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Synergism analysis of biochemical systems. I. Conceptual framework

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Abstract

The detection of synergisms – deviations from additive or linear behaviour – is often an important step in uncovering mechanisms of biochemical processes. Yet, a theoretical background for systemic analysis of synergisms in metabolic networks is lacking. Based on suitable mathematical models, such a theoretical approach should allow predicting synergisms and analysing what mechanistic features contribute to specific synergisms. This work presents a conceptual framework and formalism that fulfil these purposes. The synergism between perturbations of a pair of parameters is quantified as the difference between the response to the simultaneous perturbation of both parameters and the sum of the individual responses to the perturbations of each parameter. A generalisation measures deviations from multiplicative or power-law behaviour. These deviations were called log-synergisms, as in logarithmic coordinates they are quantified in the same way as the synergisms are in Cartesian coordinates. For small perturbations, synergisms and log-synergisms are approximately proportional to the second derivatives (in Cartesian and logarithmic coordinates, respectively) of the observable to the perturbed parameter(s). These derivatives, here called synergism or log-synergism coefficients, measure how steeply the responses diverge from linearity/additivity or power-law/multiplicativity. The formalism now presented allows evaluating (log-)synergism coefficients for systemic steady-state responses, and relates these coefficients to intrinsic kinetic properties of the underlying processes. A robust homeostasis of metabolite concentrations requires that these have moderate systemic log- and relative-synergism coefficients.

Keywords: Additivity; Antagonism; Mathematical model; Metabolic networks; Power law; Sensitivity analysis

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1. Introduction

A pair of stimuli is said to be synergistic when the response to the joint stimuli is greater than the sum of the responses to each individual stimulus. When the response to the joint stimuli is less than the latter sum, the stimuli are usually called antagonistic. In the present work, however, we will refer to a synergism as any deviation from additive or linear behaviour and consider an antagonism as a negative synergism.

A synergism between two factors demonstrates interdependency between their mechanisms of action. Thus, the detection of synergisms is often an important step in the investigation of complex networks of interactions, such as metabolic networks. Numerous examples in experimental biochemical literature illustrate this claim (see [1–9], to name but a few). Less understood are the biochemical implications of departures from power-law/multiplicative behaviour, although the latter is most prevalent in Nature [10,11]. Mechanistically, multiplicative behaviour is the simplest type of non-linearity. It arises when the effect of one perturbation independently builds on the effect of another one and the response is not subject to conservation relationships or other constraints. The best known example is perhaps the response of the rate of a mass action bimolecular reaction to joint changes of reactant concentrations. Power-law behaviour reflects multiplicativity towards distinct perturbations of a single parameter. Deviations from power-law/multiplicative behaviour – here called log-synergisms – often result from more complex mechanisms.

Experimental studies of synergisms [1–9] usually have a restricted scope, inasmuch as they study the interaction between a single pair of factors and, in favourable cases, trace the synergism to a specific interaction between a couple of system components. A systemic analysis should permit addressing cases where various mechanisms contribute for synergistic or log-synergistic behaviour, or even inferring mechanisms from the examination of (log-)synergisms between larger sets of factors. Such analysis requires a suitable theoretical background. Yet, to the author's knowledge, no formal approach has been published that has the explicit intent of relating mechanistic features of biological systems to their (log-)synergistic behaviour. Although numerical simulation allows one to predict synergisms and log-synergisms in mathematical models, this approach is impractical where studying interactions between many parameters. The present work addresses some of these methodological issues by developing a formalism for synergism and log-synergism analysis of the steady-state behaviour of kinetic models.

A synergism between two stimuli can be quantified as the difference between the response to the joint stimuli and the sum of the responses to each stimulus applied independently (Section 3). Section 4 considers differential approximations of synergisms. Second-order derivatives, here called synergism coefficients, measure how steeply the response of the observable departs from linear/additive behaviour as the perturbations increase. The synergism coefficients are equivalent to second-order differential sensitivities. Methods of general sensitivity theory [12–15] can thus be applied to compute systemic synergism coefficients of steady-state properties appearing in kinetic models (Section 5). In this way, the formalism relates the synergistic behaviour of a metabolic network as a whole to its topology and to intrinsic kinetic properties of its reaction steps.

A logarithmic transformation converts power-law relationships into linear relationships and multiplicative relationships into additive relationships. Thus, once observables and parameters are represented in logarithmic coordinates, log-synergisms are quantified in the same way as the

synergisms are in Cartesian coordinates. The analogy carries on to the differential approximations of log-synergisms, and so log-synergism coefficients are defined as second derivatives in logarithmic space that measure how steeply the responses deviate from power-law/multiplicative behaviour. The formalism for log-synergism analysis thus derives directly from the formalism for synergisms.

High systemic log- and relative-synergism coefficients are a symptom of poor homeostasis, and should therefore be rare in biochemical systems under physiological conditions (Section 6).

2. Notation

Scalar quantities are represented in normal type, vectors and matrices in bold. Lower- and uppercase subscripts refer to single elements and sets of elements, respectively. Unless otherwise specified, generic parameters, the concentrations of internal metabolites and the rates of individual processes are represented by λ, X and v, respectively. Elementary reactions, enzyme-catalysed reactions and aggregated fluxes, can all be considered as unit processes, as long as the stoichiometric matrix ($\boldsymbol{N}$, in Section 5) is accordingly defined. Concentrations of conserved moieties (e.g., total concentrations of enzymes) and of external metabolites are treated as any other parameters.

The operators of non-normalised and logarithmic first-order sensitivities are represented by $J(\cdot,\cdot)$ and $S(\cdot,\cdot)$, respectively

$$J(F,x)=\frac{\partial F}{\partial x},\qquad S(F,x)=\frac{x}{F}\frac{\partial F}{\partial x}=\frac{\partial \log|F|}{\partial \log|x|}.$$

The operators for non-normalised, relative- and log-synergism coefficients are indicated as

$$J^{[2]}(F,x_1,x_2)=\frac{\partial^{[2]}F}{\partial x_1\partial x_2},\qquad W^{[2]}(F,x_1,x_2)=\frac{x_1x_2}{F}\frac{\partial^2 F}{\partial x_1\partial x_2}$$

and

$$S^{[2]}(F,x_1,x_2)=\frac{\partial^2 \log|F|}{\partial \log|x_1|\,\partial \log|x_2|},$$

respectively.

Constrained derivatives are indicated by listing the variables or parameters that are kept constant as subscripts of the operators. For instance,

$$J^{[2]}_{(x_2,x_3),(x_1,x_3)}(F,x_1,x_2)=\left(\frac{\partial(\partial F/\partial x_1)_{x_2,x_3}}{\partial x_2}\right)_{x_1,x_3},$$

that is, the derivative with respect to x_1 is taken at constant x_2 and x_3, and the derivative with respect to x_2 is taken at constant x_1 and x_3. Over this series of papers, constrained differentiations are often made with respect to various alternative sets of variables (i.e., dependent variables, independent variables, parameters), while variables that belong to other sets are kept constant. In all these cases, it is assumed that all other variables belonging to the same set as the differentiation variable are kept constant. To simplify notation, those variables are omitted from the list of

constants. For instance, the matrix of logarithmic sensitivities of the rates to the concentrations at constant parameters is denoted by $\mathbf{S}_{\boldsymbol{\lambda}}(\mathbf{v}, \boldsymbol{X})$, though each element i, j of this matrix assumes as well the constancy of all other concentrations except X_j. In this formalism, constrained rate derivatives correspond to intrinsic kinetic properties of each process considered. Hence, they are called intrinsic sensitivities or intrinsic synergism coefficients. Operators without a subscript refer to unconstrained derivatives. These correspond to systemic sensitivities or synergism coefficients, which account for the responses of the system as a whole.

The symbol $\delta_{i,j}$ stands for Kronecker's delta, which takes on the value 1 if $i = j$ and 0 otherwise.

A reaction is said to follow generalised mass action kinetics if its rate is proportional to a product of (not necessarily integer or positive) powers of the concentrations of reactants and effectors [16].

3. Quantification of synergisms and log-synergisms

The synergism between two stimuli is measured by comparing the response to the joint stimuli to the sum of the responses to each stimulus applied independently. To formalise the concept, consider an observable $P(\lambda_1, \lambda_2)$ (e.g., a concentration or a flux) and perturbations $\boldsymbol{\Delta\lambda} = [\Delta\lambda_1, \Delta\lambda_2]$ to $\boldsymbol{\lambda} = [\lambda_1, \lambda_2]$ (e.g. changes in the concentrations of two external metabolites) around an operating point $\boldsymbol{\lambda}_o$. If only the jth and kth components of $\boldsymbol{\Delta\lambda}_{j,k}$ are non-zero, the responses of P (Fig. 1(a)) are defined as

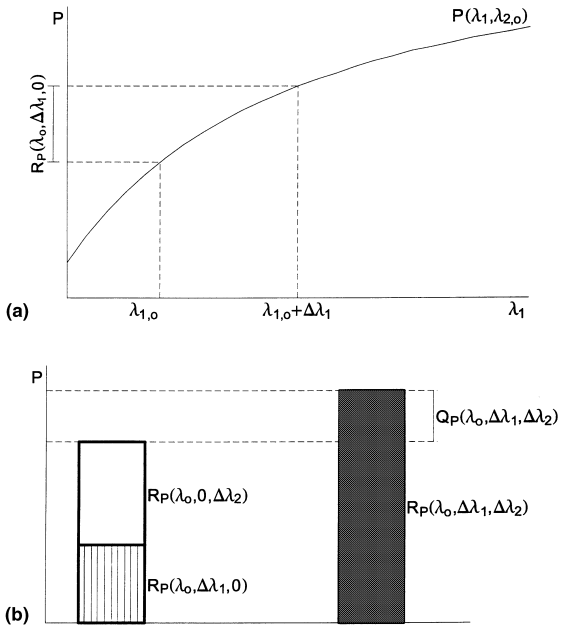


Fig. 1. Calculation of the synergism between two perturbations ($\Delta\lambda_1$,$\Delta\lambda_2$) referred to a property P: (a) response of P to $\Delta\lambda_1$; (b) synergism between $\Delta\lambda_1$ and $\Delta\lambda_2$, referred to $P(Q_P(\lambda_o, \Delta\lambda_1, \Delta\lambda_2))$.

