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Cooperativity and Saturation in Biochemical Networks: A Saturable Formalism Using Taylor Series Approximations

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ABSTRACT: Cooperative and saturable systems are common in molecular biology. Nevertheless, common canonical formalisms for kinetic modeling that are theoretically well justified do not have a saturable form. Modeling and fitting data from saturable systems are widely done using Hill-like equations. In practice, there is no theoretical justification for the generalized use of these equations, other than their ability to fit experimental data. Thus it is important to find a canonical formalism that is (a) theoretically well supported, (b) has a saturable functional form, and (c) can be justifiably applicable to any biochemical network. Here we derive such a formalism using Taylor approximations in a special transformation space defined by power-inverses and logarithms of power-inverses. This formalism is generalized for processes with n-variables, leading to a useful mathematical representation for molecular biology: *the Saturable and Cooperative Formalism* (*SC formalism*). This formalism provides an appropriate representation that can be used for modeling processes with cooperativity and saturation. We also show that the Hill equation can be seen as a special case within this formalism. Parameter estimation for the SC formalism requires information that is also necessary to build Power-Law models, Metabolic Control Analysis descriptions or (log)linear and Lin-log models. In addition, the saturation fraction of the relevant processes at the operating point needs to be considered. The practical use of the SC formalism for modeling is illustrated with a few examples. Similar models are built using different formalisms and compared to emphasize advantages and limitations of the different approaches.

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Introduction

Mathematical formalisms based on approximated representations have a long tradition in science. Application of approximation theory to obtain a practical non-lineal approximation to complicated kinetic functions was first proposed by Savageau in the late 1960s and led to the Power-Law formalism (Savageau, 1969a,b, 1970). This formalism is derived by using a Taylor series approximation to a kinetic function in logarithmic coordinates. In brief, the resulting representation for a velocity v_i that depends on different metabolites and effectors, $X_1,\ldots,X_n$, is

$$v_i(X_i,\ldots X_n) = \gamma_i \prod_{j=1}^{n} X_j^{f_{ij}} \tag{1}$$

where

$$f_{ij} = \left(\frac{\partial v_i}{\partial X_j}\frac{X_j}{v_i}\right)_0 \tag{2}$$

is the apparent kinetic order (local sensibility) of v_i with respect to X_j. The subindex $_0$ indicates evaluation at a given

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operating point $X_{10},\ldots,X_{n0}$. The apparent rate constant γ_i is calculated in order to give a velocity v_{i0} when the concentrations of all the metabolites are at their operating point. As it has been shown elsewhere (see for instance Curto et al., 1995, 1997, 1998b), the kinetic-orders in the Power-Law formalism are equivalent to the elasticities of Metabolic Control Analysis (MCA). Mathematical models based on the Power-Law formalisms have been extensively used for investigating different classes of problems in Systems Biology through a set of methods known as Biochemical Systems Theory (Voit, 2000). Among others, the most relevant results concern characterizing design (Alves and Savageau, 2000a,b, 2001; Atkinson et al., 2003; Hlavacek and Savageau, 1995, 1996, 1997; Savageau, 1974, 1977; Schwacke and Voit, 2004; Wall et al., 2003, 2004) and operational principles in metabolism (Almeida and Voit, 2003; Alvarez-Vasquez et al., 2004, 2005; Sims et al., 2004; Vilaprinyo et al., 2006; Voit, 2003; Voit and Radivoyevitch, 2000), identifying network structures (Alvarez-Vasquez et al., 2005; Alves et al., 2004a,b; Berg et al., 1996; Curto et al., 1998a,b; Ferreira et al., 2003; Sims et al., 2004; Voit, 2002, 2003; Voit and Riley, 2003; Voit et al., 2006), and optimizing metabolic pathways in biotechnological applications (Hatzimanikatis et al., 1996; Thomas et al., 2004; Torres and Voit, 2002; Voit, 1992). The mathematical models provided by the Power-Law formalism are suited for dynamical simulations as well. This allows parameter estimation from time course data, which is an important issue in System Biology (Mocek et al., 2005; Polisetty et al., 2006; Schwacke and Voit, 2005; Voit and Almeida, 2004; Voit et al., 2004, 2005, 2006).

Recently, other authors have proposed approaches closely related to the Power-Law formalism. This is the case of the *(log)linear* (Hatzimanikatis and Bailey, 1996; Hatzimanikatis et al., 1998) and *Lin-log* (Visser and Heijnen, 2002, 2003) approaches. Although the resulting mathematical approximations are different, the fundamental parameters, MCA elasticities in the case of (log)linear and Lin-log representations, are equivalent. However, a recent comparison of methods has introduced some confusion regarding this equivalence (Heijnen, 2005), and the global advantages of the alternative formalisms, if any, remain to be established by appropriate case studies.

In general, the current approximated representations have a low range of accuracy when saturation and cooperativity are to be represented. Piece-wise Power-Law representations can deal with this problem at the cost of some additional computations (Igoshin et al., 2006; Savageau, 2002). However, because none of the formalisms mentioned above provides saturable rate expressions, if a model needs to include saturation and cooperativity, one may feel that approximation theory is not appropriate and thus switch to more complicated kinetic representations.

Under conditions where the rate has a sigmoid dependence with respect to some substrate(s) or modifier(s), a preferred mathematical function is the Hill equation (Hill, 1910). Its most common form is

$$v = \frac{V_m x^{n_H}}{K_m^{n_H} + x^{n_H}} \tag{3}$$

In its original derivation, the so-called Hill coefficient n_H corresponds to the number of binding sites in the molecule that catalyzes the process. Often, when processes are catalyzed by multi-subunit protein complexes, there is only one binding site per subunit. In such cases, the theoretical Hill coefficient would correspond to the number of subunits in the complex. However, in most cases, the actual mechanism is different from that for which the original Hill equation has been derived. Furthermore, in many cases, cooperativity is weak and the Hill coefficient that is obtained after fitting Equation (1) to the experimental data is different from the number of the existing binding sites (Weiss, 1997). For example, in the original article by Hill (1910) on the binding of oxygen to tetrameric hemoglobin, the measured values for n_H range from 1.7 to 3.2, well below the theoretical value of 4. In practice, a measured value of $n_H > 1$ is taken as an indication of cooperativity in the underlying mechanism.

Rounding off non-integer values for the Hill coefficient found while fitting data and using the derived integer as a measure of the number of binding sites is in general not correct. The non-integer values cannot be attributed exclusively to experimental error, because such values also depend upon the experimental conditions. For example, in a study on *Dictyostelium discoideum* mutants, the enzyme phosphofructokinase (EC. 2.7.1.11) shows a cooperative kinetics with respect to Fructose-6-P (F6P). The Hill coefficient for F6P is 2.8 at pH 8.0, 3.8 at pH 7.2, and 3.7 at pH 6.4 (Santamaria et al., 2002). In a different study, the rate of hydrolysis of 1-naphthyl phosphate, phenyl phosphate, and of phosphotyrosine as catalyzed by human prostatic acid phosphatase (EC 3.1.3.2) was measured and fitted to Hill equations. The corresponding Hill coefficients vary between 1.08 and 3.59 depending on the substrate and the enzyme concentration used in the experiment (see Table I in Luchter-Wasylewska, 2001).

In all these examples, the Hill equation is used as an appropriate phenomenological model that provides good fitting to the available data. When it comes to considering the simultaneous effect of different metabolites no general simple model similar to the Hill equation has been derived. Although some equations that account for reversibility and include the effect of two modifiers have been derived (Hofmeyr and Cornish-Bowden, 1997), the Hill equation is mainly used to capture the cooperative of individual metabolites at fixed values of all other metabolites that are involved in the process.

Moreover, cooperativity is not a phenomenon exclusively related to the number of active subunits (sites) in multimeric enzymes. Phenomena such as ultrasensitivity, a special case of cooperativity as an emergent property of a

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Table I. Steady-state values for the reference system at different X_{50} values.

	X_{50}		
	2.5	1	0.4
X_1	3.6768	0.9531	0.2898
X_2	1.4150	1.3975	1.2105
X_3	1.4150	1.3975	1.2105
X_4	5.3549	0.7156	0.0244
v_1	11.4286	8	4.5714
v_2	11.4286	8	4.5714
v_3	4.6874	4.6632	4.3809
v_4	4.6874	4.6632	4.3809
v_5	6.7411	3.3368	0.1905
v_6	6.7411	3.3368	0.1905

These are the three operating points for computing the Power-Law, Lin-Log/(log)linear, and SC approximations. Units are arbitrary.

simple system, arises in pairs of inactive/active (usually related to regulation by phosphorylation) enzymes that have simple Michaelis–Menten kinetics (Goldbeter and Koshland, 1981). Also, signal amplification through cascade systems can result in cooperativity-like relationships (Markevich et al., 2004). In addition, it has been suggested that simple reaction schemes, such as the classical one-substrate Michaelis–Menten mechanism, can show cooperative kinetics if the reaction takes place in micro-heterogeneous environments (Savageau, 1993, 1995, 1998). In such a situation, the elementary chemical kinetics description may involve fractional kinetic orders. Finally, if the enzyme concentration is comparable to the concentration of substrate, the common assumptions of the Michaelis–Menten equation may be invalid in situ (Tzafriri, 2003). This is so because the quasi-steady-state assumption that allows the derivation of the Michaelis–Menten expression is violated. Such experimental conditions can lead to a non-hyperbolic dependence of the rate with respect to the substrate. Although the mechanism underlying this situation may be simple, in general it is not possible to obtain an explicit rate expression that can be fitted to non-hyperbolic kinetic data. Thus, the use of the Hill equation to describe the rates of all these processes cannot be justified in terms of mechanism, although it is a convenient function for fitting the experimental data (Angeli et al., 2004).

Finding a rationale that justifies (a) using the Hill equation for fitting any experimental data and (b) extending the use of the Hill equation to fit data for several variables simultaneously is, thus, an open question. Such a generalized representation would provide a useful tool for modeling purposes when no alternative kinetic functions were available. This suggests investigating the problem from the point of view of approximation theory. Following the rationale behind the development of the Power-Law formalism (Savageau, 1969a,b, 1970), in this article we present a novel approximation leading to a formalism that complements the properties of the Power-Law representation. By deriving a Taylor series approximation to a function of m variables in a space of power-inverse transformations we obtain a special non-linear representation of the target kinetic function: the *Saturable and Cooperative approximation* (*SC formalism*). We show that the Hill equation can be obtained as a special case of the SC formalism, when there is a single metabolite involved in the process of interest. This could explain the success of the equation in fitting kinetic data of processes with unknown mechanisms (Cornish-Bowden and Koshland, 1975).

