometric constraints, if they exist, will also be filtered out by eqn. (5). However, in order to avoid unnecessary complications in the regression, it is advisable to delete one of the metabolites so that the mass-balance equation fails while eqn. (4) still holds. If a variable is deleted in the regression, one needs to delete the corresponding row in the stoichiometric matrix used in eqn. (5).

Another collinearity is caused by missing the fast transient of the system. If some of the reactions respond faster than one can measure, the data will contain additional collinearities (mode relaxation). One way to solve this problem is to lump the substrates and products of the fast reactions into common pools, so that the fast transient in the individual pools does not have to be measured. By doing so, one loses the information about the Flux Control Coefficients of the fast reactions. However, these reactions are not rate-controlling and their Flux Control Coefficients are very close to zero. If lumping is used, the rows corresponding to the metabolites in a pool must be combined (added up) as a single row, and the columns corresponding to the reactions in the common pool must be deleted. This situation is illustrated in Example 2 below.

Example 1. Consider the linear pathway with feedback inhibition depicted in Fig. 2. The stoichiometric matrix is given by:

$$\mathbf{A} = \begin{bmatrix} -1 & 0 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 1 & -1 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \qquad (7)$$

The kinetic rate laws are approximated by:

$$v_1 = -0.4x_2+0.1 \qquad (8a)$$
$$v_2 = 0.5x_2-0.8x_3-0.3x_5+0.01 \qquad (8b)$$
$$v_3 = 0.6x_3-0.2x_4+0.01 \qquad (8c)$$
$$v_4 = 0.15x_4-0.12x_5+0.01 \qquad (8d)$$
$$v_5 = 0.2x_5+0.01 \qquad (8e)$$

Knowing the above equations, one can calculate the steady-state concentrations for all the metabolites and all the Elasticity Coefficients. We can then use the Summation and Connectivity Theorems to calculate the Flux Control Coefficients (Fell & Sauro, 1985; Westerhoff & Kell, 1987). The results are:

$$[C^J_{e_1}\ C^J_{e_2}\ C^J_{e_3}\ C^J_{e_4}\ C^J_{e_5}] = [0.16\ 0.13\ 0.17\ 0.22\ 0.32] \qquad (9)$$

Note that this calculation is possible only if we know the kinetic rate laws or the Elasticity Coefficients.

In practice, however, one does not have the kinetic rate laws or the Elasticity Coefficients. We therefore measure the metabolite concentrations as a function of time. This is done here by computer simulations, and we take ten points (with three significant digits) before the system reaches the quasi-steady state (Fig. 3).

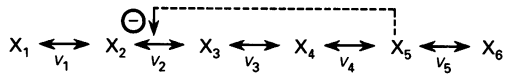

Fig. 2. Unbranched pathway with feedback inhibition used in Example 1

In this example, eqn. (4) takes the following form:

$$\sum_{i=1}^{6} \alpha_i[x_i(t)-x_i(0)] = t \qquad (10)$$

We then determine α_i from these simulated data. Here one may be tempted to use $x_i(t)-x_i(0)$ as regressors and t as the dependent variable and apply linear-regression techniques to estimate α_i. However, one has to be extremely careful because linear-regression techniques assume that the regressors are independent. In fact, the regressors here are not independent: they are related by the mass-balance equation as discussed above. Although the mass-balance equation will not cause a problem in most cases, it is advisable to avoid this additional collinearity by deleting one variable from the equation. One usual way to select the best variables to be deleted from the set of regressors is to choose those with large standard errors or large variance inflation factors (Myers, 1990). Another possibility is to use the variance-decomposition proportions matrix discussed by Belsley *et al.* (1980). In may cases, however, a few trial deletions will yield satisfactory results. In this case, we delete X_1, and use X_2 to X_6 as regressors. The resulting constraint is:

$$t = 6.66[x_2(t)-x_2(0)]+10.92[x_3(t)-x_3(0)]+19.25[x_4(t)-x_4(0)] +26.95[x_5(t)-x_5(0)]+41.38[x_6(t)-x_6(0)] \qquad (11)$$

The coefficients of the above equations are the values of α_2, α_3, α_4, α_5 and α_6 respectively. The first coefficient, α_1, is equal to zero since the variable $x_1(t)-x_1(0)$ does not appear in the above equation. The steady-state flux, J, can be calculated from the steady-state accumulation of the product, X_6. In this case, $J = 0.0242$. Using eqn. (5) and the stoichiometric matrix, one obtains:

$$[C^J_{e_1}\ C^J_{e_2}\ C^J_{e_3}\ C^J_{e_4}\ C^J_{e_5}] = [0.16\ 0.10\ 0.20\ 0.19\ 0.35] \qquad (12)$$

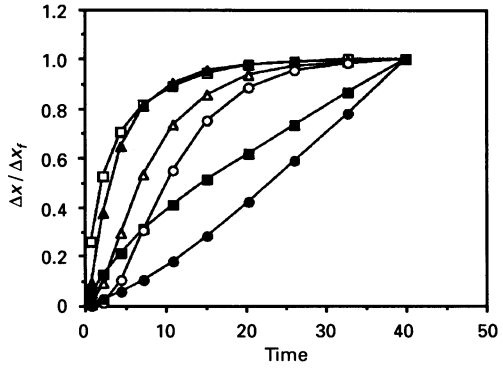

Fig. 3. Metabolite concentration profiles for Example 1

Vertical axis is $\Delta x/\Delta x_f$, where $\Delta x = x - x_{initial}$ and $\Delta x_f = x_{final} - x_{initial}$. Symbols represent hypothetical experimental data points: ■, X_1; □, X_2; ▲, X_3; △, X_4; ○, X_5; ●, X_6.

which is in good agreement with the true values [eqn. (9)] calculated from the known enzyme kinetics. For practical purposes, the differences between the estimates and the true values are negligible. In this case, if one did not measure the steady-state flux, $J = 1$ can be used in eqn. (5). The resulting coefficients are then normalized so that their summation is unity.