$$R_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(j,k)}) = P(\boldsymbol{\lambda}_o + \Delta\boldsymbol{\lambda}_{(j,k)}) - P(\boldsymbol{\lambda}_o), \quad j,k \in \{1,2\}, \tag{1}$$

with the convention that $\Delta\boldsymbol{\lambda}_{(j,j)}$ means the same as $\Delta\boldsymbol{\lambda}_{(j)}$. The synergism, $Q_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(1,2)})$, between stimuli $\Delta\boldsymbol{\lambda}_1$ and $\Delta\boldsymbol{\lambda}_2$ with respect to P (Fig. 1(b)) can now be defined as

$$Q_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(1,2)}) = R_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(1,2)}) - [R_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(1)}) + R_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(2)})], \tag{2}$$

and extended to single-parameter perturbations as

$$Q_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(j)}) = R_P(\boldsymbol{\lambda}_o, 2\Delta\boldsymbol{\lambda}_{(j)})/2 - R_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(j)}), \quad j \in \{1,2\}. \tag{3}$$

A positive (negative) $Q_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(1,2)})$ implies a supra-additive (respectively sub-additive) response of P to the joint perturbation of λ_1 and λ_2. In turn, a positive (negative) $Q_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(j)})$ implies a supra-linear (respectively sub-linear) response of P to the perturbation of λ_j. To ascertain the significance of a synergism and to compare synergisms it is convenient to use relative-synergisms, defined as

$$\Psi_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(j,k)}) = \frac{Q_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(j,k)})}{P(\boldsymbol{\lambda}_o)}, \quad j,k \in \{1,2\}.$$

To quantify log-synergisms, consider the perturbations $(\varphi_j\lambda_{j,o})$ that change a positive parameter λ_j by a positive factor φ_j from $\lambda_{j,o}$. For a given pair $\boldsymbol{\varphi} = [\varphi_1, \varphi_2]$, the *proportional* responses of the (positive) magnitude P are

$$\Gamma_P(\boldsymbol{\lambda}_o, \boldsymbol{\varphi}_{(j,k)}) = P(\mathrm{diag}(\boldsymbol{\varphi}_{(j,k)}) \cdot \boldsymbol{\lambda}_o)/P(\boldsymbol{\lambda}_o), \quad j,k \in \{1,2\}. \tag{4}$$

The log-synergism between both stimuli with respect to P can then be quantified as

$$\Theta_P(\boldsymbol{\lambda}_o, \boldsymbol{\varphi}_{(1,2)}) = \frac{\Gamma_P(\boldsymbol{\lambda}_o, \boldsymbol{\varphi}_{(1,2)})}{\Gamma_P(\boldsymbol{\lambda}_o, \boldsymbol{\varphi}_{(1)})\,\Gamma_P(\boldsymbol{\lambda}_o, \boldsymbol{\varphi}_{(2)})}, \tag{5}$$

for dual-parameter perturbations, and as

$$\Theta_P(\boldsymbol{\lambda}_o, \boldsymbol{\varphi}_{(j)}) = \frac{\sqrt{\Gamma_P(\boldsymbol{\lambda}_o, \boldsymbol{\varphi}^2_{(j)})}}{\Gamma_P(\boldsymbol{\lambda}_o, \boldsymbol{\varphi}_{(j)})} \quad j \in \{1,2\}, \tag{6}$$

for single-parameter perturbations. As log-synergisms are dimensionless quantities, no further normalisation is required. In absence of log-synergism – i.e., if the response is power-law ($j = k$) or multiplicative ($j \neq k$) – $\Theta_P(\boldsymbol{\lambda}_o, \boldsymbol{\varphi}_{(j,k)}) = 1$; otherwise $\Theta_P(\boldsymbol{\lambda}_o, \boldsymbol{\varphi}_{(j,k)}) \neq 1$.

Although (2) and (3) are the most natural ways of expressing log-synergisms, the comparison with the corresponding relative-synergisms is facilitated by considering relative differences instead of ratios. To achieve this, one may first notice that if a multiplicative response to a dual-parameter perturbation is assumed, the predicted relative response is

$$\frac{R_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(j,k)})}{P(\boldsymbol{\lambda}_o)} = \frac{R_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(j)})}{P(\boldsymbol{\lambda}_o)} + \frac{R_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(k)})}{P(\boldsymbol{\lambda}_o)} + \frac{R_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(j)})}{P(\boldsymbol{\lambda}_o)}\,\frac{R_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(k)})}{P(\boldsymbol{\lambda}_o)},$$

with $\Delta\boldsymbol{\lambda}_j = (\varphi_j - 1)\lambda_{j,o}$. So, the difference, $\boldsymbol{\Omega}_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(j,k)})$, between the first and the second members of this expression is also a measure of deviation from multiplicative behaviour. Using the definition of relative-synergism (Ψ_P) above, one can then write

$$\Omega_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(j,k)}) = \Psi_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(j,k)}) - \frac{R_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(j)})}{P(\boldsymbol{\lambda}_o)} \frac{R_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(k)})}{P(\boldsymbol{\lambda}_o)}. \tag{7}$$

A similar reasoning for single-parameter perturbations yields

$$\Omega_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(j)}) = \sqrt{\frac{R_P(\boldsymbol{\lambda}_o, \Delta^*\boldsymbol{\lambda}_{(j)})}{P(\boldsymbol{\lambda}_o)} + 1} - \frac{R_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(j)})}{P(\boldsymbol{\lambda}_o)} - 1, \tag{8}$$

with $\Delta^*\lambda_j = (\varphi^2 - 1)\lambda_{j,o}$.

Synergisms and log-synergisms have simple geometrical interpretations in Cartesian and logarithmic coordinates, respectively. If (Fig. 2(a)) the synergism $Q_P(\boldsymbol{\lambda}, \Delta\boldsymbol{\lambda}_{(1,2)})$ is 0, then point P_{11} – which represents the value of P after perturbation of both λ_1 and λ_2 – is coplanar with P_{00}, P_{10} and P_{01}, which correspond to the value of P before perturbation and after perturbation of each

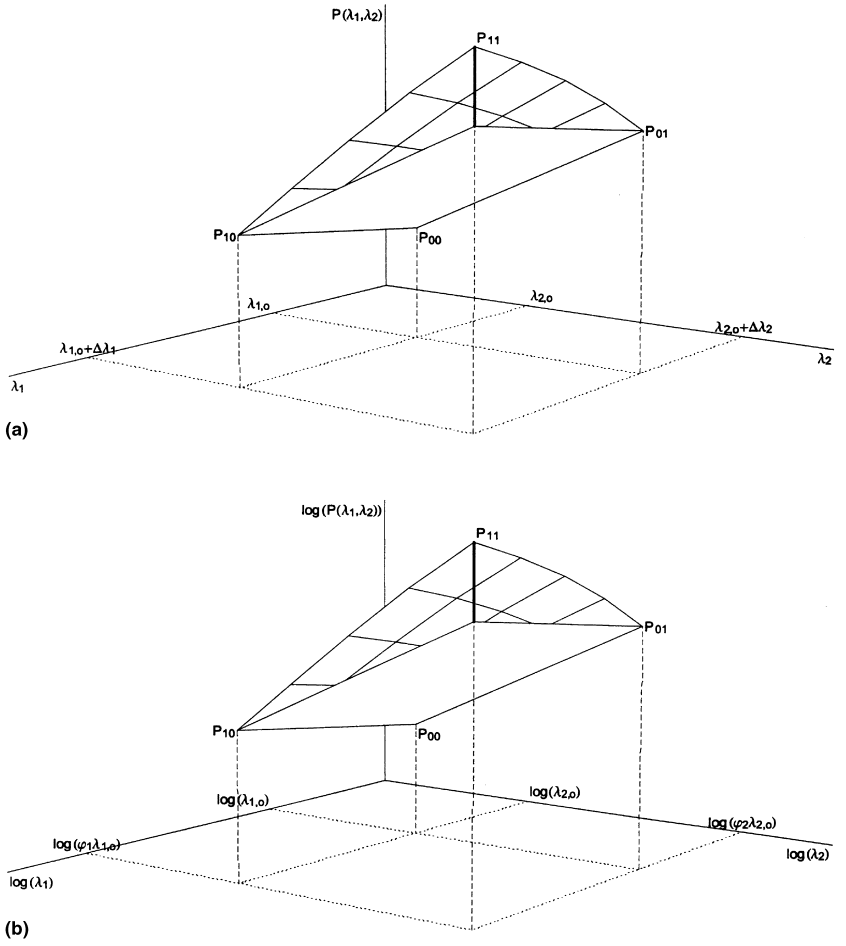


Fig. 2. Geometrical representation of a non-normalised synergism (a) and of a log-synergism (b) between two perturbations ($\Delta\lambda_1$,$\Delta\lambda_2$), referred to a property P. The magnitude of the synergism in (a) and of the logarithm of the log-synergism in (b) is given by the (highlighted) vertical distance of point P_{11} to the plane defined by P_{00}, P_{01} and P_{10}.

parameter. If the synergism $Q_P(\boldsymbol{\lambda}, \Delta\boldsymbol{\lambda}_{(1,2)})$ is positive (negative), P_{11} lies above (respectively below) the plane defined by P_{10}, P_{01} and P_{00}. Likewise (Fig. 2(b)), if the log-synergism $\Theta_P(\boldsymbol{\lambda}_o, \boldsymbol{\varphi}_{(1,2)})$ is 1, point P_{11} is coplanar with P_{00}, P_{10} and P_{01}, and if $\Theta_P(\boldsymbol{\lambda}_o, \boldsymbol{\varphi}_{(1,2)})$ is higher (lower) than 1, P_{11} lies above (respectively below) this plane.

Note that additivity and multiplicativity are just relationships between responses to dual-parameter perturbations and responses to single-parameter perturbations, without anything being assumed about the latter responses. Translated into properties of the observable P, additivity holds for any operating point and amplitude of perturbation if $P(\lambda_1, \lambda_2) \equiv f_1(\lambda_1) + f_2(\lambda_2)$, with f_1, f_2 *arbitrary* functions. Multiplicativity holds if $P(\lambda_1, \lambda_2) \equiv f_1(\lambda_1) \times f_2(\lambda_2)$.

