Multistationarity in the Space of Total Concentrations for Systems that Admit a Monomial Parametrization

Carsten Conradi1 · Alexandru Iosif2 · Thomas Kahle3

Received: 1 November 2018 / Accepted: 2 July 2019 / Published online: 22 July 2019
© Society for Mathematical Biology 2019

Abstract
We apply tools from real algebraic geometry to the problem of multistationarity of chemical reaction networks. A particular focus is on the case of reaction networks whose steady states admit a monomial parametrization. For such systems, we show that in the space of total concentrations multistationarity is scale invariant: If there is multistationarity for some value of the total concentrations, then there is multistationarity on the entire ray containing this value (possibly for different rate constants)—and vice versa. Moreover, for these networks it is possible to decide about multistationarity independent of the rate constants by formulating semi-algebraic conditions that involve only concentration variables. These conditions can easily be extended to include total concentrations. Hence, quantifier elimination may give new insights into multistationarity regions in the space of total concentrations. To demonstrate this, we show that for the distributive phosphorylation of a protein at two binding sites multistationarity is only possible if the total concentration of the substrate is larger than either the total concentration of the kinase or the total concentration of the phosphatase. This result is enabled by the chamber decomposition of the space of total concentrations from polyhedral geometry. Together with the corresponding sufficiency result of Bihan et al., this yields a characterization of multistationarity up to lower-dimensional regions.

Keywords Polynomial systems in biology · Chemical reaction networks · Steady states · Multistationarity

Carsten Conradi
carsten.conradi@htw-berlin.de
Alexandru Iosif
iosif@aices.rwth-aachen.de
Thomas Kahle
thomas.kahle@ovgu.de

1 Hochschule für Technik und Wirtschaft, Berlin, Germany
2 Joint Research Center for Computational Biomedicine, Aachen, Germany
3 Otto-von-Guericke Universität, Magdeburg, Germany
1 Introduction

The dynamics of many biochemical processes can be described by systems of ordinary differential equations (ODEs). Already the steady states of such ODEs contain important information, for example, about the long-term behavior of a process. In particular, in modeling signal transduction and cell cycle control, one is often interested in the existence of multiple steady states, multistationarity for short (Conradi and Flockerzi 2012; Conradi et al. 2017).

As measurement data are often noisy and realistic models tend to be large, parameter values often come with large uncertainties or are not known at all. Hence, given an ODE system, one asks whether or not there exist parameter values such that the system admits multistationarity. This is a mathematically challenging problem, even in the simplest case when all kinetics are of mass-action form and the ODEs have polynomial right-hand sides. In this case, one has to identify those parameter values for which a parametrized family of polynomials admits at least two positive real solutions.

A variety of necessary conditions for multistationarity in mass-action networks are known in the literature, for example graph-based conditions (e.g., Schlosser and Feinberg 1994; Banaji and Craciun 2009, 2010 and the references therein), conditions based on the determinant of the Jacobian (e.g., Wiuf and Feliu 2013; Feliu and Wiuf 2012 and the references therein) or conditions based on network concordance (e.g., Shinar and Feinberg 2012, 2013).

Conditions that are both necessary and sufficient can usually only be found if the network satisfies additional conditions, for example, encoded in the network deficiency (Feinberg 1995a, b; Ellison 1998; Ellison and Feinberg 2000; Ellison et al. 2000), in the stoichiometric matrix (Conradi and Flockerzi 2012), the steady-state ideal (Pérez-Millán et al. 2012) and Müller et al. (2016, Section 3.1) or the Brouwer degree of the polynomial map defining the steady-state ideal (Conradi et al. 2017; Dickenstein et al. 2019). The last two references are similar in spirit to the results presented here: While we require a monomial parametrization of the positive steady states, their results require a rational parametrization. Moreover, our results are derived independent of the Brouwer degree.

Some of the aforementioned results allow determining rate constants where multistationarity occurs. It is, however, currently not possible to directly infer total concentrations (a different, but equally important set of parameters) based on these results. The notable exception is Bihan et al. (2018), where results similar to the ones obtained in Sect. 4 are presented: If the total concentrations satisfy a linear inequality, then there exist rate constants such that multistationarity is possible. The linear inequalities, however, are not arbitrarily imposed by the authors of Bihan et al. (2018) but arise from the system itself. It is therefore currently not possible to decide whether or not multistationarity is possible for arbitrary polynomial inequalities in the total concentrations.
As the total concentrations are experimentally more accessible than the rate constants, conditions directly incorporating total concentrations are desirable. Here, we initiate the study of such conditions with a focus on systems whose positive steady states admit a monomial parametrization (Definition 3.1). These systems are closely related to systems with toric steady states described in Pérez-Millán et al. (2012), that is, to systems whose steady-state ideal is binomial (i.e., the ideal is generated by polynomials with at most two terms). One way to establish this property is to find a Gröbner basis that is binomial. The results in Sadeghimanesh and Feliu (2019a) allow for the efficient computation of such Gröbner bases for the enzymatic systems frequently used in modeling intracellular signaling and control. Multistationarity conditions are described in Sadeghimanesh and Feliu (2019b). The systems discussed in Sadeghimanesh and Feliu (2019a, b) belong to the larger class of MESSI systems (Pérez-Millán and Dickenstein 2018). However, while the former systems always admit a monomial parametrization this need not be the case for the latter.

For systems that admit a monomial parametrization, we show that in the space of total concentrations multistationarity is scale invariant: From Theorems 3.18 and 3.19, it follows that if there is multistationarity for some vector \( c \) of the total concentrations \( \{c, k\} \), then for any \( \alpha > 0 \) there is multistationarity for \( \alpha c \), albeit for a different \( k \). And vice versa: If for some \( c \) there is no \( k \) such that multistationarity is possible, then there is no \( k \) such that multistationarity is possible for \( \alpha c \), \( \alpha > 0 \).

In Theorem 3.15 and Corollary 3.16, we formulate semi-algebraic conditions for multistationarity that use only variables representing concentrations. Such conditions can be extended to incorporate constraints on the total concentrations. Hence, for such systems it is possible to decide about multistationarity without knowing the rate constants.