Note that, if the original method proposed by Delgado & Liao (1991) were used, the Flux Control Coefficients determined would be

$$[C^J_{e_1}\ C^J_{e_2}\ C^J_{e_3}\ C^J_{e_4}\ C^J_{e_5}] = [-10.9\ 41.1\ -38.6\ 21.7\ -12.3] \quad (13)$$

which are not satisfactory compared with the theoretical values. The poor performance of the original method is due to the differentiation of the transient metabolite concentrations, which requires much more information (in the form of more data points and more significant digits) to be successful. Here we used only ten data points in the transient state, and three significant digits for the estimation.

Example 2. In this case we consider again the pathway shown in Fig. 2, but now assume that one knows *a priori* that reactions 3 and 4 are fast-equilibrium reactions and that they are not rate-controlling. It is shown below how one can incorporate this additional information into the analysis.

The reaction rates are approximated by:

$$v_1 = -1.1x_2 + 0.01 \quad (14a)$$
$$v_2 = 0.5x_2 - 0.8x_3 - 0.3x_5 + 0.00001 \quad (14b)$$
$$v_3 = 6.0x_3 - 2.0x_4 + 0.00001 \quad (14c)$$
$$v_4 = 10.0x_4 - 7.0x_5 + 0.00001 \quad (14d)$$
$$v_5 = 0.6x_5 + 0.00001 \quad (14e)$$

The Flux Control Coefficients calculated from the above rate laws are:

$$[C^J_{e_1}\ C^J_{e_2}\ C^J_{e_3}\ C^J_{e_4}\ C^J_{e_5}] = [0.19\ 0.41\ 0.06\ 0.01\ 0.33] \quad (15)$$

Now assume that the kinetic parameters are unknown, but it is known that enzymes 3 and 4 are not rate-controlling so that the Flux Control Coefficients are close to zero compared with the others. To determine the Flux Control Coefficients for the other enzymes, we measure the metabolite concentrations in a transient state. Again, this is done by using computer simulation, and the data are shown in Fig. 4. It has to be noted that this time we used only two significant digits in the simulation, and again ten data points.

Since reactions 3 and 4 are fast, they reach quasi-equilibrium before the first measurement. Therefore if we use X_1 to X_6 as regressors, there will be three collinearities present among the transient metabolite concentrations: a mass-balance constraint and two others due to the quasi-equilibrium of reactions 3 and 4. Regression using all the variables or even deleting one variable will still encounter difficulties. To solve this problem, we take advantage of the known fact that the reactions 3 and 4 are mediated by fast-equilibrium enzymes, and postulate that the transients between X_3, X_4 and X_5 are not important. Therefore, we can lump X_3, X_4 and X_5 into a common pool, which is denoted by P. The lumped pathway then is shown in Fig. 5.

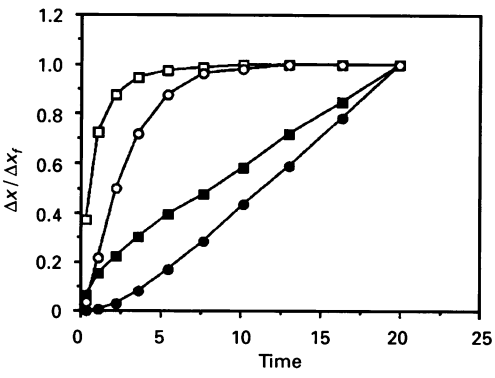

Fig. 4. Metabolite concentration profiles for Example 2

Vertical axis is $\Delta x/\Delta x_f$, where $\Delta x = x - x_{initial}$ and $\Delta x_f = x_{final} - x_{initial}$. Symbols represent hypothetical experimental data points: ■, X_1; □, X_2; ○, P; ●, X_6.

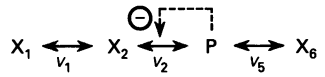

Fig. 5. Lumped form of the pathway shown in Fig. 2 and used in Example 2

P represents X_3, X_4 and X_5 combined into a common pool.

One can now treat this case as in Example 1: use X_2, P ($= X_3 + X_4 + X_5$) and X_6 as regressors, and obtain:

$$t = 103.31[x_2(t) - x_2(0)] + 331.41[p - p(0)] + 537.4[x_6(t) - x_6(0)] \quad (16)$$

Note that X_1 was deleted in order to avoid collinearity due to the mass balance. Moreover, because of the lumping the stoichiometric matrix **A** is now:

$$\mathbf{A} = \begin{bmatrix} -1 & 0 & 0 \\ 1 & -1 & 0 \\ 0 & 1 & -1 \\ 0 & 0 & 1 \end{bmatrix} \quad (17)$$

The Flux Control Coefficients for enzymes 1, 2 and 5 can now be calculated by using eqn. (5):

$$[C^J_{e_1}\ C^J_{e_2}\ C^J_{e_5}] = [0.19\ 0.43\ 0.39] \quad (18)$$

which are very good estimations for practical purposes. Note that $C^J_{e_3}$ and $C^J_{e_4}$ are determined to be zero, reflecting the assumptions made *a priori*. The estimation would deteriorate as the values of $C^J_{e_3}$ and $C^J_{e_4}$ differ from zero.

The way metabolites 3, 4 and 5 were lumped is no coincidence. It has been shown that the presence of non-controlling enzymes in metabolic pathways is related to the characteristic reaction path and that lumping has to be done in a stoichiometric fashion (Liao & Lightfoot, 1988). Non-controlling enzymes result in the relaxation of modes of the system. If these non-controlling enzymes are not identified *a priori* (i.e. they are not lumped) an increased number of linear dependencies will appear among the transient metabolite concentrations, and this situation would complicate the regression analysis.