4. Differential approximation of synergisms and log-synergisms

4.1. Definition of (log-)synergism coefficients

If an explicit formula for $P(\boldsymbol{\lambda})$ is unavailable, expressions (1)–(6) are not directly applicable for computing synergisms and log-synergisms. In many of these cases, suitable differential approximations can still be achieved. Namely, if $P(\boldsymbol{\lambda})$ is twice-differentiable around $\boldsymbol{\lambda}_o$, the replacement of $\boldsymbol{\lambda}_o$-centred Taylor series approximations of $P(\lambda_{1,o}, \lambda_{2,o}), P(\lambda_{1,o} + \Delta\lambda_1, \lambda_{2,o}), P(\lambda_{1,o}, \lambda_{2,o} + \Delta\lambda_2)$ and $P(\lambda_{1,o}, +\Delta\lambda_1, \lambda_{2,o} + \Delta\lambda_2)$ up to second-order terms into (2) and (3) yields

$$Q_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(i,j)}) \approx (1 - \tfrac{1}{2}\delta_{i,j}) J^{[2]}(P, \lambda_i, \lambda_j)\, \Delta\lambda_i\, \Delta\lambda_j. \tag{9}$$

The coefficients $J^{[2]}(P, \lambda_i, \lambda_j)$ will be called 'non-normalised synergism coefficients'. For $i \neq j$, they quantify how steeply the non-normalised response of P to 'small' joint perturbations of λ_i and λ_j diverges from the sum of the responses to independent perturbations of λ_i and λ_j as the perturbations increase. The coefficients $J^{[2]}(P, \lambda_i, \lambda_i)$ quantify how steeply the response of P to perturbations of λ_i diverges from linearity.

By normalising both members of expression (9) by $P(\lambda_o)$ and the perturbations $\Delta\lambda_i$ by λ_i, it follows

$$\Psi_P(\boldsymbol{\lambda}_o, \Delta\boldsymbol{\lambda}_{(i,j)}) \approx (1 - \tfrac{1}{2}\delta_{i,j}) W^{[2]}(P, \lambda_i, \lambda_j) \frac{\Delta\lambda_i}{\lambda_{i,o}} \frac{\Delta\lambda_j}{\lambda_{j,o}}. \tag{10}$$

The coefficients $W^{[2]}(P, \lambda_i, \lambda_j)$ will be called 'relative-synergism coefficients'. Their meaning is similar to that of the non-normalised synergism coefficients, except for both responses and perturbations being normalised. For instance, if λ_1 and λ_2 are *both* increased by 5% and the relative-synergism coefficient $W^{[2]}(P, \lambda_1, \lambda_2)$ is 1, then the response of P is approximately $1 \times 0.05 \times 0.05 = 0.25\%$ higher than the sum of the responses to 5% increases in each parameter alone.

The replacement of the Taylor-series expansions of $P(\boldsymbol{\lambda})$ in logarithmic space into (4)–(6) yields

$$\Theta_P(\boldsymbol{\lambda}_o, \boldsymbol{\varphi}_{(i,j)}) \approx \exp\left((1 - \tfrac{1}{2}\delta_{i,j}) S^{[2]}(P, \lambda_i, \lambda_j) \log(\varphi_i) \log(\varphi_j)\right). \tag{11}$$

We call the coefficients $S^{[2]}(F, \lambda_1, \lambda_2)$ 'log-synergism coefficients'. For $i \neq j$, these coefficients measure how steeply the *proportional* response of P to 'small' joint *proportional* perturbations of λ_i and λ_j diverges from the *product* of the *proportional* responses to independent perturbations of λ_i

and λ_j. In turn, $S^{[2]}(P, \lambda_i, \lambda_i)$ quantifies how steeply the proportional response of P to proportional perturbations of λ_i diverges from a power function of λ_i. Power-law/multiplicative responses, which yield $\Theta_P(\boldsymbol{\lambda}_o, \boldsymbol{\varphi}_{(i,j)}) = 1$, translate into null log-synergism coefficients.

Though expression (11) is formally different from expressions (9) and (10), for small perturbations it can be shown [17] that

$$\Omega_P(\boldsymbol{\lambda}_o, \boldsymbol{\Delta\lambda}_{(i,j)}) \approx (1 - \tfrac{1}{2}\delta_{i,j}) S^{[2]}(P, \lambda_i, \lambda_j) \frac{\boldsymbol{\Delta\lambda}_i}{\boldsymbol{\lambda}_{i,o}} \frac{\boldsymbol{\Delta\lambda}_j}{\boldsymbol{\lambda}_{j,o}}. \tag{12}$$

So, if $S^{[2]}(P, \lambda_i, \lambda_j) = 1$ and λ_1 and λ_2 are *both* increased by 5%, then the response of P to the perturbation will be approximately $1 \times 0.05 \times 0.05 = 0.25\%$ higher than the *product* of the responses to 5% increases of each parameter alone.

The equivalence between logarithmic and relative first-order sensitivities does not hold between log- and relative-synergism coefficients. The latter can be related through

$$S^{[2]}(P, \lambda_i, \lambda_j) = W^{[2]}(P, \lambda_i, \lambda_j) - S(P, \lambda_i) S(P, \lambda_j) + \delta_{i,j} S(P, \lambda_i). \tag{13}$$

4.1.1. Example 1

In models of metabolic pathways, enzyme-catalysed reactions are often treated as single processes, with rate expressions derived from their steady-state kinetics. Michaelis–Menten and Hill rate expressions are the simplest of these, and perhaps the most often used. The analyses performed in Biochemical Systems Theory [18–20], Metabolic Control Analysis [21,22] or Flux-Oriented Theory [23] approximate these rate expressions as linear functions of the concentrations or as products of powers of the concentrations. These are first-order Taylor-series approximations in Cartesian or logarithmic coordinates, respectively. Because relative- and log-synergism coefficients derive from the corresponding second-order approximations, below we use these coefficients to estimate the local accuracy of the former approximations.

After normalising rates and concentrations, Michaelis–Menten and Hill rate-expressions can be written in the general form $z = x^n/(1 + x)^n$, with $z = v/V_{max}$, $x = X/K_M$ and n standing for the Hill number. The log-synergism coefficient of z,

$$S^{[2]}_{\cdot,\cdot}(z, x, x) = -\frac{n^2 x^n}{(1 + x^n)^2}$$

is always non-positive, indicating sub-power-law behaviour of z. That is, for small perturbations, power-law approximations always overestimate the rate. In turn, the relative-synergism coefficient is

$$W^{[2]}_{\cdot,\cdot}(z, x, x) = -\frac{n((n+1)x^n - n + 1)}{(1 + x^n)^2}.$$

This is positive if $x < ((n-1)/(n+1))^{1/n}$, which never happens if $x \geqslant 1$ or $n \leqslant 1$, and negative otherwise. So, linear approximations may either underestimate or overestimate the rate, depending on the operating point.

Fig. 3 shows that $|S^{[2]}_{\cdot,\cdot}(z, x, x)|$ is maximal at $x = 1 (X = K_M)$, whereas $S^{[3]}_{\cdot,\cdot,\cdot}(z, x, x, x)$ is zero at this point. Hence, whereas the power approximation is least accurate at operating points near K_M, the

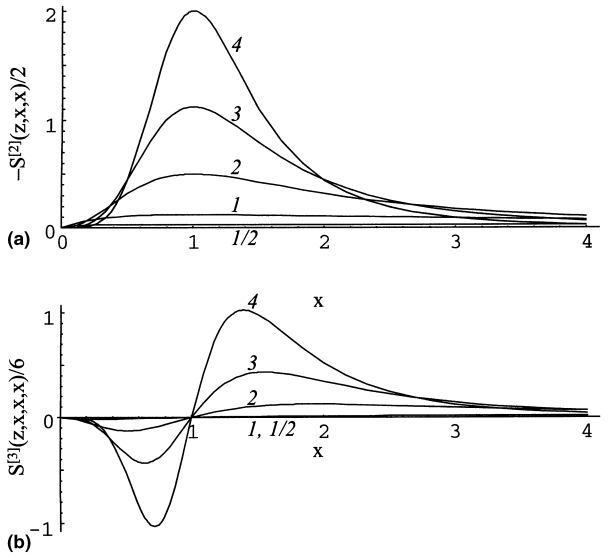


Fig. 3. Concentration-dependence of logarithmic sensitivities of the rate of enzyme-catalysed reactions (following Michaelis–Menten or Hill kinetics) to the normalised concentration: (a) second-order sensitivities; (b) third-order sensitivities. The Hill numbers of each kinetics are indicated in italics near the respective curves.

second-order logarithmic approximation is maximally accurate there. These are probably the operating points that best represent typical physiological conditions (see below).

To compare the accuracy of linear and power approximations, we consider the ratio between $S^{[2]}_{\cdot,\cdot,\cdot}(z,x,x)$ and $W^{[2]}_{\cdot,\cdot,\cdot}(z,x,x)$,

$$q = \frac{n\,x^n}{1-n+(1+n)x^n}.$$

For Michaelis–Menten kinetics ($n = 1$), this is 1/2, irrespective of operating point. For $x = 1$ and arbitrary n, one finds $q = n/2$. Thus, for small perturbations around $x = 1$, a power-law approximation is expected to be more accurate than a linear approximation if $n < 2$, being the reverse true if $n > 2$. The general rule is: (a) for $n \leqslant 1$, a power approximation is more accurate locally than a linear approximation at any operating point; (b) for $n > 1$, a *linear* approximation becomes more accurate than a power approximation at operating points in the range

$$x \in \left]\left(\frac{n-1}{2n-1}\right)^{1/n}, (n-1)^{1/n}\right[.$$

The relative errors of the first- and second-order approximations, $z^*(x)$, of $z(x)$ around $x = 1$ are defined as $\varepsilon(x) = |z^*(x) - z(x)|/z(x)$. The ranges over which $\varepsilon(x) < 0.1$, for various ns (Table 1), further strengthen the previous assertions: (a) the second-order approximations in logarithmic space are accurate over wider ranges than the first-order approximations; (b) for $n > 2$ the linear approximations are accurate over wider ranges than the power-law approximations, as found by Voit and Savageau [24]. At low n, the log-space second-order approximations stay within 10% relative error over several orders of magnitude.

Table 1
Ranges of normalised concentrations where the relative errors of approximations of Michaelis–Menten or Hill rate expressions around the K_M stay below 10%[a]

n_H	First-order approximations		Second-order approximations	
	Cartesian space	Logarithmic space	Cartesian space	Logarithmic space
1/2	0.39–2.3 (5.8)	0.17–5.9 (35.0)	0.27–2.5 (9.2)	0.010–95 (9091)
1	0.54–1.9 (3.5)	0.41–2.4 (5.9)	0.35–2.2 (6.4)	0.10–9.8 (95)
2	0.64–1.6 (2.4)	0.64–1.6 (2.4)	0.46–2.2 (4.7)	0.32–3.1 (9.8)
3	0.65–1.4 (2.2)	0.74–1.3 (1.8)	0.66–2.3 (3.5)	0.47–2.1 (4.6)
4	0.61–1.3 (2.2)	0.80–1.2 (1.6)	0.74–2.5 (3.4)	0.57–1.8 (3.1)

[a] The values in parentheses are the ratios between the highest and the lowest values of the ranges.

