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Multistationarity in the activation of a MAPK: Parametrizing the relevant region in parameter space

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Abstract

Mathematical models of biochemical reaction networks in the form of ordinary differential equations can exhibit all sorts of complex dynamical behaviour. It is for example known, that even a single layer of a MAPK cascade can exhibit bistability (i.e. there exist multiple (positive) steady state solutions). It is almost a common-place that bistability or some other form of multistationarity are observed in many biochemical reaction networks, especially if the focus is on signal transduction or cell cycle regulation. However, multistationarity is only exhibited if the parameter vector is located in an appropriate region of parameter space. To find these regions, for example by using numerical tools like bifurcation analysis, is a non-trivial task as it amounts to searching the whole parameter space. In this paper we show that for a model of a single layer of a MAPK cascade it is possible to derive analytical descriptions of these regions, if mass action kinetics are used. Moreover, our results give rise to a straightforward explanation for the 'robust yet fragile' behaviour in the activation of a MAPK.

Keywords: Activation of a MAPK; Multistationarity; Robust yet fragile; Systems of ordinary differential equations

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1. Introduction

Mitogen-activated protein kinase (MAPK) cascades are well studied systems in cell biology. The most common form of a MAPK cascade is that of a three-tiered cascade, as depicted in Fig. 1. Its building blocks are a MAPK, a MAPK kinase (MAPKK) and a MAPKK kinase (MAPKKK) together with the respective mono- and double-phosphorylated forms (-P denoting single phosphorylation and -PP double phosphorylation). Mono-phosphorylated MAPKKK catalyzes phosphorylation of MAPKK, whose double-phosphorylated form in turn catalyzes phosphorylation of MAPK. E_1 stands for a stimulus that triggers the cascade, MAPKKK′ase, MAPKK′ase and E_2 are phosphatases. (If the mammalian ERK-cascade were considered, E_1, for example, would be RAS, the MAPKKK Raf-1 and the MAPK ERK-1 [29]).

Mathematical models describing the dynamics of a MAPK cascade have been known for some time (see e.g. [14,18] or [27] for a more general overview of quantitative models for signal transduction networks in general, as well as [20] and the references therein). These models have been extensively studied in the literature using numerical tools like bifurcation analysis. It is therefore known that models of a MAPK cascade can exhibit all sorts of complex dynamical behaviour like bistability and oscillations (see e.g. [2,4] or [19,20]). Only recently, it was shown in [23] that, surprisingly, bistability can even occur on layer two or three of Fig. 1 alone, provided a distributive,

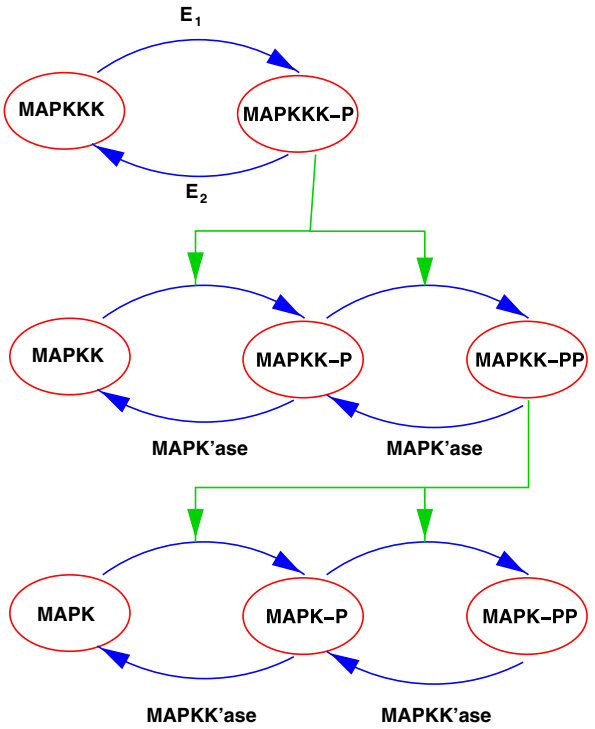

Fig. 1. Scheme of a MAPK cascade (c.f. [18]). MAPK denotes the mitogen-activated protein kinase, MAPKK the MAPK kinase and MPAKKK the MAPKK kinase, -P mono- and -PP double-phosphorylation. E_1 is a stimulus, *MAPK′ase*, *MAPKK′ase* and E_2 are phosphatases.

multi-collision mechanism is used for both, phosphorylation and dephosphorylation of the MAPK or the MAPKK [20,23].

For a MAPK cascade, as for any biochemical reaction network, only non-negative steady states are of interest, as only non-negative concentrations are meaningful. Bistability in this context therefore always refers to the existence of at least two positive or at least non-negative steady states. Multistationarity, a prerequisite for bistability, is only exhibited for certain combinations of parameter values (i.e. reaction rates and conserved moieties), that is, if the parameter vector is located in an appropriate region of parameter space. To find these regions numerically, e.g. by bifurcation analysis, is a non-trivial task as it amounts to searching the whole parameter space. Thus, an analytical method to determine these regions is desirable, as it would greatly simplify this task.

Due to the importance of positive steady states we focus in this paper on the existence of multiple positive steady states. We furthermore restrict our attention to systems endowed with mass action kinetics. To be more precise, for a particular reaction network corresponding to the activation of a MAPKK or MAPK we derive (i) a representation of two positive steady states a and b and (ii) a representation of all parameter vectors that ensure that a and b are steady state solutions to the ODEs derived from the reaction network.

It is possible to show that any pair of positive steady states together with the corresponding parameter vector can be obtained using this representation. As multistationarity requires the existence of at least two steady states we conclude that we have in fact found a parametrization of the region in parameter space where multistationarity can occur. It seems noteworthy that the method used to derive these results is not restricted to the system considered here. A suitable generalization to arbitrary biochemical reaction networks endowed with mass action kinetics seems possible and is under way.

As a consequence of our results it is possible to show that for the activation of a MAPK the property *multistationarity* is robust with respect to variation of parameters in a certain region of parameter space, whereas it is extremely sensitive to variations in an other, different region of parameter space. These results therefore give an explanation for the 'robust yet fragile' behaviour observed in many biochemical reaction networks (see e.g. [8,9]), at least for the activation of a MAPK.

The approach presented in this paper has been motivated by an in depth analysis of Feinberg's Chemical Reaction Network Theory (CRNT). CRNT connects the structure of a biochemical reaction network endowed with mass action kinetics to the ability of the network to admit at least two positive steady states. CRNT provides two algorithms for networks with different structural properties, called Deficiency One Algorithm (see e.g. [11,12]) and Advanced Deficiency Algorithm (see e.g. [10], both algorithms are implemented in a software called CRNT-toolbox, obtainable via [13]). These algorithms can be used to decide whether or not a given reaction network can admit multiple steady states at all. If this is the case, the algorithms determine two positive steady states a and b together with a parameter vector k such that a and b are steady state solutions to the system of ODEs derived from the network. If this is not the case, CRNT guarantees that no such parameter vector exists.

However, if multistationarity is possible, the parameter vector must be located in an appropriate region of parameter space and every element of this region will also lead to multistationarity. CRNT describes a formalism to construct distinct elements of this region, but it is not possible to obtain a representation of the region itself. Thus, the results obtained in this paper cannot be obtained using either the Deficiency One Algorithm or the Advanced Deficiency Algorithm. Nonetheless, as our approach was inspired by CRNT, we make in some cases use of the notation introduced

by CRNT. The development of our results greatly profited from the notation used to describe biochemical reaction networks introduced by Karin Gatermann [16,17] and Eduardo Sontag [30].

The paper is organized as follows: in Section 2, we introduce some notation, followed by a presentation of the reaction network and the associated mathematical model for the activation of a MAPK/MAPKK in Section 3. In Section 4, the main results are presented, while in Section 5, the implication for robustness and fragility of *multistationarity* are discussed. The paper closes with a discussion in Section 6. The algorithms used to obtain the representations of the steady and the parameter vector are given in Appendix A.

2. Notation

The following symbols are used frequently throughout the paper:

$\mathbb{R}^n$ ... the n-dimensional Euclidian space,
$\mathbb{R}^n_{>0}$ ... the positive orthant of $\mathbb{R}^n$,
$\mathbb{R}^n_{\geqslant 0}$ ... the non-negative orthant of $\mathbb{R}^n$.

Let p and q be positive integers. Then $\mathbb{R}^{p\times q}$ is used to denote the set of all $p\times q$ real valued matrices. For vectors $u, v \in \mathbb{R}^n$, we use $\langle u, v\rangle = \sum_{i=1}^n u_i v_i$ to denote the standard scalar product in $\mathbb{R}^n$ and v' to denote the transpose of vector v.

3. Activation of a MAPK(K)

The biochemical reaction network depicted in ($\mathcal{N}_1$) belongs to a family of network structures postulated as a model for a single layer of a MAPK cascade. This family of network structures has been extensively studied, see e.g. [20,23] or [6,7]. For notational convenience, we use A as a placeholder for either a MAPKK or a MAPK, E_1 for mono-phosphorylated MAPKKK or double-phosphorylated MAPKK and E_2 for MAPKK′ase or MAPK′ase.

$$\begin{aligned} &A + E_1 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AE_1 \overset{k_3}{\to} A_p + E_1 \underset{k_5}{\overset{k_4}{\rightleftharpoons}} A_pE_1 \overset{k_6}{\to} A_{pp} + E_1, \\ &A_{pp} + E_2 \underset{k_8}{\overset{k_7}{\rightleftharpoons}} A_{pp}E_2 \overset{k_9}{\to} A_p + E_2 \underset{k_{11}}{\overset{k_{10}}{\rightleftharpoons}} A_pE_2 \overset{k_{12}}{\to} A + E_2. \end{aligned} \qquad (\mathcal{N}_1)$$

This network involves the species A, A_p, A_{pp}, E_1, E_2, AE_1, A_pE_1, $A_{pp}E_2$, and A_pE_2. Each species can be associated with a continuous variable representing its concentration. We choose the following variables: x_1 for A, x_2 for E_1, x_3 for AE_1, x_4 for A_p, x_5 for A_pE_1, x_6 for A_{pp}, x_7 for E_2, x_8 for $A_{pp}E_2$ and x_9 for A_pE_2. The following ODEs can be derived from this reaction network (where $\dot{x}_i$ is used to denote the derivative with respect to time t and the argument t of the variables $x_i(t)$ is omitted for sake of brevity)

$$\dot{x}_1 = -k_1x_1x_2 + k_2x_3 + k_{12}x_9, \qquad (1a)$$
$$\dot{x}_2 = -k_1x_1x_2 + (k_2 + k_3)x_3 - k_4x_2x_4 + (k_5 + k_6)x_5, \qquad (1b)$$
$$\dot{x}_3 = k_1x_1x_2 - (k_2 + k_3)x_3, \qquad (1c)$$

$$\dot{x}_4 = k_3x_3 - k_4x_2x_4 + k_5x_5 + k_9x_8 - k_{10}x_4x_7 + k_{11}x_9, \tag{1d}$$
$$\dot{x}_5 = k_4x_2x_4 - (k_5 + k_6)x_5, \tag{1e}$$
$$\dot{x}_6 = k_6x_5 - k_7x_6x_7 + k_8x_8, \tag{1f}$$
$$\dot{x}_7 = -k_7x_6x_7 + (k_8 + k_9)x_8 - k_{10}x_4x_7 + (k_{11} + k_{12})x_9, \tag{1g}$$
$$\dot{x}_8 = k_7x_6x_7 - (k_8 + k_9)x_8, \tag{1h}$$
$$\dot{x}_9 = k_{10}x_4x_7 - (k_{11} + k_{12})x_9. \tag{1i}$$

The total concentrations of E_1, E_2 and A are constant. This leads to the conservation relation:

$$x_2 + x_3 + x_5 = c_1, \tag{2a}$$

for E_1, AE_1 and A_pE_1,

$$x_7 + x_8 + x_9 = c_2, \tag{2b}$$

for E_2, $A_{pp}E_2$ and A_pE_2 and

$$x_1 + x_3 + x_4 + x_5 + x_6 + x_8 + x_9 = c_3, \tag{2c}$$

for A, A_p, A_{pp}, AE_1, A_pE_1, A_pE_2 and $A_{pp}E_2$. Using vectors $x' = (x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8, x_9)$ and

$$w_1' = (0,1,1,0,1,0,0,0,0), \tag{3a}$$
$$w_2' = (0,0,0,0,0,0,1,1,1), \tag{3b}$$
$$w_3' = (1,0,1,1,1,1,0,1,1), \tag{3c}$$

the ODEs Eqs. (1a)–(1e), (1g)–(1i) and Eqs. (2a)–(2c) can be represented in the following compact form:

$$\begin{aligned} &\dot{x} = Nv(k,x), \\ &\langle w_i, x\rangle = c_i, \quad i = 1,2,3, \end{aligned} \tag{4}$$

where $N \in \mathbb{R}^{9\times 12}$ and $v(k,x) \in \mathbb{R}^{12}_{>0}$ are sometimes called the stoichiometric matrix and the vector of reaction rates. For the ODEs given in Eqs. (1a)–(1e), (1g)–(1i), N and $v(k,x)$ are

$$N = \begin{bmatrix} -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ -1 & 1 & 1 & -1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & 1 & -1 & 1 & 0 \\ 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 1 & -1 & 1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 \end{bmatrix} \tag{5a}$$

and

$$v(k,x)' = (k_1x_1x_2, k_2x_3, k_3x_3, k_4x_2x_4, k_5x_5, k_6x_5, k_7x_6x_7, k_8x_8, k_9x_8, k_{10}x_4x_7, k_{11}x_9, k_{12}x_9). \tag{5b}$$

As $k = (k_1, \ldots, k_{12})' \in \mathbb{R}^r_{>0}$, positive values of x will result in positive values of $v(k,x)$ (i.e. $x \in \mathbb{R}^9_{>0} \Rightarrow v(k,x) \in \mathbb{R}^{12}_{>0}$).

