Synergism analysis of biochemical systems. II. Tensor formulation and treatment of stoichiometric constraints

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Abstract

The previous paper outlined a conceptual and mathematical framework for synergism analysis of kinetic models. Though the formalism presented there is adequate for studying simple models, the analysis of large-scale models benefits from the more effective formulation achieved in this work. The present formulation is based on simple tensor operations and takes advantage of the analogy between the formalisms for synergism and log-synergism analysis presented before. Well-known relationships of first-order sensitivity analysis and new relationships for (log-)synergism coefficients of various steady-state properties are cast in the new formal setting. The formalism is then extended to models that are subject to constraints between variables, fluxes and/or parameters. This treatment, which generalises Reder's concept of link matrices, is applied to networks that include moiety conservation cycles [C. Reder, Metabolic control theory: a structural approach, J. Theor. Biol. 135 (1988) 175]. It is also used to take advantage of flux conservation at steady-state to simplify synergism analysis. Issues of numerical effectiveness are briefly discussed, and the theory illustrated with the study of synergistic behaviour in the metabolism of reactive oxygen species and of a scheme of dynamic channelling. © 2000 Elsevier Science Inc. All rights reserved.

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1. Introduction

The need to integrate large collections of kinetic data and the availability of powerful computational resources led to a resurgent interest in large-scale integrative models of metabolic

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processes [1–5]. Owing to mathematical complexity, closed-form analytical steady-state solutions of these models are seldom available, while extensive numerical simulations are impractical for analysing responses to large sets of parameters. Differential sensitivity analysis [6–9] – as conveyed to biochemistry by various formalisms [10–15] – usually becomes the method of choice in these circumstances, owing to various factors. First, the concept of sensitivity is adequate for describing the control properties of metabolic networks [16], and the values of the sensitivities can be used as indexes of metabolic performance [17] or model robustness [18]. Second, the sensitivities *condense* this information into scalar quantities, making the characterisation of the steady-state behaviour of whole systems more intelligible. Third, computer implementation of differential sensitivity analysis is relatively straightforward, and even detailed analyses of large-scale models require modest computational resources by present standards.

The formalism presented in the previous paper [19] extends the first two features (physical meaning and condensed information) to multifactorial analyses, and refines the characterisation of responses to single-parameter perturbations. Ease of implementation and computational economy drove the gradual replacement of earlier scalar formulations [10–15] of sensitivity analysis of biochemical systems by matrix formulations [11,20–24]. The ensuing gains in formal conciseness and numerical effectiveness argue for similar improvements of the methodology for synergism analysis [19]. Yet, while the first-order sensitivities of a set of properties to a set of parameters are logically accommodated in a matrix, the (log-)synergism coefficients for the response of a set of properties to all single and dual perturbations of a set of parameters should be accommodated in a rank-3 tensor. The general results of [19] are thus recast in a tensor formulation (Section 3), and formulas for non-normalised, relative- and log-synergism coefficients of various properties are derived (Appendix B). All the relevant expressions make use of only the simplest tensor operations, which are explained in Appendix A. The theory is illustrated by the analysis of a simple model of the metabolism of reactive oxygen species in the mitochondrial matrix of rat hepatocytes (Section 3.1).

The calculation of systemic generalised-synergism coefficients of systems that include moiety conservation cycles requires a special procedure. The concept of link matrix [25] permitted overcoming the corresponding problem in first-order sensitivity analysis of metabolic networks. Upon multiplication of a vector of a suitably chosen subset of 'independent' sensitivities of a system, link matrices yield a vector of sensitivities that can be expressed as linear combinations of the former non-normalised [25] or logarithmic [26] sensitivities. Section 4.1 extends this concept to synergism analysis and to general constraints between variables, fluxes and/or parameters that may apply to biochemical systems. This general framework replaces the concept of link matrix by that of link tensor, with a similar meaning but applying also to synergism analysis. The technique is then applied (a) to synergism analysis of systems that include moiety conservation cycles (Section 4.2.1) and (b) to simplifying synergism analysis of fluxes by accounting for flux conservation at steady-state (Section 4.2.2).

Often, sensitivity and synergism analyses of kinetic models subject to conservation relationships can be performed with less computation than necessary for other kinetic models of the same dimensionality. This computational advantage is especially important for large-scale integrative models (for instance, [1–5], see Section 5).

2. Notation and conventions

The notation and conventions introduced in the previous papers are adopted here, with extensions as described below.

The term 'generalised-synergism' will be used to denote a measure of deviation from a predefined pattern of behaviour. This includes non-normalised synergisms, relative synergisms and log-synergisms [19]. The differential measures of steepness of deviation from the predefined patterns will be denoted by 'generalised-synergism coefficients'. To simplify language, where there is no ambiguity the term 'sensitivities' will be used to refer to both first-order sensitivities and generalised-synergism coefficients (second-order sensitivities).

It seems natural to represent the generalised-synergisms of all single- and dual-parameter perturbations with respect to a vector $\mathbf{P}(\lambda)$ of n_P functions of a vector λ of p parameters as a $n_P \times p \times p$ tensor. For instance,

$$\mathbf{\Psi}_P(\lambda_o, \Delta\lambda) = \{\Psi_{P_i}(\lambda_o, \Delta\lambda_{(j,k)})\}, \qquad i \in \{1, 2, \ldots, n_P\}, \quad j, k \in \{1, 2, \ldots, p\}.$$

When the generalised-synergism coefficients are similarly arranged, the calculation of the systemic coefficients requires only simple tensor operations. These operations and the corresponding notation are explained in Appendix A. Additional notation is: $\mathbf{I}_k$, the $k \times k$ identity matrix; $\mathbf{0}_{i \times j \times k}$, the $i \times j \times k$ null tensor; $\ker(\mathbf{A})$, the kernel of matrix $\mathbf{A}$; $\mathbf{x}^\backslash$, $\mathrm{diag}(\mathbf{x})$, the diagonal matrix whose main diagonal is formed by the elements of $\mathbf{x}$. To avoid ambiguity, one must distinguish matrix-rank (the number of linearly independent rows of a matrix) from tensor-rank (the number of indexes necessary to specify an element of a tensor). This notation casts Eq. (10) of [19] in tensor form as

$$\mathbf{\Psi}_P(\lambda_o, \Delta\lambda) \approx \frac{1}{2}\left(\mathbf{W}^{[2]}(\mathbf{P}, \lambda, \lambda)_{\dot{2}}\,\Delta\lambda\right) \cdot \Delta\lambda,$$

similar expressions apply for the other generalised-synergisms.

If $\mathbf{P}$, λ_A and λ_B are vectors, the following relationships hold:

$$\mathbf{S}(\mathbf{P}, \lambda) = \mathbf{P}^{\backslash-1} \cdot \mathbf{J}(\mathbf{P}, \lambda) \cdot \lambda^\backslash, \tag{1}$$

$$\mathbf{W}^{[2]}(\mathbf{P}, \lambda_A, \lambda_B) = \mathbf{P}^{\backslash-1} \cdot (\mathbf{J}^{[2]}\left(\mathbf{P}, \lambda_A, \lambda_B\right)_{\dot{2}}\lambda_A^\backslash) \cdot \lambda_B^\backslash, \tag{2}$$

$$\mathbf{S}^{[2]}(\mathbf{P}, \lambda_A, \lambda_B) = \mathbf{J}^{[2]}(\log|\mathbf{P}|, \log|\lambda_A|, \log|\lambda_B|), \tag{3}$$

$$\mathbf{S}^{[2]}(\mathbf{P}, \lambda_A, \lambda_B) = \mathbf{W}^{[2]}(\mathbf{P}, \lambda_A, \lambda_B) - \left(\mathbf{I}^*_{n_P\,\dot{2}}\mathbf{S}(\mathbf{P}, \lambda_A)\right) \cdot \mathbf{S}(\mathbf{P}, \lambda_B) + \mathbf{S}(\mathbf{P}, \lambda_A) \cdot \mathbf{I}^*_{n_A} \cdot \mathbf{S}(\lambda_A, \lambda_B), \tag{4}$$

with n_P and n_A standing for the dimensions of the vectors $\mathbf{P}$ and λ_A, respectively.

The $\mathbf{J}$ and $\mathbf{S}$ operators also follow the chain rule when operating over composite functions. That is, if $\mathbf{F} \equiv \mathbf{F}(\mathbf{P}(\lambda), \lambda)$ and $\mathbf{F}$ and $\mathbf{P}$ are both differentiable, then

$$\mathbf{J}(\mathbf{F}, \lambda) = \mathbf{J}_\lambda(\mathbf{F}, \mathbf{P}) \cdot \mathbf{J}(\mathbf{P}, \lambda) + \mathbf{J}_{\mathbf{P}}(\mathbf{F}, \lambda) \tag{5}$$

and

$$\mathbf{S}(\mathbf{F}, \lambda) = \mathbf{S}_\lambda(\mathbf{F}, \mathbf{P}) \cdot \mathbf{S}(\mathbf{P}, \lambda) + \mathbf{S}_{\mathbf{P}}(\mathbf{F}, \lambda). \tag{6}$$

This shared property between $\mathbf{J}$ and $\mathbf{S}$ allows developing the formalisms for the Cartesian and logarithmic spaces in parallel. To avoid redundancy, the symbol $\mathbf{O}$ is used for the operator in relationships that are formally similar for various sensitivity operators. Unless otherwise stated, in such relationships $\mathbf{O}$ can be $\mathbf{J}$, $\mathbf{S}$ or $\mathbf{W}$. For first-order sensitivities, the operators $\mathbf{W}$ (relative sensitivities) and $\mathbf{S}$ (logarithmic sensitivities) have the same meaning, but this identity does not hold for generalised-synergism coefficients [19].

The symbols $\mathbf{V}_+$ and $\mathbf{U}$ stand for aggregated rates of production of a metabolite and turnover numbers, respectively.

3. The tensor formulation of synergism analysis

To clarify the relationships of the tensor formulation with the previous formulation [19], and to examine the mathematical assumptions upon which the formalism relies, the basic formulas for synergism analysis are re-derived in tensor form.

An autonomous kinetic model can be expressed as

$$\dot{\mathbf{X}} = \mathbf{N} \cdot \mathbf{v}(\mathbf{X}_0, \mathbf{X}, \boldsymbol{\lambda}), \tag{7}$$

with $\mathbf{N}$ the stoichiometric matrix and $\mathbf{X}_0$ the initial concentrations of the internal species. The fluxes, $\mathbf{v}$, may have various meanings. Each process could be an aggregate of all reactions producing or consuming each internal species, an enzyme-catalysed transformation, an elementary reaction, etc. Though the initial conditions, $\mathbf{X}_0$, determine the steady-state to which the system will converge if (7) allows coexisting stable steady-states, they lack any other influence on the steady-state. So, if (a) the kinetic model is structurally stable, (b) the operating point is not poised at a bifurcation, and (c) $\mathbf{v}$ is differentiable around the operating point, one obtains

$$\mathbf{J}(\dot{\mathbf{X}}, \boldsymbol{\lambda}) = \mathbf{N} \cdot (\mathbf{J}_{\boldsymbol{\lambda}}(\mathbf{v}, \mathbf{X}) \cdot \mathbf{J}(\mathbf{X}, \boldsymbol{\lambda}) + \mathbf{J}_{\mathbf{X}}(\mathbf{v}, \boldsymbol{\lambda})) = \mathbf{0}, \tag{8}$$

or, by normalising the sensitivities,

$$\mathbf{N} \cdot \mathbf{v}^{\backslash} \cdot (\mathbf{S}_{\boldsymbol{\lambda}}(\mathbf{v}, \mathbf{X}) \cdot \mathbf{S}(\mathbf{X}, \boldsymbol{\lambda}) + \mathbf{S}_{\mathbf{X}}(\mathbf{v}, \boldsymbol{\lambda})) = \mathbf{0}. \tag{9}$$

If (d) the matrix $\mathbf{N} \cdot \mathbf{J}_{\boldsymbol{\lambda}}(\mathbf{v}, \mathbf{X})$ is non-singular, one can solve (8) for the matrix of systemic sensitivities:

$$\mathbf{J}(\mathbf{X}, \boldsymbol{\lambda}) = \hat{\mathbf{J}}(\mathbf{X}, \mathbf{v}) \cdot \mathbf{J}_{\mathbf{X}}(\mathbf{v}, \boldsymbol{\lambda}) \tag{10}$$

with

$$\hat{\mathbf{J}}(\mathbf{X}, \mathbf{v}) = -(\mathbf{N} \cdot \mathbf{J}_{\boldsymbol{\lambda}}(\mathbf{v}, \mathbf{X}))^{-1} \cdot \mathbf{N}. \tag{11}$$

Furthermore, if (d′) the matrix $\mathbf{N} \cdot \mathbf{v}^{\backslash} \cdot \mathbf{S}_{\boldsymbol{\lambda}}(\mathbf{v}, \mathbf{X})$ is non-singular, then solving (9) for the systemic logarithmic sensitivities gives

$$\mathbf{S}(\mathbf{X}, \boldsymbol{\lambda}) = \hat{\mathbf{S}}(\mathbf{X}, \mathbf{v}) \cdot \mathbf{S}_{\mathbf{X}}(\mathbf{v}, \boldsymbol{\lambda}) \tag{12}$$

with

$$\hat{\mathbf{S}}(\mathbf{X}, \mathbf{v}) = -\left(\mathbf{N} \cdot \mathbf{v}^{\backslash} \cdot \mathbf{S}_{\boldsymbol{\lambda}}(\mathbf{v}, \mathbf{X})\right)^{-1} \cdot \mathbf{N} \cdot \mathbf{v}^{\backslash}. \tag{13}$$

We call systemic sensitivities of the type $\hat{\mathbf{O}}(\mathbf{P}, \mathbf{v})$, which cannot be obtained through explicit differentiation of the steady-state solution, 'implicit sensitivities', to distinguish them from the systemic parameter sensitivities, $\mathbf{O}(\mathbf{P}, \boldsymbol{\lambda})$.

If the model includes moiety conservation cycles, conditions (d) and (d′) are violated, making the treatment presented in Section 4.2.1 necessary. To compute first-order sensitivities of properties that are functions of concentrations (derived properties) one applies expressions (5) and (6). Table 1 lists various such relationships. There is a direct correspondence between these and similar relationships in biochemical systems theory [30], metabolic control analysis [22–24] and/or flux-oriented theory [21].

