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Phenomenological Definition of Response Times with Application to Metabolic Reactions

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The metabolic response time, i.e. the delay the system introduces in the response to an input flux, is considered. A novel phenomenological definition is presented, which is valid for any kind of behavior, including transitory or permanent oscillatory responses. In order to calculate the response time of single-input systems, output fluxes have to be deconvoluted with the input flux. The bases for this are established. The resulting function (unit impulse response in time-invariant linear systems) is transformed by subtracting its final state, taking the absolute value and normalizing by the resulting area, so that a norm can be applied that weights the response at every time. This response time can also be interpreted as an average. It coincides with the transition (characteristic) time of an output flux, provided that the input is performed instantaneously (step function). A strictly nonnegative response function is needed for the response time to be interpreted as a mass balance. A simple example is used to study the deviation otherwise. The method is advantageous in that it provides clues on the phenomenological behavior of biochemical systems. For example, deconvolution reveals the intrinsic oscillation-generating mechanism of an allosteric enzyme, which becomes hidden when the input flux increases in a slow way. This is illustrated by means of a model.

I. Introduction

The description of the metabolic response to perturbations of the system can be tackled from different theoretical approaches. The most exhaustive one involves posing a set of differential equations, starting from kinetic parameters and rate equations. Its main advantage is the detailed predictions it provides, even when no analytical solutions can be derived from the equations. On the other hand, the mechanism of all processes must be known beforehand. In practice, the values of some parameters are missing and an incomplete model is used to fit experiments, which may render it unreliable under different conditions. An additional problem is the parameters determined from subsystems *in vitro* might be inappropriate for characterizing the behavior *in vivo*. A recent study has been conducted to assess such a possibility in yeast glycolysis (Teusink *et al.*, 2000), concluding that some enzyme parameters change up to one order of magnitude in the *in vivo* system.

Whereas such detailed models are desirable to check our understanding of biochemistry, some practicalities of the response can be dealt with by means of simplified analysis. Compartmental models fall in this category (Brown, 1980; Cutler, 1978d; Jacquez & Simon, 1993): A

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complex system is represented by a few interconnected subsystems (compartments), the interpretation and properties of which depend on the specific aspect under study. For some applications, even purely phenomenological approaches are useful. Pharmacologists have been able to measure the bioavailability of a drug upon supply in this way (Cutler, 1978e; Tett *et al.*, 1992), as well as some important average times, including the residence time in a compartment (Hearon, 1981a–c) and the response time of the system to perturbations (Hak *et al.*, 1992; van Beek *et al.*, 1998). Provided that a few general assumptions are met, the properties of distinct compartments are model independent. For example, by assuming that some relationships between subsystems are linear one need not specify any further details, even if other processes of the system are nonlinear. Thus, although metabolism is generally nonlinear, the cardiac mitochondrial response time of oxygen consumption has been measured in rabbit by assuming or checking that, under particular circumstances, such process is linear (Eijgelshoven *et al.*, 1993; Hak *et al.*, 1992; van Beek *et al.*, 1998; van Beek & Westerhof, 1991). Essentially, a transfer or response function, the unit impulse response, can be calculated attending to the linearity between the input and the response, by numerical deconvolution of experimental data. From that, a definition has been introduced for the response time, which is interpreted as the first statistical moment of the area-normalized impulse response function (van Beek & Westerhof, 1991). This definition presents two main disadvantages.

First, the response time calculated as an average of the response function is suitable only if the latter can be normalized to a probability density, that is, if it is positive at all times and its integral is finite. In that case, the response time is identical with the mass balance between the input and the output fluxes of matter (van Beek & Westerhof, 1991). Because the mass balance is proportional to the area enclosed between those two fluxes, the numerically cumbersome deconvolution of noisy data is usually avoided. However, the definition is no longer valid for response functions that become negative over some time interval. This is the case for several kinds of metabolic behavior, e.g. overshoot in the accumulation of a metabolite or sustained oscillations.

Second, linearity is assumed to be required for the approach to be valid. The reason for this is that the response function is obtained by deconvolution. However, if some kinetic parameters are functions of time, that is if the system is time-variant (Cutler, 1978a), deconvolution produces, as we shall show, an apparent time-invariant response function, which is representative only for the particular excitation being considered. On the other hand, most metabolic reactions are nonlinear. Whether the response time calculated from deconvolution in time-variant linear systems or in nonlinear systems should be dismissed as invalid or, on the contrary, it is a reasonable measure of delay needs further investigation.

Because the response time is instrumental in establishing causal relationships between the components of a metabolic system, a definition of response time acceptable for all kinds of metabolic responses, including sustained and damped oscillations, would be extremely useful. The aim of this work is giving a feasible candidate. In order to do so, a recent definition of transition time will be used as a basis (Lloréns *et al.*, 1999). In this context, a transition is the measurable profile of a concentration or reaction rate. We start with a brief derivation of the usual definition of response time, stressing its implications in metabolism. The difficulties of that definition will be illustrated with an example and an alternative one will be suggested. The concept of response time as a mass balance will be discussed. Further, the idea that the response time calculated from the function obtained by deconvolution is also valid for nonlinear systems will be commented and tested by means of a model for an allosteric enzyme.

2. Theoretical Framework

Given a reference state (*ref*) of a biological system with a single input, a variation in the input flux, $\Delta J_{in}(t) = J_{in}(t) - J_{in}^{ref}(t)$, induces a variation or response in an output flux, $\Delta J_{out}(t) = J_{out}(t) - J_{out}^{ref}(t)$. The reference state may depend on time. However, a steady state

will be assumed in what follows, since it is usually possible and convenient to choose one. Current definitions of response time derive from the assumption that the output flux can be expressed as a convolution between the input flux and a given response function of real time only, $r(t)$. Causal convolution is defined as $\Delta J_{out}(t) = \int_0^t \Delta J_{in}(t-\tau) r(\tau)\,\mathrm{d}\tau$ and is conveniently denoted by $\Delta J_{out}(t) = \Delta J_{in}(t) * r(t)$ (Oppenheim *et al.*, 1997). The area under the response function must be finite and thus $r(t)$ must tend to zero at long times. In addition, it must be non-negative, for the reasons stated in the introduction. Let us assume that the input flux is non-decreasing and asymptotically tends to a steady state. This implies that the output flux is also non-decreasing, because the response function is positively valued, the integral of a positive function is positive and

$$\frac{\mathrm{d}\Delta J_{out}(t)}{\mathrm{d}t} = \Delta J_{in}(0) r(t) + \frac{\mathrm{d}\Delta J_{in}(t)}{\mathrm{d}t} * r(t), \quad (1)$$

where Leibniz's integral formula has been used.