The SC formalism can be a practical tool from a modeler's point of view. The examples we present suggest that this formalism may be accurate over a wide range about the operating point of the local representation. The SC model could be used in tandem with the Power-Law model in the following way. The Power-Law model in its S-system representation can be used for closed form analysis of the steady-state properties while the SC model can be used for numerical analysis. For a process that depends upon several metabolites and regulators, the SC formalism provides a well-structured representation that can be set-up directly from a metabolic scheme in a similar way to Power-Law models and other alternative representations. This is particularly useful when parts of the problem are represented as a single aggregated process (for instance a black box, e.g., Hooshangi et al., 2005).

The Saturable and Cooperative Formalism: A Taylor Series Approximation After a Power-Inverse Transformation

Transformation to the Space of Power-Inverses

Our goal is to develop a mathematical formalism that provides an accurate representation for the rate of saturable and cooperative processes that depend on several variables. These processes may either be elementary reactions, or aggregated sets of reactions considered as a black box. We start by considering the rationale behind the Power-Law formalism within BST. The rate expressions are approximated using Taylor series after a logarithmic transformation of the original function and variables. This approximation becomes a Power-Law representation after a back transformation to a linear space (see Voit, 2000 and references therein). Using alternative transformations is theoretically possible, as far as we can reverse the transformation and obtain a useful representation (Salvador and Savageau, unpublished work). One possibility is to use inverse transformations, as these are commonly used to linearize simple kinetic rate-laws. The inverse transformation leads to linear representations in Michaelis–Menten kinetics and has been extensively used for obtaining the corresponding kinetic parameters (e.g., Lineweaver–Burk plot):

$$v = \frac{V_m S}{K_m + S} \quad w = \frac{1}{v} = \frac{K_m}{V_m}\frac{1}{S} + \frac{1}{V_m} \rightarrow w = a + bS^{-1} \qquad (4)$$

A simple inverse transformation does not provide a linear result for other types of enzyme mechanisms. For example, if we consider the competitive inhibition:

$$v = \frac{V_m S}{K_m\left(1+\frac{I}{K_i}\right)+S} = \frac{V_m S I^{-1}}{K_m\left(I^{-1}+\frac{1}{K_i}\right)+SI^{-1}}$$
$$w = \frac{1}{v} = \frac{K_m}{V_m}\frac{1}{S} + \frac{K_m}{K_i}\frac{1}{V_m}\frac{1}{S}\frac{1}{I^{-1}} + \frac{1}{V_m} \quad (5)$$

If, in Equation (5), we fix $S = S_0$, where the subscript $_0$ indicates evaluation of this expression at the operating point, we obtain

$$w = \frac{1}{v} = \frac{K_m}{V_m}\frac{1}{S_0} + \frac{K_m}{K_i}\frac{1}{V_m}\frac{1}{S_0}\frac{1}{I^{-1}} + \frac{1}{V_m} \rightarrow w = a_s + b_s I^1$$
$$a_s = \frac{K_m}{V_m}\frac{1}{S_0} + \frac{1}{V_m}$$
$$b_s = \frac{K_m}{K_i} + \frac{1}{V_m}\frac{1}{S_0} \quad (6)$$

If we now fix $I = I_0$, we obtain

$$w = \frac{1}{v} = \frac{K_m}{V_m}\frac{1}{S} + \frac{K_m}{K_i}\frac{1}{V_m}\frac{1}{S}\frac{1}{I_0^{-1}} + \frac{1}{V_m} \rightarrow w = a_1 + b_1 S^{-1}$$
$$a_I = \frac{1}{V_m}$$
$$b_I = \frac{K_m}{V_m} + \frac{K_m}{K_i}\frac{1}{V_m}\frac{1}{I_0^{-1}} \quad (7)$$

Thus, in the case of the substrate, an inverse transformation leads to a linear function for a fixed value of the inhibitor. In the case of the inhibitor, an inverse–inverse transformation is required. This is common to other one-substrate one-inhibitor simple mechanisms. In terms of approximating the rate-law, these transformations provide an accurate representation as far as we do not move too far away from the selected S_0 or I_0. Thus, using different transformations for each variable is an option for obtaining a useful approximation in the general case.

With this in mind, we explore transformations of the form $z_i = x_i^{-n_i}$ (i.e., a power-inverse transformation) where n_i can be different for each of the metabolites involved in the considered mechanism. This is a generalization of the approach used to derive the Power-Law formalism, were all the variables undertake the same (logarithmic) transformation.

We start by considering a kinetic function that depends on m concentration variables $(x_i,\ldots,x_m)$, namely

$$v = F(x_i, \ldots, x_m) \quad (8)$$

Here, F is a non-linear function that depends on the underlying mechanism of the process. This function is monotonically growing with respect to each substrate or positive effector, while it is monotonically decreasing with respect to each product or negative effector. Furthermore, F is a rational function that may saturate at different values for each variable. The saturation value for one variable will most likely depend on the fixed values that the other variables take. This set of conditions is common to most kinetic processes to be considered in metabolic pathways.

We now approximate F (unknown or not) in a similar way to that used to derive models under the Power-Law formalism. We start by transforming the original function into power-inverse coordinates of the form

$$w = v^{-1}, \quad z_i = x_i^{-ni}, \quad i = 1, \ldots, m \quad (9)$$

where n_i are real exponents, with values determined to provide the best transformation of F in terms defined below. Using this transformation, the resulting functional dependence (G) in the new variables can be written as:

$$w = G(z_i, \ldots z_m) \quad (10)$$

We can now use a Taylor series to approximate Equation (10) at a selected operating point.

First-Order Taylor Approximation in a Power-Inverse Space

Assume that we are interested in approximating the function G in the neighborhood of an operational point $z_0 = (z_{01},\ldots,z_{0m})$. Then, by means of a standard Taylor expansion we have:

$$w = w_0 + \sum_{i=1}^{m} \left.\frac{\partial G}{\partial z_i}\right|_{z_0} (z_i - z_{0i}) + h.o.t. \quad (11)$$

Truncating the Taylor expansion at the first order term we get

$$w \approx a + \sum_{i=1}^{m} b_i z_i$$
$$a = w_0 - \sum_{i=1}^{m} \left.\frac{\partial G}{\partial z_i}\right|_{z_{0i}}$$
$$b_i = \left.\frac{\partial G}{\partial z_i}\right|_{z_0} = \left.\frac{\partial v^{-1}}{\partial x_i^{-n_i}}\right|_{z_0} \quad (12)$$

Hereafter the symbol $\approx$ shall denote equality to first order in the sense of the Taylor expansion around the specified point. Once the transformation is reversed we obtain a

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rational approximation for the rate with the following non-linear functional form:

$$v \approx \frac{1}{a + \sum_{i=1}^{m} \frac{b_i}{x_i^{n_i}}} = \frac{\prod_{j=1}^{m} x_j^{n_j}}{a \prod_{j=1}^{m} x_j^{n_j} + \sum_{i=1}^{m} b_i \prod_{\substack{j=1, \\ j \neq i}}^{m} x_j^{n_j}}$$

$$= \frac{a^{-1} \prod_{j=1}^{m} x_j^{n_j}}{\prod_{j=1}^{m} x_j^{n_j} + a^{-1} \sum_{i=1}^{m} b_i \prod_{\substack{j=1, \\ j \neq i}}^{m} x_j^{n_j}} \qquad (13)$$

In this approximation the x_j are internal metabolites, regulators, enzymes, or other variables, while a and b are parameters. To better understand the physical meaning of the parameters for this approximation, let us consider the case with a single variable, that is

$$v = F(x) \approx \frac{1}{a + \frac{b}{x^n}} = \frac{x^n}{ax^n + b} = \frac{a^{-1}x^n}{x^n + a^{-1}b} \qquad (14)$$

If $n > 0$ in Equation (14), v saturates at $V_a = a^{-1}$. Furthermore, the rate will be half of its saturation value when the concentration of x is $x = \sqrt[n]{b/a}$. The case $n < 0$ will be discussed together with the m-variable function, below.

Let us now consider the local sensitivity (*kinetic-order* in the Power-Law formalism, *elasticity* in MCA) of the original function to changes in the dependent variable at the operational point, that is

$$f = \left(\frac{\partial v}{\partial x}\frac{x}{v}\right)_0 \qquad (15)$$

Computing the local sensitivity of Equation (14) we obtain

$$f = \left(\frac{\partial v}{\partial x}\frac{x}{v}\right)_0 = \frac{a^{-1}b\,n}{a^{-1}b + x_0^n} \qquad (16)$$

where the subscript $_0$ indicates evaluation of this expression at the operating point. Using Equation (16) we can now rewrite the parameters in Equation (12) as

$$b = \left(\frac{\partial w}{\partial z}\right)_0 = \left(\frac{\partial v}{\partial x^{-n}}\right)_0 = v_0^{-1} f \frac{x_0^n}{n} \qquad (17)$$

and

$$a = V_a^{-1} = v_0^{-1}\left(\frac{1-f}{n}\right) \qquad (18)$$

Because the space of the transformation is defined by the value of n, we obtain different approximations if we consider an inverse transformation with $n = 1$, an inverse square transformation with $n = 2$, or any other real value for n. Once we fix the value for n, Equation (14) has the following properties: (i) it has a value of v_0 at the operating point, (ii) its sensitivity is f at the operating point, and (iii) it will saturate at $v_0(1 - f/n)^{-1}$ (Fig. 1). Thus, choosing a value for n will fix the saturation value of Equation (14).

