Subnetwork Analysis for Multistationarity in Mass Action Kinetics

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Abstract. Since quantitative knowledge of the complex (bio)chemical reaction networks is often very limited, formal methods that connect network structure and dynamic behavior are needed in mathematical modeling and analysis. Feinberg’s Chemical Reaction Network Theory allows for the classification of the potential network behavior, for instance, with respect to the existence of multiple steady states, but is computationally limited to small systems. Here, we show that by analyzing subnetworks associated to stoichiometric generators, the applicability of the theory can be extended to more complex networks. Moreover, based on mild conditions regarding multistationarity of such subnetworks, we present an algorithm which establishes multistationarity in the overall network.

For example-networks inspired by cell cycle control in budding yeast, the approach allows for identification of key mechanisms for multistationarity, for model discrimination and for robustness analysis. The present paper continues and extends our work that has appeared in PNAS (cf. [6]).

1. Introduction

In building mathematical models of intracellular processes, network topology as well as parameter values are often uncertain. Frequently, one can constrain the set of feasible network topologies by biological intuition, but this is only of limited help when it comes to parameter estimation. Thus, the question whether or not there exists a set of parameter values such that a given network can express a certain qualitative behavior arises naturally. In analyzing biological switches in, for example, developmental processes, the existence of multiple positive steady states (multistationarity) is of primary interest [2]. Therefore, the connection between network topology and multistationarity has been discussed in a variety of contributions. In [29, 31], necessary conditions for bistability—the existence of two stable steady states—are given. A switching surface, a threshold, might be given by the stable manifold of a saddle point.

Feinberg’s Chemical Reaction Network Theory (CRNT) gives necessary and sufficient conditions for multistationarity, provided that all kinetics are of the mass action form and that the network satisfies certain structural conditions. CRNT has recently received some attention in (systems) biology [9, 7, 8] because it connects questions about multistationarity for a system of ordinary differential equations (ODEs) derived from a biochemical reaction network to the network structure alone. Its assertions do not depend on parameter values and it only assumes mass action kinetics for all reactions. In principle, CRNT is a powerful, however CRNT requires the analysis of potentially large numbers of inequality systems, with many unknowns and many
(possibly nonlinear) inequalities. Current algorithms practically limit the application of CRNT to networks of at most 20 complexes where the inequality systems are linear.

The complexity in cellular networks might be reduced by restricting to smaller subunits—

motifs [28] or modules [21]—that one can analyze separately. We argue that the analysis of certain subnetworks can be sufficient to confirm multistationarity. The subnetworks of interest are elementary flux modes, important tools in systems biology [24, 27]. So we propose to analyze subnetworks defined by a special class of flux modes, called stoichiometric generators. Such a subnetwork presents a powerful reduction of the overall network, since there are less CRNT–inequalities to be taken into account and—as we show under some mild conditions on the stoichiometric generators the inequality systems for the subnetwork are guaranteed to be linear. In case multistationarity in a subnetwork, induced by a stoichiometric generator, can be established (by CRNT or by other means (cf. [5]), we prove—under some (computationally) easy to verify conditions—the sufficiency for multistationarity in the overall network. In summary, we extend the class of networks where multistationarity can be investigated with the help of linear inequality systems.

As a proof-of-concept, we investigate reaction networks that are inspired by the control of the G1/S transition in the cell cycle of Saccharomyces cerevisiae.

Outline:

In the following subsections of Section 1 we introduce some terminology, including the notion of multistationarity with respect to a subspace $V$, and recall some facts of CRNT and stoichiometric generators. Section 2 is dedicated to a special class of subnetworks. Lemma 2.3 provides the crucial fact that the kernel of the stoichiometric matrix of a subnetwork, that is defined by a stoichiometric generator, is one-dimensional. Theorem 2.1 presents sufficient conditions for such a subnetwork to allow the application of the deficiency one algorithm.

In Section 3, sufficient conditions are presented that guarantee that (multiple) steady states in a subnetwork can be extended to (multiple) steady states in the overall network. The main result, stated as Theorem 3.6, is reformulated in terms of an algorithm that can be applied easily and without reference to the underlying mathematical details (cf. Algorithm 3.1).

Finally, in Section 4 we consider a switching device in yeast cell cycle regulation as an ideal test case for our approach. The translation of the corresponding biology-inspired reaction scheme into the formal network notation of CRNT is shown for the ternary complex model and for the binary complex model (see networks $N_1$ and $N_2$ in subsections 5.1 and 5.2 resp.). In each network, the extension of multistationarity from the subnetworks under consideration to the overall network is possible. where we’d like to point that CRNT just works for the ternary complex model.

1.1. Network Definitions

We consider mass action models for biochemical reaction networks in the form of ODEs and start with a brief presentation of the corresponding notation introduced in [12, 13] and [19].

Consider a simple reaction network of the form

$$ A + B \xrightleftarrows_{k_1 \atop k_2} C \quad A \xrightarrow{k_3 \atop k_4} 0 $$

(1.1)
the standard form defined in [12, 13]: node labels are unique; that is, each complex is displayed only once. When the species concentrations \(x_1, \ldots, x_n\) for species \(A, \ldots, B\) are collected in a vector \(x = (x_1, \ldots, x_n)^T\), each \(x_i\) can be associated to a unit vector \(e_i\) of \(\mathbb{R}^n\), the \(n\)-dimensional Euclidean space. Each complex can be related to a vector \(y\) corresponding to the sum of its constituents: \(A + B\) with \(y_1 = e_1 + e_2\), \(C\) with \(y_2 = e_3\), \(A\) with \(y_3 = e_1\), and 0 with \(y_4 = 0\), the 3-dimensional zero vector.

Let \(Y = [y_1, \ldots, y_m]\) be a matrix whose column vectors are the complexes of the network, i.e. \(Y \in \mathbb{R}^m \times n\). The reactions are represented by the incidence matrix \(I \in \{-1, 0, 1\}^{m \times r}\). Each column of \(I\) represents a reaction and has exactly one entry \(-1\) for the educt complex and one entry \(1\) for the product complex, i.e. one entry \(-1\) for the input and one entry \(1\) for the output of a reaction. The remaining entries are zero. According to the mass-action law, each reaction has a reaction rate \(v_i\) consisting of a rate constant \(k_i \in \mathbb{R}_{>0}\) and a monomial \(x^{y_i} = x_1^{y_{1,i}} \cdots x_n^{y_{n,i}}\), where \(x\) is the vector of concentrations and \(y\) the vector of concentrations corresponding to the educt complex of the reaction. Let \(\mathcal{Y} := [y_1, \ldots, y_r] \in \mathbb{R}^n \times r\) be a collection of column vectors of \(Y\) with the following property: the \(i\)-th column vector of \(\mathcal{Y}\) corresponds to the educt of the \(i\)-th reaction (i.e. \(v_i = k_i x^{y_i}\)). When several reactions share the same educt, \(\mathcal{Y}\) will contain several copies of the corresponding complex vector \(\tilde{y}_i\). With the columns \(\tilde{y}_i\) of \(\mathcal{Y}\) one obtains the monomial vector
\[
\phi(x) := (x^{\tilde{y}_1}, \ldots, x^{\tilde{y}_r})^T
\]
and the vector of reaction rates
\[
v(k, x) = \text{diag}(k) \phi(x),
\]
where \(k := (k_1, \ldots, k_r)^T\) is the vector of rate constants. Then the ODEs describing the dynamics of a reaction networks are given as
\[
\dot{x}(t) = N v(k, x(t)), \quad N := Y I \in \mathbb{R}^n \times r.
\]
In general, the stoichiometric matrix \(N\) does not have maximal row rank. For \(s := \text{rank}(N)\) there exist \(n - s\) conservation relations.

In the literature on Chemical Reaction Network Theory, the linear subspace \(S \equiv \text{im}(N)\) of dimension \(s\), spanned by the columns of \(N\), is called stoichiometric subspace. The corresponding \(n - s\) conservation relations can be characterized as follows: Let \(W \in \mathbb{R}^n \times (n-s)\) be a matrix of full column rank with \(W^T N = 0\) so that the column space \(\text{im}(W)\) of \(W\) satisfies
\[
\text{im}(W) = S^\perp.
\]
Then the level sets \(\{x \in \mathbb{R}^n : W^T x = \text{const}\}\) are invariant under the flow of (1.3) due to \(W^T x(t) = W^T x(0)\) along solutions \(x(t)\) of (1.3). These level sets are equivalent to the conservation relations
\[
W^T x = c.
\]
One might add the requirement that the columns of \(W\) form an orthonormal basis of the left-nullspace of \(N\) so that \(W^T W\) becomes the \((n-s) \times (n-s)\) dimensional identity matrix. Every biochemical reaction network endowed with mass action kinetics defines a system of the form presented in eq. (1.3), (1.5).

For the small example in (1.1) we obtain
\[
Y = \begin{bmatrix} 1 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \end{bmatrix}, \quad I = \begin{bmatrix} -1 & 1 & 0 & 0 \\ 1 & -1 & 0 & 0 \\ 0 & 0 & -1 & 1 \\ 0 & 0 & 1 & -1 \end{bmatrix}
\]
and \( v(k, x) = (k_1 x_1 x_2, k_2 x_3, k_3 x_1, k_4)^T \) where e.g. the first two columns of \( Y \) and the first column of \( \mathcal{I} \) refer to the reaction \( A + B \overset{k_1}{\rightarrow} C \) with \( A + B \) as educt complex and \( C \) as product complex. Thus (1.3) takes the form

\[
\begin{align*}
\dot{x}_1 &= -k_1 x_1 x_2 + k_2 x_3 - k_3 x_1 + k_4 \\
\dot{x}_2 &= -k_1 x_1 x_2 + k_2 x_3 \\
\dot{x}_3 &= k_1 x_1 x_2 - k_2 x_3
\end{align*}
\]

For the conservation relation (1.5) we obtain \( x_2 + x_3 = c \) for \( W = (0, 1, 1) \).

Finally, we want to describe the notion of multistationarity precisely.

**Definition 1.1** Given a subspace \( \mathcal{V} \subset \mathbb{R}^n \), the system \( \dot{x} = N v(k, x) \) from (1.3) with stoichiometric subspace \( \mathcal{S} = \text{im}(N) \) is said to exhibit \( \mathcal{V} \)-multistationarity if and only if there exist a positive vector \( k \in \mathbb{R}_{>0}^l \) and at least two distinct positive vectors \( a, b \in \mathbb{R}_{>0}^n \) with

\[
\begin{align*}
N v(k, a) &= 0, \quad N v(k, b) = 0, \\
b - a &\in \mathcal{V}.
\end{align*}
\]

In the special case \( \mathcal{V} = \mathcal{S} \) one rediscovers the familiar concept of multistationarity. Later on, we will be interested in \( \mathcal{S} \)-multistationarity for subnetworks of (1.3) where the stoichiometric subspace \( \mathcal{S}_{\text{sub}} \) of the subnetwork might be a proper subspace of \( \mathcal{S} \).

### 1.2. Chemical Reaction Network Theory

The distinguishing feature of CRNT is its ability to connect the structure of a reaction network and the existence of (multiple) equilibria for the corresponding system of ODEs. The general idea can be summarized in the following way: for any network, a non-negative integer \( \delta \) called the deficiency can be derived from the network structure alone. For its formal definition one more concept is needed – the linkage class. Network (1.1) consists of two sets of complexes: \( \{A + B, C\} \) and \( \{A, 0\} \). 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. With the number of complexes, \( m \), and the rank of \( N, s \), the network deficiency is defined as the non-negative integer

\[
\delta = m - l - s, \tag{1.7}
\]

(see [12]). Note that the deficiency only depends on the network structure and thus, in particular, \( \delta \) is independent of parameter values. For the small example it is easy to check that \( \delta = 4 - 2 = 0 \).

If the deficiency \( \delta \) is zero for a particular network, then no system of ODEs endowed with mass action kinetics that can be derived from the network admits multiple steady states (or sustained oscillations), regardless of the rate constants [12, 14]. If \( \delta = 1 \) and the network satisfies some mild additional conditions, the deficiency one algorithm [13, 15] can be applied to decide whether or not the network can admit multiple steady states. If the deficiency is greater than one then, under certain conditions, the advanced deficiency theory and corresponding algorithm can be used to decide about multistationarity [10, 11].