Using eqn (1) and the easily checked product by time $t(f_1 * f_2) = (tf_1) * f_2 + f_1 * (tf_2)$, where f_1 and f_2 are two arbitrary functions of time, the following equation results:

$$t\frac{\mathrm{d}\Delta J_{out}(t)}{\mathrm{d}t} = \Delta J_{in}(0)\, tr(t) + \left(t\frac{\mathrm{d}\Delta J_{in}(t)}{\mathrm{d}t}\right) * r(t) + \frac{\mathrm{d}\Delta J_{in}(t)}{\mathrm{d}t} * (tr(t)), \quad (2)$$

The total area under a convolution is the product of areas under the convoluted functions (Bracewell, 1986), and thus

$$\int_0^\infty t\frac{\mathrm{d}\Delta J_{out}(t)}{\mathrm{d}t}\,\mathrm{d}t = \int_0^\infty t\frac{\mathrm{d}\Delta J_{in}(t)}{\mathrm{d}t}\mathrm{d}t \int_0^\infty r(t)\,\mathrm{d}t + \left[\Delta J_{in}(0) + \int_0^\infty \frac{\mathrm{d}\Delta J_{in}(t)}{\mathrm{d}t}\,\mathrm{d}t\right] \int_0^\infty tr(t)\,\mathrm{d}t.$$

Analogously, the area under the derivative, eqn (1), yields

$$\int_0^\infty \frac{\mathrm{d}\Delta J_{out}(t)}{\mathrm{d}t}\,\mathrm{d}t = \left[\Delta J_{in}(0) + \int_0^\infty \frac{\mathrm{d}\Delta J_{in}(t)}{\mathrm{d}t}\,\mathrm{d}t\right] \int_0^\infty r(t)\,\mathrm{d}t. \quad (4)$$

Importantly, the ratio between the final states of the output and the input fluxes (with respect to the reference fluxes) is the area under the response function,

$$\chi \equiv \frac{\Delta J_{out}^{ss}}{\Delta J_{in}^{ss}} = \int_0^\infty r(t)\,\mathrm{d}t \quad (5)$$

with $\Delta J^{ss} = \lim_{t\to\infty} \Delta J(t) = J^{ss} - J^{ref(ss)}$, where *ss* denotes the final steady state. This result follows from eqn (4) and is related to the stoichiometry of the metabolic transformation, which determines all admissible fluxes at steady state. Note that the convolution integral implies that $\Delta J_{out}(0) = 0$, no matter what the value of $\Delta J_{in}(0)$ is. If the output flux reaches a steady state, the response function can be normalized, as required, since the integral on the right-hand member of eqn (5) converges.

It can be proven, integrating eqn (3) by parts and using eqn (5), that

$$\tau_R = \frac{\int_0^\infty [\chi \Delta J_{in}(t) - \Delta J_{out}(t)]\,\mathrm{d}t}{\Delta J_{out}^{ss}}, \quad (6)$$

where the response time is denoted here by τ_R, and defined as:

$$\tau_R \equiv \int_0^\infty tr(t)\,\mathrm{d}t \Big/ \int_0^\infty r(t)\,\mathrm{d}t. \quad (7)$$

Since eqns (5) and (6) do not depend on the value of $\Delta J_{in}(0)$, it follows that this can be chosen, without loss of generality, to be zero. This choice can also be justified by observing that any relevant biochemical system is causal and $\Delta J_{in}(t) = \Delta J_{out}(t) = 0$ when $t < 0$, which implies that $\Delta J = \Delta J \cdot h(t)$. The value of Heaviside's step function, $h(t)$, at initial time is arbitrary, as it affects neither its integral (a ramp function) nor its derivative (a Dirac's delta). Consequently, eqns (1)–(4) can be simplified by omitting all terms that include $\Delta J_{in}(0)$. In

addition, capital deltas (Δ) will also be omitted in the rest of the paper, to simplify the notation.

Normalization by eqn (4) transforms eqn (3) into a difference of transition times:

$$\tau_R = T_{c(out)} - T_{c(in)}, \tag{8}$$

where $T_{c(in)}$ and $T_{c(out)}$ are transition (characteristic) times for the input and output fluxes, respectively, and are defined as

$$T_c \equiv \int_0^\infty t\frac{\mathrm{d}J(t)}{\mathrm{d}t}\,\mathrm{d}t \Bigg/ \int_0^\infty \frac{\mathrm{d}J(t)}{\mathrm{d}t}\,\mathrm{d}t. \tag{9}$$

The reasons for using the derivative of a transition to calculate transition times, instead of the transition itself, have been discussed elsewhere (Lloréns *et al.*, 1999). On the contrary, the response time directly results from the response function.

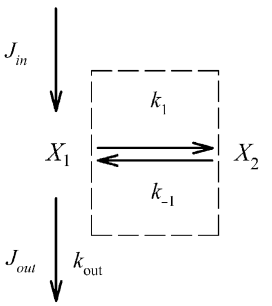
It can be proven, integrating by parts, that the transition time is a mass balance between the normalized transition and a step function, $h(t)$. Therefore, the difference between the output and input transition times is the overall mass balance of the metabolic transformation, normalized by the variation of the output flux, and coincides with eqn (6), which is identical in meaning to eqn (8). This is the rationale for calculating the area between transitions, instead of deconvoluting and applying eqn (7). Of course, the mass balance is a positive quantity in biochemical systems.

3. Definition of Response Time

3.1. SHORTCOMINGS OF PREVIOUS DEFINITIONS

As recognized by some authors, definition (7) is limited to nonnegative response functions, as is its estimation via the mass balance. Usually, when the area enclosed by the negative part of the function is small, it is simply neglected (van Beek *et al.*, 1998; van Beek & Westerhof, 1991). However, this contribution can be important in metabolic systems.

Consider, for example, the rate of formation of a metabolite from a second one that is irreversibly introduced to and extracted from the system, as shown in Scheme 1. First-order kinetics is assumed.



SCHEME 1. Dead-end pathway with linear kinetics. Constants k_i, $i=1$, -1, *out*, are rate constants, X_1 and X_2 are two metabolites. Concentrations are denoted by the same symbols.

If the system is initially at rest, a constant input flux of one species, X_1, induces the accumulation of the other one, X_2, up to the equilibrium value. Its velocity of formation is $v_1 = k_1X_1 - k_{-1}X_2$ (squared in Scheme 1), where $k_{\pm 1}$ are rate constants and $X_{1,2}$ are concentrations. It initially increases and, after reaching a maximum, decreases to zero. Clearly, from the definition of convolution, the deconvolution of this velocity with the constant input flux is just its normalized time derivative,

$$r(t) = \frac{1}{J_{in}}\frac{\mathrm{d}v_1(t)}{\mathrm{d}t} \tag{10}$$

and, consequently, the response function has a negative part. An example is depicted in Fig. 1, along with the velocity v_1. Since the final velocity vanishes, it is evident that the area under the response is zero. The mathematical details are presented in Appendix A. Hence, eqn (7) cannot be applied. Since the system is linear and time-invariant, the response function is unique. Therefore, the conclusion is general for any input flux.