4.1.2. Example 2

Consider an enzyme acting through an irreversible Ping-Pong Bi-Bi mechanism according to Scheme 1, where X_1, X_2 stand for substrates, X_3, X_5 for free forms of the enzyme, X_4, X_6 for enzyme-substrate complexes and X_7, X_8 for products.

The normalised steady-state rate of the catalysed reaction can be written in terms of normalised concentrations $x_1 = X_1/K_1$ and $x_2 = X_2/K_2$ (with K_i the Michaelis–Menten constant for each substrate) as

$$f = \frac{v}{V} = \frac{x_1 x_2}{x_1 x_2 + x_1 + x_2}. \tag{14}$$

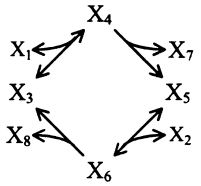
Log- and relative-synergism coefficients are invariant upon this scaling. So, for interactions between the substrate concentrations X_1 and X_2, one obtains

$$S^{[2]}_{\cdot,\cdot}(f, x_1, x_2) = \frac{x_1 x_2}{(x_1 x_2 + x_1 + x_2)^2}$$

and

$$W^{[2]}_{\cdot,\cdot}(f, x_1, x_2) = \frac{2x_1 x_2}{(x_1 x_2 + x_1 + x_2)^2}.$$

As both coefficients are positive, the effect of perturbations on X_1 and X_2 is always supra-multiplicative and supra-additive. Besides, as the log-synergism coefficient is half the relative-synergism coefficient for all x_1 and x_2, the response of the rate to dual-substrate perturbations is always more nearly multiplicative than additive. The synergistic interaction between X_1 and X_2 follows from the fact that increasing the concentration of X_1 increases the amount of X_5 available for reaction with X_2, thus potentiating the effect of increasing X_2, and vice versa. On the other hand, the rate becomes less responsive to the concentration of X_1 as this approaches saturation, thus



Scheme 1.

preventing synergistic effects. The same effect occurs at very low X_1 concentrations, where the binding of X_1 becomes limiting for the catalytic cycle. Hence, except for the particular case $x_1 = x_2$, both the synergism and the log-synergism should be non-monotonic functions of the concentrations. The analysis of the previous formulas confirms this assertion by showing that the maximal (log-)synergisms for each particular concentration of one of the substrates lie at the parametric curves $(x_1, x_1/(1+x_1))$ and $(x_2/(1+x_2), x_2)$. Therefore, if the concentration of, say, X_1 matches its K_M (i.e., $x_1 = 1$), the (log-)synergism between the concentrations of both substrates as regards v is maximised when the concentration of X_2 is $K_M/2$. The log- and relative-synergism coefficients will then be 0.125 and 0.25, respectively. Lets now suppose that from this operating point we increase the concentrations of both metabolites by 10%. The response of v would then be approximately $0.125 \times 0.1 \times 0.1 \times 100 = 0.125\%$ higher than the product and $0.25 \times 0.1 \times 0.1 \times 100 = 0.25\%$ higher than the sum of the responses elicited by applying the same perturbations individually to each substrate.

The single-parameter log- and relative-synergism coefficients for X_1 are

$$S^{[2]}_{\cdot,\cdot}(f, x_1, x_1) = -\frac{x_1 x_2 (1+x_2)}{(x_1 x_2 + x_1 + x_2)^2}$$

and

$$W^{[2]}_{\cdot,\cdot}(f, x_1, x_1) = -\frac{2 x_1 x_2 (1+x_2)}{(x_1 x_2 + x_1 + x_2)^2},$$

respectively. The negative values indicate that the response of rate of the reaction to changes in the concentration of X_1 is always sub-power-law and sub-linear. This prediction is readily verified for a particular operating point (Fig. 4).

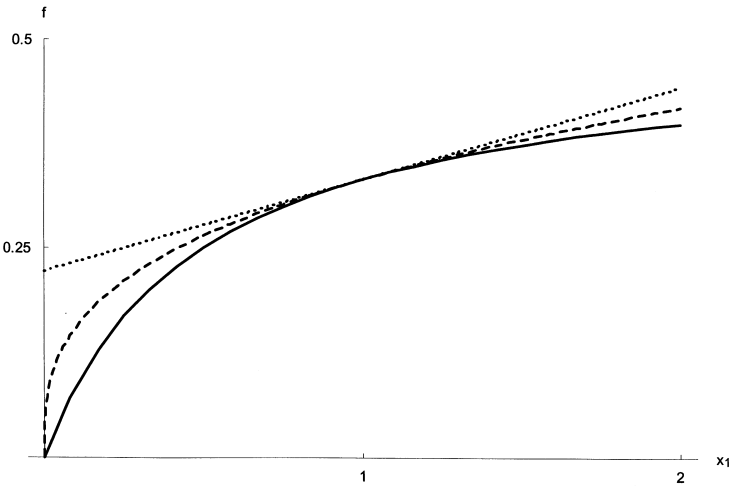


Fig. 4. Linear (dotted line) and power (dashed line) approximation of f (continuous line) around the operating point $x_1 = x_2 = 1$.

4.2. Typical values of intrinsic log- and relative-synergism coefficients

Hyperbolic kinetics tend to yield modest log- and relative-synergism coefficients (Tables 2 and 3). Perturbations of pairs of substrate concentrations yield relative-synergism coefficients in the range $[-1/8,\ 1]$. Negative values, though possible for the ordered mechanism, require near-saturating substrates and $\kappa > 2$. For individual perturbations of substrate or inhibitor concentrations, the relative-synergism coefficients fall in the ranges $[-1/2,\ 0]$ and $[0,\ 6[$, respectively, whereas the coefficients for joint inhibitor-substrate perturbations fall over $]-2,\ 1/8[$. Note that according to expressions (10) and (12) the log- and relative-synergism coefficients for single-parameter perturbations should be multiplied by 1/2 when compared to those for dual-parameter perturbations. The intrinsic log-synergism coefficients of hyperbolic kinetics are usually in the ranges $[-1/4,\ 0]$, for single-substrate or single-inhibitor perturbations, and $[-1/4,\ 1/4]$, for dual-substrate or substrate-inhibitor perturbations. Of the hyperbolic kinetics in Tables 2 and 3, only mixed inhibition can reach relative-synergism coefficients above 2 or log-synergism coefficients below $-1/4$. These values are achieved at near-saturating inhibitor concentrations.

Hill kinetics may also yield high log- and relative-synergism coefficients which may reach −4 and 12, respectively, for $n = 4$. Generalised mass action kinetics [16] always yields null intrinsic log-synergism coefficients, and intrinsic relative-synergism coefficients that are constant and determined by the kinetic orders. High kinetic orders yield high relative-synergism coefficients.

Table 2
Ranges of relative synergism coefficients for current biochemical kinetics[a]

	Normalised rate expression	$W^2(v,x_1,x_1)$	$W^2(v,x_2,x_2)$	$W^2(v,x_1,x_2)$
Generalised mass action	$x_1^{f_1}x_2^{f_2}$	$f_1(f_1-1)$	$f_2(f_2-1)$	f_1f_2
Hill/Michaelis–Menten	$\frac{x_1^n}{1+x_1^n}$	$[-\frac{(n+1)^2}{8}, n(n-1)]$		
		$(-n/2)$		
Competitive inhibition	$\frac{x_1}{1+x_1+x_2}$	$[-1/2,\ 0]$	$[0,\ 2[$	$]-1,\ 1/8[$
		$(-4/9)$	$(2/9)$	$(-1/9)$
Non-competitive inhibition	$\frac{x_1}{(1+x_1)(1+x_2)}$	$[-1/2,\ 0]$	$[0,\ 2[$	$]-1,\ 0]$
		$(-1/2)$	$(1/2)$	$(-1/4)$
Mixed inhibition	$\frac{x_1}{(1+x_1)(1+x_1+x_2)}$	$[-1/2,\ 0]$	$[0,\ 6[$	$]-2,\ 0]$
		$(-4/9)$	$(19/18)$	$(-4/9)$
Uncompetitive inhibition	$\frac{x_1}{1+(1+x_2)x_1}$	$[-1/2,\ 0]$	$[0,\ 2[$	$]-1/2,\ 0]$
		$(-4/9)$	$(2/9)$	$(-2/9)$
Ping–Pong	$\frac{x_1x_2}{x_1+x_2+x_1x_2}$	$[-1/2,\ 0]$	$[-1/2,\ 0]$	$[0,\ 1/2]$
		$(-4/9)$	$(-4/9)$	$(2/9)$
Ordered	$\frac{x_1x_2}{\kappa+x_1+x_2+x_1x_2}$	$[-1/2,\ 0]$	$[-1/2,\ 0]$	$[-1/8,\ 1]$
		$\left(-\frac{4(\kappa+1)}{(\kappa+3)^2}\right)$	$\left(-\frac{4(\kappa+1)}{(\kappa+3)^2}\right)$	$\left(\frac{\kappa(\kappa+1)+2}{(\kappa+3)^2}\right)$

[a] Only irreversible mechanisms are considered. In the inhibition kinetics, x_1 and x_2 stand for substrate and inhibitor, respectively. The relative synergism coefficients for substrate and inhibitor concentrations matching their K_Ms and K_Is (i.e., $x_1 = x_2 = 1$) are shown in parentheses below the ranges. Relative synergism coefficients for generalised mass action kinetics are constant.