4. Steady states

Using the form presented in Eq. (4) the network depicted in Eq. ($\mathcal{N}_1$) is said to exhibit multistationarity, if two distinct positive vectors $a, b \in \mathbb{R}^9_{>0}$, a positive vector $k \in \mathbb{R}^{12}_{>0}$ and positive values c_i, $i = 1,2,3$, can be found such that the following conditions hold:

$$Nv(k,a) = 0, \tag{6a}$$

$$\langle w_i, a\rangle = c_i, \quad i = 1,2,3 \tag{6b}$$

and

$$Nv(k,b) = 0, \tag{7a}$$

$$\langle w_i, b\rangle = c_i, \quad i = 1,2,3. \tag{7b}$$

In the remainder of this section we solve Eqs. (6a), (6b) and (7a), (7b) for a, b, k and c_i. This is done in a stepwise manner by first processing Eqs. (6a) and (7a) to parametrize the k_i. Then Eqs. (6b) and (7b) are used to enforce $\langle w_i, a\rangle = \langle w_i, b\rangle$. Finally the resulting equations are solved and a, b, k and c_i are derived from this solution.

4.1. Processing Eqs. (6a) and (7a)

We are only interested in positive solutions to Eqs. (6a), (6b) and (7a), (7b). The definition of $v(\cdot,\cdot)$ then guarantees $v(k,a) \in \mathbb{R}^{12}_{>0}$ and $v(k,b) \in \mathbb{R}^{12}_{>0}$. The equations $Nv(k,a) = 0$ and $Nv(k,b) = 0$ are then equivalent to

$$v(k,a) \in \operatorname{int}(\ker(N) \cap \mathbb{R}^{12}_{\geqslant 0}), \tag{8a}$$

$$v(k,b) \in \operatorname{int}(\ker(N) \cap \mathbb{R}^{12}_{\geqslant 0}), \tag{8b}$$

where $\ker(N) \cap \mathbb{R}^{12}_{\geqslant 0}$ is the pointed polyhedral cone defined by the intersection of the null space of N, $\ker(N)$, with the non-negative orthant of $\mathbb{R}^{12}$, $\mathbb{R}^{12}_{\geqslant 0}$. As any pointed polyhedral cone, it can be represented by non-negative linear combinations of a finite set of generators or extreme rays (see [26]). The calculation of these generators is in general computationally hard, however, there exists a variety of algorithms and software tools (see e.g. [15,28]) and thus systems of up to a hundred reactions are manageable.

The aforementioned papers are part of the huge body of literature concerned with *metabolic flux analysis*, where each element of $\ker(N) \cap \mathbb{R}^{12}_{\geqslant 0}$ is interpreted as a particular *flux*, an allocation of values $v_i \in \mathbb{R}$ to each reaction of the network, such that the overall network is in steady state. An *elementary flux* is a an element $v \in \ker(N) \cap \mathbb{R}^{12}_{\geqslant 0}$ with a maximum number of zero entries. If every reaction in the network is 'irreversible' (in the terminology used in metabolic flux analysis), then elementary fluxes are equivalent to extreme rays generating $\ker(N) \cap \mathbb{R}^{12}_{\geqslant 0}$ (see [15]). (In the literature on metabolic flux analysis the term *irreversible reaction* is defined differently from the context of this paper: forward and backward reaction are represented as a *single edge in the direc-*

ted graph representing the reaction network. Thus, in particular, negative reaction rates $v_i(k,x)$ are possible. These correspond to reactions 'in opposite direction' (relative to the edge in the directed graph.) Therefore, in this setup, $x \in \mathbb{R}^n_{>0} \Rightarrow v_i(k,x) > 0$ is required only for *irreversible reactions*. In our setup on the other hand $x \in \mathbb{R}^n_{>0} \Rightarrow v_i(k,x) > 0$ for every reaction, thus all *reactions are irreversible in the sense of metabolic flux analysis*. As a consequence, it is possible to use the software tools from metabolic flux analysis to calculate the generators of $\ker(N) \cap \mathbb{R}^{12}_{\geqslant 0}$).

Let $\operatorname{supp}(E_j) = \{i \in \{1,\dots,r\} | E_{j_i} > 0,\}$ denote the support of vector E_j, i.e. the set of indices where E_j has non-zero values. Then, in accordance with [15], the generators E_i of $\ker(N) \cap \mathbb{R}^{12}_{\geqslant 0}$ are defined as follows: let $E_i, E_j \in \ker(N) \cap \mathbb{R}^{12}_{\geqslant 0}$ and

$$\operatorname{supp}(E_i) \subseteq \operatorname{supp}(E_j) \Rightarrow E_i = 0 \quad \text{or} \quad E_j = \alpha E_i, \quad \alpha \in \mathbb{R}_{>0}. \tag{9}$$

Generators are unique up to scalar multiplication: if E_i is an extreme ray, then αE_i, $\alpha \in \mathbb{R}_{>0}$ is also an extreme ray.

The vectors displayed in Eq. (10) correspond to generators $E_1,\dots,E_6$ for $\ker(N) \cap \mathbb{R}^{12}_{\geqslant 0}$ with N as in Eq. (5a).

$$\begin{aligned}
E'_1 &= (1,1,0,0,0,0,0,0,0,0,0,0),\\
E'_2 &= (0,0,0,1,1,0,0,0,0,0,0,0),\\
E'_3 &= (0,0,0,0,0,0,1,1,0,0,0,0),\\
E'_4 &= (0,0,0,0,0,0,0,0,0,0,1,1,0),\\
E'_5 &= (1,0,1,0,0,0,0,0,0,1,0,1),\\
E'_6 &= (0,0,0,1,0,1,1,0,1,0,0,0).
\end{aligned} \tag{10}$$

Using $E_1,\dots,E_6$ as columns of a matrix $E = [E_1,\dots,E_6]$, the conditions $v(k,a) \in \operatorname{int}(\ker(N) \cap \mathbb{R}^{12}_{\geqslant 0})$ and $v(k,b) \in \operatorname{int}(\ker(N) \cap \mathbb{R}^{12}_{\geqslant 0})$ can be recast as

$$v(k,a) = E\lambda, \quad \lambda \in \mathbb{R}^6_{>0}, \tag{11a}$$

$$v(k,b) = Ev, \quad v \in \mathbb{R}^6_{>0}. \tag{11b}$$

As $v_i > 0$, the application of $\ln(\cdot)$ to both sides of each equation is well defined. For Eq. (11a) we get

$$\ln a_1 + \ln a_2 + \ln k_1 = \ln(\lambda_1 + \lambda_5), \tag{12a}$$

$$\ln a_3 + \ln k_2 = \ln \lambda_1, \tag{12b}$$

$$\ln a_3 + \ln k_3 = \ln \lambda_5, \tag{12c}$$

$$\ln a_2 + \ln a_4 + \ln k_4 = \ln(\lambda_2 + \lambda_6), \tag{12d}$$

$$\ln a_5 + \ln k_5 = \ln \lambda_2, \tag{12e}$$

$$\ln a_5 + \ln k_6 = \ln \lambda_6, \tag{12f}$$

$$\ln a_6 + \ln a_7 + \ln k_7 = \ln(\lambda_3 + \lambda_6), \tag{12g}$$

$$\ln a_8 + \ln k_8 = \ln \lambda_3, \tag{12h}$$

$$\ln a_8 + \ln k_9 = \ln \lambda_6, \tag{12i}$$

$$\ln a_4 + \ln a_7 + \ln k_{10} = \ln(\lambda_4 + \lambda_5), \tag{12j}$$

$$\ln a_9 + \ln k_{11} = \ln \lambda_4, \tag{12k}$$
$$\ln a_9 + \ln k_{12} = \ln \lambda_5, \tag{12l}$$

and similar equations involving b_i instead of a_i and v_i instead of λ_i in the equations derived from Eq. (11b). Subtracting each of Eqs. (12a)–(12l) from its counterpart derived from Eq. (11b) and using $\mu_i := \ln \frac{b_i}{a_i}$ yields the equations

$$\mu_1 + \mu_2 = \ln \frac{v_1 + v_5}{\lambda_1 + \lambda_5}, \tag{13a}$$
$$\mu_3 = \ln \frac{v_1}{\lambda_1}, \tag{13b}$$
$$\mu_3 = \ln \frac{v_5}{\lambda_5}, \tag{13c}$$
$$\mu_2 + \mu_4 = \ln \frac{v_2 + v_6}{\lambda_2 + \lambda_6}, \tag{13d}$$
$$\mu_5 = \ln \frac{v_2}{\lambda_2}, \tag{13e}$$
$$\mu_5 = \ln \frac{v_6}{\lambda_6}, \tag{13f}$$
$$\mu_6 + \mu_7 = \ln \frac{v_3 + v_6}{\lambda_3 + \lambda_6}, \tag{13g}$$
$$\mu_8 = \ln \frac{v_3}{\lambda_3}, \tag{13h}$$
$$\mu_8 = \ln \frac{v_6}{\lambda_6}, \tag{13i}$$
$$\mu_4 + \mu_7 = \ln \frac{v_4 + v_5}{\lambda_4 + \lambda_5}, \tag{13j}$$
$$\mu_9 = \ln \frac{v_4}{\lambda_4}, \tag{13k}$$
$$\mu_9 = \ln \frac{v_5}{\lambda_5}. \tag{13l}$$

This set of 12 equations is solvable, if and only if

$$\ln \frac{v_1 + v_5}{\lambda_1 + \lambda_5} = \ln \frac{v_1}{\lambda_1} = \ln \frac{v_5}{\lambda_5} = \ln \frac{v_4}{\lambda_4} = \ln \frac{v_4 + v_5}{\lambda_4 + \lambda_5}$$

and

$$\ln \frac{v_2 + v_6}{\lambda_2 + \lambda_6} = \ln \frac{v_2}{\lambda_2} = \ln \frac{v_6}{\lambda_6} = \ln \frac{v_3}{\lambda_3} = \ln \frac{v_3 + v_6}{\lambda_3 + \lambda_6}.$$

Using $\ln\frac{v_3}{\lambda_3}$ and $\ln\frac{v_4}{\lambda_4}$ to represent the right hand side of the equations and solving for μ finally yields

$$\mu = \mu_7(-1,1,0,-1,0,-1,1,0,0)' + \ln\frac{v_3}{\lambda_3}(-1,1,0,0,1,1,0,1,0)' + \ln\frac{v_4}{\lambda_4} \times (2,-1,1,1,0,0,0,0,1)'. \tag{14}$$

If in Eq. (14) $\ln\frac{v_3}{\lambda_3}$ and $\ln\frac{v_4}{\lambda_4}$ are interpreted as free parameters, then any element μ of the corresponding linear subspace $\mathcal{M}$ can be associated with the difference of two positive steady state solutions: if a satisfies $Nv(k,a)=0$ and $v(k,a)\in\mathbb{R}^{12}_{\geqslant 0}$ (i.e. $v(k,a)=E\alpha$) and if $\mu\in\mathcal{M}$ then $b=\mathrm{diag}(\mathrm{Exp}(\mu))a$ satisfies $Nv(k,b)=0$, with $v(k,b)=E\beta$, where $\beta_i=\frac{v_4}{\lambda_4}\alpha_i$ and $\beta_j=\frac{v_3}{\lambda_3}\alpha_j$, $i=1,4,5$, $j=2,3,6$ and $\mathrm{Exp}(\mu)=(e^{\mu_1},\ldots,e^{\mu_9})'$.

