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Multistationarity in mass action networks with applications to ERK activation

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Abstract Ordinary Differential Equations (ODEs) are an important tool in many areas of Quantitative Biology. For many ODE systems multistationarity (i.e. the existence of at least two positive steady states) is a desired feature. In general establishing multistationarity is a difficult task as realistic biological models are large in terms of states and (unknown) parameters and in most cases poorly parameterized (because of noisy measurement data of few components, a very small number of data points and only a limited number of repetitions). For *mass action networks* establishing multistationarity hence is equivalent to establishing the existence of at least two positive solutions of a large polynomial system with unknown coefficients. For mass action networks with certain structural properties, expressed in terms of the stoichiometric matrix and the reaction rate-exponent matrix, we present necessary and sufficient conditions for multistationarity that take the form of *linear inequality systems*. Solutions of these inequality systems define pairs of steady states and parameter values. We also present a sufficient condition to identify networks where the aforementioned conditions hold. To show the applicability of our results we analyse an ODE system that is defined by the mass action network describing the extracellular signal-regulated kinase (ERK) cascade (i.e. ERK-activation).

Keywords Mass action kinetics · Multistationarity · Linear inequalities

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1 Introduction

Ordinary Differential Equations (ODEs) have become an important tool in many areas of (quantitative) Biology like Systems Biology, Computational Biology or Synthetic Biology. For many ODE systems, multistationarity (i.e. the existence of at least two positive steady states) is a desired feature, e.g. in modeling the cell cycle (see e.g. Sha et al. 2003; Battogtokh and Tyson 2004; Chen et al. 2004), in signal transduction (see e.g. Markevich et al. 2004; Kapuy et al. 2009) or cellular differentiation (see e.g. Thomas and Kaufman 2001a,b). Establishing multistationarity, however, is a difficult task: on the one hand realistic biological models (especially in Systems Biology) contain a large number of ODEs and an even larger number of parameters. Because of noisy measurement data of few components, because of a very small number of data points and rather limited number of repetitions parameter identification is challenging, often yielding poorly parameterized models. Hence one often faces the question whether or not a given system of ODEs can exhibit multistationarity at all (Conradi et al. 2005).

In the present paper we address the question of multistationarity for ODE systems defined by mass action networks. Such an ODE system is defined by two matrices: (i) the *stoichiometric matrix* encoding the reactions and (ii) the reaction *rate-exponent matrix* whose columns define the monomials describing the rate of a reaction. For certain networks necessary and sufficient conditions are presented that allow to decide about multistationarity and that take the form of *linear inequality systems* (Theorem 2, Sect. 4; note that in general multistationarity for mass action systems requires two positive solutions of a polynomial system). Moreover, we present a sufficient condition to identify networks where the aforementioned necessary and sufficient conditions hold (Remark 11, Sect. 4). This condition is stated in terms of the stoichiometric matrix and the rate-exponent matrix; it exploits the pointed polyhedral cone, defined by the intersection of the nullspace of the stoichiometric matrix with the nonnegative orthant, and the interconnection of certain educt complexes where each such educt complex belongs to more than one reaction. Hence we view our sufficient condition as a *network property*. Furthermore, our results are constructive in the sense that they allow the computation of pairs of steady states and corresponding parameter values (i.e. rate constants).

To demonstrate the usefulness of these results we analyse an ODE system describing the extracellular signal-regulated kinase (ERK) cascade (see e.g. the review article Shaul and Seger 2007) of the family of Mitogen Activated Kinase cascades. Besides the three-tiered core cascade that has been frequently analysed and where bistability and hence multistationarity has been reported (see e.g. Huang and Ferrell 1996; Qiao et al. 2007) we also include several feedback mechanisms. Using the aforementioned necessary and sufficient conditions one can obtain multistationarity via linear inequality systems.

Due to its relative importance multistationarity has received continuing attention since the 1970s (at least), where most of the research originated in Chemical Engineering. In the context of the results presented the approach of Feinberg and co-workers is relevant (see e.g. Feinberg 1995b; Ellison and Feinberg 2000; Ellison et al. 2000; Ellison 1998). Of these references the work concerning so-called deficiency zero and

one networks is not related to the approach presented here, as our results are independent of the network deficiency (a network property Feinberg 1995a,b, 1987, 1988). The results presented in Ellison (1998), however, are comparable to our results: Ellison (1998) contains for certain networks necessary and sufficient conditions for multistationarity that take the form of linear inequality systems. Ellison (1998) also gives sufficient conditions to identify networks where the linear inequalities are necessary and sufficient for multistationarity. From a technical point of view, the main difference to the approach presented here is that we work with the generators of the aforementioned pointed polyhedral cone, while Ellison (1998) is based on the nullspace of the stoichiometric matrix only. Both approaches do not depend on any particular choice of coordinates and thus describe a property of the reaction network itself. As there are networks where the approach of Feinberg and co-workers is inconclusive and ours yields positive results (as for the network $\mathcal{N}_2$ below, cf. Sect. 4) and vice versa we view our results as *complementary*.

A second approach from Chemical Engineering, mostly due to Feinberg and Craciun is based on the Jacobian derived from a mass action network and gives *necessary conditions* for the injectivity of the dynamical system and hence the *absence* of multistationarity (Craciun and Feinberg 2005, 2006; Banaji and Craciun 2010). In a certain sense this approach can therefore be seen as complementary to our results and the aforementioned work of Feinberg and co-workers alike.

Closely related are approaches where one exploits the connections between the network graph and the coefficients of the characteristic polynomial of the Jacobian to establish a singular Jacobian and arising saddle node bifurcations: Mincheva and Roussel (2007), e.g. use graph theoretic concepts for this purpose, while Domijan and Kirkilionis (2009) employ graph theory and algebraic geometry, very much in the line of Gatermann et al. (2005), albeit not restricted to Hopf bifurcations as in Gatermann et al. (2005). Our present approach does not incorporate the Jacobian and results in linear inequality systems.

The paper is organised as follows. In Sect. 2 mass action networks are defined and some very basic properties are derived. In Sect. 3 a transformation is introduced that yields necessary and sufficient conditions for multistationarity that are—in general—not based on linear systems. Section 4 contains the main result of our paper: necessary and sufficient conditions that take the form of linear inequality systems and a sufficient condition that identifies networks were the results are applicable. In Sect. 5 an analysis of ERK activation demonstrates that there exist real-world examples where our results are applicable and Sect. 6, finally, contains the proofs of the results presented in Sect. 4.

2 Mass action networks

We consider mass action models for biochemical reaction networks in the form of ODEs and start with a brief presentation of the corresponding notation introduced in Feinberg (1987, 1988) and Gatermann and Wolfrum (2005). For this purpose network $\mathcal{N}_1$ given below, a system that has been analysed in (Conradi et al. 2008), will be revisited. For network $\mathcal{N}_1$, establishing multistationarity has been reduced to a

linear problem in Conradi et al. (2008). Sections 3 and 4 will make the underlying mechanism transparent, where it is shown that this reduction to a linear problem is a consequence of Lemma 4.

$$\begin{aligned} &\mathbf{A}+\mathbf{E_1} \underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} \mathbf{A\,E_1} \xrightarrow{\mathbf{k_3}} \mathbf{A_p}+\mathbf{E_1} \underset{\mathbf{k_5}}{\overset{\mathbf{k_4}}{\rightleftharpoons}} \mathbf{A_p\,E_1} \xrightarrow{\mathbf{k_6}} \mathbf{A_{pp}}+\mathbf{E_1} \\ &\mathbf{A_{pp}}+\mathbf{E_2} \underset{\mathbf{k_8}}{\overset{\mathbf{k_7}}{\rightleftharpoons}} \mathbf{A_{pp}\,E_2} \xrightarrow{\mathbf{k_9}} \mathbf{A_p}+\mathbf{E_2} \underset{\mathbf{k_{11}}}{\overset{\mathbf{k_{10}}}{\rightleftharpoons}} \mathbf{A_p\,E_2} \xrightarrow{\mathbf{k_{12}}} \mathbf{A}+\mathbf{E_2} \end{aligned} \tag{$\mathcal{N}_1$}$$

Network $\mathcal{N}_1$ consists of nine *species* A, E_1, $A\,E_1$, A_p, $A_p\,E_1$, A_{pp}, E_2, $A_{pp}\,E_2$ and $A_p\,E_2$ and ten *complexes*: $A+E_1$, $A\,E_1$, A_p+E_1, $A_p\,E_1$, $A_{pp}+E_1$, $A_{pp}+E_2$, $A_{pp}\,E_2$, A_p+E_2, $A_p\,E_2$ and $A+E_2$. Throughout this paper we assume that the node labels are unique, i.e. that every complex is displayed only once (this layout is called 'standard form' in Feinberg 1995a,b). These complexes are connected by 12 *reactions*, where each reaction is associated with a *rate constant* k_i. In the ordering chosen here one has, e.g. reaction 1 as $A+E_1 \overset{k_1}{\to} A\,E_1$ with rate constant k_1. In this reaction the complex $A+E_1$ reacts to the complex $A\,E_1$, hence $A+E_1$ is called *educt complex* and $A\,E_1$ *product complex*.

Variables x_i are used to denote the species concentrations. Use, e.g. x_1 for A, x_2 for E_1, x_3 for $A\,E_1$, x_4 for A_p, x_5 for $A_p\,E_1$, x_6 for A_{pp}, x_7 for E_2, x_8 for $A_{pp}\,E_2$ and x_9 for $A_p\,E_2$. Associate to each species the corresponding unit vector of Euclidean 9-space, i.e. e_1 to A, e_2 to E_1 and so on. Then every complex can be represented by the sum of its constituent species (use y_i to denote *complex vectors*): $y_1 = e_1 + e_2$ for $A+E_1$, and so on. Collect all complex vectors in the matrix $Y \in \mathbb{R}^{9\times 10}$ and thus obtain

$$\begin{aligned} Y &= \left[y_1 \ \ldots \ y_{10}\right] \\ &= \left[e_1+e_2,\ e_3,\ e_4+e_2,\ e_5,\ e_6+e_2,\ e_6+e_7,\ e_8,\ e_4+e_7,\ e_9,\ e_1+e_7\right]. \end{aligned} \tag{2.1a}$$

The network $\mathcal{N}_1$ defines the *incidence matrix* $\mathcal{I} \in \mathbb{R}^{10\times 12}$. Each column of $\mathcal{I}$ represents a reaction and has exactly one entry -1 for the educt complex and one entry 1 for the product complex. The remaining entries are zero. For $\mathcal{I}$ of $\mathcal{N}_1$ one thus obtains:

$$\mathcal{I} = \begin{bmatrix} -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}. \tag{2.1b}$$

A *reaction rate* is associated to every reaction. In mass action networks, each reaction rate is composed of a rate constant multiplied by the products of the concentrations of their educt complexes. With the notation

$$x^v = \prod_{i=1}^{n} x_i^{v_i}$$

for n-vectors x and v and the vectors $y_1, \ldots, y_{10}$ defined in (2.1a) one has $v_1 = k_1\, x_1\, x_2 = k_1\, x^{y_1}$, for the reaction $A + E_1 \overset{k_1}{\to} A\, E_1$. The remaining reaction rates are $v_2 = k_2\, x_3 = k_2\, x^{y_2}$, $v_3 = k_3\, x_3 = k_3\, x^{y_2}$, $v_4 = k_4\, x_2\, x_4 = k_4\, x^{y_3}$, $v_5 = k_5\, x_5 = k_5\, x^{y_4}$, $v_6 = k_6\, x_5 = k_6\, x^{y_4}$, $v_7 = k_7\, x_6\, x_7 = k_7\, x^{y_6}$, $v_8 = k_8\, x_8 = k_8\, x^{y_7}$, $v_9 = k_9\, x_8 = k_9\, x^{y_7}$, $v_{10} = k_{10}\, x_4\, x_7 = k_{10}\, x^{y_8}$, $v_{11} = k_{11}\, x_9 = k_{11}\, x^{y_9}$, $v_{12} = k_{12}\, x_9 = k_{12}\, x^{y_9}$. Using the vector of rate constants $k := (k_1, \ldots, k_{12})^T$ and the monomial vector

$$\phi(x) = (x_1\, x_2,\ x_3,\ x_3,\ x_2\, x_4,\ x_5,\ x_5,\ x_6\, x_7,\ x_8,\ x_8,\ x_4\, x_7,\ x_9,\ x_9)^T$$

one can write the vector of reaction rates as

$$v(k,\ x) = \mathrm{diag}(k)\, \phi(x).$$

Note that $\phi(x)$ is completely defined by exponent vectors y_i. Hence we collect those in the reaction *rate-exponent matrix* $\mathcal{Y}$, which, in case of network $\mathcal{N}_1$, is given by

$$\mathcal{Y} = \left[y_1,\ y_2,\ y_2,\ y_3,\ y_4,\ y_4,\ y_6,\ y_7,\ y_7,\ y_8,\ y_9,\ y_9\right] \in \mathbb{R}^{9\times 12}. \tag{2.1c}$$

The ODEs describing the dynamics of network $\mathcal{N}_1$ are then given with the help of the *stoichiometric matrix* S that is defined as the product $S := Y\, \mathcal{I}$ as

$$\dot{x}(t) = S\, v(k,\ x(t))$$

and hence

$$\begin{aligned}
\dot{x}_1 &= -k_1\, x_1\, x_2 + k_2\, x_3 + k_{12}\, x_9\\
\dot{x}_2 &= -k_1\, x_1\, x_2 + (k_2 + k_3)\, x_3 - k_4\, x_2\, x_4 + (k_5 + k_6)\, x_5\\
\dot{x}_3 &= k_1\, x_1\, x_2 - (k_2 + k_3)\, x_3\\
\dot{x}_4 &= k_3\, x_3 - k_4\, x_2\, x_4 + k_5\, x_5 + k_9\, x_8 - k_{10}\, x_4\, x_7 + k_{11}\, x_9\\
\dot{x}_5 &= k_4\, x_2\, x_4 - (k_5 + k_6)\, x_5\\
\dot{x}_6 &= k_6\, x_5 - k_7\, x_6\, x_7 + k_8\, x_8\\
\dot{x}_7 &= -k_7\, x_6\, x_7 + (k_8 + k_9)\, x_8 - k_{10}\, x_4\, x_7 + (k_{11} + k_{12})\, x_9\\
\dot{x}_8 &= k_7\, x_6\, x_7 - (k_8 + k_9)\, x_8\\
\dot{x}_9 &= k_{10}\, x_4\, x_7 - (k_{11} + k_{12})\, x_9
\end{aligned}$$

For $\mathcal{N}_1$ one has $S \in \mathbb{R}^{9\times 12}$, a matrix of rank 6. Hence there is a matrix $Z \in \mathbb{R}^{9\times 3}$ of rank 3 with $Z^T\, S = 0$. The matrix Z defines three *conservation relations* of the form

$Z^T x(t) = c$:

$$x_2 + x_3 + x_5 = c_1,$$

for E_1, $A\,E_1$ and $A_p\,E_1$,

$$x_7 + x_8 + x_9 = c_2$$

for E_2, $A_{pp}\,E_2$ and $A_p\,E_2$ and

$$x_1 + x_3 + x_4 + x_5 + x_6 + x_8 + x_9 = c_3$$

for A, A_p, A_{pp}, $A\,E_1$, $A_p\,E_1$, $A_p\,E_2$ and $A_{pp}\,E_2$.

Generally speaking, we assume a mass action network of n species, m complexes and r reactions and use the symbols $Y, \mathcal{I}, \mathcal{Y}$ and S to describe the corresponding dynamical system in an analogous way: $Y \in \mathbb{R}^{n\times m}$ denotes a matrix whose column vectors are the complexes of the network and $\mathcal{I} \in \{-1, 0, 1\}^{m\times r}$ denotes the incidence matrix of the graph. These two define:

1. the *stoichiometric matrix*

$$S := Y\,\mathcal{I} \tag{2.2a}$$

2. the *rate exponent matrix*

$$\mathcal{Y} := \left[\tilde{y}_1, \ldots, \tilde{y}_r\right], \tag{2.2b}$$

consisting of r columns, where the ith column is given by the educt complex vector of the ith reaction. Hence $\mathcal{Y}$ can contain multiple copies of the same complex vector, a fact that we will exploit later on.

With the columns $\tilde{y}_i$ of $\mathcal{Y}$ one obtains the monomial vector

$$\phi(x) := \left(x^{\tilde{y}_1}, \ldots, x^{\tilde{y}_r}\right)^T \tag{2.3a}$$

and the vector of reaction rates

$$v(k,\ x) = \mathrm{diag}(k)\,\phi(x), \tag{2.3b}$$

where $k := (k_1, \ldots, k_r)^T$ is the vector of rate constants. Then the ODEs describing the dynamics of a mass-action network are given as

$$\dot{x}(t) = S\,v(k, x(t)). \tag{2.4}$$

In general, the stoichiometric matrix S does not have maximal row rank. For $s := \mathrm{rank}(S)$ there exist $n - s$ conservation relations

$$Z^T x = c \tag{2.5}$$

with $Z^T S = 0$ for a $Z \in \mathbb{R}^{n\times(n-s)}$ of rank $n - s$. Note that level sets $\{x \in \mathbb{R}^n : Z^T x = const\}$ are invariant under the flow of (2.4) due to $Z^T x(t) = Z^T x(0)$ along solutions $x(t)$ of (2.4). Every biochemical reaction network endowed with mass action kinetics defines a system of the form presented in (2.4) and (2.5).

Remark 1 The stoichiometric matrix S and the rate-exponent matrix $\mathcal{Y}$ completely define the ODE system (2.4). In working with S and $\mathcal{Y}$ we will derive conditions for multistationarity in the following sections. The results presented therein make no use of the fact that the columns of $\mathcal{Y}$ correspond to complexes of the network. Hence these results hold for arbitrary rate-exponent matrices and hence for systems where each reaction rate is proportional to a monomial not necessarily defined by the educt complex of the reaction (such systems are sometimes called *generalized mass action systems*).

Throughout this paper we focus on strictly positive steady state solutions and define multistationarity as follows:

Definition 1 *(Multistationarity)* The system $\dot{x} = S\,v(k, x)$ from (2.4) is said to exhibit multistationarity if and only if there exist a positive vector $k \in \mathbb{R}^r_{>0}$ and at least two distinct positive vectors $a, b \in \mathbb{R}^n_{>0}$ with

$$S\,v(k, a) = 0 \tag{2.6a}$$
$$S\,v(k, b) = 0 \tag{2.6b}$$
$$Z^T a = Z^T b. \tag{2.6c}$$

□

Thus, for a common $k > 0$, a and b are distinct positive equilibria of (2.4) with the difference $a - b$ belonging to the subspace $\mathrm{im}(S)$. In other words, a and b belong to one and the same affine invariant manifold

$$L_a = \{x \in \mathbb{R}^n_{>0} : Z^T x = Z^T a\}.$$

Remark 2 In Definition 1 multistationarity is defined with respect to the linear subspace $\mathrm{im}(S)$, which is the standard definition in the chemical engineering literature (see e.g. Feinberg 1995a,b). Depending on the precise (bio)chemical question it might be useful to investigate multistationarity with respect to different linear subspaces (Flockerzi and Conradi 2008). □

Remark 3 (The monomial function ϕ with $\phi(e^x) = e^{\mathcal{Y}^T x}$) Note the following properties of $\phi(x)$ from (2.3a) and v from (2.3b):

(i) One has $\mathrm{diag}(\alpha)\, x = (\alpha_1 x_1, \ldots, \alpha_n x_n)^T$ and thus

$$(\mathrm{diag}(\alpha)\, x)^y = (\alpha_1 x_1)^{y_1} (\alpha_2 x_2)^{y_2} \ldots (\alpha_n x_n)^{y_n} = \alpha^y x^y,$$

for $\alpha, x \in \mathbb{R}^n_{>0}$, $y \in \mathbb{R}^n_{\geq 0}$ implying

$$\phi(\mathrm{diag}(\alpha)\, x) = \mathrm{diag}(\phi(\alpha))\, \phi(x). \tag{2.7}$$

(ii) Using the abbreviation $e^x := (e^{x_1}, \ldots, e^{x_n})^T$ for vectors $x \in \mathbb{R}^n$ one obtains the following convenient representation:

$$\begin{aligned} \phi(e^x) &= ((e^x)^{\tilde{y}_1}, \ldots, (e^x)^{\tilde{y}_r})^T \\ &= (e^{\tilde{y}_1 x}, \ldots, e^{\tilde{y}_r x})^T = e^{\mathcal{Y}^T x}. \end{aligned} \tag{2.8}$$

(iii) Let $x \in \mathbb{R}^n$ and $x \neq 0$. One has

$$\phi(x^{-1}) = (x^{\tilde{y}_1})^{-1}, \ldots, (x^{\tilde{y}_r})^{-1})^T =: \frac{1}{\phi(x)}. \tag{2.9}$$

(iv) As $k \in \mathbb{R}^r_{>0}$, positive values of x correspond to positive values of:

$$v(k,\, x) = \mathrm{diag}(k)\, \phi(x) = \mathrm{diag}(\phi(x))\, k \in \mathbb{R}^r_{>0}. \tag{2.10}$$

□

3 Conditions for multistationarity

In a first step the linear constraints $Z^T a = Z^T b$ [Eq. (2.6c)] are discarded and a transformation of the remaining polynomials is introduced (Sect. 3.1). Then the transformed equations are analysed (Sect. 3.2) and later on the linear constraints are again taken into account (Sect. 3.3). Finally network $\mathcal{N}_1$ is revisited in Sect. 3.4.

3.1 Transforming the polynomial equations $S\, v(k,\, a) = 0$ and $S\, v(k,\, b) = 0$

In a first step the linear constraints $Z^T a = Z^T b$ are discarded and we focus on the polynomial equations $S\, v(k,\, a) = 0$ and $S\, v(k,\, b) = 0$. Here positivity of a, b and k implies $v(k,\, a) > 0$ and $v(k,\, b) > 0$. Thus we are interested in the pointed polyhedral cone $\ker(S) \cap \mathbb{R}^r_{\geq 0}$ defined by the intersection of the null space of S with the nonnegative orthant $\mathbb{R}^r_{\geq 0}$ and its interior

$$\ker_+(S) := \ker S \cap \mathbb{R}^r_{>0}. \tag{3.1a}$$

This cone is a well studied object, due to its importance in the (bio)chemical literature, starting with the classical work of Clarke (1988). As a pointed polyhedral cone it can be represented by non-negative linear combinations of a finite set of extreme rays (Rockafellar 1970) (called *extremal currents* and *elementary flux modes* in Clarke 1988 and Klamt et al. 2007, resp.). The calculation of these rays is in general computationally hard, however, due its importance, there exists a variety of algorithms and software tools, e.g. Klamt et al. (2007) and Terzer and Stelling (2008).