There are many biologically meaningful networks that admit a monomial parametrization, see, for example, the networks discussed in Conradi and Shiu (2018). We apply our results to one of those, the well-known sequential distributive phosphorylation of a protein at two binding sites (Conradi et al. 2008) (see Holstein et al. 2013 for proteins with an arbitrary number of phosphorylation sites). These networks are arguably among the best studied systems when it comes to multistationarity: In Markevich et al. (2004), multistationarity has been shown numerically, in Conradi et al. (2005) via sign patterns. This analysis also allows to study the effect of parameter variations on multistationarity (Conradi et al. 2008). In Conradi and Mincheva (2014), conditions on a subset of the rate constants called catalytic constants have been derived: If the catalytic constants satisfy this condition, then multistationarity is possible for some values of the total concentrations. Here, we describe a similar result for the total concentrations: Applying Corollary 3.16, we show in Theorem 4.12 and Corollary 4.13 that multistationarity is possible only if the total concentration of the substrate is at least as large as either the total concentration of the kinase or the total concentration of the phosphatase. A result of Bihan et al. (2018) shows the converse up to lower-dimensional regions: Multistationarity occurs if the substrate concentration is strictly larger than the total concentration of kinase or the total concentration of phosphatase. Corollary 4.13 summarizes the situation. The description is complete up to lower-dimensional boundary cases. In particular, multistationarity occurs in the
Michaelis–Menten regime, where the total concentration of the substrate exceeds those of both enzymes by orders of magnitude.

To arrive at this condition, we make use of the chamber decomposition of the cone of total concentrations: In Theorem 4.3 we show that, independent of the number of phosphorylation sites, this cone consists of five full-dimensional sub-cones called chambers. These chambers are determined by subsets of linearly independent columns of a matrix defining the conservation relations. In Theorem 4.7, we show that for two sites, multistationarity is only possible in four of these chambers.

The paper is organized as follows: We close this section with an introduction of some basic mathematical notation used throughout this paper. In Sect. 2, we introduce the ODEs that arise from chemical reaction networks with mass-action kinetics and formally define multistationarity. We also comment on the relationship between steady states and rate constants. In Sect. 3, we formally define systems that admit a monomial parametrization and discuss the consequences concerning steady states and multistationarity. In Sect. 4, we apply the results of Sect. 3 to a reaction network describing the distributive phosphorylation of a protein at two binding sites. And in Sect. 5, we comment on conditions for the existence of monomial parametrizations. The paper closes with a brief discussion of the main findings in Sect. 6.

1.1 Notation

For any $m \times n$ matrix $A$, we write $\text{im}(A) = \{Ax | x \in \mathbb{R}^n\}$ for the right image and $\text{rowspace}(A) = \{yA | y \in \mathbb{R}^m\}$ for the rowspace (left image). If $A$ and $B$ are two matrices of the same dimensions, then $A \star B$ denotes their Hadamard product, that is, $(A \star B)_{ij} = A_{ij}B_{ij}$. If $x$ is a vector of length $m$ and $A$ is an $m \times n$ matrix, then we write $x^A$ for the $n$-vector with entries

$$(x^A)_j = \prod_{i=1}^{m} x_i^{A_{ij}}, \quad j = 1, \ldots, n.$$ 

Slightly deviating from the matrix-vector product notation, this operation is possible independent of whether $x$ is a row or column vector and should always return the same type of vector. We also apply scalar functions to vectors which always means coordinate-wise application. Using this, for example, one can check that

$$\ln x^A = (\ln x)A \quad \text{if } x \text{ is a row vector},$$

and

$$\ln x^A = A^T(\ln x) \quad \text{if } x \text{ is a column vector}.$$ 

A vector which has 1 in every entry is denoted by $1$. If $I \subseteq k[x_1, \ldots, x_n]$ is an ideal of $n$-variate polynomials with coefficients in a field $k$, then the variety $\mathbb{V}(I) \subseteq k^n$ is the set of all points where all elements of $I$ simultaneously vanish. See Cox et al. (1996, Chapter 4) for basics on computational algebraic geometry.
2 Chemical Reaction Networks

A chemical reaction network is a finite directed graph whose vertices are labeled by chemical complexes and whose edges are labeled by positive parameters called the rate constants (cf. (N\(_1\)) of Example 2.1). The digraph is denoted by \(N = ([m], E)\), with vertex set \([m]\) and edge set \(E\). Each chemical complex \(i \in [m]\) has the form \(\sum_{j=1}^{n} (y_i)_j X_j\) for some \(y_i \in \mathbb{Z}_{\geq 0}^n\), where \(X_1, \ldots, X_n\) are chemical species. The vectors \(y_i\) are the complex-species incidence vectors, and they are gathered as the columns of the complex-species incidence matrix \(Y = (y_1, \ldots, y_m)\). Throughout this article, the integers \(n, m,\) and \(r\) denote the number of species, complexes, and reactions, respectively. Each finite directed graph has an incidence matrix \(I\) with \(I_{jl} = -I_{li} = 1\) whenever the \(\ell\)th edge points from the \(i\)th vertex to the \(j\)th vertex and 0 otherwise. A complex which is the source of a reaction is an educt complex and a complex which is the sink of a reaction is a product complex. Each complex can be an educt and product for several reactions. For each reaction network, one has a matrix \(Y\) whose columns are the complex-species incidence vectors corresponding to the educt complexes for each reaction:

\[
Y = (\tilde{y}_1, \ldots, \tilde{y}_r), \text{ where } \tilde{y}_i = y_k \text{ when reaction } i \text{ has educt complex } k. \quad (1)
\]

We exemplify the above notation in Example 2.1 as follows. The system serves as a running example in Sects. 2 and 3 to illustrate our definitions and results.