To find all $a,b\in\mathbb{R}^9_{>0}$ that satisfy Eqs. (6a) and (6b) as well as Eqs. (7a) and (7b) two steps remain: (i) to ensure that a is indeed a steady state solution (i.e. to show for a particular a, that $\exists k$ such that $Nv(a,k)=0$) and (ii) to ensure that Eqs. (6b) and (7b) hold (i.e. that $\langle w_i,a\rangle=c_i$ and $\langle w_i,b\rangle=c_i$, $i=1,2,3$). (i) is easy. Simply solve Eqs. (12a)–(12l) for the k_i

$$k_1=\frac{\lambda_1+\lambda_5}{a_1a_2}, \tag{15a}$$

$$k_2=\frac{\lambda_1}{a_3}, \tag{15b}$$

$$k_3=\frac{\lambda_5}{a_3}, \tag{15c}$$

$$k_4=\frac{\lambda_2+\lambda_6}{a_2a_4}, \tag{15d}$$

$$k_5=\frac{\lambda_2}{a_5}, \tag{15e}$$

$$k_6=\frac{\lambda_6}{a_5}, \tag{15f}$$

$$k_7=\frac{\lambda_3+\lambda_6}{a_6a_7}, \tag{15g}$$

$$k_8=\frac{\lambda_3}{a_8}, \tag{15h}$$

$$k_9=\frac{\lambda_6}{a_8}, \tag{15i}$$

$$k_{10}=\frac{\lambda_4+\lambda_5}{a_4a_7}, \tag{15j}$$

$$k_{11}=\frac{\lambda_4}{a_9}, \tag{15k}$$

$$k_{12}=\frac{\lambda_5}{a_9}. \tag{15l}$$

Given $a>0$, $\lambda>0$ define k as in Eqs. (15a)–(15l). Then $Nv(a,k)=0$ and $v(a,k)\in\mathbb{R}^{12}_{>0}$ (i.e. $v(a,k)=E\lambda$). Choose μ as in Eq. (14) and define $b=\mathrm{diag}(\mathrm{Exp}(\mu))a$. Then $Nv(b,k)=0$ and

$v(b,k) \in \mathbb{R}^{12}_{>0}$ (i.e. $v(b,k) = Ev$). Thus it remains to find all those a and b that satisfy $\langle w_i, a\rangle = \langle w_i, b\rangle = c_i > 0$, $i = 1,2,3$.

4.2. Processing Eqs. (6b) and (7b)

In the previous section an expression related to the difference between any pair of positive steady state solutions was derived. Here we want to pave the way for an answer to the question which of these pairs satisfy the conservation relations Eqs. (6b) and (7b). As it turns out, the answer to this question is related to a more general question that can be stated and answered in $\mathbb{R}^n$. To this end, in a first step, the conservation relations are transformed in the following way: subtracting Eq. (6b) from Eq. (7b) yields three equations

$$\langle w_i, b - a\rangle = 0, \quad i = 1,2,3.$$

Thus the w_i, $i = 1,2,3$, are orthogonal to $b - a$. Using U to denote the linear subspace spanned by w_i, $i = 1,2,3$ and S to denote its orthogonal complement (i.e. $S = \{v \in \mathbb{R}^9 | \langle v, w_i\rangle = 0,\ i = 1,2,3\}$), the following condition can be derived from Eqs. (6b) and (7b) (in the literature on Chemical Reaction Network Theory S is called the *stoichiometric subspace*, see e.g. [11,12]):

$$b - a \in S. \tag{16}$$

Now two linear subspaces $\mathcal{M}$ and S of $\mathbb{R}^9$ are given and we are interested in determining vectors $a \in \mathbb{R}^9_{>0}$ and $b \in \mathbb{R}^9_{>0}$ such that $\mu \in \mathcal{M}$, where $\mu_i := \ln \frac{b_i}{a_i}$, $i = 1,\ldots,9$, and $b - a \in S$ hold. This question can be posed in a more general setting: let M_1 and M_2 be two subsets of $\mathbb{R}^n$, not necessarily linear subspaces. The following lemma gives necessary and sufficient conditions for the existence of two vectors $p \in \mathbb{R}^n_{>0}$ and $q \in \mathbb{R}^n_{>0}$ with the following properties:

$$w \in M_1, \tag{17a}$$

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

$$q - p \in M_2. \tag{17b}$$

To state the lemma, we need to introduce some additional notation: for any vector $u \in \mathbb{R}^n$ denote by σ(u) the sign pattern of u. Then $v = \sigma(u)$ is a vector with entries $v_i \in \{+, -, 0\}$ depending on whether $u_i > 0$, $u_i < 0$ or $u_i = 0$, respectively, i.e. v is a sign vector.

Lemma 1. *Let $M_1 \subseteq \mathbb{R}^n$ and $M_2 \subseteq \mathbb{R}^n$ be two non-trivial subsets of $\mathbb{R}^n$ and define $M_3 := \{(m_1, m_2) \in M_1 \times M_2 | \sigma(m_1) = \sigma(m_2)\}$ 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 the properties given in Eqs. (17a) and (17b) exist, if and only if $M_3 \neq \emptyset$. Then p and q are given by*

$$p_i = \begin{cases} \frac{m_{2i}}{e^{m_{1i}} - 1}, & \text{if } m_{1i} \neq 0 \\ \bar{p}_i > 0, & \text{if } m_{1i} = 0 \end{cases} \tag{18}$$

where $\bar{p}_i$ denotes an arbitrary positive number and

$$q_i = e^{m_{1i}} p_{i,i=1,\ldots,n.} \tag{19}$$

Proof. Suppose p and q with the properties given in Eqs. (17a) and (17b) exist. Recall that $w_i = \ln\frac{q_i}{p_i}$. Then it remains to show that $M_3 \neq \emptyset$. As $w_i < 0$ iff $q_i - p_i < 0$, $w_i > 0$ iff $q_i - p_i > 0$ and $w_i = 0$ iff $q_i - p_i = 0$ one has $\sigma(w) = \sigma(q-p)$. As, by assumption, $w \in M_1$ and $q - p \in M_2$ the desired result $M_3 \neq \emptyset$ follows.

Suppose, on the other hand, $M_3 \neq \emptyset$. Then it remains to show that p and q as defined in Eqs. (18) and (19) have the properties defined in Eqs. (17a) and (17b). Pick any pair $(m_1, m_2) \in M_3$ and suppose $m_{1i} \neq 0$, $i = 1, \ldots, n$. Then

$$q_i - p_i = e^{m_{1i}} p_i - p_i = p_i(e^{m_{1i}} - 1) = m_{2i} \quad \text{and} \quad \ln\frac{q_i}{p_i} = m_{1i}$$

Otherwise, if $m_{1i} = 0$ for some $i \in \{1, \ldots, n\}$, then $q_i = p_i = \bar{p}_i$ and $m_{2i} = q_i - p_i = 0$.

Thus $q - p = m_2 \in M_2$ and $(\ln\frac{q_i}{p_i})_{i=1,\ldots,n} = m_1 \in M_1$. $p \in \mathbb{R}^n_{>0}$ follows from $\sigma(m_2) = \sigma(m_1)$ and the fact that $\sigma(e^{m_{1i}} - 1) = \sigma(m_{1i})$, $i = 1, \ldots, n$, positivity of q follows from positivity of p. □

Based on Lemma 1, it is, at least in principle, possible to prove that a certain network structure cannot admit multiple (positive) steady state solutions. Due to the importance of this consequence we state it here as the following corollary:

Corollary 1. *Suppose it is possible to derive a representation of the difference of any pair of positive steady states, similar to μ in Eq. (14) for network ($\mathcal{N}_1$). Identify this set with M_1 and the conservation relations with M_2. If $M_3 = \emptyset$, then, as a consequence of Lemma 1, multistationarity is excluded (in the sense, that no pair of positive steady state solutions contained in M_1 satisfies the conservation relations for the same right hand side, represented by M_2).*

Results similar to Lemma 1 have been known in the literature on Feinberg's Chemical Reaction Network Theory for some time [11]. In fact, both the Deficiency One Algorithm and the Advanced Deficiency Algorithm utilize the way p and q are constructed in Eqs. (18) and (19) (see [11,12] for the Deficiency One Algorithm and [10] for the Advanced Deficiency Algorithm).

4.3. Multiple positive steady states

Now the results obtained in Sections 4.1 and 4.2 can be combined to derive the desired analytical expression for the steady state solutions a and b and the corresponding parameter vector k. As a consequence of Lemma 1, a, b and k depend crucially on the vector μ and its sign pattern. For the system under study all μ that can be derived from the conditions $Nv(k,a) = 0$ and $Nv(k,b) = 0$ are given in Eq. (14). That is, if a and b are solutions to $Nv(k,a) = 0$ and $Nv(k,b) = 0$ for some parameter vector k, then the vector μ with $\mu_i := \ln\frac{b_i}{a_i}$ is an element of the subspace displayed in Eq. (14). Thus we identify M_1 with the linear subspace $\mathcal{M}$ and M_2 with the linear subspace S. Eqs. (18) and (19) point out how a particular $\mu \in \mathcal{M}$ can be used to derive the (positive) steady states a and b: choose any vector $v \in S$ with $\sigma(v) = \sigma(\mu)$ and define as in Eq. (18):

$$\begin{aligned} &a = (a_i), \quad i = 1, \ldots, 9, \\ &a_i = \begin{cases} \frac{v_i}{e^{\mu_i} - 1}, & \text{if } \mu_i \neq 0, \\ \bar{a}_i > 0, & \text{if } \mu_i = 0. \end{cases} \end{aligned} \tag{20}$$

Once a is determined, b and the vector k of reaction rates are determined as well. As in Eq. (19) define

$$b = \mathrm{diag}(\mathrm{Exp}(\mu))a. \tag{21}$$

Once a and b are determined, the c_i's are determined as well. They can be derived from the conservation relations given in Eq. (6b) or Eq. (7b) (note that $\langle w_i, a\rangle = \langle w_i, b\rangle$ holds as a consequence of Lemma 1)

$$c_i = \langle w_i, a\rangle = \langle w_i, b\rangle, \quad i = 1, 2, 3.$$

Eqs. (20) and (21) define parametrizations of all positive a and b in terms of $v \in S$ and μ as defined in Eq. (14). Since μ, defined by Eq. (14), has three and $v \in S$ has six degrees of freedom, a and b are parameterized using nine parameters in total. However, μ and v are not independent, the condition $\sigma(\mu) = \sigma(v)$ must be satisfied.

Due to the condition $\sigma(\mu) = \sigma(v)$ this parametrization is somewhat cumbersome: suppose $\mu \in \mathcal{M}$ is given. To determine a $v \in S$ with $\sigma(\mu) = \sigma(v)$, a system of linear equations and inequalities has to be solved. Moreover, the condition $\sigma(\mu) = \sigma(v)$ will be satisfied by infinitely many vectors: suppose, for a particular μ, a vector $v \in S$ with $\sigma(v) = \sigma(\mu)$ can be determined. Then all vectors αv with $\alpha > 0$ also satisfy $\sigma(v) = \sigma(\mu)$.

Thus, to obtain a more practical parametrization, the condition $\sigma(\mu) = \sigma(v)$ is restated using pointed polyhedral cones. To formalize the discussion, some additional notation is necessary: let $\delta \in \{-1, 0, 1\}^9$ be a vector composed of entries $+1$, 0 and -1. It is used to denote a particular orthant $\mathbb{R}^9_\delta := \{x \in \mathbb{R}^9 | \sigma(x) = \delta\}$. In this notation the positive orthant, for example, is represented by the vector $\delta' = (1, 1, 1, 1, 1, 1, 1, 1, 1)$, and the hyperplane $x_1 = 0$ separating $(1, 1, 1, 1, 1, 1, 1, 1, 1)$ from $(-1, 1, 1, 1, 1, 1, 1, 1, 1)$ is denoted by $(0, 1, 1, 1, 1, 1, 1, 1, 1)$.