If the target function saturates at V_m, the most reasonable value for n is such that $V_a = V_m$. This fixes the value of a. Let $p = v_0/V_m$ define the fraction of saturation at the operating point. Then one can prove that

$$\begin{aligned} n &= \tfrac{f}{(1-p)} \\ a^{-1}b &= \tfrac{(1-p)}{p} x_0^n \end{aligned} \qquad (19)$$

For example, in Figure 1, the function saturates at $V_m = 10$, leading to $p = 0.51$ and $n = 3.5$ (which are the values corresponding to the reference curve shown in a continuous line in Fig. 1). If one chooses different values for n, the resulting function would saturate at different value than the reference function.

In the case of a single variable, Equation (14) can also be written as

$$v = \frac{V_m x^n}{\frac{(1-p)}{p} x_0^n + x^n} \rightarrow \frac{V_m x^n}{K + x^n} \qquad (20)$$

This is formally equivalent to a Hill equation, although it is derived from a completely different perspective. Equation (20) arises from a Taylor series approximation

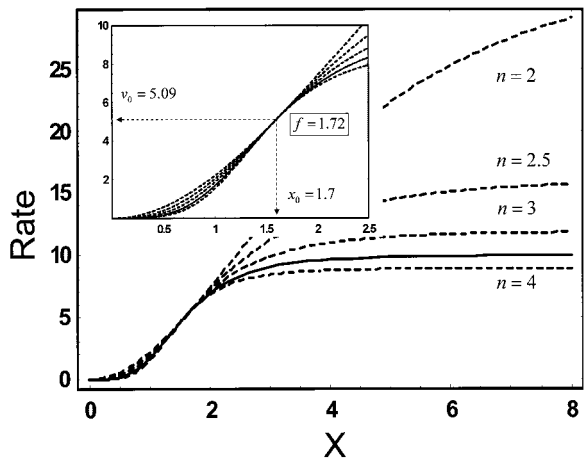

Figure 1. Approximation of a function using the power-inverse transformation. All functions cross and have the same sensitivity (slope) at the operating point. Different values of n lead to different saturating values for the approximation functions (dashed lines). The target function is indicated by a continuous line. The inset figure is an amplification showing the correspondence of the different approximations at the operating point.

in a power-inverse transformation space that fulfils three requisites: (i) it crosses the original function at (x_0, v_0), (ii) it has a sensitivity equal to that of the target function at the operating point, and (iii) it saturates with the same value as the target function. Furthermore, it is interesting to note that the expression for n obtained above is formally equivalent to that formulated by Hill in its seminal article of 1910 (Hill, 1910). The classical definition is:

$$n_{\mathrm{H}} = \frac{\mathrm{dLn}(f_{\mathrm{sat}})}{\mathrm{dLn}(L)} \frac{1}{(1-f_{\mathrm{sat}})} \tag{21}$$

Where f_{sat} is the fractional saturation of the protein and L is the concentration of the free ligand. In terms of an enzyme reaction, and using the nomenclature introduced in our article, we can write:

$$\begin{aligned} n_{\mathrm{H}} &= \frac{\mathrm{dLn}(p)}{\mathrm{dLn}(x)} \frac{1}{(1-p)} = \frac{(1/V_m)\mathrm{d}v}{\mathrm{d}x} \frac{x}{v/V_m} \frac{1}{(1-p)} \\ &= \frac{\mathrm{d}v}{\mathrm{d}x} \frac{x}{v} \frac{1}{(1-p)} = \frac{f}{(1-p)} \end{aligned} \tag{22}$$

Hence, the use of a truncated Taylor series in a power-inverse space to derive a non-linear approximation of the unknown target one-substrate function produces a Hill-like representation. Thus, our derivation provides a theoretical justification for the success of the Hill equation in fitting experimental data as it shows that the equation is a non-linear approximation to the underlying unknown rate-law.

The case with m variables can be discussed using similar arguments to those for the one-variable case. Consider Equation (13). If all variables are at the operating point except x_{k}, we can write

$$v = \frac{a^{-1}\prod_{j=1}^{m} x_j^{n_j}}{\prod_{j=1}^{m} x_j^{n_j} + a^{-1}\prod_{i=1}^{m} b_i \prod_{\substack{j=1 \\ j\neq 1}}^{m} x_j^{n_j}} \xrightarrow{(x_{10},\ldots,x_{\mathrm{k}},\ldots,x_{m0})} \frac{V_{\mathrm{k}} x_{\mathrm{k}}^{n_{\mathrm{k}}}}{K_{\mathrm{k}} + x_{\mathrm{k}}^{n_{\mathrm{k}}}} \tag{23}$$

V_{k} is the saturating value of v when $x_{\mathrm{k}} \to \infty$ and all the other variables are at their operating point values in the case $n > 0$. If $n < 0$, that is when x_{k} is an inhibitor, then V_{k} is the value of v when $x_{\mathrm{k}} \to 0$. Thus, if we define

$$\begin{aligned} p_{\mathrm{k}} &= v_0 / V_{\mathrm{k}} \\ f_{\mathrm{k}} &= \left(\frac{\partial v}{\partial x_{\mathrm{k}}} \frac{x_{\mathrm{k}}}{v}\right)_0 \end{aligned} \tag{24}$$

we obtain:

$$\begin{aligned} n_{\mathrm{k}} &= \frac{f_{\mathrm{k}}}{(1-p_{\mathrm{k}})} \\ K_{\mathrm{k}} &= \frac{(1-p_{\mathrm{k}})}{p_{\mathrm{k}}} x_{k0}^{n_{\mathrm{k}}} \end{aligned} \tag{25}$$

Furthermore

$$b_{\mathrm{k}} = \left(\frac{\partial w}{\partial z_{\mathrm{k}}}\right)_0 = \left(\frac{\partial v^{-1}}{\partial x_{\mathrm{k}}^{-n_{\mathrm{k}}}}\right)_0 = v_0^{-1} f_{\mathrm{k}} \frac{x_{\mathrm{k0}}^{n_{\mathrm{k}}}}{n_{\mathrm{k}}} \tag{26}$$

The parameters for the other $(m-1)$ variables in Equation (11) can be easily derived using a similar procedure. The information needed to calculate the parameter values is contained in the flux and metabolite concentrations at the operating point, and in the saturation fractions for each relevant metabolite. The value for a^{-1} is such that the value of Equation (13) equals the operating point value of the flux when all the variables take their nominal operating point values (see below).

First-Order Taylor Approximation in a Logarithmic Transformation of a Power-Inverse Space

The accuracy of the previous approximation can be further improved if we apply a logarithmic transformation to the power-inverse space. Because the power-inverse transformation does not guarantee a linearization of the target function, we can further transform it to logarithmic coordinates, thus obtaining a transformation that may be more appropriate for linear approximation. Our starting point is the equality

$$\log(w) = \log(G(z_1, \ldots z_m)) \tag{27}$$

with similar notation to Equation (10). Now making use of Proposition 1 in the Appendix on this expression, we get:

$$\begin{aligned} &\log(G(z_1, \ldots, z_m)) \\ &\quad \approx \log(\tilde{G}_1(z_1)) + \log(\tilde{G}_2(z_2)) + \ldots + \log(\tilde{G}_m(z_m)) \\ &\qquad + (1-m)\log(G(z_{01}, \ldots, z_{0m})) \end{aligned} \tag{28}$$

where $\tilde{G}_i(z_i) \equiv G(z_{01}, \ldots, z_{0,i-1}, z_i, z_{0,i+1}, \ldots, z_{0m})$ for all $i = 1,\ldots,m$. Proposition 2 (Appendix) can be used to convert Equation (27) into:

$$\begin{aligned} &\log(G(z_1, \ldots, z_m)) \\ &\quad \approx \log(\tilde{G}_1(z_{01}) + \tilde{G}_1'(z_{01})(z_1 - z_{01})) \\ &\qquad + \log(\tilde{G}_2(z_{02}) + \tilde{G}_2'(z_{02})(z_2 - z_{02})) + \ldots \\ &\qquad + \log(\tilde{G}_m(z_{0m}) + \tilde{G}_m'(z_{0m})(z_m - z_{0m})) \\ &\qquad + (1-m)\log(G(z_{01}, \ldots z_{0m})) \end{aligned}$$

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which can be written as

$$\log(G(z_1,\ldots,z_m)) \approx \log\left(G(z_{01},\ldots,z_{0m}) + \left.\frac{\partial G}{\partial z_1}\right|_{z_0}(z_1 - z_{01})\right) + \log\left(G(z_{01},\ldots,z_{0m}) + \left.\frac{\partial G}{\partial z_2}\right|_{z_0}(z_2 - z_{02})\right) + \cdots$$
$$+ \log\left(G(z_{01},\ldots,z_{0m}) + \left.\frac{\partial G}{\partial z_m}\right|_{z_0}(z_m - z_{0m})\right) + (1-m)\log(G(z_{01},\ldots z_{0m}))$$
$$= \log\left(G(z_{01},\ldots,z_{0m}))^{1-m}\prod_{i=1}^{m}\left(G(z_{01},\ldots,z_{0m}) + \left.\frac{\partial G}{\partial z_i}\right|_{z_0}(z_i - z_{0i})\right)\right) \quad (29)$$

Proposition 3 in the Appendix allows us to approximate Equation (28) as:

$$w = G(z_1,\ldots,z_m) \approx (G(z_{01},\ldots,z_{0m}))^{1-m}\prod_{i=1}^{m}\left(G(z_{01},\ldots,z_{0m}) + \left.\frac{\partial G}{\partial z_i}\right|_{z_0}(z_i - z_{0i})\right) \quad (30)$$

This is the foundation for a second kind of approximation, which is also based on a first-order Taylor expansion around an operating point. When the changes of variables are reversed, the result is an approximation in terms of functions that can be concisely written in the form

$$v \approx \frac{V\prod_{i=1}^{m} x_i^{n_i}}{\prod_{i=1}^{m}(K_i + x_i^{n_i})} \quad (31)$$

Where V, K_i, and n_i are real-valued constants. Following the same arguments and notation used previously, we have

$$n_i = \frac{f_i}{(1-p_i)}$$
$$K_i = \frac{(1-p_i)}{p_i}x_{i0}^{n_i} \quad (32)$$
$$V = v_0\frac{\prod_{i=1}^{m}(K_i + x_{i0}^{n_i})}{\prod_{i=1}^{m} x_{i0}^{n_i}}$$

For the general case, it is easily proven that Equation (13) is a particular case of Equation (31). As both approximations use the same information, that is: the operating point values of concentrations and fluxes, the local sensitivities, and the saturation fraction for each variable at the operating point, the approximation in Equation (31) is more general. Thus, hereafter, we use this approximation, unless otherwise stated. We call it *Saturating and Cooperative formalism* (*SC formalism*). In the next section, we present some examples of its use and further discuss its potential advantages.