If $\mathbf{v}$ is twice-differentiable around the operating point and $\boldsymbol{\lambda}_A$ and $\boldsymbol{\lambda}_B$ are two vectors of parameters, the application of chain rule (5) to Eq. (8) gives

$$
\begin{aligned}
\mathbf{N} \cdot [(\mathbf{J}_{\cdot,\cdot}^{[2]}(\mathbf{v}, \mathbf{X}, \mathbf{X})_{\dot{2}} \mathbf{J}(\mathbf{X}, \boldsymbol{\lambda}_A)) \mathbf{J}(\mathbf{X}, \boldsymbol{\lambda}_B) + \mathbf{J}_{\cdot,\cdot}^{[2]}(\mathbf{v}, \mathbf{X}, \boldsymbol{\lambda}_A)^{\mathrm{T}_{1,3,2}} \cdot \mathbf{J}(\mathbf{X}, \boldsymbol{\lambda}_B) \\
+ \mathbf{J}_{\cdot,\cdot}^{[2]}(\mathbf{v}, \mathbf{X}, \boldsymbol{\lambda}_B)_{\dot{2}} \mathbf{J}(\mathbf{X}, \boldsymbol{\lambda}_A) + \mathbf{J}_{\boldsymbol{\lambda}_A, \boldsymbol{\lambda}_B}(\mathbf{v}, \mathbf{X}) . \mathbf{J}^{[2]}(\mathbf{X}, \boldsymbol{\lambda}_A, \boldsymbol{\lambda}_B) + \mathbf{J}_{\cdot,\cdot}^{[2]}(\mathbf{v}, \boldsymbol{\lambda}_A, \boldsymbol{\lambda}_B)] = \mathbf{0}.
\end{aligned}
\tag{14}
$$

The solutions of (14) and of its counterpart for logarithmic co-ordinates share the form

$$
\mathbf{O}^{[2]}(\mathbf{X}, \boldsymbol{\lambda}_A, \boldsymbol{\lambda}_B) = \hat{\mathbf{O}}(\mathbf{X}, \mathbf{v}) \cdot (\tilde{\mathbf{O}}_{[\mathbf{X}],[\mathbf{X}]}^{[2]}(\mathbf{v}, \boldsymbol{\lambda}_A, \boldsymbol{\lambda}_B) + \tilde{\mathbf{O}}_{[\mathbf{v},\mathbf{X}],[\mathbf{v},\mathbf{X}]}^{[2]}(\mathbf{v}, \boldsymbol{\lambda}_A, \boldsymbol{\lambda}_B)).
\tag{15}
$$

The subscripts of $\tilde{\mathbf{O}}_{[\mathbf{X}],[\mathbf{X}]}^{[2]}$ and $\tilde{\mathbf{O}}_{[\mathbf{v},\mathbf{X}],[\mathbf{v},\mathbf{X}]}^{[2]}$ indicate variables that are allowed first-order variations (linear, for $\mathbf{J}$, or power-law for $\mathbf{W}$ and $\mathbf{S}$) with the parameters. So

$$
\begin{aligned}
\tilde{\mathbf{O}}_{[\mathbf{X}],[\mathbf{X}]}^{[2]}(\mathbf{v}, \boldsymbol{\lambda}_A, \boldsymbol{\lambda}_B) = (\mathbf{O}_{(\boldsymbol{\lambda}),(\boldsymbol{\lambda})}^{[2]}(\mathbf{v}, \mathbf{X}, \mathbf{X})_{\dot{2}} \mathbf{O}(\mathbf{X}, \boldsymbol{\lambda}_A)) \cdot \mathbf{O}(\mathbf{X}, \boldsymbol{\lambda}_B) + \mathbf{O}_{(\boldsymbol{\lambda}),(\mathbf{X})}^{[2]}(\mathbf{v}, \mathbf{X}, \boldsymbol{\lambda}_A)^{T_{1,3,2}} \cdot \mathbf{O}(\mathbf{X}, \boldsymbol{\lambda}_B) \\
+ \mathbf{O}_{(\boldsymbol{\lambda}),(\mathbf{X})}^{[2]}(\mathbf{v}, \mathbf{X}, \boldsymbol{\lambda}_B)_{\dot{2}} \mathbf{O}(\mathbf{X}, \boldsymbol{\lambda}_A) + \mathbf{O}_{(\mathbf{X}),(\mathbf{X})}^{[2]}(\mathbf{v}, \boldsymbol{\lambda}_A, \boldsymbol{\lambda}_B)
\end{aligned}
\tag{16}
$$

represents the generalised-synergism coefficients that would be observed for rates of reaction if the concentrations responded linearly (for $\mathbf{J}$) or as a power-law (for $\mathbf{W}$ and $\mathbf{S}$) to single-parameter perturbations. These magnitudes are considered as *semi-intrinsic generalised-synergism coefficients*, which take the *first-order* systemic responses of the concentrations into account but assume that the deviations of each flux from the first-order approximations do not interact.

The term $\tilde{\mathbf{O}}_{[\mathbf{v},\mathbf{X}],[\mathbf{v},\mathbf{X}]}^{[2]}(\mathbf{v}, \boldsymbol{\lambda}_A, \boldsymbol{\lambda}_B)$ is null when $\mathbf{O}$ stands for $\mathbf{J}$ or $\mathbf{W}$, or takes the value

$$
\tilde{\mathbf{S}}_{[\mathbf{v},\mathbf{X}],[\mathbf{v},\mathbf{X}]}^{[2]}(\mathbf{v}, \boldsymbol{\lambda}_A, \boldsymbol{\lambda}_B) = (\mathbf{I}_{r\,\dot{2}}^* \mathbf{S}(\mathbf{v}, \boldsymbol{\lambda}_A)) \cdot \mathbf{S}(\mathbf{v}, \boldsymbol{\lambda}_B) - \mathbf{S}(\mathbf{v}, \boldsymbol{\lambda}_A) \cdot \mathbf{I}_{p_A}^* \cdot \mathbf{S}(\boldsymbol{\lambda}_A, \boldsymbol{\lambda}_B).
\tag{17}
$$

Table 1
Systemic first-order sensitivities of derived steady-state properties[a]

Cartesian space	Logarithmic space
$\hat{\mathbf{O}}(\mathbf{v}, \mathbf{v}) = \mathbf{O}_{\boldsymbol{\lambda}}(\mathbf{v}, \mathbf{X}) \cdot \hat{\mathbf{O}}(\mathbf{X}, \mathbf{v}) + \mathbf{I}$	
$\hat{\mathbf{J}}(\mathbf{V}_+, \mathbf{v}) = \mathbf{N}_+ \cdot \hat{\mathbf{J}}(\mathbf{v}, \mathbf{v})$	$\hat{\mathbf{S}}(\mathbf{V}_+, \mathbf{v}) = \mathbf{S}_{X,\boldsymbol{\lambda}}(\mathbf{V}_+, \mathbf{v}) \cdot \hat{\mathbf{S}}(\mathbf{v}, \mathbf{v})$
	$\quad = \mathbf{V}_+^{\setminus -1} \cdot \mathbf{N}_+ \cdot \mathbf{v}^{\setminus} \cdot \hat{\mathbf{S}}(\mathbf{v}, \mathbf{v})$
$\hat{\mathbf{J}}(\mathbf{U}, \mathbf{v}) = \mathbf{X}^{\setminus -1} \cdot \left(\hat{\mathbf{J}}(\mathbf{V}_+, \mathbf{v}) - \mathbf{U}^{\setminus} \cdot \hat{\mathbf{J}}(\mathbf{X}, \mathbf{v}) \right)$	$\hat{\mathbf{S}}(\mathbf{U}, \mathbf{v}) = \hat{\mathbf{S}}(\mathbf{V}_+, \mathbf{v}) - \hat{\mathbf{S}}(\mathbf{X}, \mathbf{v})$
$\mathbf{O}(\mathbf{v}, \boldsymbol{\lambda}) = \hat{\mathbf{O}}(\mathbf{v}, \mathbf{v}) \cdot \mathbf{O}_X(\mathbf{v}, \boldsymbol{\lambda})$	
$\mathbf{J}(\mathbf{V}_+, \boldsymbol{\lambda}) = \mathbf{N}_+ \cdot \mathbf{J}(\mathbf{v}, \boldsymbol{\lambda})$	$\mathbf{S}(\mathbf{V}_+, \boldsymbol{\lambda}) = \mathbf{S}_{X,\boldsymbol{\lambda}}(\mathbf{V}_+, \mathbf{v}) \cdot \mathbf{S}(\mathbf{v}, \boldsymbol{\lambda})$
	$\quad = \mathbf{V}_+^{\setminus -1} \cdot \mathbf{N}_+ \cdot \mathbf{v}^{\setminus} \cdot \mathbf{S}(\mathbf{v}, \boldsymbol{\lambda})$
$\mathbf{J}(\mathbf{U}, \boldsymbol{\lambda}) = \mathbf{X}^{\setminus -1} \cdot \left(\mathbf{J}(\mathbf{V}_+, \boldsymbol{\lambda}) - \mathbf{U}^{\setminus} \cdot \mathbf{J}(\mathbf{X}, \boldsymbol{\lambda}) \right)$	$\mathbf{S}(\mathbf{U}, \boldsymbol{\lambda}) = \mathbf{S}(\mathbf{V}_+, \boldsymbol{\lambda}) - \mathbf{S}(\mathbf{X}, \boldsymbol{\lambda})$

[a] $\mathbf{N}_+$ is obtained from $\mathbf{N}$ by setting the negative elements to 0.

The elements of $\tilde{\mathbf{O}}^{[2]}_{[\mathbf{v},\mathbf{X}],[\mathbf{v},\mathbf{X}]}(\mathbf{v},\boldsymbol{\lambda}_A,\boldsymbol{\lambda}_B)$ are also semi-intrinsic generalised-synergism coefficients. As $\hat{\mathbf{S}}(\mathbf{X},\mathbf{v})\cdot\mathbf{S}(\mathbf{v},\boldsymbol{\lambda}_A) = \mathbf{0}$, for log-synergism coefficients (15) simplifies to

$$\mathbf{S}^{[2]}(\mathbf{X},\boldsymbol{\lambda}_A,\boldsymbol{\lambda}_B) = \hat{\mathbf{S}}(\mathbf{X},\mathbf{v})\cdot\lfloor\tilde{\mathbf{S}}^{[2]}_{[\mathbf{X}],[\mathbf{X}]}(\mathbf{v},\boldsymbol{\lambda}_A,\boldsymbol{\lambda}_B) + \left(\mathbf{I}^*_{r\dot{2}}\mathbf{S}(\mathbf{v},\boldsymbol{\lambda}_A)\right)\cdot\mathbf{S}(\mathbf{v},\boldsymbol{\lambda}_B)\rfloor. \tag{18}$$

Systemic generalised-synergism coefficients for fluxes, turnover numbers and other derived steady-state properties, $\mathbf{P}\equiv\mathbf{P}(\mathbf{X}(\boldsymbol{\lambda}),\boldsymbol{\lambda})$, can be computed from the systemic concentration generalised-synergism coefficients through

$$\begin{aligned}
\mathbf{O}^{[2]}(\mathbf{P},\boldsymbol{\lambda}_A,\boldsymbol{\lambda}_B) &= (\mathbf{O}^{[2]}_{(\boldsymbol{\lambda}),(\boldsymbol{\lambda})}(\mathbf{P},\mathbf{X},\mathbf{X})_{\dot{2}}(\mathbf{O},\mathbf{X},\boldsymbol{\lambda}_A))\cdot\mathbf{O}(\mathbf{X},\boldsymbol{\lambda}_B) + \mathbf{O}^{[2]}_{(\boldsymbol{\lambda}),(\mathbf{X})}(\mathbf{P},\mathbf{X},\boldsymbol{\lambda}_A)^{\mathbf{T}_{1,3,2}}\cdot\mathbf{O}(\mathbf{X},\boldsymbol{\lambda}_B) \\
&\quad + \mathbf{O}_{\boldsymbol{\lambda}}(\mathbf{P},\mathbf{X})\cdot\mathbf{O}^{[2]}(\mathbf{X},\boldsymbol{\lambda}_A,\boldsymbol{\lambda}_B) + \mathbf{O}^{[2]}_{(\boldsymbol{\lambda}),(\mathbf{X})}(\mathbf{P},\mathbf{X},\boldsymbol{\lambda}_B)_{\dot{2}}\mathbf{O}(\mathbf{X},\boldsymbol{\lambda}_A) \\
&\quad + \mathbf{O}^{[2]}_{(\mathbf{X}),(\mathbf{X})}(\mathbf{P},\boldsymbol{\lambda}_A,\boldsymbol{\lambda}_B).
\end{aligned} \tag{19}$$

By considering $\boldsymbol{\lambda}$ a vector of r reaction-specific parameters such that $\mathbf{O}(\mathbf{v},\boldsymbol{\lambda}) = \mathbf{I}$, one can use (15) to compute intrinsic $[\hat{\mathbf{O}}^{[2]}(\mathbf{X},\mathbf{v},\mathbf{v})]$ and mixed [intrinsic-parameter, $\hat{\mathbf{O}}^{[2]}(\mathbf{X},\mathbf{v},\boldsymbol{\lambda})$] generalised-synergism coefficients. Appendix B presents the derivation of systemic, intrinsic and mixed generalised-synergism coefficients for various steady-state properties.

3.1. Example

Below the tensor formulation is applied to the synergism analysis of a simple model of the metabolism of reactive oxygen species in the mitochondrial matrix of rat hepatocytes (Fig. 1). Superoxide ($O_2^{\cdot-}$) is produced as subproduct of the respiratory chain (reaction 1), and its dismutation – quickly catalysed by superoxide dismutase (SOD, reaction 2) – produces hydrogen peroxide (H_2O_2). Though the latter is mostly reduced to water through a glutathione-peroxidase-catalysed reaction (reaction 4, GPx), a small fraction reacts with Fe^{2+} (reaction 3), producing the extremely reactive hydroxyl radical ($HO^{\cdot}$), which damages proteins and DNA (reaction 6). Superoxide may also damage proteins and DNA – though with much lower rate constants than $HO^{\cdot}$ – and, once protonated, reacts also with unsaturated lipids in bilayers. The estimation of the parameters (Table 2) is discussed in Ref. [5]. The present model takes $X_1 = [O_2^{\cdot-}]$, $X_2 = [H_2O_2]$ and $X_3 = [HO^{\cdot}]$ as dependent variables, and $X_4 = [O_2]$, $X_5 = [SOD]$, $X_6 = [Fe^{2+}]$ and $X_7 = [GPx]$ as parameters of interest (vector $\boldsymbol{\lambda}$). The rate $v_d = v_5 + v_6 = k_5 X_1 + k_6 X_3$ is a coarse index of mitochondrial damage caused by superoxide generation. We are interested in evaluating the type of interaction and degree of synergism and/or antagonism between the various
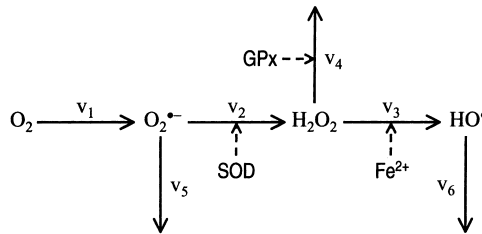

Fig. 1. Simple scheme of the metabolism of reactive oxygen species in the matrix of rat liver mitochondria. Reactions 5 and 6 account for damage to mitochondrial components. The abbreviations are: GPx, 'classic' glutathione peroxidase, GSH, glutathione, SOD, Mn-dependent superoxide dismutase.

Table 2
Rate expressions and parameters considered in the example (see Fig. 1) for identification of the reactions. Parameter estimations are discussed in [5]

Rate expression	Parameters
$v_1 = k_1[O_2]$	$k_1 = 0.16 s^{-1}$, $[O_2] = 1.0 \times 10^{-4}$ M (in lipid bilayers)
$v_2 = k_2[SOD][O_2^-]$	$k_2 = 4.7 \times 10^9$ M^{-1} s^{-1}, $[SOD] = 1.1 \times 10^{-5}$ M
$v_3 = k_3[Fe^{2+}][H_2O_2]$	$k_3 = 2.0 \times 10^4$ M^{-1} s^{-1}, $[Fe^{2+}] = 1.0 \times 10^{-7}$ M
$v_4 = \frac{[GPx]}{(\phi_1/[H_2O_2]) + (\phi_2/[GSH])}$	$\phi_1 = 4.8 \times 10^{-8}$ M s, $\phi_2 = 2.5 \times 10^{-5}$ M s, $[GPx] = 2.5 \times 10^{-6}$ M, $[GSH] = 1.1 \times 10^{-2}$ M
$v_5 = k_5[O_2^-]$	$k_5 = 5.9$ s^{-1}
$v_6 = k_6[HO^{\cdot}]$	$k_6 = 3.1 \times 10^9$ s^{-1}

antioxidant defences and pro-oxidant agents as regards v_d. For that we compute the log- and relative-synergism coefficients of v_d, through the steps described below:

1. Compute the steady-state concentrations and rates. This gives $X_1 = 3.1 \times 10^{-10}$ M, $X_2 = 1.5 \times 10^{-7}$ M, $X_3 = 9.9 \times 10^{-20}$ M, $v_1 = 1.6 \times 10^{-5}$ M s^{-1}, $v_2 = 8.0 \times 10^{-6}$ M s^{-1}, $v_3 = 3.1 \times 10^{-10}$ M s^{-1}, $v_4 = 8.0 \times 10^{-6}$ M s^{-1}, $v_5 = 1.8 \times 10^{-9}$ M s^{-1}, $v_6 = 3.1 \times 10^{-10}$ M s^{-1}.

