Control Analysis of Stationary Forced Oscillations

Oleg V. Demin,[†,‡] Hans V. Westerhoff,*[,§,||] and Boris N. Kholodenko[†,⊥]

A. N. Belozersky Institute of Physical-Chemical Biology, Moscow State University, 119899 Moscow, Russia, M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia, E. C. Slater Institute, Biocentrum Amsterdam, University of Amsterdam, Department of Molecular Cell Physiology, Free University, De Boelelaan 1087, NL-1081 HV Amsterdam, European Union, and Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, 1020 Locust Street, Philadelphia, Pennsylvania 19107

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Metabolic control analysis is here extended to stationary dynamic phenomena arising from a steadily oscillating external force. The extension focuses on the form of the oscillations, in terms of the discrete spectrum of the frequencies, as obtained by expansion into a Fourier series. The control of each oscillating metabolite concentration (or reaction rate) by any enzyme in the system is described by (i) periodic control coefficients referring to the control on the time dependence of that concentration and (ii) Fourier control coefficients. One for each Fourier frequency, the latter refer to the control of the waveform (and total amplitude) of the oscillations. It is shown how both types of control coefficient can be expressed in terms of elasticity coefficients (which comprise the relevant enzyme kinetics) and network structure. Importantly, integrals of the elasticity coefficients and reaction rates enter the expressions for the control coefficients; enzyme kinetic information along the entire oscillation route is important for the distribution of the control over the pathway enzymes. For both types of control coefficient, summation and connectivity theorems are derived. Including the control by the external frequency in the summation, the sums equal 0 and 1 for all the Fourier components of concentrations and reaction rates. An example illustrates the application of this control analysis.

Introduction

The cell or a metabolic system within it may be exposed to a periodic external force, such as an oscillating concentration of a pathway substrate (hormone),[1−3] or to an alternating electric field [ref 4, cf., refs 5 and 6]. As a consequence, forced periodic or nonperiodic oscillations of metabolic fluxes and concentrations occur. Such systems may serve to extract free energy[7] or information.[8] In some cellular systems, oscillations arise spontaneously.[3,9−14] The control aspects of such oscillations differ greatly from those of externally forced oscillations. A coefficient quantifying the control of an oscillating property cannot be defined for an autonomously oscillating system whereas it can be for an oscillation driven by an external force.[15,16] This suggests that metabolic control theory aspects should be derived separately for autonomous and enforced oscillations. This paper will be limited to the latter.

The cell cycle can be viewed as resulting from an autonomously oscillating subsystem[17,18] ("clock") that drives a large number of cellular processes into an oscillation. In view of the subtle balances known to exist in intracellular metabolism,[19] free-energy transduction,[20] and signal transduction,[21] it seems important that the amplitudes, phases, and wave forms of all the driven oscillations are in concordance. The properties of the driven oscillations are not only determined by the driving oscillations but also by the kinetic properties of the responding components. Because those kinetic properties are nonlinear, the driven oscillations may differ substantially from the driving oscillation.[22] We shall here address how the extent to which the various intracellular oscillation processes control the driven oscillations relates to the nonlinear kinetic properties.

In metabolic control analysis (MCA), control coefficients quantify the control of steady-state fluxes and concentrations by the enzymes in the system. Summation laws[19,23−26] limit the magnitudes of these control coefficients. Connectivity laws[25−27] relate control coefficients to enzyme kinetic properties. Together with the network structure, these laws enable one to express the degree to which the enzymes control steady-state fluxes and concentrations into those enzyme kinetic properties.[28−30] This suggested to us that it might be quite useful to develop an approach analogous to MCA for forced oscillations.

We have defined the periodic control coefficient as the logarithmic derivative of any periodic metabolic variable with respect to an enzyme concentration (or the enzyme's V_{max}).[15] Such periodic control coefficients quantify how stationary forced oscillations are determined by the enzymes' activities. The "transient" control coefficient[15] describes the control an enzyme exerts over the transition to the new periodic trajectory after a small but persistent perturbation in its concentration. These "transient" control coefficients tend to the corresponding periodic control coefficients as time tends to infinity.

Theorems have been derived for the control of characteristics of a forced periodic motion that do not depend on the phase. These theorems showed that the sum of all the control exerted by all enzymes in the system and the external oscillator equals 0 and 1, for amplitude of concentrations and fluxes, respectively. However, such summation relationship has not been derived for the control coefficients over phase-dependent characteristics, such as the oscillating metabolite concentrations and fluxes themselves. Calculation of the control of these properties has not yet been possible for systems with forced oscillations.

[†] A. N. Belozersky Institute of Physical-Chemical Biology.
[‡] M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry.
[§] E. C. Slater Institute.
[||] Department of Molecular Cell Physiology.
[⊥] Department of Pathology.

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For the case of forced oscillations, we shall here develop a direct method for calculating the periodic control coefficients from the kinetic properties of the participating enzymes and we shall derive summation and connectivity laws for the control of phase-dependent properties.

A. Theoretical Background

A.1. Periodically Oscillating Metabolite Concentrations and Fluxes and Their Fourier Series. Kinetic equations for a metabolic network, homogeneous in all its compartments, and exposed to a periodic external influence ("force"), are

$$\mathrm{d}\mathbf{x}/\mathrm{d}t = \mathbf{N}\cdot\mathbf{v}(p(t),\mathbf{x}) \tag{1}$$

$\mathbf{x} = [x_1, ..., x_m]^T$ and $\mathbf{v} = [v_1, ..., v_n]^T$ are vector columns of the m metabolite concentrations and the n reaction and transport rates, respectively. $\mathbf{N}$ is the $m \times n$ stoichiometry matrix.[25] The rates of the individual reactions, $v_j(t,\mathbf{x}) = v_j(\mathbf{p}(t),\mathbf{x})$, may depend on time explicitly, i.e., through $\mathbf{p}(t)$ and implicitly, i.e., through $\mathbf{x}(t)$. We shall limit this paper to stationary oscillations:

$$\mathbf{p}(t) = \mathbf{p}(t+T), \quad \mathbf{v}(t+T,\mathbf{x}) = \mathbf{v}(t,\mathbf{x}) \tag{2}$$

T is the period of the external force, related to the actual frequency (ω) by $T = 2\cdot\pi/\omega$. Reaction rates, v_j, are held proportional to the concentrations (e_j) of the corresponding enzymes (e_j will denote the maximal catalytic activity (V_{max})[33,34]):

$$v_j = e_j\cdot w_j(t,\mathbf{x}) \tag{3}$$

The turnover rates w_j depend on time periodically, i.e., $w_j(t+T,\mathbf{x}) = w_j(t,\mathbf{x})$. In matrix form,

$$\mathbf{v}(t,\mathbf{x}) = (\mathrm{diag}\ \mathbf{e})\cdot\mathbf{w}(t,\mathbf{x}),\ \mathbf{w}(t+T,\mathbf{x}) = \mathbf{w}(t,\mathbf{x}) \tag{4}$$

$\mathbf{e} = [e_1, ..., e_n]^T$ represents the enzyme concentrations and $\mathbf{w} = [w_1, ..., w_n]^T$ the turnover rates. (diag $\mathbf{y}$) designates the square matrix with diagonal elements given by $(\mathrm{diag}\ \mathbf{y})_{ii} = y_i$, and all off-diagonal elements equal to zero.

If the rank (m_0) of the stoichiometry matrix ($\mathbf{N}$) is less than the number of metabolites m, the metabolite concentrations are subject to $m-m_0$ linearly independent relationships:

$$\mathbf{M}\cdot\mathbf{x} = \mathbf{T} \tag{5}$$

$\mathbf{M}$ is an $m-m_0$ by m matrix, $\mathbf{T}$ is an $(m-m_0)$-dimensional vector, determined by the initial conditions. Often the coefficients M_{ki} can be interpreted as the number of moieties of type k in the metabolite x_i. For the choice of the matrix $\mathbf{M}$ and its relation to the link matrix[28] see, e.g., ref 35.

This paper will be limited to systems for which eq 1 has a unique, asymptotically stable, periodic solution, $\mathbf{x}^{per}(t,\mathbf{e})$ at and around the enzyme concentrations $\mathbf{e}$. We expand the concentration vector $\mathbf{x}^{per}(t,\mathbf{e})$, the flux vector $\mathbf{v}^{per}(t,\mathbf{e})$, and vector of kinetic parameters $\mathbf{p}(t)$ into their Fourier series:

$$\mathbf{x}^{per}(t,\mathbf{e}) = \sum_{h=-\infty}^{\infty} \mathbf{x}^h\cdot\exp(\mathrm{i}h\omega t)$$

$$\mathbf{v}^{per}(t,\mathbf{e}) = \mathbf{v}(t,\mathbf{x}^{per}(t,\mathbf{e}),\mathbf{e}) = \sum_{h=-\infty}^{\infty} \mathbf{v}^h\cdot\exp(\mathrm{i}h\omega t)$$

$$\mathbf{p}(t) = \sum_{h=-\infty}^{\infty} \mathbf{p}^h\cdot\exp(\mathrm{i}h\omega t) \tag{6}$$

The Fourier coefficients $\mathbf{x}^h$ and $\mathbf{v}^h$ are functions of $\mathbf{e}$:

$$\mathbf{x}^h(\mathbf{e}) = (1/T)\cdot\int_0^T \mathbf{x}(t,\mathbf{e})\cdot\exp(-\mathrm{i}h\omega t)\,\mathrm{d}t$$

$$\mathbf{v}^h(\mathbf{e}) = (1/T)\cdot\int_0^T \mathbf{v}(t,\mathbf{x}^{per}(t,\mathbf{e}),\mathbf{e})\cdot\exp(-\mathrm{i}h\omega t)\,\mathrm{d}t, \quad h = 0, \pm1, \pm2, ... \tag{7}$$

$\mathbf{p}^h$ are Fourier coefficients which do not depend on $\mathbf{e}$:

$$\mathbf{p}^h = (1/T)\int_0^T \mathbf{p}(t)\cdot\exp(-\mathrm{i}h\omega t)\,\mathrm{d}t, \quad h = 0, \pm1, \pm2, ...$$

$\mathbf{x}^h$ and $\mathbf{x}^{-h}$, $\mathbf{v}^h$ and $\mathbf{v}^{-h}$, $\mathbf{p}^h$ and $\mathbf{p}^{-h}$ are the complex conjugates of each other, such that $\mathbf{x}^{per}$, $\mathbf{v}^{per}$, and $\mathbf{p}(t)$ are always real. The Fourier coefficients of zero order ($h = 0$) are just the T-average values of the corresponding functions. The Fourier coefficients of the rates, $\mathbf{v}^h$, are related to Fourier coefficients of the turnover rates, i.e., $\mathbf{w}^h$ through:

$$\mathbf{v}^h(\mathbf{e}) = (\mathrm{diag}\ \mathbf{e})\cdot\mathbf{w}^h(\mathbf{e}), \quad h = 0, \pm1, \pm2, ... \tag{8}$$

Fourier transformation (eq 6) constitutes a one-to-one correspondence between the periodic concentrations $\mathbf{x}^{per}(t,\mathbf{e})$ and a potentially infinite series of (Fourier) coefficients ($\mathbf{x}^h$). Matrix $\mathbf{x}^{\mathbf{F}}$ consists of m rows, each of which corresponds to the concentration of a metabolite x_i^{per}. Each column corresponds to a list of Fourier coefficients of a certain order h, x_i^h; one coefficient for each independent metabolite:

$$\mathbf{x}^{\mathbf{F}}(\mathbf{e}) = (x_i^h(\mathbf{e})), \quad i = 1, ..., m; \quad h = 0, \pm1, \pm2, ... \tag{9}$$

Superscript $\mathbf{F}$ denotes Fourier series. When the oscillations are virtually sinusoidal,[36] $\mathbf{x}^{\mathbf{F}}$ approaches an $m_0 \times 3$ matrix and the oscillation in each concentration a point in a three-dimensional Fourier space. Each row of the matrix $\mathbf{x}^{\mathbf{F}}$ can also be called the discrete Fourier spectrum of the corresponding metabolite concentration. When the oscillation is nearly sinusoidal with the same frequency as the frequency of the forcing oscillation (i.e., ω), then this spectrum peaks at $h = \pm1$.

Introducing $\mathbf{v}^{\mathbf{F}}$ and $\mathbf{p}^{\mathbf{F}}$, for the matrix of Fourier coefficients of the periodic fluxes (v_i^{per}) and parameter vector $\mathbf{p}(t)$:

$$\mathbf{v}^{\mathbf{F}}(\mathbf{e}) = (v_j^h(\mathbf{e})), \quad j = 1, ..., n; \quad h = 0, \pm1, \pm2, ...$$
$$\mathbf{p}^{\mathbf{F}} = (\mathbf{p}^h), \quad h = 0, \pm1, \pm2, ... \tag{10}$$

In a periodic trajectory of an enforced oscillation, the dependence of reaction rates ($\mathbf{v}$) on metabolite concentrations ($\mathbf{x}^{per}$) and periodic force ($\mathbf{p}(t)$) can be translated in terms of the dependence of the Fourier coefficients of the former on those of the latter. In terms of turnover rates:

$$\mathbf{w}^h(\mathbf{e}) = \mathbf{w}^h(\mathbf{p}^{\mathbf{F}},\mathbf{x}^{\mathbf{F}}(\mathbf{e})), \quad h = 0, \pm1, \pm2, ...$$

In this manuscript we consider sensitivity of periodic fluxes $\mathbf{v}^{per}$ and metabolite concentrations $\mathbf{x}^{per}$ with respect to enzyme concentrations $\mathbf{e}$ and Fourier coefficients $\mathbf{p}^h$ do not depend on $\mathbf{e}$. Consequently, the designation $\mathbf{p}^{\mathbf{F}}$ can be eliminated from the above equation:

$$\mathbf{w}^h(\mathbf{e}) = \mathbf{w}^h(\mathbf{x}^{\mathbf{F}}(\mathbf{e})), \quad h = 0, \pm1, \pm2, ... \tag{11}$$

A.2. Matrices of the Periodic Control and Elasticity Coefficients and Their Fourier Series. We designate by $\mathbf{C}_{\mathbf{e}}^{\mathbf{x}}$ the $m \times n$ matrix of the periodic control coefficients that quantify the control any enzyme may exert on any concentration.

The coefficient at row i and column j of the matrix $\mathbf{C}^{\mathbf{x}}_{\mathbf{e}}$ is the control coefficient of x_i^{per} with respect to the concentration of the enzyme j, as defined in ref 15:

$$(\mathbf{C}^{\mathbf{x}}_{\mathbf{e}})_{ij} = \mathrm{d}\ln x_i^{\mathrm{per}}(t,\mathbf{e})/\mathrm{d}\ln e_j \tag{12}$$

Similarly, we designate by $\mathbf{C}^{\mathbf{v}}_{\mathbf{e}}$ the $n \times n$ matrix of the enzyme control coefficients over all the reaction rates, $\mathbf{v}(t,\mathbf{x}^{\mathrm{per}}(t,\mathbf{e}),\mathbf{e})$; its coefficient at row i and column j is the control coefficient of the enzyme j with respect to the rate υ_i, considered as a function of the periodic variation of the metabolite concentrations:

$$(\mathbf{C}^{\mathbf{v}}_{\mathbf{e}})_{ij} = \mathrm{d}\ln \upsilon_i^{\mathrm{per}}(t,\mathbf{x}^{\mathrm{per}}(t,\mathbf{e}),e_i)/\mathrm{d}\ln e_j \tag{13}$$

Due to the moiety-conservation constraints (eq 5), there are only m_0 linearly independent metabolite concentrations, hence, only m_0 linearly independent rows of the matrix $\mathbf{C}^{\mathbf{x}}_{\mathbf{e}}(t)$. Without loss of generality these can be chosen as its first m_0 rows. All the rates $\upsilon_i(t,\mathbf{x}^{\mathrm{per}}(t,\mathbf{e}),e_i)$ and hence all the rows of $\mathbf{C}^{\mathbf{v}}_{\mathbf{e}}(t)$ are linearly independent, since the time dependencies of concentrations do not equal zero at all times. Both matrices $\mathbf{C}^{\mathbf{x}}_{\mathbf{e}}(t)$ and $\mathbf{C}^{\mathbf{v}}_{\mathbf{e}}(t)$ depend on time periodically.[15]

Because net reaction rates $\mathbf{v}$ can become zero, we shall use nonnormalized control coefficients ($\Gamma^{\mathbf{x}}_{\mathbf{e}}$ and $\Gamma^{\mathbf{v}}_{\mathbf{e}}$), with definitions adjusted accordingly

$$\Gamma^{\mathbf{x}}_{\mathbf{e}} = (\mathrm{diag}\ \mathbf{x})\cdot\mathbf{C}^{\mathbf{x}}_{\mathbf{e}}\cdot(\mathrm{diag}\ \mathbf{e})^{-1},\ \Gamma^{\mathbf{v}}_{\mathbf{e}} = (\mathrm{diag}\ \mathbf{v})\cdot\mathbf{C}^{\mathbf{v}}_{\mathbf{e}}\cdot(\mathrm{diag}\ \mathbf{e})^{-1} \tag{14}$$

When in eqs 12 and 13, the periodic concentrations $\mathbf{x}^{\mathrm{per}}(t,\mathbf{e})$ and fluxes $\mathbf{v}^{\mathrm{per}}(t,\mathbf{e})$ are expanded into their Fourier series (see eq 6), the differentiation of each term with respect to enzyme concentrations results in a Fourier series for the control matrices [cf. ref 16]:

$$\Gamma^{\mathbf{x}}_{\mathbf{e}}(t) = \sum_{h=-\infty}^{\infty} \Gamma^{\mathbf{x},h}_{\mathbf{e}}\cdot\exp(\mathrm{i}h\omega t),\quad \Gamma^{\mathbf{x},h}_{\mathbf{e}}(\mathbf{e}) = (\mathrm{d}\mathbf{x}^h/\mathrm{d}\mathbf{e})$$

$$\Gamma^{\mathbf{v}}_{\mathbf{e}}(t) = \sum_{h=-\infty}^{\infty} \Gamma^{\mathbf{v},h}_{\mathbf{e}}\cdot\exp(\mathrm{i}h\omega t),\quad \Gamma^{\mathbf{v},h}_{\mathbf{e}}(\mathbf{e}) = (\mathrm{d}\mathbf{v}^h/\mathrm{d}\mathbf{e})$$

$$h = 0, \pm1, \pm2, \ldots \tag{15}$$

$\Gamma^{\mathbf{x},h}_{\mathbf{e}}$ is an $n \times m_0$ matrix of so called "Fourier control coefficients" of hth order. It quantifies how the enzymes in the system control the Fourier spectra that characterize the oscillations of the metabolites. Equation 15 shows that the periodic concentration control coefficients $\Gamma^{\mathbf{x}}_{\mathbf{e}}(t)$ can be obtained by first Fourier transforming the periodic concentrations, then determining the control in Fourier space, and then reverse Fourier transforming the Fourier control coefficients. The analogous operation yields the flux control coefficients.

