Multistationarity in (bio)chemical reaction networks with mass action kinetics: model discrimination, robustness and beyond

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Magdeburg, 2008 Carsten Conradi

To my teacher: Karin Gatermann (1961–2005)

Contents

Chapter 1

Introduction

Multistationarity means the existence of at least two positive steady state solutions to a system of Ordinary Differential Equation (ODEs) derived from a biochemical reaction network. It is an interesting question in its own right: one is looking for the number of positive solutions to a system of polynomial equations in the unknown concentrations with, in most application cases, unknown rate constants. From a mathematical point of view this question is still unanswered. There exists a variety of results that gives an *upper bound* for the number of (positive) solutions to a system of polynomials. However, whether or not a given system of polynomials actually has the predicted number of zeros can usually not be decided (among these results are *Descartes rule of signs* for positive solutions and Bernstein's and Bezout's Theorem for all solutions, including negative and complex; see, for example, [13, 14] and [62]). Moreover, all of these results hold for systems of polynomials with *known* coefficients. The question whether or not a system of polynomial equations with unknown coefficients can admit at least two positive solutions has received little attention in mathematics.

In Chemical Engineering, in the so-called Chemical Reaction Network Theory (CRNT) by Martin Feinberg and his co-workers this question has received some attention [30, 24, 25, 26, 55, 28, 22, 21]. Here, the special structure of equations defined by a (bio)chemical reaction network with mass action kinetics is used to derive necessary and sufficient conditions for the existence of at least two positive solutions. These results, however, require that that the network under consideration has certain structural properties. Only recently these results have been recognized in the mathematical literature, for example in [33, 35].

The approach taken in this thesis is inspired by CRNT, even though it has been derived independent of the aforementioned references. In particular the results used to decide about multistationarity presented in Chapter 3 are not based on CRNT (albeit resulting from an attempt to give a self-contained proof of the results given in [25, 27] and [23]). The results described in Chapter 3 are in fact complementary to those presented in the aforementioned references: they can be successfully applied, where CRNT fails, while CRNT might be successfully applied, where the results of Chapter 3 fail (see Chapter 5, where multistationarity is confirmed for two networks, for which CRNT is inconclusive).

Only recently CRNT has been applied to biochemical reaction networks, in a Systems Biology inspired context, that is, in a context, where the analysis of large and complex reaction networks is required and where parameter uncertainty is predominant. Often, in this context, qualitative knowledge about the dynamics of the system exists; for example, it might be known from experimental observations that the system exhibits bistability or some other form of multistationarity. Suppose there are different hypotheses, each corresponding to a different reaction network with uncertain parameters, related to a specific biological process. Suppose, furthermore, that a certain qualitative behaviour has been observed in experiments. It is then natural to ask which of the postulated networks can, for some conceivable parameter vector, exhibit the observed behaviour. To this end, CRNT has been applied in [15, 16, 17, 18]: suppose experimental evidence suggests that the system under consideration admits different steady states, as is, for example, the case in signal transduction networks and cell cycle regulation. Further suppose, all hypotheses lead to reaction networks that can be analysed using CRNT. Then all networks corresponding to hypotheses where multistationarity is excluded for any conceivable parameter vector can be discarded, as these networks can never reproduce the experimental behaviour. With a similar goal CRNT has been applied in [10, 11, 12] (see also Chapter 4, where a similar analysis is performed using the results presented in Chapter 3).

In many cases, a certain qualitative behaviour, like multistationarity has been observed for rate constants that vary over a wide range of values. Traditionally, one therefore concludes that the system is robust with respect to changes in these rate constants. Using the results of Chapter 3, it is, for certain network structures, possible to obtain a parameterization of rate constants that can serve as an explanation of this robustness. Moreover, in these cases, it is possible to obtain analytical expressions of 'critical points' and 'critical parameters', that is points where certain bifurcations occur.
The outline of this thesis is a follows: in Chapter 2, the notation used to describe biochemical reaction networks with mass action kinetics is introduced. In Chapter 3 conditions for multistationarity are derived. Furthermore, an algorithm is presented, whose steps can be applied to any biochemical reaction network to test for multistationarity (but may be inconclusive). If multistationarity is possible, the results of Chapter 3 allow the computation of a pair of steady states and the corresponding vector of rate constants. In some cases, depending on the network structure, the conditions for multistationarity derived in Chapter 3 are necessary and sufficient, while in other cases only sufficient conditions can be obtained. However, a precise description of those network properties that facilitate necessary and sufficient conditions remains open.
In Chapters 4 – 7 the results obtained in Chapter 3 are applied to a variety of reaction networks in order to tackle the questions of multistationarity, robustness of multistationarity and bifurcation points: Chapter 4 deals with model discrimination for a double-phosphorylation mechanism, while in Chapter 5 two reaction networks proposed in cell cycle regulation are analysed with respect to the ability to admit multistationarity. Chapter 6 deals with robustness and Chapter 7 with bifurcation points of the double-phosphorylation mechanism discussed in Chapter 4. The appendices contain the structural data of all networks analysed in this thesis, as well the matlab code of some of the algorithms developed in this thesis to verify/falsify multistationarity.

Chapter 2

Biochemical reaction networks with mass action kinetics

2.1 Notation

The following symbols will be used throughout this work:
$\mathbb{R}^n$ ... the n-dimensional Euclidian space,
$\mathbb{R}^n_{>0}$ ... the positive orthant of $\mathbb{R}^n$,
$\mathbb{R}^n_{\geq 0}$ ... the nonnegative 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. If $A \in \mathbb{R}^{p\times q}$ then A^T is used to denote its transpose. Vectors $v \in \mathbb{R}^n$ are considered as column vectors and, in most cases, denoted as transposed row vectors, e.g. $v = \left(\begin{smallmatrix}1\\0\\1\end{smallmatrix}\right)$ will be displayed as $v = (1, 0, 1)^T$. For vectors $u, v \in \mathbb{R}^n$, the standard scalar product in $\mathbb{R}^n$ is denoted by $\langle u, v\rangle = \sum_{i=1}^n u_i v_i$. Furthermore, for vectors $u \in \mathbb{R}^n$, the following abbreviations are used:

$$u > 0 \quad \Leftrightarrow u_1 > 0, \ldots, u_n > 0, \tag{2.1a}$$

$$\frac{1}{u} = \left(\frac{1}{u_1}, \ldots, \frac{1}{u_n}\right)^T, \ u_i \neq 0 \tag{2.1b}$$

$$\ln u = (\ln u_1, \ldots, \ln u_n)^T, u > 0 \tag{2.1c}$$

$$e^u = (e^{u_1}, \ldots, e^{u_n})^T \tag{2.1d}$$

The symbol $\operatorname{diag}(u)$ is used to denote the $n \times n$-diagonal matrix:

$$\operatorname{diag}(u) := \begin{bmatrix} u_1 & 0 & 0 \\ 0 & \ddots & 0 \\ 0 & 0 & u_n \end{bmatrix}. \tag{2.1e}$$

Let $A \in \mathbb{R}^{p\times q}$ be a matrix. Then $[A]$ is used to denote the range (image) of A:

$$[A] := \operatorname{im}(A). \tag{2.1f}$$

2.2 Modeling of biochemical reaction networks with mass action kinetics

In this section the notation used to describe biochemical reaction networks with mass action kinetics is introduced. Consider reaction network $\mathcal{N}_1$:

$$\begin{gathered} A \underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftarrows}} 0 \underset{\mathbf{k_4}}{\overset{\mathbf{k_3}}{\rightleftarrows}} B \\ 2\,A + B \underset{\mathbf{k_6}}{\overset{\mathbf{k_5}}{\rightleftarrows}} 3\,A \end{gathered} \tag{$\mathcal{N}_1$}$$

This network consists of two *species* A and B. The symbol n is used to denote the number of species. Thus $n = 2$ for network $\mathcal{N}_1$. Network $\mathcal{N}_1$ contains five *complexes*: A, 0, B, $2\,A + B$ and $3\,A$, that is, in graph-theoretical terms, the nodes of the graph representing the reaction network are called complexes. The symbol m is used to denote the number of complexes, thus $m = 5$ for network $\mathcal{N}_1$. The *zero complex* 0 is used to denote that a system is open with respect to a certain species: A and B can enter and leave the system. Throughout this work, it is assumed that the reaction network is in the *standard form of CRNT*, as defined in, for example, [27]: *node labels are unique* (i.e. complexes appear exactly once). The complexes are linked by *reactions*: some of them are $A \to 0$, or $2\,A + B \to 3\,A$. The symbol r is used to denote the number of reactions, thus $r = 6$ for $\mathcal{N}_1$. Associated to every reaction, there is a *rate constant*: $k_1, \ldots, k_6$ for $\mathcal{N}_1$.

To every species belongs a concentration variable, x_1 for A and x_2 for B. Thus, in general, $x \in R^n$, the n-dimensional Euclidian space. Associate to each species the corresponding unit vector, that is e_1 to A and e_2 to B. Then every complex can be represented by a vector y_i, which is the sum of its constituent species. In the above example, $y_1 = e_1$ for A, $y_2 = 0$, the two dimensional zero vector for 0, $y_3 = e_2$ for B, $y_4 = 2\,e_1 + e_2$ for $2\,A + B$ and $y_5 = 3\,e_1$ for $3\,A$. Collect all y_i in a matrix Y, thus

$$\begin{aligned} Y &= \begin{bmatrix} y_1 & \ldots & y_5 \end{bmatrix} \\ &= \begin{bmatrix} 1 & 0 & 0 & 2 & 3 \\ 0 & 0 & 1 & 1 & 0 \end{bmatrix}. \end{aligned} \tag{2.2a}$$

Thus $Y \in \mathbb{R}^{2\times 5}$ for $\mathcal{N}_1$ and $Y \in \mathbb{R}^{n\times m}$, in general. The graph of the reaction network is represented by its *incidence matrix* I_a. Each colum of I_a represents a reaction and contains exactly two nonzero entries: $+1$ for the product complex and -1 for the educt complex. Thus $I_a \in \mathbb{R}^{5\times 6}$ for $\mathcal{N}_1$ and $I_a \in \mathbb{R}^{m\times r}$, in general. Consider, for example, the reaction $2\,A + B \to 3\,A$. Here the product complex is $3\,A$ and the educt complex is $2\,A + B$. Thus I_a contains the column vector $(0,\, 0,\, 0,\, -1,\, 1)^T$. For $\mathcal{N}_1$ one obtains:

$$I_a = \begin{bmatrix} -1 & 1 & 0 & 0 & 0 & 0 \\ 1 & -1 & -1 & 1 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 0 & 1 & -1 \end{bmatrix}. \tag{2.2b}$$

Up to now, the structure of the reaction network has been described. To describe dynamics, a reaction rate is associated to each reaction. As only mass-action kinetics are considered in this thesis, each reaction rate consists of the rate constant multiplied by the product of the species concentrations corresponding to its educt complex. Hence one obtains $v_1 := k_1\, x_1$, $v_2 := k_2$, $v_3 := k_3$, $v_4 := k_4\, x_2$, $v_5 := k_5\, x_1^2\, x_2$ and $v_6 = k_6\, x_1^3$ for $\mathcal{N}_1$. To formalize this, let $Y^{(L)}$ be the *matrix of educt complexes*, where each educt complex is listed as often, as it is an educt complex of a reaction. For example, for $\mathcal{N}_1$, one obtains (using y_i, $i = 1, \ldots, 5$, as defined above):

$$\begin{aligned} Y^{(L)} &= \begin{bmatrix} y_1 & y_2 & y_2 & y_3 & y_4 & y_5 \end{bmatrix} \\ &= \begin{bmatrix} 1 & 0 & 0 & 0 & 2 & 3 \\ 0 & 0 & 0 & 1 & 1 & 0 \end{bmatrix}. \end{aligned} \tag{2.2c}$$

Thus one obtains $Y^{(L)} \in \mathbb{R}^{2\times 6}$ for $\mathcal{N}_1$ and $Y^{(L)} \in \mathbb{R}^{n\times r}$, in general. Using $y_i^{(L)}$ to denote column vectors of $Y^{(L)}$ and the definition

$$x^y := x_1^{y_1}\, x_2^{y_2}\, \ldots,\, x_n^{y_n} = \prod_{i=1}^{n} x_i^{y_i}, \tag{2.3a}$$

for vectors x, $y \in \mathbb{R}^n$, one obtains the monomial vector

$$\phi\,(x) := \begin{pmatrix} x^{y_1^{(L)}} \\ \vdots \\ x^{y_r^{(L)}} \end{pmatrix} \tag{2.3b}$$

and the vector of reaction rates

$$\begin{aligned} v(k,\, x) &= \operatorname{diag}\,(k)\ \phi\,(x)\,, \\ &= \operatorname{diag}\,(\phi\,(x))\ k, \end{aligned} \tag{2.3c}$$

where $k := (k_1, \ldots, k_r)^T$, the vector of rate constants. For the reaction network $\mathcal{N}_1$ $\phi(x) = (\, x_1,\, 1,\, 1,\, x_2,\, x_1^2\, x_2,\, x_1^3\,)^T$ and thus $v(k,\, x) = \left(k_1\, x_1,\, k_2,\, k_3,\, k_4\, x_2,\, k_5\, {x_1}^2\, x_2,\, k_6\, {x_1}^3\right)$. Then the ODEs describing the dynamics of a reaction networks are given as

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

Note the $n \times r$-matrix $Y\, I_a$ is sometimes called *stoichiometric matrix* and denoted by $N := Y\, I_a$. Consequently, in the literature on Chemical Reaction Network Theory, the linear subspace spanned by the columns of N is called *stoichiometric subspace*. Usually n (the number of species) is less than r (the number of reactions) and this will be a standing assumption in this thesis. The symbol s is used to denote its dimension, that is $s := \operatorname{rank}(N)$. The symbol $\mathcal{S}$ is used to denote the stoichiometric subspace, that is $\mathcal{S} := \operatorname{im}(N)$. If $s < n$, then the system (2.4) is subject to *conservation relations*. Let $W \in I\!R^{n\times(n-s)}$, with $W^T N = 0$. Then the flow of (2.4) is invariant under $[W]$ [58, 34, 35]. Thus one has to consider

$$W^T x = c, \tag{2.5}$$

for some $c \in I\!R^{n-s}_{>0}$. Often c is considered as a parameter that has to be determined. Observe that, by elementary linear algebra

$$[W] = \mathcal{S}^{\perp}, \tag{2.6}$$

that is, the range of W is the orthogonal complement of N (which follows naturally from the property $W^T N = 0$, [61]). Observe that the ODEs (2.4) are linear in the rate constants, as

$$\dot{x}\,(t) = Y\, I_a\, v(k,\, x(t)) = Y\, I_a\, \operatorname{diag}(\phi\,(x\,(t)))\; k.$$

For network $\mathcal{N}_1$, the stoichiometric matrix is

$$N = \begin{bmatrix} -1 & 1 & 0 & 0 & 1 & -1 \\ 0 & 0 & 1 & -1 & -1 & 1 \end{bmatrix} \tag{2.7}$$

and $s = 2$. The ODEs defined by the network are

$$\begin{aligned} \dot{x}_1 &= k_2 - k_1\, x_1 - k_6\, {x_1}^3 + k_5\, {x_1}^2\, x_2 \\ \dot{x}_2 &= k_3 + k_6\, {x_1}^3 - k_4\, x_2 - k_5\, {x_1}^2\, x_2. \end{aligned}$$

2.2.1 Properties of $\phi(x)$ and $v(k,\, x)$

Recall the definition of $\phi(x)$:

$$\phi(x) = \begin{pmatrix} x^{y_1^{(L)}} \\ \vdots \\ x^{y_r^{(L)}} \end{pmatrix}.$$

Let $\alpha \in I\!R^n$ and observe that $(\operatorname{diag}(\alpha)\; x) = (\alpha_1\, x_1,\, \ldots,\, \alpha_n\, x_n)^T$ and thus

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

for $\alpha,\, x,\, y \in I\!R^n$. Thus one obtains

$$\begin{aligned} \phi(\operatorname{diag}(\alpha)\; x) &= \begin{pmatrix} (\operatorname{diag}(\alpha)\; x)^{y_1^{(L)}} \\ \vdots \\ (\operatorname{diag}(\alpha)\; x)^{y_r^{(L)}} \end{pmatrix} = \begin{pmatrix} \alpha^{y_1^{(L)}}\, x^{y_1^{(L)}} \\ \vdots \\ \alpha^{y_r^{(L)}}\, x^{y_r^{(L)}} \end{pmatrix} \\ &= \operatorname{diag}\left(\alpha^{y_1^{(L)}},\, \ldots,\, \alpha^{y_r^{(L)}}\right) \begin{pmatrix} x^{y_1^{(L)}} \\ \vdots \\ x^{y_r^{(L)}} \end{pmatrix} = \operatorname{diag}(\phi(\alpha))\; \phi(x). \end{aligned} \tag{2.8}$$

Using the abbreviation $e^x := (e^{x_1}, \ldots, e^{x_n})^T$ for vectors $x \in I\!R^n$, one obtains in particular (for $\mu \in I\!R^n$)

$$\phi\left(e^{\mu}\right) = \begin{pmatrix} \left(e^{\mu}\right)^{y_1^{(L)}} \\ \vdots \\ \left(e^{\mu}\right)^{y_r^{(L)}} \end{pmatrix} = \begin{pmatrix} e^{\langle y_1^{(L)}, \mu \rangle} \\ \vdots \\ e^{\langle y_r^{(L)}, \mu \rangle} \end{pmatrix} = e^{Y^{(L)T}\mu}. \tag{2.9}$$

Further note

$$\frac{1}{\phi\left(x\right)} = \begin{pmatrix} \left(x^{y_1^{(L)}}\right)^{-1} \\ \vdots \\ \left(x^{y_r^{(L)}}\right)^{-1} \end{pmatrix} = \begin{pmatrix} \left(x^{-1}\right)^{y_1^{(L)}} \\ \vdots \\ (x-1)^{y_r^{(L)}} \end{pmatrix} = \phi\left(x^{-1}\right). \tag{2.10}$$

As $k \in I\!R^r_{>0}$, positive values of x will result in positive values of $v(k, x)$, that is

$$x \in I\!R^n_{>0} \Rightarrow v(k, x) \in I\!R^r_{>0}$$

and vice versa.

2.3 Chemical Reaction Network Theory (CRNT)

The distinguishing feature of CRNT is its ability to make a connection between the structure of a reaction network and the existence of (multiple) equilibria for the corresponding system of ODEs. Its general idea can be summarised in the following way: for any network, a non-negative integer δ called the deficiency can be derived from the network structure alone. For its formal definition one more concept is needed – the linkage class. Network $\mathcal{N}_1$ consists of two sets of complexes: $\{A, 0, B\}$ and $\{2\,A + B, 3\,A\}$. Both sets are internally connected by reactions, while no reactions exist between elements of distinct sets. Sets of complexes that are internally connected by reactions are called linkage classes. Let ℓ be the number of linkage classes in an arbitrary network. Then the deficiency of this network is defined as the non-negative integer [24, 25]

$$\delta = m - \ell - s \tag{2.11}$$

Note that the deficiency of the network only depends on the network structure and thus, in particular, δ is independent of parameter values. For network $\mathcal{N}_1$, $\delta = 1$. If δ is zero for a particular network, then no system of ODEs endowed with mass action kinetics that can be derived from the network can admit multiple steady states, regardless of the rate constants. Furthermore, sustained periodic oscillations are impossible [24, 27]. If δ is one and the network satisfies some mild additional conditions, the so-called Deficiency One Algorithm can be applied to decide whether or not the network can admit multiple steady states. If the deficiency is greater than one, under certain conditions the so-called Advanced Deficiency Theory can be used to decide about the possibility of multistationarity (for the Deficiency One Algorithm see [25, 28] and for Advanced Deficiency Theory see [23, 21, 22]).
For certain networks with $\delta = 1$, the deficiency one algorithm can be used to decide about the existence of a parameter vector k such that the corresponding system of ODEs endowed with mass action kinetics admits at least two positive steady-states. For certain networks of higher deficiency, the so-called Advanced Deficiency Theory can be applied. The principle idea of both the Deficiency One Algorithm and the Advanced Deficiency Theory is as follows: for each network where the algorithm or the theory is applicable, several systems of linear equalities and inequalities can be formulated (inequality systems, for short). (Note, whether or not the algorithm or the theory are applicable depends on the network structure alone; see the aforementioned references.) These inequality systems only depend on the network structure and the complexes, that is, the way the species are combined in the complexes and the way the complexes interact. If, for any of these systems, a solution exists and if this solution is sign compatible with the subspace $\mathcal{S}$, then multistationarity is possible (and a set of rate constants together with two distinct steady-states can be calculated from this solution). If no such solution exists, then multistationarity is impossible. (Note that, following the notation used in [24], a vector is sign compatible with a linear subspace if this subspace contains at least one vector with the same sign pattern; that is, the vector (1, -1) is sign compatible with the subspace spanned by (1, 0) and (1, -2) because (1, -2) has the same sign pattern as (1, -1), namely (+, -).)

Chapter 3

Conditions for multistationarity

Consider the Ordinary Differential Equations describing the dynamics of a biochemical reaction network as introduced in (2.4), (2.5) in Chapter 2:

$$\begin{aligned} \dot{x}(t) &= Y\, I_a\, v(k,\, x(t)) \\ W^T x &= c \end{aligned}$$

In this thesis only positive solutions x are considered, thus the corresponding reaction network is said to exhibit multistationarity, if two distinct positive vectors $a,\, b \in \mathbb{R}^n_{>0}$, a positive vector $k \in \mathbb{R}^r_{>0}$ and values c_i, $i = 1, \ldots, n-s$, can be found such that the following conditions hold:

$$N\, v(k,\, a) = 0 \tag{3.1a}$$

$$W^T a = c \tag{3.1b}$$

and

$$N\, v(k,\, b) = 0 \tag{3.2a}$$

$$W^T b = c. \tag{3.2b}$$

In the remainder of this chapter (3.1a), (3.1b) and (3.2a), (3.2b) are analysed and transformed into equivalent conditions that are in most cases easier to handle: in a first step the polynomial equations (3.1a) and (3.2a) are transformed and the rate constants are eliminated. This is described in Section 3.1. Solvability of the resulting system of equations is analysed in Section 3.2. As an arbitrary solution obviously can only be expected to satisfy (3.1a) and (3.2a), conditions are derived that guarantee that (3.1b) and (3.2b) hold as well. This is described in Section 3.3. Section 3.4 contains a discussion of the connection between multistationarity in a subnetwork and multistationarity in the overall network. This chapter closes with an algorithm to decide about multistationarity in a biochemical reaction network with mass action kinetics in Section 3.5. This algorithm incorporates the results obtained in Section 3.1 – 3.4.

3.1 Positive solutions to the polynomial equations

For biochemical reaction networks only positive solutions a, b to (3.1a), (3.1b) and (3.2a), (3.2b) are of interest. As discussed in the previous chapter, if $k > 0$, this requirement is equivalent to $v(k,\, a)$ and $v(k,\, b)$ being positive. The equations $Y\, I_a\, v(k,\, a) = 0$ and $Y\, I_a\, v(k,\, b) = 0$ with the positivity constraint can be simplified using the pointed polyhedral cone $\ker(Y\, I_a) \cap \mathbb{R}^r_{\geq 0}$ defined by the intersection of the null space of $Y\, I_a$, $\ker(Y\, I_a)$, with the nonnegative orthant of $\mathbb{R}^r$, $\mathbb{R}^r_{\geq 0}$. For this purpose some properties of $\ker(Y\, I_a) \cap \mathbb{R}^r_{\geq 0}$ are discussed in Section 3.1.1, before the equations are transformed in Section 3.1.2.

3.1.1 The cone $\ker(Y\, I_a) \cap \mathbb{R}^r_{\geq 0}$

The cone $\ker(Y\, I_a) \cap \mathbb{R}^r_{\geq 0}$ is a well studied object, due to its importance in the (bio)chemical literature, starting with the classical work of Clarke [8]. As a pointed polyhedral cone it can be represented by

non-negative linear combinations of a finite set of extreme rays [52]. The calculation of these rays is in general computationally hard, however, due to the importance of $\ker(Y\,I_a) \cap \mathbb{R}^r_{\geq 0}$, there exists a variety of algorithms and software tools, for example, [56, 32].
The aforementioned papers are part of the huge body of literature concerned with *metabolic flux analysis*, where each element of $\ker(Y\,I_a) \cap \mathbb{R}^r_{\geq 0}$ 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 $v \in \ker(Y\,I_a) \cap \mathbb{R}^r_{\geq 0}$ 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 $\ker(Y\,I_a) \cap \mathbb{R}^r_{\geq 0}$ [32].
In the literature on metabolic flux analysis the term *irreversible reaction* is defined differently than in the context of this work: forward and backward reaction are represented as a *single edge in the directed 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 the setup considered in this thesis, $x \in \mathbb{R}^n_{>0} \Rightarrow v_i(k, x) > 0$ for every reaction, thus all *reactions are irreversible in the context of metabolic flux analysis*. As a consequence, it is possible to use the software tools from metabolic flux analysis to calculate the extreme rays of $\ker(Y\,I_a) \cap \mathbb{R}^r_{\geq 0}$.
As defined in [32], extreme rays E_i of $\ker(Y\,I_a) \cap \mathbb{R}^r_{\geq 0}$ satisfy (3.3a), (3.3b) and (3.3c) given below:

$$Y\,I_a\,E_i = 0, \tag{3.3a}$$

$$E_i \in \mathbb{R}^r_{\geq 0}. \tag{3.3b}$$

Given E_i, E_j with $Y\,I_a\,E_i = 0$, $Y\,I_a\,E_j = 0$ and E_i, $E_j \in \mathbb{R}^r_{\geq 0}$. Then

$$\operatorname{supp}(E_i) \subseteq \operatorname{supp}(E_j) \Rightarrow E_i \;=\; 0 \text{ or } E_j \;=\; \alpha\,E_i,\, \alpha \;\in\; \mathbb{R}_{>0}, \tag{3.3c}$$

where $\operatorname{supp}(E_j) = \{\, i \in \{1, \ldots, r\} \mid E_{j_i} > 0, \}$ denotes the support of vector E_j, i.e. the set of indices where E_j has nonzero values. Let p be the number of extreme rays of $\ker(Y\,I_a) \cap \mathbb{R}^r_{\geq 0}$. (Note that extreme rays need not be linearly independent.) If a set of extreme rays $\{E_1, \ldots, E_p\}$ is complete, in the sense that their nonnegative linear combination is all of $\ker(Y\,I_a) \cap \mathbb{R}^r_{\geq 0}$, its elements are called *generators* of $\ker(Y\,I_a) \cap \mathbb{R}^r_{\geq 0}$.
In metabolic flux analysis the importance of the generators of $\ker(Y\,I_a) \cap \mathbb{R}^r_{\geq 0}$ stems from their one-to-one correspondence to the reactions: nonzero entries can be interpreted as 'active' reactions, zero entries as 'inactive' (in steady state). In this sense every generator defines a subnetwork of the original reaction network consisting of all 'active' reactions.

3.1.2 Parametrizing positive solutions – the equation $Y^{(L)^T}\mu = \ln \frac{E\,\nu}{E\,\lambda}$

Let E be a matrix, whose columns are generators for $\ker(Y\,I_a) \cap \mathbb{R}^r_{\geq 0}$, as described in Section 3.1.1. Then the equations $Y\,I_a\,v(k,\,a) = 0$ and $Y\,I_a\,v(k,\,b) = 0$ with the positivity constraint are equal to:

$$v(k,\,a) = E\,\lambda,\; \lambda \in \mathbb{R}^p_{\geq 0},\; E\,\lambda > 0, \tag{3.4a}$$

$$v(k,\,b) = E\,\nu,\; \nu \in \mathbb{R}^p_{\geq 0},\; E\,\nu > 0, \tag{3.4b}$$

where p denotes the number of extreme rays of $\ker(Y\,I_a) \cap \mathbb{R}^r_{\geq 0}$. Equations (3.4a) and (3.4b) guarantee that $v(k,\,a) \in \mathbb{R}^r_{>0}$ and $v(k,\,b) \in \mathbb{R}^r_{>0}$. The definition of $v(k,\,\cdot)$ in (2.3c) in turn ensures that $v(k,\,a) \in \mathbb{R}^r_{>0}$ and $v(k,\,b) \in \mathbb{R}^r_{>0}$ imply $a,\, b \in \mathbb{R}^n_{>0}$.
To streamline the discussion define the set of all nonnegative vectors $x \in \mathbb{R}^p_{\geq 0}$ such that $E\,x$ is positive:

$$\Lambda(E) := \left\{\, x \in \mathbb{R}^p_{\geq 0} \mid E\,x > 0 \,\right\}. \tag{3.5}$$

Then (3.4a), (3.4b) can be rewritten as

$$\begin{aligned} v(k,\,a) &= E\,\lambda, \\ v(k,\,b) &= E\,\nu, \\ \nu,\, \lambda &\in \Lambda(E)\,. \end{aligned} \tag{3.6}$$

Remark 1. ***Some comments about*** $\Lambda(E)$*:* $\Lambda(E)$ *is the union of cones. It consists of the positive orthant* $\mathbb{R}^p_{>0}$ *and some of its boundaries. To see this, observe the following points:*

(1) As $E \in \mathbb{R}^{r\times p}_{\geq 0}$, $x \in \mathbb{R}^p_{>0}$ *implies* $E\,x > 0$, *that is* $\mathbb{R}^p_{>0} \subseteq \Lambda(E)$.

(2) Let $x \in \mathbb{R}^p_{\geq 0}$ *with one zero component and the remaining components positive. Denote the zero component with* x_i *and let* $n_i^T \in \mathbb{R}^p_{\geq 0}$, $i = 1, \ldots, r$ *be the row vectors of* E. *Then*

$$E\,x = \begin{pmatrix} \langle n_1,\, x\rangle \\ \vdots \\ \langle n_r,\, x\rangle \end{pmatrix} > 0,$$

if and only if no scalar product $\langle n_j,\, x\rangle$ *is a multiple of* x_i *(i.e. if and only if* $\langle n_j,\, x\rangle = \sum_{l=1}^{p} n_{jl}\, x_l \neq n_{ji}\, x_i$ *). That is, if and only if* $i \in \operatorname{supp}(n_j)$ *implies* $|\operatorname{supp}(n_j| > 1)$, $j = 1, \ldots, r$. *A similar argument can be made for* $x \in \mathbb{R}^p_{>0}$ *with several components equal to zero.*

(3) In general the constraint $\nu,\, \lambda \in \Lambda(E)$ *is not hard to test: one can, for example, start with looking for positive* $\nu,\, \lambda$. *Only if those cannot be established, one might have to consider* $\nu,\, \lambda$ *with some components equal to zero. As such* $\Lambda(E)$ *is easily obtained once* E *is obtained . It is a standing assumption of this thesis that* $\Lambda(E)$ *is known.*

To proceed with the transformation, let $n_i^T \in \mathbb{R}^p_{\geq 0}$, $i = 1, \ldots, r$ be the row vectors of E, that is $E = \begin{bmatrix} n_1^T \\ \vdots \\ n_r^T \end{bmatrix}$. Then (3.4a), (3.4b) are

$$\begin{aligned} k_1\, a^{y_1^{(L)}} &= \langle n_1,\, \lambda\rangle & \qquad k_1\, b^{y_1^{(L)}} &= \langle n_1,\, \nu\rangle \\ &\vdots & &\vdots \\ k_r\, a^{y_r^{(L)}} &= \langle n_r,\, \lambda\rangle & \qquad k_r\, b^{y_r^{(L)}} &= \langle n_r,\, \nu\rangle \end{aligned}$$

As all terms are positive, application of ln to both sides of each equation is well justified (using for vectors $v \in \mathbb{R}^n$ the abbreviation $\ln v := (\ln v_1, \ldots, \ln v_n)^T$):

$$\begin{aligned} \ln k_1 + \langle y_1^{(L)},\, \ln a\rangle &= \ln\langle n_1,\, \lambda\rangle & \qquad \ln k_1 + \langle y_1^{(L)},\, \ln b\rangle &= \ln\langle n_1,\, \nu\rangle \\ &\vdots & &\vdots \\ \ln k_r + \langle y_r^{(L)},\, \ln a\rangle &= \ln\langle n_r,\, \lambda\rangle & \qquad \ln k_r + \langle y_r^{(L)},\, \ln b\rangle &= n\langle n_r,\, \nu\rangle \end{aligned}$$

In short:

$$\ln k_i + \langle y_i^{(L)},\, \ln a\rangle = \ln\langle n_i,\, \lambda\rangle \qquad \ln k_i + \langle y_i^{(L)},\, \ln b\rangle = \ln\langle n_i,\, \nu\rangle,\ i = 1, \ldots, r.$$

Subtracting equations in a from equations in b yields (using the definition $\mu := \left(\ln \frac{b_1}{a_1}, \ldots, \ln \frac{b_n}{a_n}\right)^T$):

$$\langle y_i^{(L)},\, \mu\rangle = \ln \frac{\langle n_i,\, \nu\rangle}{\langle n_i,\, \lambda\rangle},\ i = 1, \ldots, r. \tag{3.7}$$

Further note that the equations (3.7) are independent of the parameter vector k. These equations can be used to state a lemma connecting the existence of $a,\, b \in \mathbb{R}^n_{>0}$ and $k \in \mathbb{R}^r_{>0}$ to the existence of vectors $\lambda,\, \nu,\, \in \mathbb{R}^p_{>0}$ and $\mu \in \mathbb{R}^n$. With the definition

$$\ln \frac{E\,\nu}{E\,\lambda} := \left(\ln \frac{\langle n_1,\, \nu\rangle}{\langle n_1,\, \lambda\rangle}, \ldots, \ln \frac{\langle n_r,\, \nu\rangle}{\langle n_r,\, \lambda\rangle}\right)^T \tag{3.8a}$$

equation (3.7) can be written as

$$Y^{(L)^T}\,\mu = \ln \frac{E\,\nu}{E\,\lambda}. \tag{3.8b}$$

Lemma 1. *Let $\dot{x}(t) = Y\, I_a\, v(k,\, x(t))$ be a set of ordinary differential equations derived from a biochemical reaction network endowed with mass action kinetics. Further let $E = [E_1,\, \ldots,\, E_p]$, $E \in \mathbb{R}^{r\times p}_{\geq 0}$ be a matrix, whose columns are generators of $\ker(Y\, I_a) \cap \mathbb{R}^r_{>0}$ and let $\Lambda\,(E)$ be the set of all nonnegative vectors $x \in \mathbb{R}^p_{\geq 0}$ such that $E\, x > 0$, as defined in (3.5). Then the following statements are equivalent:*

(I) There exist two vectors $a, b \in \mathbb{R}^n_{>0}$, $a \neq b$ and a vector $k \in \mathbb{R}^r_{>0}$ with

$$Y\, I_a\, v(k,\, a) = 0$$
$$Y\, I_a\, v(k,\, b) = 0$$

(II) There exist two vectors $\lambda, \nu \in \Lambda\,(E)$ and a vector $\mu \in \mathbb{R}^n$ with

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

Proof. (I) $\Rightarrow$ (II): Let $a,\, b \in \mathbb{R}^n_{>0}$ with $k \in \mathbb{R}^r_{>0}$ with

$$Y\, I_a\, v(k,\, a) = 0 \tag{3.9a}$$
$$Y\, I_a\, v(k,\, b) = 0. \tag{3.9b}$$

To obtain $\mu \in \mathbb{R}^n$ and $\nu, \lambda \in \Lambda\,(E)$ proceed as above: (3.9a), (3.9b) and positivity of k imply $v(k,\, a) > 0$, $v(k,\, b) > 0$ and $v(k,\, a) \in \ker(Y\, I_a) \cap \mathbb{R}^r_{\geq 0}$, $v(k,\, b) \in \ker(Y\, I_a) \cap \mathbb{R}^r_{\geq 0}$. Thus there exists $\lambda, \nu \in \Lambda\,(E)$ such that

$$v(k,\, a) = E\, \lambda$$
$$v(k,\, b) = E\, \nu.$$

These equations can be transformed in the same process as described above:

$$Y^{(L)^T}\, \mu = \ln \frac{E\, \nu}{E\, \lambda},$$

where $\mu := \ln \frac{b}{a}$.

(II) $\Rightarrow$ (I): Assume $\lambda,\, \nu \in \Lambda\,(E)$ and $\mu \in \mathbb{R}^n$ are given such that $Y^{(L)^T}\, \mu = \ln \frac{E\, \nu}{E\, \lambda}$ holds. Then one has to show the existence of $a, b \in \mathbb{R}^n_{>0}$ and $k \in \mathbb{R}^r_{>0}$ such that $Y\, I_a\, v(k,\, a) = 0$ and $Y\, I_a\, v(k,\, b) = 0$. To this end choose any vector $a \in \mathbb{R}^n_{>0}$ and observe that a is a steady state if a positive vector k exists, such that $v(k,\, a) = E\, \lambda$, where $\lambda \in \Lambda\,(E)$ is known by assumption. Recall that $v(k,\, a) = \operatorname{diag}(k)\, \phi\,(a) = \operatorname{diag}(\phi\,(a))\, k$. Thus it is straightforward to obtain k:

$$k = \operatorname{diag}\left(\phi\left(a^{-1}\right)\right)\, E\, \lambda.$$

Then $b = \operatorname{diag}(e^{\mu})\, a$ is a positive steady state as well, where $\mu \in \mathbb{R}^n$ is fixed by assumption. To see this, one has to show that $v(k,\, b) = E\, \nu$, with k as above. Note that $\nu \in \Lambda\,(E)$ is known by assumption. Recall, that, by assumption as well, ν, λ and μ satisfy $Y^{(L)^T}\, \mu = \ln \frac{E\, \nu}{E\, \lambda}$. Observe the following:

$$\begin{aligned}
v(k,\, b) = \operatorname{diag}(k)\, \phi\,(b) &= \operatorname{diag}\left(\phi\left(a^{-1}\right)\right)\, \operatorname{diag}(E\, \lambda)\, \operatorname{diag}(\phi\,(e^{\mu}))\, \phi\,(a) \\
&= \operatorname{diag}(\phi\,(e^{\mu}))\, \operatorname{diag}(E\, \lambda)\, \underbrace{\operatorname{diag}\left(\phi\left(a^{-1}\right)\right)\, \phi\,(a)}_{=\underline{1}} \\
&= \operatorname{diag}(\phi\,(e^{\mu}))\, E\, \lambda \\
&= \operatorname{diag}\left(e^{Y^{(L)^T}\, \mu}\right)\, E\, \lambda \\
&= \operatorname{diag}\left(\frac{E\, \nu}{E\, \lambda}\right)\, E\, \lambda \\
&= E\, \nu.
\end{aligned}$$

■

Solutions $\mu \in \mathbb{R}^n$ and $\nu, \lambda \in \Lambda(E)$ can be associated with the difference of two positive steady state solutions: if a satisfies $Y\,I_a\,v(k,\,a) = 0$ (i.e. $v(k,\,a) = E\,\lambda$) then $b = \mathrm{diag}\,(e^{\mu})\;a$ satisfies $Y\,I_a\,v(k,\,b) = 0$ (i.e. $v(k,\,b) = E\,\nu$). Note that, as is shown in the proof of Lemma 1, any positive vector $a \in \mathbb{R}^n_{>0}$ can be a steady state solution. Simply solve $v(k,\,a) = \mathrm{diag}\,(k)\;\phi\,(a)$ for the parameter vector $k \in \mathbb{R}^r_{>0}$ (note that this equation is *linear in* k):

$$k = \mathrm{diag}\left(\phi\left(a^{-1}\right)\right)\;E\,\lambda. \tag{3.10a}$$

Thus, a solution $(\mu,\,\nu,\,\lambda)$ defines in fact a continuum of solutions parameterized by $a \in \mathbb{R}^n_{>0}$: for a given solution $(\mu,\,\nu,\,\lambda)$ choose $a \in \mathbb{R}^n_{>0}$ and fix k as in (3.10a). Then, by Lemma 1,

$$b = \mathrm{diag}\,(\mathrm{Exp}\,(\mu))\;a \tag{3.10b}$$

is a steady state solution as well. However, a and b need not satisfy the conservation relations (3.1b) and (3.2b) for the same values c_i. To find pairs a and b that satisfy these constraints as well, see Section 3.3.

3.2 Solvability of $Y^{(L)^T}\mu = \ln\frac{E\,\nu}{E\,\lambda}$

In this Section (3.8b) is analysed. Note that

$$Y^{(L)^T}\mu = \ln\frac{E\,\nu}{E\,\lambda}$$

is solvable for given ν, λ, if and only if

$$U^T\,Y^{(L)^T} = 0 \Rightarrow U^T\,\ln\frac{E\,\nu}{E\,\lambda} = 0. \tag{3.11}$$

This is a consequence of the fact that (3.8b) is only solvable, if the right hand side is contained in the image of $Y^{(L)^T}$, that is, if $\ln\frac{E\,\nu}{E\,\lambda} \in \left[Y^{(L)^T}\right]$. This is the case, if and only if the orthogonal complement of $\left[Y^{(L)^T}\right]$, $\left[Y^{(L)^T}\right]^{\perp}$, is orthogonal to $\ln\frac{E\,\nu}{E\,\lambda}$. Note that by the 'fundamental theorem of linear algebra $\left[Y^{(L)^T}\right]^{\perp} = \ker\left(Y^{(L)}\right)$ [61]. In Section 3.2.1 and Section 3.2.2 two solution strategies based on condition (3.11) are discussed. Recall that $Y^{(L)} \in \mathbb{R}^{n\times r}$, where n is the number of species and r the number of reactions. As, by assumption, $r > n$, condition (3.11) will exist for almost any biochemical reaction network.

3.2.1 Solvability I

Let U be a basis for $\ker\left(Y^{(L)}\right)$ with integer coefficients (such a basis can always be obtained by an adequate scaling of basis-vectors, as $Y^{(L)} \in \mathbb{N}_0^{n\times r}$). Consider basis-vector u_i and let $u_i = u_i^{\oplus} - u_i^{\ominus}$, where $u_i^{\oplus}$, $u_i^{\ominus}$ are vectors with nonnegative integer coefficients. Then

$$u_i^T\,\ln\frac{E\,\nu}{E\,\lambda} = 0$$

evaluates to

$$u_i^{\oplus T}\,\ln\frac{E\,\nu}{E\,\lambda} = u_i^{\ominus T}\,\ln\frac{E\,\nu}{E\,\lambda}$$

$$\sum_{j\in\mathrm{supp}\left(u_i^{\oplus}\right)} u_{ij}^{\oplus}\,\ln\frac{\langle n_j,\,\nu\rangle}{\langle n_j,\,\lambda\rangle} = \sum_{l\in\mathrm{supp}\left(u_i^{\ominus}\right)} u_{il}^{\ominus}\,\ln\frac{\langle n_l,\,\nu\rangle}{\langle n_l,\,\lambda\rangle}$$

$$\sum_{j\in\mathrm{supp}\left(u_i^{\oplus}\right)} \ln\left(\frac{\langle n_j,\,\nu\rangle}{\langle n_j,\,\lambda\rangle}\right)^{u_{ij}^{\oplus}} = \sum_{l\in\mathrm{supp}\left(u_i^{\ominus}\right)} \ln\left(\frac{\langle n_l,\,\nu\rangle}{\langle n_l,\,\lambda\rangle}\right)^{u_{il}^{\ominus}}.$$

Using the summation rule for ln one obtains

$$\ln \prod_{j\in\operatorname{supp}\left(u_i^{\oplus}\right)} \left(\frac{\langle n_j,\, \nu\rangle}{\langle n_j,\, \lambda\rangle}\right)^{u_{ij}^{\oplus}} = \ln \prod_{l\in\operatorname{supp}\left(u_i^{\ominus}\right)} \left(\frac{\langle n_l,\, \nu\rangle}{\langle n_l,\, \lambda\rangle}\right)^{u_{il}^{\ominus}}.$$

By removing ln (by taking both sides to $e^{\cdot}$), multiplying with numerators and rearranging terms one finally obtains

$$\prod_{j\in\operatorname{supp}\left(u_i^{\oplus}\right)} (\langle n_j,\, \nu\rangle)^{u_{ij}^{\oplus}} \prod_{l\in\operatorname{supp}\left(u_i^{\ominus}\right)} (\langle n_l,\, \lambda\rangle)^{u_{il}^{\ominus}} - \prod_{l\in\operatorname{supp}\left(u_i^{\ominus}\right)} (\langle n_l,\, \nu\rangle)^{u_{il}^{\ominus}} \prod_{j\in\operatorname{supp}\left(u_i^{\oplus}\right)} (\langle n_j,\, \lambda\rangle)^{u_{ij}^{\oplus}} = 0, \qquad i = 1,\, \ldots,\, r - \operatorname{rank}\left(Y^{(L)}\right). \tag{3.12}$$

There are $r - \operatorname{rank}\left(Y^{(L)}\right)$ basis-vectors for $\ker\left(Y^{(L)}\right)$ and thus $r - \operatorname{rank}\left(Y^{(L)}\right)$ polynomials of the form given in (3.12). If a solution to these polynomials exists, then it is straightforward to solve (3.8b) in terms of the μ_i. To see this, let $\tilde{\nu},\, \tilde{\lambda} \in \mathbb{R}^p_{>0}$ be such that (3.12) holds. Then $\ln \frac{E\,\tilde{\nu}}{E\,\tilde{\lambda}} \in \left[Y^{(L)^T}\right]$. Thus

$$Y^{(L)^T}\,\mu = \ln \frac{E\,\tilde{\nu}}{E\,\tilde{\lambda}}$$

is solvable and, as these equations are linear in μ, it is straightforward to obtain μ. In this case $\mu = M\,\kappa$, where M is a matrix of appropriate dimension and $[M] \subseteq \mathbb{R}^n$ is a linear subspace of $\mathbb{R}^n$.

Remark 2. *In this Chapter the question of the existence of more than one positive solution to the system of polynomials $Y\,I_a\,v(k,\,x) = 0$ has been analysed. In general $Y\,I_a\,v(k,\,x) = 0$ is a system of n polynomials with $r > n$* unknown *coefficients k_i in the n unknowns x_i. Lemma 1 and the preceeding discussion show that this question is equivalent to the question of the existence of* at least one *solution $\nu,\, \lambda \in \Lambda(E)$ to a system of $r - \operatorname{rank}\left(Y^{(L)}\right)$ polynomials with* known *coefficients in the $2\,p$ unknowns ν and λ. Thus it can be expected that these polynomials are easier to handle (in fact, it is possible to examine these polynomials* numerically*, even though the rate constants k_i are unknown). However, even though the polynomials are now accessible numerically it will in general not be easy to find positive solutions, let alone all of them.*

3.2.1.1 Special form of polynomials

The matrix $Y^{(L)}$ as described in Section 2.2 contains all reactant complexes of the network. If a complex is reactant in several reactions (say in γ reactions) then $Y^{(L)}$ contains exactly γ copies of this complex vector. Then a basis for $\ker\left(Y^{(L)}\right)$, the kernel of $Y^{(L)}$, exists, that contains $\gamma - 1$ vectors with exactly one entry $+1$, one entry -1 and the remaining entries 0. Let $Y^{(R)} \in \mathbb{R}^{n\times \text{nr. of reactant complexes}}$ be the matrix of reactant complexes (i.e. the matrix that contains the vectors of each reactant complexes exactly once). If this matrix is of full rank and the number of reactant complexes is less than n, the number of species, then $\ker\left(Y^{(L)}\right)$ is spanned only by vectors of this form; this is, for example, the case for the networks related to the activation of an MAPK, see Chapter 4. Vectors of this form lead to a special system of polynomials that will be discussed here.
Let $u_i = (\,0,\, \ldots,\, 1,\, 0,\, \ldots,\, -1, 0,\, \ldots)^T$ (with $\operatorname{supp}(u_i) = \{j,\, l\}$). Then (3.12) simplifies to

$$\langle n_j,\, \nu\rangle\,\langle n_l,\, \lambda\rangle - \langle n_l,\, \nu\rangle\,\langle n_j,\, \lambda\rangle = 0$$

as $u_{ij}^{\oplus} = 1$ and $u_{il}^{\ominus} = 1$. This is equivalent to

$$\left(\sum_{s\in\operatorname{supp}(n_j)} n_{js}\,\nu_s\right)\left(\sum_{t\in\operatorname{supp}(n_l)} n_{lt}\,\lambda_t\right) - \left(\sum_{s\in\operatorname{supp}(n_j)} n_{js}\,\lambda_s\right)\left(\sum_{t\in\operatorname{supp}(n_l)} n_{lt}\,\nu_t\right) = 0.$$

Let $\Gamma_i := \operatorname{supp}(n_j) \times \operatorname{supp}(n_l)$, be the set of all ordered pairs of elements of $\operatorname{supp}(n_j)$ and $\operatorname{supp}(n_l)$. Then

$$\left(\sum_{s\in\operatorname{supp}(n_j)} n_{js}\,\nu_s\right)\left(\sum_{t\in\operatorname{supp}(n_l)} n_{lt}\,\lambda_t\right) = \sum_{(s,t)\in\Gamma_i} n_{js}\,n_{lt}\,\nu_s\,\lambda_t$$

and

$$\left(\sum_{s\in\mathrm{supp}(n_j)} n_{js}\,\lambda_s\right)\left(\sum_{t\in\mathrm{supp}(n_l)} n_{lt}\,\nu_t\right) = \sum_{(s,t)\in\Gamma_i} n_{js}\,n_{lt}\,\nu_t\,\lambda_s.$$

This leads to the following special form for (3.12):

$$\langle n_j,\,\nu\rangle\,\langle n_l,\,\lambda\rangle - \langle n_l,\,\nu\rangle\,\langle n_j,\,\lambda\rangle = \sum_{(s,t)\in\Gamma_i} n_{js}\,n_{lt}\,\left(\nu_s\,\lambda_t - \nu_t\,\lambda_s\right). \tag{3.13}$$

Let $\alpha_i = (n_{js}\,n_{lt})_{(s,t)\in\Gamma_i}$ and $\beta_i\,(\nu,\lambda) = (\nu_s\,\lambda_t - \nu_t\,\lambda_s)_{(s,t)\in\Gamma_i}$. Then (3.13) is equivalent to $\langle\alpha_i,\,\beta_i\,(\nu,\lambda)\rangle = 0$. Clearly each u_i of the form discussed here yields vectors α_i and β_i. By collecting all binomials of the form $\nu_s\,\lambda_t - \nu_t\,\lambda_s$ in a vector $\beta\,(\nu,\lambda)$ and, after an appropriate filling with zeros, of all α_i as row vectors of a matrix A one obtains the following compact representation:

$$A\,\beta\,(\nu,\lambda) = 0. \tag{3.14}$$

3.2.2 Solvability II

Instead of solving the polynomials associated with $U^T\,\ln\frac{E\,\nu}{E\,\lambda} = 0$ as described in the previous section the equation $Y^{(L)^T}\mu = \ln\frac{E\,\nu}{E\,\lambda}$ is transformed in the following way: note that (3.8b) is equivalent to

$$\mathrm{diag}\left(e^{Y^{(L)^T}\mu}\right)\,E\,\lambda = E\,\nu$$

and, after rearranging terms,

$$\left[E \quad -\mathrm{diag}\left(e^{Y^{(L)^T}\mu}\right)\,E\right]\begin{pmatrix}\nu\\ \lambda\end{pmatrix} = 0\,,\nu,\,\lambda\in\Lambda\,(E)\,. \tag{3.15}$$

Recall that $E\in\mathbb{R}^{r\times p}_{\geq 0}$, r the number of reactions and note that in general $r > p$. Thus, for the remainder of Section 3.2.2 the following assumption will be made:

Assumption 1. *The number of reactions r is greater than p the number of generators of* $\ker(Y\,I_a)\cap\mathbb{R}^r_{\geq 0}$.

Recall that $\nu,\,\lambda\in\Lambda\,(E)$, as they are coordinates of points in the interior of a pointed polyhedral cone. Let let $U\in\mathbb{R}^{r\times r}$ be a matrix such that $U\,E$ is in row reduced echelon form:

$$U\,E = \begin{bmatrix} I_o & E_o\\ 0 & 0\end{bmatrix},$$

where $o = \mathrm{rank}\,(E)$ and $\begin{bmatrix} E_0\\ -I_{r-o}\end{bmatrix}$ is a basis for $\ker(E)$. The row reduced echelon form is a result of *Gauss-Jordan elimination* (after a possible reordering of variables). Thus, such a matrix U can always be obtained [61]. Let $U = \begin{bmatrix} U_1\\ U_2\end{bmatrix}$ and multiply (3.15) by U to obtain

$$\underbrace{\begin{bmatrix} I_o & E_0 & -U_1\,\mathrm{diag}\left(e^{Y^{(L)^T}\mu}\right)E\\ 0 & 0 & -U_2\,\mathrm{diag}\left(e^{Y^{(L)^T}\mu}\right)E\end{bmatrix}}_{=:Q(\mu)}\begin{pmatrix}\nu\\ \lambda\end{pmatrix} = 0\,,\nu,\,\lambda\in\Lambda\,(E)\,. \tag{3.16}$$

Observe the following facts:

(i) Equation (3.16) is feasible, only if $Q_2\,(\mu) := U_2\,\mathrm{diag}\left(e^{Y^{(L)^T}\mu}\right)\,E$ has a kernel vector $\lambda\in\Lambda\,(E)$, that is only if

$$Q_2\,(\mu)\,\lambda = 0,\ \lambda\in\Lambda\,(E)$$

is feasible for some $\mu\in\mathbb{R}^n$.

(ii) However $\nu \in \Lambda(E)$ has to hold as well. Thus, in general, one has to consider the complete matrix $Q(\mu)$. There might be cases, where one must not consider all of $Q(\mu)$: if $Q_1(\mu) := \left[E_0 \; -U_1 \operatorname{diag}\left(e^{Y^{(L)^T}\mu}\right) E\right]$ contains nonpositive rows. Let $Q_1(\mu) = \begin{bmatrix} q_1(\mu) \\ \vdots \\ q_o(\mu) \end{bmatrix}$ and let $\mathcal{I} \subseteq \{1, \ldots, o\}$ be the set of indices such that $q_i(\mu) \leq 0$, $q_i(\mu) \neq 0$, for all $\mu \in \mathbb{R}^n$. That is, $q_i(\mu)$ is a nonpositive row vector of $Q_1(\mu)$, not identically zero. Split $\binom{\nu}{\lambda} = \begin{pmatrix} \tilde{\nu} \\ \nu_0 \\ \lambda \end{pmatrix}$ accordingly. Then

$$\tilde{\nu} = -\left[E_0 \quad -U_1 \operatorname{diag}\left(e^{Y^{(L)^T}\mu}\right) E\right] \begin{pmatrix} \nu_0 \\ \lambda \end{pmatrix} = -Q_1(\mu) \begin{pmatrix} \nu_0 \\ \lambda \end{pmatrix}.$$

Obviously, if $q_i(\mu) \leq 0$, $q_i(\mu) \neq 0$ then $\tilde{\nu}_i = -q_i(\mu)\binom{\nu_0}{\lambda} > 0$, if $\binom{\nu_0}{\lambda} > 0$. Thus the row $q_i(\mu)$ can be excluded, as $\tilde{\nu}_i$ will be positive, if $\binom{\nu_0}{\lambda} > 0$.

(iii) Suppose $Q_1(\mu)$ as defined above contains a nonnegative row (for all $\mu \in \mathbb{R}^n$), that is, $q_j(\mu) \geq 0$, for some $j \in \{1, \ldots, o\}$. Then $\tilde{\nu}_i = -q_i(\mu)\binom{\nu_0}{\lambda} \leq 0$, if $\binom{\nu_0}{\lambda} > 0$ and multistationarity is excluded, as by Lemma 1, the system cannot admit two positive steady state solutions.

As a consequence of the previous discussion, for the remainder of Section 3.2.2, the following assumption will be made:

Assumption 2. *No row of $Q_1(\mu)$ as defined above is nonnegative (for all $\mu \in \mathbb{R}^n$).*

As a consequence of and the above assumption, it suffices to consider those rows of $Q_1(\mu)$ that contain positive and negative elements. Let $\mathcal{I}^{\pm} \subseteq \{1, \ldots, o\}$ be the set of indices that correspond to rows of $Q_1(\mu)$ containing positive and negative entries and let $pm := |\mathcal{I}^{\pm}|$. Then one has to examine the following submatrix of $Q(\mu)$:

$$\tilde{Q}(\mu) := \begin{bmatrix} I_{pm} & (q_i(\mu))_{i \in \mathcal{I}^{\pm}} \\ 0 & Q_2(\mu) \end{bmatrix}.$$

Note that this submatrix is defined mainly for convenience. The algorithms presented in the remainder of this Chapter depend on the size of this matrix. Thus a removal of those rows of $Q(\mu)$ that always have a positive null vector can simplify the analysis. Thus, from now on, $Q(\mu)$ will be used to denote the matrix, where all nonpositive rows of $Q_1(\mu)$ gave been removed.
Further note that (3.16) is *linear* in ν and λ. For fixed values of μ the computation of ν, λ is straightforward, by determining the extreme rays of $\ker(Q(\mu)) \cap \mathbb{R}^{2p}_{\geq 0}$. However, $\ker(Q(\mu)) \cap \mathbb{R}^{2p}_{\geq 0}$ might be the empty set for a particular μ. In some cases it is known, that for any choice of μ the cone $\ker(Q(\mu)) \cap \mathbb{R}^{2p}_{\geq 0}$ has *nonempty interior*, that is there exists a positive kernel vector for $Q(\mu)$. This is the case, if $Q(\mu)$ is a so-called L^+-matrix [44].

3.2.2.1 L^+-matrices matrices and sign-central matrices

In some cases it is possible to decide about the existence of positive kernel vectors by the *sign pattern* of a matrix. In many applications a system of linear equations of the form $Q\,y = 0$ has to be solved, where the entries Q_{ij} of Q are known only approximately, if at all. It is however frequently the case that the sign of the Q_{ij} is known. There exist certain matrices Q where the *sign pattern* of the solutions y to $Q\,y = 0$ can be determined by the sign pattern of Q (see e.g. [6] for a general introduction, [40] and [41] or [3] for nonnegative solutions and [44] for positive solutions). In the context of this section, the results of [44] and of [3] are of the most importance: in [44] L^+-matrices are introduced and in [3] sign-central matrices are introduced. If Q is an L^+-matrix, then every matrix with *the same sign pattern* has a positive kernel vector. If Q is a sign-central matrix, then every matrix with *the same sign pattern* has a nonnegative kernel vector.
To state the main results, some additional notation is necessary: let $A \in \{-1, 0, 1\}^{m \times n}$ be a sign pattern. A nonzero vector $\sigma \in \{-1, 0, 1\}^m$ is called a *signing* and a nonzero vector $\sigma \in \{-1, 1\}^m$ is called a *strict signing*. Furthermore, in [44, 3], the 'requires' and 'allows' terminology is used: let P be a property that a matrix can or cannot have. Then the sign pattern A *requires* P if each matrix with *the same sign pattern has property P*, and A *allows* P if there *exists some matrix with the same sign pattern with property P*. Consider [44, Theroem 2.4, p. 6]:

Theorem 1. *Let A be an m by n sign pattern. Then the following are equivalent:*

(a) A is an L^+-matrix.

(b) A requires a positive null vector and A has no zero row.

(c) For each signing σ, some column of $\mathrm{diag}(\sigma)$ *A is nonzero and nonnegative.*

(d) For each signing σ, some column of $\mathrm{diag}(\sigma)$ *A is nonzero and nonpositive.*

and [3, Theorem 2.1, p. 286]:

Theorem 2. *Let A be an m by n sign pattern. Then the following are equivalent:*

(a) A is a sign-central matrix.

(b) For every strict signing, σ, some column of $\mathrm{diag}(\sigma)$ *A is nonzero and nonnegative.*

(c) For each strict signing, σ, some column of $\mathrm{diag}(\sigma)$ *A is nonzero and nonpositive.*

Recall that by definition a sign-central matrix has a nonnegative kernel vector [3, p. 284]. Note that Theorem 1 and 2 already contain primitive algorithms to determine whether or not a given sign pattern is an L^+- or a sign-central matrix, respectively.
In general $Y^{(L)^T}\mu$ and thus the sign of the entries Q_{ij} of $Q(\mu)$ is unknown, however, in certain cases, the signs of the Q_{ij} can be determined *by systems of linear inequalities*. In the remainder of this section it is in a first step shown under what conditions the sign-pattern of $Q(\mu)$ can be determined by linear inequality systems, before in a second step L^+-matrices are used to solve $Q(\mu)\begin{pmatrix}\nu\\ \lambda\end{pmatrix} = 0$, $\nu, \lambda \in \Lambda(E)$.