2. Compute the tensors of intrinsic sensitivities and log-synergism coefficients by differentiating the rate expressions according to the definitions of the respective operators [19] and replacing the operating values. This yields

$$
S_\lambda(v, X) = \begin{bmatrix} 0 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0.99 & 0 \\ 1 & 0 & 0 \\ 0 & 0 & 1 \end{bmatrix}, \qquad
S_X(v, \lambda) = \begin{bmatrix} I_4 \\ 0_{2 \times 4} \end{bmatrix},
$$

$$
S^{[2]}_{(\lambda),(\lambda)}(v, X, X) = \left\{ \begin{matrix} 0_{3 \times 3 \times 3} \\ \begin{bmatrix} 0 & 0 & 0 \\ 0 & -7.2 \times 10^{-3} & 0 \\ 0 & 0 & 0 \end{bmatrix} \\ 0_{2 \times 3 \times 3} \end{matrix} \right\}, \qquad
S^{[2]}_{(\lambda),(X)}(v, X, \lambda) = 0_{6 \times 3 \times 4},
$$

$$
S^{[2]}_{(X),(X)}(v, \lambda, \lambda) = 0_{6 \times 4 \times 4}.
$$

3. Compute the implicit sensitivities, using (13):

$$
S(X, v) = \begin{bmatrix} 1.0 & -1.0 & 0 & 0 & -1.1 \times 10^{-4} & 0 \\ 1.0 & 1.2 \times 10^{-4} & -3.9 \times 10^{-5} & -1.0 & -1.2 \times 10^{-4} & 0 \\ 1.0 & 1.2 \times 10^{-4} & 0 & -1.0 & -1.2 \times 10^{-4} & -1.0 \end{bmatrix}.
$$

4. Compute the systemic first-order sensitivities (log gains) of concentrations and fluxes, using (12) and the expression for $S(v, \lambda)$ in Table 1:

$$
S(X, \lambda) = \begin{bmatrix} 1.0 & -1.0 & 0 & 0 \\ 1.0 & 1.2 \times 10^{-4} & -3.9 \times 10^{-5} & -1.0 \\ 1.0 & 1.2 \times 10^{-4} & 1.0 & -1.0 \end{bmatrix},
$$

$$\mathbf{S}(\mathbf{v}, \lambda) = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 1.0 & 1.1 \times 10^{-4} & 0 & 0 \\ 1.0 & 1.2 \times 10^{-4} & 1.0 & -1.0 \\ 1.0 & 1.1 \times 10^{-4} & -3.8 \times 10^{-5} & 3.9 \times 10^{-5} \\ 1.0 & -1.0 & 0 & 0 \\ 1.0 & 1.2 \times 10^{-4} & 1.0 & -1.0 \end{bmatrix}.$$

5. Compute $\tilde{\mathbf{S}}^{[2]}_{[\mathbf{X}],[\mathbf{X}]}(\mathbf{v}, \lambda, \lambda)$, through (16), and $\left(\mathbf{I}^*_{r_2} \mathbf{S}(\mathbf{v}, \lambda)\right).\mathbf{S}(\mathbf{v}, \lambda)$

$$\tilde{\mathbf{S}}^{[2]}_{[\mathbf{X}],[\mathbf{X}]}(\mathbf{v}, \lambda, \lambda) = \left\{ \begin{matrix} \mathbf{0}_{3 \times 4 \times 4} \\ \begin{bmatrix} -7.3 \times 10^{-3} & -8.4 \times 10^{-7} & 7.3 \times 10^{-3} & 2.8 \times 10^{-7} \\ -8.4 \times 10^{-7} & -9.6 \times 10^{-11} & 8.4 \times 10^{-7} & 3.2 \times 10^{-11} \\ 7.3 \times 10^{-3} & 8.4 \times 10^{-7} & -7.3 \times 10^{-3} & -2.8 \times 10^{-7} \\ 2.8 \times 10^{-7} & 3.2 \times 10^{-11} & -2.8 \times 10^{-7} & -1.1 \times 10^{-11} \end{bmatrix} \\ \mathbf{0}_{2 \times 4 \times 4} \end{matrix} \right\},$$

$$\left(\mathbf{I}^*_{r_2} \mathbf{S}(\mathbf{v}, \lambda)\right).\mathbf{S}(\mathbf{v}, \lambda) = \left\{ \begin{matrix} \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix} \\ \begin{bmatrix} 0 & 1.1 \times 10^{-4} & 0 & 0 \\ 1.1 \times 10^{-4} & -1.1 \times 10^{-4} & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix} \\ \begin{bmatrix} 7.4 \times 10^{-3} & 1.2 \times 10^{-4} & 1.0 & -1.0 \\ 1.2 \times 10^{-4} & -1.2 \times 10^{-4} & 1.2 \times 10^{-4} & -1.2 \times 10^{-4} \\ 1.0 & 1.2 \times 10^{-4} & -3.9 \times 10^{-5} & -1.0 \\ -1.0 & -1.2 \times 10^{-4} & -1.0 & 2.0 \end{bmatrix} \\ \begin{bmatrix} -2.8 \times 10^{-7} & 1.1 \times 10^{-4} & -3.8 \times 10^{-5} & 3.9 \times 10^{-5} \\ 1.1 \times 10^{-4} & -1.1 \times 10^{-4} & -4.4 \times 10^{-9} & 4.4 \times 10^{-9} \\ -3.8 \times 10^{-5} & -4.4 \times 10^{-9} & 3.8 \times 10^{-5} & -1.5 \times 10^{-9} \\ 3.9 \times 10^{-5} & 4.4 \times 10^{-9} & -1.5 \times 10^{-9} & -3.9 \times 10^{-5} \end{bmatrix} \\ \begin{bmatrix} 0 & -1.0 & 0 & 0 \\ -1.0 & 2.0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix} \\ \begin{bmatrix} 7.4 \times 10^{-3} & 1.2 \times 10^{-4} & 1.0 & -1.0 \\ 1.2 \times 10^{-4} & -1.2 \times 10^{-4} & 1.2 \times 10^{-4} & -1.2 \times 10^{-4} \\ 1.0 & 1.2 \times 10^{-4} & -3.9 \times 10^{-5} & -1.0 \\ -1.0 & -1.2 \times 10^{-4} & -1.0 & 2.0 \end{bmatrix} \end{matrix} \right\}.$$

6. Compute the systemic log-synergism coefficients, through (18), which yields

$$
\mathbf{S}^{[2]}(\mathbf{X}, \lambda, \lambda) = \left\{
\begin{bmatrix}
0 & 0 & 0 & 0 \\
0 & -1.1 \times 10^{-4} & 0 & 0 \\
0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0
\end{bmatrix}
\begin{bmatrix}
7.4 \times 10^{-3} & 8.5 \times 10^{-7} & -7.4 \times 10^{-3} & 5.7 \times 10^{-7} \\
8.5 \times 10^{-7} & -1.2 \times 10^{-4} & -8.5 \times 10^{-7} & -6.5 \times 10^{-11} \\
-7.4 \times 10^{-3} & -8.5 \times 10^{-7} & 7.3 \times 10^{-3} & 3.9 \times 10^{-5} \\
5.7 \times 10^{-7} & -6.5 \times 10^{-11} & 3.9 \times 10^{-5} & -3.9 \times 10^{-5}
\end{bmatrix}
\begin{bmatrix}
7.4 \times 10^{-3} & 8.5 \times 10^{-7} & -7.4 \times 10^{-3} & 5.7 \times 10^{-7} \\
8.5 \times 10^{-7} & -1.2 \times 10^{-4} & -8.5 \times 10^{-7} & -6.5 \times 10^{-11} \\
-7.4 \times 10^{-3} & -8.5 \times 10^{-7} & 7.3 \times 10^{-3} & 3.9 \times 10^{-5} \\
5.7 \times 10^{-7} & -6.5 \times 10^{-11} & 3.9 \times 10^{-5} & -3.9 \times 10^{-5}
\end{bmatrix}
\right\}.
$$

To compute the systemic relative-synergism coefficients one may just apply expression (4):

$$
\mathbf{W}^{[2]}(\mathbf{X}, \lambda, \lambda) = \left\{
\begin{bmatrix}
0 & -1.0 & 0 & 0 \\
-1.0 & 2.0 & 0 & 0 \\
0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0
\end{bmatrix}
\begin{bmatrix}
1.5 \times 10^{-2} & 1.2 \times 10^{-4} & -1.0 & -4.0 \times 10^{-5} \\
1.2 \times 10^{-4} & -2.3 \times 10^{-4} & -1.2 \times 10^{-4} & -4.5 \times 10^{-9} \\
-1.0 & -1.2 \times 10^{-4} & 2.0 & 7.8 \times 10^{-5} \\
-4.0 \times 10^{-5} & -4.5 \times 10^{-9} & 7.8 \times 10^{-5} & 3.0 \times 10^{-9}
\end{bmatrix}
\begin{bmatrix}
1.5 \times 10^{-2} & 1.2 \times 10^{-4} & -1.0 & 1.0 \\
1.2 \times 10^{-4} & -2.3 \times 10^{-4} & -1.2 \times 10^{-4} & 1.2 \times 10^{-4} \\
-1.0 & 1.2 \times 10^{-4} & 2.0 & -1.0 \\
1.0 & 1.2 \times 10^{-4} & -1.0 & -3.9 \times 10^{-5}
\end{bmatrix}
\right\}.
$$

7. Compute the logarithmic gains of the rate of damage, through (6):

$$S(\mathbf{v}_d, \lambda) = [1.0 \quad -0.86 \quad 0.14 \quad -0.14].$$

8. Finally, by applying expression (19) obtain the log-synergism coefficients of the total rate of damage (v_d) and – again through (4) – the corresponding relative-synergism coefficients:

$$
\mathbf{S}^{[2]}(\mathbf{v}_d, \lambda, \lambda) =
\begin{bmatrix}
1.1 \times 10^{-3} & 9.0 \times 10^{-4} & -2.0 \times 10^{-3} & 9.0 \times 10^{-4} \\
9.0 \times 10^{-4} & 0.12 & -0.12 & 0.12 \\
-2.0 \times 10^{-3} & -0.12 & 0.13 & -0.12 \\
9.0 \times 10^{-4} & 0.12 & -0.12 & 0.12
\end{bmatrix},
$$

$$
\mathbf{W}^{[2]}(\mathbf{v}_d, \lambda, \lambda) =
\begin{bmatrix}
2.1 \times 10^{-3} & -0.86 & 0.14 & -0.14 \\
-0.86 & 1.7 & -0.25 & 0.25 \\
0.14 & -0.25 & 2.9 \times 10^{-3} & -0.14 \\
-0.14 & 0.25 & -0.14 & 0.29
\end{bmatrix}.
$$

Analysis of the latter pair of matrices yields the following inferences. First, positive synergisms between pro-oxidant agents (O_2, Fe^{2+}) and between antioxidant defences (SOD, GPx), as well as

antagonisms (negative relative-synergism coefficients) between pro-oxidants and antioxidant defences should hold. The strongest antagonism predicted is between oxygen and SOD $[\mathbf{W}^{[2]}(\mathbf{v}_d, X_4, X_5) = -0.86]$ and the strongest synergism between SOD and GPx $[\mathbf{W}^{[2]}(\mathbf{v}_d, X_5, X_7) = 0.25]$. Second, combined changes of oxygen concentration and any of the other factors should yield nearly multiplicative responses (log-synergism coefficients near 0). Joint perturbations of [SOD] and [GPx] yield a slightly supra-multiplicative response $[\mathbf{S}^{[2]}(\mathbf{v}_d, X_5, X_7) = 0.12]$, whereas perturbations of $[Fe^{2+}]$ combined with [SOD] or [GPx] yield slightly sub-multiplicative responses. Third, $\mathbf{v}_d$ is nearly proportional to $[O_2]$ (unit log gain and both log- and relative-synergism coefficients near 0) and varies almost linearly with $[Fe^{2+}]$ (relative-synergism coefficients near 0). The response to [SOD] is clearly supra-linear $[\mathbf{W}^{[2]}(\mathbf{v}_d, X_5, X_5) = 1.7]$ and slightly supra-power-law $[\mathbf{S}^{[2]}(\mathbf{v}_d, X_5, X_5) = 0.12]$, indicating diminishing returns of increasing the concentration of this enzyme. The same holds for the response to [GPx].

4. Treatment of models subject to constraints

4.1. Conversion between sensitivities of transformed and non-transformed models – the general case

4.1.1. Transformations of concentrations and parameters

Consider a metabolic process described by the kinetic model $\dot{\mathbf{X}} = \mathbf{N} \cdot \mathbf{v}(\mathbf{X}, \boldsymbol{\lambda})$. In some cases – e.g., in presence of moiety conservation or micro-reversibility constraints – it is desirable to describe the behaviour of the system in terms of transformed vectors of variables $(\mathbf{X}_N)$ and parameters $(\boldsymbol{\lambda}_N)$ instead of the 'natural' concentrations and parameters $(\mathbf{X}$ and $\boldsymbol{\lambda})$. Usually, it is not too restrictive to assume that the transformation of $\mathbf{X}(\boldsymbol{\lambda})$ into $\mathbf{X}_N(\boldsymbol{\lambda}_N)$ is such that $\mathbf{X}$ and $\boldsymbol{\lambda}$ can be expressed as double-differentiable functions of $\mathbf{X}_N$ and $\boldsymbol{\lambda}_N$, respectively: $\boldsymbol{\lambda} \equiv \boldsymbol{\lambda}(\boldsymbol{\lambda}_N)$, $\mathbf{X} \equiv \mathbf{X}(\mathbf{X}_N(\boldsymbol{\lambda}_N(\boldsymbol{\lambda})), \boldsymbol{\lambda}_N(\boldsymbol{\lambda}))$. The chain rule of differentiation then gives

$$\mathbf{O}(\mathbf{X}, \boldsymbol{\lambda}) = (\mathscr{X}_O^X \cdot \mathbf{O}(\mathbf{X}_N, \boldsymbol{\lambda}_N) + \mathscr{X}_O^{\boldsymbol{\lambda}}) \cdot \mathscr{P}_O, \tag{20}$$

$$\mathbf{O}(\mathbf{X}, \boldsymbol{\lambda}_N) = \mathscr{X}_O^X \cdot \mathbf{O}(\mathbf{X}_N, \boldsymbol{\lambda}_N) + \mathscr{X}_O^{\boldsymbol{\lambda}}, \tag{21}$$

$$\begin{aligned}
\mathbf{O}^{[2]}(\mathbf{X}, \boldsymbol{\lambda}, \boldsymbol{\lambda}) = &\Big\{ (\mathscr{X}_{O^{[2]}}^{X,X} \dot{2} \mathbf{O}(\mathbf{X}_N, \boldsymbol{\lambda}_N)) \cdot \mathbf{O}(\mathbf{X}_N, \boldsymbol{\lambda}_N) + \mathscr{X}_{O^{[2]}}^{X,\boldsymbol{\lambda}} \dot{2} \mathbf{O}(\mathbf{X}_N, \boldsymbol{\lambda}_N) \\
&+ \mathscr{X}_O^X \cdot \mathbf{O}^{[2]}(\mathbf{X}_N, \boldsymbol{\lambda}_N, \boldsymbol{\lambda}_N) + \mathscr{X}_{O^{[2]}}^{X, \boldsymbol{\lambda}^{T_{1,3,2}}} \cdot \mathbf{O}(\mathbf{X}_N, \boldsymbol{\lambda}_N) + \mathscr{X}_{O^{[2]}}^{\boldsymbol{\lambda},\boldsymbol{\lambda}}]\dot{2} \mathscr{P}_O \Big\} \cdot \mathscr{P}_O \\
&+ \big(\mathscr{X}_O^X \cdot \mathbf{O}(X_N, \boldsymbol{\lambda}_N) + \mathscr{X}_O^{\boldsymbol{\lambda}} \big) \cdot \mathscr{P}_{O^{[2]}},
\end{aligned} \tag{22}$$

$$\begin{aligned}
\mathbf{O}^{[2]}(\mathbf{X}, \boldsymbol{\lambda}_N, \boldsymbol{\lambda}_N) = &(\mathscr{X}_{O^{[2]}}^{X,X} \dot{2} \mathbf{O}(\mathbf{X}_N, \boldsymbol{\lambda}_N)) \cdot \mathbf{O}(\mathbf{X}_N, \boldsymbol{\lambda}_N) + (\mathscr{X}_{O^{[2]}}^{X,\boldsymbol{\lambda}} \dot{2} \mathbf{O}(\mathbf{X}_N, \boldsymbol{\lambda}_N) + \mathscr{X}_O^X \\
&\cdot \mathbf{O}^{[2]}(\mathbf{X}_N, \boldsymbol{\lambda}_N, \boldsymbol{\lambda}_N) + (\mathscr{X}_{O^{[2]}}^{X,\boldsymbol{\lambda}^{T_{1,3,2}}} \cdot \mathbf{O}(X_N, \boldsymbol{\lambda}_N) + \mathscr{X}_{O^{[2]}}^{\boldsymbol{\lambda},\boldsymbol{\lambda}},
\end{aligned} \tag{23}$$

where

$$\mathcal{X}_O^X = \mathbf{O}_{\lambda_N}(\mathbf{X}, \mathbf{X}_N), \quad \mathcal{X}_O^\lambda = \mathbf{O}_{\mathbf{X}_N}(\mathbf{X}, \lambda_N), \quad \mathcal{P}_O = \mathbf{O}(\lambda_N, \lambda),$$

$$\mathcal{X}_{O^{[2]}}^{X,X} = \mathbf{O}^{[2]}(\mathbf{X}, \mathbf{X}_N, \mathbf{X}_N), \quad \mathcal{X}_{O^{[2]}}^{X,\lambda} = \mathbf{O}^{[2]}(\mathbf{X}, \mathbf{X}_N, \lambda_N), \quad \mathcal{X}_{O^{[2]}}^{\lambda,\lambda} = \mathbf{O}^{[2]}(\mathbf{X}, \lambda_N, \lambda_N),$$

$$\mathcal{P}_{O^{[2]}} = \mathbf{O}^{[2]}(\lambda_N, \lambda, \lambda) \tag{24}$$

and $\mathbf{O}$ stands for $\mathbf{J}$, $\mathbf{W}$ or $\mathbf{S}$. Whereas $\mathbf{O}(\mathbf{X}_N, \lambda_N)$ and $\mathbf{O}^{[2]}(\mathbf{X}_N, \lambda_N, \lambda_N)$ refer to the properties of the system, $\mathcal{X}_O^X, \mathcal{X}_O^\lambda, \mathcal{P}_O, \mathcal{X}_{O^{[2]}}^{X,X}, \mathcal{X}_{O^{[2]}}^{X,\lambda}, \mathcal{X}_{O^{[2]}}^{\lambda,\lambda}$ and $\mathcal{P}_{O^{[2]}}$ refer to the properties of the *transformation*. We call the latter rank-2 and rank-3 tensors ($\mathcal{X}_.$, $\mathcal{P}_.$, etc.) '*link tensors*', by similarity to Reder's [25] link matrices.