Let p be the number of extreme rays of $\ker(S) \cap \mathbb{R}^r_{\geq 0}$. A set of extreme rays $\{E_1, \ldots, E_p\}$ that is minimal and generates $\ker(S) \cap \mathbb{R}^r_{\geq 0}$ via nonnegative linear combinations is called a set of *generators* of $\ker(S) \cap \mathbb{R}^r_{\geq 0}$. Let $E \in \mathbb{R}^{r \times p}_{\geq 0}$ be a matrix,

whose columns are generators for $\ker(S) \cap \mathbb{R}^r_{\geq 0}$ and *whose rows we denote by* n_j. We define the set of nonnegative λ with positive $E\,\lambda$ as

$$\Lambda(E) := \{\lambda \in \mathbb{R}^p_{\geq 0} \mid E\,\lambda > 0\}. \tag{3.1b}$$

We'd like to emphasize that the conditions $S\,v(k,\,a) = 0$, $S\,v(k,\,b) = 0$ and the positivity constraints $v(k,\,a) \in \mathbb{R}^r_{>0}$, $v(k,\,b) \in \mathbb{R}^r_{>0}$ can be rewritten as

$$V := v(k,a) \in \ker_+(S), \quad W := v(k,b) \in \ker_+(S), \tag{3.2a}$$

or, equivalently, as

$$v(k,\,a) = E\,\lambda, \quad \lambda \in \Lambda(E), \quad v(k,\,b) = E\,\nu, \quad \nu \in \Lambda(E). \tag{3.2b}$$

Further note that as $(v(k,\,x))_i = k_i\,x^{\tilde{y}_i}$ one has positive a, b, if and only if none of the rows n_i of E is a zero row. All in all we make the following assumptions regarding the generator matrix E:

Assumption 1 (i) We speak of the generator matrix E (with rows n_i) by assuming a fix order of the columns of fixed 1-norms.
(ii) The matrix E does not have a zero row. □

In Sect. 4, we will redefine the order of the reactions in the network (2.4) and thus the order of the rows of E so that the desired network structure of (2.4) is reflected in the block structure (4.1) of E. Concerning elements of $\Lambda(E)$ we establish the following simple result:

Remark 4 (Properties of $\Lambda(E)$)

(1) $\Lambda(E)$ contains $\mathbb{R}^p_{>0}$, the interior of the nonnegative orthant $\mathbb{R}^p_{\geq 0}$.
(2) $\Lambda(E)$ can additionally contain faces of $\mathbb{R}^p_{\geq 0}$ and thus $\lambda \in \Lambda(E)$ with $\lambda_i = 0$ for some index i.
(3) Let $\lambda, \tilde{\lambda} \in \mathbb{R}^p_{\geq 0}$ be given with $\operatorname{sign}(\lambda) = \operatorname{sign}(\tilde{\lambda})$. Then $\lambda \in \Lambda(E)$ implies $\tilde{\lambda} \in \Lambda(E)$.
(4) Let $\lambda, \tilde{\lambda} \in \mathbb{R}^p_{\geq 0}$. Then $\lambda \in \Lambda(E)$ implies $\lambda + \tilde{\lambda} \in \Lambda(E)$.

Proof (1) and (2) follow from the fact that $\ker(S) \cap \mathbb{R}^r_{\geq 0}$ is generated by nonnegative linear combinations of the columns of E and from the fact that E is a nonnegative matrix without zero rows. Concerning (3) let $\lambda, \tilde{\lambda} \in \mathbb{R}^p_{\geq 0}$ with $\lambda \neq \tilde{\lambda}$ and $\operatorname{sign}(\lambda) = \operatorname{sign}(\tilde{\lambda})$. Split $\lambda = (\lambda_{(0)},\,\lambda_{(1)})$ and $\tilde{\lambda} = (\tilde{\lambda}_{(0)},\,\tilde{\lambda}_{(1)})$ and assume w.l.o.g. that $\lambda_{(0)}, \tilde{\lambda}_{(0)}$ contain all zero elements of $\lambda, \tilde{\lambda}$, respectively. Assume $\lambda \in \Lambda(E)$ and $\tilde{\lambda} \notin \Lambda(E)$, that is $E\,\lambda > 0$ and $E\,\tilde{\lambda} \not> 0$. Then there is a row n_i in E such that $n_i\,\lambda > 0$ and $n_i\,\tilde{\lambda} = 0$. Split $n_i = (\hat{n}_i,\,\check{n}_i)$, according to λ and observe that $n_i\,\lambda = \check{n}_i\,\lambda_{(1)} > 0$ and $n_i\,\tilde{\lambda} = \check{n}_i\,\tilde{\lambda}_{(1)} = 0$, a contradiction, as $\check{n}_i \gneq 0$ and $\tilde{\lambda}_{(1)} > 0$($\check{n}_i$ contains at least one nonzero element, as $\check{n}_i\,\lambda_{(1)} > 0$). Hence $\tilde{\lambda} \in \Lambda(E)$, which proves the claim. Concerning (4) note that $E\,(\lambda + \tilde{\lambda}) = E\,\lambda + E\,\tilde{\lambda}$. Clearly $E\,\lambda > 0$ implies $E\,(\lambda + \tilde{\lambda}) > 0$, as $E\,\tilde{\lambda} \geq 0$. □

Next consider positive pairs (k, a) and (ℓ, b) with $V := v(k, a) \in \ker(S) \cap \mathbb{R}^r_{>0}$ and $W := v(\ell, b) \in \ker(S) \cap \mathbb{R}^r_{>0}$ so that V and W can be written as $V = E\,\lambda$ and $W = E\,\nu$ for suitable $(\nu, \lambda) \in \Lambda^2(E) := \Lambda(E) \times \Lambda(E)$ (cf. (3.2a)–(3.2b)). We note that b can be written in a unique way as

$$b = \operatorname{diag}(e^{\mu})\, a, \quad \mu := \ln \frac{b}{a}. \tag{3.3}$$

With the notation

$$K(V, a) := \operatorname{diag}(\phi(a^{-1}))\, V = \operatorname{diag}(\phi(a^{-1}))\, E\lambda$$

the rate constant vectors k and ℓ satisfy $k = K(V, a)$ and $\ell = K(W, b)$ together with

$$v(K(V, a), a) = V, \quad v(K(W, b), b) = W.$$

Note that, given $a > 0$, the vector λ determines k and thus takes on the role of the rate constants. Recall that multistationarity requires that a, b are steady states for the same rate constants. Thus, for ℓ to equal k the equation

$$\operatorname{diag}(\phi(a^{-1}))\, V = \operatorname{diag}(\phi(\operatorname{diag}(e^{-\mu})a^{-1}))\, W$$

is necessary and sufficient. Due to Remark 3, this is equivalent to

$$W = \operatorname{diag}(\phi(e^{\mu}))\, V \tag{3.4}$$

and, because of $\phi(e^x) = e^{\mathcal{Y}^T x}$, also to the *determining equation*

$$\mathcal{Y}^T \mu = \ln \frac{W}{V} = \ln \frac{E\,\nu}{E\,\lambda} \quad \text{with } \nu,\ \lambda \in \Lambda(E) \tag{3.5}$$

(with the abbreviation for n-vectors $x, y \neq 0$: $\ln \frac{x}{y} := (\ln \frac{x_1}{y_1}, \ldots, \ln \frac{x_n}{y_n})^T$). We'd like to point out that b from (3.3) and W from (3.4) satisfy

$$K(W, b) = K(\operatorname{diag}(\phi(e^{\mu}))\, V, \quad \operatorname{diag}(e^{\mu})\, a) = K(V, a)$$

and that the relation

$$K(E\nu, e^{\mu} a) = K(E\lambda, a),$$

which is equivalent to (3.5), characterizes the fact that $b = e^{\mu} a$ and a are positive steady states for the same $k > 0$. Any nontrivial solution $\mu = \mu(\lambda, \nu)$ of (3.5) with $\lambda, \nu \in \Lambda(E)$ gives rise to a positive steady state b as in (3.3) for the rate constant k with $b \neq a$. Of course, the solution μ can be considered as a function of $(V, W) \in \ker^2_+(S) = \ker_+(S) \times \ker_+(S)$ too. By the Fredholm alternative (Strang 1976), the solvability of (3.5) can be formulated equivalently with the help of a basis of the left kernel of $\mathcal{Y}^T$.

Fact 1 (Fredholm: (3.5) $\Leftrightarrow$(3.6)) *Let U be a matrix whose columns form a basis of* $\ker(\mathcal{Y})$. *Then, for given ν and λ in $\Lambda(E)$, (3.5) is solvable if and only if the alternative determining equation*

$$U^T \ln \frac{W}{V} = U^T \ln \frac{E\,\nu}{E\,\lambda} = 0 \tag{3.6}$$

holds. If (3.6) is satisfied, i.e. if

$$(\lambda, \nu) \in \left\{ (\lambda, \nu) \in \Lambda(E) \times \Lambda(E) : U^T \ln \frac{E\,\nu}{E\,\lambda} = 0 \right\}$$

then the solution set of (3.5) can be stated with the help of the Moore-Penrose inverse $\mathcal{Y}'$ of $\mathcal{Y}^T$ as

$$\left\{ \mu = \mathcal{Y}' \ln \frac{E\,\nu}{E\,\lambda} + \xi : \xi \in \ker(\mathcal{Y}^T) \right\}.$$

In terms of $(W, V) \in \ker_+(S)^2$ one has $\{\mu = \mathcal{Y}' \ln \frac{W}{V} + \xi : \xi \in \ker(\mathcal{Y}^T)\}$ as the solution set of (3.5) under condition (3.6). □

3.2 Analysis of the determining equation

We now elaborate on Eq. (3.5). Given (3.5), one necessarily has $\ln \frac{E\,\nu}{E\,\lambda} \in \mathrm{im}(\mathcal{Y}^T)$, a linear subspace of dimension $n - rank(\mathcal{Y})$. In many cases however, the range of the r-dimensional mapping

$$(\nu, \lambda) \in \Lambda^2(E) \;\mapsto\; \kappa(\nu, \lambda) := \ln \frac{E\,\nu}{E\,\lambda} \in \mathbb{R}^r \tag{3.7}$$

may be contained in a linear subspace of smaller dimension $\gamma \leq r$ within $\mathrm{im}(\mathcal{Y}^T)$. We will employ the same symbol $\kappa(\nu, \lambda)$ for *all* $(\nu, \lambda) \in \mathbb{R}^p \times \mathbb{R}^p$ for which $\ln \frac{E\,\nu}{E\,\lambda}$ is meaningful. Of course, this mapping $(\nu, \lambda) \mapsto \ln \frac{E\,\nu}{E\,\lambda}$ could be equivalently considered as a mapping $\tilde{\kappa} : (W, V) \mapsto \ln \frac{W}{V}$ on $\ker_+^2(S)$.

Note that in many cases the matrix $\mathcal{Y}$ contains multiple copies of the same column vector $\tilde{y}_i$ (this is the case if the complex $\tilde{y}_i$ is educt of more than one reaction, e.g. the complexes $A\,E_1$, $A_p\,E_1$, $A_{pp}\,E_2$ and $A_p\,E_2$ in $\mathcal{N}_1$). In this case $\ker(\mathcal{Y})$ will contain vectors of the form

$$u = (0, \ldots, 0, 1, 0, \ldots, 0, -1, 0, \ldots, 0)^T = e_i - e_k,$$

where e_i, e_k are suitable unit vectors. From hereon we will assume that there are d such vectors that are linear independent and will exploit the consequences for the nonlinear system (3.6). To be more precise:

Assumption 2 (*Doubling vectors*) We assume that the matrix $\mathcal{Y}$ contains a certain number of columns $\tilde{y}_i$ corresponding to educt complexes belonging to more than one reaction. In precise terms, we assume the following:

(i) There exists pairwise different $u_1, \ldots, u_d, d > 0$, of the form

$$u = e_i - e_k \quad (i \neq k) \tag{3.8a}$$

with $\mathcal{Y}\, u_j = 0$. These will be called doubling vectors of $\mathcal{Y}$.

(ii) The matrix $U^{doub} = (u_1, \ldots, u_d)$ is extended by an appropriate matrix $\widetilde{U}$ such that

$$U = [u_1, \ldots, u_d \,|\, \widetilde{U}] = [U^{doub} \,|\, \widetilde{U}] \tag{3.8b}$$

is a basis of $\ker(\mathcal{Y})$ where $\widetilde{U}$ is absent in case $\dim(\ker(\mathcal{Y})) = d$.

The doubling vectors force two entries of $\ln \frac{W}{V}$ to be equal. Moreover one has for $u = e_i - e_k$ from (3.8a):

$$u^T \ln \frac{W}{V} = 0 \Rightarrow \frac{W_i}{V_i} - \frac{W_k}{V_k} = 0. \tag{3.8c}$$

Such a condition can imply

$$\frac{W_i}{V_i} - \frac{W_j}{V_j} = 0$$

for some $j \in \{1, \ldots, r\}$ different from i and k. This is formalized in the following easy observation.

Fact 2 (Clusters J_i)

(a) *Given a doubling vector $u_i = e_\ell - e_k, i \in \{1, \ldots, d\}$ (where we suppress the dependence of ℓ and k on i for simplicity), the equality*

$$\frac{n_\ell \nu}{n_\ell \lambda} = \frac{n_k \nu}{n_k \lambda} = e^\kappa \quad \textit{for } (\nu, \lambda) \in \Lambda^2(E)$$

implies

$$\frac{n_j \nu}{n_j \lambda} = e^\kappa \quad \textit{for all } j \textit{ with } n_j \in \operatorname{span}\{n_\ell, n_k\}.$$

(b) *The doubling vector $u_i = e_\ell - e_k, i \in \{1, \ldots, d\}$, is said to induce the cluster J_i, where J_i is given by the row indices j for which $\frac{n_\ell \nu}{n_\ell \lambda} = \frac{n_j \nu}{n_j \lambda}$ holds on $\Lambda^2(E)$. The common value $\frac{n_j \nu}{n_j \lambda}$ for $j \in J_i$ will be denoted by $e^{\kappa_{J_i}}$.*

□

Next we propose an algorithm to group the elements of $\ln \frac{E\nu}{E\lambda}$ or of $\ln \frac{W}{V}$ according to Fact 2 (under Assumption 2). For later convenience, we formulate this algorithm for $\ln \frac{E\nu}{E\lambda}$ and the mapping κ from (3.7). It reduces the r-dimensional $\kappa \in \mathrm{im}(\mathcal{Y}^T)$ to a γ-dimensional ψ over $\Lambda^2(E)$ (cf. Lemma 1 below). Of course, there is the analogous result for $\ln \frac{W}{V}$ and the mapping $\tilde{\kappa} : (W, V) \mapsto \ln \frac{W}{V}$ on $\ker_+^2(S)$.

Algorithm 1 (*Reduction from dimension r to γ over $\Lambda^2(E)$*) *Given $\kappa = \ln \frac{E\nu}{E\lambda} \in \mathbb{R}^r \cap \mathrm{im}(\mathcal{Y}^T)$ with $\kappa_i = \ln \frac{n_i\nu}{n_i\lambda}$ for $i = 1, \ldots, r$ (cf. (3.7)):*

- *initialize $k := 1$, $I_k := \{1, \ldots, r\}$,*
- *when $I_k \neq \emptyset$ set $s_k := \min(I_k)$ and obtain all row vectors n_j of E with*

$$\frac{n_j\nu}{n_j\lambda} \equiv \frac{n_{s_k}\nu}{n_{s_k}\lambda} \quad \text{on } \Lambda^2(E),$$

collect all corresponding indices j in the set

$$J_k := \left\{ j \in \{1, \ldots, r\} \;\middle|\; \frac{n_j\nu}{n_j\lambda} \equiv \frac{n_{s_k}\nu}{n_{s_k}\lambda} =: e^{\psi_{J_k}} \right\}$$

- *set $I_k := I_k \backslash J_k$ and $k := k + 1$,*
- *record as outputs the pairwise different clusters $J_1, J_2 \ldots$ of row indices of E with cardinalities $|J_1|, |J_2| \ldots$ and pairwise different functions $\psi_{J_i} = \psi_{J_i}(\nu, \lambda), i = 1, \ldots, \gamma$.*

Remark 5 The set I_1 contains r elements, thus the algorithm will terminate after at most r steps, resulting in at most r sets $J_1, J_2, \ldots$. If Assumption 2 holds, then some of the J_i will contain more than one element. And each such J_i forces $(W, V) \in \ker_+(S)^2$ into the subset

$$\{(W, V) \mid W = E\nu,\ V = E\lambda, (\nu, \lambda) \in \Lambda^2(E),\ E_{J_i}\,(\nu - e^{\psi_{J_i}}\lambda) = 0\}$$

of $\ker_+(S)^2$ for $E_{J_i} := (n_j)_{j \in J_i}$. In general, the condition $E_{J_i}\,(\nu - e^{\psi_{J_i}}\lambda) = 0$ imposes an active constraint. □

The clusters J_i are associated to the network, they do not depend on a chosen representation of $\ker_+(S)$. Before we proceed, the results of Algorithm 1 for network $\mathcal{N}_1$ are demonstrated in the following example:

Example 1 (The generator matrix E for network $\mathcal{N}_1$ and derivation of the set J_1) Using $\epsilon_1, \ldots, \epsilon_6$ to denote the unit vectors of $\mathbb{R}^6$ and $e_1, \ldots, e_{12}$ to denote the unit vectors of $\mathbb{R}^{12}$ one obtains for E and $\ker(\mathcal{Y})$:

Generator matrix: Basis for $\ker(\mathcal{Y})$:

$$E = \begin{bmatrix} \epsilon_1^T + \epsilon_6^T \\ \epsilon_1^T \\ \epsilon_6^T \\ \epsilon_2^T + \epsilon_5^T \\ \epsilon_2^T \\ \epsilon_5^T \\ \epsilon_3^T + \epsilon_5^T \\ \epsilon_3^T \\ \epsilon_5^T \\ \epsilon_4^T + \epsilon_6^T \\ \epsilon_4^T \\ \epsilon_6^T \end{bmatrix}$$

$$U = [e_2 - e_3,\ e_5 - e_6,\ e_8 - e_9,\ e_{11} - e_{12}].$$

Thus one has (from the kernel vectors $e_2 - e_3$ and $e_{11} - e_{12}$)

$$\ln \frac{\epsilon_1^T \nu}{\epsilon_1^T \lambda} = \ln \frac{\epsilon_6^T \nu}{\epsilon_6^T \lambda} \quad \text{and} \quad \ln \frac{\epsilon_4^T \nu}{\epsilon_4^T \lambda} = \ln \frac{\epsilon_6^T \nu}{\epsilon_6^T \lambda}.$$

This implies for

$$\ln \frac{(\epsilon_1^T + \epsilon_6^T)\, \nu}{(\epsilon_1^T + \epsilon_6^T)\, \lambda} = \ln \frac{\epsilon_6^T \nu}{\epsilon_6^T \lambda} \quad \text{and} \quad \ln \frac{(\epsilon_4^T + \epsilon_6^T)\, \nu}{(\epsilon_4^T + \epsilon_6^T)\, \lambda} = \ln \frac{\epsilon_6^T \nu}{\epsilon_6^T \lambda}.$$

Thus one obtains the set $J_1 = \{1, 2, 3, 10, 11, 12\}$ (and, by analogous arguments involving $e_5 - e_6$ and $e_8 - e_9$, the set $J_2 = \{4, 5, 6, 7, 8, 9\}$).

The sets J_1 and J_2 define the functions

$$\psi_{J_1}(\nu, \lambda) = \ln \frac{\nu_1}{\lambda_1} = \ln \frac{\nu_4}{\lambda_4} = \ln \frac{\nu_6}{\lambda_6} = \ln \frac{\nu_1 + \nu_6}{\lambda_1 + \lambda_6} = \ln \frac{\nu_4 + \nu_6}{\lambda_4 + \lambda_6}, \tag{3.9a}$$

$$\psi_{J_2}(\nu, \lambda) = \ln \frac{\nu_2}{\lambda_2} = \ln \frac{\nu_3}{\lambda_3} = \ln \frac{\nu_5}{\lambda_5} = \ln \frac{\nu_2 + \nu_5}{\lambda_2 + \lambda_5} = \ln \frac{\nu_3 + \nu_5}{\lambda_3 + \lambda_5}. \tag{3.9b}$$

Hence, as a consequence of the Fredholm Alternative one arrives at the conclusion that meaningful $\kappa = \ln \frac{E\,\nu}{E\,\lambda}$ is equivalent to

$$\ln \frac{E\,\nu}{E\,\lambda} = \underbrace{\begin{bmatrix} 1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 \\ 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 & 1 & 0 & 0 & 0 \end{bmatrix}^T}_{=:\Pi} \begin{pmatrix} \psi_{J_1}(\nu, \lambda) \\ \psi_{J_2}(\nu, \lambda) \end{pmatrix} \tag{3.10}$$

□

The output of Algorithm 1 furnishes the following *reduction result* concerning the solvability condition (3.6) and an equivalent reformulation of (3.6) which we state in the following lemma.

Lemma 1 ((3.6) $\Leftrightarrow$ (3.11a) and (3.12) and (3.13b)) *Let U from (3.8b) be a basis for* $\ker(\mathcal{Y})$ *and assume, under Assumption 2, Algorithm 1 has been applied to* $\ln \frac{E\,\nu}{E\,\lambda}$ *on* $\Lambda^2(E)$. *Let $\gamma \leq r$ be the number of pairwise different clusters J_i obtained from Algorithm 1 and let $\pi_i \in \mathbb{R}^r$ be the indicator vector for J_i (with $\pi_i^{(j)} = 1$ for $j \in J_i$ and $\pi_i^{(j)} = 0$ otherwise). For $\nu, \lambda \in \Lambda(E)$ and $i = 1, \ldots, \gamma$ define the pairwise different functions*

$$\psi_{J_i}(\nu,\lambda) := \ln \frac{n_j \nu}{n_j \lambda}, \quad \forall j \in J_i, \tag{3.11a}$$

and collect them as

$$\psi(\nu,\lambda) := (\psi_{J_i}(\nu,\lambda))_{i=1,\ldots,\gamma} \in \mathbb{R}^\gamma. \tag{3.11b}$$

For $(\nu,\lambda) \in \Lambda^2(E)$ *the alternative determining equation* $U^T \ln \frac{E\,\nu}{E\,\lambda} = 0$ *implies that* $\kappa = \ln \frac{E\,\nu}{E\,\lambda}$ *is of the form*:

$$\kappa = \ln \frac{E\,\nu}{E\,\lambda} = [\pi_1, \ldots, \pi_\gamma]\,\psi(\nu,\lambda) =: \Pi\,\psi(\nu,\lambda). \tag{3.12}$$

Hence the alternative determining equation holds if and only if there exists a vector $\varkappa \in \mathbb{R}^\gamma$ *such that*

$$\widetilde{U}^T \Pi\,\varkappa = 0 \tag{3.13a}$$

$$\varkappa = \psi(\nu,\lambda). \tag{3.13b}$$

In Example 1, the representation 3.12 of κ is given by Eq. (3.10). Equation (3.13b) represents a *nonlinear condition* for $\varkappa$ to belong to the range of the mapping $\psi : \Lambda^2(E) \to \mathbb{R}^\gamma$ from (3.11b).