3.2.2.2 Sign patterns of $Q(\mu)$ determined by linear inequalities

Consider

$$Q(\mu) = \begin{bmatrix} I_o & E_0 & -U\ \mathrm{diag}\left(e^{Y^{(L)^T}\mu}\right) E \end{bmatrix}$$

as defined above. Split $Q(\mu) = \begin{bmatrix} Q_f & -Q_v(\mu) \end{bmatrix}$, $Q_f \in \mathbb{R}^{r\times p}_{>0}$, $Q_v(\mu) \in \mathbb{R}^{r\times p}$, where

$$Q_f := \begin{bmatrix} I_o & E_0 \\ 0 & 0 \end{bmatrix},\ Q_v(\mu) := U\ \mathrm{diag}\left(e^{Y^{(L)^T}\mu}\right) E. \tag{3.17}$$

Obviously $\mathrm{sign}(Q_f)$, the sign pattern of Q_f, is fixed, while $\mathrm{sign}(Q_v(\mu))$, the sign pattern of $Q_v(\mu)$, depends on the vector μ. Under some conditions it is possible to determine all sign patterns $\mathrm{sign}(Q_v(\mu))$, that $Q_v(\mu)$ can admit, for any conceivable vector $\mu \in \mathbb{R}^n$. This is examined in this section.
Consider $Q_v(\mu) = U\ \mathrm{diag}\left(e^{Y^{(L)^T}\mu}\right) E$ and observe that

$$Q_{ij} = U_i^T\ \mathrm{diag}\left(e^{Y^{(L)^T}\mu}\right) E_j,\ i,j = 1,\dots,r,$$

where U_i^T is the i-th row vector of U and E_j the j-th column vector of E. Then

$$Q_{ij} = \sum_{l=1}^{r} e^{\langle y_l^{(L)},\mu\rangle} U_{il} E_{jl},\ i,j = 1,\dots,r.$$

Obviously $e^{\langle y_l^{(L)},\mu\rangle} U_{il} E_{jl} = 0$, if $U_{il} = 0$ or $E_{jl} = 0$. Thus

$$Q_{ij} = \sum_{l\in \mathrm{supp}\left(U_i^T\right)\cap \mathrm{supp}(E_j)} e^{\langle y_l^{(L)},\mu\rangle} U_{il} E_{jl}.$$

Now assume $\mathrm{supp}\left(U_i^T\right)\cap\mathrm{supp}(E_j) = \{s_{ij}, t_{ij}\}$, that is $\mathrm{supp}\left(U_i^T\right)$ and $\mathrm{supp}(E_j)$ have only two elements in common. Then

$$Q_{ij} = e^{\langle y_{s_{ij}}^{(L)},\mu\rangle} U_{is_{ij}} E_{js_{ij}} + e^{\langle y_{t_{ij}}^{(L)},\mu\rangle} U_{it_{ij}} E_{jt_{ij}}.$$

If either

$$e^{\langle y^{(L)}_{s_{ij}},\,\mu\rangle}\,U_{is_{ij}}\,E_{js_{ij}} > 0 \quad \text{and } e^{\langle y^{(L)}_{t_{ij}},\,\mu\rangle}\,U_{it_{ij}}\,E_{jt_{ij}} > 0$$

or

$$e^{\langle y^{(L)}_{s_{ij}},\,\mu\rangle}\,U_{is_{ij}}\,E_{js_{ij}} < 0 \quad \text{and } e^{\langle y^{(L)}_{t_{ij}},\,\mu\rangle}\,U_{it_{ij}}\,E_{jt_{ij}} < 0,$$

then trivially $Q_{ij} > 0$ or $Q_{ij} < 0$, respectively. Thus assume

$$e^{\langle y^{(L)}_{s_{ij}},\,\mu\rangle}\,U_{is_{ij}}\,E_{js_{ij}} > 0 \quad \text{and } e^{\langle y^{(L)}_{t_{ij}},\,\mu\rangle}\,U_{it_{ij}}\,E_{jt_{ij}} < 0.$$

Then

$$\operatorname{sign}(Q_{ij}) = \begin{cases} > 0, \text{ if } e^{\langle y^{(L)}_{s_{ij}},\,\mu\rangle}\,U_{is_{ij}}\,E_{js_{ij}} > e^{\langle y^{(L)}_{t_{ij}},\,\mu\rangle}\,U_{it_{ij}}\,E_{jt_{ij}} \\ < 0, \text{ if } e^{\langle y^{(L)}_{s_{ij}},\,\mu\rangle}\,U_{is_{ij}}\,E_{js_{ij}} < e^{\langle y^{(L)}_{t_{ij}},\,\mu\rangle}\,U_{it_{ij}}\,E_{jt_{ij}} \\ = 0, \text{ if } e^{\langle y^{(L)}_{s_{ij}},\,\mu\rangle}\,U_{is_{ij}}\,E_{js_{ij}} = e^{\langle y^{(L)}_{t_{ij}},\,\mu\rangle}\,U_{it_{ij}}\,E_{jt_{ij}} \end{cases} \tag{3.18}$$

It is easy to see that the conditions in (3.18) are linear: simply apply $\ln(\cdot)$ to obtain

$$\begin{aligned} Q_{ij} < 0, \text{ if } \langle y^{(L)}_{s_{ij}},\,\mu\rangle + \ln\left(U_{is_{ij}}\,E_{js_{ij}}\right) &< \langle y^{(L)}_{t_{ij}},\,\mu\rangle + \ln\left(U_{it_{ij}}\,E_{jt_{ij}}\right). \\ Q_{ij} = 0, \text{ if } \langle y^{(L)}_{s_{ij}},\,\mu\rangle + \ln\left(U_{is_{ij}}\,E_{js_{ij}}\right) &= \langle y^{(L)}_{t_{ij}},\,\mu\rangle + \ln\left(U_{it_{ij}}\,E_{jt_{ij}}\right). \\ Q_{ij} > 0, \text{ if } \langle y^{(L)}_{s_{ij}},\,\mu\rangle + \ln\left(U_{is_{ij}}\,E_{js_{ij}}\right) &> \langle y^{(L)}_{t_{ij}},\,\mu\rangle + \ln\left(U_{it_{ij}}\,E_{jt_{ij}}\right). \end{aligned}$$

Thus one obtains

$$\operatorname{sign}(Q_{ij}) = \operatorname{sign}\left(\langle v_{ij},\,\mu\rangle + \beta_{ij}\right), \tag{3.19a}$$

where

$$v_{ij} := y^{(L)}_{s_{ij}} - y^{(L)}_{t_{ij}}, \tag{3.19b}$$

$$\beta_{ij} := \ln\frac{U_{is_{ij}}\,E_{is_{ij}}}{U_{is_{ij}}\,E_{is_{ij}}}. \tag{3.19c}$$

This motivates the following Lemma:

Lemma 2. *Let* $U = \begin{bmatrix} U_1^T \\ \vdots \\ U_r^T \end{bmatrix}$ *and* $E = \begin{bmatrix} E_1 & \dots & E_p \end{bmatrix}$. *Then the sign pattern of* $Q(\mu)$ *as defined in (3.16) can be determined by linear inequality systems if* $\operatorname{supp}\left(U_i^T\right) \cap \operatorname{supp}(E_j) \leq 2$, $\forall i, j = 1, \dots, r$.

Remark 3. *Note that if for* Q_{ij} *either all terms* $e^{\langle\cdot,\,\cdot\rangle}$ *have the same sign or* $\operatorname{supp}\left(U_i^T\right) \cap \operatorname{supp}(E_j) \leq 1$, *it is straightforward to determine the sign of* Q_{ij}.

Next determine all entries Q_{ij}, where the sign has to be determined by a linear inequality, that is define the index set

$$\begin{aligned} \mathcal{I} := \Big\{ (i,j) \in \{1,\dots,r\} \times \{1,\dots,r\} \,|\, &\operatorname{supp}(U_i) \cap \operatorname{supp}(E_j) = \{s_{ij}, t_{i,j}\} \\ &\text{and } \left(U_{is_{ij}}\,E_{js_{ij}}\right)\left(U_i t_{ij}\,E_{jt_{ij}}\right) < 0 \Big\}. \end{aligned} \tag{3.20}$$

Let s_0 be the number of entries Q_{ij}, where the sign has to be determined by a linear inequality (i.e. $s_0 = |\mathcal{I}|$) and collect all v_{ij} as defined in (3.19b) as row vectors in a matrix $V \in \mathbb{R}^{s_0 \times n}$:

$$V := \left[\; v_{ij}^T \;\right]_{(i,j)\in\mathcal{I}} \tag{3.21a}$$

and all β_{ij} as defined in (3.19c) in a vector $\beta \in \mathbb{R}^{s_0}$:

$$\beta := (\beta_{ij})_{(i,j)\in\mathcal{I}}, \tag{3.21b}$$

using $\mathcal{I}$ as in (3.20). Note that one can determine all possible values of $\mathrm{sign}(V\mu+\beta)$: let $\sigma \in \{-1, 0, 1\}^{s_0}$ be a signing and observe that $\mathrm{sign}(V\mu+\beta) = \sigma$, if and only if

$$V\mu + \beta - \mathrm{diag}(\sigma)\, s = 0, s > 0 \tag{3.22}$$

has a solution (μ, s), $s > 0$. Thus, by checking feasibility of (3.22), for every $\sigma \in \{-1, 0, 1\}^{s_0}$, one can determine all signings that $\mathrm{sign}(Q(\mu))$ can assume. A signing σ, such that (3.22) is feasible is called a *feasible signing*. Observe the following fact:

Fact 1. *Suppose $\beta_{ij} = 0$, $\forall (i,j) \in \mathcal{I}$. In this case, if $(\bar{\mu}, \bar{s})$ is solution to (3.22) for σ, then $(-\bar{\mu}, \bar{s})$ is solution for $-\sigma$. Thus, if σ is a feasible signing for (3.22), then $-\sigma$ a feasible signing as well.*

To see this, suppose $(\bar{\mu}, \bar{s})$ has been established as a solution to (3.22), that is

$$V\bar{\mu} - \mathrm{diag}(\sigma)\, \bar{s} = 0$$

holds. Observe that

$$V(-\bar{\mu}) - \mathrm{diag}(-\sigma)\, \bar{s} = -V\bar{\mu} + \mathrm{diag}(\sigma)\, \bar{s} = 0.$$

The following algorithm can be used to determine all feasible signings:

Algorithm 1.

(1) Let s_0 be the number of inequalities. Create all signings $\Sigma := \{-1, 0, 1\}^{s_0}$.

(2) $\forall \sigma \in \Sigma$:

(a) Solve

$$\begin{bmatrix} V & \beta & -\mathrm{diag}\,\sigma \end{bmatrix} \begin{pmatrix} \mu \\ \bar{s} \\ s \end{pmatrix} = 0, \quad s > 0, \bar{s} = 1.$$

(b) If $(\mu, \bar{s}, s)$, with $\bar{s} = 1$, $s > 0$ exists, then σ is a feasible signing.

Remark 4. *For Algorithm 1 the system (3.22) has to be solved $(3^{s_0} - 1)$ times (if the signing $\sigma = 0$ is excluded). If $\beta = 0$, the system has only to be solved $\frac{1}{2}(3^{s_0} - 1)$ times (as in this case (μ, s) is a solution to (3.22) for σ implies by Fact 1 that $(-\mu, s)$ is a solution to (3.22) for $-\sigma$).*

Remark 5. *The existence of a signing such that the sign pattern of $Q(\mu)$ is an L^+-matrix is necessary, but not sufficient for the existence of a positive solution to (3.16).*

Remark 6. *If one cannot establish signings, such that $Q(\mu)$ is an L^+-matrix, it might still be possible to establish signings, such that $Q(\mu)$ is a sign-central matrix. In this case some elements of ν and λ and thus of the vector of rate constants k can equal zero. In this case one can try to find values for the zero elements of k using the results discussed in Section 3.4 (This approach has been successfully applied to one of the models for cell cycle regulation discussed in Chapter 5).*

3.2.2.3 An example: application to network $\mathcal{N}_1$

As an illustration the matrix $Q(\mu)$ for network $\mathcal{N}_1$ is determined. Recall that for the example network

$$Y\, I_a = \begin{bmatrix} -1 & 1 & 0 & 0 & 1 & -1 \\ 0 & 0 & 1 & -1 & -1 & 1 \end{bmatrix}$$

The generators of $\ker(Y\, I_a) \cap \mathbb{R}^6_{\geq 0}$ are given as the column vectors of the matrix

$$E = \begin{bmatrix} 1 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 & 1 \\ 0 & 0 & 1 & 1 & 0 \\ 0 & 0 & 1 & 0 & 1 \end{bmatrix}.$$

For $\Lambda(E)$ as defined in (3.5) one obtains

$$\begin{aligned}\Lambda(E) = \{x \in \mathbb{R}^5_{\geq 0} \mid x_1 = 0 \& (x_2 = 0 \& (x_3 = 0 \& x_4 > 0 \& x_5 > 0 || x_3 > 0 \& x_4 > 0 \& x_5 > 0) || \\ x_2 > 0 \& (x_3 = 0 \& x_4 > 0 \& x_5 > 0 || x_3 > 0 \& x_4 > 0 \& x_5 > 0)) || \\ x_1 > 0 \& (x_2 = 0 \& (x_3 = 0 \& x_4 > 0 \& x_5 > 0 || x_3 > 0 \& x_4 > 0 \& x_5 > 0) || \\ x_2 > 0 \& (x_3 = 0 \& x_4 > 0 \& x_5 > 0 || x_3 > 0 \& x_4 \geq 0 \& x_5 \geq 0))\}.\end{aligned}$$

And the transformation matrix U, with $U\,E = \left[\begin{smallmatrix} I_o & E_o \\ 0 & 0 \end{smallmatrix}\right]$ is given by

$$U = \begin{bmatrix} 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 1 & -1 \\ 1 & -1 & 0 & 0 & -1 & 1 \\ 0 & 0 & 1 & -1 & -1 & 1 \end{bmatrix}.$$

Therefore one obtains

$$\begin{aligned} Q_{\mathcal{N}_1} &= \begin{bmatrix} I_o & E_0 & -U\ \mathrm{diag}\left(e^{Y^{(L)^T}\mu}\right) E \end{bmatrix} \\ &= \left[\begin{array}{cccc|c|ccccc} 1 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & -1 \\ 0 & 1 & 0 & 0 & 1 & 0 & -e^{\mu_2} & 0 & 0 & -e^{\mu_2} \\ 0 & 0 & 1 & 0 & 1 & 0 & 0 & -e^{3\,\mu_1} & 0 & -e^{3\,\mu_1} \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & q_1 & -e^{2\,\mu_1+\mu_2} & e^{3\,\mu_1} \\ \hline 0 & 0 & 0 & 0 & 0 & q_2 & 0 & -q_1 & q_3 & q_4 \\ 0 & 0 & 0 & 0 & 0 & 0 & q_5 & -q_1 & q_6 & q_7 \end{array}\right], \end{aligned}$$

with

$$\begin{aligned} q_1 &= e^{3\,\mu_1} - e^{2\,\mu_1+\mu_2} \\ q_2 &= 1 - e^{\mu_1} \\ q_3 &= -e^{\mu_1} + e^{2\,\mu_1+\mu_2} \\ q_4 &= 1 - e^{3\,\mu_1} \\ q_5 &= -1 + e^{\mu_2} \\ q_6 &= -1 + e^{2\,\mu_1+\mu_2} \\ q_7 &= -e^{3\,\mu_1} + e^{\mu_2}. \end{aligned}$$

As every row of $Q(\mu)$contains positive and negative entries, no row can be discarded. To determine the sign pattern of $Q(\mu)$, the signs of the q_i have to be determined. This can be done by solving systems of linear inequalities: $\mathrm{sign}(q_1) = \mathrm{sign}(\mu_1 - \mu_2)$, $\mathrm{sign}(q_2) = \mathrm{sign}(-\mu_1)$, $\mathrm{sign}(q_3) = \mathrm{sign}(\mu_1 + \mu_2)$, ... Let $q = (q_1, \ldots, q_7)^T$. Then

$$\mathrm{sign}(q) = \mathrm{sign}(V\,\mu),$$

where

$$V = \begin{bmatrix} 1 & -1 \\ -1 & 0 \\ 1 & 1 \\ -3 & 0 \\ 0 & 1 \\ 2 & 1 \\ -3 & 1 \end{bmatrix}.$$

Let $\sigma \in \{-1, 0, 1\}^7$. The signing $\sigma = (1, 1, 1, 1, 1, 1)^T$, for example, corresponds to $q_1 > 0, \ldots, q_7 > 0$ and thus $V\,\mu > 0$. Each signing defines an inequality system that is feasible if an only if the system

$$\begin{bmatrix} V & -\mathrm{diag}(\sigma) \end{bmatrix} \begin{pmatrix} \mu \\ s \end{pmatrix} = 0, s > 0 \tag{3.23}$$

is feasible. (Note that $\beta = 0$ in this case.) Solutions to (3.23) form a pointed polyhedral cone, whose extreme rays can be computed by efficient algorithms. Let the columns of $E = \left[\begin{smallmatrix} A_1 \\ A_2 \end{smallmatrix}\right]$ be generators of this cone (where A_1 and A_2 are matrices of appropriate dimension). Then $\mu = A_1\,\kappa$, $\kappa > 0$ is a parametrization of all μ that satisfy (3.23) for a particular signing.

The inequality system defined by $\sigma = (1,\ 1,\ 1,\ 1,\ 1,\ 1)^T$ has no solution, as the inequality $-\mu_2 > 0$ (a consequence of the first two inequalities $\mu_1 - \mu_2 > 0$ and $-\mu_1 > 0$) is not compatible with the fifth inequality $\mu_2 > 0$. Thus the sign pattern defined by $\sigma = (1,\ 1,\ 1,\ 1,\ 1,\ 1)^T$

$$\mathrm{sign}\left(Q\left(\mu\right)\right) = \begin{bmatrix} 1 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & -1 \\ 0 & 1 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & -1 \\ 0 & 0 & 1 & 0 & 1 & 0 & 0 & -1 & 0 & -1 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 1 & -1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & -1 & 1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & 1 \end{bmatrix}$$

is not a feasible sign pattern for $Q\left(\mu\right)$. The sign pattern

$$\mathrm{sign}\left(Q\left(\mu\right)\right) = \begin{bmatrix} 1 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & -1 \\ 0 & 1 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & -1 \\ 0 & 0 & 1 & 0 & 1 & 0 & 0 & -1 & 0 & -1 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & -1 & -1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & -1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & -1 & 1 \end{bmatrix}$$

is feasible. It corresponds to the signing $\sigma^* = (-1,\ 1,\ -1,\ 1,\ 1,\ -1,\ 1)$ that defines the feasible inequality system

$$\begin{aligned} \mu_1 - \mu_2 &< 0 \\ -\mu_1 &> 0 \\ \mu_1 + \mu_2 &< 0 \\ -3\,\mu_1 &> 0 \\ \mu_2 &> 0 \\ 2\,\mu_1 + \mu_2 &< 0 \\ -3\,\mu_1 + \mu_2 &> 0 \end{aligned}$$

The pointed polyhedral cone defined by the corresponding equalities

$$\begin{bmatrix} V & -\,\mathrm{diag}\left(\sigma^*\right) \end{bmatrix} \begin{pmatrix} \mu \\ s \end{pmatrix} = 0, s > 0 \tag{3.24}$$

is generated by

$$E_{\sigma^*} = \left[\begin{array}{cc} -1 & -1 \\ 0 & 1 \\ \hline 1 & 2 \\ 1 & 1 \\ 1 & 0 \\ 3 & 3 \\ 0 & 1 \\ 2 & 1 \\ 3 & 4 \end{array}\right].$$

Thus all $\mu = A_1\,\kappa$, $\kappa > 0$, with

$$A_1 = \begin{bmatrix} -1 & -1 \\ 0 & 1 \end{bmatrix}. \tag{3.25}$$

are solutions to the inequality system. Equation (3.24) is feasible for the following signings $\sigma \in$

$\{-1,0,1\}^7$

$$\begin{array}{rl}
\sigma_1 =& (-1,\ -1,\ \ 1,\ -1,\ \ 1,\ \ 1,\ -1)^T \\
\sigma_2 =& (-1,\ -1,\ \ 1,\ -1,\ \ 1,\ \ 1,\ \ 0)^T \\
\sigma_3 =& (-1,\ -1,\ \ 1,\ -1,\ \ 1,\ \ 1,\ \ 1)^T \\
\sigma_4 =& (-1,\ \ 1,\ -1,\ \ 1,\ -1,\ -1,\ \ 1)^T \\
\sigma_5 =& (-1,\ \ 1,\ -1,\ \ 1,\ \ 0,\ -1,\ \ 1)^T \\
\sigma_6 =& (-1,\ \ 1,\ -1,\ \ 1,\ \ 1,\ -1,\ \ 1)^T \\
\sigma_7 =& (-1,\ \ 1,\ \ 0,\ \ 1,\ \ 1,\ -1,\ \ 1)^T \\
\sigma_8 =& (-1,\ \ 1,\ \ 1,\ \ 1,\ \ 1,\ -1,\ \ 1)^T \\
\sigma_9 =& (-1,\ \ 1,\ \ 1,\ \ 1,\ \ 1,\ \ 1,\ \ 1)^T \\
\sigma_{10} =& (0,\ -1,\ \ 1,\ -1,\ \ 1,\ \ 1,\ -1)^T \\
\sigma_{11} =& (0,\ \ 1,\ -1,\ \ 1,\ -1,\ -1,\ \ 1)^T \\
\sigma_{12} =& (1,\ -1,\ -1,\ -1,\ -1,\ -1,\ -1)^T \\
\sigma_{13} =& (1,\ -1,\ -1,\ -1,\ -1,\ \ 1,\ -1)^T \\
\sigma_{14} =& (1,\ -1,\ \ 0,\ -1,\ -1,\ \ 1,\ -1)^T \\
\sigma_{15} =& (1,\ -1,\ \ 1,\ -1,\ -1,\ \ 1,\ -1)^T \\
\sigma_{16} =& (1,\ -1,\ \ 1,\ -1,\ \ 0,\ \ 1,\ -1)^T \\
\sigma_{17} =& (1,\ -1,\ \ 1,\ -1,\ \ 1,\ \ 1,\ -1)^T \\
\sigma_{18} =& (1,\ \ 1,\ -1,\ \ 1,\ -1,\ -1,\ -1)^T \\
\sigma_{19} =& (1,\ \ 1,\ -1,\ \ 1,\ -1,\ -1,\ \ 0)^T \\
\sigma_{20} =& (1,\ \ 1,\ -1,\ \ 1,\ -1,\ -1,\ \ 1)^T
\end{array} \tag{3.26}$$

Of these twenty signings only the signings

$$\begin{array}{rl}
\sigma_6 =& (-1,\ \ 1,\ -1,\ \ 1,\ \ 1,\ -1,\ \ 1)^T \\
\sigma_{15} =& (1,\ -1,\ \ 1,\ -1,\ -1,\ \ 1,\ -1)^T
\end{array} \tag{3.27}$$

define sign patterns of $Q(\mu)$ that are L^+-matrices. This is easy to verify using Theorem 1. The signings given in (3.27) correspond to the following sign patterns of $Q(\mu)$:

$$\operatorname{sign}(Q(\mu)) = \begin{bmatrix}
1 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & -1 \\
0 & 1 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & -1 \\
0 & 0 & 1 & 0 & 1 & 0 & 0 & -1 & 0 & -1 \\
0 & 0 & 0 & 1 & -1 & 0 & 0 & -1 & -1 & 1 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & -1 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & -1 & 1
\end{bmatrix}$$

for σ_6 and

$$\operatorname{sign}(Q(\mu)) = \begin{bmatrix}
1 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & -1 \\
0 & 1 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & -1 \\
0 & 0 & 1 & 0 & 1 & 0 & 0 & -1 & 0 & -1 \\
0 & 0 & 0 & 1 & -1 & 0 & 0 & 1 & -1 & 1 \\
0 & 0 & 0 & 0 & 0 & -1 & 0 & -1 & 1 & -1 \\
0 & 0 & 0 & 0 & 0 & 0 & -1 & -1 & 1 & -1
\end{bmatrix}$$

for σ_{15}. Consider σ_6, the solutions to the corresponding inequality systems are given above as $\mu_1 = -\kappa_1 - \kappa_2$, $\mu_2 = \kappa_2$, $\kappa_1, \kappa_2 > 0$. Choose, for example, $\kappa_1 = \kappa_2 = 1$. Then $\mu = (-2,1)^T$ and

$$Q(-2,1) = \begin{bmatrix}
1 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & -1 \\
0 & 1 & 0 & 0 & 1 & 0 & -e & 0 & 0 & -e \\
0 & 0 & 1 & 0 & 1 & 0 & 0 & -\frac{1}{e^6} & 0 & -\frac{1}{e^6} \\
0 & 0 & 0 & 1 & -1 & 0 & 0 & \frac{1}{e^6} - \frac{1}{e^3} & -\frac{1}{e^3} & \frac{1}{e^6} \\
0 & 0 & 0 & 0 & 0 & 1-\frac{1}{e^2} & 0 & -\frac{1}{e^6} + \frac{1}{e^3} & \frac{1}{e^3} - \frac{1}{e^2} & 1 - \frac{1}{e^6} \\
0 & 0 & 0 & 0 & 0 & 0 & -1+e & -\frac{1}{e^6} + \frac{1}{e^3} & -1 + \frac{1}{e^3} & -\frac{1}{e^6} + e
\end{bmatrix}$$

The cone $\ker(Q(-2,1)) \cap \mathbb{R}^{10}_{\geq 0}$ is generated by

$$\begin{bmatrix}
4.0342879e+02 & 0.0000000e+00 & 1.0000000e+00 & 4.0242879e+02 & 0.0000000e+00 & 0.0000000e+00 \\
6.4346646e+03 & 3.1936309e+02 & 1.5193499e+01 & 6.4336646e+03 & 3.1936309e+02 & 3.0516959e+02 \\
1.0000000e+00 & 0.0000000e+00 & 0.0000000e+00 & 0.0000000e+00 & 0.0000000e+00 & 1.0000000e+00 \\
2.3320418e+02 & 3.1696090e+01 & 5.0321472e-01 & 2.3420418e+02 & 3.1696090e+01 & 3.0192875e+01 \\
0.0000000e+00 & 1.0000000e+00 & 0.0000000e+00 & 1.0000000e+00 & 1.0000000e+00 & 0.0000000e+00 \\
0.0000000e+00 & 0.0000000e+00 & 1.0000000e+00 & 0.0000000e+00 & 1.0000000e+00 & 0.0000000e+00 \\
1.9637520e+03 & 1.1685499e+02 & 5.5893760e+00 & 1.9637520e+03 & 1.1785499e+02 & 1.1226562e+02 \\
0.0000000e+00 & 4.0242879e+02 & 0.0000000e+00 & 0.0000000e+00 & 4.0342879e+02 & 4.0342879e+02 \\
4.7041168e+03 & 2.3420418e+02 & 1.0107338e+01 & 4.7041168e+03 & 2.3320418e+02 & 2.2309685e+02 \\
4.0342879e+02 & 1.0000000e+00 & 0.0000000e+00 & 4.0342879e+02 & 0.0000000e+00 & 0.0000000e+00
\end{bmatrix}$$

A solution for ν, λ is

$$\nu = (8.0685759e+01,\ 1.3827419e+03,\ 2.0000000e-01,\ 5.6149664e+01,\ 3.0000000e-01)^T \tag{3.28a}$$

$$\lambda = (2.0000000e-01,\ 4.2800691e+02,\ 1.2092864e+02,\ 1.0108846e+03,\ 8.0785759e+01)^T \tag{3.28b}$$

A pair of steady states is given by $a = (1,1)$ and $b = \left(e^{-2}, e^{1}\right)$. The corresponding parameter vector is given by

$$k = \big(1.0110846e+03,\ 8.0985759e+01,\ 1.4388915e+03,\ 5.0879266e+02,\ 1.1318133e+03,\ 2.0171440e+02\big)^T. \tag{3.28c}$$

3.3 Positive solutions satisfying the conservation relations

Recall the present situation: the goal is to find two positive steady state solutions $a, b \in \mathbb{R}^n_{>0}$ and a parameter vector $k \in \mathbb{R}^r_{>0}$, such that $Y\, I_a\, v(k,\, a) = Y\, I_a\, v(k,\, b) = 0$ and $W^T a = W^T b$. As a result of Section 3.1, it is known how $a, b \in \mathbb{R}^n_{>0}$ and $k \in \mathbb{R}^r_{>0}$ can be established: by obtaining solutions to

$$Y^{(L)^T} \mu = \ln \frac{E\,\lambda}{E\,\nu},$$

where $\nu,\ \lambda \in \Lambda(E)$ and $\mu \in \mathbb{R}^n$. If a solution ν, λ, μ exists, then

$$\begin{aligned}
a &\in \mathbb{R}^n_{>0}, \text{ arbitrary} \\
b &= \operatorname{diag}\left(e^{\mu}\right) a \\
k &= \operatorname{diag}\left(\phi\left(a^{-1}\right)\right)\ E\,\lambda
\end{aligned}$$

satisfy $Y\, I_a\, v(k,\, a) = Y\, I_a\, v(k,\, b) = 0$. However, the condition $W^T a = W^T b$ will in general not hold for arbitrary vectors a. But, as a can be chosen freely, $W^T a = W^T b$ can be checked independent of the rate constants k. Note that $W^T a = W^T b$ is equivalent to $W^T\ (b - a)$ and thus to $b - a \in \mathcal{S}$, where $\mathcal{S} = [Y\, I_a]$. 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 positive vectors $p \in \mathbb{R}^n_{>0}$ and $q \in \mathbb{R}^n_{>0}$ with the following properties:

$$\ln \frac{q}{p} \in M_1, \tag{3.29a}$$

where, as usual, $\ln \frac{q}{p} := \left(\ln \frac{q_1}{p_1}, \ldots, \ln \frac{q_n}{p_n}\right)^T$ and

$$q - p \in M_2. \tag{3.29b}$$

The following Lemma formalizes the discussion in [25], or [23, p. 187-188]:

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 $M_3 := \left\{ (m_1, m_2) \in M_1 \times M_2 \middle|\ \operatorname{sign}(m_1) = \operatorname{sign}(m_2) \right\}$ 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 (3.29a) *and* (3.29b) *exist, if and only if $M_3 \neq \emptyset$. Moreover, any pair $(m_1, m_2) \in M_3$ defines a pair p, q:*

$$(p_i)_{i=1,\ldots,n} = \begin{cases} \frac{m_{2i}}{e^{m_{1i}}-1}, \text{ if } m_{1i} \neq 0 \\ \bar{p}_i > 0, \text{ arbitrary, if } m_{1i} = 0 \end{cases} \tag{3.30a}$$

and

$$(q_i)_{i=1,\ldots,n} = e^{m_{1i}}\, p_i. \tag{3.30b}$$

Proof. Suppose p and q with the properties given in (3.29a) and (3.29b) exist. One has to show that $M_3 \neq \emptyset$. As $\ln \frac{q_i}{p_i} < 0$ iff $q_i - p_i < 0$, $\ln \frac{q_i}{p_i} > 0$ iff $q_i - p_i > 0$ and $\ln \frac{q_i}{p_i} = 0$ iff $q_i - p_i = 0$ one has $\operatorname{sign}\left(\ln \frac{q}{p}\right) = \operatorname{sign}(q-p)$. As, by assumption, $\ln \frac{q}{p} \in M_1$ 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 (3.30a) and (3.30b) have the properties defined in (3.29a) and (3.29b). Pick any pair $(m_1, m_2) \in M_3$ and suppose $m_{1i} \neq 0$, $i = 1, \ldots, n$. Then

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

Otherwise, if $m_{1i} = 0$ for some $i \in \{1, \ldots, n\}$, then $q_i = p_i = \bar{p}_i$. Moreover, $\operatorname{sign}(m_1) = \operatorname{sign}(m_2)$ ensures $m_{2i} = q_i - p_i = 0$ and $\ln \frac{q_i}{p_i} = m_{1i} = 0$.
Thus $q - p = m_2 \in M_2$ and $\frac{q}{p} = m_1 \in M_1$, by definition. $p \in \mathbb{R}^n_{>0}$ follows from $\operatorname{sign}(m_2) = \operatorname{sign}(m_1)$ and the fact that $\operatorname{sign}(e^{m_{1i}} - 1) = \operatorname{sign}(m_{1i})$, $i = 1, \ldots, n$, positivity of q follows from positivity of p. ■

As an easy consequence of Lemma 3 necessary and sufficient conditions for the existence of multiple positive steady state solutions can be derived:

Theorem 3. *Consider a biochemical reaction network with mass action kinetics and the matrices $Y \in \mathbb{R}^{n\times m}$, $I_a \in \mathbb{R}^{m\times r}$ and $Y^{(L)} \in \mathbb{R}^{n\times r}$ as defined in Chapter 2. Let the columns of $E \in \mathbb{R}^{r\times p}$ be generators of $\ker(Y\, I_a) \cap \mathbb{R}^r_{\geq 0}$. Obtain*

$$\mathcal{M} = \left\{ \mu \in \mathbb{R}^n | \exists \nu, \lambda \in \Lambda(E) \text{ such that } {Y^{(L)}}^T \mu = \ln \frac{E\,\nu}{E\,\lambda} \right\}. \tag{3.31}$$

Let $\mathcal{S} = [Y\, I_a]$ denote the stoichiometric subspace and define the set of all ordered pairs (μ, v) of elements $\mu \in \mathcal{M}$ and $v \in \mathcal{S}$ with the same sign pattern:

$$\mathcal{T} := \{(\mu, v) \in \mathcal{M} \times \mathcal{S} | \operatorname{sign}(\mu) = \operatorname{sign}(v)\}. \tag{3.32}$$

The ODEs derived from the biochemical reaction network admit multistationarity, if and only if $\mathcal{T} \neq \emptyset$. Moreover, any element of T defines a pair of steady state solutions $a, b \in \mathbb{R}^n_{>0}$ and a vector of rate constants $k \in \mathbb{R}^r_{>0}$ with

$$\begin{aligned} Y\, I_a\, v(k, a) = Y\, I_a\, v(k, b) &= 0 \\ W^T b = W^T a & \end{aligned}$$

in the following way:

$$(a_i)_{i=1,\ldots,n} = \begin{cases} \frac{v_i}{e^{\mu_i}-1}, \text{ if } \mu_i \neq 0 \\ \bar{a}_i > 0, \text{ arbitrary, if } \mu_i = 0 \end{cases} \tag{3.33a}$$

$$b = \operatorname{diag}(e^{\mu})\, a \tag{3.33b}$$

$$k = \operatorname{diag}\left(\phi\left(a^{-1}\right)\right) E\, \lambda, \tag{3.33c}$$

for some $\lambda \in \Lambda(E)$, such that ${Y^{(L)}}^T \mu = \ln \frac{E\,\nu}{E\,\lambda}$.

Proof.

$\Rightarrow$ Suppose $\mathcal{T} \neq \emptyset$. Therefore $\mathcal{M} \neq \emptyset$. Then there exist $\mu \in \mathbb{R}^n$ and $\nu, \lambda \in \Lambda(E)$ such that ${Y^{(L)}}^T \mu = \ln \frac{E\,\nu}{E\,\lambda}$. Thus, by Lemma 1, for arbitrary positive a, vectors $b \in \mathbb{R}^n_{>0}$ as in (3.33b) and $k \in \mathbb{R}^r_{>0}$ as in (3.33c) satisfy $Y\,I_a\,v(k,\,a) = Y\,I_a\,v(k,\,b) = 0$. As $\mathcal{T} \neq 0$, Lemma 3 implies that $b - a \in \mathcal{S}$ and thus $W^T a = W^T b$, if a as in (3.33a).

$\Leftarrow$ Suppose $a, b \in \mathbb{R}^n_{>0}$ and $k \in \mathbb{R}^r_{>0}$ exist with $Y\,I_a\,\mathrm{diag}\,(k)\,\phi\,(a) = Y\,I_a\,\mathrm{diag}\,(k)\,\phi\,(b) = 0$ and $W^T a = W^T b$. As $a, b > 0$, the assumption $Y\,I_a\,\mathrm{diag}\,(k)\,\phi\,(a) = Y\,I_a\,\mathrm{diag}\,(k)\,\phi\,(b) = 0$ implies $\mathrm{diag}\,(k)\,\phi\,(a) = E\,\lambda$ and $\mathrm{diag}\,(k)\,\phi\,(b) = E\,\nu$, for some $\nu,\, \lambda \in \Lambda(E)$. From Lemma 1 follows that $\mu := \ln \frac{b}{a}$ satisfies ${Y^{(L)}}^T \mu = \ln \frac{E\,\nu}{E\,\lambda}$, for $\nu,\, \lambda$ as above. Thus $\mathcal{M} \neq \emptyset$. Observe that $W^T a = W^T b$ implies $b - a \in \mathcal{S}$ and thus $\mathcal{T} \neq \emptyset$ by Lemma 3.

■

Corollary 1. *Suppose* $\dim(\mathcal{S}) = n$*, that is, the system has no conservation relations. Then the ODEs derived from the biochemical reaction network admit multistationarity, if and only if* $\mathcal{M} \neq \emptyset$*, with* $\mathcal{M}$ *as defined in* (3.31)*. Any vector* $\mu \in \mathcal{M}$ *defines* $a, b \in \mathbb{R}^n_{>0}$ *and* $k \in \mathbb{R}^r_{>0}$ *with*

$$Y\,I_a\,v(k,\,a) = Y\,I_a\,v(k,\,b) = 0$$

in the following way:

$$\begin{aligned} &a \in \mathbb{R}_{>0},\ \textit{arbitrary} \\ &b = \mathrm{diag}\,(e^{\mu})\ a \\ &k = \mathrm{diag}\left(\phi\left(a^{-1}\right)\right)\ E\,\lambda, \end{aligned}$$

for some $\lambda \in \Lambda(E)$*, such that* ${Y^{(L)}}^T \mu = \ln \frac{E\,\nu}{E\,\lambda}$.

Moreover, based on Lemma 3, it is, at least in principle, possible to prove that a certain network structure cannot admit multiple (positive) steady state solutions. This motivates the following corollary:

Corollary 2. *Suppose* $\mathcal{M} \neq \emptyset$*,* $\mathcal{M}$ *as in* (3.31) *and* $\mathcal{T} = \emptyset$*,* $\mathcal{T}$ *as in* (3.32)*. Then multistationarity is* ***impossible*** *(in the sense, that no pair* $a, b \in \mathbb{R}^n_{>0}$ *of positive steady state solutions satisfies* $W^T b = W^T a$*).*

Results similar to Lemma 3 have been known in the literature on Feinberg's Chemical Reaction Network Theory for some time (see, for example, the informal discussion in Ellison's Ph.D. thesis [23, p. 187-188]). In fact, both the Deficiency One Algorithm and the Advanced Deficiency Algorithm utilize the way p and q are constructed in (3.30a) and (3.30b). Note that multistationarity does not depend on specific parameter values. This motivates the following fact.

Fact 2. *For a biochemical reaction network with mass action kinetics multistationarity is a* ***network property****, that is, it can be tested* ***without any knowledge*** *of* ***parameter values*** *whatsoever. Conclusiveness of a test depends on the existence of vectors* $\nu,\, \lambda \in \Lambda(E)$ *and a vector* μ *that satisfy the nonlinear equation* (3.8b) *and a vector* $v \in [Y\,I_a]$ *with* $\mathrm{sign}\,(v) = \mathrm{sign}\,(\mu)$*.*

3.3.1 The linear subspace case

Suppose that $\mathcal{M}$ as given in (3.31) defines a linear subspace of $\mathbb{R}^n$. In this case it is straightforward (but may be computationally demanding) to check whether or not $\mathcal{T} \neq \emptyset$ (i.e. to check whether or not there exists $\mu \in \mathcal{M}$ and $v \in \mathcal{S}$ with $\mathrm{sign}\,(\mu) = \mathrm{sign}\,(v)$): to see this, suppose, for a particular $\mu \in \mathcal{M}$, a vector $v \in \mathcal{S}$ with $\mathrm{sign}\,(v) = \mathrm{sign}\,(\mu)$ can be determined. Then all vectors $\alpha\,v$ with $\alpha > 0$ also satisfy $\mathrm{sign}\,(v) = \mathrm{sign}\,(\mu)$. In fact, every vector $v \in \mathcal{S}$ that is contained in the same orthant as μ also satisfies $\mathrm{sign}\,(v) = \mathrm{sign}\,(\mu)$. And vice versa: suppose for a given vector $v \in \mathcal{S}$ there exists a $\mu \in \mathcal{M}$ with $\mathrm{sign}\,(\mu) = \mathrm{sign}\,(v)$. Then every vector $\mu \in \mathcal{M}$ that is contained in the same orthant as v also satisfies $\mathrm{sign}\,(\mu) = \mathrm{sign}\,(v)$. Thus, to show that $\mathcal{T} \neq \emptyset$, it suffices to find an orthant that intersects $\mathcal{M}$ as well as $\mathcal{S}$ (provided that $\mathcal{M}$ is a linear subspace of $\mathbb{R}^n$).
To formalize this discussion, let $\delta \in \{-1, 0, 1\}^n$ be a vector composed of entries $+1$, 0 and -1. It is used to denote a particular orthant $\mathbb{R}^n_\delta := \left\{x \in \mathbb{R}^n \,|\, \delta = \mathrm{sign}\,(x),\ j = 1,\ \ldots, n\right\}$, with δ the

signature of the orthant. In this notation the positive orthant, for example, is represented by the vector $\delta = (1, \ldots, 1)^T$, entirely composed of entries +1, and $\mathbb{R}^n_\delta$, $\delta = (0, 1, \ldots, 1)^T$ is the part of the hyperplane $x_1 = 0$ separating $\mathbb{R}^n_\delta$ with $\bar{\delta} = (1, \ldots, 1)^T$ from $\mathbb{R}^n_{\tilde{\delta}}$ with $\tilde{\delta} = (-1, 1, \ldots, 1)^T$. Using this notation the previous discussion can be summarized as follows:

Fact 3. *Recall that $\mathcal{S}$ is a linear subspace of $\mathbb{R}^n$. Suppose $\mathcal{M} \subseteq \mathbb{R}^n$ as given in* (3.31) *is a linear subspace of $\mathbb{R}^n$. Then $\mathcal{T}$ as defined in* (3.32)*, has $\mathcal{T} \neq \emptyset$, if and only if there exists an orthant $\mathbb{R}^n_\delta$ as defined above with $\mathcal{M} \cap \mathbb{R}^n_\delta \neq \emptyset$ and $\mathcal{S} \cap \mathbb{R}^n_\delta \neq \emptyset$. Note that in this case $\mathcal{M} \cap \mathbb{R}^n_{\delta_o} \neq \emptyset$ and $\mathcal{S} \cap \mathbb{R}^n_{\delta_o} \neq \emptyset$, with $\delta_o = -\delta$ as well.*

Proof. Recall that $\mathcal{T} \neq \emptyset$, if there exists $v \in \mathcal{S}$ and $\mu \in \mathcal{M}$ with $\operatorname{sign}(v) = \operatorname{sign}(\mu) = \delta$, which implies $\mathcal{M} \cap \mathbb{R}^n_\delta \neq \emptyset$ and $\mathcal{S} \cap \mathbb{R}^n_\delta \neq \emptyset$. As $\mathcal{S}$ and $\mathcal{M}$ are linear vector spaces, $-v \in \mathcal{S}$ and $-\mu \in \mathcal{M}$ holds. Obviously $\operatorname{sign}(-v) = \operatorname{sign}(-\mu) = -\delta =: \delta_o$. Thus $\mathcal{M} \cap \mathbb{R}^n_{\delta_o} \neq \emptyset$ and $\mathcal{S} \cap \mathbb{R}^n_{\delta_o} \neq \emptyset$. ■

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

$$\mu^\delta = E^{\mathcal{M}^\delta}\, \alpha^\delta,\ \alpha^\delta \in \mathbb{R}^{p_{\mathcal{M}^\delta}}_{>0}, \tag{3.34a}$$

and all $v \in \mathcal{S} \cap \mathbb{R}^n_\delta$ can be represented by

$$v^\delta = E^{\mathcal{S}^\delta}\, \beta^\delta,\ \beta^\delta \in \mathbb{R}^{p_{\mathcal{S}^\delta}}_{>0}. \tag{3.34b}$$

Using (3.34a) and (3.34b) in (3.33a) a can be parametrized in terms of α^δ and β^δ. The symbol a^δ is used to denote this parametrization of a for each $\delta \in \Delta$. Using a^δ in (3.33b) and (3.33c) yields parametrizations of b^δ and k^δ in terms of α^δ and β^δ. Thus, for each orthant $\delta \in \Delta$ a different representation of a, b and k can be derived.
It seems worthwhile to discuss the following property of $\mathcal{M}$ and S: whenever $v \in \mathcal{S}$ ($\mu \in \mathcal{M}$) holds, $-v \in \mathcal{S}$ ($-\mu \in \mathcal{M}$) 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 $-\delta$, as all representations $a^{-\delta}$, $b^{-\delta}$, $k^{-\delta}$ can be obtained from representations a^δ, b^δ, k^δ. To be more precise, let $\mu_1^\delta \in \mathcal{M} \cap \mathbb{R}^n_\delta$, $v_1^\delta \in \mathcal{S} \cap \mathbb{R}^n_\delta$ and $\mu_2^{-\delta} \in \mathcal{M} \cap \mathbb{R}^n_{-\delta}$, $v_2^{-\delta} \in \mathcal{S} \cap \mathbb{R}^n_{-\delta}$ with $\mu_2^{-\delta} = -\mu_1^\delta$ and $v_2^{-\delta} = -v_1^\delta$. Without loss of generality assume $\mu_{1i}^\delta \neq 0$, $\mu_{2i}^{-\delta} \neq 0$, $v_{1i}^\delta \neq 0$, $v_{2i}^{-\delta} \neq 0$, $i = 1, \ldots, n$. 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

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

In a similar way $b_{2i}^{-\delta} = a_{1i}^\delta$ can be derived. As k is determined by a, 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 $k^{-\delta} = k^\delta$.
Let B and W be two matrices of appropriate dimension with $[B] = \mathcal{M}^\perp$ and $[W] = \mathcal{S}^\perp$ and note that $x \in \mathbb{R}^n_\delta$ is equivalent to $x = \operatorname{diag}(\delta)\, \xi$, $\xi \in \mathbb{R}^n_{>0}$. Observe that $\mathcal{M} \cap \mathbb{R}^n_\delta \neq \emptyset$ and $\mathcal{S} \cap \mathbb{R}^n_\delta \neq \emptyset$ hold for a particular δ, if and only if the system

$$B^T \operatorname{diag}(\delta)\, \xi = 0 \tag{3.35a}$$

$$W^T \operatorname{diag}(\delta)\, \xi = 0 \tag{3.35b}$$

has a positive solution $\xi \in \mathbb{R}^n_{>0}$. The following algorithm can be used to obtain orthants with $\mathcal{M} \cap \mathbb{R}^n_\delta \neq \emptyset$ and $\mathcal{S} \cap \mathbb{R}^n_\delta \neq \emptyset$ (see Appendix D, where (3.35b) and (3.35a) are used):

Algorithm 2.

(1) Assumptions:

(i) In solving (3.8b)*, ν, $\lambda \in \Lambda(E)$ can be determined independently of $\mu \in \mathbb{R}^n$.*

(ii) $\mathcal{M}$ as defined in (3.31) *is a linear subspace of $\mathbb{R}^n$.*

(2) Determine all $\delta \in \{-1, 0, 1\}^n$ with $\mathcal{S} \cap \mathbb{R}^n_\delta \neq \emptyset$ (e.g. using the algorithms discussed in Appendix D):

$$\Delta^{\mathcal{S}} := \{\delta \in \{-1, 0, 1\}^n \,|\mathcal{S} \cap \mathbb{R}^n_\delta \neq \emptyset\} \tag{3.36a}$$

(3) Determine all $\delta \in \{-1, 0, 1\}^n$ with $\mathcal{M} \cap \mathbb{R}^n_\delta \neq \emptyset$ (e.g. using the algorithms discussed in Appendix D).

$$\Delta^{\mathcal{M}} := \{\delta \in \{-1, 0, 1\}^n \,|\mathcal{M} \cap \mathbb{R}^n_\delta \neq \emptyset\} \tag{3.36b}$$

(4) Determine $\Delta := \Delta^{\mathcal{S}} \cap \Delta^{\mathcal{M}}$. If and only if

$$\Delta \neq \emptyset \tag{3.36c}$$

multistationarity is possible. This is a consequence of Fact 3 and Theorem 3.

(5) If $\Delta \neq \emptyset$, use (3.34a) *and* (3.34b) *in* (3.33a), (3.33b) *and* (3.33c) *to obtain parametrizations for a pair of steady state a^δ, b^δ and the corresponding vector of rate constants k^δ. Note that one obtains a different parameterization for each $\delta \in \Delta$.*

There exists a variety of algorithms and software tools to check feasibility of (3.35b) and (3.35a). However, as up to $\frac{1}{2}3^n$ inequality systems have to be checked, it is computationally hard, to use Fact 3 to check sufficient conditions for multistationarity. For systems of moderate size, as those presented in Section 4, the algorithms presented in Appendix D can be used.

3.4 Multistationarity in subnetworks

Consider again the pointed polyhedral cone $\ker(Y\,I_a) \cap \mathbb{R}^r_{\geq 0}$ and its generators. Let E_i be a generator. Then two different types of generators can be distinguished: *trivial generators* with $I_a\,E_i = 0$ and *stoichiometric generators* with $I_a\,E_i \neq 0$.[1,2] As explained in Section 3.1.1 every generator corresponds to a subnetwork of the overall network. The ODEs corresponding to any subnetwork can be obtained from the ODEs of the overall network by setting those rate constants to zero, that correspond to reactions not contained in the subnetwork. Thus, if $J \subseteq \{1, \ldots, r\}$ is the index set of the reactions contained in a subnetwork, then $k_i = 0$, $i \notin J$ (if the subnetwork is defined by a stoichiometric generator E_j, this is equivalent to $k_i = 0$, if $i \notin \operatorname{supp}(E_j)$).

The motivation of this section is based on the idea that steady states in a subnetwork are connected to steady states in the overall network. Intuitively, if $x^{(1)}$, $x^{(2)}$ are steady states of a subnetwork and if in the overall network those k_i previously set to zero are assigned arbitrarily small numbers, then the overall network should have steady states 'close' to $x^{(1)}$, $x^{(2)}$. In Section 3.4.1 multistationarity in subnetworks defined by a stoichiometric generator are examined. For these subnetworks it is particularly easy to establish multistationarity, as this can often be done using the Deficiency One Algorithm of CRNT. This means, in particular, that multistationarity can be established by analysis of systems of linear inequalities. Furthermore, as a consequence of the results presented in Section 3.1 and 3.3 it is straightforward to obtain a parametrization of pairs of steady states and the corresponding parameter vectors.

[35] describes how steady states of the overall network deform to steady states of subnetworks defined by stoichiometric generators, if the k_i not contained in the subnetwork approach zero. In Section 3.4.2 the other direction is pursued: starting with (multiple) equilibria in a subnetwork, conditions are derived that guarantee that these steady states are steady states of the overall network as well. Moreover, by testing these conditions for a specific (pair of) steady state(s) it is possible to obtain values for those parameters of the overall network that are not contained in the subnetwork.

[1] Note that trivial generators correspond to cycles in the directed graph defined by I_a.

[2] The term *stoichiometric generators* is used in [35]

3.4.1 Multistationarity in subnetworks defined by stoichiometric generators

In this Section a subnetwork of a (bio)chemical reaction network that is defined by a stoichiometric generator of the larger network is considered. To distinguish subnetwork and overall network the symbol $\hat{}$ is used to mark all entities that belong to the overall network: $\hat{I}_a$ for the incidence matrix, $\hat{E}$ for the stoichiometric generator that defines the subnetwork and $\hat{Y}^{(L)}$ for the matrix of exponent vectors. Both subnetwork and overall network involve the same complexes, thus Y is used for both networks. Note that the incidence matrix I_a and the matrix of exponent vectors of the subnetwork consist of column vectors $\hat{I}_{a,i}$ and $\hat{y}_i^{(L)}$ of the overall network, namely those with $i \in \operatorname{supp}\left(\hat{E}\right)$. Let $\hat{r}$ be the number of reactions contained in the overall network and r be the number of reactions in the subnetwork. Let E be the vector obtained from $\hat{E}$ by deleting all zero entries; obviously $E > 0$. It follows that $Y\, I_a\, E = 0$ and $I_a\, E \neq 0$.
This Section contains two parts. In the first part algebraic properties of subnetworks defined by a stoichiometric generator are derived, before in the second part parametrizations of pairs of steady states and the corresponding parameter vectors are derived.

3.4.1.1 Algebraic properties of subnetworks defined by a stoichiometric generator

Recall the properties of generators E_i, E_j of $\ker(Y\, I_a) \cap \mathbb{R}^r_{\geq 0}$:

$$Y\, I_a\, E_i = 0, \quad Y\, I_a\, E_j = 0$$
$$E_i \in \mathbb{R}^r_{\geq 0}.$$

Given E_i, E_j with $Y\, I_a\, E_i = 0$ and $Y\, I_a\, E_j = 0$. Then

$$\operatorname{supp}(E_i) \subseteq \operatorname{supp}(E_j) \Rightarrow E_i \;=\; 0 \text{ or } E_j \;=\; \alpha\, E_i.$$

Further note the well-known result from algebraic graph theory concerning incidence matrices of arbitrary (oriented) graphs (for details see, for example, [36, Theorem 8.3.1, p. 168]). It is given here using the notation introduced in [24, 25].

Lemma 4. *Let I_a be the incidence matrix of a graph with m vertices and l connected components (i.e. linkage classes). Then* $\operatorname{rank} I_a = m - l$.

The following facts can be established for the matrix product $Y\, I_a$ (the matrices associated to the subnetwork defined by $\hat{E}$):

Lemma 5. *Let $\hat{E}$ be a stoichiometric generator and E a vector obtained by deleting all zero entries of $\hat{E}$. Further let Y and I_a be the matrices associated with the subnetwork, as described above. Then the following holds:*

(i) E is a generator of $\ker(Y\, I_a) \cap \mathbb{R}^r_{\geq 0}$ *and it is the only generator, that is*

$$\ker(Y\, I_a) \cap \mathbb{R}^r_{\geq 0} = \alpha\, E,\ \alpha \geq 0$$

(ii) $\dim(\ker(Y\, I_a)) = 1$

(iii) I_a has full column rank

(iv) I_a contains $m - l$ columns

(v) $\operatorname{rank}(Y\, I_a) = m - l - 1$

Proof. (i.) Follows from the fact that $\hat{E}$ is a generator of $\ker\left(\hat{Y}\, \hat{I}_a\right) \cap \mathbb{R}^{\hat{r}}_{\geq 0}$ and positivity of E. Let E_0 be a nonzero nonnegative vector that satisfies (3.3a) and (3.3b). Positivity of E implies $\operatorname{supp}(E_0) \subseteq \operatorname{supp}(E)$. Let $\hat{E}_0 \in \mathbb{R}^{\hat{r}}_{\geq 0}$ be obtained from E_0 by an appropriate padding with zeros. Then $\hat{Y}\, \hat{I}_a \hat{E}_0 = 0$ and $\operatorname{supp}\left(\hat{E}_0\right) \subseteq \operatorname{supp}\left(\hat{E}\right)$ follow from the construction of $\hat{E}_0$. As $\hat{E}$ is by definition a generator of $\ker\left(\hat{Y}\, \hat{I}_a\right) \cap \mathbb{R}^{\hat{r}}_{\geq 0}$ we conclude that $\hat{E}_0 = \alpha\, \hat{E}$, $\alpha > 0$ and thus $E_0 = \alpha\, E$, $\alpha > 0$. Thus E is a generator of $\ker(Y\, I_a) \cap \mathbb{R}^r_{\geq 0}$.

That E is the only generator follows again from positivity of E: any nonzero nonnegative vector E_0 that satisfies (3.3a) and (3.3b) will inevitably satisfy $\mathrm{supp}\,(E_0) \subseteq \mathrm{supp}\,(E)$. As E is a generator of $\ker(Y\,I_a) \cap \mathbb{R}^r_{\geq 0}$ all E_0 satisfying (3.3a) and (3.3b) must satisfy $E_0 = \alpha\,E$, $\alpha > 0$. Thus E is the only generator of $\ker(Y\,I_a) \cap \mathbb{R}^r_{\geq 0}$.

(ii.) The proof is similar to the one for (i): suppose there exists a nonzero vector $E_0 \in \mathbb{R}^r$, $E_0 \notin [E]$ with $Y\,I_a\,E_0 = 0$. That E_0 contains negative entries follows from (i): otherwise $E_0 = \alpha\,E$, $\alpha > 0$ and $\dim(\ker(Y\,I_a)) = 1$ from (i). Let $\tilde{E} = \alpha\,E + E_0$. Then $\tilde{E} > 0$, for sufficiently large α and $\tilde{E} = \beta\,E$ by (i), a contradiction. Thus $\dim(\ker(Y\,I_a)) = 1$.

(iii.) As $I_a\,E \neq 0$ by definition of E and $\dim(\ker(Y\,I_a)) = 1$ by (ii), I_a must have full column rank.

(iv.) By Lemma 4 $\mathrm{rank}\,(I_a) = m - l$. By (iii) I_a has full column rank. Thus I_a must have $m - l$ columns.

(v.) By a standard result of linear algebra for any matrix A (see e.g. [61]) $\dim(\ker(A))$ = nr. of columns− $\mathrm{rank}\,(A)$. As $\dim(\ker(Y\,I_a)) = 1$ by (ii) and nr. of columns $= m - l$ by (iv) the desired result $\mathrm{rank}\,(Y\,I_a) = m - l - 1$ follows.

■

Suppose the subnetwork defined by $\hat{E}$ contains several linkage classes and let ℓ be the number of linkage classes of the subnetwork with $\ell \geq 2$. Let $J_i \subseteq 1, \ldots, m$ be the set of indices whose complexes are part of linkage class i. Each linkage class can be considered as a graph of its own with an incidence matrix $I_a^{J_i}$, that contains those columns of I_a that are indexed by J_i. The following result can be established for the rank of the matrix product $Y\,I_a^{J_i}$:

Corollary 3. *Assume the subnetwork defined by $\hat{E}$ has $\ell \geq 2$ linkage classes. Consider one of these linkage classes. Let $J_i \subseteq 1, \ldots, m$ be the set of indices whose complexes are part of that linkage class and let $I_a^{J_i}$ the incidence matrix associated to the linkage class (as described above, $I_a^{J_i}$, that contains those columns of I_a that are indexed by J_i). Let m_i be the number of complexes in the linkage class. Then*

$$\mathrm{rank}\left(Y\,I_a^{J_i}\right) = m_i - 1.$$

Proof. By assumption the linkage class contains m_i nodes. By definition a linkage class is a graph with one connected component. Thus $\mathrm{rank}\left(I_a^{J_i}\right) = m_i - 1$ by Lemma 4. By Lemma 5, (ii) one has for the subnetwork defined by $\hat{E}$, that $\dim(\ker(Y\,I_a)) = 1$. As a consequence $Y\,I_a^{J_i}$ must have full column rank. To see this, assume that $Y\,I_a^{J_i}$ does not have full column rank, that is, there exists a vector E_0 of appropriate dimension with $Y\,I_a^{J_i}\,E_0 = 0$. Let $\tilde{E}_0$ be a suitable padding of E_0 with zeros. One has $Y\,I_a\,\tilde{E}_0 = 0$ for the complete subnetwork and $\tilde{E}_0 \notin [E]$ (as E is a positive vector and $\tilde{E}_0$ contains zero entries). Thus $\dim(\ker(Y\,I_a)) = 2$, a contradiction. Thus $Y\,I_a^{J_i}$ has full column rank. As $Y\,I_a^{J_i}$ has full column rank and as $\mathrm{rank}\left(I_a^{J_i}\right) = m_i - 1$ one obtains $\mathrm{rank}\left(Y\,I_a^{J_i}\right) = m_i - 1$. ■

Subnetworks defined by stoichiometric generators are intimately connected to the Deficiency One Theorem of CRNT. To see this, recall the definition of network deficiency $\mathcal{D}$, as given in, for example, [24, 25, 27, 28]:

$$\mathcal{D} = m - \ell - s.$$

Recall that s is the dimension of the stoichiometric subspace, that is $s = \mathrm{rank}\,(Y\,I_a)$ for the subnetwork defined by the stoichiometric generator $\hat{E}$. As a consequence of Lemma 5 and Corollary 3 the following result can be established:

Corollary 4. *Consider a subnetwork with $\ell \geq 2$ linkage classes defined by a stoichiometric generator $\hat{E}$. For the deficiency $\mathcal{D}$ of the subnetwork as well as for the deficiencies $\mathcal{D}_i$ of the linkage classes of the subnetwork the following holds:*

(i.) $\mathcal{D} = 1$

(ii.) $\mathcal{D}_i = 0$, $i = 1, \ldots, \ell$

Proof. Lemma 5 (v) and (2.11) imply (i), Corollary 3 and (2.11) imply (ii). ■

In CRNT the Deficiency One Algorithm can be used to decide about multistationarity. It is applicable to biochemical reaction networks with deficiency $\mathcal{D} = 1$ that satisfy certain additional constraints. To state these a concept of graph theory is needed: the strongly connected component. A directed graph is called strongly connected if for every pair of nodes u and v there is a path from u to v and a path from v to u. The *strongly connected components* of a directed graph are its maximal strongly connected subgraphs. In the notation introduced in [27, 28], strongly connected components are called *strong linkage classes*. If no edge from a node inside a strong linkage classes to a node outside exists, this strong linkage class is called *terminal*. The Deficiency One Algorithm is applicable to biochemical reaction networks satisfying the the following requirements [25, 28]:

(I) The network deficiency is $\mathcal{D} = 1$

(II) The deficiency of the linkage classes is $\mathcal{D}_i = 0$, $i = 1, \ldots, \ell$

(III) There exists a positive vector E with $Y\, I_a\, E = 0$.

(IV) The terminal strong linkage classes do not contain any cycles

(V) Each linkage class contains only one terminal strong linkage class

Note that (I) and (II) hold by Corollary 4 and that (III) holds by Lemma 5. Further note that the graph cannot contain any cycles, as by Lemma 5 I_a has full column rank. Thus (IV) holds as well. However, no information about (V) is obtainable using Lemma 5. This motivates the following fact:

Fact 4. *Consider a biochemical reaction network that is a subnetwork of a larger network defined by a stoichiometric generator of the larger network. Assume that the subnetwork is displayed in the standard form of CRNT. Then the following holds: if every linkage class of the subnetwork contains only one terminal strong linkage class, the Deficiency One Algorithm is applicable. Thus, in particlar, only systems of* ***linear inequalities*** *have to be considered to decide about multistationarity.*

Remark 7. *Suppose the subnetwork defined by $\hat{E}$ consists of a single linkage class (i.e. $\ell = 1$). Clearly Corollary 3 does not hold in this case. However the deficiency of the subnetwork is still one. To see this, note that by Lemma 5* $\operatorname{rank}(Y\, I_a) = m - \ell - 1 = m - 2$ *in this case. And for the deficiency $\mathcal{D}$ one obtains*

$$\mathcal{D} = m - \ell - s = m - 1 - (m - 2) = 1.$$

3.4.1.2 Steady states for subnetworks defined by a stoichiometric generator

From Lemma 5 it follows that $\ker(Y\, I_a) \cap \mathbb{R}^r_{\geq 0}$ is spanned by a single positive vector E, that is $\ker(Y\, I_a) \cap \mathbb{R}^r_{\geq 0} = \alpha\, E$, $E \in \mathbb{R}^r_{>0}$, $\alpha > 0$. Then the conditions $v(k,\, a) = E\, \lambda$ and $v(k,\, b) = E\, \nu$ become

$$k_i\, a^{y_i^{(L)}} = n_i\, \lambda \qquad\qquad k_i\, b^{y_i^{(L)}} = n_i\, \nu,\ i = 1, \ldots, r,$$

where n_i is the i-th component of E. Apply $\ln()$

$$\ln k_i + \langle y_i^{(L)},\, \ln a\rangle = \ln n_i + \ln \lambda \qquad\qquad \ln k_i + \langle y_i^{(L)},\, \ln b\rangle = \ln n_i + \ln \nu,\ i = 1, \ldots, r.$$

Subtracting equations in a from those in b one obtains (using, as before, $\mu := \ln \frac{b}{a}$):

$$\langle y_i^{(L)},\, \mu\rangle = \ln \frac{\nu}{\lambda},\ i = 1, \ldots, r.$$

Using $\underline{1} = (1, \ldots, 1)^T$, (3.8b) becomes

$$Y^{(L)^T}\, \mu = \ln \frac{\nu}{\lambda}\, \underline{1}, \tag{3.37}$$

$\nu,\, \lambda > 0$. Solvability of (3.37) can be established by means of linear algebra. Let $\tilde{\mu} = \left(\ln \frac{\nu}{\lambda},\, \mu\right)$ and transform (3.37) to

$$\begin{bmatrix} -\underline{1} & Y^{(L)^T} \end{bmatrix} \tilde{\mu} = 0. \tag{3.38}$$

Any solution $\tilde{\mu} \neq 0$ to (3.38) can be used to determine two vectors $a,\, b \in \mathbb{R}^n_{>0}$ and $k \in \mathbb{R}^r_{>0}$ with $Y\, I_a\, v(k,\, a) = Y\, I_a\, v(k,\, b) = 0$. Note that the only requirement is that $\tilde{\mu} \neq 0$ and not that $\ln \frac{\nu}{\lambda} \neq 0$. Thus, in particular, $\ln \frac{\nu}{\lambda} = 0$ and thus $\nu = \lambda$ is allowed. One obtains the following Lemma:

Lemma 6. *Consider equation* (3.8b)

$$Y^{(L)^T}\mu = \ln\frac{\nu}{\lambda}\underline{1},$$

with $\nu,\ \lambda > 0$. *Let* $\tilde{\mu} = \left(\ln\frac{\nu}{\lambda}, \mu_0\right)$ *be a nonzero solution to*

$$\left[-\underline{1} \quad Y^{(L)^T}\right]\tilde{\mu} = 0.$$

Let $\alpha > 0$ *and* $a \in \mathbb{R}^n_{>0}$. *Choose*

$$k = \operatorname{diag}\left(\phi\left(a^{-1}\right)\right)\ (\alpha\, E) \tag{3.39a}$$

$$b = \operatorname{diag}\left(e^{\mu_0}\right)\ a. \tag{3.39b}$$

Then $Y\, I_a\, v(k,\, a) = Y\, I_a\, v(k,\, b) = 0$.