For each network where such an algorithm is applicable, several systems of equalities and inequalities (inequality systems, for short) can be formulated. These inequality systems only depend on the network structure and the complexes. For the deficiency one algorithm it is guaranteed that the inequality systems are linear [15]. The advanced deficiency algorithm might have to consider nonlinear inequalities [11]. If one of these systems has a solution \( s \in \mathbb{R}^m \) and
if the stoichiometric subspace $S = \text{im} (N)$ contains a vector $d \in \mathbb{R}^n$ with the same sign pattern as this solution (i.e. $s_i d_i > 0$ for $i = 1, \ldots, n$), then multistationarity is possible. Also, 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.

1.3. Positive Steady States and Elementary Fluxes

Under mass-action law, positive species concentrations $x \in \mathbb{R}^n_+$ and positive rate constants $k \in \mathbb{R}^r_+$ imply positive reaction rates $v(k, x) > 0$. Then, the steady state form of (1.3), $Y I v(k, x) = 0$, implies that all reaction rates lie in a pointed polyhedral cone defined by the intersection of the kernel of the stoichiometric matrix $Y I$ with the cone of vectors with nonnegative components, that is, $v(k, x) \in \ker(Y I) \cap \mathbb{R}^r_+$. Starting with the classical work of Clarke [3], this cone has become a well studied object because it characterizes the steady-state flux space for a reaction network. Each element of the cone can be interpreted as a particular flux distribution, an allocation of values $v_i$ to each reaction of the network, such that the overall network is in steady state.

As a pointed polyhedral cone, the flux space can be represented by non-negative linear combinations of a finite set of generators or extreme rays [25]. An elementary flux mode is a feasible flux distribution (an element $v \in \ker(Y I) \cap \mathbb{R}^r_+$) with a maximum number of zero entries. If, as in our setup, we consider only positive reaction rates, elementary flux modes are equivalent to extreme rays [18]. Formally, the extreme rays $E_i$ of $\ker(Y I) \cap \mathbb{R}^r_+$ are defined as follows:

$$Y I E_i = 0 \quad \text{and} \quad E_i \in \mathbb{R}^r_+$$

and,

for given $E_i$, $E_j$ with $Y I E_i = 0$ and $Y I E_j = 0$,

$$\text{supp} (E_i) \subseteq \text{supp} (E_j) \Rightarrow E_i = 0 \quad \text{or} \quad E_j = \alpha E_i$$

where $\text{supp} (E_j) = \{ i \in \{ 1, \ldots, r \} | E_{j_i} > 0, \}$ denotes the support of vector $E_j$, i.e. the set of indices where $E_j$ has nonzero values [18]. We call a set of nonnegative vectors $\{ E_1, \ldots, E_p \}$ generators of $\ker(Y I) \cap \mathbb{R}^r_+$.

The importance of the generators 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. Depending on whether or not a particular generator $E_i \in \mathbb{R}^r_+$ is contained in the kernel of $I$ ($I E_i = 0$), two kinds of generators can be distinguished: generators with $I E_i = 0$ and stoichiometric generators with $I E_i \neq 0$ [19]. In general, calculating the generators is computationally difficult, but there exists a variety of corresponding algorithms and software tools [26, 18].

2. Subnetworks by Stoichiometric Generators

2.1. Statement of the Result

Applications of CRNT are limited both by the network sizes and by the need to analyze nonlinear inequalities. Subnetwork analysis obviously reduces the first limitation. Here, we show that investigating subnetworks defined by stoichiometric generators $E$, under weak additional conditions, also enables a decision on multistationarity by analyzing linear inequality systems.
In brief, the deficiency one algorithm imposes five conditions on the subnetwork structure (for details and proofs we refer to the subsection 2.2). For a stoichiometric generator, four conditions are always fulfilled. For instance, we establish that for any $E$, $s = m - l - 1$, leading to the deficiency $\delta = m - l - s = 1$. The fifth condition states that each linkage class contains only one so-called terminal strong linkage class. In graph theory 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 vice versa. The strongly connected components—strong linkage classes in CRNT notation [14, 15]—are the maximal strongly connected subgraphs of a directed graph. If no edge from a node inside a strong linkage class to a node outside exists, we have a terminal strong linkage class. However, we can not make statements on the number of terminal strong linkage classes for a general $E$, which leads us to the following fact:

**Theorem 2.1** Consider a biochemical reaction network that is a subnetwork, defined by a stoichiometric generator of the overall network. Assume that the subnetwork is displayed in the standard form of CRNT, as defined above. 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 particular, only systems of linear inequalities have to be considered to decide about multistationarity.

Note that as a reaction network defined by a stoichiometric generator can not contain any cycles (apart from the one induced by the generator itself, see below), each terminal strong linkage class consists of a single complex. Thus, it is straightforward to check whether or not a linkage class contains more than one terminal strong linkage class.

2.2. Proof of Theorem 2.1

In this subsection we consider a (bio)chemical reaction network that is a subnetwork of a larger network and that is defined by a stoichiometric generator of that larger network. Throughout this subsection it is understood that any (bio)chemical reaction network is in the standard form defined in, for example, [12, 13]: node labels are unique; that is, each complex is displayed only once.

To distinguish subnetwork and overall network the symbol ‘hat’ to mark all entities that belong to the overall network: $\hat{I}$ for the incidence matrix, $\hat{E}$ for the stoichiometric generator that defines the network and $\hat{Y}$ 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 $I$ and the matrix of exponent vectors of the subnetwork consist of column vectors $\hat{I}_i$ and $\tilde{y}_i^\wedge$ of the overall network, namely those with $i \in \text{supp}(E)$. Let $r$ be the number of reactions contained in the overall network and $\check{r}$ 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 $Y I E = 0$ and $I E \neq 0$ for the subnetwork.

Let $m$, $\hat{m}$ and $l$, $\hat{l}$ be the number of complexes and the number of linkage classes of the subnetwork and the overall network, respectively. Recall that a stoichiometric generator is a generator of $\ker(Y I) \cap \mathbb{R}_{\geq 0}^r$ that contains more than one linkage class. Thus the network defined by $Y$ and $I$ will contain at least two linkage classes.

We further note the following fact concerning incidence matrices of arbitrary (oriented) graphs. We state it here without proof using the notation introduced in [12, 13]. For details see, for example, [20, Theorem 8.3.1, p. 168]

**Lemma 2.2** Let $I$ be the incidence matrix of a graph with $m$ vertices and $l$ connected components (i.e. linkage classes). Then $\text{rank} I = m - l$.

The following facts can be established for the matrix product $Y I$, where $I$ is the adjacency matrix associated with the subnetwork defined by the given stoichiometric generator $E$: 
Lemma 2.3 Let $\hat{E}$ be a stoichiometric generator and $E$ a vector obtained by deleting all zero entries of $\hat{E}$. Further let $Y$ and $I$ be the matrices associated with the subnetwork, as described above. Then the following holds:

(i) $E$ is a generator of $\ker(Y I) \cap R^r_{\geq 0}$ and it is the only generator, that is
$$\ker(Y I) \cap R^r_{\geq 0} = \{ \alpha E : \alpha \geq 0 \} .$$

(ii) $\dim(\ker(Y I)) = 1$.

(iii) $I$ has full column rank.

(iv) $I$ contains $m-l$ columns.

(v) $\text{rank}(Y I) = m-l-1$.

Proof:

(i.) Part (i) follows from the fact that $\hat{E}$ is a generator of $\ker\left( Y \hat{I} \right) \cap R^r_{\geq 0}$ and positivity of $E$.

Let $E_0$ be a nonzero nonnegative vector that satisfies eq. (1.8a). Positivity of $E$ implies $\text{supp}(E_0) \subseteq \text{supp}(E)$. Let $\hat{E}_0 \in R^r_{\geq 0}$ be obtained from $E_0$ by an appropriate filling with zeros. Then $Y \hat{I} \hat{E}_0 = 0$ and $\text{supp}\left( \hat{E}_0 \right) \subseteq \text{supp}(\hat{E})$ follow from the construction of $\hat{E}_0$.

As $\hat{E}$ is by definition a generator of $\ker\left( Y \hat{I} \right) \cap R^r_{\geq 0}$ 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 $\ker(Y I) \cap R^r_{\geq 0}$.

That $E$ is the only generator follows again from positivity of $E$: any nonzero nonnegative vector $E_0$ that satisfies eq. (1.8a) has $\text{supp}(E_0) \subseteq \text{supp}(E)$ and thus $E_0 = \alpha E$, $\alpha > 0$ (as $E$ is a generator of $\ker(Y I) \cap R^r_{\geq 0}$). Thus $E$ is the only generator of $\ker(Y I) \cap R^r_{\geq 0}$.

(ii.) The proof is similar to that for (i): suppose there exists a nonzero vector $E_0 \in R^r_{\geq 0}$, $E_0 \notin [E]$ with $Y I E_0 = 0$. That $E_0$ contains negative entries follows from (i): otherwise $E_0 = \alpha E$, $\alpha > 0$ and we are done. We will show that the existence of a vector $E_0$ with negative entries implies that $E$ is not a generator of $\ker(Y I) \cap R^r_{\geq 0}$. To see this let $\tilde{E} = \alpha E + E_0$. Then $\tilde{E} > 0$, for sufficiently large $\alpha$ and $\tilde{E} = \beta E$ by (i), a contradiction. Thus $\dim(\ker(Y I)) = 1$.

(iii.) We have $IE \neq 0$ by definition of $E$ and $\dim(\ker(Y I)) = 1$ by (ii). Thus $I$ must have full column rank.

(iv.) By Lemma 2.2 $\text{rank}(I) = m-l$. By (iii) $I$ has full column rank. Thus $I$ must have $m-l$ columns.

(v.) By a standard result of linear algebra for any matrix $A$ (see e.g. [30]) $\dim(\ker(A)) = \text{nr. of columns} - \text{rank}(A)$. As $\dim(\ker(Y I)) = 1$ by (ii) and nr. of columns $= m-l$ by (iv) the desired result $\text{rank}(Y I) = m-l-1$ follows.

The subnetwork defined by $\hat{E}$ will contain several linkage classes. Let $\ell$ be the number of linkage classes of the subnetwork. 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 $I^{J_i}$, that contains those columns of $I$ that are indexed by $J_i$. The following result can be established for the rank of the matrix product $Y I^{J_i}$:

Corollary 2.4 Consider a linkage class of the subnetwork defined by $\hat{E}$. Let $J_i \subseteq \{1, \ldots, m\}$ be the set of indices whose complexes are part of the linkage class and $I^{J_i}$ the incidence matrix associated with the linkage class (as described above, $I^{J_i}$, that contains those columns of $I$ that are indexed by $J_i$). Let $m^{J_i}$ be the number of complexes in the linkage class. Then
$$\text{rank}(Y I^{J_i}) = m^{J_i} - 1$$
Proof:
By assumption the linkage class contains \( m^j_i \) nodes. By definition a linkage class is a graph with one connected component. Thus \( \text{rank}(I^j_i) = m^j_i - 1 \) by Lemma 2.2. As by Lemma 2.3 (ii), \( Y I^j_i \) has full column rank the result \( \text{rank}(Y I^j_i) = m^j_i - 1 \) follows. \( \square \)

As a consequence of Lemma 2.3 the following Corollary gives the various deficiencies from (1.7):

**Corollary 2.5** Consider a subnetwork with \( l \) linkage classes defined by a stoichiometric generator \( \hat{E} \). For the deficiency \( \delta \) of the subnetwork as well as for the deficiencies \( \delta_i \) of the linkage classes of the subnetwork the following holds:

(i.) \( \delta = 1 \)
(ii.) \( \delta_i = 0, i = 1, \ldots, l \)

Proof: Lemma 2.3 (v) and eq. (1.7) imply (i), Lemma 2.3 (vi) and eq. (1.7) imply (ii). \( \square \)

In graph theory 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 [14, 15], 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.

In [13, 15] the deficiency one algorithm is defined for networks that satisfy the following requirements:

(I) The network deficiency is \( \delta = 1 \)
(II) The deficiency of the linkage classes is \( \delta_i = 0, i = 1, \ldots, l \)
(III) There exists a positive vector \( E \) with \( Y I E = 0 \).
(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 2.5 and that (III) holds by Lemma 2.3. Further note that the graph can not contain any cycles, as by Lemma 2.3 \( I \) has full column rank. Thus (IV) holds as well. This leads to Theorem 2.1.