By considering vectors of reaction-specific parameters to which the rates of reaction are proportional one can derive formulas for the conversion of implicit and mixed sensitivities:

$$\hat{\mathbf{O}}(\mathbf{X}, \mathbf{v}) = \mathcal{X}_O^X \cdot \hat{\mathbf{O}}(\mathbf{X}_N, \mathbf{v}), \tag{25}$$

$$\hat{\mathbf{O}}^{[2]}(\mathbf{X}, \mathbf{v}, \mathbf{v}) = (\mathcal{X}_{O^{[2]}}^{X,X} \,\dot{}_2\, \hat{\mathbf{O}}(\mathbf{X}_N, \mathbf{v})) \cdot \hat{\mathbf{O}}(\mathbf{X}_N, \mathbf{v}) + \mathcal{X}_O^X \cdot \hat{\mathbf{O}}^{[2]}(\mathbf{X}_N, \mathbf{v}, \mathbf{v}), \tag{26}$$

$$\hat{\mathbf{O}}^{[2]}(\mathbf{X}, \mathbf{v}, \lambda) = \left[(\mathcal{X}_{O^{[2]}}^{X,X} \,\dot{}_2\, \hat{\mathbf{O}}(\mathbf{X}_N, \mathbf{v})) \cdot \mathbf{O}(\mathbf{X}_N, \lambda) + \mathcal{X}_{O^{[2]}}^{X,\lambda} \,\dot{}_2\, \hat{\mathbf{O}}(\mathbf{X}_N, v) + \mathcal{X}_O^X \cdot \hat{\mathbf{O}}^{[2]}(\mathbf{X}_N, \mathbf{v}, \lambda) \right] \cdot \mathcal{P}_O. \tag{27}$$

4.1.2. Transformations of fluxes

If a transformation changes the set of rates of reaction, one can write the modified kinetic model as

$$\dot{\mathbf{X}}_N = \mathbf{N}_N \cdot \mathbf{v}_N(\mathbf{X}_N, \lambda_N), \tag{28}$$

and, considering $\mathbf{v} \equiv \mathbf{v}(\mathbf{v}_N, \lambda)$, find

$$\mathbf{O}(\mathbf{v}, \lambda) = \mathcal{V}_O^v \cdot \mathbf{O}(\mathbf{v}_N, \lambda_N) \cdot \mathcal{P}_O + \mathcal{V}_O^\lambda, \tag{29}$$

$$\hat{\mathbf{O}}^{[2]}(\mathbf{v}, \lambda, \lambda) = \left(\mathcal{V}_{O^{[2]}}^{v,v} \,\dot{}_2\, (\mathbf{O}(\mathbf{v}_N, \lambda_N) \cdot \mathcal{P}_O) \right) \cdot \mathbf{O}(\mathbf{v}_N, \lambda_N) \cdot \mathcal{P}_O + \mathcal{V}_O^v \cdot (\mathbf{O}^{[2]}(\mathbf{v}_N, \lambda_N, \lambda_N) \,\dot{}_2\, \mathcal{P}_O)$$
$$\cdot \mathcal{P}_O + \mathcal{V}_O^v \cdot \mathbf{O}(\mathbf{v}_N, \lambda_N) \cdot \mathcal{P}_{O^{[2]}} + \mathcal{V}_{O^{[2]}}^{v,\lambda} \,\dot{}_2\, (\mathbf{O}(\mathbf{v}_N, \lambda_N) \cdot \mathcal{P}_O) + \mathcal{V}_{O^{[2]}}^{\mathbf{v},\lambda^{T_{1,3,2}}}$$
$$\cdot \mathbf{O}(\mathbf{v}_N, \lambda_N) \cdot \mathcal{P}_O + \mathcal{V}_{O^{[2]}}^{\lambda,\lambda}, \tag{30}$$

with the flux link tensors defined as

$$\mathcal{V}_O^v = \mathbf{O}_\lambda(\mathbf{v}, \mathbf{v}_N), \quad \mathcal{V}_O^\lambda = \mathbf{O}_{\mathbf{v}_N}(\mathbf{v}, \lambda),$$
$$\mathcal{V}_{O^{[2]}}^{v,v} = \mathbf{O}_{(\lambda),(\lambda)}^{[2]}(\mathbf{v}, \mathbf{v}_N, \mathbf{v}_N), \quad \mathcal{V}_{O^{[2]}}^{v,\lambda} = \mathbf{O}_{(\lambda),(\mathbf{v}_N)}^{[2]}(\mathbf{v}, \mathbf{v}_N, \lambda), \quad \mathcal{V}_{O^{[2]}}^{\lambda,\lambda} = \mathbf{O}_{(\mathbf{v}_N),(\mathbf{v}_N)}^{[2]}(\mathbf{v}, \lambda, \lambda). \tag{31}$$

The following expressions allow calculating the sensitivities for the *transformed* set of parameters:

$$\mathbf{O}(\mathbf{v}, \lambda_N) = \mathcal{V}_O^v \cdot \mathbf{O}(\mathbf{v}_N, \lambda_N) + \mathcal{V}_O^\lambda \cdot \overleftarrow{\mathcal{P}}_O, \tag{32}$$

$$\mathbf{O}^{[2]}(\mathbf{v}, \lambda, \lambda_N) = \left(\mathcal{V}_{O^{[2]}}^{v,v} \,\dot{}_2\, \mathbf{O}(\mathbf{v}_N, \lambda_N) \right) \cdot \mathbf{O}(\mathbf{v}_N, \lambda_N) + \mathcal{V}_O^v \cdot \mathbf{O}^{[2]}(\mathbf{v}_N, \lambda_N, \lambda_N)$$
$$+ (\mathcal{V}_{O^{[2]}}^{\lambda,\lambda} \,\dot{}_2\, \mathbf{O}(\mathbf{v}_N, \lambda_N)) \cdot \overleftarrow{\mathcal{P}}_O + (\mathcal{V}_{O^{[2]}}^{v,\lambda^{T_{1,3,2}}} \,\dot{}_2\, \overleftarrow{\mathcal{P}}_O) \cdot \mathbf{O}(\mathbf{v}_n, \lambda_N)$$
$$+ (\mathcal{V}_{O^{[2]}}^{\lambda,\lambda} \,\dot{}_2\, \overleftarrow{\mathcal{P}}_O) \cdot \overleftarrow{\mathcal{P}}_O + \mathcal{V}_\lambda \cdot \overleftarrow{\mathcal{P}}_{O^{[2]}}, \tag{33}$$

with $\overleftarrow{\mathcal{P}} = \mathbf{O}(\lambda, \lambda_N)$ and $\overleftarrow{\mathcal{P}}_{O^{[2]}} = \mathbf{O}^{[2]}(\lambda, \lambda_N, \lambda_N)$.

By replacing (20), (22), (29) and (30) into the pertinent expressions in Section 3 and Appendix B.2, one obtains relationships between systemic sensitivities of derived magnitudes of the original system and systemic sensitivities of the transformed system.

4.2. Applications

4.2.1. Treatment of structural conservation relationships

4.2.1.1. Derivation of the link tensors. If the stoichiometric matrix $\mathbf{N}$ has n rows and rank $n_0 < n$, then there are $n - n_0$ independent structural conservation relationships, which are time-invariant linear combinations of dynamical concentrations. Identifying each of these linear combinations with a new parameter, one can express $n - n_0$ variables (sub-vector $\mathbf{X}_E$, below) as function of the latter parameters and of the other variables involved in the conservation relationships. So, once the variables in $\mathbf{X}_E$ are substituted into the kinetic model, the subset of n_0 differential equations corresponding to the remaining n_0 dynamical variables (vector $\mathbf{X}_N$) is sufficient to describe the kinetics of the system. The systemic sensitivities of the latter variables can be found by applying the approach in Section 3 to the transformed system, and the sensitivities of the original variables are then computed through (21) and (23), by using the link tensors derived in Appendix C

$$
\mathbf{O}(\mathbf{X}, \boldsymbol{\lambda}_N) = \left[\frac{\mathbf{O}(\mathbf{X}_N, \boldsymbol{\lambda}_N)}{\tilde{\mathcal{X}}_O^X \cdot \mathbf{O}(X_N, \boldsymbol{\lambda}_N) + \tilde{\mathcal{X}}_O^\lambda} \right] \tag{34}
$$

and

$$
\mathbf{O}^{[2]}(\mathbf{X}, \boldsymbol{\lambda}_N, \boldsymbol{\lambda}_N) = \mathcal{X}_O^X \cdot \mathbf{O}^{[2]}(\mathbf{X}_N, \boldsymbol{\lambda}_N, \boldsymbol{\lambda}_N) = \left\{ \frac{\mathbf{O}^{[2]}(\mathbf{X}_N, \boldsymbol{\lambda}_N, \boldsymbol{\lambda}_N)}{\tilde{\mathcal{X}}_O^X \cdot \mathbf{O}^{[2]}(\mathbf{X}_N, \boldsymbol{\lambda}_N, \boldsymbol{\lambda}_N)} \right\} \tag{35}
$$

for $\mathbf{O}$ standing for $\mathbf{J}$ or $\mathbf{W}$; or

$$
\mathbf{S}^{[2]}(\mathbf{X}, \boldsymbol{\lambda}_N, \boldsymbol{\lambda}_N) = \mathcal{X}_S^X \cdot \mathbf{S}^{[2]}(\mathbf{X}_N, \boldsymbol{\lambda}_N, \boldsymbol{\lambda}_N) + (\mathcal{X}_{S^{[2]}}^{X,X} \, \dot{2} \, \mathbf{S}(\mathbf{X}_N, \boldsymbol{\lambda}_N) \cdot \mathbf{S}(\mathbf{X}_N, \boldsymbol{\lambda}_N) + \mathcal{X}_{S^{[2]}}^{\mathbf{X},\lambda} \, \dot{2} \, \mathbf{S}(\mathbf{X}_N, \boldsymbol{\lambda}_N)
$$
$$
+ \mathcal{X}_{S^{[2]}}^{\mathbf{X},\lambda \, T_{1,3,2}} \cdot \mathbf{S}(\mathbf{X}_N, \boldsymbol{\lambda}_N) + \mathcal{X}_{S^{[2]}}^{\lambda,\lambda}. \tag{36}
$$

This procedure extends the synergism analysis described in [19] to systems subject to structural conservation relationships.

For computing the systemic sensitivities $\mathbf{O}(\mathbf{X}_N, \boldsymbol{\lambda}_N)$ and $\mathbf{O}^{[2]}(\mathbf{X}_N, \boldsymbol{\lambda}_N, \boldsymbol{\lambda}_N)$, note that the matrices $\mathbf{N}_N \cdot \mathbf{J}_{\boldsymbol{\lambda}_N}(\mathbf{v}, \mathbf{X}_N)$ and $\mathbf{N}_N \cdot \mathbf{v}^{\backslash} \cdot S_{\boldsymbol{\lambda}_N}(\mathbf{v}, \mathbf{X}_N)$ are non-singular. ($\mathbf{N}_N$ is formed by the n_0 rows of $\mathbf{N}$ that correspond to the variables in $\mathbf{X}_N$.) Hence,

$$
\hat{\mathbf{J}}(\mathbf{X}_N, \mathbf{v}) = -(\mathbf{N}_N \cdot \mathbf{J}_{\boldsymbol{\lambda}_N}(\mathbf{v}, \mathbf{X}_N))^{-1} \cdot \mathbf{N}_N \tag{37}
$$

and

$$\hat{\mathbf{S}}(\mathbf{X}_N, \mathbf{v}) = -(\mathbf{N}_N \cdot \mathbf{v}^{\backslash} \cdot \mathbf{S}_{\lambda_N}(\mathbf{v}, \mathbf{X}_N))^{-1} \cdot \mathbf{N}_N \cdot \mathbf{v}^{\backslash} \tag{38}$$

with

$$\mathbf{O}_{\lambda_N}(\mathbf{v}, \mathbf{X}_N) = \mathbf{O}_\lambda(\mathbf{v}, \mathbf{X}) \cdot \mathscr{X}_O^X. \tag{39}$$

The intrinsic sensitivities of the fluxes to the transformed set of parameters are also straightforwardly obtained from the corresponding sensitivities to the original parameters

$$\begin{aligned}
\mathbf{O}_{\mathbf{X}_N}(\mathbf{v}, \lambda_N) &= \mathbf{O}_{\mathbf{X}_N, \lambda}(\mathbf{v}, \mathbf{X}_E) \cdot \tilde{\mathscr{X}}_O^\lambda \cdot \mathbf{O}(\lambda_A, \lambda_N) + \mathbf{O}_X(\mathbf{v}, \lambda) \cdot \overline{\mathscr{P}} \\
&= [\, \mathbf{O}_X(\mathbf{v}, \lambda) \,|\, \mathbf{O}_{\mathbf{X}_N, \lambda}(\mathbf{v}, \mathbf{X}_E)] \cdot \begin{bmatrix} \mathbf{I}_p & | & \mathbf{0} \\ \hline \mathbf{0} & | & \tilde{\mathscr{X}}_O^\lambda \end{bmatrix}.
\end{aligned} \tag{40}$$

In turn, the intrinsic second-order sensitivities for the original system and those of the transformed system can be related through

$$\mathbf{O}^{[2]}_{(\lambda_N),(\lambda_N)}(\mathbf{v}, \mathbf{X}_N, \mathbf{X}_N) = \mathbf{O}_\lambda(\mathbf{v}, \mathbf{X}) \cdot \mathscr{X}_{O^{[2]}}^{X,X} + (\mathbf{O}^{[2]}_{(\lambda),(\lambda)}(\mathbf{v}, \mathbf{X}, \mathbf{X})_{\dot{2}} \, \mathscr{X}_O^X) \cdot \mathscr{X}_O^X, \tag{41}$$

$$\begin{aligned}
\mathbf{O}^{[2]}_{(\lambda_N),(\mathbf{X}_N)}(\mathbf{v}, \mathbf{X}_N, \lambda_N) &= \mathbf{O}_\lambda(\mathbf{v}, \mathbf{X}) \cdot \mathscr{X}_{O^{[2]}}^{X,\lambda} + (\mathbf{O}^{[2]}_{(\lambda),(\mathbf{X}_N,\lambda)}(\mathbf{v}, \mathbf{X}, \mathbf{X}_E)_{\dot{2}} \mathscr{X}_O^X) \tilde{\mathscr{X}}_O^\lambda \cdot \mathbf{O}(\lambda_A, \lambda_N) \\
&\quad + (\mathbf{O}^{[2]}_{(\lambda),(\mathbf{X})}(\mathbf{v}, \mathbf{X}, \lambda)_{\dot{2}} \mathscr{X}_O^X) \cdot \overline{\mathscr{P}},
\end{aligned} \tag{42}$$

and

$$\begin{aligned}
\mathbf{O}^{[2]}_{\mathbf{X}_N, \mathbf{X}_N}(\mathbf{v}, \lambda_N, \lambda_N) &= \mathbf{O}_{\mathbf{X}_N, \lambda}(\mathbf{v}, \mathbf{X}_E) \cdot (\tilde{\mathscr{X}}^{\lambda,\lambda}_{O^{[2]}\dot{2}} \mathbf{O}(\lambda_A, \lambda_N)) \cdot \mathbf{O}(\lambda_A, \lambda_N) \\
&\quad + [\mathbf{O}^{[2]}_{(\mathbf{X}_N,\lambda),(\mathbf{X}_N,\lambda)}(\mathbf{v}, \mathbf{X}_E, \mathbf{X}_E)_{\dot{2}} \; (\tilde{\mathscr{X}}_O^\lambda \cdot \mathbf{O}(\lambda_A, \lambda_N))] \cdot \tilde{\mathscr{X}}_O^\lambda \cdot \mathbf{O}(\lambda_A, \lambda_N) \\
&\quad + [\mathbf{O}^{[2]}_{(\mathbf{X}_N,\lambda),(\mathbf{X})}(\mathbf{v}, \mathbf{X}_E, \lambda)_{\dot{2}} (\tilde{\mathscr{X}}_O^\lambda \cdot \mathbf{O}(\lambda_A, \lambda_N))] \cdot \overline{\mathscr{P}} \\
&\quad + (\mathbf{O}^{[2]}_{(\mathbf{X}_N,\lambda),(\mathbf{X})}(\mathbf{v}, \mathbf{X}_E, \lambda)^{\mathrm{T}_{1,3,2}}_{\dot{2}} \overline{\mathscr{P}}) \cdot \tilde{\mathscr{X}}_O^\lambda \cdot \mathbf{O}(\lambda_A, \lambda_N) \\
&\quad + (\mathbf{O}^{[2]}_{(\mathbf{X}),(\mathbf{X})}(\mathbf{v}, \lambda, \lambda)_{\dot{2}} \overline{\mathscr{P}}) \cdot \overline{\mathscr{P}}.
\end{aligned} \tag{43}$$

Here, $[\mathbf{0}_{(n-n_0) \times p} \,|\, \mathbf{I}_{(n-n_0)}]$. All the intrinsic sensitivities of the transformed system might as well be obtained through differentiation of the rate expressions at the operating point, after replacing the eliminated variables. The systemic sensitivities of the transformed system follow from applying expression (15) or (18) to (41)–(43).