Proof. Suppose the first component of $\tilde{\mu}$ is nonzero. Then $\ln\frac{\nu}{\lambda} = \beta$, $\beta \in \mathbb{R} \setminus (0)$. Let $\nu = e^{\beta}\lambda$, $\lambda > 0$ and choose $\alpha = \lambda$ in (3.39a). In this case the desired result follows from Lemma 1.
Suppose the first component of $\tilde{\mu}$ is zero. Then $\ln\frac{\nu}{\lambda} = 0$ and $Y^{(L)^T}\mu_0 = 0$ (as, by assumption $\tilde{\mu}$ is a solution to (3.38)). As a consequence, $\phi\left(e^{\mu_0}\right) = e^{Y^{(L)^T}\mu_0} = \underline{1}$. Consider the steady state equations

$$\begin{aligned}
Y\, I_a\, v(k,\, a) &= Y\, I_a\, \operatorname{diag}(\alpha\, E)\ \underbrace{\operatorname{diag}\left(\phi\left(a^{-1}\right)\right)\ \phi\,(a)}_{=\underline{1}} \\
&= Y\, I_a\ (\alpha\, E) \quad = 0 \\
Y\, I_a\, v(k,\, b) &= Y\, I_a\, \operatorname{diag}(\alpha\, E)\ \operatorname{diag}\left(\phi\left(a^{-1}\right)\right)\ \phi\left(\operatorname{diag}\left(e^{\mu_0}\right) a\right) \\
&= Y\, I_a\, \operatorname{diag}(\alpha\, E)\ \underbrace{\operatorname{diag}\left(\phi\left(a^{-1}\right)\right)\ \operatorname{diag}\left(\phi\,(a)\right)}_{=I_r}\ \phi\left(e^{\mu_0}\right) \\
&= Y\, I_a\, \operatorname{diag}(\alpha\, E)\ \phi\left(e^{\mu_0}\right) \\
&= Y\, I_a\, \operatorname{diag}(\alpha\, E)\ \underline{1} \\
&= Y\, I_a\ (\alpha\, E) \quad = 0.
\end{aligned}$$

■

Let $\ker\left(\left[-\underline{1}\ Y^{(L)^T}\right]\right) = \left[\begin{smallmatrix} M_1 \\ M_2 \end{smallmatrix}\right]$. Then $\mu = M_2\,\kappa$, κ a vector of appropriate dimension, is a representation of all vectors that can satisfy (3.38) (i.e. all vectors $\mu \in \mathbb{R}^n$, with $\exists\alpha \in \mathbb{R}$ such that $(\alpha,\ \mu)^T$ is solution to (3.38)). To determine $a, b \in \mathbb{R}^n_{>0}$ and $k \in \mathbb{R}^n_{>0}$ that satisfy the conservation relations as well (if $\operatorname{rank}(Y\, Ia) < n$, otherwise $a \in \mathbb{R}^n_{>0}$ is free) Lemma 3 has to be considered. Thus one has to find vectors $v \in \mathcal{S}$ and $\mu \in [M_2]$ with $\operatorname{sign}(\mu) = \operatorname{sign}(v)$. If such a pair μ, v can exists, equation (3.33a) from Theorem 3 and (3.39a), (3.39b) yield the desired $a,\, b \in \mathbb{R}^n_{>0}$ and $k \in \mathbb{R}^r_{>0}$. Note that by Lemma 6 the parameter $\alpha > 0$ can be chosen independent of μ.

Remark 8. *Note that* $\ln\frac{\nu}{\lambda} = 0$ *will always occur, if the subnetwork defined by a stoichiometric generator is open with respect to certain species. If a species enters the system, then* $Y^{(L)}$ *contains the zero column. Assume that only one species can enter the system. Then* $Y^{(L)} = \left[\begin{smallmatrix} 0 \\ \tilde{Y} \end{smallmatrix}\right]$, *where* $\tilde{Y}$ *contains only nonzero column vectors. Equation* (3.37) *becomes*

$$\begin{bmatrix} 0 \\ \tilde{Y}^T \end{bmatrix}\mu = \ln\frac{\nu}{\lambda}\, E.$$

and $\ln\frac{\nu}{\lambda} = 0$ *follows immediately.*

As an example consider the following network involving a protein A, its phosphorylated form A_p, a catalyst C and various combinations of these species (network $\mathcal{N}_2$ is structurally equivalent to a

subnetwork of network $\mathcal{N}_{11}$ discussed in Section 5):

$$
\begin{aligned}
&\mathbf{A_p} \xrightarrow{\mathbf{k_3}} 0 \underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} \mathbf{A} \\
&\mathbf{A} + \mathbf{C} \underset{\mathbf{k_5}}{\overset{\mathbf{k_4}}{\rightleftharpoons}} \mathbf{A\,C} \\
&\mathbf{A\,C} + \mathbf{C} \underset{\mathbf{k_7}}{\overset{\mathbf{k_6}}{\rightleftharpoons}} \mathbf{C\,A\,C} \xrightarrow{\mathbf{k_8}} \mathbf{A_p\,C} + \mathbf{C} \\
&\mathbf{A_p\,C} \underset{\mathbf{k_{10}}}{\overset{\mathbf{k_9}}{\rightleftharpoons}} \mathbf{A_p} + \mathbf{C}
\end{aligned}
\qquad (\mathcal{N}_2)
$$

Network $\mathcal{N}_2$ involves the species A, A_p, C, $A\,C$, $C\,A\,C$ and $A_p\,C$. Use x_1 for the concentration of A, x_2 for the concentration of A_p, x_3 for the concentration of C, x_4 for the concentration of $A\,C$, x_5 for the concentration of $C\,A\,C$ and x_6 for the concentration of $A_p\,C$. The complexes are the zero complex $y_1 = 0$, A with $y_2 = e_1$, A_p with $y_3 = e_2$, $A + C$ with $y_4 = e_1 + e_3$, $A\,C$ with $y_5 = e_4$, $A\,C + C$ with $y_6 = e_4 + e_3$, $C\,A\,C$ with $y_7 = e_5$, $A_p\,C + C$ with $y_8 = e_3 + e_6$, $A_p\,C$ with $y_9 = e_6$ and $A_p + C$ with $y_{10} = e_2 + e_3$. The columns of the 6×10-matrix Y are:

$$
Y = \begin{bmatrix} 0 & e_1 & e_2 & e_1 + e_3 & e_4 & e_4 + e_3 & e_5 & e_3 + e_6 & e_6 & e_2 + e_3 \end{bmatrix},
$$

the columns of the 6×10-matrix $Y^{(L)}$ are

$$
\hat{Y}^{(L)} = \begin{bmatrix} 0 & e_1 & e_2 & e_1 + e_3 & e_4 & e_4 + e_3 & e_5 & e_5 & e_6 & e_2 + e_3 \end{bmatrix}
$$

and the 10×10-matrix $\hat{I}_a$ is given by

$$
\hat{I}_a = \operatorname{diag}\left(I_a^{(1)}, I_a^{(2)}, I_a^{(3)}, I_a^{(2)} \right)
$$

with

$$
\begin{aligned}
\hat{I}_a^{(1)} &= \begin{bmatrix} -1 & 1 & 1 \\ 1 & -1 & 0 \\ 0 & 0 & -1 \end{bmatrix} \\
\hat{I}_a^{(2)} &= \begin{bmatrix} -1 & 1 \\ 1 & -1 \end{bmatrix} \\
\hat{I}_a^{(3)} &= \begin{bmatrix} -1 & 1 & 0 \\ 1 & -1 & -1 \\ 0 & 0 & 1 \end{bmatrix}.
\end{aligned}
$$

The stoichiometric matrix is

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

and the conservation relation is given by

$$
W = \begin{pmatrix} 0, & 0, & 1, & 1, & 2, & 1 \end{pmatrix}^T
$$

The cone $\ker(Y\ I_a) \cap \mathbb{R}^r_{\geq 0}$ is generated by the column vectors of

$$E_{\mathcal{N}_2} = \begin{bmatrix} 1 & 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 1 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 & 1 \\ 0 & 0 & 0 & 1 & 0 \end{bmatrix}$$

The first for columns are trivial generators, while the fifth column vector is the only stoichiometric generator of network $\mathcal{N}_2$, thus $\hat{E} = (1, 0, 1, 1, 0, 1, 0, 1, 1, 0)^T$. The subnetwork defined by $\hat{E}$ is displayed below:

$$\begin{aligned} &\mathbf{A_p} \xrightarrow{\mathbf{k_3}} 0 \xrightarrow{\mathbf{k_1}} \mathbf{A} \\ &\mathbf{A} + \mathbf{C} \xrightarrow{\mathbf{k_4}} \mathbf{A\,C} \\ &\mathbf{A\,C} + \mathbf{C} \xrightarrow{\mathbf{k_6}} \mathbf{C\,A\,C} \xrightarrow{\mathbf{k_8}} \mathbf{A_p\,C} + \mathbf{C} \\ &\mathbf{A_p\,C} \xrightarrow{\mathbf{k_9}} \mathbf{A_p} + \mathbf{C} \end{aligned} \qquad (\mathcal{N}_3)$$

The matrix $Y^{(L)}$ of the subnetwork $\mathcal{N}_3$ is given by

$$Y^{(L)} = \begin{bmatrix} 0 & e_2 & e_1 + e_3 & e_3 + e_4 & e_5 & e_6 \end{bmatrix}$$

Thus (3.37) is given by

$$\begin{bmatrix} 0 \\ \mu_2 \\ \mu_1 + \mu_3 \\ \mu_3 + \mu_4 \\ \mu_5 \\ \mu_6 \end{bmatrix} = \ln \frac{\nu}{\lambda} \begin{bmatrix} 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \end{bmatrix}$$

Obviously any solution must have $\ln \frac{\nu}{\lambda} = 0$. All solutions are given by

$$\mu = \kappa \begin{pmatrix} 1 \\ 0 \\ -1 \\ 1 \\ 0 \\ 0 \end{pmatrix}$$

There are two orthants, where $\operatorname{sign}(\mu) = \operatorname{sign}(v)$, $v \in [S]$ and μ as defined above:

$$\delta = (1\,, 0,\ -1,\ 1,\ 0,\ 0)^T$$

and $-\delta$. Using the generators of $\mathcal{S} \cap \mathbb{R}^6_\delta$ all pairs of positive steady states and the corresponding vectors of rate constants are can be parameterized by $\alpha_1, \ldots, \alpha_5 > 0$ and $\kappa \in \mathbb{R}$, $\kappa \neq 0$ (where, as usual, $\mathcal{S} = [Y\ I_a]$, the stoichiometric subspace of the subnetwork defined by $\hat{E}$):

$$\begin{aligned} a &= \left(\frac{\alpha_1}{e^\kappa - 1},\ \alpha_3,\ -\frac{\alpha_2}{e^{-\kappa} - 1},\ \frac{\alpha_2}{e^\kappa - 1},\ \alpha_4,\ \alpha_5 \right)^T \\ b &= \left(\frac{e^\kappa \alpha_1}{e^\kappa - 1},\ \alpha_3,\ -\frac{e^{-\kappa} \alpha_2}{e^{-\kappa} - 1},\ \frac{e^\kappa \alpha_2}{e^\kappa - 1},\ \alpha_4,\ \alpha_5 \right)^T \\ k &= \left(1,\ {\alpha_3}^{-1},\ -\frac{(e^\kappa - 1)\,(e^{-\kappa} - 1)}{\alpha_1\,\alpha_2},\ -\frac{(e^\kappa - 1)\,(e^{-\kappa} - 1)}{{\alpha_2}^2},\ {\alpha_4}^{-1},\ {\alpha_5}^{-1} \right)^T \end{aligned}$$

3.4.2 Extension of multistationarity to the overall network

If multistationarity can be established for a subnetwork, one is of course interested in extending those solutions to the overall network. In this section conditions are presented that guarantee that (multiple) steady states in a subnetwork can be extended to (multiple) steady states in the overall network. Recall that the ODEs defined by a subnetwork can be obtained from those of the overall network by setting certain rate constants to zero. To carry over a pair of steady states and a vector of rate constants from a subnetwork to the overall network one therefore has to determine values for those rate constants that are zero in the subnetwork.

Remark 9. *All results given in this subsection and especially Theorem 4 stem from a cooperation with D. Flockerzi and have been published in [9]. The proof of Theorem 4 is entirely D. Flockerzi's work and is given here only for the sake of completeness.*

To begin with, consider a system of ODEs in the form $\dot{x} = N\,v(k,\,x)$, with $N \in \mathbb{R}^{n\times\hat{r}}$, $x \in \mathbb{R}^n$ and $v, k \in \mathbb{R}^{\hat{r}}$ (using, as before, the symbol $\hat{}$ to denote quantities belonging to the overall network). Recall that $v(k,\,x) = \operatorname{diag}(k)\,\phi(x) = \operatorname{diag}(\phi(x))\,k$, where $\phi(x) = \left(x^{y_1^{(L)}}, \ldots, x^{y_{\hat{r}}^{(L)}}\right)^T$. Let $J \subseteq \{1, \ldots, \hat{r}\}$ be the set of reactions defining the subnetwork (if, for example, the subnetwork is defined by a stoichiometric generator $\hat{E}$ of the overall network, then $J = \operatorname{supp}\left(\hat{E}\right)$). J can be used to split the parameter vector: let $\hat{k}_E,\ \hat{k}_c \in \mathbb{R}^{\hat{r}}_{>0}$ and collect all parameters belonging to reactions contained in the subnetwork in $\hat{k}_E$ (i.e. $\hat{k}_{E,i} = k_i$ for $i \in \operatorname{supp}(E)$ and $\hat{k}_{E,i} = 0$ otherwise) and the remaining ones in $\hat{k}_c$ (i.e. $\hat{k}_c = k - \hat{k}_E$). For example, for the subnetwork $\mathcal{N}_3$ of the overall network $\mathcal{N}_2$ one has $J = \{\,1,\,3,\,4,\,6,\,8,\,9\,\}$ and thus $\hat{k}_E = (k_1,\,0,\,k_3,\,k_4,\,0,\,k_6,\,0,\,k_8,\,k_9,\,0)^T$ and $\hat{k}_c = (0,\,k_2,\,0,\,0,\,k_5,\,0,\,k_7,\,0,\,0,\,k_{10})^T$. Since v is linear in k one obtains

$$\dot{x} = N\,v(k,\,x) = N\,v(\hat{k}_E,\,x) + N\,v(\hat{k}_c,\,x). \tag{3.40}$$

Obtain vectors $k_E \in \mathbb{R}^r_{>0}$ and $k_c \in \mathbb{R}^{\hat{r}-r}_{>0}$ by removing zero elements from $\hat{k}_E$, $\hat{k}_c$ as

$$k_E = (k_i)_{i\in J} \quad \text{and} \quad k_c = (k_i)_{i\notin J} \quad \text{resp. .}$$

For the subnetwork $\mathcal{N}_3$ of the overall network $\mathcal{N}_2$ one has $k_E = (k_1,\,k_3,\,k_4,\,k_6,\,k_8,\,k_9)^T$ and $k_c = (k_2,\,k_5,\,k_7,\,k_{10})^T$. Let $v_E(k_E,\,x) \in \mathbb{R}^r$ and $v_c(k_c,\,x) \in \mathbb{R}^{\hat{r}-r}$ be given by

$$v_E(k_E,\,x) := \left(v(\hat{k}_E,\,x)\right)_{i\in J} = \operatorname{diag}\phi_E(x)\,k_E\ ,$$

$$v_c(k_c,\,x) := \left(v(\hat{k}_c,\,x)\right)_{i\notin J} = \operatorname{diag}\phi_c(x)\,k_c\ .$$

Let $N_i \in \mathbb{R}^n$ denote the columns of N and define

$$N_E = (N_i)_{i\in J} \quad \text{and } N_c = (N_i)_{i\notin J}.$$

Then the ODE (3.40) can be rewritten as

$$\dot{x} = N_E\,v_E(k_E,\,x) + N_c\,v_c(k_c,\,x) \tag{3.41}$$

and the ODEs for the subnetwork defined by J are given by

$$\dot{x} = N_E\,v_E(k_E,\,x)\,. \tag{3.42}$$

For the subnetwork $\mathcal{N}_3$ of the overall network $\mathcal{N}_2$ one has $v_E = (k_1,\,k_3\,x_2,\,k_4\,x_1\,x_3,\,k_6\,x_3\,x_4,\,k_8\,x_5,\,k_9\,x_3\,x_6)^T$ and $v_c = (k_2\,x_1,\,k_5\,x_4,\,k_7\,x_5,\,k_1 0\,x_6)^T$ and the matrices

$$N_E = \begin{bmatrix} 1 & 0 & -1 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 1 \\ 0 & 0 & -1 & -1 & 1 & 1 \\ 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 \end{bmatrix} \quad \text{and} \quad N_c = \begin{bmatrix} -1 & 1 & 0 & 0 \\ 0 & 0 & 0 & -1 \\ 0 & 1 & 1 & -1 \\ 0 & -1 & 1 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}.$$

The ODEs defined by the overall network $\mathcal{N}_2$ and by the subnetwork $\mathcal{N}_3$ are (the terms on the right hand side of the ODEs defined by the subnetwork are marked with a gray background):

$$\dot{x} = N_E\, v_E\,(k_E,\, x) + N_c\, v_c\,(k_c,\, x)$$

$$= \begin{array}{|c|}\hline k_1 - k_4\, x_1\, x_3 \\ -k_3\, x_2 + k_9\, x_3\, x_6 \\ -k_4\, x_1\, x_3 - k_6\, x_3\, x_4 + k_8\, x_5 + k_9\, x_3\, x_6 \\ k_4\, x_1\, x_3 - k_6\, x_3\, x_4 \\ k_6\, x_3\, x_4 - k_8\, x_5 \\ k_8\, x_5 - k_9\, x_3\, x_6 \\ \hline \end{array} \quad \begin{array}{c} -k_2\, x_1 + k_5\, x_4 \\ -k_{10}\, x_6 \\ k_5\, x_4 + k_7\, x_5 - k_{10}\, x_6 \\ -k_5\, x_4 + k_7\, x_5 \\ -k_7\, x_5 \\ k_{10}\, x_6 \end{array}$$

Let $\hat{s}$ denote $\operatorname{rank}(N)$ and s denote $\operatorname{rank}(N_E)$. Observe that $\operatorname{rank}(N)$ need not equal $\operatorname{rank}(N_E)$, that is, in general $\operatorname{rank}(N_E) \leq \operatorname{rank}(N)$ and thus $s \leq \hat{s}$ holds. Let $W \in \mathbb{R}^{(n-\hat{s})\times n}$ be an orthonormal basis for the left kernel of N and let $W_E \in \mathbb{R}^{(n-s)\times n}$ with $W_E^T = \begin{pmatrix} W_{add}^T \\ W^T \end{pmatrix}$ be an orthonormal basis for the left kernel of N_E (using W_{add} to accommodate additional basis vectors, if $s < \hat{s}$; W_{add} empty if $s = \hat{s}$). Let S_E be an orthonormal basis for $\operatorname{im}(N_E)$ and let $T = (S_E,\, W_{add},\, W)$ be an orthonormal transformation with $x = x(\xi,\, \eta,\, \rho) = T \begin{pmatrix} \xi \\ \eta \\ \rho \end{pmatrix}$ and $\xi = S_E^T\, x$, $\eta = W_{add}^T\, x$, $\rho = W^T\, x$. Then (3.40) reads

$$\begin{aligned} \dot{\xi} &= S_E^T\, N_E\, v_E\,(k_E,\, x) + S_E^T\, N_c\, v_c\,(k_c,\, x) \\ \dot{\eta} &= W_{add}^T\, N_c\, v_c\,(k_c,\, x) \\ \dot{\rho} &= 0. \end{aligned} \tag{3.43}$$

Suppose a $k_E^* > 0$ and steady states $x_{1,2}^* > 0$ for the subnetwork (3.42) defined by J have been established, that is

$$\begin{aligned} N_E\, v_E\left(k_E^*,\, x_{1,2}^*\right) &= 0 \\ W_E^T\, x_1^* &= W_E^T\, x_2^*. \end{aligned}$$

Let $\left(\xi_{1,2}^*,\, \eta^*,\, \rho^*\right)^T = T^T\, x_{1,2}^*$ and observe that

$$\begin{aligned} \dot{\xi} &= \underbrace{S_E^T\, N_E\, v_E\left(k_E^*,\, x\left(\xi_{1,2}^*,\, \eta^*,\, \rho^*\right)\right)}_{=0} + S_E^T\, N_c\, v_c\left(k_c,\, x\left(\xi_{1,2}^*,\, \eta^*,\, \rho^*\right)\right) \\ \dot{\eta} &= W_{add}^T\, N_c\, v_c\left(k_c,\, x\left(\xi_{1,2}^*,\, \eta^*,\, \rho^*\right)\right) \\ \dot{\rho} &= 0. \end{aligned}$$

Fix $k_E^* > 0$ and ρ^*, define $\hat{k}_E^*$ as

$$\left(\hat{k}_E^*\right)_{i\notin J} = 0 \quad \text{and} \quad \left(\hat{k}_E^*\right)_{i\in J} = k_E^*, \tag{3.44}$$

so that $\hat{k}_E^*$ is obtained from k_E^* by a suitable padding with zeros and let $k := \hat{k}_E^* + \epsilon\, \hat{k}_c$. For steady states of (3.43) one needs to determine ξ, η and $\hat{k}_c$ such that

$$\begin{aligned} S_E^T\, N_E\, v_E\left(k_E^*,\, x\left(\xi,\, \eta,\, \rho^*\right)\right) + \epsilon\, S_E^T\, N_c\, v_c\left(k_c,\, x\left(\xi,\, \eta,\, \rho^*\right)\right) &= 0 \\ \epsilon\, W_{add}^T\, N_c\, v_c\left(k_c,\, x\left(\xi,\, \eta,\, \rho^*\right)\right) &= 0. \end{aligned}$$

Observe that the last equation is equivalent to

$$\epsilon\, W_{add}^T\, N_c\, \operatorname{diag}\left(\phi_c\left(x\left(\xi,\, \eta,\, \rho^*\right)\right)\right)\, k_c = 0.$$

With $F(k, x) := Nv(k,\, x)$ and its Jacobian $F_x := D_x F(k,\, x)$ one can outline the following program:

Algorithm 3. *(1) Compute* $S_E^T\, F_x\left(\hat{k}_E^*,\, x_{1,2}^*\right)\,(S_E,\, W_{add}) =: \left(A_{1,2}^*,\, B_{1,2}^*\right)$,

ask for

$$\text{regular } A_{1,2}^* \tag{3.45a}$$

and solve

$$A_{1,2}^*\, X_{1,2}^* + B_{1,2}^* = 0 \tag{3.45b}$$

for $X_{1,2}^* = -[A_{1,2}^*]^{-1} B_{1,2}^*$.

(2) Compute a positive κ_0 *with*

$$\mathcal{G}_c^*\,\kappa_0 = 0 \tag{3.45c}$$

for

$$\mathcal{G}_c^* := \begin{bmatrix} W_{add}^T\, N_c\, \mathrm{diag}\,(\phi_c\,(x_1^*)) \\ W_{add}^T\, N_c\, \mathrm{diag}\,(\phi_c\,(x_2^*)) \end{bmatrix}. \tag{3.45d}$$

and define $\hat{k}_c^* \in \mathbb{R}^{\hat{r}}$ *by*

$$\left(\hat{k}_c^*\right)_{i\in\mathrm{supp}(E)} = 0 \quad \textit{and} \quad \left(\hat{k}_c^*\right)_{i\notin\mathrm{supp}(E)} = \kappa_0. \tag{3.45e}$$

(3) Compute $W_{add}^T\, F_x\left(\hat{k}_c^*,\, x_{1,2}^*\right)\,(S_E,\, W_{add}) =: \left(C_{1,2}^*,\, D_{1,2}^*\right)$ *and ask for*

$$\textit{regular } \mathcal{D}_{1,2}^* := D_{1,2}^* + C_{1,2}^*\, X_{1,2}^*. \tag{3.45f}$$

The following Theorem 4 shows that this program leads to a pair of positive steady states $\tilde{x}_{1,2}$ of (3.40) near $x_{1,2}^*$ for $\hat{k}_E^* + \epsilon\,\hat{k}_c^*$ if $\epsilon > 0$ is sufficiently small. To obtain values for $\tilde{x}_{1,2}$, fix a sufficiently small ϵ and solve $N\,v(\hat{k}_E^* + \epsilon\,\hat{k}_c^*,\, \tilde{x}_{1,2}) = 0$ for $\tilde{x}_{1,2}$ near $x_{1,2}^*$.

Theorem 4. *Suppose the following conditions are fulfilled:*

(i) There exist $x_{1,2}^* > 0$, $k_E^* > 0$ *with* $N_E\, v_E\left(k_E^*,\, x_{1,2}^*\right) = 0$.

(ii) There exists a $\kappa_0 > 0$ *with* $\mathcal{G}_c^*\,\kappa_0 = 0$, *cf.* (3.45d).

(iii) Both A_1^* *and* A_2^* *are regular, cf.* (3.45a).

(iv) Both $\mathcal{D}_1^*$ *and* $\mathcal{D}_2^*$ *are regular, cf.* (3.45f).

Then there exist $\epsilon_0 > 0$ *and* $\delta_0 > 0$ *such that*

$$0 < \epsilon < \epsilon_0 \quad \textit{and } |\rho - \rho^*| < \delta_0$$

imply the existence of different positive hyperbolic steady states

$$x_{1,2}\,(\rho,\, \epsilon) = (S_E,\, W_{add},\, W) \begin{pmatrix} \Xi_{1,2}\,(\rho,\, \epsilon) \\ H_{1,2}\,(\rho,\, \epsilon) \\ \rho \end{pmatrix}$$

of (3.40) *with*

$$W^T\, x_1\,(\rho,\, \epsilon) = W^T\, x_2\,(\rho,\, \epsilon)$$

for the positive $k(\epsilon) = \hat{k}_E^* + \epsilon\,\hat{k}_c^*$ – *cf.* (3.44) *and* (3.45e).

Proof. Apply the orthonormal transformation $\xi = S_E^T\, x$, $\eta = W_{add}^T\, x$, $\rho = W^T\, x$ to obtain

$$\begin{aligned} \dot{\xi} &= S_E^T\, N\, v(\hat{k}_E^* + \epsilon\,\hat{k}_c^*,\, x) \\ &= S_E^T\, N\, v(\hat{k}_E^*,\, x) + \epsilon\, S_E^T\, N\, v(\hat{k}_c^*,\, x) \\ \dot{\eta} &= W_{add}^T\, N\, v(\hat{k}_E^* + \epsilon\,\hat{k}_c^*,\, x) = \epsilon\, W_{add}^T\, N\, v(\hat{k}_c^*,\, x) \\ \dot{\hat{\eta}} &= 0. \end{aligned}$$

For fixed x_j^*, k_E^* and regular A_j^* (see assumption (i) and (iii)) the equation $\dot{\xi} = 0$ has a locally unique solution $\xi_j = \Xi_j\left(\eta,\, \rho,\, \epsilon\,\hat{k}_c^*\right)$ near $(\eta^*, \rho^*, 0)$ with $A_j^*\, X_j + B_j^* = 0$, $X_j := \frac{\partial}{\partial\eta}\Xi_j\,(\eta^*,\, \rho^*,\, 0)$, by the implicit function theorem. To find steady states with common ρ–components we need to solve

$$\begin{aligned} W_{add}^T\, N\, v(\hat{k}_c^*,\, S_E\, \Xi_1\left(\eta,\, \rho,\, \epsilon\,\hat{k}_c^*\right) + W_{add}\,\eta + W\,\rho) &= 0 \\ W_{add}^T\, N\, v(\hat{k}_c,\, S_E\, \Xi_2\left(\tilde{\eta},\, \rho,\, \epsilon\,\hat{k}_c^*\right) + W_{add}\,\tilde{\eta} + W\,\rho) &= 0 \end{aligned}$$

for $\binom{\eta}{\bar{\eta}} = \binom{H_1(\rho,\,\epsilon)}{H_2(\rho,\,\epsilon)}$ near the solution $(\eta^*,\,\eta^*,\rho^*,0)$ (cf. assumption (ii) and (3.45e)). By assumption (iv) and the implicit function theorem such functions H_1 and H_2 exist. Thus locally, there exist steady states

$$\begin{aligned}\xi_1 &= \Xi_1\left(H_1\left(\rho,\,\epsilon\right),\,\rho,\,\epsilon\right) =: \Xi_1\left(\rho,\,\epsilon\right),\ \eta = H_1\left(\rho,\,\epsilon\right),\,\rho\\ \xi_2 &= \Xi_2\left(H_2\left(\rho,\,\epsilon\right),\,\rho,\,\epsilon\right) =: \Xi_2\left(\rho,\,\epsilon\right),\ \eta = H_2\left(\rho,\,\epsilon\right),\,\rho\end{aligned}$$

of (3.43) for sufficiently small $\epsilon > 0$. The corresponding positive steady states of (3.40) near $x^*_{1,2}$ are given by the $x_{1,2}\left(\rho,\,\epsilon\right)$ of the theorem. ■

Remark 10. *Note that Theorem 4 makes no use of the fact, that the subnetwork is defined by a stoichiometric generator. Thus it can be applied to any subnetwork. The same holds of course for Algorithm 3.*

3.5 Resume: a program to decide about multistationarity

In this section the results of this chapter are combined to a program that can be applied to decide about multistationarity. One has to distinguish two cases: (i) ν, $\lambda\Lambda\left(E\right)$ can be determined independent of $\mu \in \mathbb{R}^n$ (see Section 3.2.1) and (ii) ν, $\lambda \in \Lambda\left(E\right)$ and $\mu \in \mathbb{R}^n$ have to be determined together (see Section 3.2.2). In the first case Algorithm 2 given in Section 3.3.1, in the second case Algorithm 4 given below can be applied.

Algorithm 4.

(1) Assumptions:

(i) The sign of the elements of Q_{ij} the matrix $Q_v\left(\mu\right)$, $\operatorname{sign}\left(Q_{ij}\right)$ can be determined by linear inequalities (i.e. the conditions given in Lemma 2 hold).

(ii) Let s_0 be the number of linear inequalities that determine the sign pattern of $Q\left(\mu\right)$.

(iii) Let $V \in \mathbb{R}^{s_0 \times n}$ be the coefficient matrix of the inequalities that determine the sign pattern of $Q\left(\mu\right)$, as defined in (3.21a).

(iv) Let $\beta \in \mathbb{R}^{s_0}$ be as defined in (3.21b).

(2) Determine all $\delta \in \{-1,\,0,\,1\}^n$ with $\mathcal{S} \cap \mathbb{R}^n_\delta \neq \emptyset$:

$$\Delta^{\mathcal{S}} := \{\delta \in \{-1,\,0,\,1\}^n \,|\mathcal{S} \cap \mathbb{R}^n_\delta \neq \emptyset\} \tag{3.46}$$

(3) Determine all feasible signings $\sigma \in \{-1,\,0,\,1\}^{s_0}$ for $Q\left(\mu\right)$:

$$\Sigma := \left\{\sigma \in \{-1,\,0,\,1\}^n \,|\, \begin{bmatrix} V & \beta & -\operatorname{diag}\left(\sigma\right)\end{bmatrix} \begin{pmatrix}\mu\\ \bar{s}\\ s\end{pmatrix} = 0,\ \bar{s} = 1,\ s > 0,\ \textit{has a solution}\right\} \tag{3.47}$$

(4) (a) Determine all $\sigma \in \Sigma$ such that $Q\left(\mu\right)$ is an L^+ -matrix (e.g. using Theorem 1):

$$\Sigma^+ := \left\{\sigma \in \Sigma | Q\left(\mu\right) \textit{ is an } L^+\textit{-matrix}\right\} \tag{3.48}$$

(b) Determine all $\sigma \in \Sigma$ such that $Q\left(\mu\right)$ is a sign-central matrix (e.g. using Theorem 2):

$$\Sigma^0 := \{\sigma \in \Sigma | Q\left(\mu\right) \textit{ is an sign-central matrix}\} \tag{3.49}$$

(5) Determine all orthants $\delta \in \Delta^{\mathcal{S}}$ that contain a feasible signing $\sigma \in \Sigma^+$ (or, if $\Sigma^+ = \emptyset$, $\sigma \in \Sigma^0$). That is, identify all orthants, with $\mathcal{S} \cap \mathbb{R}^n_\delta \neq \emptyset$ that contain a solution to $V\,\mu + \beta - \operatorname{diag}\left(\sigma\right)\,s = 0$, $s > 0$:

$$\mathcal{P}^{+,0} := \left\{\left(\sigma,\,\delta\right) \in \Sigma^{+,0} \times \Delta | \begin{bmatrix} V\,\operatorname{diag}\left(\delta\right) & \beta & -\operatorname{diag}\left(\sigma\right)\end{bmatrix} \begin{pmatrix}\xi\\ \bar{s}\\ s\end{pmatrix} = 0,\ \xi,\,s > 0,\ \bar{s} = 1\right\} \tag{3.50}$$

If $\mathcal{P}^+ \neq \emptyset$ then multistationarity is guaranteed. If $\mathcal{P}^+ = \emptyset$ and $\mathcal{P}^0 \neq \emptyset$, then multistationarity is guaranteed for a subnetwork. Observe that $\mathcal{P}^0 \neq \emptyset$ means that $Q(\mu)$ is a sign-central matrix, thus it is only guaranteed that $Q(\mu)$ has a ***nonnegative*** *kernel vector. That is, some components of ν, $\lambda \in \mathbb{R}^p_{>0}$ might be zero. In this case one can still try to establish multistationarity for the overall network using the results given in Section 3.4; see (vi) below.*

(6) If $\mathcal{P}^+ \neq \emptyset$ obtain a pair of steady states in the following way:

(i) Solve

$$\underbrace{\begin{bmatrix} V\ \mathrm{diag}(\delta) & \beta & -\mathrm{diag}(\sigma) \end{bmatrix}}_{=:H} \begin{pmatrix} \xi \\ \bar{s} \\ s \end{pmatrix} = 0,\ \xi, s > 0,\ \bar{s} = 1$$

to obtain generators of $\ker(H) \cap \mathbb{R}^{n+1+s_0}_{>0}$. Let the columns of $M = \begin{bmatrix} M_1 \\ \bar{m} \\ M_2 \end{bmatrix}$ be generators. Then $\mu = \mathrm{diag}(\delta)\ M_1\,\kappa$, $\kappa > 0$ is a representation of all $\mu \in \mathbb{R}^n_\delta$ with $\mathrm{sign}(V\,\mu + \beta) = \sigma$.

(ii) Fix $\bar{\kappa} > 0$ to obtain $\bar{\mu} = M_1\,\bar{\kappa}$.

(iii) Solve $Q(\bar{\mu}) \begin{pmatrix} \nu \\ \lambda \end{pmatrix} = 0$, $\nu, \lambda \in \Lambda(E)$ for ν, λ.

(iv) Choose any $v \in \mathcal{S} \cap \mathbb{R}^n_\delta$.

(v) Use (3.33a), (3.33b) *and* (3.33c) *to obtain values for a, b, k (using $\bar{\mu}$ and ν, λ obtained above).*

(vi) If $\mathcal{P}^+ = \emptyset$ and $\mathcal{P}^0 \neq \emptyset$ use the Algorithm 3 to try to extend multistationarity to the overall network.

Remark 11. *The condition $\mathcal{P}^+ \neq \emptyset$ is necessary for multistationarity, the conditions $\mathcal{P}^+ = \emptyset$ and $\mathcal{P}^0 \neq \emptyset$ are necessary for multistationarity in a subnetwork (and can be extended to necessary conditions for the overall network if combined with the conditions given in Algorithm 3). As a consequence $\mathcal{P}^+ = \emptyset$ and $\mathcal{P}^0 = \emptyset$ does not imply that multistationarity cannot occur in a biochemical reaction network.*

Chapter 4

Multistationarity in the activation of an MAPK(K)

Mitogen-activated protein kinase (MAPK) cascades are well studied systems in cell biology. The most common form of an MAPK cascade is that of a three tiered cascade, as depicted in Fig. 4.1. Its building blocks are an MAPK, an MAPK kinase (MAPKK) and an 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 was considered, E_1, for example, would be RAS, the MAPKKK Raf-1 or Mos and the MAPK ERK-1 [57]).
Mathematical models describing the dynamics of an MAPK cascade have been known for some time (see e.g. [31, 37] or [54] for a more general overview of quantitative models for signal transduction networks in general, as well as [39] 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 an MAPK cascade can exhibit all sorts of complex dynamical behaviour like bistability and oscillations (see e.g. [4, 5] or [38, 39]). Only recently [45] showed that, surprisingly, bistability can even occur on layer two or three of Fig. 4.1 alone, provided a distributive, multi-collision mechanism is used for both, phosphorylation and dephosphorylation of the MAPK or the MAPKK [39, 45].
In principle, both phosphorylation and dephosphorylation could follow either a distributive or processive mechanism (see Section 4.1 for details). One way to decide which mechanism is employed is to experimentally verify multiple steady states on a single layer. This idea was already suggested in [45, 50]. However, to **safely discard** different candidate mechanisms, a **mathematical proof** is required that these systems cannot exhibit multistationarity *for any conceivable parameter vector*. This can be accomplished using CRNT, as shown in [10, 11, 12]. However, using the approach presented in Chapter 3 will not only provide additional information (that is discussed in Chapter 6) but will also lead to a better understanding of the phenomenon multistationarity itself.
This chapter is organised as follows: in Section 4.1 distributive and processive mechanisms are introduced. In Section 4.2 and 4.3 the methods developed in Chapter 3 are applied to the reaction networks presented in Section 4.1. In Section 4.4 the influence of the assumption that some species are **not** subject to a conservation relation on the existence of multiple steady states is examined, while Section 4.5 contains a brief discussion of the implications of the results obtained in Section 4.2 and 4.3 for model discrimination. The chapter closes with a discussion of the results obtained in Chapter 3 in the light of the results obtained for the activation of an MAPK(K).

4.1 Processive vs. distributive phosphorylation

In this section three candidate network structures for a single layer of the reaction scheme displayed in Fig. 4.1 are discussed. Every scheme is a realization of the double-phosphorylation process sketched in Fig. 4.2. Both phosphorylation and dephosphorylation can follow either a distributive or processive mechanism. By a processive mechanism the kinase (phosphatase) carries out two phosphorylation or

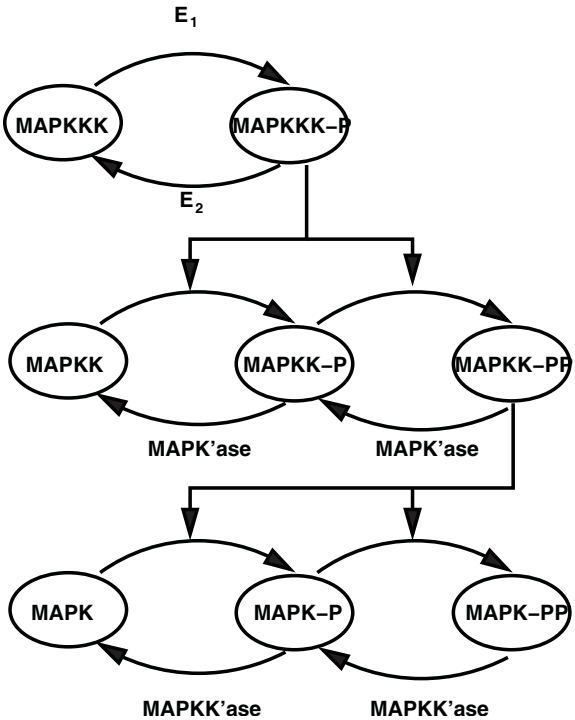


Figure 4.1: Scheme of an MAPK cascade (c.f. [37]). MAPK denotes the mitogen-activated protein kinase, MAPKK the MAPK kinase and MPAKKK the MAPKK kinase, -P mono- and -PP double-phosphorylation. E_1 is a stimulus, $MAPK'ase$, $MAPKK'ase$ and E_2 are phosphatases.

dephosphorylation steps, before the final product is released. In a distributive mechanism, monophosphorylated intermediates are released before conversion to the final product occurs, after a second binding of kinase (phosphatase) and intermediate. In the following scenarios, A is the MAPK, subscript $_p$ and $_{pp}$ denote single and double phosphorylation, E_1 corresponds to MEK and E_2 to the phosphatase. The following networks involving different combinations of processive and distributive mechanisms are analyzed:

- A distributive mechanism both for phosphorylation and dephosphorylation:

$$\begin{aligned} \mathbf{A}+\mathbf{E_1} \underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} \mathbf{A\,E_1} \xrightarrow{\mathbf{k_3}} \mathbf{A_p}+\mathbf{E_1} \underset{\mathbf{k_5}}{\overset{\mathbf{k_4}}{\rightleftharpoons}} \mathbf{A_p\,E_1} \xrightarrow{\mathbf{k_6}} \mathbf{A_{pp}}+\mathbf{E_1} \\ \mathbf{A_{pp}}+\mathbf{E_2} \underset{\mathbf{k_8}}{\overset{\mathbf{k_7}}{\rightleftharpoons}} \mathbf{A_{pp}\,E_2} \xrightarrow{\mathbf{k_9}} \mathbf{A_p}+\mathbf{E_2} \underset{\mathbf{k_{11}}}{\overset{\mathbf{k_{10}}}{\rightleftharpoons}} \mathbf{A_p\,E_2} \xrightarrow{\mathbf{k_{12}}} \mathbf{A}+\mathbf{E_2} \end{aligned} \qquad (\mathcal{N}_4)$$

- A processive mechanism for phosphorylation and a distributive mechanism for dephosphorylation:

$$\begin{aligned} \mathbf{A}+\mathbf{E_1} \underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} \mathbf{A\,E_1} \underset{\mathbf{k_4}}{\overset{\mathbf{k_3}}{\rightleftharpoons}} \mathbf{A_p\,E_1} \xrightarrow{\mathbf{k_5}} \mathbf{A_{pp}}+\mathbf{E_1} \\ \mathbf{A_{pp}}+\mathbf{E_2} \underset{\mathbf{k_7}}{\overset{\mathbf{k_6}}{\rightleftharpoons}} \mathbf{A_{pp}\,E_2} \xrightarrow{\mathbf{k_8}} \mathbf{A_p}+\mathbf{E_2} \underset{\mathbf{k_{10}}}{\overset{\mathbf{k_9}}{\rightleftharpoons}} \mathbf{A_p\,E_2} \xrightarrow{\mathbf{k_{11}}} \mathbf{A}+\mathbf{E_2} \end{aligned} \qquad (\mathcal{N}_5)$$

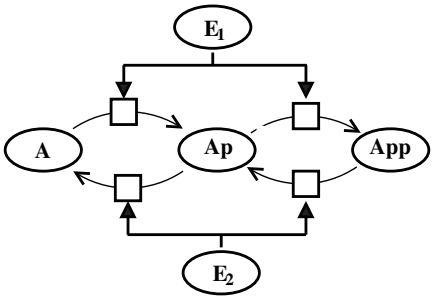


Figure 4.2: Scheme of activation of an MAPK (A). Enzyme E_1 (e.g. the kinase MEK) double phosphorylates the MAPK A, enzyme E_2 (a phosphatase) dephosphorylates both mono- and double- phosphorylated MAPK (i.e. A_p and A_{pp}).

From a mathematical perspective this network is structurally equal to a network incorporating a distributive mechanism for phosphorylation and a processive mechanism for dephosphorylation (as the latter one would be the result of a mere change of names: $A \to A_{pp}$, $E_1 \to E_2$ and $E_2 \to E_1$).

- A processive mechanism both for phosphorylation and dephosphorylation:

$$\begin{aligned} \mathbf{A} + \mathbf{E_1} &\underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} \mathbf{A\,E_1} \underset{\mathbf{k_4}}{\overset{\mathbf{k_3}}{\rightleftharpoons}} \mathbf{A_p\,E_1} \xrightarrow{\mathbf{k_5}} \mathbf{A_{pp}} + \mathbf{E_1} \\ \mathbf{A_{pp}} + \mathbf{E_2} &\underset{\mathbf{k_7}}{\overset{\mathbf{k_6}}{\rightleftharpoons}} \mathbf{A_{pp}\,E_2} \underset{\mathbf{k_9}}{\overset{\mathbf{k_8}}{\rightleftharpoons}} \mathbf{A_p\,E_2} \xrightarrow{\mathbf{k_{10}}} \mathbf{A} + \mathbf{E_2} \end{aligned} \qquad (\mathcal{N}_6)$$

The structural data and the ODEs for each network are given in Appendix A.

4.2 Positive solutions for the polynomial equations

In this Section the equation ${Y^{(L)}}^T \mu = \ln \frac{E\,\nu}{E\,\lambda}$ (see (3.8b) in Chapter 3) is derived and solved for $\mathcal{N}_4$, $\mathcal{N}_5$ and $\mathcal{N}_6$.

Remark 12. *From hereon, when analyzing the equation*

$${Y^{(L)}}^T \mu = \ln \frac{E\,\nu}{E\,\lambda},$$

*the constraint ν, $\lambda > 0$ is considered instead of the constraint ν, $\lambda \in \Lambda(E)$. This is done because the constraint enhances ν, $\lambda > 0$ readability and because for **all but one** of the networks considered in the remaining chapters $\Lambda(E)$ is equal to the positive orthant. The only exception is network $\mathcal{N}_{10}$. For this network however, it was possible to establish multistationarity by considering ν, $\lambda > 0$. Thus there was no need to consider all of ν, $\lambda \in \Lambda(E)$ (recall that $\Lambda(E)$ is a union of cones, multistationarity was established for one cone, thus it was not necessary to consider the remaining ones).*

4.2.1 Positive solutions for $\mathcal{N}_4$

For this network the equation

$${Y^{(L)}}^T \mu = \ln \frac{E\,\nu}{E\,\lambda}$$

reads

$$\mu_1 + \mu_2 = \ln \frac{\nu_1 + \nu_5}{\lambda_1 + \lambda_5} \tag{4.1a}$$
$$\mu_3 = \ln \frac{\nu_1}{\lambda_1} \tag{4.1b}$$
$$\mu_3 = \ln \frac{\nu_5}{\lambda_5} \tag{4.1c}$$
$$\mu_2 + \mu_4 = \ln \frac{\nu_2 + \nu_6}{\lambda_2 + \lambda_6} \tag{4.1d}$$
$$\mu_5 = \ln \frac{\nu_2}{\lambda_2} \tag{4.1e}$$
$$\mu_5 = \ln \frac{\nu_6}{\lambda_6} \tag{4.1f}$$
$$\mu_6 + \mu_7 = \ln \frac{\nu_3 + \nu_6}{\lambda_3 + \lambda_6} \tag{4.1g}$$
$$\mu_8 = \ln \frac{\nu_3}{\lambda_3} \tag{4.1h}$$
$$\mu_8 = \ln \frac{\nu_6}{\lambda_6} \tag{4.1i}$$
$$\mu_4 + \mu_7 = \ln \frac{\nu_4 + \nu_5}{\lambda_4 + \lambda_5} \tag{4.1j}$$
$$\mu_9 = \ln \frac{\nu_4}{\lambda_4} \tag{4.1k}$$
$$\mu_9 = \ln \frac{\nu_5}{\lambda_5}. \tag{4.1l}$$

These equations are solvable, if and only if

$$\ln \frac{\nu_1}{\lambda_1} = \ln \frac{\nu_5}{\lambda_5}, \qquad \ln \frac{\nu_2}{\lambda_2} = \ln \frac{\nu_6}{\lambda_6}, \qquad \ln \frac{\nu_3}{\lambda_3} = \ln \frac{\nu_6}{\lambda_6}, \qquad \ln \frac{\nu_4}{\lambda_4} = \ln \frac{\nu_5}{\lambda_5}$$

holds (note that this is equivalent to condition (3.11)). Thus one obtains the following polynomials (as $\nu_i > 0$ and $\lambda_i > 0$, $i = 1, \ldots, 6$):

$$\lambda_5 \nu_1 - \lambda_1 \nu_5 = 0, \qquad \lambda_6 \nu_2 - \lambda_2 \nu_6 = 0$$
$$\lambda_6 \nu_3 - \lambda_3 \nu_6 = 0, \qquad \lambda_5 \nu_4 - \lambda_4 \nu_5 = 0.$$

Solve for ν_1, ν_2, ν_5 and ν_6 to obtain:

$$\nu_1 = \lambda_1 \frac{\nu_4}{\lambda_4}, \qquad \nu_2 = \lambda_2 \frac{\nu_3}{\lambda_3}, \qquad \nu_5 = \lambda_5 \frac{\nu_4}{\lambda_4}, \qquad \nu_6 = \lambda_6 \frac{\nu_3}{\lambda_3}.$$

This yields a representation of the vector ν in terms of $\lambda_1, \ldots, \lambda_6$ and ν_3 and ν_4:

$$\nu^T = \left(\lambda_1 \frac{\nu_4}{\lambda_4}, \lambda_2 \frac{\nu_3}{\lambda_3}, \nu_3, \nu_4, \lambda_5 \frac{\nu_4}{\lambda_4}, \lambda_6 \frac{\nu_3}{\lambda_3} \right). \tag{4.4}$$

Thus it is possible to determine $\nu, \lambda \in \mathbb{R}^6_{>0}$ independent of $\mu \in \mathbb{R}^9$. Note that for ν as in (4.4), one obtains

$$\ln \frac{\nu_1 + \nu_5}{\lambda_1 + \lambda_5} = \ln \frac{\nu_1}{\lambda_1} = \ln \frac{\nu_5}{\lambda_5} = \ln \frac{\nu_4 + \nu_5}{\lambda_4 + \lambda_5} = \ln \frac{\nu_4}{\lambda_4}$$

and

$$\ln \frac{\nu_2 + \nu_6}{\lambda_2 + \lambda_6} = \ln \frac{\nu_2}{\lambda_2} = \ln \frac{\nu_6}{\lambda_6} = \ln \frac{\nu_3 + \nu_6}{\lambda_3 + \lambda_6} = \ln \frac{\nu_3}{\lambda_3}.$$

And thus the following system of linear equations:

$$\begin{aligned}
\mu_1 + \mu_2 &= \ln \frac{\nu_4}{\lambda_4} \\
\mu_3 &= \ln \frac{\nu_4}{\lambda_4} \\
\mu_2 + \mu_4 &= \ln \frac{\nu_3}{\lambda_3} \\
\mu_5 &= \ln \frac{\nu_3}{\lambda_3} \\
\mu_6 + \mu_7 &= \ln \frac{\nu_3}{\lambda_3} \\
\mu_8 &= \ln \frac{\nu_3}{\lambda_3} \\
\mu_4 + \mu_7 &= \ln \frac{\nu_4}{\lambda_4} \\
\mu_9 &= \ln \frac{\nu_4}{\lambda_4}
\end{aligned}$$

Solving for μ finally yields:

$$\begin{aligned}
\mu = \mu_7 \, (-1, 1, 0, -1, 0, -1, 1, 0, 0)^{'} + \ln \frac{\nu_3}{\lambda_3} \, (-1, 1, 0, 0, 1, 1, 0, 1, 0)^{'} \\
+ \ln \frac{\nu_4}{\lambda_4} \, (2, -1, 1, 1, 0, 0, 0, 0, 1)^{'} .
\end{aligned} \tag{4.5}$$

If in (4.5) $\kappa_1 := \ln \frac{\nu_3}{\lambda_3}$ and $\kappa_2 := \ln \frac{\nu_4}{\lambda_4}$ are interpreted as free parameters μ is given by

$$\mu = M_{\mathcal{N}_4} \begin{pmatrix} \mu_7 \\ \kappa_1 \\ \kappa_2 \end{pmatrix}$$

where

$$M_{\mathcal{N}_4} := \begin{bmatrix} -1 & -1 & 2 \\ 1 & 1 & -1 \\ 0 & 0 & 1 \\ -1 & 0 & 1 \\ 0 & 1 & 0 \\ -1 & 1 & 0 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} . \tag{4.6a}$$

Consider the column space of $M_{\mathcal{N}_4}$: $\mathcal{M}_{\mathcal{N}_4} = [M_{\mathcal{N}_4}]$. From Chapter 3 it is known that any element $\mu \in \mathcal{M}_{\mathcal{N}_4}$ can be associated with the difference of two positive steady state solutions: if a satisfies $Y\, I_a\, v(a,\, k) = 0$ and if $\mu \in \mathcal{M}_{\mathcal{N}_4}$, then $b = \mathrm{diag}\,(\mathrm{Exp}\,(\mu))\; a$ satisfies $Y\, I_a\, v(b,\, k) = 0$. As discussed in Section 3.1, any vector $a \in \mathbb{R}^9_{>0}$ is a steady state solution for the vector of rate constants k defined in (3.10a). For network $\mathcal{N}_4$ one obtains the following rate constants:

$$k_1 = \frac{\lambda_1 + \lambda_5}{a_1\, a_2} \qquad k_2 = \frac{\lambda_1}{a_3} \tag{4.7a}$$

$$k_3 = \frac{\lambda_5}{a_3} \qquad k_4 = \frac{\lambda_2 + \lambda_6}{a_2\, a_4} \tag{4.7b}$$

$$k_5 = \frac{\lambda_2}{a_5} \qquad k_6 = \frac{\lambda_6}{a_5} \tag{4.7c}$$

$$k_7 = \frac{\lambda_3 + \lambda_6}{a_6\, a_7} \qquad k_8 = \frac{\lambda_3}{a_8} \tag{4.7d}$$

$$k_9 = \frac{\lambda_6}{a_8} \qquad k_{10} = \frac{\lambda_4 + \lambda_5}{a_4\, a_7} \tag{4.7e}$$

$$k_{11} = \frac{\lambda_4}{a_9} \qquad k_{12} = \frac{\lambda_5}{a_9}. \tag{4.7f}$$

4.2.2 Positive solutions for $\mathcal{N}_5$

For this network the equation

$$Y^{(L)^T}\,\mu = \ln\frac{E\,\nu}{E\,\lambda}$$

reads

$$\mu_1+\mu_2 = \ln\frac{\nu_1+\nu_5}{\lambda_1+\lambda_5} \tag{4.8a}$$
$$\mu_3 = \ln\frac{\nu_1}{\lambda_1} \tag{4.8b}$$
$$\mu_3 = \ln\frac{\nu_2+\nu_5}{\lambda_2+\lambda_5} \tag{4.8c}$$
$$\mu_4 = \ln\frac{\nu_2}{\lambda_2} \tag{4.8d}$$
$$\mu_4 = \ln\frac{\nu_5}{\lambda_5} \tag{4.8e}$$
$$\mu_5+\mu_6 = \ln\frac{\nu_3+\nu_5}{\lambda_3+\lambda_5} \tag{4.8f}$$
$$\mu_7 = \ln\frac{\nu_3}{\lambda_3} \tag{4.8g}$$
$$\mu_7 = \ln\frac{\nu_5}{\lambda_5} \tag{4.8h}$$
$$\mu_6+\mu_8 = \ln\frac{\nu_4+\nu_5}{\lambda_4+\lambda_5} \tag{4.8i}$$
$$\mu_9 = \ln\frac{\nu_4}{\lambda_4} \tag{4.8j}$$
$$\mu_9 = \ln\frac{\nu_5}{\lambda_5} \tag{4.8k}$$

These equations are solvable, if and only if

$$\ln\frac{\nu_1}{\lambda_1} = \ln\frac{\nu_2+\nu_5}{\lambda_2+\lambda_5}, \qquad \ln\frac{\nu_2}{\lambda_2} = \ln\frac{\nu_5}{\lambda_5}, \qquad \ln\frac{\nu_3}{\lambda_3} = \ln\frac{\nu_5}{\lambda_5}, \qquad \ln\frac{\nu_4}{\lambda_4} = \ln\frac{\nu_5}{\lambda_5}.$$

One obtains

$$\nu_2 = \lambda_2\,\frac{\nu_5}{\lambda_5}, \qquad \nu_3 = \lambda_3\,\frac{\nu_5}{\lambda_5}, \qquad \nu_4 = \lambda_4\,\frac{\nu_5}{\lambda_5}$$

and thus $\ln\frac{\nu_2+\nu_5}{\lambda_2+\lambda_5} = \frac{\nu_5}{\lambda_5}$. Therefore $\nu_1 = \lambda_1\,\frac{\nu_5}{\lambda_5}$ and

$$\nu = \frac{\nu_5}{\lambda_5}\,\lambda,$$

$\lambda \in \mathbb{R}^5_{>0}$ and $\nu_5 > 0$ free; thus, as for network $\mathcal{N}_4$, vectors ν and $\lambda \in \mathbb{R}^5_{>0}$ can be determined independent of $\mu \in \mathbb{R}^8$. Further note that

$$\ln\frac{\nu_1+\nu_5}{\lambda_1+\lambda_5} = \ln\frac{\nu_3+\nu_5}{\lambda_3+\lambda_5} = \ln\frac{\nu_4+\nu_5}{\lambda_4+\lambda_5} = \ln\frac{\nu_5}{\lambda_5}.$$

That is, all terms on the right hand side of (4.8a) – (4.8k) equal

$$\kappa_1 := \ln\frac{\nu_5}{\lambda_5}. \tag{4.9}$$

Solving (4.8a) – (4.8k) for μ yields

$$\mu = M_{\mathcal{N}_5}\begin{pmatrix}\mu_2\\ \mu_8\\ \kappa_1\end{pmatrix} \tag{4.10a}$$

where

$$M_{\mathcal{N}_5} := \begin{bmatrix} 0 & -1 & 1 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 1 \\ 1 & 0 & 0 \\ -1 & 0 & 1 \\ 0 & 0 & 1 \\ 1 & 0 & 0 \\ 0 & 0 & 1 \end{bmatrix}. \tag{4.10b}$$

Again, any element of the linear subspace $\mathcal{M}_{\mathcal{N}_5} = [M_{\mathcal{N}_5}]$ can be interpreted as the difference of two steady states: for any $\mu \in \mathcal{M}_{\mathcal{N}_5}$, follows from Chapter 3 that $b = \operatorname{diag}(e^{\mu})\ a$, $a \in \mathbb{R}^9_{>0}$, free, is a steady state solution, if a is a steady state solution. Note that any vector $a \in \mathbb{R}^9_{>0}$ is a steady state solution, if the following $k \in \mathbb{R}^{11}_{>0}$:

$$k_1 = \frac{\lambda_1 + \lambda_5}{a_1\, a_2} \qquad k_2 = \frac{\lambda_1}{a_3} \tag{4.11a}$$

$$k_3 = \frac{\lambda_2 + \lambda_5}{a_3} \qquad k_4 = \frac{\lambda_2}{a_4} \tag{4.11b}$$

$$k_5 = \frac{\lambda_5}{a_4} \qquad k_6 = \frac{\lambda_3 + \lambda_5}{a_5\, a_6} \tag{4.11c}$$

$$k_7 = \frac{\lambda_3}{a_7} \qquad k_8 = \frac{\lambda_5}{a_7} \tag{4.11d}$$

$$k_9 = \frac{\lambda_4 + \lambda_5}{a_6\, a_8} \qquad k_{10} = \frac{\lambda_4}{a_9} \tag{4.11e}$$

$$k_{11} = \frac{\lambda_5}{a_9}. \tag{4.11f}$$

4.2.3 Positive solutions for $\mathcal{N}_6$

For this network the equation

$$Y^{(L)^T}\, \mu = \ln \frac{E\, \nu}{E\, \lambda}$$

reads

$$\mu_1 + \mu_2 = \ln \frac{\nu_1 + \nu_5}{\lambda_1 + \lambda_5} \tag{4.12a}$$

$$\mu_3 = \ln \frac{\nu_1}{\lambda_1} \tag{4.12b}$$

$$\mu_3 = \ln \frac{\nu_2 + \nu_5}{\lambda_2 + \lambda_5} \tag{4.12c}$$

$$\mu_4 = \ln \frac{\nu_2}{\lambda_2} \tag{4.12d}$$

$$\mu_4 = \ln \frac{\nu_5}{\lambda_5} \tag{4.12e}$$

$$\mu_5 + \mu_6 = \ln \frac{\nu_3 + \nu_5}{\lambda_3 + \lambda_5} \tag{4.12f}$$

$$\mu_7 = \ln \frac{\nu_3}{\lambda_3} \tag{4.12g}$$

$$\mu_7 = \ln \frac{\nu_4 + \nu_5}{\lambda_4 + \lambda_5} \tag{4.12h}$$

$$\mu_8 = \ln \frac{\nu_4}{\lambda_4} \tag{4.12i}$$

$$\mu_8 = \ln \frac{\nu_5}{\lambda_5} \tag{4.12j}$$

A solution exists, if and only if

$$\ln\frac{\nu_1}{\lambda_1}=\ln\frac{\nu_2+\nu_5}{\lambda_2+\lambda_5},\qquad \ln\frac{\nu_2}{\lambda_2}=\ln\frac{\nu_5}{\lambda_5},\qquad \ln\frac{\nu_3}{\lambda_3}=\frac{\nu_4+\nu_5}{\lambda_4+\lambda_5},\qquad \ln\frac{\nu_4}{\lambda_4}=\ln\frac{\nu_5}{\lambda_5}.$$

One immediately obtains $\nu_2=\lambda_2\,\frac{\nu_5}{\lambda_5}$ and $\nu_4=\lambda_4\,\frac{\nu_5}{\lambda_5}$. Note that this implies

$$\ln\frac{\nu_2+\nu_5}{\lambda_2+\lambda_5}=\ln\frac{\nu_4+\nu_5}{\lambda_4+\lambda_5}=\ln\frac{\nu_5}{\lambda_5},$$

which in turn implies $\ln\frac{\nu_1}{\lambda_1}=\ln\frac{\nu_3}{\lambda_3}=\ln\frac{\nu_5}{\lambda_5}$. Thus one obtains $\nu_1=\lambda_1\,\frac{\nu_5}{\lambda_5}$, $\nu_3=\lambda_3\,\frac{\nu_5}{\lambda_5}$ and thus

$$\nu=\frac{\nu_5}{\lambda_5}\,\lambda,$$

$\lambda\in\mathbb{R}^5_{>0}$ and $\nu_5>0$, free. That is, one can obtain ν, $\lambda\in\mathbb{R}^5_{>0}$ independent of $\mu\in\mathbb{R}^9$. Note that all terms on the right hand side of (4.12a) – (4.12j) are equal to

$$\kappa_1:=\ln\frac{\nu_5}{\lambda_5}. \tag{4.13}$$

Solving (4.12a) – (4.12j) for μ yields:

$$\mu=M_{\mathcal{N}_6}\begin{pmatrix}\mu_2\\ \mu_6\\ \kappa_1\end{pmatrix} \tag{4.14a}$$

where

$$M_{\mathcal{N}_6}:=\begin{bmatrix}-1&0&1\\1&0&0\\0&0&1\\0&0&1\\0&-1&1\\0&1&0\\0&0&1\\0&0&1\end{bmatrix} \tag{4.14b}$$

As in the previous sections, any element of the linear subspace $\mathcal{M}_{\mathcal{N}_6}=[M_{\mathcal{N}_6}]$ can be interpreted as the difference of two steady states: for any $\mu\in\mathcal{M}_{\mathcal{N}_6}$, follows from Chapter 3 that $b=\operatorname{diag}(e^\mu)\,a$, $a\in\mathbb{R}^8_{>0}$, free, is a steady state solution, if a is a steady state solution. Note that any vector $a\in\mathbb{R}^8_{>0}$ is a steady state solution, if the following $k\in\mathbb{R}^{10}_{>0}$:

$$k_1=\frac{\lambda_1+\lambda_5}{a_1\,a_2}\qquad k_2=\frac{\lambda_1}{a_3} \tag{4.15a}$$

$$k_3=\frac{\lambda_2+\lambda_5}{a_3}\qquad k_4=\frac{\lambda_2}{a_4} \tag{4.15b}$$

$$k_5=\frac{\lambda_5}{a_4}\qquad k_6=\frac{\lambda_3+\lambda_5}{a_5\,a_6} \tag{4.15c}$$

$$k_7=\frac{\lambda_3}{a_7}\qquad k_8=\frac{\lambda_4+\lambda_5}{a_7} \tag{4.15d}$$

$$k_9=\frac{\lambda_4}{a_8}\qquad k_{10}=\frac{\lambda_5}{a_8} \tag{4.15e}$$

4.3 Positive solutions satisfying the conservation relations

Recall that for networks $\mathcal{N}_4$, $\mathcal{N}_5$ and $\mathcal{N}_6$ it is possible to determine ν and λ independent of μ. Thus Algorithm 2 can be applied to decide about multistationarity. As a consequence of Theorem 3, the existence of an orthant δ with $\mathcal{S}_i\cap\mathbb{R}^n_\delta\neq\emptyset$ and $\mathcal{M}_i\cap\mathbb{R}^n_\delta\neq\emptyset$ (i.e. with $\mathcal{T}\neq\emptyset$, $\mathcal{T}$ as defined in (3.32)) is necessary and sufficient for multistationarity, $i=\mathcal{N}_4,\mathcal{N}_5,\mathcal{N}_6$. For network $\mathcal{N}_4$ 14 orthants were found with $\mathcal{S}_{\mathcal{N}_4}\cap\mathbb{R}^n_\delta\neq\emptyset$ and $\mathcal{M}_{\mathcal{N}_4}\cap\mathbb{R}^n_\delta\neq\emptyset$, while for network $\mathcal{N}_5$ and $\mathcal{N}_6$ no orthants were found. This leads to the following conclusion:

Fact 5. *Only network $\mathcal{N}_4$ can admit multistationarity. That is, in particular, for $\mathcal{N}_5$ and $\mathcal{N}_6$ no parameter vector exists, such that the corresponding ODEs exhibit multistationarity.*

Using the algorithms presented in Appendix D all orthants with $\mathcal{S}_{\mathcal{N}_4} \cap \mathbb{R}^9_\delta \neq \emptyset$ and $\mathcal{M}_{\mathcal{N}_4} \cap \mathbb{R}^9_\delta \neq \emptyset$ have been determined:

$$\begin{array}{lllllllll}
\delta_1=(-1, & -1, & -1, & 1, & 1, & 1, & -1, & 1, & -1)^T \\
\delta_2=(-1, & 0, & -1, & 1, & 1, & 1, & -1, & 1, & -1)^T \\
\delta_3=(-1, & 1, & -1, & -1, & 1, & 1, & -1, & 1, & -1)^T \\
\delta_4=(-1, & 1, & -1, & -1, & 1, & 1, & 0, & 1, & -1)^T \\
\delta_5=(-1, & 1, & -1, & -1, & 1, & 1, & 1, & 1, & -1)^T \\
\delta_6=(-1, & 1, & -1, & 0, & 1, & 1, & -1, & 1, & -1)^T \\
\delta_7=(-1, & 1, & -1, & 1, & 1, & 1, & -1, & 1, & -1)^T
\end{array} \tag{4.16}$$

Recall that if $\mathcal{S}_{\mathcal{N}_4} \cap \mathbb{R}^9_\delta \neq \emptyset$ and $\mathcal{M}_{\mathcal{N}_4} \cap \mathbb{R}^9_\delta \neq \emptyset$, then this holds for $-\delta$ as well; thus there are in fact 14 orthants where $S_{\mathcal{N}_4}$ and $\mathcal{M}_{\mathcal{N}_4}$ are sign compatible.