Let $\mu \in \mathcal{M}$, $v \in S$ and suppose $\sigma(\mu) = \sigma(v)$. Obviously μ and v are contained in the same orthant. Let δ denote this orthant. Then every element $\tilde{v}$ of the pointed polyhedral cone $S \cap \mathbb{R}^9_\delta$ satisfies $\sigma(\tilde{v}) = \sigma(\mu) = \delta$. Obviously this also works the other way around: every element $\tilde{\mu}$ of the pointed polyhedral cone $\mathcal{M} \cap \mathbb{R}^9_\delta$ satisfies $\sigma(\tilde{\mu}) = \sigma(v) = \delta$ as well.

As a consequence a, b and k can be recast using the aforementioned cones $\mathcal{M} \cap \mathbb{R}^9_\delta$ and $S \cap \mathbb{R}^9_\delta$. Let $\Delta := \{\delta \in \{-1, 0, 1\}^9 | \mathcal{M} \cap \mathbb{R}^9_\delta \neq \emptyset \text{ and } S \cap \mathbb{R}^9_\delta \neq \emptyset\}$. Using elements $\delta \in \Delta$, a representation of a^δ, b^δ, k^δ can be derived in terms of the generators of $\mathcal{M} \cap \mathbb{R}^9_\delta$ and $S \cap \mathbb{R}^9_\delta$: let $E_1^{S^\delta}, \ldots, E_{p_{S^\delta}}^{S^\delta}$ be a set of generators for the cone $S \cap \mathbb{R}^9_\delta$ and define the matrix $E^{S^\delta} = [E_1^{S^\delta}, \ldots, E_{p_{S^\delta}}^{S^\delta}]$. Further let $E_1^{\mathcal{M}^\delta}, \ldots, E_{p_{\mathcal{M}^\delta}}^{\mathcal{M}^\delta}$ be a set of generators for the cone $\mathcal{M} \cap \mathbb{R}^9_\delta$ and define the matrix $E^{\mathcal{M}^\delta} = [E_1^{\mathcal{M}^\delta}, \ldots, E_{p_{\mathcal{M}^\delta}}^{\mathcal{M}^\delta}]$. Then, for a particular orthant δ, all $\mu \in \mathbb{R}^9_\delta$ can be represented by

$$\mu^\delta = E^{\mathcal{M}^\delta} \alpha^\delta, \quad \alpha^\delta \in \mathbb{R}^{p_{\mathcal{M}^\delta}}_{\geqslant 0} \tag{22}$$

all $v \in S \cap \mathbb{R}^9_\delta$ can be represented by

$$v^\delta = E^{S^\delta} \beta^\delta, \quad \beta^\delta \in \mathbb{R}^{p_{S^\delta}}_{\geqslant 0}. \tag{23}$$

Using Eqs. (22) and (23) in Eq. (20) the vector a can be parameterized in terms of α^δ and β^δ. The symbol a^δ is used to denote this parametrization of a using $\mathcal{M} \cap \mathbb{R}^9_\delta$ and $S \cap \mathbb{R}^9_\delta$. Using a^δ in Eq. (21) and Eqs. (15a)–(15l) yields parametrizations of b^δ and k_j^δ, $j = 1, \ldots, 12$, in terms of α^δ and β^δ. Thus, for each orthant $\delta \in \Delta$ (i.e. each orthant containing $\mathcal{M}$ as well as S) a different representation of a, b and k can be derived.

Before Δ corresponding to network $(\mathcal{N}_1)$ is specified it seems worthwhile to discuss the following property of $\mathcal{M}$ and S: obviously, whenever $v \in S(\mu \in \mathcal{M})$ holds, $-v \in S(-\mu \in \mathcal{M})$ holds as well. In terms of orthants this is equivalent to the fact, that whenever $\delta \in \Delta$, then $-\delta \in \Delta$ as well. It is sufficient to consider either δ or $-\delta$: all representations $a^{-\delta}$, $b^{-\delta}$, $k^{-\delta}$ can be obtained from representations a^{δ}, b^{δ}, k^{δ} as $a^{-\delta} = b^{\delta}$, $b^{-\delta} = a^{\delta}$ and $k^{-\delta} = k^{\delta}$. To see this, let $\mu_1^{\delta} \in \mathcal{M} \cap \mathbb{R}^9_{\delta}$, $v_1^{\delta} \in S \cap \mathbb{R}^9_{\delta}$ and $\mu_2^{-\delta} \in \mathcal{M} \cap \mathbb{R}^9_{-\delta}$, $v_2^{-\delta} \in S \cap \mathbb{R}^9_{-\delta}$ with $\mu_2^{-\delta} = -\mu_1^{\delta}$ and $v_2^{-\delta} = -v_1^{\delta}$. Without loss of generality assume $\mu_{1i}^{\delta} \neq 0$, $\mu_{2i}^{-\delta} \neq 0$, $v_{1i}^{\delta} \neq 0$, $v_{2i}^{-\delta} \neq 0$, $i = 1, \ldots, 9$. Then $a_{1i}^{\delta} = \frac{v_{1i}^{\delta}}{e^{\mu_{1i}^{\delta}} - 1}$ and $b_{1i} = e^{\mu_{1i}^{\delta}} a_{1i}$. Using $v_{2i}^{-\delta} = -v_{1i}^{\delta}$ and $\mu_{2i}^{-\delta} = -\mu_{1i}^{\delta}$ in $a_{2i}^{-\delta}$ yields

$$a_{2i}^{-\delta} = \frac{v_{2i}^{-\delta}}{e^{\mu_{2i}^{-\delta}} - 1} = \frac{-v_{1i}^{\delta}}{e^{-\mu_{1i}^{\delta}} - 1} = \frac{-v_{1i}^{\delta}}{e^{-\mu_{1i}^{\delta}}(1 - e^{\mu_{i1}^{\delta}})} = \frac{1}{e^{-\mu_{1i}^{\delta}}} \frac{v_{1i}^{\delta}}{e^{\mu_{1i}^{\delta}} - 1} = e^{\mu_{1i}^{\delta}} \frac{v_{1i}^{\delta}}{e^{\mu_{1i}^{\delta}} - 1} = b_{1i}^{\delta}.$$

In a similar way $b_{2i}^{-\delta} = a_{1i}^{\delta}$ can be derived. As $k_j^{(a^{\delta})} = k_j^{(b^{\delta})}$, $j = 1, \ldots, 12$, we conclude, that in fact all representations $a^{-\delta}$, $b^{-\delta}$, $k^{-\delta}$ can be derived from a^{δ}, b^{δ}, k^{δ} as $a^{-\delta} = b^{\delta}$, $b^{-\delta} = a^{\delta}$ and $k^{-\delta} = k^{\delta}$.

Using $\mathcal{M}$ and S for our example, the elements of Δ have been determined (note that $\delta \in \Delta$ implies $-\delta \in \Delta$ as well; thus Δ contains in fact 14 elements)

$$\begin{aligned}
\delta_1 &= (-1,-1,-1,1,1,1,-1,1,-1)', \\
\delta_2 &= (-1,0,-1,1,1,1,-1,1,-1)', \\
\delta_3 &= (-1,1,-1,-1,1,1,-1,1,-1)', \\
\delta_4 &= (-1,1,-1,-1,1,1,0,1,-1)', \\
\delta_5 &= (-1,1,-1,-1,1,1,1,1,-1)', \\
\delta_6 &= (-1,1,-1,0,1,1,-1,1,-1)', \\
\delta_7 &= (-1,1,-1,1,1,1,-1,1,-1)'.
\end{aligned} \tag{24}$$

For each orthant $\mathbb{R}^9_{\delta_i}$, the vectors μ^{δ_i} and v^{δ_i} have been determined using the generators of $\mathcal{M} \cap \mathbb{R}^9_{\delta_i}$ and $S \cap \mathbb{R}^9_{\delta_i}$ given in Eq. (24). Table 1 contains the μ^{δ_i}, $i = 1, \ldots, 7$, and Table 2 the v^{δ_i}, $i = 1, \ldots, 7$. Using these data, a set $(a^{\delta_i}, b^{\delta_i}, k^{\delta_i})$ of steady states and rate constants can be assembled for each orthant $\mathbb{R}^9_{\delta_i}$.

Using the data given in Tables 1 and 2 it is therefore possible to give analytical expressions for any pair of positive steady states a^{δ_i}, b^{δ_i} and, for each pair, all parameter vectors k^{δ_i} that ensure that the ODEs displayed in Eqs. (1a)–(1e), (1g)–(1i) and the conservation relations given in Eqs. (2a)–(2c) admit a^{δ_i} and b^{δ_i} as steady state solutions. The algorithms used to obtain the results

Table 1
Representations of μ^{δ_i} in the orthants $\mathbb{R}^9_{\delta_i}$ that contain $\mathcal{M}$ and S

$\mathbb{R}^9_{\delta_i}$	$p_{\mathcal{M}^{\delta i}}$	Representation of $\mu^{\delta_i} \in \mathcal{M} \cap \mathbb{R}^9_{\delta_i}$
$\mathbb{R}^9_{\delta_1}$	3	$\mu^{\delta_1} = (-\alpha_1, -\alpha_2, -\alpha_1 - \alpha_2, \alpha_2 + \alpha_3, \alpha_3, \alpha_1 + 2\alpha_2 + 2\alpha_3, -\alpha_1 - 2\alpha_2 - \alpha_3, \alpha_3, -\alpha_1 - \alpha_2)'$
$\mathbb{R}^9_{\delta_2}$	3	$\mu^{\delta_2} = (-\alpha_3, 0, -\alpha_3, \alpha_2, \alpha_2, 2\alpha_2 + \alpha_3, -\alpha_2 - \alpha_3, \alpha_2, -\alpha_3)'$
$\mathbb{R}^9_{\delta_3}$	3	$\mu^{\delta_3} = (-2\alpha_1 - \alpha_2 - \alpha_3, \alpha_1 + \alpha_2, -\alpha_1 - \alpha_3, -\alpha_1, \alpha_2, \alpha_2 + \alpha_3, -\alpha_3, \alpha_2, -\alpha_1 - \alpha_3)'$
$\mathbb{R}^9_{\delta_4}$	3	$\mu^{\delta_4} = (-\alpha_1 - 2\alpha_3, \alpha_1 + \alpha_3, -\alpha_3, -\alpha_3, \alpha_1, \alpha_1, 0, \alpha_1, -\alpha_3)'$
$\mathbb{R}^9_{\delta_5}$	3	$\mu^{\delta_5} = (-\alpha_1 - 2\alpha_2 - 2\alpha_3, \alpha_1 + 2\alpha_2 + \alpha_3, -\alpha_3, -\alpha_2 - \alpha_3, \alpha_1 + \alpha_2, \alpha_1, \alpha_2, \alpha_1 + \alpha_2, -\alpha_3)'$
$\mathbb{R}^9_{\delta_6}$	3	$\mu^{\delta_6} = (-\alpha_1 - \alpha_3, \alpha_1, -\alpha_3, 0, \alpha_1, \alpha_1 + \alpha_3, -\alpha_3, \alpha_1, -\alpha_3)'$
$\mathbb{R}^9_{\delta_7}$	3	$\mu^{\delta_7} = (-\alpha_1 - \alpha_3, \alpha_1, -\alpha_3, \alpha_2, \alpha_1 + \alpha_2, \alpha_1 + 2\alpha_2 + \alpha_3, -\alpha_2 - \alpha_3, \alpha_1 + \alpha_2, -\alpha_3)'$

Table 2
Representations of $v^{\delta_i} \in S \cap \mathbb{R}^9_{\delta_i}$ in orthants $\mathbb{R}^9_{\delta_i}$ that contain both, $\mathcal{M}$ and S

$\mathbb{R}^9_{\delta_i}$	$p_{S^{\delta_i}}$	Representation of $v^{\delta_i} \in S \cap \mathbb{R}^9_{\delta_i}$
$\mathbb{R}^9_{\delta_1}$	6	$v^{\delta_1} = (-\beta_1 - \beta_3 - \beta_4 - \beta_5, -\beta_1, -\beta_2, \beta_3, \beta_1 + \beta_2, \beta_4, -\beta_5, \beta_5 + \beta_6, -\beta_6)'$
$\mathbb{R}^9_{\delta_2}$	6	$v^{\delta_2} = (-\beta_3 - \beta_4 - \beta_5, 0, -\beta_2, \beta_3, \beta_2, \beta_4, -\beta_5, \beta_5 + \beta_6, -\beta_6)'$
$\mathbb{R}^9_{\delta_3}$	8	$v^{\delta_3} = (-\beta_3 - \beta_6, \beta_4 + \beta_7, -\beta_1 - \beta_4 - \beta_7, -\beta_5 - \beta_8, \beta_1, \beta_6 + \beta_7 + \beta_8, -\beta_3 - \beta_4 - \beta_5, \beta_2 + \beta_3 + \beta_4 + \beta_5, -\beta_2)'$
$\mathbb{R}^9_{\delta_4}$	6	$v^{\delta_4} = (-\beta_1, \beta_2, -\beta_2 - \beta_4, -\beta_3, \beta_4, \beta_1 + \beta_2 + \beta_3, 0, \beta_6, -\beta_6)'$
$\mathbb{R}^9_{\delta_5}$	6	$v^{\delta_5} = (-\beta_1, \beta_2, -\beta_2 - \beta_4, -\beta_3, \beta_4, \beta_1 + \beta_2 + \beta_3 + \beta_5, \beta_5, \beta_6, -\beta_5 - \beta_6)'$
$\mathbb{R}^9_{\delta_6}$	7	$v^{\delta_6} = (-\beta_4 - \beta_6, \beta_5 + \beta_7, -\beta_2 - \beta_5 - \beta_7, 0, \beta_2, \beta_6 + \beta_7, -\beta_4 - \beta_5, \beta_3 + \beta_4 + \beta_5, -\beta_3)'$
$\mathbb{R}^9_{\delta_7}$	8	$v^{\delta_7} = (-\beta_3 - \beta_5 - \beta_6, \beta_4 + \beta_7 + \beta_8, -\beta_1 - \beta_4 - \beta_7 - \beta_8, \beta_5 + \beta_7, \beta_1, \beta_6 + \beta_8, -\beta_3 - \beta_4, \beta_2 + \beta_3 + \beta_4, -\beta_2)'$

presented in Tables 1 and 2 are given in the supplementary material to this article. The matlab M-files are available upon request.