As the best algorithms for inversion of an $n \times n$ matrix are of order n^3 [27], the computational cost of the calculation of the systemic first-order sensitivities for large-scale models is dominated by the matrix inversion. Cascante et al. [26] presented a strategy that requires the inversion of an $r \times r$ matrix for obtaining simultaneously the flux control coefficients and the concentration control coefficients of a system. Yet, by calculating the systemic flux sensitivities through $\mathbf{O}(\mathbf{v}, \lambda_N) = \mathbf{O}_\lambda(\mathbf{v}, \mathbf{X}).\mathbf{O}(\mathbf{X}, \lambda_N) + \mathbf{O}_{\mathbf{X}_N}(\mathbf{v}, \lambda_N)$, one obtains the same result by inverting a smaller $(n_0 \times n_0)$ matrix. Any kinetic model with a non-trivial steady-state has $r > n \geqslant n_0$. If all processes are considered reversible, then $r > 2n$; and, as cycles are prevalent in metabolic networks, r can be

much higher than $2n$. The computational cost of the two alternative strategies for computing the systemic sensitivities may then differ by orders of magnitude.

4.2.1.2. Example. Scheme 1 represents a simple mechanism of dynamic metabolic channelling [28]. X_1 stands for the substrate of the pathway, X_3 for free intermediate, X_6 and X_7 for free enzymes, X_2 and X_4 for $X_6{:}X_1$ and $X_7{:}X_3$ complexes (respectively), X_5 for $X_6{:}X_3{:}X_7$ complex. Reactions 7 and 9 are the reverse of reactions 6 and 8, respectively. All reactions follow mass action kinetics, and a physiologically plausible dimensionless operating point is considered: $k_1 = X_{10} = X_{11} = 1$, $k_2 = 0.35$, $k_3 = 3.5$, $k_4 = 100$, $k_5 = 2$, $k_6 = k_7 = k_9 = 10^4$, $k_8 = 10^5$. The dimensionless concentrations are then

$$\mathbf{X} = \begin{bmatrix} 9.51 \times 10^{-1} & 4.83 \times 10^{-1} & 1.02 \times 10^{-1} & 5.00 \times 10^{-1} & 8.31 \times 10^{-3} & 5.09 \times 10^{-1} & 4.92 \times 10^{-1} \end{bmatrix}^{\mathrm{T}}.$$

The dimensionless fluxes are

$$\mathbf{v} = \begin{bmatrix} 1 & 0.169 & 0.831 & 0.831 & 1 & 484. & 483. & 500.2 & 500.0 \end{bmatrix}^{\mathrm{T}},$$

so that 49% of the first enzyme and 51% of the second are in the free form, and 83% of the flux is channelled.

To find the systemic sensitivities of the concentrations, one proceeds as follows:

(1) Find the conservation matrix and the conservation relationships, through the procedure in Appendix C or following [29]. As the stoichiometric matrix

$$\mathbf{N} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 \\ 0 & -1 & -1 & 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 1 & -1 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 1 & 0 & -1 & 1 & 0 & 0 \\ 0 & 0 & -1 & 0 & 1 & 0 & 0 & -1 & 1 \end{bmatrix}$$

has $n = 7$ rows and rank $n_0 = 5$, there are two conservation relationships. As follows from

$$\mathscr{L}^{\mathrm{T}} = \begin{bmatrix} 0 & 1 & 0 & 0 & 1 & 1 & 0 \\ 0 & 0 & 0 & 1 & 1 & 0 & 1 \end{bmatrix},$$
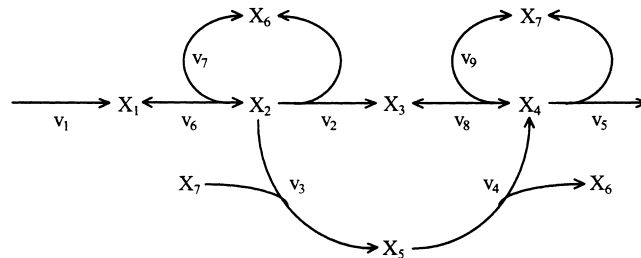
Scheme 1.

they are

$$X_2 + X_5 + X_6 = X_{10}, \quad X_4 + X_5 + X_7 = X_{11},$$

where X_{10} and X_{11} are new parameters accounting for the total concentration of each enzyme.

(2) Choose the concentration variables to eliminate and compute the link tensors accordingly, through the procedure in Appendix C. Eliminating X_6 and X_7 and considering $\lambda_N = \begin{bmatrix} k_1 & \cdots & k_9 & X_{10} & X_{11} \end{bmatrix}^{\mathrm{T}}$ it follows from (C.1) and (C.3):

$$\tilde{\mathscr{X}}_S^\lambda = \begin{bmatrix} \frac{X_{10}}{X_6} & 0 \\ 0 & \frac{X_{11}}{X_7} \end{bmatrix}, \tag{44}$$

$$\tilde{\mathscr{X}}_S^X = \begin{bmatrix} & & \mathbf{I} & & \\ \hline 0 & -\frac{X_2}{X_6} & 0 & 0 & -\frac{X_5}{X_6} \\ 0 & 0 & 0 & -\frac{X_4}{X_7} & -\frac{X_5}{X_7} \end{bmatrix}. \tag{45}$$

For the second-order link tensors, (C.6) and (C.10) give

$$\mathscr{X}_{S[2]}^{X,X} = \left\{ \begin{bmatrix} & & & \mathbf{0} & & \\ \hline 0 & 0 & 0 & 0 & 0 \\ 0 & -\frac{X_2(X_6+X_2)}{X_6^2} & 0 & 0 & -\frac{X_2 X_5}{X_6^2} \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & -\frac{X_2 X_5}{X_6^2} & 0 & 0 & -\frac{X_2(X_6+X_5)}{X_6^2} \end{bmatrix} \\ \begin{bmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -\frac{X_4(X_7+X_4)}{X_7^2} & -\frac{X_4 X_5}{X_7^2} \\ 0 & 0 & 0 & -\frac{X_4 X_5}{X_7^2} & -\frac{X_4(X_7+X_5)}{X_7^2} \end{bmatrix} \end{right\}, \tag{46}$$

(C.7) gives

$$
\mathscr{X}^{X,\lambda}_{S[2]} = \left\{
\begin{matrix}
\mathbf{0} \\
\begin{bmatrix}
0 & 0 \\
\frac{X_2 X_{10}}{X_6^2} & 0 \\
0 & 0 \\
0 & 0 \\
\frac{X_5 X_{10}}{X_6^2} & 0
\end{bmatrix} & \\
\begin{bmatrix}
0 & 0 \\
0 & 0 \\
0 & 0 \\
0 & \frac{X_4 X_{11}}{X_7^2} \\
0 & \frac{X_5 X_{11}}{X_7^2}
\end{bmatrix}
\end{matrix}
\right\}
\tag{47}
$$

and (C.8) gives

$$
\mathscr{X}^{\lambda,\lambda}_{S[2]} = \left\{
\begin{bmatrix}
\mathbf{0} & \mathbf{0} \\
\mathbf{0} & -\frac{X_{10}(X_{10}-X_6)}{X_6^2} \quad 0 \\
& 0 \qquad 0 \\
\mathbf{0} & \mathbf{0} \\
\mathbf{0} & 0 \qquad 0 \\
& 0 \quad -\frac{X_{11}(X_{11}-X_7)}{X_7^2}
\end{bmatrix}
\right\}.
\tag{48}
$$

(3) Compute the systemic (implicit and parameter) first-order sensitivities of the transformed system. Start by the intrinsic flux sensitivities, through (39) and (40); then replace $\mathbf{S}_{\lambda_N}(\mathbf{v},\mathbf{X}_N)$ into (38), to find

$$
\hat{\mathbf{S}}(\mathbf{X}_N,\mathbf{v}) =
\begin{bmatrix}
3.63 & -0.336 & -1.61 & -0.0436 & -1.64 & -1 & 0.988 & 0 & 0 \\
1.86 & -0.171 & -0.829 & -0.0140 & -0.843 & 0 & 0 & 0 & 0 \\
2.03 & -0.00257 & 0.00257 & -0.0169 & -2.01 & 0 & 0 & -1 & 1.00 \\
1 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 \\
0.826 & -0.169 & 0.169 & -0.997 & 0.171 & 0 & 0 & 0 & 0
\end{bmatrix},
\tag{49}
$$

and apply (12) to compute $\mathbf{S}(\mathbf{X}_N, \lambda_N)$.

(4) Compute the systemic first-order sensitivities of the original set of concentrations, through (21)

$$\mathbf{S}(\mathbf{X}, \lambda_N) = \left[\begin{array}{cccccccc|cc} & & & & & & & & -1.97 & -3.28 \\ & & & & & & & & 0 & -1.69 \\ & & & \mathbf{S}(\mathbf{X}_N, \lambda) & & & & & 0 & -2.03 \\ & & & & & & & & 0 & 0 \\ & & & & & & & & 0 & 0.343 \\ \hline -1.78 & 0.165 & 0.784 & 0.0296 & 0.797 & 0 & 0 & 0 & 0 & 1.97 & 1.59 \\ -1.03 & 0.00285 & -0.00285 & 0.0169 & 1.01 & 0 & 0 & 0 & 0 & 0 & 2.03 \end{array} \right].$$

$$(50)$$

Here $\mathbf{S}(\mathbf{X}_N, \lambda) = \hat{\mathbf{S}}(\mathbf{X}_N, \mathbf{v})$, as λ is the vector of rate constants.

(5) Compute the systemic log-synergism coefficients of the transformed system. As the reactions follow mass action kinetics, the intrinsic log-synergism coefficients of the original system are null. So, (41)–(43) simplify to

$$\mathbf{S}^{[2]}_{(\lambda_N),(\lambda_N)}(\mathbf{v}, \mathbf{X}_N, \mathbf{X}_N) = \mathbf{S}_\lambda(\mathbf{v}, \mathbf{X}) \cdot \mathcal{X}^{X,X}_{S^{[2]}},$$

$$\mathbf{S}^{[2]}_{(\lambda_N),(\mathbf{X}_N)}(\mathbf{v}, \mathbf{X}_N, \lambda_N) = \mathbf{S}_\lambda(\mathbf{v}, \mathbf{X}) \cdot \mathcal{X}^{X,\lambda}_{S^{[2]}},$$

and

$$\mathbf{S}^{[2]}_{(\mathbf{X}_N),(\mathbf{X}_N)}(\mathbf{v}, \lambda_N, \lambda_N) = \mathbf{S}_{\mathbf{X}_N,\lambda}(\mathbf{v}, \mathbf{X}_E) \cdot \left(\tilde{\mathcal{X}}^{\lambda,\lambda}_{S^{[2]} \, 2} \, \mathbf{S}(\lambda_A, \lambda_N) \right) \mathbf{S}(\lambda_A, \lambda_N),$$

respectively. The replacement of these expressions into (18) yields $\mathbf{S}^{[2]}(\mathbf{X}_N, \lambda_N, \lambda_N)$. For conciseness, only the log-synergism coefficients for $\lambda_C = [\, k_1 \quad X_{10} \quad X_{11} \,]^{\mathrm{T}}$ are shown

$$\mathbf{S}^{[2]}(\mathbf{X}_N, \lambda_C, \lambda_C) = \left\{ \begin{array}{c} \begin{bmatrix} 9.51 & -3.49 & -8.57 \\ -3.49 & 1.90 & 3.13 \\ -8.57 & 3.13 & 7.45 \end{bmatrix} \\ \begin{bmatrix} 1.58 & 0 & -1.43 \\ 0 & 0 & 0 \\ -1.43 & 0 & 1.14 \end{bmatrix} \\ \begin{bmatrix} 2.08 & 0 & -2.08 \\ 0 & 0 & 0 \\ -2.08 & 0 & 2.07 \end{bmatrix} \\ \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix} \\ \begin{bmatrix} -0.501 & 0 & 0.644 \\ 0 & 0 & 0 \\ 0.644 & 0 & -0.927 \end{bmatrix} \end{array} \right\}.$$

$$(51)$$

(6) Compute the systemic log-synergism coefficients for the original set of variables, by replacing $\mathbf{S}^{[2]}(\mathbf{X}_N, \lambda_N, \lambda_N)$ into (23)

$$\mathbf{S}^{[2]}(\mathbf{X}, \lambda_C, \lambda_C) = \left\{ \begin{array}{c} \mathbf{S}^{[2]}(\mathbf{X}_N, \lambda_N, \lambda_N) \\ \hline \begin{bmatrix} -7.93 & 3.49 & 7.14 \\ 3.49 & -1.90 & -3.13 \\ 7.14 & -3.13 & -6.30 \end{bmatrix} \\ \begin{bmatrix} -2.08 & 0 & 2.07 \\ 0 & 0 & 0 \\ 2.07 & 0 & -2.07 \end{bmatrix} \end{array} \right\}. \tag{52}$$

Note the high log-synergism coefficients, even as compared to the first-order sensitivities. The relative-synergism coefficients (not shown) reach even higher values. Such high log- and relative-synergism coefficients indicate strong deviations from both power-law/multiplicative and linear/additive behaviour [19]. Hence, descriptions of this system that are based on first-order approximations either in Cartesian or logarithmic co-ordinates may be very inaccurate and may overlook, for instance, the high synergism between both enzymes.