Definition 2 (Λ-*range of* $\psi(\nu,\lambda)$) We call a *numerical value* $\varkappa$ *realizable by* ψ if and only if there exists $\nu, \lambda \in \Lambda^2(E)$ with

$$\varkappa_i = \psi_{J_i}(\nu,\lambda) = \ln \frac{n_j\,\nu}{n_j\,\lambda} \quad \forall j \in J_i, \quad i = 1,\ldots,\gamma.$$

The set of all realizable vectors $\varkappa \in \mathbb{R}^\gamma$, called the Λ-range of ψ, will be denoted by

$$\mathcal{K} := \{\varkappa \in \mathbb{R}^\gamma \,|\, \exists\,(\nu,\lambda) \in \Lambda^2(E) \text{ such that } \varkappa = \psi(\nu,\lambda)\}. \tag{3.14}$$

□

We note that the diagonal $\{\alpha\,\underline{1} : \alpha \in \mathbb{R}\}$ is realizable by $(e^\alpha\,\lambda, \lambda)$ with $\lambda > 0$ implying $\mathcal{K} \neq \emptyset$.

Observe that our considerations to this point successively incorporated more and more information provided by the biochemical reaction network: the fact that we are only interested in *positive solutions* led to the use of $\ker(S) \cap \mathbb{R}^r_{\geq 0}$ and the determining Eq. (3.5). As a first step towards solving the determining equation we employed Fredholm's alternative which motivated Algorithm 1. Before proceeding with an analysis based on the output of Algorithm 1 it seems worthwhile to collect our considerations in the following remark:

Remark 6 (Objects associated to a mass action network) Consider a mass action network with n species and r reactions. This network defines the following objects:

(1) the stoichiometric matrix $S \in \mathbb{R}^{n\times r}$ (cf. Eq. (2.2a)),
(2) the rate-exponent matrix $\mathcal{Y} \in \mathbb{R}^{n\times r}$ (cf. Eq. (2.2b)).
The matrix S in turn determines uniquely (up to scalar multiplication and column order) the generators of the pointed polyhedral cone $\ker(S) \cap \mathbb{R}^r_{\geq 0}$ and thus
(3) the matrix E, with generators of $\ker(S) \cap \mathbb{R}^r_{\geq 0}$ as column vectors,
(4) the set $\Lambda(E)$ with $\lambda \in \Lambda(E) \Leftrightarrow E\,\lambda > 0$.
The matrices S and $\mathcal{Y}$ define
(5) the clusters J_i where for $j, \ell \in J_i$ and $(W, V) \in \ker^2_+(S)$ one has $\frac{W_j}{V_j} = \frac{W_\ell}{V_\ell}$, equivalently, where for $j, \ell \in J_i$ and $(\nu, \lambda) \in \Lambda^2(E)$ one has $\frac{n_j\,\nu}{n_j\,\lambda} = \frac{n_\ell\,\nu}{n_\ell\,\lambda}$,
(6) the indicator vectors $\pi_i \in \mathbb{R}^r$ for J_i with

$$\pi_i^{(j)} = 1 \quad \text{for } j \in J_i, \quad \pi_i^{(j)} = 0 \text{ otherwise},$$

(7) the reduced mapping $\psi : \Lambda^2(E) \to \mathbb{R}^\gamma$ from (3.11b) with the components

$$\psi_{J_i}(\nu, \lambda) := \ln \frac{n_j\,\nu}{n_j\,\lambda} \quad \forall j \in J_i$$

together with the associated Λ-range

$$\mathcal{K} := \{\varkappa \in \mathbb{R}^\gamma \,|\, \exists\,(\nu, \lambda) \in \Lambda^2(E) \text{ such that } \varkappa = \psi(\nu, \lambda)\}.$$

from (3.14).

Here, we'd like to recall our standing Assumptions 1 and 2. Observe that, as the columns of E are uniquely defined by a network (up to scalar multiplication), the functions $\psi_{J_i}(\nu, \lambda)$ are uniquely defined by a reaction network. □

In summary we have derived the following equivalent formulations:

Lemma 2 (Equivalent Formulations of (2.6a) and (2.6b)) *Consider a mass action network and recall the objects collected in Remark 6. Then the following statements are equivalent:*

(I) $\exists a, b \in \mathbb{R}^n_{>0}$, $a \neq b$ *and* $\exists k \in \mathbb{R}^r_{>0}$, *with*

$$S\,v(k,\,a) = 0, \quad S\,v(k,\,b) = 0.$$

(II) $\exists \mu \in \mathbb{R}^n$, $\mu \neq 0$, *and* $\exists (\nu, \lambda) \in \Lambda^2(E)$ *with*

$$\mathcal{Y}^T\,\mu = \ln \frac{E\,\nu}{E\,\lambda}.$$

(III) $\exists \mu \in \mathbb{R}^n$, $\mu \neq 0$, *and* $\exists \varkappa \in \mathcal{K}$ *with*

$$\mathcal{Y}^T\,\mu = \Pi\,\varkappa, \quad \tilde{U}^T\,\Pi\,\varkappa = 0.$$

3.3 Incorporating the linear constraints $Z^T a = Z^T b$

Now we take the linear constraints $Z^T a = Z^T b$ back into account. Let $(\nu, \lambda, \mu) \in \Lambda^2(E) \times \mathbb{R}^n$ be a triplet satisfying the determining Eq. (3.5). Based on the following result from (Conradi et al. 2008) it is straightforward to decide about the existence of a and $b = \mathrm{diag}(e^\mu)\, a$ that satisfy additional $Z^T a = Z^T b$:

Lemma 3 *Let $M_1 \subseteq \mathbb{R}^n$ and $M_2 \subseteq \mathbb{R}^n$ be two nontrivial subsets of $\mathbb{R}^n$ and define $M_3 := \{(m_1, m_2) \in M_1 \times M_2 | \, \mathrm{sign}(m_1) = \mathrm{sign}(m_2)\}$ as the set of all ordered pairs (m_1, m_2) of elements $m_1 \in M_1$ and $m_2 \in M_2$ with the same sign pattern. Two positive vectors p and q with*

$$w := \ln \frac{q}{p} \in M_1, \tag{3.15a}$$

with components $w_i := \ln \frac{q_i}{p_i}$, $i = 1, \ldots, n$, and

$$q - p \in M_2 \tag{3.15b}$$

exist if and only if $M_3 \neq \emptyset$. For nonempty M_3, p and q given by

$$p_i = \begin{cases} \frac{m_{2i}}{e^{m_{1i}} - 1}, & \text{if } m_{1i} \neq 0 \\ \bar{p}_i > 0, & \text{if } m_{1i} = 0 \end{cases}, \quad q_i = e^{m_{1i}}\, p_i, \tag{3.16}$$

where $\bar{p}_i$ denotes an arbitrary positive number, fulfill (3.15a) and (3.15b).

Lemma 3 is the basis for the following result:

Theorem 1 (Conditions for multistationarity) *Consider a biochemical reaction network with mass action kinetics and recall the objects collected in Remark 6. Then there exist $a, b \in \mathbb{R}^n_{>0}$, $a \neq b$ and $k \in \mathbb{R}^r_{>0}$ with*

$$Sv(k, a) = 0, \quad Sv(k, b) = 0 \quad \text{and} \quad Z^T a = Z^T b,$$

if and only if

$$\exists\, \mu \in \mathbb{R}^n \backslash \{0\}, z \in \mathbb{R}^n \quad \text{and} \quad \varkappa \in \mathcal{K} \text{ with} \tag{3.17a}$$

$$\mathcal{Y}^T \mu = \Pi\, \varkappa, \quad \tilde{U}^T \Pi\, \varkappa = 0, \quad Z^T z = 0, \quad \mathrm{sign}(z) = \mathrm{sign}(\mu). \tag{3.17b}$$

Proof From Lemma 2 follows that μ, ν, λ and $\varkappa$ with (3.17a) and (3.17b) are necessary and sufficient for the existence of positive a, b and k with $Sv(k, a) = 0$ and $Sv(k, b) = 0$. And by Lemma 3 the condition $\mathrm{sign}(\mu) = \mathrm{sign}(z)$ is necessary and sufficient for $Z^T a = Z^T b$. To see this recall first that $\mu = \ln \frac{b}{a}$ and observe that $Z^T a = Z^T b$ is equivalent to $Z^T (b - a) = 0$. Then identify M_1 with the solution set of the determining Eq. (3.5) and M_2 with $\ker(Z^T)$ and apply Lemma 3. □

Remark 7 (Computing steady states and rate constants) Given μ, $\varkappa$ and z satisfying (3.17a) and (3.17b), we define $a = (a_i)_{i=1,\dots,n}$ and b via

$$a_i = \begin{cases} \frac{z_i}{e^{\mu_i}-1}, & \text{if } \mu_i \neq 0 \\ \bar{a}_i > 0, & \text{arbitrary, if } \mu_i = 0, \end{cases} \tag{3.18a}$$

and

$$b = \operatorname{diag}(e^{\mu})\, a. \tag{3.18b}$$

By Definition 2 of $\mathcal{K}$, the condition $\varkappa \in \mathcal{K}$ implies the existence of $\nu, \lambda \in \Lambda^2(E)$ with $\varkappa = \psi(\nu, \lambda)$. Together with the vector a from Eq. (3.18a) such a vector λ defines a vector

$$k = \operatorname{diag}(\phi(a^{-1}))\, E\, \lambda \tag{3.18c}$$

of rate constants so that a, b and k fulfill the requirements of Theorem 1. □

Remark 8 In Definition 1 the columns of the matrix Z are a basis of the left kernel of the stoichiometric matrix S. Hence Theorem 1 establishes multistationarity with respect to the linear subspace $\operatorname{im}(S)$. This is the standard definition from Chemical Engineering. For Theorem 1, however, Z is just a matrix of appropriate dimension. Thus one can use Theorem 1 (and all results derived from it in the following sections) to establish multistationarity with respect to arbitrary linear subspaces (see e.g. Flockerzi and Conradi 2008). □

3.4 Multistationarity for network $\mathcal{N}_1$

Next we turn again to network $\mathcal{N}_1$. We will reexamine multistationarity in the light of the results obtained so far. For network $\mathcal{N}_1$ one has $\Lambda(E) \equiv \mathbb{R}^6_{>0}$. As described in Example 1 above, one obtains two functions

$$\begin{aligned} \psi_{J_1}(\nu, \lambda) &= \ln \frac{\nu_1}{\lambda_1} = \ln \frac{\nu_4}{\lambda_4} = \ln \frac{\nu_6}{\lambda_6} = \ln \frac{\nu_1 + \nu_6}{\lambda_1 + \lambda_6} = \ln \frac{\nu_4 + \nu_6}{\lambda_4 + \lambda_6}, \\ \psi_{J_2}(\nu, \lambda) &= \ln \frac{\nu_2}{\lambda_2} = \ln \frac{\nu_3}{\lambda_3} = \ln \frac{\nu_5}{\lambda_5} = \ln \frac{\nu_2 + \nu_5}{\lambda_2 + \lambda_5} = \ln \frac{\nu_3 + \nu_5}{\lambda_3 + \lambda_5}. \end{aligned}$$

We claim

Fact 3 *Let* $\psi(\nu, \lambda) = (\psi_{J_1}(\nu, \lambda), \psi_{J_2}(\nu, \lambda))^T$ *and let* $\varkappa \in \mathbb{R}^2$. *Then there exists* $\nu, \lambda \in \mathbb{R}^6_{>0}$ *such that* $\varkappa = \psi(\nu, \lambda)$.

Proof Let $\varkappa \in \mathbb{R}^2$. Pick $\lambda \in \mathbb{R}^6_{>0}$, arbitrary. Then $\nu = (e^{\varkappa_1}\lambda_1, e^{\varkappa_2}\lambda_2, e^{\varkappa_2}\lambda_3, e^{\varkappa_1}\lambda_4, e^{\varkappa_2}\lambda_5, e^{\varkappa_1}\lambda_6)^T$ gives $\varkappa = \psi(\nu, \lambda)$. □

From Theorem 1 follows that multistationarity in the sense of Definition 1 requires feasibility of (recall the definition of the matrix Π in Example 1 and Lemma 1)

$$\begin{aligned} \mathcal{Y}^T \mu = \Pi \varkappa, \quad Z^T z = 0, \quad \operatorname{sign}(\mu) = \operatorname{sign}(z) \\ \varkappa = \psi(\nu, \lambda), \quad (\nu, \lambda) \in \Lambda^2(E). \end{aligned}$$

Note that as a consequence of Fact 3 the vector $\varkappa \in \mathbb{R}^2$ is unconstrained (there is a *guarantee*, that, no matter what value $\varkappa$ takes, one will always find positive ν, λ that *realize* $\varkappa$). Thus we can *discard the nonlinear constraints* and solve instead the leftover linear system

$$\mathcal{Y}^T \mu = [\pi_1, \pi_2]\, \varkappa, \quad Z^T z = 0, \quad \operatorname{sign}(\mu) = \operatorname{sign}(z).$$

4 Multistationarity by analysis of linear inequality systems

First, we introduce some convenient notation: vectors v will be split in sub-vectors denoted by indices in brackets, that is we will use the notation $v = \operatorname{col}(v_{(1)}, \ldots, v_{(\omega)})$ to denote that v consists of ω sub-vectors $v_{(i)}$. If $\mathcal{A}^{(i,j)}$ are matrices of dimension $d_i \times d_j$, then $A = [\mathcal{A}^{(i,j)}]_{\substack{i=1,\ldots,p_1 \\ j=1,\ldots,p_2}}$ denotes the block matrix

$$A = \begin{bmatrix} \mathcal{A}^{(1,1)} & \mathcal{A}^{(1,2)} & \ldots & \mathcal{A}^{(1,p_2)} \\ \vdots & & & \vdots \\ \mathcal{A}^{(p_1,1)} & & \ldots & \mathcal{A}^{(p_1,p_2)} \end{bmatrix}$$

of block size $p_1 \times p_2$.

From hereon we will assume a certain ordering of the reactions in the network (2.4) so that the first $|J_1|$ rows of the generator matrix E are those indexed by cluster J_1, followed by the $|J_2|$ rows indexed by cluster J_2 and so on. Then we use the representation

$$E = \left[\begin{array}{c|c|c} \mathcal{E}^{(1,1)} & \mathcal{E}^{(1,2)} & \ldots \\ \hline \mathcal{E}^{(2,1)} & \mathcal{E}^{(2,2)} & \ldots \\ \hline \vdots & \vdots & \vdots \\ \hline \mathcal{E}^{(\gamma,1)} & \mathcal{E}^{(\gamma,2)} & \ldots \end{array}\right] \tag{4.1}$$

where each block matrix $\mathcal{E}^{(i,j)}$ contains $|J_i|$ rows and a certain number of columns. (See Example 2 for an explanation of how the number of columns is determined.) We split

$$\nu = \operatorname{col}(\nu_{(1)}, \nu_{(2)}, \ldots) \quad \text{and} \quad \lambda = \operatorname{col}(\lambda_{(1)}, \lambda_{(2),\ldots})$$

accordingly. We recall that this block structure of E is a structural property of the given network (2.4) since it is derived from the data $\ker_+(S)$ and $\mathcal{Y}$ by the induced clustering without passing to ambiguous coordinate choices.

We will consider situations where many blocks $\mathcal{E}^{(i,j)}$ are zero-blocks. Consider the rows n_j of E indexed by a particular cluster J_i and recall that these n_j define the function $\psi(\nu, \lambda)$. Denoting the *support* of a vector $v \in \mathbb{R}^p$ by

$$\mathrm{supp}(v) = \{\ell \in \{1, \ldots, p\} \mid v_\ell \neq 0\},$$

we define the union of the support of all row vectors n_j of E, $j \in J_i$, as

$$\mathcal{U}_i := \cup_{j \in J_i} \mathrm{supp}(n_j)$$

This leads to the following definition:

Definition 3 (Isolation and Bridging Property)

1. We say that a pair (ν_j, λ_j) has the *Isolation Property*, if it is used in exactly one function $\psi_{J_i}(\nu, \lambda)$, that is, there exists a unique set $\mathcal{U}_i$ with $j \in \mathcal{U}_i$.
2. We say that a pair (ν_j, λ_j) has the *Bridging Property*, if it is used in exactly two functions $\psi_{J_{i_1}}(\nu, \lambda)$ and $\psi_{J_{i_2}}(\nu, \lambda)$, that is, there are exactly two, distinct sets $\mathcal{U}_{i_1}$ and $\mathcal{U}_{i_2}$ with $j \in \mathcal{U}_{i_1}$ and $j \in \mathcal{U}_{i_2}$.

In network $\mathcal{N}_1$ all pairs have the Isolation property: each (ν_j, λ_j) is either used in $\psi_1(\nu, \lambda)$ or in $\psi_2(\nu, \lambda)$, but no (ν_i, λ_i) is used in both. In the sequel, we will consider systems of γ functions ψ_{J_i} where each pair (ν_j, λ_j) has either the Isolation or the Bridging Property. To illustrate the definitions and results, the following network $\mathcal{N}_2$ will be used:

$$\begin{aligned}
\mathbf{A} + \mathbf{E_1} &\underset{\mathbf{k_{14}}}{\overset{\mathbf{k_{13}}}{\rightleftharpoons}} \mathbf{A\,E_1} \xrightarrow{\mathbf{k_{15}}} \mathbf{A_p} + \mathbf{E_1} \underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} \mathbf{A_p\,E_1} \xrightarrow{\mathbf{k_3}} \mathbf{A_{pp}} + \mathbf{E_1} \\
\mathbf{A_{pp}} + \mathbf{E_2} &\underset{\mathbf{k_5}}{\overset{\mathbf{k_4}}{\rightleftharpoons}} \mathbf{A_{pp}\,E_2} \xrightarrow{\mathbf{k_6}} \mathbf{A_p} + \mathbf{E_2} \underset{\mathbf{k_{17}}}{\overset{\mathbf{k_{16}}}{\rightleftharpoons}} \mathbf{A_p\,E_2} \xrightarrow{\mathbf{k_{18}}} \mathbf{A} + \mathbf{E_2} \\
\mathbf{A_p} + \mathbf{E_3} &\underset{\mathbf{k_8}}{\overset{\mathbf{k_7}}{\rightleftharpoons}} \mathbf{A_p\,E_3} \xrightarrow{\mathbf{k_9}} \mathbf{A_{pp}} + \mathbf{E_3} \\
\mathbf{A_{pp}} + \mathbf{E_4} &\underset{\mathbf{k_{11}}}{\overset{\mathbf{k_{10}}}{\rightleftharpoons}} \mathbf{A_{pp}\,E_4} \xrightarrow{\mathbf{k_{12}}} \mathbf{A_p} + \mathbf{E_4}.
\end{aligned} \tag{$\mathcal{N}_2$}$$

Example 2 (The matrix E, the Isolation and Bridging property for network $\mathcal{N}_2$) For network $\mathcal{N}_2$, the ordering of species and complexes of Table 5 in Appendix A has been used. With the ordering of reactions displayed in $\mathcal{N}_2$ one obtains the following generator matrix E and kernel matrix U, where ϵ_i denote the unit vectors of $\mathbb{R}^{11}$ and e_i the unit vectors of $\mathbb{R}^{18}$.

$$\begin{aligned}
E^T = [&\epsilon_1 + \epsilon_3 + \epsilon_5,\ \epsilon_5,\ \epsilon_1 + \epsilon_3,\ \epsilon_3 + \epsilon_4 + \epsilon_6,\ \epsilon_6,\ \epsilon_3 + \epsilon_4, \\
&\epsilon_2 + \epsilon_4 + \epsilon_7,\ \epsilon_7,\ \epsilon_2 + \epsilon_4,\ \epsilon_1 + \epsilon_2 + \epsilon_8,\ \epsilon_8,\ \epsilon_1 + \epsilon_2, \\
&\epsilon_9 + \epsilon_{10},\ \epsilon_9,\ \epsilon_{10},\ \epsilon_{10} + \epsilon_{11},\ \epsilon_{11},\ \epsilon_{10}] \\
U = [&e_2 - e_3, e_5 - e_6, e_8 - e_9, e_{11} - e_{12}, e_{14} - e_{15}, e_{17} - e_{18}]\,.
\end{aligned}$$

For example, from $e_{14} - e_{15}, e_{17} - e_{18}$ one has

$$\frac{\epsilon_9^T\ \nu}{\epsilon_9^T\ \lambda} = \frac{\epsilon_{10}^T\,\nu}{\epsilon_{10}^T\,\lambda} \quad \text{and} \quad \frac{\epsilon_{11}^T\ \nu}{\epsilon_{11}^T\ \lambda} = \frac{\epsilon_{10}^T\,\nu}{\epsilon_{10}^T\,\lambda}.$$

This implies

$$\frac{(\epsilon_9 + \epsilon_{10})^T\ \nu}{(\epsilon_9 + \epsilon_{10})^T\ \lambda} = \frac{\epsilon_{10}^T\,\nu}{\epsilon_{10}^T\,\lambda} \quad \text{and} \quad \frac{(\epsilon_{10} + \epsilon_{11})^T\ \nu}{(\epsilon_{10} + \epsilon_{11})^T\ \lambda} = \frac{\epsilon_{10}^T\,\nu}{\epsilon_{10}^T\,\lambda}.$$

Hence one obtains sets

$$J_5 = \{13, 14, 15, 16, 17, 18\} \quad \text{and} \quad \mathcal{U}_5 = \{9,\ 10,\ 11\}$$

implying

$$\begin{aligned}\psi_{J_5}(\nu, \lambda) &= \ln \frac{\nu_{10} + \nu_9}{\lambda_{10} + \lambda_9} = \ln \frac{\nu_9}{\lambda_9} = \ln \frac{\nu_{10}}{\lambda_{10}} \\ &= \ln \frac{\nu_{10} + \nu_{11}}{\lambda_{10} + \lambda_{11}} = \ln \frac{\nu_{11}}{\lambda_{11}} = \ln \frac{\nu_{10}}{\lambda_{10}}.\end{aligned}$$