Using the SC Formalism as a Modeling Tool

Mainly, the SC formalism can be very useful in situations in which we need to define a rate-law for a saturable process but no detailed information exists about the underlying mechanism. A Taylor approximation to the unknown function in transformed coordinates, first used to derive a Power-Law formalism (Savageau, 1969a,b, 1970; Voit, 2000), provides a solution by considering the operating point values and the sensitivity (kinetic order) of the rate with respect to the relevant variables. The SC formalism requires a supplementary piece of information: the saturation fraction of the process with respect to the relevant variable.

As an illustrative example, consider a process that has a kinetic order $f = 1.2$ at the operating point $x_0 = 10$, with $v_0 = 3$. Using this information, the Power-Law approximation to the rate of the process is

$$v = 0.19\,x^{1.2} \quad (33)$$

If we use the SC formalism given in Equation (31) we obtain the following result, which depends on the value for the saturation fraction (p)

$$n = \frac{f}{(1-p)} = \frac{1.2}{1-p}$$
$$K = \frac{(1-p)}{p}x_0^n = \frac{(1-p)}{p}10^n \quad (34)$$
$$V_m = v_0/p = 3/p$$
$$v = \frac{3/p\,x^{1.2/(1-p)}}{(1-p)p10^{1.2/(1-p)} + x^{1.2/(1-p)}}$$

In Figure 2 we compare both representations for different values of the saturation fraction. It is important to note that the same information leading to a given Power-Law representation leads to different saturating approximations because different values for the saturation fraction p change

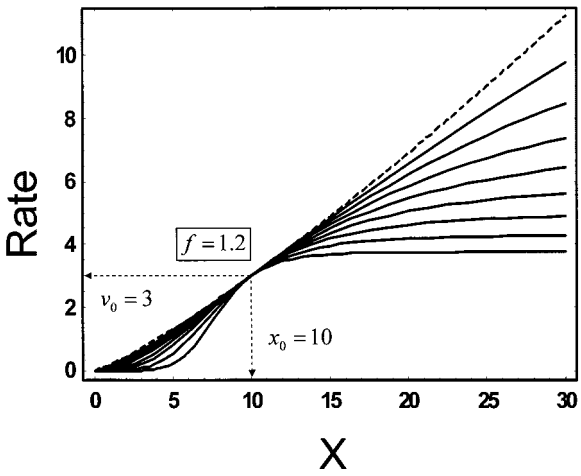

Figure 2. SC representation at different saturation fractions. Using the same information at the operating point, we obtain different representations in function of the saturation fraction considered (Continuous lines. Value of p from 0.1 (upper line) to 0.8 (lower line) in 0.1 steps). The Power-Law representation is indicated by a dashed line.

the resulting SC representation but not the Power-Law representation. By taking into account the saturation fraction, the SC results in a Hill-like representation that exhibits cooperativity and saturation.

For illustrative purposes, assume that we determine that at the operating point $p = 0.5$. In this case, Equation (34) becomes:

$$\left.\begin{array}{c} n = \dfrac{f}{(1-p)} = \dfrac{1.2}{1-0.5} = 2.4 \\ K = \dfrac{(1-p)}{p} x_0^n = \dfrac{(1-0.5)}{0.5} 10^{2.4} = 251.9 \\ V_m = v_0/p = 3/0.5 = 6 \end{array}\right\} \rightarrow v$$
$$= \frac{6x^{2.4}}{251.19 + x^{2.4}} \tag{35}$$

This representation can now be used in a model for computational and numerical goals. The mathematical derivation of the SC formalism ensures that this is a theoretically sound approximation to the unknown rate function and that both share the same sensitivity at the selected operating point and the same saturation for $x \rightarrow \infty$.

Let us consider now a process that depends on more than one variable. Assume that the process depends upon one substrate X_1 and one inhibitor X_2 and that $(X_{10}, X_{20}) = (10, 2)$, $v_0 = 5$, and $f_1 = 0.5$, $f_2 = -0.8$ at the operating point. Kinetic orders could have been estimated by any of the methods established for either BST or MCA (see for instance Voit, 2000). Finally, assume that the saturation fractions have been determined to be $p_1 = 0.7$ and $p_2 = 0.5$. Using this information, we obtain:

$$n_1 = \frac{0.5}{1-0.7} = 1.67 \quad n_2 = \frac{-0.8}{1-0.5} = -1.6$$
$$K_1 = \frac{1-0.7}{0.7} 10^{1.67} = 19.89 \quad K_2 = \frac{1-0.5}{0.5} 2^{-1.6} = 0.33 \tag{36}$$

and $V = 14.29$. Then, the function we shall use to model this process is:

$$v = \frac{14.29\, X_1^{1.67}\, X_2^{-1.6}}{(19.89 + X_1^{1.67})(0.33 + X_2^{-1.6})} \tag{37}$$

Parameter Estimation

Parameter estimation is a central issue in any formalism. In our case, we have shown how to calculate the parameter values once we have measures for: (i) the operating point values of the dependent variables and fluxes, (ii) the local sensitivity (kinetic orders or elasticities), and (iii) the saturation fraction. The values for the dependent variables and fluxes at the operating point can be experimentally measured. As stated above, local sensitivities can be determined by the methods used by BST and MCA for obtaining kinetic orders (elasticities). Estimations of the saturation fractions require additional experiments. In any case, if the operating point and the elasticity are known, we can consider different values of the saturation fraction and obtain alternative representations. We can then analyze the effect of different saturation fractions on the model behavior.

There are alternatives to estimate the parameters using the information of a single given operating point. We can instead use non-linear regression to obtain the parameters from a series of measurements that sample a given range of metabolite values (Hernández-Bermejo et al., 1999, 2000). The wide use of the Hill equation as a phenomenological model corresponds to this alternative. As an example of this approach, consider the mechanism shown in Figure 3.

If the usual simplifications of classical enzyme kinetics hold, this mechanism corresponds to the rate-law

$$v = \frac{V_m S}{K_m + S\left(1 + \frac{I}{K_i}\right)} \tag{38}$$

For illustrative purposes, consider the following parameter values: $V_m = 10$, $K_m = 5$, $K_i = 3$, and the operating point: $S_0 = 2$, $I_0 = 1$. Under these conditions, we obtain the following SC representation

$$v = \frac{8.21\, S^1 I^{-1}}{(3.75 + S^1)(0.095 + I^{-1})} \tag{39}$$

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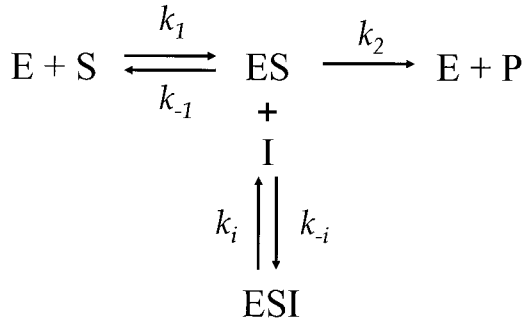

Figure 3. Uncompetitive inhibition mechanism. *k*s are elemental rate constants. E, free enzyme; ES, enzyme-substrate complex; ESI, enzyme-substrate-inhibitor complex; P, product.

Now assume that an experimental data series is available. We have simulated such a series by generating noisy data points for different values of S and I. The noise was introduced by adding a random number from a normal distribution with mean 0 and standard deviation 0.2. Adjusting a two-variable SC representation to this data series with non-linear regression we obtain

$$v = \frac{8.40\, S^{0.98} I^{-0.99}}{(3.16 + S^{0.98})(0.18 + I^{-0.99})} \qquad (40)$$

As expected, both representations are different and approximate with different accuracy the actual rate-law (Fig. 4). Both strategies use different information. The operating point characteristics are exactly represented by the first strategy, although the overall error in approximating a wider range of variation of the velocity is greater than the one corresponding to the least-squares strategy (see Hernández-Bermejo et al., 1999, 2000 for details). In practice, the least-squares strategy will provide a better approximation to the actual rate-law in the considered range because it uses information over the whole range of variation of the involved the variables.

The accuracy of the representation provided by the SC formalism depends on the selected operating point. In the

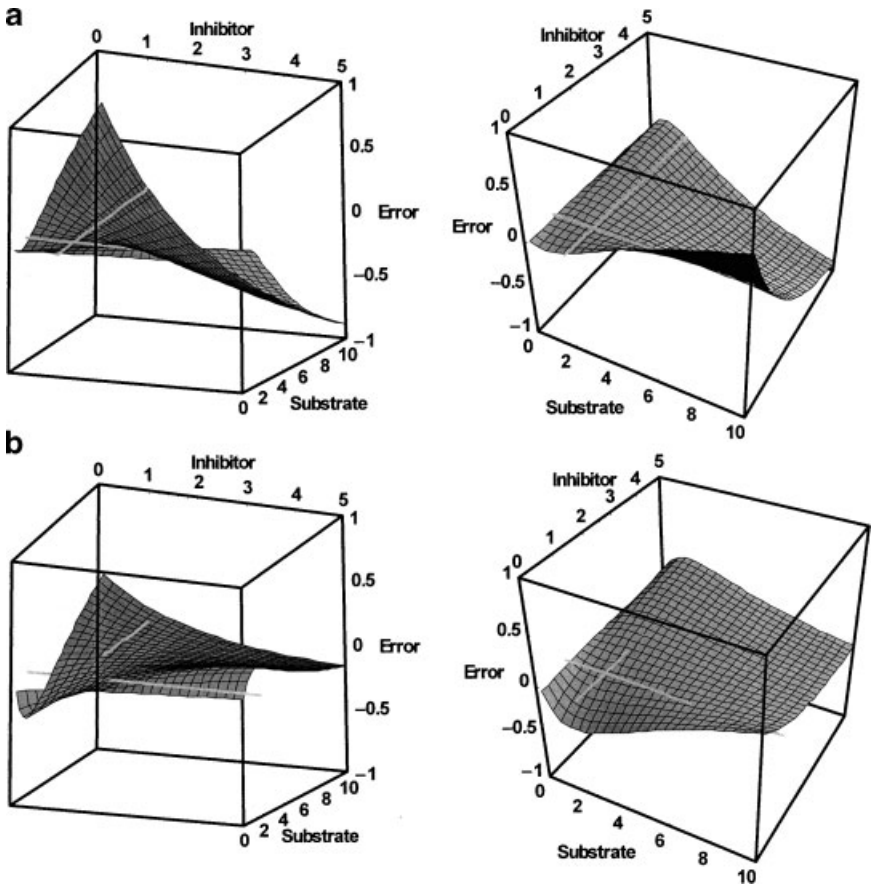

Figure 4. Approximating an uncompetitive mechanism with the SC formalism. The kinetic parameters for the uncompetitive mechanism are: $V_m = 10$, $K_m = 5$, $K_i = 3$. **a**: Error surface using the saturating approximation at the operating point $S_0 = 2$, $I_0 = 1$. **b**: Error surface using the saturating approximation obtained by mean-squares (see text for details).

case of the example used above, we can find the optimal point for making the minimum error when representing the target rate-law by the SC formalism. The optimal operating point is obtained through the following procedure:

1. Select a point.
2. Compute the SC approximation at this point.
3. Compute the squared sum of errors (SSE).
4. Run a minimization procedure until an operating point that minimizes SSE is found.