As an example the parametrizations a^{δ_5}, b^{δ_5} and $k_1^{\delta_5}, \ldots, k_{12}^{\delta_5}$ are given. From Table 1 the cone $\mathcal{M} \cap \mathbb{R}^9_{\delta_5}$ can be parameterized as $\mu^{\delta_5} = (-\alpha_1 - 2\alpha_2 - 2\alpha_3, \alpha_1 + 2\alpha_2 + \alpha_3, -\alpha_3, -\alpha_2 - \alpha_3, \alpha_1 + \alpha_2, \alpha_1, \alpha_2, \alpha_1 + \alpha_2, -\alpha_3)'$. From Table 2 it follows that $v \in S \cap \mathbb{R}^9_{\delta_5}$ can be parameterized as $v^{\delta_5} = (-\beta_1, \beta_2, -\beta_2 - \beta_4, -\beta_3, \beta_4, \beta_1 + \beta_2 + \beta_3 + \beta_5, \beta_5, \beta_6, -\beta_5 - \beta_6)'$. Inserting μ^{δ_5} and v^{δ_5} in Eq. (20) yields a parametrization of a^{δ_5} in terms of α_1, α_2, α_3 and $\beta_1, \ldots, \beta_6$, $\alpha_i \geqslant 0$, $\beta_i \geqslant 0$:

$$a^{\delta_5} = \left(-\frac{\beta_1}{-1 + e^{-\alpha_1 - 2\alpha_2 - 2\alpha_3}}, \frac{\beta_2}{-1 + e^{\alpha_1 + 2\alpha_2 + \alpha_3}}, \frac{-\beta_2 - \beta_4}{-1 + e^{-\alpha_3}}, \right.$$
$$\left. -\frac{\beta_3}{-1 + e^{-\alpha_2 - \alpha_3}}, \frac{\beta_4}{-1 + e^{\alpha_1 + \alpha_2}}, \frac{\beta_1 + \beta_2 + \beta_3 + \beta_5}{-1 + e^{\alpha_1}}, \frac{\beta_5}{-1 + e^{\alpha_2}}, \frac{\beta_6}{-1 + e^{\alpha_1 + \alpha_2}}, \frac{-\beta_5 - \beta_6}{-1 + e^{-\alpha_3}} \right)'. \tag{25}$$

Using Eq. (25) and μ^{δ_5} in Eq. (21) yields the following parametrization of b^{δ_5}:

$$b^{\delta_5} = \left(-\frac{\beta_1 e^{-\alpha_1 - 2\alpha_2 - 2\alpha_3}}{-1 + e^{-\alpha_1 - 2\alpha_2 - 2\alpha_3}}, \frac{\beta_2 e^{\alpha_1 + 2\alpha_2 + \alpha_3}}{-1 + e^{\alpha_1 + 2\alpha_2 + \alpha_3}}, \frac{(-\beta_2 - \beta_4) e^{-\alpha_3}}{-1 + e^{-\alpha_3}}, \right.$$
$$\left. -\frac{\beta_3 e^{-\alpha_2 - \alpha_3}}{-1 + e^{-\alpha_2 - \alpha_3}}, \frac{\beta_4 e^{\alpha_1 + \alpha_2}}{-1 + e^{\alpha_1 + \alpha_2}}, \frac{(\beta_1 + \beta_2 + \beta_3 + \beta_5) e^{\alpha_1}}{-1 + e^{\alpha_1}}, \frac{\beta_5 e^{\alpha_2}}{-1 + e^{\alpha_2}}, \frac{\beta_6 e^{\alpha_1 + \alpha_2}}{-1 + e^{\alpha_1 + \alpha_2}}, \frac{(-\beta_5 - \beta_6) e^{-\alpha_3}}{-1 + e^{-\alpha_3}} \right)'. \tag{26}$$

Using Eq. (25) in Eqs. (15a)–(15l) yields a parametrization of $k_1^{\delta_5}, \ldots, k_{12}^{\delta_5}$ in terms of α_1, α_2, α_3, $\beta_1, \ldots, \beta_6$, $\alpha_i \geqslant 0$, $\beta_i \geqslant 0$ and $\lambda_1, \ldots, \lambda_6$, $\lambda_i > 0$:

$$k_1^{\delta_5} = -\frac{(-1 + e^{-\alpha_1 - 2\alpha_2 - 2\alpha_3})(-1 + e^{\alpha_1 + 2\alpha_2 + \alpha_3})(\lambda_1 + \lambda_5)}{\beta_1 \beta_2}, \tag{27a}$$

$$k_2^{\delta_5} = \frac{(-1 + e^{-\alpha_3})\lambda_1}{-\beta_2 - \beta_4}, \tag{27b}$$

$$k_3^{\delta_5} = \frac{(-1 + e^{-\alpha_3})\lambda_5}{-\beta_2 - \beta_4}, \tag{27c}$$

$$k_4^{\delta_5} = -\frac{(-1+e^{-\alpha_2-\alpha_3})(-1+e^{\alpha_1+2\alpha_2+\alpha_3})(\lambda_2+\lambda_6)}{\beta_2\beta_3}, \tag{27d}$$

$$k_5^{\delta_5} = \frac{(-1+e^{\alpha_1+\alpha_2})\lambda_2}{\beta_4}, \tag{27e}$$

$$k_6^{\delta_5} = \frac{(-1+e^{\alpha_1+\alpha_2})\lambda_6}{\beta_4}, \tag{27f}$$

$$k_7^{\delta_5} = \frac{(-1+e^{\alpha_1})(-1+e^{\alpha_2})(\lambda_3+\lambda_6)}{\beta_5(\beta_1+\beta_2+\beta_3+\beta_5)}, \tag{27g}$$

$$k_8^{\delta_5} = \frac{(-1+e^{\alpha_1+\alpha_2})\lambda_3}{\beta_6}, \tag{27h}$$

$$k_9^{\delta_5} = \frac{(-1+e^{\alpha_1+\alpha_2})\lambda_6}{\beta_6}, \tag{27i}$$

$$k_{10}^{\delta_5} = -\frac{(-1+e^{\alpha_2})(-1+e^{-\alpha_2-\alpha_3})(\lambda_4+\lambda_5)}{\beta_3\beta_5}, \tag{27j}$$

$$k_{11}^{\delta_5} = \frac{(-1+e^{-\alpha_3})\lambda_4}{-\beta_5-\beta_6}, \tag{27k}$$

$$k_{12}^{\delta_5} = \frac{(-1+e^{-\alpha_3})\lambda_5}{-\beta_5-\beta_6}. \tag{27l}$$

5. Robust yet fragile nature of multistationarity in the activation a MAPK

A variety of biochemical reaction networks give rise to qualitative properties that are robust with respect to certain parameter variations (see [21,24,32] and the references therein). Nowadays robustness is often considered as an inherent principle of cellular functions (and, of course, of the biochemical reaction networks that implement this functionality). However, robustness never comes alone. It is almost always accompanied by 'fragility' with respect to variations of different parameter sets: in [31], for example, it has been shown that the existence of cyclic trajectories is robust to large variations in one group of parameters, whereas small variations in a second, different group of parameters lead to a destruction of this qualitative property. This '*robust yet fragile*' nature has been observed frequently both in biochemical and in man-made networks like the Internet (see [8,9]).

Along with the variety of published models for the MAPK-cascade as a whole (c.f. Section 1), there exists a variety of contributions concerned with robustness and fragility; especially of bistability, which is most important in the context of this work (e.g. [5,3]). Bistability on the single layer and its influence on robustness of bistability in the overall cascade has been described in [22]. Robustness of bistability on the single layer has already been analyzed in [25]: here a mechanism using Michaelis–Menten kinetics has been analyzed and the regions in parameter space, where bistability occurs, have been identified analytically.

Throughout this paper we have not considered stability of steady states, thus when discussing robustness and stability of *multistationarity*, bistability will only be considered implicitly, as

multistationarity is a prerequisite. This, however, is not the major difference between the aforementioned references and the present contribution. That difference is twofold: (i) we show that the existence of a *specific pair of steady states* a and b is robust to certain variations in the rate constants (that is, a and b do not change even though the rate constants change). And (ii) we do not merely state the robustness and fragility of *multistationarity* by showing that there exists a region in parameter space where this phenomenon occurs, but we explain it as a consequence of the results presented in Section 4 and thus, ultimately, as a consequence of the reaction network structure.

To be more specific, in the following subsections we show that it is straightforward to obtain, *for a particular pair of steady states*, sets of rate constants that vary significantly, thus showing robustness of *multistationarity*. The fragility follows from the fact that it is easy to identify combinations of rate constants, where minimal changes result in an immediate loss of *multistationarity*. Robustness and fragility of *multistationarity* are consequences of the network structure whenever the vector of rate constants is chosen as described in Section 4. We created just one pair of steady states and the corresponding vector of rate constants for demonstration purposes only and did not focus on physiological meaningful values for the rate constants.

5.1. Multistationarity – a robust yet fragile property

Multistationarity in the activation of a MAPK is a qualitative property with a *robust yet fragile* nature. This is a direct consequence of the results presented here, in particular of the definition of k, the parameter vector, as displayed in Eqs. (15a)–(15l). Even for a specific steady state a (belonging to, e.g. an experimentally obtained pair of steady states) the k_i are not unique, as the vector $\lambda \in \mathbb{R}^6_{>0}$ can still be chosen freely. Thus, a specific pair of steady states is compatible with an infinite set of parameter values .

Multistationarity is robust with respect to variations in the k_i, as long as the k_i satisfy the equations Eqs. (15a)–(15l) for positive λ_i. Note that for a fixed vector a Eqs. (15a)–(15l) are linear. In this case the right hand side of the equations defines a pointed polyhedral cone in $\mathbb{R}^{12}_{\geqslant 0}$. The generators of this 'multistationarity cone' can be obtained from the generators of $\ker(N) \cap \mathbb{R}^{12}_{\geqslant 0}$ via the linear transformation

$$P = \operatorname{diag}(v^{-1}(\underline{1}, a))E, \tag{28}$$

where E is a matrix composed of the generators of $\ker(N) \cap \mathbb{R}^{12}_{\geqslant 0}$ as given in Eq. (10) and

$$v^{-1}(\underline{1}, a)' = \left(\frac{1}{a_1 a_2}, \frac{1}{a_3}, \frac{1}{a_3}, \frac{1}{a_2 a_4}, \frac{1}{a_5}, \frac{1}{a_5}, \frac{1}{a_6 a_7}, \frac{1}{a_8}, \frac{1}{a_8}, \frac{1}{a_4 a_7}, \frac{1}{a_9}, \frac{1}{a_9}\right)$$

is the reciprocal of $v(k, a)$ as given in Eq. (5b) evaluated at $k = \underline{1}$. For a fixed vector a the parameter vector is given by

$$k = P\lambda, \quad \lambda \in \mathbb{R}^6_{>0}. \tag{29}$$

Thus we conclude that the property *multistationarity* is robust with respect to variations of k within the 'multistationarity cone' defined by P.