Similarly one has

$$\begin{aligned}\psi_{J_1}(\nu, \lambda) &= \ln \frac{\nu_1 + \nu_3 + \nu_5}{\lambda_1 + \lambda_3 + \lambda_5} = \ln \frac{\nu_5}{\lambda_5} = \ln \frac{\nu_1 + \nu_3}{\lambda_1 + \lambda_3} \\ \psi_{J_2}(\nu, \lambda) &= \ln \frac{\nu_3 + \nu_4 + \nu_6}{\lambda_3 + \lambda_4 + \lambda_6} = \ln \frac{\nu_6}{\lambda_6} = \ln \frac{\nu_3 + \nu_4}{\lambda_3 + \lambda_4} \\ \psi_{J_3}(\nu, \lambda) &= \ln \frac{\nu_2 + \nu_4 + \nu_7}{\lambda_2 + \lambda_4 + \lambda_7} = \ln \frac{\nu_7}{\lambda_7} = \ln \frac{\nu_2 + \nu_4}{\lambda_2 + \lambda_4} \\ \psi_{J_4}(\nu, \lambda) &= \ln \frac{\nu_1 + \nu_2 + \nu_8}{\lambda_1 + \lambda_2 + \lambda_8} = \ln \frac{\nu_8}{\lambda_8} = \ln \frac{\nu_1 + \nu_2}{\lambda_1 + \lambda_2}.\end{aligned}$$

Note that the pair (ν_1, λ_1) is only used in the pair (ψ_{J_1}, ψ_{J_4}) of functions ψ_{J_1} and ψ_{J_4}, (ν_2, λ_2) only in the pair (ψ_{J_3}, ψ_{J_4}), (ν_3, λ_3) only in the pair (ψ_{J_1}, ψ_{J_2}) and (ν_4, λ_4) only in the pair (ψ_{J_2}, ψ_{J_3}), whereas each of (ν_i, λ_i), $i = 5, \ldots, 11$, is used in a single function. That is, (ν_i, λ_i) have the Bridging Property for $i = 1, \ldots, 4$ and the Isolation Property for $i = 5, \ldots, 11$. As shown in Table 1, each pair (ν_i, λ_i) is either associated with a unique function or a unique pair of functions. A subvector is designated to the variables contained in each function and each pair of functions, respectively. Thus the columns of Table 1 define the following partition of ν, λ in subvectors:

$$\begin{aligned}\nu_{(i)} &= (\nu_i), \quad i = 1, \ldots, 8, \quad \nu_{(9)} = \text{col}(\nu_9,\ \nu_{10},\ \nu_{11}), \\ \lambda_{(i)} &= (\lambda_i), \quad i = 1, \ldots, 8, \quad \lambda_{(9)} = \text{col}(\lambda_9,\ \lambda_{10},\ \lambda_{11}),\end{aligned}$$

where the $\nu_{(i)}$ and $\lambda_{(i)}$ are scalar quantities for $i = 1, \ldots, 8$. □

Table 1 Pairs (ν_i, λ_i) and associated functions/pairs of functions

(ν_1, λ_1)	(ν_2, λ_2)	(ν_3, λ_3)	(ν_4, λ_4)	(ν_5, λ_5)	(ν_6, λ_6)	(ν_7, λ_7)	(ν_8, λ_8)	(ν_9, λ_9) (ν_{10}, λ_{10}) (ν_{11}, λ_{11})
ψ_{J_1}, ψ_{J_4}	ψ_{J_3}, ψ_{J_4}	ψ_{J_1}, ψ_{J_2}	ψ_{J_2}, ψ_{J_3}	ψ_{J_1}	ψ_{J_2}	ψ_{J_3}	ψ_{J_4}	ψ_{J_5}

We first consider systems like $\mathcal{N}_1$, where all (ν, λ) have the Isolation Property. First assume there is just one nonlinear constraint (i.e. $\gamma = 1$). In this case $\mathcal{K} \equiv \mathbb{R}$. To see this, fix $\varkappa \in \mathbb{R}$ and solve the equations

$$\varkappa = \psi(\nu, \lambda) = \ln \frac{n_j\, \nu}{n_j\, \lambda}, \quad \forall j \in J$$

for $(\nu, \lambda) \in \Lambda^2(E)$. Recall that the n_j are row vectors of the matrix E. Thus one has to solve

$$E\, \nu = e^{\varkappa}\, E\, \lambda \Leftrightarrow E\, (\nu - e^{\varkappa}\, \lambda) = 0$$

for $(\nu, \lambda) \in \Lambda^2(E)$. Let $\mathcal{C}$ be a basis for $\ker(E)$. Then the general solution can be given as

$$\nu = e^{\varkappa}\, \lambda + \mathcal{C}\, \beta,$$

where β is a vector of appropriate dimension. As we only have to show the existence of one pair $(\nu, \lambda) \in \Lambda^2(E)$, we can—w.l.o.g.—set $\beta = 0$. Observe that, due to positivity of $e^{\varkappa}$, one has $\operatorname{sign}(e^{\kappa}\, \lambda) = \operatorname{sign}(\lambda)$. Thus $\lambda \in \Lambda(E)$ implies $e^{\varkappa}\, \lambda \in \Lambda(E)$. Hence, for arbitrary $\varkappa$, the pair

$$(\nu = e^{\varkappa}\, \lambda, \lambda) \in \Lambda^2(E)$$

solves the equation $\varkappa = \psi(\nu, \lambda)$, which proves the claim $\mathcal{K} \equiv \mathbb{R}$. We can extend this to systems of γ functions with the Isolation Property:

Lemma 4 (Multistationarity for systems with the Isolation Property) *Consider a (bio)chemical reaction network with mass action kinetics and recall the objects given in Remark 6. Assume there are γ functions ψ_{J_i} and further assume that all (ν_i, λ_i) have the Isolation Property (cf. Definition 3). Then there exists $a, b \in \mathbb{R}^n_{>0}$, $a \neq b$ and $k \in \mathbb{R}^r_{>0}$ with*

$$S\, v(k,\, a) = 0, \quad S\, v(k,\, b) = 0 \quad \textit{and} \quad Z^T\, a = Z^T\, b$$

if and only if there exists $z, \mu \in \mathbb{R}^n$, $\mu \neq 0$ and $\varkappa \in \mathbb{R}^\gamma$ such that the following linear inequality system is feasible:

$$\mathcal{Y}^T\, \mu = \Pi\, \varkappa, \quad \tilde{U}^T\, \Pi\, \varkappa = 0, \quad Z^T\, z = 0, \quad \operatorname{sign}(\mu) = \operatorname{sign}(z).$$

Every solution $\mu, z, \varkappa$ defines a, b and k via (3.18a), (3.18b) and (3.18c), where $\lambda \in \Lambda(E)$ in (3.18c) is free.

Now we turn to the more general (mixed) case where each (ν_i, λ_i) either has the Isolation or the Bridging Property. We will split $\nu = \mathrm{col}(\nu_{(1)}, \ldots, \nu_{(\omega)})$ and $\lambda = \mathrm{col}(\lambda_{(1)}, \ldots, \lambda_{(\omega)})$ in sub-vectors $\nu_{(j)}, \lambda_{(j)}$, where all elements of $\nu_{(j)}, \lambda_{(j)}$ are used in the same function $\psi_{J_i}(\nu, \lambda)$ (in case all elements of $(\nu_{(j)}, \lambda_{(j)})$ have the Isolation Property) or the same pair of functions $\psi_{J_{i_1}}(\nu, \lambda)$ and $\psi_{J_{i_2}}(\nu, \lambda)$ (in case all elements of $(\nu_{(j)}, \lambda_{(j)})$ have the Bridging Property). Then E can be written as

$$E = [\mathcal{E}^{(i,j)}]_{\substack{i=1,\ldots,\gamma \\ j=1,\ldots,\omega}}$$

where the first index i identifies the function $\psi_{J_i}(\nu, \lambda)$ and the second index j identifies the sub-vectors $\nu_{(j)}$ and $\lambda_{(j)}$ ($\gamma = 5$ and $\omega = 9$ in Example 2). Observe that the assumption that every variable either has the Isolation or the Bridging Property implies that for a fixed j there are at most two nonzero block matrices $\mathcal{E}^{(i,j)}$. We demonstrate this procedure for network $\mathcal{N}_2$ in the following Example 3.

Example 3 (Matrix E for network $\mathcal{N}_2$—block structure induced by $\psi_{J_i}(\nu, \lambda)$)

The generator matrix E:

$$\left[\begin{array}{c|c|c|c|c|c|c|c|c} 1\,0\,1 & 0 & 1 & 0\,0\,0 & 0 & 0 & 0 & 0 & 0\,0\,0 \\ 0\,0\,0 & 0 & 1 & 0\,0\,0 & 0 & 0 & 0 & 0 & 0\,0\,0 \\ 1\,0\,1 & 0 & 0 & 0\,0\,0 & 0 & 0 & 0 & 0 & 0\,0\,0 \\ \hline 0\,0\,1 & 1 & 0 & 1\,0\,0 & 0 & 0 & 0 & 0 & 0\,0\,0 \\ 0\,0\,0 & 0 & 0 & 1\,0\,0 & 0 & 0 & 0 & 0 & 0\,0\,0 \\ 0\,0\,1 & 1 & 0 & 0\,0\,0 & 0 & 0 & 0 & 0 & 0\,0\,0 \\ \hline 0\,1\,0 & 1 & 0 & 0\,1\,0 & 0 & 0 & 1 & 0 & 0\,0\,0 \\ 0\,0\,0 & 0 & 0 & 0\,1\,0 & 0 & 0 & 0 & 0 & 0\,0\,0 \\ 0\,1\,0 & 1 & 0 & 0\,0\,0 & 0 & 0 & 0 & 0 & 0\,0\,0 \\ \hline 1\,1\,0 & 0 & 0 & 0\,0\,0 & 0 & 1 & 0 & 0 & 0\,0\,0 \\ 0\,0\,0 & 0 & 0 & 0\,0\,0 & 0 & 1 & 0 & 0 & 0\,0\,0 \\ 1\,1\,0 & 0 & 0 & 0\,0\,0 & 0 & 0 & 0 & 0 & 0\,0\,0 \\ \hline 0\,0\,0 & 0 & 0 & 0\,0\,0 & 0 & 0 & 0 & 1 & 1\,1\,0 \\ 0\,0\,0 & 0 & 0 & 0\,0\,0 & 0 & 0 & 0 & 1 & 0\,0\,0 \\ 0\,0\,0 & 0 & 0 & 0\,0\,0 & 0 & 0 & 0 & 0 & 0\,1\,0 \\ 0\,0\,0 & 0 & 0 & 0\,0\,0 & 0 & 0 & 0 & 0 & 0\,1\,1 \\ 0\,0\,0 & 0 & 0 & 0\,0\,0 & 0 & 0 & 0 & 0 & 0\,0\,1 \\ 0\,0\,0 & 0 & 0 & 0\,0\,0 & 0 & 0 & 0 & 0 & 0\,1\,0 \end{array}\right]$$

Block partition of E

$$\left[\begin{array}{c|c|c|c|c|c|c|c|c} \mathcal{E}^{(1,1)} & 0 & \mathcal{E}^{(1,3)} & 0 & \mathcal{E}^{(1,5)} & 0 & 0 & 0 & 0 \\ \hline 0 & 0 & \mathcal{E}^{(2,3)} & \mathcal{E}^{(2,4)} & 0 & \mathcal{E}^{(2,6)} & 0 & 0 & 0 \\ \hline 0 & \mathcal{E}^{(3,2)} & 0 & \mathcal{E}^{(3,4)} & 0 & 0 & \mathcal{E}^{(3,7)} & 0 & 0 \\ \hline \mathcal{E}^{(4,1)} & \mathcal{E}^{(4,2)} & 0 & 0 & 0 & 0 & 0 & \mathcal{E}^{(4,8)} & 0 \\ \hline 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \mathcal{E}^{(5,9)} \end{array}\right]$$

$$\mathcal{E}^{(5,9)} = \begin{bmatrix} 1 & 1 & 0 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 1 & 1 \\ 0 & 0 & 1 \\ 0 & 1 & 0 \end{bmatrix}$$

$$\mathcal{E}^{(i,j)} = \begin{bmatrix} 1 \\ 1 \\ 0 \end{bmatrix}, \quad (i,j) \in \{(1,5),(2,6),(3,7),(4,8)\}$$

$$\mathcal{E}^{(i,j)} = \begin{bmatrix} 1 \\ 0 \\ 1 \end{bmatrix}, \quad (i,j) \in \left\{ \begin{array}{c} (1,1),(1,3),(2,3),(2,4), \\ (3,2),(3,4)(4,1),(4,2) \end{array} \right\}$$

So, the generator matrix E is of block size 5×9 and the first 4 block columns correspond to the Bridging Property. □

Observe that after a reordering of columns one may assume that the first $\bar{\omega}$ sub-vectors $\nu_{(j)}, \lambda_{(j)}$ correspond to the Bridging Property, while all sub-vectors $\nu_{(j)}, \lambda_{(j)}$ with $j > \bar{\omega}$ correspond to the Isolation Property ($\bar{\omega} = 4$ in Example 3).

Assumption 3 (*Ordering of* ν, λ) Let $\nu = \mathrm{col}(\nu_{(1)}, \ldots, \nu_{(\omega)})$, $\lambda = \mathrm{col}(\lambda_{(1)}, \ldots, \lambda_{(\omega)})$ be ordered such that

- all entries of the subvectors $(\nu_{(j)}, \lambda_{(j)})$ have the Bridging Property, if $j \in 1, \ldots, \bar{\omega}$
- all entries of the subvectors $(\nu_{(j)}, \lambda_{(j)})$ have the Isolation Property, if $j \in \bar{\omega} + 1, \ldots, \omega$.

In the following we will construct new matrices from block matrices like $\mathcal{E}^{(i,j)}$ whereby we will make use of the following notations:

Let $A^{(i,j_1)}, A^{(i,j_2)}, \ldots$ be block matrices and let $J = \{j_1, j_2, \ldots\}$. Then we define

$$[\ldots\ A^{(i,j)}\ \ldots]_{j\in J} := \left[\, A^{(i,j_1)} \middle| A^{(i,j_2)} \middle| \ldots \right], \tag{4.2a}$$

$$\begin{bmatrix} \vdots \\ A^{(i,j)} \\ \vdots \end{bmatrix}_{j\in J} := \left[\begin{array}{c} A^{(i,j_1)} \\ \hline A^{(i,j_2)} \\ \hline \vdots \end{array}\right]. \tag{4.2b}$$

For each function $\psi_{J_i}(\nu, \lambda)$ and hence each block row we collect the indices of the nonzero blocks in the set

$$\mathcal{M}_i := \{j \in \{1, \ldots, \omega\} | \mathcal{E}^{(i,j)} \neq \mathbf{0}\}$$

To establish $\varkappa = \psi(\nu, \lambda)$ (i.e. $\varkappa_i = \psi_{J_i}(\nu, \lambda), i = 1, \ldots, \gamma$) one has to solve

$$[\ldots\ \mathcal{E}^{(i,j)}\ \ldots]_{j\in\mathcal{M}_i}\ (\nu_{(j)} - e^{\varkappa_i}\ \lambda_{(j)})_{j\in\mathcal{M}_i} = 0. \tag{4.3a}$$

We compute a basis of $\ker([\mathcal{E}^{(i,j)}]_{j\in\mathcal{M}_i})$ and partition it according to the size of the sub-vectors $\nu_{(j)}, \lambda_{(j)}$:

$$\ker([\mathcal{E}^{(i,j)}]_{j\in\mathcal{M}_i}) = \begin{bmatrix} \vdots \\ \mathcal{C}^{(i,j)} \\ \vdots \end{bmatrix}_{j\in\mathcal{M}_i} \tag{4.3b}$$

with blocks $\mathcal{C}^{(i,j)}$ of appropriate dimensions. In case $[\ldots\ \mathcal{E}^{(i,j)}\ \ldots]_{j\in\mathcal{M}_i}$ has full column rank, we set $\mathcal{C}^{(i,j)}$ equal to the zero vector 0. Then Eq. (4.3a) is equivalent to

$$\nu_{(j)} - e^{\varkappa_i}\ \lambda_{(j)} = \mathcal{C}^{(i,j)}\ \beta_{(i)}, \quad j \in \mathcal{M}_i, \tag{4.3c}$$

where the $\beta_{(i)}$ are vectors of appropriate length. For each pair of sub-vectors $\nu_{(j)}, \lambda_{(j)}$ we collect the indices of the (at most two) nonzero blocks in triplets $(i_1(j), i_2(j), j)$ where we set $i_1(j) = i_2(j)$, if $(\nu_{(j)}, \lambda_{(j)})$ has the Isolation Property. We collect all triplets in the set

$$\mathcal{M}^C := \{(i_1(j), i_2(j), j) | \mathcal{E}^{(i_1(j),j)} \neq \mathbf{0}, \mathcal{E}^{(i_2(j),j)} \neq \mathbf{0}\}. \tag{4.4}$$

Fact 4 *With the triplets* $(i_1(j), i_2(j), j) \in \mathcal{M}^C$ *and with the abbreviations*

$$x_{(j)} := \mathcal{C}^{(i_2(j),j)}\, \beta_{(i_2(j))}, \quad y_{(j)} := \mathcal{C}^{(i_1(j),j)}\, \beta_{(i_1(j))} \tag{4.5a}$$

the equations $\varkappa = \psi(\nu, \lambda)$ *can be reformulated as follows*:

– *if* $j = 1, \ldots, \bar{\omega}$:

$$\left(e^{\varkappa_{i_1(j)}} - e^{\varkappa_{i_2(j)}}\right) \lambda_{(j)} = x_{(j)} - y_{(j)} \tag{4.5b}$$

$$\nu_{(j)} = e^{\varkappa_{i_1(j)}}\, \lambda_{(j)} + y_{(j)}. \tag{4.5c}$$

– *if* $j = \bar{\omega} + 1, \ldots, \omega$:

$$\nu_{(j)} = e^{\varkappa_{i_2(j)}}\, \lambda_{(j)} + x_{(j)}, \quad \lambda_{(j)}\ \textit{free}. \tag{4.5d}$$

□

Recall that, by assumption, every $\nu_{(j)}, \lambda_{(j)}$ is involved in at most two equations $\nu_{(j)} - e^{\varkappa_{i_1(j)}}\, \lambda_{(j)} = y_{(j)}$, $\nu_{(j)} - e^{\varkappa_{i_2(j)}}\, \lambda_{(j)} = x_{(j)}$. This motivates the following result concerning the solvability of $\varkappa = \psi(\nu, \lambda)$ under our standing Assumptions 1–3.

Lemma 5 (Solvability of $\varkappa = \psi(\nu, \lambda)$ for general (ν, λ)) *Assume that each* (ν_i, λ_i) *has either the Isolation or the Bridging Property and recall the set* $\mathcal{M}^C$ *and the matrices* $\mathcal{C}^{(i,j)}$ *[cf. Eq. (4.4) and (4.3b)]. Fix (a numerical value of)* $\varkappa \in \mathbb{R}^\gamma$. *Then the equation* $\varkappa = \psi(\nu, \lambda)$ *has (not necessarily nonnegative) solutions* $\nu, \lambda \in \mathbb{R}^p$ *if and only if the following linear system is feasible:*

$$0 = x_{(j)} - y_{(j)}, \quad \forall j = 1, \ldots, \omega \textit{ with } (i_1(j), i_2(j), j) \in \mathcal{M}^C \textit{ and } \varkappa_{i_1(j)} = \varkappa_{i_2(j)}. \tag{4.6}$$

In case (4.6) *is feasible,* ν *and* λ *are given by*

– $j = 1, \ldots, \bar{\omega}$ *and* $\varkappa_{i_1(j)} \neq \varkappa_{i_2(j)}$:

$$\rho_j := e^{\varkappa_{i_1(j)} - \varkappa_{i_2(j)}} \tag{4.7a}$$

$$\nu_{(j)} = \frac{e^{\varkappa_{i_2(j)}}}{e^{\varkappa_{i_1(j)}} - e^{\varkappa_{i_2(j)}}} \left[\rho_j\, x_{(j)} - y_{(j)}\right] \tag{4.7b}$$

$$\lambda_{(j)} = \frac{1}{e^{\varkappa_{i_1(j)}} - e^{\varkappa_{i_2(j)}}} \left[\quad x_{(j)} - y_{(j)}\right]. \tag{4.7c}$$

– $j = 1, \ldots, \bar{\omega}$ *and* $\varkappa_{i_1(j)} = \varkappa_{i_2(j)}$:

$$\nu_{(j)} = e^{\varkappa_{i_2(j)}}\, \lambda_{(j)} + x_{(j)}, \quad \lambda_{(j)}\ \textit{free}. \tag{4.7d}$$

$$0 = x_{(j)} - y_{(j)} \quad \forall (i_1(j), i_2(j), j) \in \mathcal{M}^C \textit{ with } \varkappa_{i_1(j)} = \varkappa_{i_2(j)}. \tag{4.7e}$$

Table 2 Solutions of $\varkappa = \Psi(\nu, \lambda)$ for general $\varkappa$

$\varkappa_{i_1(j)} - \varkappa_{i_2(j)}$	$x_{(j)} - y_{(j)}$	$\rho_j\, x_{(j)} - y_{(j)}$	Solution $\nu_{(j)}, \lambda_{(j)}$
$= 0$	$= 0$	$= 0$	$\lambda_{(j)}$ free, $\nu_{(j)} = e^{\varkappa_{i_2(j)}}\, \lambda_{(j)} + y_{(j)}$
$= 0$	$= 0$	$\neq 0$	Impossible as $\varkappa_{i_1(j)-\varkappa_{i_2(j)}=0}$ and $x_{(j)} - y_{(j)} = 0$ imply $\rho_j\, x_{(j)} - y_{(j)} = 0$
$= 0$	$\neq 0$	$= 0$	Not solvable
$= 0$	$\neq 0$	$\neq 0$	Not solvable
$\neq 0$	$= 0$	$= 0$	$\nu_{(j)} = 0, \lambda_{(j)} = 0$ as $\varkappa_{i_1(j)} - \varkappa_{i_2(j)} \neq 0$, $x_{(j)} - y_{(j)} = 0$ and $\rho_j\, x_{(j)} - y_{(j)} = 0$ imply $x_{(j)} = 0$ and $y_{(j)} = 0$
$\neq 0$	$= 0$	$\neq 0$	$\nu_{(j)} = y_{(j)},\ \lambda_{(j)} = 0$
$\neq 0$	$\neq 0$	$= 0$	$\nu_{(j)} = 0,\ \lambda_{(j)} = -e^{-\varkappa_{i_2(j)}}\, x_{(j)}$
$\neq 0$	$\neq 0$	$\neq 0$	$\rho_j := e^{\varkappa_{i_1(j)} - \varkappa_{i_2(j)}}$ $\nu_{(j)} = \frac{e^{\varkappa_{i_2(j)}}}{e^{\varkappa_{i_1(j)}} - e^{\varkappa_{i_2(j)}}} [\rho_j\, x_{(j)} - y_{(j)}]$ $\lambda_{(j)} = \frac{1}{e^{\varkappa_{i_1(j)}} - e^{\varkappa_{i_2(j)}}} [x_{(j)} - y_{(j)}]$

– $j = \bar{\omega} + 1, \ldots, \omega$:

$$\nu_{(j)} = e^{\varkappa_{i_2(j)}}\, \lambda_{(j)} + x_{(j)}, \quad \lambda_{(j)}\ \textit{free}. \tag{4.7f}$$

Proof Observe that for $j = \bar{\omega} + 1, \ldots, \omega$ one always has solution (4.7f). Hence we focus on $j = 1, \ldots, \bar{\omega}$ and Eqs. (4.5b) and (4.5c). Table 2 gives the solutions of $\varkappa = \psi(\nu, \lambda)$ in dependence of $\varkappa$. From this table follows that the equation $\varkappa = \psi(\nu, \lambda)$ has solutions ν, λ unless (i) $\varkappa_{i_1(j)} - \varkappa_{i_2(j)} = 0$ and $x_{(j)} - y_{(j)} \neq 0$ or (ii) $\rho_j\, x_{(j)} - y_{(j)} \neq 0$. This proves the claim.