We shall examine whether elasticity coefficients[20,25,26] are the local kinetic properties that determine stationary oscillations, as they do steady-state properties. We employ nonnormalized elasticity coefficients,[28]

$$\mathbf{D} = \partial\mathbf{v}/\partial\mathbf{x} = (\mathrm{diag}\ \mathbf{e})\cdot\partial\mathbf{w}/\partial\mathbf{x} \tag{16}$$

The elasticity matrix $\mathbf{D}$ considered under influence of periodic force $p(t)$ at the periodic trajectory $\mathbf{x}^{\mathrm{per}}(t,\mathbf{e})$ is a periodic function of time:

$$\mathbf{D}(p(t),\mathbf{x}^{\mathrm{per}}(t)) = \mathbf{D}(t+T) = \mathbf{D}(t)$$

$$\mathbf{D}(t) = \sum_{h=-\infty}^{\infty} \mathbf{D}^h\cdot\exp(\mathrm{i}h\omega t)$$

$$\mathbf{D}^h = (1/T)\cdot\int_0^T \mathbf{D}(t)\cdot\exp(-\mathrm{i}h\omega t)\,\mathrm{d}t,\quad h = 0, \pm1, \pm2, \ldots \tag{17}$$

$\mathbf{D}^h$ is the matrix of Fourier elasticity coefficients. The latter can be related to the derivatives of some Fourier coefficients of the reaction rates with respect to different Fourier coefficients of the concentrations (cf. Appendix A):

$$\partial\mathbf{v}^h/\partial\mathbf{x}^k = \mathbf{D}^{h-k},\ h = 0, \pm1, \pm2, \ldots \quad k = 0, \pm1, \pm2, \ldots \tag{18}$$

When kinetics are linear in terms of concentrations and the system has a linear response, only $\mathbf{D}^0$ differs from zero. It becomes equal to the classical nonnormalized elasticity coefficient. Now the aim of the present paper can be formulated to find the relationships connecting the matrices of the periodic control coefficients (systemic properties) to the matrix of the periodic elasticity coefficients ("enzyme" or "local" properties).

In this paper we shall generalize the perturbation method of[29] to stationary systems subject to a forced oscillation. We shall first summarize the steady-state version of this method. At steady state the enzyme concentrations are characterized by the vector $\mathbf{e}$, and the steady-state concentrations and rates (fluxes) by the vector $\mathbf{z} = (\mathbf{x}_{\mathrm{SS}},\mathbf{v}_{\mathrm{SS}})$. All enzyme concentrations are modulated simultaneously by $\Delta\mathbf{e}$, which leads to a change in steady-state concentrations and rates denoted by $\Delta\mathbf{z}$. $\Delta\mathbf{e}$ can be such that only enzyme **1** is modulated, or such that all enzymes are modulated to the same relative extent, or can represent any other spectrum of modulations. The corresponding $\Delta\mathbf{e}$'s differ in "direction" (as evident in the space of enzyme activities). The second aspect of $\Delta\mathbf{e}$ is the magnitude of the modulation, which may differ between two modulations of the same direction. Because we need to consider modulations of various directions but of infinitely small magnitude, we factor out the magnitude of $\Delta\mathbf{e}$:

$$\Delta\mathbf{e} = \mathbf{a}\cdot u \tag{19}$$

Here $\mathbf{a}$ is a vector of unit length with the (arbitrary) direction of the modulation. Scalar u is the absolute magnitude of the modulation. We shall consider u to be infinitesimal:

$$\mathbf{a} = \mathrm{d}\mathbf{e}/\mathrm{d}u \tag{20}$$

As a function of u, the steady-state concentrations and rates describe some curve in the phase space of state variables. The vector $\mathrm{d}\mathbf{z}/\mathrm{d}u$ gives the direction of the change of the state variables. The components of this vector are given by χ_i for the concentrations and ρ_i for the rates, such that

$$\mathrm{d}\mathbf{z}/\mathrm{d}u = (\mathrm{d}\mathbf{x}/\mathrm{d}u,\mathrm{d}\mathbf{v}/\mathrm{d}u) = (\chi,\rho) \tag{21}$$

In terms of the control coefficients, one may write

$$\mathrm{d}\mathbf{z}/\mathrm{d}u = (\Gamma^{\mathbf{x}}_{\mathbf{e}},\Gamma^{\mathbf{v}}_{\mathbf{e}})\cdot\mathbf{a} \tag{22}$$

so that

$$\chi = \Gamma^{\mathbf{x}}_{\mathbf{e}}\cdot\mathbf{a} \tag{23}$$

$$\rho = \Gamma^{\mathbf{v}}_{\mathbf{e}}\cdot\mathbf{a} \tag{24}$$

$\Gamma^{\mathbf{x}}_{\mathbf{e}}$ and $\Gamma^{\mathbf{v}}_{\mathbf{e}}$ are the nonnormalized concentration and flux control coefficients of eq 14 and **a** parameterizes the arbitrary direction of the modulation of the enzyme concentrations.

The changes in reaction rates are partly directly due to the modulation of the enzyme concentrations and partly due to changes in turnover number. The latter are due to the changes in metabolite (e.g., substrate and product) concentrations that result from the changes in the enzyme concentrations[25] (using eq 3):

$$\mathrm{d}\mathbf{v}/\mathrm{d}u = (\mathrm{diag}\ \mathbf{a})\cdot\mathbf{w} + (\mathrm{diag}\ \mathbf{e})\cdot(\mathrm{d}\mathbf{w}/\mathrm{d}u) \tag{25}$$

Equation 16 allows one to rewrite this in terms of the nonnormalized elasticity coefficients ((diag **f**)·**g** = (diag **g**)·**f** for any vectors **f** and **g**; cf., eqs 21 and 16):

$$\rho = \mathrm{d}\mathbf{v}/\mathrm{d}u = (\mathrm{diag}\ \mathbf{w})\cdot\mathbf{a} + \mathbf{D}\cdot\chi \tag{26}$$

The above equations are valid for arbitrary directions of **a**. From eqs 26 and 23, 24:

$$\mathbf{a} = (\mathrm{diag}\ \mathbf{w})^{-1}\cdot(\rho - \mathbf{D}\cdot\chi) \tag{27}$$

$$\chi = \Gamma^{\mathbf{x}}_{\mathbf{e}}\cdot(\mathrm{diag}\ \mathbf{w})^{-1}\cdot(\rho - \mathbf{D}\cdot\chi) \tag{28}$$

$$\rho = \Gamma^{\mathbf{v}}_{\mathbf{e}}\cdot(\mathrm{diag}\ \mathbf{w})^{-1}\cdot(\rho - \mathbf{D}\cdot\chi) \tag{29}$$

The modulations of vector **e** can have any direction **a** in the *n*-dimensional space of enzyme concentrations. However, the vector **z** in the $n + m$ dimensional space of state variables (concentrations and rates) and in particular $\mathbf{x}_{SS}$, and hence χ, must remain consistent with the $m-m_0$ moiety conservation relationships (eq 5) and the steady-state condition:

$$0 = \mathrm{d}\mathbf{x}/\mathrm{d}t = \mathbf{N}\cdot\mathbf{v} \tag{30}$$

These two requirements translate to

$$\mathbf{M}\cdot\chi = 0 \tag{31}$$

$$\mathbf{N}\cdot\rho = \mathrm{d}(\mathrm{d}\mathbf{x}/\mathrm{d}t)/\mathrm{d}u = \mathrm{d}(0)/\mathrm{d}u = 0 \tag{32}$$

The control coefficients are the unique solution of eqs 28 and 29 consistent with the conditions for χ and ρ (eqs 31 and 32).

Assuming that the system description has been transformed to truly independent variables *x*, such that **M** is absent and **N** has full rank ($m=m_0$), combination of eqs 26 and 32 leads to

$$0 = \mathbf{N}\cdot\rho = \mathbf{N}\cdot(\mathrm{diag}\ \mathbf{w})\cdot\mathbf{a} + \mathbf{N}\cdot\mathbf{D}\cdot\chi \tag{33}$$

so that for arbitrary d**e**:

$$\chi = -(\mathbf{N}\cdot\mathbf{D})^{-1}\cdot\mathbf{N}\cdot(\mathrm{diag}\ \mathbf{w})\cdot\mathbf{a}$$

$$\Gamma^{\mathbf{x}}_{\mathbf{e}}\cdot\mathrm{d}\mathbf{e} = -(\mathbf{N}\cdot\mathbf{D})^{-1}\cdot\mathbf{N}\cdot(\mathrm{diag}\ \mathbf{w})\cdot\mathrm{d}\mathbf{e} \tag{34}$$

Hence one obtains expressions for the control coefficients in terms of the elasticity coefficients **D** and network structure **N**:[28]

$$\Gamma^{\mathbf{x}}_{\mathbf{e}} = -(\mathbf{N}\cdot\mathbf{D})^{-1}\cdot\mathbf{N}\cdot(\mathrm{diag}\ \mathbf{w}) \tag{35}$$

$$\rho = (\mathbf{I} - \mathbf{D}\cdot(\mathbf{N}\cdot\mathbf{D})^{-1}\cdot\mathbf{N})\cdot(\mathrm{diag}\ \mathbf{w})\cdot\mathbf{a} \tag{36}$$

$$\Gamma^{\mathbf{v}}_{\mathbf{e}} = (\mathbf{I} - \mathbf{D}\cdot(\mathbf{N}\cdot\mathbf{D})^{-1}\cdot\mathbf{N})\cdot(\mathrm{diag}\ \mathbf{w}) \tag{37}$$

Kacser and Burns[25] noted that there are two ways of modulating the enzyme and metabolite concentrations that lead to special effects. The first of these leaves all metabolite concentrations unaltered and changes all reaction rates proportionally:

$$\chi = 0 \tag{38}$$

$$\rho = \alpha\cdot\mathbf{v}\ (\alpha \text{ is a scalar}) \tag{39}$$

These values of χ and ρ comply with eqs 31 and 32. Substitution of these expressions for χ and ρ (eqs 38 and 39) in eqs 28 and 29 leads to the summation theorems for the concentration and flux control coefficients, respectively,

$$\mathbf{C}^{\mathbf{x}}_{\mathbf{e}}\cdot\mathbf{1} = (\mathrm{diag}\ \mathbf{x})^{-1}\cdot\Gamma^{\mathbf{x}}_{\mathbf{e}}\cdot\mathbf{e} = 0 \tag{40}$$

$$\mathbf{C}^{\mathbf{v}}_{\mathbf{e}}\cdot\mathbf{1} = (\mathrm{diag}\ \mathbf{v})^{-1}\cdot\Gamma^{\mathbf{v}}_{\mathbf{e}}\cdot\mathbf{e} = 1 \tag{41}$$

i.e., the sums of the normalized control coefficients on a concentration or flux equal 0 and 1, respectively.

The other special modulation is in such a direction in **e** space that the steady-state rates remain the same:

$$\rho = 0 \tag{42}$$

Insertion into eqs 28 and 29 leads to the connectivity theorems:

$$\mathbf{I}_m = -\mathbf{C}^{\mathbf{x}}_{\mathbf{e}}\cdot\epsilon_{\mathbf{x}}, \quad \mathbf{0} = \mathbf{C}^{\mathbf{v}}_{\mathbf{e}}\cdot\epsilon_{\mathbf{x}}$$

$\epsilon_{\mathbf{x}}$ is the normalized elasticity matrix, and the concentrations have been assumed to be not constrained by moiety conservations.

B. Results

B.1. Generalizing the Perturbation Method to Forced Oscillations. In the case of forced oscillations, a perturbation $\Delta\mathbf{e}$ results in a change in periodic functions (i.e., in the functions that describe oscillating metabolites and fluxes). Our expansion of the periodic solution into a Fourier series (above) makes it possible to specify the changes in the periodic functions in terms of changes of the Fourier coefficients. As in MCA, the consideration of infinitesimally small changes should then allow one to descend to linearized changes, i.e., to vectors of the tangent space. Let us designate by $\mathbf{z}^{\mathbf{F}}(\mathbf{e})$ the infinite matrix involving both the infinite matrix of the Fourier series of oscillating concentrations $\mathbf{x}^{\mathbf{F}}$ and that of oscillating fluxes $\mathbf{v}^{\mathbf{F}}$ (see eq 9). Due to the precondition that eq 1 has a unique periodic solution, there is a correspondence between the "points" **e** and their images ("points" in Hilbert space) in the space of the Fourier coefficients $\mathbf{z}^{\mathbf{F}}(\mathbf{e})$.

Let us consider the two sets of enzyme concentrations **e** and $\mathbf{e} + \Delta\mathbf{e}$ and their corresponding images $\mathbf{z}^{\mathbf{F}}$ and $\mathbf{z}^{\mathbf{F}} + \Delta\mathbf{z}^{\mathbf{F}}$. We shall again decompose the modulation $\Delta\mathbf{e}$ in terms of its direction (vector **a**) and its magnitude *u* (see eq 19). When the direction **a** is kept constant, eq 19 corresponds to a line. Every point of this line in enzyme space corresponds to a condition in which the system exhibits a sustained forced oscillation in concentration-rate space. Accordingly, the points $\mathbf{z}^{\mathbf{F}}$ corresponding to the line (eq 19) form some "curve" (in the Hilbert space) that begins at the point $\mathbf{z}^{\mathbf{F}}$ and finishes at the point $\mathbf{z}^{\mathbf{F}} + \Delta\mathbf{z}^{\mathbf{F}}$. The direction of the curve $\mathbf{z}^{\mathbf{F}}(\mathbf{e}(u))$ is given by the vector $\mathrm{d}\mathbf{z}^{\mathbf{F}}/\mathrm{d}u$. The (complex) components of this vector will be denoted by $(\mathbf{l}^h_i, \mathbf{r}^h_i)$:

$$\mathrm{d}\mathbf{z}^{\mathbf{F}}/\mathrm{d}u|_{u=0} = (\mathrm{d}\mathbf{x}^h(u)/\mathrm{d}u|_{u=0}, \quad \mathrm{d}\mathbf{v}^h(u)/\mathrm{d}u|_{u=0}) = (\mathbf{l}^h,\mathbf{r}^h) = (l^h_i,r^h_j),\ i = 1, ..., m,\ j = 1, ..., n, \quad h = 0, \pm1, \pm2, ... \tag{43}$$

$d\mathbf{z}^F/du|_{u=0}$ varies with direction $\mathbf{a}$. l_i^h quantifies how the Fourier spectrum of metabolite x_i changes under an infinitesimal modulation of the enzymes in the system in direction $\mathbf{a}$. r_j^h does the same for the Fourier spectrum of rate v_j. l_i^h and r_j^h depend on $\mathbf{a}$ but not on u. The components of the directing vector $d\mathbf{z}^F/du$ can also be specified in terms of the Fourier series of the control matrix $\Gamma_{\mathbf{e}}^{\mathbf{x}}$ and $\Gamma_{\mathbf{e}}^{\mathbf{v}}$:

$$d\mathbf{z}^F/du|_{u=0} = (\partial\mathbf{z}^F/\partial\mathbf{e})\cdot d\mathbf{e}/du|_{u=0} = (\Gamma_{\mathbf{e}}^{\mathbf{x},h}, \Gamma_{\mathbf{e}}^{\mathbf{v},h})\cdot\mathbf{a} \quad (44)$$