Suppose, however, that the overall area under $r(t)$, A, is positive. For example, let us construct a series of response functions, as follows. Decompose the response function of the above system into the difference of its positive and negative parts, $r(t) = r_+(t) - r_-(t)$. The functions $r_\pm(t)$ are positively valued, with areas $A^+ = \int_0^\infty r_+(t)\,\mathrm{d}t$, and $A^- = \int_0^\infty r_-(t)\,\mathrm{d}t$, respectively, so that $A = A^+ - A^-$. Partial response times can be calculated as $\tau_R^+ = \int_0^\infty t(r_+(t)/A^+)\,\mathrm{d}t$ and $\tau_R^- = \int_0^\infty t(r_-(t)/A^-)\,\mathrm{d}t$. It is easy to check that the overall response time,

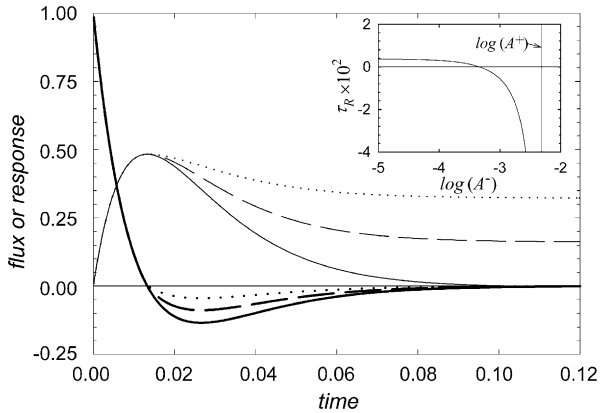


FIG. 1. Response function of the velocity of formation of X_2 (cf. Scheme 1; heavy solid line) and its corresponding velocity for a constant input flux of 100 (light solid line). The parameters used were $k_1 = 1$, $k_{-1} = 75$ and $k_{out} = 75$. The response displays a negative part, responsible for the non-monotonic velocities. Its overall area is zero, which determines the final state (equilibrium). Dashed lines are response/velocity pairs (heavy/light lines) for responses constructed progressively reducing the area of their negative part, without changing the aspect (see text for further details). Their overall areas are positive, leading to a positive final state for the velocities (proportional to the area). The inset shows the evolution of the average time τ_R of such functions as the negative area approaches the positive one ($A^+ = 0.005$). The response times of the positive and the negative parts of the response are $\tau_R^+ = 0.004$ and $\tau_R^- = 0.004$, respectively. Arbitrary units are used.

τ_R, results from the following expression:

$$\tau_R = \frac{\tau_R^+ A^+ - \tau_R^- A^-}{A^+ - A^-}. \qquad (11)$$

Now, substitute the negative part of $r(t)$ by a proportional function (Fig. 1), satisfying the condition $A^+ > A^-$ so that the final value of v_1 is positive. In these constructs, τ_R^+, τ_R^- and A^+ are constant. The variation of τ_R as A^- tends to A^+ is shown in Fig. 1 (inset). For areas greater than $A^+\tau_R^+/\tau_R^-$, eqn (11) takes negative values, which is non-sensical for a consistent definition of response time. Hence the need for some alternative definition.

3.2. GENERALIZATION OF THE DEFINITION

Definition (7) is usually interpreted as an average. However, as we have seen a response function is not a properly defined probability density. Even when it is a nonnegative function, it must be normalized. Similarly, only the normalized derivative of a transition can be averaged. Hence, definition (9) cannot be applied to non-monotonic transitions. A general method for determining the transition time was found by constructing a monotonic function from the original curve (Lloréns *et al.*, 1999). The new function preserves the aspect of the actual transition in that their respective derivatives are identical in absolute value.

An additional difficulty occurs when the final state is non-stationary, i.e. a sustained oscillation. Since the derivative does not tend to zero at long times, the new function cannot be normalized. The realization that a transition approaches the final state in an asymptotic fashion helps overcome this problem by computing the difference of the transition with this final, though time dependent, state, $\bar{J}(t)$ [cf. Lloréns *et al.* (1999) for a more detailed discussion]. The resulting general definition is

$$T_c \equiv \int_0^\infty t\left|\frac{\mathrm{d}(J(t) - \bar{J}(t))}{\mathrm{d}t}\right| \mathrm{d}t \Bigg/ \int_0^\infty \left|\frac{\mathrm{d}(J(t) - \bar{J}(t))}{\mathrm{d}t}\right| \mathrm{d}t. \qquad (12)$$

As we have seen, the response function of a linear system under a constant input flux can be computed by taking the time derivative of the output flux and normalizing [eqn (10)]. Since the transition time for a constant input (in fact, a step function), is zero, one expects that the response time of the system be equal to the transition time of the corresponding output. For this reason, and on the basis of definitions (7) and (12), an obvious generalization of the response time is

$$T_R \equiv \int_0^\infty t|r(t) - \bar{r}(t)|\,\mathrm{d}t \Bigg/ \int_0^\infty |r(t) - \bar{r}(t)|\,\mathrm{d}t, \qquad (13)$$

where, again, a bar over a symbol denotes the final state. The convention T_R is used from now on to distinguish the general definition from the particular case τ_R. Note that if an oscillatory output results as a consequence of the constant input flux, $\bar{r}(t)$ will be the derivative of

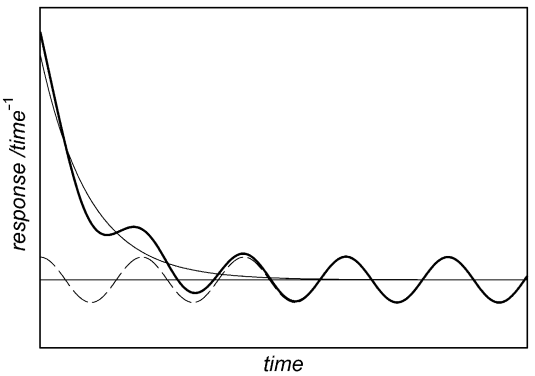


FIG. 2. Calculation of the response time for responses with a sustained oscillatory final state. An extension of the final state to all times (dashed line) is subtracted from the actual response function (heavy solid line). The resulting function (light solid line) can be normalized by the area to a density function, from which the response function can be obtained.

that sustained oscillation (Fig. 2), that is, an oscillation around the time axis. Otherwise, the final value of the response function is zero.

Expressions (12) and (13) can be interpreted as average times. Nevertheless, we suggest an alternative interpretation, based on the method for computing them: First, a function is transformed to the vectorial space of functions $\phi_0(t)$ with final value zero, Φ, by means of a difference with the final-state function and, in its case, a derivative. Then the transformed function is normalized by the area of its absolute value. Finally, integral (14) is calculated as

$$\|\phi_0\|_t = \int_0^\infty t|\phi_0(t)|\,\mathrm{d}t. \tag{14}$$

The motivation of this procedure is as follows: The members of the space Φ can be used to represent all possible ways of approaching a given state. Such functions vanish at long times, indicating that the final state has been reached. Since the space is vectorial, we can define the norm

$$\|\phi_0\|_1 = \int_0^\infty |\phi_0(t)|\,\mathrm{d}t. \tag{15}$$

This is used as a normalization factor so that functions of different "sizes" can be compared. The norm $\|\cdot\|_t$ weights the value of the function with the variable (time), thus giving a measure of delay for functions of the same "size", $\|\cdot\|_1$. Consequently, the ratio between these two norms is the response time (or, in its case, the transition time). The advantage of this explanation is that no ad hoc interpretation of the probability densities is needed.