### 3. Extension to the Overall Network

If switching or multiple steady states can be established for a subnetwork, one is of course interested in extending those features 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. We start by noting that the ODEs defined by a subnetwork can be obtained from the ODEs of the overall biochemical reaction network by assigning zero to all those rate constants associated with reactions not contained in the subnetwork. To see this, consider the following subnetwork of (1.1):

\[
A + B \xrightarrow{\begin{array}{c} k_1 \\ k_2 \end{array}} C
\]

with ODEs \( \dot{x}_1 = \dot{x}_2 = -k_1 x_1 x_2 + k_3 x_3 \) and \( \dot{x}_3 = -\dot{x}_1 \) and conservation relations \( x_1 + x_3 = c_1 \) and \( x_2 + x_3 = c_2 \) (network (3.1) imposes an additional conservation relation). The vector of rate constants of (3.1) is \( k_E = (k_1, k_2)^T \) and the 'complementary vector' of rate constants not
in the subnetwork is \( k_c = (k_3, k_4)^T \). To obtain the ODEs directly from those of network (1.1), the vector \( \dot{k}_E = (k_1, k_2, 0, 0)^T \) can be used to define the subnetwork \( \dot{x} = N v(\dot{k}_E, x) \) of the overall network (1.1). Using \( \dot{k}_c = (0, 0, k_3, k_4)^T \) one obtains the terms not contained in the ODEs defined by the subnetwork as \( N v(\dot{k}_c, x) \).

Let \( E \) be a stoichiometric generator with \( p \) nonzero entries. As for network (3.1), we use the decomposition \( k = \dot{k}_E + \dot{k}_c \in \mathbb{R}^r_{\geq 0} \) given by

\[
\begin{align*}
(\dot{k}_E)_i &= k_i, \; i \in \text{supp}(E), \quad (\dot{k}_E)_i = 0, \; i \notin \text{supp}(E), \\
(\dot{k}_c)_i &= 0, \; i \in \text{supp}(E), \quad (\dot{k}_E)_i = k_i, \; i \notin \text{supp}(E)
\end{align*}
\]

and define the vectors of rate constants contained in the subnetwork and the complementary rate constants by

\[
k_E = (k_i)_{i \in \text{supp}(E)} \in \mathbb{R}^{p}_{\geq 0} \quad \text{and} \quad k_c = (k_i)_{i \notin \text{supp}(E)} \in \mathbb{R}^{r-p}_{\geq 0}
\]

respectively.

In the following we propose a way to extend multistationarity from a subnetwork, defined by a stoichiometric generator, with stoichiometric subspace \( S_{\text{sub}} \) contained in the stoichiometric subspace \( S = \text{im}(N) \), to \( S \)-multistationarity of the overall network. Suppose \( S \)- or \( S_{\text{sub}} \)-multistationarity have been established for the subnetwork defined by the stoichiometric generator \( E \). That is, there exists a vector \( k_E^* \in \mathbb{R}^{p}_{\geq 0} \) of rate constants and two positive steady states \( x_1^*, x_2^* \) of the subnetwork in the same level set of the overall system (in case of \( S \)-multistationarity), i.e.

\[
x_2^* \in x_1^* + S \quad (\text{i.e.} \; W^T x_1^* = W^T x_2^*)
\]

or in the same level set of the subnetwork (in case of \( S_{\text{sub}} \)-multistationarity), i.e.

\[
x_2^* \in x_1^* + S_{\text{sub}} \quad (\text{i.e.} \; \begin{bmatrix} W^T \\ W_{\text{add}} \end{bmatrix} x_1^* = \begin{bmatrix} W^T \\ W_{\text{add}} \end{bmatrix} x_2^*),
\]

where the subspace \( S_{\text{sub}} \) of \( S \) satisfies \( \text{im} \left( \begin{bmatrix} W & W_{\text{add}} \end{bmatrix} \right) = S_{\text{sub}}^\perp \) (cf. subsection 1.1). Note that if \( k_E^* \in \mathbb{R}^{p}_{\geq 0} \) and \( x_1^*, x_2^* \) are obtained by the CRNT toolbox, one necessarily has the more restrictive condition \( x_2^* \in x_1^* + S_{\text{sub}} \).

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. Based on the implicit function theorem and on the special structure of the ODEs defined by a biochemical reaction network we derive computationally simple tests to decide whether the positive stationary pairs \( (k_E^*, x_i^*) \) of the subnetwork can be continued to curves of positive stationary pairs \( (k(\epsilon), x_i(\epsilon)) \) of the overall network. To not obscure the algorithm with more mathematical detail than necessary, the relevant results and proofs are relegated to the subsections to follow.

The following algorithm, based on Theorem 3.6 below, can be applied without reference to the underlying mathematical details. It is based on the data \( (k_E^*, x_1^*) \) with (3.2) of the subnetwork and the Jacobian \( J(x, k) := D_x N v(k, x) \) of the overall system.

**Algorithm 3.1** Collect those columns of \( I \) corresponding to reactions contained in the subnetwork in a matrix \( I^{(E)} \), the remaining ones in a matrix \( I^{(c)} \) and define the matrices

\[
N_E := Y I^{(E)} \in \mathbb{R}^{n \times p} \quad \text{and} \quad N_c := Y I^{(c)} \in \mathbb{R}^{n \times (r-p)}
\]
Let $S_E$ be orthonormal basis for $\text{im} (N_E)$ with

$$S_{\text{sub}} = \text{im} (S_E) = \text{im} (N_E) \subset \text{im} (N) = S$$

and let $W_{\text{add}}$ be orthonormal basis for the subspace defined by the additional conservation relations imposed by the subnetwork (so that the columns of $W$ from (1.5) and $W_{\text{add}}$ form a basis of the left-kernel of $N_E$). Let $\phi_c(x)$ be the vector of monomials defined by the educt complexes of those reactions not contained in the subnetwork.

(1) Define for $i = 1, 2$

$$A_i := S_E^T J (k_E^*, x_i^*) S_E, \quad B_i := S_E^T J (k_E^*, x_i^*) W_{\text{add}}.$$ 

If both $A_i$ are regular, solve the linear matrix equations $A_i X_i + B_i = 0$ for matrices $X_i$.

(2) Obtain a positive vector of complementary rate constants $k^*_c$ by solving the linear equations

$$\begin{bmatrix} W_{\text{add}}^T N_c \text{ diag} (\phi_c (x_1^*)) \\ W_{\text{add}}^T N_c \text{ diag} (\phi_c (x_2^*)) \end{bmatrix} k^*_c = 0.$$

If a positive $k^*_c \in \mathbb{R}_{>0}^c$ exists, define $\hat{k}_c^* \in \mathbb{R}_{\geq 0}^c$ as described above (by filling in 0’s).

(3) Given a positive $k^*_c$ (from step (2)), define

$$C_i := W_{\text{add}}^T J (\hat{k}_c^*, x_i^*) S_E, \quad D_i := W_{\text{add}}^T J (\hat{k}_c^*, x_i^*) W_{\text{add}}$$

and $D_i := C_i X_i + D_i$ (using $X_i$ obtained in step (1)).

If both matrices $D_i$ are regular, then there exist a line segment of positive rate constants

$$k^*(\epsilon) = \hat{k}_c^* + \epsilon \hat{k}_c^*.$$ 

and a pair of smooth one-parameter curves of positive steady states $x_1(\epsilon), x_2(\epsilon)$ of (1.3) with

$$W^T x_1(\epsilon) = W^T x_2(\epsilon), \quad \text{i.e.} \quad x_2(\epsilon) \in x_1(\epsilon) + \mathcal{S},$$

and $x_i(0) = x_i^*$ as long as $\epsilon > 0$ is sufficiently small.

In summary, the following steps can be used to establish multistationarity for the overall network: first, obtain $k^*_E$ and $x_1^*, x_2^*$ with (3.2) for the subnetwork. Second, use Algorithm 3.1 to test the continuability of $(k^*_E, x^*_i)$ to the overall network for positive rate constants given explicitly by (3.4) (cf. step (2)). The regularity assumptions in step (1) and step (3) entail the continuability by the Implicit function Theorem.

Section 3 is organized as follows:

We first introduce some notations for subnetworks and then turn to the continuation of a single steady state of the subnetwork (where some rate constants vanish) to a steady state of the overall network (where all rate constants are positive). Subsection 3.3 presents the extension of the $\mathcal{S}$-multistationarity of the subnetwork to the $\mathcal{S}$-multistationarity of the overall network and thus provides the basis for Algorithm 3.1. The more restricted continuation problem from $\mathcal{S}_{\text{sub}}$-multistationarity of the subnetwork to the $\mathcal{S}_{\text{sub}}$-multistationarity of the overall network is addressed in subsection 3.4. In analogy to Algorithm 3.1, an algorithm for this kind of $\mathcal{S}_{\text{sub}}$-multistationarity, might be based on Theorem 3.7.

We’d like to point out that subsection 3.5 offers an alternative condition for one of the crucial regularity conditions in the Theorems 3.2, 3.6 and 3.7. Conditions for multistationarity
in biochemical reaction networks endowed with mass action kinetics have been derived using algebraic geometry in [19], where it is shown that, under genericity conditions, multistationarity in a subnetwork defined by a stoichiometric generator implies multistationarity in the overall network. As we determine whether or not multistationarity indeed takes place in the overall network, we shed some new light on those results of [19] in providing a computationally simple procedure to check sufficient conditions for multistationarity (see subsection 3.5).

In the concluding subsection 3.6 we offer a comparing summary.

3.1. Setup for Subnetworks

To begin with, consider a system of ODEs given in the form \( \dot{x} = N v(k, x) \), with \( N \in \mathbb{R}^{n \times r} \), \( x \in \mathbb{R}^n \) and \( v, k \in \mathbb{R}^r \). Note that \( v(k, x) = \text{diag}(k) \phi(x) = \text{diag}(\phi(x))k \), where \( \phi(x) = (x^{y_1}, \ldots, x^{y_p})^T \). Let \( E \in \mathbb{R}^r_{\geq 0} \) be a stoichiometric generator of the overall network. \( E \) can be used to split the parameter vector: let \( k_E, k_c \in \mathbb{R}^r_{\geq 0} \) and collect all parameters belonging to reactions contained in the subnetwork in \( \hat{k}_E \) (i.e. \( \hat{k}_{E,i} = k_i \) for \( i \in \text{supp}(E) \) and \( \hat{k}_{E,i} = 0 \) otherwise) and the remaining ones in \( \hat{k}_c \) (i.e. \( \hat{k}_c = k - \hat{k}_E \)). Since \( v \) is linear in \( k \) we have

\[
\dot{x} = N v(k, x) = N v(\hat{k}_E, x) + N v(\hat{k}_c, x). \tag{3.6}
\]

Let \( |\text{supp}(E)| = \rho \), define \( k_E \in \mathbb{R}^{r}_{\geq 0} \) and \( k_c \in \mathbb{R}^{r-\rho}_{\geq 0} \) by

\[
k_E = (k_i)_{i \in \text{supp}(E)} \text{ and } k_c = (k_i)_{i \not\in \text{supp}(E)} \text{ resp. .}
\]

Let \( v_E(k_E, x) \in \mathbb{R}^\rho \) and \( v_c(k_c, x) \in \mathbb{R}^{r-\rho} \) be given by

\[
\begin{align*}
(v(\hat{k}_E, x))_{i \in \text{supp}(E)} &= v_E(k_E, x) = \text{diag}_E(x)k_E, \\
(v(\hat{k}_c, x))_{i \not\in \text{supp}(E)} &= v_c(k_c, x) = \text{diag}_c(x)k_c.
\end{align*}
\]

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

\[
N_E = (N_i)_{i \in \text{supp}(E)} \text{ and } N_c = (N_i)_{i \not\in \text{supp}(E)}.
\]

Then the ODE (3.6) can be rewritten as

\[
\dot{x} = N_E v_E(k_E, x) + N_c v_c(k_c, x) \tag{3.7}
\]

and the ODEs for the subnetwork defined by \( E \) are given by

\[
\dot{x} = N_E v_E(k_E, x). \tag{3.8}
\]

As \( E \) is a stoichiometric generator Lemma 2.3 implies \( \text{rank}(N_E) = \rho - 1 \) and \( \ker(N_E) = [\pi_E] \), with \( \pi_E = (E_j)_{j \in \text{supp}(E)} \).