$\Gamma_{\mathbf{e}}^{\mathbf{x},h}$ and $\Gamma_{\mathbf{e}}^{\mathbf{v},h}$ are given by eq 15, and $\mathbf{a}$ is the unit vector that describes the direction of the modulation ($\Delta\mathbf{e}$). Through e_j in eq 3 the modulation $\Delta\mathbf{e}$ will have a direct as well as an indirect effect on the rates of the perturbed enzymes by affecting $\mathbf{x}$ and hence turnover numbers $\mathbf{w}$. Changes in the Fourier coefficients $\mathbf{v}^h$ of the rates $\mathbf{v}$ and in the Fourier coefficients $\mathbf{w}^h$ of the functions $\mathbf{w}$, corresponding to the modulation ($\Delta\mathbf{e}$), are related through vector $\mathbf{a}$ of eq 8. Differentiating eq 8 with respect to u and using eq 19, one obtains

$$d\mathbf{v}^h/du = (\text{diag } \mathbf{a})\cdot\mathbf{w}^h + (\text{diag } \mathbf{e})\cdot d\mathbf{w}^h/du, \quad h = 0, \pm1, \pm2, \ldots \quad (45)$$

According to eq 11 the Fourier coefficients $\mathbf{w}^h$ of the function $\mathbf{w}$ can be presented as functions of the Fourier coefficients $\mathbf{x}^k$ of the periodic concentration vector. Therefore,

$$d\mathbf{w}^h/du = \sum_{k=-\infty}^{\infty} (\partial\mathbf{w}^h/\partial\mathbf{x}^k)\cdot d\mathbf{x}^k/du \quad (46)$$

Representing $(\text{diag } \mathbf{a})\cdot\mathbf{w}^h$ as $(\text{diag } \mathbf{w}^h)\cdot\mathbf{a}$ in eq 45, using eqs 46 and 43 and rearranging, one finds the following relationship between the direction of the modulation and the dependencies of the Fourier coefficients on the magnitude of the modulation:

$$\Gamma^{\mathbf{v},h}\cdot\mathbf{a} = \mathbf{r}^h = (\text{diag } \mathbf{w}^h)\cdot\mathbf{a} + \sum_{k=-\infty}^{\infty} \mathbf{D}^{hk}\cdot\Gamma^{\mathbf{x},k}\cdot\mathbf{a} = (\text{diag } \mathbf{w}^h)\cdot\mathbf{a} + \sum_{k=-\infty}^{\infty} \mathbf{D}^{hk}\cdot\mathbf{l}^k \quad (47)$$

$$\mathbf{a} = (\text{diag } \mathbf{w}^h)^{-1}\cdot(d\mathbf{v}^h/du|_{u=0} - (\text{diag } \mathbf{e})\cdot d\mathbf{w}^h/du|_{u=0}) = (\text{diag } \mathbf{w}^h)^{-1}\cdot(\mathbf{r}^h - \sum_{k=-\infty}^{\infty} (\partial\mathbf{v}^h/\partial\mathbf{x}^k)\cdot\mathbf{l}^k), \quad h = 0, \pm1, \pm2, \ldots \quad (48)$$

$\mathbf{D}^{hk}$, defined as $\partial\mathbf{v}^h/\partial\mathbf{x}^k$, quantifies the capability of the enzymes that are influenced by periodic force to deform the wave form of the concentration oscillations as it transduces the latter to oscillations in the reaction rates.

Equations 47 and 48 relate the Fourier flux control coefficients $\Gamma^{\mathbf{v},h}$ and the Fourier concentration control coefficients $\Gamma^{\mathbf{x},k}$ to the kinetic characteristics of the enzymes, i.e., $\mathbf{D}^{hk}$. They are the analogue of eq 26 for steady-state conditions. For systems with linear kinetics and linear response to periodic influences,

$$\mathbf{D}^{hk} = \mathbf{D}^0\cdot\delta_k^h, \ (\delta_k^h = 0 \text{ if } h \neq k, \quad \delta_k^h = 1 \text{ if } h = k)$$

and eq 47 reduces to

$$\Gamma^{\mathbf{v},h}\cdot\mathbf{a} = \mathbf{r}^h = (\text{diag } \mathbf{w}^h)\cdot\mathbf{a} + \mathbf{D}^0\cdot\Gamma^{\mathbf{x},k}\cdot\mathbf{a}$$

which is very similar to the relationship between the flux control coefficients and concentration control coefficients at steady state (eq 26).

The direction of the enzyme modulation is the same for all Fourier coefficients. This allows elimination of the direction of the modulation by inserting the effect of the modulation on the average turnover numbers and average elasticity coefficients. Substituting the expression of eq 48 for $h = 0$, for $\mathbf{a}$ in eq 44, and equating the right-hand sides of eqs 43 and 44 yields

$$\mathbf{l}^h = \Gamma_{\mathbf{e}}^{\mathbf{x},h}\cdot(\text{diag } \mathbf{w}^0)^{-1}\cdot(\mathbf{r}^0 - \sum_{k=-\infty}^{\infty} \mathbf{D}^{-k}\cdot\mathbf{l}^k),$$

$$\mathbf{r}^h = \Gamma_{\mathbf{e}}^{\mathbf{v},h}\cdot(\text{diag } \mathbf{w}^0)^{-1}\cdot(\mathbf{r}^0 - \sum_{k=-\infty}^{\infty} \mathbf{D}^{-k}\cdot\mathbf{l}^k), \quad h = 0, \pm1, \pm2, \ldots \quad (49)$$

$\mathbf{D}^{-k}$ is the derivative of the zero-order Fourier coefficient of the rate with respect to the kth order Fourier coefficient of the concentration (they relate to the derivative $\partial\mathbf{w}^0/\partial\mathbf{x}^k$ by eqs 8 and 18). Comparison of eqs 49 to their steady-state analogue (eqs 28 and 29) reveals a substantial similarity. The crucial difference is that the higher order Fourier components of the control coefficients, i.e., $\Gamma_{\mathbf{e}}^{\mathbf{v},h}$ and $\Gamma_{\mathbf{e}}^{\mathbf{x},h}$, are not only determined by the modulation direction of the same order ($\mathbf{l}^h$,$\mathbf{r}^h$) but also by the modulation direction of the other orders ($\mathbf{l}^k$,$\mathbf{r}^k$) which need not be the same. This distinction disappears in the case of linear kinetics.

The relationships (eq 49) contain the components of the Fourier direction vector, ($\mathbf{l}^h$,$\mathbf{r}^h$) (see eq 43), which are in part arbitrary because the modulation can be arbitrary. There are only n linearly independent components to the modulation, since the perturbation vector $\Delta\mathbf{e}$ has n (independent) components. This was also implied by eq 48 because its left-hand side depends on the n components of the directing vector $\mathbf{a}$ and must be identical for all $h = 0,\pm1,\pm2,\ldots$. Equating the right-hand side of eq 48 with $h = 0$ to those with $h \neq 0$ and rearranging, one obtains the essence of these constraints between the components of ($\mathbf{l}^h$,$\mathbf{r}^h$):

$$(\text{diag } \mathbf{w}^h)\cdot\mathbf{r}^0 - (\text{diag } \mathbf{w}^0)\cdot\mathbf{r}^h + \sum_{k=-\infty}^{\infty} [(\text{diag } \mathbf{w}^0)\cdot(\partial\mathbf{v}^h/\partial\mathbf{x}^k) - (\text{diag } \mathbf{w}^h)\cdot(\partial\mathbf{v}^0/\partial\mathbf{x}^k)]\cdot\mathbf{l}^k = 0, \quad h = \pm1, \pm2, \ldots \quad (50)$$

In addition, the components of the Fourier direction vector ($\mathbf{l}^h$,$\mathbf{r}^h$) correspond to the infinitesimal difference of two periodic solutions to the system of differential equations (see eq 43) and are hence constrained by the latter. These constraints can be explicited by substituting the Fourier series for the periodic functions (eq 6) ($\mathbf{x}^{\text{per}}(t;\mathbf{e})$, $\mathbf{v}^{\text{per}}(t;\mathbf{e})$) in eqs 1 and 5. Equating terms of the same Fourier order (frequency), one finds[36]

$$\mathbf{M}\cdot\mathbf{x}^0 = \mathbf{T}, \quad \mathbf{N}\cdot\mathbf{v}^0 = 0 \quad (51)$$

$$\mathbf{M}\cdot\mathbf{x}^h = 0, \quad \mathbf{N}\cdot\mathbf{v}^h = i\omega h\mathbf{x}^h, \quad h = \pm1, \pm2, \ldots \quad (52)$$

The eq 51 corresponding to the zero-order Fourier coefficients, i.e., to the average values, $\mathbf{x}^0$ and $\mathbf{v}^0$ (see eq 7), are identical to the equations delimiting the control of steady-state systems: the average values of metabolite concentrations and fluxes over T satisfy exactly the same equations as do their steady-state values. Yet, the zero-order Fourier coefficients do not always coincide with the time averages because $\mathbf{v}^0$ may depend on all the Fourier coefficients $\mathbf{x}^h$ and not just on $\mathbf{x}^0$.

Indeed, enzymes can be driven by the oscillating components of a driving force.[4,7] Identity of the average and steady-state values does occur in systems with linear responses to any external force.[22,38] Differentiating eqs 51 and 52 with respect to u at $u = 0$, one obtains for the zero-order Fourier coefficients of $\mathbf{r}^0$ and $\mathbf{l}^0$ the same constraint as in the steady-state case (eqs 31 and 32):

$$\mathbf{M}\cdot\mathbf{l}^0 = 0 \tag{53}$$

$$\mathbf{N}\cdot\mathbf{r}^0 = 0 \tag{54}$$

$$\mathbf{N}\cdot\mathbf{r}^h = \mathrm{i}\omega h\mathbf{l}^h, \quad \mathbf{M}\cdot\mathbf{l}^h = 0, \quad h = \pm1, \pm2, \ldots \tag{55}$$

Equations 50 and 53−55 constitute all the relationships that constrain the coefficients ($\mathbf{l}^h$,$\mathbf{r}^h$): these relationships allow the dimension of the tangent space, to which the directing vector $\mathrm{d}\mathbf{z}^{\mathbf{F}}/\mathrm{d}u|_{u=0}$ belongs, to be equal to n, the number of enzymes. Hence, n elements suffice to constitute a basis of this tangent space.

Equation 55 allows one to express the coefficients $\mathbf{l}^h$, $h \neq 0$, into $\mathbf{r}^h$, hence diminishing the number of unknown coefficients. Applying this to eq 49 and, in addition, substituting the Fourier coefficients of the elasticities (see eq 18) for the derivatives, $\partial\mathbf{v}^0/\partial\mathbf{x}^k$ yields

$$\mathbf{l}^0 = \Gamma_{\mathbf{e}}^{\mathbf{x},0}\cdot(\mathrm{diag}\ \mathbf{w}^0)^{-1}\cdot(\mathbf{r}^0 - \sum_{k=-\infty}^{\infty}\mathbf{D}^{-k}\cdot\mathbf{l}^k)$$

$$\omega h\mathbf{l}^h = -\mathbf{N}\cdot\mathrm{i}\mathbf{r}^h = \Gamma_{\mathbf{e}}^{\mathbf{x},h}\cdot(\mathrm{diag}\ \mathbf{w}^0)^{-1}\cdot(\mathbf{r}^0 + \mathbf{D}^0\cdot\mathbf{l}^0 + \sum_{\substack{k=-\infty\\k\neq0}}^{\infty}\mathbf{D}^{-k}\cdot\mathbf{N}\cdot\mathrm{i}\mathbf{r}^k/\omega k)\cdot\omega h, \quad h = \pm1, \pm2, \ldots$$

$$\mathbf{r}^h = \Gamma_{\mathbf{e}}^{\mathbf{v},h}\cdot(\mathrm{diag}\ \mathbf{w}^0)^{-1}\cdot(\mathbf{r}^0 + \mathbf{D}^0\cdot\mathbf{l}^0 + \sum_{k=-\infty}^{\infty}\mathbf{D}^{-k}\cdot\mathbf{N}\cdot\mathrm{i}\mathbf{r}^k/\omega k), \quad h = 0, \pm1, \pm2, \ldots \tag{56}$$

Together with the constraints on $\mathbf{r}^h$ (eqs 50 and 53−55), eq 56 determines the Fourier control coefficients and hence the periodic control coefficients in terms of the elasticity coefficients. Equations 56 are the ultimate analogues of eqs 28 and 29.

B.2. Expressing Control into Elasticity. To express control coefficients into elasticity coefficients, one might be tempted to follow the same route as for systems at steady state. Multiplying eq 47 from the left by $\mathbf{N}$, one obtains the analogue of eq 33, using also eqs 43, 44, and 55:

$$\mathbf{N}\cdot\sum_{k=-\infty}^{\infty}\mathbf{D}^{hk}\cdot\Gamma^{\mathbf{x},k}\cdot\mathbf{a} = -\mathbf{N}\cdot(\mathrm{diag}\ \mathbf{w}^h)\cdot\mathbf{a} + \mathrm{i}\omega h\mathbf{l}^h, \quad h = 0, \pm1, \pm2, \ldots$$

However, although eq 56 together with eqs 50 and 53−55 determines the periodic control coefficients, it is impossible to find an exact general solution because of the infinitely large number of the equations. In most actual cases, the wave forms of the oscillations are such that they can be approximated by a few terms of the Fourier series. Then the above equation system may be solved directly.[34] In the case of linear kinetics and linear response to a periodic external force, the equation contains a single $\mathbf{D}^{hh}$ term, so that, since $\mathbf{a}$ is arbitrary:

$$\Gamma_{\mathbf{e}}^{\mathbf{x},h} = -(\mathbf{N}\cdot\mathbf{D}^{hh} - \mathrm{i}\omega h\mathbf{I}_m)^{-1}\cdot\mathbf{N}\cdot(\mathrm{diag}\ \mathbf{w}^h)$$

i.e., the Fourier control coefficients relate to the elasticity coefficients in a form that is similar to that for the steady-state control coefficients (cf. eq 35). Note, however, that the higher than first order coefficients contain the phase shift term $\mathrm{i}\omega h$.

To solve the problem for the general case we shall return from the Fourier coefficients to the corresponding periodic functions. First, by reverse Fourier transformation, the infinite number of equations for Fourier coefficients of the control matrices (eq 56), will be rearranged into single equations for the control matrices as periodic functions of time. Multiplying both sides of eq 56 by $\exp(\mathrm{i}h\omega t)$, and summing over h from minus to plus infinity, yields

$$\mathbf{l}^0 - \sum_{h=-\infty,h\neq0}^{\infty}\mathbf{N}\cdot(\mathrm{i}\mathbf{r}^h/\omega h)\cdot\exp(\mathrm{i}h\omega t) = \Gamma_{\mathbf{e}}^{\mathbf{x}}(t)\cdot(\mathrm{diag}\ \mathbf{w}^0)^{-1}\cdot(\mathbf{r}^0 + \mathbf{D}^0\cdot\mathbf{l}^0 + \sum_{\substack{k=-\infty\\k\neq0}}^{\infty}\mathbf{D}^{-k}\cdot\mathbf{N}\cdot\mathrm{i}\mathbf{r}^k/\omega k) \tag{57}$$

$$\mathbf{r}(t) = \Gamma_{\mathbf{e}}^{\mathbf{v}}(t)\cdot(\mathrm{diag}\ \mathbf{w}^0)^{-1}\cdot(\mathbf{r}^0 + \mathbf{D}^0\cdot\mathbf{l}^0 + \sum_{\substack{k=-\infty\\k\neq0}}^{\infty}\mathbf{D}^{-k}\cdot\mathbf{N}\cdot\mathrm{i}\mathbf{r}^k/\omega k) \tag{58}$$

Equations 57 and 58 enable one to estimate the control matrices $\Gamma_{\mathbf{e}}^{\mathbf{x}}$ and $\Gamma_{\mathbf{e}}^{\mathbf{v}}$ if the sums of Fourier coefficients present in these equations can be estimated. The coefficients $\mathbf{r}^h$ relate to the periodic function $\mathbf{r}(t)$ defined by the reverse Fourier transformation:

$$\mathbf{r}(t,\mathbf{e}) = \sum_{h=-\infty}^{\infty}\mathbf{r}^h(\mathbf{e})\cdot\exp(\mathrm{i}h\omega t) \tag{59}$$

The real valued function $\mathbf{r}(t)$ has the operational meaning of the linear approximation of the differences between the fluxes at the two periodic trajectories that correspond to the (infinitesimally) different enzyme concentrations, $\mathbf{e}$ and $\mathbf{e} + \Delta\mathbf{e}$ (see eqs 20 and 43):

$$\mathbf{r}(t,\mathbf{e}) = \lim_{t\to0}[\mathbf{v}(t,\mathbf{x}^{\mathrm{per}}(t;\mathbf{e}+\Delta\mathbf{e}(u)),\mathbf{e}+\Delta\mathbf{e}(u)) - \mathbf{v}(t,\mathbf{x}^{\mathrm{per}}(t;\mathbf{e}),\mathbf{e})]/u$$