4.3.1 Parameterizing Multistationarity for $\mathcal{N}_4$

As $\mathcal{M}_{\mathcal{N}_4}$ is a linear subspace of $\mathbb{R}^9$, (3.34a) and (3.34b) can be used to represent $\mu \in \mathcal{M}_{\mathcal{N}_4}$ and $v \in \mathcal{S}_{\mathcal{N}_4}$ in the orthants given in (4.16). Thus vectors μ^{δ_i} and v^{δ_i} have been determined using generators of $\mu \cap \mathbb{R}^9_{\delta_i}$ and $S \cap \mathbb{R}^9_{\delta_i}$ for each orthant $\mathbb{R}^9_{\delta_i}$ given by (4.16). Table 4.1 contains the μ^{δ_i}, $i = 1, \ldots, 7$, Table 4.2 the v^{δ_i}, $i = 1, \ldots, 7$. Using this 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 Table 4.1 and 4.2 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 (A.1a) – (A.1i) and the conservation relations (A.2a) – (A.2c) given in Appendix A.1.2 admit a^{δ_i} and b^{δ_i} as steady state solutions.

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 4.1 the cone $\mathcal{M}_{\mathcal{N}_4} \cap \mathbb{R}^9_{\delta_5}$ can be parametrized as $\mu^{\delta_5} = \big(-\alpha_1 - 2\,\alpha_2 - 2\,\alpha_3, \alpha_1 + 2\alpha_2 + \alpha_3, -\alpha_3, -\alpha_2 - \alpha_3, \alpha_1 + \alpha_2, \alpha_1, \alpha_2, \alpha_1 + \alpha_2, -\alpha_3\big)^T$. From Table 4.2 follows that $v \in \mathcal{S}_{\mathcal{N}_4} \cap \mathbb{R}^9_{\delta_5}$ can be parametrized as $v^{\delta_5} = \big(-\beta_1, \beta_2, -\beta_2 - \beta_4, -\beta_3, \beta_4, \beta_1 + \beta_2 + \beta_3 + \beta_5, \beta_5, \beta_6, -\beta_5 - \beta_6\big)^T$. Inserting μ^{δ_5} in (3.33a) yields a parametrization of a^{δ_5} in terms of α_1, α_2, α_3 and $\beta_1, \ldots, \beta_6$, $\alpha_i > 0$, $\beta_i > 0$:

$$\begin{aligned}
a^{\delta_5} = \big(& -\frac{\beta_1}{-1 + e^{-\alpha_1 - 2\,\alpha_2 - 2\,\alpha_3}}, \frac{\beta_2}{-1 + e^{\alpha_1 + 2\,\alpha_2 + \alpha_3}}, \frac{-\beta_2 - \beta_4}{-1 + e^{-\alpha_3}} \\
& -\frac{\beta_3}{-1 + e^{-\alpha_2 - \alpha_3}}, \frac{\beta_4}{-1 + e^{\alpha_1 + \alpha_2}}, \frac{\beta_1 + \beta_2 + \beta_3 + \beta_5}{-1 + e^{\alpha_1}} \\
& \frac{\beta_5}{-1 + e^{\alpha_2}}, \frac{\beta_6}{-1 + e^{\alpha_1 + \alpha_2}}, \frac{-\beta_5 - \beta_6}{-1 + e^{-\alpha_3}}\big)^T
\end{aligned} \tag{4.17a}$$

Using (4.17a) and μ^{δ_5} in (3.33b) yields the following parametrization of b^{δ_5}:

$$\begin{aligned}
b^{\delta_5} = \Big(& -\frac{\beta_1\, e^{-\alpha_1 - 2\,\alpha_2 - 2\,\alpha_3}}{-1 + e^{-\alpha_1 - 2\,\alpha_2 - 2\,\alpha_3}}, \frac{\beta_2\, e^{\alpha_1 + 2\,\alpha_2 + \alpha_3}}{-1 + e^{\alpha_1 + 2\,\alpha_2 + \alpha_3}}, \frac{(-\beta_2 - \beta_4)\, e^{-\alpha_3}}{-1 + e^{-\alpha_3}} \\
& -\frac{\beta_3\, e^{-\alpha_2 - \alpha_3}}{-1 + e^{-\alpha_2 - \alpha_3}}, \frac{\beta_4\, e^{\alpha_1 + \alpha_2}}{-1 + e^{\alpha_1 + \alpha_2}}, \frac{(\beta_1 + \beta_2 + \beta_3 + \beta_5)\, e^{\alpha_1}}{-1 + e^{\alpha_1}} \\
& \frac{\beta_5\, e^{\alpha_2}}{-1 + e^{\alpha_2}}, \frac{\beta_6\, e^{\alpha_1 + \alpha_2}}{-1 + e^{\alpha_1 + \alpha_2}}, \frac{(-\beta_5 - \beta_6)\, e^{-\alpha_3}}{-1 + e^{-\alpha_3}}\Big)^T
\end{aligned} \tag{4.17b}$$

Using (4.17a) in (3.33c) yields a parametrization of $k_1^{\delta_5}, \ldots, k_{12}^{\delta_5}$ in terms of α_1, α_2, α_3, $\beta_1, \ldots, \beta_6$,

$\alpha_i \geq 0$, $\beta_i \geq 0$ and $\lambda_1, \ldots, \lambda_6$, $\lambda_i > 0$:

$$k_1^{\delta_5} = -\frac{(-1+e^{-\alpha_1-2\,\alpha_2-2\,\alpha_3})\,(-1+e^{\alpha_1+2\,\alpha_2+\alpha_3})\,(\lambda_1+\lambda_5)}{\beta_1\,\beta_2} \tag{4.18a}$$

$$k_2^{\delta_5} = \frac{(-1+e^{-\alpha_3})\,\lambda_1}{-\beta_2-\beta_4} \tag{4.18b}$$

$$k_3^{\delta_5} = \frac{(-1+e^{-\alpha_3})\,\lambda_5}{-\beta_2-\beta_4} \tag{4.18c}$$

$$k_4^{\delta_5} = -\frac{(-1+e^{-\alpha_2-\alpha_3})\,(-1+e^{\alpha_1+2\,\alpha_2+\alpha_3})\,(\lambda_2+\lambda_6)}{\beta_2\,\beta_3} \tag{4.18d}$$

$$k_5^{\delta_5} = \frac{(-1+e^{\alpha_1+\alpha_2})\,\lambda_2}{\beta_4} \tag{4.18e}$$

$$k_6^{\delta_5} = \frac{(-1+e^{\alpha_1+\alpha_2})\,\lambda_6}{\beta_4} \tag{4.18f}$$

$$k_7^{\delta_5} = \frac{(-1+e^{\alpha_1})\,(-1+e^{\alpha_2})\,(\lambda_3+\lambda_6)}{\beta_5\,(\beta_1+\beta_2+\beta_3+\beta_5)} \tag{4.18g}$$

$$k_8^{\delta_5} = \frac{(-1+e^{\alpha_1+\alpha_2})\,\lambda_3}{\beta_6} \tag{4.18h}$$

$$k_9^{\delta_5} = \frac{(-1+e^{\alpha_1+\alpha_2})\,\lambda_6}{\beta_6} \tag{4.18i}$$

$$k_{10}^{\delta_5} = -\frac{(-1+e^{\alpha_2})\,(-1+e^{-\alpha_2-\alpha_3})\,(\lambda_4+\lambda_5)}{\beta_3\,\beta_5} \tag{4.18j}$$

$$k_{11}^{\delta_5} = \frac{(-1+e^{-\alpha_3})\,\lambda_4}{-\beta_5-\beta_6} \tag{4.18k}$$

$$k_{12}^{\delta_5} = \frac{(-1+e^{-\alpha_3})\,\lambda_5}{-\beta_5-\beta_6} \tag{4.18l}$$

$\mathbb{R}^9_{\delta_i}$	$p_{\mu^{\delta_i}}$	Representation of $\mu^{\delta_i} \in \mathcal{M}_{\mathcal{N}_4} \cap \mathbb{R}^9_{\delta_i}$
$\mathbb{R}^9_{\delta_1}$	3	$\mu^{\delta_1} = \big(-\alpha_1, -\alpha_2, -\alpha_1-\alpha_2, \alpha_2+\alpha_3, \alpha_3, \alpha_1+2\alpha_2+2\alpha_3, -\alpha_1-2\alpha_2-\alpha_3, \alpha_3, -\alpha_1-\alpha_2\big)^T$
$\mathbb{R}^9_{\delta_2}$	3	$\mu^{\delta_2} = \big(-\alpha_3, 0, -\alpha_3, \alpha_2, \alpha_2, 2\alpha_2+\alpha_3, -\alpha_2-\alpha_3, \alpha_2, -\alpha_3\big)^T$
$\mathbb{R}^9_{\delta_3}$	3	$\mu^{\delta_3} = \big(-2\alpha_1-\alpha_2-\alpha_3, \alpha_1+\alpha_2, -\alpha_1-\alpha_3, -\alpha_1, \alpha_2, \alpha_2+\alpha_3, -\alpha_3, \alpha_2, -\alpha_1-\alpha_3\big)^T$
$\mathbb{R}^9_{\delta_4}$	3	$\mu^{\delta_4} = \big(-\alpha_1-2\alpha_3, \alpha_1+\alpha_3, -\alpha_3, -\alpha_3, \alpha_1, \alpha_1, 0, \alpha_1, -\alpha_3\big)^T$
$\mathbb{R}^9_{\delta_5}$	3	$\mu^{\delta_5} = \big(-\alpha_1-2\alpha_2-2\alpha_3, \alpha_1+2\alpha_2+\alpha_3, -\alpha_3, -\alpha_2-\alpha_3, \alpha_1+\alpha_2, \alpha_1, \alpha_2, \alpha_1+\alpha_2, -\alpha_3\big)^T$
$\mathbb{R}^9_{\delta_6}$	3	$\mu^{\delta_6} = \big(-\alpha_1-\alpha_3, \alpha_1, -\alpha_3, 0, \alpha_1, \alpha_1+\alpha_3, -\alpha_3, \alpha_1, -\alpha_3\big)^T$
$\mathbb{R}^9_{\delta_7}$	3	$\mu^{\delta_7} = \big(-\alpha_1-\alpha_3, \alpha_1, -\alpha_3, \alpha_2, \alpha_1+\alpha_2, \alpha_1+2\alpha_2+\alpha_3, -\alpha_2-\alpha_3, \alpha_1+\alpha_2, -\alpha_3\big)^T$

Table 4.1: Representations of $\mu^{\delta_i} \in \mathcal{M}_{\mathcal{N}_4}$ in the orthants $\mathbb{R}^9_{\delta_i}$ that contain both, $\mathcal{M}_{\mathcal{N}_4}$ and $\mathcal{S}_{\mathcal{N}_4}$ ($i = 1, \ldots, 7$, as defined in (4.16)).

4.4 An extension: open systems

Common to network $\mathcal{N}_4$ – $\mathcal{N}_6$ is that all concentrations are subject to at least one conservation relation. But in reality, none of these reaction networks can be observed in isolation, as $\mathcal{N}_4$ – $\mathcal{N}_6$ each describe a single layer of a signal transduction network that consists of several similar layers and E_1 and E_2 are subject to regulation from different layers. Thus, the assumption that the total concentration of E_1 and E_2 is constant, is debatable and the question, whether or not conservation of moiety with respect to enzymes E_1 and E_2 is necessary for multistationarity is natural – especially, if the focus is on model discrimination. (If conservation of moiety with respect to enzymes E_1 and E_2 is necessary for multistationarity, this phenomenon might be hard to observe experimentally).

$\mathbb{R}^9_{\delta_i}$	$p_{S^{\delta_i}}$	Representation of $v^{\delta_i} \in S_{\mathcal{N}_4} \cap \mathbb{R}^9_{\delta_i}$
$\mathbb{R}^9_{\delta_1}$	6	$v^{\delta_1} = \big(-\beta_1-\beta_3-\beta_4-\beta_5, -\beta_1, -\beta_2, \beta_3, \beta_1+\beta_2, \beta_4, -\beta_5, \beta_5+\beta_6, -\beta_6\big)^T$
$\mathbb{R}^9_{\delta_2}$	6	$v^{\delta_2} = \big(-\beta_3-\beta_4-\beta_5, 0, -\beta_2, \beta_3, \beta_2, \beta_4, -\beta_5, \beta_5+\beta_6, -\beta_6\big)^T$
$\mathbb{R}^9_{\delta_3}$	8	$v^{\delta_3} = \big(-\beta_3-\beta_6, \beta_4+\beta_7, -\beta_1-\beta_4-\beta_7, -\beta_5-\beta_8, \beta_1, \beta_6+\beta_7+\beta_8, -\beta_3-\beta_4-\beta_5, \beta_2+\beta_3+\beta_4+\beta_5, -\beta_2\big)^T$
$\mathbb{R}^9_{\delta_4}$	6	$v^{\delta_4} = \big(-\beta_1, \beta_2, -\beta_2-\beta_4, -\beta_3, \beta_4, \beta_1+\beta_2+\beta_3, 0, \beta_6, -\beta_6\big)^T$
$\mathbb{R}^9_{\delta_5}$	6	$v^{\delta_5} = \big(-\beta_1, \beta_2, -\beta_2-\beta_4, -\beta_3, \beta_4, \beta_1+\beta_2+\beta_3+\beta_5, \beta_5, \beta_6, -\beta_5-\beta_6\big)^T$
$\mathbb{R}^9_{\delta_6}$	7	$v^{\delta_6} = \big(-\beta_4-\beta_6, \beta_5+\beta_7, -\beta_2-\beta_5-\beta_7, 0, \beta_2, \beta_6+\beta_7, -\beta_4-\beta_5, \beta_3+\beta_4+\beta_5, -\beta_3\big)^T$
$\mathbb{R}^9_{\delta_7}$	8	$v^{\delta_7} = \big(-\beta_3-\beta_5-\beta_6, \beta_4+\beta_7+\beta_8, -\beta_1-\beta_4-\beta_7-\beta_8, \beta_5+\beta_7, \beta_1, \beta_6+\beta_8, -\beta_3-\beta_4, \beta_2+\beta_3+\beta_4, -\beta_2\big)^T$

Table 4.2: Representations of $v^{\delta_i} \in S_{\mathcal{N}_4} \cap \mathbb{R}^9_{\delta_i}$ in orthants $\mathbb{R}^9_{\delta_i}$ that contain both, $\mathcal{M}_{\mathcal{N}_4}$ and $\mathcal{S}_{\mathcal{N}_4}$ ($i = 1, \ldots, 7$, as defined in (4.16)).

To tackle this question the assumption that the system is open with respect to total concentrations of E_1 and/or E_2 is considered, by introducing 0, the 'zero'–complex In this Section the same strategy as in Section 4.2 and 4.3 is used to establish multistationarity.
The following networks are analysed, where

$$0 \underset{\kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} E_1, \tag{$\mathcal{N}_7$}$$

for example, incorporates the fact that E_1 is fed into the system with a constant rate κ_1 and is removed from the system with a rate that is proportional to its concentration (κ_2 being the rate constant). Thus, in this case, the 'zero'–complex represents the effect of the other layers of the signal transduction network on E_1. In a similar way

$$0 \underset{\kappa_4}{\overset{\kappa_3}{\rightleftharpoons}} E_2, \tag{$\mathcal{N}_8$}$$

and

$$E_1 \underset{\kappa_1}{\overset{\kappa_2}{\rightleftharpoons}} 0 \underset{\kappa_4}{\overset{\kappa_3}{\rightleftharpoons}} E_2 \tag{$\mathcal{N}_9$}$$

cover the fact that either E_2 alone or E_1 and E_2 together are introduced to the system. In the remainder of this Section the nine networks that can be composed by combining one network of $\mathcal{N}_4$ – $\mathcal{N}_6$ with one network of $\mathcal{N}_7$ – $\mathcal{N}_9$ will be analysed with respect to multistationarity.
Adding one of the networks $\mathcal{N}_7$ – $\mathcal{N}_9$ to one of the networks $\mathcal{N}_4$ – $\mathcal{N}_6$ will affect the number of conserved moieties in the combined network: in the case of network $\mathcal{N}_7$ or $\mathcal{N}_8$ only two conserved moieties exist: total concentration A_{tot} of A and E_{1tot} of E_1 for combinations with $\mathcal{N}_8$ and A_{tot} and E_{2tot}, the total concentration of E_2, for combinations with $\mathcal{N}_7$. For all combinations involving $\mathcal{N}_9$, only one conserved moiety remains, A_{tot}. Using the same steps as in Section 4.2, the equation $Y^{(L)^T}\mu = \ln \frac{E\,\nu}{E\,\lambda}$ was analysed for each of the nine networks. As in Chapter 3, let p be the number of generators of $\ker(Y\,I_a) \cap \mathbb{R}^r_{\geq 0}$. As in Section 4.2, ν and $\lambda \in \mathbb{R}^p_{>0}$ can be determined independent of $\mu \in \mathbb{R}^n$ and $\mathcal{M}$, as defined in (3.31) is a linear subspace of $\mathbb{R}^n$, for each network. Table 4.3 contains for each network a matrix M whose image is $\mathcal{M}$ (i.e. $\mathcal{M} = [M]$). If multistationarity is possible, Table 4.3 also contains the orthant where $\mathcal{M} \cap \mathbb{R}^n_\delta \neq \emptyset$ and $\mathcal{S}_i \cap \mathbb{R}^n_\delta \neq \emptyset$. In Appendix A.4 the equations $Y^{(L)^T}\mu = \ln \frac{E\,\nu}{E\,\lambda}$ are solved for each network in Table 4.3.
Not very surprisingly, for all networks involving $\mathcal{N}_5$ and $\mathcal{N}_6$ multistationarity is excluded and for networks $\mathcal{N}_4$+$\mathcal{N}_7$ and $\mathcal{N}_4$+$\mathcal{N}_8$ multistationarity is possible. Interestingly for network $\mathcal{N}_4$+$\mathcal{N}_9$ multistationarity is **impossible**, even though the difference to $\mathcal{N}_4 + \mathcal{N}_7$ and $\mathcal{N}_4 + \mathcal{N}_8$ seems very small. This result is obvious, however, if $\mathcal{M}_{\mathcal{N}_4+\mathcal{N}_9}$ and $S_{\mathcal{N}_4+\mathcal{N}_9}$ are analysed: $S_{\mathcal{N}_4+\mathcal{N}_9}$ is defined by

$$W^T_{\mathcal{N}_4+\mathcal{N}_9}\, x = 0 \tag{4.19a}$$

with

$$W_{\mathcal{N}_4+\mathcal{N}_9} = (\,1,\,0,\,1,\,1,\,1,\,1,\,0,\,1,\,1\,)^T. \tag{4.19b}$$

Network	M_i with $[M_i] = \mathcal{M}_i$	Sign compatible orthants δ	multistationarity
$\mathcal{N}_4 + \mathcal{N}_7$	$M^T = \begin{bmatrix} 1,0,1,0,0,-1,1,0,1 \\ 0,0,0,1,1,2,-1,1,0 \end{bmatrix}$	$\delta^T = [\ -1,0,-1,1,1,1,-1,1,-1\ ]$	Yes
$\mathcal{N}_4 + \mathcal{N}_8$	$M^T = \begin{bmatrix} 2,-1,1,1,0,0,0,0,1 \\ -1,1,0,0,1,1,0,1,0 \end{bmatrix}$	$\delta^T = [\ -1,1,-1,-1,1,1,0,1,-1\ ]$	Yes
$\mathcal{N}_4$+$\mathcal{N}_9$	$M^T = [\ 1,0,1,1,1,1,0,1,1\ ]$	$\delta = \emptyset$	No
$\mathcal{N}_5 + \mathcal{N}_7$	$M^T = \begin{bmatrix} 0,0,0,0,1,-1,0,1,0 \\ 1,0,1,1,0,1,1,0,1 \end{bmatrix}$	$\delta = \emptyset$	No
$\mathcal{N}_5$+$\mathcal{N}_8$	$M^T = \begin{bmatrix} -1,1,0,0,0,0,0,0,0 \\ 1,0,1,1,1,0,1,1,1 \end{bmatrix}$	$\delta = \emptyset$	No
$\mathcal{N}_5$+$\mathcal{N}_9$	$M^T = [\ 1,0,1,1,1,0,1,1,1\ ]$	$\delta = \emptyset$	No
$\mathcal{N}_6$+$\mathcal{N}_7$	$M^T = \begin{bmatrix} 0,0,0,0,-1,1,0,0 \\ 1,0,1,1,1,0,1,1 \end{bmatrix}$	$\delta = \emptyset$	No
$\mathcal{N}_6$+$\mathcal{N}_8$	$M^T = \begin{bmatrix} -1,1,0,0,0,0,0,0 \\ 1,0,1,1,1,0,1,1 \end{bmatrix}$	$\delta = \emptyset$	No
$\mathcal{N}_6$+$\mathcal{N}_9$	$M^T = [\ 1,0,1,1,1,0,1,1\ ]$	$\delta = \emptyset$	No

Table 4.3: Solutions for all combinations of $\mathcal{N}_4$, $\mathcal{N}_5$ and $\mathcal{N}_6$ with $\mathcal{N}_7$, $\mathcal{N}_8$ and $\mathcal{N}_9$.

From Table 4.3 it follows that $[W_{\mathcal{N}_4+\mathcal{N}_9}] = \mathcal{M}_{\mathcal{N}_4+\mathcal{N}_9}$ and thus $\mathcal{M}_{\mathcal{N}_4+\mathcal{N}_9} \perp S_{\mathcal{N}_4+\mathcal{N}_9}$. Thus $\mathcal{M}_{\mathcal{N}_4+\mathcal{N}_9}$ and $S_{\mathcal{N}_4+\mathcal{N}_9}$ **cannot** be sign compatible: recall that $\mathcal{M}_{\mathcal{N}_4+\mathcal{N}_9}$ and $S_{\mathcal{N}_4+\mathcal{N}_9}$ are sign compatible, if and only if $\exists \mu \in \mathcal{M}_{\mathcal{N}_4+\mathcal{N}_9}$ and $\exists v \in S_{\mathcal{N}_4+\mathcal{N}_9}$ with $\operatorname{sign}(\mu) = \operatorname{sign}(v)$. In this case $\langle \mu, v \rangle \geq 0$ must hold (and equality holds iff $\mu = 0$ and/or $v = 0$). Thus, as $\langle \mu, v \rangle = 0$, for all $\mu \in \mathcal{M}_{\mathcal{N}_4+\mathcal{N}_9}$, $v \in S_{\mathcal{N}_4+\mathcal{N}_9}$ and μ, $v \neq 0$, μ and v cannot be sign compatible, and multistationarity is excluded by Corollary 2.

4.5 Model discrimination using steady state information

Up to this point it has been established that only network $\mathcal{N}_4$ can exhibit multistationarity. In this section a method to use this result for model discrimination is discussed, based on Theorem 3. Theorem 3 can on the one hand be used to discard $\mathcal{N}_5$ and $\mathcal{N}_6$, while on the other hand it can be used to falsify $\mathcal{N}_4$, given the necessary experimental data. However, the results obtained in Section 4.4 can explain, why this data might be hard to obtain.

Using Theorem 3 for model discrimination Already [45] have argued that the fact that among $\mathcal{N}_4, \mathcal{N}_5, \mathcal{N}_6$ only $\mathcal{N}_4$ can exhibit multistationarity can be used for model discrimination: their suggestion and a consequence of Section 4.3 is that if multistationarity was found experimentally for the activation of an MAPK(K), the mechanism involved has to be the one represented by $\mathcal{N}_4$ (as the other hypotheses **cannot** give rise to multistationarity). However, [45] use numerical tools to show multistationarity for $\mathcal{N}_4$ and fail to show multistationarity for $\mathcal{N}_5$ and $\mathcal{N}_6$ using numerical tools as well. Thus their conclusion is only valid because of the results obtained in Theorem 3: as for $\mathcal{N}_5$ and $\mathcal{N}_6$ no orthant with $\mathcal{M}_j \cap \mathbb{R}^n_\delta \neq \emptyset$ and $S_j \cap \mathbb{R}^n_\delta \neq \emptyset$, $j = \mathcal{N}_5, \mathcal{N}_6$ exists, it is guaranteed that multistationarity can be excluded for these networks **for any conceivable parameter vector**. Using numerical tools alone, this result cannot be obtained.
But even if multistationarity is verified experimentally, it is still possible to falsify $\mathcal{N}_4$ using Theorem 3: to this end let p and q be two positive steady states for $\mathcal{N}_4$, with $W^T_{\mathcal{N}_4}\, p = W^T_{\mathcal{N}_4}\, q$. From Section 4.2.1 follows that the vector μ defined by p and q as $\mu_i = \ln \frac{q_i}{p_i}$, $i = 1, \ldots, 9$ is an element of the linear subspace $\mathcal{M}_{\mathcal{N}_4}$. From Theorem 3 follows that there exists a vector $v \in \mathcal{S}_{\mathcal{N}_4}$ with $\operatorname{sign}(v) = \operatorname{sign}(\mu)$.
Now suppose two steady states a and b have been obtained experimentally for $\mathcal{N}_4$ **with** $W^T_{\mathcal{N}_4}\, a = W^T_{\mathcal{N}_4}\, b$. Then, as mentioned in [45] and discussed in [11, 12] neither phosphorylation nor dephosphorylation nor both can be realized using a processive mechanism. However, the conclusion that in this case a distributive mechanism is used for both, phosphorylation and dephosphorylation, as suggested in the aforementioned references, is only valid if there exists a vector $v \in \mathcal{S}_{\mathcal{N}_4}$ with $\operatorname{sign}(v) = \operatorname{sign}(\mu)$ for μ defined as $\mu_i = \ln \frac{b_i}{a_i}$, $i = 1, \ldots, 9$. If this was not the case, $\mathcal{N}_4$ would not be compatible with experimental data and could thus also be discarded. A similar argument has been used to discard hypotheses regarding catalytic mechanisms in [21, 22, 23].

Consequences of Section 4.4 Section 4.4 implies that multistationarity might be hard to observe experimentally: for multistationarity to occur, total concentration of either kinase E_1 or phosphatase E_2 has to be constant, that is the system must be closed either with respect to the kinase or the phosphatase.
Classically, signal transduction has been considered as a process driven by kinases, while phosphatases just act as passive, housekeeping enzymes and most mathematical models are set up accordingly. According to this paradigm total concentration of E_2 is constant and multistationarity is thus possible (if the mechanism involved is the one described by $\mathcal{N}_4$). However, recent studies indicate that phosphatases play a major role, and that they are tightly and actively regulated [2, 49]. This implies that the system has to be open with respect to E_2 as well, thus challenging the possibility of the existence of multistationarity.

Chapter 5

Multistationarity in cell cycle regulation

In this chapter two network hypotheses regarding control of the transition from G1 to S phase in the cell cycle of *Saccharomyces cerevisiae* are analysed. The key components and their proposed interaction are displayed in Fig. 5.1. In a very simple form, the biochemical network consists of two regulators that

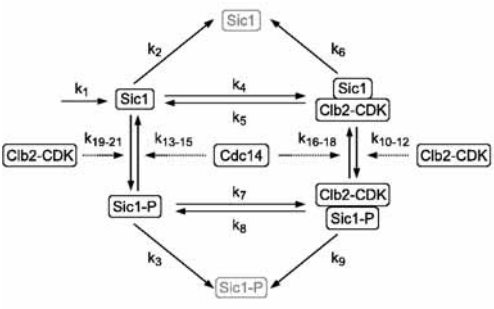


Figure 5.1: Network structure for the G1/S model. Arrows indicate biochemical reactions between cell cycle regulators (boxes). Solid lines represent elementary reactions and dotted lines catalyzed reactions (composite reactions). Kinetic parameters k_i are displayed next to the arrows. Free Clb2-CDK complexes have been omitted. Components in gray denote degradation products. An unspecified external signal controls the activity of Cdc14p phosphatase. Clb2-CDK phosphorylates the inhibitor Sic1p either in its unbound form (left, k_{19-21}), or when it is bound to a second molecule of the kinase complex (right, k_{10-12}).

mutually inhibit each other. Cyclin-dependent kinase (CDK), when associated with the mitotic cyclin Clb2p, promotes entry into mitosis through phosphorylation of its target proteins. Simultaneously, this activity prevents the exit from mitosis and subsequent passage to the G1 phase of the cell cycle. The competitive CDK inhibitor Sic1p is one component responsible for inactivating Clb2-CDK at the end of mitosis. Mitotic kinase activity, however, can phosphorylate Sic1p, thus targeting the inhibitor for rapid, proteasome-dependent degradation. The transition to a G1 state with high Sic1p concentration and low Clb2-CDK activity therefore requires activation of the phosphatase Cdc14p and concomitant stabilization of Sic1p [46].
Importantly, the transition between the cell cycle phases requires only transient Cdc14 activity as an input (trigger) signal for the bi-stable switch. Hysteresis, which means that at least two stable steady-state output signals of the system exist for an identical input signal, underlies many cellular switches [63]. It depends on the system's history, whether, for instance, low or high Sic1p concentration will be established. For the G1/S system, a transient activation of the phosphatase Cdc14p should move

the system from the mitotic branch of low Sic1p concentration to the upper branch representing a G1 state with high Sic1p abundance and, consequently, low mitotic kinase activity. Any potentially valid network hypothesis has to represent this qualitative behavior, that is the ODEs must admit two stable positive steady states; one representing the G1- and one representing the S-phase. Thus the ODEs derived from any valid network hypothesis have to admit multistationarity for some conceivable parameter vector (recall that multistationarity in the sense of this work requires the existence of at least two positive steady states).

Here, two similar network structures are considered. In one alternative, Clb2-CDK phosphorylates free Sic1p (binary complex model, see network $\mathcal{N}_{10}$), whereas in the other alternative, the already bound inhibitor is a substrate for a second kinase molecule (ternary complex model, see network $\mathcal{N}_{11}$): that is, in network $\mathcal{N}_{10}$ phosphorylated $Sic1$ is produced by the reaction $Clb+Sic1 \leftrightharpoons Sic1\cdot Clb \to Sic1P+Clb$, while in $\mathcal{N}_{11}$ phosphorylated $Sic1$ is produced by the reactions $Clb\cdot Sic1 + Clb \leftrightharpoons Clb\cdot Sic1\cdot Clb \to Clb\cdot Sic1P + Clb$ and $Clb + Sic1P \leftrightharpoons Clb\cdot Sic1P$. (Note that even though $\mathcal{N}_{10}$ contains the reaction $Clb + Sic1P \leftrightharpoons Clb\cdot Sic1P$ as well, the source of $Sic1P$-production is the reaction $Sic1\cdot Clb \to Clb + Sic1P$.) Both alternatives are biologically plausible, yet hard to distinguish experimentally.

Each network has $n = 9$ species and $m = 17$ complexes in $r = 18$ reactions. Note that the zero-complex 0 is associated with a nine dimensional zero vector $y_1 = (0, \dots, 0)$. It incorporates that each system is open with respect to $Sic1$ and its phosphorylated form $Sic1P$: $Sic1$ can enter and leave the system, $Sic1P$ can leave the system (see Fig. 5.1).

(A) Binary complex model:

$$
\begin{array}{l}
[\mathbf{Sic1P}] \xrightarrow{\mathbf{k_3}} [\mathbf{0}] \underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} [\mathbf{Sic1}] \\[2ex]
[\mathbf{Sic1\cdot Clb}] \underset{\mathbf{k_5}}{\overset{\mathbf{k_4}}{\rightleftharpoons}} [\mathbf{Clb}] + [\mathbf{Sic1}] \underset{\mathbf{k_7}}{\overset{\mathbf{k_6}}{\rightleftharpoons}} [\mathbf{Clb\cdot Sic1}] \\
\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \searrow^{\mathbf{k_8}} \\
\qquad\qquad \swarrow^{\mathbf{k_9}} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad [\mathbf{Clb}] \\
\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \nearrow_{\mathbf{k_{12}}} \\
[\mathbf{Clb}] + [\mathbf{Sic1P}] \underset{\mathbf{k_{11}}}{\overset{\mathbf{k_{10}}}{\rightleftharpoons}} [\mathbf{Clb\cdot Sic1P}] \\[2ex]
[\mathbf{Sic1P}] + [\mathbf{Cdc14}] \underset{\mathbf{k_{14}}}{\overset{\mathbf{k_{13}}}{\rightleftharpoons}} [\mathbf{Sic1P\cdot Cdc14}] \xrightarrow{\mathbf{k_{15}}} [\mathbf{Sic1}] + [\mathbf{Cdc14}] \\[2ex]
[\mathbf{Clb\cdot Sic1P}] + [\mathbf{Cdc14}] \underset{\mathbf{k_{17}}}{\overset{\mathbf{k_{16}}}{\rightleftharpoons}} [\mathbf{Clb\cdot Sic1P\cdot Cdc14}] \xrightarrow{\mathbf{k_{18}}} [\mathbf{Clb\cdot Sic1}] + [\mathbf{Cdc14}]
\end{array}
\qquad (\mathcal{N}_{10})
$$

(B) Ternary complex model:

$$
\begin{array}{l}
[\mathbf{Sic1P}] \xrightarrow{\mathbf{k_3}} [\mathbf{0}] \underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} [\mathbf{Sic1}] \\[2ex]
[\mathbf{Clb}] + [\mathbf{Sic1}] \underset{\mathbf{k_5}}{\overset{\mathbf{k_4}}{\rightleftharpoons}} [\mathbf{Clb\cdot Sic1}] \\
\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \searrow^{\mathbf{k_6}} \\
\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad [\mathbf{Clb}] \\
\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \nearrow_{\mathbf{k_9}} \\
[\mathbf{Clb}] + [\mathbf{Sic1P}] \underset{\mathbf{k_8}}{\overset{\mathbf{k_7}}{\rightleftharpoons}} [\mathbf{Clb\cdot Sic1P}] \\[2ex]
[\mathbf{Clb\cdot Sic1}] + [\mathbf{Clb}] \underset{\mathbf{k_{11}}}{\overset{\mathbf{k_{10}}}{\rightleftharpoons}} [\mathbf{Clb\cdot Sic1\cdot Clb}] \xrightarrow{\mathbf{k_{12}}} [\mathbf{Clb\cdot Sic1P}] + [\mathbf{Clb}] \\[2ex]
[\mathbf{Sic1P}] + [\mathbf{Cdc14}] \underset{\mathbf{k_{14}}}{\overset{\mathbf{k_{13}}}{\rightleftharpoons}} [\mathbf{Sic1P\cdot Cdc14}] \xrightarrow{\mathbf{k_{15}}} [\mathbf{Sic1}] + [\mathbf{Cdc14}] \\[2ex]
[\mathbf{Clb\cdot Sic1P}] + [\mathbf{Cdc14}] \underset{\mathbf{k_{17}}}{\overset{\mathbf{k_{16}}}{\rightleftharpoons}} [\mathbf{Clb\cdot Sic1P\cdot Cdc14}] \xrightarrow{\mathbf{k_{18}}} [\mathbf{Clb\cdot Sic1}] + [\mathbf{Cdc14}]
\end{array}
\qquad (\mathcal{N}_{11})
$$

The ternary complex network as defined above has deficiency $\delta = 5$, the binary complex model has deficiency $\delta = 4$. Hence, the advanced deficiency algorithm has to be applied for its analysis. As it turns out, the implementation in the *Chemical Reaction Network Toolbox* cannot decide about multistationarity. The algorithm returns that the system may or may not have multiple steady states, as nonlinear inequalities have to be considered in both cases. Thus, in a first step, the subnetworks defined by the stoichiometric generators of $\mathcal{N}_{10}$ and $\mathcal{N}_{11}$ are analysed to decide about multistationarity. This is described in Section 5.1. In Section 5.2 and 5.3 networks $\mathcal{N}_{10}$ and $\mathcal{N}_{11}$ are analysed using the methods described in Chapter 3. This chapter closes with some concluding remarks in Section 5.4.

Remark 13. *Note that even though the CRNT toolbox cannot decide about multistationarity as it needs to solve nonlinear equations, the methods described in Chapter 3 can,* ***by analysis of linear inequalities****.*

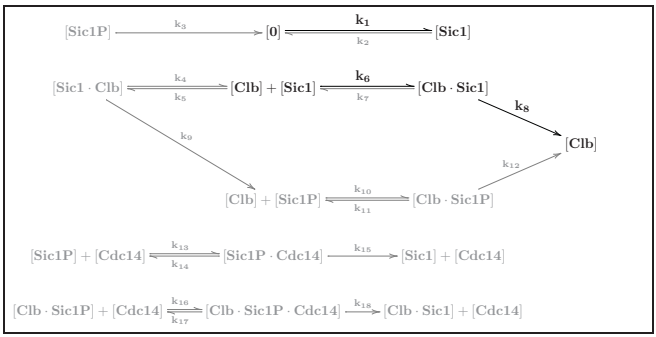
5.1 Subnetwork analysis

The generators of $\ker(Y\, I_a) \cap \mathbb{R}^{18}_{\geq 0}$ for network $\mathcal{N}_{10}$ and $\mathcal{N}_{11}$ are given in (5.1) and (5.2), respectively. Visual inspection shows that $E_{\mathcal{N}_{10}}$ contains six *stoichiometric generators* (columns 7–12), while $E_{\mathcal{N}_{11}}$ contains five *stoichiometric generators* (columns 7–11). Figures 5.2 and 5.3 contain the subnetworks defined by the stoichiometric generators of network $\mathcal{N}_{10}$, while Figures 5.4 and 5.5 depict those of network $\mathcal{N}_{11}$. Visual inspection of the subnetworks confirms that each linkage class contains exactly one terminal strong linkage class. Thus, by Fact 4, the *Deficiency One Algorithm* is applicable. Using the CRNT toolbox [29] one can establish that for network $\mathcal{N}_{10}$ no subnetwork admits multistationarity, while for network $\mathcal{N}_{11}$ the subnetworks corresponding to generators E_9, E_{10} and E_{11} (c.f. (5.2) and Figure 5.5) can have multiple steady states. In contrast, it is guaranteed that those networks defined by E_7 and E_8 cannot admit multistationarity. Thus, by analyzing the subnetworks defined by stoichiometric generators one can establish that multistationarity is possible for network $\mathcal{N}_{11}$, while this is still unclear for network $\mathcal{N}_{10}$. To decide about multistationarity for network $\mathcal{N}_{10}$ the methods described in Chapter 3 are applied in Section 5.2 (for completeness, these methods are also applied to network $\mathcal{N}_{11}$ in Section 5.3).

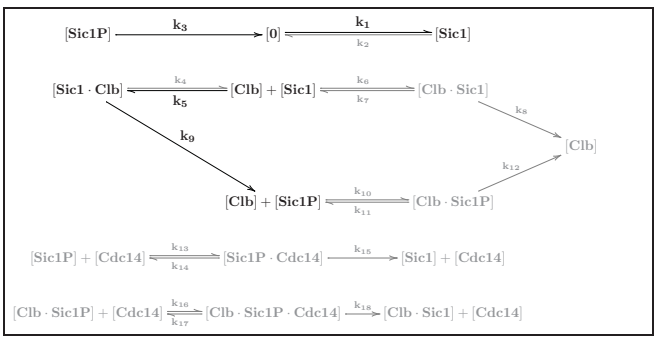
$$E_{\mathcal{N}_{10}} = \begin{bmatrix}
1 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 1 & 1 \\
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 \\
0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1
\end{bmatrix} \tag{5.1}$$

$$E_{\mathcal{N}_{11}} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 1 & 1 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 1 & 1 & 1 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \end{bmatrix} \tag{5.2}$$

$E_7 = (1, 0, 0, 0, 0, 1, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)^T$



$E_8 = (1, 0, 1, 0, 1, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0)^T$



$E_9 = (0, 0, 0, 0, 1, 0, 0, 0, 1, 0, 0, 0, 1, 0, 1, 0, 0, 0)^T$

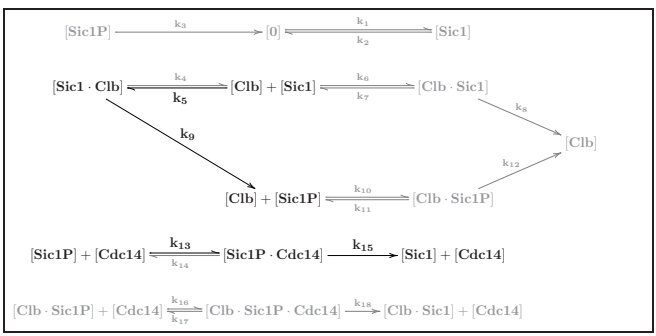
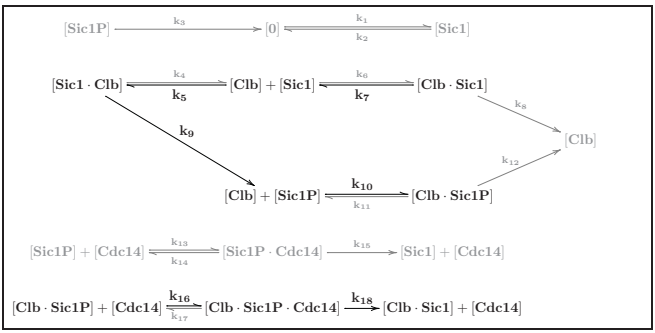
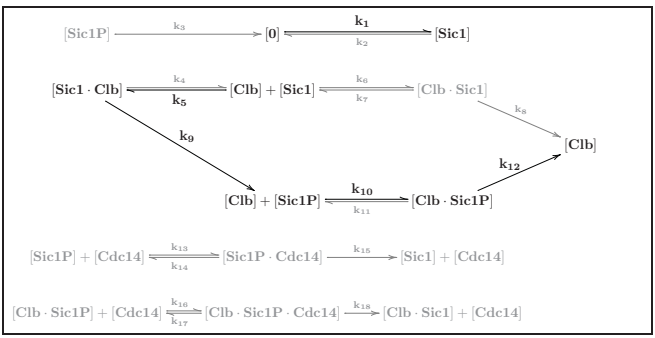


Figure 5.2: Subnetworks corresponding to stoichiometric generators E_7 - E_9 for the binary complex model. Subnetworks are denoted in black, while gray color indicates reactions not used.

$E_{10} = (0,\ 0,\ 0,\ 0,\ 1,\ 0,\ 1,\ 0,\ 1,\ 1,\ 0,\ 0,\ 0,\ 0,\ 0,\ 1,\ 0,\ 1)^T$



$E_{11} = (1,\ 0,\ 0,\ 0,\ 1,\ 0,\ 0,\ 0,\ 1,\ 1,\ 0,\ 1,\ 0,\ 0,\ 0,\ 0,\ 0,\ 0)^T$



$E_{12} = (1,\ 0,\ 0,\ 0,\ 1,\ 0,\ 0,\ 1,\ 1,\ 1,\ 0,\ 0,\ 0,\ 0,\ 0,\ 1,\ 0,\ 1)^T$

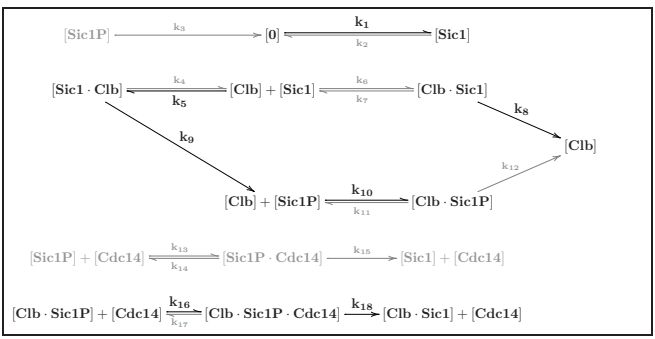
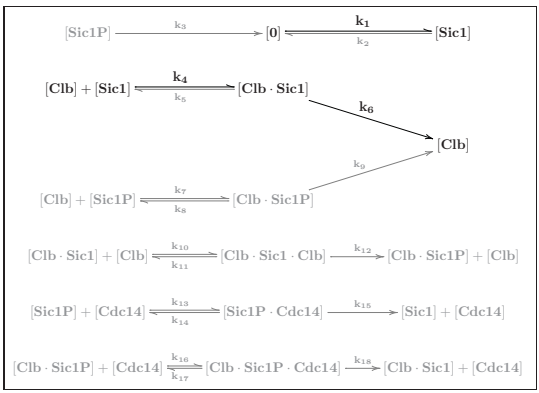


Figure 5.3: Subnetworks corresponding to stoichiometric generators E_{10} – E_{12} for the binary complex model. Subnetworks are denoted in black, while gray color indicates reactions not used.

$E_7 = (1,\ 0,\ 0,\ 1,\ 0,\ 1,\ 0,\ 0,\ 0,\ 0,\ 0,\ 0,\ 0,\ 0,\ 0,\ 0,\ 0,\ 0,\ 0,\ 0)^T$



$E_8 = (0,\ 0,\ 0,\ 0,\ 0,\ 0,\ 0,\ 0,\ 0,\ 1,\ 0,\ 1,\ 0,\ 0,\ 0,\ 1,\ 0,\ 1)^T$

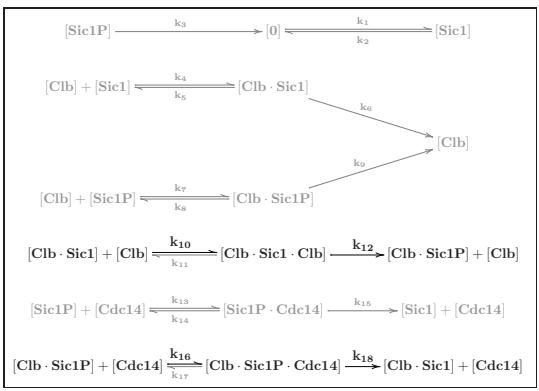
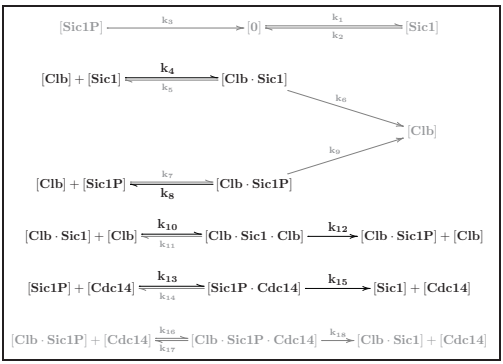
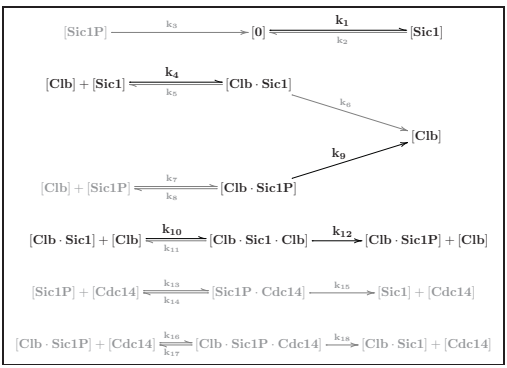


Figure 5.4: Subnetworks corresponding to stoichiometric generators E_7 and E_8 for the ternary complex model. Subnetworks are denoted in black, while gray color indicates reactions not used.

$E_9 = (0, 0, 0, 1, 0, 0, 0, 1, 0, 1, 0, 1, 1, 0, 1, 0, 0, 0)^T$



$E_{10} = (1, 0, 0, 1, 0, 0, 0, 0, 1, 1, 0, 1, 0, 0, 0, 0, 0, 0)^T$



$E_{11} = (1, 0, 1, 1, 0, 0, 0, 1, 0, 1, 0, 1, 0, 0, 0, 0, 0, 0)^T$

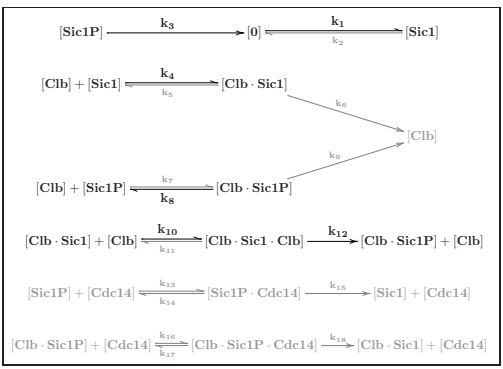


Figure 5.5: Subnetworks corresponding to the stoichiometric generators E_9 – E_{11} for the ternary complex model. Subnetworks are denoted in black, while gray color indicates reactions not used.

5.2 Analysis of the complete network $\mathcal{N}_{10}$

As described in Section 5.1, analysis of the subnetworks of $\mathcal{N}_{10}$ defined by stoichiometric generators using CRNT established that multistationarity is excluded for all subnetworks. As multistationarity is still possible for the overall network, this is analysed using the methods described in Chapter 3. For network $\mathcal{N}_{10}$ it was not possible to determine $\nu, \lambda \in \mathbb{R}^{12}_{>0}$ independent of $\mu \in \mathbb{R}^9$. (Note that this is no guarantee, that no such solution exists.) Thus the equation

$$Y^{(L)^T}\mu = \ln \frac{E\,\lambda}{E\,\nu}$$

is considered in the following form:

$$\begin{bmatrix} E & -\operatorname{diag}\left(e^{Y^{(L)^T}\mu}\right) E \end{bmatrix} \begin{pmatrix} \nu \\ \lambda \end{pmatrix}, \ \begin{pmatrix} \nu \\ \lambda \end{pmatrix} > 0.$$

Using an unimodular matrix $U \in \mathbb{R}^{18\times 18}$ with $U\,E = \begin{bmatrix} I_{11} & v \\ 0_{7\times 11} & 0_{7\times 1} \end{bmatrix}$, where $\ker(E) = \operatorname{span}\left(\begin{pmatrix} v \\ -1 \end{pmatrix}\right)$, one obtains the following system of equations

$$\underbrace{U \begin{bmatrix} E & -\operatorname{diag}\left(e^{Y^{(L)^T}\mu}\right) E \end{bmatrix}}_{=:H(\mu)} \begin{pmatrix} \nu \\ \lambda \end{pmatrix} = 0, \ \begin{pmatrix} \nu \\ \lambda \end{pmatrix} > 0,$$

where $H(\mu)$ is given at the end of this Section on page 67. Note that the nonzero elements of rows 14, 17 and 18 of $H(\mu)$ always have the same sign, for every μ. Thus, positive ν, λ only exists for those values of μ, where rows 14, 17 and 18 of $H(\mu)$ are identical to zero, thus μ must satisfy

$$\mu_9 = \mu_1 + \mu_3 \tag{5.3a}$$
$$\mu_7 = \mu_2 + \mu_6 \tag{5.3b}$$
$$\mu_8 = \mu_5 + \mu_6 \tag{5.3c}$$

Note that (5.3a), (5.3b) and (5.3c) are the only restrictions on the μ_i. Thus μ can be represented as (using new variables $\kappa \in \mathbb{R}^6$):

$$\mu = M_{\mathcal{N}_{10}}\,\kappa, \tag{5.4a}$$

where

$$M_{\mathcal{N}_{10}} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 1 & 1 \\ 1 & 0 & 1 & 0 & 0 & 0 \end{bmatrix} \kappa. \tag{5.4b}$$

Apart from $h_{12,23} = -1 + e^{\mu_4} - e^{\mu_8} + e^{\mu_9}$ the signs of all nonzero elements of $H(\mu)$ can be determined by linear inequalities in the μ_i. By subtracting column 23 from column 20 one obtains a matrix $\tilde{H}$, where the signs of all elements can be determined by linear inequalities. Note that this corresponds to a change of variables $\begin{pmatrix} \nu \\ \lambda \end{pmatrix} = T \begin{pmatrix} \tilde{\nu} \\ \tilde{\lambda} \end{pmatrix}$ where

$$T = \begin{bmatrix} e_1 & \dots & e_{22} & -e_{23} + e_{20} & e_{24} \end{bmatrix}$$

Thus

$$H(\mu)\ T \begin{pmatrix} \tilde{\nu} \\ \tilde{\lambda} \end{pmatrix} = 0,\ T \begin{pmatrix} \tilde{\nu} \\ \tilde{\lambda} \end{pmatrix} > 0,$$

where $T\begin{pmatrix}\tilde{\nu}\\ \tilde{\lambda}\end{pmatrix} > 0$ is equivalent to $\nu,\, \lambda > 0$ and

$$\lambda_8 - \lambda_{11} > 0. \tag{5.5}$$

Note that this condition can easily be included by adding a new variable $s_0 > 0$. Then one has to determine positive solutions to

$$\underbrace{\begin{bmatrix} H(\mu)\; T & 0_{24\times 1}\\ e_{20}^T - e_{23}^T & -1\end{bmatrix}}_{=:\tilde{H}(\mu)} \begin{pmatrix}\nu\\ \lambda\\ s_0\end{pmatrix} = 0$$

The matrix $\tilde{H}(\mu)$ is given at the end of this section on page 68. Note that equations (5.3a) – (5.3c) have been incorporated $\tilde{H}(\mu)$. Further note that each of row $1, 2, 4, 5, 6, 9, 10$ contains exactly two nonzero entries, thus they are equal to

$$\nu_i - e^{\langle y_j^{(L)},\, \mu\rangle}\, \lambda_i = 0,$$

for some linear form $\langle y_j^{(L)},\, \mu\rangle$. Further note that rows 13–18 are independent of the ν_i, $i = 1, \ldots, 12$. Thus it is straightforward to obtain

$$\nu_1 = e^{\mu_1}\, \lambda_1 \tag{5.6a}$$
$$\nu_2 = e^{\mu_1+\mu_3}\, \lambda_2 \tag{5.6b}$$
$$\nu_4 = e^{\mu_5}\, \lambda_4 \tag{5.6c}$$
$$\nu_5 = e^{\mu_2+\mu_6}\, \lambda_5 \tag{5.6d}$$
$$\nu_6 = e^{\mu_5+\mu_6}\, \lambda_6 \tag{5.6e}$$
$$\nu_9 = e^{\mu_2+\mu_6}\, \lambda_9 \tag{5.6f}$$
$$\nu_{10} = e^{\mu_5}\, \lambda_{10} \tag{5.6g}$$

Further note that, if μ satisfies (5.3a) – (5.3c), then λ_2, λ_5 and λ_6 only occur in row 2, 5 and 6, respectively. That is, λ_2, λ_5 and λ_6 are only involved in equations (5.6b), (5.6d) and (5.6e). Thus λ_2, λ_5 and λ_6 can be chosen freely in $\mathbb{R}_{>0}$. Therefore it remains to determine $x = (\, \nu_3,\, \nu_7,\, \nu_8,\, \nu_{11},\, \nu_{12},\, \lambda_1,\, \lambda_3,\, \lambda_4,\, \lambda_7,\, \lambda_8,\, \lambda_9,\, \lambda_{10},\, \lambda_{11},\, \lambda_{12},\, s_0\,)^T$. This amounts to solving a system of equations defined by row 3, 7, 8, 11–19 and columns 3, 7, 8, 11, 12, 13, 15, 16, 19–26 of matrix $\tilde{H}(\mu)$. The resulting submatrix $Q(\mu)$ is given on page 69 at the end of this Section. Using the unimodular transformation matrix

$$TT = \begin{bmatrix}
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0\\
0 & 1 & 1 & 1 & 1 & 0 & 0 & 0 & 0\\
0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0\\
1 & 1 & 0 & 1 & 0 & 0 & 1 & 0 & 0\\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1
\end{bmatrix} \tag{5.7}$$

one obtains the much simpler matrix $Q_s(\mu) := TT\, Q(\mu)$, displayed on page 69. Using

$$q_1 = -e^{\mu_1+\mu_3} + e^{\mu_2+\mu_6} \tag{5.8a}$$
$$q_2 = -e^{\mu_1+\mu_3} + e^{\mu_5} \tag{5.8b}$$
$$q_3 = -e^{\mu_1+\mu_3} + e^{\mu_5+\mu_6} \tag{5.8c}$$
$$q_4 = -1 + e^{\mu_1} \tag{5.8d}$$
$$q_5 = -1 + e^{\mu_5} \tag{5.8e}$$
$$q_6 = -e^{\mu_2+\mu_3} + e^{\mu_5} \tag{5.8f}$$

$Q_s(\mu)$ can be represented as:

$$Q_s(\mu) = \begin{bmatrix} 1 & 0 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 \\ 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & q_1 & q_2 & 1 & q_3 & 0 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & -1 & 0 \\ 0 & 1 & 1 & 1 & 0 & q_4 & 0 & 0 & -1 & -1 & 0 & q_5 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 1 & 0 & 0 \\ 1 & 1 & 0 & 1 & 1 & 0 & -1 & 0 & -1 & 0 & 0 & 0 & -1 & -1 & 0 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 & q_6 & 0 & 0 & 0 & q_6 & -1 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & -1 & 0 & -1 \end{bmatrix} \tag{5.9}$$

Thus any feasible inequality system

$$\begin{bmatrix} V & -\operatorname{diag}(\sigma) \end{bmatrix} \begin{pmatrix} \kappa \\ s \end{pmatrix} = 0,\ s > 0 \tag{5.10}$$

with $\sigma \in \{-1,\, 0,\, 1\}^6$ and

$$V := \begin{bmatrix} -1 & 1 & -1 & 0 & 0 & 1 \\ -1 & 0 & -1 & 0 & 1 & 0 \\ -1 & 0 & -1 & 0 & 1 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & -1 & -1 & 0 & 1 & 0 \end{bmatrix}, \tag{5.11}$$

defines a feasible signing for $Q_s(\mu)$. No signing was found such that $Q_s(\mu)$ is an L^+-matrix. However, $Q_s(\mu)$ is a sign-central matrix for some signings. Thus the results of Section 3.2.2 and 3.4 can be applied.