In our example, we obtain the best operating point at $S_0 = 7.33$, $I_0 = 3.20$. When we use the operating point values to estimate the parameters for the SC representation and then calculate the accumulated error of that representation with respect to the actual function over a range of substrate from 0 to 14, and a range of inhibitor from 0 to 14, we get an accumulated error with SSE = 4.1463. When we use the data points within the considered region to estimate the parameter values for the SC representation using non-linear regression and we calculate the accumulated error of that representation with respect to the actual function over the same range we get a SSE = 4.0998 (Fig. 5). Figure 5 makes it clear that the error increases rapidly for the parameter values estimated using a given operating point as the concentrations of substrate and inhibitor at the operating point decrease. On the contrary, the error of the least-squares approximation has a fixed value inside the concentration region used to estimate the parameters, when parameters are calculated using the SSE minimization strategy. This is so because all points within that region are used to estimate the parameter values. As a rule of thumb, non-linear regression would be preferable to estimate parameter values because it takes into account information over a range of the state space. Nevertheless, the choice between either strategy will depend upon the available information.

The SC formalism also provides a practical representation for cases in which no alternative kinetic formalism is available. Assume that the reactions shown in Figure 3 take place in an environment of restricted diffusion, for instance a membrane. In such conditions, it has been suggested that the elemental reactions may have fractional kinetics that are different from those observed in free diffusion (Savageau, 1993, 1995, 1998). Under conditions that restrict diffusion, we assume the following mechanism:

$$\begin{aligned}\frac{\mathrm{dES}}{\mathrm{d}t} &= k_{-i}\,\mathrm{ESI} + k_1 S^{g_s}(\mathrm{ET} - \mathrm{ES} - \mathrm{ESI})^{g_e} \\ &\quad - (k_2 + k_i + k_{-1})\mathrm{ES} \\ \frac{\mathrm{dESI}}{\mathrm{d}t} &= k_i I^{gi}\mathrm{ES}^{g_{es}} - k_{-i}\mathrm{ESI} \\ \mathrm{ET} &= \mathrm{E} + \mathrm{ES} + \mathrm{ESI} \end{aligned} \tag{41}$$

Here, we have considered that the bimolecular reactions follow fractional kinetics with exponents that can be different from one. If this is so, the quasi-steady-state equations of the mechanism cannot be solved explicitly. Assume a case where the values for the elemental rate constants are: $k_1 = 2$, $k_{-1} = 0.1$, $k_i = 0.2$, $k_{-1} = 0.1$, $k_2 = 3$. The fractional kinetic orders for the elemental mechanisms are: $g_e = 1$, $g_s = 2.8$, $g_i = 3.5$, $g_{es} = 1$. Assume also that ET = 5.

We can now use the kinetic rate-law for the classical uncompetitive inhibition mechanism and Equation (31) to approximate the rate of production of *P*. We estimate the parameters for both equations from the data points. Figure 6 shows that the SC formalism provides a more accurate approximation to the pseudo-experimental data points than the use of the classical enzyme kinetics rate expression. This is so because, although the mechanism corresponds to

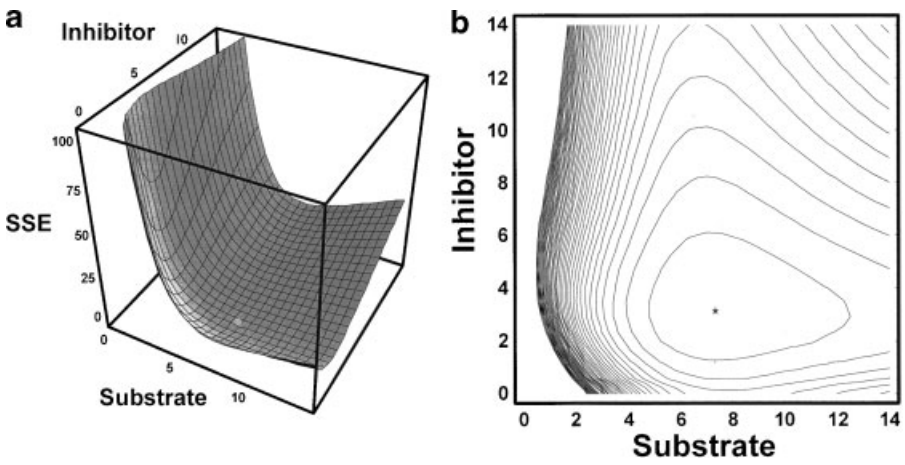

Figure 5. Approximating an uncompetitive mechanism with the SC formalism at different operating points. The kinetic parameters are the same as in Figure 4. **a**: Sum of square error as a function of the selected operating point, (**b**) Contour plot identifying the optimal point.

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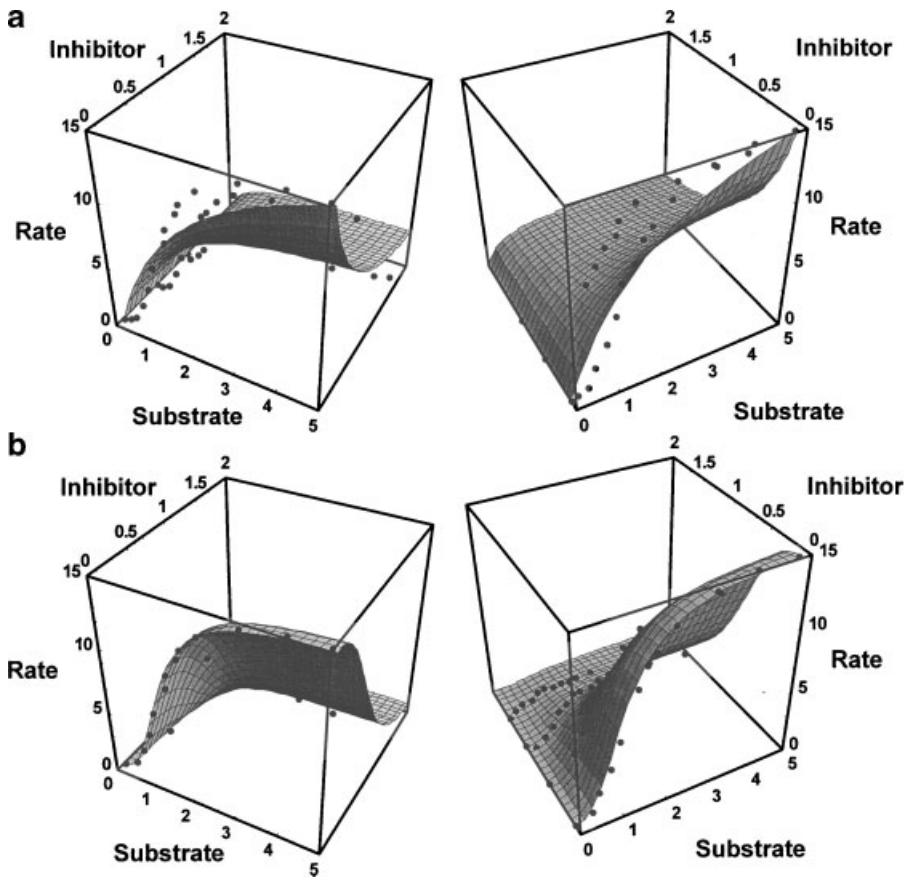

Figure 6. Fractional kinetics reflect an environment of restricted diffusion. Dots represent rate of product formation obtained by numerical computation using the equations for the detailed enzyme mechanism (see text), under different concentrations of substrate and inhibitor. **a**: Fitting obtained with the classical uncompetitive formalism. **b**: Fitting obtained with the SC formalism.

uncompetitive inhibition, the existence of fractional kinetics does not conform to the assumptions that allow the derivation of the classical rate expression.

Use of Approximate Representations to Build Mathematical Models for Systems Biology Applications

The possibility of obtaining different approximate representations to an unknown kinetic function leads to some confusion regarding which alternative should be used in a given application. The use of different nomenclature and the lack of an appropriate comparison (e.g., in Heijnen, 2005) introduces a high level of noise to an issue that should be centered on technical arguments. If we focus on a system's representation and dynamic simulations, two different approaches are now widely used: the Power-Law formalism, in either its S-system or Generalized Mass Action (GMA) forms, and the Lin-log or (log)linear representations. In this article, we provide an additional possibility with the SC formalism.

In order to illustrate the differences and similarities between the different approximations, we shall focus on an example that helps clarifying the limitations of the various representations. The Lin-log and (log)linear representations are fundamentally equivalent when enzyme concentrations are constant. This is the case in the example below. Therefore, we shall refer to them as Lin-Log/(log)linear from now on. Although more elaborated models and comparisons are required to generalize the statements regarding the advantages of using each of the formalisms, this simple example highlights some differences that are worth bearing in mind. Consider the model shown in Figure 7. The rate of each process in that model is described using kinetic equations that we assume accurate (see Appendix). We then derive the system of differential equations that describes the dynamical behavior of the system, using (a) the accurate equations, and (b) each of the alternative formalisms to approximate those equations. We do the approximations at three different operating points (see also the Appendix). Then we compare the steady-state prediction of each of the formalisms and the resulting time courses to those for the original model under various conditions.