The term ωk entering the denominators of the Fourier coefficients in the sums of eqs 57 and 58 implies the integration of the corresponding functions. As to the sum in the left-hand side of eq 57 (see Appendix B),

$$\sum_{h=-\infty,h\neq0}^{\infty}\mathbf{N}\cdot(\mathrm{i}\mathbf{r}^h/\omega h)\cdot\exp(\mathrm{i}h\omega t) = -\mathbf{N}\cdot(\int_0^t\mathbf{r}(\tau,\mathbf{e})\,\mathrm{d}\tau + (1/T)\cdot\int_0^T\tau\cdot\mathbf{r}(\tau,\mathbf{e})\,\mathrm{d}\tau) \tag{60}$$

The sum in the right-hand sides of eqs 57 and 58 is related to both the functions $\mathbf{r}(t)$ and $\mathbf{D}(t)$. According to Appendix B,

$$\mathbf{D}^0\cdot\mathbf{l}^0 + \sum_{\substack{k=-\infty\\k\neq0}}^{\infty}\mathbf{D}^{-k}\cdot\mathbf{N}\cdot\mathrm{i}\mathbf{r}^k/\omega k = -(1/T)\cdot\int_0^T\mathbf{G}(\tau)\cdot\mathbf{N}\cdot\mathbf{r}(\tau)\,\mathrm{d}\tau + \mathbf{D}^0\cdot\mathbf{l}^0 \tag{61}$$

$\mathbf{G}(\tau)$ is determined by (nonnormalized) elasticity coefficients (eq 14) integrated over a remaining part of the periodic trajectory:

$$\mathbf{G}(\tau) = \tau\cdot\mathbf{D}^0 + \int_\tau^T \mathbf{D}(t)\,\mathrm{d}t, \quad \mathbf{D}^0 = (1/T)\cdot\int_0^T \mathbf{D}(t)\,\mathrm{d}t \tag{62}$$

Substituting expressions 60 and 61 for the sums in eqs 57 and 58:

$$\mathbf{l}^0 + \mathbf{N}\cdot\left(\int_0^t \mathbf{r}(\tau)\,\mathrm{d}\tau + (1/T)\cdot\int_0^T \tau\cdot\mathbf{r}(\tau)\,\mathrm{d}\tau\right) = \Gamma^{\mathbf{x}}_{\mathbf{e}}(t)\cdot (\mathrm{diag}\ \mathbf{w}^0)^{-1}\cdot\left(\mathbf{r}^0 - (1/T)\cdot\int_0^T \mathbf{G}(\tau)\cdot\mathbf{N}\cdot\mathbf{r}(\tau)\,\mathrm{d}\tau + \mathbf{D}^0\cdot\mathbf{l}^0\right) \tag{63}$$

$$\mathbf{r}(t) = \Gamma^{\mathbf{v}}_{\mathbf{e}}(t)\cdot(\mathrm{diag}\ \mathbf{w}^0)^{-1}\cdot\left(\mathbf{r}^0 - (1/T)\cdot\int_0^T \mathbf{G}(\tau)\cdot\mathbf{N}\cdot\mathbf{r}(\tau)\,\mathrm{d}\tau + \mathbf{D}^0\cdot\mathbf{l}^0\right) \tag{64}$$

To calculate the desired periodic control coefficients from eqs 63 and 64, it suffices to calculate the function $\mathbf{r}(t)$. However, eqs 50 and 53−55 constrain the Fourier coefficients $\mathbf{r}^h$. Indeed, in view of the infinitely large number of linked equations, it is not possible directly to evaluate $\mathbf{r}(t)$. To calculate $\mathbf{r}(t)$, Appendix C applies a similar strategy as was done above when rearranging eq 56 into eqs 63 and 64. An integral equation with respect to $\mathbf{r}(t)$ results (cf. eq C37).

There will be n such linearly independent functions $\mathbf{r}(t)$, which differ in terms of the contribution of the n independent components among the $m + n$ components of the vectors $\mathbf{l}^0$ and $\mathbf{r}^0$ (see eqs 53 and 54). This dependence may be explicited as $\mathbf{r}(t,\mathbf{l}^0,\mathbf{r}^0)$. The same n independent parameters enter (as $\mathbf{l}^0$ and $\mathbf{r}^0$) the eqs 63 and 64 that allow to find the $m \times n$ control coefficients with respect to metabolite concentrations and the $n \times n$ control coefficients with respect to fluxes.

B.3. Connectivity and Summation Theorems. A special choice of the vectors $\mathbf{l}^0$ and $\mathbf{r}^0$ (eqs 38 and 39 and eq 42) can again uncover laws that the control coefficients of oscillations must obey. Equation 54 shows that the vector $\mathbf{r}^0$ satisfies the same constraints as the steady-state fluxes do; i.e., it belongs to the kernel of $\mathbf{N}$. Since the dimension of that kernel is $n-m_0$ (m_0, the dimension of metabolite space visited by the system, is the rank of the $m \times n$ matrix $\mathbf{N}$), one can choose $n-m_0$ linearly independent vectors $\mathbf{r}^{0,k}$, $k = 1, ..., n-m_0$, as a basis for that kernel. Equation 53 shows that the components of the vector $\mathbf{l}^0$ are to be chosen in such a way that vectors $\mathbf{x}^0 + u\cdot\mathbf{l}^0$ continue to satisfy the moiety-conservation relationships of eq 51. Since the rank of the $m-m_0 \times m$ matrix $\mathbf{M}$ is $m-m_0$, the dimension of its kernel is m_0; i.e., one can choose m_0 linearly independent vectors $\mathbf{l}^{0,k}$, $k = 1, ..., m_0$, that satisfy eq 53.

The zero vector $\mathbf{l}^0 = 0$ satisfies the above requirement; it corresponds to a modulation d$\mathbf{e}$ such that the time averages of the metabolite concentrations are not affected by the modulation. (The interdependence of the $\mathbf{l}^h$ and $\mathbf{r}^h$ (eqs 55 and 56) disqualifies the condition that $\mathbf{l}^h = 0$ for all values of h. A modulation causing $\mathbf{l}^0 = 0$ will cause $\mathbf{l}^l$ to differ from zero. A modulation that keeps all frequency components in the concentration oscillations the same is only possible for systems with linear kinetics and linear response to periodic external force. Then summation and connectivity theorems identical to those for the steady state are obtained.) Employing $n-m_0$ linearly independent vectors $\mathbf{r}^{0,k}$, $k = 1, ..., n-m_0$, one obtains the additional $n-m_0$ linearly independent functions $\mathbf{r}(t,0,\mathbf{r}^{0,k})$ (the two last arguments specify a particular choice of the vectors $\mathbf{l}^0$ (equal to 0 here) and $\mathbf{r}^0$ in the integral equation determining the function $\mathbf{r}(t)$; see Appendix C). Introducing these $n-m_0$ linearly independent functions $\mathbf{r}(t,0,\mathbf{r}^{0,k})$ into eqs 63 and 64, one arrives at $n-m_0$ linearly independent relationships that play the role of generalized summation theorems for periodic control coefficients (cf. ref 29):

$$(\mathrm{diag}\ \mathbf{x}^{\mathrm{per}}(t))^{-1}\cdot\mathbf{N}\cdot\left(\int_0^t \mathbf{r}(\tau,0,\mathbf{r}^{0,k})\,\mathrm{d}\tau + (1/T)\cdot\int_0^T \tau\cdot\mathbf{r}(\tau,0,\mathbf{r}^{0,k})\,\mathrm{d}\tau\right) = \mathbf{C}^{\mathbf{x}}_{\mathbf{e}}(t)\cdot(\mathrm{diag}\ \mathbf{v}^0)^{-1}\cdot\left(\mathbf{r}^{0,k} - (1/T)\cdot\int_0^T \mathbf{G}(\tau)\cdot\mathbf{N}\cdot\mathbf{r}(\tau,0,\mathbf{r}^{0,k})\,\mathrm{d}\tau\right), \quad k = 1, ..., n-m_0$$

and $n-m_0$ summation theorems for each flux:

$$(\mathrm{diag}\ \mathbf{v}(t))^{-1}\cdot\mathbf{r}(t,0,\mathbf{r}^{0,k}) = \mathbf{C}^{\mathbf{v}}_{\mathbf{e}}(t)\cdot(\mathrm{diag}\ \mathbf{v}^0)^{-1}\cdot\left(\mathbf{r}^{0,k} - (1/T)\cdot\int_0^T \mathbf{G}(\tau)\cdot\mathbf{N}\cdot\mathbf{r}(\tau,0,\mathbf{r}^{0,k})\,\mathrm{d}\tau\right), \quad k = 1, ..., n-m_0 \tag{65}$$

Alternatively, one may consider a perturbation in which the average values are not affected. Taking the vector $\mathbf{r}^0 = 0$ and employing m_0 linearly independent vectors $\mathbf{l}^{0,k}$, $k = 1, ..., m_0$, one obtains m_0 linearly independent functions $\mathbf{r}(t,\mathbf{l}^{0,k},0)$. Using these m_0 linearly independent functions, i.e., $\mathbf{r}(t,\mathbf{l}^{0,k},0)$ in eqs 63 and 64, one arrives at m_0 linearly independent relationships that play the role of generalized connectivity theorems for periodic control coefficients (cf. ref 29):

$$(\mathrm{diag}\ \mathbf{x}^{\mathrm{per}}(t))^{-1}\cdot\left(\mathbf{l}^{0,k} + \mathbf{N}\cdot\left(\int_0^t \mathbf{r}(\tau,\mathbf{l}^{0,k},0)\,\mathrm{d}\tau + (1/T)\cdot\int_0^T \tau\cdot\mathbf{r}(\tau,\mathbf{l}^{0,k},0)\,\mathrm{d}\tau\right)\right) = \mathbf{C}^{\mathbf{x}}_{\mathbf{e}}(t)\cdot(\mathrm{diag}\ \mathbf{v}^0)^{-1}\cdot\left(\mathbf{D}^0\cdot\mathbf{l}^{0,k} - (1/T)\cdot\int_0^T \mathbf{G}(\tau)\cdot\mathbf{N}\cdot\mathbf{r}(\tau,\mathbf{l}^{0,k},0)\,\mathrm{d}\tau\right)$$

$$(\mathrm{diag}\ \mathbf{v}(t))\cdot\mathbf{r}(t,\mathbf{l}^{0,k},0) = \mathbf{C}^{\mathbf{v}}_{\mathbf{e}}(t)\cdot(\mathrm{diag}\ \mathbf{v}^0)^{-1}\cdot\left(\mathbf{D}^0\cdot\mathbf{l}^{0,k} - (1/T)\cdot\int_0^T \mathbf{G}(\tau)\cdot\mathbf{N}\cdot\mathbf{r}(\tau,\mathbf{l}^{0,k},0)\,\mathrm{d}\tau\right), \quad k = 1, ..., m_0 \tag{66}$$

The generalized summation and connectivity theorems for forced oscillations obtained here (eqs 65 and 66) make it possible to calculate the periodic control coefficients (global regulatory indicators) in terms of the periodic elasticity coefficients quantifying enzyme regulation. For instance, the mn control coefficients that constitute the matrix $\mathbf{C}^{\mathbf{x}}_{\mathbf{e}}(t)$ satisfy the $m(n-m_0)$ summation relationships (eq 65) and the mm_0 connectivity relationships (eq 66), i.e., mn linearly independent equations in total [cf. refs 30, 38, and 39].

B.4. Connectivity and Summation Theorems for Control of the Averages of Fluxes and Concentrations. Connectivity and summation theorems simplify greatly when they refer to the control of oscillating concentrations and fluxes, averaged over the entire period. For nonnormalized derivatives (see eq 15) the control coefficients over the average values coincide with the Fourier control coefficients of zero order of the control matrices, i.e., with the average of the control coefficients $\Gamma^{\mathbf{x}}_{\mathbf{e}}(t)$ and $\Gamma^{\mathbf{v}}_{\mathbf{e}}(t)$. However, for scaled derivatives, i.e., for the control matrices $\mathbf{C}^{\mathbf{x}}_{\mathbf{e}}(t)$ and $\mathbf{C}^{\mathbf{v}}_{\mathbf{e}}(t)$, the corresponding time averages of the Fourier coefficients differ from the control coefficients over the averages. Equation 67 shows that the normalized control coefficients over the average values ($\mathbf{C}^{\bar{\mathbf{v}}}_{\mathbf{e}}$, $\mathbf{C}^{\bar{\mathbf{x}}}_{\mathbf{e}}$) are identical to the control coefficients with respect to the Fourier coefficients of zero order for metabolite concentrations ($\mathbf{x}^0$) and fluxes ($\mathbf{v}^0$) (see eq 7):

$$(\mathbf{C}^{\bar{\mathbf{v}}}_{\mathbf{e}})_{ij} = \mathrm{d}\ln\bar{v}_i(\mathbf{e})/\partial\ln e_j = (\mathbf{C}^{\bar{\mathbf{v}}^0}_{\mathbf{e}})_{ij} \quad i,j = 1, ..., n, \quad \bar{\mathbf{x}} = \mathbf{x}^0$$

$$(\mathbf{C}^{\bar{\mathbf{x}}}_{\mathbf{e}})_{lj} = \mathrm{d}\ln\bar{x}_l(\mathbf{e})/\partial\ln e_j = (\mathbf{C}^{\bar{\mathbf{x}}^0}_{\mathbf{e}})_{ij} \quad j = 1, ..., n, \quad l = 1, ..., m \qquad \bar{\mathbf{v}} = \mathbf{v}^0 \tag{67}$$

Using eq 65, the summation theorems for the control coefficients over the average rates and concentrations $\mathbf{C}^{\bar{\mathbf{v}}}_{\mathbf{e}}$ and

$\mathbf{C}_{\mathbf{e}}^{\bar{\mathbf{x}}}$ are obtained:

$$0 = \mathbf{C}_{\mathbf{e}}^{\bar{\mathbf{x}}}\cdot(\mathrm{diag}\ \mathbf{v}^0)^{-1}\cdot(\mathbf{r}^{0,k} - (1/T)\cdot\int_0^T \mathbf{G}(\tau)\cdot\mathbf{N}\cdot\mathbf{r}(\tau,0,\mathbf{r}^{0,k})\ \mathrm{d}\tau)$$

$$(\mathrm{diag}\ \mathbf{v}^0)^{-1}\cdot\mathbf{r}^{0,k} = \mathbf{C}_{\mathbf{e}}^{\bar{\mathbf{v}}}\cdot(\mathrm{diag}\ \mathbf{v}^0)^{-1}\cdot(\mathbf{r}^{0,k} - (1/T)\cdot\int_0^T \mathbf{G}(\tau)\cdot \mathbf{N}\cdot\mathbf{r}(\tau,0,\mathbf{r}^{0,k})\ \mathrm{d}\tau), \quad k = 1, ..., n-m_0 \quad (68)$$

Similarly, eq 66 leads to the connectivity theorems for control coefficients over the average values:

$$(\mathrm{diag}\ \mathbf{x}^0)^{-1}\cdot\mathbf{l}^{0,k} = \mathbf{C}_{\mathbf{e}}^{\bar{\mathbf{x}}}\cdot(\mathrm{diag}\ \mathbf{v}^0)^{-1}\cdot(\mathbf{D}^0\cdot\mathbf{l}^{0,k} - (1/T)\cdot\int_0^T \mathbf{G}(\tau)\cdot\mathbf{N}\cdot\mathbf{r}(\tau,\mathbf{l}^{0,k},0)\ \mathrm{d}\tau)$$

$$0 = \mathbf{C}_{\mathbf{e}}^{\bar{\mathbf{v}}}\cdot(\mathrm{diag}\ \mathbf{v}^0)^{-1}(\mathbf{D}^0\cdot\mathbf{l}^{0,k} - (1/T)\cdot\int_0^T \mathbf{G}(\tau)\cdot\mathbf{N}\cdot\mathbf{r}(\tau,\mathbf{l}^{0,k},0)\ \mathrm{d}\tau), \quad k = 1, ..., m_0 \quad (69)$$

Differences with steady state are limited to the integral term in the right-hand sides of eqs 68 and 69. In the cases when the function $\mathbf{G}(t)$ is constant (i.e., independent of τ), the corresponding integrals equal zero as one can factor out the function $\mathbf{G}$ (and stoichiometry matrix $\mathbf{N}$) from the integral. Using eq 53 yields

$$\int_0^T \mathbf{G}(\tau)\cdot\mathbf{N}\cdot\mathbf{r}(\tau)\ \mathrm{d}\tau = \mathbf{G}\cdot\mathbf{N}\cdot\int_0^T \mathbf{r}(\tau)\ \mathrm{d}\tau = T\cdot\mathbf{G}\cdot\mathbf{N}\cdot\mathbf{r}^0 = 0 \quad (70)$$

Differentiating eq 62 with respect to τ one may see that the derivative of $\mathbf{G}$ equals zero; hence, $\mathbf{G}$ is constant if and only if $\mathbf{D}(\tau)$ is independent of time:

$$\mathbf{D}(\tau) = \partial\mathbf{v}/\partial\mathbf{x} = \mathbf{D}^0$$

i.e., if (i) all rate equations are linear in terms of all metabolite concentrations or (ii) the amplitude of the forced oscillation is so small that the rate equations can effectively be approximated by such linear rate equations. In case of nonlinearity in the dependence of the reaction rates on the metabolite concentrations and a substantial amplitude of the oscillations in the external force, the integral in eqs 68 and 69 is (usually) not equal to zero. Then the normalized control coefficients on average values do not obey the classical summation and connectivity theorems.