Table 3
Lower stable steady state $x^{(1)}$, unstable steady state x^* and upper stable steady state $x^{(2)}$ (x^* and $x^{(2)}$ have been published in [6])

	$x^{(1)}$	x^*	$x^{(2)}$
x_1	1.3199	1.7047	4.6339
x_2	0.41185	0.42618	1.1585
x_3	0.085761	0.11462	0.84691
x_4	0.89271	0.84691	0.11462
x_5	2.3601	2.3169	0.85236
x_6	1.058	0.73726	0.0049676
x_7	0.0096985	0.013662	0.74596
x_8	4.0441	3.9701	1.4605
x_9	0.20815	0.27818	2.0555

To make explicit the *robust* nature a random set of 197 parameter vectors was created and simulations for each of these vectors were performed using the initial conditions given in Table 4. The results are displayed in Fig. 2 (for the initial condition $x_0^{(1)}$ in the upper half and for the initial condition $x_0^{(2)}$ in the lower half). The figure shows the temporal evolution of $x_1(t)$ and $x_6(t)$. In the upper half all trajectories converge to $x^{(1)}$ and in the lower half all trajectories converge to $x^{(2)}$, the stable steady states obtained for k^* (c.f. Table 5). Table 3 contains the stable steady states $x^{(1)}$ and $x^{(2)}$ and the unstable steady state x^* obtained for k^*. Changing λ (and thus changing k within P) does not change the steady states of the ODEs displayed in Eqs. (1a)–(1i). It seems worthwhile to highlight once again that the preceding discussion of robustness of *multistationarity* holds for *a specific pair* of steady states, that is, the value of the steady states does not change for variation of the rate constants. This differs from all aforementioned references where parameter ranges of bistability were approximated.

5.2. The fragility of multistationarity

Multistationarity is fragile with respect to variations of k transversal to P, for example to variations of k in the orthogonal complement of the subspace of $\mathbb{R}^{12}$ that contains P. To demonstrate this, we perturbed the parameter vector in the following way:

Table 4
Initial conditions for the dynamic simulations in Fig. 2

	$x_0^{(1)}$	$x_0^{(2)}$	x_0^{up}
x_{10}	1.4047	4.1339	1.0199
x_{20}	0.12618	2.1585	0.11185
x_{30}	0.41462	0.54691	0.38576
x_{40}	0.84691	0.91462	0.89271
x_{50}	2.3169	0.15236	2.3601
x_{60}	0.73726	0.70497	1.058
x_{70}	0.013662	0.74596	0.0096985
x_{80}	3.9701	1.4605	4.0441
x_{90}	0.27818	2.0555	0.20815

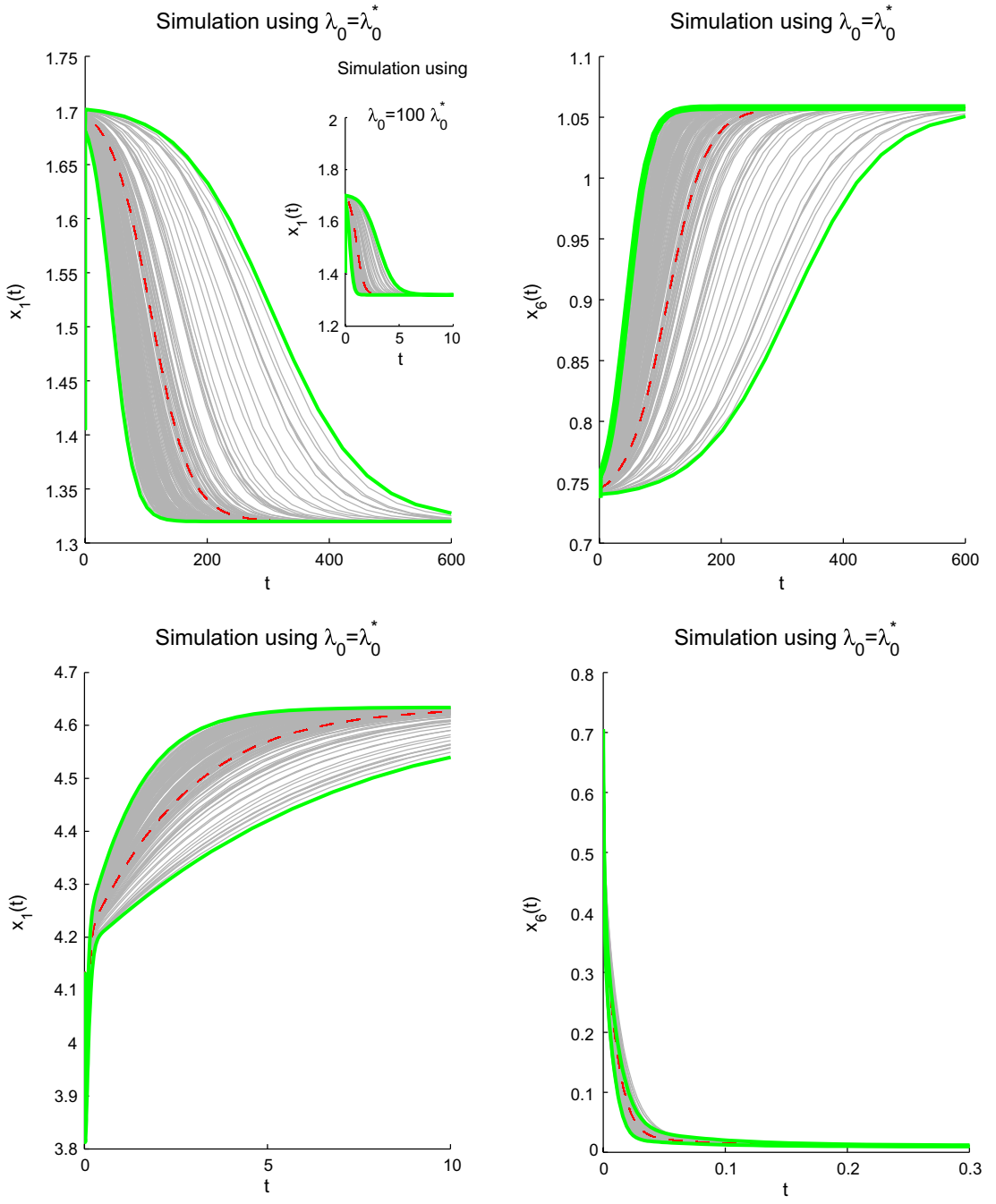

Fig. 2. Temporal evolution of $x_1(t)$ and $x_6(t)$ for a set of randomly generated parameter vectors, using the initial conditions $x_0^{(1)}$ (upper half) and $x_0^{(2)}$ (lower half). The dashed (red) line indicates the temporal evolution obtained for the reference parameter vector k^*, the thick (green) delimiting trajectories correspond to parameter sets labeled 'upper' and 'lower'. (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

$$k = k^* + \epsilon u^{\perp}.$$

where

$$u^{\perp} = (-1, 1, 1, -1, 1, 1, -1, 1, 1, -1, 1, 1)'.$$

Note that $\langle u^{\perp}, E_i \rangle = 0$, thus changing k^* along $u^{\perp}$ corresponds to changes transversal to P.

To illustrate this, a bifurcation analysis was performed using ϵ as bifurcation parameter (the bifurcation was performed using *Matcont*, see e.g. [1]). The result is displayed in Fig. 3. This analysis established upper and lower bifurcation parameters (at $\epsilon \approx 0.0017$ and $\epsilon \approx -0.1993$, respectively). Visual inspection alone shows that ϵ can vary only in a very small interval, without losing multistationarity. The parameter vectors $\tilde{k}^{\text{up}}$ (corresponding to $\epsilon \approx 0.0017$) and $\tilde{k}^{\text{low}}$ (corresponding to $\epsilon \approx -0.1993$) are displayed in Table 5, as well.

Using this idea it is in principle possible to obtain directions in parameter space, where *multistationarity* is extremely fragile: take any vector $u \in P^{\perp}$. Tiny changes along u will result in the loss of *multistationarity*. However, changes have to occur in $P^{\perp}$ and thus usually affect several rate constants at once in a correlated way.

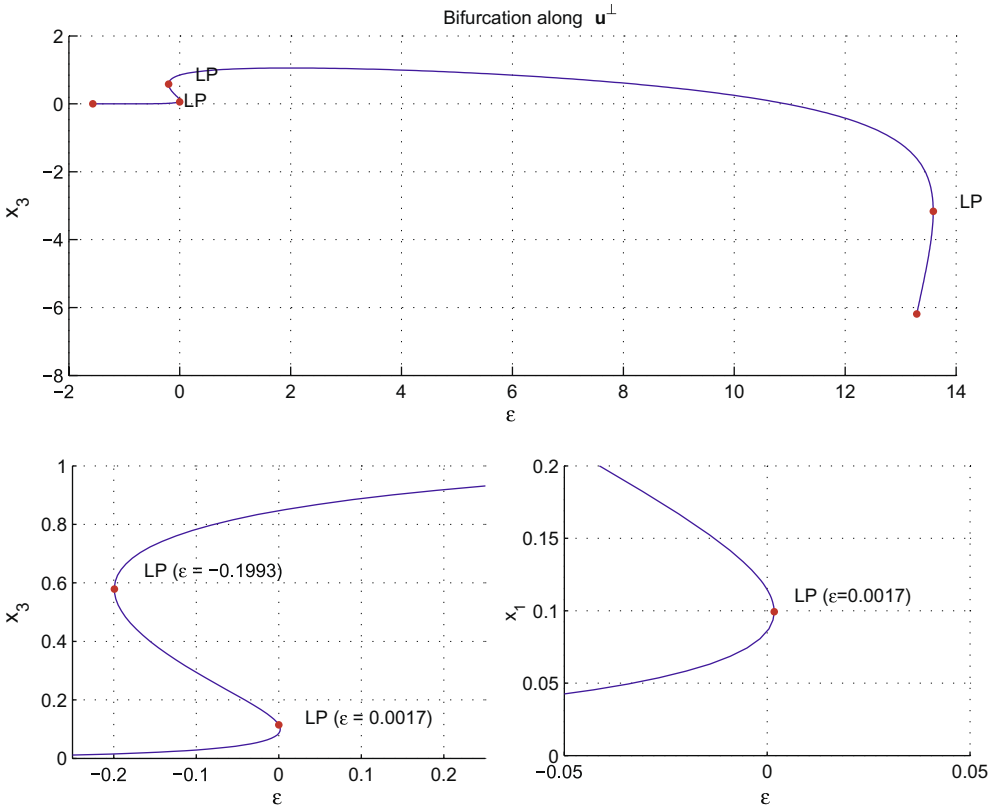

Fig. 3. Bifurcation along $u^{\perp}$.