Remark 9 (Solutions (ν, λ) with vanishing components) For a given $\varkappa \in \mathbb{R}^\gamma$ one has the following boundary solutions (cf. Table 2):

$$\nu_{(j)} = \lambda_{(j)} = 0, \tag{4.8a}$$

where, on the one hand, $x_{(j)} = y_{(j)} = 0$ is necessary and, on the other hand, $x_{(j)} = y_{(j)} = 0$ *and* $\varkappa_{i_1(j)} - \varkappa_{i_2(j)} \neq 0$ is sufficient for (4.8a);

$$\nu_{(j)} = y_{(j)}, \quad \lambda_{(j)} = 0, \tag{4.8b}$$

where, on the one hand $x_{(j)} = y_{(j)} \neq 0$ is necessary and, on the other hand, $x_{(j)} = y_{(j)} \neq 0$ *and* $\varkappa_{i_1(j)} - \varkappa_{i_2(j)} \neq 0$ is sufficient for (4.8b);

$$\nu_{(j)} = 0, \quad \lambda_{(j)} = -e^{-\bar{\varkappa}_{i_2(j)}}\, x_{(j)}, \tag{4.8c}$$

where, on the one hand $\rho_j\, x_{(j)} = y_{(j)} \neq 0$ is necessary and, on the other hand, and $\rho_j\, x_{(j)} = y_{(j)} \neq 0$ *and* $\varkappa_{i_1(j)} - \varkappa_{i_2(j)} \neq 0$ is sufficient for (4.8c). □

Example 4 (Computation of v, λ for network $\mathcal{N}_2$)
For $i = 1$ one has $\mathcal{M}_1 = \{1, 3, 5\}$. Hence

$$[\mathcal{E}^{(i,j)}]_{j\in\mathcal{M}_i}\,(v_{(j)} - e^{\varkappa_i}\,\lambda_{(j)})_{j\in\mathcal{M}_i} = 0$$

reads

$$\begin{bmatrix} 1 & 1 & 1 \\ 0 & 0 & 1 \\ 1 & 1 & 0 \end{bmatrix} \begin{pmatrix} v_{(1)} - e^{\varkappa_1}\,\lambda_{(1)} \\ v_{(3)} - e^{\varkappa_1}\,\lambda_{(3)} \\ v_{(5)} - e^{\varkappa_1}\,\lambda_{(5)} \end{pmatrix} = 0 \Leftrightarrow \begin{pmatrix} v_{(1)} - e^{\varkappa_1}\,\lambda_{(1)} \\ v_{(3)} - e^{\varkappa_1}\,\lambda_{(3)} \\ v_{(5)} - e^{\varkappa_1}\,\lambda_{(5)} \end{pmatrix} = \beta_1 \begin{pmatrix} -1 \\ 1 \\ 0 \end{pmatrix}.$$

Similarly:

$$\begin{pmatrix} v_{(3)} - e^{\varkappa_2}\,\lambda_{(3)} \\ v_{(4)} - e^{\varkappa_2}\,\lambda_{(4)} \\ v_{(6)} - e^{\varkappa_2}\,\lambda_{(6)} \end{pmatrix} = \beta_2 \begin{pmatrix} -1 \\ 1 \\ 0 \end{pmatrix}, \quad \begin{pmatrix} v_{(2)} - e^{\varkappa_3}\,\lambda_{(2)} \\ v_{(4)} - e^{\varkappa_3}\,\lambda_{(4)} \\ v_{(7)} - e^{\varkappa_3}\,\lambda_{(7)} \end{pmatrix} = \beta_3 \begin{pmatrix} -1 \\ 1 \\ 0 \end{pmatrix},$$
$$\begin{pmatrix} v_{(1)} - e^{\varkappa_4}\,\lambda_{(1)} \\ v_{(2)} - e^{\varkappa_4}\,\lambda_{(2)} \\ v_{(8)} - e^{\varkappa_4}\,\lambda_{(8)} \end{pmatrix} = \beta_4 \begin{pmatrix} -1 \\ 1 \\ 0 \end{pmatrix}.$$

Note that $\mathcal{E}^{(5,9)}$ has full column rank. Hence $v_{(9)} = e^{\varkappa_5}\,\lambda_{(9)}$. One also obtains

$$v_{(5)} = e^{\varkappa_1}\,\lambda_{(5)}, \quad v_{(6)} = e^{\varkappa_2}\,\lambda_{(6)}, \quad v_{(7)} = e^{\varkappa_3}\,\lambda_{(7)}, \quad v_{(8)} = e^{\kappa_4}\,\lambda_{(8)}.$$

For the remaining $v_{(i)}, \lambda_{(i)}$ one obtains:

$$\begin{aligned} v_{(1)} &= e^{\varkappa_1}\,\lambda_{(1)} - \beta_1 \quad v_{(2)} = e^{\varkappa_3}\,\lambda_{(2)} - \beta_3 \\ v_{(3)} &= e^{\varkappa_1}\,\lambda_{(3)} + \beta_1 \quad v_{(4)} = e^{\varkappa_2}\,\lambda_{(4)} + \beta_2 \end{aligned}$$

with $(y_{(1)}, y_{(2)}, y_{(3)}, y_{(4)}) = (\beta_1, \beta_3, -\beta_1, -\beta_2)$ and

$$\begin{aligned} (e^{\varkappa_1} - e^{\varkappa_4})\,\lambda_{(1)} &= -\beta_4 - (-\beta_1) \quad (e^{\varkappa_3} - e^{\varkappa_4})\,\lambda_{(2)} = \beta_4 - (-\beta_3) \\ (e^{\varkappa_1} - e^{\varkappa_2})\,\lambda_{(3)} &= -\beta_2 - (\beta_1) \quad (e^{\varkappa_2} - e^{\varkappa_3})\,\lambda_{(4)} = \beta_3 - (\beta_2) \end{aligned}$$

with $(x_{(1)}, x_{(2)}, x_{(3)}, x_{(4)}) = (-\beta_4, \beta_4, -\beta_2, \beta_3)$. For $\varkappa_1 \neq \varkappa_4, \varkappa_3 \neq \varkappa_4, \varkappa_1 \neq \varkappa_2$ and $\varkappa_2 \neq \varkappa_3$ one has

$$\begin{aligned} v_{(1)} &= \frac{e^{\varkappa_4}}{e^{\varkappa_1} - e^{\varkappa_4}}\,[-e^{\varkappa_1-\varkappa_4}\,\beta_4 + \beta_1], \quad v_{(2)} = \frac{e^{\varkappa_4}}{e^{\varkappa_3} - e^{\varkappa_4}}\,[e^{\varkappa_3-\varkappa_4}\,\beta_4 + \beta_3], \\ v_{(3)} &= \frac{e^{\varkappa_2}}{e^{\varkappa_1} - e^{\varkappa_2}}\,[-e^{\varkappa_1-\varkappa_2}\,\beta_2 - \beta_1], \quad v_{(4)} = \frac{e^{\varkappa_3}}{e^{\varkappa_2} - e^{\varkappa_3}}\,[e^{\varkappa_2-\varkappa_3}\,\beta_3 - \beta_2]. \end{aligned}$$

□

Lemma 5 addresses the solvability of $\varkappa = \psi(\nu, \lambda)$ for a given numerical value $\varkappa \in \mathbb{R}^\gamma$ in an extended set-up. We now turn to the realization problem $\varkappa \in \mathcal{K}$ which asks for a solution $(\nu, \lambda) \in \Lambda^2(E)$, in particular for a solution (ν, λ) with $\nu_{(j)} \gtrsim 0,\ \lambda_{(j)} \gtrsim 0$. First observe that if $(\nu_{(j)}, \lambda_{(j)})$ has the Isolation Property, then $\lambda_{(j)}$ is free by (4.5d). Hence, for any given $\beta_{(i_2(j))}$ one can choose $\lambda_{(j)} > 0$ such that $\nu_{(j)}$ is positive as well. Hence, in Fact 4, we need not worry about $\nu_{(j)}, \lambda_{(j)}$, $j = \bar{\omega}+1, \ldots, \omega$.

For $j = 1, \ldots, \bar{\omega}$ we collect all *coupling conditions* (4.5b) of Fact 4 in a *coupling matrix*:

$$\mathcal{C}_\# = \begin{bmatrix} \vdots \\ \mathcal{C}_\#^{(j)} \\ \vdots \end{bmatrix}_{j=1,\ldots,\bar{\omega}} \tag{4.9a}$$

with

$$\mathcal{C}_\#^{(j)} = [\mathbf{0}, \ldots, -\mathcal{C}^{(i_1(j),j)}, \mathbf{0}, \ldots, \mathcal{C}^{(i_2(j),j)}, \mathbf{0}, \ldots]. \tag{4.9b}$$

$\mathcal{C}_\#$ is thus of block size $\bar{\omega} \times \gamma$. We write $\beta = (\beta_{(i)})_{i=1,\ldots,\gamma}$ accordingly (recall that to every function ψ_{J_i} there is associated a $\beta_{(i)}$). Moreover we observe that $\mathcal{C}_\#^{(\cdot,i)}$ is a block column consisting of zeros if a function ψ_{J_i} contains only variables with the Isolation Property or if $[\mathcal{E}^{(i,j)}]_{j\in\mathcal{M}_i}$ has full column rank. Observe that $\mathcal{C}_\#$ may contain zero block rows. In this case one has either $\varkappa_{J_{i_1}} - \varkappa_{J_{i_2}} = 0$ or $\nu_{(j)} = \lambda_{(j)} = 0$.

Example 5 (Coupling matrix for network $\mathcal{N}_2$)

$$\mathcal{C}_\# = \begin{bmatrix} 1 & 0 & 0 & -1 & 0 \\ 0 & 0 & 1 & 1 & 0 \\ -1 & -1 & 0 & 0 & 0 \\ 0 & -1 & 1 & 0 & 0 \end{bmatrix}, \quad \beta = \operatorname{col}(\beta_1,\ \beta_2,\ \beta_3,\ \beta_4,\ \beta_5)\,.$$

Observe that ψ_{J_5} contains only variables with the Isolation Property and that $\mathcal{E}^{(5,9)}$ has full column rank. Hence the fifth column $\mathcal{C}_\#^{(\cdot,5)}$ contains only zeros (i.e. a block column of zeros consisting of a single column). □

By the expressions in (4.7a)–(4.7c), we are interested in vectors $\xi = \mathcal{C}_\#\,\beta \in \operatorname{im}(\mathcal{C}_\#)$, in particular in their sign structure. We split such ξ according to the rows of the $\mathcal{C}_\#^{(j)}$ to obtain $\xi = \operatorname{col}(\xi_{(1)}, \ldots, \xi_{(\bar{\omega})})$ with the decomposition [cf. (4.5a)]

$$\xi_{(j)} = \mathcal{C}^{(i_2(j),j)}\,\beta_{(i_2(j))} - \mathcal{C}^{(i_1(j),j)}\,\beta_{(i_1(j))} = x_{(j)} - y_{(j)}. \tag{4.10}$$

The following simple but convenient fact illustrates the consequences of the decomposition (4.10) on the nonnegativity requirement $\nu_{(j)} \gtrsim 0$ and $\lambda_{(j)} \gtrsim 0$.

Fact 5 (Introducing $\bar{\rho}_j$ and $\underline{\rho}_j$) *We consider* $(\nu_{(j)}, \lambda_{(j)})$ *with the Bridging Property and take* $\varkappa_{i_1(j)} \neq \varkappa_{i_2(j)}$. *Then* (4.7c) *implies that* $\lambda_{(j)} \gtrsim 0$ *requires either* $\varkappa_{i_1(j)} -$

$\varkappa_{i_2(j)} > 0$ and $\xi_{(j)} \gtrsim 0$ or $\varkappa_{i_1(j)} - \varkappa_{i_2(j)} < 0$ and $\xi_{(j)} \lesssim 0$. First, we consider the case $\varkappa_{i_1(j)} - \varkappa_{i_2(j)} > 0$ and $\xi_{(j)} = x_{(j)} - y_{(j)} \gtrsim 0$ with $\rho_j > 1$ [cf. (4.7a)]. Then $v_{(j)} \gtrsim 0$ is equivalent to $\rho_j\, x_{(j)} - y_{(j)} \gtrsim 0$. With the definitions

$$\bar{\rho}_j := \begin{cases} \min_l \frac{y_{(j)}^{(l)}}{x_{(j)}^{(l)}} & \forall l \text{ with } x_{(j)}^{(l)} < 0 \\ \infty & \text{if } x_{(j)} \geq 0. \end{cases} \tag{4.11a}$$

$$\underline{\rho}_j := \begin{cases} \max_l \frac{y_{(j)}^{(l)}}{x_{(j)}^{(l)}} & \forall l \text{ with } y_{(j)}^{(l)} < 0 \\ 0 & \text{if } y_{(j)} \geq 0. \end{cases} \tag{4.11b}$$

one has $\rho\, x_{(j)} - y_{(j)} \gtrsim 0$ if and only if $\rho \leq \bar{\rho}_j$. Because of $x_{(j)} - y_{(j)} \gtrsim 0$, one necessarily has $\bar{\rho}_j \geq 1$. We point out that $\bar{\rho}_j = 1$ if and only if there exists a negative $x_{(j)}^{(l)}$ that equals $y_{(j)}^{(l)}$. In other words, for $\xi_{(j)} = x_{(j)} - y_{(j)} \gtrsim 0$ one has

$$\bar{\rho}_j > 1 \quad \text{if and only if } \xi_{(j)}^{(l)} = 0 \Rightarrow x_{(j)}^{(l)} \geq 0. \tag{4.11c}$$

If $\rho_j = e^{\varkappa_{i_1(j)} - \varkappa_{i_2(j)}}$ can be chosen in $(1, \bar{\rho}_j)$ then $v_{(j)} \gtrsim 0$ in case $\lambda_{(j)} \gtrsim 0$. In case of strict positivity $\xi_{(j)} > 0$ one always can choose a $\rho_j > 1$ with $\lambda_{(j)} > 0$ and $v_{(j)} > 0$. Secondly, we treat the case $\varkappa_{i_1(j)} - \varkappa_{i_2(j)} < 0$ and $\xi_{(j)} = x_{(j)} - y_{(j)} \lesssim 0$ with $\rho_j \in (0, 1)$ [cf. (4.7a)] in complete analogy. One has $\rho\, x_{(j)} - y_{(j)} \lesssim 0$ if and only if $\rho \geq \underline{\rho}_j$. Note that for $\xi_{(j)} = x_{(j)} - y_{(j)} \lesssim 0$ one has

$$\underline{\rho}_j < 1 \quad \text{if and only if } \xi_{(j)}^{(l)} = 0 \Rightarrow y_{(j)}^{(l)} \geq 0. \tag{4.11d}$$

If $\rho_j = e^{\varkappa_{i_1(j)} - \varkappa_{i_2(j)}}$ can be chosen in $(\underline{\rho}_j, 1)$ then $v_{(j)} \gtrsim 0$ in case $\lambda_{(j)} \gtrsim 0$. In case of strict negativity $\xi_{(j)} < 0$ one always can choose a $\rho_j \in (0, 1)$ with $\lambda_{(j)} > 0$ and $v_{(j)} > 0$. Hence, the $\bar{\rho}_j$ and $\underline{\rho}_j$ are optimal values of certain fractional linear programs (see e.g. Frenk and Schaible 2004). □

Example 6 (Computation of positive v and λ for a given β for network $\mathcal{N}_2$) Consider $\beta = (6, -4, -2, -2)^T$. Then, by Example 4, one has

$$\begin{aligned}
v_{(1)} &= \frac{e^{\varkappa_4}}{e^{\varkappa_1} - e^{\varkappa_4}} \left[e^{\varkappa_1 - \varkappa_4}\, 2 + 6 \right] \quad \lambda_{(1)} = \frac{2+6}{e^{\varkappa_1} - e^{\varkappa_4}} \\
v_{(2)} &= \frac{e^{\varkappa_4}}{e^{\varkappa_3} - e^{\varkappa_4}} \left[e^{\varkappa_3 - \varkappa_4}\, (-2) - 2 \right] \quad \lambda_{(2)} = \frac{-2-2}{e^{\varkappa_3} - e^{\varkappa_4}} \\
v_{(3)} &= \frac{e^{\varkappa_2}}{e^{\varkappa_1} - e^{\varkappa_2}} \left[e^{\varkappa_1 - \varkappa_2}\, 4 - 6 \right] \quad \lambda_{(3)} = \frac{4-6}{e^{\varkappa_1} - e^{\varkappa_2}} \\
v_{(4)} &= \frac{e^{\varkappa_3}}{e^{\varkappa_2} - e^{\varkappa_3}} \left[e^{\varkappa_2 - \varkappa_3}\, (-2) + 4 \right] \quad \lambda_{(4)} = \frac{-2+4}{e^{\varkappa_2} - e^{\varkappa_3}}
\end{aligned}$$

Clearly $\lambda_{(1)} > 0$ iff $\varkappa_1 - \varkappa_4 > 0$, $\lambda_{(2)} > 0$ iff $\varkappa_3 - \varkappa_4 < 0$, $\lambda_{(3)} > 0$ iff $\varkappa_1 - \varkappa_2 < 0$ and $\lambda_{(4)} > 0$ iff $\varkappa_2 - \varkappa_3 > 0$. Observe that $\lambda_{(j)} > 0\, (j = 1, \ldots, 4)$ implies $v_{(j)} >$

$0\,(j = 1, 2, 3)$ and, in addition, $\nu_{(4)} > 0$ iff $\varkappa_2 - \varkappa_3 < \ln 2 =: \bar{\rho}_4$. So, for the above chosen β, one has positive λ, ν if $\varkappa$ satisfies

$$\begin{bmatrix} 1 & 0 & 0 & -1 \\ 0 & 0 & -1 & 1 \\ -1 & 1 & 0 & 0 \\ 0 & 1 & -1 & 0 \end{bmatrix} \begin{pmatrix} \varkappa_1 \\ \varkappa_2 \\ \varkappa_3 \\ \varkappa_4 \end{pmatrix} > 0 \quad \text{and} \quad \begin{bmatrix} 1 & 0 & 0 & -1 \\ 0 & 0 & -1 & 1 \\ -1 & 1 & 0 & 0 \\ 0 & 1 & -1 & 0 \end{bmatrix} \begin{pmatrix} \varkappa_1 \\ \varkappa_2 \\ \varkappa_3 \\ \varkappa_4 \end{pmatrix} < \begin{pmatrix} \infty \\ \infty \\ \infty \\ \ln 2 \end{pmatrix}$$

where the first inequality ensures $\lambda > 0$ and the subsequent linear inequality $\nu > 0$ so that one arrives at $(\nu, \lambda) \in \Lambda^2(E)$.

So, given the coupling matrix $\mathcal{C}_\#$ and the decomposition of $\xi = \mathcal{C}_\#\beta$ from (4.10), the condition $\nu > 0, \lambda > 0$ constrains the vector $\varkappa$ by two inequality systems that are *linear in* $\varkappa$. Write the first inequality system as $V\varkappa > 0$ and the second system as $V\varkappa < \theta$ where θ depends on the $x_{(j)}, y_{(j)}$ and hence on β ($\theta = \theta(\beta)$). Observe that the first inequality system is only determined by the sign pattern of $\mathcal{C}_\#\beta$. There is only a finite number of conditions of the form $V\varkappa > 0$, however there is a continuum of conditions $V\varkappa < \theta(\beta)$. □

Theorem 2, the main result of this paper, states that it is necessary and sufficient for positive ν, λ (and hence ultimately for such multistationarity) to check the linear inequality systems of the form $V\varkappa > 0$. We emphasize that, in general, one does not have to ensure positivity of ν and λ. But (ν, λ) has to be in $\Lambda^2(E)$, that is, (ν, λ) must have a certain *zero pattern*. This means we need to find a vector $\xi = \mathcal{C}_\#\beta$ with the decomposition (4.10) possessing a zero pattern that is compatible with $\Lambda(E)$.