B.5. Summation Theorems for Control over Wave Form. If, unlike above, the driving oscillation is modulated simultaneously, simpler theorems result. Since the reactions rates depend linearly on the enzyme concentrations (activities), the following transformation of these concentrations, of the time and of the frequency of a periodic external force, leads to a new equation system which coincides with the initial system after eliminating the superscript (*):

$$e_i^* = \lambda\cdot e_i, \quad t^* = t/\lambda, \quad \omega^* = \lambda\cdot\omega \quad (71)$$

Therefore, if the initial conditions are the same, metabolite concentrations of the transformed system at the moment t/λ will coincide with concentrations of the initial system at the moment t, whereas the fluxes will increase by factor λ (proportional to the new enzyme activities):

$$x_i(t/\lambda,\lambda\cdot\mathbf{e},\lambda\cdot\omega) = x_i(t,\mathbf{e},\omega)$$

$$v_j(t/\lambda,\lambda\cdot\mathbf{e},\lambda\cdot\omega) = \lambda\cdot v(t,\mathbf{e},\omega) \quad (72)$$

Using eqs 72 and eq 7 yields

$$x_i^h(\lambda\cdot\mathbf{e},\lambda\cdot\omega) = x_i^h(\mathbf{e},\omega)$$

$$v_j^h(\lambda\cdot\mathbf{e},\lambda\cdot\omega) = \lambda\cdot v_j^h(\mathbf{e},\omega) \quad (73)$$

Applying to eq 73 Euler's theorem on homogeneous functions, one arrives at the following summation theorems:

$$\sum_j C_j^{x^h} + C_\omega^{x^h} = 0, \quad \sum_j C_j^{v^h} + C_\omega^{v^h} = 1 \quad (74)$$

It follows from eq 74 that the sum of the enzyme control coefficients over any Fourier coefficient of any concentration or rate equals 0 or 1 minus the corresponding control by the external driving oscillator, respectively.

Equation 74 enables us to calculate Fourier control coefficients with respect to frequency via those with respect to enzyme concentrations. The simplest way to demonstrate this is to consider a linear response system. Assuming $\mathbf{r}^{0,k}$ equal to $\mathbf{v}^0$ and employing eqs 65 and 70,

$$(\mathrm{diag}\ \mathbf{v}(t))^{-1}\cdot\mathbf{r}(t,0,\mathbf{v}^0) = \mathbf{C}_{\mathbf{e}}^{\mathbf{x}}(t)\cdot\mathbf{1}$$

Using this equation, one obtains

$$C_\omega^{v^h} = \mathbf{1} - \sum_j C_j^{v^h} = \mathbf{1} - \mathbf{C}_{\mathbf{e}}^{\mathbf{x}}(t)\cdot\mathbf{1} = \mathbf{1} - (\mathrm{diag}\ \mathbf{v}^h)^{-1}\cdot\int_0^T \mathbf{r}(t,0,\mathbf{v}^0)\cdot\exp(-\mathrm{i}\omega h t)\ \mathrm{d}t$$

where $\mathbf{1} = [1, ..., 1]^{\mathrm{T}}$ is the unit vector of length n.

C. Example

In Scheme 1, S is the initial substrate, X is the pathway

SCHEME 1

$$\mathrm{S} \xrightarrow{\mathbf{1}} \mathrm{X} \xrightarrow{\mathbf{2}} \mathrm{P}$$

intermediate, and P is the product. Reaction 1 is supposed to follow linear kinetics, whereas reaction 2 is assumed to be saturable:

$$v_1 = e_1\cdot(k_1\cdot S - k_{-1}\cdot x), \quad v_2 = e_2 k_{22} x/[(k_{-21} + k_{22})/k_{21} + x] \quad (75)$$

e_1 and e_2 are the concentrations of enzymes **1** and **2**, and $k_{\pm1}$, $k_{\pm21}$, and k_{22} are the kinetic constants. When the substrate and product concentrations are maintained constant (S is independent of what happens further down in the pathway, $P = P_0$), there is a single stable steady state (Appendix D). The control coefficients quantifying the control by either enzyme over the steady-state flux (J_0) are inversely proportional to the elasticities of these enzymes with respect to the intermediate X:[25]

$$C_1^{J_0} = -D_2^0/(D_1^0 - D_2^0), \quad C_2^{J_0} = D_1^0/(D_1^0 - D_2^0) \quad (76)$$

Here D_1^0 and D_2^0 are the (nonnormalized) elasticity coefficients, defined as

$$D_1^0 = (\partial v_1/\partial X)| \quad D_2^0 = (\partial v_2/\partial X)|$$
$$S = S_0\cdot(1 + \alpha\cdot\sin(\omega t)) \quad (77)$$

Here $\omega = 2\pi/T$ is the radial frequency, T is the period, and $\alpha < 1$ is the amplitude of oscillation. In this case, the fluxes

through the first and the second reaction will differ and oscillate with the frequency ω and the amplitudes A_1 and A_2. We shall focus on the control exerted by pathway enzymes on the amplitudes of oscillation of fluxes through either reaction,

$$C_{e_j}^{A_i} = \frac{d \ln A_i}{d \ln e_j}, \quad i = 1, 2; \quad j = 1, 2 \tag{78}$$

To calculate these control coefficients using the summation and connectivity theorems derived above, one needs to know the periodic solution to the kinetic equation determining how the intermediate concentration X changes with time. This kinetic equation reads:

$$dx/dt = e_1 \cdot [k_1 S_0 \cdot (1 + \alpha \sin(\omega t)) - k_{-1} x] - e_2 k_{22} x / [(k_{-21} + k_{22})/k_{21} + x] \tag{79}$$

To simplify the solutions of eq 79, we shall make three assumptions: (a) the amplitude of substrate oscillation is small as compared to the average concentration, $\alpha \ll 1$; (b) $d = k_{21}/(S_0 k_{22}) \ll 1$; (c) the ratios of the enzyme concentrations e_2/e_1 and the rate constants k_1/k_{-1}, k_1/k_{21}, and $k_{21}S_0/k_{-21}$ are all of order 1. Appendix D shows that the assumptions a, b, and c allow one to approximate the (complex) nonlinearity in the rate v_2 on the periodic trajectory by a second order polynomial with respect to x, neglecting the terms of higher than first order with respect to d. Calculating the elasticity coefficients on the periodic solution and substituting them in the summation and connectivity theorems (see Appendix E):

$$C_1^{A_1} = \frac{C_1^{J_0} + \nu^2}{1 + \nu^2}, \quad C_2^{A_1} = \frac{C_2^{J_0} + (\tilde{D}_1/D_2^*)\nu^2}{(1 + \nu^2)\{1 + (\tilde{D}_2/D_2^*)^2 (\nu/C_1^{J_0})^2\}},$$
$$C_1^{A_2} = \frac{C_1^{J_0} + (\tilde{D}_2/D_2^*)\nu^2}{1 + \nu^2}, \quad C_2^{A_2} = \frac{C_2^{J_0} + \nu^2}{1 + \nu^2} \tag{80}$$

Here, $C_i^{J_0}$, $i = 1, 2$, are the enzyme control coefficients over the pathway flux (J^0) at *the steady state*; $\tilde{D}_i$ are the (approximate) expressions for the elasticity coefficients (D_i^0) at *the steady state*, determined with an accuracy of the linear terms with respect to α and d; and ν is the reduced frequency $\nu = \omega/(\tilde{D}_2 - \tilde{D}_1)$,

$$\tilde{D}_1 = -k_{-1} e_1, \quad \tilde{D}_2 = D_2^* \cdot (1 - d \cdot \sigma)$$

$$D_2^* = k_{21} e_2, \quad \sigma = k_{-21}/(k_{21} S_0) + 2 k_1 e_1/(k_{-1} e_1 + k_{21} e_2) \tag{81}$$

Equation 80 shows that, at very low ν, control approaches the control over the pathway flux at the steady state:

$$C_1^{A_1} = C_1^{A_2} \simeq C_1^{J_0}, C_2^{A_1} = C_2^{A_2} \simeq C_2^{J_0} \tag{82}$$

At very high ν, the control coefficients differ dramatically from the corresponding values at the steady state:

$$C_1^{A_1} \simeq 1, \quad C_2^{A_1} \simeq 0, \quad C_1^{A_2} \simeq \tilde{D}_2/D_2^* \simeq 1, \quad C_2^{A_2} \simeq 1 \tag{83}$$

Figure 1 illustrates the dependence of the control coefficients on the oscillation frequency. Interestingly, at high frequencies, the total control exerted by the enzymes on the amplitude of the flux oscillation through the second reaction is as high as 2.

Having estimated the enzyme control coefficients (eq 80), one can estimate the control coefficients ($C_\omega^{A_i}$) with respect to the frequency ω (using eq 17 of ref 15):

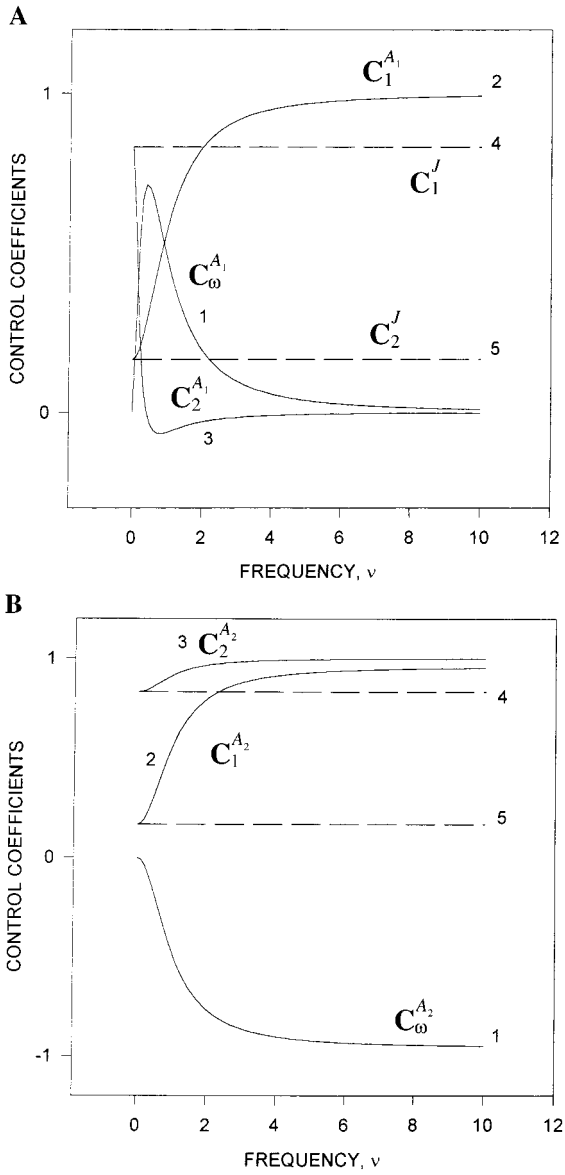

Figure 1. Comparison of the flux−amplitude control coefficients with steady-state control coefficients for the linear pathway depicted in Scheme 1. (A) The curves 1, 2, and 3 show the frequency dependencies of the control coefficients over the amplitude A_1 of the periodic flux J_1 with respect to frequency of external periodic force and concentrations of enzymes **1** and **2**, respectively. The dashed curves 4 and 5 are the steady-state control coefficients with respect to enzymes **1** and **2**, respectively. (B) Curves 1, 2, and 3 are the frequency dependencies of control coefficients over the amplitude A_2 of the periodic flux J_2 with respect to frequency of external periodic force and concentrations of enzymes **1** and **2**, respectively. The dashed curves 4 and 5 are steady-state control coefficients with respect to enzymes **1** and **2**, respectively, as calculated for Scheme 1 and equations given in the text. The magnitudes of the rate constants were $k_1 = 35$, $k_{21} = 10$, $k_{-21} = 10$, $k_{22} = 30$, $S_0 = 20$, $e_1 = 0.1$, and $e_2 = 0.05$.

$$C_\omega^{A_1} = 1 - (C_1^{A_1} + C_2^{A_1}) = \frac{\nu^2 \{[C_2^{J_0}/(C_1^{J_0})^2](\tilde{D}_2/D_2^*)^2 + \tilde{D}_1/D_2^*\}}{(1 + \nu^2)\{1 + (\tilde{D}_2/D_2^*)^2 \cdot (\nu/C_1^{J_0})^2\}}$$

$$C_\omega^{A_2} = 1 - (C_1^{A_2} + C_2^{A_2}) = \frac{\nu^2 \cdot (\tilde{D}_2/D_2^*)}{(1 + \nu^2)} \tag{84}$$

It follows from eqs 82 and 83 that at very low frequencies the

control coefficients with respect to the frequency ($C_\omega^{A_1}$ and $C_\omega^{A_2}$) are close to 0. At very high frequencies the control coefficient $C_\omega^{A_1}$ returns to 0, whereas $C_\omega^{A_2}$ is close to -1. This is confirmed by the simulations in Figure 1a and b.

A metabolic pathway of Scheme 1, but with linear reaction rates, was analyzed in our previous paper.[15] We shall now compare the regulation of a periodic oscillation in the pathway with linear kinetics to the corresponding pathway with nonlinear kinetics at equal elasticity coefficients (D_i^0), and hence, at equal control coefficients at steady state. We shall indicate by left-hand superscript "*l*" the control coefficients over the amplitudes of oscillation in the system with linear reaction rates. Using Appendix C of ref 15 yields

$$ {}^{l}C_1^{A_1} = {}^{l}C_1^{A_2} = \frac{C_1^{J_0} + \nu^2}{1 + \nu^2}, {}^{l}C_2^{A_1} = \frac{C_2^{J_0} + (D_1^0/D_2^0)\cdot\nu^2}{(1 + \nu^2)\cdot\{1 + (\nu/C_1^{J_0})^2\}} \quad (85) $$

$$ {}^{l}C_2^{A_2} = \frac{C_2^{J_0} + \nu^2}{1 + \nu^2} $$

Here $\nu = \omega/(D_2^0 - D_1^0)$ is the reduced frequency (cf. eq 84). Comparing eqs 80 and 85 one can see that the approximate magnitudes of the control coefficients in the nonlinear system differ from those in the linear case by the terms depending on the frequency of oscillation. At low frequencies these differences tend to zero, whereas at high frequencies they approach σd. For instance, for the control exerted by enzyme **1** (with the linear kinetics) and enzyme **2** (with nonlinear kinetics) on the amplitudes A_2 and A_1, respectively, one has (neglecting the terms of higher orders with respect to d):

$$ C_1^{A_2} = {}^{l}C_1^{A_2} - d\cdot\frac{\sigma\nu^2}{1 + \nu^2} $$

$$ C_2^{A_1} = {}^{l}C_2^{A_1} + d\cdot\frac{\nu^2\sigma[\nu^2 + 2(D_2^0/D_1^0)C_2^{J_0} - (C_1^{J_0})^2]}{[\nu^2 + (C_1^{J_0})^2][\nu^2 + (D_2^0/D_1^0)C_2^{J_0}]} $$

D. Discussion

Some important biological processes are essentially time-dependent.[3,12] The more convincing examples are the cell cycle,[18] Ca^{2+} oscillation,[41] and synchronizing metabolic oscillations.[11,42] Because of the absence of a steady state, the existing MCA could not be applied to those systems. Numerical integration and stability analysis were the remaining options, but these are not directed at understanding how the processes are controlled. Attempts to develop MCA for the analysis of the control of the time dependence of metabolite concentrations and fluxes have been made.[15,31,32] In ref 15 it was shown that it was impossible to analyze the control of time dependence of metabolic concentrations and fluxes in an autonomously oscillating system in terms of well-defined control coefficients, because the latter tended to infinity as time proceeds (see also ref 16). On the other hand, for the case of forced oscillations, time-dependent control coefficients were defined for both periodic and transient states of the system. The so-called "transient control coefficients" quantify the control over a transition from the initial periodic trajectory to the trajectory by an enzyme concentration. "Periodic control coefficients" refer to the dependence of the stationary time-dependent oscillation on the concentration of an enzyme. It was proven that the transient control coefficients tended to the periodic control coefficients as time tended to infinity. Similar relationships between transient and stationary control coefficients had been discovered by Heinrich and Reder for the case of the steady state as stable attractor (ref 43, see also ref 6). A method was developed to calculate both steady-state and transient control coefficients from elasticity coefficients.[15]

In the present paper we extended MCA to systems subject to forced oscillations. For the purpose of quantitatively describing the properties of individual enzyme reactions we introduced (periodic) elasticity coefficients that were defined as derivatives of reaction rates with respect to metabolite concentrations on the periodic trajectory under the influence of a periodic force. Extending the "perturbation method",[29] we derived the complete system of relationships linking (periodic) control coefficients to (periodic) elasticity coefficients. The relationships were similar to the summation and connectivity theorems which have been obtained for systems at steady state.[28,29]

The control of systems at steady state is described by MCA in terms of the sensitivities of the steady-state concentrations and fluxes to changes in enzyme activities. The information present in stationary oscillations is richer in principle; it refers to the complete time dependence of all fluxes and concentrations during each period of the oscillation. In principle, the control of this time dependence can be defined in terms of the response of concentrations and fluxes at any phase of the oscillations to changes in enzyme activities. However, that control may be described more efficiently in terms of the dependence of the wave form of the oscillations on enzyme activities. In the present manuscript we have developed the latter approach, i.e., the periodic control coefficients were considered as Fourier series. Each coefficient of that series refers to the control of an aspect of the wave form of the oscillations by an enzyme in the system. This Fourier type of metabolic control analysis was elaborated to the same level that has been achieved for metabolic control analysis of the steady state, i.e., summation laws and connectivity laws were derived and the Fourier control coefficients were expressed in terms of the nonlinear kinetic properties of the enzymes in the system. It was found that by including the Fourier control coefficient with respect to external frequency the sum of the *h*th-order Fourier control coefficients over concentration and reaction rates are equal to 0 and 1, respectively. Importantly, Larter et al.[16] have defined periodic response coefficients, but these were focused on limit cycles, lacked our focus on control by the originators of biochemical activity, i.e., the enzymes, and did not lead to the discovery of summation and connectivity laws. Also, control coefficients over average values of fluxes and concentrations were introduced. The summation and connectivity theorems for periodic control coefficients derived here were employed to obtain similar theorems but for control coefficients over average values of fluxes and concentrations. These theorems appeared to be quite similar to those for steady state.