Branched pathways

Consider now the branched pathway shown in Fig. 6. There are three sets of Flux Control Coefficients, one for each of the three branches:

$$C^{J_K}_{e_i} = \frac{e_i}{J_K}\left(\frac{\partial J_K}{\partial e_i}\right)_{ss} \quad K = A, B, C \quad (19)$$

where the index K denotes on which branch the Flux Control Coefficients are based.

Since the derivation of eqn. (5) is not restricted to any of the

Flux Control Coefficients, one can write an equation for each of them:

$$\sum_{i=1}^{6} \alpha_i^A[x_i(t)-x_i(0)] = t \quad (20a)$$

$$\sum_{i=1}^{6} \alpha_i^B[x_i(t)-x_i(0)] = t \quad (20b)$$

and

$$\sum_{i=1}^{6} \alpha_i^C[x_i(t)-x_i(0)] = t \quad (20c)$$

where α_i^A, α_i^B and α_i^C are coefficients leading to the Flux Control Coefficients of branches A, B and C respectively.

However, only two of these three constraints are linearly independent, as one can show that:

$$J_A C_{e_i}^{J_A} = J_B C_{e_i}^{J_B} + J_C C_{e_i}^{J_C} \quad (21)$$

where J_A, J_B and J_C are the steady-state fluxes through branches A, B and C respectively.

Therefore, if we use all the variables in the pathways as regressors and t as the independent variable to estimate the coefficients $C_{e_i}^{J_A}$, $C_{e_i}^{J_B}$ and $C_{e_i}^{J_C}$, one will find three collinearities: a mass-balance constraint and two out of eqns. (20a) to (20c). To avoid the problem caused by the multicollinearities, one has to delete variables in the regression. If the Flux Control Coefficients based on branch A are to be calculated, one possibility is to delete the terminal metabolites in the other two branches, namely X_5 and X_6. If branch B is of interest, one deletes X_1 and X_6. Similarly, if one wants to estimate the coefficients based on branch C, X_1 and X_5 are to be deleted. The procedure is illustrated in Example 3 below and the reasons why these particular deletions yield the desired Flux Control Coefficients are shown in Appendix D.

Once the coefficients in eqns. (20a) to (20c) are determined, the corresponding Flux Control Coefficients can be determined from the following equations:

$$[C_{e_1}^{J_A} C_{e_2}^{J_A} C_{e_3}^{J_A} C_{e_4}^{J_A} C_{e_5}^{J_A}] = [\alpha_1^A \alpha_2^A \alpha_3^A \alpha_4^A 0\,0]\mathbf{AJ} \quad (22a)$$

$$[C_{e_1}^{J_B} C_{e_2}^{J_B} C_{e_3}^{J_B} C_{e_4}^{J_B} C_{e_5}^{J_B}] = [0\,\alpha_2^B \alpha_3^B \alpha_4^B \alpha_5^B 0]\mathbf{AJ} \quad (22b)$$

$$[C_{e_1}^{J_C} C_{e_2}^{J_C} C_{e_3}^{J_C} C_{e_4}^{J_C} C_{e_5}^{J_C}] = [0\,\alpha_2^C \alpha_3^C \alpha_4^C 0\,\alpha_6^C]\mathbf{AJ} \quad (22c)$$

In this case, since the steady-state fluxes in different branches are different, **J** is now a diagonal matrix:

$$\mathbf{J} = \begin{bmatrix} J_1 & 0 & 0 & 0 & 0 \\ 0 & J_2 & 0 & 0 & 0 \\ 0 & 0 & J_3 & 0 & 0 \\ 0 & 0 & 0 & J_4 & 0 \\ 0 & 0 & 0 & 0 & J_5 \end{bmatrix} \quad (23)$$

where J_i is the steady-state flux though reaction i.

Note that, when one calculates the Flux Control Coefficients based on branch A, $\alpha_5^A = \alpha_6^A = 0$ since X_5 and X_6 were deleted for the regression analysis. The same argument holds when one calculates the Flux Control Coefficients based on branches B and C.

Example 3. Consider the branched pathway shown in Fig. 6. The stoichiometric matrix is:

$$\mathbf{A} = \begin{bmatrix} -1 & 0 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & -1 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \quad (24)$$

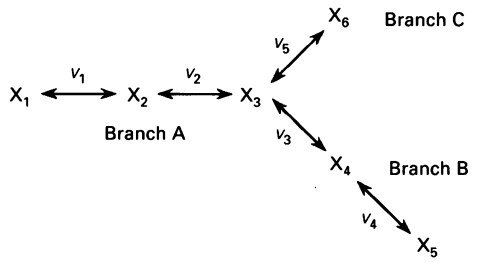

Fig. 6. Branched pathway

X_1 is an extracellular substrate and X_5 and X_6 are extracellular products.

and the reaction rate laws are approximated by:

$$v_i = -0.8x_2 + 0.1 \quad (25a)$$
$$v_2 = x_2 - x_3 + 0.001 \quad (25b)$$
$$v_3 = 0.1x_3 - 0.15x_4 + 0.001 \quad (25c)$$
$$v_4 = 0.1x_4 + 0.001 \quad (25d)$$
$$v_5 = 0.18x_3 + 0.001 \quad (25e)$$

The theoretical values for the Flux Control Coefficients are:

$$[C_{e_1}^{J_A} C_{e_2}^{J_A} C_{e_3}^{J_A} C_{e_4}^{J_A} C_{e_5}^{J_A}] = [0.18\ 0.15\ 0.06\ 0.09\ 0.53] \quad (26a)$$

$$[C_{e_1}^{J_B} C_{e_2}^{J_B} C_{e_3}^{J_B} C_{e_4}^{J_B} C_{e_5}^{J_B}] = [0.16\ 0.13\ 0.38\ 0.56\ -0.22] \quad (26b)$$

$$[C_{e_1}^{J_C} C_{e_2}^{J_C} C_{e_3}^{J_C} C_{e_4}^{J_C} C_{e_5}^{J_C}] = [0.19\ 0.15\ -0.03\ -0.04\ 0.73] \quad (26c)$$