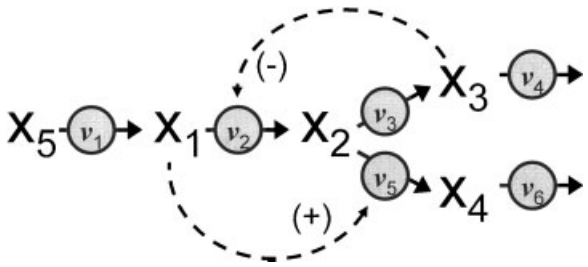

Figure 7. Metabolic network with one positive feedforward and a negative feedback. All enzymes follow a Michaelis–Menten kinetics except those corresponding to v_2 and v_5 that follow an Irreversible General Hyperbolic modifier kinetics and an Irreversible Hill kinetics with one modifier, respectively. The kinetic expressions are given in the Appendix.

The steady-state predictions obtained with each of the formalisms are compared with the reference steady-state curve in Figure 8. Clearly, the SC formalism can accurately predict the results almost independently of the operating point. When the operating point corresponds to low values of the independent variable, all the approximations produce worst predictions than the SC formalism. With the parameters chosen for the reference model, the system has no finite steady-state solution for high values of the independent variable. The SC also captures this important feature, while all the other alternative representations predict a finite steady-state. The S-system and the Lin-log/(log)linear models are equivalent with regards to the steady-state calculations, predicting the same straight line in log–log coordinates. Although the S-system model focus on aggregated fluxes, and the Lin-log/(log)linear model is build without aggregation by using a different function for each velocity, the particular structure of the Lin-log/(log)linear approximation leads to the same steady-state solution as the S-system model. This observation, that can be easily shown analytically, demonstrates that some of the arguments against the S-system form and in favor of the Lin-log/(log)linear approach were not appropriately founded (Heijnen, 2005). The GMA model generates a steady-state curve that predicts a non-linear dependence of the steady-state concentrations upon the values of the independent variables in log–log coordinates. These predictions, in the example, are more accurate than those generated using the S-system and Lin-log/(log)linear models. The generalization of this conclusion would require further investigation as previous analysis suggested that the S-system form can sometimes be more accurate (Voit and Savageau, 1987).

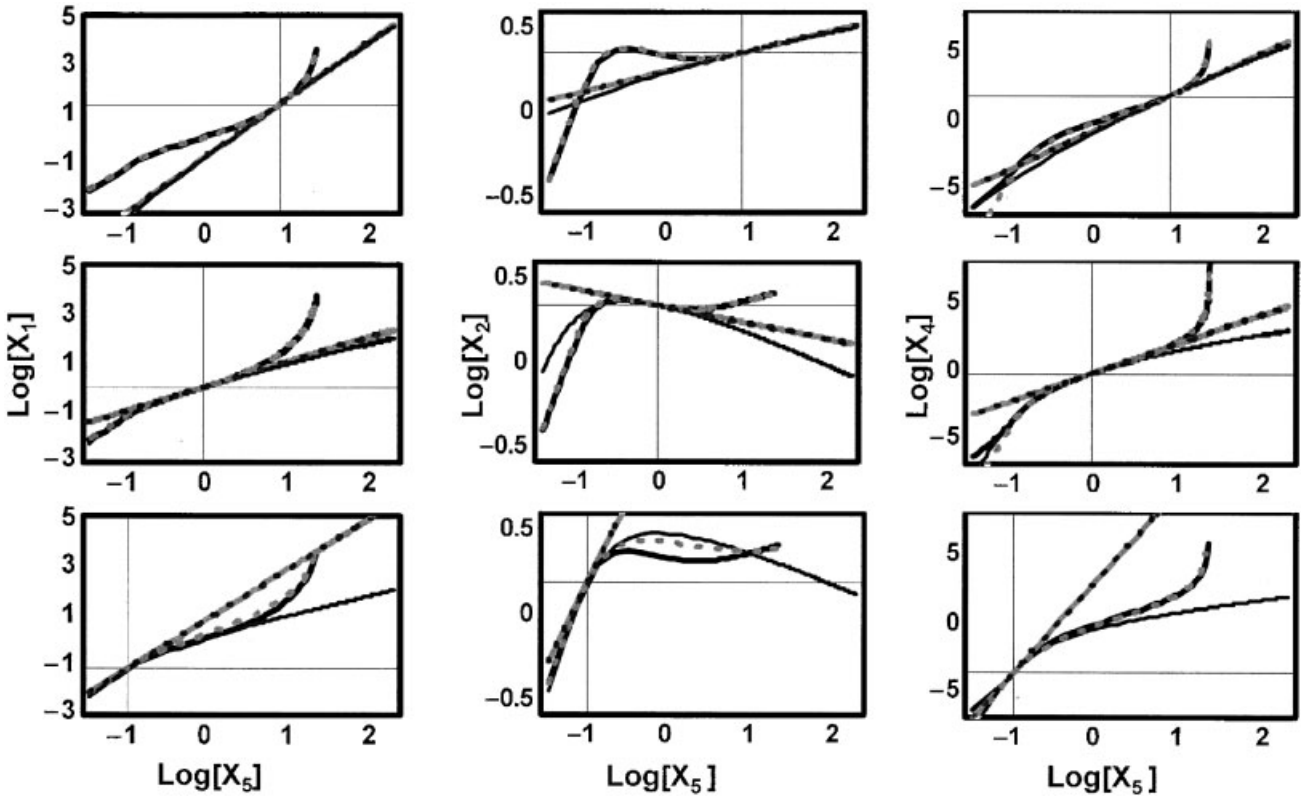

Figure 8. Steady-state curves of the dependent variables for the model in Figure 7 as a function of the independent variable (X_5). Results for X_2 and for X_3 are equal due to the particular parameter values selected. Lines: reference model (dark line), S-System (light line), GMA (slim dark line), Lin-Log/(log)linear (dashed light line), and SC formalism (dashed light line). The approximations were built at three different operating points (X_5 equal to 2.5, 1, and 0.4, from top to bottom rows).

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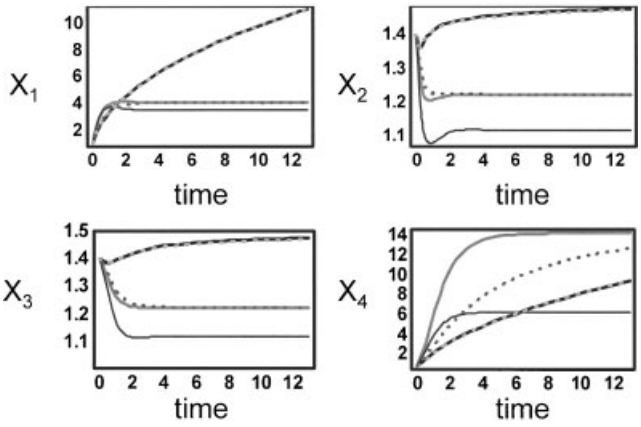

Figure 9. Dynamic response for the system of Figure 7 as the concentration of the independent variable (X_5) changes from 1 to 4. Lines follow the same pattern as in Figure 8.

The SC formalism is also able to capture an important qualitative feature of our example. The reference model shows a characteristic steady-state curve: the slope of the curve changes sign for different values of X_5. Of all representations, only the SC formalism can predict all these changes.

The dynamic response of the reference model has also been analyzed in order to compare the accuracy of each of the formalisms. For large increases in the independent variable, the SC is the only model that accurately reproduces the reference time-course (Figs. 9–11). Furthermore, all other alternative representations predict a decrease in X_2 and X_3, where both should increase. Although the time-course of the Lin-log/(log)linear model is similar to that of the S-system model, it is instructive to analyze the evolution of the rates of each reaction (Figs. 10 and 11). The Lin-log/(log)linear formalism fails in appropriately reproducing the changes in rates, predicting negative rates when the metabolites have values well below the operating point.

Although more general comparisons are needed, the present example shows that the SC formalism extends the accuracy of the local representation provided by Power-Law models and may be seen as a complementary representation. In that sense, the predictions resulting from the analysis of S-system models can further be investigated by exploring, for example, the effect of different saturation factors by means of the SC formalism. The obtained results may be more realistic when it comes to discuss the system response to large changes.

Discussion

Mathematical models play a central role in the analysis of complex metabolic networks by providing a tool for reproducing, understanding, and predicting experimental observations. They are invaluable for testing hypotheses about systemic design and operational principles. Identification of such principles is one of the main goals of Systems Biology. In pursuing such a goal, models must allow for testing hypotheses concerning evolutionary processes, regulatory influences, non-linear behaviors, dynamic responses, and so on. Thus, the models require the use of an adequate mathematical formalism that is able to successfully reproduce important dynamical features of metabolic networks. Furthermore, as the available experimental information may lack important details on the underlying mechanisms, the selected formalism should be able to provide insights under such conditions.

In this work we develop a mathematical formalism that meets those requirements. The Saturating and Cooperative formalism is derived as an approximate representation that provides certain advantages over other existing alternative formalisms. The most interesting feature is that, being a local

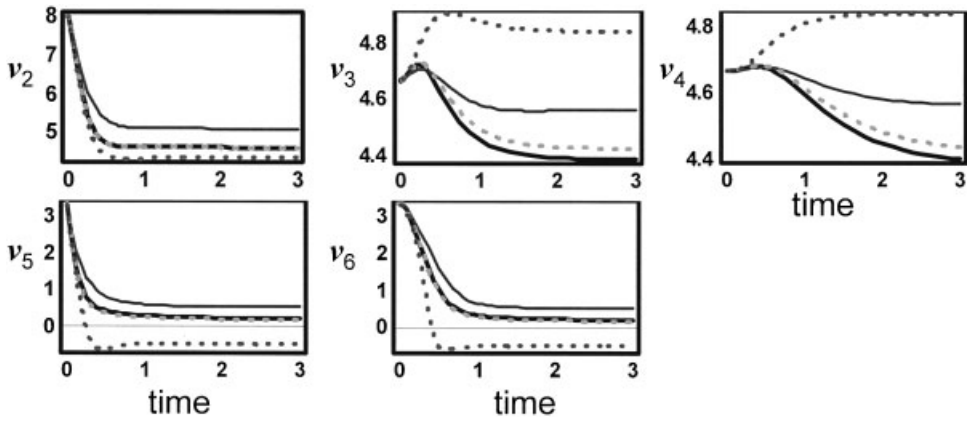

Figure 10. Changes in the rates of the reference system as the concentration of the independent variable (X_5) changes from 1 to 4. The initial values for the dependent variables are the corresponding concentrations at steady-state of X_5 equal to 1. Lines follow the same pattern as in Figure 8.