In a simple example, the control coefficients over amplitudes and flux oscillations with respect to identical enzymes coincided with one another and with flux control coefficients with respect to the same enzymes at steady state. For larger frequencies the control coefficients deviated strongly from the corresponding control coefficients in the steady-state system.

In earlier work, we showed that enzymes that are subject to oscillating external influences can transduce aspects of this oscillation to their average flux.[4,7,44] In the context of the present paper the nonlinear elasticity coefficients D_k^0 should be expected to differ from zero for most enzyme-catalyzed reactions. Indeed, the very fact that most biological reactions are catalyzed brings them into the nonlinear realm. Accordingly, whenever

external oscillations occur in biochemical systems, the control analysis derived here should become relevant. In principle, the analysis presented here should also be applicable to nonlinear dielectric spectroscopy of biological material, in as far at the latter aims at life processes. In this sense, the present paper extends an analysis focusing on single enzyme kinetics.[22]

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Appendix A

Expressing the Derivatives $\partial \mathbf{v}^0/\partial \mathbf{x}^k$ and $\partial \mathbf{v}^h/\partial \mathbf{x}^0$ in Terms of the Fourier Coefficients $\mathbf{D}^h$ of the Elasticity Matrix. We express variation of the turnover numbers of the enzymes $\mathbf{w}(t,\mathbf{x})$ as the metabolite concentrations undergo their oscillations into Fourier coefficients $\mathbf{w}^h$:

$$\mathbf{w}(t,\mathbf{x}^{\mathrm{per}}) = \sum_{h=-\infty}^{\infty} \mathbf{w}^h \cdot \exp(\mathrm{i}h\omega t) \tag{A1}$$

When the vector of enzyme concentrations $\mathbf{e}$ is modulated by a perturbation $\Delta\mathbf{e}$, as defined by eq 19 of the main text, the function $\mathbf{w}(t,\mathbf{x}^{\mathrm{per}})$ and, consequently, its Fourier coefficients $\mathbf{w}^h$ depend on the extent of perturbation u. Differentiating eq A1 with respect to the perturbation parameter u at $u = 0$ and taking spillover between the various Fourier frequencies into account through eq 46 yields

$$\mathrm{d}\mathbf{w}(t,\mathbf{x}^{\mathrm{per}})/\mathrm{d}u|_{u=0} = \sum_{h=-\infty}^{\infty} \sum_{k=-\infty}^{\infty} (\partial \mathbf{w}^h/\partial \mathbf{x}^k) \cdot \mathbf{l}^k \cdot \exp(\mathrm{i}h\omega t) \tag{A2}$$

$$\mathbf{l}^k = \mathrm{d}\mathbf{x}^k/\mathrm{d}u|_{u=0} \tag{A3}$$

Using eqs 8 and A2 yields

$$\mathrm{d}\mathbf{w}(t,\mathbf{x}^{\mathrm{per}})/\mathrm{d}u|_{u=0} = (\mathrm{diag}\ \mathbf{e})^{-1} \cdot \sum_{h=-\infty}^{\infty} \sum_{k=-\infty}^{\infty} (\partial \mathbf{v}^h/\partial \mathbf{x}^k) \cdot \mathbf{l}^k \cdot \exp(\mathrm{i}h\omega t) \tag{A4}$$

Let us present the derivative $\mathrm{d}\mathbf{w}(t,\mathbf{x}^{\mathrm{per}})/\mathrm{d}u$ as the derivative of a composite function:

$$\mathrm{d}\mathbf{w}(t,\mathbf{x})/\mathrm{d}u|_{u=0} = (\partial \mathbf{w}/\partial \mathbf{x}) \cdot \mathrm{d}\mathbf{x}/\mathrm{d}u|_{u=0} = (\mathrm{diag}\ \mathbf{e})^{-1} \cdot \mathbf{D}(t) \cdot \mathrm{d}\mathbf{x}/\mathrm{d}u|_{u=0} \tag{A5}$$

Using eq 6 of the main text and eq A3 yields

$$\mathrm{d}\mathbf{x}/\mathrm{d}u|_{u=0} = \sum_{h=-\infty}^{\infty} \mathbf{l}^h \cdot \exp(\mathrm{i}h\omega t) \tag{A6}$$

Supplementing eq 17 of the main text as well as eq A6 into eq A5 yields

$$\mathrm{d}\mathbf{w}(t,\mathbf{x})/\mathrm{d}u|_{u=0} = (\mathrm{diag}\ \mathbf{e})^{-1} \cdot \sum_{h=-\infty}^{\infty} \mathbf{D}^h \cdot \exp(\mathrm{i}h\omega t) \cdot \sum_{k=-\infty}^{\infty} \mathbf{l}^k \cdot \exp(\mathrm{i}k\omega t) = (\mathrm{diag}\ \mathbf{e})^{-1} \cdot \sum_{h=-\infty}^{\infty} \sum_{k=-\infty}^{\infty} \mathbf{D}^{h-k} \cdot \mathbf{l}^k \cdot \exp(\mathrm{i}h\omega t) \tag{A7}$$

Equations A4 and A7 present two expressions for the same derivative $\mathrm{d}\mathbf{w}(t,\mathbf{x}^{\mathrm{per}})/\mathrm{d}u|_{u=0}$. They include the coefficients $\partial \mathbf{v}^h/\partial \mathbf{x}^k$ and the Fourier coefficients of the elasticity matrix $\mathbf{D}^h$, respectively. Since the vectors $\mathbf{l}^k$ are entirely determined by the vector $\mathbf{l}^0$, their components can be chosen arbitrarily (see section 2.1). As in section 2.4 one may chose modulations that do not affect the average concentrations or rates. By equating the terms with the same h and k in the right-hand sides of eqs A4 and A7 one then obtains eq 18 of the main text.

Appendix B

Toward Equations 60 and 61. The function $\mathbf{N}\cdot\mathbf{r}(t)$ can be written as (cf. eq 59, main text):

$$\mathbf{N}\cdot\mathbf{r}(t) = \sum_{h=-\infty}^{\infty} \mathbf{N}\cdot\mathbf{r}^h \cdot \exp(\mathrm{i}h\omega t) \tag{B1}$$

Integrating eq B1 from 0 to t, using eq 55 of the main text, taking into account that, in accordance with eq 53 $\mathbf{N}\cdot\mathbf{r}^0 = 0$, yields

$$\sum_{h=-\infty,h\neq 0}^{\infty} \mathbf{N}\cdot(\mathrm{i}\mathbf{r}^h/\omega h)\cdot\exp(\mathrm{i}h\omega t) = -\mathbf{N}\cdot\int_0^t \mathbf{r}(\xi)\mathrm{d}\xi + \mathbf{N}\cdot \sum_{h=-\infty,h\neq 0}^{\infty} (\mathrm{i}\mathbf{r}^h/\omega h) \tag{B2}$$

the latter term stemming from the boundary value at $t = 0$.

The left-hand side of eq B2 equals that of eq 60 of the main text. Using an explicit expression for the Fourier coefficients, $\mathbf{r}^h$ (the reverse Fourier transformation), the sum in the right-hand side of eq B2 can be presented as

$$\sum_{h=-\infty,h\neq 0}^{\infty} \mathbf{N}\cdot(\mathrm{i}\mathbf{r}^h/\omega h) = (\mathrm{i}/\omega\sqrt{T})\mathbf{N}\cdot\int_0^T \mathbf{r}(\xi)\cdot[\sum_{h=-\infty,h\neq 0}^{\infty} \exp(-\mathrm{i}h\omega\xi)/h]\mathrm{d}\xi \tag{B3}$$

Expanding the function $\ln(1-1/z)$ in powers of $1/z$ and the function $\ln(1-z)$ in powers of z in the complex plane with $z = \exp(-\mathrm{i}\omega t)$ and subtracting the latter from the former, we obtain

$$\sum_{h=-\infty,h\neq 0}^{\infty} \exp(-\mathrm{i}h\omega\xi)/h = \mathrm{i}\cdot(\omega\xi + \pi) \tag{B4}$$

Substituting eq B4 into eq B3 and then into eq B2, one arrives at eq 60 of the main text.

In order to check eq 61 one integrates over time from 0 to T the product of Fourier series of function $\mathbf{D}(t)$ (see eq 17 of the main text) by that of function $\mathbf{N}\cdot\int_0^t \mathbf{r}(\xi)\mathrm{d}\xi$ (see eq B2). Using the fact that functions $\exp(\mathrm{i}h\omega t)$ are periodic so that their integral over period T equals zero and taking into account eqs B3 and B4, one obtains

$$\int_0^T \mathbf{D}(t)\cdot\mathbf{N}\cdot\int_0^t \mathbf{r}(\xi)\mathrm{d}\xi\mathrm{d}t = -T\cdot\sum_{k=-\infty\ k\neq 0}^{\infty} \bar{\mathbf{D}}^k\cdot\mathbf{N}\cdot(\mathrm{i}\mathbf{r}^k/\omega k) - \mathbf{D}^0\cdot\mathbf{N}\cdot\int_0^T \xi\cdot\mathbf{r}(\xi)\mathrm{d}\xi\mathrm{d}t$$

Rearranging this equation and introducing the function $\mathbf{G}(\xi)$, completely determined by the elasticity matrix $\mathbf{D}(t)$, one obtains

$$\mathbf{G}(\xi) = \xi\cdot\mathbf{D}^0 + \int_\xi^T \mathbf{D}(t)\mathrm{d}t, \quad \mathbf{D}^0 = (1/T)\cdot\int_0^T \mathbf{D}(t)\mathrm{d}t$$

and one arrives at the required result:

$$\sum_{k=-\infty}^{\infty} \mathbf{D}^{-k}\cdot\mathbf{N}\cdot i\mathbf{r}^k/\omega k = -(1/T)\cdot\int_0^T \mathbf{G}(\xi)\cdot\mathbf{N}\cdot\mathbf{r}(\xi)d\xi + \mathbf{D}^0\cdot\mathbf{l}^0$$

Appendix C

r(*t*). Transforming eq 27 to an integral equation, using eqs 18, 50, and 55, yields

$$(\text{diag } \mathbf{w}^h)\cdot\mathbf{r}^0 - (\text{diag } \mathbf{w}^0)\cdot\mathbf{r}^h + [(\text{diag } \mathbf{w}^0)\cdot\mathbf{D}^h - (\text{diag } \mathbf{w}^h)\cdot\mathbf{D}^0]\cdot\mathbf{l}^0 - (\text{diag } \mathbf{w}^0)\cdot\sum_{k=-\infty\, k\neq 0}^{\infty}(\partial\mathbf{v}^h/\partial\mathbf{x}^k)\cdot\mathbf{N}\cdot(i\cdot\mathbf{r}^k/\omega\cdot k) + (\text{diag } \mathbf{w}^h)\cdot\sum_{k=-\infty\, k\neq 0}^{\infty}\mathbf{D}^{-h}\cdot\mathbf{N}\cdot(i\cdot\mathbf{r}^k/\omega\cdot k) = 0, \quad h = 0, \pm1, \pm2, \ldots \quad \text{(C1)}$$

Multiplying eq C1 by $\exp(ih\omega t)\cdot(\text{diag } \mathbf{e})$, and summing over h from $-\infty$ to $+\infty$, yields

$$\sum^1(t) + \sum^2(t) - \sum^3(t) + \sum^4(t) = 0 \quad \text{(C2)}$$

$\Sigma^1(t)$, $\Sigma^2(t)$, $\Sigma^3(t)$, $\Sigma^4(t)$ represent

$$\sum^1(t) = \sum_{h=-\infty}^{\infty}((\text{diag } \mathbf{v}^h)\cdot\mathbf{r}^0 - (\text{diag } \mathbf{v}^0)\cdot\mathbf{r}^h)\exp(ih\omega t) \quad \text{(C3)}$$

$$\sum^2(t) = \sum_{h=-\infty}^{\infty}((\text{diag } \mathbf{v}^0)\cdot\mathbf{D}^h - (\text{diag } \mathbf{v}^h)\cdot\mathbf{D}^0)\cdot\mathbf{l}^0\exp(ih\omega t) \quad \text{(C4)}$$

$$\sum^3(t) = (\text{diag } \mathbf{v}^0)\cdot\sum_{h=-\infty}^{\infty}\sum_{k=-\infty\, k\neq 0}^{\infty}(d\mathbf{v}^h/d\mathbf{x}^k)\cdot\mathbf{N}\cdot(i\mathbf{r}^k/\omega\cdot k)\exp(ih\omega t) \quad \text{(C5)}$$

$$\sum^4(t) = \sum_{h=-\infty}^{\infty}(\text{diag } \mathbf{v}^h)\sum_{k=-\infty\, k\neq 0}^{\infty}\mathbf{D}^{-k}\cdot\mathbf{N}\cdot(i\mathbf{r}^k/\omega\cdot k)\exp(ih\omega t) \quad \text{(C6)}$$

Using the Fourier expansions of the vectors $\mathbf{v}(t)$ $\mathbf{r}(t)$ and the matrix of elasticity coefficients $\mathbf{D}(t)$ (see eqs 6, 17, 59 of the main text), eqs C3 and C4 can be written as

$$\sum^1(t) = (\text{diag } \mathbf{v}(t))\cdot\mathbf{r}^0 - (\text{diag } \mathbf{v}^0)\cdot\mathbf{r}(t) \quad \text{(C7)}$$

$$\sum^2(t) = ((\text{diag } \mathbf{v}^0)\cdot\mathbf{D}(t) - (\text{diag } \mathbf{v}(t))\cdot\mathbf{D}^0)\cdot\mathbf{l}^0 \quad \text{(C8)}$$

Using eq A2 for the derivative $d\mathbf{w}(t,\mathbf{x}^{per})/du|_{u=0}$ (see Appendix A), eqs 8 and 55 of the main text, expression C5 can be rearranged:

$$-\sum^3(t) = (\text{diag } \mathbf{v}^0)\cdot[d\mathbf{v}(t,\mathbf{x})/du|_{u=0} - \sum_{h=-\infty}^{\infty}\mathbf{D}^h\cdot\mathbf{l}^0\cdot\exp(ih\omega t)] = (\text{diag } \mathbf{v}^0)\cdot[d\mathbf{v}(t,\mathbf{x})/du|_{u=0} - \mathbf{D}(t)\cdot\mathbf{l}^0] \quad \text{(C9)}$$

Using eq A5 (see Appendix A) and taking into account eq 4 of the main text that relates function $\mathbf{w}(t,\mathbf{x})$ to vector of periodic fluxes, $\mathbf{v}(t,\mathbf{x})$, one finds for the first term on the right-hand side of the equation:

$$d\mathbf{v}(t,\mathbf{x})/du|_{u=0} = \mathbf{D}(t)\cdot d\mathbf{x}/du|_{u=0} \quad \text{(C10)}$$

Here, vector $d\mathbf{x}/du|_{u=0}$ can be presented as Fourier series with coefficients equal to $\mathbf{l}^k$ (see eq A3 of Appendix A):

$$d\mathbf{x}/du|_{u=0} = \sum_{k=-\infty}^{\infty}\mathbf{l}^k\cdot\exp(ik\omega t) \quad \text{(C11)}$$