Transformations to the space Φ are not new in definitions of metabolic times. For example, Heinrich & Rapoport (1975) proposed a definition similar to eqn (13), differing only in the absence of the absolute values. However, their definition is not valid for transitions, since it violates relationship (10) between transitions and the response function in the particular case that the system is linear and the input is a step function. More importantly, the definition is not based on a proper notion either of probability density or of norm. The latter bestows a convenient measure of distance, that is, delay, and is probably more suitable when the microscopic aspects of the system are not considered.

4. Response Time Deviation from the Mass Balance

Definition (13) challenges the interpretation of the response time as a mass balance. Consider a system with one input and one output. The mass balance is given by eqn (7). On the contrary, the response time results from eqn (13), which is generally different. Additionally, it follows from eqn (1) that, for a monotonic input, a non-monotonic output implies the sign of $r(t)$ changes at least once. However, the opposite is not necessarily true, that is, the output might be monotonic even if the sign of the response function changes. Should this be the case, the estimation of the response time from the area enclosed by the input and output transitions would be erroneous. This has important consequences that are worth analysing.

If both the input and output transitions are monotonic the difference of transition times [eqn (12)], $\Delta T_c = T_{c(out)} - T_{c(in)}$, equals the mass balance. Hence, studying the variation of ΔT_c for different monotonic inputs helps understand by how much the response time differs from the mass balance. Let us assume that the system is linear and that the sign of the response function

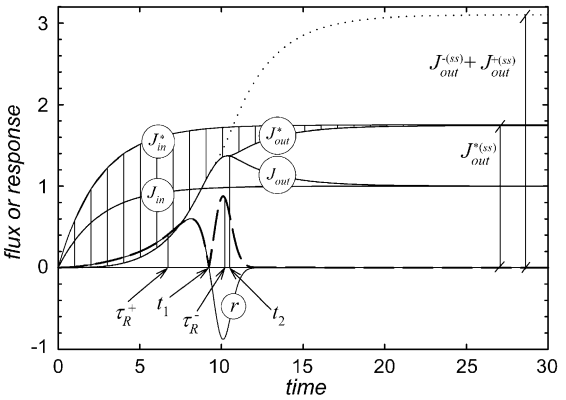


FIG. 3. Analysis of damping-generating responses. A sign-changing response function, r, describes a system with monotonic input, J_{in}, and critically damped oscillation at the output, J_{out}. The change of sign of r occurs at time $t_1 = 9.25$ and a maximum J_{out} appears at $t_2 = 10.37$. The difference between the output and input transition times is obtained by constructing equivalent transitions, J^*_{in} and J^*_{out}, and normalizing the hatched area by the height $J^{*(ss)}_{out}$. Convolution of the absolute value of the response (dashed line) with the input flux, J_{in}, leads to a transition (dotted line), the height of which represents the maximum absolute variation of flux that can be obtained under the response r. The response function can be decomposed into a positive ($\tau_R^+ = 6.72$) and a negative part ($\tau_R^- = 10.26$). Convolution of the input with these two parts generates monotonic transitions towards steady states with $J^{+(ss)}_{out} = J^{(ss)}_{in}A^+$ and $J^{-(ss)}_{out} = J^{(ss)}_{in}A^-$ as final fluxes, respectively (areas: $A^+ = 2.05$, $A^- = 1.05$). All units are arbitrary.

changes at a single time, t_1 (Fig. 3). The derivation of the response time is as that of eqn (11), except that now the absolute value $|r(t)| = r_+(t) + r_-(t)$ is used, whose overall area is $A^+ + A^-$:

$$T_R = \frac{A^+\tau_R^+ + A^-\tau_R^-}{A^+ + A^-}. \tag{16}$$

Since the mass balance must be nonnegative, and according to eqn (11), the response function fulfils the condition $A^+/A^- \geqslant \tau_R^-/\tau_R^+$, with $\tau_R^- > \tau_R^+$ (Fig. 3). In general, the output of the system for a monotonic input will be a critically damped oscillation towards a steady state, reaching a maximum at $t_2 \geqslant t_1$.

Geometrically, the difference of transition times is the hatched area in Fig. 3, normalized by the height $J^{*(ss)}_{out}$ [cf. Lloréns *et al.* (1999) for details]. This height is the total variation of the output from the reference state to the steady state (ss), irrespective of its sign. Additionally, we can calculate ΔT_c from

$$\left|\frac{\mathrm{d}J_{out}(t)}{\mathrm{d}t}\right| = \left|\frac{\mathrm{d}J_{in}(t)}{\mathrm{d}t} * r(t)\right| = \frac{\mathrm{d}J_{in}(t)}{\mathrm{d}t} * |r(t)| + \Delta(t). \tag{17}$$

The difference function $\Delta(t)$ is derived in Appendix B. $T_{c(out)}$ follows from eqn (17) by simply multiplying by time and integrating over time (norm $\|\cdot\|_t$), normalizing by $\int_0^\infty (\mathrm{d}J_{in}/\mathrm{d}t)\,\mathrm{d}t \int_0^\infty |r(t)|\mathrm{d}t$ and rearranging. The resulting equation,

$$T_{c(out)} = T_{c(in)} + T_R + (1-\zeta)(T_{c(out)} - T_\Delta), \tag{18}$$

is an extension of eqn (8), T_Δ being the ratio of $\|\cdot\|_t$ and $\|\cdot\|_1$ for the difference function, $\Delta(t)$, and

$$\zeta = \frac{\int_0^\infty |(\mathrm{d}J_{in}(t)/\mathrm{d}t) * r(t)|\mathrm{d}t}{\int_0^\infty (\mathrm{d}J_{in}(t)/\mathrm{d}t)\,\mathrm{d}t \int_0^\infty |r(t)|\mathrm{d}t} = \frac{J^{*(ss)}_{out}}{J^{*(ss)}_{in}(A^+ + A^-)}. \tag{19}$$

The constant ζ is less than or equal to unity, since $J^{*(ss)}_{out} = J^{*(ss)}_{in}(A^- - A^+) + 2J_{out}(t_2)$ and $J_{out}(t_2)$ cannot be greater than $J^{*(ss)}_{in}A^+$. It is the fraction of total possible variation of the output, irrespective of its sign, that is actually produced under a particular input. In the specific case that the input flux is constant ($h(t)$):

$$\begin{aligned} &J_{in}(t) = J^{(ss)}_{in}h(t) \Rightarrow \frac{\mathrm{d}J_{in}(t)}{\mathrm{d}t} = J^{(ss)}_{in}\delta(t), \\ &\left|\frac{\mathrm{d}J_{out}}{\mathrm{d}t}\right| = |J^{(ss)}_{in}\delta(t) * r(t)| = J^{(ss)}_{in}|r(t)| \Rightarrow \zeta = 1. \end{aligned} \tag{20}$$

Thus, the last term in eqn (18), $\eta = \Delta T_c - T_R = (1-\zeta)(T_{c(out)} - T_\Delta)$, vanishes, along with the transition time for the input, leading to the identity $T_R = T_{c(out)}$, as expected.