Example 2.1 The following reaction network is the 1-site phosphorylation network:

\[
\begin{align*}
X_1 + X_2 \xrightarrow{k_1} X_3 \xrightarrow{k_3} X_1 + X_4 \\
X_4 + X_5 \xrightarrow{k_4} X_6 \xrightarrow{k_6} X_2 + X_5.
\end{align*}
\]

The chemical species are \(X_1, X_2, X_3, X_4, X_5,\) and \(X_6,\) and the chemical complexes are \(X_1 + X_2, X_3, X_1 + X_4, X_4 + X_5, X_6,\) and \(X_2 + X_5.\) The species \(X_1\) is a catalyst for the phosphorylation of \(X_2\) which goes through an intermediate state \(X_3\) before becoming the phosphorylated \(X_4.\) Similarly, \(X_5\) catalyzes the dephosphorylation. The network has 6 reactions, each one labeled by a rate constant \(k_1, k_2, k_3, k_4, k_5\) or \(k_6.\) The matrix \(Y\) of this network is

\[
Y = \begin{bmatrix}
1 & 0 & 0 & 0 & 0 & 0 \\
1 & 0 & 0 & 0 & 0 & 0 \\
0 & 1 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 1
\end{bmatrix}.
\]
2.1 Dynamical Systems Defined by Mass-Action Networks

Every chemical reaction network defines a dynamical system of the form

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

(2)

where $S = Y \mathcal{I}$ is the stoichiometric matrix and $\nu(k, x)$ is the vector of reaction rates. It depends on the vector of concentrations $x$ and the vector of rate constants $k$.

In this paper, we are concerned with mass-action networks for which the kinetics is of mass-action form, i.e., the rate of each reaction is proportional to the product of the concentrations of its educt complex. Thus, for mass-action networks,

$$\nu(k, x) = k \cdot \phi(x),$$

where $\phi(x) = (x^{\tilde{y}_1}, \ldots, x^{\tilde{y}_r})^T = (x^J)$, and $k = (k_1, \ldots, k_r)^T$ is a vector of parameters.

**Example 2.2** The stoichiometric matrix and the monomial vector $\phi(x)$ of $\mathcal{N}_1$ are

$$S = \begin{bmatrix}
-1 & 1 & 1 & 0 & 0 & 0 \\
-1 & 1 & 0 & 0 & 0 & 1 \\
1 & -1 & -1 & 0 & 0 & 0 \\
0 & 0 & 1 & -1 & 1 & 0 \\
0 & 0 & 0 & -1 & 1 & 1 \\
0 & 0 & 0 & 1 & -1 & -1
\end{bmatrix} \quad \text{and} \quad \phi(x) = \begin{bmatrix}
x_1 x_2 \\
x_3 \\
x_4 x_5 \\
x_6
\end{bmatrix}.$$

The reaction rates are then

$$v_1 = k_1 x_1 x_2, \ v_2 = k_2 x_3, \ v_3 = k_3 x_3, \ v_4 = k_4 x_4 x_5, \ v_5 = k_5 x_6, \ \text{and} \ v_6 = k_6 x_6.$$  

Consequently, the dynamics of $\mathcal{N}_1$ is given by the following system of ODEs:

$$\begin{align*}
\dot{x}_1 &= -k_1 x_1 x_2 + (k_2 + k_3) x_3, \\
\dot{x}_2 &= -k_1 x_1 x_2 + k_2 x_3 + k_6 x_6, \\
\dot{x}_3 &= k_1 x_1 x_2 - (k_2 + k_3) x_3, \\
\dot{x}_4 &= k_3 x_3 - k_4 x_4 x_5 + k_5 x_6, \\
\dot{x}_5 &= -k_4 x_4 x_5 + (k_5 + k_6) x_6, \\
\dot{x}_6 &= k_4 x_4 x_5 - (k_5 + k_6) x_6.
\end{align*}$$

2.2 Conservation Relations and Total Concentrations

For many reaction networks, there are linear dependencies among $\dot{x}_1, \ldots, \dot{x}_n$: They are relations of the form $z \dot{x} = 0$, where $z$ is an element of the left kernel of $S$. If $z \dot{x} = 0$ for $z^T \in \mathbb{R}^n$ then, by integrating with respect to time, $zx$ is constant along trajectories. These constants $zx$ are the total concentrations or conserved moieties. As, by (2), every $z^T \in \mathbb{R}^n$ with $zS = 0$ yields $z \dot{x} = 0$, the left kernel of the stoichiometric matrix is called the conservation space $\mathcal{L}_{\text{cons}}$. A matrix $Z$ whose rows are a basis of $\mathcal{L}_{\text{cons}}$ is a conservation matrix. In general, every conservation matrix defines total concentrations via

$$c = Zx.$$

(3)
As in our setting the elements of \( x \) represent chemical species, we are usually only interested in those elements \( c \) of (3) associated with \( x \in \mathbb{R}_n^+ \). We use the following notation to refer to these:

\[
\text{im}_+(Z) = \left\{ c \in \mathbb{R}^{n-s} | \exists x \in \mathbb{R}_n^+ \text{ such that } c = Zx \right\}.
\]

(4)

Let \( x(0) \in \mathbb{R}_n^+ \) with corresponding \( c = Zx(0) \). If \( x(0) \in \mathbb{R}_n^+ \) is the initial condition of the trajectory \( \{x(t) | t > 0\} \), then, under mass-action kinetics, the above discussion implies that \( x(t) \) is constrained to the following polyhedron associated with \( c \):

\[
\mathcal{P}_c = \{ x \in \mathbb{R}_n^+ | Zx = c \}.
\]

(5)

The set \( \mathcal{P}_c \) is known as the invariant polyhedron with respect to \( x(0) \) (Craciun et al. 2009), or the stoichiometric compatibility class of \( x(0) \) (Feinberg 1995a, b).

**Remark 2.3** For a given \( c \in \mathbb{R}^{n-s} \), one has \( \mathcal{P}_c \neq \emptyset \) if and only if \( c \in \text{im}_+(Z) \).

**Example 2.4** The conservation space \( \mathcal{L} \text{cons} \) of \( \mathcal{N}_1 \) is spanned by the rows of the matrix

\[
Z = \begin{bmatrix}
1 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 1 \\
0 & 1 & 1 & 1 & 0 & 1.
\end{bmatrix}
\]

Consequently, \( \mathcal{N}_1 \) has three linearly independent conservation relations and three total concentrations \( c_1, c_2 \) and \( c_3 \):

\[
\begin{align*}
x_1 + x_3 &= c_1, \\
x_5 + x_6 &= c_2, \\
x_2 + x_3 + x_4 + x_6 &= c_3.
\end{align*}
\]

The values \( c_1, c_2 \) and \( c_3 \) can be interpreted as total amount of kinase, phosphatase and substrate, respectively. Further examples can be found in Conradi and Pantea (2019) and Shiu (2010).