Definition 4 *(Λ-signature of the coupling matrix $\mathcal{C}_\#$)* The Λ-signature of the coupling matrix $\mathcal{C}_\#$ is the subset $\Sigma = \{\sigma = (\sigma_j)_{j=1,\dots,\bar{\omega}}\}$ of $\{1, -1, 0, \emptyset\}^{\bar{\omega}}$ defined as follows: $\sigma = (\sigma_j)_{j=1,\dots,\bar{\omega}}$ is an element of Σ if and only if there exist a $\beta = (\beta_{(j)})_{j=1,\dots,\gamma}$ and a $\eta = (\eta_{(j)})_{j=1,\dots,\bar{\omega}} \in \Lambda(E)$ such that

$$\sigma_j := \begin{cases} 1 & \text{in case (4.14a)}, \\ -1 & \text{in case (4.14b)}, \\ 0 & \text{in case (4.14c)}, \\ \emptyset & \text{in case (4.14d)} \end{cases} \tag{4.12}$$

where the decomposition of $\mathcal{C}_\#\beta$ according to (4.10), i.e.

$$\mathcal{C}^{(i_2(j),j)}\,\beta_{(i_2(j))} - \mathcal{C}^{(i_1(j),j)}\,\beta_{(i_1(j))} = x_{(j)} - y_{(j)} =: \xi_{(j)}, \tag{4.13}$$

is used in the definition of the four cases

$$\xi_{(j)} \gtrsim 0 \quad \& \ \operatorname{sign}(\eta_{(j)}) = \quad \operatorname{sign}(\xi_{(j)}) \ \& \ \xi^{(l)}_{(j)} = 0 \Rightarrow x^{(l)}_{(j)} = y^{(l)}_{(j)} \geq 0, \tag{4.14a}$$

$$\xi_{(j)} \lesssim 0 \ \& \ \operatorname{sign}(\eta_{(j)}) = -\operatorname{sign}(\xi_{(j)}) \ \& \ \xi^{(l)}_{(j)} = 0 \Rightarrow x^{(l)}_{(j)} = y^{(l)}_{(j)} \geq 0, \tag{4.14b}$$

$$\xi_{(j)} = 0 \ \& \ \eta_{(j)} \gtrsim 0, \tag{4.14c}$$

$$\xi_{(j)} = 0 \ \& \ \eta_{(j)} = 0 \ \& \ x_{(j)} = y_{(j)} \geq 0. \tag{4.14d}$$

We then say that σ is *realized* by such a pair (β, η). □

We'd like to emphasize that a pair (β, η), realizing a certain signature $\sigma \in \Sigma$, imposes certain *sign conditions* on the differences $e^{\varkappa_{i_1(j)}} - e^{\varkappa_{i_2(j)}}$ in Eq. (4.5b) of Fact 4. Moreover, for such a pair (β, η), one can construct a solution $(\lambda, \nu) \in \Lambda^2(E)$ of Eqs. (4.5b) and (4.5c) provided the sign conditions on the differences $\varkappa_{i_1(j)} - \varkappa_{i_2(j)}$ are fulfilled.

To formulate these sign conditions on $\varkappa_{i_1(j)} - \varkappa_{i_2(j)}$ for a given Λ-signature $\sigma \in \Sigma$ we introduce the matrices

$$V^\sigma := [\sigma_j\,(e^T_{i_1(j)} - e^T_{i_2(j)})]_{j=1,\ldots,\bar\omega} \quad \text{and } \sigma_j = \pm 1 \tag{4.15a}$$

$$V^\sigma_0 := [e^T_{i_1(j)} - e^T_{i_2(j)}]_{j=1,\ldots,\bar\omega} \quad \text{and } \sigma_j = 0\,. \tag{4.15b}$$

Moreover, given a Λ-signature σ and a pair (β, η) realizing it, we associate the vector

$$\theta^\sigma(\beta) \;=\; (\theta^\sigma(\beta_{(j)}))_{j=1,\ldots,\bar\omega} \quad \text{and} \quad \sigma_j = \pm 1\,,$$
$$\text{where } \theta^\sigma(\beta_{(j)}) := \begin{cases} \ln \bar\rho_j, & \text{if } \sigma_j = \;\; 1, \\ -\ln \underline{\rho}_j, & \text{if } \sigma_j = -1 \end{cases} \tag{4.15c}$$

with the $\bar\rho_j, \underline{\rho}_j$ from Fact 5 (cf. (4.11a) and (4.11b)). We observe the following:

Fact 6 (Positivity of $\theta^\sigma(\beta)$) *By Fact 5,* (4.14a) *and* (4.14b) *imply* $\ln \bar\rho_j > 0$ *and* $-\ln \underline{\rho}_j > 0$ *and thus* $\theta^\sigma(\beta) > 0$. *Moreover,* $\theta^\sigma(\beta)$ *depends just on the direction of* β: $\theta^\sigma(\beta) = \theta^\sigma(\ell\beta)$ *for* $\ell > 0$. □

Before formulating the main result of our paper we illustrate the above concepts by the following example.

Example 7 (Λ-signatures for network $\mathcal{N}_2$) The set $\Lambda(E)$ for network $\mathcal{N}_2$ consists of the union of the following five subsets—recall $\lambda \in \mathbb{R}^{11}$:

$$\Lambda(E) \;=\; \{\eta_1 = 0,\ \eta_i > 0,\ i \neq 1\} \cup \{\eta_1 = 0,\ \eta_4 = 0,\ \eta_i > 0,\ 1 \neq i \neq 4\}$$
$$\cup\, \{\eta_3 = 0,\ \eta_i > 0,\ i \neq 3\} \cup \{\eta_4 = 0,\ \eta_i > 0,\ i \neq 4\} \cup \{\eta_i > 0,\ i = 1, \ldots, 11\}\,.$$

Recall that (ν_j, λ_j) have the Bridging Property for $j = 1, \ldots, 4$, hence one has $\sigma = (\sigma_i)_{i=1,\ldots,4}$. Pick $\tilde\eta$ with $\tilde\eta_1 = 0$ and $\tilde\eta_i = 1, i = 2, \ldots, 11$. Then $\sigma_1 = (\emptyset, 1, 1, 1)^T$ is realized by the pair $(\beta, \tilde\eta)$, where β is any solution to the linear system

$$(\mathcal{C}_\# \beta)_1 = 0 \quad \text{and} \quad x_{(1)} = y_{(1)} \geq 0, \quad (\mathcal{C}_\# \beta)_2 > 0, \quad (\mathcal{C}_\# \beta)_3 > 0, \quad (\mathcal{C}_\# \beta)_4 > 0,$$

that is to

$$\beta_1 - \beta_4 = 0 \quad \text{and} \quad \beta_1 = \beta_4 \geq 0, \quad \beta_3 + \beta_4 > 0, \quad -\beta_1 - \beta_2 > 0, \quad -\beta_2 + \beta_3 > 0.$$

The signature $\sigma_2 = (\emptyset, 1, 1, -1)^T$ is not realizable, due to infeasibility of the linear system:

$$(\mathcal{C}_\# \beta)_1 = 0 \quad \text{and} \quad x_{(1)} = y_{(1)} \geq 0, \quad (\mathcal{C}_\# \beta)_2 > 0, \quad (\mathcal{C}_\# \beta)_3 > 0, \quad (\mathcal{C}_\# \beta)_4 < 0,$$

that is

$$\beta_1 - \beta_4 = 0 \quad \text{and} \quad \beta_1 = \beta_4 \geq 0, \quad \beta_3 + \beta_4 > 0, \quad -\beta_1 - \beta_2 > 0, \quad -\beta_2 + \beta_3 < 0.$$

Hence one has $\sigma_1 \in \Sigma$ and $\sigma_2 \notin \Sigma$. □

Now we can present the main result of our paper and its application to network $\mathcal{N}_2$. In Sect. 5 we present an application of this theorem to ERK-Signalling. Theorem 2 will be proven in Sect. 6. It will be a consequence of Lemma 4 and Theorem 3 therein.

Theorem 2 (Multistationary Systems with the Isolation and/or the Bridging Property) *Consider a mass action network and recall the objects collected in Remark 6. Assume that each (ν_i, λ_i) has either the Isolation or the Bridging Property (cf. Definition 3) and that the (ν_i, λ_i) are ordered as in Assumption 3. Let Σ be the Λ-signature of $\mathcal{C}_\#$ from (4.9a) (cf. Definition 4) and let V^σ and V_0^σ be the matrices associated to elements $\sigma \in \Sigma$ (cf. Eqs. (4.15a), (4.15b)). In this set-up one has the following equivalence: There exist $a, b \in \mathbb{R}^n_{>0}$, $a \neq b$, and $k \in \mathbb{R}^r_{>0}$ with*

$$S\, v(k,\, a) = 0, \quad S\, v(k,\, b) = 0 \quad \text{and} \quad Z^T a = Z^T b$$

if and only if there exist a Λ-signature $\sigma \in \Sigma$ and vectors $\mu \in \mathbb{R}^n_{\neq 0}$, $z \in \mathbb{R}^n$ and $\varkappa \in \mathbb{R}^\gamma$ satisfying the linear inequality system

$$\begin{aligned} &\mathcal{Y}^T \mu = \Pi\, \varkappa, \quad \tilde{U}^T \varkappa = 0, \quad Z^T z = 0, \quad \operatorname{sign}(\mu) = \operatorname{sign}(z) \\ &V^\sigma \varkappa > 0, \quad V_0^\sigma \varkappa = 0. \end{aligned} \tag{4.16}$$

Remark 10 (Computing a, b and k given σ) Let σ be a Λ-signature, being realized by some β and $\eta \in \Lambda(E)$, such that the above system is feasible. Define the decomposition (4.13) of $\mathcal{C}_\#\beta$ and obtain the positive vector $\theta^\sigma(\beta)$ from (4.15c) (cf. Fact 6). Then the system

$$\begin{aligned} &\mathcal{Y}^T \mu = \Pi\, \varkappa, \quad \tilde{U}^T \Pi\, \varkappa = 0, \quad Z^T z = 0, \quad \operatorname{sign}(\mu) = \operatorname{sign}(z), \\ &V^\sigma \varkappa > 0, \quad V_0^\sigma \varkappa = 0, \quad V^\sigma \varkappa < \theta^\sigma(\beta) \end{aligned}$$

is feasible. The feasibility is a trivial consequence of the feasibility of (4.16) since θ is positive and (4.16) is linear in $\varkappa$. That (ν, λ) belongs to $\Lambda^2(E)$ will be proven in Sect. 6 as a consequence of Theorem 3 therein.

Now choose any solution $\mu \neq 0, z, \varkappa$ and use μ and z to define $a = (a_i)_{i=1,\ldots,n}$ via (3.18a) and b via (3.18b).

- For $j = 1, \ldots, \bar{\omega}$ and $\sigma_j = \emptyset$ obtain $(\nu, \lambda) \in \Lambda^2(E)$ via (4.8b),
- for $j = 1, \ldots, \bar{\omega}$ and $\varkappa_{i_1(j)} \neq \varkappa_{i_2(j)}$ obtain $(\nu, \lambda) \in \Lambda^2(E)$ via (4.7a)–(4.7c),
- for $j = 1, \ldots, \bar{\omega}$ and $\varkappa_{i_1(j)} = \varkappa_{i_2(j)}$ obtain $(\nu, \lambda) \in \Lambda^2(E)$ via (4.7d) and (4.7e),
- for $j = \bar{\omega} + 1, \ldots, \omega$ via (4.7f).

Using λ and a one obtains the rate constants k via (3.18c). □

Remark 11 (Multistationarity by linear inequality systems) Observe that the condition

each (v_i, λ_i) *has either the Isolation or the Bridging Property*

implies that multistationarity can be established by analysis of linear inequality systems. Hence it is *sufficient* for the fact that multistationarity can be established by analysis of linear inequality systems. □

Example 8 (Theorem 2 applied to network $\mathcal{N}_2$) For network $\mathcal{N}_2$ the kernel of $\mathcal{Y}$ is spanned by doubling vectors only (cf. Example 3). Hence there is no condition of the form $\tilde{U}\,\Pi\,\varkappa = 0$. For $\mathcal{Y}^T\,\mu = \Pi\,\varkappa$, $Z^T\,z = 0$ one obtains

$$\begin{aligned}
&\mu_2+\mu_4=\kappa_1,\quad \mu_5=\kappa_1,\quad \mu_6+\mu_7=\kappa_2,\quad \mu_8=\kappa_2,\quad \mu_{10}+\mu_4=\kappa_3,\quad \mu_{11}=\kappa_3,\\
&\mu_{12}+\mu_6=\kappa_4,\quad \mu_{13}=\kappa_4,\quad \mu_1+\mu_2=\kappa_5,\quad \mu_3=\kappa_5,\quad \mu_4+\mu_7=\kappa_5,\quad \mu_9=\kappa_5,\\
&z_2+z_3+z_5=0,\quad z_7+z_8+z_9=0,\quad z_{10}+z_{11}=0,\quad z_{12}+z_{13}=0,\\
&z_1+z_{11}+z_{13}+z_3+z_4+z_5+z_6+z_8+z_9=0.
\end{aligned} \tag{4.17a}$$

Using $\sigma = (1,-1,-1,1) \in \Sigma$-based on the sign structure of $\mathcal{C}_\#\beta$ with $\mathcal{C}_\#$ and $\beta = (6,-4,-2,-2)^T$ from Example 5 and Example 6 resp.—one has for $V^\sigma\,\varkappa > 0$ and $V^\sigma\,\varkappa < \theta^\sigma(\beta)$:

$$x_1 - x_4 > 0,\quad x_3 - x_4 < 0,\quad x_1 - x_2 < 0,\quad x_2 - x_3 > 0,\quad x_2 - x_3 < \ln 2. \tag{4.17b}$$

The condition $\operatorname{sign}(\mu) = \operatorname{sign}(z)$ can be expressed as, e.g.

$$\begin{aligned}
&\mu_1>0,\quad \mu_2>0,\quad \mu_3>0,\quad -\mu_4>0,\quad -\mu_5>0,\quad -\mu_6>0,\quad \mu_7>0,\quad -\mu_8>0,\\
&\mu_9>0,\quad \mu_{10}>0,\quad -\mu_{11}>0,\quad \mu_{12}>0,\quad -\mu_{13}>0,\\
&z_1>0,\quad z_2>0,\quad z_3>0,\quad -z_4>0,\quad -z_5>0,\quad -z_6>0,\quad z_7>0,\quad -z_8>0,\\
&\qquad z_9>0,\quad z_{10}>0,\quad -z_{11}>0,\quad z_{12}>0,\quad -z_{13}>0.
\end{aligned} \tag{4.18}$$

The system (4.17a), (4.17b), (4.18) is feasible. A particular solution is given in Table 3. From this solution one can calculate steady states a and b (also given in Table 3) and the rate constants given in Table 4 (using a and λ from Table 3). Table 3 also contains ν and λ as determined in Example 4 for the particular choice of β and $\varkappa$. □

5 Application of Theorem 2 to ERK-Signalling

The ERK1/2-cascade is the 'best characterized MAPK pathway' (Yao and Seger 2009). The three-tiered core cascade consists of Raf, MEK and ERK and describes the activation of Raf by Ras, of MEK by Raf-p (mono-phosphorylated Raf) and of ERK by MEK-pp (doubly-phosphorylated MEK), see Fig. 1. It has been the subject of many studies, both theoretical and experimental (see e.g. Huang and Ferrell 1996; Seger and Krebs 1995, to name just two references).

Table 3 A solution $(\varkappa, \mu, z)$ to (4.17a)–(4.18)

	$\varkappa$	μ	z	a	b	λ	ν
1	$-\frac{9}{8}$	$\frac{1}{32}$	6	$\frac{6}{-1+e^{1/32}}$	$\frac{6e^{1/32}}{-1+e^{1/32}}$	$\frac{8}{-\frac{1}{e^{73/64}}+\frac{1}{e^{9/8}}}$	$\frac{2\left(3+e^{1/64}\right)}{-1+e^{1/64}}$
2	-1	$\frac{3}{64}$	1	$\frac{1}{-1+e^{3/64}}$	$\frac{e^{3/64}}{-1+e^{3/64}}$	$-\frac{4}{\frac{1}{e^{37/32}}-\frac{1}{e^{73/64}}}$	$\frac{2\left(1+e^{1/64}\right)}{-1+e^{1/64}}$
3	$-\frac{37}{32}$	$\frac{5}{64}$	1	$\frac{1}{-1+e^{5/64}}$	$\frac{e^{5/64}}{-1+e^{5/64}}$	$-\frac{2}{\frac{1}{e^{9/8}}-\frac{1}{e}}$	$\frac{-4+6e^{1/8}}{-1+e^{1/8}}$
4	$-\frac{73}{64}$	$-\frac{75}{64}$	-1	$-\frac{1}{-1+\frac{1}{e^{75/64}}}$	$\frac{1}{-1+e^{75/64}}$	$\frac{2}{-\frac{1}{e^{37/32}}+\frac{1}{e}}$	$-\frac{2\left(-2+e^{5/32}\right)}{-1+e^{5/32}}$
5	$\frac{5}{64}$	$-\frac{9}{8}$	-2	$-\frac{2}{-1+\frac{1}{e^{9/8}}}$	$\frac{2}{-1+e^{9/8}}$	1	$\frac{1}{e^{9/8}}$
6		$-\frac{9}{4}$	-1	$-\frac{1}{-1+\frac{1}{e^{9/4}}}$	$\frac{1}{-1+e^{9/4}}$	1	$\frac{1}{e}$
7		$\frac{5}{4}$	1	$\frac{1}{-1+e^{5/4}}$	$\frac{e^{5/4}}{-1+e^{5/4}}$	1	$\frac{1}{e^{37/32}}$
8		-1	-2	$-\frac{2}{-1+\frac{1}{e}}$	$\frac{2}{-1+e}$	1	$\frac{1}{e^{73/64}}$
9		$\frac{5}{64}$	1	$\frac{1}{-1+e^{5/64}}$	$\frac{e^{5/64}}{-1+e^{5/64}}$	1	$e^{5/64}$
10		$\frac{1}{64}$	1	$\frac{1}{-1+e^{1/64}}$	$\frac{e^{1/64}}{-1+e^{1/64}}$	1	$e^{5/64}$
11		$-\frac{37}{32}$	-1	$-\frac{1}{-1+\frac{1}{e^{37/32}}}$	$\frac{1}{-1+e^{37/32}}$	1	$e^{5/64}$
12		$\frac{71}{64}$	1	$\frac{1}{-1+e^{71/64}}$	$\frac{e^{71/64}}{-1+e^{71/64}}$		
13		$-\frac{73}{64}$	-1	$-\frac{1}{-1+\frac{1}{e^{73/64}}}$	$\frac{1}{-1+e^{73/64}}$		

Steady states a and b were computed from μ and z using (3.18a) and (3.18b). Vectors λ and ν where computed using $\varkappa$ and $\beta = (6, -4, -2, -2)^T$ for λ_i, ν_i, $i = 1, \ldots, 4$ as given in Example 6. λ_i for $i > 4$ are free and hence set to 1

Table 4 Rate constants for network $\mathcal{N}_2$ for the steady states a and b given in Table 3

k_1	k_2	k_3	k_4	k_5	k_6	k_7	k_8	k_9
54.2139	0.337674	552.33	189.035	0.31606	26.5018	9.19336	0.685336	578.979
k_{10}	k_{11}	k_{12}	k_{13}	k_{14}	k_{15}	k_{16}	k_{17}	k_{18}
54.2139	0.337674	552.33	189.035	0.31606	26.5018	9.19336	0.685336	578.979

The values were computed using (3.18c) and λ from Table 3

Here an extended system is analysed, where ERK activation is embedded in two negative feedback loops: one where ERK-pp phosphorylates Raf-p hence inhibiting its own phosphorylation and one where ERK-pp activates MKP, which in turn dephosphorylates ERK-pp. The network is derived from Shaul and Seger (2007), apart from the negative feedback involving MKP, that is described in Chang et al. (2009). The network also includes phosphatases PP2A and PTPs which dephosphorylate Raf-p, Raf-pp, MEK-p, MEK-pp, ERK-p and ERK-pp (cf. Fig. 1). Apart from the negative feedback involving MKP, activation/deactivation is described by sequential distribu-

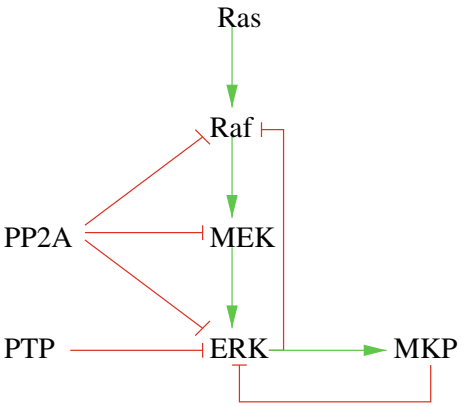


Fig. 1 Cartoon of ERK activation: $\rightarrow$ indicates activation (phosphorylation) and $\dashv$ inhibition (dephosphorylation)

tive phosphorylation/dephosphorylation, see Fig. 2, where the cartoon from Fig. 1 is expanded to a mass action network.

The mass action network of Fig. 2 consists of 29 species and 46 reactions. The stoichiometric matrix S and the vector of reaction rates $v(k,\ x)$ are given in Appendix B (where S and $v(k,\ x)$ are derived under the ordering of reactions, species and complexes given in Fig. 2 and Table 6). The matrix $\mathcal{Y} \in \mathbb{R}^{29\times 46}$ has rank 28. One obtains a basis of $\ker(\mathcal{Y})$ that consists of 15 doubling and three additional kernel vectors.

For the stoichiometric matrix from Appendix B one obtains the 24 generators of $\ker(S) \cap \mathbb{R}^r_{\geq 0}$ displayed as column vectors of the matrix E in Fig. 3. With the help of Algorithm 1 one obtains 11 functions $\varkappa_{J_i}(v, \lambda)$. In Fig. 3, the shading is used to highlight the correspondence between the rows of E and the $\psi_{J_i}(v, \lambda)$.

From Fig. 3 follows immediately that each (v_i, λ_i) either has the Bridging Property ($i = 1, \ldots, 4$) or the Isolation Property ($i = 5, \ldots, 24$, in the column ordering used in Fig. 3).