Suppose now that the output is monotonic. In this case $\zeta = \chi/(A^+ + A^-)$ and the difference of transition times is the mass balance. By subtracting eqn (16) from eqn (11), a minimum η is obtained, which is the difference between the

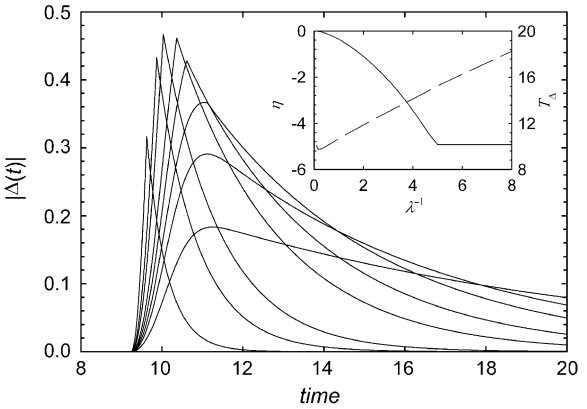


FIG. 4. Difference function, $\Delta(t)$, for the response in Fig. 3 and inputs of the form $1-e^{\lambda t}$, with decreasing values of the positive parameter λ. All of them are null before the time at which the response sign changes ($t_1 = 9.25$). The inset displays the average time of $\Delta(t)$ (dashed line) as a function of the inverse of the parameter (equal to the input transition time). It also shows, $\eta = T_{c(out)} - T_{c(in)} - T_R$, the divergence of the response time from the difference of transition times (solid line). The final constant value of η corresponds to monotonic output transitions and is the difference of the mass balance ($\tau_R = 3.00$) and the response time. ($T_R = 7.92$). At this particular extreme situation, the mass balance equals the difference of transition times, indicating that it may underestimate the response time even if the input and output transitions are both monotonic. All time units are arbitrary.

mass balance and the response time:

$$\eta_{min} = \frac{2A^+A^-(\tau_R^+ - \tau_R^-)}{(A^+)^2 - (A^-)^2}. \tag{21}$$

Therefore, ΔT_c ranges between those two magnitudes ($T_R \geqslant \Delta T_c \geqslant \tau_R$).

An example of the evolution of $\Delta(t)$ for input fluxes of the form $1-e^{\lambda t}$, $\lambda > 0$ is depicted in Fig. 4. Remarkably, η_{min} is not reached asymptotically but abruptly, indicating that the output can in fact be monotonic. Consequently, the difference of transition times reaches a constant value. At this limit, t_2 tends to infinity and $T_\Delta = T_{c(in)} + J_{in}^{(ss)}A^-$, as can be deduced from the difference function $\Delta(t)$ (Appendix B).

The conclusion of this section is that, for the calculation of the response time, explicit deconvolution is required. The area enclosed between the output and the input transitions may underestimate the response time even when both transitions are monotonic. Special care is needed when the input varies slowly towards a final state, i.e. when the transition time of the input is high.

5. Deconvolution in Time-variant Linear Systems and Nonlinear Systems

5.1. TIME-VARIANT LINEAR SYSTEMS

It is usually assumed that calculating the response time from the deconvolution of an output with an input is acceptable only for linear systems. Moreover, the system must be time-invariant, that is, the response must be the same regardless of the moment of perturbation, except for a translation on the time axis. We will show in the next section that a nonlinear system can *always* be represented, *for any given input*, as a time-variant linear system. Hence, the effort must concentrate in justifying that the response time can reasonably be calculated from the deconvolution of an output with the input when the system is linear and time-variant. In this case, the general output for any variation of an input, $J_{in}(t)$, is

$$v(t) = \int_0^t J_{in}(\tau) r_v(t;\tau)\,\mathrm{d}\tau. \tag{22}$$

The function $r_v(t;\tau)$ is a time-variant response function, characteristic of unit impulse perturbations at time τ, $\delta(t-\tau)$.

As an example, let us consider the degradation of a metabolite S following linear kinetics [eqn (23)] with a decaying first-order rate coefficient $(2+t)/(1+t)$ (i.e. its dimension is $time^{-1}$). The coefficient takes values from 1 to 2, t being real time:

$$\frac{\mathrm{d}S}{\mathrm{d}t} = -v(t) = -\frac{2+t}{1+t}S. \tag{23}$$

The velocity of degradation, $v(t)$, is the absolute value of the right-hand side member of eqn (23), and may describe the partial loss of activity of an enzyme operating at low saturation.

In order to obtain the response function, we solve eqn (23) for the "initial" condition $S(\tau) = 1$. This is based on the fact that equations $\mathrm{d}X(t)/\mathrm{d}t = 0, X(\tau) = 1$ and $\mathrm{d}X(t)/\mathrm{d}t = \delta(t-\tau), X(\tau) = 0$ are equivalent, since both of them Laplace transform to, $sx(s) = \mathrm{e}^{-\tau s}X(\tau+)$, with $x(s) = \int_0^\infty X(t)\mathrm{e}^{-st}\,\mathrm{d}t$, where

$X(\tau+)$ is a positive lateral limit. The resulting function is

$$r_v(t;\tau) = \frac{(t+2)(\tau+1)}{(t+1)^2} e^{\tau - t} h(t-\tau). \quad (24)$$

Once more, $h(t)$ is the standard function of Heaviside.

For an impulse excitation at time t_p, $J_{in}(t) = \delta(t - t_p)$, the velocity is just $v(t) = r_v(t; t_p)$. Its deconvolution with the input is straightforward, $r(t) = r_v(t + t_p; t_p)$. It is obviously positive at all times. Furthermore, it is clear that eqn (7) provides a representative response time, since $r_v(t;\tau)$ has been derived from unit impulse perturbations. Hence:

$$\tau_R = T_R = (t_p + 1) e^{t_p + 1} \Gamma(0, t_p + 1). \quad (25)$$

The function $\Gamma(a,b) = \int_b^\infty t^{a-1} e^{-t}\, dt$ is the incomplete gamma function. Contrarily to time-invariant linear systems, this response time depends on when the system is perturbed. Since the initial rate coefficient is 2, one expects the response time for $t_p = 0$ to be close to 0.5 (the actual initial value is 0.596). On the other hand, the limit of the response time at long times is one, since $\lim_{\tau \to \infty} r_v(t;\tau) = e^{-\Delta t} h(\Delta t)$, with $\Delta t = t - \tau$ (as can be checked by substituting $t = \Delta t + \tau$ in $r_v(t;\tau)$ and passing to the limit).

If, instead of a unit impulse, the perturbation is a unit step, $h(t - t_p)$, the velocity becomes

$$v(t) = \frac{(t+2)(t - t_p e^{t_p - t})}{(t+1)^2} h(t - t_p), \quad (26)$$

The response time is now expected to be constant, since the derivative of the velocity is strictly positive,

$$\frac{dv}{dt} = \frac{2 + t_p(t^2 + 4t + 5) e^{t_p - t}}{(t+1)^3} h(t - t_p), \quad (27)$$

and, consequently, $T_{c(v)} - t_p$ equals the mass balance. Because the input is a step function at time t_p, the relationship $T_R = T_{c(v)} - t_p$ should hold. That this is the case when deconvolution is used to calculate the response time can be checked by substituting eqn (27) into eqn (7), shifted to the origin of times, which yields $T_R = 1$ for any t_p. Therefore, deconvolution produces reasonable results for this kind of perturbation as well.