Table 5
Reference parameter vector k^* (as given in [6]), parameter vectors k^{up} and k^{low} obtained by random variations λ, with $\sum_i \lambda_i = \lambda_0^*$ and parameter vectors $\tilde{k}^{\mathrm{up}}$ and $\tilde{k}^{\mathrm{low}}$ obtained by varying k along $\epsilon u^{\perp}$

	k^*	k^{up}	k^{low}	$\tilde{k}^{\mathrm{up}}$	$\tilde{k}^{\mathrm{low}}$
k_1	10.9293	8.3595	12.6521	10.9275	11.1285
k_2	34.6381	30.44	46.1592	34.6399	34.4389
k_3	34.6381	22.5474	34.0373	34.6399	34.4389
k_4	21.9991	31.4783	14.6527	21.9973	22.1983
k_5	1.7135	1.3566	1.604	1.7153	1.5143
k_6	1.7135	3.5471	0.67864	1.7153	1.5143
k_7	788.272	1211.7563	727.2734	788.2703	788.4713
k_8	1	1.0042	1.4491	1.0017	0.80077
k_9	1	2.0701	0.39605	1.0017	0.80077
k_{10}	686.2147	430.6848	647.1921	686.2129	686.4139
k_{11}	14.2718	8.6237	12.8949	14.2736	14.0726
k_{12}	14.2718	9.2901	14.0243	14.2736	14.0726

5.3. Quantification of robustness

To quantify 'robustness' of *multistationarity* (or lack thereof) the parameter sets corresponding to the thick (green) delimiting trajectories in Fig. 2 were used to approximate the parameter range were *multistationarity* can occur. (This is, of course, a poor approximation of the parameter range, where multistationarity can occur. But it is sufficient to back our argument.) For each parameter we defined an *elasticity coefficient* that measures the difference between the upper and the lower value of the parameter in relation to the 'nominal' value k_i^*

$$\eta_i := \frac{k_i^{\mathrm{up}} - k_i^{\mathrm{low}}}{k_i^*}. \tag{30}$$

Here k^{up} corresponds to the upper trajectory and k^{low} to the lower trajectory in Fig. 2. Both parameter vectors are given in Table 5. Using $\tilde{k}^{\mathrm{up}}$ and $\tilde{k}^{\mathrm{low}}$ in formula (30), the elasticities for perturbations along $u^{\perp}$ have been determined as well (see Table 6, second Column). The results are

Table 6
Elasticities obtained for a random sample of parameter vectors (column one) and for variation along $u^{\perp}$ (column two)

	$\frac{k_i^{\mathrm{up}} - k_i^{\mathrm{low}}}{k_i^*}$	$\frac{k_{\epsilon i}^{\mathrm{up}} - k_{\epsilon i}^{\mathrm{low}}}{k_i^*}$
η_1	−0.39276	−0.018391
η_2	−0.45381	0.0058029
η_3	−0.33171	0.0058029
η_4	0.76483	−0.0091367
η_5	−0.14438	0.1173
η_6	1.674	0.1173
η_7	0.61461	−0.00025499
η_8	−0.4449	0.20093
η_9	1.6741	0.20093
η_{10}	−0.31551	−0.00029291
η_{11}	−0.29927	0.014084
η_{12}	−0.33172	0.014084

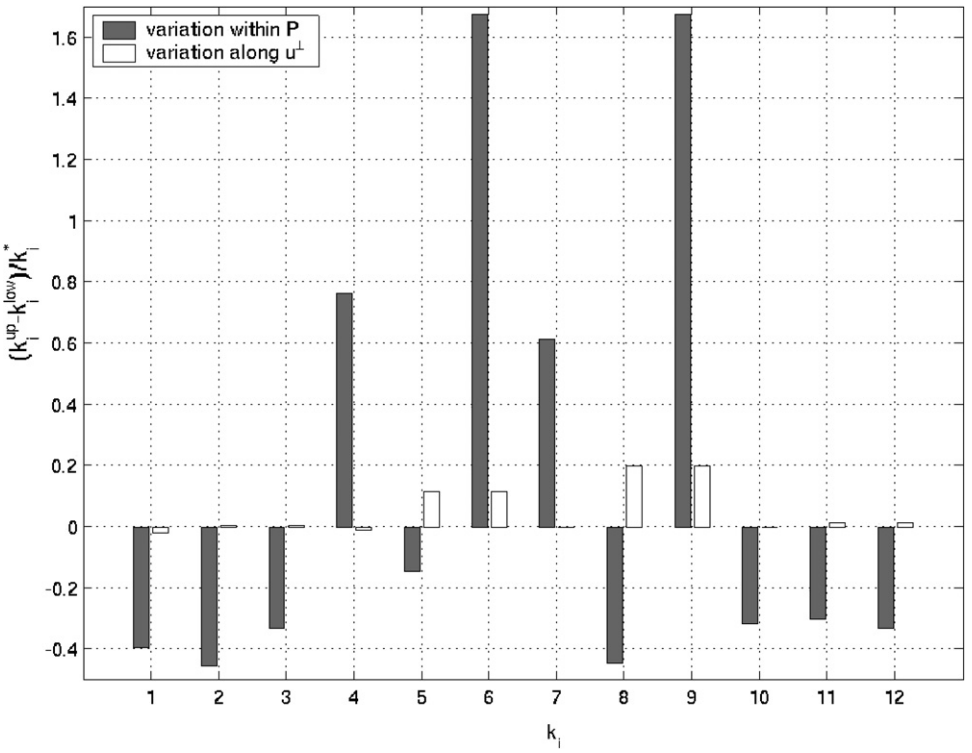

Fig. 4. Robustness vs. fragility of *multistationarity* for the activation of a MAPK. *Filled bars:* Elasticities obtained for a random sample of parameters contained in the *multistationarity cone P*. *Empty bars*: Elasticities obtained for a set of parameters created along $\epsilon u^{\perp}$.

displayed in Fig. 4: the elasticities corresponding to variations within p as filled bars and those corresponding to variations along $u^{\perp}$ as empty bars. This figure nicely reflects the robust yet fragile nature of *multistationarity*: on the one hand, each k_i can vary by large amounts, if variation occurs within the multistationarity cone P. On the other hand, if the multistationarity cone P is left (e.g. by adding $\epsilon u^{\perp}$), then, for relatively small variations of the k_i, multistationarity is lost. This is best illustrated using k_7. From Table 5 follows that k_7 can vary between 727.2734 and 1211.7563 if variation occurs within P (i.e. k_7 can vary by at least 60% of its nominal value $k_7^* = 788.272$ and *multistationarity* is retained). If k_7 is varied along $u^{\perp}$, then again by Table 5 it can vary between 788.2703 and 788.4713 (i.e. by at most *0.025*% relative to $k_7^* = 788.272$) without losing *multistationarity*. Fig. 4 nicely illustrates this fact.

5.4. A note on quantifying robustness

When quantifying 'robustness', some care has to be exercised: as P is a pointed polyhedral cone it is in principle possible to obtain deviations from k^* that are arbitrarily large without losing *multistationarity*. To see this, consider two vectors $\tilde{k}^{\mathrm{up}}$, $\tilde{k}^{\mathrm{low}}$ and the vector k^*. Let $\tilde{k}^{\mathrm{up}} = \lambda_0 k^*$ and $\tilde{k}^{\mathrm{low}} = \frac{1}{\lambda_0} k^*$, where $\lambda_0 \gg 1$. As P is a cone and $k^* \in P$, any positive scalar multiple of k^* is in the

cone as well. Thus the system will exhibit multistationarity for $\tilde{k}^{\text{up}} = \lambda_0 k^*$ and $\tilde{k}^{\text{low}} = \frac{1}{\lambda_0} k^*$ and the elasticity coefficients will be $\tilde{\eta}_i = \frac{\lambda_o k_i^* - \frac{1}{\lambda_0} k_i^*}{k_i^*} = \lambda_0 - \frac{1}{\lambda_0} \approx \lambda_0$ (using $\tilde{k}^{\text{low}}$, $\tilde{k}^{\text{up}}$ and k^* in formula (30)).

However the $\tilde{\eta}_i$ are not meaningful, as $\tilde{k}^{\text{up}}$ and $\tilde{k}^{\text{low}}$ correspond to a mere scaling of time that does not change the qualitative dynamic behaviour of the system (i.e. it is 'obvious' that the system retains *multistationarity*): to see this, let $k_1 = \lambda_0 \tilde{k}_1$, $k_2 = \lambda_0 \tilde{k}_2$ and $k_3 = \lambda_0 \tilde{k}_3$. Inserting this into Eq. (1a) yields

$$\dot{x}_1 = \lambda_0(-\tilde{k}_1 x_1 x_2 + \tilde{k}_2 x_3 + \tilde{k}_{12} x_9).$$

Clearly this corresponds to a scaling of time t with λ_0. A similar argument can be made for the remaining ODEs.

Thus, to circumvent this scaling of time and thus to exclude this trivial robustness, these parameter deviations have to be ruled out. To do this, note that if $k^* \in P$, then there exists a $\lambda^* \in \mathbb{R}^6_{>0}$, such that $k^* = P\lambda^*$. Then $\lambda_0 k^* = \lambda_0 P\lambda^* = P(\lambda_0 \lambda^*)$ (i.e. multiplying k^* by λ_0 is equivalent to multiplying λ^* by λ_0). Thus, to deal with the effect of time scaling we consider only λ_i contained in the affine linear subspace $\langle \underline{1}, \lambda \rangle = \lambda_0$ for a fixed value λ_0. Elements of this subspace are given by $\lambda = \lambda_0(\lambda_1, \ldots, \lambda_5, 1 - \sum_{i=1}^5 \lambda_i)$, $\lambda_i \in [0,1]$, $i = 1, \ldots, 5$. Then λ_0 is fixed as $\lambda_0^* = 23.8207$, the value calculated for k^*, as given in Table 5 (using e.g. the steady state x^* from Table 3, the vector λ can be calculated as $\lambda = \lambda_0^*(0.1667, 0.1667, 0.1667, 0.1667, 0.1667, 0.1667)$). To demonstrate the effect of λ_0, Fig. 2 contains the trajectories of $x_1(t)$ obtained using $100\lambda_0^*$.

6. Discussion

We have shown that it is possible to characterize analytically the region in parameter space where the biochemical reaction network $(\mathcal{N}_1)$ allows more than one positive steady state. In particular, an analytical expression for any pair of positive steady states a, b, as well as for all parameter vectors k that ensure that a and b are indeed steady state solutions for $(\mathcal{N}_1)$ have been derived. These expressions require different parametrizations for different orthants of $\mathbb{R}^9$. The data given in Tables 1 and 2 can be used to write down any of these parametrizations. For demonstration purposes this has been done for a particular orthant in Eqs. (25) and (26) for a and b and in Eqs. (27a)–(27l) for the parameter vector.

Last but not least we like to mention two limitations of this approach. The first is the obvious fact that the approach presented here is only valid for positive steady states. That is, no information is given about boundary equilibria (i.e. steady states $\tilde{x}$ with $\tilde{x}_i = 0$ for some $i \in \{1, \ldots, 9\}$). The second is the fact that no information about stability of steady states is obtained. As this information is of great interest we like to recall a potential way to circumvent this limitation, as we have shown in [6,7]: using the approach presented here or Feinberg's Chemical Reaction Network Toolbox [13] a set of parameters that guarantee multistationarity can be established. Then bifurcation analysis can be used to establish and track stability of the steady states. Furthermore, as a, b and k are parameterized, it is in principle possible to derive the Jacobian at a and b and to numerically calculate and track its eigenvalues.

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Appendix A. Calculating the generators for all cones $\mathcal{M} \cap \boldsymbol{R}^{\boldsymbol{9}}_{\boldsymbol{\delta_i}}$ and $\boldsymbol{S} \cap \boldsymbol{R}^{\boldsymbol{9}}_{\boldsymbol{\delta_i}}$

A.1. Preliminary ideas

We have available a software to calculate the extreme rays of a pointed polyhedral cone $\ker(A) \cap \mathbb{R}^n_{>0}$, where $A \in \mathbb{R}^{m\times n}$ is an arbitrary $m \times n$ matrix. It is possible to use this software in order to calculate extreme rays of a different cone $\ker(A) \cap \mathbb{R}^n_{\delta_i}$. To see this, recall the definition of an extreme ray v of $\ker(A) \cap \mathbb{R}^n_{\delta_i}$ (see Section 4.1)

$$v \in \mathbb{R}^n_{\delta_i},$$
$$Av = 0.$$

Given u, v with $Au = 0$ and $Av = 0$. Then

$$\operatorname{supp}(u) \subseteq \operatorname{supp}(v) \;\Rightarrow\; u = 0 \quad \text{or} \quad v = \alpha u, \quad \alpha \in \mathbb{R}_{>0}.$$

Further note that any $v \in \mathbb{R}^n_{\delta_i}$ can be expressed as

$$v = \operatorname{diag}(\delta_i) w, \quad w \in \mathbb{R}^n_{\geqslant 0}, \quad \text{with } w_i = |v_i|, \quad i = 1, \ldots, n. \tag{31}$$

Using Eq. (31) in $Av = 0$ yields

$$A \operatorname{diag}(\delta_i) w = 0.$$

Then extreme rays of a cone $\ker(A) \cap \mathbb{R}_{\delta_i^n}$ can be defined as

$$v \in \mathbb{R}^n_{\delta_i} \tag{32a}$$

given by $v = \operatorname{diag}(\delta_i) w$ with

$$w \in \mathbb{R}^n_{\geqslant 0}, \tag{32b}$$
$$\widetilde{A} w = 0, \tag{32c}$$

where $\widetilde{A} := A \operatorname{diag}(\delta_i)$. Given $w^{(1)}$, $w^{(2)}$ with $Aw^{(1)} = 0$ and $Aw^{(2)} = 0$. Then

$$\operatorname{supp}(w^{(1)}) \subseteq \operatorname{supp}(w^{(2)}) \;\Rightarrow\; w^{(1)} = 0 \quad \text{or} \quad w^{(2)} = \alpha u, \quad \alpha \in \mathbb{R}_{>0}. \tag{32d}$$

If $\widetilde{E}_1, \ldots, \widetilde{E}_p$ are generators of $\ker(\widetilde{A}) \cap \mathbb{R}^n_{\geqslant 0}$, then $E_i = \operatorname{diag}(\delta_i)\widetilde{E}_i$, $i = 1, \ldots, p$ are generators of $\ker(A) \cap \mathbb{R}^n_{\delta_i}$. To see this, consider $AE_i = A\operatorname{diag}(\delta_i)\widetilde{E}_i = \widetilde{A}\widetilde{E}_i = 0$, $i = 1, \ldots, p$. Using this definition it is thus possible to calculate generators of $\ker(A) \cap \mathbb{R}^n_{\delta_i}$ using software designed to calculate generators of $\ker(A) \cap \mathbb{R}^n_{\geqslant 0}$.