To estimate these Flux Control Coefficients without knowing the kinetic parameters, one again measures the metabolite concentrations in a transient state. The computer-simulated data are shown in Fig. 7. The steady-state fluxes in the three branches are: $J_A = 0.0199$, $J_B = 0.00425$ and $J_C = 0.0156$, so the steady-state flux matrix **J** is given by:

$$\mathbf{J} = \begin{bmatrix} 0.0199 & 0 & 0 & 0 & 0 \\ 0 & 0.0199 & 0 & 0 & 0 \\ 0 & 0 & 0.00425 & 0 & 0 \\ 0 & 0 & 0 & 0.00425 & 0 \\ 0 & 0 & 0 & 0 & 0.0156 \end{bmatrix} \quad (27)$$

We first use X_1 to X_4 as regressors and obtain:

$$t = -50.29[x_1(t)-x_1(0)] - 41.38[x_2(t)-x_2(0)] - 33.13[x_3(t)-x_3(0)] - 20.12[x_4(t)-x_4(0)] \quad (28)$$

Substituting these coefficients into eqn. (22a) yields:

$$[C_{e_1}^{J_A} C_{e_2}^{J_A} C_{e_3}^{J_A} C_{e_4}^{J_A} C_{e_5}^{J_A}] = [0.18\ 0.16\ 0.06\ 0.09\ 0.52] \quad (29)$$

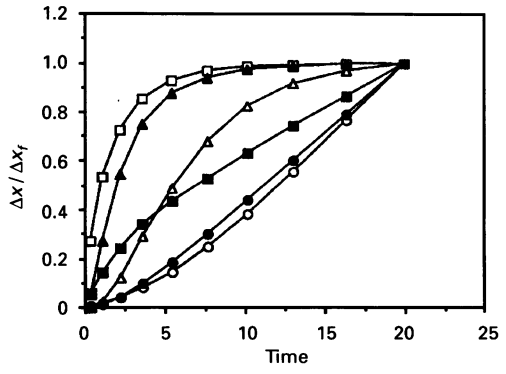

Fig. 7. Metabolite concentration profiles for Example 3

Vertical axis is $\Delta x/\Delta x_f$, where $\Delta x = x - x_{initial}$ and $\Delta x_f = x_{final} - x_{initial}$. Symbols represent hypothetical experimental data points: ■, X_1; □, X_2; ▲, X_3; △, X_4; ○, X_5; ●, X_6.

Similarly, we use X_2 to X_5 as the regressors to obtain:

$$t = 7.54[x_2(t)-x_2(0)]+14.93[x_3(t)-x_3(0)] + 100.67[x_4(t)-x_4(0)]+235.68[x_5(t)-x_5(0)] \quad (30)$$

Substituting these values into eqn. (22b) gives:

$$[C^{J_B}_{e_1}\ C^{J_B}_{e_2}\ C^{J_B}_{e_3}\ C^{J_B}_{e_4}\ C^{J_B}_{e_5}] = [0.15\ 0.15\ 0.36\ 0.57\ -0.23] \quad (31)$$

If the Flux Control Coefficients for branch C are to be calculated, one uses X_2, X_3, X_4 and X_6 as the regressors to obtain:

$$t = 9.56[x_2(t)-x_2(0)]+17.38[x_3(t)-x_3(0)] + 10.91[x_4(t)-x_4(0)]+63.96[x_5(t)-x_5(0)] \quad (32)$$

and the Flux Control Coefficients for branch C can be calculated from eqn. (22c) as:

$$[C^{J_C}_{e_1}\ C^{J_C}_{e_2}\ C^{J_C}_{e_3}\ C^{J_C}_{e_4}\ C^{J_C}_{e_5}] = [0.19\ 0.16\ -0.03\ -0.05\ 0.73] \quad (33)$$

Note that the estimated Control Coefficients are very close to the theoretical values.

DISCUSSION

The Flux Control Coefficient is a useful index for determining the rate-controlling capacity of an enzyme in a pathway. In theory, it quantifies the change in the steady-state flux caused by an infinitesimal change in enzyme concentration or related kinetic parameter. However, no practical method exists to change enzyme concentration or kinetic parameter infinitesimally. Existing approaches for determining this coefficient can be roughly classified into the following categories: (1) direct perturbation of enzyme activity or concentration and measuring the change in the steady-state flux (e.g. Flint *et al.*, 1981; Groen *et al.*, 1982; Mazat *et al.*, 1986; Salter *et al.*, 1986; Torres *et al.*, 1986, 1989; Brand *et al.*, 1988; Kruckeberg *et al.*, 1989), and (2) calculation from the Elasticity Coefficients, which in turn require comprehensive information on individual enzyme kinetics (e.g. Groen *et al.*, 1986; Fell & Snell, 1988). We have now presented a methodology that enables one to determine the Flux Control Coefficients from transient metabolite concentrations. This approach can potentially reduce the amount of experimentation by an order of magnitude, if it is used in conjunction with n.m.r. measurements. Although the accuracy of the methodology has been greatly improved from our previous results, this approach still requires relatively accurate measurements (about 5–10% maximum error).

The central concept of our approach is that the transient metabolite concentrations are determined by the enzyme kinetics, and that the transient state contains much more information than the steady state. It is therefore possible, in principle at least, to extract kinetic information from the transient state. However, such practice is so sensitive to measurement noise that it becomes almost impossible to extract all the kinetic parameters from the transient data. Instead of estimating all the kinetic parameters, here we limit the scope to extract some useful information, namely the Flux Control Coefficients, from the transient metabolite data.