It must not be implied from a positive response function, $r_v(t;\tau) > 0$, that the deconvolution of any output/input pair will be a positive function. For example, if the rate coefficient in eqn (23) is t instead of $(2 + t)/(1 + t)$, the time-variant response is positive:

$$r_v(t;\tau) = t e^{(\tau^2 - t^2)/2} h(t - \tau). \quad (28)$$

However, unit step inputs, $h(t - t_p)$, produce critically damped oscillations towards the steady state. Unfortunately, explicit expressions are difficult to derive. Nevertheless, it is clear that, again, $r(t)$ results from direct derivation of the output. Consequently, the sign of the response function changes (Fig. 5) and the more general definition (13) must be used. Since the value of the rate coefficient increases without bound, the concentration of the metabolite tends to vanish (though the velocity tends to the input flux). Thus, the mass balance is zero, as can be checked by applying eqn (6). On the contrary, the response time decreases from the initial value (1.54) to zero as $r(t)$ tends to a Dirac's delta (Fig. 5). This is more reasonable, as the response is clearly not instantaneous.

The key point that follows from these examples is that deconvolution produces an apparent time-invariant response function, $r(t)$,

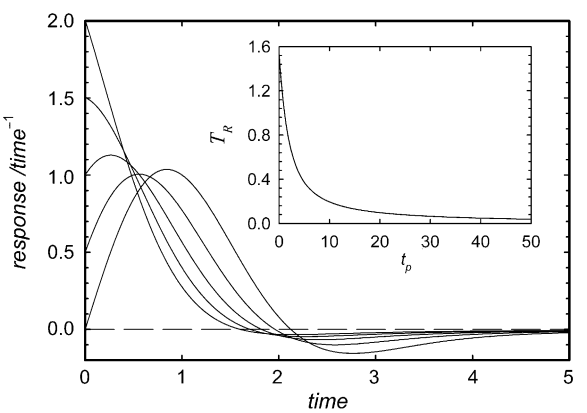


FIG. 5. Deconvolution of the velocity $v(t) = tS$, where S is the substrate concentration, with a unit step input flux at time t_p. The value of t_p for each curve corresponds to the origin ordinate. The response function tends to a Dirac's delta as t_p increases. Correspondingly, the response time [eqn (13)] tends to zero (inset).

which is suitable for every $J_{in}(t)$ in that it represents the delay that mediates between the input and the output. This is so because, for a particular pair $\{J_{in}(t), r(t) = r[J_{in}]\}$, time-invariant convolution of the input with the apparent $r(t)$ applies, and so do the conclusions of Sections 2 and 3. A more technical discussion can be found in Section 7.

5.2. NONLINEAR SYSTEMS

In nonlinear systems the output cannot be generated by superposition of responses. Consider, however, the variation of a metabolite concentration, X_i, which is a balance between two velocities, $\mathrm{d}X_i(t)/\mathrm{d}t = J_{in}(t) - v(X_i, X_{j\neq i})$. This equation could be coupled to others. Now, the velocity $v(t)$ may be highly nonlinear and depend not only on X_i but also on the concentrations of any other species present, $X_{j\neq i}$. Giving a solution to the dynamics of the system is not the aim of this analysis. On the contrary, the evolution of every concentration (the solution of the equations), $\left\{X_i^{sol}(t), X_{j\neq i}^{sol}(t)\right\}$, is supposed to be known by an appropriate method (very likely, an experimental one). The variation of the metabolite concentration can be restated as

$$\frac{\mathrm{d}X_i(t)}{\mathrm{d}t} = J_{in}(t) - \frac{v(X_i^{sol}(t), X_{j\neq i}^{sol}(t))}{X_i^{sol}(t)} X_i(t)$$

$$= J_{in}(t) - K(t)X_i(t), \tag{29}$$

where $K(t)$ is a *known* function of time that possibly depends on every concentration. Hence, even though the dynamics is nonlinear, the system can be *represented*, for any given input, as a time-variant linear system [cf. a related argument in Cutler (1978a)]. An apparent time-variant response for the velocity $v(t)$, $r_v(t;\tau) = K(t)G(t;\tau)$, can be obtained, where $G(t;\tau)$ results from solving the equation $\mathrm{d}G(t)/\mathrm{d}t = -K(t)G(t)$ with the initial condition $G(\tau) = 1$. In this way, velocity $v(t)$ is recovered by time-dependent superposition [eqn (22)]. Unlike linear systems, this response does depend on the input, i.e. $r_v(t;\tau) = r_v[J_{in}]$. However, if the response time calculated from deconvolution is representative in time-variant linear systems, it is so too in nonlinear systems. Thus, input dependence must be expected as a feature of nonlinear response time.

6. Response of an Allosteric Enzyme under Slowly Increasing Input Fluxes

In order to illustrate the conclusion of Section 5 that the response time (13) calculated from the deconvolution of an output with the input is representative also in nonlinear systems, we employ Goldbeter's model of phosphofructokinase, a glycolytic enzyme (Goldbeter & Lefever, 1972; Goldbeter, 1996; Goldbeter & Dupont, 1990). This allosteric enzyme presents complex dynamics such as critically damped oscillations, damped oscillations and sustained oscillations, depending on the input flux to the enzyme (Goldbeter, 1996). The evolution equations for the normalized concentrations of substrate α (fructose 6-phosphate or ATP) and product γ (fructose 1,6-biphosphate or ADP) are, according to the model (Goldbeter, 1996)

$$\begin{cases} \dfrac{\mathrm{d}\alpha}{\mathrm{d}t} = v(t) - \varphi\phi(\alpha,\gamma), \\ \dfrac{\mathrm{d}\gamma}{\mathrm{d}t} = q\varphi\phi(\alpha,\gamma) - k\gamma. \end{cases} \tag{30}$$

Restricting the model to a dimeric enzyme with no affinity of the inactive form for the substrate, the velocity ϕ is given by

$$\phi(\alpha,\gamma) = \frac{\alpha(1+\alpha)(1+\gamma)^2}{L + (1+\alpha)^2(1+\gamma)^2}. \tag{31}$$

To assess the possible influence of slowly increasing inputs on the response, a substrate input flux of the form $v(t) = v(1 - \mathrm{e}^{-\lambda t})$, with $\lambda, v > 0$, was simulated. The product is irreversibly extracted with a constant rate k. The maximum rate of the enzyme is given by φ. The constant q is a scale factor, needed as a weight of the ratio of dissociation constants for the substrate and product. Finally, cooperativity effects are included as an allosteric constant, L. The following set of parameters was used in the simulations: $\varphi = 4\ \mathrm{s}^{-1}, q = 1, L = 5 \times 10^6, k = 0.1\ \mathrm{s}^{-1}$. A final flux for which transitory (damped) oscillatory behavior occurs was selected, namely $v = 0.1\ \mathrm{s}^{-1}$.

Whereas the response time of the enzyme velocity under constant input fluxes directly results from computing the transition time, time-dependent input fluxes require, for the reasons discussed in section 4, deconvoluting the enzyme velocity with the actual input flux. Figure 6(a) depicts a series of such deconvolutions corresponding to decreasing values of the parameter λ. The enzymic response functions vary as the input flux profile does, thus reflecting the nonlinearities present in the equations of the model. The oscillatory behavior is progressively reduced, eventually reaching the final steady state through a monotonic transition [Fig. 6(b)]. However, the response functions make obvious that the enzyme possesses a mechanism able to generate damped oscillations since, up to the value of the parameter analysed, they display at least one change of sign, even when the output flux does not oscillate.