Let \( W^T \in \mathbb{R}^{\rho \times \rho} \) be an orthonormal basis for the left kernel of \( N \). Let

\[
W^T_E = \begin{pmatrix} W^T_{add} & W^T_T \end{pmatrix} \in \mathbb{R}^{(n-(\rho-1)+p) \times n}
\]

be an orthonormal basis for the left kernel of \( N_E \) with \( W^T_E W_E = I_{n-(\rho-1)} \). Let \( S_E \) be an orthonormal basis for \( \text{im}(N_E) \) and let \( T = (S_E, W_{add}, W) \) be an orthonormal transformation with

\[
x = x(\xi, \eta, \hat{\eta}) = T \begin{pmatrix} \xi \\ \eta \\ \hat{\eta} \end{pmatrix} \quad \text{and} \quad \xi = S^T_E x, \ \eta = W^T_{add} x, \ \hat{\eta} = W^T T x.
\]
Then (3.6) reads
\[
\begin{align*}
\dot{\xi} &= S_E^T N_E v_E (k_E, x) + S_E^T N_c v_c (k_c, x) \\
\dot{\eta} &= W_{add}^T N_c v_c (k_c, x) \\
\dot{v} &= 0.
\end{align*}
\tag{3.9}
\]

### 3.2. Continuation of One Steady State

Suppose a $k_E^* > 0$ and a steady state $x_0^* > 0$ for the subnetwork (3.8) defined by $E$ have been established, i.e.
\[
N_E v_E (k_E^*, x_0^*) = 0, \quad (\xi_0^*, \eta_0^*, \tilde{\eta}_0^*)^T = T^T x_0^*
\]

Observe that the last equation is equivalent to
\[
\begin{align*}
\dot{\xi} &= S_E^T N_E v_E (k_E^*, x (\xi_0^*, \eta_0^*, \tilde{\eta}_0^*)) + S_E^T N_c v_c (k_c, x (\xi_0^*, \eta_0^*, \tilde{\eta}_0^*)) \\
\dot{\eta} &= W_{add}^T N_c v_c (k_c, x (\xi_0^*, \eta_0^*, \tilde{\eta}_0^*)) \\
\dot{\tilde{v}} &= 0.
\end{align*}
\]

With $F (k, x) := N v(k, x)$ and its Jacobian $F_x := D_x F (k, x)$ we can outline the following program:

1. Compute $S_E^T F_x \left( \hat{k}_E^*, x_0^* \right) \left( S_E, W_{add} \right) =: (A_0^*, B_0^*)$.
2. Compute a positive $\kappa_0$ such that
\[
\begin{align*}
G_c^* \kappa_0 &= 0
\end{align*}
\]
and define $\hat{k}_c^* \in \mathbb{R}^r$ by
\[
\begin{align*}
\left( \hat{k}_c^* \right)_{i \in \supp(E)} &= 0 \quad \text{and} \quad \left( \hat{k}_c^* \right)_{i \notin \supp(E)} = \kappa_0.
\end{align*}
\]
(3) Compute \( W^T_{add} F_x \left( \hat{k}_c^*, x_0^* \right) (S_E, W_{add}) =: (C_0^*, D_0^*) \) and ask for
\[
\text{regular } D_0^* := D_0^* + C_0^* X_0^*.
\] (3.18)

Concerning the regularity of \( A_0^* \) we also refer to Lemma 3.8 below. The following Theorem 3.2 shows that this program leads to a steady state \( \hat{x}_0 \) of (3.6) near \( x_0^* \) for \( \hat{k}_E^* + \epsilon \hat{k}_c^* \) if \( \epsilon > 0 \) is sufficiently small.

**Theorem 3.2** Suppose the following conditions are fulfilled:

(i) There exist \( x_0^* > 0, k_E^* > 0 \) with \( N_E v_E (k_E^*, x_0^*) = 0 \).

(ii) There exists a \( \kappa_0 > 0 \) with \( G_E^* \kappa_0 = 0 \), cf. (3.16).

(iii) \( A_0^* \) is regular, cf. (3.13).

(iv) \( D_0^* \) is regular, cf. (3.18).

Then there exist \( \epsilon_0 > 0 \) and \( \delta_0 > 0 \) such that
\[
0 < \epsilon < \epsilon_0 \quad \text{and} \quad |\hat{\eta} - \hat{\eta}^*| < \delta_0
\]

imply the existence of a steady state
\[
x_0(\hat{\eta}, \epsilon) = (S_E, W_{add}, W) \begin{pmatrix} \Xi_0(\hat{\eta}, \epsilon) \\ H_0(\hat{\eta}, \epsilon) \end{pmatrix}
\]
of (3.6) for the positive \( k(\epsilon) = \hat{k}_E^* + \epsilon \hat{k}_c^* \) – cf. (3.10) and (3.17). That is in particular true for \( \hat{\eta} = \hat{\eta}_0^* \) where \( x_0(\hat{\eta}_0^*, \epsilon) \) represents a continuation of \( x_0^* \).

The eigenvalues of the linearization of (3.6) at the steady state \( x_0(\hat{\eta}, \epsilon) \) are given in leading order by the eigenvalues of the matrices \( A_0^* \) and \( \epsilon D_0^* \).

Before turning to the proof of Theorem 3.2 we’d like to add some remarks.

Switching in \( n \) dimensions might be caused by the existence of a hypersurface acting as a threshold, e.g. by the boundary of a domain of attraction for some invariant set (attractor) or by the \((n-1)\)-dimensional stable manifold of a saddle point. One might call the switching surface *fast* in cases where solutions are repelled at an exponential rate of order \( \gamma t \) and *slow* in cases where they are repelled at an exponential rate of order \( \epsilon \gamma t \) for a finite positive \( \gamma \) (with \( \epsilon \to 0^+ \)).

**Remark 3.3 (Reduction by Singular Perturbations)** We’d like to describe the geometric setup for the network \( \dot{x} = F(k, x) = Nv(k, x) \) from (3.6) in its coordinate form of (3.9). Suppose (3.11) with \( k_c = 0 \), i.e. suppose
\[
S^T_E N(S_E \xi + W_{add} \eta + W \hat{\eta}, k_E^*) = 0
\]
has a smooth solution \( \xi = \Xi(\eta, \hat{\eta}) \) with its Jacobian
\[
A(\eta, \hat{\eta}) = S^T_E F_x (S_E \Xi(\eta, \hat{\eta}) + W_{add} \eta + W \hat{\eta}, k_E^*) S_E
\] (3.19)
having its spectrum bounded away from the imaginary axis \( i\mathbb{R} \) for \( (\eta, \hat{\eta}) \) in a compact ball \( \mathcal{C} \) around \( (\eta_0^*, \hat{\eta}_0^*) \).

Then, for sufficiently small \( \epsilon > 0 \), there exists a smooth invariant manifold
\[
\xi = \sigma(\eta, \hat{\eta}, \epsilon) = \Xi(\eta, \hat{\eta}) + \mathcal{O}(\epsilon)
\] (3.20)
over $C$ for (3.9) having

$$\dot{\eta} = W_{add}^T F(\sigma(\eta, \dot{\eta}, \epsilon), \eta, \dot{\eta}, k_c^* \epsilon) =: \epsilon h(\eta, \dot{\eta}, \epsilon) \tag{3.21}$$

as an exact reduced subsystem. For any such fixed $\dot{\eta} = \dot{\eta}_f$, any solution $\eta = \eta(t, \dot{\eta}_f, \epsilon)$ of (3.21) within $C$ gives rise to a solution $(\xi(t, \dot{\eta}_f, \epsilon), \eta(t, \dot{\eta}_f, \epsilon))$ of the overall system with

$$\xi(t, \dot{\eta}_f, \epsilon) = \sigma(\eta(t, \dot{\eta}_f, \epsilon), \dot{\eta}_f, \epsilon),$$

in particular, stationary solutions or periodic solutions over $C$. We’d like to point out that, in the setup of Theorem 3.2, $h(\eta, \dot{\eta}, \epsilon)$ vanishes at $H_0(\dot{\eta}, \epsilon)$. For periodic solutions we refer to Remark 3.4.

For a proof one might use e.g. Fenichel’s results ([17]) for the scaled time $\tau = \epsilon t$ and

$$\frac{d\xi}{d\tau} = S_E^T N_E v_E (k_c^* E, x) + \epsilon S_E^T N_c v_c (k_c^*, x),$$

$$\frac{d\eta}{d\tau} = W_{add}^T N_c v_c (k_c^*, x),$$

$$\frac{d\dot{\eta}}{d\tau} = 0.$$ \quad \tag{3.22}

**Remark 3.4 (Slow Hopf Bifurcations)** In the framework of Remark 3.3 we might use $\dot{\eta}$ as a bifurcation parameter near $\dot{\eta}^*$ while keeping $\epsilon$ fixed. Eigenvalues $\alpha(\dot{\eta}) \pm i \beta(\dot{\eta})$ of

$$D^*(\dot{\eta}, \epsilon) = (D_0^* + C_0 X_0^*)(\dot{\eta}, \epsilon),$$

for $X_0^*$ from (3.14) and $W_{add}^T F_x \left(\dot{k}_c^*, x_0(\dot{\eta}, \epsilon)\right) (S_E, W_{add}) =: (C_0^*, D_0^*)(\dot{\eta}, \epsilon)$, crossing the imaginary axis ($\beta \neq 0$) might introduce a Hopf-bifurcation. Under the regularity assumption (iii) for $A_0^*$ the theory of singular perturbation allows the reduction of the overall network (3.6) to an ODE (3.21) for the slow $\eta$–variables on a slow invariant manifold $\xi = \sigma(\eta, \dot{\eta}, \epsilon)$, possibly on a quasi–stationary manifold $\xi = \sigma(\eta, \dot{\eta}, 0)$ with vectorfield $ch(\eta, \dot{\eta}, 0)$. Hopf-bifurcations in the reduced slow system then give rise to periodic solutions in the overall network. See the example studied in the section 4 for $\epsilon \in (0, 6 \cdot 10^{-4})$.

**Remark 3.5 (Switching Surfaces)**

(a) In the case where all eigenvalues $\alpha_i(A_0^*)$ of $A_0^*$ and $\delta_j(D_0^*)$ of $D_0^*$ have negative real part, the continued steady state is exponentially stable within its level set (i.e. for fixed $\dot{\eta}$).

(b) In the case where there is just one algebraically simple positive eigenvalue among the $\delta_j(D_0^*)$ (with all other eigenvalues of $D_0^*$ and $A_0^*$ having negative real part) the overall system has a slow acting switching surface generated by the global stable manifold of the saddle-type steady state $x_0^*(\dot{\eta}, \epsilon)$ within its level set. Here, the steady state $x_0^*$ is exponentially stable with respect to the subnetwork and separation (in the overall network) occurs on an exponential time-scale $O(\epsilon t)$.

(c) In the case where there is just one algebraically simple positive eigenvalue among the $\alpha_i(A_0^*)$ (with all other eigenvalues of of $A_0^*$ and $D_0^*$ having negative real part) the overall system has a fast acting switching surface generated by the global stable manifold of the saddle-type steady state $x_0^*(\dot{\eta}, \epsilon)$ within its level set. The steady state $x_0^*$ is a saddle already in the subnetwork and separation (in the overall network) accrues on an exponential time-scale $O(t)$.
(d) In the case where there is just one algebraically simple vanishing eigenvalue among the \(\alpha_i(A^*_0)\) (with all other eigenvalues of \(A^*_0\) and \(D^*_0\) having negative real part) and where the subnetwork undergoes a (generic) saddle-node bifurcation to an exponentially stable positive steady state and a hyperbolic positive saddle (with one-dimensional unstable manifold) the bifurcated steady states of the subnetwork may be continued to the overall network (cf. subsection 3.3).

We now turn to the proof of Theorem 3.2 and the consequent remarks.