Table 3
Ranges of log-synergism coefficients for current biochemical kinetics[a]

	Normalised rate expression	$S^2(v,x_1,x_1)$	$S^2(v,x_2,x_2)$	$S^2(v,x_1,x_2)$
Generalised mass action	$x_1^{f_1}x_2^{f_2}$	0	0	0
Hill/Michaelis–Menten	$\frac{x_1^n}{1+x_1^n}$	$[-n^2/4, 0]$ $(-n^2/4)$		
Competitive inhibition	$\frac{x_1}{1+x_1+x_2}$	$[-1/4,\ 0]$ $(-2/9)$	$[-1/4,\ 0]$ $(-2/9)$	[0, 1/4[(1/9)
Non-competitive inhibition	$\frac{x_1}{(1+x_1)(1+x_2)}$	$[-1/4,\ 0]$ $(-1/4)$	$[-1/4,\ 0]$ $(-1/4)$	0
Mixed inhibition	$\frac{x_1}{(1+x_1)(1+x_1+x_2)}$	$[-1/4,\ 0]$ $(-2/9)$	$[-1/2, 0]$ $(-17/36)$	[0, 1/4[(1/9)
Uncompetitive inhibition	$\frac{x_1}{1+(1+x_2)x_1}$	$[-1/4,\ 0]$ $(-2/9)$	$[-1/4,\ 0]$ $(-2/9)$	$[-1/4,\ 0]$ $(-1/9)$
Ping–pong	$\frac{x_1x_2}{x_1+x_2+x_1x_2}$	$[-1/4,\ 0]$ $(-2/9)$	$[-1/4,\ 0]$ $(-2/9)$	[0, 1/4] (1/9)
Ordered	$\frac{x_1x_2}{\kappa+x_1+x_2+x_1x_2}$	$[-1/4,\ 0]$ $\left(-\frac{2(\kappa+1)}{(\kappa+3)^2}\right)$	$[-1/4,\ 0]$ $\left(-\frac{2(\kappa+1)}{(\kappa+3)^2}\right)$	$[-1/4,\ 1/4]$ $\left(\frac{1-\kappa}{(\kappa+3)^2}\right)$

[a] Only irreversible mechanisms are considered. In the inhibition kinetics, x_1 and x_2 stand for substrate and inhibitor, respectively. The log-synergism coefficients for substrate and inhibitor concentrations matching their K_Ms and K_Is (i.e., $x_1 = x_2 = 1$) are shown in parentheses below the ranges.

It is conjectured that most enzymes have K_Ms near the physiological concentrations of their substrates, to avoid hypersensitivity of substrate concentrations to perturbations while maximising reaction rates (see [25,26] and references therein for theoretical rationale, and [27] for experimental evidence). If this conjecture is valid, the most frequent log- and relative-synergism coefficients should be those applying at substrate and inhibitor concentrations near the respective K_Ms and K_Is. Typical absolute values of the relative-synergism coefficients at these concentrations are 1/2 or lower. However, the same conditions yield relative-synergism coefficients near 1 for dual-substrate perturbations of the ordered mechanism (with $\kappa \gg 1$) and inhibitor perturbations of mixed inhibition, and -2 for Hill kinetics with $n = 4$. For the same operating points, single-substrate and single-inhibitor perturbations yield log-synergism coefficients towards the lower extremes of the ranges in Table 4, whereas dual-substrate and substrate-inhibitor perturbations yield log-synergism coefficients in the middle of those ranges. Hill kinetics can yield log-synergism coefficients as large as -4 at K_M, for $n = 4$.

The intrinsic log-synergism coefficients of hyperbolic kinetics (Table 3) usually have lower absolute values and narrower ranges of variation than the corresponding relative-synergism coefficients (Table 2). Example 1 demonstrated that single-substrate perturbations of hyperbolic kinetics always yield log-synergism coefficients that are half the corresponding relative-synergism coefficients. The same applies for dual-substrate perturbations of Ping-Pong Bi-Bi kinetics (Example 2). These results support the superior accuracy of power-product approximations of enzyme kinetics [18,24] over linear approximations. Exceptions occur for Hill kinetics [24] (Example 1), and for dual-substrate perturbations of the ordered mechanism when $\kappa > 1$ and at least one of the substrates is nearly saturating.

The (log-)synergism coefficients discussed in this section are those that would be observed were all the non-perturbed parameters and variables kept constant. They compound in a complex manner to yield the systemic responses. The next section analyses how the intrinsic (log-)synergism coefficients relate to the (log-)synergism coefficients of systemic responses.

5. Calculation of systemic (log-)synergism coefficients

By definition, the synergism coefficients are second-order differential sensitivities. So, one can base a systemic synergism analysis of kinetic models on the implicit methods from general sensitivity theory [12–15]. The application of these methods to the first-order sensitivity analysis of metabolic networks by Cascante et al. [28,29] will serve as starting point. In this formal setting, the systemic first-order sensitivities are derived from implicit differentiation of the steady-state conditions

$$\dot{\mathbf{X}} = \mathbf{N}.\mathbf{v}(\mathbf{X}, \boldsymbol{\lambda}) = 0. \tag{15}$$

Here $\mathbf{N}$ is the stoichiometric matrix, whose element i, j is the difference between the stoichiometric coefficient of X_i as product and reactant in process j. By applying the chain rule of differentiation, we find

$$J(\dot{X}_i, \lambda_j) = \sum_{a=1}^{r} v_{i,a} \sum_{b=1}^{n} J_{\boldsymbol{\lambda}}(v_a, X_b) J(X_b, \lambda_j) + \sum_{a=1}^{r} v_{i,a} J_{\mathbf{X}}(v_a, \lambda_j) = 0, \quad i \in \{1, \ldots, n\}, \tag{16}$$

where n and r stand for the numbers of internal metabolites and processes, respectively, and $v_{i,a}$ stands for the element i, a of $\mathbf{N}$. The systemic first-order sensitivities, $J(X_i, \lambda_j)$, can then be found by solving the algebraic system (16), which yields

$$J(X_i, \lambda_j) = \sum_{a=1}^{r} c_{i,a} J_{\mathbf{X}}(v_a, \lambda_j), \tag{17}$$

with $c_{i,j}$ standing for the element i, j of the matrix

$$\mathbf{C} = -(\mathbf{N} \cdot \mathbf{J}_{\boldsymbol{\lambda}}(\mathbf{v}, \mathbf{X}))^{-1} \cdot \mathbf{N}. \tag{18}$$

A formally similar expression holds for relative sensitivities:

$$S(X_i, \lambda_j) = \sum_{a=1}^{r} c^*_{i,a} S_{\mathbf{X}}(v_a, \lambda_j), \tag{19}$$

with $c^*_{i,j}$ the element i, j of

$$\mathbf{C}^* = -(\mathbf{N} \cdot \mathrm{diag}(\mathbf{v}) \cdot \mathbf{S}_{\boldsymbol{\lambda}}(\mathbf{v}, \mathbf{X}))^{-1} \cdot \mathbf{N} \cdot \mathrm{diag}(\mathbf{v}). \tag{20}$$

To obtain the synergism coefficients, we apply again the chain rule of differentiation to (16):

$$J^{[2]}(\dot{X}_i,\lambda_j,\lambda_k)=\sum_{a=1}^{r}c_{i,a}\left(\sum_{b=1}^{n}J_{\boldsymbol{\lambda}}(v_a,X_b)J^{[2]}(X_b,\lambda_j,\lambda_k)\right.$$
$$+\sum_{b=1}^{n}\sum_{c=1}^{n}J^{[2]}_{(\boldsymbol{\lambda}),(\boldsymbol{\lambda})}(v_a,X_b,X_c)J(X_b,\lambda_j)J(X_c,\lambda_k)$$
$$+\sum_{b=1}^{n}J^{[2]}_{(\boldsymbol{\lambda}),(\mathbf{X})}(v_a,X_b,\lambda_k)J(X_b,\lambda_j)$$
$$\left.+\sum_{b=1}^{n}J^{[2]}_{(\mathbf{X}),(\boldsymbol{\lambda})}(v_a,\lambda_j,X_b)J(X_b,\lambda_k)+J^{[2]}_{(\mathbf{X}),(\mathbf{X})}(v_a,\lambda_j,\lambda_k)\right)=0 \tag{21}$$

with $J(X_\cdot,\lambda_\cdot)$ given by (17). By solving this system of algebraic equations for the $J^{[2]}(X_i,\lambda_j,\lambda_k)$ it follows

$$J^{[2]}(X_i,\lambda_j,\lambda_k)=\sum_{a=1}^{r}c_{i,a}\left(\sum_{b=1}^{n}\sum_{c=1}^{n}J^{[2]}_{(\boldsymbol{\lambda}),(\boldsymbol{\lambda})}(v_a,X_b,X_c)J(X_b,\lambda_j)J(X_c,\lambda_k)\right.$$
$$+\sum_{b=1}^{n}J^{[2]}_{(\boldsymbol{\lambda}),(\mathbf{X})}(v_a,X_b,\lambda_k)J(X_b,\lambda_j)$$
$$\left.+\sum_{b=1}^{n}J^{[2]}_{(\mathbf{X}),(\boldsymbol{\lambda})}(v_a,\lambda_j,X_b)J(X_b,\lambda_k)+J^{[2]}_{(\mathbf{X}),(\mathbf{X})}(v_a,\lambda_j,\lambda_k)\right). \tag{22}$$

An equation relating relative-synergism coefficients and relative sensitivities can then be derived from (22):

$$W^{[2]}(X_i,\lambda_j,\lambda_k)=\sum_{a=1}^{r}c^{*}_{i,a}\left(\sum_{b=1}^{n}\sum_{c=1}^{n}W^{[2]}_{(\boldsymbol{\lambda}),(\boldsymbol{\lambda})}(v_a,X_b,X_c)S(X_b,\lambda_j)S(X_c,\lambda_k)\right.$$
$$+\sum_{b=1}^{n}W^{[2]}_{(\boldsymbol{\lambda}),(\mathbf{X})}(v_a,X_b,\lambda_k)S(X_b,\lambda_j)$$
$$\left.+\sum_{b=1}^{n}W^{[2]}_{(\mathbf{X}),(\boldsymbol{\lambda})}(v_a,\lambda_j,X_b)S(X_b,\lambda_k)+W^{[2]}_{(\mathbf{X}),(\mathbf{X})}(v_a,\lambda_j,\lambda_k)\right). \tag{23}$$

Finally, by replacing Eq. (13) into (23) and noting that $\sum_{a=1}^{r}c^{*}_{i,a}S(v_a,\lambda)=0$, one obtains

$$S^{[2]}(X_i,\lambda_j,\lambda_k)=\sum_{a=1}^{r}c^{*}_{i,a}\left(\sum_{b=1}^{n}\sum_{c=1}^{n}S^{[2]}_{(\boldsymbol{\lambda}),(\boldsymbol{\lambda})}(v_a,X_b,X_c)S(X_b,\lambda_j)S(X_c,\lambda_k)\right.$$
$$+\sum_{b=1}^{n}S^{[2]}_{(\boldsymbol{\lambda}),(\mathbf{X})}(v_a,X_b,\lambda_k)S(X_b,\lambda_j)$$
$$\left.+\sum_{b=1}^{n}S^{[2]}_{(\mathbf{X}),(\boldsymbol{\lambda})}(v_a,\lambda_j,X_b)S(X_b,\lambda_k)+S^{[2]}_{(\mathbf{X}),(\mathbf{X})}(v_a,\lambda_j,\lambda_k)+S(v_a,\lambda_j)S(v_a,\lambda_k)\right). \tag{24}$$

Formulas (22)–(24) do not apply if the model includes moiety conservation cycles. Reference [30] presents a technique to overcome this problem and a more effective formalism for calculating synergism coefficients in large-scale kinetic models.