Note that all μ as defined in (5.4a) are contained in the linear subspace $\mathcal{M}_{\mathcal{N}_{10}} = [M_{\mathcal{N}_{10}}]$. Thus, with Fact 3 in mind, all orthants with $\mathcal{S}_{\mathcal{N}_{10}} \cap \mathbb{R}^9_\delta \neq \emptyset$ were determined. Of those all orthants with $\mathcal{M}_{\mathcal{N}_{10}} \cap \mathbb{R}^9_\delta \neq \emptyset$ and (5.10) is feasible for some σ have been determined. That is, all orthants, where

$$\begin{bmatrix} \operatorname{diag}(\delta) & -M_{\mathcal{N}_{10}} & 0 \\ 0 & V & -\operatorname{diag}(\sigma) \end{bmatrix} \begin{pmatrix} \xi \\ \kappa \\ s \end{pmatrix} = 0,\ \xi,\ s > 0 \tag{5.12}$$

is feasible. Finally all signings, where (5.10) is feasible for some orthant and $Q(\mu)$ is a sign-central matrix were determined. The orthants are given in (5.13a):

$$\begin{aligned}
\delta_1 &= (-1, \ -1, \ 1, \ -1, \ 1, \ -1, \ -1, \ 1, \ -1)^T \\
\delta_2 &= (-1, \ -1, \ 1, \ -1, \ 1, \ -1, \ -1, \ 1, \ 1)^T \\
\delta_3 &= (-1, \ -1, \ 1, \ -1, \ 1, \ 0, \ -1, \ 1, \ -1)^T \\
\delta_4 &= (-1, \ -1, \ 1, \ -1, \ 1, \ 0, \ -1, \ 1, \ 1)^T \\
\delta_5 &= (-1, \ -1, \ 1, \ -1, \ 1, \ 1, \ -1, \ 1, \ -1)^T \\
\delta_6 &= (-1, \ -1, \ 1, \ -1, \ 1, \ 1, \ -1, \ 1, \ 1)^T \\
\delta_7 &= (-1, \ -1, \ 1, \ 1, \ 1, \ -1, \ -1, \ 1, \ -1)^T \\
\delta_8 &= (-1, \ -1, \ 1, \ 1, \ 1, \ 0, \ -1, \ 1, \ -1)^T \\
\delta_9 &= (-1, \ -1, \ 1, \ 1, \ 1, \ 1, \ -1, \ 1, \ -1)^T \\
\delta_{10} &= (-1, \ 0, \ 1, \ -1, \ 1, \ -1, \ -1, \ 1, \ -1)^T \\
\delta_{11} &= (-1, \ 0, \ 1, \ -1, \ 1, \ -1, \ -1, \ 1, \ 1)^T \\
\delta_{12} &= (-1, \ 0, \ 1, \ 1, \ 1, \ -1, \ -1, \ 1, \ -1)^T \\
\delta_{13} &= (-1, \ 1, \ 1, \ -1, \ 1, \ -1, \ -1, \ 1, \ -1)^T \\
\delta_{14} &= (-1, \ 1, \ 1, \ -1, \ 1, \ -1, \ -1, \ 1, \ 1)^T \\
\delta_{15} &= (-1, \ 1, \ 1, \ -1, \ 1, \ -1, \ 1, \ 1, \ 1)^T \\
\delta_{16} &= (-1, \ 1, \ 1, \ 1, \ 1, \ -1, \ -1, \ 1, \ -1)^T
\end{aligned} \tag{5.13a}$$

the signings in (5.13b):

$$\begin{array}{l} \sigma_1{=}(-1,\ \ 1,\ \ \ 1,\ -1,\ \ 1,\ \ 0)^T \\ \sigma_2{=}(-1,\ \ 0,\ -1,\ -1,\ \ 1,\ \ 0)^T \\ \sigma_3{=}(-1,\ \ 1,\ -1,\ -1,\ \ 1,\ \ 0)^T \\ \sigma_4{=}(-1,\ \ 1,\ \ \ 0,\ -1,\ \ 1,\ \ 0)^T \\ \sigma_5{=}(-1,\ \ 0,\ \ \ 1,\ -1,\ \ 1,\ \ 0)^T \end{array} \tag{5.13b}$$

Note that if (5.10) is feasible for (δ, σ), then it is also feasible for $(-\delta, -\sigma)$. Thus there are in fact 32 orthants and five signings. Further note, that not all signings need to hold in every orthant. It is only guaranteed that in every orthant some signing holds. Recall that a sign-central matrix has the property that every matrix with the same sign pattern has a nonnegative nullvector. A nonnegative nullvector yields a nonnegative parameter vector, where some rate constants are zero. In some cases it is possible to derive values for these rate constants using the ideas discussed in Section 3.4. This is the strategy pursued in the remainder of this section. Further analysis of these signings reveals that $Q_s(\mu)$ contains sub-matrices that are L^+-matrices. Obviously, by an appropriate filling with zeros, a positive nullvector of a submatrix yields a nonnegative nullvector of the overall matrix. Thus, for each submatrix that is an L^+-matrix, one obtains a nullvector of $Q_s(\mu)$, with known zero pattern. To see this, let $\mathcal{I}^+ \subseteq 1, \ldots, 15$ contain the indices of those columns of $Q(\mu)$ that form an L^+-matrix and let $\bar{Q} := Q_s(\mu)[\mathcal{I}^+]$ be the corresponding submatrix (after a removal of zero rows, if necessary). Recall that an L^+-matrix has a positive nullvector, thus let x be a vector of appropriate dimension with $\bar{Q}x = 0$. As $\bar{Q}$ is an L^+ matrix $\operatorname{supp}(x) = \mathcal{I}^+$ follows. Let $\hat{x} \in \mathbb{R}^{15}_{\geq 0}$ with $\hat{x}_i = x_i$, if $i \in \mathcal{I}^+$ and $\hat{x}_i = 0$, otherwise. Clearly $Q_s(\mu)\,\hat{x} = 0$ and $\operatorname{supp}(\hat{x}) = \mathcal{I}^+$, thus the zero pattern of $\hat{x}$ is known. Table 5.1 contains, for each signing, the column indices that form L^+-matrices, after a removal of zero

Column indices	σ_1	σ_2	σ_3	σ_4	σ_5	σ_6	σ_7	σ_8	σ_9	σ_{10}
{3, 6, 8, 10, 11, 12, 15}	+		+	+		+	+	+		
{3, 6, 10, 11, 12, 15}	+		+	+		+	+	+		
{6, 8, 11, 12}	+		+	+		+	+	+		
{6, 11, 12}	+		+	+		+	+	+		
{6, 8, 12}		+			+				+	+
{6, 12}		+			+				+	+

Table 5.1: Signings and column indices that are L^+-matrices

rows, were necessary. In total, there are six different sub-matrices that are L^+-matrices. Consider, for example, $\sigma_1 = (-1, 1, 1, -1, 1, 0)^T$. Here the following sub-matrices are L^+-matrices (note that for each matrix all zero rows have been removed):

$$Q(\mu)[3, 6, 8, 10, 11, 12, 15] = \begin{bmatrix} 1 & 0 & 0 & -1 & -1 & 1 & 0 \\ 1 & -1 & 0 & -1 & 0 & 1 & 0 \\ 1 & 0 & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & -1 \end{bmatrix}$$

$$Q(\mu)[3, 6, 10, 11, 12, 15] = \begin{bmatrix} 1 & 0 & -1 & -1 & 1 & 0 \\ 1 & -1 & -1 & 0 & 1 & 0 \\ 1 & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & -1 \end{bmatrix}$$

$$Q(\mu)[6, 8, 11, 12] = \begin{bmatrix} 0 & 0 & -1 & 1 \\ -1 & 0 & 0 & 1 \end{bmatrix}$$

$$Q(\mu)[6, 11, 12] = \begin{bmatrix} 0 & -1 & 1 \\ -1 & 0 & 1 \end{bmatrix}$$

For the signing $\sigma_2 = (-1, 0, -1, -1, 1, 0)$ the following sub-matrices are L^+-matrices:

$$Q(\mu)[6, 8, 12] = \begin{bmatrix} -1 & 0 & 1 \end{bmatrix} \qquad Q(\mu)[6, 12] = \begin{bmatrix} -1 & 1 \end{bmatrix}$$

To obtain multistationarity, consider, for example, signing σ_1. There exists a vector x with $Q_s(\mu)\,x = 0$, with $\operatorname{supp}(x) = \{3, 6, 8, 10, 11, 12, 15\}$, no matter what values the q_i take. Recall that $x = (\nu_3,$

$\nu_7,\ \nu_8,\ \nu_{11},\ \nu_{12},\ \lambda_1,\ \lambda_3,\ \lambda_4,\ \lambda_7,\ \lambda_8,\ \lambda_9,\ \lambda_{10},\ \lambda_{11},\ \lambda_{12},\ s_0\ \big)^T$ and that λ_2, λ_5 and λ_6 are free. Thus one obtains

$$\lambda_i \neq 0, \quad \nu_i \neq 0,\ i = 1, 2, 4, 5, 6, 8, 9, 10.$$

Recall that $k = \operatorname{diag}\left(\phi\left(a^{-1}\right)\right)\ E\,\lambda$. Using this definition one obtains for k:

$$k = \Big(\lambda_1 + \lambda_8 + \lambda_{10},\ \frac{\lambda_1}{a_1},\ \frac{\lambda_8}{a_2},\ \frac{\lambda_2}{a_9},\ \frac{\lambda_2 + \lambda_8 + \lambda_9 + \lambda_{10}}{a_1\, a_3},\ 0,\ 0,\ 0,\ \frac{\lambda_8 + \lambda_9 + \lambda_{10}}{a_9},$$
$$\frac{\lambda_4 + \lambda_{10}}{a_2\, a_3},\ \frac{\lambda_4}{a_5},\ \frac{\lambda_{10}}{a_5},\ \frac{\lambda_5 + \lambda_9}{a_2\, a_6},\ \frac{\lambda_5}{a_7},\ \frac{\lambda_9}{a_7},\ \frac{\lambda_6}{a_5\, a_6},\ \frac{\lambda_6}{a_8},\ 0\Big)^T.$$

Thus $k_i = 0$, $i = 6, 7, 8, 18$. To find values for these k_i, the procedure described in Section 3.4 is applied. To this end it is necessary to find a positive vector v with

$$G_c\, v = 0$$
$$G_c = \begin{bmatrix} W_{add}^T\, N_c\, \phi\,(a) \\ W_{add}^T\, N_c\, \phi\,(b), \end{bmatrix}$$

where $b = \operatorname{diag}\,(e^{\mu})\ a$, with $\mu = M_{\mathcal{N}_{10}}\,\kappa$ and $W_{add}^T = (0,\ 0,\ 0,\ 1,\ 0,\ 0,\ 0,\ 0,\ 0)$. Then

$$G_c = \begin{bmatrix} a_1\, a_3 & -a_4 & -a_4 & a_8 \\ a_1\, a_3\, e^{\mu_1+\mu_3} & -e^{\mu_4}\, a_4 & -e^{\mu_4}\, a_4 & e^{\mu_5+\mu_6}\, a_8 \end{bmatrix}.$$

Solutions to $G_c\, v = 0$ are defined by

$$a_1\, a_3\, v_1 - a_4\, v_2 - a_4\, v_3 + a_8\, v_4 = 0$$
$$a_1\, a_3\, e^{\mu_1+\mu_3}\, v_1 - a_4\, e^{\mu_4}\, v_2 - a_4\, e^{\mu_4}\, v_3 + a_8\, e^{\mu_5+\mu_6}\, v_4 = 0$$

The first equation is equivalent to

$$a_4\ (v_2 + v_3) = a_1\, a_3\, v_1 + a_8\, v_4.$$

Inserting in the second equation yields

$$a_1\, a_3\, (e^{\mu_1+\mu_3} - e^{\mu_4})\, v_1 + a_8\, (-e^{\mu_4} + e^{\mu_5+\mu_6})\, v_4 = 0.$$

The last equation has a positive solution, if and only if either

$$e^{\mu_1+\mu_3} - e^{\mu_4} > 0 \text{ and } e^{\mu_5+\mu_6} - e^{\mu_4} < 0 \tag{5.14a}$$

or

$$e^{\mu_1+\mu_3} - e^{\mu_4} < 0 \text{ and } e^{\mu_5+\mu_6} - e^{\mu_4} > 0. \tag{5.14b}$$

Note that (5.14a) and (5.14b) are equivalent to the linear inequalities

$$\mu_1 + \mu_3 - \mu_4 > 0 \text{ and } -\mu_4 + \mu_5 + \mu_6 < 0 \tag{5.15a}$$

or

$$\mu_1 + \mu_3 - \mu_4 < 0 \text{ and } -\mu_4 + \mu_5 + \mu_6 > 0. \tag{5.15b}$$

Thus multistationarity can be extended to the overall network, if μ satisfies the inequalities defined by the signing σ_1 together with these additional constraints. For the other sub-matrices given in Table 5.1 it is possible to show that multistationarity can be extended to the overall network, if (5.15a) and (5.15b) hold. There exists exactly one signing such that (5.15a) and (5.15b) hold in some of the orthants given in (5.13a):

$$\sigma_1 = (-1,\quad 1,\quad 1,\quad -1,\quad 1,\quad 0.)' \tag{5.16}$$

σ_1 holds in the following orthants:

$$\begin{array}{ll}
\delta_1 = & (-1,\ -1,\ \ 1,\ -1,\ \ 1,\ -1,\ -1,\ \ 1,\ -1)^T \\
\delta_2 = & (-1,\ -1,\ \ 1,\ -1,\ \ 1,\ \ 1,\ -1,\ \ 1,\ -1)^T \\
\delta_3 = & (-1,\ -1,\ \ 1,\ \ 1,\ \ 1,\ -1,\ -1,\ \ 1,\ -1)^T \\
\delta_4 = & (-1,\ -1,\ \ 1,\ \ 1,\ \ 1,\ \ 1,\ -1,\ \ 1,\ -1)^T \\
\delta_5 = & (-1,\ \ 1,\ \ 1,\ -1,\ \ 1,\ -1,\ -1,\ \ 1,\ -1)^T \\
\delta_6 = & (-1,\ \ 1,\ \ 1,\ \ 1,\ \ 1,\ -1,\ -1,\ \ 1,\ -1)^T \\
\delta_7 = & (\ 1,\ -1,\ -1,\ -1,\ -1,\ \ 1,\ \ 1,\ -1,\ \ 1)^T \\
\delta_8 = & (\ 1,\ -1,\ -1,\ \ 1,\ -1,\ \ 1,\ \ 1,\ -1,\ \ 1)^T \\
\delta_9 = & (\ 1,\ \ 1,\ -1,\ -1,\ -1,\ -1,\ \ 1,\ -1,\ \ 1)^T \\
\delta_{10} = & (\ 1,\ \ 1,\ -1,\ -1,\ -1,\ \ 1,\ \ 1,\ -1,\ \ 1)^T \\
\delta_{11} = & (\ 1,\ \ 1,\ -1,\ \ 1,\ -1,\ -1,\ \ 1,\ -1,\ \ 1)^T \\
\delta_{12} = & (\ 1,\ \ 1,\ -1,\ \ 1,\ -1,\ \ 1,\ \ 1,\ -1,\ \ 1)^T
\end{array} \tag{5.17}$$

Consider $\delta_5 = (-1,\ 1,\ 1,\ -1,\ 1,\ -1,\ -1,\ 1,\ -1)^T$. To determine μ, the following equations are solved for nonnegative ξ, s and unconstraint κ:

$$\begin{bmatrix} \operatorname{diag}(\delta) & -M & 0_{9\times 8} \\ 0_{8\times 9} & \begin{bmatrix} V \\ V_{add} \end{bmatrix} & -\operatorname{diag}((\sigma_1,\ -1,\ 1)) \end{bmatrix} \begin{pmatrix} \xi \\ \kappa \\ s \end{pmatrix} = 0,$$

with V as in (5.11) and

$$V_{add} = \begin{bmatrix} 1 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & -1 & 1 & 1 \end{bmatrix}. \tag{5.18}$$

A solution for κ is given by

$$\kappa = \begin{bmatrix} 0 & -2 & -1 & -1 & -2 \\ 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 1 & 1 \\ 0 & -1 & 0 & 0 & 0 \\ 1 & 1 & 1 & 1 & 1 \\ -1 & -1 & 0 & -1 & -1 \end{bmatrix} \alpha, \quad \alpha \in \mathbb{R}^5_{>0}. \tag{5.19}$$

Choose $\alpha_i = 1$ to obtain $\bar{\mu} = (-6,\ 1,\ 4,\ -1,\ 5,\ -4,\ -3,\ 1,\ -2)$ and thus

$$Q(\bar{\mu}) = \begin{bmatrix}
1 & 0 & 0 & 0 & 1 & 0 & -\frac{1}{e} & 0 & 0 & 0 & 0 & 0 & 0 & -\frac{1}{e} & 0 \\
0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & -\frac{1}{e} & 0 & 0 & 0 & -\frac{1}{e} & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -\frac{1}{e^2} & \frac{1}{e^3}-\frac{1}{e^2} & -\frac{1}{e^2}+e^5 & e & -\frac{1}{e^2}+e & 0 \\
0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -e & -e & 0 \\
0 & 1 & 1 & 1 & 0 & -1+\frac{1}{e^6} & 0 & 0 & -1 & -1 & 0 & -1+e^5 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -e & 0 & 0 & e & 0 & 0 \\
1 & 1 & 0 & 1 & 1 & 0 & -\frac{1}{e^2} & 0 & -\frac{1}{e^2} & 0 & 0 & 0 & -e & -e & 0 \\
0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -e^5 & -e^5 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & -1 & 0 & -1
\end{bmatrix}$$

The cone $\ker(Q(\bar{\mu})) \cap \mathbb{R}^{15}_{\geq 0}$ is generated by

$$E^T = \begin{bmatrix}
0 & 0 & e & 0 & 0 & -\frac{-1+e}{-1+\frac{1}{e^6}} & 0 & 0 & 0 & 1 & e(1+e+e^2) & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & -\frac{-1+e^5}{-1+\frac{1}{e^6}} & 0 & 0 & 0 & 0 & e(1+e+e^2+e^3+e^4+e^5+e^6) & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}.$$

Choose, for example,

$$\begin{aligned}
x &= E \begin{pmatrix} 1 \\ 1 \\ 1 \end{pmatrix} \\
&= \Bigg(0,\ 0,\ e,\ 0,\ 0,\ -\frac{-1+e}{-1+\frac{1}{e^6}} - \frac{-1+e^5}{-1+\frac{1}{e^6}},\ 0,\ 1,\ 0,\ 1, \\
&\qquad e(1+e+e^2) + e(1+e+e^2+e^3+e^4+e^5+e^6),\ 1,\ 0,\ 0,\ 1\Bigg)^T.
\end{aligned}$$

Thus one obtains

$$\begin{aligned}\lambda_1 &= -\frac{-1+e}{-1+\frac{1}{e^6}} - \frac{-1+e^5}{-1+\frac{1}{e^6}}\\ \lambda_4 &= 1,\\ \lambda_8 &= 1,\\ \lambda_9 &= e\,(1+e+e^2) + e\,(1+e+e^2+e^3+e^4+e^5+e^6),\\ \lambda_{10} &= 1.\end{aligned}$$

Recall that $\lambda_2, \lambda_5, \lambda_6$ are free, thus choose $\lambda_2 = \lambda_5 = \lambda_6 = 1$ to obtain

$$\begin{aligned}\hat{k}_E^* = \Big(&2 - \frac{-1+e}{-1+\frac{1}{e^6}} - \frac{-1+e^5}{-1+\frac{1}{e^6}},\ \frac{e^6\,(2+e+e^2+e^3+e^4)}{a_1\,(1+e+e^2+e^3+e^4+e^5)},\ \frac{1}{a_2},\ \frac{1}{a_9},\\ &\frac{3+2\,e+2\,e^2+2\,e^3+e^4+e^5+e^6+e^7}{a_1\,a_3},\ 0,\ 0,\ 0,\ \frac{2+2\,e+2\,e^2+2\,e^3+e^4+e^5+e^6+e^7}{a_9},\\ &\frac{2}{a_2\,a_3},\ \frac{1}{a_5},\ \frac{1}{a_5},\ \frac{1+2\,e+2\,e^2+2\,e^3+e^4+e^5+e^6+e^7}{a_2\,a_6},\ \frac{1}{a_7},\\ &\frac{e\,(2+2\,e+2\,e^2+e^3+e^4+e^5+e^6)}{a_7},\ \frac{1}{a_5\,a_6},\ \frac{1}{a_8},\ 0\Big)^T.\end{aligned}$$

Using $v \in S \cap \mathbb{R}^9_{\delta_5}$, $v = \big(-1,\ 1,\ 2,\ -4,\ 2,\ -2,\ -2,\ 4,\ -4\big)^T$ one obtains

$$\begin{aligned}x_1 = (&\tfrac{e^6}{-1+e^6},\ \tfrac{1}{-1+e},\ \tfrac{2}{-1+e^4},\ \tfrac{4\,e}{-1+e},\ \tfrac{2}{-1+e^5},\ \tfrac{2\,e^4}{-1+e^4},\ \tfrac{2\,e^3}{-1+e^3},\ \tfrac{4}{-1+e},\ \tfrac{4\,e^2}{-1+e^2})^T\\ x_2 = (&\tfrac{1}{-1+e^6},\ \tfrac{e}{-1+e},\ \tfrac{2\,e^4}{-1+e^4},\ \tfrac{4}{-1+e},\ \tfrac{2\,e^5}{-1+e^5},\ \tfrac{2}{-1+e^4},\ \tfrac{2}{-1+e^3},\ \tfrac{4\,e}{-1+e},\ \tfrac{4}{-1+e^2})^T\end{aligned}$$

and

$$G_c = \begin{bmatrix} \frac{2\,e^6}{1-e^4-e^6+e^{10}} & -\frac{4\,e}{-1+e} & -\frac{4\,e}{-1+e} & \frac{4}{-1+e} \\ \frac{2\,e^4}{1-e^4-e^6+e^{10}} & -\frac{4}{-1+e} & -\frac{4}{-1+e} & \frac{4\,e}{-1+e} \end{bmatrix}.$$

For the nullspace of G_c one obtains

$$\ker(G_c) = \begin{bmatrix} \frac{2\,(-1+e)\,(1+e)^3\,(1+2\,e^2+2\,e^4+e^6)}{e^5} & 1+\frac{1}{e}+e & 0 & 1 \\ 0 & -1 & 1 & 0 \end{bmatrix}$$

Choosing

$$\kappa_0 = \big(\frac{2\,(-1+e)\,(1+e)^3\,(1+2\,e^2+2\,e^4+e^6)}{e^5},\ \frac{1}{e}+e,\ 1,\ 1\big)^T$$

one obtains for $\hat{k}_c$:

$$\begin{aligned}\hat{k}_c = \big(&0,\ 0,\ 0,\ 0,\ 0,\ \frac{2\,(-1+e)\,(1+e)^3\,(1+2\,e^2+2\,e^4+e^6)}{e^5},\\ &\frac{1}{e}+e,\ 1,\ 0,\ 0,\ 0,\ 0,\ 0,\ 0,\ 0,\ 0,\ 1\big)^T\end{aligned}$$

Using $k(\epsilon) = \hat{k}_E^* + \epsilon\,\hat{k}_c$ as parameter vector and x_1 as initial value for a numerical continuation with, for example, c_1 (total concentration of $Cdc14$) as a bifurcation parameter one obtains results displayed in Fig. 5.6.

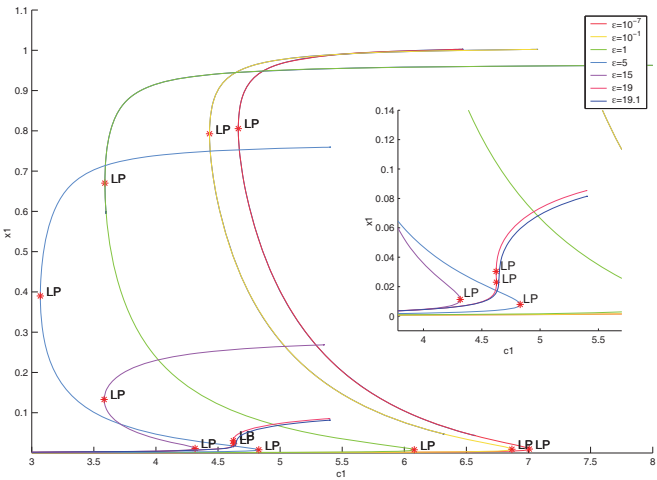


Figure 5.6: Numerical Continuation of x_1 using c_1 (total concentration of $Cdc14$) as bifurcation parameter. Parameter vector $k(\epsilon) = k^* + \epsilon\, k_c$, as given in the text. For $\epsilon \in [0, 19]$ the system shows multistationarity.

$$H = \begin{bmatrix} H_1 & H_2 \end{bmatrix}$$

$$H_1 = \begin{bmatrix}
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -e^{\mu_1} & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -e^{\mu_1+\mu_3} & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & -e^{\mu_4} & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -e^{\mu_5} \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1+e^{\mu_1} & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & e^{\mu_1+\mu_3} - e^{\mu_9} & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -e^{\mu_1+\mu_3} + e^{\mu_4} & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -e^{\mu_2+\mu_3} + e^{\mu_5} \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}$$

$$H_2 = \begin{bmatrix}
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & -e^{\mu_1+\mu_3} + e^{\mu_9} & -e^{\mu_1+\mu_3} + e^{\mu_9} & -e^{\mu_1+\mu_3} + e^{\mu_9} & -e^{\mu_1+\mu_3} + e^{\mu_9} & -e^{\mu_1+\mu_3} + e^{\mu_9} \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & -e^{\mu_4} \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
-e^{\mu_7} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & -e^{\mu_8} & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -e^{\mu_4} & 0 & 0 & 0 & -e^{\mu_4} + e^{\mu_8} & e^{\mu_8} \\
0 & 0 & 0 & -e^{\mu_9} & e^{\mu_7} - e^{\mu_9} & e^{\mu_5} - e^{\mu_9} & e^{\mu_8} - e^{\mu_9} & e^{\mu_8} - e^{\mu_9} \\
0 & 0 & 0 & 0 & -e^{\mu_7} & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & -e^{\mu_5} & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & -e^{\mu_8} & -e^{\mu_8} \\
0 & 0 & -1 + e^{\mu_4} & -1 + e^{\mu_9} & -e^{\mu_7} + e^{\mu_9} & -1 + e^{\mu_9} & -1 + e^{\mu_4} - e^{\mu_8} + e^{\mu_9} & -e^{\mu_8} + e^{\mu_9} \\
0 & 0 & 0 & -e^{\mu_2} + e^{\mu_9} & -e^{\mu_7} + e^{\mu_9} & -e^{\mu_5} + e^{\mu_9} & -e^{\mu_8} + e^{\mu_9} & -e^{\mu_8} + e^{\mu_9} \\
0 & 0 & 0 & e^{\mu_1+\mu_3} - e^{\mu_9} & e^{\mu_1+\mu_3} - e^{\mu_9} & e^{\mu_1+\mu_3} - e^{\mu_9} & e^{\mu_1+\mu_3} - e^{\mu_9} & e^{\mu_1+\mu_3} - e^{\mu_9} \\
0 & 0 & -e^{\mu_1+\mu_3} + e^{\mu_4} & 0 & 0 & 0 & e^{\mu_4} - e^{\mu_8} & e^{\mu_4} - e^{\mu_8} \\
0 & 0 & 0 & 0 & 0 & -e^{\mu_2+\mu_3} + e^{\mu_5} & -e^{\mu_2+\mu_3} + e^{\mu_8} & -e^{\mu_2+\mu_3} + e^{\mu_8} \\
-e^{\mu_2+\mu_6} + e^{\mu_7} & 0 & 0 & 0 & -e^{\mu_2+\mu_6} + e^{\mu_7} & 0 & 0 & 0 \\
0 & -e^{\mu_5+\mu_6} + e^{\mu_8} & 0 & 0 & 0 & 0 & -e^{\mu_5+\mu_6} + e^{\mu_8} & -e^{\mu_5+\mu_6} + e^{\mu_8}
\end{bmatrix}$$

$$H(\mu) = \begin{bmatrix} H_1 & H_2 \end{bmatrix}$$

$$\tilde{H}_1 = \begin{bmatrix}
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -e^{\mu_1} & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -e^{\mu_1+\mu_3} & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & -e^{\mu_4} & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -e^{\mu_5} \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1+e^{\mu_1} & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -e^{\mu_1+\mu_3}+e^{\mu_4} & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -e^{\mu_2+\mu_3}+e^{\mu_5} \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}$$

$$\tilde{H}_2 = \begin{bmatrix}
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & -e^{\mu_4} & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
-e^{\mu_2+\mu_6} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & -e^{\mu_5+\mu_6} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -e^{\mu_4} & 0 & 0 & 0 & -e^{\mu_4}+e^{\mu_5+\mu_6} & e^{\mu_5+\mu_6} & 0 \\
0 & 0 & 0 & -e^{\mu_1+\mu_3} & -e^{\mu_1+\mu_3}+e^{\mu_2+\mu_6} & -e^{\mu_1+\mu_3}+e^{\mu_5} & e^{\mu_5+\mu_6} & -e^{\mu_1+\mu_3}+e^{\mu_5+\mu_6} & 0 \\
0 & 0 & 0 & 0 & -e^{\mu_2+\mu_6} & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & -e^{\mu_5} & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & -e^{\mu_5+\mu_6} & -e^{\mu_5+\mu_6} & 0 \\
0 & 0 & -1+e^{\mu_4} & -1+e^{\mu_1+\mu_3} & e^{\mu_1+\mu_3}-e^{\mu_2+\mu_6} & -1+e^{\mu_1+\mu_3} & e^{\mu_4}-e^{\mu_5+\mu_6} & e^{\mu_1+\mu_3}-e^{\mu_5+\mu_6} & 0 \\
0 & 0 & 0 & -e^{\mu_2}+e^{\mu_1+\mu_3} & e^{\mu_1+\mu_3}-e^{\mu_2+\mu_6} & e^{\mu_1+\mu_3}-e^{\mu_5} & e^{\mu_2}-e^{\mu_5+\mu_6} & e^{\mu_1+\mu_3}-e^{\mu_5+\mu_6} & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -e^{\mu_1+\mu_3}+e^{\mu_4} & 0 & 0 & 0 & e^{\mu_4}-e^{\mu_5+\mu_6} & e^{\mu_4}-e^{\mu_5+\mu_6} & 0 \\
0 & 0 & 0 & 0 & 0 & -e^{\mu_2+\mu_3}+e^{\mu_5} & -e^{\mu_2+\mu_3}+e^{\mu_5+\mu_6} & -e^{\mu_2+\mu_3}+e^{\mu_5+\mu_6} & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & -1 & 0 & -1
\end{bmatrix}$$

$$
Q = \begin{bmatrix} Q_1 & Q_2 \end{bmatrix}
$$

$$
Q_1 = \begin{bmatrix}
1 & 0 & 0 & 0 & 1 & 0 \\
0 & 1 & 0 & 0 & -1 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}
$$

$$
Q_2 = \begin{bmatrix}
0 & 0 & 0 & -e^{\mu 4} & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & -e^{\mu 1+\mu 3} & 0 & 0 & -e^{\mu 1+\mu 3} & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -e^{\mu 4} & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & -1+e^{\mu 1+\mu 3} & 0 & 0 & 0 & -e^{\mu 1+\mu 3} & 0 & 0 & 0 & 0 \\
0 & -e^{\mu 2}+e^{\mu 1+\mu 3} & 0 & 0 & -1 & 0 & -e^{\mu 2} & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0
\end{bmatrix}
$$

$$
Q_3 = \begin{bmatrix}
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -e^{\mu 4} & 0 \\
0 & -e^{\mu 1+\mu 3} & 0 & -e^{\mu 1+\mu 3+\mu 6} & -e^{\mu 1+\mu 3}+e^{\mu 5} & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & -e^{\mu 4} & 0 & 0 & 0 & 0 \\
0 & -1+e^{\mu 1+\mu 3} & 0 & -e^{\mu 2+\mu 6} & -1+e^{\mu 1+\mu 3} & 0 & -e^{\mu 2} & 0 & 0 & 0 \\
0 & -e^{\mu 2}+e^{\mu 1+\mu 3} & 0 & -e^{\mu 2+\mu 6} & e^{\mu 1+\mu 3}-e^{\mu 5} & 0 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & -e^{\mu 2}+e^{\mu 5} & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1
\end{bmatrix}
$$

$$
Q_4 = \begin{bmatrix}
0 & 0 & 0 & -e^{\mu 4} & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & -e^{\mu 1+\mu 3} & 0 & 0 & -e^{\mu 1+\mu 3}+e^{\mu 2+\mu 6} & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -e^{\mu 4} & 0 & 0 & -e^{\mu 1+\mu 3} & 0 & 0 & 0 & 0 \\
0 & -1+e^{\mu 1+\mu 3} & 0 & -e^{\mu 2+\mu 6} & -1+e^{\mu 1+\mu 3} & 0 & -e^{\mu 2} & 0 & 0 & 0 \\
0 & -e^{\mu 2}+e^{\mu 1+\mu 3} & 0 & -e^{\mu 2+\mu 6} & e^{\mu 1+\mu 3}-e^{\mu 5} & 0 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & -e^{\mu 2}+e^{\mu 5} & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1
\end{bmatrix}
$$

$$
Q_5 = \begin{bmatrix}
0 & 0 & 0 & -e^{\mu 4} & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & -e^{\mu 1+\mu 3} & 0 & 0 & -e^{\mu 1+\mu 3}+e^{\mu 2+\mu 6} & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -e^{\mu 4} & 0 & 0 & -e^{\mu 1+\mu 3} & 0 & 0 & 0 & 0 \\
0 & -1+e^{\mu 1+\mu 3} & 0 & -e^{\mu 2+\mu 6} & -1+e^{\mu 1+\mu 3}-e^{\mu 5} & 0 & -e^{\mu 2} & 0 & 0 & 0 \\
0 & -e^{\mu 2}+e^{\mu 1+\mu 3} & 0 & -e^{\mu 2+\mu 6} & e^{\mu 1+\mu 3}-e^{\mu 5} & 0 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & -e^{\mu 2}+e^{\mu 5} & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1
\end{bmatrix}
$$

$$
Q_6 = \begin{bmatrix}
0 & 0 & 0 & -e^{\mu 4} & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & -e^{\mu 1+\mu 3} & 0 & 0 & -e^{\mu 1+\mu 3}+e^{\mu 2+\mu 6} & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -e^{\mu 4} & 0 & 0 & -e^{\mu 1+\mu 3} & 0 & 0 & 0 & 0 \\
0 & -1+e^{\mu 1+\mu 3} & 0 & -e^{\mu 2+\mu 6} & -1+e^{\mu 1+\mu 3}-e^{\mu 5} & 0 & -e^{\mu 2} & 0 & 0 & 0 \\
0 & -e^{\mu 2}+e^{\mu 1+\mu 3} & 0 & -e^{\mu 2+\mu 6} & e^{\mu 1+\mu 3}-e^{\mu 5} & 0 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & -e^{\mu 2}+e^{\mu 5} & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1
\end{bmatrix}
$$

$$
Q_7 = \begin{bmatrix}
0 & 0 & 0 & -e^{\mu 4} & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & -e^{\mu 1+\mu 3} & 0 & 0 & -e^{\mu 1+\mu 3}+e^{\mu 2+\mu 6} & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -e^{\mu 4} & 0 & 0 & -e^{\mu 1+\mu 3} & 0 & 0 & 0 & 0 \\
0 & -1+e^{\mu 1+\mu 3} & 0 & -e^{\mu 2+\mu 6} & -1+e^{\mu 1+\mu 3}-e^{\mu 5} & 0 & -e^{\mu 2} & 0 & 0 & 0 \\
0 & -e^{\mu 2}+e^{\mu 1+\mu 3} & 0 & -e^{\mu 2+\mu 6} & e^{\mu 1+\mu 3}-e^{\mu 5} & 0 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & -e^{\mu 2}+e^{\mu 5} & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1
\end{bmatrix}
$$

$$
Q_8 = \begin{bmatrix}
1 & 0 & 0 & 0 & 1 & 0 \\
0 & 1 & 0 & 0 & -1 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1
\end{bmatrix}
$$

5.3 Analysis of the complete network $\mathcal{N}_{11}$

In Section 5.1 it has been established that multistationarity is possible for three subnetworks of $\mathcal{N}_{11}$. For the sake of completeness the overall network is analysed using the methods established in Chapter 3. As for network $\mathcal{N}_{10}$, it was not possible for network $\mathcal{N}_{11}$ to determine $\nu,\ \lambda \in \mathbb{R}^{11}_{>0}$ independent of $\mu \in \mathbb{R}^9$. Thus the equation

$$Y^{(L)^T}\,\mu = \ln \frac{E\,\lambda}{E\,\nu}$$

is considered in the following form:

$$\begin{bmatrix} E & -\operatorname{diag}\left(e^{Y^{(L)^T}\mu}\right) E \end{bmatrix} \begin{pmatrix} \nu \\ \lambda \end{pmatrix},\ \begin{pmatrix} \nu \\ \lambda \end{pmatrix} > 0.$$

Using an unimodular matrix $U \in \mathbb{R}^{18\times 18}$ with $U\,E = \begin{bmatrix} I_{11} \\ 0_{7\times 11} \end{bmatrix}$ one obtains the following system of equations

$$\underbrace{U \begin{bmatrix} E & -\operatorname{diag}\left(e^{Y^{(L)^T}\mu}\right) E \end{bmatrix}}_{=:H(\mu)} \begin{pmatrix} \nu \\ \lambda \end{pmatrix} = 0,\ \begin{pmatrix} \nu \\ \lambda \end{pmatrix} > 0,$$

where $H(\mu)$ is given at the end of this Section on page 73. Note that the nonzero elements of rows 16 – 18 of $H(\mu)$ always have the same sign, for every μ. Thus, positive ν, λ only exists for those values of μ, where rows 16 – 18 of $H(\mu)$ are identical to zero, that is, for μ that satisfy

$$\mu_9 = \mu_3 + \mu_4 \tag{5.20a}$$
$$\mu_7 = \mu_2 + \mu_6 \tag{5.20b}$$
$$\mu_8 = \mu_5 + \mu_6. \tag{5.20c}$$

Note that (5.20a), (5.20b) and (5.20c) are the only restriction on $\mu \in \mathbb{R}^9$. Thus μ can be represented as (using new variables $\kappa \in \mathbb{R}^6$):

$$\mu = M_{\mathcal{N}_{11}} \tag{5.21a}$$

where

$$M_{\mathcal{N}_{11}} := \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 1 & 1 \\ 0 & 0 & 1 & 1 & 0 & 0 \end{bmatrix}. \tag{5.21b}$$

Note that each of rows $1, 2, 4, 5, 6, 8, 9, 10, 11$ contains exactly two entries, thus they are equal to

$$\nu_i - e^{\langle y_j^{(L)},\,\mu\rangle}\,\lambda_i = 0,$$

for some linear form $\langle y_j^{(L)},\,\mu\rangle$. Further note that rows 12–18 are independent of the ν_i, $i = 1, \ldots, 11$. Thus it is straightforward to obtain

$$\nu_1 = e^{\mu_1}\,\lambda_1 \tag{5.22a}$$
$$\nu_2 = e^{\mu_4}\,\lambda_2 \tag{5.22b}$$
$$\nu_4 = e^{\mu_9}\,\lambda_4 \tag{5.22c}$$
$$\nu_5 = e^{\mu_7}\,\lambda_5 \tag{5.22d}$$
$$\nu_6 = e^{\mu_8}\,\lambda_6 \tag{5.22e}$$
$$\nu_8 = e^{\mu_4}\,\lambda_8 \tag{5.22f}$$
$$\nu_9 = e^{\mu_5}\,\lambda_9 \tag{5.22g}$$
$$\nu_{10} = e^{\mu_7}\,\lambda_{10} \tag{5.22h}$$
$$\nu_{11} = e^{\mu_8}\,\lambda_{11}. \tag{5.22i}$$

Thus it remains to determine $\nu_3,\ \nu_7,\ \lambda_1,\ \ldots,\ \lambda_{11}$. This corresponds to a system of equations that involves rows 3, 7 and 12 – 15 and columns 3, 7 and 12 – 22 of $H(\mu)$. Note that λ_4, λ_5 and λ_6 do not occur in these equations anymore, thus the corresponding columns of $H(\mu)$ can be left out. Then the following system of equations remains:

$$Q(\mu)\,\tilde{\lambda} = 0,\ \tilde{\lambda} > 0,$$

where $Q(\mu) = H(\mu)[\{3,7,12,13,14,15\},\{12,13,14,18,19,20,21,22\}]$ and $\tilde{\lambda} = \left(\nu_3,\ \nu_7,\ \lambda_1,\ \lambda_2,\ \lambda_3,\ \lambda_7,\ \lambda_8,\ \lambda_9,\ \lambda_{10},\ \lambda_{11}\right)^T$. $Q\left(\mu\right)$ is of the form

$$Q(\mu) = \begin{bmatrix} 1 & 0 & 0 & 0 & -1 & q_1 & 0 & q_1 & q_1 & q_2 \\ 0 & 1 & 0 & 0 & 0 & -1 & 0 & -q_1 & q_3 & -q_2 \\ 0 & 0 & q_4 & 0 & 0 & q_5 & q_6 & q_5 & -q_3 & q_2 \\ 0 & 0 & 0 & 0 & 0 & q_7 & 0 & q_1 & -q_3 & q_2 \\ 0 & 0 & 0 & q_8 & 0 & q_9 & q_8 & q_9 & q_9 & q_2 \\ 0 & 0 & 0 & 0 & q_{10} & -q_1 & 0 & -q_1 & -q_1 & -q_2 \end{bmatrix} \tag{5.23}$$

with

$$\begin{aligned} q_1 &= e^{\mu_3+\mu_4} - e^{\mu_5}, & q_2 &= e^{\mu_3+\mu_4} - e^{\mu_5+\mu_6}, & q_3 &= -e^{\mu_3+\mu_4} + e^{\mu_2+\mu_6}, \\ q_4 &= -1 + e^{\mu_1}, & q_5 &= -1 + e^{\mu_3+\mu_4}, & q_6 &= -1 + e^{\mu_4}, \\ q_7 &= -e^{\mu_2} + e^{\mu_3+\mu_4}, & q_8 &= -e^{\mu_1+\mu_3} + e^{\mu_4}, & q_9 &= -e^{\mu_1+\mu_3} + e^{\mu_3+\mu_4}, \\ q_{10} &= -e^{\mu_2+\mu_3} + e^{\mu_5}. \end{aligned}$$

Thus any feasible inequality system

$$\begin{bmatrix} V & -\operatorname{diag}(\sigma) \end{bmatrix} \begin{pmatrix} \kappa \\ s \end{pmatrix} = 0, s > 0$$

with $\sigma \in \{-1,\ 0,\ 1\}^{10}$ and

$$V = \begin{bmatrix} 0 & 1 & 0 & 0 & -1 & 1 \\ 0 & 0 & -1 & -1 & 1 & 0 \\ 0 & 1 & -1 & -1 & 0 & 1 \\ 0 & 0 & -1 & -1 & 1 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ -1 & 0 & -1 & 1 & 0 & 0 \\ -1 & 0 & 0 & 1 & 0 & 0 \\ 0 & -1 & -1 & 0 & 1 & 0 \end{bmatrix} \tag{5.24}$$

defines a feasible signing for $Q\left(\mu\right)$. Following Algorithm 4, first $\Delta^{\mathcal{S}_{\mathcal{N}_{11}}}$, the set of all orthants $\delta \in \{-1,0,1\}^9$ with $\mathcal{S}_{\mathcal{N}_{11}} \cap \mathbb{R}^9_\delta \neq \emptyset$ has been obtained. Then the set $\Delta^+ = \left\{\ \delta \in \{-1,0,1\}^9 | \mathcal{M}_{\mathcal{N}_{11}} \cap \mathbb{R}^9_\delta \neq \emptyset\right.$ and $\exists \sigma \in \{-1,0,1\}^{10}$ such that $Q\left(\mu\right)$ is an L^+-matrix $\left.\ \right\}$ has been obtained. As $\Delta^{\mathcal{S}_{\mathcal{N}_{11}}} \cap \Delta^+ \neq \emptyset$, multistationarity has been established by Algorithm 4. The elements of $\Delta^{\mathcal{S}_{\mathcal{N}_{11}}} \cap \Delta^+$ are

$$\begin{array}{llllllllll} \delta_1{=}(-1, & 0, & 1, & -1, & 1, & -1, & -1, & 1, & 0)^T \\ \delta_2{=}(-1, & 1, & 1, & -1, & 1, & -1, & -1, & 1, & 0)^T \\ \delta_3{=}(-1, & 1, & 1, & -1, & 1, & -1, & -1, & 1, & 1)^T \\ \delta_4{=}(-1, & 1, & 1, & -1, & 1, & -1, & 1, & -1, & 1)^T \\ \delta_5{=}(-1, & 1, & 1, & -1, & 1, & -1, & 1, & 1, & 1)^T \\ \delta_6{=}(-1, & 1, & 1, & 1, & 1, & -1, & 1, & -1, & 1)^T \end{array} \tag{5.25}$$

All signings such that $Q(\mu)$ is an L^+ in one of the orthants given in (5.25), are

$$\begin{aligned}
\sigma_1 &= (-1, -1, -1, -1, 0, -1, 0, -1, 1, -1)^T \\
\sigma_2 &= (-1, -1, -1, -1, 0, -1, -1, -1, 1, -1)^T \\
\sigma_3 &= (-1, -1, -1, -1, 1, -1, -1, -1, 1, -1)^T \\
\sigma_4 &= (-1, -1, -1, -1, 1, -1, 0, -1, 1, -1)^T \\
\sigma_5 &= (-1, 0, -1, -1, 1, -1, -1, -1, 1, -1)^T \\
\sigma_6 &= (-1, 0, -1, -1, 1, -1, 0, -1, 1, -1)^T \\
\sigma_7 &= (-1, 1, -1, -1, 1, -1, -1, -1, 0, -1)^T \\
\sigma_8 &= (-1, 1, -1, -1, 1, -1, -1, -1, 1, -1)^T \\
\sigma_9 &= (-1, 1, -1, -1, 1, -1, 0, -1, 0, -1)^T \\
\sigma_{10} &= (-1, 1, -1, -1, 1, -1, 0, -1, 1, -1)^T \\
\sigma_{11} &= (-1, 1, 0, -1, 1, -1, -1, -1, 0, -1)^T \\
\sigma_{12} &= (-1, 1, 0, -1, 1, -1, -1, -1, 1, -1)^T \\
\sigma_{13} &= (-1, 1, -1, -1, 1, 1, -1, -1, 1, -1)^T \\
\sigma_{14} &= (-1, 1, 0, -1, 1, 1, -1, -1, 1, -1)^T
\end{aligned} \tag{5.26}$$

Table 5.2 shows, for which orthant δ as in (5.25) and which signing σ as in (5.26), the system

$$\begin{bmatrix} \operatorname{diag}(\delta) & -M_{\mathcal{N}_{11}} & 0_{10\times 10} \\ 0_{9\times 9} & V & -\operatorname{diag}(\sigma) \end{bmatrix} \begin{pmatrix} \xi \\ \kappa \\ s \end{pmatrix},\ \xi,\ s > 0$$

is feasible.

	σ_1	σ_2	σ_3	σ_4	σ_5	σ_6	σ_7	σ_8	σ_9	σ_{10}	σ_{11}	σ_{12}	σ_{13}	σ_{14}
δ_1	+													
δ_2		+												
δ_3			+	+	+	+	+	+	+	+				
δ_4							+	+			+	+		
δ_5			+	+	+	+	+	+	+	+	+	+		
δ_6													+	+

Table 5.2: Orthants and sign conditions for the ternary complex model

$$H_{tc}(\mu) = \begin{bmatrix} H_1 & H_2 \end{bmatrix}$$

$$H_1 = \left[\begin{array}{cccccccccccccccccccccccccccccccccccc}
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 & 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 & 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 & 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 & 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 & 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 & 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 & 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 & 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 & 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 & 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 & 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 & 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 & 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 & 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 & 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 & 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 & 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 & 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 & 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 & 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 & 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 & 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 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
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\end{array}\right]$$

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\end{array}\right]$$

5.4 Conclusions

In this chapter two equally plausible hypotheses describing the G1/S transition in *Saccharomyces cerevisiae* have been analysed. For both network structures multistationarity has been established, however, there is a difference: in contrast to the ternary complex model, where individual subunits can establish the desired switch–like behavior, the integrity of the binary complex model needs to be preserved for this function. This has important consequences for the robustness of the two models. Robustness, often, is defined as the resistance of qualitative network behavior to perturbations, for instance, in network structure or parameter values [60]. Clearly, the ternary model is more robust in this sense than the binary model, although capturing this effect quantitatively is difficult. Previously, it has been proposed that robustness can also serve as a measure of plausibility for biological network models because, in fluctuating environments, robustness of functions should have been key for evolutionary selection [47]. In this regard, subnetwork analysis could be used for model discrimination—the ternary complex mechanism would be considered as the more plausible one.

Chapter 6

Robustness of Multistationarity

A variety of biochemical reaction networks give rise to qualitative properties that are robust with respect to certain parameter variations (see [42, 48] and [60] 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 [59], 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 [19, 20].
In this chapter robustness of multistationarity in the Activation of an MAPK(K) and in a variety of other signal transduction motifs is analysed. For the Activation of an MAPK(K) robustness (and fragility) of multistationarity with respect to variations in the rate constants is examined in Section 6.1. For a variety of signal transduction motifs, which are the building blocks of many signal transduction networks robustness (and fragility) of multistationarity with respect to fluctuations in the total concentrations is examined in Section 6.2.

6.1 Robustness against variations in the rate constants

Multistationarity in the activation of an MAPK is a qualitative property with a *robust yet fragile* nature. This is a direct consequence of the results presented in Chapter 4, in particular of the definition of k, the parameter vector, as given in (4.7a) – (4.7f). Even for a specific steady state a 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 (4.7a) – (4.7f) for positive λ_i. Note that for a fixed vector a (4.7a) – (4.7f) are linear. In this case the right hand side of the equations defines a pointed polyhedral cone in $\mathbb{R}^{12}_{\geq 0}$. The generators of this 'parameter cone' can be obtained from the generators of $\ker(Y\, I_a) \cap \mathbb{R}^{12}_{\geq 0}$ via the linear transformation

$$P = \operatorname{diag}\left(\phi\left(a^{-1}\right)\right) E, \tag{6.1}$$

where E is a matrix composed of the generators of $\ker(Y\, I_a) \cap \mathbb{R}^{12}_{\geq 0}$. For a given vector a the vector of rate constants is given by

$$k = P\,\lambda,\ \lambda \in \mathbb{R}^6_{>0}. \tag{6.2}$$

Thus one can conclude that the property multistationarity is robust with respect to variations of k within the 'parameter cone' P. Moreover, as long as k is in the interior of P, the values of the steady state pair a, b are not affected. 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 6.1[1]. The results are displayed in Fig. 6.1 (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 $x_1(t)$

[1]Tables and Figures in this chapter are reprinted from [65] with permission from Elsevier

	$x_0^{(1)}$	$x_0^{(2)}$	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

Table 6.1: Initial conditions for the dynamic simulations in Fig. 6.1.

and $x_6(t)$. In 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^*. Table 6.2 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 (A.1a) – (A.1i).

	$x^{(1)}$	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

Table 6.2: 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 [11]).

The fragility of multistationarity Multistationarity is fragile with respect to variations of k perpendicular to P (or, more precise, to variations of k in the orthogonal complement of the subspace of $\mathbb{R}^{12}$ that contains P). To demonstrate this, the parameter vector was perturbed in the following way:

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

where

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

Note that $\langle u^{\perp},\, E_i\rangle = 0$, $i = 1, \ldots, 6$, 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. 6.2. 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 k_ϵ^{up} (corresponding to $\epsilon \approx 0.0017$) and k_ϵ^{low} (corresponding to $\epsilon \approx -0.1993$) are displayed in Table 6.3, as well. Dynamic simulations displayed in Fig. 6.3 confirm that multistationarity vanishes, once ϵ is outside the interval $(-0.1993, 0.0017)$. As long as the parameter vector is 'sufficiently close' to P, the system retains multistationarity (albeit for different steady states). If the parameter vector is 'far' from P multistationarity is no longer exhibited. Fig. 6.3 shows the effect of changing ϵ on $x_1(t)$. In the upper half ϵ varies from 0 to -0.25 and the initial condition $x_0^{(2)}$ is used. As long as $\epsilon > -0.1993$ the trajectories reach a steady state that is close to $x^{(2)}$ (i.e. the trajectories converge to the upper branch of the steady state solutions displayed in Fig. 6.2). In the lower half ϵ varies from 0 to 0.0017 and the initial condition x_0^{up} is used. As long as $\epsilon < 0.0017$ the trajectories

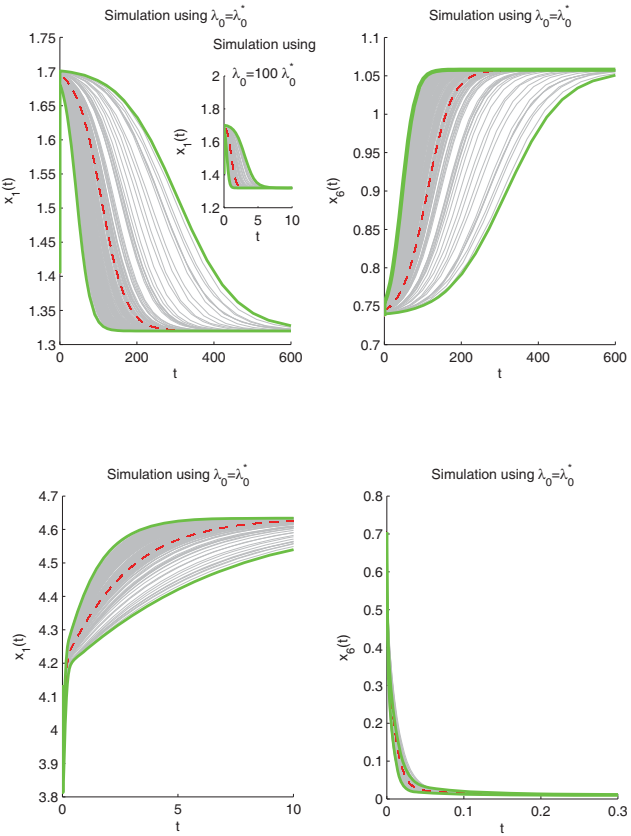


Figure 6.1: Temporal evolution of $x_1(t)$ and $x_6(t)$ for a set of randomly generated parameter vectors, using the initial condition $x_0^{(1)}$. The dashed red line indicates the temporal evolution obtained for the reference parameter vector k^*, the delimiting trajectories correspond to parameter sets labeled 'upper' and 'lower'.

reach a steady state that is close to $x^{(1)}$ (i.e. the trajectories converge to the lower branch of the steady state solutions displayed in Fig. 6.2). (Note that when integrating the ODEs using matlab®'s `ode15s` the bifurcation occurs somewhere between $\epsilon = 0.0018$ and $\epsilon = 0.0019$, whereas the bifurcation software *Matcont* locates the bifurcation at $\epsilon = 0.0017$.)

Quantification of robustness To quantify 'robustness' of multistationarity (or lack thereof) the parameter sets corresponding to those trajectories marked green in Fig. 6.1 were used to approximate the parameter range where multistationarity can occur. (This is, of course a poor approximation. But it is sufficient to back the argument). For each parameter an *elasticity coefficient* was defined that measures the difference between the upper and the lower value of the parameter in relation to the

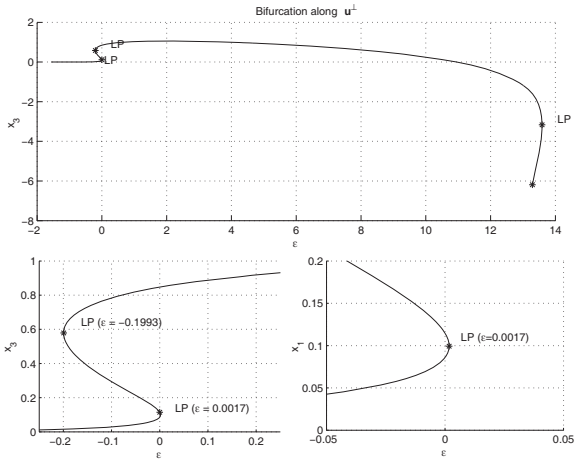


Figure 6.2: Bifurcation along $u^{\perp}$.

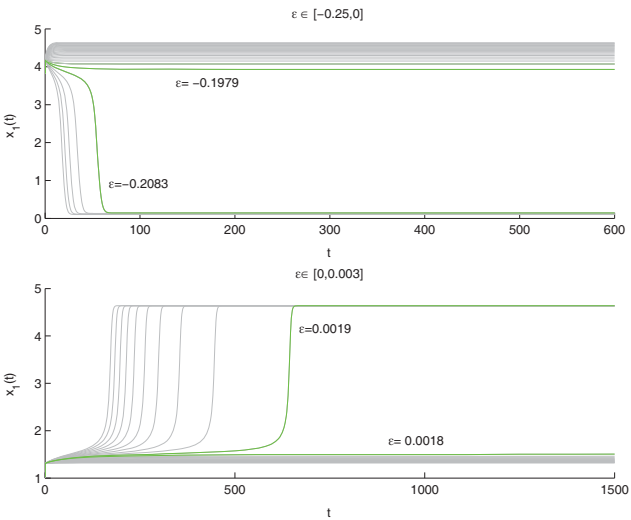


Figure 6.3: Dynamic simulation 'close' to bifurcation points for $\epsilon \approx -0.1993$ and $\epsilon \approx 0.0017$. The bifurcation occurs between the temporal evolution displayed in green.

'nominal' value k_i^*:

$$\eta_i := \frac{k_i^{up} - k_i^{low}}{k_i^*} \tag{6.3}$$

	k^*	k^{up}	k^{low}	k_ϵ^{up}	k_ϵ^{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

Table 6.3: Reference parameter vector k^* (as given in [11]), parameter vectors k^{up} and k^{low} obtained by random variations λ, with $\sum_i \lambda_i = \lambda_0^*$ and parameter vectors k_ϵ^{up} and k_ϵ^{low} obtained by varying k along $\epsilon\, u^\perp$.

Here k^{up} corresponds to the upper delimiting trajectory in the upper left part of Fig. 6.1 and k^{low} to the lower delimiting trajectory in the upper left part of Fig. 6.1. Both parameter vectors are given in Table 6.3. Using k_ϵ^{up} and k_ϵ^{low} in formula (6.3), the elasticities for perturbations along $u^\perp$ have been determined as well (see Table 6.4, second colum). The results are displayed in Fig. 6.4: the elasticities corresponding to variations within P as empty bars and those corresponding to variations along $u^\perp$ as filled 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 parameter cone P. On the other hand, if the parameter 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 6.3 it 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 6.3 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.

	$\frac{k_i^{up}-k_i^{low}}{k_i^*}$	$\frac{k_{\epsilon_i}^{up}-k_{\epsilon_i}^{low}}{k_i^*}$
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

Table 6.4: Elasticities obtained for a random sample of parameter vectors (column one) and for variation along $u^\perp$ (column two).

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 $\tilde{k}^{up}$, $\tilde{k}^{low}$ and the vector k^*. Let $\tilde{k}^{up} = \lambda_0\, k^*$ and $\tilde{k}^{low} = \frac{1}{\lambda_0}\, k^*$, 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

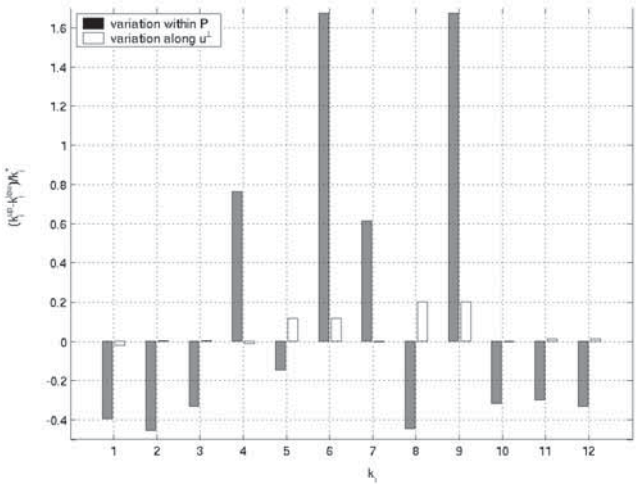


Figure 6.4: Robustness vs. fragility of multistationarity for the activation of an MAPK. Empty bars: Elasticities obtained for a random sample of parameters contained in the *parameter cone* P. Filled bars Elasticities obtained for a set of parameters created along $\epsilon u^{\perp}$.

$\tilde{k}^{up} = \lambda_0\, k^*$ and $\tilde{k}^{low} = \frac{1}{\lambda_0}\, k^*$ 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 $\tilde{k}^{low}$, $\tilde{k}^{up}$ and k^* in formula (6.3)).
However such an $\tilde{\eta}_i$ is not meaningful, as $\tilde{k}^{up}$ and $\tilde{k}^{low}$ correspond to a mere scaling of time that does not change the qualitative dynamic behaviour of the system, hence it is 'obvious' that the system retains multistationarity: to see this, let $k = \lambda_0\, \tilde{k}$ and recall that the ODEs derived from any biochemical reaction network endowed with mass action kinetics are linear in the vector of rate constants k:

$$\dot{x} = Y\, I_a\ \mathrm{diag}\,(k)\ \phi\,(x) = Y\, I_a\ \mathrm{diag}\,(\phi\,(x))\ k.$$

Thus using $k = \lambda_0\, \tilde{k}$ yields

$$\dot{x} = \lambda_0\, Y\, I_a\ \mathrm{diag}\,(\phi\,(x))\ \tilde{k}.$$

Clearly this corresponds to a scaling of time t with λ_0. 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 $\lambda^* \in I\!R^6_{>0}$, 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 6.3 (using e.g. the steady state x^* from Table 6.2, 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. 6.1 contains the trajectories of $x_1(t)$ obtained using $100\,\lambda_0^*$.

6.2 Robustness against concentration fluctuations for different signal transduction motifs

In [53] a variety of small reaction networks called motifs that can be found as subnetworks in many signal transduction networks is presented and analysed. Among them are network $\mathcal{N}_4$ described in Section 4 and $\mathcal{N}_{12}$, $\mathcal{N}_{13}$ and $\mathcal{N}_{14}$ given below. Here * is used to denote the activated form of protein A and E_i denotes activating or deactivating enzymes.

- Autocatalytic activation:

$$\begin{aligned} A + E_1 &\underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} AE_1 \xrightarrow{\mathbf{k_3}} A^* + E_1 \\ A + A^* &\underset{\mathbf{k_5}}{\overset{\mathbf{k_4}}{\rightleftharpoons}} AA^* \xrightarrow{\mathbf{k_6}} A^* + A^* \\ A^* + E_2 &\underset{\mathbf{k_8}}{\overset{\mathbf{k_7}}{\rightleftharpoons}} A^*E_2 \xrightarrow{\mathbf{k_9}} A + E_2 \end{aligned} \qquad (\mathcal{N}_{12})$$

- Distributive activation by enzyme E_1 and distributive deactivation by two different enzymes E_2 and E_3:

$$\begin{aligned} A + E_1 &\underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} A\,E_1 \xrightarrow{\mathbf{k_3}} A^* + E_1 \underset{\mathbf{k_5}}{\overset{\mathbf{k_4}}{\rightleftharpoons}} A^*\,E_1 \xrightarrow{\mathbf{k_6}} A^{**} + E_1 \\ A^{**} + E_3 &\underset{\mathbf{k_8}}{\overset{\mathbf{k_7}}{\rightleftharpoons}} A^{**}\,E_3 \xrightarrow{\mathbf{k_9}} A^* + E_3 \\ A^* + E_2 &\underset{\mathbf{k_{11}}}{\overset{\mathbf{k_{10}}}{\rightleftharpoons}} A^*\,E_2 \xrightarrow{\mathbf{k_{12}}} A + E_2 \end{aligned} \qquad (\mathcal{N}_{13})$$

- Autocatalytic activation and distributive deactivation by two different enzymes E_2 and E_3:

$$\begin{aligned} A + E_1 &\underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} A\,E_1 \xrightarrow{\mathbf{k_3}} A^* + E_1 \\ A^* + E_3 &\underset{\mathbf{k_5}}{\overset{\mathbf{k_4}}{\rightleftharpoons}} A^*\,E_3 \xrightarrow{\mathbf{k_6}} A + E_3 \\ 2\,A^* &\underset{\mathbf{k_8}}{\overset{\mathbf{k_7}}{\rightleftharpoons}} A^*\,A^* \xrightarrow{\mathbf{k_9}} A^{**} + A^* \underset{\mathbf{k_{11}}}{\overset{\mathbf{k_{10}}}{\rightleftharpoons}} A^*\,A^{**} \xrightarrow{\mathbf{k_{12}}} 2\,A^{**} \\ A^{**} + E_2 &\underset{\mathbf{k_{14}}}{\overset{\mathbf{k_{13}}}{\rightleftharpoons}} A^{**}\,E_2 \xrightarrow{\mathbf{k_{15}}} A^* + E_2 \end{aligned} \qquad (\mathcal{N}_{14})$$

Network $\mathcal{N}_4$, $\mathcal{N}_{12}$, $\mathcal{N}_{13}$ and $\mathcal{N}_{14}$ were the only networks considered in [53] that showed multistationarity. While in Section 6.1 robustness against variations in the k_i has been analysed, in this section robustness against variations in total concentrations is compared among networks $\mathcal{N}_4$, $\mathcal{N}_{12}$, $\mathcal{N}_{13}$ and $\mathcal{N}_{14}$. This is based on the results of Section 3. In Section 6.2.1 the steady state equations are analysed in the way described in Section 3 and in Section 6.2.2 robustness against variations in total concentrations is compared.