4.2.2. Steady-state constraints between fluxes

4.2.2.1. Derivation of the link tensors. Suppose that the $(n \times r)$ stoichiometric matrix of a kinetic model has matrix-rank $n_0 < r$. If $\mathbf{N}$ is full rank $(n_0 = n)$, one can rearrange it as $\mathbf{N} = \begin{bmatrix} \mathbf{N}_D & \vdots & \mathbf{N}_E \end{bmatrix}$, where $\mathbf{N}_E$ is a square sub-matrix formed by n_0 linearly independent columns (corresponding to n_0 eliminated rates of reaction) and $\mathbf{N}_D$ is formed by the remaining columns. If $n_0 < n$, $\mathbf{N}$ should be replaced for the matrix $\mathbf{N}_N$ of the previous section. After the concomitant rearrangement of the vector of reaction rates, one finds, at steady-state,

$$\mathbf{N}.\mathbf{v} = [\mathbf{N}_D \vdots \mathbf{N}_E].\begin{bmatrix} \mathbf{v}_N \\ \hline \mathbf{v}_E \end{bmatrix} = \mathbf{N}_D.\mathbf{v}_N + \mathbf{N}_E.\mathbf{v}_E = \mathbf{0},$$

which leads to $\mathbf{v}_E = \tilde{\mathcal{V}}_J^v \cdot \mathbf{v}_N$, with $\tilde{\mathcal{V}}_J^v \doteq \mathbf{J}_\lambda(\mathbf{v}_E, \mathbf{v}_N) = -\mathbf{N}_E^{-1} \cdot \mathbf{N}_D$. So, $\mathbf{v} = \mathcal{V}_J^v \cdot \mathbf{v}_N$, with

$$\mathcal{V}_J^v = \mathbf{J}_\lambda(\mathbf{v}, \mathbf{v}_N) = \begin{bmatrix} \mathbf{I} \\ \hline -\tilde{\mathcal{V}}_J^v \end{bmatrix}. \tag{53}$$

It also follows that $\mathcal{V}_O^\lambda = \mathbf{0}$, $\mathcal{V}_{O^{[2]}}^{\lambda,\lambda} = \mathbf{0}$, $\mathcal{V}_{O^{[2]}}^{v,\lambda} = \mathbf{0}$, $\mathcal{V}_{J^{[2]}}^{v,v} = \mathbf{0}$, $\mathcal{V}_{W^{[2]}}^{v,v} = \mathbf{0}$.
The non-trivial logarithmic link tensors are

$$\mathcal{V}_S^v = \mathbf{v}^{\backslash -1} \cdot \mathcal{V}_J^v \cdot \mathbf{v}_N^\backslash = \begin{bmatrix} \mathbf{I} \\ \hline -\tilde{\mathcal{V}}_S^v \end{bmatrix}, \tag{54}$$

$$\mathcal{V}_{S^{[2]}}^{v,v} = \mathbf{J}_{\mathbf{x},\lambda}(\mathcal{V}_S^v, \mathbf{v}_N) \cdot \mathbf{v}_N^\backslash = \mathcal{V}_S^v \cdot \mathbf{I}_{r_0}^* - \left(\mathbf{I}_r^* \, _2\mathcal{V}_S^v \right) \cdot \mathcal{V}_S^v = \left\{ \begin{array}{c} 0 \\ \hline -\tilde{\mathcal{V}}_{S^{[2]}}^{v,v} \end{array} \right\} \tag{55}$$

with

$$\tilde{\mathscr{V}}_S^v = \mathbf{v}^{\backslash -1} \cdot \tilde{\mathscr{V}}_J^v \cdot \mathbf{v}_N^{\backslash}, \tag{56}$$

$$\tilde{\mathscr{V}}_{S^{[2]}}^{v,v} = \tilde{\mathscr{V}}_S^v \cdot \mathbf{I}_{r_0}^* - \left(\mathbf{I}_{r_0 - \dot{r}2}^* \, \tilde{\mathscr{V}}_S^v\right) \cdot \tilde{\mathscr{V}}_S^v. \tag{57}$$

Because this transformation does not change the variables nor the parameters, the $\mathscr{X}$ and $\mathscr{P}$ link tensors for $\mathbf{J}$, $\mathbf{W}$ and $\mathbf{S}$ are trivial. So, the relationships between the systemic flux sensitivities of the original system and those of the transformed system simplify to

$$\mathbf{O}(\mathbf{v}, \lambda) = \mathscr{V}_O^v \cdot \mathbf{O}(\mathbf{v}_N, \lambda)$$

and

$$\mathbf{O}^{[2]}(\mathbf{v}, \lambda, \lambda) = \mathscr{V}_O^v \cdot \mathbf{O}^{[2]}(\mathbf{v}_N, \lambda, \lambda)$$

for $\mathbf{J}$ or $\mathbf{W}$, or

$$\mathbf{S}^{[2]}(\mathbf{v}, \lambda, \lambda) = (\mathscr{V}_{S^{[2]} \dot{2}}^{v,v} \, \mathbf{S}(\mathbf{v}_N, \lambda)) \cdot \mathbf{S}(\mathbf{v}_N, \lambda) + \mathscr{V}_S^v \cdot \mathbf{S}^{[2]}(\mathbf{v}_N, \lambda, \lambda).$$

4.2.2.2. Example. Consider the example in Section 4.2.1.2. The stoichiometric matrix of the transformed system is

$$\mathbf{N}_N = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 \\ 0 & -1 & -1 & 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 1 & -1 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}.$$

The analysis of $\mathbf{N}_N$ or of Scheme 1 shows that one can express five of the steady-state fluxes as a linear combination of the remaining four steady-state fluxes. Choosing to eliminate v_3, v_4, v_5, v_7 and v_9, one finds

$$\mathbf{N}_E = \begin{bmatrix} 0 & 0 & 0 & 1 & 0 \\ -1 & 0 & 0 & -1 & 0 \\ 1 & 0 & 0 & 0 & 1 \\ 0 & 1 & -1 & 0 & -1 \\ 0 & -1 & 0 & 0 & 0 \end{bmatrix}, \quad \mathbf{N}_D = \begin{bmatrix} 1 & 0 & -1 & 0 \\ 0 & -1 & 1 & 0 \\ 0 & 1 & 0 & 1 \\ 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 \end{bmatrix}$$

from which follows

$$v_3 = v_1 - v_2, \quad v_4 = v_1 - v_2, \quad v_5 = v_1, \quad v_7 = v_6 - v_1, \quad v_9 = v_8 - v_2.$$

Hence, according to (54),

$$\mathscr{V}_S^v = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ \frac{v_1}{v_1-v_2} & -\frac{v_2}{v_1-v_2} & 0 & 0 \\ \frac{v_1}{v_1-v_2} & -\frac{v_2}{v_1-v_2} & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ -\frac{v_1}{v_6-v_1} & 0 & \frac{v_6}{v_6-v_1} & 0 \\ 0 & 0 & 0 & 1 \\ 0 & -\frac{v_2}{v_8-v_2} & 0 & \frac{v_8}{v_8-v_2} \end{bmatrix}.$$

The relationship $\mathbf{S}(\mathbf{v}, \boldsymbol{\lambda}_N) = \mathscr{V}_S^v \cdot \mathbf{S}(\mathbf{v}_N, \boldsymbol{\lambda}_N)$ permits computing the sensitivities of all fluxes from the knowledge of the sensitivities of v_1, v_2, v_6 and v_8.

5. Discussion

This work presents a tensor formulation for synergism analysis of kinetic models. Like matrix formulations for first-order sensitivity analysis [11,20–24,26], the tensor formulation permits a concise formal representation and a more effective computation of sets of second-order sensitivities. The tensor operations involved are not more complex than conventional matrix multiplication.

First-order sensitivity analysis is intrinsic to synergism analysis, as the computation of gener-alised-synergism coefficients (Eq. (15)) requires knowing the first-order sensitivities. Furthermore, because the formalism of (generalised-)synergism analysis integrates operations in both Cartesian and logarithmic space, it embeds the mathematical structure that underlies current approaches [10–15] to sensitivity analysis of metabolic networks. To avoid combinatorial explosion of new symbols, this article and its companion [19] use a new notation. This notation conveys immediate information about the mathematical meaning of the sensitivities being represented, makes it easier to remember relationships, requires a lower number of different symbols, and is more readily generalisable for higher orders or other coordinate systems.

As synergism analyses of large models are computationally demanding, it is important to optimise the computational strategies. For a model with n internal metabolites, r reactions and p parameters, with $n, r, p \gg 1$ and $p > n$, straight application of the formulas (15) through (18) would require $rnp(n + 3p)$ multiplications and $rnp(n + 3p)$ sums. However, if $\boldsymbol{\lambda}_A = \boldsymbol{\lambda}_B$ the matrix $\mathbf{O}^{[2]}(\mathrm{P}_i, \lambda_A, \lambda_B)$ of generalised-synergism coefficients for each scalar property P_i is symmetric (i.e., $\mathrm{O}^{[2]}(\mathrm{P}_i, \lambda_j, \lambda_k) = \mathrm{O}^{[2]}(\mathrm{P}_i, \lambda_k, \lambda_j)$), and so a tensor $\mathbf{O}^{[2]}(\mathbf{P}, \boldsymbol{\lambda}, \boldsymbol{\lambda})$ has at most $n_P p(p - 1)/2$ different coefficients. The symmetry of the tensors $\mathbf{O}^{[2]}(\mathbf{X}, \boldsymbol{\lambda}, \boldsymbol{\lambda})$, $\mathbf{O}^{[2]}_{(\lambda),(\lambda)}(\mathbf{v}, \mathbf{X}, \mathbf{X})$, $\mathbf{O}^{[2]}_{(\mathbf{X}),(\mathbf{X})}(\mathbf{v}, \boldsymbol{\lambda}, \boldsymbol{\lambda})$ and $\mathbf{O}^{[2]}_{(\lambda),(\mathbf{X})}(\mathbf{v}, \mathbf{X}, \boldsymbol{\lambda})_2 \mathbf{O}(\mathbf{X}, l)$ allows computing $\mathbf{O}^{[2]}(\mathbf{X}, \boldsymbol{\lambda}, \boldsymbol{\lambda})$ with about $rnp(n + 4p)/2$ multiplications and $rnp(n + 2p)$ sums – an improvement of about 30%. The nature of the rate expressions may also permit simplifications where both log- and relative-synergism coefficients are of interest. As $\tilde{\mathbf{W}}^{[2]}_{[\mathbf{v},\mathbf{X}],[\mathbf{v},\mathbf{X}]}(\mathbf{v}, \lambda_A, \lambda_B) = \mathbf{0}$, the systemic relative-synergism coefficients may be easier to compute through (15) than the corresponding log-synergism coefficients. However, generalised mass action [31] rate expressions yield null intrinsic log-synergism coefficients. So, if the model includes many

of those rate expressions, it is more effective to compute first the systemic log-synergism coefficients through (15) and obtaining then the systemic relative-synergism coefficients by applying (4).

Flux and concentration conservation relationships permit additional numerical shortcuts in sensitivity and synergism analysis. For formal simplicity, the link tensors in Section 4 refer to conversions between full sets of transformed and non-transformed variables, fluxes or parameters; but often the transformations affect only a small subset of variables, fluxes and/or parameters. Then it is preferable to deal separately with the subsets of sensitivities that are changed by the transformations, and to use the appropriate link *sub*-tensors instead of the full tensors. Important gains in numerical effectiveness of the treatment of moiety conservation cycles also ensue from applying the procedure in Section 4.2.1.1 instead of that in [26]. Algorithms for generalised-synergism analysis are implemented in the software package PARSYS [32,33].

The tensor formulation was illustrated by the study of interactions of pro-oxidant agents and anti-oxidant defences in a simple model of the metabolism of reactive oxygen species in the mitochondrial matrix. This analysis highlighted (a) SODs importance in antagonising the effects of oxygen activation and (b) the synergistic effect of SOD and GPx activities in decreasing the rate of damage to mitochondrial components by oxygen radicals. The analysis also indicates that although increasing the concentration of either SOD or GPx alone decreases the rate of damage, there are diminishing returns from increasing each of these concentrations separately. Despite these conclusions being accessible through examination of the analytical steady-state solution of the model, the generalised-synergism coefficients remain convenient indexes of relevance of multifactorial effects.

As more data becomes available, the need for large-scale integrative approaches to biochemistry is growing. Present models with such aims (as for instance [1–5]) include dozens of variables and hundreds of parameters. Methods that provide condensed, physically meaningful, information about systemic responses to such large sets of parameters will likely contribute towards a more integrated understanding of how biochemical processes are coordinated.

Acknowledgements

Appendix A. Tensors

Tensors generalise the concept of vectors (rank-1 tensors) and matrices (rank-2 tensors). A rank-3 tensor can be considered as a vector of matrices, a rank-4 tensor as a matrix of matrices, etc. So, for instance, a $2 \times 2 \times 2$ tensor, $\mathbf{A}$, can be written as

$$\mathbf{A} = \{a_{i,j,k}\} = \left\{ \begin{bmatrix} a_{1,1,1} & a_{1,1,2} \\ a_{1,2,1} & a_{1,2,2} \end{bmatrix} \\ \begin{bmatrix} a_{2,1,1} & a_{2,1,2} \\ a_{2,2,1} & a_{2,2,2} \end{bmatrix} \right\}.$$

Transposing a tensor corresponds to exchanging its indexes. A rank-n tensor can be transposed in $n!$ ways, which are distinguished by the indexes of the transposition symbol: $\mathbf{T}^{T_{i_1, i_2, \dots, i_n}}$ is the tensor obtained by turning the first index of $\mathbf{T}$ into the i_1th index, the second index into the i_2th, etc. For instance

$$\mathbf{A}^{T_{1,3,2}} = \left\{ \begin{bmatrix} a_{1,1,1} & a_{1,2,1} \\ a_{1,1,2} & a_{1,2,2} \\ a_{2,1,1} & a_{2,2,1} \\ a_{2,1,2} & a_{2,2,2} \end{bmatrix} \right\}.$$

Contracted products are the generalisation of matrix dot products. A tensor admits contracted products over each of its indexes. The index is indicated under the dot: $\mathbf{T}_i \mathbf{B}$ is the contracted product over the ith index of $\mathbf{T}$ and the first index of $\mathbf{B}$. For simplicity, the indication of the index is omitted in contracted products over the last index of the right hand tensor. For instance, if $\mathbf{B}$ is 2×2, the contracted products of $\mathbf{A}$ by $\mathbf{B}$ over the third and over the second indexes of $\mathbf{A}$ are

$$\mathbf{A} \cdot \mathbf{B} = \mathbf{A}_{\dot{3}} \, \mathbf{B} = \left\{ \begin{bmatrix} a_{1,1,1}b_{1,1} + a_{1,1,2}b_{2,1} & a_{1,1,1}b_{1,2} + a_{1,1,2}b_{2,2} \\ a_{1,2,1}b_{1,1} + a_{1,2,2}b_{2,1} & a_{1,2,1}b_{1,2} + a_{1,2,2}b_{2,2} \\ a_{2,1,1}b_{1,1} + a_{2,1,2}b_{2,1} & a_{2,1,1}b_{1,2} + a_{2,1,2}b_{2,2} \\ a_{2,2,1}b_{1,1} + a_{2,2,2}b_{2,1} & a_{2,2,1}b_{1,2} + a_{2,2,2}b_{2,2} \end{bmatrix} \right\}$$

and

$$\mathbf{A}_{\dot{2}} \, \mathbf{B} = (\mathbf{A}^{T_{1,3,2}} \cdot \mathbf{B})^{T_{1,3,2}} = \left\{ \begin{bmatrix} a_{1,1,1}b_{1,1} + a_{1,2,1}b_{2,1} & a_{1,1,2}b_{1,1} + a_{1,2,2}b_{2,1} \\ a_{1,1,1}b_{1,2} + a_{1,2,1}b_{2,2} & a_{1,1,2}b_{1,2} + a_{1,2,2}b_{2,2} \\ a_{2,1,1}b_{1,1} + a_{2,2,1}b_{2,1} & a_{2,1,2}b_{1,1} + a_{2,2,2}b_{2,1} \\ a_{2,1,1}b_{1,2} + a_{2,2,1}b_{2,2} & a_{2,1,2}b_{1,2} + a_{2,2,2}b_{2,2} \end{bmatrix} \right\},$$

respectively. The multiplication of $\mathbf{B}$ by $\mathbf{A}$ over the first index of $\mathbf{A}$ is

$$\mathbf{B} \cdot \mathbf{A} = \left\{ \begin{bmatrix} b_{1,1}a_{1,1,1} + b_{1,2}a_{2,1,1} & b_{1,1}a_{1,1,2} + b_{1,2}a_{2,1,2} \\ b_{1,1}a_{1,2,1} + b_{1,2}a_{2,2,1} & b_{1,1}a_{1,2,2} + b_{1,2}a_{2,2,2} \\ b_{2,1}a_{1,1,1} + b_{2,2}a_{2,1,1} & b_{2,1}a_{1,1,2} + b_{2,2}a_{2,1,2} \\ b_{2,1}a_{1,2,1} + b_{2,2}a_{2,2,1} & b_{2,1}a_{1,2,2} + b_{2,2}a_{2,2,2} \end{bmatrix} \right\}.$$

In this work, these types of contracted products arise from the differentiation of matrix products which, for $\mathbf{B}$ and $\mathbf{C}$ matrices of compatible dimensions, follows the rule $\mathbf{J}(\mathbf{B} \cdot \mathbf{C}, \boldsymbol{\lambda}) = \mathbf{J}(\mathbf{B}, \boldsymbol{\lambda})_{\dot{2}} \mathbf{C} + \mathbf{B} \cdot \mathbf{J}(\mathbf{C}, \boldsymbol{\lambda})$.