Systemic log- and relative-synergism coefficients for steady-state properties that are functions of the concentrations are derived by applying the chain rule of differentiation:

$$\begin{aligned} S^{[2]}(P,\lambda_j,\lambda_k) = &\sum_{a=1}^{n}\sum_{b=1}^{n} S^{[2]}_{(\boldsymbol{\lambda}),(\boldsymbol{\lambda})}(P,X_a,X_b)S(X_a,\lambda_j)S(X_b,\lambda_k) \\ &+ \sum_{a=1}^{n} S^{[2]}_{(\boldsymbol{\lambda}),(\mathbf{X})}(P,X_a,\lambda_k)S(X_a,\lambda_j) + \sum_{a=1}^{n} S(P,X_a)S^{[2]}(X_a,\lambda_j,\lambda_k) \\ &+ \sum_{a=1}^{n} S^{[2]}_{(\mathbf{X}),(\boldsymbol{\lambda})}(P,\lambda_j,X_a)S(X_a,\lambda_k) + S^{[2]}_{(\mathbf{X}),(\mathbf{X})}(P_a,\lambda_j,\lambda_k), \end{aligned} \tag{25}$$

$$\begin{aligned} W^{[2]}(P,\lambda_j,\lambda_k) = &\sum_{a=1}^{n}\sum_{b=1}^{n} W^{[2]}_{(\boldsymbol{\lambda}),(\boldsymbol{\lambda})}(P,X_a,X_b)S(X_a,\lambda_j)S(X_b,\lambda_k) \\ &+ \sum_{a=1}^{n} W^{[2]}_{(\boldsymbol{\lambda}),(\mathbf{X})}(P,X_a,\lambda_k)S(X_a,\lambda_j) + \sum_{a=1}^{n} S(P,X_a)W^{[2]}(X_a,\lambda_j,\lambda_k) \\ &+ \sum_{a=1}^{n} W^{[2]}_{(\mathbf{X}),(\boldsymbol{\lambda})}(P,\lambda_j,X_a)S(X_a,\lambda_k) + W^{[2]}_{(\mathbf{X}),(\mathbf{X})}(P_a,\lambda_j,\lambda_k). \end{aligned} \tag{26}$$

By replacing the systemic coefficients into Eqs. (9)–(11), we approximate synergisms, relative-synergisms or log-synergisms (respectively) that could be measured through the following minimal protocol:

1. Measure the observable (e.g., the concentration of a metabolite) at the reference state.
2. Make a small change to one of the parameters (e.g., the concentration of an external metabolite), allow time for the observable to relax to stationary values and measure again.
3. Repeat the procedure for the other parameter.
4. Change *both* parameters simultaneously, each by the same amount as in the previous steps, and measure the observable after allowing time for relaxation.
5. Apply expressions (1) and (2) to compute the synergism, or (4) and (5) for the log-synergism.

For single-parameter perturbations, after Step 2 increase again the parameter by the same amount, allow for relaxation and measure. Then apply expression (3) or (6). The relative-synergism is obtained by normalising the synergism by the value of the observable at the reference state.

5.1. Example 3

To gain insight on the mechanistic features that originate additive or multiplicative behaviour, one may consider the two schemes in Fig. 5. In scheme (a) it is assumed that v_1 and v_2 are arbitrary functions of X_1 and X_2, and that v_3 is proportional to X_4 with rate constant γ_3. At steady-state, one then obtains $X_4 = (1/\gamma_3)(v_1(X_1) + v_2(X_2))$. Hence, joint perturbations of X_1 and X_2 have an additive effect on X_4. Accordingly, the log- and relative-synergism coefficients are

$$S^{[2]}(X_4,X_1,X_2) = -S(X_4,X_1)\,S(X_4,X_2) \quad \text{and} \quad W^{[2]}(X_4,X_1,X_2) = 0.$$

For scheme (b), by considering v_1 as an arbitrary function of X_1, and $v_3 = X_4^{f_{34}}u(X_3)$, with u an arbitrary function of X_3 only, one obtains the steady-state

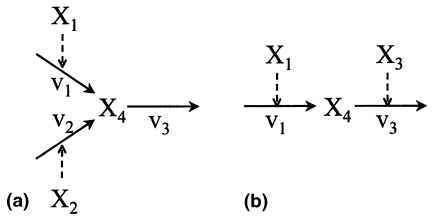


Fig. 5. Configurations of dual-parameter perturbations originating (a) additive or (b) multiplicative responses of the steady-state value of X_4. It is assumed that v_3 responds linearly to X_4 in (a) and as a power function of X_4 in (b). See Example 3 for discussion.

$$X_4 = \left(v_1(X_1) \frac{1}{u(X_3)} \right)^{1/f_{34}}.$$

Joint perturbations of X_1 and X_3 have, in this case, a multiplicative effect on X_4, and the log- and relative-synergism coefficients become

$$S^{[2]}(X_4, X_1, X_3) = 0 \quad \text{and} \quad W^{[2]}(X_4, X_1, X_3) = S(X_4, X_1)\, S(X_4, X_3).$$

The next example will show that more complex networks or kinetics blur these relationships between perturbation configurations and response patterns.

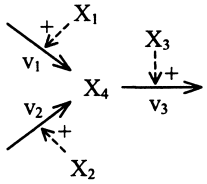
5.2. Example 4

Consider three genes, each coding for one of the enzymes that catalyses the pathway in Scheme 2. For simplicity, we assume the expression of each gene to be controlled independently and the maximal rate of each reaction to be a power of the concentration of the gene transcript coding for the respective enzyme. These concentrations, which play here the role of the vector $\boldsymbol{\lambda}$ of parameters, will be represented by the vector of independent variables $\mathbf{X_I} = [X_1 \quad X_2 \quad X_3]$. X_4 is the only dependent variable in this system. If the consumption of X_4 obeys Michaelis–Menten kinetics and the substrates of the pathway are present at constant levels it follows

$$\frac{dX_4}{dt} = \gamma_1 X_1^{f_{11}} + \gamma_2 X_2^{f_{22}} - \frac{\gamma_3 X_3^{f_{33}} X_4}{K_M + X_4}. \tag{27}$$

Though (27) can be solved straightforwardly for steady-state, disregarding the solution simplifies the analysis and permits illustrating the theory.

To compute the systemic synergism coefficients, we first calculate $\mathbf{C}^*$. From the reaction scheme we find $\mathbf{N} = [1 \quad 1 \quad -1]$; and differentiation of the rate expressions yields



Scheme 2.

$$\mathbf{S}_{\mathbf{X}_\mathrm{I}}(\mathbf{v}, X_4) = \begin{bmatrix} 0 \\ 0 \\ 1-\phi \end{bmatrix},$$

where $\phi = X_4/(K_\mathrm{M} + X_4)$ is the fraction of saturation of enzyme 3. Replacing $\mathbf{N}$ and $S_{\mathbf{X}_\mathrm{I}}(\mathbf{v}, X_4)$ into (20) gives

$$\mathbf{C}^* = \frac{1}{1-\phi}[\,\theta \quad 1-\theta \quad -1\,],$$

with $\theta = v_1/v_3$ the fraction of flux coming from branch 1. Given that

$$\mathbf{S}_{X_4}(\mathbf{v}, \mathbf{X}_\mathrm{I}) = \begin{bmatrix} f_{11} & 0 & 0 \\ 0 & f_{22} & 0 \\ 0 & 0 & f_{33} \end{bmatrix},$$

the logarithmic gains (systemic sensitivities) for concentrations and fluxes become

$$\mathbf{S}(X_4, \mathbf{X}_\mathrm{I}) = \frac{1}{1-\phi}[\,f_{11}\theta \quad f_{22}(1-\theta) \quad -f_{33}\,] \tag{28}$$

and

$$\mathbf{S}(v_3, \mathbf{X}_\mathrm{I}) = [\,f_{11}\theta \quad f_{22}(1-\theta) \quad 0\,], \tag{29}$$

respectively. From the definition of the $W^{[2]}$ operator we find the intrinsic relative-synergism coefficients:

$$W^{[2]}_{(\mathbf{X}_\mathrm{I}),(\mathbf{X}_\mathrm{I})}(\mathbf{v}, X_4, X_4) = \begin{bmatrix} 0 \\ 0 \\ -2\phi(1-\phi) \end{bmatrix},$$

$$W^{[2]}_{(\mathbf{X}_\mathrm{I}),(X_4)}(\mathbf{v}, X_4, \mathbf{X}_\mathrm{I}) = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & f_{33}(1-\phi) \end{bmatrix},$$

$$W^{[2]}_{(X_4),(X_4)}(v_1, \mathbf{X}_\mathrm{I}, \mathbf{X}_\mathrm{I}) = \begin{bmatrix} f_{11}(f_{11}-1) & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix},$$

$$W^{[2]}_{(X_4),(X_4)}(v_2, \mathbf{X}_\mathrm{I}, \mathbf{X}_\mathrm{I}) = \begin{bmatrix} 0 & 0 & 0 \\ 0 & f_{22}(f_{22}-1) & 0 \\ 0 & 0 & 0 \end{bmatrix},$$

$$W^{[2]}_{(X_4),(X_4)}(v_3, \mathbf{X}_\mathrm{I}, \mathbf{X}_\mathrm{I}) = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & f_{33}(f_{33}-1) \end{bmatrix}.$$

Because the rate expressions of reactions 1 and 2 are powers of the concentrations, their intrinsic log-synergism coefficients are null. The rate expression for reaction 3 is a product of a power of X_3

by a function of X_4. So, the only non-zero log-synergism coefficient is that for changes in X_4. It thus follows that $S^{[2]}_{(\mathbf{X}_\mathrm{I}),(X_4)}(\mathbf{v}, X_4, \mathbf{X}_\mathrm{I})$ and $S^{[2]}_{(X_4),(X_4)}(v_\cdot, \mathbf{X}_\mathrm{I}, \mathbf{X}_\mathrm{I})$ are null, whereas

$$S^{[2]}_{(\mathbf{X}_\mathrm{I}),(\mathbf{X}_\mathrm{I})}(v, X_4, X_4) = [\,0 \quad 0 \quad -\phi(1-\phi)\,].$$