6.2.1 Analysis of the steady state equations

6.2.1.1 Network $\mathcal{N}_{12}$

The equations

$$Y^{(L)^T}\mu = \ln\frac{E\,\nu}{E\,\lambda}$$

for this network are

$$\mu_1 + \mu_2 = \ln \frac{\nu_1 + \nu_4}{\lambda_1 + \lambda_4} \tag{6.4a}$$
$$\mu_3 = \ln \frac{\nu_1}{\lambda_1} \tag{6.4b}$$
$$\mu_3 = \ln \frac{\nu_4}{\lambda_4} \tag{6.4c}$$
$$\mu_1 + \mu_4 = \ln \frac{\nu_2 + \nu_5}{\lambda_2 + \lambda_5} \tag{6.4d}$$
$$\mu_5 = \ln \frac{\nu_2}{\lambda_2} \tag{6.4e}$$
$$\mu_5 = \ln \frac{\nu_5}{\lambda_5} \tag{6.4f}$$
$$\mu_4 + \mu_6 = \ln \frac{\nu_3 + \nu_4 + \nu_5}{\lambda_3 + \lambda_4 + \lambda_5} \tag{6.4g}$$
$$\mu_7 = \ln \frac{\nu_3}{\lambda_3} \tag{6.4h}$$
$$\mu_7 = \ln \frac{\nu_4 + \nu_5}{\lambda_4 + \lambda_5} \tag{6.4i}$$

These equations are solvable, if and only if

$$\ln \frac{\nu_1}{\lambda_1} = \ln \frac{\nu_4}{\lambda_4}, \qquad \ln \frac{\nu_2}{\lambda_2} = \ln \frac{\nu_5}{\lambda_5}, \qquad \ln \frac{\nu_3}{\lambda_3} = \ln \frac{\nu_4 + \nu_5}{\lambda_4 + \lambda_5}.$$

Thus one obtains $\nu_1 = \lambda_1 \frac{\nu_4}{\lambda_4}$, $\nu_2 = \lambda_2 \frac{\nu_5}{\lambda_5}$ and $\nu_3 = \lambda_3 \frac{\nu_4+\nu_5}{\lambda_4+\lambda_5}$ and thus

$$\nu = \left(\lambda_1 \frac{\nu_4}{\lambda_4}, \lambda_2 \frac{\nu_5}{\lambda_5}, \lambda_3 \frac{\nu_4 + \nu_5}{\lambda_4 + \lambda_5}, \nu_4, \nu_5 \right), \tag{6.5}$$

with $\lambda \in \mathbb{R}^5_{>0}$ and $\nu_4, \nu_5 > 0$. Using ν as above, one obtains

$$\ln \frac{\nu_1 + \nu_4}{\lambda_1 + \lambda_4} = \ln \frac{\nu_4}{\lambda_4}, \qquad \ln \frac{\nu_2 + \nu_5}{\lambda_2 + \lambda_5} = \ln \frac{\nu_5}{\lambda_5}, \qquad \ln \frac{\nu_3 + \nu_4 + \nu_5}{\lambda_3 + \lambda_4 + \lambda_5} = \ln \frac{\nu_4 + \nu_5}{\lambda_4 + \lambda_5}.$$

Using the definitions

$$\kappa_1 := \ln \frac{\nu_4}{\lambda_4} \tag{6.6a}$$
$$\kappa_2 := \ln \frac{\nu_5}{\lambda_5} \tag{6.6b}$$
$$\kappa_3 := \frac{\nu_4 + \nu_5}{\lambda_4 + \lambda_5} \tag{6.6c}$$

and solving for μ, one obtains

$$\mu = \underbrace{\begin{bmatrix} 1 & 0 & 0 & 0 \\ -1 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ -1 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 \\ 1 & 0 & -1 & 1 \\ 0 & 0 & 0 & 1 \end{bmatrix}}_{=:M_{\mathcal{N}_{12}}} \begin{pmatrix} \mu_1 \\ \kappa_1 \\ \kappa_2 \\ \kappa_3 \end{pmatrix}. \tag{6.7}$$

If the κ_i are considered as free variables, (6.7) defines a linear subspace of $\mathbb{R}^7$. Note that in this case ν_4, ν_5, λ_4 and λ_5 are determined by (6.6a) – (6.6c): from (6.6a) follows $\nu_4 = \lambda_4 e^{\kappa_1}$ and from (6.6b) $\nu_5 = \lambda_5 e^{\kappa_2}$. Inserting in (6.6c) and solving for λ_5 yields

$$\lambda_5 = \lambda_4 \frac{e^{\kappa_1} - e^{\kappa_3}}{e^{\kappa_3} - e^{\kappa_2}}. \tag{6.8a}$$

Note that $\lambda_5 > 0$, if one of the following conditions holds

$$\kappa_1 > \kappa_3 \ > \ \kappa_2 \tag{6.8b}$$
$$\kappa_1 < \kappa_3 \ < \ \kappa_2. \tag{6.8c}$$

Note that (6.7) together with either (6.8b) or (6.8c) is a parametrization of positive steady states in the following sense: suppose (i) the κ_i are chosen such that either (6.8b) or (6.8c) holds, (ii) λ_5 is chosen such that (6.8a) holds and (iii) the remaining λ_i are assigned some positive values. Further suppose the vector a is a steady state. Then, by Lemma 1, $b = \operatorname{diag}(\operatorname{Exp}(\mu))\ a$ is a steady state as well.
To ensure that a is indeed a steady state apply again (3.10a) to obtain:

$$k_1 = \frac{\lambda_1 + \lambda_4}{a_1\, a_2} \qquad k_2 = \frac{\lambda_1}{a_3} \qquad k_3 = \frac{\lambda_4}{a_3} \tag{6.9a}$$
$$k_4 = \frac{\lambda_2 + \lambda_5}{a_1\, a_4} \qquad k_5 = \frac{\lambda_2}{a_5} \qquad k_6 = \frac{\lambda_5}{a_5} \tag{6.9b}$$
$$k_7 = \frac{\lambda_3 + \lambda_4 + \lambda_5}{a_4\, a_6} \qquad k_8 = \frac{\lambda_3}{a_7} \qquad k_9 = \frac{\lambda_4 + \lambda_5}{a_7} \tag{6.9c}$$

To find pairs of steady states that satisfy the conservation relations for the same c_i, Lemma 3 is used. To this end, let $\kappa := (\mu_2,\, \kappa_1,\, \kappa_2,\, \kappa_3)^T$ and $\mathcal{M}_{\mathcal{N}_{12}} := \left\{ \mu \in \mathbb{R}^7 \mid \mu = M_{\mathcal{N}_{12}}\, \kappa \text{ and either } \kappa_1 > \kappa_3 > \kappa_2 \text{ or } \kappa_1 < \kappa_3 < \kappa_2 \right\}$, with $M_{\mathcal{N}_{12}}$ as in (6.7). Further let $\mathcal{S}_{\mathcal{N}_{12}}$ be the stoichiometric subspace for $\mathcal{N}_{12}$. Using Algorithm 2, it is straightforward to establish that there are two orthants with $\mathcal{M}_{\mathcal{N}_{12}} \cap \mathbb{R}^7_\delta \neq \emptyset$ and $\mathcal{S}_{\mathcal{N}_{12}} \cap \mathbb{R}^7_\delta \neq \emptyset$:

$$\begin{aligned} \delta_1 &= (1, \ -1, \ \ 1, \ -1, \ -1, \ \ 1, \ -1)^T \\ \delta_2 &= (-1, \ \ 1, \ -1, \ \ 1, \ \ 1, \ -1, \ \ 1)^T \end{aligned} \tag{6.10}$$

However, $\delta_1 = -\delta_2$, thus these orthants give rise to the same pair of steady states (see Section 3.3) and only one of them need to be considered. Thus multistationarity has been established by Theorem 3. To obtain a pair of steady states, consider δ_1. All $\mu \in \mathcal{M}_{\mathcal{N}_{12}} \cap \mathbb{R}^7_{\delta_1}$ are given by

$$\mu_1 = \alpha_1 + \alpha_2 \tag{6.11a}$$
$$\mu_2 = -\alpha_1 \tag{6.11b}$$
$$\mu_3 = \alpha_2 \tag{6.11c}$$
$$\mu_4 = -\alpha_1 - \alpha_2 - \alpha_3 - \alpha_4 \tag{6.11d}$$
$$\mu_5 = -\alpha_3 - \alpha_4 \tag{6.11e}$$
$$\mu_6 = \alpha_1 + \alpha_2 + \alpha_4 \tag{6.11f}$$
$$\mu_7 = -\alpha_3, \tag{6.11g}$$

for $\alpha_i > 0$, $i = 1, 2, 3$. The κ_i, $i = 1, 2, 3$ can, of course, be expressed in terms of the α_i:

$$\kappa = \begin{pmatrix} -\alpha_1 \\ \alpha_2 \\ -\alpha_3 - \alpha_4 \\ -\alpha_3 \end{pmatrix}. \tag{6.12a}$$

Thus one obtains the following expression for λ_5:

$$\lambda_5 = \lambda_4\, \frac{(e^{\alpha_2} - e^{-\alpha_3})}{e^{-\alpha_3} - e^{-\alpha_3 - \alpha_4}}. \tag{6.12b}$$

All $v \in \mathcal{S}_{\mathcal{N}_{12}} \cap \mathbb{R}^7_{\delta_1}$ are given by

$$v_1 = \beta_1 + \beta_5 + 2\,\beta_6 \tag{6.13a}$$
$$v_2 = -\beta_2 - 2\,\beta_3 - \beta_4 \tag{6.13b}$$
$$v_3 = \beta_2 + 2\,\beta_3 + \beta_4 \tag{6.13c}$$
$$v_4 = -\beta_4 - \beta_5 \tag{6.13d}$$
$$v_5 = -\beta_3 - \beta_6 \tag{6.13e}$$
$$v_6 = \beta_1 + \beta_2 \tag{6.13f}$$
$$v_7 = -\beta_1 - \beta_2, \tag{6.13g}$$

for $\beta_i > 0$, $i = 1, \ldots, 6$. As described in Section 3.3, it is possible to state a and b using the generators of $\mathcal{M}_{\mathcal{N}_{12}} \cap \mathbb{R}^7_\delta$ and $\mathcal{SN}_{12} \cap \mathbb{R}^7_\delta$ in (3.30a). This leads to the following formulas for the steady state a

$$a_1 = \frac{\beta_1 + \beta_5 + 2\,\beta_6}{-1 + e^{\alpha_1+\alpha_2}} \qquad a_2 = \frac{-\beta_2 - 2\,\beta_3 - \beta_4}{-1 + e^{-\alpha_1}} \tag{6.14a}$$

$$a_3 = \frac{\beta_2 + 2\,\beta_3 + \beta_4}{-1 + e^{\alpha_2}} \qquad a_4 = \frac{-\beta_4 - \beta_5}{-1 + e^{-\alpha_1-\alpha_2-\alpha_3-\alpha_4}} \tag{6.14b}$$

$$a_5 = \frac{-\beta_3 - \beta_6}{-1 + e^{-\alpha_3-\alpha_4}} \qquad a_6 = \frac{\beta_1 + \beta_2}{-1 + e^{\alpha_1+\alpha_2+\alpha_4}} \tag{6.14c}$$

$$a_7 = \frac{-\beta_1 - \beta_2}{-1 + e^{-\alpha_3}} \tag{6.14d}$$

and the steady state b

$$b_1 = \frac{(\beta_1 + \beta_5 + 2\,\beta_6)\,e^{\alpha_1+\alpha_2}}{-1 + e^{\alpha_1+\alpha_2}} \qquad b_2 = \frac{(-\beta_2 - 2\,\beta_3 - \beta_4)\,e^{-\alpha_1}}{-1 + e^{-\alpha_1}} \tag{6.15a}$$

$$b_3 = \frac{(\beta_2 + 2\,\beta_3 + \beta_4)\,e^{\alpha_2}}{-1 + e^{\alpha_2}} \qquad b_4 = \frac{(-\beta_4 - \beta_5)\,e^{-\alpha_1-\alpha_2-\alpha_3-\alpha_4}}{-1 + e^{-\alpha_1-\alpha_2-\alpha_3-\alpha_4}} \tag{6.15b}$$

$$b_5 = \frac{(-\beta_3 - \beta_6)\,e^{-\alpha_3-\alpha_4}}{-1 + e^{-\alpha_3-\alpha_4}} \qquad b_6 = \frac{(\beta_1 + \beta_2)\,e^{\alpha_1+\alpha_2+\alpha_4}}{-1 + e^{\alpha_1+\alpha_2+\alpha_4}} \tag{6.15c}$$

$$b_7 = \frac{(-\beta_1 - \beta_2)\,e^{-\alpha_3}}{-1 + e^{-\alpha_3}}. \tag{6.15d}$$

6.2.1.2 Network $\mathcal{N}_{13}$

The equations

$$Y^{(L)^T}\,\mu = \ln \frac{E\,\nu}{E\,\lambda}$$

for this network are

$$\begin{aligned}
\mu_1 + \mu_2 &= \ln \frac{\nu_1 + \nu_5}{\lambda_1 + \lambda_5} \\
\mu_3 &= \ln \frac{\nu_1}{\lambda_1} \\
\mu_3 &= \ln \frac{\nu_5}{\lambda_5} \\
\mu_2 + \mu_4 &= \ln \frac{\nu_2 + \nu_6}{\lambda_2 + \lambda_6} \\
\mu_5 &= \ln \frac{\nu_2}{\lambda_2} \\
\mu_5 &= \ln \frac{\nu_6}{\lambda_6} \\
\mu_6 + \mu_7 &= \ln \frac{\nu_3 + \nu_6}{\lambda_3 + \lambda_6} \\
\mu_8 &= \ln \frac{\nu_3}{\lambda_3} \\
\mu_8 &= \ln \frac{\nu_6}{\lambda_6} \\
\mu_4 + \mu_9 &= \ln \frac{\nu_4 + \nu_5}{\lambda_4 + \lambda_5} \\
\mu_{10} &= \ln \frac{\nu_4}{\lambda_4} \\
\mu_{10} &= \ln \frac{\nu_5}{\lambda_5}
\end{aligned}$$

These equations are solvable, if and only if

$$\ln \frac{\nu_1}{\lambda_1} = \ln \frac{\nu_5}{\lambda_5}, \qquad \ln \frac{\nu_2}{\lambda_2} = \ln \frac{\nu_6}{\lambda_6}, \qquad \ln \frac{\nu_3}{\lambda_3} = \ln \frac{\nu_6}{\lambda_6}, \qquad \ln \frac{\nu_4}{\lambda_4} = \ln \frac{\nu_5}{\lambda_5}.$$

Thus one obtains

$$\nu = \left(\lambda_1 \frac{\nu_5}{\lambda_5}, \lambda_2 \frac{\nu_6}{\lambda_6}, \lambda_3 \frac{\nu_6}{\lambda_6}, \lambda_4 \frac{\nu_5}{\lambda_5}, \nu_5, \nu_6\right)^T \tag{6.16}$$

with $\lambda \in \mathbb{R}^6_{>0}$ and $\nu_5, \nu_6 > 0$, free. Note that using ν as above, one obtains

$$\ln\frac{\nu_1+\nu_5}{\lambda_1+\lambda_5} = \ln\frac{\nu_4+\nu_5}{\lambda_4+\lambda_5} = \ln\frac{\nu_5}{\lambda_5}$$

and

$$\ln\frac{\nu_2+\nu_6}{\lambda_2+\lambda_6} = \ln\frac{\nu_3+\nu_6}{\lambda_3+\lambda_6} = \ln\frac{\nu_6}{\lambda_6}.$$

Using $\kappa_1 := \ln\frac{\nu_5}{\lambda_5}$ and $\kappa_2 := \ln\frac{\nu_6}{\lambda_6}$ and solving for μ, one finally obtains

$$\mu = \underbrace{\begin{bmatrix} -1 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ -1 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 \\ 0 & -1 & 0 & 1 \\ 0 & 0 & 0 & 1 \\ 1 & 0 & 1 & -1 \\ 0 & 0 & 1 & 0 \end{bmatrix}}_{=:M_{\mathcal{N}_{13}}} \begin{pmatrix} \mu_2 \\ \mu_6 \\ \kappa_1 \\ \kappa_2 \end{pmatrix} \tag{6.17}$$

Let $\mathcal{M}_{\mathcal{N}_{13}} = [M_{\mathcal{N}_{13}}]$ and observe that $\mathcal{M}_{\mathcal{N}_{13}} \cap \mathbb{R}^{10}_\delta \neq \emptyset$ and $\mathcal{S}_{\mathcal{N}_{13}} \cap \mathbb{R}^{10}_\delta \neq \emptyset$ holds in the orthant δ_1:

$$\delta_1 = (-1, \quad 1, \quad -1, \quad -1, \quad 1, \quad 1, \quad -1, \quad 1, \quad 1, \quad -1)^T \tag{6.18}$$

Thus multistationarity has been established by Theorem 3. Using generators of $\mathcal{M}_{\mathcal{N}_{13}} \cap \mathbb{R}^{10}_\delta$ and $\mathcal{S}_{\mathcal{N}_{13}} \cap \mathbb{R}^{10}_\delta$ a parametrization of all pairs of steady states a, b that satisfy $W^T b = W^T a$ is obtained:

$$\begin{array}{llll} a_1 &= \frac{-\beta_7-\beta_8}{-1+e^{-\alpha_1-\alpha_3-2\,\alpha_4}}, & a_2 &= \frac{\beta_4+\beta_9}{-1+e^{\alpha_1+\alpha_3+\alpha_4}}, \\ a_3 &= \frac{-\beta_3-\beta_4-\beta_9}{-1+e^{-\alpha_4}}, & a_4 &= \frac{-\beta_1-\beta_2}{-1+e^{-\alpha_3-\alpha_4}}, \\ a_5 &= \frac{\beta_3}{-1+e^{\alpha_1}}, & a_6 &= \frac{\beta_2+\beta_5+\beta_7+\beta_9}{-1+e^{\alpha_1+\alpha_2}}, \\ a_7 &= \frac{-\beta_1-\beta_4-\beta_6-\beta_8}{-1+e^{-\alpha_2}}, & a_8 &= \frac{\beta_1+\beta_4+\beta_6+\beta_8}{-1+e^{\alpha_1}}, \\ a_9 &= \frac{\beta_5+\beta_6}{-1+e^{\alpha_3}} & a_{10} &= \frac{-\beta_5-\beta_6}{-1+e^{-\alpha_4}}. \end{array} \tag{6.19a}$$

$$\begin{array}{llll} b_1 &= \frac{(-\beta_7-\beta_8)\,e^{-\alpha_1-\alpha_3-2\,\alpha_4}}{-1+e^{-\alpha_1-\alpha_3-2\,\alpha_4}}, & b_2 &= \frac{(\beta_4+\beta_9)\,e^{\alpha_1+\alpha_3+\alpha_4}}{-1+e^{\alpha_1+\alpha_3+\alpha_4}}, \\ b_3 &= \frac{(-\beta_3-\beta_4-\beta_9)\,e^{-\alpha_4}}{-1+e^{-\alpha_4}}, & b_4 &= \frac{(-\beta_1-\beta_2)\,e^{-\alpha_3-\alpha_4}}{-1+e^{-\alpha_3-\alpha_4}}, \\ b_5 &= \frac{\beta_3\,e^{\alpha_1}}{-1+e^{\alpha_1}}, & b_6 &= \frac{(\beta_2+\beta_5+\beta_7+\beta_9)\,e^{\alpha_1+\alpha_2}}{-1+e^{\alpha_1+\alpha_2}}, \\ b_7 &= \frac{(-\beta_1-\beta_4-\beta_6-\beta_8)\,e^{-\alpha_2}}{-1+e^{-\alpha_2}}, & b_8 &= \frac{(\beta_1+\beta_4+\beta_6+\beta_8)\,e^{\alpha_1}}{-1+e^{\alpha_1}}, \\ b_9 &= \frac{(\beta_5+\beta_6)\,e^{\alpha_3}}{-1+e^{\alpha_3}}, & b_{10} &= \frac{(-\beta_5-\beta_6)\,e^{-\alpha_4}}{-1+e^{-\alpha_4}}. \end{array} \tag{6.19b}$$

Using the a_i given in (6.19a) one obtains a parametrization of the rate constants that ensure that $a = (a_i)$ and $b = (b_i)$, $i = 1, \ldots, 10$ are steady states. Note that here $\lambda_1 > 0, \ldots, \lambda_6 > 0$ can be can be chosen freely.

$$\begin{array}{llllll} k_1 &= \frac{\lambda_1+\lambda_5}{a_1\,a_2}, & k_2 &= \frac{\lambda_1}{a_3}, & k_3 &= \frac{\lambda_5}{a_3}, \\ k_4 &= \frac{\lambda_2+\lambda_6}{a_2\,a_4}, & k_5 &= \frac{\lambda_2}{a_5}, & k_6 &= \frac{\lambda_6}{a_5}, \\ k_7 &= \frac{\lambda_3+\lambda_6}{a_6\,a_7}, & k_8 &= \frac{\lambda_3}{a_8}, & k_9 &= \frac{\lambda_6}{a_8}, \\ k_{10} &= \frac{\lambda_4+\lambda_5}{a_4\,a_9}, & k_{11} &= \frac{\lambda_4}{a_4\,a_9}, & k_{12} &= \frac{\lambda_5}{a_{10}} \end{array} \tag{6.20a}$$

6.2.1.3 Network $\mathcal{N}_{14}$

The equations

$$Y^{(L)^T}\,\mu = \ln\frac{E\,\nu}{E\,\lambda}$$

for this network are

$$\begin{aligned}
\mu_1+\mu_2 &= \ln\frac{\nu_1+\nu_6}{\lambda_1+\lambda_6}\\
\mu_3 &= \ln\frac{\nu_1}{\lambda_1}\\
\mu_3 &= \ln\frac{\nu_6}{\lambda_6}\\
\mu_4+\mu_5 &= \ln\frac{\nu_2+\nu_6}{\lambda_2+\lambda_6}\\
\mu_6 &= \ln\frac{\nu_2}{\lambda_2}\\
\mu_6 &= \ln\frac{\nu_6}{\lambda_6}\\
2\,\mu_4 &= \ln\frac{\nu_3+\nu_7}{\lambda_3+\lambda_7}\\
\mu_7 &= \ln\frac{\nu_3}{\lambda_3}\\
\mu_7 &= \ln\frac{\nu_7}{\lambda_7}\\
\mu_4+\mu_8 &= \ln\frac{\nu_4+\nu_8}{\lambda_4+\lambda_8}\\
\mu_9 &= \ln\frac{\nu_4}{\lambda_4}\\
\mu_9 &= \ln\frac{\nu_8}{\lambda_8}\\
\mu_{10}+\mu_8 &= \ln\frac{\nu_5+\nu_7+\nu_8}{\lambda_5+\lambda_7+\lambda_8}\\
\mu_{11} &= \ln\frac{\nu_5}{\lambda_5}\\
\mu_{11} &= \ln\frac{\nu_7+\nu_8}{\lambda_7+\lambda_8}
\end{aligned}$$

These equations are solvable, if and only if

$$\ln\frac{\nu_1}{\lambda_1}=\ln\frac{\nu_6}{\lambda_6},\qquad \ln\frac{\nu_2}{\lambda_2}=\ln\frac{\nu_6}{\lambda_6},\qquad \ln\frac{\nu_3}{\lambda_3}=\ln\frac{\nu_7}{\lambda_7},\qquad \ln\frac{\nu_4}{\lambda_4}=\ln\frac{\nu_8}{\lambda_8},\qquad \ln\frac{\nu_5}{\lambda_5}=\ln\frac{\nu_7+\nu_8}{\lambda_7+\lambda_8}.$$

Thus one obtains $\nu_1=\lambda_1\frac{\nu_6}{\lambda_6}$, $\nu_2=\lambda_2\frac{\nu_6}{\lambda_6}$, $\nu_3=\lambda_3\frac{\nu_7}{\lambda_7}$, $\nu_4=\lambda_4\frac{\nu_8}{\lambda_8}$ and $\nu_5=\lambda_5\frac{\nu_7+\nu_8}{\lambda_7+\lambda_8}$. Thus one finally obtains

$$\nu=\left(\lambda_1\frac{\nu_6}{\lambda_6},\,\lambda_2\frac{\nu_6}{\lambda_6},\,\lambda_3\frac{\nu_7}{\lambda_7},\,\lambda_4\frac{\nu_8}{\lambda_8},\,\lambda_5\frac{\nu_7+\nu_8}{\lambda_7+\lambda_8},\,\nu_6,\,\nu_7,\,\nu_8\right)^T.$$

Note that using ν as above, one obtains

$$\begin{aligned}
\ln\frac{\nu_1+\nu_6}{\lambda_1+\lambda_6}=\ln\frac{\nu_2+\nu_6}{\lambda_2+\lambda_6}&=\ln\frac{\nu_6}{\lambda_6},\\
\ln\frac{\nu_3+\nu_7}{\lambda_3+\lambda_7}&=\ln\frac{\nu_7}{\lambda_7},\\
\ln\frac{\nu_4+\nu_8}{\lambda_4+\lambda_8}&=\ln\frac{\nu_8}{\lambda_8},
\end{aligned}$$

and

$$\ln\frac{\nu_5+\nu_7+\nu_8}{\lambda_5+\lambda_7+\lambda_8}=\ln\frac{\nu_7+\nu_8}{\lambda_7+\lambda_8}.$$

Using

$$\kappa_1 := \ln \frac{\nu_6}{\lambda_6} \tag{6.21a}$$

$$\kappa_2 := \ln \frac{\nu_7}{\lambda_7} \tag{6.21b}$$

$$\kappa_3 := \ln \frac{\nu_8}{\lambda_8} \tag{6.21c}$$

$$\kappa_4 := \ln \frac{\nu_7 + \nu_8}{\lambda_7 + \lambda_8} \tag{6.21d}$$

and solving for μ, one obtains

$$\mu = \underbrace{\begin{bmatrix} -1 & 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & \frac{1}{2} & 0 & 0 \\ 0 & 1 & -\frac{1}{2} & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & -\frac{1}{2} & 1 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & \frac{1}{2} & -1 & 1 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}}_{=:M_{\mathcal{N}_{14}}} \begin{pmatrix} \mu_2 \\ \kappa_1 \\ \kappa_2 \\ \kappa_3 \\ \kappa_4 \end{pmatrix} \tag{6.22}$$

If the κ_i are considered as free variables, (6.22) defines a linear subspace of $\mathbb{R}^{11}$. Note that in this case ν_7, ν_8, λ_7 and λ_8 are determined by (6.21b) – (6.21d): from (6.21b) follows $\nu_7 = \lambda_7 e^{\kappa_2}$ and from (6.21c) $\nu_8 = \lambda_8 e^{\kappa_3}$. Inserting in (6.21d) and solving for λ_8 yields

$$\lambda_8 = \frac{e^{\kappa_4} - e^{\kappa_2}}{e^{\kappa_3} - e^{\kappa_4}} \lambda_7 \tag{6.23}$$

Thus $\lambda_8 > 0$, if the κ_i have to satisfy either one of the relations

$$\kappa_3 > \kappa_4 > \kappa_2 \tag{6.24a}$$

$$\kappa_3 < \kappa_4 < \kappa_2 \tag{6.24b}$$

Let $\kappa := (\mu_2, \kappa_1, \kappa_2, \kappa_3, \kappa_4)^T$ and $\mathcal{M}_{\mathcal{N}_{14}} := \big\{\mu \in \mathbb{R}^{11} \,|\, \mu = M_{\mathcal{N}_{14}}\, \kappa$ and either $\kappa_3 > \kappa_4 > \kappa_2$ or $\kappa_3 < \kappa_4 < \kappa_2\big\}$. Observe that $\mathcal{S}_{\mathcal{N}_{14}} \cap \mathbb{R}^{11}_\delta \neq \emptyset$ and $\mathcal{M}_{\mathcal{N}_{14}} \cap \mathbb{R}^{11}_\delta \neq \emptyset$ hold for δ_1 and $-\delta_1$ as given below:

$$\delta_1 = (-1, \quad 1, \quad -1, \quad -1, \quad 1, \quad -1, \quad -1, \quad 1, \quad 1, \quad -1, \quad 1)^T \tag{6.25}$$

The pairs of steady states that satisfy the conservation relations are given by:

$$\begin{aligned}
a_1 &= \tfrac{-\beta_1-\beta_8-2\,\beta_9}{-1+e^{-\alpha_1-\alpha_3}}, & a_2 &= \tfrac{\beta_{10}+2\,\beta_{11}+\beta_2}{-1+e^{\alpha_1}}, \\
a_3 &= \tfrac{-\beta_{10}-2\,\beta_{11}-\beta_2}{-1+e^{-\alpha_3}}, & a_4 &= \tfrac{-\beta_{12}-2\,\beta_{13}-\beta_3}{-1+e^{\frac{1}{2}(-2\,\alpha_2-2\,\alpha_3)}}, \\
a_5 &= \tfrac{\beta_{14}+2\,\beta_{15}+\beta_4}{-1+e^{-\alpha_3+\frac{1}{2}(2\,\alpha_2+2\,\alpha_3)}}, & a_6 &= \tfrac{-\beta_{14}-2\,\beta_{15}-\beta_4}{-1+e^{-\alpha_3}}, \\
a_7 &= \tfrac{-\beta_5-\beta_6-\beta_7}{-1+e^{-2\,\alpha_2-2\,\alpha_3}}, & a_8 &= \tfrac{\beta_{10}+\beta_{12}+\beta_{14}+2\,\beta_7+\beta_8}{-1+e^{\frac{1}{2}(2\,\alpha_2+2\,\alpha_3)+\alpha_4+\alpha_5}}, \\
a_9 &= \tfrac{\beta_{11}+\beta_{13}+\beta_{15}+\beta_6+\beta_9}{-1+e^{\alpha_4+\alpha_5}}, & a_{10} &= \tfrac{-\beta_1-\beta_2-\beta_3-\beta_4-2\,\beta_5}{-1+e^{\frac{1}{2}(-2\,\alpha_2-2\,\alpha_3)-\alpha_5}}, \\
a_{11} &= \tfrac{\beta_1+\beta_2+\beta_3+\beta_4+2\,\beta_5}{-1+e^{\alpha_4}}.
\end{aligned} \tag{6.26a}$$

$$\begin{aligned}
b_1 &= \tfrac{(-\beta_1-\beta_8-2\,\beta_9)\,e^{-\alpha_1-\alpha_3}}{-1+e^{-\alpha_1-\alpha_3}}, & b_2 &= \tfrac{(\beta_{10}+2\,\beta_{11}+\beta_2)\,e^{\alpha_1}}{-1+e^{\alpha_1}}, \\
b_3 &= \tfrac{(-\beta_{10}-2\,\beta_{11}-\beta_2)\,e^{-\alpha_3}}{-1+e^{-\alpha_3}}, & b_4 &= \tfrac{(-\beta_{12}-2\,\beta_{13}-\beta_3)\,e^{\frac{1}{2}(-2\,\alpha_2-2\,\alpha_3)}}{-1+e^{\frac{1}{2}(-2\,\alpha_2-2\,\alpha_3)}}, \\
b_5 &= \tfrac{(\beta_{14}+2\,\beta_{15}+\beta_4)\,e^{-\alpha_3+\frac{1}{2}(2\,\alpha_2+2\,\alpha_3)}}{-1+e^{-\alpha_3+\frac{1}{2}(2\,\alpha_2+2\,\alpha_3)}}, & b_6 &= \tfrac{(-\beta_{14}-2\,\beta_{15}-\beta_4)\,e^{-\alpha_3}}{-1+e^{-\alpha_3}}, \\
b_7 &= \tfrac{(-\beta_5-\beta_6-\beta_7)\,e^{-2\,\alpha_2-2\,\alpha_3}}{-1+e^{-2\,\alpha_2-2\,\alpha_3}}, & b_8 &= \tfrac{(\beta_{10}+\beta_{12}+\beta_{14}+2\,\beta_7+\beta_8)\,e^{\frac{1}{2}(2\,\alpha_2+2\,\alpha_3)+\alpha_4+\alpha_5}}{-1+e^{\frac{1}{2}(2\,\alpha_2+2\,\alpha_3)+\alpha_4+\alpha_5}}, \\
b_9 &= \tfrac{(\beta_{11}+\beta_{13}+\beta_{15}+\beta_6+\beta_9)\,e^{\alpha_4+\alpha_5}}{-1+e^{\alpha_4+\alpha_5}}, & b_{10} &= \tfrac{(-\beta_1-\beta_2-\beta_3-\beta_4-2\,\beta_5)\,e^{\frac{1}{2}(-2\,\alpha_2-2\,\alpha_3)-\alpha_5}}{-1+e^{\frac{1}{2}(-2\,\alpha_2-2\,\alpha_3)-\alpha_5}}, \\
b_{11} &= \tfrac{(\beta_1+\beta_2+\beta_3+\beta_4+2\,\beta_5)\,e^{\alpha_4}}{-1+e^{\alpha_4}}.
\end{aligned} \tag{6.26b}$$

Using the a_i given in (6.19a) one obtains a parametrization of rate constants that ensure that $a = (a_i)$ and $b = (b_i)$, $i = 1, \ldots, 11$ from (6.26a) and (6.26b) are steady states. Note that $\lambda_i > 0$, $i = 1, \ldots, 7$ can be chosen freely, but λ_8 must satisfy (6.23). To this end a parametrization of the κ_i associated with $\mu \in \mathcal{S}_{\mathcal{N}_{14}} \cap I\!R^{11}_{\delta_1}$ is obtained:

$$\kappa = \begin{pmatrix} \alpha_1 \\ -\alpha_3 \\ -2\,\alpha_2 - 2\,\alpha_3 \\ \alpha_4 + \alpha_5 \\ \alpha_4 \end{pmatrix}. \tag{6.27a}$$

Then one obtains the following expression for λ_8:

$$\lambda_8 = \lambda_7 \, \frac{-e^{-2\,\alpha_2 - 2\,\alpha_3} + e^{\alpha_4}}{-e^{\alpha_4} + e^{\alpha_4 + \alpha_5}}. \tag{6.27b}$$

The rate constants are then given by

$$\begin{array}{llllll} k_1 = \frac{\lambda_1+\lambda_6}{a_1\,a_2}, & k_2 = \frac{\lambda_1}{a_3}, & k_3 = \frac{\lambda_6}{a_3}, \\ k_4 = \frac{\lambda_2+\lambda_6}{a_4\,a_5}, & k_5 = \frac{\lambda_2}{a_6}, & k_6 = \frac{\lambda_6}{a_6}, \\ k_7 = \frac{\lambda_3+\lambda_7}{a_4{}^2}, & k_8 = \frac{\lambda_3}{a_7}, & k_9 = \frac{\lambda_7}{a_7}, \\ k_{10} = \frac{\lambda_4+\lambda_8}{a_4\,a_8}, & k_{11} = \frac{\lambda_4}{a_9}, & k_{12} = \frac{\lambda_8}{a_9}, \\ k_{13} = \frac{\lambda_5+\lambda_7+\lambda_8}{a_8\,a_{10}}, & k_{14} = \frac{\lambda_5}{a_{11}}, & k_{15} = \frac{\lambda_7+\lambda_8}{a_{11}}. \end{array} \tag{6.28a}$$

6.2.2 Comparing robustness against concentration fluctuations

In this section the robustness of multistationarity with respect to variations in A_T, the total concentration of protein A is analysed. This is done to asses the usability of $\mathcal{N}_4$, $\mathcal{N}_{12}$, $\mathcal{N}_{13}$ and $\mathcal{N}_{14}$ as a switching device. Since concentrations typically fluctuate, a useful switch should maintain bistability over a wide range of concentrations. To this end, the following steps have been performed:

(1) For each of the parametrizations of steady state pairs obtained for $\mathcal{N}_4$, $\mathcal{N}_{12}$, $\mathcal{N}_{13}$ and $\mathcal{N}_{14}$ in Section 6.2.1, the vectors α and β were chosen randomly (for each network approximately 1000 vectors α and β were created). Thus one obtains a set of 1000 steady state pairs for each network: $\left(a_i^{(j)},\, b_i^{(j)}\right)$, $i = 1, \ldots, 1000$, $j = \mathcal{N}_4, \mathcal{N}_{12}, \mathcal{N}_{13}, \mathcal{N}_{14}$.

(2) Using parametrizations of the rate constants, a vector of rate constants was obtained for each pair of steady states: $k_i^{(j)}$, $i = 1, \ldots, 1000$, $j = \mathcal{N}_4, \mathcal{N}_{12}, \mathcal{N}_{13}, \mathcal{N}_{14}$. Thus one obtains triplets $\tau_i^{(j)} := \left(a_i^{(j)},\, b_i^{(j)},\, k_i^{(j)}\right)$, $i = 1, \ldots, 1000$, $j = \mathcal{N}_4, \mathcal{N}_{12}, \mathcal{N}_{13}, \mathcal{N}_{14}$ for which networks $\mathcal{N}_4$, $\mathcal{N}_{12}$, $\mathcal{N}_{13}$ and $\mathcal{N}_{14}$ show multistationarity (with $a_i^{(j)}$, $b_i^{(j)}$ as steady states and $k_i^{(j)}$ as vector of rate constants).

(3) For every network and every triplet $\tau_i^{(j)}$, a numerical continuation of steady states was performed, using A_T as bifurcation parameter. Bistability was confirmed numerically for each $\tau_i^{(j)}$.

(4) Lower and upper turning point $A_{low,i}^{(j)}$ and $A_{up,i}^{(j)}$ of the numerical continuation were determined (see Fig. 6.5, where turning points are labeled LP, limit point). Using $A_{low,i}^{(j)}$ and $A_{up,i}^{(j)}$, for each $\tau_i^{(j)}$, the relative range of bistability was determined as

$$\Delta_{A_T,i}^{(j)} = \frac{|A_{up,i}^{(j)} - A_{low,i}^{(j)}|}{\frac{A_{low,i}^{(j)} + A_{up,i}^{(j)}}{2}}. \tag{6.29}$$

(5) Finally $\bar{\Delta}_{A_T}^{(j)}$, the mean of all $\Delta_{A_T,i}^{(j)}$ was calculated for each network.

The mean $\bar{\Delta}_{A_T}^{(j)}$ can be interpreted as the average range of total concentration A_T, where bistability is possible. That is, in the average over all realisations of a particular network, where bistability was confirmed (represented by the triplets $\tau_i^{(j)}$) the relative difference between upper and lower bifurcation

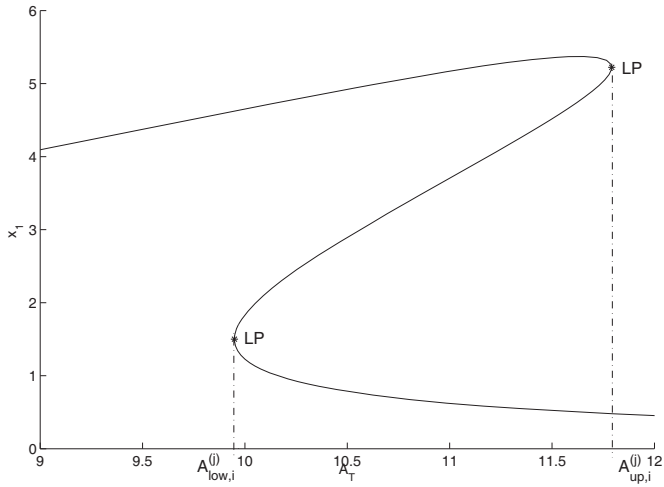


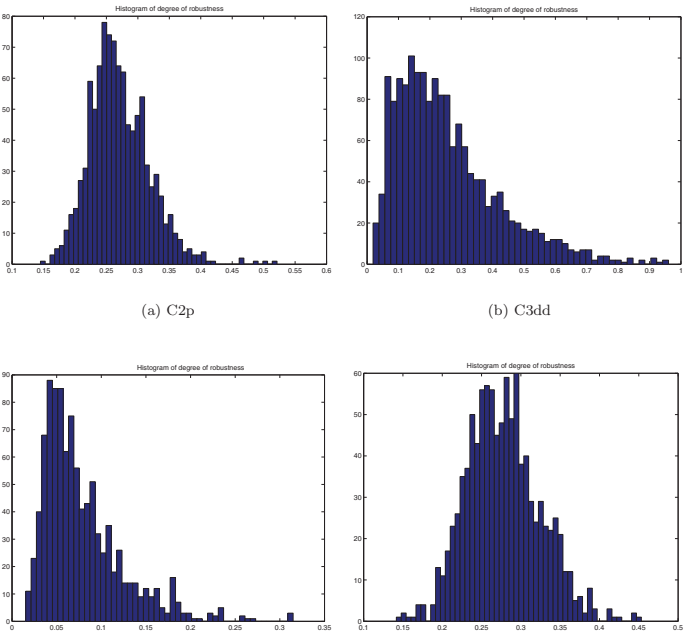
Figure 6.5: Numerical continuation for $\mathcal{N}_4$ using A_T (total concentration of A) as bifurcation parameter. Here the value of x_1 (concentration of A) is plotted against A_T.

point is given by $\bar{\Delta}^{(j)}_{A_T}$. The significance of this number is based on the following idea: if a certain network is designed to be a switch, and optimised against noise, its total concentration A_T should be approximately in the centre of the bistability region, that is close to $c_b = \frac{A^1_T + A^2_T}{2}$. If this is the case, then A_T can in the average vary by at most $\pm\frac{\bar{\Delta}^{(j)}_{A_T}}{2}$ without leaving the bistability range. Thus the $\bar{\Delta}^{(j)}_{A_T}$ seem to be well suited to asses the robustness of the switch-like behaviour against fluctuations of protein concentration. The values of $\bar{\Delta}^{(j)}_{A_T}$ for $\mathcal{N}_4$, $\mathcal{N}_{12}$, $\mathcal{N}_{13}$ and $\mathcal{N}_{14}$ are given in Table 6.5, while Figure 6.6 contains the distribution of the $\Delta^{(j)}_{A_T,i}$ for each network. Apart from network $\mathcal{N}_{13}$, all networks have

	$\mathcal{N}_4$	$\mathcal{N}_{12}$	$\mathcal{N}_{13}$	$\mathcal{N}_{14}$
$\bar{\Delta}^{(j)}_{A_T}$	0.2562	0.2708	0.0786	0.2773

Table 6.5: Mean values $\bar{\Delta}^{(j)}_{A_T}$

an average value of ≈ 25 % for $\bar{\Delta}^{(j)}_{A_T}$. Network $\mathcal{N}_{13}$ shows a much lower value (≈ 8 %, see Table 6.5). Note that in case of $\mathcal{N}_{13}$, a single distributive double-step activation mechanism is responsible for multistationarity. In the other cases either two double-step activation mechanisms (for network $\mathcal{N}_4$) or an explicit autocatalytic activation (for networks $\mathcal{N}_{12}$ and $\mathcal{N}_{14}$) are responsible for multistationarity. These results thus suggest that, as a mechanism to cause multistationarity, an autocatalytic activation is stronger (i.e. more robust to concentration fluctuations) than a distributive activation, since one of the earlier leads to similar ranges of $\bar{\Delta}^{(j)}_{A_T}$ as two of the latter.



(a) C2p (b) C3dd

(c) C3di (d) C3sr

Figure 6.6: Distribution of $\Delta^{(j)}_{A_{T,i}}$ for $\mathcal{N}_{12}$, $\mathcal{N}_4$, $\mathcal{N}_{13}$, and $\mathcal{N}_{14}$.

Chapter 7

Multistationarity and Beyond

While in the previous chapters biochemical reaction networks have been analysed with respect to the qualitative phenomenon multistationarity, the topic of this chapter is a different qualitative phenomenon: the occurrence of limit cycles. As it turns out, for some biochemical reaction networks it is possible to determine analytically points in state space ('critical states') and in parameter space ('critical parameters'), where the Jacobian has a zero eigenvalue. Thus it is, for example, possible to determine critical points (states and parameters), where necessary conditions for saddle node bifurcations and certain bifurcations of codimension one (e.g. Takens-Bogdanov type bifurcations) are satisfied. The occurrence of a Takens-Bogdanov bifurcation can lead to limit cycles, while the only qualitative behaviour associated with saddle-node bifurcations is multistationarity.
This chapter is organized as follows: in Section 7.1 the Jacobian of $f(x,k) := Y\, I_a\, v(k,\, x)$ is analyzed with respect to zero eigenvalues and necessary and sufficient conditions for the existence of a saddle-node bifurcation are given. In Section 7.2 the results are applied to network $\mathcal{N}_4$ for the activation of an MAPK, where the Takens-Bogdanov type bifurcation is used to determine states and parameters, where the ODEs defined by $\mathcal{N}_4$ exhibit limit cycles.

7.1 The Jacobian matrix

Let $J(x,k)$ denote the Jacobian matrix of $f(x,k) := Y\, I_a\, \mathrm{diag}(k)\, \phi(x)$ as defined in Chapter 2, that is $J(x,k) := D_x f(x,k)$. The structure of $f(x,k)$ leads to a special form of $J(x,k)$ [35]:

$$J(x,k) = N\ \mathrm{diag}(k)\ \mathrm{diag}(\phi(x))\ Y^{(L)^T}\ \mathrm{diag}\left(\frac{1}{x}\right), \tag{7.1}$$

for $x > 0$ where $\frac{1}{x}$ is shorthand for $\left(\frac{1}{x_1}, \ldots, \frac{1}{x_n}\right)^T$. In the remainder of this chapter the following assumption regarding $J(x,k)$ is made:

Assumption 3. *The image of $J(x,k)$ is equal to the image of the matrix N, that is* $\mathrm{im}(J(x,k)) = \mathrm{im}(N)$.

Note that the set of networks that satisfy Assumption 3 is neither empty nor academic, as it includes, for example, $\mathcal{N}_4$, $\mathcal{N}_5$ and $\mathcal{N}_6$.

7.1.1 Zero eigenvalues of the Jacobian

The following notation will be used: as usual, let $\mathcal{S} = [Y\, I_a]$ and let $S \in \mathbb{R}^{n\times s}$, $W \in \mathbb{R}^{n\times(n-s)}$ be two matrices whose columns are orthonormal bases for $\mathrm{im}(Y\, I_a)$ and $\mathrm{im}(Y\, I_a)^\perp$, respectively, with [61]:

$$\begin{aligned}\mathcal{S} &= [S] = \mathrm{im}(J(x,k)),\\ \mathcal{S}^\perp &:= [W] = \mathrm{im}(N)^\perp = \ker\left(N^T\right).\end{aligned}$$

Then x can be written as

$$x = \xi(y,z) = S\,y + W\,z$$

with $y = S^T x \in \mathbb{R}^s$, $z = W^T x \in \mathbb{R}^{n-s}$. In the new coordinates y and z the system of ODEs $\dot{x} = f(x,k)$ reads

$$\begin{aligned} \dot{y} &= S^T \dot{x} = S^T f(\xi(y,z),k) =: g(y,z,k), \\ \dot{z} &= W^T \dot{x} = 0 \end{aligned} \tag{7.2}$$

showing the invariance of the subspace $\mathrm{im}(Y\, I_a)$. Consider the reduced system

$$\dot{y} = g(y,z,k) = g(y,\nu) \tag{7.3}$$

with $\nu = (z,k)$ as parameter vector. The Jacobian $\mathbf{D}_y g$ is then given by

$$\mathbf{D}_y g(y,z,k) = S^T \mathbf{D}_x f(\xi(y,z),k)\ S \tag{7.4}$$

As $\mathrm{im}(N)$ is invariant under the flow of $\dot{x} = f(x,k)$, as the equation $\dot{z} = 0$ in (7.2) shows, $\mathbf{D}_x f(x,k)$ always has $n-s$ eigenvalues $\lambda_0 = 0$. Values of y, z and k where $\mathbf{D}_y g(y,z,k)$ has a zero eigenvalue are thus candidates for bifurcation points.
The following lemma, based on the fundamental result $\mathrm{im}(A)^\perp = \ker\left(A^T\right)$ for any $(m \times n)$–matrix A and Fredholm's Alternative [61], will be used to link zero eigenvalues of $\mathbf{D}_y g(y,z,k)$ to zero eigenvalues of $J(\xi(y,z),k)$. As for example in [64], an eigenvalue λ is called *defective*, if its *algebraic multiplicity* $m_{alg}(\lambda)$ is greater than its *geometric multiplicity* $m_{geo}(\lambda)$ (i.e. if the multiplicity of λ as a root of the characteristic polynomial is greater than the number of linear independent eigenvectors corresponding to λ). Recall

Fact 6. *$\lambda_0 = 0$ is a defective eigenvalue of a matrix $A \in \mathbb{R}^{n\times n}$ iff there exists an $x \neq 0$ with $x \in \mathrm{im}(A) \cap \ker(A)$.*

Fact 7. *$\lambda_0 = 0$ is a defective eigenvalue of a matrix $A \in \mathbb{R}^{n\times n}$ of order r iff there exists a nontrivial x – w.l.o.g. $0 \neq x \in \mathrm{im}\left(A^T\right)$ – with*

$$A^j x \neq 0,\ j = 0, 1, \ldots, r \quad \text{and } A^{r+1} x = 0.$$

*Such an x will be called **principal vector of order** r and **principal vector of maximal order** r, if, in addition, $x \notin \mathrm{im}(A)$ holds.*

Lemma 7. *Let $A \in \mathbb{R}^{n\times n}$ be a matrix of rank $s < n$. Let S and W be orthonormal bases for $\mathrm{im}(A)$ and $\ker\left(A^T\right)$, respectively, and let V be a basis for $\ker(A)$. Consider the matrix $B_1 := S^T A S \in \mathbb{R}^{s\times s}$.*

1. *$\lambda_0 = 0$ is an eigenvalue of B_1 iff $\lambda_0 = 0$ is a defective eigenvalue of A.*
2. *$\lambda_0 = 0$ is a defective eigenvalue of A iff $\Gamma = W^T V$ is singular.*
3. *If x is a principal vector of order r for A then any y with $Sy = Ax$ is a principal vector of order $r-1$ for B_1 – note that y is to be taken as $y = S^T Ax \in \mathrm{im}\left(B_1^T\right)$.*
4. *If y is a principal vector of order r for B_1 then any x with $Sy = Ax$ is a principal vector of order $r+1$ for A – note that x can be taken to be $x^\# = A^\# S\, y \in \mathrm{im}\left(A^T\right)$ with the Moore–Penrose inverse $A^\#$ of A.*
5. *$\lambda_0 = 0$ is an algebraically simple eigenvalue of B_1 if and only if $\lambda_0 = 0$ is a defective eigenvalue of A with $m_{alg}(\lambda_0) = m_{geo}(\lambda_0) + 1$.*
6. *$\lambda_0 = 0$ is an algebraically simple eigenvalue of B_1 if and only if B_1 has one–dimensional left and right nullspace $[\beta^T]$ and $[b]$, respectively, with $\beta^T b \neq 0$.*

Proof. Apply the orthonormal transformation matrix $\phi = (S, W) \in \mathbb{R}^{n\times n}$ to obtain $B = \phi^T A \phi$ with

$$B = \begin{bmatrix} S^T A S & S^T A W \\ 0 & 0 \end{bmatrix} = \begin{bmatrix} B_1 & B_2 \\ 0 & 0 \end{bmatrix}. \tag{7.5}$$

Note that one has

$$A = \phi\, B\, \phi^T = S\, B_1\, S^T + S\, B_2\, W^T \tag{7.6}$$

and thus

$$A^i S = S B_1^i. \tag{7.7}$$

ad(1) If λ_0 is a defective eigenvalue of A then there exists by Fact 6 a nontrivial $v_0 \in im\,(A) \cap \ker\,(A)$ so that $W^T\,v_0 = 0$ and $v_0 = A\,v_1 = S\,z$ for suitable nonzero v_1 and z. Thus by (7.6) $0 = A\,v_0 = S\,B_1\,S^T\,v_0 + 0 = S\,B_1 z$ (as $S^T\,S = I_s$). As S is of full rank, $B_1\,z = 0$ follows. Vice versa, suppose that there exists $\zeta \in I\!R^s$ with $B_1\,\zeta = 0$, $\zeta \neq 0$. Define $v := S\,\zeta \in \mathrm{im}\,(A)$. Then $W^T\,v = 0$ and, again by (7.6), $A\,v = S\,B_1\,S^T\,v + 0 = S\,B_1\,\zeta = 0$. Thus $v \in \ker\,(A)$ so that λ_0 is a defective eigenvalue of A by Fact 6.

ad(2) If λ_0 is a defective eigenvalue of A then we employ Fact 7. Choose a nontrivial x with $y := A\,x \neq 0$ and $A\,y = A^2\,x = 0$. Since y belongs to $\ker\,(A)$ it can be written as $y = V\,z$ with a nonzero z. Since y belongs to $im\,(A)$ it is orthogonal to $[W] = ker\,\left(A^T\right)$. Hence $\Gamma y = W^T\,V\,z = 0$ for a nonzero z. Vice versa, if z is a nullvector of $\Gamma = W^T\,V$ then $V\,z \in [V] = ker\,(A)\,, V\,z \neq 0$, and $V\,z$ is orthogonal to $[W] = \mathrm{im}\,(A)^{\perp}$. Hence the nonzero $V\,z$ belongs to $ker\,(A)$ and $\mathrm{im}\,(A)$.

ad(3) If x is given as in 3) the element $\xi := A\,x$ can be written as $\xi = S\,y$ with $S^T\,\xi = S^T\,A\,x = S^T\,S\,y = y$. Because of (7.7) we have $S\,B_1^i\,y = A^i\,S\,y = A^{i+1}\,x$. Hence y is a principal vector of order $r-1$ for B_1 (as S has full column rank).

ad(4) If y is given as in 4) the element $\eta := S\,y$ can be written as $\eta = A\,x$. Because of (7.7) we have – as above – $S B_1^i\,y = A^{i+1}\,x$ so that x (e.g. $x = x^{\#}$) is a principal vector of order $r+1$ for A.

ad(5) Assume λ_0 is a multiple eigenvalue of B_1 with two linearly independent nullvectors y_1 and y_2. Each y_j generates a corresponding principal vector x_j of order 1 for A. Since the x_j are necessarily linearly independent, A has 0 with $m_{alg}(\lambda_0) > m_{geo}(\lambda_0) + 1$. Assume now that λ_0 is a defective eigenvalue of B_1 of order $r \geq 1$. By 4), λ_0 is a defective eigenvalue of A of order $r+1 \geq 2$. Thereby we have shown $\Leftarrow$ of 5).
For $\Rightarrow$ we refer to 3) stating that a principal vector of order 2 for A yields a principal vector of order 1 for B_1. In case $\lambda_0 = 0$ is a non-defective eigenvalue of A we recall 1) stating that λ_0 is not an eigenvalue of B_1 at all.

ad(6) Note that the statement in 6) is a particular case of the one in 2). The equation $B_1\,y = b$ is solvable for y iff $z^T\,B_1 = 0$ implies $z^T\,b = 0$. Since the left nullspace of B_1 is spanned by β^T, the equivalence in 6) follows.

■

The following Corollary uses Lemma 7 to establish a link between right (left) principal vectors of A and right (left) nullvectors of B.

Corollary 5. *If B has an algebraically simple eigenvalue $\lambda_0 = 0$ or, equivalently, if A has λ_0 as a defective eigenvalue with $m_{alg}(\lambda_0) = m_{geo}(\lambda_0) + 1$, one has the following:*
A principal right nullvector a of A yields a right nullvector b of B if $b = S^T\,A\,a$ is chosen (with $A\,a = S\,b$).
A principal left nullvector α^T of A yields a left nullvector β^T of B if $\beta^T = \alpha^T\,S$ is chosen (with $\alpha^T\,A = \beta^T\,S^T\,A$).
Vice versa: Nullvectors b and β^T of B give rise to corresponding principal vectors a and α^T of A of maximal order 1 via $A\,a = S\,b$ and $\alpha^T\,A = \beta^T\,S^T\,A$ respectively, that is $a = A^{\#}\,S\,b \in \mathrm{im}\,\left(A^T\right)$ and $\alpha = S\,\beta \in \mathrm{im}\,\left(A^T\right)$.

Proof. Consult Lemma 7 for right eigenvectors and principal vectors. Turn to left eigenvectors and principal vectors.
If $\beta^T\,B = 0$ for a nonzero β^T then $\alpha^T := \beta^T\,S^T$ (satisfying $\alpha^T\,S = \beta^T$) is nonzero. The equalities $\alpha^T = \beta^T\,S^T$ and $\alpha^T\,A = \beta^T\,S^T\,A$ imply $\alpha^T\,A \neq 0$ as $S^T\,A$ has full row–rank (proof by contradiction). Furthermore one deduces $\alpha^T\,A^2 = \beta^T\,S^T\,A\,S\,S^T\,A = \beta^T\,B\,S^T\,A = 0$.
Vice versa, given a left principal vector $\alpha^T \neq 0$ with $\alpha^T\,A \neq 0$ and $\alpha^T\,A^2 = 0$ we take the nonzero $\alpha^T\,S = \beta^T$ (with $\alpha^T = \beta^T\,S^T + \sigma^T$ with $\sigma \in \ker\,\left(A^T\right)$) and verify $\beta^T\,B = \beta^T\,S^T\,A\,S = \alpha^T\,A\,S = 0$ (as $A^T\,\alpha \in \ker\,\left(A^T\right) = [S]^{\perp}$). ■

As discussed above, Lemma 7 and Corollary 5 establish a link between spectral properties of the Jacobian $A = J\,(\xi\,(y,z)\,,k)$ and the Jacobian $B = \mathbf{D}_y\,g\,(y,z,k)$ of the reduced system (7.3).

7.1.2 Saddle-node bifurcations

In this section the focus is on saddle-node bifurcations. The following well-known theorem gives necessary and sufficient conditions for a saddle-node bifurcation of the system $\dot{y} = g(y, z, k)$ as defined in (7.3). Let μ be the component of the parameter vector $\nu = (z, k)$ that will be used as bifurcation parameter.

Theorem 5 (see e.g, [51], p. 330 and [7], p. 497). *Suppose (y_0, ν_0) is a zero of g and suppose that the $s \times s$ matrix $\mathbf{D}_y\, g(y_0, \nu_0)$ has an algebraically simple eigenvalue $\lambda_0 = 0$ with right eigenvector b and left eigenvector β^T. Furthermore suppose that the following conditions are satisfied:*

$$\beta^T \mathbf{D}_\mu\, g(y_0, \nu_0) \neq 0, \quad \beta^T \left[\mathbf{D}_y^2\, g(y_0, \nu_0)(b, b)\right] \neq 0. \tag{7.8}$$

Then there is a smooth curve of zeroes of g passing through (y_0, ν_0). Depending on the signs of the expressions in (7.8), there are no or two zeroes near y_0 for $\mu \neq \mu_0$ whereas the other components of ν_0 remain fixed.

In the remainder of this section it is examined, when conditions (7.8) are satisfied for the system (7.3) in terms of the Jacobian $J(\xi(y, z), k)$. First note:

(N1) From Lemma 5 follows that $\mathbf{D}_y\, g(y_0, \nu_0)$ has an algebraically simple eigenvalue $\lambda_0 = 0$ if and only if $J(\xi(y, z), k)$ has λ_0 as a defective eigenvalue with $m_{alg}(\lambda_0) = m_{geo}(\lambda_0) + 1$.

Now, if $\mathbf{D}_y\, g(y_0, \nu_0)$ has an algebraically simple eigenvalue $\lambda_0 = 0$, observe the following:

(N2) Ad $\beta^T \mathbf{D}_\mu\, g(y_0, \mu_0) \neq 0$: if μ is any rate constant k_i, then

$$\mathbf{D}_\mu\, g(y_0, \mu_0) = S^T \mathbf{D}_{k_i}\, f(x, k) = S^T N \operatorname{diag}(\phi(x_0))\, e_i$$

and therefore

$$\beta^T \mathbf{D}_\mu\, g(y_0, \mu_0) = \phi_i(x_0)\, \beta^T S^T N\, e_i \neq 0$$

for at least one i (as $\phi_i(x_0) > 0$ and $[S] = \operatorname{im}(N)$).

(N3) Ad $\beta^T \left[\mathbf{D}_y^2\, g(y_0, \nu_0)(b, b)\right] \neq 0$: From Lemma 7 and Corollary 5 follows

$$\beta^T \left[\mathbf{D}_y^2\, g(y_0, z_0, k_0)(b, b)\right] \neq 0 \Leftrightarrow \alpha^T \left[\mathbf{D}_x^2\, f(\xi(y_0, z_0), k_0)(A\,a, A\,a)\right] \neq 0 \tag{7.9}$$

with $A := J(\xi(y, z), k)$, left and right principal vectors α^T and a of $J(\xi(y, z), k)$. To see this, note that $A\,a = S\,b$, thus the evaluation of $\mathbf{D}_y^2\, g$ at (b, b) and of $\mathbf{D}_x^2\, f$ at $(A\,a, A\,a)$ amounts to the same. Since $\alpha^T S = \beta^T$ (or equivalently $\alpha^T = \beta^T S^T + [W^T]$) and since – by the particular form of f – $\mathbf{D}_x^2\, f(\xi(y_0, z_0), k_0)(A\,a, A\,a) \subset \operatorname{im}(N)$ the equivalence (7.9) holds true.

As consequence of this discussion the following fact concerning the originally given system (2.4), (2.5) holds:

Fact 8. *The system (2.4), (2.5) has a saddle-node bifurcation at (x_0, k_0) (within the plane $W^T x = W^T x_0 =: c$) if the following conditions are satisfied:*

(a) $\lambda_0 = 0$ is a defective eigenvalue of $J(x_0, k_0)$ with $m_{alg}(\lambda_0) = m_{geo}(\lambda_0) + 1$.

(b) $\alpha^T \left[\mathbf{D}_x^2\, f(x_0, k_0)(Aa, Aa)\right] \neq 0$ is satisfied for left and right principal vectors w_0^T and v_0 of $J(x_0, k_0)$.