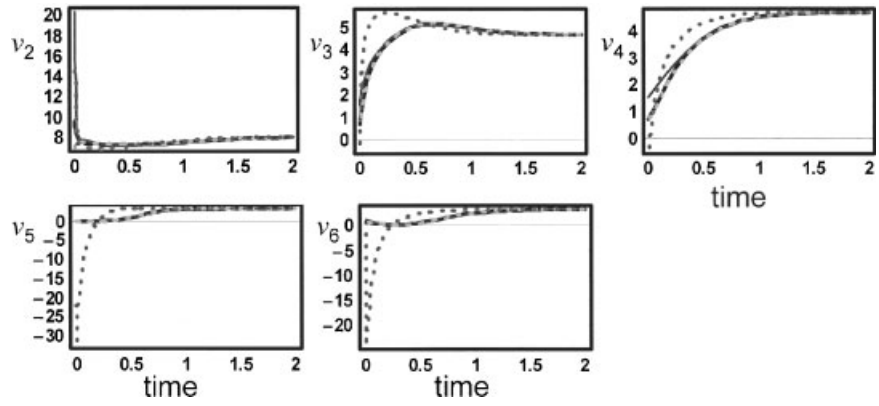

Figure 11. Changes in the rates of the reference system as the concentration of the independent variable (X_5) changes from 1 to 0.4. The initial values for the dependent variables are 0.1, simulating the situation where a system that is almost totally in the off state is activated by some external signal. Early rate values for the Lin-Log/(log)linear approximation are negative, although they recover in the time. Lines follow the same pattern as in Figure 8.

representation derived at a given operating point, it accounts for cooperativity and saturation. The SC formalism extends other existing approaches by following the ideas introduced by the Power-Law formalism. However, the straightforward algebraic analysis that can be done using S-systems models in BST is lost in models based on the SC formalism. Nevertheless, the SC formalism can be seen as a complementary extension than provides greater accuracy for numerical simulations. For instance, design principles can be analyzed using the S-system form of the Power-Law formalism to identify parameter constraints and optimal regulatory patterns. The SC formalism can then be used to explore in depth the resulting predictions in the dynamic domain by considering both the constraints corresponding to the local sensitivities and the effect of different saturation fractions.

In terms of modeling strategies, as we have shown here, a model based on the SC formalism can automatically be obtained from the scheme of the target system. Furthermore, as it happens with the Power-Law formalism as well, no precise details on the underlying mechanisms are required to obtain a model that is able to capture essential features of the involved processes. This makes the kind of approaches that use approximation theory an interesting alternative when complex networks are to be modeled and few kinetic data are available (see examples of the Power-Law approach in Alves et al., 2004a,b; Vilaprinyo et al., 2006). This is the case in almost every system in biotechnology and metabolic bioengineering.

The use of models built as systems of differential equations is a requisite for dealing with dynamic data. The results discussed in this work suggest that the SC formalism can be specially suited for this task. Our comparison of alternative approximated formalisms based on different strategies suggests that the SC performs with higher accuracy. Although more general comparisons are needed, our results show that the Lin-log/(log)linear formalism has important problems when computing dynamic changes. Arguments in favor of this formalism have been based on its appropriateness for approximating a kinetic function (Heijnen, 2005). However, this formalism leads to negative velocities when we move towards low relative values of the metabolites. This produces inappropriate predictions. Our results show that the Lin-log/(log)linear approach cannot be considered a practical alternative in all cases.

Finally, we want to indicate that parameter estimation from dynamic data is an important issue that concentrates many efforts (see Voit et al., 2005 and references therein). This problem becomes more relevant than ever with the increasing amount of available metabolomic data. As the SC formalism is a suitable approximation to non-linear cooperative and saturating functions, we expect that models based on this formalism can be used for fitting dynamic data with better accuracy. This is an unexplored problem that will concentrate our interest in the near future.

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Appendix

Complementary Technical Results

As in the main text, the symbol ≈ will always mean "equal to first order" in the sense of the Taylor expansion around the specified operating point.

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Proposition 1: Let $f(x_1,\ldots,x_m)$ be a smooth real function, and let $x_0 = (x_{01},\ldots,x_{0m}) \in R^m$. Then

$$f(x_1,\ldots,x_m) \approx \tilde{f}_1(x_1) + \tilde{f}_2(x_2) + \ldots + \tilde{f}_m(x_m) + C \quad (42)$$

around x_0, where

$$\tilde{f}_i(x_i) \equiv f(x_{01},\ldots,x_{0,i-1},x_i,x_{0,i+1},\ldots,x_{0m}) \quad (43)$$

for all $i=1,\ldots,m$, and $C=(1-m)\ f(x_{01},\ldots,x_{0m})$ is a real constant.

Proof: We have:

$$f(x_1,\ldots,x_m) \approx f(x_{01},\ldots,x_{0m}) + \sum_{i=1}^{m} \left.\frac{\partial f}{\partial x_i}\right|_{x_0} (x_i - x_{0i}) \quad (44)$$

while for the restrictions of f:

$$\begin{aligned}\tilde{f}_i(x_i) &\approx \tilde{f}_i(x_{0i}) + \left.\frac{\mathrm{d}\tilde{f}_i}{\mathrm{d}x_i}\right|_{x_{0i}} (x_i - x_{0i}) \\ &= f(x_{01},\ldots,x_{0m}) + \left.\frac{\partial f}{\partial x_i}\right|_{x_0} (x_i - x_{0i}) \end{aligned} \quad (45)$$

After this, the equality is demonstrated by direct substitution. □

Proposition 2: For every smooth and strictly positive one-variable real function $f(x)$ and for every $x_0 \in R$, we have

$$\log(f(x)) \approx \log(f(x_0) + f'(x_0)(x - x_0)) \quad (46)$$

in a neighborhood of x_0.

Proof: It can be seen that:

$$\log(f(x)) \approx \log(f(x_0)) + \frac{f'(x_0)}{f(x_0)}(x - x_0) \quad (47)$$

In particular, this equality can also be applied to the function $\log(f(x_0) + f'(x_0)(x - x_0))$, and the result leads to the establishment of the proposition. □

Proposition 3: Let $f(x_1,\ldots,x_m)$ and $g(x_1,\ldots,x_m)$ be two smooth and strictly positive real functions and let $x_0 = (x_{01},\ldots,x_{0m}) \in R^m$. Then $\log(f(x_1,\ldots,x_m)) \approx \log(g(x_1,\ldots,x_m))$ at x_0 if and only if $f(x_1,\ldots,x_m) \approx g(x_1,\ldots,x_m)$ at x_0.

Proof: After some standard calculations we have:

$$\begin{aligned}&\log(f(x_1,\ldots,x_m)) \\ &\quad\approx \log(f(x_{01},\ldots,x_{0m})) \\ &\qquad + \sum_{i=1}^{m} \frac{1}{f(x_{01},\ldots,x_{0m})} \left.\frac{\partial f}{\partial x_i}\right|_{x_0} (x_i - x_{0i})\end{aligned} \quad (48)$$

$$\begin{aligned}&\log(g(x_1,\ldots,x_m)) \\ &\quad\approx \log(g(x_{01},\ldots,x_{0m})) \\ &\qquad + \sum_{i=1}^{m} \frac{1}{g(x_{01},\ldots,x_{0m})} \left.\frac{\partial g}{\partial x_i}\right|_{x_0} (x_i - x_{0i})\end{aligned} \quad (49)$$

If $\log(f(x_i,\ldots,x_m)) \approx \log(g(x_1,\ldots,x_m))$ at x_0 then it is simple to see that $\log(f(x_{01},\ldots,x_{0m})) = \log(g(x_{01},\ldots,x_{0m}))$, namely $f(x_{01},\ldots,x_{0m}) = g(x_{01},\ldots,x_{0m})$. As a consequence $\nabla f = \nabla g$ at x_0 and therefore $f(x_1,\ldots,x_m) \approx g(x_1,\ldots,x_m)$ at x_0.

Conversely, if $f(x_1,\ldots,x_m) \approx g(x_1,\ldots,x_m)$ at x_0, then the proof follows analogous steps but in the reverse order: since $f(x_{01},\ldots,x_{0m}) = g(x_{01},\ldots,x_{0m})$ and $\nabla f = \nabla g$ at x_0, then necessarily $\log(f(x_1\ldots,x_m)) \approx \log(g(x_1\ldots,x_m))$ holds at x_0. This completes the proof. □

Reference Model

Kinetic equations for the reference model and operating point

The reference model (Fig. 7) can be described by the following set of ordinary differential equations:

$$\begin{aligned}\frac{\mathrm{d}X_1}{\mathrm{d}t} &= \nu_1 - \nu_2 \\ \frac{\mathrm{d}X_2}{\mathrm{d}t} &= \nu_2 - \nu_3 - \nu_5 \\ \frac{\mathrm{d}X_3}{\mathrm{d}t} &= \nu_3 - \nu_4 \\ \frac{\mathrm{d}X_4}{\mathrm{d}t} &= \nu_5 - \nu_6\end{aligned}$$

The considered kinetics for each of the reactions are:

$$\nu_1 = \frac{16X_5}{1+X_5} \qquad \nu_2 = \frac{65\frac{X_1}{0.3}\left(1+\frac{0.04}{2.5}\frac{X_3}{0.12}\right)}{1+\frac{X_3}{0.12}+\frac{X_1}{0.3}\left(1+\frac{X_3}{2.5\cdot 0.12}\right)}$$

$$\nu_3 = \frac{8X_2}{1+X_2} \qquad \nu_4 = \frac{8X_3}{1+X_3}$$

$$\nu_5 = \frac{58\left(\frac{X_2}{11}\right)^3}{\left(\frac{X_2}{11}\right)^3 + \frac{1+\left(\frac{X_1}{1}\right)^3}{1+63\left(\frac{X_1}{1}\right)^3}} \qquad \nu_6 = \frac{8X_4}{1+X_4}$$

The steady-state solution is computed by numerically solving the steady-state equations

$$\begin{aligned} v_1 - v_2 &= 0 \\ v_2 - v_3 - v_5 &= 0 \\ v_3 - v_4 &= 0 \\ v_5 - v_6 &= 0 \end{aligned}$$

for different values of the independent variable X_5. All the computations have been performed using Mathematica©. The resulting operating points for the X_5 values of Figure 8 are indicated in Table I.