The non-zero element obtains by applying the definition of the $S^{[2]}$ operator to v_3. From (23) and (24), one now obtains the systemic relative and log-synergism coefficients of the concentrations:

$$W^{[2]}(X_4, \mathbf{X}_\mathrm{I}, \mathbf{X}_\mathrm{I}) = \frac{1}{(1-\phi)^2}\begin{bmatrix} f_{11}\theta(2f_{11}\theta\phi + (f_{11}-1)(1-\phi)) & 2f_{11}f_{22}\theta(1-\theta)\phi & -f_{11}f_{33}\theta(1+\phi) \\ 2f_{11}f_{22}\theta(1-\theta)\phi & f_{22}(1-\theta)(2f_{22}(1-\theta)\phi + (f_{22}-1)(1-\phi)) & -f_{22}f_{33}(1-\theta)(1+\phi) \\ -f_{11}f_{33}\theta(1+\phi) & -f_{22}f_{33}(1-\theta)(1+\phi) & f_{33}(f_{33}(1+\phi)+1-\theta) \end{bmatrix} \tag{30}$$

$$S^{[2]}(X_4, \mathbf{X}_\mathrm{I}, \mathbf{X}_\mathrm{I}) = \frac{1}{(1-\phi)^2}\begin{bmatrix} f_{11}^2\theta(1-(1-\theta)\phi-\theta(1-\phi)) & 2f_{11}f_{22}\theta(1-\theta)(\phi-\frac{1}{2}) & -f_{11}f_{33}\theta\phi \\ 2f_{11}f_{22}\theta(1-\theta)(\phi-\frac{1}{2}) & f_{22}^2(1-\theta)(1-\theta\phi-(1-\theta)(1-\phi)) & -f_{22}f_{33}(1-\theta)\phi \\ -f_{11}f_{33}\theta\phi & -f_{22}f_{33}(1-\theta)\phi & f_{33}^2\phi \end{bmatrix}. \tag{31}$$

Combined modulations of the expression of genes 1 and 2 yield supra-additive

$$\left(W^{[2]}(X_4, X_1, X_2) = \frac{2f_{11}f_{22}\theta(1-\theta)\phi}{(1-\phi)^2} > 0\right)$$

responses of X_4, which are sub-multiplicative if $X_4 < K_\mathrm{M}$

$$\left(\phi < 1/2, \qquad S^{[2]}(X_4, X_1, X_2) = \frac{2f_{11}f_{22}\theta(1-\theta)(\phi-1/2)}{(1-\phi)^2} < 0\right)$$

and supra-multiplicative if $X_4 > K_\mathrm{M}$. Balancing the fluxes through branches 1 and 2 exacerbates both synergism and log-synergism. In turn, the modulation of either X_1 or X_2 in combination with X_3 has a sub-additive and sub-multiplicative effect on X_4.

The responses of X_4 to independent modulations of each gene product, which are always supra-multiplicative, are supra-linear in most conditions. Exceptions are the responses to changes in X_1, if $f_{11} \leqslant 1$ and

$$\theta \leqslant \frac{1}{2}\left(\frac{1}{\phi}-1\right)\left(\frac{1}{f_{11}}-1\right),$$

or X_2, if $f_{22} \leqslant 1$ and

$$1-\theta \leqslant \frac{1}{2}\left(\frac{1}{\phi}-1\right)\left(\frac{1}{f_{22}}-1\right).$$

Consider $X_4 = K_\mathrm{M}$ (i.e., $\phi = 1/2$), flux evenly distributed between branches 1 and 2 ($\theta = 1/2$) and enzyme concentrations proportional to the concentrations of the gene transcripts ($f_{11} = f_{22} = f_{33} = 1$). In these 'average' conditions, one finds

$$\mathbf{W}^{[2]}(X_4, \mathbf{X}_\mathrm{I}, \mathbf{X}_\mathrm{I}) = \begin{bmatrix} 1 & 1 & -3 \\ 1 & 1 & -3 \\ -3 & -3 & 8 \end{bmatrix}$$

and

$$\mathbf{S}^{[2]}(X_4, \mathbf{X}_\mathrm{I}, \mathbf{X}_\mathrm{I}) = \begin{bmatrix} 1 & 0 & -1 \\ 0 & 1 & -1 \\ -1 & -1 & 2 \end{bmatrix}.$$

This result prompts various inferences. First, the perturbations involving X_3 are expected to elicit stronger synergisms than those involving only X_1 and/or X_2. Second, the log-synergism coefficient for joint perturbations of X_1 and X_2 is zero at this operating point, in spite of the perturbations being applied in a parallel configuration. This is due to the non-linear dependence of v_3 on X_4. Third, the system exhibits significant but moderate systemic relative and log-synergism coefficients. However, expressions (30) and (31) show that as enzyme 3 approaches saturation (i.e., as $(1/(1-\phi)^2) \to +\infty$), these coefficients increase even faster than the logarithmic gains. So, near saturation the responses become dominated by strong synergisms (and log-synergisms), even for mild perturbations. Fourth, none of the log-synergism coefficients is, in absolute value, higher than the corresponding relative-synergism coefficient. For generic operating points (Table 4), higher log- than relative-synergism coefficients are only found at low saturation of enzyme 3 – $\phi < 1/4$, for joint perturbations of X_1 and X_2; and $\phi < f_{11}\theta - 1$ or $\phi < f_{22}(1-\theta) - 1$, for individual perturbations of either X_1 or X_2, respectively.

6. Discussion

The experimental detection of synergisms plays an important role in the investigation of biochemical mechanisms [1–9]. Most experiments in this context rely on the naïve assumption that the origin of a synergism can be traced to a particular interaction between system components. Although this working assumption proves justified in some cases, in many others the synergisms could arise from mechanisms that are more complex. Indeed, little of general significance is known about what conditions lead to large synergisms in metabolic networks. Even less widely appreciated are the implications of departures from multiplicative behaviour (log-synergisms, Section 3). Multiplicative responses are arguably the physically simplest non-linear responses. They are typical of perturbations applied in a series configuration (e.g., Example 3, Fig. 5(a)), so that the effect of each perturbation feeds on the effect of the other one without any other interference and without the response being limited by any constraints. Additive responses, in turn, ensue from perturbations applied in parallel (e.g., Example 3, Fig. 5(b)), so that the effect of each perturbation is completely independent of the effect of the other perturbation. This relationship to the basic configurations for applying a pair of stimuli justifies considering both the linear/additive and the power-law/multiplicative patterns of behaviour as canonical. Many non-linearities that are more complex arise from, or can be reduced to, an interplay of multiplicative and additive responses. [1] Mass action reaction networks are a well-known example. Though their kinetics are a

[1] Note that a wide class of non-linearities can be recast as canonical forms (GMA systems, *S*-systems, half systems, Lotka–Volterra systems) within the realm of the Power-Law Formalism [16]. The formal structure of these canonical forms encompasses only linear/additive and power-law/multiplicative relationships.

Table 4
Ratios between systemic log-synergism coefficients and systemic relative synergism coefficients for Example 4

	X_1	X_2	X_3
X_1	$\dfrac{f_{11}(2f_{11}\theta\phi + (f_{11}-1)(1-\phi))}{f_{11}(2f_{11}\theta\phi + (f_{11}-1)(1-\phi)) + f_{11}\theta - (1-\phi)}$	$1 - 1/2\phi$	$\dfrac{\phi}{1+\phi}$
X_2	–	$\dfrac{f_{22}(2f_{22}(1-\theta)\phi + (f_{22}-1)(1-\phi))}{f_{22}(2f_{22}(1-\theta)\phi + (f_{22}-1)(1-\phi)) + f_{22}(1-\theta) - (1-\phi)}$	$\dfrac{\phi}{1+\phi}$
X_3	–	–	$\dfrac{f_{33}\phi}{f_{33}(1+\phi) + 1 - \phi}$

combination of multiplicative (concentrations to fluxes) and additive relationships (between fluxes, and, if there are moiety-conservation cycles, between concentrations), it can originate very complex behaviour [31,32]. On the other hand, if a mass-action reaction network lacks cycles and each internal species is produced by one reaction and consumed by one other, then only multiplicative interactions are left, and the systemic responses to perturbations are always multiplicative. Such systems yield only unit log-synergisms, and their kinetic description reduces to an S system [18]. The observation of (non-unit) log-synergisms hence reveals the existence of moiety-conservation cycles and/or of multiple elementary reactions consuming or producing a chemical species.

A classification of responses in terms of relative compliance with the linear/additive or power-law/multiplicative patterns is likely to offer more useful clues about mechanistic features of a system than the examination of synergisms alone. As Example 4 illustrates, the complex reaction structure of biochemical processes produces many intermediate cases between canonical patterns of behaviour and complicates the simple interpretation outlined above.

The analysis of synergisms and log-synergisms in realistic metabolic networks requires a suitable theoretical framework. Any general theoretical approach relating intrinsic kinetic properties of individual processes to systemic properties at steady-state confronts two difficulties. First, many mathematical models of metabolic networks lack closed-form analytical steady-state solutions. Second, if the models have an ad hoc structure, steady-state solutions may have a complicated dependence on the parameters and thus be difficult to apprehend in intuitive terms. Both problems are usually dealt with by restricting the analysis to the effects of small perturbations around an operating point. A differential treatment becomes then pertinent and, given a suitable mathematical model, differential approximations of the systemic responses can be obtained from differential approximations of the kinetics of the individual processes considered. Such was the approach taken in this work. The (log-)synergisms were approximated in terms of second-order derivatives – either non-normalised, log- or relative-synergism coefficients, as defined in Section 4 – that measure how steeply the responses diverge from additivity/linearity or power-law/multiplicativity. Two types of (log-)synergism coefficients were discriminated. Intrinsic coefficients refer to the responses of the rates of each process alone; i.e., when all other parameters and variables are kept constant. Systemic coefficients refer to the responses of the system as a whole when all the measurements (before and after perturbations) are made after letting the system approach its steady-state. A similar distinction applies in first-order differential sensitivity analysis of biochemical systems [18–23]. *Systemic* (log-)synergism coefficients are computed from intrinsic (log-)synergism coefficients as explained in Section 5.

The reader acquainted to Metabolic Control Analysis [21,22] may notice that Höfer and Heinrich's [33] Eq. (16) is formally similar to Eq. (22) of the present work. One can thus interpret those authors' second-order approach to Metabolic Control Analysis in terms of synergisms, as evaluated through non-normalised synergism coefficients. A formulation in terms of relative-synergism coefficients can be rooted on expression (23). The latter formulation seems preferable, because relative-synergism coefficients, being dimensionless, can be directly compared. Moreover, as these coefficients vary over typical ranges in 'average' physiological conditions (Section 4.2), one can attach significance to the observed values (see below). The present theoretical framework permits also interpreting the $g'_{ij,k}$ and $L'_{ij,k}$ that appear in second-order *S*-systems [34], as intrinsic and systemic log-synergism coefficients, respectively.