Substituting expressions $-\mathbf{N}\cdot(i\mathbf{r}^k/\omega k)$ for $\mathbf{l}^k$ in eq C11 (see eq 55 of the main text) and using eq 60 yields

$$d\mathbf{x}/du|_{u=0} - \mathbf{l}^0 = \mathbf{N}\cdot(\int_0^t\mathbf{r}(\xi)d\xi + (1/T)\cdot\int_0^T\xi\cdot\mathbf{r}(\xi)d\xi) \quad \text{(C12)}$$

Combining eqs C9, C10, and C12 yields

$$\sum^3(t) = -(\text{diag } \mathbf{v}^0)\cdot\mathbf{D}(t)\cdot\mathbf{N}\cdot(\int_0^t\mathbf{r}(\xi)d\xi + (1/T)\cdot\int_0^T\xi\cdot\mathbf{r}(\xi)d\xi) \quad \text{(C13)}$$

Using eq 61 of the main text also, the sum $\Sigma^4(t)$ can be transformed to an integral form:

$$\sum^4(t) = -(1/T)\cdot(\text{diag } \mathbf{v}(t))\cdot\int_0^T\mathbf{G}(\xi)\cdot\mathbf{N}\cdot\mathbf{r}(\xi)d\xi \quad \text{(C14)}$$

Substituting eqs C7, C8, C13, and C14 into eq C2, one arrives at an integral equation for $\mathbf{r}(t)$:

$$\mathbf{r}(t) + \int_0^T\mathbf{P}(t,\xi)\cdot\mathbf{N}\cdot\mathbf{r}(\xi)d\xi - \int_0^t\mathbf{D}(t)\cdot\mathbf{N}\cdot\mathbf{r}(\xi)d\xi = \mathbf{q}(t) \quad \text{(C15)}$$

$$\mathbf{P}(t,\xi) = (1/T)(-\mathbf{Q}(t)\cdot\xi + \Phi(t)\cdot\int_\xi^T\mathbf{D}(\eta)d\eta)$$

$$\mathbf{Q}(t) = \mathbf{D}(t) - \Phi(t)\cdot\mathbf{D}^0$$

$$\mathbf{q}(t) = \Phi(t)\cdot\mathbf{r}^0 + \mathbf{Q}(t)\cdot\mathbf{l}^0$$

$$\Phi(t) = (\text{diag } \mathbf{v}(t))\cdot(\text{diag } \mathbf{v}^0)^{-1}$$

To find a (unique) solution of the integral equation 15, the first m_0 rows and the first m_0 column of the stoichiometric matrix are selected to be linearly independent and matrix $\mathbf{N}$ to be a block matrix

$$\mathbf{N} = \begin{pmatrix}\mathbf{N}_{m_0}^{m_0} & \mathbf{N}_{m_0}^{n-m_0}\\ \mathbf{N}_{n-m_0}^{m_0} & \mathbf{N}_{n-m_0}^{n-m_0}\end{pmatrix}, \quad \det\mathbf{N}_{m_0}^{m_0} \neq 0$$

Here $\mathbf{N}_{m_0}^{m_0}$ is an $m_0 \times m_0$ matrix, $\mathbf{N}_{m_0}^{n-m_0}$ is an $m_0 \times (n-m_0)$ matrix, $\mathbf{N}_{n-m_0}^{m}$ is an $(n-m_0) \times m_0$ matrix, and $\mathbf{N}_{n-m_0}^{n-m_0}$ is an $(n-m_0) \times (n-m_0)$ matrix. We introduce a function, $\mathbf{z}(t)$, which relates to the function $\mathbf{r}(t)$ as

$$\mathbf{r}(t) = \mathbf{U}\cdot\mathbf{z}(t) \quad \text{(C16)}$$

Here $\mathbf{U}$ is the nonsingular $n \times n$ matrix that is constructed by partitioning the stoichiometric matrix $\mathbf{N}$ (see above):

$$\mathbf{U} = \begin{pmatrix}\mathbf{I}_{m_0} & -(\mathbf{N}_{m_0}^{m_0})^{-1}\cdot\mathbf{N}_{m_0}^{n-m_0}\\ 0 & \mathbf{I}_{n-m_0}\end{pmatrix} \quad \text{(C17)}$$

Substituting the $\mathbf{U}\cdot\mathbf{z}(t)$ of eq C16 for $\mathbf{r}(t)$ into eq C15 yields

$$\mathbf{U}\cdot\mathbf{z}(t) + \int_0^T \mathbf{P}(t,\xi)\cdot\mathbf{N}\cdot\mathbf{U}\cdot\mathbf{z}(\xi)\mathrm{d}\xi - \int_0^t (t)\cdot\mathbf{N}\cdot\mathbf{U}\cdot\mathbf{z}(\xi)\mathrm{d}\xi = \mathbf{q}(t) \quad \text{(C18)}$$

$\mathbf{z}$ may be decomposed to the vectors $\mathbf{z}^{m_0}$ and $\mathbf{z}^{n-m_0}$:

$$\mathbf{z} = [\mathbf{z}^{m_0},\mathbf{z}^{n-m_0}]^{\mathrm{T}}, \mathbf{z}^{m_0} = [z_1,...,z_{m_0}]^{\mathrm{T}}, z^{n-m_0} = [z_1,...,z_{n-m_0}]^{\mathrm{T}} \quad \text{(C19)}$$

Substituting eqs C19 and C17 into C16 yields

$$\mathbf{N}\cdot\mathbf{U}\cdot\mathbf{z}(t) = \mathbf{N}^{m_0}\cdot\mathbf{z}^{m_0}(t) \quad \text{(C20)}$$

Here, $\mathbf{N}^{m_0}$ is the matrix consisting of the m_0 first columns of the stoichiometric matrix $\mathbf{N}$. Premultiplying eq C18 by matrix $\mathbf{U}^{-1}$ and using eqs C19 and C20:

$$\mathbf{z}^{m_0} + \gamma\cdot\int_0^T \mathbf{P}(t,\xi)\cdot\mathbf{N}^{m_0}\cdot\mathbf{z}^{m_0}(\xi)\mathrm{d}\xi - \gamma\cdot\int_0^t \mathbf{D}(t)\cdot\mathbf{N}^{m_0}\cdot\mathbf{z}^{m_0}(\xi)\mathrm{d}\xi = \gamma\cdot\mathbf{q}(t) \quad \text{(C21)}$$

$$\mathbf{z}^{n-m_0} + \alpha\cdot\int_0^T \mathbf{P}(t,\xi)\cdot\mathbf{N}^{m_0}\cdot\mathbf{z}^{m_0}(\xi)\mathrm{d}\xi - \alpha\cdot\int_0^t \mathbf{D}(t)\cdot\mathbf{N}^{m_0}\cdot\mathbf{z}^{m_0}(\xi)\mathrm{d}\xi = \alpha\cdot\mathbf{q}(t) \quad \text{(C22)}$$

Here, matrices γ and α consist of the first m_0 and the last $n-m_0$ rows of the matrix $\mathbf{U}^{-1}$, respectively,

$$\mathbf{U}^{-1} = \begin{pmatrix} \mathbf{I}_{m_0} & (\mathbf{N}_{m_0}^{m_0})^{-1}\cdot\mathbf{N}_{m_0}^{n-m_0} \\ 0 & \mathbf{I}_{n-m_0} \end{pmatrix} = \begin{pmatrix} \gamma \\ \alpha \end{pmatrix}, \quad \gamma = (\mathbf{I}_{m_0}|\mathbf{R}),\ \alpha = (0|\mathbf{I}_{n-m_0}), \quad \mathbf{R} = (\mathbf{N}_{m_0}^{m_0})^{-1}\cdot\mathbf{N}_{m_0}^{n-m_0} \quad \text{(C23)}$$

It follows from eqs C21 and C22 that to find the vector $\mathbf{z}(t)$ only the vector $\mathbf{z}^{m_0}(t)$ should be estimated (the vector $\mathbf{z}^{n-m_0}(t)$ is calculated directly via $\mathbf{z}^{m_0}(t)$ using eq C22). The integrals in eq C21 are taken from 0 to T (the first integral) and from 0 to t (the second integral). Therefore, eq C21 is a compound integral equation involving equations of Volterra and Fredholm types. Accordingly, solving of the equation is divided into two steps: (i) rearrangement of the original equation to an integrodifferential one and (ii) a transformation of the integrodifferential equation into an integral equation of the Fredholm type that can be solved analytically. After the following change of variables,

$$\mathbf{z}^{m_0}(t) = \mathrm{d}\mathbf{y}(t)/\mathrm{d}t, \quad \mathbf{y}(t) = \int_0^t \mathbf{z}^{m_0}(\xi)\mathrm{d}\xi \quad \text{(C24)}$$

the integrals in eq C21 take the form,

$$\gamma\cdot\int_0^t \mathbf{D}(t)\cdot\mathbf{N}^{m_0}\cdot\mathbf{z}^{m_0}(\xi)\mathrm{d}\xi = \gamma\cdot\mathbf{D}(t)\cdot\mathbf{N}^{m_0}\cdot\mathbf{y}(t),$$

$$\gamma\cdot\int_0^T \mathbf{P}(t,\xi)\cdot\mathbf{N}^{m_0}\cdot\mathbf{z}^{m_0}(\xi)\mathrm{d}\xi = \gamma\cdot\int_0^t \mathbf{P}(t,\xi)\cdot\mathbf{N}^{m_0}\cdot\mathrm{d}\mathbf{y}(\xi) = \gamma\cdot\mathbf{P}(t,T)\cdot\mathbf{N}^{m_0}\cdot\mathbf{y}(T) + \gamma\cdot\int_0^T \mathbf{F}(t,\xi)\cdot\mathbf{N}^{m_0}\cdot\mathbf{y}(\xi)\mathrm{d}\xi \quad \text{(C25)}$$

Here, $\mathbf{y}(t)$ is vector of the length m_0. The matrix $\mathbf{F}(t,\xi)$ is determined as follows:

$$\mathbf{F}(t,\xi) = -\mathrm{d}\mathbf{P}(t,\xi)/\mathrm{d}\xi = (1/T)(\mathbf{Q}(t) + \Phi(t)\cdot\mathbf{D}(\xi)) \quad \text{(C26)}$$

We shall now show that $\mathbf{y}(T) = 0$. From eq C24 it follows that

$$\mathbf{y}(T) = \int_0^T \mathbf{z}^{m_0}(\xi)\mathrm{d}\xi \quad \text{(C27)}$$

Representing $\mathbf{z}^{m_0}(t)$ via the function $\mathbf{r}(t)$ by using eqs C16 and C23 and inserting the derived expression into relationship C27:

$$\mathbf{y}(t) = \gamma\cdot\int_0^T \mathbf{r}(\xi)\mathrm{d}\xi = T\cdot\gamma\cdot\mathbf{r}^0$$

In view of eq30 vector $\mathbf{r}^0$ belongs to kernel of matrix $\mathbf{N}$. Let us prove that kernel of matrix γ coincides with that of the stoichiometry matrix. Indeed, by multiplying the matrix γ by the nonsingular square matrix $\mathbf{N}_{m_0}^{m_0}$ on the left, one obtains:

$$\mathbf{N}_{m_0}^{m_0}\cdot\gamma = (\mathbf{N}_{m_0}^{m_0}|\mathbf{N}_{m_0}^{n-m_0}) \quad \text{(C28)}$$

By virtue of the partitioning of matrix $\mathbf{N}$, the matrix C28 consists of the first m_0 rows of the stoichiometry matrix, which are linearly independent. Because of nonsingularity of the matrix $\mathbf{N}_{m_0}^{m_0}$, the kernel of the matrix $\mathbf{N}_{m_0}^{m_0}\cdot\gamma$ coincides with that of matrix γ. Consequently, the kernel of matrix $\mathbf{N}$ coincides with that of matrix γ. This implies that

$$\mathbf{y}(T) = \gamma\cdot\int_0^T \mathbf{r}(\xi)\mathrm{d}\xi = T\cdot\gamma\cdot\mathbf{r}^0 = 0 \quad \text{(C29)}$$

Applying eqs C24−C26 and C28 to eq C21, one obtains an integrodifferential equation with respect to the function $\mathbf{y}(t)$:

$$\mathrm{d}\mathbf{y}(t)/\mathrm{d}t = \gamma\cdot\mathbf{D}(t)\cdot\mathbf{N}^{m_0}\cdot\mathbf{y}(t) + \gamma\cdot(\mathbf{q}(t) - \int_0^T \mathbf{F}(t,\xi)\cdot\mathbf{N}^{m_0}\cdot\mathbf{y}(\xi)\mathrm{d}\xi) \quad \text{(C30)}$$

From eq C24 the initial conditions for this equation can be derived:

$$\mathbf{y}(0) = 0 \quad \text{(C31)}$$

Equation 30 can be considered as a linear differential equation with the nonhomogeneity equal to

$$\gamma\cdot(\mathbf{q}(t) - \int_0^T \mathbf{F}(t,\xi)\cdot\mathbf{N}^{m_0}\cdot\mathbf{y}(\xi)\mathrm{d}\xi)$$

In all nonpathological cases, the $m_0 \times m_0$ matrix $\gamma\cdot\mathbf{D}(t)\cdot\mathbf{N}^{m_0}$ is nonsingular. Therefore, a solution of eq 30 with the initial condition 31 can be written as

$$\mathbf{y}(t) = \mathbf{H}(t)\cdot\int_0^t \mathbf{H}(\eta)^{-1}\cdot\gamma\cdot(\mathbf{q}(\eta) - \int_0^T \mathbf{F}(\eta,\xi)\cdot\mathbf{N}^{m_0}\cdot\mathbf{y}(\xi)\mathrm{d}\xi)\mathrm{d}\eta \quad \text{(C32)}$$

Here, the $m_0 \times m_0$ matrix $\mathbf{H}(t)$ is the solution of the homogeneous system of equations:

$$\mathrm{d}\mathbf{H}/\mathrm{d}t = \gamma\cdot\mathbf{D}(t)\cdot\mathbf{N}^{m_0}\cdot\mathbf{H}, \quad \mathbf{H}(0) = \mathbf{I}_{m_0}$$

By reversing the order of the integrals in eq 32 and defining the following functions:

$$\mathbf{S}_1(t) = (1/T)\cdot\mathbf{H}(t)\cdot\int_0^t \mathbf{H}(\eta)^{-1}\cdot\gamma\cdot\mathbf{Q}(\eta)\mathrm{d}\eta$$

$$\mathbf{S}_2(t) = (1/T)\cdot\mathbf{H}(t)\cdot\int_0^t \mathbf{H}(\eta)^{-1}\cdot\gamma\cdot\Phi(\eta)\mathrm{d}\eta$$

one obtains an integral equation of the Fredholm type with respect to the function $\mathbf{y}(t)$

$$\mathbf{y}(t) + \int_0^T \mathbf{W}(t,\xi)\cdot\mathbf{y}(\xi)\mathrm{d}\xi = \mathbf{h}(t) \tag{C33}$$

$$\mathbf{W}(t,\xi) = \mathbf{S}_1(t)\cdot\mathbf{N}^{m_0} + \mathbf{S}_2(t)\cdot\mathbf{D}(\xi)\cdot\mathbf{N}^{m_0}$$

$$\mathbf{h}(t) = \mathbf{H}(t)\cdot\int_0^t \mathbf{H}(\eta)^{-1}\cdot\gamma\cdot\mathbf{q}(\eta)\mathrm{d}\eta$$

Since the function $\mathbf{W}(t,\xi)$, entering the integrand expression, is the sum of two terms that are products of functions of ξ and t only, the solution of eq C33 can be presented in the following form:

$$\mathbf{y}(t) = \mathbf{S}_1(t)\cdot\mathbf{b}^1 + \mathbf{S}_2(t)\cdot\mathbf{b}^2 \tag{C34}$$

Vector $\mathbf{b}^1$ of length m and vector $\mathbf{b}^2$ of length n can be found by substitution of eq C34 into eq C33. Indeed, by equating the terms involving the functions $\mathbf{S}_1(t)$ and $\mathbf{S}_2(t)$ at both sides of the equation, one obtains a linear equation system with respect to the vectors $\mathbf{b}^1$ and $\mathbf{b}^2$:

$$\begin{pmatrix} \mathbf{I}_m + (1/T)\cdot\mathbf{N}^{m_0}\cdot\mathbf{A}_1 & (1/T)\cdot\mathbf{N}^{m_0}\cdot\mathbf{A}_2 \\ (1/T)\cdot\mathbf{B}_1 & \mathbf{I}_n + \mathbf{B}_2\cdot(1/T) \end{pmatrix}\cdot\begin{pmatrix} \mathbf{b}^1 \\ \mathbf{b}^2 \end{pmatrix} = \begin{pmatrix} \mathbf{l}^0 \\ \mathbf{r}^0 \end{pmatrix}$$