The theoretical basis of our approach is presented by eqns. (3) and (4), which can be applied to pathways of any topology as long as (1) the internal metabolite concentrations are able to reach a unique and stable steady or quasi-steady state, (2) the calculation of the transient fluxes through each reaction from the transient metabolite concentration data must be theoretically possible, and (3) the metabolites are homogeneously distributed in the system. The first assumption makes the definition of the steady-state Flux Control Coefficients meaningful. The second assumption is necessary as the methodology implicitly converts the transient metabolite concentrations into transient fluxes. This is usually possible if the number of metabolites is greater than the number of enzymes. The third assumption avoids the sometimes justifiable complexity of enzyme–enzyme complexes, which will need to be treated elsewhere.

We also stated previously that the derivation of eqns. (3) and (4) is based on a linear approximation of the enzyme kinetics around the steady state. In principle, eqn. (4) is strictly true for systems described by linear kinetics. In practice, however, the regression process based on this equation to estimate the α_i is somewhat equivalent to the linearization process, which has been justifiably used in the definition of the Control Coefficients and the Elasticity Coefficients. In other words, the linearization process is applied directly to the transient-state data, instead of linearizing the kinetic rate laws, which may be unknown. Therefore this approach can be applied to systems with non-linear kinetics, and the resulting Flux Control Coefficients will be defined for the vicinity of the steady state under investigation.

The derivation of the equations used here is obtained by using an approach reported by Reder (1988) and is presented in the Appendixes. It has to be noted that the Flux Control Coefficients determined by our method are general in the sense that they do not rely on the assumption that the velocity of every isolated step is proportional to the enzyme concentration. Rather, the derivation is based on the generalized Flux Control Coefficients defined in Appendix A [eqn. (A-19)], which will reduce to the conventional definition [eqn. (1)] if the enzyme activity is proportional to the enzyme concentration. Thus eqns. (3) and (4) yield Flux Control Coefficients defined as in eqn. (1) if the above condition is met. In case the enzyme activity is not proportional to the enzyme concentration, the Flux Control Coefficients determined by the present method are the ones defined in eqn. (A-19), which are related to the conventional ones by the elasticity of the velocity of the enzyme (v_i) with respect to its concentration (e_i) (e.g. Kacser *et al.*, 1990; Meléndez-Hevia *et al.*, 1990). All of the above are consequence of the use of Reder's (1988) approach in the derivation of eqns. (3) and (4).

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APPENDIX A

This Appendix presents the derivation of eqn. (3) based on previous results reported by Reder (1988). The complete derivation of the fundamental equations is rather technical and mathematically intensive. Therefore only a brief summary useful for our derivation and to define the nomenclature is presented. Complete details of the basic equations can be found in the original paper (Reder, 1988).

Consider a biochemical system with m internal metabolites and r reactions described by:

$$\frac{d\mathbf{y}}{dt} = \mathbf{N}\mathbf{v} \tag{A-1}$$

where $\mathbf{y}$ is an $m \times 1$ column vector containing the internal metabolite concentrations, $\mathbf{N}$ is the $m \times r$ stoichiometric matrix where the element in the ith row and jth column is the stoichiometric coefficient of internal metabolite i in reaction j, and $\mathbf{v}$ is an $r \times 1$ column vector containing the rates of reaction of fluxes for each reaction of the system under analysis. Note that the stoichiometric matrix $\mathbf{N}$ is different from the full stoichiometric matrix $\mathbf{A}$ used in the main text, as $\mathbf{A}$ contains additional rows that include the external (pool) metabolites.

The stoichiometric matrix $\mathbf{N}$ can be decomposed as:

$$\mathbf{N} = \mathbf{L}\mathbf{N}_\mathbf{R} \tag{A-2}$$

where $\mathbf{N}_\mathbf{R}$ is an $m_0 \times r$ matrix formed by the first m_0 rows of $\mathbf{N}$ that constitute a basis for its row space. $\mathbf{L}$ is an $m \times m_0$ matrix that has the form:

$$\mathbf{L} = \begin{bmatrix} \mathbf{I}_{m_0} \\ \mathbf{L}_0 \end{bmatrix} \tag{A-3}$$

where $\mathbf{I}_{m_0}$ is the $m_0 \times m_0$ identity matrix and $\mathbf{L}_0$ is $(m-m_0) \times m_0$.

It has been shown that the following relation is satisfied (Theorem 5 of Reder, 1988):

$$\mathbf{Z}\mathbf{D}_\mathbf{y}\mathbf{v}\mathbf{L} = 0 \tag{A-4}$$

$\mathbf{Z}$ is defined as the $r \times r$ simple steady-state flux-control matrix that satisfies (Proposition 4 of Reder, 1988):

$$\mathbf{D}_\lambda\mathbf{J} = \mathbf{Z}\mathbf{D}_\lambda\mathbf{v} \tag{A-5}$$

where $\mathbf{D}_\lambda\mathbf{J}$ and $\mathbf{D}_\lambda\mathbf{v}$ are defined as:

$$[\mathbf{D}_\lambda\mathbf{J}]_{ij} = \frac{\partial J_i}{\partial \lambda_j} \tag{A-6a}$$

and

$$[\mathbf{D}_\lambda\mathbf{v}]_{ij} = \frac{\partial v_i}{\partial \lambda_j} \tag{A-6b}$$

The parameter λ_j may represent any external parameter (such as a fixed extracellular substrate or enzyme concentration). Note that λ_j cannot normally be an internal variable for any useful purpose. J_i is the steady-state flux through enzyme i and v_i is the velocity through enzyme i (note that $\mathbf{Z}$ was denoted as $\mathbf{C}$ by Reder, 1988). The relation between the $\mathbf{Z}$ matrix and the conventional Flux Control Coefficients is shown later below. The element of the ith row and jth column of $\mathbf{D}_\mathbf{y}\mathbf{v}$ is given by:

$$[\mathbf{D}_\mathbf{y}\mathbf{v}]_{ij} = \frac{\partial v_i}{\partial y_j} \tag{A-7}$$

where the derivative is evaluated at the steady state.