Absolute values of the intrinsic relative- and log-synergism coefficients of the rates of hyperbolic enzyme kinetics are usually lower than 2 and 1/4, respectively. Absolute values around 1/2 and 1/4 are typical of intrinsic relative and log-(respectively)synergism coefficients calculated at substrate and inhibitor concentrations matching the respective K_Ms and K_Is – an 'average' physiological situation. Mixed inhibition tends to yield higher coefficients though; and Hill kinetics can yield relative-synergism coefficients as high as 12, and log-synergism coefficients as low as -4, for $n = 4$.

Both intrinsic and systemic log-synergism coefficients tend to have lower absolute values than the corresponding relative-synergism coefficients. Examples 1 and 2 show that the intrinsic log-synergism coefficients for single-substrate perturbations of hyperbolic kinetics and dual-substrate perturbations of Ping-Pong kinetics are always half the corresponding relative-synergism coefficients. The systemic coefficients in Example 4 show that the same trend for lower log- than relative-synergisms. These results agree with earlier studies of the accuracy of various modelling strategies [24,35–37], and likewise suggest that the *S*-systems approach [18–20] tends to be more accurate than strategies based on linear approximations. On the other hand, as the S-systems approach assumes that all the responses follow power-law/multiplicative behaviour, it is inadequate to model situations where the log-synergism coefficients are large or are otherwise important features of the system. One of these situations is illustrated by Example 4 when enzyme 3 approaches saturation (i.e., as $\phi \to 1$ in Eqs. (30) and (31)). Both log- and relative-synergism coefficients become very large, indicating that models based on either power or linear approximations become inaccurate and that X_4 responds very sharply to small changes.

Such sharp responses are incompatible with homeostasis, though. In studies of metabolic design principles [10,38] this fact is usually expressed by moderate systemic first-order sensitivities [39]. High systemic first-order sensitivities are also regarded as symptom poor robustness in model assessments [40,41]. While Eqs. (23) and (24) suggest that high systemic log- and relative-synergism coefficients should often correlate with high systemic first-order sensitivities, they also allow high systemic log- and relative-synergism coefficients where systemic first-order sensitivities are low. Hence, as the present work indicates that robust steady-states should in general have moderate log- and relative-synergism coefficients, these coefficients should be used as additional indexes of robustness. As the log-synergism coefficients tend to have lower absolute values and are more intimately connected to common modelling approaches [16,18] than the relative-synergism coefficients, they are probably more convenient indexes of robustness than the latter coefficients.

Expression (13) allows converting between log- and relative-synergism coefficients with little extra computation. The comparison between log- and relative-coefficients allows classifying the local steady-state responses in terms of their relationships to the canonical patterns of behaviour, as suggested above.

Example 4 shows that the expression of genes for enzymes that catalyse the supply and the consumption of a metabolite may have significant synergistic effects on the steady-state concentration of this metabolite. This is so even in absence of other interactions between gene products. As the enzyme that catalyses the common step approaches saturation, systemic log- and relative-synergism coefficients increase even more sharply than the logarithmic gains. Hence, poor performance of the system near saturation may ensue not only from excessive responsiveness to fluctuations of the supply of each substrate, but perhaps even more from strong supra-additivity (and also supra-multiplicativity) towards fluctuations of the supply of different substrates.

A variety of applications in the analysis of biochemical processes, as well as in model assessment and improvement, suggest themselves in the discussion above. Those applications, however, would benefit from a more effective mathematical formulation and extension to models with moiety conservation. Such are the tasks undertaken in the next paper.

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References

[1] J.E. Casida, J.L. Engel, E.G. Essac, F.X. Kamienski, S. Kuwatsuka, Methylene-C14-dioxyphenyl compounds: metabolism in relation to their synergistic action, Science 153 (1966) 1130.
[2] B. Andersson, O. Westbye, Synergistic action of sodium and angiotensin on brain mechanisms controlling water and salt balance, Nature 228 (1970) 75.
[3] R.E. Viola, F.M. Raushel, A.R. Rendina, W.W. Cleland, Substrate synergism and the kinetic mechanism of yeast hexokinase, Biochemistry 21 (1982) 1295.
[4] R. Schüle, M. Muller, C. Kaltschmidt, R. Renkawitz, Many transcription factors interact synergistically with steroid receptors, Science 242 (1988) 1418.
[5] M. Carey, Y.S. Lin, M.R. Green, M.A. Ptashne, A mechanism for synergistic activation of a mammalian gene by GAL4 derivatives, Nature 345 (1990) 361.
[6] D. Herschlag, F.B. Johnson, Synergism in transcriptional activation: a kinetic view, Genes Development 7 (1993) 173.
[7] S. Chen, D.R. Tomchick, D. Wolle, P. Hu, J.L. Smith, R.L. Switzer, H. Zalkin, Mechanism of the synergistic end-product regulation of Bacillus subtilis glutamine phosphoribosylpyrophosphate amidotransferase by nucleotides, Biochemistry 36 (1997) 10718.
[8] W. Gu, X.L. Shi, R.G. Roeder, Synergistic activation of transcription by CBP and p53, Nature 387 (1997) 819.
[9] E.M. Goldberg, R. Zidovetzki, Synergistic effects of diacylglycerols and fatty acids on membrane structure and protein kinase C activity, Biochemistry 37 (1998) 5623.
[10] M.A. Savageau, Concepts relating the behaviour of biochemical systems to their underlying molecular properties, Arch. Biochem. Biophys. 145 (1971) 612.
[11] M. Schroeder, Fractals, Chaos, Power-laws. Minutes from an Infinite Paradise, Freeman, New York, 1990.
[12] H.W. Bode, Network Analysis and Feedback Amplifier Design, Van Nostrand, Princeton, NJ, 1945.
[13] R. Tomovic, M. Vukobratovic, General Sensitivity Theory, Elsevier, New York, 1972.
[14] J.B. Cruz, Ed., System Sensitivity Analysis, Dowen Hutchinson and Ross, Stroudsburg, 1973.
[15] H.K. Rabitz, M. Kramer, D. Dacol, Sensitivity analysis in chemical kinetics, Ann. Rev. Phys. Chem. 34 (1983) 419.
[16] M.A. Savageau, E.O. Voit, Recasting nonlinear differential equations as S-systems: a canonical nonlinear form, Math. Biosci. 87 (1987) 83.
[17] A. Salvador, Development of methodology and software for analysis of kinetic models of metabolic processes. Application to the mitochondrial metabolism of lipid hydroperoxides. PhD thesis. University of Lisbon, 1996, pp. 182–184.

[18] M.A. Savageau, Biochemical systems analysis: I. Some mathematical properties of the rate law for the component enzymatic reactions, J. Theor. Biol. 25 (1969) 365.
[19] M.A. Savageau, Biochemical systems analysis: II. The steady-state solutions for an *n*-pool system using a power-law approximation, J. Theor. Biol. 25 (1969) 370.
[20] M.A. Savageau, Biochemical systems analysis: III. Dynamic solutions using the power-law approximation, J. Theor. Biol. 26 (1970) 215.
[21] H. Kacser, J.A. Burns, Rate control of biological processes, Symp. Soc. Exp. Biol. XXVII, 1973, 64.
[22] R. Heinrich, T.A. Rapoport, A linear steady-state treatment of enzymatic chains – General properties, control and effector strength, Eur. J. Biochem. 42 (1974) 89.
[23] B. Crabtree, E.A. Newsholme, Sensitivity of a near-equilibrium reaction in a metabolic pathway to changes in substrate concentration, Eur. J. Biochem. 89 (1978) 19.
[24] E.O. Voit, M.A. Savageau, Accuracy of alternative representations for integrated biochemical systems, Biochemistry 26 (1987) 6869.
[25] P.W. Hochachka, G.N. Somero, Biochemical Adaptation, Princeton University, Princeton, NJ, 1984, p. 59.
[26] R. Heinrich, E. Klipp, Control analysis of unbranched enzymatic chains in states of maximal activity, J. Theor. Biol. 182 (1996) 243.
[27] O.H. Lowry, J.V. Passonneau, The relationship between substrates and enzymes of glycolysis in brain, J. Biol. Chem. 239 (1964) 31.
[28] M. Cascante, E.I. Canela, R. Franco, Use of implicit methods of general sensitivity theory to develop a systematic approach to metabolic control. I. Unbranched pathways, Math. Biosci. 94 (1989) 271.
[29] M. Cascante, E.I. Canela, R. Franco, Use of implicit methods of general sensitivity theory to develop a systematic approach to metabolic control. II. Complex systems, Math. Biosci. 94 (1989) 289.
[30] A. Salvador, Synergism analysis of biochemical systems. II. Tensor formulation and treatment of stoichiometric constraints, Math. Biosci., this issue, p. 131.
[31] A. Lotka, Undamped oscillations derived from the law of mass action, J. Am. Chem. Soc. 42 (1920) 1595.
[32] O.E. Rössler, Chaotic behaviour of simple reaction systems, Z. Naturfrssch. 31a (1976) 259.
[33] T. Höfer, R. Heinrich, A second-order approach to Metabolic control analysis, J. Theor. Biol. 164 (1993) 85.
[34] M. Cascante, A. Sorribas, R. Franco, E.I. Canela, Biochemical Systems Theory: Increasing predictive power by using second-order derivatives measurements, J. Theor. Biol. 149 (1991) 521.
[35] A. Sorribas, M.A. Savageau, Strategies for representing metabolic pathways within biochemical systems theory: reversible pathways, Math. Biosci. 94 (1989) 239.
[36] A. Sorribas, M.A. Savageau, A comparison of variant theories of intact biochemical systems. I. Enzyme–enzyme interactions and biochemical systems theory, Math. Biosci. 94 (1989) 161.
[37] A. Sorribas, M.A. Savageau, A comparison of variant theories of intact biochemical systems. II. Flux-oriented and metabolic control theories, Math. Biosci. 94 (1989) 195.
[38] M.A. Savageau, Biochemical Systems Analysis. A Study of Function and Design in Molecular Biology. Addison-Wesley, Reading, MA, 1976.
[39] M.A. Savageau, Parameter sensitivity as a criterion for evaluating and comparing the performance of biochemical systems, Nature 229 (1971) 542.
[40] F. Shiraishi, M.A. Savageau, The tricarboxylic acid cycle in *Dictyostelium discoideum*. II. Evaluation of model consistency and robustness, J. Biol. Chem. 267 (1992) 22919.
[41] T.-C. Ni, M.A. Savageau, Model assessment and refinement using strategies from Biochemical Systems Theory: application to metabolism in human red blood cells, J. Theor. Biol. 179 (1996) 329.