A.2. Deciding $\ker(A) \cap \mathbb{R}^n_{\delta_i} \neq \emptyset$

To decide $\ker(A) \cap \mathbb{R}^n_{\delta_i} \neq \emptyset$ the following algorithm is used: generate all δ_i, i.e. all combinations of n elements from $\{-1, 0, 1\}$. This is done using the m-file `combn.m`, obtainable at http://www.mathworks.com/matlabcentral. For all δ_i:

(i) Put $\widetilde{A} := \mathrm{diag}(\delta_i)A$,
(ii) calculate generators $\widetilde{E}_i$, $i = 1, \ldots, p$ of $\ker(A) \cap \mathbb{R}^n_{\delta_i}$.
(iii) If $\nexists j \in \{1, \ldots, n\}$ with $j \notin \mathrm{supp}(\widetilde{E}_i)$, $\quad i = 1, \ldots, p$,

then $\ker(A) \cap \mathbb{R}^n_{\delta_i} \neq \emptyset$. Thus keep δ_i.

The condition in (ii) requires that no entry $\widetilde{E}_{i_j}$ exists that is zero in every generator. Those correspond to generators for a different orthant, namely the one where $\delta_{i_j} = 0$.

The above mentioned algorithm is by no means efficient. It could easily be made more efficient by

1. Considering that $\mathcal{M}$ and S are subspaces, that is, that $\tilde{\mu} \in \mathcal{M} \Rightarrow -\tilde{\mu} \in \mathcal{M}$ and $\tilde{v} \in S \Rightarrow -\tilde{v} \in S$. Thus, if $\mathcal{M} \cap \mathbb{R}^n_\delta \neq \emptyset$ and $S \cap \mathbb{R}^n_\delta \neq \emptyset$, then $\mathcal{M} \cap \mathbb{R}^n_{-\delta} \neq \emptyset$ and $S \cap \mathbb{R}^n_{-\delta} \neq \emptyset$ as well.
2. Using the implicit information given in step (ii) that even though $\ker(A) \cap \mathbb{R}^n_\delta = \emptyset$, there exists an orthant $\bar{\delta}$ with $\ker(A) \cap \mathbb{R}^n_{\bar{\delta}} \neq \emptyset$. For $\bar{\delta}$ the following holds:

$$\bar{\delta}_j = \begin{cases} \delta_j, & \text{if } \exists i \in \{1, \ldots, p\} \text{ with } E_{i_j} \neq 0, \\ 0, & \text{else.} \end{cases}$$

However due to the relatively small amount of time required to perform the calculations for the example considered here, we choose to trade efficiency for ease of implementation. The algorithm is available in the matlab file `sign_comp_subs.m` displayed below

```
function E = sign_comp_subs( W )
n = length(W)
M = combn([-1 0 1], n);
[Mnrows,Mncols]=size(M);
or_count = 1;
for i = 1:Mnrows
  disp('Processing orthant:'); M(i,:)
  [elm,num_modes,revs,mode_rates,fix_rates]= ...
    elmodes_calc((diag(M(i,:))*W)',[],[], ones(1,length(W)), ...
      [],1e-10,'useMex','1');
  if length(fix_rates) ==0
    E(:,or_count) = M(i,:)';
    or_count = or_count + 1;
  end;
end;
```

The command `[elm,...]= elmodes_calc(...)` invokes a routine to calculate the generators of the cone $\mathrm{diag}(M(i,:))W \cap \mathbb{R}^n_{>0}$. It is contained in the software package *CellNetAnalyzer* (see e.g. [15]).

A.3. Determining generators

The following steps were executed to derive the generators for all orthants where $\mathcal{M} \cap \mathbb{R}^n_\delta \neq \emptyset$ and $S \cap \mathbb{R}^n_\delta \neq \emptyset$.

Step 1: Reformulate $\mathcal{M}$ and S: Let $W = [w_1, \ldots, w_s]$ be a matrix whose column vectors are a basis for $S^\perp$ and $B = [b_1, \ldots, b_p]$ be a matrix whose column vectors are a basis for $\mathcal{M}^\perp$. Then all $v \in S$ can be represented as $\{v \in \mathbb{R}^n | W'v = 0\}$ and all μ as $\{\mu \in \mathbb{R}^n | B'\mu = 0\}$, i.e. $v \in S$ and $\mu \in \mathcal{M}$ are in a form suitable for the algorithm described above (using either W or B as matrix A).
Step 2: Determine all δ_i that are sign compatible to S: Δ^S.
Step 3: Determine all δ_i that are sign compatible to $\mathcal{M}$: Δ^μ.
Step 4: Determine $\Delta = \Delta^S \cap \Delta^\mu$.
Step 5: For each $\delta_i \in \Delta$ calculate the generators of $\mathcal{M} \cap \mathbb{R}^n_{\delta_i}$ and $S \cap \mathbb{R}^n_{\delta_i}$ (see the matlab file `all_cones.m`).

The following matlab commands were used to perform these steps:

```
orthi = sign_comp_subs(W);
orthi_mu = sign_comp_subs(mu);
k = 1; for i = 1:length(orthi) for j = 1:length (orthi_mu) if
(orthi(:,i) = =orthi_mu(:,j)) Erg(:,k) = orthi(:,i);k = k + 1;end; end;end;
MuCones = all_cones(Erg,mu)
SCones=all_cones(Erg,W)
```

The matlab file `all_cones.m`:

```
function ConeSet = all_cones(orth_mat,w)
[Orows,Ocols] = size(orth_mat);
ConeSet = {};
ConeCounter = 1;
for k = 1:Ocols
  SomeCone = calculate_cone(orth_mat(:,k),w);
  ConeSet(ConeCounter) = {SomeCone};
  ConeCounter = ConeCounter + 1;
end;
```

And the matlab file `calculate_cone.m` that calculates generators for a particular cone:

```
function C = calculate_cone( orthant, w)
[C, num_modes, revs, mode_rates, fix_rates] = elmodes_calc( ...
  (diag(orthant)*w)', [], [], ones(1,length(w')), [], 1e-10, ...
  'useMex','1');
if (num_modes > 0)
  C = diag(orthant)*C';
else
  C=[];
end;
```

References

[1] Matcont – a Matlab continuation package, http://www.matcont.ugent.be/.

[2] Upinder S. Bhalla, Ravi Iyengar, Emergent properties of networks of biological signaling pathways, Science 283 (5400) (1999) 381.

[3] Upinder S. Bhalla, Ravi Iyengar, Robustness of the bistable behavior of a biological signaling feedback loop, Chaos 11 (1) (2001) 221.

[4] Upinder S. Bhalla, Prahlad T. Ram, Ravi Iyengar, MAP kinase phosphatase as a locus of flexibility in a mitogen-activated protein kinase signaling network, Science 297 (5583) (2002) 1018.

[5] Nils Blüthgen, Hanspeter Herzel, How robust are switches in intracellular signaling cascades? J. Theor. Biol. 225 (3) (2003) 293.

[6] Carsten Conradi, Julio Saez-Rodriguez, Ernst-Dieter Gilles, Jörg Raisch, Using Chemical Reaction Network Theory to discard a kinetic mechanism hypothesis, IEE Proc. Syst. Biol. 152 (4) (2005) 243.

[7] Carsten Conradi, Julio Saez-Rodriguez, Ernst-Dieter Gilles, Jörg Raisch, Chemical Reaction Network Theory ... a tool for systems biology, in: Proceedings of the 5th MATHMOD, 2006.

[8] John C. Doyle, David L. Alderson, Lun Li, Steven Low, Matthew Roughan, Stanislav Shalunov, Reiko Tanaka, Walter Willinger, The "robust yet fragile nature" of the Internet, PNAS 102 (41) (2005) 14497.

[9] H. El-Samad, H. Kurata, J.C. Doyle, C.A. Gross, M. Khammash, Surviving heat shock: control strategies for robustness and performance, PNAS 102 (8) (2005) 2736.

[10] Phillipp Ellison, The Advanced Deficiency Algorithm and its applications to mechanism discrimination, Ph.D. thesis, The University of Rochester, 1998.

[11] Martin Feinberg, Chemical reaction network structure and the stability of complex isothermal reactors – II. Multiple steady states for networks of deficiency one, Chem. Eng. Sci. 43 (1) (1988) 1.

[12] Martin Feinberg, Multiple steady states for chemical reaction networks of Deficiency One, Arch. Ration. Mech. Anal. 132 (4) (1995) 371.

[13] Martin Feinberg, Phillip Ellison. The chemical reaction network toolbox. http//www.chbmeng.ohio-state.edu/feinberg/crnt.

[14] James E. Ferrell, Self-perpetuating states in signal transduction: positive feedback, double-negative feedback and bistability, Curr. Opin. Cell Biol. 14 (2) (2002) 140.

[15] Julien Gagneur, Steffen Klamt, Computation of elementary modes: a unifying framework and the new binary approach, BMC Bioinform. 5 (2004).

[16] Karin Gatermann, Birkett Huber, A family of sparse polynomial systems arising in chemical reaction systems, J. Symbolic Comput. 33 (3) (2002) 275.

[17] Karin Gatermann, Matthias Wolfrum, Bernstein's second theorem and Viro's method for sparse polynomial systems in chemistry, Adv. Appl. Math. 34 (2) (2005) 252.

[18] Chi-Ying F. Huang, James E. Ferrell Jr., Ultrasensitivity in the mitogen-activated protein kinase cascade, PNAS 93 (19) (1996) 10078.

[19] Boris N. Kholodenko, Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades, Eur. J. Biochem. 267 (6) (2000) 1583.

[20] Boris N. Kholodenko, Cell-signalling dynamics in time and space, Nat. Rev. Mol. Cell Biol. 7 (2006) 165.
[21] Hiroaki Kitano, Biological robustness, Nat. Rev. Genet. 5 (11) (2004) 826.
[22] Stefan Legewie, Birgit Schoeberl, Nils Blüthgen, Hanspeter Herzel, Competing docking interactions can bring about bistability in the MAPK cascade. Biophys. J., 2007, biophysj.107.109132.
[23] Nick I. Markevich, Jan B. Hoek, Boris N. Kholodenko, Signaling switches and bistability arising from multisite phosphorylation in protein kinase cascades, J. Cell Biol. 164 (3) (2004) 353.
[24] Mineo Morohashi, Amanda E. Winn, Mark T. Borisuk, Hamid Bolouri, John Doyle, Hiroaki Kitano, Robustness as a measure of plausibility in models of biochemical networks, J. Theor. Biol. 216 (1) (2002) 19.
[25] Fernando Ortega, José L. Garcé, Francesc Mas, Boris N. Kholodenko, Marta Cascante, Bistability from double phosphorylation in signal transduction, FEBS J. 273 (17) (2006) 3915.
[26] Ralph Tyrell Rockafellar, Convex Analysis, Princeton University Press, 1970.
[27] Herbert M. Sauro, Boris N. Kholodenko, Quantitative analysis of signaling networks, Prog. Biophys. Mol. Biol. 86 (1) (2004) 5.
[28] S. Schuster, C. Hilgetag, J.H. Woods, D.A. Fell, Reaction routes in biochemical reaction systems: algebraic properties, validated calculation procedure and example from nucleotide metabolism, J. Math. Biol. 45 (2) (2002) 153.
[29] R. Seger, E.G. Krebs, The MAPK signaling cascade, FASEB J. 9 (1995) 726.
[30] E.D. Sontag, Structure and stability of certain chemical networks and applications to the kinetic proofreading model of T-cell receptor signal transduction, IEEE Trans. Automatic Contr. 46 (7) (2001) 1028.
[31] Jörg Stelling, Ernst-Dieter Gilles, Francis J. Doyle, Robustness properties of circadian clock architectures, PNAS 101 (36) (2004) 13210.
[32] Jörg Stelling, Uwe Sauer, Zoltan Szallasi, Francis J. Doyle, John Doyle, Robustness of cellular functions, Cell 118 (6) (2004) 675.