Power-law approximation (GMA)

The Power-Law model for the reference system is obtained by defining a Power-Law representation of each v_i in the model. Thus, we have

$$\frac{dX_1}{dt} = \gamma_1 X_5^{f_{15}} - \gamma_2 X_1^{f_{21}} X_3^{f_{31}}$$

$$\frac{dX_2}{dt} = \gamma_2 X_1^{f_{21}} X_3^{f_{31}} - \gamma_3 X_2^{f_{32}} - \gamma_5 X_1^{f_{51}} X_2^{f_{52}}$$

$$\frac{dX_3}{dt} = \gamma_3 X_2^{f_{32}} - \gamma_4 X_3^{f_{43}}$$

$$\frac{dX_4}{dt} = \gamma_5 X_1^{f_{51}} X_2^{f_{52}} - \gamma_6 X_4^{f_{64}}$$

where $f_{ij} = (\frac{\partial v_i}{\partial X_j}\frac{X_j}{v_i})_0$ and $\gamma_i = v_{i0}\prod_{j=1}^{m} X_{j0}^{-f_{ij}}$. The resulting parameters for each of the operating points in Figure 8 are indicated in Table II.

Table II. Parameters for the GMA model at the different operating points (Table I).

	X_{50}		
	2.5	1	0.4
f_{15}	0.2857	0.5	0.7143
f_{21}	0.1544	0.4130	0.6951
f_{23}	−0.6813	−0.7065	−0.7378
f_{32}	0.4141	0.4171	0.4524
f_{43}	0.4141	0.4171	0.4524
f_{51}	0.0514	1.4646	1.7386
f_{52}	2.6513	2.8274	2.9901
f_{64}	0.1573	0.5829	0.9762
γ_1	8.7962	8	8.7962
γ_2	11.8416	10.3374	12.450
γ_3	4.0598	4.0556	4.0182
γ_4	4.0598	4.0556	4.0182
γ_5	2.5113	1.3897	0.9270
γ_6	5.1768	4.0556	7.1486

Power-Law approximation (S-system)

In the S-system approximation, the differential equations are defined as a balance of aggregated fluxes of synthesis and degradation for each metabolite. In our example:

$$\frac{dX_1}{dt} = V_1^+ - V_1^- = v_1 - v_2$$

$$\frac{dX_2}{dt} = V_2^+ - V_2^- = v_2 - (v_3 + v_5)$$

$$\frac{dX_3}{dt} = V_3^+ - V_3^- = v_3 - v_4$$

$$\frac{dX_4}{dt} = V_4^+ - V_4^- = v_5 - v_6$$

In the nomenclature of S-systems, the kinetic-orders f_{ij} are called g_{ij} is they refer to V_i^+ and h_{ij} if they refer to V_i^-. The resulting parameters for each of the operating points in Figure 8 are indicated in Table III. The model is

$$\frac{dX_1}{dt} = \alpha_1 X_5^{g_{15}} - \beta_1 X_1^{h_{11}} X_3^{h_{13}}$$

$$\frac{dX_2}{dt} = \beta_1 X_1^{h_{11}} X_3^{h13} - \beta_2 X_1^{h_{21}} X_2^{h_{22}}$$

$$\frac{dX_3}{dt} = \alpha_3 X_2^{g_{32}} - \beta_3 X_3^{h_{33}}$$

$$\frac{dX_4}{dt} = \alpha_4 X_1^{g_{41}} X_2^{g_{42}} - \beta_4 X_4^{h_{44}}$$

Table III. Parameters for the S-system model at the different operating points (Table I)

	X_{50}		
	2.5	1	0.4
g_{15}	0.2857	0.5	0.7143
h_{11}	0.1544	0.4130	0.6951
h_{13}	−0.6813	−0.7065	−0.7378
h_{21}	0.0303	0.6109	0.0724
h_{22}	1.7337	1.4224	0.5581
g_{32}	0.4141	0.4171	0.4524
h_{33}	0.4141	0.4171	0.4524
g_{41}	0.0514	1.4646	1.7386
g_{42}	2.6513	2.8274	2.9901
h_{44}	0.1574	0.5829	0.9762
α_1	8.7962	8	8.7962
α_3	4.0598	4.0556	4.0182
α_4	2.5113	1.3897	0.9270
β_1	11.8416	10.337	12.4503
β_2	6.0178	5.1179	4.4949
β_3	4.0598	4.0556	4.0182
β_4	5.1768	4.0556	7.1486

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Lin-log and (log)linear approximation

The basic equations for the Lin-Log/(log)linear model are the same as for the GMA model, that is, the flux

$$\frac{dX_1}{dt} = v_1 - v_2$$

$$\frac{dX_2}{dt} = v_2 - v_3 - v_5$$

$$\frac{dX_3}{dt} = v_3 - v_4$$

$$\frac{dX_4}{dt} = v_5 - v_6$$

In that case, as no change in the enzyme levels is considered, each velocity is approximated by $v_i = v_{i0}(1 + \sum_{j=1}^{m} f_{ij}\mathrm{Ln}(\frac{X_j}{X_{j0}}))$. The corresponding parameters are, thus, the same as for the GMA model (Table II). The resulting model is:

$$\frac{dX_3}{dt} = v_{30}(1 + f_{32}\mathrm{Ln}(X_2/X_{20})) - v_{40}(1 + f_{43}Ln(X_3/X_{30}))$$

$$\frac{dX_4}{dt} = v_{50}(1 + f_{51}\mathrm{Ln}(X_1/X_{10}) + f_{52}Ln(X_2/X_{20})) - v_{60}(1 + f_{64}Ln(X_4/X_{40}))$$

$$\frac{dX_1}{dt} = v_{10}(1 + f_{51}\mathrm{Ln}(X_1/X_{10})) - v_{20}(1 + f_{21}Ln(X_1/X_{10}) + f_{23}Ln(X_3/X_{30}))$$

$$\frac{dX_2}{dt} = v_{20}(1 + f_{23}\mathrm{Ln}(X_3/X_{30})) - v_{30}(1 + f_{32}Ln(X_2/X_{20})) - v_{50}(1 + f_{51}Ln(X_1/X_{10}) + f_{52}Ln(X_2/X_{20}))$$

SC approximation

In the SC approximation, each velocity is represented as

$$v_i = \frac{V_i \prod_{j=1}^{m} X_j^{n_{ij}}}{\prod_{j=1}^{m} (K_{ij} + X_j^{n_{ij}})}$$

At a given steady-state, the parameters of this representation can be computed from the corresponding sensitivity (f_{ij}) and from the saturation fraction p_{ij} (see text for details). The parameters for the SC approximation at each operating point are indicated in Table IV. The resulting model is:

$$\frac{dX_1}{dt} = \frac{V_1 X_5^{n_{15}}}{K_{15} + X_5^{n_{15}}} - \frac{V_2 X_1^{n_{21}} X_3^{n_{32}}}{(K_{21} + X_1^{n_{21}})(K_{32} + X_3^{n_{32}})}$$

$$\frac{dX_2}{dt} = \frac{V_2 X_1^{n_{21}} X_3^{n_{32}}}{(K_{21} + X_1^{n_{21}})(K_{32} + X_3^{n_{32}})} - \frac{V_3 X_2^{n_{32}}}{K_{32} + X_2^{n_{32}}} - \frac{V_5 X_1^{n_{51}} X_5^{n_{52}}}{(K_{51} + X_1^{n_{51}})(K_{52} + X_3^{n_{52}})}$$

$$\frac{dX_3}{dt} = \frac{V_3 X_2^{n_{32}}}{K_{32} + X_2^{n_{32}}} - \frac{V_4 X_3^{n_{43}}}{K_{43} + X_3^{n_{43}}}$$

$$\frac{dX_4}{dt} = \frac{V_5 X_1^{n_{51}} X_5^{n_{52}}}{(K_{51} + X_1^{n_{51}})(K_{52} + X_3^{n_{52}})} - \frac{V_6 X_4^{n_{64}}}{K_{64} + X_4^{n_{64}}}$$

Table IV. Parameters for the S-system model at the different operating points (Table I).

	X_{50}		
	2.5	1	0.4
n_{15}	1	1	1
n_{21}	1	1	1
n_{23}	−0.8413	−0.8429	−0.8610
n_{32}	1	1	1
n_{43}	1	1	1
n_{51}	2.9990	2.9460	1.8156
n_{52}	3	3	3
n_{64}	1	1	1
K_{15}	1	1	1
K_{21}	0.6713	0.6705	0.6606
K_{23}	3.1799	3.9065	5.0782
K_{32}	1	1	1
K_{43}	1	1	1
K_{51}	0.8664	0.8581	2.3826
K_{52}	21.5452	44.7121	538.252
K_{64}	1	1	1
V_1	16	16	16
V_2	71.0686	84.2175	104.745
V_3	8	8	8
V_4	8	8	8
V_5	59.0123	115.341	1367.71
V_6	8	8	8
p_{15}	0.7143	0.5	0.2857
p_{21}	0.8456	0.5870	0.3049
p_{23}	0.1902	0.1618	0.1431
p_{32}	0.5859	0.5829	0.5476
p_{43}	0.5859	0.5829	0.5476
p_{51}	0.9828	0.5029	0.0424
p_{52}	0.1162	0.0575	0.0033
p_{64}	0.8426	0.4171	0.0238

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