### 2.3 Steady States

If \( k \) and \( x \) are such that

\[
S\nu(k, x) = 0,
\]

(6)

then \( x \) is a steady state. In the mass-action networks setting, as \( \nu(k, x) \) is a vector of monomials, Eq. (6) are algebraic; hence, tools from algebraic geometry are useful in the study of steady states. As \( x \) is a vector of concentrations of chemical species, only nonnegative \( x \) are chemically meaningful. Consequently, when talking about steady states, we mean nonnegative real solutions of Eq. (6). A steady state is positive if all its coordinates are positive real numbers. It is a boundary steady state if all coordinates are nonnegative but it is not positive. The steady-state ideal \( I \) is the
polynomial ideal generated by the entries of $S \nu(x, k)$. This ideal can be considered in different polynomial rings. The parameters $k$ can be part of the indeterminates, i.e., $I \subset \mathbb{R}[x, k]$, or appear as the variables in rational functions that serve as coefficients. In the second case, $I \subset \mathbb{R}(k)[x]$. In both cases, the steady-state variety is the zero locus of the steady-state ideal.

**Example 2.5** The equations $\dot{x}_i = 0$ define the steady-state ideal of $N_1$:

$$I = \langle -k_1 x_1 x_2 + (k_2 + k_3) x_3, -k_1 x_1 x_2 + (k_2 + k_3) x_3, k_3 x_3 - k_4 x_4 x_5 + k_5 x_6, -k_4 x_4 x_5 + (k_5 + k_6) x_6, k_4 x_4 x_5 - (k_5 + k_6) x_6 \rangle = \langle -k_1 x_1 x_2 + (k_2 + k_3) x_3, k_3 x_3 - k_4 x_4 x_5 + k_5 x_6, -k_4 x_4 x_5 + (k_5 + k_6) x_6, k_4 x_4 x_5 - (k_5 + k_6) x_6 \rangle.$$

The second equality results from elementary simplification and omitting redundant generators. While such simplifications are useful to understand the geometry of steady states, the resulting polynomials need not have a biochemical interpretation anymore.

**Remark 2.7** It would be very interesting to systematically understand the ideal $I(V^+)$ of polynomials that vanish on $V^+$. This ideal is typically much larger than the steady-state ideal. First, the steady-state ideal need not contain all functions that vanish on its real variety (i.e., it need not be a real-radical ideal). Real-radicals can be computed (Neuhaus 1998; Becker and Neuhaus 1993). The second and more severe problem is that there is no simple method to determine $I(V^+)$, the ideal of all polynomials that vanish on the strictly positive part. If the steady-state equations are binomial equations in the $x$ variables (that is if the steady-state ideal is binomial in $\mathbb{R}(k)[x]$), then a remedy of sorts is offered at the end of Sect. 5.

Often it is possible to obtain a parametrization of $V^+$ as shown in Example 2.8 as follows. Such parametrizations simplify the study of multistationarity (which we formally define after Example 2.8) and are the topic of Sect. 3.

**Example 2.8** According to Example 2.5, the steady-state ideal of $N_1$ is generated by 3 polynomials. Since we are only interested in positive $x_i$, the equations that describe $V^+$ can be rearranged as

$$\frac{x_3}{x_6} = \frac{k_6}{k_3}, \quad \frac{x_1 x_2}{x_3} = \frac{k_2 + k_3}{k_1}, \quad \frac{x_4 x_5}{x_6} = \frac{k_5 + k_6}{k_4}. \quad (7)$$
These equations can be solved as
\[
    x_3 = \frac{k_1}{k_2 + k_3}x_1x_2, \quad x_4 = \frac{k_1k_3(k_5 + k_6) x_1x_2}{(k_2 + k_3)k_4k_6} x_5, \quad x_6 = \frac{k_1k_3}{(k_2 + k_3)k_6}x_1x_2.
\] (8)

This shows that the positive steady-state variety of \(N_1\) can be parametrized by \(x_1, x_2,\) and \(x_5\) together with \(k_1, \ldots, k_6\). This parametrization uses only products (and divisions) of the \(x_i\), but no sums. This monomial parametrization is crucial for the developments of Sect. 3.

The following is the central property studied in this paper.

**Definition 2.9** A network \(\mathcal{N}\) admits multistationarity if there are \(k \in \mathbb{R}_{>0}^r\) and \(a \neq b \in \mathbb{R}_{>0}^n\) such that \((k, a) \in V^+, (k, b) \in V^+,\) and \(a, b \in \mathcal{P}_c\) for \(c = za = zb\).

Multistationarity requires the existence of a vector of rate constants \(k\) and an affine subspace \(x_0 + \text{im}(S)\) that intersects the variety \(\{x | Sv(k, x) = 0\}\) in at least two distinct positive points. Often it is useful to have a dual view of this variety: globally, as a variety in \(\mathbb{R}^r \times \mathbb{R}^n\), or as a family of varieties in \(\mathbb{R}^n\), parametrized over \(k\). The theory of multistationarity is mathematically interesting because the existential quantifier “\(\exists k \in \mathbb{R}_{>0}^r\)” can often be eliminated and equivalently expressed without quantifiers. Theorems 3.15 and 3.18 are instances of this phenomenon.