Proof:
Apply the orthonormal transformation \(\xi = S^T_E x, \eta = W^T_{add} x, \hat{\eta} = W^T x\) to obtain

\[
\dot{\xi} = S^T_E N v(k^*_E + \epsilon \hat{k}^*_c, x)
\]

\[
= S^T_E N v(k^*_E, x) + S^T_E N v(\epsilon \hat{k}^*_c, x)
\]

\[
\dot{\eta} = W^T_{add} N v(k^*_E + \epsilon \hat{k}^*_c, x) = N v(\hat{k}^*_c, x)
\]

\[
\dot{\hat{\eta}} = 0.
\]

For fixed \(x^*_0, k^*_E\) and regular \(A^*_0\) (cf. assumption (i)&(iii)) the equation \(\dot{\xi} = 0\) has a locally unique solution \(\xi = \Xi_0(\eta, \hat{\eta}, \epsilon \hat{k}^*_c)\) near \((\eta^*_0, \hat{\eta}^*_0, 0)\) with

\[
A^*_0 X_0 + B^*_0 = 0, \quad X_0 := \frac{\partial}{\partial \eta} \Xi_0(\eta^*_0, \hat{\eta}^*_0, 0),
\]

by the implicit function theorem. To find steady states we need to solve

\[
W^T_{add} N v(k^*_E, S_E \Xi_0(\eta, \hat{\eta}, \epsilon \hat{k}^*_c) + W_{add} \eta + W \hat{\eta}) = 0
\]

for \(\eta = H_0(\hat{\eta}, \epsilon)\) near the solution \((\eta^*_0, \hat{\eta}^*_0, 0)\) (cf. assumption (ii) and (3.17)). By assumption

(iv) and the implicit function theorem such a function \(H_0\) exists. Thus locally, there exist steady states

\[
\xi_0 = \Xi_0(H_0(\hat{\eta}, \epsilon), \hat{\eta}, \epsilon) = \Xi_0(\hat{\eta}, \epsilon), \quad \eta = H_0(\hat{\eta}, \epsilon), \quad \hat{\eta}
\]

of (3.9) for sufficiently small \(\epsilon > 0\). The corresponding positive steady state of (3.6) near \(x^*_0\) is given by the \(x_0(\hat{\eta}, \epsilon)\) of the theorem.

Concerning the statement about the eigenvalues we note that the linearization of the \((\xi, \eta)\)–part in (3.9) at \(x^*_0\) and \(\epsilon = 0\) is given by

\[
\mathcal{M}(0) := \begin{pmatrix} A^*_0 & B^*_0 \\ 0 & 0 \end{pmatrix}
\]

with complementary invariant subspaces spanned by the columns of

\[
P_1(0) := \begin{pmatrix} I \\ 0 \end{pmatrix} \quad \text{and} \quad P_2(0) := \begin{pmatrix} X^*_0 \\ I \end{pmatrix}
\]

leading with \(P(0) := (P_1(0), P_2(0))\) to the similarity relation

\[
\mathcal{M}(0) P(0) = \begin{pmatrix} A^*_0 & 0 \\ 0 & 0 \end{pmatrix} = P(0) \begin{pmatrix} A^*_0 & 0 \\ 0 & 0 \end{pmatrix}.
\]
The corresponding linearization of the \((\xi, \eta)\)-part in (3.9) at \(x_0(\hat{\eta}, \epsilon)\) for small \(\epsilon > 0\) is of the form
\[
\mathcal{M}(\epsilon) = \begin{pmatrix} A_0^* + \mathcal{O}(\epsilon) & B_0^* + \mathcal{O}(\epsilon) \\ \epsilon[C_0^* + \mathcal{O}(\epsilon)] & \epsilon[D_0^* + \mathcal{O}(\epsilon)] \end{pmatrix}.
\]
By the Implicit Function Theorem there exist complementary invariant subspaces leading with \(\mathcal{P}_1(\epsilon) = \begin{pmatrix} I + \mathcal{O}(\epsilon) \\ 0 + \mathcal{O}(\epsilon) \end{pmatrix}\) and \(\mathcal{P}_2(\epsilon) = \begin{pmatrix} X_0^* + \mathcal{O}(\epsilon) \\ I + \mathcal{O}(\epsilon) \end{pmatrix}\) leading with \(\mathcal{P}(\epsilon) = (\mathcal{P}_1(\epsilon), \mathcal{P}_2(\epsilon))\) to
\[
\mathcal{M}(\epsilon)\mathcal{P}(\epsilon) = \mathcal{P}(\epsilon) \begin{pmatrix} A_0^* + \mathcal{O}(\epsilon) & 0 \\ \epsilon[C_0^* + \mathcal{O}(\epsilon)] & \epsilon[D_0^* + \mathcal{O}(\epsilon)] \end{pmatrix}.
\]
Thereby the claim concerning the eigenvalues follows. As does the note about switching surfaces.
\(\square\)

3.3. Continuation of a Pair of Steady States

Suppose a \(k_E^* > 0\) and steady states \(x_{1,2}^* > 0\) within the same level set of the overall system have been established for the subnetwork (3.8) defined by \(E\), i.e.
\[
\begin{align*}
N_E v_E (k_E^*, x_{1,2}^*) &= 0, \tag{3.26a} \\
(\xi_{1,2}^*, \eta_{1,2}^*, \hat{\eta}^*)^T &= T^T x_{1,2}^*, \tag{3.26b} \\
W^T x_1^* &= W^T x_2^*. \tag{3.26c}
\end{align*}
\]
We’d like to point out that we do not require
\[
\eta_1^* = \eta_2^*, \quad \text{i.e.} \quad W_{add}^T x_1^* = W_{add}^T x_2^*. \tag{3.27}
\]
Hence we just ask for \(x_2^*\) to belong to the coset \(x_1^* + \text{im}(N)\) of \(x_1^*\) but do not ask for \(x_2^*\) to belong to \(x_1^* + \text{im}(N_E)\). So we investigate the question when \(S\)-multistationarity of the subnetwork leads to \(S\)-multistationarity of the overall network. Observe that
\[
\begin{align*}
\hat{\xi} &= S_E^T N_E v_E (k_E^*, x (\xi_{1,2}^*, \eta_{1,2}^*, \hat{\eta}^*)) + S_E^T N_c v_c \left( k_c, x (\xi_{1,2}^*, \eta_{1,2}^*, \hat{\eta}^*) \right) \\
\hat{\eta} &= W_{add}^T N_c v_c \left( k_c, x (\xi_{1,2}^*, \eta_{1,2}^*, \hat{\eta}^*) \right) \\
\hat{j} &= 0.
\end{align*}
\]
Fix \(k_E^* > 0\) and \(\hat{\eta}^*\), define \(\hat{k}_E^*\) as in (3.10)
\[
\left( \hat{k}_E^* \right)^{i \notin \text{supp}(E)} = 0 \quad \text{and} \quad \left( \hat{k}_E^* \right)^{i \in \text{supp}(E)} = k_E^*, \tag{3.28}
\]
so that \(\hat{k}_E^*\) is obtained from \(k_E^*\) by a suitable filling with zeros and let \(k := \hat{k}_E^* + \epsilon \hat{k}_c\). For steady states of (3.9) we can outline the following program (leading to Algorithm 3.1):

1. Compute \(S_E^T F_x \left( \hat{k}_E^*, x_{1,2}^* \right) \) \((S_E, W_{add}) =: (A_{1,2}^*, B_{1,2}^*)\),
   ask for
   \[
   \text{regular } A_{1,2}^*
   \tag{3.29}
   \]
   and solve
   \[
   A_{1,2}^* X_{1,2}^* + B_{1,2}^* = 0
   \tag{3.30}
   \]
   for \(X_{1,2}^* = -[A_{1,2}^*]^{-1} B_{1,2}^*\).
(2) Compute a positive $\kappa_0$ with

$$ G_c^* \kappa_0 = 0 $$

for

$$ G_c^* := \begin{bmatrix} W_{q_{add}} N_c \text{ diag} (\phi_c (x_1^*)) \\ W_{add} N_c \text{ diag} (\phi_c (x_2^*)) \end{bmatrix}. $$

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

$$ \left( \hat{k}_c^* \right)_{\xi \in \text{supp}(E)} = 0 \quad \text{and} \quad \left( \hat{k}_c^* \right)_{\xi \notin \text{supp}(E)} = \kappa_0. $$

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

$$ \text{regular} \quad D_{1,2}^* := D_{1,2}^* + C_{1,2}^* X_{1,2}. $$

Concerning the regularity of $A_{1,2}^*$ we also refer to Lemma 3.8 below. The following Theorem 3.6 shows that this program leads to a pair of positive steady states $\tilde{x}_{1,2}$ of (3.6) near $x_{1,2}^*$ for $\hat{k}_c^* + \epsilon \hat{k}_c^*$ if $\epsilon > 0$ is sufficiently small. The G1-S-transition example studied in section 4 shows multistationarity for $\epsilon \in \left[0, 12 \cdot 10^{-3}\right]$.

**Theorem 3.6** Suppose the following conditions are fulfilled:

(i) There exist $x_{1,2}^* > 0$, $k_E^* > 0$ with

$$ N_E v_E (k_E^*, x_{1,2}^*) = 0 \quad \text{and} \quad W^T x_1^* = W^T x_2^*. $$

(ii) There exists a $\kappa_0 > 0$ with $G_c^* \kappa_0 = 0$, cf. (3.32).

(iii) Both $A_1^*$ and $A_2^*$ are regular, cf. (3.29).

(iv) Both $D_1^*$ and $D_2^*$ are regular, cf. (3.34).

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

$$ 0 < \epsilon < \epsilon_0 \quad \text{and} \quad |\hat{\eta} - \hat{\eta}| < \delta_0 $$

imply the existence of different positive steady states

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

of (3.6) with

$$ W^T x_1 (\hat{\eta}, \epsilon) = W^T x_2 (\hat{\eta}, \epsilon), \quad \text{i.e.} \quad x_2 (\hat{\eta}, \epsilon) \in x_1 (\hat{\eta}, \epsilon) + \text{im} (N), $$

for the positive $k(\epsilon) = \hat{k}_E^* + \epsilon \hat{k}_c^*$ - cf. (3.28) and (3.33). That is in particular true for $\hat{\eta} = \hat{\eta}^*$ where $x_{1,2} (\hat{\eta}^*, \epsilon)$ represents a continuation of $x_{1,2}^*$.

The eigenvalues of the linearization of (3.6) at the steady states $x_{1,2} (\hat{\eta}, \epsilon)$ are given in leading order by the eigenvalues of the matrices $A_{1/2}^*$ and $\epsilon D_{1/2}^*$. 

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What can be said if (3.27) is taken as a further assumption in Theorem 3.6? Just in case

\[ H_1(\hat{\eta}, \epsilon) = H_2(\hat{\eta}, \epsilon) \]

one happens to have the difference \( x_2(\hat{\eta}, \epsilon) - x_1(\hat{\eta}, \epsilon) \) in the stoichiometric subspace \( \text{im}(N_E) \) of the subnetwork.