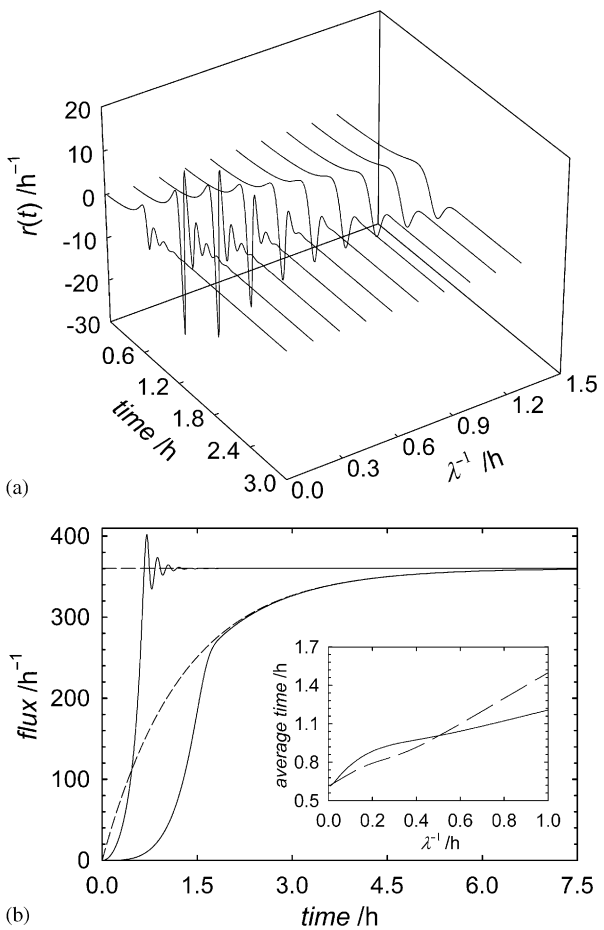


FIG. 6. Response functions for an allosteric enzyme operated under increasingly slow monotonic input fluxes. (a) Dependence on the input transition time (inverse of λ; see text for the meaning and value of this and other parameters of the model). (b) Two input/output transition pairs (dashed/solid lines): The enzyme shows damped oscillatory behavior under constant input flux and monotonic behavior when the input transition time is longer than approximately 1.0 hr ($\lambda^{-1} = 1.2$ hr shown). The inset depicts the evolution of the response time [eqn (13)] as λ^{-1} grows (solid line), along with the parallel increase of the transition time of the product (dashed line).

The response time of the enzyme velocity, evaluated according to definition (13), increases with the transition time at the input. Conversely, applying eqn (7) a constant results, indicating that the mass balance does not depend on the input transition. In fact, the steady concentrations of substrate and product are functions of the final state. This result further supports the proposition that the absolute value of the response should be considered for estimating a representative time. Moreover, the product of the reaction acts as an activator of the enzyme. Therefore, longer response times are expected when the accumulation of product occurs at slower rates, that is, with greater transition times for the product. Such a correlation is observed in the model under consideration, as shown in Fig. 6(b) (inset).

7. Discussion

The response time measures how delayed the response of a system to perturbations is. Hence, it is important for determining causal links in metabolism. In a complex metabolic network, it is difficult to distinguish between causes and effects under particular operating conditions. This is so because for proving a cause–effect relationship at least two facts must be established: the existence of a correlation between two reaction rates, or velocities, and the precedence of one velocity over the other (Dillon & Goldstein, 1984; Hume, 1973). The latter is given by the response time. The problem of determining it is twofold. First, a proper definition must be given that can be applied to a representative response function. Second, the response function must be accessible from experimental data by a method as general as possible.

As to the first problem, we note that, unlike the response function, transitions are observed immediately. After a system is perturbed, the

system moves from the initial state to a final one. Consequently, concentrations and velocities change and they can be measured as they do. One need not know what is the specific perturbation or cause that induced such transitions to calculate the time they take to occur, i.e. the transition time. Complex behaviors, such as non-monotonic transitions and damped or sustained oscillations, present some difficulties from the phenomenological point of view, but they can conveniently be addressed (Lloréns *et al.*, 1999). Thus, it has been possible to give a general definition of transition time [eqn (12)].

We argued in Section 3 that when the input is a step transition, and hence its transition time is zero, the response time must coincide with the transition time of the output. From this general observation, we propose the transition time as a basis for the definition of the response time. However, in order to provide the foundations for the latter, some assumptions are required concerning the nature of the system.

For instance, the response of a time-invariant linear system obeys a convolution law with the response function, $r(t)$. When $r(t)$ is strictly positive and both the input and the output are monotonic, the response time has previously been defined as a mass balance (van Beek & Westerhof, 1991), which is equivalent, as we show in Section 2, to a difference of transition times. However, the definition proposed for such cases [eqn (7)] may produce negative values of the response time if the sign of the response function changes. Fortunately, for step inputs, $r(t)$ is related to the output by a time derivative, a relationship that we have used to suggest, by comparison, a new definition for the response time [eqn (13)].

Because this definition should be as general as possible, the nature of the system must be taken into account by means of the response function itself. This brings us to the second problem: determining $r(t)$ from experimental data. In time-invariant linear systems, the response function is obviously obtained by deconvolution, which is computationally equivalent to discretizing and solving by substitution a triangular system of linear equations (Clough *et al.*, 1993; Verotta, 1989). In practice, such finite difference, or point-area, methods treat the impulse response as a piecewise-constant function, with or without variable step size (Yu *et al.*, 1996), and use recurrent formulas for deconvoluting. Whereas the solution is well behaved for free-of-noise data, deconvolution of real data often leads to spurious oscillations. Its stability has been analysed (Li & Cutler, 1998a, b) and inequality-constrained least-squares improvements have been introduced for nonnegative responses (Clough *et al.*, 1993; Hovorka *et al.*, 1998; Verotta, 1989).

Nevertheless, more assumptive methods are usually more stable. In essence, the response function is supposed to take a prescribed parametric form. Typical examples include sums of exponentials (Cutler, 1978c) and polynomials (Cutler, 1978b). The parameters are then determined by means of least-squares methods. These are usually nonlinear, though linear ones have also been devised (Vajda *et al.*, 1988). An alternative approach is to approximate the output to a continuous function such as a polyexponential (Veng-Pedersen, 1980) or a cubic spline (Yu *et al.*, 1997). Hence, deconvolution is experimentally feasible. However, its meaning had to be clarified in other systems.

The response of time-variant linear systems is a function of perturbation times, and thus deconvolution produces an apparent time-invariant function, $r(t)$. It is shown in Section 5, by means of examples, that this function is in fact adequate for calculating the response time, as it represents the delay mediating between the input and the outputs. Furthermore, since any nonlinear system can be represented as a time-variant linear system *for every given input*, deconvolution is arguably appropriate in general. However, we further discuss this point.