### 2.4 Steady States and Rate Constants

We revisit Eq. (6) and observe that \(Sv(k, x) = 0\) for \(k \in \mathbb{R}_{>0}^r\) and \(x \in \mathbb{R}_{>0}^n\), if and only if \(v(k, x) \in \ker(S) \cap \mathbb{R}_{>0}^r\) (as \(v(k, x)\) is nonnegative for \(k \in \mathbb{R}_{>0}^r\) and \(x \in \mathbb{R}_{>0}^n\)). As discussed in—among many other references—(Conradi and Flockerzi 2012), \(\ker(S) \cap \mathbb{R}_{>0}\) is a pointed polyhedral cone. As such, it is generated by finitely many generators that are unique up to scalar multiplication. Let \(E_1, \ldots, E_d\) denote the generators and let \(E = [E_1, \ldots, E_d]\) be the cone generator matrix. In particular, every generator \(E_i\) is nonnegative and every element of the cone can be represented by a nonnegative linear combination of the generators (Rockafellar 1970):

\[
    v(k, x) \in \ker(S) \cap \mathbb{R}_{>0}^r \iff v(k, x) = E\lambda, \text{ for some } \lambda \in \mathbb{R}_{\geq 0}^d.
\]

And \((k, x) \in V^+,\) if and only if \(v(k, x)\) is in the (relative) interior of \(\ker(S) \cap \mathbb{R}_{>0}^r\), that is if and only if \(v(k, x) \in \ker(S) \cap \mathbb{R}_{>0}^r\). As suggested in Conradi and Flockerzi (2012), the cone \(\ker(S) \cap \mathbb{R}_{>0}^r\) can be parametrized with the help of the following set that we call coefficient cone:

\[
    \Lambda(E) = \left\{ \lambda \in \mathbb{R}_{\geq 0}^d | E\lambda > 0 \right\}. \tag{9}
\]

See (Conradi and Flockerzi 2012, Remark 4) for more on \(\Lambda(E)\). For future use, we observe:

**Remark 2.10** \(\Lambda(E) = \emptyset\) if and only if \(E\) contains a zero row.
Example 2.11 The network $\mathcal{N}_1$ has the following cone generator matrix and coefficient cone:

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

and $\Lambda(E) = \mathbb{R}^3_{>0}$.

The first connected component of network, $\mathcal{N}_1$, can be considered as a reaction network in its own right with $x = (x_1, \ldots, x_4)$ the concentrations of $X_1, \ldots, X_4$. This network has the following stoichiometric matrix, cone generator matrix and vector of reaction rates:

$$S = \begin{bmatrix} -1 & 1 & 1 \\ -1 & 1 & 0 \\ 1 & -1 & -1 \\ 0 & 0 & 1 \end{bmatrix}, \quad E = \begin{bmatrix} 1 \\ 1 \\ 0 \end{bmatrix} \quad \text{and} \quad \nu(k, x) = \begin{pmatrix} k_1 x_2 x_2 \\ k_2 x_3 \\ k_3 x_3 \end{pmatrix}$$

In this case, the coefficient cone $\Lambda(E)$ is the empty set (there is no nonnegative real number $\lambda$ such that $E\lambda > 0$). Moreover, $(k, x)$ is a steady state, if and only if $\nu(k, x) = E\lambda$. From the structure of $E$, it follows that $x_3 = 0$ for every steady state. Hence, there are no positive steady states and $V^+ = \emptyset$ in this case.

The following lemma formalizes the observation of the above example.

Lemma 2.12 $V^+ \neq \emptyset$ if and only if $E$ does not have a zero row.

Proof Suppose $V^+ \neq \emptyset$, i.e., there exist $(k, x) \in V^+$, i.e., $\nu(k, x) = k \lambda = k \phi(x) \in \text{ker}(S) \cap \mathbb{R}^r_{>0}$, i.e., $k \lambda = E \lambda$, for some $\lambda \in \Lambda(E)$, and thus $\Lambda(E) \neq \emptyset$. Then, by Remark 2.10, $E$ does not have a zero row. Vice versa, suppose $E$ does not have a zero row. Then $\Lambda(E) \neq \emptyset$, again by Remark 2.10. Pick any $x \in \mathbb{R}^n_{>0}$ and define $k = \phi(x^{-1}) \star E \lambda$. Then, $k \phi(x) = E \lambda$ and $(k, x) \in V^+$, i.e., $V^+ \neq \emptyset$. □

The cone generator matrix and the coefficient cone contain important information about $V^+$:

Theorem 2.13 Let $x \in \mathbb{R}^n_{>0}$ and $k \in \mathbb{R}^r_{>0}$. If $E$ does not have any zero row; then

$$(k, x) \in V^+ \iff \exists \lambda \in \Lambda(E) \text{ such that } k = \phi(x^{-1}) \star E \lambda.$$  

Proof By Definition 2.6, $(k, x) \in V^+$ if and only if $S(k \phi(x)) = 0$ and $k \in \mathbb{R}^r_{>0}$, $x \in \mathbb{R}^n_{>0}$. Every element of $\text{ker}(S) \cap \mathbb{R}^r_{>0}$ is of the form $E \lambda$ for some $\lambda \in \Lambda(E)$. Then, if $S(k \phi(x)) = 0$, there is a $\lambda \in \Lambda(E)$ such that $k \phi(x) = E \lambda$. Hence, $k = \phi(x^{-1}) \star E \lambda$.

$\Leftarrow$ If $k = \phi(x^{-1}) \star E \lambda$ for some $\lambda \in \Lambda(E)$, then $k \phi(x) = E \lambda$. As $\forall \lambda \in \Lambda(E)$, $E \lambda \in \text{ker}(S) \cap \mathbb{R}^r_{>0}$, $S(k \phi(x)) = 0$. Hence $(k, x) \in V^+$. □
Corollary 2.14 If $E$ does not have any zero row then, for every $x \in \mathbb{R}^n_>$, there is a $k \in \mathbb{R}^r_>$ such that $(k, x) \in V^+$.

Proof Suppose $E$ does not have a zero row. Then, $V^+ \neq \emptyset$ by Lemma 2.12. Pick any $\lambda \in \Lambda(E)$ and define $k = \phi(x^{-1}) \ast E \lambda$. Then, $k \ast \phi(x) = E \lambda$ which is equivalent to $\nu(k, x) \in \ker(S) \cap \mathbb{R}^r_>$. Hence $(k, x) \in V^+$. \qed

Remark 2.15 Theorem 2.13 shows in particular that under the (very mild) assumption that $E$ does not have any zero row, for every positive $x$, one can find positive $k$ such that $(k, x) \in V^+$. If $E$ does have zero rows, then there are no positive steady states and $V^+$ is empty. Hence, from here on we only consider reaction networks $\mathcal{N}$, where the cone generator matrix $E$ does not have any zero row.

3 Monomial Parametrizations of Positive Steady States

In this section, we consider a mass-action network $\mathcal{N}$ on $n$ species and $r$ reactions, with at least one conservation relation. In Sect. 4, we use the results of this section to deduce conditions for multistationarity in the space of total concentrations for a network describing the distributive phosphorylation of a protein.