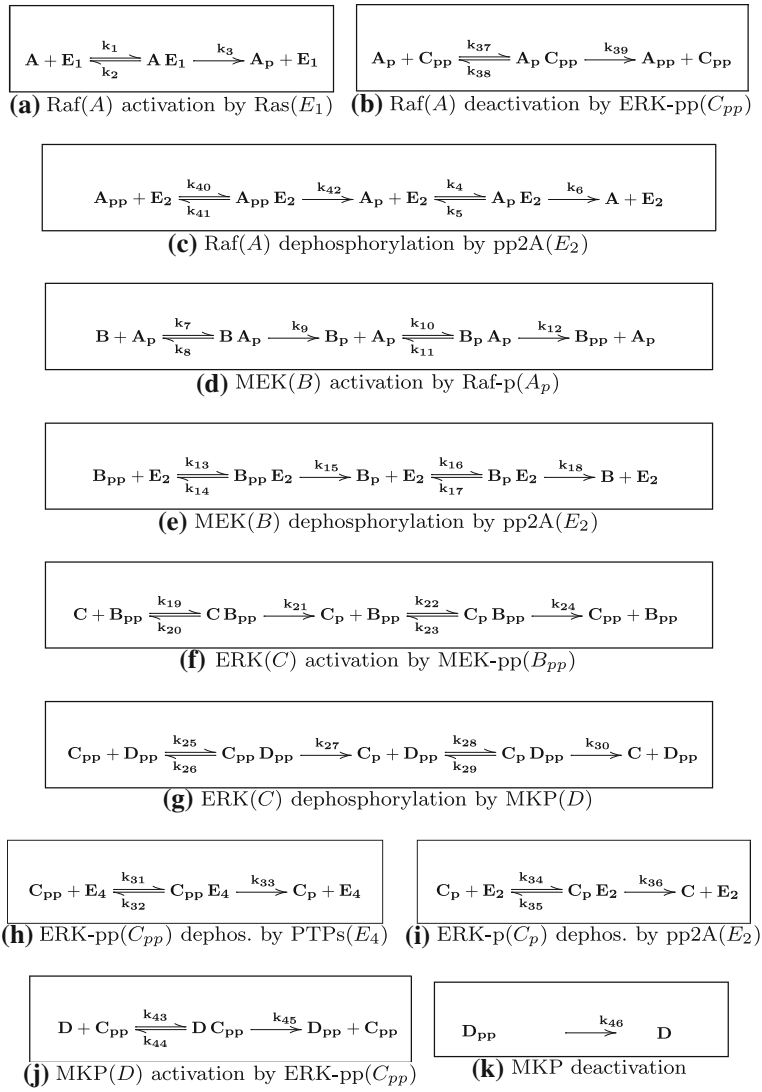
Let $\varkappa \in \mathbb{R}^{11}$ and observe $\ker\left(\begin{bmatrix} 1 & 1 & 1 \\ 0 & 0 & 1 \\ 1 & 1 & 0 \end{bmatrix}\right) = \beta \begin{pmatrix} -1 \\ 1 \\ 0 \end{pmatrix}$ and $\ker\left(\begin{bmatrix} 1 & 1 \\ 0 & 1 \\ 1 & 0 \end{bmatrix}\right) = \begin{pmatrix} 0 \\ 0 \\ 0 \end{pmatrix}$. Then by Lemma 5 $\varkappa \in \mathcal{K}$ implies for the variables with the Bridging Property

$$v_1 = e^{\varkappa_9}\lambda_1, \quad (e^{\varkappa_9} - e^{\varkappa_4})\,\lambda_1 = -\beta_1, \quad v_2 = e^{\varkappa_7}\lambda_2, \quad (e^{\varkappa_7} - e^{\varkappa_4})\,\lambda_2 = \beta_1,$$
$$v_3 = e^{\varkappa_8}\lambda_3, \quad (e^{\varkappa_8} - e^{\varkappa_5})\,\lambda_3 = -\beta_2, \quad v_4 = e^{\varkappa_6}\lambda_4, \quad (e^{\varkappa_6} - e^{\varkappa_5})\,\lambda_4 = \beta_2.$$

And for the variables with the Isolation Property

$$v_5 = e^{\varkappa_5}\lambda_5, \quad v_6 = e^{\varkappa_6}\lambda_6, \quad v_7 = e^{\varkappa_8}\lambda_7, \quad v_8 = e^{\varkappa_4}\lambda_8,$$
$$v_9 = e^{\varkappa_7}\lambda_9, \quad v_{10} = e^{\varkappa_9}\lambda_{10}, \quad v_{11} = e^{\varkappa_1}\lambda_{11}, \quad v_{12} = e^{\varkappa_1}\lambda_{12},$$
$$v_{13} = e^{\varkappa_1}\lambda_{13}, \quad v_{14} = e^{\varkappa_2}\lambda_{14}, \quad v_{15} = e^{\varkappa_2}\lambda_{15}, \quad v_{16} = e^{\varkappa_2}\lambda_{16},$$
$$v_{17} = e^{\varkappa_3}\lambda_{17}, \quad v_{18} = e^{\varkappa_3}\lambda_{18}, \quad v_{19} = e^{\varkappa_3}\lambda_{19}, \quad v_{20} = e^{\varkappa_{10}}\lambda_{20},$$
$$v_{21} = e^{\varkappa_{10}}\lambda_{21}, \quad v_{22} = e^{\varkappa_{10}}\lambda_{22}, \quad v_{23} = e^{\varkappa_{11}}\lambda_{23}, \quad v_{24} = -e^{\varkappa_{11}}\lambda_{24}.$$

Observe that $\Lambda(E) \equiv \mathbb{R}^{24}_{>0}$ and that hence $\lambda_i > 0, i = 1, \ldots, 24$ must hold. For the coupling matrix one obtains



(a) Raf(A) activation by Ras(E_1) **(b)** Raf(A) deactivation by ERK-pp(C_{pp})

(c) Raf(A) dephosphorylation by pp2A(E_2)

(d) MEK(B) activation by Raf-p(A_p)

(e) MEK(B) dephosphorylation by pp2A(E_2)

(f) ERK(C) activation by MEK-pp(B_{pp})

(g) ERK(C) dephosphorylation by MKP(D)

(h) ERK-pp(C_{pp}) dephos. by PTPs(E_4) **(i)** ERK-p(C_p) dephos. by pp2A(E_2)

(j) MKP(D) activation by ERK-pp(C_{pp}) **(k)** MKP deactivation

Fig. 2 Mass action network derived from the cartoon depicted in Fig. 1. Apart from (j) and (k) each activation/deactivation is described by a sequential distributive mechanism

$$\mathcal{C} = \begin{bmatrix} -1 & 0 \\ 1 & 0 \\ 0 & -1 \\ 0 & 1 \end{bmatrix}$$

and the set of Λ-signatures

$$\Sigma = \left\{ \begin{pmatrix} -1 \\ 1 \end{pmatrix}, \begin{pmatrix} 1 \\ -1 \end{pmatrix}, \begin{pmatrix} 0 \\ 0 \end{pmatrix} \right\} \times \left\{ \begin{pmatrix} -1 \\ 1 \end{pmatrix}, \begin{pmatrix} 1 \\ -1 \end{pmatrix}, \begin{pmatrix} 0 \\ 0 \end{pmatrix} \right\}.$$

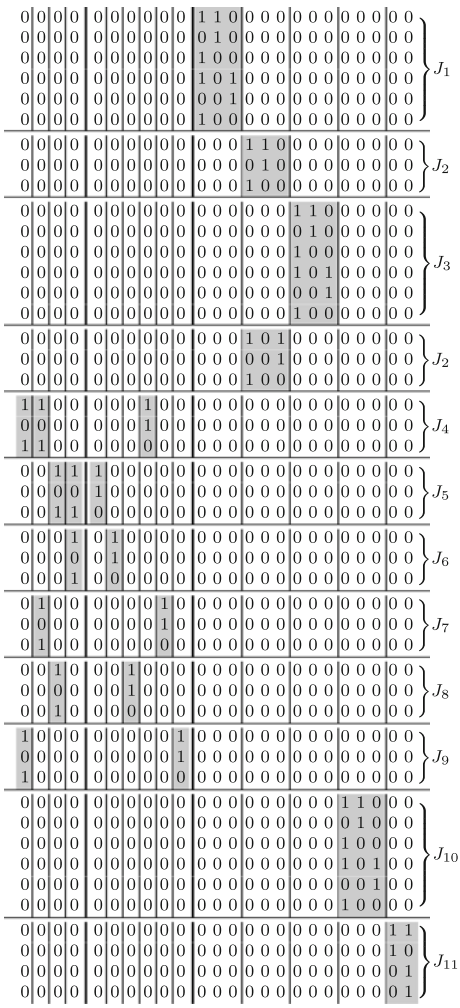


Fig. 3 Array representing the generator matrix for the network from Fig. 2. The nonzero blocks relevant for the definition of $\varkappa_{J_i}, i = 1, \ldots, 11$ are *shaded*. For each row the corresponding cluster J_i is written on the *right hand side*. *Vertical lines* separate columns that either have nonzero entries in only one cluster J_i (and hence are only relevant for one function $\varkappa_{J_i}$) or have nonzero entries in exactly two different clusters J_i and J_j. Columns $1, \ldots, 4$ correspond to the Bridging Property (each column block contains two nonzero blocks), while columns $5, \ldots, 24$ correspond to the Isolation Property (each column block contains only one nonzero block)

Let e_i be unit vectors of $\mathbb{R}^{11}$ and define V^σ according to (4.15a) as

$$V^\sigma = \begin{bmatrix} \sigma_1 \, (e_9^T - e_4^T) \\ \sigma_2 \, (e_7^T - e_4^T) \\ \sigma_3 \, (e_8^T - e_5^T) \\ \sigma_4 \, (e_6^T - e_5^T) \end{bmatrix}, \quad \sigma = (\sigma_i)_{i=1,\ldots,4} \in \Sigma.$$

Then one has $\lambda_i > 0, i = 1, \ldots, 4$, iff $\kappa \in \mathbb{R}^{11}$ satisfies $V^\sigma \, \kappa > 0$, for some $\sigma \in \Sigma$. Note that one has $\nu_i > 0$, whenever $\lambda_i > 0$. Hence there are no constraints of the form $V^\sigma < \theta(\xi)^\sigma$ and (4.16) from Theorem 2 becomes:

$$\mathcal{Y}^T \, \mu = \Pi \, \kappa, \quad \tilde{U}^T \, \Pi \, \kappa = 0, \quad Z^T \, z = 0, \quad V^\sigma \, \kappa > 0. \tag{5.1a}$$

Interpreting $\text{sign}(\mu) = \text{sign}(z)$ as

$$\begin{aligned}
&\mu_1 > 0, \quad -\mu_2 > 0, \quad \mu_3 > 0, \quad \mu_4 > 0, \quad -\mu_5 > 0, \quad \mu_6 > 0, \quad -\mu_7 > 0,\\
&-\mu_8 > 0, \quad \mu_9 > 0, \quad \mu_{10} > 0, \quad \mu_{11} > 0, \quad \mu_{12} > 0, \quad -\mu_{13} > 0, \quad \mu_{14} > 0,\\
&\mu_{15} > 0, \quad \mu_{16} > 0, \quad \mu_{17} > 0, \quad \mu_{18} > 0, \quad \mu_{19} > 0, \quad \mu_{20} > 0, \quad \mu_{21} > 0,\\
&-\mu_{22} > 0, \quad \mu_{23} > 0, \quad -\mu_{24} > 0, \quad \mu_{25} > 0, \quad \mu_{26} > 0, \quad \mu_{27} > 0, \quad -\mu_{28} > 0,\\
&\mu_{29} > 0,\\
&z_1 > 0, \quad -z_2 > 0, \quad z_3 > 0, \quad z_4 > 0, \quad -z_5 > 0, \quad z_6 > 0, \quad -z_7 > 0,\\
&-z_8 > 0, \quad z_9 > 0, \quad z_{10} > 0, \quad z_{11} > 0, \quad z_{12} > 0, \quad -z_{13} > 0, \quad z_{14} > 0,\\
&z_{15} > 0, \quad z_{16} > 0, \quad z_{17} > 0, \quad z_{18} > 0, \quad z_{19} > 0, \quad z_{20} > 0, \quad z_{21} > 0,\\
&-z_{22} > 0, \quad z_{23} > 0, \quad -z_{24} > 0, \quad z_{25} > 0, \quad z_{26} > 0, \quad z_{27} > 0, \quad -z_{28} > 0,\\
&z_{29} > 0, \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad (5.1b)
\end{aligned}$$

one finds that (5.1a) and (5.1b) is feasible for

$$\sigma = (-1,\ 1,\ -1,\ 1)^T$$

and one obtains multistationarity. Solutions μ and z together with two steady states are given in Table 8 in Appendix C. For $\beta_1 = \beta_2 = 1$ one obtains

$$\lambda_1 = 0.213209, \quad \lambda_2 = 1.12419, \quad \lambda_3 = 0.274797, \quad \lambda_4 = 0.0498965.$$

Together with $\lambda_i = 1, i = 5, \ldots, 24$ one obtains the rate constants given in Table 9 for the steady states a and b from Table 8 (cf. Appendix C).

Figure 4 visually confirms multistationarity using the rate constants from Table 9 and the b-values from Table 8 as starting point of the numerical continuation. Here the total concentration c_6 is used as a bifurcation parameter and x_1 is plotted against varying values of c_6 (cf. Table 7). There is an interval, where the system has at least two positive steady states and hence multistationarity.

6 Proof of Theorem 2

We start off with the main result of this section: necessary and sufficient conditions in the form of *linear inequalities* that replace the nonlinear condition $\varkappa \in \mathcal{K}$.

Theorem 3 *Consider a mass action network and recall the objects collected in Remark 6. Assume that each (ν_i, λ_i) has either the Isolation or the Bridging Property (cf. Definition 3) and that the (ν_i, λ_i) are ordered as in Assumption 3. Let Σ be the Λ-signature of $\mathcal{C}_\#$ from (4.9a) (cf. Definition 4) and let V^σ and V_0^σ be the matrices associated to elements $\sigma \in \Sigma$ (cf. Eqs. (4.15a) and (4.15b)). Finally, recall the vector $\theta^\sigma(\beta)$ from (4.15c) associated to a realization of σ by a pair (β, η).*

Let $\varkappa \in \mathbb{R}^\gamma$. Then there exists a positive number ϵ^* such that the line segment

$$\tilde{\varkappa} = \epsilon\, \varkappa, \quad \epsilon \in (0,\ \epsilon^*), \qquad (6.1)$$

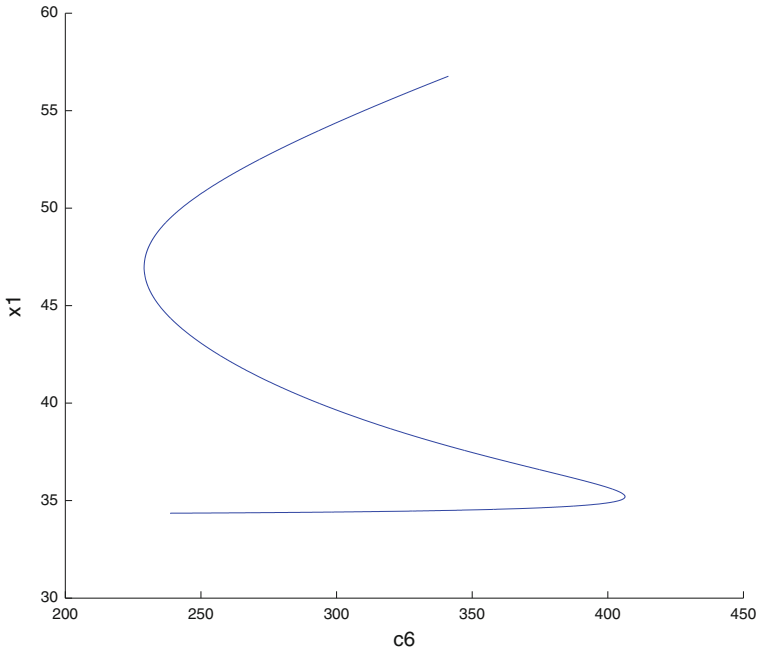


Fig. 4 Bifurcation diagram using c_6 as a bifurcation parameter

is contained in $\mathcal{K}$ if and only if there exists an element $\sigma \in \Sigma$ such that $\varkappa$ satisfies

$$V^\sigma \varkappa > 0, \quad V_0^\sigma \varkappa = 0. \tag{6.2}$$

If an element $\varkappa$ satisfies (6.2) for some $\sigma \in \Sigma$, realized by (β, η), then ϵ^* can be taken as

$$\epsilon^* := \min_i \frac{(\theta^\sigma(\beta))_i}{(V^\sigma \varkappa)_i} > 0. \tag{6.3}$$

The proof of Theorem 3 is based on the following lemmata. The first Lemma establishes sufficient conditions for $\varkappa \in \mathcal{K}$ and presents the formulas for $(\nu, \lambda) \in \Lambda^2(E)$ that realize $\varkappa \in \mathcal{K}$.

Lemma 6 (Sufficient conditions for $\varkappa \in \mathcal{K}$ and (ν, λ)-formulas) *In the set-up of Theorem 3, let $\varkappa \in \mathbb{R}^\gamma$ and let $\sigma \in \Sigma$ be realized by (β, η) with*

$$V^\sigma \varkappa > 0, \quad V^\sigma \varkappa < \theta^\sigma(\beta), \quad V_0^\sigma \varkappa = 0. \tag{6.4}$$

Define $\nu = \mathrm{col}(\nu_{(1)}, \ldots, \nu_{(\omega)})$ and $\lambda = \mathrm{col}(\lambda_{(1)}, \ldots, \lambda_{(\omega)})$ as follows:

– *If $j \in \{1, \ldots, \bar{\omega}\}$ and $\sigma_j = \emptyset$:*

$$\lambda_{(j)} = 0, \quad \nu_{(j)} = \mathcal{C}^{(i_1(j), j)}\, \beta_{i_1(j)} \tag{6.5}$$

– *If* $j \in \{1, \dots, \bar{\omega}\}$ *and* $\varkappa_{i_1(j)} \neq \varkappa_{i_2(j)}$:

$$\rho_j := e^{\varkappa_{i_1(j)} - \varkappa_{i_2(j)}} \tag{6.6a}$$

$$\nu_{(j)} = \frac{e^{\varkappa_{i_1(j)}}}{e^{\varkappa_{i_1(j)}} - e^{\varkappa_{i_2(j)}}} \left[\rho_j \, \mathcal{C}^{(i_2(j),j)} \, \beta_{(i_2(j))} - \mathcal{C}^{(i_1(j),j)} \, \beta_{(i_1(j))}\right] \tag{6.6b}$$

$$\lambda_{(j)} = \frac{1}{e^{\varkappa_{i_1(j)}} - e^{\varkappa_{i_2(j)}}} \left[\mathcal{C}^{(i_2(j),j)} \, \beta_{(i_2(j))} - \mathcal{C}^{(i_1(j),j)} \, \beta_{(i_1(j))}\right] \tag{6.6c}$$

– *If* $j \in \{1, \dots, \bar{\omega}\}$ *and* $\varkappa_{i_1(j)} = \varkappa_{i_2(j)}$:

$$\nu_{(j)} = e^{\varkappa_{i_1(j)}} \, \lambda_{(j)} + \mathcal{C}^{(i_1(j),j)} \, \beta_{(i_1(j))}, \; \lambda_{(j)} > 0 \quad \textit{large so that } \nu_{(j)} > 0. \tag{6.7}$$

– *If* $j \in \{\bar{\omega} + 1, \dots, \omega\}$:

$$\nu_{(j)} = e^{\varkappa_{i_1(j)}} \, \lambda_{(j)} + \mathcal{C}^{(i_1(j),j)} \, \beta_{(i_1(j))}, \; \lambda_{(j)} > 0 \quad \textit{large so that } \nu_{(j)} > 0. \tag{6.8}$$

Then one has

$$\varkappa = \psi(\nu, \lambda) \quad \textit{and} \quad (\nu, \lambda) \in \Lambda^2(E) \quad \textit{and thus} \quad \varkappa \in \mathcal{K}.$$

Proof That the pairs (ν, λ) with (6.5)–(6.8) (6.8) imply $\varkappa = \psi(\nu, \lambda)$ follows from Lemma 5 in Sect. 4. Thus we have to show $(\nu, \lambda) \in \Lambda^2(E)$. To this end we first show that the condition (6.4) implies that $\lambda = \mathrm{col}(\lambda_{(1)}, \dots, \lambda_{(\omega)})$ defined according to (6.5), (6.6c), (6.7) or (6.8) satisfies

(a) $\mathrm{sign}(\lambda_{(j)}) = \mathrm{sign}(\eta_{(j)})$ under the conditions of (6.6c) and (6.5),
(b) $\lambda_{(j)} > 0$ under the conditions of (6.7) and (6.8).

We start by recalling the definition of $\xi_{(j)}$ and its decomposition (4.13).

Concerning (a), we observe that the fact that σ is realized by (β, η) implies $\mathrm{sign}(\xi_{(j)}) = \mathrm{sign}(\eta_{(j)})$ in case of $\xi_{(j)} \gtrdot 0$ and $\sigma_j = 1$, $\mathrm{sign}(\xi_{(j)}) = -\mathrm{sign}(\eta_{(j)})$ in case of $\xi_{(j)} \lessdot 0$ and $\sigma_j = -1$ and, finally, $\eta_{(j)} = 0$ in case of $\xi_{(j)} = 0$ and $\sigma_j = \emptyset$.

Now $V^\sigma \varkappa > 0$ implies $e^{\varkappa_{i_1(j)}} - e^{\varkappa_{i_2(j)}} > 0$ for $\xi_{(j)} \gtrdot 0$ and $e^{\varkappa_{i_1(j)}} - e^{\varkappa_{i_2(j)}} < 0$ for $\xi_{(j)} \lessdot 0$. Thus the claim (a) follows.

Concerning (b): If $j = 1, \dots, \bar{\omega}$ and $\varkappa_{i_1(j)} = \varkappa_{i_2(j)}$ or $j = \bar{\omega} + 1, \dots, \omega$ one may choose $\lambda_{(j)} > -\mathcal{C}^{(i_1(j),j)} \, \beta_{(i_1(j))}$ such that $\nu_j > 0$. Hence one can choose $\lambda_{(j)} > 0$ (cf. Eq. (6.7)). This implies (b).

Thus one can always split $\lambda = \tilde{\lambda} + \hat{\lambda}$ such that $\mathrm{sign}(\tilde{\lambda}) = \mathrm{sign}(\eta)$ and $\hat{\lambda} \geq 0$. Then, by Remark 4 one has $\lambda \in \Lambda(E)$.

Concerning $\nu \in \Lambda(E)$, recall (6.5), (6.6b), (6.7) and (6.8) and observe that $\nu_{(j)} > 0$ for $j = 1, \dots, \bar{\omega}$ and $\varkappa_{i_1(j)} = \varkappa_{i_2(j)}$ [cf. (6.7)] and also for $j = \bar{\omega} + 1, \dots, \omega$ [cf. (6.8)]. So there are two remaining cases:

(A) $j \in \{1, \dots, \bar{\omega}\}$ and $\varkappa_{i_1(j)} \neq \varkappa_{i_2(j)}$, (B) $j \in \{1, \dots, \bar{\omega}\}$ and $\sigma_j = \emptyset$.

We will show that the strict inequality $V^\sigma \varkappa < \theta^\sigma(\beta)$ in (6.4) implies $\nu_{(j)}^{(l)} = 0 \Rightarrow \lambda_{(j)}^{(l)} = 0$. So, $\lambda \in \Lambda(E)$ will imply $\nu \in \Lambda(E)$ by Remark 4.

Ad (A): Let $\xi_{(j)} \gtrless 0$ and observe that (6.4) now implies $\varkappa_{i_1(j)} - \varkappa_{i_2(j)} > 0$ and $1 < \rho_j < \bar{\rho}_j$ and, in addition, $\nu_{(j)}^{(l)} = 0 \Leftrightarrow x_{(j)}^{(l)} - y_{(j)}^{(l)} = 0$ (cf. Fact 5). The representation $\rho_j \nu_{(j)} = e^{\varkappa_{i_1(j)}}(\rho_j x_{(j)} - y_{(j)})$ of (6.6b) thus entails the following implication:

$$\nu_{(j)}^{(l)} = 0 \Rightarrow (\rho_j - 1)x_{(j)}^{(l)} = 0 \Rightarrow \lambda_{(j)}^{(l)} = 0.$$

Clearly, there is the analogon for $\xi_{(j)} \lesssim 0$ and $\rho_j \in (0, 1)$. All in all, one arrives at $\nu_{(j)} \geq 0$ and $\nu_{(j)}^{(l)} = 0 \Rightarrow \lambda_{(j)}^{(l)} = 0$.