**Proof:**

Apply the orthonormal transformation \( \xi = S^T E x, \eta = W^T_{add} x, \hat{\eta} = W^T x \) to obtain

\[ \dot{\xi} = S^T_E N v(\hat{k}^*_E, x) \]
\[ = S^T_E N v(\hat{k}^*_E, x) + S^T_E N v(\epsilon \hat{k}^*_c, x) \]
\[ \dot{\eta} = W^T_{add} N v(\hat{k}^*_E + \epsilon \hat{k}^*_c, x) = \epsilon N v(\hat{k}^*_c, x) \]
\[ \dot{\hat{\eta}} = 0. \]

For fixed \( x_j^*, k^*_c \) and regular \( A^*_j \) (cf. assumption (i)\( \hat{k}\)(iii)) the equation \( \dot{\xi} = 0 \) has a locally unique solution \( \xi_j = \Xi_j(\eta, \hat{\eta}, \epsilon \hat{k}^*_c) \) near \( (\eta^*_1, \eta^*_2, \hat{\eta}^*, 0) \) with \( A^*_j X_j + B^*_j = 0, X_j := \frac{\partial}{\partial \eta} \Xi_j(\eta^*_1, \hat{\eta}^*, 0) \), by the implicit function theorem. To find steady states with common \( \hat{\eta}\)-components we need to solve

\[ W^T_{add} N v(\hat{k}^*_c, S E \Xi_1(\eta, \hat{\eta}, \epsilon \hat{k}^*_c) + W_{add} \eta + W \hat{\eta}) = 0 \]
\[ W^T_{add} N v(\hat{k}^*_c, S E \Xi_2(\eta, \hat{\eta}, \epsilon \hat{k}^*_c) + W_{add} \hat{\eta} + W \eta) = 0 \]

for \( \left( \frac{\eta}{\hat{\eta}} \right) \equiv \left( \frac{H_1(\hat{\eta}, \epsilon)}{H_2(\hat{\eta}, \epsilon)} \right) \) near the solution \( (\eta^*_1, \eta^*_2, \hat{\eta}^*, 0) \) (cf. assumption (ii) and (3.33)). By assumption (iv) and the implicit function theorem such functions \( H_1 \) and \( H_2 \) exist. Thus locally, there exist steady states

\[ \xi_1 = \Xi_1(H_1(\hat{\eta}, \epsilon), \hat{\eta}, \epsilon) =: \Xi_1(\hat{\eta}, \epsilon), \eta = H_1(\hat{\eta}, \epsilon), \hat{\eta} \]
\[ \xi_2 = \Xi_2(H_2(\hat{\eta}, \epsilon), \hat{\eta}, \epsilon) =: \Xi_2(\hat{\eta}, \epsilon), \eta = H_2(\hat{\eta}, \epsilon), \hat{\eta} \]

of (3.9) for sufficiently small \( \epsilon > 0 \). The corresponding positive steady states of (3.6) near \( x_{1,2}^* \) are given by the \( x_{1,2}(\hat{\eta}, \epsilon) \) of the theorem. The statement about the eigenvalues has already been shown in the proof of Theorem 3.2. \( \square \)

### 3.4. Restricted Continuation of a Pair of Steady States

We formulate the result accompanying Theorem 3.6 for the simultaneous continuation of a pair of distinct positive steady states \( x_{1,2}^* \), but now we ask for \( x_2^* \) to belong to \( x_1^* + \text{im}(N_E) \) which generally is a proper submanifold of \( x_1^* + \text{im}(N) \). So we investigate the question when \( S_{\text{sub}-}\text{multistationarity} \) of the subnetwork leads to \( S_{\text{sub}-}\text{multistationarity} \) of the overall network—always having just positive steady states in mind.

**Theorem 3.7** Let \( G^*_c \) be given by (3.32) and assume

(i) there exist \( x_{1,2}^* > 0, k^*_E > 0 \) with

\[ N_E v_E(k^*_E, x_{1,2}^*) = 0 \] and \( W^T_E x_1^* = W^T_E x_2^* \),

(ii) there exists a \( \kappa_0 > 0 \) with \( G^*_c \kappa_0 = 0 \),
(iii) both \( A_1^* = A(x_1^*) \) and \( A_2^* = A(x_2^*) \) are regular (cf. (3.29)) and
(iv) \( G^*_c \) has full row rank.

Consider parameter vectors of the form \( k (\epsilon) = \hat{k}_E^* + \epsilon \hat{k}_c \) with
\[
\left( \hat{k}_E^* \right)_{i \in \text{supp}(E)} = 0, \quad \left( \hat{k}_E^* \right)_{i \in \text{supp} E} = k_E^*.
\]

Then there exists \( \epsilon_0 > 0 \) such that \( 0 < \epsilon < \epsilon_0 \) implies the existence of different positive steady states
\[
x_{1,2} (\epsilon) = (S_E, W_{add}, W) \begin{pmatrix} \xi_{1,2} (\epsilon) \\ \eta^* \end{pmatrix}
\]
of the overall network (3.6) with
\[
W_E^T x_1 (\epsilon) = W_E^T x_2 (\epsilon), \text{ i.e. } x^*_2 \in x^*_1 + \text{im} (N_E),
\]
for suitable parameters \( k (\epsilon) = \hat{k}_E^* + \epsilon \hat{k}_c (\epsilon) \).

Proof:

Apply the orthonormal transformation \( x = T \begin{pmatrix} \xi \\ \eta \end{pmatrix} \) with \( \xi = S_E^T x, \eta = W_{add}^T x, \hat{\eta} = W^T x \) to obtain
\[
\dot{\xi} = S_E^T N v(\hat{k}_E^* + \epsilon \hat{k}_c, x) = S_E^T N v(\hat{k}_E^*, x) + S_E^T N v(\epsilon \hat{k}_c, x) \\
\dot{\eta} = W_{add}^T N v(\hat{k}_E^* + \epsilon \hat{k}_c, x) = \epsilon W_{add}^T N_c \text{ diag} (\phi_c (x)) k_c \\
\hat{\eta} = 0.
\]

To extend \( x^*_{1,2} \) with \( (\xi^*_{1,2}, \eta^*, \hat{\eta}^*) \) = \( T^T \) \( x^*_{1,2} \) to the overall network we keep \( \hat{k}_E^*, \eta^* \) and \( \hat{\eta}^* \) fixed and solve
\[
\begin{align*}
S_E^T N v(\hat{k}_E^*, x) + S_E^T N v(\epsilon \hat{k}_c, x) &= 0 \\
\epsilon W_{add}^T N_c \text{ diag} (\phi_c (x)) k_c &= 0
\end{align*}
\]
for \( \xi \) and \( \hat{k}_c \) near \( \xi^*_{1,2} \) and \( \hat{k}_c = 0 \). By assumption (iii) \( A_1^* \) is regular. Thus, by the implicit function theorem, there are smooth manifolds \( \xi_1 = \Xi_1 (\epsilon k_c) \) and \( \xi_2 = \Xi_2 (\epsilon k_c) \) through \( \xi = \xi^*_{1,2}, \epsilon k_c = 0 \) annihilating \( \dot{\xi} \) locally. For these manifolds to correspond to equilibria of the overall network for \( \epsilon k_c > 0 \) one has to satisfy
\[
G_c (x_1) k_c = 0, \quad \text{ (3.35a)}
\]
\[
G_c (x_2) k_c = 0, \quad \text{ (3.35b)}
\]
\[
x_{1,2} = S_E \Xi_{1,2} (\epsilon k_c) + W_{add} \eta^* + W \hat{\eta}^* \quad \text{ (3.35c)}
\]
(where \( x^*_{1,2} = S_E \Xi_{1,2} (0) + W_{add} \eta^* + W \hat{\eta}^* \)). To obtain a positive solution \( k_c \) of (3.35a) \& (3.35b) we use the \( \kappa_0 \) from assumption (ii) and the representation
\[
k_c = \kappa (\epsilon, \kappa_1) = \kappa_0 + \epsilon \begin{bmatrix} G_c (x_1^1) \\ G_c (x_2^2) \end{bmatrix}^T \kappa_1 = \kappa_0 + \epsilon G_c^T \kappa_1
\]
to arrive at the condition
\[ G_c := \begin{bmatrix} G_c(x_1) \\ G_c(x_2) \end{bmatrix}, \]
where \( G_c \) depends via \( x_{1,2} \) on \( \epsilon \) and \( \kappa_1 \) (cf. (3.35c)). At \( \epsilon = 0 \) we have \( G_c^* \kappa_0 = 0 \). This condition can be written as
\[ 0 = G_c \left( \kappa_0 + \epsilon G_c^T \kappa_1 \right) \]
\[ = \left( G_c^* + \epsilon G_c^* \right) \left( \kappa_0 + \epsilon G_c^T \kappa_1 \right) \]
\[ = G_c^* \kappa_0 + \epsilon \left[ G_c^* G_c^T \kappa_1 + \Gamma (\kappa_0) \kappa_0 \right] + O(\epsilon^2). \]

By assumption (iv) \( G_c^* \) is of maximal row rank. Hence \( G_c^* G_c^T \) is invertible. By the implicit function theorem one obtains a unique local solution:
\[ \kappa_1 = \kappa_1(\epsilon) = - \left( G_c^* G_c^T \right)^{-1} \Gamma (\kappa_0) \kappa_0 + O(\epsilon). \]

Thus, for fixed \( k^*_E, \eta^*, \hat{\eta}^* \) and sufficiently small \( \epsilon > 0 \) there exists a parameter vector
\[ k(\epsilon) = \hat{k}_E + \epsilon \hat{k}_c(\epsilon) \]
with \( \hat{k}_c(\epsilon) \) being derived from \( k_c(\epsilon) = \kappa_0 + \epsilon G_c^T \kappa_1(\epsilon) \) by a suitable filling with zeros and there exist state vectors \( x_{1,2}(\epsilon) = \Xi_{1,2}(\epsilon)k_c(\epsilon) \) such that
\[ x_{1,2}(\epsilon) = T \begin{pmatrix} \xi_{1,2}(\epsilon) \\ \eta^* \\ \hat{\eta}^* \end{pmatrix} \]
are positive steady states of the overall network (3.6) with \( W_E^T x_{1,2}(\epsilon) = W_E^T x_{2,2}(\epsilon). \)

3.5. Alternative Formulation
In the following Lemma 3.8 we present a characterization for the regularity conditions (iii) of the Theorems 3.2, 3.6 and 3.7. We consider the \((n \times n)\)-matrix \( A \) given by
\[ A := \begin{bmatrix} (\mathcal{Y}_E \Delta)^T \\ W_E^T \text{diag}(x) \end{bmatrix} \quad \text{with} \quad \Delta^T := (-\tilde{e}, I_{\rho-1}), \]
evaluated at a positive steady state \( x^* \) where \( \tilde{e} \) is \((1, \ldots, 1)^T \in \mathbb{R}^{\rho-1}\) and where \( \mathcal{Y}_E \in \mathbb{R}^{n \times \rho} \) is the matrix
\[ \mathcal{Y}_E = \begin{bmatrix} \tilde{y}_1, \ldots, \tilde{y}_\rho \end{bmatrix} \]
containing the exponent vectors of \( \phi_E(x) \).

Lemma 3.8 Let \( x^* \) be a positive steady state of the subnetwork \( \dot{x} = N_E v_E(k^*_E, x) \) from (3.7) with \( k_c = 0 \) and let \( \mathcal{Y}_E \) be of full column-rank. Then
\[ A = D_x S_E^T N_E v_E(k^*_E, x^*) \] is regular if and only if \( A \) is regular.
Proof:

Recall that \( x = x(\xi, \eta, \tilde{\eta}) = T \begin{pmatrix} \xi \\ \eta \\ \tilde{\eta} \end{pmatrix} \). The Jacobian \( A \) is given by

\[
A = S_E^T N_E \, \text{diag} \left( v_E(k_E, x^*) \right) Y_E^T \, \text{diag} \left( \frac{1}{x^*} \right) S_E.
\]

Note that, as \( E \) is a stoichiometric generator of \( \ker (Y I) \cap \mathbb{R}_{\geq 0}^\rho \), one has necessarily \( v_E(k_E, x^*) \in [\pi_E]_+ \). Since \( S_E \) spans \( \ker (N_E) \), \( A \) is singular iff there exists \( z \neq 0 \) with

\[
\text{diag} \left( \pi_E \right) Y_E^T \, \text{diag} \left( \frac{1}{x^*} \right) S_E \, z \in [\pi_E] \ (= \ker (N_E)).
\]

i.e. iff there exists a \( z \neq 0 \) with

\[
Y_E^T \, \text{diag} \left( \frac{1}{x^*} \right) S_E \, z \in [e], \ e = \begin{pmatrix} 1 \\ \vdots \\ 1 \end{pmatrix} \in \mathbb{R}^\rho
\]

and thus equivalently with

\[
Y_E^T \, \mu = e, \ \text{diag} \left( x^* \right) \, \mu = S_E \, z.
\]

With \( \tilde{e} = (1, \ldots, 1)^T \in \mathbb{R}^{\rho-1} \) the left equation can be written as

\[
\begin{bmatrix}
\tilde{y}_2^T \\
\vdots \\
\tilde{y}_\rho^T
\end{bmatrix} \, \mu = \tilde{e} \tilde{y}_1^T \, \mu
\]

and thus as \( \Delta^T \, Y_E^T \, \mu = 0 \) for \( \Delta^T = (-\tilde{e}, I_{\rho-1}) \). Vice versa, by the full row-rank of \( Y_E^T \), a nontrivial solution \( \mu \) of this last equation is also a nontrivial solution of \( Y_E^T \, \mu = e \). As \( [W_E] \) is the orthogonal complement of \( [S_E] \), the condition \( \text{diag} \left( x^* \right) \, \mu = S_E \, z \) is equivalent to \( W_E^T \, \text{diag} \left( x^* \right) \, \mu = 0 \). Thus, \( A \) is singular if and only if there exists a nonzero vector \( \mu \) with

\[
A \, \mu = \begin{bmatrix}
(Y_E \, \Delta)^T \\
W_E^T \, \text{diag} \left( x^* \right)
\end{bmatrix} \, \mu = 0.
\]  \hspace{1cm} (3.37)