Let $S$ and $Z$ denote the stoichiometric and a conservation matrix of $\mathcal{N}$, respectively. We study the consequences of the existence of monomial parametrizations for the positive steady-state variety of $\mathcal{N}$. Following Müller et al. (2016), the positive steady-state variety admits a monomial parametrization if suitable Laurent monomials in the concentrations can be expressed in terms of the reaction rates (cf. Müller et al. 2016, Section 3.2). Such systems can be diagonalized using monomial transformations. The following definition captures what was observed in Example 2.8.

Definition 3.1 The positive steady-state variety $V^+$ admits a monomial parametrization if there is a matrix $M \in \mathbb{Z}^{n \times d}$ of rank $p < n$ and a rational function $\gamma$ in the variables $k_1, \ldots, k_r$ with values in $\mathbb{R}^d$ such that, for all $(k, x) \in \mathbb{R}^r_+ \times \mathbb{R}^n_>$,

$$(k, x) \in V^+ \iff \gamma(k) \text{ is defined and } x^M = \gamma(k).$$

In Definition 3.1, the matrix $M$ is understood as part of saying admits a monomial parametrization. In the following, if $V^+$ admits a monomial parametrization and a matrix $M$ appears, then it is the matrix in that definition.

The existence of a monomial parametrization implies that all positive steady states can be recovered from monomial transformations of one positive steady state. In algebraic geometry, a variety which equals the closure of an algebraic torus acting on the variety is known as a toric variety. Affine toric varieties are cut out by binomial equations such as those in Definition 3.1.

Equation (7) of Example 2.8 shows that the network $\mathcal{N}_1$ admits a monomial parametrization according to Definition 3.1. By introducing two matrices $M^+$ and $M^-$ with nonnegative entries, of appropriate dimension, such that

$$M = M^+ - M^-,$$  \hfill (10)
and extracting numerators and denominators of the rational function $\gamma(k)$ as follows

$$\gamma^{\pm}(k) = (\gamma_i^{\pm}(k))_i, \text{ where } \gamma_i(k) = \frac{\gamma_i^-(k)}{\gamma_i^+(k)},$$

(11)

we can write the system of Definition 3.1 as a binomial system:

$$\gamma^+(k)\star x^{M^+} - \gamma^-(k)\star x^{M^-} = 0.$$  

(12)

**Remark 3.2** In (11), the coefficients $\gamma^{\pm}(k)$ usually have many terms, hence (11) is binomial only in $x$. As a consequence of Eisenbud and Sturmfels (1996, Theorem 2.1), the ideal $\langle x^M - \gamma(k) \rangle \subset \mathbb{R}(k)[x^\pm]$ is a complete intersection. This means that there exists a generating set of $\langle x^M - \gamma(k) \rangle$ in which $M$ has full rank. In the following, we assume that $M$ is of full rank.

**Remark 3.3** Our Definition 3.1 is equivalent to asking that the ideal $\mathbb{I}(V^+)$, considered in the ring $\mathbb{R}(k)[x]$, is generated by the binomials (12). As there may well be several generating sets of binomials, neither the coefficients $\gamma^{\pm}(k)$ nor the matrices $M^{\pm}$ need be unique. In Sadeghimanesh and Feliu (2019b), a similar situation is considered: The ideal defined by the polynomials $S\nu(k, x) = 0$ is generated by binomials in the ring $\mathbb{R}(k)[x]$. Our definition is slightly more general, as it might happen that even though $S\nu(k, x) = 0$ is not generated by binomials, the ideal $\mathbb{I}(V^+)$ is.

Given the binomials (12), we can now define the positive values of $k$ where the vector $\gamma(k)$ of Definition 3.1 is defined: The system (12) can only be satisfied by positive $k$ and $x$ if the coefficients $\gamma^{\pm}(k)$ are nonzero and of the same sign, that is, if $k$ is contained in the semi-algebraic set

$$K^+_\gamma := \{k \in \mathbb{R}_{>0}^n | \gamma^+_i(k) \cdot \gamma^-_i(k) > 0, \quad i = 1, \ldots, p \}.$$  

(13)

In particular, if $k \notin K^+_\gamma$, then there does not exist a vector $x \in \mathbb{R}_{>0}^n$ such that $(k, x) \in V^+$.

The next few lemmata make the monomial parametrization explicit in our setting.

**Lemma 3.4** If $V^+$ admits a monomial parametrization and, for $q < n$, $A \in \mathbb{Q}^{q \times n}$ is any matrix of maximal rank $q$ such that $AM = 0$, then:

(i) $(k, x) \in V^+ \iff (k, x \star (e^A)^\ast) \in V^+, \forall \xi \in \mathbb{R}_>^q$.

(ii) $(k, x) \in V^+ \iff (k, x \star (e^A)^A) \in V^+, \forall \kappa \in \mathbb{R}_>^q$.

**Proof** As $V^+$ admits a monomial parametrization, the left-hand side of (i) is equivalent to $x^M = \gamma(k)$ and the right-hand side is equivalent to $(x \star \xi^A)^M = \gamma(k)$. As $AM = 0$, these are equivalent: $(x \star (e^A)^A)^M = x^M$. Item (ii) follows from (i) by replacing $\xi$ with $e^\kappa$.

By Lemma 3.4, given a pair $(k, x) \in V^+$, one obtains all $x$ with $(k, x) \in V^+$ from $x$ with the help of the left kernel of $M$. In the following lemma, we show that by choosing a special basis of the left kernel of $M$, one can make the connection between $x$ and $k$ in the solution of $S\nu(k, x) = 0$ explicit.
Lemma 3.5 Assume \( V^+ \) admits a monomial parametrization. Then, there are
- a matrix \( A \in \mathbb{Q}^{(n-p)\times n} \) of rank \( n - p \) such that \( AM = 0 \),
- a function \( \psi : K_\gamma^+ \to \mathbb{R}^n \), and
- an exponent \( \eta \in \mathbb{Z} > 0 \),
such that \( \psi^\eta \) is a rational function and

\[
(k, x) \in V^+ \iff k \in K_\gamma^+ \text{ and } \exists \xi \in \mathbb{R}^{n-p}_{>0} \text{ such that } x = \psi(k) \star \xi^A.
\]

Proof As in Remark 3.2, consider the ideal \( \langle x^M - \gamma(k) \rangle \subset \mathbb{R}(k)[x^\pm] \). By Eisenbud and Sturmfels (1996, Theorem 2.1), this ideal is a complete intersection and we can find a generating set in which \( M \) has full rank and format \( n \times p \) for a suitable \( \gamma \).