Combining eqns. (A-1), (A-2) and (A-3) and partitioning the metabolite concentration vector $\mathbf{y}$ accordingly, one obtains:

$$\frac{d\mathbf{y}}{dt} = \frac{d}{dt}\begin{bmatrix} \mathbf{y}_\mathbf{R} \\ \mathbf{y}_\mathbf{R}' \end{bmatrix} = \begin{bmatrix} \mathbf{I}_{m_0} \\ \mathbf{L}_0 \end{bmatrix}\mathbf{N}_\mathbf{R}\mathbf{v} \tag{A-8}$$

where $\mathbf{y}_\mathbf{R}$ is $m_0 \times 1$ and $\mathbf{y}_\mathbf{R}'$ is $(m-m_0) \times 1$.

It can be shown that (Theorem 1 of Reder, 1988):

$$\frac{d}{dt}(\mathbf{y}_\mathbf{R}' - \mathbf{L}_0\mathbf{y}_\mathbf{R}) = 0 \tag{A-9}$$

Integrating between two arbitrary time points, one gets:

$$\Delta\mathbf{y}_\mathbf{R}' = \mathbf{L}_0\Delta\mathbf{y}_\mathbf{R} \tag{A-10}$$

Eqn. (A-9) or eqn. (A-10) describes all the $m-m_0$ structural conservation relationships existing among the internal metabolite concentrations.

Multiplying eqn. (A-4) by $\Delta\mathbf{y}_\mathbf{R}$:

$$\mathbf{Z}\mathbf{D}_\mathbf{y}\mathbf{v}\mathbf{L}\Delta\mathbf{y}_\mathbf{R} = 0 \tag{A-11}$$

Using the partitioned form of $\mathbf{L}$ [eqn. (A-3)]:

$$\mathbf{Z}\mathbf{D}_\mathbf{y}\mathbf{v}\begin{bmatrix} \Delta\mathbf{y}_\mathbf{R} \\ \mathbf{L}_0\Delta\mathbf{y}_\mathbf{R} \end{bmatrix} = 0 \tag{A-12}$$

By eqn. (A-10):

$$\mathbf{Z}\mathbf{D}_\mathbf{y}\mathbf{v}\begin{bmatrix} \Delta\mathbf{y}_\mathbf{R} \\ \Delta\mathbf{y}_\mathbf{R}' \end{bmatrix} = 0 \tag{A-13}$$

or

$$\mathbf{Z}\mathbf{D}_\mathbf{y}\mathbf{v}\Delta\mathbf{y} = 0 \tag{A-14}$$

The first-order approximation of the kinetic rate laws around the steady state is:

$$\Delta v_i = \sum_{j=1}^{m} \frac{\partial v_i}{\partial y_j}\Delta y_j \quad i = 1, 2, \ldots, r \tag{A-15}$$

or in matrix form:

$$\Delta\mathbf{v} = \mathbf{D}_\mathbf{y}\mathbf{v}\Delta\mathbf{y} \tag{A-16}$$

Note that in eqn. (A-15) it has been assumed that the fluxes v_i do not depend on the external (pool) metabolites. Otherwise, the

external metabolites that do not comply with this assumption have to be kept constant during the transient state.

One can substitute the term $\mathbf{D}_y\mathbf{v}\Delta\mathbf{y}$ by $\Delta\mathbf{v}$ in eqn. (A-14) and obtain:

$$\mathbf{Z}\Delta\mathbf{v} = 0 \qquad \text{(A-17)}$$

Eqn. (A-17) is the generalized version of eqn. (3). The significance of the elements of **Z** and their relation to the conventional Flux Control Coefficients defined in eqn. (1) can be seen if some simplifying assumptions are made. First, assume there are r parameters λ_j that act specifically on the rates v_j. Then, **Z** can be normalized as:

$$\mathbf{C} = \mathbf{J}^{-1}\mathbf{Z}\mathbf{J} \qquad \text{(A-18)}$$

where **J** is the steady-state flux diagonal matrix and **C** is the Flux Control Coefficients matrix, in which the elements are:

$$[C]_{ij} = \frac{J_j}{J_i}\left(\frac{\partial J_i}{\partial \lambda_j}\right)\left(\frac{\partial v_j}{\partial \lambda_j}\right)^{-1} \quad i = 1,2,\ldots,r; j = 1,2,\ldots r \qquad \text{(A-19)}$$

Substituting eqn. (A-18) into eqn. (A-17):

$$\mathbf{Z}\Delta\mathbf{v} = \mathbf{J}\mathbf{C}\mathbf{J}^{-1}\Delta\mathbf{v} = 0$$

or

$$\mathbf{C}\mathbf{J}^{-1}\Delta\mathbf{v} = 0 \qquad \text{(A-20)}$$

which is the matrix version of eqn. (3). Note that in a linear (unbranched) pathway $J_1 = J_2 = \ldots = J_r$, and all the rows in the matrix **C** are the same, Eqn. (A-20) becomes a single equation, reducing to eqn. (3). Eqns. (A-17) and (A-20) are valid for any pathway configuration as long as the system under analysis possesses a unique stable steady or quasi-steady state for the internal metabolite concentrations. This condition is usually fulfilled if the pathway kinetics do not depend on the external (pool) metabolite concentrations, or, if they do, these concentrations are buffered.