A special rank-3 tensor used in this work is $\mathbf{I}_k^*$, which has unit main diagonal elements and null non-diagonal elements. For instance

$$\mathbf{I}_3^* = \left\{ \begin{bmatrix} 1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 1 \end{bmatrix} \right\}.$$

Appendix B. Systemic parameter, implicit and mixed generalised-synergism coefficients

B.1. Implicit and mixed generalised-synergism coefficients of concentrations

By considering $\boldsymbol{\lambda}$ a vector of r reaction-specific parameters such that $\mathbf{O}_{\cdot}(\mathbf{v}, \boldsymbol{\lambda}) = \mathbf{I}$, (15) becomes

$$\hat{\mathbf{O}}^{[2]}(\mathbf{X}, \mathbf{v}, \mathbf{v}) = \hat{\mathbf{O}}(\mathbf{X}, \mathbf{v}) \cdot (\tilde{\mathbf{O}}^{[2]}_{[\mathbf{X}],[\mathbf{X}]}(\mathbf{v}, \mathbf{v}, \mathbf{v}) + \tilde{\mathbf{O}}^{[2]}_{[\mathbf{v},\mathbf{X}],[\mathbf{v},\mathbf{X}]}(\mathbf{v}, \mathbf{v}, \mathbf{v})) \tag{B.1}$$

with

$$\tilde{\mathbf{O}}^{[2]}_{(\mathbf{X}),(\mathbf{X})}(\mathbf{v}, \mathbf{v}, \mathbf{v}) = (\mathbf{O}^{[2]}_{(\boldsymbol{\lambda}),(\boldsymbol{\lambda})}(\mathbf{v}, \mathbf{X}, \mathbf{X})_{\dot{2}}\, \hat{\mathbf{O}}(\mathbf{X}, \mathbf{v})) \cdot \hat{\mathbf{O}}(\mathbf{X}, \mathbf{v}) \quad (\mathbf{O} = \mathbf{J}, \mathbf{W}) \tag{B.2}$$

or

$$\tilde{\mathbf{S}}^{[2]}_{[\mathbf{v},\mathbf{X}],[\mathbf{v},\mathbf{X}]}(\mathbf{v}, \mathbf{v}, \mathbf{v}) = (\mathbf{I}^{*}_{r\,\dot{2}}\hat{\mathbf{S}}(\mathbf{v}, \mathbf{v})) \cdot \hat{\mathbf{S}}(\mathbf{v}, \mathbf{v}) - \hat{\mathbf{S}}(\mathbf{v}, \mathbf{v}) \cdot \mathbf{I}^{*}_{r}. \tag{B.3}$$

By considering $\boldsymbol{\lambda}_A$ as the vector of reaction-specific parameters and $\boldsymbol{\lambda}_B$ as the vector of generic parameters in (15), one finds

$$\hat{\mathbf{O}}^{[2]}(\mathbf{X}, \mathbf{v}, \boldsymbol{\lambda}) = \hat{\mathbf{O}}(\mathbf{X}, \mathbf{v}) \cdot (\tilde{\mathbf{O}}^{[2]}_{[\mathbf{X}],[\mathbf{X}]}(\mathbf{v}, \mathbf{v}, \boldsymbol{\lambda}) + \tilde{\mathbf{O}}^{[2]}_{[\mathbf{v},\mathbf{X}],[\mathbf{v},\mathbf{X}]}(\mathbf{v}, \mathbf{v}, \boldsymbol{\lambda})) \tag{B.4}$$

with

$$\tilde{\mathbf{O}}^{[2]}_{[\mathbf{X}],[\mathbf{X}]}(\mathbf{v}, \mathbf{v}, \boldsymbol{\lambda}) = (\mathbf{O}^{[2]}_{(\boldsymbol{\lambda}),(\boldsymbol{\lambda})}(\mathbf{v}, \mathbf{X}, \mathbf{X})_{\dot{2}}\hat{\mathbf{O}}(\mathbf{X}, \mathbf{v})) \cdot \mathbf{O}(\mathbf{X}, \boldsymbol{\lambda}) + \mathbf{O}^{[2]}_{(\boldsymbol{\lambda}),(\mathbf{X})}(\mathbf{v}, \mathbf{X}, \boldsymbol{\lambda}) \cdot \hat{\mathbf{O}}(\mathbf{X}, \mathbf{v}) \quad (\mathbf{O} = \mathbf{J}, \mathbf{W}) \tag{B.5}$$

or

$$\tilde{\mathbf{S}}^{[2]}_{[\mathbf{v},\mathbf{X}],[\mathbf{v},\mathbf{X}]}(\mathbf{v}, \mathbf{v}, \boldsymbol{\lambda}) = (\mathbf{I}^{*}_{r\,\dot{2}}\hat{\mathbf{S}}(\mathbf{v}, \mathbf{v})) \cdot \mathbf{S}(\mathbf{v}, \boldsymbol{\lambda}). \tag{B.6}$$

B.2. Systemic generalised-synergism coefficients of derived steady-state magnitudes

Consider a steady-state property $\mathbf{P} \equiv \mathbf{P}(\mathbf{X}(\boldsymbol{\lambda}), \boldsymbol{\lambda})$ that is a twice-differentiable function of $\mathbf{X}$ and $\boldsymbol{\lambda}$. The replacement of (15) into (19) gives

$$\mathbf{O}^{[2]}(\mathbf{P}, \boldsymbol{\lambda}_A, \boldsymbol{\lambda}_B) = \mathbf{O}_{\boldsymbol{\lambda}}(\mathbf{P}, \mathbf{X}) \cdot \hat{\mathbf{O}}(\mathbf{X}, \mathbf{v}) \cdot (\tilde{\mathbf{O}}^{[2]}_{[\mathbf{X}],[\mathbf{X}]}(\mathbf{v}, \boldsymbol{\lambda}_A, \boldsymbol{\lambda}_B) + \tilde{\mathbf{O}}^{[2]}_{[\mathbf{v},\mathbf{X}],[\mathbf{v},\mathbf{X}]}(\mathbf{v}, \boldsymbol{\lambda}_A, \boldsymbol{\lambda}_B)) + \tilde{\mathbf{O}}^{[2]}_{[\mathbf{X}],[\mathbf{X}]}(\mathbf{P}, \boldsymbol{\lambda}_A, \boldsymbol{\lambda}_B) \tag{B.7}$$

and

$$\begin{aligned}\tilde{\mathbf{O}}^{[2]}_{[\mathbf{X}],[\mathbf{X}]}(\mathbf{P}, \boldsymbol{\lambda}_A, \boldsymbol{\lambda}_B) &= (\mathbf{O}^{[2]}_{(\boldsymbol{\lambda}),(\boldsymbol{\lambda})}(\mathbf{P}, \mathbf{X}, \mathbf{X})_{\dot{2}}\mathbf{O}(\mathbf{X}, \boldsymbol{\lambda}_A)) \cdot \mathbf{O}(\mathbf{X}, \boldsymbol{\lambda}_B) + \mathbf{O}^{[2]}_{(\boldsymbol{\lambda}),(\mathbf{X})}(\mathbf{P}, \mathbf{X}, \boldsymbol{\lambda}_A)^{\mathrm{T}_{1,3,2}} \cdot \mathbf{O}(\mathbf{X}, \boldsymbol{\lambda}_B) \\ &\quad + \mathbf{O}^{[2]}_{(\boldsymbol{\lambda}),(\mathbf{X})}(\mathbf{P}, \mathbf{X}, \boldsymbol{\lambda}_B)_{\dot{2}}\mathbf{O}(\mathbf{X}, \boldsymbol{\lambda}_A) + \mathbf{O}^{[2]}_{(\mathbf{X}),(\mathbf{X})}(\mathbf{P}, \boldsymbol{\lambda}_A, \boldsymbol{\lambda}_B). \end{aligned} \tag{B.8}$$

The formulas for the intrinsic and mixed generalised-synergism coefficients of $\mathbf{P}$ are formally similar to (B.7). The previous expressions allow calculating systemic generalised-synergism coefficients for fluxes

$$\mathbf{O}^{[2]}(\mathbf{v}, \lambda_A, \lambda_B) = \mathbf{O}_\lambda(\mathbf{v}, \mathbf{X}) \cdot \hat{\mathbf{O}}(\mathbf{X}, \mathbf{v}).(\tilde{\mathbf{O}}^{[2]}_{[\mathbf{X}],[\mathbf{X}]}(\mathbf{v}, \lambda_A, \lambda_B) + \tilde{\mathbf{O}}^{[2]}_{[\mathbf{v},\mathbf{X}],[\mathbf{v},\mathbf{X}]}(\mathbf{v}, \lambda_A, \lambda_B)) + \tilde{\mathbf{O}}^{[2]}_{[\mathbf{X}],[\mathbf{X}]}(\mathbf{v}, \lambda_A, \lambda_B)$$
$$= \hat{\mathbf{O}}(\mathbf{v}, \mathbf{v}) \cdot \tilde{\mathbf{O}}^{[2]}_{[\mathbf{X}],[\mathbf{X}]}(\mathbf{v}, \lambda_A, \lambda_B) + (\hat{\mathbf{O}}(\mathbf{v}, \mathbf{v}) - \mathbf{I}) \cdot \tilde{\mathbf{O}}^{[2]}_{[\mathbf{v},\mathbf{X}],[\mathbf{v},\mathbf{X}]}(\mathbf{v}, \lambda_A, \lambda_B). \tag{B.9}$$

By differentiating $\hat{\mathbf{O}}(\mathbf{v}, \mathbf{v})$ (Table 1) with respect to a vector of generic parameters or of reaction-specific parameters one can find the mixed generalised-synergism coefficients

$$\hat{\mathbf{O}}^{[2]}(\mathbf{v}, \mathbf{v}, \lambda) = (\mathbf{O}^{[2]}_{(\lambda),(\lambda)}(\mathbf{v}, \mathbf{X}, \mathbf{X})_{\dot{2}}\hat{\mathbf{O}}(\mathbf{X}, \mathbf{v})).\mathbf{O}(\mathbf{X}, \lambda) + \mathbf{O}^{[2]}_{(\lambda),(\mathbf{X})}(\mathbf{v}, \mathbf{X}, \lambda)_{\dot{2}}\hat{\mathbf{O}}(\mathbf{X}, \mathbf{v})$$
$$+ \mathbf{O}_\lambda(\mathbf{v}, \mathbf{X}) \cdot \hat{\mathbf{O}}^{[2]}(\mathbf{X}, \mathbf{v}, \lambda), \tag{B.10}$$

and the intrinsic generalised-synergism coefficients

$$\hat{\mathbf{O}}^{[2]}(\mathbf{v}, \mathbf{v}, \mathbf{v}) = (\mathbf{O}^{[2]}_{(\lambda),(\lambda)}(\mathbf{v}, \mathbf{X}, \mathbf{X})_{\dot{2}}\hat{\mathbf{O}}(\mathbf{X}, \mathbf{v})).\hat{\mathbf{O}}(\mathbf{X}, \mathbf{v}) + \mathbf{O}_\lambda(\mathbf{v}, \mathbf{X}) \cdot \hat{\mathbf{O}}^{[2]}(\mathbf{X}, \mathbf{v}, \mathbf{v}). \tag{B.11}$$

If a derived property $\mathbf{P}$ depends explicitly on the rates ($\mathbf{P} \equiv \mathbf{P}(\mathbf{v}(\mathbf{X}(\lambda), \lambda), \mathbf{X}(\lambda), \lambda)$), the systemic generalised-synergism coefficients can be expressed as

$$\mathbf{O}^{[2]}(\mathbf{P}, \lambda_A, \lambda_B) = \hat{\mathbf{O}}(\mathbf{P}, \mathbf{v}).\tilde{\mathbf{O}}^{[2]}_{[\mathbf{X}],[\mathbf{X}]}(\mathbf{v}, \lambda_A, \lambda_B) + \mathbf{O}_\lambda(\mathbf{P}, \mathbf{X}) \cdot \hat{\mathbf{O}}(\mathbf{X}, \mathbf{v}) \cdot \tilde{\mathbf{O}}^{[2]}_{[\mathbf{v},\mathbf{X}],[\mathbf{v},\mathbf{X}]}(\mathbf{v}, \lambda_A, \lambda_B)$$
$$+ \tilde{\mathbf{O}}^{[2]}_{[\mathbf{v},\mathbf{X}],[\mathbf{v},\mathbf{X}]}(\mathbf{P}, \lambda_A, \lambda_B), \tag{B.12}$$

with

$$\tilde{\mathbf{O}}^{[2]}_{[\mathbf{v},\mathbf{X}],[\mathbf{v},\mathbf{X}]}(\mathbf{P}, \lambda_A, \lambda_B) = (\mathbf{O}^{[2]}_{(\mathbf{X},\lambda),(\mathbf{X},\lambda)}(\mathbf{P}, \mathbf{v}, \mathbf{v})_{\dot{2}}\mathbf{O}(\mathbf{v}, \lambda_A)) \cdot \mathbf{O}(\mathbf{v}, \lambda_B)$$
$$+ (\mathbf{O}^{[2]}_{(\mathbf{X},\lambda),(\mathbf{v},\lambda)}(\mathbf{P}, \mathbf{v}, \mathbf{X})_{\dot{2}}\mathbf{O}(\mathbf{v}, \lambda_A)) \cdot \mathbf{O}(\mathbf{X}, \lambda_B)$$
$$+ (\mathbf{O}^{[2]}_{(\mathbf{X},\lambda),(\mathbf{v},\lambda)}(\mathbf{P}, \mathbf{v}, \mathbf{X})^{\mathrm{T}_{1,3,2}}_{\dot{2}}\mathbf{O}(\mathbf{X}, \lambda_A)) \cdot \mathbf{O}(\mathbf{v}, \lambda_B)$$
$$+ (\mathbf{O}^{[2]}_{(\mathbf{V},\lambda),(\mathbf{v},\lambda)}(\mathbf{P}, \mathbf{X}, \mathbf{X})_{\dot{2}}\mathbf{O}(X, \lambda_A)) \cdot \mathbf{O}(\mathbf{X}, \lambda_B)$$
$$+ \mathbf{O}^{[2]}_{(\mathbf{X},\lambda),(\mathbf{v},\mathbf{X})}(\mathbf{P}, \mathbf{v}, \lambda_B)_{\dot{2}}\mathbf{O}(\mathbf{v}, \lambda_A) + \mathbf{O}^{[2]}_{(\mathbf{X},\lambda),(\mathbf{v},\mathbf{X})}(\mathbf{P}, \mathbf{v}, \lambda_A)^{\mathrm{T}_{1,3,2}}$$
$$\cdot \mathbf{O}(\mathbf{v}, \lambda_B) + \mathbf{O}^{[2]}_{(\mathbf{v},\lambda),(\mathbf{v},\mathbf{X})}(\mathbf{P}, \mathbf{X}, \lambda_B)_{\dot{2}}\mathbf{O}(\mathbf{X}, \lambda_A)$$
$$+ \mathbf{O}^{[2]}_{(\mathbf{v},\lambda),(\mathbf{v},\mathbf{X})}(\mathbf{P}, \mathbf{X}, \lambda_A)^{\mathrm{T}_{1,3,2}} \cdot \mathbf{O}(\mathbf{X}, \lambda_B) + \mathbf{O}^{[2]}_{(\mathbf{v},\mathbf{X}),(\mathbf{v},\mathbf{X})}(\mathbf{P}, \lambda_A, \lambda_B). \tag{B.13}$$