The fact that $r(t)$, i.e. deconvolution, depends on the input except when the system is linear and time-invariant is expected and can be understood as follows. Mathematically, the response of *any* system is the result of the action of an operator, e.g. a convolution integral, on the input transition. Additionally, the variation of the response as the input varies is the functional derivative of the operator with respect to the input transition. In much the same way as the derivative of a straight line is a constant number, the functional derivative of a linear operator is a constant

functional (a function). On the contrary, the derivative of a nonlinear operator is a non-constant functional of the input. Thus, for systems with a single input, deconvolution is just the functional derivative of the operator that describes the system *evaluated for a particular input transition*. Due to the meaning of any derivative, it is reasonable to use it for calculating the response time. It is, however, not sufficient for describing the response of the system, as it changes with the input.

The dimensions of the derivative of a functional with respect to a function are somewhat different to those of a common derivative. They are not only the ratio of the dimensions of the functional and the function, but are also divided by the dimensions of the variable (Zeidler, 1986). Thus, in the common situation that the input is a flux of substrate and the output is a reaction rate, the dimension of the response function is $time^{-1}$. It is easy to realize that this is so by analysing the convolution integral [cf. also eqn (10)]. According to this view, the apparent time-invariant response function obtained by deconvolution is the degree of variation of the output when varying infinitesimally the actual input transition *per unit time*. This idea of sensitivity is precisely what is usually understood as response and is completely independent of the linearity of the system.

It is also shown, in Section 4, that any complex behavior that induces a change in the sign of the apparent response function leads to a deviation of response time (13) from the mass balance, which is possible even when both the input and the output are monotonic. This is illustrated by means of the nonlinear model of an allosteric enzyme. The results are reasonably compatible with the regulatory mechanisms implicit in the model, thus supporting the definition. Particularly, there is a correlation between the time of accumulation of the product (an activator) and the response time. The independence of the response time from the mass balance has an important advantage: for reaction rates for which the estimation of the latter does not make sense, like the velocity of formation of X_2 in Scheme 1, the calculation of the former is still possible and significant.

In summary, the response time of metabolic systems with influx of a single metabolite can be calculated, by means of a novel definition relying on the concept of vector norm, from a function obtained by deconvolution of the response with the input. Functional analysis considerations help demonstrate the generality of such an approach. The relationship of the response time with mass balances and transition times has also been studied. We finally observe that when several simultaneous input fluxes enter the system, functional analysis is required in order to compute explicitly the functional derivative of the response *with respect to every input*. This is beyond the scope of this paper.

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APPENDIX A

The differential equations governing Scheme 1 are:

$$\begin{cases} \dfrac{dX_1}{dt} = J_{in}(t) - (k_1 + k_{out})X_1 + k_{-1}X_2, \\ \dfrac{dX_2}{dt} = k_1X_1 - k_{-1}X_2 \end{cases} \tag{A.1}$$

with the initial conditions $X_1(0) = X_2(0) = 0$. Laplace transformation techniques lead to the following general solution for any input flux:

$$X_1(t) = J_{in}(t)* \left[\frac{(k_{-1} + \lambda_1)e^{\lambda_1 t} - (k_{-1} + \lambda_2)e^{\lambda_2 t}}{\lambda_1 - \lambda_2}\right],$$

$$X_2(t) = J_{in}(t)* \left[\frac{k_1(e^{\lambda_1 t} - e^{\lambda_2 t})}{\lambda_1 - \lambda_2}\right], \tag{A.2}$$

where $\lambda_{1,2} = (1/2)[-(k_1 + k_{out} + k_{-1}) \pm (k_1 + k_{out} + k_{-1})^2 - 4k_{-1}k_{out})^{1/2}]$ are the poles of the Laplace transform, which are negative and different.

The response function can be calculated from the deconvolution of $v_1 = k_1X_1 - k_{-1}X_2$ with the input flux. Thus

$$r_{v_1}(t) = \frac{k_1(\lambda_1 e^{\lambda_1 t} - \lambda_2 e^{\lambda_2 t})}{\lambda_1 - \lambda_2}. \tag{A.3}$$

The sign of the response $r_{v_1}(t)$ changes at time $t = \ln(\lambda_2/\lambda_1)/(\lambda_1 - \lambda_2)$.

APPENDIX B

Responses like the one depicted in Fig. 3 can be decomposed into the difference of two positive functions:

$$r_+(t) = \begin{cases} r(t), & 0 \leqslant t \leqslant t_1, \\ 0, & t > t_1, \end{cases}$$
$$r_-(t) = \begin{cases} 0, & 0 \leqslant t \leqslant t_1, \\ -r(t), & t > t_1, \end{cases} \qquad r(t) = r_+(t) - r_-(t) \tag{B.1}$$

that contribute separately to the output:

$$J^+_{out}(t) = J_{in}(t) * r_+(t), \qquad J^-_{out}(t) = J_{in}(t) * r_-(t). \tag{B.2}$$

Consequently

$$\left|\frac{\mathrm{d}J_{out}(t)}{\mathrm{dt}}\right| = \begin{cases} \dfrac{\mathrm{d}J^+_{out}(t)}{\mathrm{dt}}, & 0 \leqslant t \leqslant t_1, \\ \dfrac{\mathrm{d}J^+_{out}(t)}{\mathrm{dt}} - \dfrac{\mathrm{d}J^-_{out}(t)}{\mathrm{dt}}, & t_1 < t \leqslant t_2, \\ \dfrac{\mathrm{d}J^-_{out}(t)}{\mathrm{dt}} - \dfrac{\mathrm{d}J^+_{out}(t)}{\mathrm{dt}} & t_2 < t \end{cases} \tag{B.3}$$

and similarly

$$\frac{\mathrm{d}J_{in}(t)}{\mathrm{dt}} * |r(t)| = \begin{cases} \dfrac{\mathrm{d}J^+_{out}(t)}{\mathrm{dt}}, & 0 \leqslant t \leqslant t_1, \\ \dfrac{\mathrm{d}J^+_{out}(t)}{\mathrm{dt}} + \dfrac{\mathrm{d}J^-_{out}(t)}{\mathrm{dt}}, & t_1 < t. \end{cases} \tag{B.4}$$

Subtracting eqn (B.4) from eqn (B.3), a difference results which is not null:

$$\Delta(t) = \begin{cases} 0, & 0 \leqslant t \leqslant t_1, \\ -2\dfrac{\mathrm{d}J^-_{out}(t)}{\mathrm{dt}}, & t_1 < t \leqslant t_2, \\ -2\dfrac{\mathrm{d}J^+_{out}(t)}{\mathrm{dt}}, & t_2 < t. \end{cases} \tag{B.5}$$

This function is continuous, since $r_-(t)$ is zero before t_1 and

$$\frac{\mathrm{d}J_{out}(t_2)}{\mathrm{dt}} = \left[\frac{\mathrm{d}J^+_{out}(t)}{\mathrm{dt}} - \frac{\mathrm{d}J^-_{out}(t)}{\mathrm{dt}}\right]_{t=t_2} = 0.$$

Additionally, for monotonic output transitions it only depends on the negative part of the response, since t_2, the time at which J_{out} is maximum, tends to infinity.