Equivalently, \( A \) is regular if and only if the \((n \times n)\)-matrix \( A \) is regular. \( \square \)

**Remark 3.9** Lemma 3.8 offers a reinterpretation of some conditions in Proposition 4.8 of [19]. Note that \( S_E^T \, \mu = S_E^T \, \text{diag} \left( \frac{1}{x^*} \right) \, S_E \, z \) vanishes if and only if \( z = 0 \). In particular, if \( \mu \) is not orthogonal to \( \text{im} (N_E) \) then \( z = S_E^T \, \text{diag} \left( x^* \right) \, \mu \) is a nontrivial right nullvector of \( A \) for \( \mu \) satisfying (3.37). So the last assumption in Proposition 4.8 of [19] on the existence of a vector \( \mu \) just asks for a nonzero \( \mu \) satisfying (3.37) and hence for the singularity of the Jacobian matrix \( A \) of the subnet. Thereby a necessary condition for a saddle–node bifurcation will be fulfilled.
3.6. Comparison of Results
For both, Theorem 3.6 and Theorem 3.7, one has to determine a positive element \( \kappa_0 \) in \( \ker (G^*_c) \). Both, Theorem 3.6 and Theorem 3.7 guarantee that for sufficiently small \( \epsilon > 0 \) there exist a parameter vector \( k = k(\epsilon) > 0 \) and two positive state vectors \( x_{1,2} = x_{1,2}(\epsilon) \) with

\[
N v(k(\epsilon), x_{1,2}(\epsilon)) = 0.
\]

To apply Theorem 3.6 one starts with the orthonormal transformation \( T = (S_E, W_{add}, W) \). One has to check the regularity of \( A^*_1,2 \), to solve \( A^*_1,2 X_{1,2}^* + B_{1,2}^* = 0 \) for matrices \( X_{1,2}^* \) and to test the regularity of \( D_{1,2}^* \). In case \( A^*_1,2 \) and \( D_{1,2}^* \) are regular it is guaranteed that, for sufficiently small \( \epsilon > 0 \), the overall network admits two positive steady states \( x_{1,2}(\epsilon) \) with

\[
W^T x_1(\epsilon) = W^T x_2(\epsilon)
\]

for the explicitly given vector of reaction rates

\[
k = \hat{k}_E + \epsilon \hat{k}_c^*.
\]

To apply Theorem 3.7 all one has to do is check the regularity of \( A^*_1,2 \) and the maximal row rank of

\[
G^*_c = \left[ \begin{array}{c} W_{add}^T N_c \text{ diag} (\phi_c(x^*_1)) \\ W_{add}^T N_c \text{ diag} (\phi_c(x^*_2)) \end{array} \right]
\]

where \( W_{add}^T \) may be any complementary basis extension (to \( W^T \)) for the left kernel of \( N_E \). In case \( A^*_1,2 \) and \( G^*_c \) are of maximal row rank it is guaranteed that, for \( \epsilon > 0 \) sufficiently small, there exist a vector of reaction rates \( k^*(\epsilon) \) and positive steady states \( x_{1,2}(\epsilon) \) with

\[
W_{add}^T x_1(\epsilon) = W_{add}^T x_2(\epsilon).
\]

The parameter vector

\[
k(\epsilon) = \hat{k}_E + \epsilon \hat{k}_c + O(\epsilon^2)
\]

can in general not be computed explicitly, its existence however is guaranteed by the implicit function theorem.

4. Example: G1/S Transition in Budding Yeast
In this section 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. 4.1. In a very simple form, the biochemical network consists of two regulators that 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 \( Clb2p - CDK \) 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 \( Sic1p \)[22].

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[32]. It depends on the system’s history, whether, for instance, low or
high \textit{Sic1p} concentration will be established. For the G1/S system, a transient activation of the phosphatase \textit{Cdc14p} should move the system from the mitotic branch of low \textit{Sic1p} concentration to the upper branch representing a G1 state with high \textit{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).

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{network.png}
\caption{Adapted from [6]; 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 \textit{Clb2–CDK} complexes have been omitted. Components in gray denote degradation products. An unspecified external signal controls the activity of \textit{Cdc14p} phosphatase. \textit{Clb2–CDK} phosphorylates the inhibitor \textit{Sic1p} either in its unbound form (left, $k_{19-21}$), or when it is bound to a second molecule of the kinase complex (right, $k_{10-12}$).}
\end{figure}

Here, two similar network structures are considered. In one alternative, \textit{Clb2–CDK} phosphorylates free \textit{Sic1p} (binary complex model, see network $N_2$), whereas in the other alternative, the already bound inhibitor is a substrate for a second kinase molecule (ternary complex model, see network $N_1$): that is, in network $N_2$ phosphorylated \textit{Sic1} is produced by the reaction $\text{Clb} + \text{Sic1} \rightleftharpoons \text{Sic1} \cdot \text{Clb} \rightarrow \text{Sic1p} + \text{Clb}$, while in $N_1$ phosphorylated \textit{Sic1} is produced by the reactions $\text{Clb} \cdot \text{Sic1} + \text{Clb} \rightleftharpoons \text{Clb} \cdot \text{Sic1} \cdot \text{Clb} \rightarrow \text{Clb} \cdot \text{Sic1p} + \text{Clb}$ and $\text{Clb} + \text{Sic1p} \rightleftharpoons \text{Clb} \cdot \text{Sic1p}$. (Note that even though $N_2$ contains the reaction $\text{Clb} + \text{Sic1p} \rightleftharpoons \text{Clb} \cdot \text{Sic1p}$ as well, the source of \textit{Sic1p}-production is the reaction $\text{Sic1} \cdot \text{Clb} \rightarrow \text{Clb} + \text{Sic1p}$.) 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 $y_1 = 0 \in \mathbb{R}^9$. It incorporates that each system is open with respect to \textit{Sic1} and its phosphorylated form \textit{Sic1p}: \textit{Sic1} can enter and leave the system, \textit{Sic1p} can leave the system (see Fig. 4.1).
The corresponding ODEs can be found in the Appendix (cf. equations (5.3a) and (5.5a)). 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.

The generators of $\text{ker}(Y I) \cap R_{\geq 0}^{18}$ for network $N_1$ and $N_2$ are given by $E_{N_1}$ and $E_{N_2}$ in (5.4) and (5.6), respectively. Visual inspection shows that $E_{N_1}$ contains five stoichiometric generators (columns 7–11), while $E_{N_2}$ contains six stoichiometric generators (columns 7–12). Figure 4.2 contains the subnetworks defined by the stoichiometric generators of network $N_1$, while Figure 4.4 depicts those of network $N_2$. Visual inspection of the subnetworks confirms that each linkage class contains exactly one terminal strong linkage class. Thus, by Theorem 2.1,
the *Deficiency One Algorithm* is applicable.

\[ E_0 = (0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 1, 0, 0, 0)^T \]

\[ E_7 = (0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 1, 0, 0, 0)^T \]

\[ E_8 = (0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0)^T \]

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

**Figure 4.2.** Subnetworks defined by generators *E*_7 – *E*_11 for the ternary complex model (c.f. (5.4)). As described in the main text and in [6], the shaded networks can exhibit *S*_sub- and thus, trivially, *S*-multistationarity.

Note that it is impossible for the current implementations of CRNT to know that a given network is a subnetwork of an overall network. Thus, in the context of this paper, when a subnetwork is analysed using the toolbox [16] one can either establish *S*_sub- and thus *S*-multistationarity or exclude *S*_sub-, not *S*-multistationarity. As discussed in [6], *S*_sub-
multistationarity can be established for the subnetworks defined by the generators $E_9$, $E_{10}$ and $E_{11}$ of network $N_1$, while one fails to establish $S_{sub}$-multistationarity for any of the subnetworks of network $N_2$. But, using the ideas described in [5, 4], one can establish $S$-multistationarity for the subnetworks defined by generators $E_9$ and $E_{12}$.

In the remainder of this section we demonstrate multistationarity numerically, by analyzing the bifurcation behavior of both networks. To this end, we first apply Algorithm 3.1 to the generator

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

of network $N_1$, where variation of the total concentration of Cdc14 will lead to qualitatively different steady-state solutions.

Using the CRNT toolbox, we establish $S_{sub}$-multistationarity for the $E_{10}$-subnetwork and obtain a vector of rate constants

$$k_E^* = (1.4857376, 0.63212055, 0.37143441, 0.63212055, 0.74286883, 0.63212055)\T \quad (4.1)$$

and a pair of steady states $x_1^*$, $x_2^*$ for the subnetwork (with $x_2^* \in x_1^* + S_{sub}$). Matrices $A^*_i$ and $D^*_i$ of Algorithm 3.1 are regular, a positive $k_E^*$ exists. Thus, (4.1) can be used to obtain $k^*$ ($\epsilon$) for the overall network. Numerical analysis shows that for $\epsilon \in [0, 12 \cdot 10^{-3}$] the overall network exhibits multistationarity (Fig. 4.3(b)). Aside, for $\epsilon \in (0, 3 \cdot 10^{-3})$ we find a Hopf-bifurcation, thus indicating oscillations for the overall network.

![Bifurcation in the subnetwork defined by $E_{10}$](image1)

(a) Bifurcation in the subnetwork defined by $E_{10}$ of network $N_1$ for various values of $\epsilon$

![Bifurcation in the overall network $N_1$](image2)

(b) Bifurcation in the overall network $N_1$ for several values of $\epsilon$

**Figure 4.3.** (a): Bifurcation for for the subnetwork defined by $E_{10}$: $x_1$ (concentration of Sic1) over $c_2$ (total concentration of Cdc14). LP is an abbreviation for limit point. (b): Extension of solutions to the overall network. Bifurcation diagram for the ternary complex model for several vectors of rate constants calculated as described in Algorithm 3.1 using (4.1) and $\epsilon = 4 \cdot 10^{-3}$ (dashed line), $8 \cdot 10^{-3}$ (dotted line), $12 \cdot 10^{-3}$ (o) and $14 \cdot 10^{-3}$ (□). Turning points are limit points (LP) and separate stable and unstable branches. Note hat for $\epsilon = 14 \cdot 10^{-3}$ there is no turning point and no change of stability.

On the other hand, using the ideas described in [4] and [5], one can establish $S$-multistationarity for the network $N_2$. To demonstrate this, the subnetwork defined by the generator

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

was analysed, and a vector of rate constants

$$k_{E}^* = (0.24922, 0.32723, 17.86, 0.32723)\T \quad (4.2)$$
desired switch–like behavior, the ‘integrity’ of the binary complex model needs to be preserved.

Remark 4.1

and a pair of steady states $x_1^*$, $x_2^*$ for the $E_9$-subnetwork were obtained (with $x_2^* \in x_1^* + \mathcal{S}$), such that $E_9$-subnetwork of $\mathcal{N}_2$ shows $\mathcal{S}$-multistationarity. Again, matrices $A_1^*$ and $D_1^*$ of Algorithm 3.1 are regular and a positive $k^*$ exists. Thus, (4.2) can be used to obtain $k^* (\epsilon)$ for $\mathcal{S}$-multistationarity of the overall network. This is demonstrated in Fig. 4.5, for $\epsilon \in (5 \cdot 10^{-6}, 2 \cdot 10^{-5})$. Note that the ‘multistationarity range’ is smaller than the one obtained for network $\mathcal{N}_1$.

Remark 4.1

(i) 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. In the sense that a given subnetwork of the binary model, defined by a stoichiometric generator, does not show $\mathcal{S}_{\text{sub}}$-multistationarity, i.e. does not have different
Figure 4.5. Extension of solutions to the overall network. Bifurcation diagram for the binary complex model for several vectors of rate constants calculated as described in Algorithm 3.1 using (4.2) and $\epsilon = 5 \cdot 10^{-6}$ (solid line), $10^{-5}$ (dash-dotted line), $1.5 \cdot 10^{-5}$ (dotted line) and $2 \cdot 10^{-5}$ (dashed line). Turning points are limit points (LP) and separate stable and unstable branches. Note that for $\epsilon = 2 \cdot 10^{-5}$ there is no turning point and no change of stability.

positive steady states $x_1^*, x_2^*$ with $x_2^* \in x_1^* + S_{sub}$. This has important consequences for the robustness of the two models. Robustness, in general, is defined as the resistance of qualitative network behavior to perturbations, for instance, in network structure or parameter values. 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 [23]. In this regard, subnetwork analysis could be used for model discrimination—the ternary complex mechanism would be considered more plausible.