One can obtain mixed and intrinsic systemic generalised-synergism coefficients of properties that are functions of the rates of reaction by differentiating

$$\hat{\mathbf{O}}(\mathbf{P}, \mathbf{v}) = \mathbf{O}_{\mathbf{X},\lambda}(\mathbf{P}, \mathbf{v}) \cdot \hat{\mathbf{O}}(\mathbf{v}, \mathbf{v}) + \mathbf{O}_{\mathbf{v},\lambda}(\mathbf{P}, \mathbf{X}) \cdot \hat{\mathbf{O}}(\mathbf{X}, \mathbf{v})$$

with respect to a vector of generic parameters or of reaction-specific parameters, respectively. They take the forms

$$\hat{\mathbf{O}}^{[2]}(\mathbf{P}, \mathbf{v}, \lambda) = (\mathbf{O}^{[2]}_{(\mathbf{X},\lambda),(\mathbf{X},\lambda)}(\mathbf{P}, \mathbf{v}, \mathbf{v}) \cdot \mathbf{O}(\mathbf{v}, \lambda) + \mathbf{O}^{[2]}_{(\mathbf{X},\lambda),(\mathbf{v},\lambda)}(\mathbf{P}, \mathbf{v}, \mathbf{X}) \cdot \mathbf{O}(\mathbf{X}, \lambda)$$
$$+ \mathbf{O}^{[2]}_{(\mathbf{X},\lambda),(\mathbf{v},\mathbf{X})}(\mathbf{P}, \mathbf{v}, \lambda))_{\dot{2}}\mathbf{O}(\mathbf{v}, \mathbf{v}) + (\mathbf{O}^{[2]}_{(\mathbf{X},\lambda),(\mathbf{v},\lambda)}(\mathbf{P}, \mathbf{v}, \mathbf{X})^{\mathrm{T}_{1,3,2}} \cdot \mathbf{O}(\mathbf{v}, \lambda)$$
$$+ \mathbf{O}^{[2]}_{(\mathbf{v},\lambda),(\mathbf{v},\lambda)}(\mathbf{P}, \mathbf{X}, \mathbf{X}) \cdot \mathbf{O}(\mathbf{X}, \lambda) + \mathbf{O}^{[2]}_{(\mathbf{v},\lambda),(\mathbf{v},\mathbf{X})}(\mathbf{P}, \mathbf{X}, \lambda))_{\dot{2}}\hat{\mathbf{O}}(\mathbf{X}, \mathbf{v})$$
$$+ \mathbf{O}_{\mathbf{X},\lambda}(\mathbf{P}, \mathbf{v}) \cdot \hat{\mathbf{O}}^{[2]}(\mathbf{v}, \mathbf{v}, \lambda) + \mathbf{O}_{\mathbf{v},\lambda}(\mathbf{P}, \mathbf{X}) \cdot \hat{\mathbf{O}}^{[2]}(\mathbf{X}, \mathbf{v}, \lambda) \tag{B.14}$$

and

$$\hat{\mathbf{O}}^{[2]}(\mathbf{P}, \mathbf{v}, \mathbf{v}) = (\mathbf{O}^{[2]}_{(\mathbf{X},\lambda),(\mathbf{v},\lambda)}(\mathbf{P}, \mathbf{v}, \mathbf{v}) \cdot \hat{\mathbf{O}}(\mathbf{v}, \mathbf{v}) + \mathbf{O}^{[2]}_{(\mathbf{X},\lambda),(\mathbf{v},\lambda)}(\mathbf{P}, \mathbf{v}, \mathbf{X}) \cdot \hat{\mathbf{O}}(\mathbf{X}, \mathbf{v}))_{\dot{2}} \hat{\mathbf{O}}(\mathbf{v}, \mathbf{v})$$
$$+ (\mathbf{O}^{[2]}_{(\mathbf{X},\lambda),(\mathbf{v},\lambda)}(\mathbf{P}, \mathbf{v}, \mathbf{X})^{\mathrm{T}_{1,3,2}} \cdot \hat{\mathbf{O}}(\mathbf{v}, \mathbf{v}) + \mathbf{O}^{[2]}_{(\mathbf{v},\lambda),(\mathbf{v},\lambda)}(\mathbf{P}, \mathbf{X}, \mathbf{X})$$
$$\cdot \hat{\mathbf{O}}(\mathbf{X}, \mathbf{v}))_{\dot{2}} \hat{\mathbf{O}}(\mathbf{X}, \mathbf{v}) + \mathbf{O}_{\mathbf{X},\lambda}(\mathbf{P}, \mathbf{v}) \cdot \hat{\mathbf{O}}^{[2]}(\mathbf{v}, \mathbf{v}, \mathbf{v}) + \mathbf{O}_{\mathbf{v},\lambda}(\mathbf{P}, \mathbf{X})$$
$$\cdot \hat{\mathbf{O}}^{[2]}(\mathbf{X}, \mathbf{v}, \mathbf{v}), \tag{B.15}$$

respectively.

Relationships between systemic generalised-synergism coefficients of various derived steady-state properties are shown below. The systemic generalised-synergism coefficients of the aggregated fluxes of production $(\mathbf{V}_+)$ follow from applying (5) or (6) to $\mathbf{O}(\mathbf{V}_+, \mathbf{v})$:

$$\mathbf{J}^{[2]}(\mathbf{V}_+, \lambda_A, \lambda_B) = \mathbf{N}_+ \cdot \mathbf{J}^{[2]}(\mathbf{v}, \lambda_A, \lambda_B), \tag{B.16}$$

$$\mathbf{W}^{[2]}(\mathbf{V}_+, \lambda_A, \lambda_B) = \mathbf{S}_{\mathbf{X},\lambda}(\mathbf{V}_+, \mathbf{v}) \cdot \mathbf{W}^{[2]}(\mathbf{v}, \lambda_A, \lambda_B), \tag{B.17}$$

$$\mathbf{S}^{[2]}(\mathbf{V}_+, \lambda_A, \lambda_B) = (\mathbf{R}_{\dot{2}} \mathbf{S}(\mathbf{v}, \lambda_A)) \cdot \mathbf{S}(\mathbf{v}, \lambda_B) + \mathbf{S}_{\mathbf{X},\lambda}(\mathbf{V}_+, \mathbf{v}) \cdot \mathbf{S}^{[2]}(\mathbf{v}, \lambda_A, \lambda_B) \tag{B.18}$$

with

$$\mathbf{S}_{\mathbf{X},\lambda}(\mathbf{V}_+, \mathbf{v}) = \mathbf{V}^{\backslash -1}_+ \cdot \mathbf{N}_+ \cdot \mathbf{v}^{\backslash}$$

and

$$\mathbf{R} = \mathbf{S}^{[2]}_{(\mathbf{X},\lambda),(\mathbf{X},\lambda)}(\mathbf{V}_+, \mathbf{v}, \mathbf{v}) = \mathbf{S}_{\mathbf{X},\lambda}(\mathbf{V}_+, \mathbf{v}) \cdot \mathbf{I}^*_r - (\mathbf{I}^*_{n\dot{2}} \mathbf{S}_{\mathbf{X},\lambda}(\mathbf{V}_+, \mathbf{v})) \cdot \mathbf{S}_{\mathbf{X},\lambda}(\mathbf{V}_+, \mathbf{v}). \tag{B.19}$$

Similar operations on $\mathbf{O}(\mathbf{U}, \mathbf{v})$ yield:

$$\mathbf{J}^{[2]}(\mathbf{U}, \lambda_A, \lambda_B) = \mathbf{X}^{\backslash -1} \cdot \{\mathbf{J}^{[2]}(\mathbf{V}_+, \lambda_A, \lambda_B) - \mathbf{U}^{\backslash} \cdot \mathbf{J}^{[2]}(\mathbf{X}, \lambda_A, \lambda_B) - (\mathbf{I}^*_{n\dot{2}} \mathbf{J}(\mathbf{U}, \lambda_A)) \cdot \mathbf{J}(\mathbf{X}, \lambda_B)$$
$$- (\mathbf{I}^*_{n\dot{2}} \mathbf{J}(\mathbf{X}, \lambda_A)) \cdot \mathbf{J}(\mathbf{U}, \lambda_B)\}, \tag{B.20}$$

$$\mathbf{W}^{[2]}(\mathbf{U}, \lambda_A, \lambda_B) = \mathbf{W}^{[2]}(\mathbf{V}_+, \lambda_A, \lambda_B) - \mathbf{W}^{[2]}(\mathbf{X}, \lambda_A, \lambda_B) - (\mathbf{I}^*_{p\dot{2}} \mathbf{S}(\mathbf{U}, \lambda_A)) \cdot \mathbf{S}(\mathbf{X}, \lambda_B)$$
$$- (\mathbf{I}^*_{p\dot{2}} \mathbf{S}(\mathbf{X}, \lambda_A)) \cdot \mathbf{S}(\mathbf{U}, \lambda_B), \tag{B.21}$$

$$\mathbf{S}^{[2]}(\mathbf{U}, \lambda_A, \lambda_B) = \mathbf{S}^{[2]}(\mathbf{V}_+, \lambda_A, \lambda_B) - \mathbf{S}^{[2]}(\mathbf{X}, \lambda_A, \lambda_B). \tag{B.22}$$

The corresponding relationships between mixed generalised-synergism coefficients of concentrations, rates of reaction, aggregated fluxes and turnover numbers are formally similar to (B.16)–(B.18) and (B.20)–(B.22). The same holds for the cognate relationships between intrinsic generalised-synergism coefficients.

Appendix C. Link tensors for systems subject to concentration conservation relationships

From the definition (Section 4.2.1.1) it follows that any linear combination of concentrations of internal species, $\ell^{\mathrm{T}} \cdot \mathbf{X}$, such that $\ell^{\mathrm{T}} \cdot \dot{\mathbf{X}} = 0$ for any $\mathbf{v} \equiv \mathbf{v}(\mathbf{X}, \lambda)$, is a structural conservation relationship. The latter condition is fulfilled if and only if $\mathbf{N}^{\mathrm{T}} \cdot \ell = 0$. Therefore, a set of $n - n_0$ independent structural conservation relationships can be readily obtained as $\mathcal{L}^{\mathrm{T}} \cdot \mathbf{X}$, where

$\mathscr{L}$ – the so-called *conservation matrix* – is a $n \times (n - n_0)$ matrix whose columns form a basis for $\ker(\mathbf{N}^T)$. The physiological interpretation of these conservation relationships is frequently complicated by the occurrence of negative elements in $\mathscr{L}$. However, as any linear combination of columns of $\mathscr{L}$ still belongs to $\ker(\mathbf{N}^T)$, one can often rearrange the conservation relationships as non-negative linear combinations of concentrations. (For a systematic procedure see [29].) In this form, the conservation relationships represent total concentrations of conserved moieties. The choice of conservation matrix is irrelevant for the derivations below.

By assigning a vector $\boldsymbol{\lambda}_A$ of $n - n_0$ new parameters to the conserved magnitudes and by reordering the rows of $\mathscr{L}$ and the variables in $\mathbf{X}$, one can write

$$\boldsymbol{\lambda}_A = \left[\frac{\mathscr{L}_N}{\mathscr{L}_E} \right]^{\mathrm{T}} \cdot \left[\frac{\mathbf{X}_N}{\mathbf{X}_E} \right] = \mathscr{L}_N^{\mathrm{T}} \cdot \mathbf{X}_N + \mathscr{L}_E^{\mathrm{T}} \cdot \mathbf{X}_E.$$

Here, $\mathbf{X}_E$ and $\mathbf{X}_N$ stand for the sub-vectors of $\mathbf{X}$ corresponding to the $n - n_0$ eliminated variables and to the remaining n_0 variables, respectively; and $\mathscr{L}_E$ and $\mathscr{L}_N$ stand for the corresponding $(n - n_0) \times (n - n_0)$ and $n_0 \times (n - n_0)$ sub-matrices of $\mathscr{L}$. So, assuming that $\mathscr{L}_E$ is non-singular, the values of the eliminated variables at any time can be calculated as $\mathbf{X}_E = \mathscr{L}_E^{\mathrm{T}^{-1}} \cdot \boldsymbol{\lambda}_A - \mathscr{L}_E^{\mathrm{T}^{-1}} \cdot \mathscr{L}_N^{\mathrm{T}} \cdot \mathbf{X}_N$. This permits identifying:

$$\tilde{\mathscr{X}}_J^X \doteq \mathbf{J}_{\boldsymbol{\lambda}_N}(\mathbf{X}_E, \mathbf{X}_N) = -\mathscr{L}_E^{T^{-1}} \cdot \mathscr{L}_N^T, \quad \tilde{\mathscr{X}}_J^\lambda \doteq \mathbf{J}_{\mathbf{X}_N}(\mathbf{X}_E, \boldsymbol{\lambda}_A) = \mathscr{L}_E^{T^{-1}} \tag{C.1}$$

and

$$\tilde{\mathscr{X}}_S^X \doteq \mathbf{S}_{\boldsymbol{\lambda}_N}(\mathbf{X}_E, \mathbf{X}_N) = \mathbf{X}_E^{\backslash^{-1}} \cdot \tilde{\mathscr{X}}_J^X \cdot \mathbf{X}_N^\backslash, \tilde{\mathscr{X}}_S^\lambda \doteq \mathbf{S}_{\mathbf{X}_N}(\mathbf{X}_E, \boldsymbol{\lambda}_A) = \mathbf{X}_E^{\backslash^{-1}} \cdot \tilde{\mathscr{X}}_J^\lambda \cdot \boldsymbol{\lambda}_A^\backslash. \tag{C.2}$$

So, considering $\mathbf{X} = \left[\frac{\mathbf{X}_N}{\mathbf{X}_E} \right]$ and $\boldsymbol{\lambda}_N = \left[\frac{\boldsymbol{\lambda}}{\boldsymbol{\lambda}_A} \right]$, it follows

$$\mathscr{X}_O^X \doteq \mathbf{O}_{\boldsymbol{\lambda}_N}(\mathbf{X}, \mathbf{X}_N) = \left[\frac{\mathbf{I}}{\tilde{\mathscr{X}}_0^X} \right] \tag{C.3}$$

and

$$\mathscr{X}_O^\lambda \doteq \mathbf{O}_{\mathbf{X}_N}(\mathbf{X}, \boldsymbol{\lambda}_N) = \left[\begin{array}{c|c} \mathbf{0} & \mathbf{0} \\ \hline \mathbf{0} & \tilde{\mathscr{X}}_0^\lambda \end{array} \right]. \tag{C.4}$$

From the definition of $\boldsymbol{\lambda}_N$ it follows

$$\mathscr{P}_O \doteq \mathbf{O}(\boldsymbol{\lambda}_N, \boldsymbol{\lambda}) = \left[\frac{\mathbf{I}_p}{\mathbf{0}_{(n-n_0) \times p}} \right]. \tag{C.5}$$

All these link matrices only depend on the (constant) stoichiometric coefficients of the kinetic model. Therefore, the second-order link tensors $\mathscr{X}_{O^{[2]}}^{\cdots}$ and $\mathscr{P}_{O^{[2]}}$, for $\mathbf{O}$ standing for $\mathbf{J}$ or $\mathbf{W}$, are null, whereas the second-order logarithmic link tensors are

$$\mathscr{X}_{S^{[2]}}^{X,X} = \mathscr{X}_S^X \cdot \mathbf{I}_{n_0}^* - (\mathbf{I}_{n}^* \dot{2} \mathscr{X}_S^X) \cdot \mathscr{X}_S^X = \left\{ \frac{\mathbf{0}}{\tilde{\mathscr{X}}_{S^{[2]}}^{X,X}} \right\}, \tag{C.6}$$

$$\mathscr{X}^{X,\lambda}_{S^{[2]}} = -\left(\mathbf{I}^*_{n\hat{2}}\mathscr{X}^X_S\right) \cdot \mathscr{X}^\lambda_S, \tag{C.7}$$

$$\mathscr{X}^{\lambda,\lambda}_{S^{[2]}} = \mathscr{X}^\lambda_S \cdot \mathbf{I}^*_{p_N} - \left(\mathbf{I}^*_{n\hat{2}}\mathscr{X}^\lambda_S\right) \cdot \mathscr{X}^\lambda_S, \tag{C.8}$$

$$\mathscr{P}_{S^{[2]}} = \mathbf{0}. \tag{C.9}$$

Here, p_N stands for the number of parameters in the transformed system, and

$$\tilde{\mathscr{X}}^{X,X}_{S^{[2]}} \doteq \mathbf{S}^{[2]}_{(\lambda_N),(\lambda_N)}(\mathbf{X}_E, \mathbf{X}_N, \mathbf{X}_N) = \tilde{\mathscr{X}}^X_S \cdot \mathbf{I}^*_{n_0} - \left(\mathbf{I}^*_{n-n_0\hat{2}}\tilde{\mathscr{X}}^X_S\right) \cdot \tilde{\mathscr{X}}^X_S. \tag{C.10}$$

All the elements of $\mathscr{X}^{\mathbf{X},\lambda}_{S^{[2]}}$ are null except those in the sub-tensor

$$\tilde{\mathscr{X}}^{X,\lambda}_{S^{[2]}} \doteq \mathbf{S}^{[2]}_{(\lambda_N),(\mathbf{X}_N)}(\mathbf{X}_E, \mathbf{X}_N, \lambda_A) = -\left(\mathbf{I}^*_{n-n_0\hat{2}}\tilde{\mathscr{X}}^X_S\right) \cdot \tilde{\mathscr{X}}^\lambda_S. \tag{C.11}$$

Likewise, all the elements of $\mathscr{X}^{\lambda,\lambda}_{S^{[2]}}$ are null except those in the sub-tensor

$$\tilde{\mathscr{X}}^{\lambda,\lambda}_{S^{[2]}} \doteq \mathbf{S}^{[2]}_{(\mathbf{X}_N),(\mathbf{X}_N)}(\mathbf{X}_E, \lambda_A, \lambda_A) = \tilde{\mathscr{X}}^\lambda_S \cdot \mathbf{I}^*_{n-n_0} - \left(\mathbf{I}^*_{n-n_0\hat{2}}\tilde{\mathscr{X}}^\lambda_S\right) \cdot \tilde{\mathscr{X}}^\lambda_S. \tag{C.12}$$

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