Finally, if λ_j denotes enzyme concentration, $\lambda_j = e_j$ and v_j is proportional to e_j, then the generalized Flux Control Coefficient defined in eqn. (A-19) reduces to the conventional Flux Control Coefficient defined in eqn. (1).

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APPENDIX B

This Appendix presents the derivation of eqns. (4), (20a), (20b) and (20c), the integral versions of eqn. (3). Substituting $\Delta\mathbf{v} = \mathbf{v} - \mathbf{v}_{ss}$ into eqn. (A-17) yields:

$$\mathbf{Z}\mathbf{v} = \mathbf{Z}\mathbf{v}_{ss} \qquad \text{(B-1)}$$

where $\mathbf{v}_{ss}$ is the $r \times 1$ steady-state flux vector.

The fluxes **v** can be expressed in terms of the metabolite concentrations (internal and external) by using the pseudo-inverse of the (full) stoichiometric matrix **A**:

$$\mathbf{v} = \mathbf{A}^{+}\frac{d\mathbf{x}}{dt} \qquad \text{(B-2)}$$

Here the concentration vector **x** includes both internal and external (pool) metabolites as they are needed to obtain the transient fluxes **v**. Matrix $\mathbf{A}^{+}$ denotes the pseudo-inverse of **A**. Eqn. (B-2) is also known as the least-squares solution for **v**. For general $p \times q$ matrix **A**, the pseudo-inverse $\mathbf{A}^{+}$ is the $q \times p$ matrix whose jth column $\mathbf{a}_j$ is the unique minimum-length solution of the least-squares problem $\mathbf{A}\mathbf{x}_j \simeq \mathbf{e}_j$, where $\mathbf{e}_j$, is the jth column of the identity matrix $\mathbf{I}_p$. Further details can be found in Lawson & Hanson (1974).

Substituting eqn. (B-2) into eqn. (B-1):

$$\mathbf{Z}\mathbf{A}^{+}\frac{d\mathbf{x}}{dt} = \mathbf{Z}\mathbf{v}_{ss} \qquad \text{(B-3)}$$

Since $\mathbf{v}_{ss}$ belongs to the kernel of the (reduced) stoichiometric matrix **N** (defined in Appendix A), one can write (Reder, 1988):

$$\mathbf{Z}\mathbf{v}_{ss} = \mathbf{v}_{ss} \qquad \text{(B-4)}$$

Thus:

$$\mathbf{Z}\mathbf{A}^{+}\frac{d\mathbf{x}}{dt} = \mathbf{v}_{ss} \qquad \text{(B-5)}$$

Eqn. (B-5) can now be integrated between an initial instant $t_0 = 0$ and any arbitrary time point t to give:

$$\mathbf{Z}\mathbf{A}^{+}\Delta\mathbf{x} = \mathbf{v}_{ss}t \qquad \text{(B-6)}$$

Eqn. (B-6) is the generalized matrix version of eqn. (4). It can be expressed in terms of the normalized Flux Control Coefficients if the assumptions stated in the Appendix A about **Z** hold. Using eqn. (A-18), the above equation becomes:

$$\mathbf{C}\mathbf{J}^{-1}\mathbf{A}^{+}\Delta\mathbf{x} = \begin{bmatrix} 1 \\ 1 \\ . \\ 1 \end{bmatrix} t \qquad \text{(B-7)}$$

If one considers a single row of the steady-state flux-control matrix, eqn. (B-7) can be written as:

$$[\alpha_1\,\alpha_2 \ldots \alpha_n]\,\Delta\mathbf{x} = t \qquad \text{(B-8)}$$

where

$$[\alpha_1\,\alpha_2 \ldots \alpha_n] = \mathbf{C}^{\mathbf{K}}\mathbf{J}^{-1}\mathbf{A}^{+} \qquad \text{(B-9)}$$

or

$$[\alpha_1\,\alpha_2 \ldots \alpha_n]\,\mathbf{A}\mathbf{J} = \mathbf{C}^{\mathbf{K}} \qquad \text{(B-10)}$$

Here $\mathbf{C}^{\mathbf{K}}$ is a row vector whose elements are $C^{J_K}_{\lambda_j}$. Eqn. (B-8) is the matrix version of eqns. (4), (20a), (20b) and (20c) in the main paper. Also, from eqns. (B-9) and (B-10) one can see that the coefficients in eqn. (B-8) are related to the stoichiometric coefficients, the steady-state fluxes and the Flux Control Coefficients. Thus eqns. (5), (22a), (22b) and (22c) can be readily seen.

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APPENDIX C

In this Appendix we show how the mass balance or other stoichiometric constraints vanish from the constraints among the transient metabolite concentrations after post-multiplying by the (full) stoichiometric matrix **A**. Assuming **A** has dimension $n \times r$, where r is the total number of reactions in the pathway and n is the total number of metabolites (internal plus external), we follow the same approach as in Appendix A and decompose **A** as:

$$\mathbf{A} = \mathbf{L}\mathbf{A}_\mathbf{R} \tag{C-1}$$

where $\mathbf{A}_\mathbf{R}$ is an $n_0 \times r$ matrix formed by the first n_0 rows of **A** that constitute a basis for its row space, and **L** is an $n \times n_0$ matrix that has the form:

$$\mathbf{L} = \begin{bmatrix} \mathbf{I}_{n_0} \\ \mathbf{L}_0 \end{bmatrix} \tag{C-2}$$

where $\mathbf{L}_0$ is $(n-n_0) \times n_0$ and $\mathbf{I}_{n_0}$ is the $n_0 \times n_0$ identity matrix.

With these definitions, one can easily show that all the structural conservation relationships among all metabolites can be expressed by:

$$[\mathbf{L}_0 - \mathbf{I}_{n-n_0}]\,\Delta\mathbf{x} = 0 \tag{C-3}$$

where the $n \times 1$ concentration change vector $\Delta\mathbf{x}$ includes all external and internal metabolites.