Proof. By the present assumptions, $\mathbf{D}_y\, g(y_0, z_0, k_0)$ has $\lambda_0 = 0$ as a simple eigenvalue at $y_0 = S^T x_0, z_0 = W^T x_0$ and k_0. By assumption (a) and Note (N2) from above the condition $\beta^T \mathbf{D}_\mu\, g(y_0, \mu_0) \neq 0$ is satisfied for at least one choice of $\mu = k_i$. Combined with Note (N3) and the assumption (b), all conditions of Theorem 5 are satisfied and the reduced system (7.3) has thus a saddle-node bifurcation at $y_0 = S^T x_0, z_0 = W^T x_0$ and k_0. As system (7.3) is on $\{x : W^T x = W^T x_0 = z_0\}$ equivalent to the original system (2.4), this has a saddle-node bifurcation at (x_0, k_0) as well. ■

7.2 Application to a double-phosphorylation mechanism

In this section the consequences of Fact 8 for network $\mathcal{N}_4$ are determined. As in Section 4.2.1, let $E \in \mathbb{R}^{9\times 6}$ be a set of generators for the pointed polyhedral cone $\ker(Y\,I_a) \cap \mathbb{R}^9_{\geq 0}$. Then, as in Section 3.1, the following relation must hold for a solution (x_0, k_0) to $Y\,I_a\,v(k_0,\,x_0) = 0$ (with $x_0 > 0, k_0 > 0$):

$$\operatorname{diag}(k_0)\ \phi(x_0) = E\,\lambda \tag{7.10}$$

for some positive $\lambda \in \mathbb{R}^6$. By (7.1) and (7.10) the Jacobian $J(x_0, k_0)$ evaluated at this zero state is thus $N\ \operatorname{diag}(E\,\lambda)\ {Y^{(L)}}^T\ \operatorname{diag}\left(x_0^{-1}\right)$. One has to determine nonzero vectors v with $J(x_0, k_0)\ v = 0$. With $\gamma := \operatorname{diag}\left(x_0^{-1}\right)\ v$ one is thus looking for γ's with

$$J(\lambda)\ \gamma = 0, \quad J(\lambda) := N\ \operatorname{diag}(E\,\lambda)\ {Y^{(L)}}^T.$$

Thus $\operatorname{diag}(E\,\lambda)\,{Y^{(L)}}^T\gamma \in \ker(N)$ and therefore ${Y^{(L)}}^T\,\gamma = \operatorname{diag}\left((E\,\lambda)^{-1}\right)\ E\,\alpha$, for some $\alpha \in \mathbb{R}^6$. This leads to the following set of equations (note the similarity to (4.1a)–(4.1l)):

$$\gamma_1 + \gamma_2 = \frac{\alpha_1 + \alpha_5}{\lambda_1 + \lambda_5}, \quad \gamma_3 = \frac{\alpha_1}{\lambda_1}, \quad \gamma_3 = \frac{\alpha_5}{\lambda_5} \tag{7.11a}$$

$$\gamma_2 + \gamma_4 = \frac{\alpha_2 + \alpha_6}{\lambda_2 + \lambda_6}, \quad \gamma_5 = \frac{\alpha_2}{\lambda_2}, \quad \gamma_5 = \frac{\alpha_6}{\lambda_6} \tag{7.11b}$$

$$\gamma_6 + \gamma_7 = \frac{\alpha_3 + \alpha_6}{\lambda_3 + \lambda_6}, \quad \gamma_8 = \frac{\alpha_3}{\lambda_3}, \quad \gamma_8 = \frac{\alpha_6}{\lambda_6} \tag{7.11c}$$

$$\gamma_4 + \gamma_7 = \frac{\alpha_4 + \alpha_5}{\lambda_4 + \lambda_5}, \quad \gamma_9 = \frac{\alpha_4}{\lambda_4}, \quad \gamma_9 = \frac{\alpha_5}{\lambda_5} \tag{7.11d}$$

The system (7.11a) – (7.11d) is solvable, if

$$\frac{\alpha_1}{\lambda_1} = \frac{\alpha_5}{\lambda_5} = \frac{\alpha_1 + \alpha_5}{\lambda_1 + \lambda_5} = \frac{\alpha_4}{\lambda_4} = \frac{\alpha_4 + \alpha_5}{\lambda_4 + \lambda_5} =: \kappa_1$$

and

$$\frac{\alpha_2}{\lambda_2} = \frac{\alpha_6}{\lambda_6} = \frac{\alpha_2 + \alpha_6}{\lambda_2 + \lambda_6} = \frac{\alpha_3}{\lambda_3} = \frac{\alpha_4 + \alpha_5}{\lambda_4 + \lambda_5} =: \kappa_2.$$

Using κ_1 and κ_2 and solving for γ yields:

$$\begin{aligned}\gamma = {} & \gamma_1\ (1, -1, 0, 1, 0, 1, -1, 0, 0)^T \\ & + \kappa_1\ (0, 1, 1, -1, 0, -2, 2, 0, 1)^T \\ & + \kappa_2\ (0, 0, 0, 1, 1, 2, -1, 1, 0)^T.\end{aligned} \tag{7.12}$$

Note that γ as defined in (7.12) is equivalent to μ as in (4.5): both define the same linear subspace $\mathcal{M}_{\mathcal{N}_4}$. Denote this by

$$\gamma = M_{\mathcal{N}_4} \begin{bmatrix} \gamma_1 \\ \kappa_1 \\ \kappa_2 \end{bmatrix},$$

with $M_{\mathcal{N}_4}$ as in (4.6a). Now the right null space of $J(x_0, k_0)$ can be represented as $[V(x_0)] = [\operatorname{diag}(x_0)\ M_{\mathcal{N}_4}]$ with $V(x_0)$ given by

$$V(x_0) = \operatorname{diag}(x_0)\ M_{\mathcal{N}_4} = \begin{bmatrix} x_{10} & 0 & 0 \\ -x_{20} & 0 & x_{20} \\ 0 & 0 & x_{30} \\ x_{40} & x_{40} & -x_{40} \\ 0 & x_{50} & 0 \\ x_{60} & 2\,x_{60} & -2\,x_{60} \\ -x_{70} & -x_{70} & 2\,x_{70} \\ 0 & x_{80} & 0 \\ 0 & 0 & x_{90} \end{bmatrix}. \tag{7.13}$$

Since $J(x_0, k_0) \in \mathbb{R}^{9\times 9}$ has a 3–dimensional nullspace and thus a 6–dimensional range and since, by (7.1), the range of $J(x_0, k_0)$ belongs to $\operatorname{im}(N)$, Assumption 3 is satisfied for $\mathcal{N}_4$.

7.2.1 Zero eigenvalues

To check whether $J(x_0, k_0)$ has $\lambda_0 = 0$ has a defective eigenvalue consider the matrix Γ in Lemma 7, now given by $\Gamma(x_0) = W^T V(x_0)$. It can be obtained as

$$\Gamma(x_0) = \begin{bmatrix} -x_{20} & x_{50} & x_{20} + x_{30} \\ -x_{70} & -x_{70} + x_{80} & 2\,x_{70} + x_{90} \\ x_{10} + x_{40} + x_{60} & x_{40} + x_{50} + 2\,x_{60} + x_{80} & x_{30} - x_{40} - 2\,x_{60} + x_{90} \end{bmatrix}. \tag{7.14}$$

$\Gamma(x_0)$ is singular if and only if

$$\Gamma(x_0)\ \pi_0 = W^T V(x_0)\ \pi_0 = 0 \tag{7.15}$$

has a nontrivial solution $\pi_0 \in \mathbb{R}^3$. Note that

$$\Gamma(x_0)\ \pi_0 = W^T\ \mathrm{diag}(x_0)\ M_{\mathcal{N}_4}\,\pi_0 = W^T\ \mathrm{diag}(M_{\mathcal{N}_4}\,\pi_0)\ x_0$$

and that $[W]$ is the orthogonal complement of the stoichiometric subspace $\mathcal{S}_{\mathcal{N}_4}$, that is $W^T S = 0$, where $S \in \mathbb{R}^{9\times 6}$ is an orthonormal basis for $\mathcal{S}_{\mathcal{N}_4}$. To obtain x_0, the following observation is useful: if $u \in \mathbb{R}^9$ is a solution to $W^T u = 0$, then $\tilde{u} = \mathrm{diag}\left(\frac{1}{M\,\pi}\right) u$ satisfies $W^T\ \mathrm{diag}(M\,\pi)\ \tilde{u} = 0$ (for nonzero $\pi \in \mathbb{R}^3$). The following results establish parametrizations of points where $\Gamma(x_0)$ is singular:

Corollary 6. *Points $x_0 \in \mathbb{R}^9$ where $\Gamma(x_0)$ is singular are given by*

$$x_0 = \frac{S\,\rho_0}{M_{\mathcal{N}_4}\,\pi_0}, \tag{7.16a}$$

with $(M_{\mathcal{N}_4}\,\pi_0)_i \neq 0$, $i = 1, \ldots, 9$. Right Eigenvectors γ_0 of $J(x, \lambda)$ with $W^T\gamma_0 = 0$ are given by

$$\gamma_0 = S\,\rho_0 \tag{7.16b}$$

Proof. $x_0 = \frac{S\,\rho_0}{M_{\mathcal{N}_4}\,\pi_0}$ follows from the previous discussion. Recall that by (7.12) $\gamma_0 = diag(x_0)\ M_{\mathcal{N}_4}\,\pi_0$ and thus $\gamma_0 = diag\left(\frac{S\,\rho_0}{M_{\mathcal{N}_4}\,\pi_0}\right) M_{\mathcal{N}_4}\,\pi_0 = S\,\rho_0$. Note that $W^T\gamma_0 = 0$ by $[W] = \mathcal{S}_{\mathcal{N}_4}^{\perp}$. ■

For biochemical reaction networks only *positive* x_0 are of interest. To obtain those, the condition

$$W^T\ \mathrm{diag}(M_{\mathcal{N}_4}\,\pi_0)\ x_0 = 0$$

must hold for positive x_0. The following Corollary can be established:

Corollary 7. *The equation*

$$W^T\ \mathrm{diag}(M_{\mathcal{N}_4}\,\pi_0)\ x_0 = 0$$

has a positive solution $x_0 \in \mathbb{R}^9_{>0}$ (for some $\pi_0 \in \mathbb{R}^3$), if and only if an orthant δ of $\mathbb{R}^9$ exists, where

(i) $\mathcal{S}_{\mathcal{N}_4} \cap \mathbb{R}^9_\delta \neq \emptyset$

(ii) $\mathcal{M}_{\mathcal{N}_4} \cap \mathbb{R}^9_\delta \neq \emptyset$.

In this case collect generators of $\mathcal{S}_{\mathcal{N}_4} \cap \mathbb{R}^9_\delta$ and $\mathcal{M}_{\mathcal{N}_4} \cap \mathbb{R}^9_\delta$ in $E^\delta_{\mathcal{S}_{\mathcal{N}_4}}$ and $E^\delta_{\mathcal{M}_{\mathcal{N}_4}}$. Then

$$x_0 = \frac{E^\delta_{\mathcal{S}_{\mathcal{N}_4}}\,\beta}{E^\delta_{\mathcal{M}_{\mathcal{N}_4}}\,\alpha}, \tag{7.17}$$

for $\alpha,\ \beta > 0$ of appropriate dimension and $\left(E^\delta_{\mathcal{M}_{\mathcal{N}_4}}\,\alpha\right)_i \neq 0$, $i = 1, \ldots, 9$.

Note that a different representation of x_0 exists for each orthant, where $\mathcal{M}^\delta_{(\mathcal{N}_4)} \cap \mathbb{R}^9_\delta \neq \emptyset$ and $\mathcal{S}_{\mathcal{N}_4} \cap \mathbb{R}^9_\delta \neq \emptyset$. For network $\mathcal{N}_4$, 14 such orthants exist (see Section 4.3.1, in particular (4.16) and Table 4.1 for representations of $\mathcal{M}_{\mathcal{N}_4} \cap \mathbb{R}^9_{\delta_i}$ and Table 4.2 representations of $\mathcal{S}_{\mathcal{N}_4} \cap \mathbb{R}^9_{\delta_i}$).
From Table 4.1 and Table 4.2 follows, that, for example for δ_1, all solutions x_0 to (7.2.1) can be obtained as:

$$\begin{aligned} x_0^{\delta_1} = \big(&\frac{\beta_1 + \beta_3 + \beta_4 + \beta_5}{\alpha_1}, \frac{\beta_1}{\alpha_2}, \frac{\beta_2}{\alpha_1 + \alpha_2}, \frac{\beta_3}{\alpha_2 + \alpha_3}, \frac{\beta_1 + \beta_2}{\alpha_3}, \\ &\frac{\beta_4}{\alpha_1 + 2\,(\alpha_2 + \alpha_3)}, \frac{\beta_5}{\alpha_1 + 2\,\alpha_2 + \alpha_3}, \frac{\beta_5 + \beta_6}{\alpha_3}, \frac{\beta_6}{\alpha_1 + \alpha_2}\big)^T. \end{aligned} \tag{7.18}$$

Finally one obtains the following Corollary:

Corollary 8. *Given a positive zero (x_0, k_0) of $Y\, I_a\, v(k_0,\, x_0) = 0$, the Jacobian $\mathbf{D}_\mu\, g(y_0, \nu_0)$ has the eigenvalue $\lambda_0 = 0$ with $m_{geo}(\lambda_0) = 1$, if and only if x_0 can be parametrized as in (7.17) for some orthant δ. For a x_0 as in (7.17) to be a component of a zero of $Y\, I_a\, v(k_0,\, x_0) = 0$, the vector of rate constants k_0 needs to be of the form (see (3.10a)):*

$$k_0 = \operatorname{diag}\left(\phi\left(x_0^{-1}\right)\right)\, E\, \lambda. \tag{7.19}$$

Remark 14. *Equation (7.16a) and equation (7.19) give an explicit representation of the manifold of potential critical points (in the sense of the Jacobian $\mathbf{D}_\mu\, g(y_0, \nu_0)$ having a geometrically simple eigenvalue 0). The eigenvalue $\lambda_0 = 0$ will have algebraic multiplicity 1 too, iff part (6) of Lemma 7 holds true, i.e. if the one–dimensional left nullspace $[\beta^T]$ is not orthogonal to the right nullspace spanned by $S^T\, V\, \rho$:*

$$\frac{1}{|\beta|\, |S^T\, V\, \rho|}\; \beta^T\, S^T\, V\, \rho \neq 0\ . \tag{7.20}$$

In case the additional hypotheses (7.8) of Theorem 5 are satisfied, saddle-node bifurcations do occur in the reduced system (7.3) and hence in the full system (2.4).
By Fact 8, these bifurcation conditions can be checked with the right eigenvector $V\rho$ of J and the left principal vector α^T of J satisfying $\alpha^T = \beta^T\, S^T$. ■

7.2.2 Saddle-node bifurcations

To illustrate the consequences of Fact 8 and Remark 14, the positive steady states for $\mathcal{N}_4$ obtained in [11] were analyzed. To this end, the parameter vector

$$\begin{aligned} k = \big(&10.929273,\, 34.638118,\, 34.638118,\, 21.999084,\, 1.7135134,\, 1.7135134,\, 788.27203,\\ &1,\, 1,\, 686.21469,\, 14.271831,\, 14.271831\big)^T. \end{aligned} \tag{7.21}$$

and the steady state

$$\begin{aligned} x^{(1)} = \big(&1.7047107,\, 0.42617769,\, 0.11461683,\, 0.84691022,\, 2.3169421,\, 0.73726101,\, 1.3662e-2,\\ &3.9701114,\, 0.27817813\big)^T \end{aligned}$$

were used. Numerical continuation of $x^{(1)}$ using the rate constant k_1 as bifurcation parameter revealed the existence of two positive limit points (c.f. Fig. 7.1): ξ_1 at $k_1^{(1)} = 10.78047065415938$ and ξ_2 at $k_1^{(2)} = 16.92069563203855$:

$$\xi_1 = \begin{bmatrix} 1.5081503832748009e{+}00 \\ 4.1794279971945869e{-}01 \\ 9.8087786724483220e{-}02 \\ 8.7282751801919412e{-}01 \\ 2.3417060335560578e{+}00 \\ 8.9735230240123864e{-}01 \\ 1.1345163975775674e{-}02 \\ 4.0125446673591174e{+}00 \\ 2.3806169866510707e{-}01 \end{bmatrix}, \xi_2 = \begin{bmatrix} 3.2452663058283555e{+}00 \\ 6.7851703331421120e{-}01 \\ 5.3783000287414795e{-}01 \\ 3.7684592311080473e{-}01 \\ 1.6413895838116406e{+}00 \\ 4.9527633841469319e{-}02 \\ 1.4408058946641877e{-}01 \\ 2.8125430464816645e{+}00 \\ 1.3053278940519168e{+}00 \end{bmatrix}$$

Evaluated at each of these points, $\operatorname{rank}(\Gamma\,(\xi_i)) = 2$ (as expected). Corresponding kernel vectors ρ_1 and ρ_2 are:

$$\rho_1 = \begin{bmatrix} +1.1303636028285451e{+}00 \\ -8.0163523743329912e{-}02 \\ +1.2792783092819293e{+}00 \end{bmatrix}, \rho_2 = \begin{bmatrix} +3.5343229854352359e{-}01 \\ -5.0805188443278348e{-}01 \\ +8.8274223950851360e{-}01 \end{bmatrix}$$

The observation $\operatorname{rank}(\Gamma\,(\xi_i)) = 2$ implies that $A_i := J\left(\xi_i, k^{(i)}\right)$ has λ_0 as a defective eigenvalue. (The saddle-node bifurcation occurs at $(\xi_i, k^{(i)})$, where $k^{(i)} = k$ as (7.21), apart from $k_1^{(i)}$, which is the value of the bifurcation parameter given above.) Thus $\mathbf{D}_y\, g\,(y, \nu)$ has λ_0 as an eigenvalue of geometric multiplicity 1 (Corollary 8). To show that $m_{alg} = 1$ as well, we use Remark 14 and (7.20): left and right eigenvectors β_1 and b_1 of $S^T\, A_1\, S$ and β_2 and b_2 of $S^T\, A_2\, S$ are

$$\beta_1 = \begin{bmatrix} -4.1964341933838814e{-}02 \\ -2.5294006493730586e{-}01 \\ -1.2501904579758152e{-}01 \\ -3.0246504922821843e{-}01 \\ -3.8819920238436935e{-}01 \\ +8.2246387702299073e{-}01 \end{bmatrix}, b_1 = \begin{bmatrix} -7.2991592453109961e{-}04 \\ +1.7089171544296726e{-}02 \\ -5.3917073343408051e{-}01 \\ +1.2651324391400867e{-}01 \\ -6.8437325239016328e{-}02 \\ +8.2964635795940922e{-}01 \end{bmatrix}$$

$$\beta_2 = \begin{bmatrix} -4.2053043375917150e{-}02 \\ -2.5397520447083777e{-}01 \\ -1.2652496096167992e{-}01 \\ -3.2740827851964677e{-}01 \\ -3.8987406404680935e{-}01 \\ +8.1149344091673992e{-}01 \end{bmatrix}, b_2 = \begin{bmatrix} -4.2053043375917150e{-}02 \\ -2.5397520447083777e{-}01 \\ -1.2652496096167992e{-}01 \\ -3.2740827851964677e{-}01 \\ -3.8987406404680935e{-}01 \\ +8.1149344091673992e{-}01 \end{bmatrix}$$

It is straightforward to verify that $\beta_1^T b_1 \neq 0$ and $\beta_2^T b_2 \neq 0$. Thus $m_{alg}(\lambda_0) = m_{geo}(\lambda_0) = 1$ for λ_0 as an eigenvalue of $S^T A_i S$ (and thus $m_{alg}(\lambda_0) = m_{geo}(\lambda_0) + 1$ for λ_0 as an eigenvalue of A_i). To check hypothesis (b) of Fact 8 note that $\mathbf{D}_y^2 f(x,k)(v,v)$ evaluated at $v \in \mathbb{R}^9$ is given by

$$\mathbf{D}_y^2 f(x,k)(v,v) = \big(2\,v_1\,v_2,\ 0,\ 0,\ 2\,v_2\,v_4,\ 0,\ 0,\ 2\,v_6\,v_7,\\ 0,\ 0,\ 2\,v_4\,v_7,\ 0,\ 0\big)^T.$$

Using this equation together with $\alpha_i = S\,\beta_i$ and $J\left(\xi_i, k^{(i)}\right) a_i = S\,b_i$ in (7.9), it is straightforward to establish that condition (b) is satisfied for $(\xi_1, k^{(1)})$ and $(\xi_1, k^{(2)})$.
To see the consequences of Corollary 8, choose $\rho_0 \in \mathbb{R}^6_{>0}$ and $\pi_0 \in \mathbb{R}^3_{>0}$ and verify numerically that a saddle-node bifurcation occurs at the corresponding pair $(x^{(a)}, k_0)$ (with $x^{(a)}$ as in (7.16a) and k_0 as in (7.19)): choose, for example, $x^{(a)} = (6,\,1,\,1,\,1,\,1,\,1,\,1,\,3,\,1)^T$ and $k_0 = (\frac{1}{3},\,1,\,1,\,2,\,1,\,1,\,2,\,\frac{1}{3},\,\frac{1}{3},\,2,\,1,\,1)^T$. Numerical continuation of $x^{(a)}$ using rate constant k_1 as bifurcation parameter confirms a saddle-node bifurcation at $x^{(a)}$ (see Fig. 7.2, where x_3 is plotted against k_1).

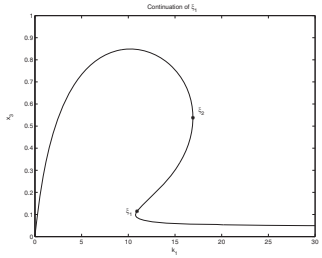


Figure 7.1: Continuation of $x^{(1)}$ using k_1. The figure shows x_3, the third component of the state vector. Along the continuation two positive saddle-node points have been identified (ξ_1 and ξ_2).

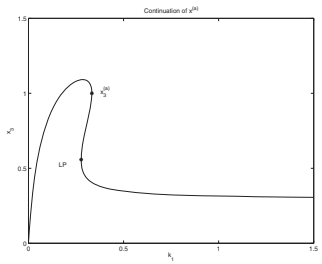


Figure 7.2: Continuation of $x^{(a)}$, using rate constant k_1 as bifurcation parameter. The figure shows x_3, the third component of the state vector. The starting point of the continuation $x^{(a)}$ can be identified as saddle-node point.

7.2.3 Bifurcations of higher codimension: Bogdanov-Takens

The ideas discussed in the previous section can be used to determine points where bifurcations of higher codimension can occur. For bifurcations of Bogdanov-Takens type the Jacobian of the reduced system must have a Jordan block of the form $\left[\begin{smallmatrix} 0 & 1 \\ 0 & 0 \end{smallmatrix}\right]$, see e.g. [51, p. 456] or [43, p. 272]).
From Lemma 7 follows that this is the case, iff the Jacobian of the original system has a zero eigenvalue with algebraic multiplicity three and geometric multiplicity one, that is, the following vectors must

exist:

$$J(x,k)\ \gamma_0 = 0$$
$$J(x,k)\ \gamma_1 = \gamma_0$$
$$J(x,k)\ \gamma_2 = \gamma_1$$

These are equivalent to the condition

$$J(x,k)\ \gamma_0 = 0,\ W^T \gamma_0 = 0 \text{ and } W^T \gamma_1 = 0. \tag{7.22}$$

From Section 7.2.1 it is known that the first condition is equivalent to $\Gamma(x_0)\ \pi_0 = 0$ (in this case $\gamma_0 = \mathrm{diag}(x_0)\ M_{\mathcal{N}_4}\ \pi_0$). For the remaining conditions recall the special form of the Jacobian

$$J(\lambda,\ x_0) = J(\lambda)\ \mathrm{diag}\left(\frac{1}{x_0}\right) \quad \text{with } J(\lambda) = N\ \mathrm{diag}(E\,\lambda)\ {Y^{(L)}}^T$$

Using Corollary 6 one obtains $x_0 = \frac{S\,\rho_0}{M_{\mathcal{N}_4}\,\pi_0}$ and $\gamma_0 = S\,\rho_0$. Thus a solution γ_1 to

$$J(\lambda)\ \mathrm{diag}\left(\frac{1}{x_0}\right)\ \gamma_1 = S\,\rho_0 \tag{7.23}$$

with $W^T \gamma_1 = 0$ and $\lambda > 0$ is required. By definition of $J(\lambda)$

$$[S]^{\perp} \subseteq [J(\lambda)]^{\perp},$$

thus (7.23) has a solution. Using $v := \mathrm{diag}\left(\frac{1}{x_0}\right)\ \gamma_1$ one obtains the equations

$$J(\lambda)\ v = S\,\rho_0 \tag{7.24a}$$
$$W^T\ \mathrm{diag}(x_0)\ v = 0 \tag{7.24b}$$

Using the transformation matrix

$$U(\lambda) = \begin{bmatrix}
-\frac{1}{\lambda_5} & 0 & -\frac{\lambda_1}{\lambda_5\,(\lambda_1+\lambda_5)} & 0 & -\frac{1}{\lambda_2+\lambda_6} & -\frac{1}{\lambda_6} & 0 & -\frac{1}{\lambda_6} & \frac{1}{\lambda_4+\lambda_5} \\
0 & 0 & 0 & 0 & \frac{1}{\lambda_2+\lambda_6} & \frac{1}{\lambda_6} & 0 & \frac{1}{\lambda_6} & -\frac{1}{\lambda_4+\lambda_5} \\
-\frac{1}{\lambda_5} & 0 & -\frac{1}{\lambda_5} & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{1}{\lambda_4+\lambda_5} \\
0 & 0 & 0 & 0 & 0 & \frac{1}{\lambda_6} & 0 & \frac{1}{\lambda_6} & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & \frac{1}{\lambda_3+\lambda_6} & 0 \\
0 & \frac{1}{\lambda_2+\lambda_6} & \frac{1}{\lambda_2+\lambda_6} & 0 & \frac{1}{\lambda_2+\lambda_6} & 0 & 0 & 0 & 0 \\
\frac{1}{\lambda_6} & 0 & \frac{1}{\lambda_6} & \frac{1}{\lambda_6} & \frac{1}{\lambda_6} & \frac{1}{\lambda_6} & 0 & \frac{1}{\lambda_6} & \frac{1}{\lambda_6} \\
0 & 0 & 0 & 0 & 0 & 0 & \frac{1}{\lambda_3+\lambda_6} & \frac{1}{\lambda_3+\lambda_6} & \frac{1}{\lambda_3+\lambda_6}
\end{bmatrix}$$

one obtains for (7.24a)

$$\begin{bmatrix} I_6 & \tilde{K} \\ 0_{3\times 6} & 0_{3\times 3} \end{bmatrix} v = U\,S\,\rho_0,$$

where

$$\ker(J(\lambda)) = \begin{bmatrix} -\tilde{K} \\ I_3 \end{bmatrix} = M_{\mathcal{N}_4}\,\pi_1.$$

Thus one obtains

$$\gamma_1 = \mathrm{diag}(x_0)\ [U(\lambda)\ S\,\rho_0 + M_{\mathcal{N}_4}\,\pi_1]. \tag{7.25}$$

Note that $U(\lambda)$ is invertible for $\lambda_i > 0$, as $\det(U(\lambda)) = \frac{1}{\lambda_5\,(\lambda_1+\lambda_5)\,(\lambda_4+\lambda_5)\,{\lambda_6}^2\,(\lambda_2+\lambda_6)^2\,(\lambda_3+\lambda_6)^2}$. Thus multiplication with $U(\lambda)$ is justified. Condition (7.24b) now becomes

$$W^T\ \mathrm{diag}(x_0)\ U(\lambda)\ S\,\rho_0 + W^T\ \mathrm{diag}(x_0)\ M_{\mathcal{N}_4}\,\pi = 0,$$

that is one has to find $\rho_0 \in \mathbb{R}^6$ and $\pi_1 \in \mathbb{R}^3$ such that

$$Q(x_0,\lambda)\,\rho_0 + \Gamma(x_0)\ \pi_1 = 0, \tag{7.26}$$

where $\Gamma(x_0)$ as in (7.14) and

$$Q(x_0, \lambda) := W^T \operatorname{diag}(x_0)\, U(\lambda)\, S. \tag{7.27}$$

Recall that x_0 depends on $\pi_0 \in \mathbb{R}^3$, $\rho_0 \in \mathbb{R}^6$ via (7.16a). Equation (7.26) has the following form

$$\begin{bmatrix} q_1^T \\ 0 \\ q_3^T \end{bmatrix} \rho_0 + \begin{bmatrix} g_1^T \\ g_2^T \\ g_3^T \end{bmatrix} \pi_1 = 0, \tag{7.28}$$

where $q_i^T = (q_{i,1}, \ldots, q_{i,6})$ and $g_i^T = (g_{i_1},\ g_{i_2}, g_{i_3})$ are vectors of rational functions of ρ_{0_i}, $i = 1, 2, 3$, π_{0_i}, $i = 1, \ldots, 6$ and λ_i, $i = 1, \ldots, 6$. Solve in a first step $g_2^T\, \pi_1 = 0$ to obtain a solution for π_1:

$$\pi_1 = \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ c_1 & c_2 \end{bmatrix} \begin{pmatrix} \pi_{1_1} \\ \pi_{1_2} \end{pmatrix},$$

with $c_{1,2}$ rational functions of ρ_{0_i}, $i = 1, 2, 3$ and π_{0_i}, $i = 1, \ldots, 6$ and $\pi_{1_{1,2}} \in \mathbb{R}$. The resulting system is

$$\begin{bmatrix} q_1^T \\ 0 \\ q_3^T \end{bmatrix} \rho_0 + \begin{bmatrix} \tilde{g}_1^T \\ 0 \\ \tilde{g}_3^T \end{bmatrix} \begin{pmatrix} \pi_{1_1} \\ \pi_{1_2} \end{pmatrix} = 0,$$

with

$$\begin{bmatrix} \tilde{g}_1^T \\ 0 \\ \tilde{g}_3^T \end{bmatrix} := \begin{bmatrix} g_1^T \\ g_2^T \\ g_3^T \end{bmatrix} \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ c_1 & c_2 \end{bmatrix} \begin{pmatrix} \pi_{1_1} \\ \pi_{1_2} \end{pmatrix}.$$

That is

$$\begin{bmatrix} \tilde{g}_1^T & q_1^T \\ \tilde{g}_3^T & q_3^T \end{bmatrix} \begin{pmatrix} \pi_{1_1} \\ \pi_{1_2} \\ \rho_0 \end{pmatrix} = 0.$$

Multiplication with an invertible matrix, yields

$$\begin{bmatrix} 1 & g_0 & \tilde{q}_1^T \\ 0 & 0 & \tilde{q}_3^T \end{bmatrix} \begin{pmatrix} \pi_{1_1} \\ \pi_{1_2} \\ \rho_0 \end{pmatrix} = 0.$$

Thus $\tilde{q}_3^T\, \rho_0 = 0$ must hold. Note that $\tilde{q}_3^T\, \rho_0 = 0$ is a lengthy rational functional of the 15 unknowns ρ_{0_i}, $i = 1, \ldots, 6$, the π_{0_i}, $i = 1, \ldots, 3$ and the λ_i, $i = 1, \ldots, 6$ that fills several rows. If one is interested in positive x_0, the analysis described above is required for each δ where $\mathcal{M}_{\mathcal{N}_4} \cap \mathbb{R}^9_\delta \neq \emptyset$ and $\mathcal{S}_{\mathcal{N}_4} \cap \mathbb{R}^9_\delta \neq \emptyset$: in this case $x_0^\delta = \frac{E^\delta_{\mathcal{S}_{\mathcal{N}_4}}\, \rho_0}{E^\delta_{\mathcal{M}_{\mathcal{N}_4}}\, \pi_0}$ from (7.17) and $\gamma_0^\delta = E_{\mathcal{S}}^\delta\, \rho_0$ have to be used. Note that in this case one has to look for **positive** π_0 and ρ and **arbitrary** $\pi_{1_{1,2}}$.
To demonstrate the usefulness of this approach, consider $\pi_{0_i} = 1$, $i = 1, \ldots, 3$, $\rho_{0_i} = 1$, $i = 2, \ldots 6$ and $\lambda_i = 1$, $i = 1, \ldots, 5$. The resulting rational function in ρ_{0_1} and λ_6 is given by:

$$\rho_{0_1} = -\frac{1908 + 2937\,\lambda_6 + 977\,{\lambda_6}^2 + 40\,{\rho_{0_1}}^2\,(24 + 36\,\lambda_6 + 11\,{\lambda_6}^2) + 8\,\rho_{0_1}\,(417 + 686\,\lambda_6 + 231\,{\lambda_6}^2)}{4\,\lambda_6\,(72 + 57\,\lambda_6 + 10\,{\rho_{0_1}}^2\,(4 + 3\,\lambda_6) + 8\,\rho_{0_1}\,(23 + 15\,\lambda_6))} \tag{7.29a}$$

Solving for λ_6 as a function of ρ_{0_1} one obtains (as one root):

$$\lambda_6 = \frac{-2937 - 5776\,\rho_{0_1} - 2176\,{\rho_{0_1}}^2 - 160\,{\rho_{0_1}}^3 - \sqrt{(1169505 + 5047104\,\rho_{0_1} + 7668736\,{\rho_{0_1}}^2 + 4912832\,{\rho_{0_1}}^3 + 1449216\,{\rho_{0_1}}^4 + 235520\,{\rho_{0_1}}^5 + 25600\,{\rho_{0_1}}^6)}}{2\,(977 + 2076\,\rho_{0_1} + 920\,{\rho_{0_1}}^2 + 120\,{\rho_{0_1}}^3)} \tag{7.29b}$$

For $\rho_{0_1} \in (-5, 5)$ equation (7.29b) is plotted in Fig. 7.3. For example, in the interval $(-0.721918, -0.634033)$ one obtains positive values of λ_6. Choosing $\rho_{0_1} = -0.7$ yields $\lambda_6 = 0.363018$ and

$$x_0 = \left(2.3,\ -0.7,\ \frac{1}{2},\ \frac{1}{2},\ 0.3,\ \frac{1}{5},\ \frac{1}{4},\ 2,\ \frac{1}{2}\right)^T \tag{7.30a}$$

and thus

$$k_0 = (-1.24224,\ 2,\ 2,\ -3.89434,\ 3.33333,\ 1.21006,\ 27.2604,\ 0.5,\ 0.181509,\ 16,\ 2,\ 2)^T. \tag{7.30b}$$

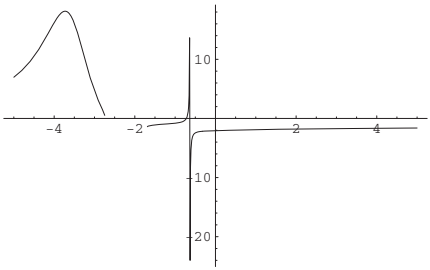


Figure 7.3: Plot of (7.29b) for $\rho_{1_1} \in (-5, 5)$

Using this data for a numerical continuation with respect to the conserved moieties, one finds that at $(x_0,\ k_0)$ indeed a Takens-Bogdanov bifurcation occurs (that is sufficient conditions for a Takens-Bogdanov bifurcations are satisfied as well; see Figure 7.4, where $(x_0,\ k_0)$ are used as initial point of the continuation. The numerical continuation software Matcont finds a Takens-Bogdanov bifurcation after one step.). Note that the bifurcation diagram depicted in Fig. 7.4 is quite involved: in the c_1-c_3 plane

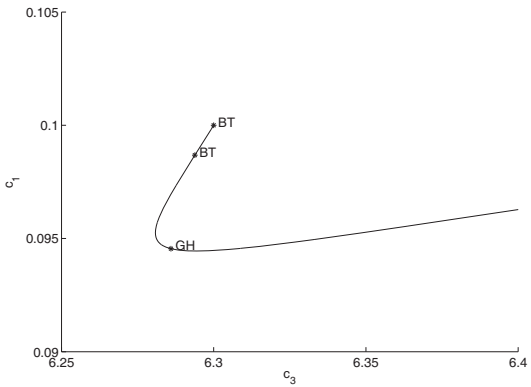


Figure 7.4: Continuation of $(x_0,\ k_0)$ as given in (7.30a) and (7.30b)with respect c_1 (total concentration of E_1) and c_3 (total concentration of A)

the Takens-Bogdanov bifurcation at (0.1,6.3) is followed by another Takens-Bogdanov bifurcation at (0.098678,6.2938) and a Generalized-Hopf bifurcation at (0.09455,6.2859). To gather further numerical evidence for the Takens-Bogdanov bifurcation an initial point in the parameter region where sustained oscillations occur was chosen:
Figure 7.5 shows the numerical integration of the ODES $x_0,\ k_0$ as given above and $c_1 = 0.099603388$. Fig. 7.5 suggest a limit cycle with period $T = 0.4$. Using this cycle as an initial point for a numerical continuation of limit cycles in Matcont, the bifurcation diagram depicted in Fig. 7.6 was obtained. Here two bifurcations of limit cycles occur: a limit point of cycles (LPC) or fold bifurcation of cycles (see e.g. [43, p. 149]) at $(c_1,\ c_3) = (0.099225,\ 6.3101)$ and at $(c_1,\ c_3) = (0.09852,\ 6.3099)$. The second fold bifurcation of cycles is immediately followed by a neutral saddle (of limit cycles labeled NS) at $(c_1,\ c_3) = (0.098457,\ 6.3099)$.
Note that for obtaining Fig. 7.6 the continuation of limit cycles was performed using c_1 and c_3 as bifurcation parameters.

Remark 15. *For the numerical analysis used to obtain Fig. 7.5 and Fig. 7.6 each ODE was scaled by*

a factor $s = 100$. *This corresponds to a time scale* $t := s\,\tau$. *Then* $x(t) = x(s\,\tau)$ *and* $x' := \frac{d}{d\,\tau}\,x(s\,\tau) = \frac{d}{d\,t}\,x(s\,\tau)\,\frac{d}{d\,\tau}\,t = s\,\dot{x}(t) = s\,Y\,I_a\,v(k,\,x(t))$.

Remark 16. *Equation* (7.29a) *has been obtained using generators of* $\mathcal{M}_{\mathcal{N}_4} \cap R^9_{\delta_1}$ *and* $\mathcal{S}_{\mathcal{N}_4} \cap \mathbb{R}^9_{\delta_1}$ *for* δ_1 *as in* (4.16). *Obtaining negative values for* ρ_{0_1} *is justified in this case, as generators of a cone are a basis for the linear subspace that contains the cone, in this case* $\mathcal{M}_{\mathcal{N}_4}$. *The idea was to try to find solutions to* (7.29a) *with as many positive components as possible.*

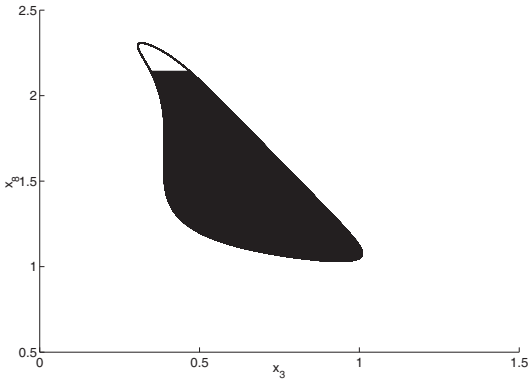


Figure 7.5: Numerical integration starting at x^1 using k_0

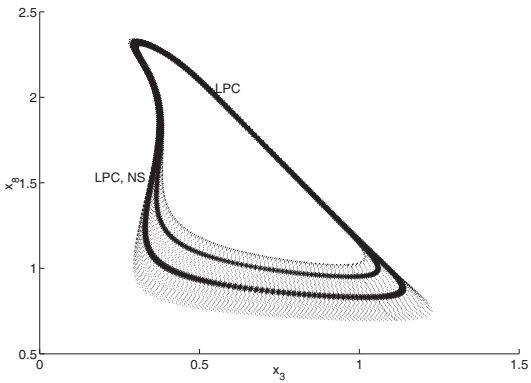


Figure 7.6: Continuation of limit cycles starting with the limit cycle shown in Fig. 7.5

Chapter 8

Conclusions

In this thesis multistationarity in (bio)chemical reaction networks with mass action kinetics is analysed. As variables typically describe concentrations of chemical species, only positive steady state solutions are of interest. In general one therefore has to look for positive solutions to a system of polynomial equations with unknown coefficients. In its most general form, these equations are given by (see Chapter 2):

$$\begin{aligned} Y\, I_a\, v(k,\, x) &= 0 \\ W^T\, x &= c. \end{aligned}$$

From these it is possible to derive a system of nonlinear equations with known coefficients:

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

Vectors μ and $\nu > 0$,$\lambda > 0$, that are solutions to these equations, define, in general, a parameterization of pairs of steady states a, b with $Y\, I_a\, v(k,\, a) = Y\, I_a\, v(k,\, b) = 0$ (Lemma 1, Chapter 3). That is, one has established an infinite set of positive solutions to the polynomial equations $Y\, I_a\, v(k,\, x) = 0$. But, in general, these solutions do not satisfy the conservation relations $W^T\, a = W^T\, b$. Once solutions $\mu \in I\!R^n$ and ν, $\lambda \in I\!R^p_{>0}$ to the nonlinear equation $Y^{(L)^T}\, \mu = \ln \frac{E\, \nu}{E\, \lambda}$ have been established, one has to find a vector $v \in \mathcal{S}$ with $\mathrm{sign}\,(v) = \mathrm{sign}\,(\mu)$ (Lemma 3, Theorem 3) in order to find solutions with $W^T\, a = W^T\, b$ (Lemma 3, Theorem 3).

Note that in special cases Theorem 3 contains in fact necessary and sufficient conditions for multistationarity in a given (bio)chemical reaction network. This is the case, if vectors ν, $\lambda \in I\!R^n_{>0}$ can be determined independently of vector μ (as is, for example the case for the networks discussed in Chapter 4 and 6). In this case one obtains an explicit representation of μ: $\mu \in \mathcal{M}$, a linear subspace of $I\!R^n$. Thus one can test all orthants δ of $I\!R^n$ for $\mathcal{S} \cap I\!R^n_\delta \neq \emptyset$ and $\mathcal{M} \cap I\!R^n_\delta \neq \emptyset$. If a δ can be established with $\mathcal{S} \cap I\!R^n_\delta \neq \emptyset$ and $\mathcal{M} \cap I\!R^n_\delta \neq \emptyset$, then multistationarity is established. If no δ exists, then multistationarity is excluded by Theorem 3. However, if vectors $\nu > 0$ and $\lambda > 0$ cannot be determined independently of vector μ (as, for example, for the networks discussed in Section 5), then Theorem 3 gives only sufficient conditions for multistationarity: if, for a given μ, one can establish a $v \in \mathcal{S}$ with $\mathrm{sign}\,(\mu) = \mathrm{sign}\,(v)$, then multistationarity is established, if no such $v \in \mathcal{S}$ can be established, there is no guarantee that there does not exist a different μ that satisfies this condition.

The usefulness of the methodology developed in Chapter 3 has been demonstrated by applying it to (bio)chemical reaction networks from signal transduction in Chapter 4 and cell cycle control in Chapter 5. Of particular interest in a systems biology oriented context is the potential application for model discrimination (as discussed in Chapter 4.5 and 5.1) and as an explanation for robustness (as discussed in Chapter 6). Note that both is possible, if vectors $\nu > 0$ and $\lambda > 0$ can be determined independently of vector μ. In this case it is furthermore possible to determine potential bifurcation points analytically (as discussed in Chapter 7). Thus, this thesis closes with one question for further research: which structural properties of a (bio)chemical reaction network allow to determine vectors ν, $\lambda \in I\!R^p_{>0}$ can be independently of vector μ?

Appendix A

Models for the activation of an MAPK(K)

A.1 Distributive mechanism for phosphorylation and dephosphorylation

A.1.1 Species and complexes of network $\mathcal{N}_4$

Species	x_i	Complex	y_i
A	x_1	$A + E_1$	y_1
E_1	x_2	$A\,E_1$	y_2
$A\,E_1$	x_3	$A_p + E_1$	y_3
A_p	x_4	$A_p\,E_1$	y_4
$A_p\,E_1$	x_5	$A_{pp} + E_1$	y_5
A_{pp}	x_6	$A_{pp} + E_2$	y_6
E_2	x_7	$A_{pp}\,E_2$	y_7
$A_{pp}\,E_2$	x_8	$A_p + E_2$	y_8
$A_p\,E_2$	x_9	$A_p\,E_2$	y_9
		$A + E_2$	y_{10}

A.1.2 Ordinary differential equations

$$\dot{x}_1 = -k_1\,x_1\,x_2 + k_2\,x_3 + k_{12}\,x_9 \tag{A.1a}$$
$$\dot{x}_2 = -k_1\,x_1\,x_2 + (k_2 + k_3)\,x_3 - k_4\,x_2\,x_4 + (k_5 + k_6)\,x_5 \tag{A.1b}$$
$$\dot{x}_3 = k_1\,x_1\,x_2 + (-k_2 - k_3)\,x_3 \tag{A.1c}$$
$$\dot{x}_4 = k_3\,x_3 - k_4\,x_2\,x_4 + k_5\,x_5 + k_9\,x_8 - k_{10}\,x_4\,x_7 + k_{11}\,x_9 \tag{A.1d}$$
$$\dot{x}_5 = k_4\,x_2\,x_4 + (-k_5 - k_6)\,x_5 \tag{A.1e}$$
$$\dot{x}_6 = k_6\,x_5 - k_7\,x_6\,x_7 + k_8\,x_8 \tag{A.1f}$$
$$\dot{x}_7 = -k_7\,x_6\,x_7 + (k_8 + k_9)\,x_8 - k_{10}\,x_4\,x_7 + (k_{11} + k_{12})\,x_9 \tag{A.1g}$$
$$\dot{x}_8 = k_7\,x_6\,x_7 + (-k_8 - k_9)\,x_8 \tag{A.1h}$$
$$\dot{x}_9 = k_{10}\,x_4\,x_7 + (-k_{11} - k_{12})\,x_9 \tag{A.1i}$$

$$x_2 + x_3 + x_5 = c_1 \tag{A.2a}$$
$$x_7 + x_8 + x_9 = c_2 \tag{A.2b}$$
$$x_1 + x_3 + x_4 + x_5 + x_6 + x_8 + x_9 = c_3 \tag{A.2c}$$

A.1.3 Structural data

The ordinary differential equations (A.1a) – (A.1i) can be written as

$$\dot{x} = Y\, I_a\, \mathrm{diag}\,(k)\, \phi\,(x) \tag{A.3a}$$

using matrices

$$Y = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ 1 & 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \end{bmatrix}, \tag{A.3b}$$

$$I_a = \begin{bmatrix} -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix} \tag{A.3c}$$

and the monomial vector

$$\phi\,(x) = (\, x_1\, x_2,\, x_3,\, x_3,\, x_2\, x_4,\, x_5,\, x_5,\, x_6\, x_7,\, x_8,\, x_8,\, x_4\, x_7,\, x_9,\, x_9\,)' \tag{A.3d}$$

A representation $\phi\,(x) = \left(x^{y_1^{(L)}}, \ldots, x^{y_r^{(L)}}\right)'$ can be obtained using

$$Y^{(L)} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \end{bmatrix} \tag{A.3e}$$

The conservation relations (A.2a) – (A.2c) can be written as

$$W^T x = c \tag{A.4a}$$

using the matrix

$$W^T = \begin{bmatrix} 0 & 1 & 1 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 \\ 1 & 0 & 1 & 1 & 1 & 1 & 0 & 1 & 1 \end{bmatrix} \tag{A.4b}$$

and the vector $c = (\, c_1,\, c_2,\, c_3\,)^T$.

A.2 A processive mechanism for phosphorylation and a distributive mechanism for dephosphorylation

A.2.1 Species and complexes of network $\mathcal{N}_5$

Species	x_i	Complex	y_i
A	x_1	$A + E_1$	y_1
E_1	x_2	$A\,E_1$	y_2
$A\,E_1$	x_3	$A_p\,E_1$	y_3
$A_p\,E_1$	x_4	$A_{pp} + E_1$	y_4
A_{pp}	x_5	$A_{pp} + E_2$	y_5
E_2	x_6	$A_{pp}\,E_2$	y_6
$A_{pp}\,E_2$	x_7	$A_p + E_2$	y_7
A_p	x_8	$A_p\,E_2$	y_8
$A_p\,E_2$	x_9	$A + E_2$	y_9

A.2.2 Ordinary differential equations

$$\dot{x}_1 = -k_1\,x_1\,x_2 + k_2\,x_3 + k_{11}\,x_9 \tag{A.5a}$$
$$\dot{x}_2 = -k_1\,x_1\,x_2 + k_2\,x_3 + k_5\,x_4 \tag{A.5b}$$
$$\dot{x}_3 = k_1\,x_1\,x_2 + (-k_2 - k_3)\,x_3 + k_4\,x_4 \tag{A.5c}$$
$$\dot{x}_4 = k_3\,x_3 + (-k_4 - k_5)\,x_4 \tag{A.5d}$$
$$\dot{x}_5 = k_5\,x_4 - k_6\,x_5\,x_6 + k_7\,x_7 \tag{A.5e}$$
$$\dot{x}_6 = -k_6\,x_5\,x_6 + (k_7 + k_8)\,x_7 - k_9\,x_6\,x_8 + (k_{10} + k_{11})\,x_9 \tag{A.5f}$$
$$\dot{x}_7 = k_6\,x_5\,x_6 + (-k_7 - k_8)\,x_7 \tag{A.5g}$$
$$\dot{x}_8 = k_8\,x_7 - k_9\,x_6\,x_8 + k_{10}\,x_9 \tag{A.5h}$$
$$\dot{x}_9 = k_9\,x_6\,x_8 + (-k_{10} - k_{11})\,x_9 \tag{A.5i}$$

$$x_2 + x_3 + x_4 = c_1 \tag{A.6a}$$
$$x_6 + x_7 + x_9 = c_2 \tag{A.6b}$$
$$x_1 + x_3 + x_4 + x_5 + x_7 + x_8 + x_9 = c_3 \tag{A.6c}$$

A.2.3 Structural data

The ordinary differential equations (A.9a) – (A.9h) can be written as

$$\dot{x} = Y\,I_a\,\mathrm{diag}\,(k)\,\phi\,(x) \tag{A.7a}$$

using matrices

$$Y = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \end{bmatrix}, \tag{A.7b}$$

$$I_a = \begin{bmatrix} -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & -1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix} \tag{A.7c}$$

and the monomial vector

$$\phi(x) = (\, x_1\, x_2,\, x_3,\, x_3,\, x_4,\, x_4,\, x_5\, x_6,\, x_7,\, x_7,\, x_6\, x_8,\, x_9,\, x_9\,)^{\cdot} \tag{A.7d}$$

A representation $\phi(x) = \left(x^{y_1^{(L)}}, \ldots, x^{y_r^{(L)}}\right)^{'}$ can be obtained using

$$Y^{(L)} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \end{bmatrix}. \tag{A.7e}$$

The conservation relations (A.10a) – (A.10c) can be written as

$$W^T x = c \tag{A.8a}$$

using the matrix

$$W^T = \begin{bmatrix} 0 & 1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 1 \\ 1 & 0 & 1 & 1 & 1 & 0 & 1 & 1 & 1 \end{bmatrix} \tag{A.8b}$$

and the vector $c = (\, c_1,\, c_2,\, c_3\,)^T$.

A.3 Processive mechanism for phosphorylation and dephosphorylation

A.3.1 Species and complexes of network $\mathcal{N}_6$

Species	x_i	Complex	y_i
A	x_1	$A + E_1$	y_1
E_1	x_2	$A\,E_1$	y_2
$A\,E_1$	x_3	$A_p\,E_1$	y_3
$A_p\,E_1$	x_4	$A_{pp} + E_1$	y_4
A_{pp}	x_5	$A_{pp} + E_2$	y_5
E_2	x_6	$A_{pp}\,E_2$	y_6
$A_{pp}\,E_2$	x_7	$A_p\,E_2$	y_7
$A_p\,E_2$	x_8	$A + E_2$	y_8

A.3.2 Ordinary differential equations

$$\dot{x}_1 = -k_1\,x_1\,x_2 + k_2\,x_3 + k_{10}\,x_8 \tag{A.9a}$$
$$\dot{x}_2 = -k_1\,x_1\,x_2 + k_2\,x_3 + k_5\,x_4 \tag{A.9b}$$
$$\dot{x}_3 = k_1\,x_1\,x_2 - (k_2 + k_3)\,x_3 + k_4\,x_4 \tag{A.9c}$$
$$\dot{x}_4 = k_3\,x_3 - (k_4 + k_5)\,x_4 \tag{A.9d}$$
$$\dot{x}_5 = k_5\,x_4 - k_6\,x_5\,x_6 + k_7\,x_7 \tag{A.9e}$$
$$\dot{x}_6 = -k_6\,x_5\,x_6 + k_7\,x_7 + k_{10}\,x_8 \tag{A.9f}$$
$$\dot{x}_7 = k_6\,x_5\,x_6 - (k_7 + k_8)\,x_7 + k_9\,x_8 \tag{A.9g}$$
$$\dot{x}_8 = k_8\,x_7 - (k_9 + k_{10})\,x_8 \tag{A.9h}$$

$$x_2 + x_3 + x_4 = c_1 \tag{A.10a}$$
$$x_6 + x_7 + x_8 = c_2 \tag{A.10b}$$
$$x_1 + x_3 + x_4 + x_5 + x_7 + x_8 = c_3 \tag{A.10c}$$

A.3.3 Structural data

The ordinary differential equations (A.9a) – (A.9h) can be written as

$$\dot{x} = Y\,I_a\ \mathrm{diag}\,(k)\ \phi\,(x) \tag{A.11a}$$

using matrices

$$Y = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \end{bmatrix} \tag{A.11b}$$

$$I_a = \begin{bmatrix} -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & -1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix} \tag{A.11c}$$

and the monomial vector

$$\phi(x) = (\, x_1 x_2,\, x_3,\, x_3,\, x_4,\, x_4,\, x_5 x_6,\, x_7,\, x_7,\, x_8,\, x_8 \,)' \tag{A.11d}$$

A representation $\phi(x) = \left(x^{y_1^{(L)}}, \ldots, x^{y_r^{(L)}}\right)'$ can be obtained using

$$Y^{(L)} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \end{bmatrix} \tag{A.11e}$$

The conservation relations (A.10a) – (A.10c) can be written as

$$W^T x = c \tag{A.12a}$$

using the matrix

$$W^T = \begin{bmatrix} 0 & 1 & 1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 \\ 1 & 0 & 1 & 1 & 1 & 0 & 1 & 1 \end{bmatrix} \tag{A.12b}$$

and the vector $c = (\, c_1,\, c_2,\, c_3 \,)^T$.