In the following, we assume that \( M \) is ordered such that the first \( p \) rows are linearly independent. (Note that this can always be achieved by a suitable reordering of the variables \( x \).) Then, there is an invertible matrix \( U \in \mathbb{Q}^{p \times p} \) such that

\[
MU = \begin{bmatrix} I_p & -W \end{bmatrix},
\]

where \( W \) is of format \( (n-p) \times p \). Let

\[
A = [W|I_{n-p}] \text{ and } \psi(k) = \gamma(k)^{U[0_{p \times n-p}]},
\]

We now argue that \( A \) and \( \psi(k) \) have the properties stated above: First, \( AM = 0 \) since \( AMU = 0 \) and \( U \) is invertible. Second, only if \( k \in K_\gamma^+ \), there exists \( x \in \mathbb{R}^n_{>0} \) such that \( (k, x) \in V^+ \). Hence, we only need to consider \( k \in K_\gamma^+ \). As \( \gamma_i^\pm(k) \neq 0 \) for \( k \in K_\gamma^+ \) by (13) and as powers of the entries \( \psi_i(k) \) are products of \( \gamma_i^\pm \), the function \( \psi(k) \) is well defined on \( K_\gamma^+ \). And third, as \( \gamma(k) \) is rational, the coordinate-wise power \( \psi^\eta(k) \) is rational when \( \eta \) is the least common multiple of the denominators in \( U \).

Next, we turn to the equivalence: according to Definition 3.1,

\[
(k, x) \in V^+ \iff x^M = \gamma(k) \text{ and } k \in K_\gamma^+.
\]

In the following calculations, we take logarithms on both sides of the equation above. This is well defined, if we require \( k \in K_\gamma^+ \) which implies \( \gamma(k) > 0 \). Taking logarithms, we get

\[
M^T (\ln x) = \ln \gamma(k) \iff U^T M^T (\ln x) = U^T (\ln \gamma(k)).
\]

Decompose \( x \) into \( x' = (x_1, \ldots, x_p)^T \) and \( \xi = (x_{p+1}, \ldots, x_n)^T \). As \( U^T M = [I_p - W^T] \), the above equivalence is

\[
(k, x) \in V^+ \iff \ln x' - W^T (\ln \xi) = U^T (\ln \gamma(k)) \iff x' = \gamma(k)^U \star \xi^W.
\]

Using \( x = (x', \xi) \) and the above matrix \( A \) together with the vector \( \psi(k) \), we obtain the final equivalence \( (k, x) \in V^+ \iff x = \psi(k) \star \xi^A \). \( \square \)
**Remark 3.6**

(i) For fixed $k \in K^+$, the matrix $A$ in Lemma 3.5 captures all information about the parametrization. We call $A$ the **exponent matrix** of the parametrization.

(ii) Choosing $\xi_i = 1$ for all $i$, one obtains $(k, \psi(k)) \in V^+$, i.e., the vector $\psi(k)$ is a (positive) solution of the equation $S\nu(k, x) = 0$ for a given vector $k$.

(iii) In the proof of Lemma 3.5, the coordinates $\xi$ are elements of $x$, i.e., the $\xi_i$ correspond to variables of the system and thus have a biological meaning. Moreover, usually there are several orderings of variables one can choose from when constructing the matrix $A$ in the proof of Lemma 3.5. One strategy would be to choose an ordering that yields $\xi_i$ that correspond to measurable species.

The following example is an illustration of Lemma 3.5 and the steps taken in its proof.

**Example 3.7** Going back to Example 2.8, Eq. (7) can be expressed as

\[ x^M = \gamma(k), \]

where

\[
M = \begin{bmatrix}
0 & 0 & 1 & 0 & 0 & -1 \\
1 & 1 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & 1 & -1
\end{bmatrix}^T \quad \text{and} \quad \gamma(k) = \left( \frac{k_6}{k_3}, \frac{k_2 + k_3}{k_1}, \frac{k_5 + k_6}{k_4} \right)^T.
\]

As numerators and denominators of $\gamma(k)$ are sums of positive monomials, one has $K^+_{\gamma} = \mathbb{R}^6_{\geq 0}$, that is, the monomial parametrization is valid for all positive $k$. For example, for the matrix

\[
U = \begin{bmatrix}
0 & -1 & -1 \\
-1 & -1 & -1 \\
0 & 0 & 1
\end{bmatrix}, \quad \text{one obtains} \quad MU = \begin{bmatrix}
-1 & -1 & -1 \\
-1 & -1 & -1 \\
1 & 0 & 0 \\
0 & 0 & 1 \\
0 & 0 & 1
\end{bmatrix},
\]

which in the ordering $(x_3, x_6, x_4, x_1, x_2, x_5)^T$ is equivalent to

\[
\begin{bmatrix}
I_3 \\
-W
\end{bmatrix} \quad \text{with} \quad W = \begin{bmatrix}
1 & 1 & 1 \\
1 & 1 & 1 \\
0 & 0 & -1
\end{bmatrix}.
\]

As in the proof of Lemma 3.5, we obtain

\[
A = [W|I_3] = \begin{bmatrix}
1 & 1 & 1 & 1 & 0 & 0 \\
1 & 1 & 1 & 0 & 1 & 0 \\
0 & 0 & -1 & 0 & 0 & 1
\end{bmatrix}.
\]
For \( \psi(k) \), we obtain

\[
\psi(k) = \gamma(k)[U|0_{3\times3}] = \left( \begin{array}{cccc} k_1 & k_3 & k_1 & k_5 + k_6 \\ k_2 + k_3 & k_6 & k_2 + k_3 & k_4 \\ k_6 & k_2 + k_3 & k_4 & 1, 1, 1 \end{array} \right)^T.
\]