(ii) For the analysis of metabolic networks, elementary flux modes are often employed to identify essential reactions or subsystems, for example, for cell growth [27]. Reactions are essential when they are used in all (relevant) flux modes because any steady-state flux solution in the network can be constructed from linear combination of the subnetworks. We can follow a similar line of argument for the analysis of dynamic features such as multistationarity. In this case, reaction participation in all elementary subnetworks that, on their own, have the potential to perform a certain function may identify critical reactions. For the ternary complex model, all subnetworks with potential multistationarity contain the formation of the trimeric complex between Sic1 and cyclin-dependent kinase, and subsequent phosphorylation of Sic1 (cf. fourth row of the shaded subnetworks in Fig. 4.2 corresponding to $E_9$, $E_{10}$ and $E_{11}$). This indicates that for a robust switch-like function of the system, these reactions are important. However, they might not be essential, as analysis of the binary complex model revealed. Comparison of these three subnetworks also reveals that action of the phosphatase Cdc14 is not necessary for multistability, which can arise from cyclin–inhibitor interactions alone (cf. fifth row of the subnetworks in Fig. 4.2 corresponding to $E_{10}$, $E_{11}$). Yet, to accommodate the experimental observation that transient activation of Cdc14 is critical in vivo, one needs to consider the third subnetwork (Fig. 4.2 for $E_9$) as well.
5. Appendix
5.1. Model Involving Ternary Complex

The reaction network in the standard form defined by [12, 13] is given by network $N_1$, where the concentration variables and complex vectors are given in Table 5.1, Table 5.2 and Table 5.3. Complexes between two proteins $[X]$ and $[Y]$ are denoted by $[X \cdot Y]$. Using mass action kinetics, the following ODEs can be derived from network $N_1$:

\begin{align*}
\dot{x}_1 &= k_1 - k_2 x_1 - k_4 x_1 x_3 + k_5 x_4 + k_15 x_7 \\
\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 \\
\dot{x}_3 &= -k_4 x_1 x_3 - k_7 x_2 x_3 + k_5 x_4 + k_6 x_4 - k_10 x_3 x_4 + \\
&\quad k_8 x_5 + k_9 x_5 + k_11 x_9 + k_12 x_9 \\
\dot{x}_4 &= k_4 x_1 x_3 - k_5 x_4 - k_6 x_4 - k_10 x_3 x_4 + k_18 x_8 + k_11 x_9 \\
\dot{x}_5 &= k_7 x_2 x_3 - k_8 x_5 - k_9 x_5 - k_16 x_5 x_6 + k_17 x_8 + k_12 x_9 \\
\dot{x}_6 &= -k_13 x_2 x_6 - k_16 x_5 x_6 + k_14 x_7 + k_15 x_7 + k_17 x_8 + k_18 x_8 \\
\dot{x}_7 &= k_13 x_2 x_6 - k_14 x_7 - k_15 x_7 \\
\dot{x}_8 &= k_16 x_5 x_6 - k_17 x_8 - k_18 x_8 \\
\dot{x}_9 &= k_10 x_3 x_4 - k_11 x_9 - k_12 x_9
\end{align*}

The conservation relations are given by

\begin{align*}
x_6 + x_7 + x_8 &= c_1 \\
x_3 + x_4 + x_5 + x_6 + x_7 + 2 x_8 + 2 x_9 &= c_2.
\end{align*}

The elements $Y, \mathcal{Y}, \mathcal{I}$ and $v(k, x)$ of the representation $\dot{x} = Y I v(k, x)$ are given on the following pages. The generators of $\ker(Y I) \cap \mathbb{R}_{\geq 0}^{18}$ for network $N_1$ are given by the column vectors of the matrix

\begin{equation}
E_{N_1} = \begin{bmatrix}
1 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\
1 & 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 & 1 & 1 & 1 & 0 \\
0 & 1 & 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 & 1 & 0 & 0 & 1 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 1 & 1 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 1 & 1 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 & 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 & 0 & 0 & 0 & 1
\end{bmatrix}
\end{equation}
| State variable | Substance |
|---------------|-----------|
| $x_1$         | Sic1      |
| $x_2$         | Sic1p     |
| $x_3$         | Clb       |
| $x_4$         | Clb · Sic1 |
| $x_5$         | Clb · Sic1p |
| $x_6$         | Cdc14     |
| $x_7$         | Sic1p · Cdc14 |
| $x_8$         | Clb · Sic1p · Cdc14 |
| $x_9$         | Clb · Sic1 · Clb |

**Table 5.1.** The state variables of the system ($N_1$)

| Complex          | Name | Realization |
|------------------|------|-------------|
| 0                | $y_1$| 0           |
| Sic1             | $y_2$| $e_1$       |
| Sic1p            | $y_3$| $e_2$       |
| Clb + Sic1       | $y_4$| $e_1 + e_3$ |
| Clb · Sic1       | $y_5$| $e_4$       |
| Clb              | $y_6$| $e_3$       |
| Clb + Sic1p      | $y_7$| $e_2 + e_3$ |
| Clb · Sic1p      | $y_8$| $e_5$       |
| Clb · Sic1 + Clb | $y_9$| $e_3 + e_4$ |
| Clb · Sic1 · Clb | $y_{10}$| $e_5$ |
| Clb · Sic1p + Clb| $y_{11}$| $e_3 + e_5$ |
| Sic1p + Cdc14    | $y_{12}$| $e_2 + e_6$ |
| Sic1p · Cdc14    | $y_{13}$| $e_7$ |
| Sic1 + Cdc14     | $y_{14}$| $e_1 + e_6$ |
| Clb · Sic1p + Cdc14| $y_{15}$| $e_5 + e_6$ |
| Clb · Sic1p · Cdc14| $y_{16}$| $e_8$ |
| Clb · Sic1 + Cdc14| $y_{17}$| $e_4 + e_6$ |

**Table 5.2.** The complexes of the system ($N_1$)

**Table 5.3.** ODE-data for ($N_1$)
\[
Y = \begin{bmatrix}
0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\end{bmatrix}
\]

\[v(k, x)^T = (k_1, k_2 x_1, k_3 x_2, k_4 x_1 x_3, k_5 x_4, k_6 x_4, k_7 x_2 x_3, k_8 x_5, k_9 x_5, k_{10} x_3 x_4, k_{11} x_9, k_{12} x_9, k_{13} x_2 x_6, k_{14} x_7, k_{15} x_7, k_{16} x_5 x_6, k_{17} x_8, k_{18} x_8)^T\]

Table 5.3: ODE-data for \(\mathcal{N}_1\)
5.2. Model Involving Binary Complex

The reaction network in the standard form defined by [12, 13] is given by network $N_2$, where the concentration variables and complex vectors are given in Table 5.4, Table 5.5 and Table 5.6. Using mass action kinetics, the following ODEs can be derived from network $N_2$:

$$\dot{x}_1 = k_1 - k_2 x_1 - k_5 x_1 x_3 - k_6 x_1 x_3 + k_7 x_4 + k_{15} x_7 + k_4 x_9$$  \hspace{1cm} (5.5a)

$$\dot{x}_2 = -k_3 x_2 - k_{10} x_2 x_3 + k_{11} x_5 - k_{13} x_2 x_6 + k_{14} x_7 + k_9 x_9$$  \hspace{1cm} (5.5b)

$$\dot{x}_3 = -k_5 x_1 x_3 - k_6 x_1 x_3 - k_{10} x_2 x_3 + k_7 x_4 + k_8 x_4 + k_{11} x_5 + k_{12} x_5 + k_4 x_9 + k_9 x_9$$  \hspace{1cm} (5.5c)

$$\dot{x}_4 = k_6 x_1 x_3 - k_7 x_4 - k_8 x_4 + k_{18} x_8$$  \hspace{1cm} (5.5d)

$$\dot{x}_5 = k_{10} x_2 x_3 - k_{11} x_5 - k_{12} x_5 - k_{16} x_5 x_6 + k_{17} x_8$$  \hspace{1cm} (5.5e)

$$\dot{x}_6 = -k_{13} x_2 x_6 - k_{16} x_5 x_6 + k_{14} x_7 + k_{15} x_7 + k_{17} x_8 + k_{18} x_8$$  \hspace{1cm} (5.5f)

$$\dot{x}_7 = k_{13} x_2 x_6 - (k_{14} + k_{15}) x_7$$  \hspace{1cm} (5.5g)

$$\dot{x}_8 = k_{16} x_5 x_6 - (k_{17} + k_{18}) x_8$$  \hspace{1cm} (5.5h)

$$\dot{x}_9 = k_5 x_1 x_3 - (k_4 + k_9) x_9$$  \hspace{1cm} (5.5i)

The conservation relations are given by

$$x_3 + x_4 + x_5 + x_8 + x_9 = c_1$$  \hspace{1cm} (5.5k)

$$x_6 + x_7 + x_8 = c_2$$  \hspace{1cm} (5.5l)

The elements $Y$, $Y^*$, $I$ and $v(k, x)$ of the representation $\dot{x} = Y I v(k, x)$ are given on the following pages. The generators of $\ker(Y I) \cap \mathbb{R}^{18}_{\geq 0}$ for network $N_2$ are given by the column vectors of the matrix

$$E_{N_2} = \begin{bmatrix}
1 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 1 & 1 & 0 \\
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 \\
0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 & 1 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1
\end{bmatrix}$$  \hspace{1cm} (5.6)
Table 5.4. The state variables of the system ($N_2$)

| State variable | Substance        |
|----------------|------------------|
| $x_1$          | Sic1             |
| $x_2$          | Sic1p            |
| $x_3$          | Clb              |
| $x_4$          | Clb · Sic1       |
| $x_5$          | Clb · Sic1p      |
| $x_6$          | Cdc14            |
| $x_7$          | Sic1p · Cdc14    |
| $x_8$          | Clb · Sic1p · Cdc14 |
| $x_9$          | Sic1 · Clb       |

Table 5.5. The complexes of the system ($N_2$)

| Complex | Name          | Realization |
|---------|---------------|-------------|
| $y_1$   | 0             | 0           |
| $y_2$   | Sic1          | $e_1$       |
| $y_3$   | Sic1p         | $e_2$       |
| $y_4$   | Sic1 · Clb    | $e_9$       |
| $y_5$   | Clb + Sic1    | $e_1 + e_3$ |
| $y_6$   | Clb · Sic1    | $e_4$       |
| $y_7$   | Clb           | $e_3$       |
| $y_8$   | Clb + Sic1p   | $e_2 + e_3$ |
| $y_9$   | Clb · Sic1p   | $e_5$       |
| $y_{10}$| Sic1p + Cdc14 | $e_2 + e_6$ |
| $y_{11}$| Sic1p · Cdc14 | $e_7$       |
| $y_{12}$| Sic1 + Cdc14  | $e_1 + e_6$ |
| $y_{13}$| Clb · Sic1p + Cdc14 | $e_6 + e_7$ |
| $y_{14}$| Clb · Sic1p · Cdc14 | $e_8$       |
| $y_{15}$| Clb · Sic1 + Cdc14 | $e_4 + e_6$ |

Table 5.6. ODE-data for ($N_2$)
\[ Y = \begin{bmatrix}
0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & 0 & 1 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\end{bmatrix} \]

\[ \mathcal{Y} = \begin{bmatrix}
y_1, y_2, y_3, y_4, y_5, y_6, y_7, y_8, y_9, y_{10}, y_{11}, y_{12}, y_{13}, y_{14}, y_{15} \end{bmatrix} \]

\[ \mathcal{I} = \begin{bmatrix}
-1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\end{bmatrix} \]

\[ v(k, x)' = (k_1, k_2 x_1, k_3 x_2, k_4 x_9, k_5 x_1 x_3, k_6 x_1 x_3, k_7 x_4, k_8 x_4, k_9 x_9, k_{10} x_2 x_3, k_{11} x_5, k_{12} x_5, k_{13} x_2 x_6, k_{14} x_7, k_{15} x_7, k_{16} x_5 x_6, k_{17} x_8, k_{18} x_8) \]

Table 5.6 ODE-data for \((N_2)\)
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