Ad (B) with $j \in \{1, \ldots, \bar{\omega}\}$: In case $\sigma_j = \emptyset$ one has $\lambda_{(j)} = 0$ and $\nu_{(j)} = y_{(j)} \geq 0$. Hence the implication $\nu_{(j)}^{(j)} = 0 \Rightarrow \lambda_{(j)}^{(l)} = 0$ holds. Moreover, for $j \in \{1, \ldots, \bar{\omega}\}$ and $\varkappa_{i_1(j)} - \varkappa_{i_2(j)} = 0$, i.e. in case $\sigma_j = 0$, and also for $j \in \{\bar{\omega} + 1, \ldots, \omega\}$ one has $\lambda_{(j)} > 0$ and $\nu_{(j)} > 0$. □

Next we present necessary conditions for $\varkappa \in \mathcal{K}$.

Lemma 7 (Necessary conditions for $\varkappa \in \mathcal{K}$) *In the set-up of Theorem 3, suppose an element $\varkappa$ belongs to $\mathcal{K}$. Then there exists an element $\sigma \in \Sigma$, realized by some pair (β, η), with*

$$V^\sigma \varkappa > 0, \quad V^\sigma \varkappa \leq \theta^\sigma(\beta), \quad V_0^\sigma \varkappa = 0 \tag{6.9}$$

for positive $\theta^\sigma(\beta)$.

Proof Let $\varkappa \in \mathcal{K}$ and pick $(\nu, \lambda) \in \Lambda^2(E)$ with $\varkappa = \psi(\nu, \lambda)$. By Lemma 5 [see also (4.5b–4.5d)] one has $\nu = \mathrm{col}(\nu_{(1)}, \ldots, \nu_{(\omega)})$ and $\lambda = \mathrm{col}(\lambda_{(1)}, \ldots, \lambda_{(\omega)})$ with

$$\nu_{(j)} = e^{\varkappa_{i_1(j)}} \lambda_{(j)} + \mathcal{C}^{(i_1(j), j)} \beta_{(i_1(j))} \tag{6.10a}$$

$$(e^{\varkappa_{i_1(j)}} - e^{\varkappa_{i_2(j)}}) \lambda_{(j)} = \mathcal{C}^{(i_2(j), j)} \beta_{(i_2(j))} - \mathcal{C}^{(i_1(j), j)} \beta_{(i_1(j))} = \xi_{(j)} \tag{6.10b}$$

for $j = 1, \ldots, \bar{\omega}$ and

$$\nu_{(j)} = e^{\varkappa_{i_2(j)}} \lambda_{(j)}, \quad \lambda_{(j)} > 0, \quad j = \bar{\omega} + 1, \ldots, \omega, \tag{6.10c}$$

for appropriate $\beta = \mathrm{col}(\beta_{(1)}, \ldots, \beta_{(\gamma)})$. Observe that this implies $\xi_{(j)} = 0$ if and only if $\varkappa_{i_1(j)} = \varkappa_{i_2(j)}$ or $\lambda_{(j)} = 0$ holds (cf. Lemma 5). In case $\lambda_{(j)} \gtrless 0$ one necessarily has $\varkappa_{i_1(j)} = \varkappa_{i_2(j)}$ and, in defining a candidate signature $\sigma \in \Sigma$, we put $\sigma_j = 0$ (leading to $V_0^\sigma \varkappa = 0$ in the end). To the j with $\lambda_{(j)} = 0$ we associate $\sigma_j = \emptyset$. Furthermore, $\lambda \in \Lambda(E)$ implies $\varkappa_{i_1(j)} - \varkappa_{i_2(j)} > 0$ for $\xi_{(j)} \gtrless 0$ and $\varkappa_{i_1(j)} - \varkappa_{i_2(j)} < 0$ for $\xi_{(j)} \lesssim 0$. So, in defining a candidate signature $\sigma \in \Sigma$ we put $\sigma_j = 1$ and $\sigma_j = -1$ respectively (leading to $V^\sigma \varkappa > 0$ in the end).

The representation (6.10a) of $\nu \in \Lambda(E)$ implies that the pair (β, λ) realizes the above σ in the sense of Definition 4 with $V^\sigma \varkappa > 0$ and $V_0^\sigma \varkappa = 0$. We now turn to the second condition in (6.9).

We assume that $\varkappa_{i_1(j)} \neq \varkappa_{i_2(j)}$ for some $j \in \{1, \ldots, \bar{\omega}\}$. Then $\xi_{(j)} = x_{(j)} - y_{(j)}$ gives

$$\nu_{(j)} = \frac{e^{\varkappa_{i_2(j)}}}{e^{\varkappa_{i_1(j)}} - e^{\varkappa_{i_2(j)}}} [\rho_j \, x_{(j)} - y_{(j)}], \quad \rho_j := e^{\varkappa_{i_1(j)} - \varkappa_{i_2(j)}},$$

with $x_{(j)} - y_{(j)} \gtrsim 0$ for $\varkappa_{i_1(j)} - \varkappa_{i_2(j)} > 0$ and $x_{(j)} - y_{(j)} \lesssim 0$ for $\varkappa_{i_1(j)} - \varkappa_{i_2(j)} < 0$. By Fact 5, $\nu \in \Lambda(E)$ then implies $\rho_j \leq \bar{\rho}_j$ for $x_{(j)} - y_{(j)} \gtrsim 0$ and $\rho_j \geq \underline{\rho}_j$ for $x_{(j)} - y_{(j)} \lesssim 0$. This implies $\varkappa_{i_1(j)} - \varkappa_{i_2(j)} \leq \ln \bar{\rho}_j$ for $\xi_{(j)} \gtrsim 0$ and $\varkappa_{i_1(j)} - \varkappa_{i_2(j)} \geq \ln \underline{\rho}_j$ for $\xi_{(j)} \lesssim 0$. Thus will lead to $V^\sigma \varkappa \leq \theta^\sigma(\beta)$. □

Finally we emphasize that $V^\sigma \varkappa > 0$ and $V_0^\sigma \varkappa = 0$ imply (6.4) and (6.9), respectively, for *positive* $\theta^\sigma(\beta)$:

Fact 7 *Suppose there is an element $\sigma \in \Sigma$, realized by (β, η), such that $\varkappa \in \mathbb{R}^\gamma$ satisfies*

$$V^\sigma \varkappa > 0, \quad V_0^\sigma \varkappa = 0 \tag{6.2}$$

with V^σ and V_0^σ as in (4.15a), (4.15b). Compute the (positive)

$$\epsilon^* := \min_i \frac{(\theta^\sigma(\beta))_i}{(V^\sigma \varkappa)_i}. \tag{6.3}$$

Then $\bar{\varkappa} = \epsilon \varkappa$ from (6.1) satisfies (6.4), i.e.

$$V^\sigma \bar{\varkappa} > 0, \quad V_0^\sigma \bar{\varkappa} = 0, \quad V^\sigma \bar{\varkappa} < \theta^\sigma(\beta) \quad \text{for } \epsilon \in (0, \epsilon^*).$$

In case of a finite ϵ^* one has $(V^\sigma \epsilon^* \varkappa)_j = (\theta^\sigma(\beta))_j$ for all j where the above minimum is assumed. □

Now we can prove Theorem 3.

Proof (Theorem 3) Suppose $\varkappa \in \mathcal{K}$. Then by Lemma 7 there exists an element $\sigma \in \Sigma$ such that $\varkappa$ satisfies (6.9) and hence (6.2). Clearly any $\epsilon \varkappa, \epsilon > 0$, then also satisfies (6.2), thus, in particular, the line segment (6.1).

Vice versa, suppose there exists an element $\sigma \in \Sigma$, realized by (β, η), such that an element $\varkappa$ satisfies (6.2). Then, by Fact 7, the line segment (6.1) satisfies (6.4) and hence is an element of $\mathcal{K}$ by Lemma 6. □

Finally, we turn to the proof of Theorem 2:

Proof (Theorem 2) Suppose $\exists a, b \in \mathbb{R}^n_{>0}$ and $k \in \mathbb{R}^r_{>0}$ with $Sv(k, a) = 0$, $Sv(k, b) = 0$ and $Z^T a = Z^T b$. Then by Theorem 1 there exist $\mu \in \mathbb{R}^n, \mu \neq 0, \varkappa \in \mathbb{R}^\gamma$ and $\nu, \lambda \in \mathbb{R}^n_{\geq 0}$ with

$$\mathcal{Y}^T \mu = \Pi \varkappa, \quad \tilde{U}^T \Pi \varkappa = 0, \quad Z^T z = 0, \quad \operatorname{sign}(z) = \operatorname{sign}(\mu),$$
$$\varkappa = \varkappa(\nu, \lambda), \quad \nu, \lambda \in \Lambda^2(E).$$

Thus $\varkappa \in \mathcal{K}$ and, by Theorem 3, there exists a $\sigma \in \Sigma$, realized by (β, η), and associated V^σ, V_0^σ such that $V^\sigma \varkappa > 0$, $V_0^\sigma \varkappa = 0$.

Suppose $\exists \sigma \in \Sigma$ and $\exists \mu \in \mathbb{R}^n,\ \mu \neq 0,\ \varkappa \in \mathbb{R}^\gamma$ and $z \in \mathbb{R}^n$ such that

$$\mathcal{Y}^T \mu = \Pi \varkappa, \quad \tilde{U}^T \varkappa = 0, \quad Z^T z = 0, \quad \operatorname{sign}(\mu) = \operatorname{sign}(z)$$
$$V^\sigma \varkappa > 0, \quad V_0^\sigma \varkappa = 0.$$

Choose any realization (β, η) of σ and obtain $\theta^\sigma(\beta)$. Compute

$$\epsilon^* := \min_i \frac{(\theta^\sigma(\beta))_i}{(V^\sigma \varkappa)_i}$$

and choose $\epsilon \in (0, \epsilon^*)$. Then $(\bar{z}, \bar{\mu}, \bar{\varkappa})$ with $\bar{z} = z$, $\bar{\mu} = \epsilon \mu$ and $\bar{\varkappa} = \epsilon \varkappa$ satisfy

$$\mathcal{Y}^T \bar{\mu} = \Pi \bar{\varkappa}, \quad \tilde{U}^T \Pi \bar{\varkappa} = 0, \quad Z^T \bar{z} = 0, \quad \operatorname{sign}(z) = \operatorname{sign}(\mu),$$

and—cf. Fact 7

$$V^\sigma \bar{\varkappa} > 0, \quad V_0^\sigma \bar{\varkappa} = 0, \quad V^\sigma \bar{\varkappa} < \theta^\sigma(\beta).$$

By Theorem 3 one has $\bar{\varkappa} \in \mathcal{K}$. Hence Theorem 1 implies the existence of a, b, k. □

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Appendix A: Data for network $\mathcal{N}_2$

See Appendix Table 5.

Table 5 Species and complexes for the network $\mathcal{N}_2$ (u_i denote the unit vectors of $\mathbb{R}^{13}$)

Variable x_i	Species	Unit vector u_i	Complex vector y_i	Complex	$\sum u_i$
x_1	A	u_1	y_1	$A + E_1$	$u_1 + u_2$
x_2	E_1	u_2	y_2	$A\ E_1$	u_3
x_3	$A\ E_1$	u_3	y_3	$A_p + E_1$	$u_4 + u_2$
x_4	A_p	u_4	y_4	$A_p\ E_1$	u_5
x_5	$A_p\ E_1$	u_5	y_5	$A_{pp} + E_1$	$u_6 + u_2$
x_6	A_{pp}	u_6	y_6	$A_{pp} + E_2$	$u_6 + u_7$
x_7	E_2	u_7	y_7	$A_{pp}\ E_2$	u_8
x_8	$App\ E_2$	u_8	y_8	$A_p + E_2$	$u_4 + u_7$
x_9	$A_p\ E_2$	u_9	y_9	$A_p\ E_2$	u_9
x_{10}	E_3	u_{10}	y_{10}	$A + E_2$	$u_1 + u_7$
x_{11}	$A_p\ E_3$	u_{11}	y_{11}	$A_p + E_3$	$u_4 + u_{10}$
x_{12}	E_4	u_{12}	y_{12}	$A_p\ E_3$	u_{11}

Table 5 continued

Variable x_i	Species	Unit vector u_i	Complex vector y_i	Complex	$\sum u_i$
x_{13}	$A_{pp}\,E_4$	u_{13}	y_{13}	$A_{pp}+E_3$	u_6+u_{10}
			y_{14}	$A_{pp}+E_4$	u_6+u_{12}
			y_{15}	$A_{pp}\,E_4$	u_{13}
			y_{16}	A_p+E_4	u_4+u_{12}

The values were computed using (3.18c) and λ from Table 3

Appendix B: Data for ERK activation network given in Fig. 2

See Appendix Tables 6, 7.

Table 6 Species and complexes for the network given in Fig. 2

Species	Variable x_i	Biochemical compound	Complex vector y_i	Complex	$\sum_i e_i$
x_1	A	Raf	y_1	$A+E_1$	e_1+e_2
x_2	E_1	Ras	y_2	$A\cdot E_1$	e_3
x_3	$A\,E_1$		y_3	A_p+E_1	e_2+e_4
x_4	A_p	Active Raf	y_4	A_p+E_2	e_4+e_5
x_5	E_2	*PP2A*	y_5	$A_p\cdot E_2$	e_6
x_6	$A_p\cdot E_2$		y_6	$A+E_2$	e_1+e_5
x_7	B	*MEK*1/2	y_7	$B+A_p$	e_4+e_7
x_8	$B\cdot A_p$		y_8	$B\cdot A_p$	e_8
x_9	B_p	Monophosphorylated MEK	y_9	B_p+A_p	e_4+e_9
x_{10}	$B_p\cdot A_p$		y_{10}	$B_p\cdot A_p$	e_{10}
x_{11}	B_{pp}	Double phosphorylated MEK	y_{11}	$B_{pp}+A_p$	e_4+e_{11}
x_{12}	$B_{pp}\cdot E_2$		y_{12}	$B_{pp}+E_2$	e_5+e_{11}
x_{13}	$B_p\cdot E_2$		y_{13}	$B_PP\cdot E_2$	e_{12}
x_{14}	C	*ERK*1/2	y_{14}	B_P+E_2	e_5+e_9
x_{15}	$C\cdot B_{pp}$		y_{15}	$B_p\cdot E_2$	e_{13}
x_{16}	C_p	Monophosphorylated ERK	y_{16}	$B+E_2$	e_5+e_7
x_{17}	$C_p\cdot B_{pp}$		y_{17}	$C+B_{pp}$	$e_{11}+e_{14}$
x_{18}	C_{pp}	Double phosphorylated ERK	y_{18}	$C\cdot B_{pp}$	e_{15}
x_{19}	D_{pp}	Active (double phosphorylated) MKP	y_{19}	C_p+B_{pp}	$e_{11}+e_{16}$
x_{20}	$C_{pp}\cdot D_{pp}$		y_{20}	C_p+B_{pp}	e_{17}
x_{21}	$C_p\cdot D_{pp}$		y_{21}	$C_{pp}+B_{pp}$	$e_{11}+e_{18}$
x_{22}	E_4	PTPs	y_{22}	$C_{pp}+E_3$	$e_{18}+e_{19}$
x_{23}	$C_{pp}\cdot E_4$		y_{23}	$C_{pp}\cdot E_3$	e_{20}
x_{24}	$C_p\cdot E_2$		y_{24}	C_p+E_3	$e_{16}+e_{19}$
x_{25}	$A_p\cdot C_{pp}$		y_{25}	$C_p\cdot E_3$	e_{21}
x_{26}	A_{pp}	Double phosphorylated Raf	y_{26}	$C+E_3$	$e_{14}+e_{19}$

Table 6 continued

Species	Variable x_i	Biochemical compound	Complex vector y_i	Complex	$\sum_i e_i$
x_{27}	$A_{pp} \cdot E_2$		y_{27}	$C_{pp} + E_4$	$e_{18} + e_{22}$
x_{28}	D	*MKP*	y_{28}	$C_{pp} \cdot E_4$	e_{23}
x_{29}	$D \cdot C_{pp}$		y_{29}	$C_p + E_4$	$e_{16} + e_{22}$
			y_{30}	$C_p + E_2$	$e_5 + e_{16}$
			y_{31}	$C_p \cdot E_2$	e_{24}
			y_{32}	$C + E_2$	$e_5 + e_{14}$
			y_{33}	$A_p + C_{pp}$	$e_4 + e_{18}$
			y_{34}	$A_p \cdot C_{pp}$	e_{25}
			y_{35}	$A_{pp} + C_{pp}$	$e_{18} + e_{26}$
			y_{36}	$A_{pp} + E_2$	$e_5 + e_{26}$
			y_{37}	$A_{pp} \cdot E_2$	e_{27}
			y_{38}	$D + C_{pp}$	$e_{28} + e_{18}$
			y_{39}	$D \cdot C_{pp}$	e_{29}
			y_{40}	$D_{pp} + C_{pp}$	$e_{19} + e_{18}$
			y_{41}	D_{pp}	e_{19}
			y_{42}	D	e_{28}

Table 7 For the network of Fig. 2

Stoichiometric matrix S :

$$\begin{bmatrix}
-1 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
-1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & -1 & 1 & 0 & -1 & 1 & 1 & -1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & -1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 1 & -1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
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0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0
\end{bmatrix}$$

Vector $v(k, x)$ of reaction rates:

$$\big(k_1x_1x_2,\ k_2x_3,\ k_3x_3,\ k_4x_4x_5,\ k_5x_6,\ k_6x_6,\ k_7x_4x_7,\ k_8x_8,\ k_9x_8,\ k_{10}x_4x_9,\ k_{11}x_{10},\ k_{12}x_{10},\ k_{13}x_{11}x_5,\ k_{14}x_{12},\ k_{15}x_{12},\ k_{16}x_5x_9,\ k_{17}x_{13},\ k_{18}x_{13},\ k_{19}x_{11}x_{14},\ k_{20}x_{15},\ k_{21}x_{15},\ k_{22}x_{11}x_{16},\ k_{23}x_{17},\ k_{24}x_{17},\ k_{25}x_{18}x_{19},\ k_{26}x_{20},\ k_{27}x_{20},\ k_{28}x_{16}x_{19},\ k_{29}x_{21},\ k_{30}x_{21},\ k_{31}x_{18}x_{22},\ k_{32}x_{23},\ k_{33}x_{23},\ k_{34}x_{16}x_5,\ k_{35}x_{24},\ k_{36}x_{24},\ k_{37}x_{18}x_4,\ k_{38}x_{25},\ k_{39}x_{25},\ k_{40}x_{26}x_5,\ k_{41}x_{27},\ k_{42}x_{27},\ k_{43}x_{18}x_{28},\ k_{44}x_{29},\ k_{45}x_{29},\ k_{46}x_{19}\big)^T$$

Conservation relations and total concentrations:

$c_1 := x_2 + x_3$

$c_2 := x_{10} + x_{11} + x_{12} + x_{13} + x_{15} + x_{17} + x_7 + x_8 + x_9$

$c_3 := x_{22} + x_{23}$

$c_4 := x_1 + x_{10} + x_{25} + x_{26} + x_{27} + x_3 + x_4 + x_6 + x_8$

$c_5 := x_{12} + x_{13} + x_{24} + x_{27} + x_5 + x_6$

$c_6 := x_{19} + x_{20} + x_{21} + x_{28} + x_{29}$

$c_7 := x_{14} + x_{15} + x_{16} + x_{17} + x_{18} + x_{20} + x_{21} + x_{23} + x_{24} + x_{25} + x_{29}$

Appendix C: Rate constants and steady states for the ERK-activation network

See Appendix Tables 8, 9.

Table 8 Vectors μ, z and steady states a, b for the network given in Fig. 2

	μ	z	a	b
1	0.3002	14	39.9853	53.9853
2	−0.1	−14	147.117	133.117
3	0.2002	14	63.1635	77.1635
4	0.7117	14	13.4946	27.4946
5	−0.5115	−13	32.4672	19.4672
6	0.2002	30	135.35	165.35
7	−0.8832	−14	23.8687	9.8687
8	−0.1715	−122	774.113	652.113
9	0.34	29	71.6142	100.614
10	1.0517	1	0.536909	1.53691
11	1.5632	29	7.684	36.684
12	1.0517	68	36.5098	104.51
13	−0.1715	−5	31.7259	26.7259
14	0.1402	13	86.3765	99.3765
15	1.7034	7	1.55812	8.55812
16	0.2913	13	38.4427	51.4427
17	1.8545	7	1.29906	8.29906
18	1.7123	13	2.86237	15.8624
19	1.5622	1	0.265301	1.2653
20	3.2745	13	0.511207	13.5112
21	1.8535	13	2.41541	15.4154
22	−0.7009	−13	25.8007	12.8007
23	1.0114	13	7.43092	20.4309
24	−0.2202	−110	556.563	446.563
25	2.424	5	0.485865	5.48586
26	2.9355	14	0.785153	14.7852
27	2.424	30	2.91519	32.9152
28	−0.1501	−40	286.989	246.989
29	1.5622	13	3.44891	16.4489

Table 9 Rate constants for the network given in Fig. 2

k_1	k_2	k_3	k_4	k_5	k_6	k_7	k_8	k_9	k_{10}
0.000339991	0.0158319	0.0158319	0.00456484	0.00738824	0.00738824	0.00620928	0.0012918	0.0012918	0.00206953
k_{11}	k_{12}	k_{13}	k_{14}	k_{15}	k_{16}	k_{17}	k_{18}	k_{19}	k_{20}
1.86251	1.86251	0.00801674	0.0273899	0.0273899	0.000860174	0.0315199	0.0315199	0.00352168	0.641799
k_{21}	k_{22}	k_{23}	k_{24}	k_{25}	k_{26}	k_{27}	k_{28}	k_{29}	k_{30}
0.858342	0.0044845	0.769786	0.249944	1.38255	1.95615	0.0976052	0.208277	0.414009	0.465426
k_{31}	k_{32}	k_{33}	k_{34}	k_{35}	k_{36}	k_{37}	k_{38}	k_{39}	k_{40}
0.0172617	0.134573	0.0369802	0.000972026	0.00179674	0.000383082	0.0517779	2.05819	2.05819	0.0784569
k_{41}	k_{42}	k_{43}	k_{44}	k_{45}	k_{46}				
0.343031	0.343031	0.00243466	0.289946	0.289946	3.7693				

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