All constraints among the transient metabolite concentrations will be a linear combination of eqns. (B-6) and (C-3). Then one can write:

$$\mathbf{B}\Delta\mathbf{x} = \mathbf{v}_{ss}t \tag{C-4}$$

where

$$\mathbf{B} = \mathbf{Z}\mathbf{A}^+ + \beta[\mathbf{L}_0 - \mathbf{I}_{n-n_0}] \tag{C-5}$$

and β is an arbitrary constant.

To determine the Flux Control Coefficients and according to eqn. (B-10) one has to post-multiply the coefficients in the constraint [eqn. (C-5)] by the full stoichiometric matrix **A**:

$$\mathbf{B}\mathbf{A} = \mathbf{Z}\mathbf{A}^+\mathbf{A} + \beta[\mathbf{L}_0 - \mathbf{I}_{n-n_0}]\,\mathbf{A} \tag{C-6}$$

By eqn. (C-1):

$$\mathbf{B}\mathbf{A} = \mathbf{Z} + \beta[\mathbf{L}_0 - \mathbf{I}_{n-n_0}]\,\mathbf{L}\mathbf{A}_\mathbf{R} \tag{C-7}$$

Using the partitioned form of **L** [eqn. (C-2)]:

$$\mathbf{B}\mathbf{A} = \mathbf{Z} + \beta[\mathbf{L}_0 - \mathbf{I}_{n-n_0}]\begin{bmatrix} \mathbf{I}_{n_0} \\ \mathbf{L}_0 \end{bmatrix}\mathbf{A}_\mathbf{R} \tag{C-8}$$

Multiplying the partitioned matrices:

$$\mathbf{B}\mathbf{A} = \mathbf{Z} + \beta[\mathbf{L}_0\mathbf{I}_{n_0} - \mathbf{I}_{n-n_0}\mathbf{L}_0]\,\mathbf{A}_\mathbf{R} \tag{C-9}$$

or

$$\mathbf{B}\mathbf{A} = \mathbf{Z} + \beta[\mathbf{L}_0 - \mathbf{L}_0]\,\mathbf{A}_\mathbf{R} = \mathbf{Z} \tag{C-10}$$

and the structural or stoichiometric conservation equations vanish when one post-multiplies by **A**.

APPENDIX D

As shown in eqn. (B-10), the Flux Control Coefficients are given by:

$$[C^{J_\mathrm{K}}_{e_1}\, C^{J_\mathrm{K}}_{e_2}\, C^{J_\mathrm{K}}_{e_3} \ldots C^{J_\mathrm{K}}_{e_r}] = [\alpha_1^\mathrm{K}\, \alpha_2^\mathrm{K}\, \alpha_3^\mathrm{K} \ldots \alpha_n^\mathrm{K}]\,\mathbf{A}\mathbf{J} \tag{D-1}$$

where the K denotes the branch on which the Flux Control Coefficients are based.

In linear (unbranched) pathways there are no special relationships among the Flux Control Coefficients. On the other hand, in branched pathways some relationships can be extracted and their number and form will depend on the topology of the reaction network. For the configuration depicted in Fig. 6, the stoichiometric matrix **A** is given by eqn. (24). In this case eqn. (D-1) can be explicitly written as:

$$[C^{J_\mathrm{K}}_{e_1}\, C^{J_\mathrm{K}}_{e_2}\, C^{J_\mathrm{K}}_{e_3}\, C^{J_\mathrm{K}}_{e_4}\, C^{J_\mathrm{K}}_{e_5}] = [(\alpha_2^\mathrm{K} - \alpha_1^\mathrm{K})J_1\ (\alpha_3^\mathrm{K} - \alpha_2^\mathrm{K})J_2\ (\alpha_4^\mathrm{K} - \alpha_3^\mathrm{K})J_3\ (\alpha_5^\mathrm{K} - \alpha_4^\mathrm{K})J_4\ (\alpha_6^\mathrm{K} - \alpha_3^\mathrm{K})J_5] \tag{D-2}$$

where $J_1 = J_2 = J_\mathrm{A}$, $J_3 = J_4 = J_\mathrm{B}$ and $J_5 = J_\mathrm{C}$.

It can be shown that the following relation among the Flux Control Coefficients holds (e.g. Fell & Sauro, 1985):

$$J_\mathrm{C}(C^{J_\mathrm{A}}_{e_3} + C^{J_\mathrm{A}}_{e_4}) = J_\mathrm{B}C^{J_\mathrm{A}}_{e_5} \tag{D-3}$$

Substituting $C^{J_\mathrm{A}}_{e_3}$, $C^{J_\mathrm{A}}_{e_4}$ and $C^{J_\mathrm{A}}_{e_5}$ using eqn. (D-2) we obtain:

$$\alpha_5^\mathrm{A} = \alpha_6^\mathrm{A} \tag{D-4}$$

Thus the coefficients of $[x_5(t) - x_5(0)]$ and $[x_6(t) - x_6(0)]$ in eqn. (20a) must be the same. Since we must delete variables so that the mass-balance constraint fails, deletion of $[x_5(t) - x_5(0)]$ {or $[x_6(t) - x_6(0)]$} leads to $\alpha_5^\mathrm{A} = \alpha_6^\mathrm{A} = 0$. Following the same argument, one can show that deletion of X_1 and X_6 ($\alpha_1^\mathrm{B} = \alpha_6^\mathrm{B} = 0$) leads to the calculation of $C^{J_\mathrm{B}}_{e_i}$, and that deletion of X_1 and X_5 ($\alpha_1^\mathrm{C} = \alpha_5^\mathrm{C} = 0$) leads to the calculation of $C^{J_\mathrm{C}}_{e_i}$.

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