A.4 Open systems

A.4.1 Network $\mathcal{N}_4$+ $\mathcal{N}_7$

The network is given by

$$\begin{aligned} \mathbf{A} + \mathbf{E_1} \underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} \mathbf{A\,E_1} \xrightarrow{\mathbf{k_3}} \mathbf{A_p} + \mathbf{E_1} \underset{\mathbf{k_5}}{\overset{\mathbf{k_4}}{\rightleftharpoons}} \mathbf{A_p\,E_1} \xrightarrow{\mathbf{k_6}} \mathbf{A_{pp}} + \mathbf{E_1} \\ \mathbf{A_{pp}} + \mathbf{E_2} \underset{\mathbf{k_8}}{\overset{\mathbf{k_7}}{\rightleftharpoons}} \mathbf{A_{pp}\,E_2} \xrightarrow{\mathbf{k_9}} \mathbf{A_p} + \mathbf{E_2} \underset{\mathbf{k_{11}}}{\overset{\mathbf{k_{10}}}{\rightleftharpoons}} \mathbf{A_p\,E_2} \xrightarrow{\mathbf{k_{12}}} \mathbf{A} + \mathbf{E_2} \\ \mathbf{E_1} \underset{\mathbf{k_{14}}}{\overset{\mathbf{k_{13}}}{\rightleftharpoons}} 0 \end{aligned} \tag{$\mathcal{N}_{15}$}$$

For network $\mathcal{N}_{15}$ the equation

$$Y^{(L)^T} \mu = \ln \frac{E\,\nu}{E\,\lambda}$$

reads

$$\mu_1 + \mu_2 = \ln \frac{\nu_1 + \nu_6}{\lambda_1 + \lambda_6} \tag{A.13}$$
$$\mu_3 = \ln \frac{\nu_1}{\lambda_1} \tag{A.14}$$
$$\mu_3 = \ln \frac{\nu_6}{\lambda_6} \tag{A.15}$$
$$\mu_2 + \mu_4 = \ln \frac{\nu_2 + \nu_7}{\lambda_2 + \lambda_7} \tag{A.16}$$
$$\mu_5 = \ln \frac{\nu_2}{\lambda_2} \tag{A.17}$$
$$\mu_5 = \ln \frac{\nu_7}{\lambda_7} \tag{A.18}$$
$$\mu_6 + \mu_7 = \ln \frac{\nu_3 + \nu_7}{\lambda_3 + \lambda_7} \tag{A.19}$$
$$\mu_8 = \ln \frac{\nu_3}{\lambda_3} \tag{A.20}$$
$$\mu_8 = \ln \frac{\nu_7}{\lambda_7} \tag{A.21}$$
$$\mu_4 + \mu_7 = \ln \frac{\nu_4 + \nu_6}{\lambda_4 + \lambda_6} \tag{A.22}$$
$$\mu_9 = \ln \frac{\nu_4}{\lambda_4} \tag{A.23}$$
$$\mu_9 = \ln \frac{\nu_6}{\lambda_6} \tag{A.24}$$
$$0 = \ln \frac{\nu_5}{\lambda_5} \tag{A.25}$$
$$\mu_2 = \ln \frac{\nu_5}{\lambda_5}. \tag{A.26}$$

These equations are solvable, if and only if

$$\ln \frac{\nu_1}{\lambda_1} = \ln \frac{\nu_6}{\lambda_6} \qquad \ln \frac{\nu_2}{\lambda_2} = \ln \frac{\nu_7}{\lambda_7} \qquad \ln \frac{\nu_3}{\lambda_3} = \ln \frac{\nu_7}{\lambda_7} \qquad \ln \frac{\nu_4}{\lambda_4} = \ln \frac{\nu_6}{\lambda_6} \qquad \nu_5 = \lambda_5.$$

That is, if

$$\nu = \left(\lambda_1 \frac{\nu_6}{\lambda_6}, \lambda_2 \frac{\nu_7}{\lambda_7}, \lambda_3 \frac{\nu_7}{\lambda_7}, \lambda_4 \frac{\nu_6}{\lambda_6}, \lambda_5, \nu_6, \nu_7 \right)^T,$$

with $\lambda \in \mathbb{R}^7_{>0}$ and $\nu_6, \nu_7 > 0$ free. Note that it is possible to determine ν and $\lambda \in \mathbb{R}^7_{>0}$ independent of $\mu \in \mathbb{R}^9$. Using ν as above, one obtains

$$\ln \frac{\nu_1 + \nu_6}{\lambda_1 + \lambda_6} = \ln \frac{\nu_4 + \nu_4}{\lambda_4 + \lambda_6} = \ln \frac{\nu_6}{\lambda_6}$$

and

$$\ln \frac{\nu_2 + \nu_7}{\lambda_2 + \lambda_7} = \ln \frac{\nu_3 + \nu_7}{\lambda_3 + \lambda_7} = \ln \frac{\nu_7}{\lambda_7}.$$

Using $\kappa_1 := \ln \frac{\nu_6}{\lambda_6}$ and $\kappa_2 := \ln \frac{\nu_7}{\lambda_7}$ in the right hand side of (A.13) – (A.26) and solving for μ yields

$$\mu = \begin{bmatrix} 1 & 0 \\ 0 & 0 \\ 1 & 0 \\ 0 & 1 \\ 0 & 1 \\ -1 & 2 \\ 1 & -1 \\ 0 & 1 \\ 1 & 0 \end{bmatrix} \begin{pmatrix} \kappa_1 \\ \kappa_2 \end{pmatrix}$$

A.4.2 Network $\mathcal{N}_4$+ $\mathcal{N}_8$

The network is given by

$$\begin{aligned}
&\mathbf{A}+\mathbf{E_1} \underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} \mathbf{A}\,\mathbf{E_1} \xrightarrow{\mathbf{k_3}} \mathbf{A_p}+\mathbf{E_1} \underset{\mathbf{k_5}}{\overset{\mathbf{k_4}}{\rightleftharpoons}} \mathbf{A_p}\,\mathbf{E_1} \xrightarrow{\mathbf{k_6}} \mathbf{A_{pp}}+\mathbf{E_1}\\
&\mathbf{A_{pp}}+\mathbf{E_2} \underset{\mathbf{k_8}}{\overset{\mathbf{k_7}}{\rightleftharpoons}} \mathbf{A_{pp}}\,\mathbf{E_2} \xrightarrow{\mathbf{k_9}} \mathbf{A_p}+\mathbf{E_2} \underset{\mathbf{k_{11}}}{\overset{\mathbf{k_{10}}}{\rightleftharpoons}} \mathbf{A_p}\,\mathbf{E_2} \xrightarrow{\mathbf{k_{12}}} \mathbf{A}+\mathbf{E_2} \qquad (\mathcal{N}_{16})\\
&\mathbf{E_2} \underset{\mathbf{k_{14}}}{\overset{\mathbf{k_{13}}}{\rightleftharpoons}} 0
\end{aligned}$$

For network $\mathcal{N}_{16}$ the equation

$$Y^{(L)^T}\,\mu = \ln\frac{E\,\nu}{E\,\lambda}$$

reads

$$\begin{aligned}
\mu_1+\mu_2 &= \ln\frac{\nu_1+\nu_6}{\lambda_1+\lambda_6}\\
\mu_3 &= \ln\frac{\nu_1}{\lambda_1}\\
\mu_3 &= \ln\frac{\nu_6}{\lambda_6}\\
\mu_2+\mu_4 &= \ln\frac{\nu_2+\nu_7}{\lambda_2+\lambda_7}\\
\mu_5 &= \ln\frac{\nu_2}{\lambda_2}\\
\mu_5 &= \ln\frac{\nu_7}{\lambda_7}\\
\mu_6+\mu_7 &= \ln\frac{\nu_3+\nu_7}{\lambda_3+\lambda_7}\\
\mu_8 &= \ln\frac{\nu_3}{\lambda_3}\\
\mu_8 &= \ln\frac{\nu_7}{\lambda_7}\\
\mu_4+\mu_7 &= \ln\frac{\nu_4+\nu_6}{\lambda_4+\lambda_6}\\
\mu_9 &= \ln\frac{\nu_4}{\lambda_4}\\
\mu_9 &= \ln\frac{\nu_6}{\lambda_6}\\
0 &= \ln\frac{\nu_5}{\lambda_5}\\
\mu_7 &= \ln\frac{\nu_5}{\lambda_5}
\end{aligned}$$

These equations are solvable, if and only if

$$\ln\frac{\nu_1}{\lambda_1}=\ln\frac{\nu_6}{\lambda_6} \qquad \ln\frac{\nu_2}{\lambda_2}=\ln\frac{\nu_7}{\lambda_7} \qquad \ln\frac{\nu_3}{\lambda_3}=\ln\frac{\nu_7}{\lambda_7} \qquad \ln\frac{\nu_4}{\lambda_4}=\ln\frac{\nu_6}{\lambda_6} \qquad \nu_5=\lambda_5$$

That is, if

$$\nu = \left(\lambda_1\frac{\nu_6}{\lambda_6},\,\lambda_2\frac{\nu_7}{\lambda_7},\,\lambda_3\frac{\nu_7}{\lambda_7},\,\lambda_4\frac{\nu_6}{\lambda_6},\,\lambda_5,\,\nu_6,\,\nu_7\right)^T,$$

with $\lambda \in \mathbb{R}^7_{>0}$ and $\nu_6,\,\nu_7 > 0$ free. (Note that ν as given above is equal to the vector ν obtained for network $\mathcal{N}_{15}$ in Section A.4.1). As network $\mathcal{N}_{15}$ it is possible to determine ν and $\lambda \in \mathbb{R}^7_{>0}$ independent of $\mu \in \mathbb{R}^9$. Using ν as above, one obtains

$$\ln\frac{\nu_1+\nu_6}{\lambda_1+\lambda_6} = \ln\frac{\nu_4+\nu_6}{\lambda_4+\lambda_6} = \ln\frac{\nu_6}{\lambda_6}$$

and

$$\ln \frac{\nu_2 + \nu_7}{\lambda_2 + \lambda_7} = \ln \frac{\nu_3 + \nu_7}{\lambda_3 + \lambda_7} = \ln \frac{\nu_7}{\lambda_7}.$$

Using $\kappa_1 := \ln \frac{\nu_6}{\lambda_6}$ and $\kappa_2 := \ln \frac{\nu_7}{\lambda_7}$ in the right hand side of (A.13) – (A.26) and solving for μ yields

$$\mu = \begin{bmatrix} 2 & -1 \\ -1 & 1 \\ 1 & 0 \\ 1 & 0 \\ 0 & 1 \\ 0 & 1 \\ 0 & 0 \\ 0 & 1 \\ 1 & 0 \end{bmatrix} \begin{pmatrix} \kappa_1 \\ \kappa_2 \end{pmatrix}$$

A.4.3 Network $\mathcal{N}_4$+ $\mathcal{N}_9$

The network is given by

$$\begin{aligned} \mathbf{A} + \mathbf{E_1} &\underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} \mathbf{A\,E_1} \xrightarrow{\mathbf{k_3}} \mathbf{A_p} + \mathbf{E_1} \underset{\mathbf{k_5}}{\overset{\mathbf{k_4}}{\rightleftharpoons}} \mathbf{A_p\,E_1} \xrightarrow{\mathbf{k_6}} \mathbf{A_{pp}} + \mathbf{E_1} \\ \mathbf{A_{pp}} + \mathbf{E_2} &\underset{\mathbf{k_8}}{\overset{\mathbf{k_7}}{\rightleftharpoons}} \mathbf{A_{pp}\,E_2} \xrightarrow{\mathbf{k_9}} \mathbf{A_p} + \mathbf{E_2} \underset{\mathbf{k_{11}}}{\overset{\mathbf{k_{10}}}{\rightleftharpoons}} \mathbf{A_p\,E_2} \xrightarrow{\mathbf{k_{12}}} \mathbf{A} + \mathbf{E_2} \\ \mathbf{E_1} &\underset{\mathbf{k_{14}}}{\overset{\mathbf{k_{13}}}{\rightleftharpoons}} 0 \underset{\mathbf{k_{16}}}{\overset{\mathbf{k_{15}}}{\rightleftharpoons}} \mathbf{E_2} \end{aligned} \qquad (\mathcal{N}_{17})$$

For network $\mathcal{N}_{17}$ the equation

$$Y^{(L)^T} \mu = \ln \frac{E\,\nu}{E\,\lambda}$$

reads

$$\begin{aligned}
\mu_1 + \mu_2 &= \ln \frac{\nu_1 + \nu_7}{\lambda_1 + \lambda_7} \\
\mu_3 &= \ln \frac{\nu_1}{\lambda_1} \\
\mu_3 &= \ln \frac{\nu_7}{\lambda_7} \\
\mu_2 + \mu_4 &= \ln \frac{\nu_2 + \nu_8}{\lambda_2 + \lambda_8} \\
\mu_5 &= \ln \frac{\nu_2}{\lambda_2} \\
\mu_5 &= \ln \frac{\nu_8}{\lambda_8} \\
\mu_6 + \mu_7 &= \ln \frac{\nu_3 + \nu_8}{\lambda_3 + \lambda_8} \\
\mu_8 &= \ln \frac{\nu_3}{\lambda_3} \\
\mu_8 &= \ln \frac{\nu_8}{\lambda_8} \\
\mu_4 + \mu_7 &= \ln \frac{\nu_4 + \nu_7}{\lambda_4 + \lambda_7} \\
\mu_9 &= \ln \frac{\nu_4}{\lambda_4} \\
\mu_9 &= \ln \frac{\nu_7}{\lambda_7} \\
\mu_2 &= \ln \frac{\nu_5}{\lambda_5} \\
0 &= \ln \frac{\nu_5}{\lambda_5} \\
0 &= \ln \frac{\nu_6}{\lambda_6} \\
\mu_7 &= \ln \frac{\nu_6}{\lambda_6}
\end{aligned}$$

These equations are solvable, if and only if

$$\ln \frac{\nu_1}{\lambda_1} = \ln \frac{\nu_7}{\lambda_7} \qquad \ln \frac{\nu_2}{\lambda_2} = \ln \frac{\nu_8}{\lambda_8} \qquad \ln \frac{\nu_3}{\lambda_3} = \ln \frac{\nu_8}{\lambda_8} \qquad \ln \frac{\nu_4}{\lambda_4} = \ln \frac{\nu_7}{\lambda_7} \qquad \nu_5 = \lambda_5 \qquad \nu_6 = \lambda_6.$$

Thus one obtains

$$\begin{aligned}
\nu_1 &= \lambda_1 \frac{\nu_7}{\lambda_7} & \qquad \nu_2 &= \lambda_2 \frac{\nu_8}{\lambda_8} \\
\nu_3 &= \lambda_3 \frac{\nu_8}{\lambda_8} & \qquad \nu_4 &= \lambda_4 \frac{\nu_8}{\lambda_8}.
\end{aligned}$$

Note that $\mu_2 = 0$ and $\mu_7 = 0$ and thus $\mu_4 = \ln \frac{\nu_2+\nu_8}{\lambda_2+\lambda_8}$ and $\mu_4 = \ln \frac{\nu_4+\nu_7}{\lambda_4+\lambda_7}$ follows. Thus one finally obtains $\frac{\nu_7}{\lambda_7} = \frac{\nu_8}{\lambda_8}$ and therefore

$$\nu = \left(\lambda_1 \frac{\nu_8}{\lambda_8},\, \lambda_2 \frac{\nu_8}{\lambda_8},\, \lambda_3 \frac{\nu_8}{\lambda_8},\, \lambda_4 \frac{\nu_8}{\lambda_8},\, \lambda_5,\, \lambda_6,\, \lambda_7 \frac{\nu_8}{\lambda_8}, \nu_8 \right)^T,$$

with $\lambda \in \mathbb{R}^8_{>0}$, $\nu_8 > 0$, free. As network $\mathcal{N}_{15}$ it is possible to determine ν and $\lambda \in \mathbb{R}^8_{>0}$ independent of $\mu \in \mathbb{R}^9$. Using ν as above, one obtains

$$\ln \frac{\nu_1 + \nu_7}{\lambda_1 + \lambda_7} = \ln \frac{\nu_2 + \nu_8}{\lambda_2 + \lambda_8} = \ln \frac{\nu_3 + \nu_8}{\lambda_3 + \lambda_8} = \ln \frac{\nu_4 + \nu_7}{\lambda_4 + \lambda_7} = \ln \frac{\nu_8}{\lambda_8}$$

Using $\kappa_1 := \ln \frac{\nu_8}{\lambda_8}$ and solving for μ yields

$$\mu = \left[\begin{array}{cccccccc} 1, & 0, & 1, & 1, & 1, & 1, & 0, & 1, & 1 \end{array} \right]^T \kappa_1$$

A.4.4 Network $\mathcal{N}_5$+ $\mathcal{N}_7$

The network is given by

$$\begin{array}{c} \mathbf{A}+\mathbf{E_1} \underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} \mathbf{A}\,\mathbf{E_1} \underset{\mathbf{k_4}}{\overset{\mathbf{k_3}}{\rightleftharpoons}} \mathbf{A_p}\,\mathbf{E_1} \xrightarrow{\mathbf{k_5}} \mathbf{A_{pp}}+\mathbf{E_1} \\ \mathbf{A_{pp}}+\mathbf{E_2} \underset{\mathbf{k_7}}{\overset{\mathbf{k_6}}{\rightleftharpoons}} \mathbf{A_{pp}}\,\mathbf{E_2} \xrightarrow{\mathbf{k_8}} \mathbf{A_p}+\mathbf{E_2} \underset{\mathbf{k_{10}}}{\overset{\mathbf{k_9}}{\rightleftharpoons}} \mathbf{A_p}\,\mathbf{E_2} \xrightarrow{\mathbf{k_{11}}} \mathbf{A}+\mathbf{E_2} \\ \mathbf{E_1} \underset{\mathbf{k_{13}}}{\overset{\mathbf{k_{12}}}{\rightleftharpoons}} 0 \end{array} \qquad (\mathcal{N}_{18})$$

For network $\mathcal{N}_{18}$ the equation

$$Y^{(L)^T}\,\mu = \ln \frac{E\,\nu}{E\,\lambda}$$

reads

$$\begin{aligned} \mu_1+\mu_2 &= \ln \frac{\nu_1+\nu_6}{\lambda_1+\lambda_6} \\ \mu_3 &= \ln \frac{\nu_1}{\lambda_1} \\ \mu_3 &= \ln \frac{\nu_2+\nu_6}{\lambda_2+\lambda_6} \\ \mu_4 &= \ln \frac{\nu_2}{\lambda_2} \\ \mu_4 &= \ln \frac{\nu_6}{\lambda_6} \\ \mu_5+\mu_6 &= \ln \frac{\nu_3+\nu_6}{\lambda_3+\lambda_6} \\ \mu_7 &= \ln \frac{\nu_3}{\lambda_3} \\ \mu_7 &= \ln \frac{\nu_6}{\lambda_6} \\ \mu_6+\mu_8 &= \ln \frac{\nu_4+\nu_6}{\lambda_4+\lambda_6} \\ \mu_9 &= \ln \frac{\nu_4}{\lambda_4} \\ \mu_9 &= \ln \frac{\nu_6}{\lambda_6} \\ 0 &= \ln \frac{\nu_5}{\lambda_5} \\ \mu_2 &= \ln \frac{\nu_5}{\lambda_5} \end{aligned}$$

These equations are solvable, if and only if

$$\ln \frac{\nu_1}{\lambda_1} = \ln \frac{\nu_2+\nu_6}{\lambda_2+\lambda_6} \qquad \ln \frac{\nu_2}{\lambda_2} = \ln \frac{\nu_6}{\lambda_6} \qquad \ln \frac{\nu_3}{\lambda_3} = \ln \frac{\nu_6}{\lambda_6} \qquad \ln \frac{\nu_4}{\lambda_4} = \ln \frac{\nu_6}{\lambda_6} \qquad \nu_5 = \lambda_5$$

Thus one obtains

$$\nu_2 = \lambda_2 \frac{\nu_6}{\lambda_6}, \qquad \nu_3 = \frac{\nu_6}{\lambda_6}, \qquad \nu_4 = \lambda_4 \frac{\nu_6}{\lambda_6}.$$

Note that $\frac{\nu_2+\nu_6}{\lambda_2+\lambda_6} = \frac{\nu_6}{\lambda_6}$ and thus $\nu_1 = \lambda_1 \frac{\nu_6}{\lambda_6}$, as well. Therefore

$$\nu = \left(\lambda_1 \frac{\nu_6}{\lambda_6},\, \lambda_2 \frac{\nu_6}{\lambda_6},\, \frac{\nu_6}{\lambda_6},\, \lambda_4 \frac{\nu_6}{\lambda_6},\, \lambda_5, \nu_6 \right)^T$$

with $\lambda \in I\!R^6_{>0}$, $\nu_6 > 0$, free. As for network $\mathcal{N}_{15}$ it is possible to determine ν and $\lambda \in I\!R^6_{>0}$ independent of $\mu \in I\!R^9$. Using ν as above, one obtains

$$\frac{\nu_1+\nu_6}{\lambda_1+\lambda_6} = \frac{\nu_3+\nu_6}{\lambda_3+\lambda_6} = \ln \frac{\nu_4+\nu_6}{\lambda_4+\lambda_6} = \ln \frac{\nu_6}{\lambda_6}.$$

Using $\kappa_1 := \ln \frac{\nu_6}{\lambda_6}$ and solving for μ yields

$$\mu = \begin{bmatrix} 0 & 1 \\ 0 & 0 \\ 0 & 1 \\ 0 & 1 \\ 1 & 0 \\ -1 & 1 \\ 0 & 1 \\ 1 & 0 \\ 0 & 1 \end{bmatrix} \cdot \begin{pmatrix} \mu_8 \\ \kappa_1 \end{pmatrix}.$$

A.4.5 Network $\mathcal{N}_5$+ $\mathcal{N}_8$

The network is given by

$$\begin{aligned} \mathbf{A} + \mathbf{E_1} &\underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} \mathbf{A}\,\mathbf{E_1} \underset{\mathbf{k_4}}{\overset{\mathbf{k_3}}{\rightleftharpoons}} \mathbf{A_p}\,\mathbf{E_1} \xrightarrow{\mathbf{k_5}} \mathbf{A_{pp}} + \mathbf{E_1} \\ \mathbf{A_{pp}} + \mathbf{E_2} &\underset{\mathbf{k_7}}{\overset{\mathbf{k_6}}{\rightleftharpoons}} \mathbf{A_{pp}}\,\mathbf{E_2} \xrightarrow{\mathbf{k_8}} \mathbf{A_p} + \mathbf{E_2} \underset{\mathbf{k_{10}}}{\overset{\mathbf{k_9}}{\rightleftharpoons}} \mathbf{A_p}\,\mathbf{E_2} \xrightarrow{\mathbf{k_{11}}} \mathbf{A} + \mathbf{E_2} \qquad (\mathcal{N}_{19}) \\ \mathbf{E_2} &\underset{\mathbf{k_{13}}}{\overset{\mathbf{k_{12}}}{\rightleftharpoons}} 0 \end{aligned}$$

For network $\mathcal{N}_{19}$ the equation

$$Y^{(L)^T}\,\mu = \ln \frac{E\,\nu}{E\,\lambda}$$

reads

$$\begin{aligned} \mu_1 + \mu_2 &= \ln \frac{\nu_1 + \nu_6}{\lambda_1 + \lambda_6} \\ \mu_3 &= \ln \frac{\nu_1}{\lambda_1} \\ \mu_3 &= \ln \frac{\nu_2 + \nu_6}{\lambda_2 + \lambda_6} \\ \mu_4 &= \ln \frac{\nu_2}{\lambda_2} \\ \mu_4 &= \ln \frac{\nu_6}{\lambda_6} \\ \mu_5 + \mu_6 &= \ln \frac{\nu_3 + \nu_6}{\lambda_3 + \lambda_6} \\ \mu_7 &= \ln \frac{\nu_3}{\lambda_3} \\ \mu_7 &= \ln \frac{\nu_6}{\lambda_6} \\ \mu_6 + \mu_8 &= \ln \frac{\nu_4 + \nu_6}{\lambda_4 + \lambda_6} \\ \mu_9 &= \ln \frac{\nu_4}{\lambda_4} \\ \mu_9 &= \ln \frac{\nu_6}{\lambda_6} \\ 0 &= \ln \frac{\nu_5}{\lambda_5} \\ \mu_6 &= \ln \frac{\nu_5}{\lambda_5} \end{aligned}$$

These equations are solvable, if and only if

$$\ln \frac{\nu_1}{\lambda_1} = \ln \frac{\nu_2 + \nu_6}{\lambda_2 + \lambda_6} \qquad \ln \frac{\nu_2}{\lambda_2} = \ln \frac{\nu_6}{\lambda_6} \qquad \ln \frac{\nu_3}{\lambda_3} = \ln \frac{\nu_6}{\lambda_6} \qquad \ln \frac{\nu_4}{\lambda_4} = \ln \frac{\nu_6}{\lambda_6} \qquad \nu_5 = \lambda_5$$

Note, that these conditions are equivalent to those obtained for A.4.4. Thus one obtains the same solution $\nu \in I\!R^6_{>0}$, as in Section A.4.4:

$$\nu = \left(\lambda_1 \frac{\nu_6}{\lambda_6}, \lambda_2 \frac{\nu_6}{\lambda_6}, \frac{\nu_6}{\lambda_6}, \lambda_4 \frac{\nu_6}{\lambda_6}, \lambda_5, \nu_6 \right)^T$$

with $\lambda \in I\!R^6_{>0}$, $\nu_6 > 0$, free. As for network $\mathcal{N}_{15}$ it is possible to determine ν and $\lambda \in I\!R^6_{>0}$ independent of $\mu \in I\!R^9$. Using ν as above, one obtains

$$\frac{\nu_1 + \nu_6}{\lambda_1 + \lambda_6} = \frac{\nu_3 + \nu_6}{\lambda_3 + \lambda_6} = \ln \frac{\nu_4 + \nu_6}{\lambda_4 + \lambda_6} = \ln \frac{\nu_6}{\lambda_6}.$$

Using $\kappa_1 := \ln \frac{\nu_6}{\lambda_6}$ and solving for μ yields

$$\mu = \begin{bmatrix} -1 & 1 \\ 1 & 0 \\ 0 & 1 \\ 0 & 1 \\ 0 & 1 \\ 0 & 0 \\ 0 & 1 \\ 0 & 1 \\ 0 & 1 \end{bmatrix} \begin{pmatrix} \mu_2 \\ \kappa_1 \end{pmatrix}.$$

A.4.6 Network $\mathcal{N}_5$+ $\mathcal{N}_9$

The network is given by

$$\begin{aligned} \mathbf{A} + \mathbf{E_1} &\underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} \mathbf{A}\,\mathbf{E_1} \underset{\mathbf{k_4}}{\overset{\mathbf{k_3}}{\rightleftharpoons}} \mathbf{A_p}\,\mathbf{E_1} \xrightarrow{\mathbf{k_5}} \mathbf{A_{pp}} + \mathbf{E_1} \\ \mathbf{A_{pp}} + \mathbf{E_2} &\underset{\mathbf{k_7}}{\overset{\mathbf{k_6}}{\rightleftharpoons}} \mathbf{A_{pp}}\,\mathbf{E_2} \xrightarrow{\mathbf{k_8}} \mathbf{A_p} + \mathbf{E_2} \underset{\mathbf{k_{10}}}{\overset{\mathbf{k_9}}{\rightleftharpoons}} \mathbf{A_p}\,\mathbf{E_2} \xrightarrow{\mathbf{k_{11}}} \mathbf{A} + \mathbf{E_2} \qquad (\mathcal{N}_{20}) \\ \mathbf{E_1} &\underset{\mathbf{k_{13}}}{\overset{\mathbf{k_{12}}}{\rightleftharpoons}} 0 \underset{\mathbf{k_{15}}}{\overset{\mathbf{k_{14}}}{\rightleftharpoons}} \mathbf{E_2} \end{aligned}$$

For network $\mathcal{N}_{20}$ the equation

$$Y^{(L)^T} \mu = \ln \frac{E\,\nu}{E\,\lambda}$$

reads

$$\begin{aligned}
\mu_1 + \mu_2 &= \ln \frac{\nu_1 + \nu_7}{\lambda_1 + \lambda_7} \\
\mu_3 &= \ln \frac{\nu_1}{\lambda_1} \\
\mu_3 &= \ln \frac{\nu_2 + \nu_7}{\lambda_2 + \lambda_7} \\
\mu_4 &= \ln \frac{\nu_2}{\lambda_2} \\
\mu_4 &= \ln \frac{\nu_7}{\lambda_7} \\
\mu_5 + \mu_6 &= \ln \frac{\nu_3 + \nu_7}{\lambda_3 + \lambda_7} \\
\mu_7 &= \ln \frac{\nu_3}{\lambda_3} \\
\mu_7 &= \ln \frac{\nu_7}{\lambda_7} \\
\mu_6 + \mu_8 &= \ln \frac{\nu_4 + \nu_7}{\lambda_4 + \lambda_7} \\
\mu_9 &= \ln \frac{\nu_4}{\lambda_4} \\
\mu_9 &= \ln \frac{\nu_7}{\lambda_7} \\
\mu_2 &= \ln \frac{\nu_5}{\lambda_5} \\
0 &= \ln \frac{\nu_5}{\lambda_5} \\
0 &= \ln \frac{\nu_6}{\lambda_6} \\
\mu_6 &= \ln \frac{\nu_6}{\lambda_6}
\end{aligned}$$

These equations are solvable, if and only if

$$\ln \frac{\nu_1}{\lambda_1} = \ln \frac{\nu_2 + \nu_7}{\lambda_2 + \lambda_7} \qquad \ln \frac{\nu_2}{\lambda_2} = \ln \frac{\nu_7}{\lambda_7} \qquad \ln \frac{\nu_3}{\lambda_3} = \ln \frac{\nu_7}{\lambda_7} \qquad \ln \frac{\nu_4}{\lambda_4} = \ln \frac{\nu_7}{\lambda_7} \qquad \nu_5 = \lambda_5 \qquad \nu_6 = \lambda_6.$$

Thus one obtains

$$\nu_2 = \lambda_2 \frac{\nu_7}{\lambda_7}, \qquad \nu_3 = \frac{\nu_7}{\lambda_7}, \qquad \nu_4 = \lambda_4 \frac{\nu_7}{\lambda_7}.$$

Note that $\frac{\nu_2+\nu_7}{\lambda_2+\lambda_7} = \frac{\nu_7}{\lambda_7}$ and thus $\nu_1 = \lambda_1 \frac{\nu_7}{\lambda_7}$, as well.

$$\nu = \left(\lambda_1 \frac{\nu_7}{\lambda_7},\, \lambda_2 \frac{\nu_7}{\lambda_7},\, \frac{\nu_7}{\lambda_7},\, \lambda_4 \frac{\nu_7}{\lambda_7},\, \lambda_5, \lambda_6,\, \nu_7 \right)^T$$

with $\lambda \in \mathbb{R}^7_{>0}$, $\nu_7 > 0$, free. As for network $\mathcal{N}_{15}$ it is possible to determine ν and $\lambda \in \mathbb{R}^7_{>0}$ independent of $\mu \in \mathbb{R}^9$. Using ν as above, one obtains

$$\frac{\nu_1 + \nu_7}{\lambda_1 + \lambda_7} = \frac{\nu_3 + \nu_7}{\lambda_3 + \lambda_7} = \ln \frac{\nu_4 + \nu_7}{\lambda_4 + \lambda_7} = \ln \frac{\nu_7}{\lambda_7}.$$

Using $\kappa_1 := \ln \frac{\nu_7}{\lambda_7}$ and solving for μ yields

$$\mu = \left[\ 1, 0, 1, 1, 1, 0, 1, 1, 1\ \right]^T \kappa_1.$$

A.4.7 Network $\mathcal{N}_6$+ $\mathcal{N}_7$

The network is given by

$$\begin{array}{c} \mathbf{A}+\mathbf{E_1} \underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} \mathbf{A}\,\mathbf{E_1} \underset{\mathbf{k_4}}{\overset{\mathbf{k_3}}{\rightleftharpoons}} \mathbf{A_p}\,\mathbf{E_1} \xrightarrow{\mathbf{k_5}} \mathbf{A_{pp}}+\mathbf{E_1} \\ \mathbf{A_{pp}}+\mathbf{E_2} \underset{\mathbf{k_7}}{\overset{\mathbf{k_6}}{\rightleftharpoons}} \mathbf{A_{pp}}\,\mathbf{E_2} \underset{\mathbf{k_9}}{\overset{\mathbf{k_8}}{\rightleftharpoons}} \mathbf{A_p}\,\mathbf{E_2} \xrightarrow{\mathbf{k_{10}}} \mathbf{A}+\mathbf{E_2} \\ \mathbf{E_1} \underset{\mathbf{k_{14}}}{\overset{\mathbf{k_{13}}}{\rightleftharpoons}} 0 \end{array} \qquad (\mathcal{N}_{21})$$

For network $\mathcal{N}_{21}$ the equation

$$Y^{(L)^T}\,\mu = \ln\frac{E\,\nu}{E\,\lambda}$$

reads

$$\begin{aligned} \mu_1+\mu_2 &= \ln\frac{\nu_1+\nu_6}{\lambda_1+\lambda_6} \\ \mu_3 &= \ln\frac{\nu_1}{\lambda_1} \\ \mu_3 &= \ln\frac{\nu_2+\nu_6}{\lambda_2+\lambda_6} \\ \mu_4 &= \ln\frac{\nu_2}{\lambda_2} \\ \mu_4 &= \ln\frac{\nu_6}{\lambda_6} \\ \mu_5+\mu_6 &= \ln\frac{\nu_3+\nu_6}{\lambda_3+\lambda_6} \\ \mu_7 &= \ln\frac{\nu_3}{\lambda_3} \\ \mu_7 &= \ln\frac{\nu_4+\nu_6}{\lambda_4+\lambda_6} \\ \mu_8 &= \ln\frac{\nu_4}{\lambda_4} \\ \mu_8 &= \ln\frac{\nu_6}{\lambda_6} \\ 0 &= \ln\frac{\nu_5}{\lambda_5} \\ \mu_2 &= \ln\frac{\nu_5}{\lambda_5} \end{aligned}$$

These equations are solvable, if and only if

$$\ln\frac{\nu_1}{\lambda_1} = \ln\frac{\nu_2+\nu_6}{\lambda_2+\lambda_6} \qquad \ln\frac{\nu_2}{\lambda_2} = \ln\frac{\nu_6}{\lambda_6} \qquad \ln\frac{\nu_3}{\lambda_3} = \ln\frac{\nu_4+\nu_6}{\lambda_4+\lambda_6} \qquad \ln\frac{\nu_4}{\lambda_4} = \ln\frac{\nu_6}{\lambda_6} \qquad \nu_5 = \lambda_5.$$

Thus one obtains

$$\nu_2 = \lambda_2\,\frac{\nu_6}{\lambda_6}, \qquad\qquad \nu_4 = \lambda_4\,\frac{\nu_6}{\lambda_6}.$$

Note that therefore $\frac{\nu_2+\nu_6}{\lambda_2+\lambda_6} = \frac{\nu_4+\nu_6}{\lambda_4+\lambda_6} = \frac{\nu_6}{\lambda_6}$ and thus $\nu_1 = \lambda_1\,\frac{\nu_6}{\lambda_6}$ and $\nu_3 = \lambda_3\,\frac{\nu_6}{\lambda_6}$. Thus one finally obtains

$$\nu = \left(\lambda_1\,\frac{\nu_6}{\lambda_6},\, \lambda_2\,\frac{\nu_6}{\lambda_6},\, \frac{\nu_6}{\lambda_6},\, \lambda_4\,\frac{\nu_6}{\lambda_6},\, \lambda_5, \nu_6\right)^T$$

with $\lambda \in \mathbb{R}^6_{>0}$, $\nu_6 > 0$, free. As for network $\mathcal{N}_{15}$ it is possible to determine ν and $\lambda \in \mathbb{R}^6_{>0}$ independent of $\mu \in \mathbb{R}^9$. Using ν as above, one obtains

$$\frac{\nu_1+\nu_6}{\lambda_1+\lambda_6} = \frac{\nu_3+\nu_6}{\lambda_3+\lambda_6} = \frac{\nu_6}{\lambda_6}.$$

Using $\kappa_1 := \ln \frac{\nu_6}{\lambda_6}$ and solving for μ yields

$$\mu = \begin{bmatrix} 0 & 1 \\ 0 & 0 \\ 0 & 1 \\ 0 & 1 \\ -1 & 1 \\ 1 & 0 \\ 0 & 1 \\ 0 & 1 \end{bmatrix} \begin{pmatrix} \mu_6 \\ \kappa_1 \end{pmatrix}$$

A.4.8 Network $\mathcal{N}_6$+ $\mathcal{N}_8$

The network is given by

$$\begin{aligned} \mathbf{A} + \mathbf{E_1} &\underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} \mathbf{A\,E_1} \underset{\mathbf{k_4}}{\overset{\mathbf{k_3}}{\rightleftharpoons}} \mathbf{A_p\,E_1} \xrightarrow{\mathbf{k_5}} \mathbf{A_{pp}} + \mathbf{E_1} \\ \mathbf{A_{pp}} + \mathbf{E_2} &\underset{\mathbf{k_7}}{\overset{\mathbf{k_6}}{\rightleftharpoons}} \mathbf{A_{pp}\,E_2} \underset{\mathbf{k_9}}{\overset{\mathbf{k_8}}{\rightleftharpoons}} \mathbf{A_p\,E_2} \xrightarrow{\mathbf{k_{10}}} \mathbf{A} + \mathbf{E_2} \\ \mathbf{E_2} &\underset{\mathbf{k_{14}}}{\overset{\mathbf{k_{13}}}{\rightleftharpoons}} 0 \end{aligned} \qquad (\mathcal{N}_{22})$$

For network $\mathcal{N}_{22}$ the equation

$$Y^{(L)^T}\,\mu = \ln \frac{E\,\nu}{E\,\lambda}$$

reads

$$\begin{aligned} \mu_1 + \mu_2 &= \ln \frac{\nu_1 + \nu_6}{\lambda_1 + \lambda_6} \\ \mu_3 &= \ln \frac{\nu_1}{\lambda_1} \\ \mu_3 &= \ln \frac{\nu_2 + \nu_6}{\lambda_2 + \lambda_6} \\ \mu_4 &= \ln \frac{\nu_2}{\lambda_2} \\ \mu_4 &= \ln \frac{\nu_6}{\lambda_6} \\ \mu_5 + \mu_6 &= \ln \frac{\nu_3 + \nu_6}{\lambda_3 + \lambda_6} \\ \mu_7 &= \ln \frac{\nu_3}{\lambda_3} \\ \mu_7 &= \ln \frac{\nu_4 + \nu_6}{\lambda_4 + \lambda_6} \\ \mu_8 &= \ln \frac{\nu_4}{\lambda_4} \\ \mu_8 &= \ln \frac{\nu_6}{\lambda_6} \\ 0 &= \ln \frac{\nu_5}{\lambda_5} \\ \mu_6 &= \ln \frac{\nu_5}{\lambda_5} \end{aligned}$$

These equations are solvable, if and only if

$$\ln \frac{\nu_1}{\lambda_1} = \ln \frac{\nu_2 + \nu_6}{\lambda_2 + \lambda_6} \qquad \ln \frac{\nu_2}{\lambda_2} = \ln \frac{\nu_6}{\lambda_6} \qquad \ln \frac{\nu_3}{\lambda_3} = \ln \frac{\nu_4 + \nu_6}{\lambda_4 + \lambda_6} \qquad \ln \frac{\nu_4}{\lambda_4} = \ln \frac{\nu_6}{\lambda_6} \qquad \nu_5 = \lambda_5.$$

Note, that these conditions are equivalent to those obtained for A.4.7. Thus one obtains the same solution $\nu \in I\!R^6_{>0}$, as in Section A.4.7:

$$\nu = \left(\lambda_1 \frac{\nu_6}{\lambda_6}, \lambda_2 \frac{\nu_6}{\lambda_6}, \frac{\nu_6}{\lambda_6}, \lambda_4 \frac{\nu_6}{\lambda_6}, \lambda_5, \nu_6 \right)^T$$

with $\lambda \in I\!R^6_{>0}$, $\nu_6 > 0$, free. As for network $\mathcal{N}_{15}$ it is possible to determine ν and $\lambda \in I\!R^6_{>0}$ independent of $\mu \in I\!R^8$. Using ν as above, one obtains

$$\frac{\nu_1 + \nu_6}{\lambda_1 + \lambda_6} = \frac{\nu_3 + \nu_6}{\lambda_3 + \lambda_6} = \frac{\nu_6}{\lambda_6}.$$

Using $\kappa_1 := \ln \frac{\nu_6}{\lambda_6}$ and solving for μ yields

$$\mu = \begin{bmatrix} -1 & 1 \\ 1 & 0 \\ 0 & 1 \\ 0 & 1 \\ 0 & 1 \\ 0 & 0 \\ 0 & 1 \\ 0 & 1 \end{bmatrix} \begin{pmatrix} \mu_2 \\ \kappa_1 \end{pmatrix}.$$

A.4.9 Network $\mathcal{N}_6$+ $\mathcal{N}_9$

The network is given by

$$\begin{aligned} \mathbf{A} + \mathbf{E_1} &\underset{\mathbf{k_2}}{\overset{\mathbf{k_1}}{\rightleftharpoons}} \mathbf{A}\,\mathbf{E_1} \underset{\mathbf{k_4}}{\overset{\mathbf{k_3}}{\rightleftharpoons}} \mathbf{A_p}\,\mathbf{E_1} \xrightarrow{\mathbf{k_5}} \mathbf{A_{pp}} + \mathbf{E_1} \\ \mathbf{A_{pp}} + \mathbf{E_2} &\underset{\mathbf{k_7}}{\overset{\mathbf{k_6}}{\rightleftharpoons}} \mathbf{A_{pp}}\,\mathbf{E_2} \underset{\mathbf{k_9}}{\overset{\mathbf{k_8}}{\rightleftharpoons}} \mathbf{A_p}\,\mathbf{E_2} \xrightarrow{\mathbf{k_{10}}} \mathbf{A} + \mathbf{E_2} \qquad (\mathcal{N}_{23}) \\ \mathbf{E_1} &\underset{\mathbf{k_{12}}}{\overset{\mathbf{k_{11}}}{\rightleftharpoons}} 0 \underset{\mathbf{k_{14}}}{\overset{\mathbf{k_{13}}}{\rightleftharpoons}} \mathbf{E_2} \end{aligned}$$

For network $\mathcal{N}_{23}$ the equation

$$Y^{(L)^T} \mu = \ln \frac{E\,\nu}{E\,\lambda}$$

reads

$$\begin{aligned}
\mu_1 + \mu_2 &= \ln \frac{\nu_1 + \nu_7}{\lambda_1 + \lambda_7} \\
\mu_3 &= \ln \frac{\nu_1}{\lambda_1} \\
\mu_3 &= \ln \frac{\nu_2 + \nu_7}{\lambda_2 + \lambda_7} \\
\mu_4 &= \ln \frac{\nu_2}{\lambda_2} \\
\mu_4 &= \ln \frac{\nu_7}{\lambda_7} \\
\mu_5 + \mu_6 &= \ln \frac{\nu_3 + \nu_7}{\lambda_3 + \lambda_7} \\
\mu_7 &= \ln \frac{\nu_3}{\lambda_3} \\
\mu_7 &= \ln \frac{\nu_4 + \nu_7}{\lambda_4 + \lambda_7} \\
\mu_8 &= \ln \frac{\nu_4}{\lambda_4} \\
\mu_8 &= \ln \frac{\nu_7}{\lambda_7} \\
\mu_2 &= \ln \frac{\nu_5}{\lambda_5} \\
0 &= \ln \frac{\nu_5}{\lambda_5} \\
0 &= \ln \frac{\nu_6}{\lambda_6} \\
\mu_6 &= \ln \frac{\nu_6}{\lambda_6}
\end{aligned}$$

These equations are solvable, if and only if

$$\ln \frac{\nu_1}{\lambda_1} = \ln \frac{\nu_2 + \nu_7}{\lambda_2 + \lambda_7} \quad \ln \frac{\nu_2}{\lambda_2} = \ln \frac{\nu_7}{\lambda_7} \quad \ln \frac{\nu_3}{\lambda_3} = \ln \frac{\nu_4 + \nu_7}{\lambda_4 + \lambda_7} \quad \ln \frac{\nu_4}{\lambda_4} = \ln \frac{\nu_7}{\lambda_7} \quad \nu_5 = \lambda_5 \quad \nu_6 = \lambda_6.$$

Thus one obtains

$$\nu_2 = \lambda_2 \frac{\nu_7}{\lambda_7}, \qquad \nu_3 = \lambda_3 \frac{\nu_7}{\lambda_7}, \qquad \nu_4 = \lambda_4 \frac{\nu_7}{\lambda_7}.$$

and therefore

$$\nu = \left(\lambda_1 \frac{\nu_7}{\lambda_7}, \lambda_2 \frac{\nu_7}{\lambda_7}, \lambda_3 \frac{\nu_7}{\lambda_7}, \lambda_4 \frac{\nu_7}{\lambda_7}, \lambda_5, \lambda_6, \nu_7 \right)^T,$$

with $\lambda \in \mathbb{R}^7_{>0}$, $\nu_7 > 0$, free. As for network $\mathcal{N}_{15}$ it is possible to determine ν and $\lambda \in \mathbb{R}^7_{>0}$ independent of $\mu \in \mathbb{R}^9$. Using ν as above, one obtains

$$\frac{\nu_1 + \nu_7}{\lambda_1 + \lambda_7} = \frac{\nu_3 + \nu_7}{\lambda_3 + \lambda_7} = \frac{\nu_7}{\lambda_7}.$$

Using $\kappa_1 := \ln \frac{\nu_7}{\lambda_7}$ and solving for μ yields

$$\mu = \begin{bmatrix} 1, 0, 1, 1, 1, 0, 1, 1 \end{bmatrix}^T \kappa_1.$$

Appendix B

Models for cell cycle regulation

B.1 Binary complex model

B.1.1 Species and complexes of network $\mathcal{N}_{10}$

Species	x_i	Complex	y_i
$Sic1$	x_1	0	y_1
$Sic1P$	x_2	$Sic1$	y_2
Clb	x_3	$Sic1P$	y_3
$Clb \cdot Sic1$	x_4	$Sic1 \cdot Clb$	y_4
$Clb \cdot Sic1P$	x_5	$Clb + Sic1$	y_5
$Cdc14$	x_6	$Clb \cdot Sic1$	y_6
$Sic1P \cdot Cdc14$	x_7	Clb	y_7
$Clb \cdot Sic1P \cdot Cdc14$	x_8	$Clb + Sic1P$	y_8
$Sic1 \cdot Clb$	x_9	$Clb \cdot Sic1P$	y_9
		$Sic1P + Cdc14$	y_{10}
		$Sic1P \cdot Cdc14$	y_{11}
		$Sic1 + Cdc14$	y_{12}
		$Clb \cdot Sic1P + Cdc14$	y_{13}
		$Clb \cdot Sic1P \cdot Cdc14$	y_{14}
		$Clb \cdot Sic1 + Cdc14$	y_{15}

B.1.2 Ordinary differential equations

$$\dot{x}_1 = k_1 - k_2\,x_1 + k_4\,x_9 - k_5\,x_1\,x_3 - k_6\,x_1\,x_3 + k_7\,x_4 + k_{15}\,x_7 \tag{B.1a}$$
$$\dot{x}_2 = -k_3\,x_2 + k_9\,x_9 - k_{10}\,x_2\,x_3 + k_{11}\,x_5 - k_{13}\,x_2\,x_6 + k_{14}\,x_7 \tag{B.1b}$$
$$\dot{x}_3 = k_4\,x_9 - k_5\,x_1\,x_3 - k_6\,x_1\,x_3 + k_7\,x_4 + k_8\,x_4 + k_9\,x_9 - k_{10}\,x_2\,x_3 + k_{11}\,x_5 + k_{12}\,x_5 \tag{B.1c}$$
$$\dot{x}_4 = k_6\,x_1\,x_3 - k_7\,x_4 - k_8\,x_4 + k_{18}\,x_8 \tag{B.1d}$$
$$\dot{x}_5 = k_{10}\,x_2\,x_3 - k_{11}\,x_5 - k_{12}\,x_5 - k_{16}\,x_5\,x_6 + k_{17}\,x_8 \tag{B.1e}$$
$$\dot{x}_6 = -k_{13}\,x_2\,x_6 + k_{14}\,x_7 + k_{15}\,x_7 - k_{16}\,x_5\,x_6 + k_{17}\,x_8 + k_{18}\,x_8 \tag{B.1f}$$
$$\dot{x}_7 = k_{13}\,x_2\,x_6 - k_{14}\,x_7 - k_{15}\,x_7 \tag{B.1g}$$
$$\dot{x}_8 = k_{16}\,x_5\,x_6 - k_{17}\,x_8 - k_{18}\,x_8 \tag{B.1h}$$
$$\dot{x}_9 = -k_4\,x_9 + k_5\,x_1\,x_3 - k_9\,x_9 \tag{B.1i}$$

$$x_6 + x_7 + x_8 = c_1 \tag{B.2a}$$
$$x_3 + x_4 + x_5 + x_8 + x_9 = c_2 \tag{B.2b}$$

B.1.3 Structural data

The ordinary differential equations (B.1a) – (B.1i) can be written as

$$\dot{x} = Y\, I_a\, \mathrm{diag}\,(k)\; \phi\,(x)$$

using matrices

$$Y = \left[\begin{array}{cccccccccccccccc}
0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 1 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{array}\right],$$

$$I_a = \left[\begin{array}{cccccccccccccccccc}
-1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & -1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1
\end{array}\right]$$

and the monomial vector

$$\phi\,(x) = (\,1,\, x_1,\, x_2,\, x_9,\, x_1\,x_3,\, x_1\,x_3,\, x_4,\, x_4,\, x_9,\, x_2\,x_3,\, x_5,\, x_5,\, x_2\,x_6,\, x_7,\, x_7,\, x_5\,x_6,\, x_8,\, x_8\,)^T$$

A representation $\phi\,(x) = \left(x^{y_1^{(L)}}, \ldots, x^{y_r^{(L)}}\right)'$ can be obtained using

$$Y^{(L)} = \left[\begin{array}{cccccccccccccccccc}
0 & 1 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{array}\right]$$

The conservation relations (B.2a) and (B.2b) can be written as

$$W^T\, x = c$$

using the matrix

$$W^T = \left[\begin{array}{ccccccccc}
0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 0 \\
0 & 0 & 1 & 1 & 1 & 0 & 0 & 1 & 1
\end{array}\right]$$

and the vector $c^T = (\,c_1,\, c_2)$.

B.2 Ternary complex model

B.2.1 Species and complexes of network $\mathcal{N}_{11}$

Species	x_i	Complex	y_i
$Sic1$	x_1	0	y_1
$Sic1P$	x_2	$Sic1$	y_2
Clb	x_3	$Sic1P$	y_3
$Clb \cdot Sic1$	x_4	$Clb + Sic1$	y_4
$Clb \cdot Sic1P$	x_5	$Clb \cdot Sic1$	y_5
$Cdc14$	x_6	Clb	y_6
$Sic1P \cdot Cdc14$	x_7	$Clb + Sic1P$	y_7
$Clb \cdot Sic1P \cdot Cdc14$	x_8	$Clb \cdot Sic1P$	y_8
$Clb \cdot Sic1 \cdot Clb$	x_9	$Clb \cdot Sic1 + Clb$	y_9
		$Clb \cdot Sic1 \cdot Clb$	y_{10}
		$Clb \cdot Sic1P + Clb$	y_{11}
		$Sic1P + Cdc14$	y_{12}
		$Sic1P \cdot Cdc14$	y_{13}
		$Sic1 + Cdc14$	y_{14}
		$Clb \cdot Sic1P + Cdc14$	y_{15}
		$Clb \cdot Sic1P \cdot Cdc14$	y_{16}
		$Clb \cdot Sic1 + Cdc14$	y_{17}

B.2.2 Ordinary differential equations

$$\dot{x}_1 = k_1 - k_2\,x_1 - k_4\,x_1\,x_3 + k_5\,x_4 + k_{15}\,x_7 \tag{B.5a}$$
$$\dot{x}_2 = -k_3\,x_2 - k_7\,x_2\,x_3 + k_8\,x_5 - k_{13}\,x_2\,x_6 + k_{14}\,x_7 \tag{B.5b}$$
$$\dot{x}_3 = -k_4\,x_1\,x_3 + k_5\,x_4 + k_6\,x_4 - k_7\,x_2\,x_3 + k_8\,x_5 + k_9\,x_5 - k_{10}\,x_3\,x_4 + k_{11}\,x_9 + k_{12}\,x_9 \tag{B.5c}$$
$$\dot{x}_4 = k_4\,x_1\,x_3 - k_5\,x_4 - k_6\,x_4 - k_{10}\,x_3\,x_4 + k_{11}\,x_9 + k_{18}\,x_8 \tag{B.5d}$$
$$\dot{x}_5 = k_7\,x_2\,x_3 - k_8\,x_5 - k_9\,x_5 + k_{12}\,x_9 - k_{16}\,x_5\,x_6 + k_{17}\,x_8 \tag{B.5e}$$
$$\dot{x}_6 = -k_{13}\,x_2\,x_6 + k_{14}\,x_7 + k_{15}\,x_7 - k_{16}\,x_5\,x_6 + k_{17}\,x_8 + k_{18}\,x_8 \tag{B.5f}$$
$$\dot{x}_7 = k_{13}\,x_2\,x_6 - k_{14}\,x_7 - k_{15}\,x_7 \tag{B.5g}$$
$$\dot{x}_8 = k_{16}\,x_5\,x_6 - k_{17}\,x_8 - k_{18}\,x_8 \tag{B.5h}$$
$$\dot{x}_9 = k_{10}\,x_3\,x_4 - k_{11}\,x_9 - k_{12}\,x_9 \tag{B.5i}$$

$$x_6 + x_7 + x_8 = c_1 \tag{B.6a}$$
$$x_3 + x_4 + x_5 + x_8 + 2\,x_9 = c_2 \tag{B.6b}$$

B.2.3 Structural data

The ordinary differential equations (B.5a) – (B.5i) can be written as

$$\dot{x} = Y\,I_a\,\mathrm{diag}\,(k)\;\phi\,(x)$$

using matrices

$$Y = \begin{bmatrix}
0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 1 & 1 & 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 1 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix},$$

$$I_a = \left[\begin{array}{cccccccccccccccccc}
-1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1
\end{array}\right]$$

and the monomial vector

$$\phi\,(x) = (\,1,\, x_1,\, x_2,\, x_1\,x_3,\, x_4,\, x_4,\, x_2\,x_3,\, x_5,\, x_5,\, x_3\,x_4,\, x_9,\, x_9,\, x_2\,x_6,\, x_7,\, x_7,\, x_5\,x_6,\, x_8,\, x_8\,)\,T$$

A representation $\phi\,(x) = \left(x^{y_1^{(L)}}, \ldots, x^{y_r^{(L)}}\right)'$ can be obtained using

$$Y^{(L)} = \left[\begin{array}{cccccccccccccccccc}
0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0
\end{array}\right]$$

The conservation relations (B.6a) and (B.6b) can be written as

$$W^T\,x = c$$

using the matrix

$$W^T = \left[\begin{array}{ccccccccc}
0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 0 \\
0 & 0 & 1 & 1 & 1 & 0 & 0 & 1 & 2
\end{array}\right]$$

and the vector $c^T = (\,c_1,\, c_2)$.
A matrix whose columns are generators of $\ker(Y\,I_a) \cap \mathbb{R}^{18}_{\geq 0}$ is

$$E = \left[\begin{array}{cccccccccccc}
1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 1 \\
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 1 & 1 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 1 & 1 & 1 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0
\end{array}\right]. \tag{B.9}$$

Appendix C

Models of the signal transduction motifs

C.1 Network $\mathcal{N}_{12}$

C.1.1 Species and complexes of network $\mathcal{N}_{12}$

Species	x_i	Complex	y_i
A	x_1	$A + E_1$	y_1
E_1	x_2	$A\,E_1$	y_2
$A\,E_1$	x_3	$A^* + E_1$	y_3
A^*	x_4	$A + A^*$	y_4
$A\,A^*$	x_5	$A\,A^*$	y_5
E_2	x_6	$A^* + A^*$	y_6
$A^*\,E_2$	x_7	$A^* + E_2$	y_7
		$A^*\,E_2$	y_8
		$A + E_2$	y_9

C.1.2 Ordinary differential equations

$$\dot{x}_1 = -k_1\,x_1\,x_2 + k_2\,x_3 - k_4\,x_1\,x_4 + k_5\,x_5 + k_9\,x_7 \tag{C.1a}$$
$$\dot{x}_2 = -k_1\,x_1\,x_2 + k_2\,x_3 + k_3\,x_3 \tag{C.1b}$$
$$\dot{x}_3 = k_1\,x_1\,x_2 - k_2\,x_3 - k_3\,x_3 \tag{C.1c}$$
$$\dot{x}_4 = k_3\,x_3 - k_4\,x_1\,x_4 + k_5\,x_5 + 2\,k_6\,x_5 - k_7\,x_4\,x_6 + k_8\,x_7 \tag{C.1d}$$
$$\dot{x}_5 = k_4\,x_1\,x_4 - k_5\,x_5 - k_6\,x_5 \tag{C.1e}$$
$$\dot{x}_6 = -k_7\,x_4\,x_6 + k_8\,x_7 + k_9\,x_7 \tag{C.1f}$$
$$\dot{x}_7 = k_7\,x_4\,x_6 - k_8\,x_7 - k_9\,x_7 \tag{C.1g}$$

$$x_2 + x_3 = c_1 \tag{C.2a}$$
$$x_6 + x_7 = c_2 \tag{C.2b}$$
$$x_1 + x_3 + x_4 + 2\,x_5 + x_7 = c_3 \tag{C.2c}$$

C.1.3 Structural data

The ordinary differential equations (C.1a) – (C.1g) can be written as

$$\dot{x} = Y\,I_a\,\operatorname{diag}(k)\,\phi(x)$$

using matrices

$$Y = \begin{bmatrix} 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 \\ 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 1 & 0 & 2 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \end{bmatrix},$$

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

and the monomial vector

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

A representation $\phi\,(x) = \left(x^{y_1^{(L)}}, \ldots, x^{y_r^{(L)}}\right)^T$ can be obtained using

$$Y^{(L)} = \begin{bmatrix} 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \end{bmatrix}$$

The conservation relations (C.2a) – (C.2c) can be written as

$$W^T\, x = c$$

using the matrix

$$W^T = \begin{bmatrix} 0 & 1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 1 \\ 1 & 0 & 1 & 1 & 2 & 0 & 1 \end{bmatrix}$$

and the vector $c^T = (\, c_1,\, c_2,\, c_3)$.

C.2 Network $\mathcal{N}_{13}$

C.2.1 Species for and complexes of network $\mathcal{N}_{13}$

Species	x_i	Complex	y_i
A	x_1	$A + E_1$	y_1
E_1	x_2	$A\,E_1$	y_2
$A\,E_1$	x_3	$A^* + E_1$	y_3
A^*	x_4	$A^*\,E_1$	y_4
$A^*\,E_1$	x_5	$A^{**} + E_1$	y_5
A^{**}	x_6	$A^{**} + E_3$	y_6
E_3	x_7	$A^{**}\,E_3$	y_7
$A^{**}\,E_3$	x_8	$A^* + E_3$	y_8
E_2	x_9	$A^* + E_2$	y_9
$A^*\,E_2$	x_{10}	$A^*\,E_2$	y_{10}
		$A + E_2$	y_{11}

C.2.2 Ordinary differential equations

$$\dot{x}_1 = -k_1\,x_1\,x_2 + k_2\,x_3 + k_{12}\,x_{10} \tag{C.5a}$$
$$\dot{x}_2 = -k_1\,x_1\,x_2 + k_2\,x_3 + k_3\,x_3 - k_4\,x_2\,x_4 + k_5\,x_5 + k_6\,x_5 \tag{C.5b}$$
$$\dot{x}_3 = k_1\,x_1\,x_2 - k_2\,x_3 - k_3\,x_3 \tag{C.5c}$$
$$\dot{x}_4 = k_3\,x_3 - k_4\,x_2\,x_4 + k_5\,x_5 + k_9\,x_8 - k_{10}\,x_4\,x_9 + k_{11}\,x_{10} \tag{C.5d}$$
$$\dot{x}_5 = k_4\,x_2\,x_4 - k_5\,x_5 - k_6\,x_5 \tag{C.5e}$$
$$\dot{x}_6 = k_6\,x_5 - k_7\,x_6\,x_7 + k_8\,x_8 \tag{C.5f}$$
$$\dot{x}_7 = -k_7\,x_6\,x_7 + k_8\,x_8 + k_9\,x_8 \tag{C.5g}$$
$$\dot{x}_8 = k_7\,x_6\,x_7 - k_8\,x_8 - k_9\,x_8 \tag{C.5h}$$
$$\dot{x}_9 = -k_{10}\,x_4\,x_9 + k_{11}\,x_{10} + k_{12}\,x_{10} \tag{C.5i}$$
$$\dot{x}_{10} = k_{10}\,x_4\,x_9 - k_{11}\,x_{10} - k_{12}\,x_{10} \tag{C.5j}$$

$$x_2 + x_3 + x_5 = c_1 \tag{C.6a}$$
$$x_7 + x_8 = c_2 \tag{C.6b}$$
$$x_9 + x_{10} = c_3 \tag{C.6c}$$
$$x_1 + x_3 + x_4 + x_5 + x_6 + x_8 + x_{10} = c_4 \tag{C.6d}$$

C.2.3 Structural data

The ordinary differential equations (C.5a) – (C.5j) can be written as

$$\dot{x} = Y\,I_a\,\mathrm{diag}\,(k)\,\phi\,(x)$$

using matrices

$$Y = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ 1 & 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \end{bmatrix},$$

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

and the monomial vector

$$\phi\,(x) = (\,x_1\,x_2,\; x_3,\; x_3,\; x_2\,x_4,\; x_5,\; x_5,\; x_6\,x_7,\; x_8,\; x_8,\; x_4\,x_9,\; x_{10},\; x_{10}\,)^T$$

A representation $\phi\,(x) = \left(x^{y_1^{(L)}}, \ldots, x^{y_r^{(L)}}\right)^T$ can be obtained using

$$Y^{(L)} = \left[\begin{array}{cccccccccccc} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ \hline 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ \hline 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \end{array}\right]$$

The conservation relations (C.6a) – (C.6d) can be written as

$$W^T\,x = c$$

using the matrix

$$W^T = \begin{bmatrix} 0 & 1 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \\ 1 & 0 & 1 & 1 & 1 & 1 & 0 & 1 & 0 & 1 \end{bmatrix}$$

and the vector $c^T = (\,c_1,\; c_2,\; c_3,\; c_4)$.

C.3 Network $\mathcal{N}_{14}$

C.3.1 Species and complexes of network $\mathcal{N}_{14}$

Species	x_i	Complex	y_i
A	x_1	$A + E_1$	y_1
E_1	x_2	$A\,E_1$	y_2
$A\,E_1$	x_3	$A^* + E_1$	y_3
A^*	x_4	$A^* + E_3$	y_4
E_3	x_5	$A^*\,E_3$	y_5
$A^*\,E_3$	x_6	$A + E_3$	y_6
$A^*\,A^*$	x_7	$2\,A^*$	y_7
A^{**}	x_8	$A^*\,A^*$	y_8
$A^*\,A^{**}$	x_9	$A^{**} + A^*$	y_9
E_2	x_{10}	$A^*\,A^{**}$	y_{10}
$A^{**}\,E_2$	x_{11}	$2\,A^{**}$	y_{11}
		$A^{**} + E_2$	y_{12}
		$A^{**}\,E_2$	y_{13}
		$A^* + E_2$	y_{14}

C.3.2 Ordinary differential equations

$$\dot{x}_1 = -k_1\,x_1\,x_2 + k_2\,x_3 + k_6\,x_6 \tag{C.9a}$$
$$\dot{x}_2 = -k_1\,x_1\,x_2 + k_2\,x_3 + k_3\,x_3 \tag{C.9b}$$
$$\dot{x}_3 = k_1\,x_1\,x_2 - k_2\,x_3 - k_3\,x_3 \tag{C.9c}$$
$$\dot{x}_4 = k_3\,x_3 - k_4\,x_4\,x_5 + k_5\,x_6 - 2\,k_7\,x_4^2 + 2\,k_8\,x_7 + k_9\,x_7 - k_{10}\,x_4\,x_8 + k_{11}\,x_9 + k_{15}\,x_{11} \tag{C.9d}$$
$$\dot{x}_5 = -k_4\,x_4\,x_5 + k_5\,x_6 + k_6\,x_6 \tag{C.9e}$$
$$\dot{x}_6 = k_4\,x_4\,x_5 - k_5\,x_6 - k_6\,x_6 \tag{C.9f}$$
$$\dot{x}_7 = k_7\,x_4^2 - k_8\,x_7 - k_9\,x_7 \tag{C.9g}$$
$$\dot{x}_8 = k_9\,x_7 - k_{10}\,x_4\,x_8 + k_{11}\,x_9 + 2\,k_{12}\,x_9 - k_{13}\,x_8\,x_{10} + k_{14}\,x_{11} \tag{C.9h}$$
$$\dot{x}_9 = k_{10}\,x_4\,x_8 - k_{11}\,x_9 - k_{12}\,x_9 \tag{C.9i}$$
$$\dot{x}_{10} = -k_{13}\,x_8\,x_{10} + k_{14}\,x_{11} + k_{15}\,x_{11} \tag{C.9j}$$
$$\dot{x}_{11} = k_{13}\,x_8\,x_{10} - k_{14}\,x_{11} - k_{15}\,x_{11} \tag{C.9k}$$

$$x_2 + x_3 = c_1 \tag{C.10a}$$
$$x_5 + x_6 = c_2 \tag{C.10b}$$
$$x_{10} + x_{11} = c_3 \tag{C.10c}$$
$$x_1 + x_3 + x_4 + x_6 + 2\,x_7 + x_8 + 2\,x_9 + x_{11} = c_4 \tag{C.10d}$$

C.3.3 Structural data

The ordinary differential equations (C.9a) – (C.9k) can be written as

$$\dot{x} = Y\,I_a\ \mathrm{diag}\,(k)\ \phi\,(x)$$

using matrices

$$Y = \begin{bmatrix}
1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & 1 & 0 & 0 & 2 & 0 & 1 & 0 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 2 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0
\end{bmatrix},$$

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

and the monomial vector

$$\phi\,(x) = \left(\, x_1\,x_2,\ x_3,\ x_3,\ x_4\,x_5,\ x_6,\ x_6,\ x_4^2,\ x_7,\ x_7,\ x_4\,x_8,\ x_9,\ x_9,\ x_8\,x_{10},\ x_{11},\ x_{11} \,\right)^T$$

A representation $\phi(x) = \left(x^{y_1^{(L)}}, \ldots, x^{y_r^{(L)}}\right)^T$ can be obtained using

$$Y^{(L)} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 2 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \end{bmatrix}$$

The conservation relations (C.10a) – (C.10d) can be written as

$$W^T x = c$$

using the matrix

$$W^T = \begin{bmatrix} 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \\ 1 & 0 & 1 & 1 & 0 & 1 & 2 & 1 & 2 & 0 & 1 \end{bmatrix}$$

and the vector $c^T = (\, c_1,\, c_2,\, c_3,\, c_4)$.

Appendix D

An Algorithm to check Lemma 3

D.1 Preliminary ideas

The starting point is 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 Chapter 3):

$$\begin{aligned} v &\in \mathbb{R}^n_{\delta_i}. \\ A\,v &= 0, \end{aligned}$$

Given u, v with $A\,u = 0$ and $A\,v = 0$. Then

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

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

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

Using eq. (D.1) in $A\,v = 0$ yields

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

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

$$v \in \mathbb{R}^n_{\delta_i} \tag{D.2a}$$

given by $v = diag\,(\delta_i)\; w$ with

$$w \in \mathbb{R}^n_{\geq 0} \tag{D.2b}$$

$$\widetilde{A}\,w = 0, \tag{D.2c}$$

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

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

If $\widetilde{E}_1, \ldots \widetilde{E}_p$ are generators of $\ker\left(\widetilde{A}\right) \cap \mathbb{R}^n_{\geq 0}$, 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 $A\,E_i = A\operatorname{diag}(\delta_i)\;\widetilde{E}_i = \widetilde{A}\,\widetilde{E}_i = 0,\; i = 1, \ldots, p$. 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 $\ker(A) \cap \mathbb{R}^n_{\geq 0}$.

D.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) $\widetilde{A} := \operatorname{diag}(\delta_i)\, A$

(ii) calculate generators $\widetilde{E}_i$, $i = 1, \ldots, p$ of $\ker(A) \cap \mathbb{R}^n_{\delta_i}$. If

$$\nexists j \in \{1, \ldots, n\} \text{ with } j \notin \operatorname{supp}\left(\widetilde{E}_i\right),\ i = 1, \ldots, p$$

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 $\mathcal{S}$ are subspaces, that is, that $\mu \in \mathcal{M} \Rightarrow -\mu \in \mathcal{M}$ and $v \in \mathcal{S} \Rightarrow -v \in \mathcal{S}$. Thus, if $\mathcal{M} \cap \mathbb{R}^n_\delta \neq \emptyset$ and $\mathcal{S} \cap \mathbb{R}^n_\delta \neq \emptyset$, then $\mathcal{M} \cap \mathbb{R}^n_{-\delta} \neq \emptyset$ and $\mathcal{S} \cap \mathbb{R}^n_{-\delta} \neq \emptyset$ 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 $\bar{\delta}$ holds the following

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

The algorithm is available in the matlab file `sign_comp_subs.m` displayed below:

```
function E=sign_comp_subs( W )
% E=sign_comp_subs( W ) returns a matrix E whose columns correspond
% to those orthants of R^n that are sign compatible to the linear
% subspace that is defined by the matrix W: W is the orthogonal
% complement of the subspace under consideration. Each column
% vector e entirely consits of entries e_i=1, e_i=0 or e_i=-1, indicating
% x_i>0, x_i=0 and x_i<0, respectively. Thus each e defines an
% orthant.

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 calculated the generators of the cone $diag(M(i,:)) * W \cap \mathbb{R}^n_{>0}$. It is contained in the software package *CellNetAnalyzer* [32].

D.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 $\mathcal{S} \cap \mathbb{R}^n_\delta \neq \emptyset$.

Step 1: Reformulate $\mathcal{M}$ and $\mathcal{S}$: Let $W = [w_1, \ldots, w_s]$ be a matrix whose column vectors are a basis for $\mathcal{S}^\perp$ and $B = [b_1, \ldots, b_p]$ be a matrix whose column vectors are a basis for $\mathcal{M}^\perp$. Then all $v \in \mathcal{S}$ can be represented as $\{v \in \mathbb{R}^n \mid W^T v = 0\}$ and all $\mu \in \mathcal{M}$ as $\{\mu \in \mathbb{R}^n \mid B^{'} \mu = 0\}$, i.e. $v \in \mathcal{S}$ and $\mu \in \mathcal{M}$ are in a form suitable for the algorithm described above (using either W or B as matrix A).

Step 1: Determine all δ_i that are sign compatible to $\mathcal{S}$: $\Delta^{\mathcal{S}}$.

Step 1: Determine all δ_i that are sign compatible to $\mathcal{M}$: $\Delta^{\mathcal{M}}$.

Step 1: Determine $\Delta = \Delta^{\mathcal{S}} \cap \Delta^{\mathcal{M}}$.

Step 1: For each $\delta_i \in \Delta$ calculate the generators of $\mathcal{M} \cap \mathbb{R}^n_{\delta_i}$ and $\mathcal{S} \cap \mathbb{R}^n_{\delta_i}$ (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;
```

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