Here, the matrices $\mathbf{A}_i$ and $\mathbf{B}_i$, $i = 1$ and 2, are determined in the following manner:

$$\mathbf{A}_1 = \int_0^T \mathbf{H}(t)\cdot\int_0^t \mathbf{H}^{-1}(\xi)\cdot\gamma\cdot\mathbf{D}(\xi)\mathrm{d}\xi\mathrm{d}t - \mathbf{A}_2\cdot\mathbf{D}^0$$

$$\mathbf{A}_2 = \int_0^T \mathbf{H}(t)\cdot\int_0^t \mathbf{H}^{-1}(\xi)\cdot\gamma\cdot\Phi(\xi)\mathrm{d}\xi\mathrm{d}t$$

$$\mathbf{B}_1 = \int_0^T \mathbf{D}(t)\cdot\mathbf{N}^{\mathrm{r}}\cdot\mathbf{H}(t)\cdot\int_0^t \mathbf{H}^{-1}(\xi)\cdot\gamma\cdot\mathbf{D}(\xi)\mathrm{d}\xi\mathrm{d}t - \mathbf{B}_2\cdot\mathbf{D}^0$$

$$\mathbf{B}_2 = \int_0^T \mathbf{D}(t)\cdot\mathbf{N}^{\mathrm{r}}\cdot\mathbf{H}(t)\cdot\int_0^t \mathbf{H}^{-1}(\xi)\cdot\gamma\cdot\Phi(\xi)\mathrm{d}\xi\mathrm{d}t$$

Using eq C24 to express the function $\mathbf{z}^{m_0}(t)$ via the function $\mathbf{y}(t)$:

$$\mathbf{z}^{m_0}(t) = \gamma\cdot\mathbf{D}(t)\cdot\mathbf{N}^{m_0}\cdot\mathbf{H}(t)\cdot\int_0^t \mathbf{H}^{-1}(\xi)\cdot\gamma\cdot\mathbf{d}(\xi)\mathrm{d}\xi + \gamma\cdot\mathbf{d}(t) \tag{C35}$$

$$\mathbf{d}(t) = (1/T)\cdot\mathbf{Q}(t)\cdot\mathbf{b}^1 + \Phi(t)\cdot\mathbf{b}^2$$

Substituting eq C35 into eq C22, rearranging and collecting corresponding terms, one arrives at the following expressions for the function $\mathbf{z}^{n-m_0}(t)$

$$\mathbf{z}^{n-m_0}(t) = \alpha\cdot\mathbf{D}(t)\cdot\mathbf{N}^{m_0}\cdot\mathbf{H}(t)\cdot\int_0^t \mathbf{H}^{-1}(\xi)\cdot\gamma\cdot\mathbf{d}(\xi)\mathrm{d}\xi + \alpha\cdot\mathbf{d}(t) \tag{C36}$$

By substituting eqs C35 and C36 into eq C16, one arrives at the final expression of the function $\mathbf{r}(t)$ in terms of the elasticity matrix $\mathbf{D}$:

$$\mathbf{r}(t) = \mathbf{D}(t)\cdot\mathbf{N}^{m_0}\cdot\mathbf{H}(t)\cdot\int_0^t \mathbf{H}^{-1}(\xi)\cdot\gamma\cdot\mathbf{d}(\xi)\mathrm{d}\xi + \mathbf{d}(t) \tag{C37}$$

Appendix D

An Approximate Periodic Solution to Equation 75. Equating the rates v_1 and v_2 at a steady state yields

$$x_0 = (1/2e_1k_{-1}k_{21})\cdot\{-(e_2k_{22}k_{21} + e_1k_{-1}\cdot(k_{-21} + k_{22}) - e_1k_1k_{21}S_0) + [(e_2k_{22}k_{21} + e_1k_{-1}\cdot(k_{-21} + k_{22}) - e_1k_1k_{21}S_0)^2 + 4e_1^2k_{-1}k_1(k_{-21} + k_{22})S_0]^{1/2}\} \tag{D1}$$

Correspondingly, the elasticity coefficients at the steady state read:

$$D_1^0 = -e_1k_{-1},$$
$$D_2^0 = e_2k_{22}\cdot[(k_{-21} + k_{22})/k_{21}]/[(k_{-21} + k_{22})/k_{21} + x^0] \tag{D2}$$

Multiplying eq 79 by $1/(e_1k_1S_0)$, and using the following designations for dimensionless variables and the combinations of parameters, yields

$$y = x/S_0, \quad \tau = e_1k_1t, \quad K = k_{-1}/k_1, \quad V = e_2k_{21}/(e_1k_1),$$
$$b = k_{-21}/(k_{21}S_0), \quad \Omega = \omega/(e_1k_1), \quad d = k_{21}S_0/k_{22} \tag{D3}$$

and one arrives at

$$\mathrm{d}y/\mathrm{d}\tau = 1 + \alpha\sin(\Omega\tau) - Ky - Vy/[1 + d(b + y)] \tag{D4}$$

The (complex) nonlinearity of Michaelis−Menten type in eq D4 can be expressed as a Taylor series provided that $d(b + y) < 1$. Due to the assumed small amplitude (α) of the oscillation (see the assumption a in the main text), the concentration x is of the same order of magnitude as x_0, and therefore, y is of the same order of magnitude as that of the steady-state value y_0. $b + y_0$ is of the order of 1, if the ratios of the enzyme concentrations e_1/e_2 and the rate constants k_1/k_{-1}, k_1/k_{21}, and $k_{21}S_0/k_{-21}$ are of the order of 1 (see the assumption c in the main text). Assuming that (see the assumption b in the main text)

$$d \ll 1 \tag{D5}$$

one can expand the right-hand side of eq D4, neglecting the terms of the second and higher order with respect to α and $d(b + y_0)$:

$$\mathrm{d}y/\mathrm{d}\tau = 1 + \alpha\sin(\Omega\tau) - (K + V)y + dVy(b + y) + \ldots \tag{D6}$$

A periodic solution of eq D6 can be expanded into the Taylor series,

$$y(\tau) = y_0(\tau) + \alpha\cdot y_1(\tau) + d\cdot y_2(\tau) + \ldots \tag{D7}$$

Substituting eq D7 in eq D6 and equating the terms of the same order with respect to α and d, respectively, one obtains a set of differential equations (to explain more why the derivatives of the term with a small parameter (e.g., y_2) cannot be so high as the term of the order of y_1):

$$\mathrm{d}y_0/\mathrm{d}\tau = 1 - (K + V)y_0, \quad \mathrm{d}y_1/\mathrm{d}\tau = -(K + V)y_1 + \sin(\Omega\tau)$$
$$\mathrm{d}y_2/\mathrm{d}\tau = -(K + V)y_2 + V(b + y_0)y_0, \ldots \tag{D8}$$

As all the equations in eq D8 are linear, the solution can be found in terms of the same harmonics that constitute an external force:

$$y_0(\tau) = y_0 = 1/(K + V), \quad y_1(\tau) =$$
$$[(K + V)\cdot\sin(\Omega\tau) - \Omega\cdot\cos(\Omega\tau)]/[(K + V)^2 + \Omega^2]$$
$$y_2(\tau) = y_2 = V[b(K + V) + 1]/(K + V)^3 \tag{D9}$$

Substituting eq D9 in eq D7 and returning to dimensional variables (see eq D3) one obtains the following approximation for x, where the terms of the second and higher orders with respect to α and d are neglected:

$$x(t) \simeq S_0 e_1 k_1/(k_{-1}e_1 + k_{21}e_2) + d\cdot[e_1 e_2 k_{21} k_1/(k_{-1}e_1 + k_{21}e_2)^2]$$
$$[k_{-21}/k_{21} + S_0 e_1 k_1/(k_{-1}e_1 + k_{21}e_2)] + \alpha\cdot S_0 e_1 k_1\cdot[(k_{-1}e_1 + k_{21}e_2)^2 + \omega^2]^{-1/2}\cdot\sin(\omega t - \varphi)$$
$$\varphi = \arcsin\{e_1 k_1 \omega/[(k_{-1}e_1 + k_{21}e_2)^2 + \omega^2]\} \quad \text{(D10)}$$

Correspondingly, the approximation for the reaction rates on the periodic solution reads:

$$v_i(t) \simeq v_0 + v_i^s\cdot\sin(\omega t) + v_i^c\cdot\cos(\omega t), \quad i = 1, 2 \quad \text{(D11)}$$

$$v_0 = S_0 k_1 e_1 D_2^*/(D_2^* - D_1)\cdot\{1 + d[D_1/(D_2^* - D_1)]\cdot[k_{-21}/(k_{21}S_0) + k_1 e_1/(k_{-1}e_1 + k_{21}e_2)]$$
$$v_1^s = v_2^s = \alpha S_0 k_1 e_1\cdot[D_2^*(D_2^* - D_1) + \omega^2]/[(D_2^* - D_1)^2 + \omega^2]$$
$$v_1^c = -\alpha S_0 k_1 e_1 D_1 \omega/[(D_2^* - D_1)^2 + \omega^2], \quad v_2^c = v_1^c(D_2^*/D_1)$$

The amplitudes of the periodic fluxes are given by

$$A_i^2 = (v_i^s)^2 + (v_i^c)^2 \quad \text{(D12)}$$

The elasticity coefficients are calculated using eq 16

$$D_1 = -k_{-1}e_1,$$
$$D_2 \simeq D_2^*\{1 - d[k_{-21}/(k_{21}S_0) + 2k_1 e_1/(k_{-1}e_1 + k_{21}e_2)]\}$$
$$D_2^* = k_{21}e_2 \quad \text{(D13)}$$

Appendix E

Control Coefficients over the Oscillation Amplitudes. We shall neglect terms of higher than the first order with respect to d and α. Using eqs D11 and D12, one obtains

$$A_i^2 = 2\cdot[(1/T)\int_0^T v_i^2(t)\mathrm{d}t - v_0^2], \quad i = 1, 2 \quad \text{(E1)}$$

Differentiating eq E1 with respect to e_j, $j = 1$ and 2, one expresses (nonnormalized) amplitude control coefficients, $\Gamma_{e_j}^{A_i} = \mathrm{d}A_i/\mathrm{d}e_j$ through (nonnormalized) periodic control coefficients $\Gamma_{e_j}^{v_i}(t)$ as follows:

$$A_i\Gamma_{e_j}^{A_i} = 2[(1/T)\int_0^T v_i(t)\Gamma_{e_j}^{v_i}(t)\mathrm{d}t - v_0\Gamma_{e_j}^{v_0}], \quad i, j = 1, 2 \quad \text{(E2)}$$

Here, v_0 is the flux at the steady state and $\Gamma_{e_j}^{v_0}$ is the control coefficient at steady state. Introducing the following designations:

$$\mathrm{K}_{e_j}^{v_i} = (1/T)\int_0^T v_i(t)\cdot\Gamma_{e_j}^{v_i}(t)\mathrm{d}t, \quad \mathrm{K}_{\mathbf{e}}^{\mathbf{v}} = (\mathrm{K}_{e_j}^{v_i})\begin{matrix} i = 1, 2 \\ j = 1, 2 \end{matrix}$$
$$\mathbf{A} = [A_1, A_2]^{\mathrm{T}}, \quad \Gamma_{\mathbf{e}}^{\mathbf{A}} = (\Gamma_{e_j}^{A_i})\begin{matrix} i = 1, 2 \\ j = 1, 2 \end{matrix} \quad \text{(E3)}$$

one rewrites eq E2 in matrix form as follows:

$$(\mathrm{diag}\ \mathbf{A})\cdot\Gamma_{\mathbf{e}}^{\mathbf{A}} = 2\cdot(\mathrm{K}_{\mathbf{e}}^{\mathbf{A}} - v_0\cdot\Gamma_{\mathbf{e}}^{v_0}) \quad \text{(E4)}$$

Using eqs E2−E4, the summation and connectivity theorems for control coefficients over amplitudes will now be derived from the corresponding theorems for periodic control coefficients (see eqs 65 and 66 of the main text). Since linear approximations of the elasticities do not depend on time (see eq D13), in eqs 65 and 66 the integral term equals zero with an accuracy of the linear terms. Then, eqs 65 and 66 can be simplified as

$$\mathbf{r}(t,0,\mathbf{r}^{0,k}) = \Gamma_{\mathbf{e}}^{\mathbf{v}}(t)\cdot(\mathrm{diag}\ \mathbf{w}^0)^{-1}\cdot\mathbf{r}^{0,k}, \quad k = 1, ..., n - m_0 \quad \text{(E5)}$$

$$\mathbf{r}(t,\mathbf{l}^{0,k},0) = \Gamma_{\mathbf{e}}^{\mathbf{v}}(t)\cdot(\mathrm{diag}\ \mathbf{w}^0)^{-1}\cdot\mathbf{D}\cdot\mathbf{l}^{0,k}, \quad k = 1, ..., m_0 \quad \text{(E6)}$$

Premultiplying these equations by the diagonal matrix $(1/T)\cdot(\mathrm{diag}\ \mathbf{v}(t))$ and integrating from time 0 to T, using eq C37:

$$v_0\cdot\mathbf{r}^{0,k} + (1/2v_0)\cdot\mathbf{B}\cdot\mathbf{r}^{0,k} = \mathrm{K}_{\mathbf{e}}^{\mathbf{v}}\cdot(\mathrm{diag}\ \mathbf{w}^0)^{-1}\cdot\mathbf{r}^{0,k}, \quad k = 1, ..., n - m_0 \quad \text{(E7)}$$

$$(1/2v_0)\cdot\mathbf{B}\cdot\mathbf{D}\cdot\mathbf{l}^{0,k} = \mathrm{K}_{\mathbf{e}}^{\mathbf{v}}\cdot(\mathrm{diag}\ \mathbf{w}^0)^{-1}\cdot\mathbf{D}\cdot\mathbf{l}^{0,k}, \quad k = 1, ..., m_0 \quad \text{(E8)}$$

$$\mathbf{B} = (\mathrm{diag}\ \mathbf{v}^s)\cdot P\cdot(\mathrm{diag}\ \mathbf{v}^s) + (\mathrm{diag}\ \mathbf{v}^c)\cdot P\cdot(\mathrm{diag}\ \mathbf{v}^c) + (1/\omega)\cdot[(\mathrm{diag}\ \mathbf{v}^s)\cdot P\cdot\mathbf{D}\cdot\mathbf{N}\cdot(\mathrm{diag}\ \mathbf{v}^s) + (\mathrm{diag}\ \mathbf{v}^c)\cdot P\cdot\mathbf{D}\cdot\mathbf{N}\cdot(\mathrm{diag}\ \mathbf{v}^c)],$$
$$P = \omega^2\cdot[\omega^2\cdot\mathrm{I}_2 + (\mathbf{D}\cdot\mathbf{N})^2]^{-1} \quad \text{(E9)}$$

Steady-state control coefficients $\Gamma_{\mathbf{e}}^{v_0}$ satisfy the following summation and connectivity theorems (see ref 35):

$$\mathbf{r}^{0,k} = \Gamma_{\mathbf{e}}^{v_0}\cdot(\mathrm{diag}\ \mathbf{w}^0)^{-1}\cdot\mathbf{r}^{0,k}, \quad k = 1, ..., n - m_0 \quad \text{(E10)}$$

$$0 = \Gamma_{\mathbf{e}}^{v_0}\cdot(\mathrm{diag}\ \mathbf{w}^0)^{-1}\cdot\mathbf{D}\cdot\mathbf{l}^{0,k}, \quad k = 1, ..., m_0 \quad \text{(E11)}$$

$$(\mathrm{diag}\ \mathbf{w}^0)^{-1} = (1/v_0)\cdot(\mathrm{diag}\ \mathbf{e}) \quad \text{(E12)}$$

By subtracting eqs E10 and E11 from eqs E8 and E9, respectively, premultiplying by $(\mathrm{diag}\ \mathbf{A})^{-2}$ and taking into account eq E4, one obtains

$$\mathbf{B}\cdot\mathbf{r}^{0,k} = (\mathrm{diag}\ \mathbf{A})\cdot\Gamma_{\mathbf{e}}^{\mathbf{A}}\cdot(\mathrm{diag}\ \mathbf{e})\cdot\mathbf{r}^{0,k}, \quad k = 1, ..., n - m_0$$
$$\mathbf{B}\cdot\mathbf{D}\cdot\mathbf{l}^{0,k} = (\mathrm{diag}\ \mathbf{A})\cdot\Gamma_{\mathbf{e}}^{\mathbf{A}}\cdot(\mathrm{diag}\ \mathbf{e})\cdot\mathbf{D}\cdot\mathbf{l}^{0,k}, \quad k = 1, ..., m_0 \quad \text{(E13)}$$

As for the example considered, $n = 2$, $m_0 = 1$, i.e., $\mathbf{r}^{0,k} = \mathbf{r}^{0,1} = \mathbf{r}^0$, $\mathbf{l}^{0,k} = \mathbf{l}^{0,1} = \mathbf{l}^0$, one arrives at the following summation and connectivity theorems:

$$(\mathrm{diag}\ \mathbf{A})^{-2}\cdot\mathbf{B}\cdot\mathbf{r}^0 = \mathrm{C}_{\mathbf{e}}^{\mathbf{A}}\cdot\mathbf{r}^0, (\mathrm{diag}\ \mathbf{A})^{-2}\cdot\mathbf{B}\cdot\mathbf{l}^0 = \mathrm{C}_{\mathbf{e}}^{\mathbf{A}}\cdot\mathbf{l}^0 \quad \text{(E14)}$$

Equation 80 for amplitude control coefficients is the solution to the linear equation system, eq E14.

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