In this case, \( \psi(k) \) is already a rational function, as the matrix \( U \) contains only integer entries and the least common multiple of the denominators in \( U \) therefore is \( \eta = 1 \). If \( U \) was not an integer matrix but contained rational entries, then \( \psi(k) \) would not be a rational function as it would contain entries with rational exponents. In this case, only \( \psi(k)^n \) would be rational as taking entries of \( \psi \) with rational exponents to the power \( \eta \) yields integer exponents. Then, with \( \xi = (\xi_1, \xi_2, \xi_3)^T \),

\[
(x_3, x_6, x_4, x_1, x_2, x_5)^T = \psi(k) \star \xi^A
\]

\[
= \left( \frac{k_1}{k_2 + k_3} \xi_1 \xi_2, \frac{k_1 k_3}{(k_2 + k_3)k_6} \xi_1 \xi_2, \frac{k_1 k_3 (k_5 + k_6)}{(k_2 + k_3)k_4 k_6} \xi_3, \xi_1, \xi_2, \xi_3 \right)^T.
\]

As explained above, if \( V^+ \) admits a monomial parametrization and if \( (k, x) \in V^+ \), then there are infinitely many \( \tilde{x} \) with \( (k, \tilde{x}) \in V^+ \). The following result describes the connection between an arbitrary pair \( a \) and \( b \) of these.

**Lemma 3.8** If \( V^+ \) admits a monomial parametrization with exponent matrix \( A \in \mathbb{Q}^{(n-p)\times n} \) and \( k \in \mathcal{K}_\gamma^n \) and \( a \neq b \in \mathbb{R}_>^n \) are such that \( (k, a) \in V^+ \) and \( (k, b) \in V^+ \), then

(i) \( \exists \xi \neq 1 \in \mathbb{R}_>^{n-p} \) such that \( b = a \star \xi^A \),

(ii) \( \exists 0 \neq \mu \in \text{rowspace}(A) \) such that \( b = a \star e^\mu \).

**Proof** (i) It follows from Lemma 3.5 that there are \( \xi_1, \xi_2 \in \mathbb{R}_>^{n-p} \) such that \( a = \psi(k) \star \xi_1^A \) and \( b = \psi(k) \star \xi_2^A \). Then, \( \psi(k) = a \star \xi_1^{-A} \) and \( b = a \star \xi_2^{-A} \star \xi_2^A = a \star \xi^A \) with \( \xi = \frac{\xi_2}{\xi_1} \). Item (ii) follows from (i) by replacing \( \xi^A \) with \( (e^{\ln(\xi)})^A \).

This final corollary summarizes the development so far.

**Corollary 3.9** If \( V^+ \) admits a monomial parametrization with exponent matrix \( A \in \mathbb{Q}^{(n-p)\times n} \), then for every positive \( x \in \mathbb{R}_>^n \) there exists a vector \( k \in \mathcal{K}_\gamma^n \) such that the following equivalent conditions hold:

(i) \( (k, x) \in V^+ \),

(ii) \( x^M = \gamma(k) \),

(iii) \( \exists \xi \in \mathbb{R}_>^{n-p} \) such that \( x = \psi(k) \star \xi^A \).

**Proof** This is Lemma 3.5 together with Theorem 2.13 and Corollary 2.14.

### 3.1 Multistationarity

This section collects results concerning multistationarity under the assumption that \( V^+ \) admits a monomial parametrization. Some conditions involve sign patterns similar
to Conradi and Flockerzi (2012) and Müller et al. (2016). For a scalar \( u \), we use \( \text{sign}(u) \) to denote its sign, for a vector \( v \in \mathbb{R}^n \) we use \( \text{sign}(v) = (\text{sign}(v_1), \ldots, \text{sign}(v_n)) \) to denote its sign pattern. Theorem 3.14 appeared in a different formulation in Müller et al. (2016). In this subsection, we frequently refer to \( Z \), the conservation matrix of a reaction network and the set \( \text{im}_+(Z) \). Our first result exploits a monomial parametrization of \( V^+ \) to formulate conditions for multistationarity that are independent of the rate constants.

**Lemma 3.10** If \( V^+ \) admits a monomial parametrization with exponent matrix \( A \in \mathbb{Q}^{(n-p) \times n} \), then the following are equivalent:

(i) \( \mathcal{N} \) admits multistationarity,
(ii) \( \exists x \in \mathbb{R}^{n_0} \) and \( \xi \in \mathbb{R}^{n-p} \setminus \{1\} \), such that \( Z(x - x^*\xi^A) = 0 \),
(iii) \( \exists x \in \mathbb{R}^{n_0} \) and \( \kappa \in \mathbb{R}^{n-p} \setminus \{0\} \), such that \( Z(x - x^*(e^\kappa)^A) = 0 \).

**Proof** Items (ii) and (iii) are equivalent as for any \( \xi \in \mathbb{R}^{n-p} \), there is a \( \kappa \in \mathbb{R}^{n-p} \) such that \( \xi = e^\kappa \). Now assume (ii) holds for some \( x \) and \( \xi \). We prove that (i) holds. By Lemma 3.5, there exists a \( k \in \mathcal{K}_+^\gamma \) such that \( (k, x) \in V^+ \) and by Lemma 3.4 \( (k, x^*\xi^A) \in V^+ \) as well. Since \( Zx = Z(\xi^A^*x) = c \) by assumption one has \( x, \xi^A^*x \in \mathcal{P}_c \), that is, \( \mathcal{N} \) admits multistationarity. When (i) holds, we have \( x \neq x' \) and \( k \) such that \( Z(x - x') = 0 \), and \( (k, x) \in V^+ \) and \( (k, x') \in V^+ \). Now, Lemma 3.8 implies \( x' = x^*\xi^A \) and thus (ii).

As discussed in Pérez-Millán et al. (2012) and Müller et al. (2016) for systems with toric steady states, multistationarity can be established by analysis of sign patterns. Theorems 3.11–3.14 below translate this to our setting. Theorems 3.11 and 3.12 show that the existence of a pair of nontrivial vectors \( \mu \in \text{rowspace}(A) \) and \( z \in \text{im}(S) \) with \( \text{sign}(\mu) = \text{sign}(z) \) is both necessary and sufficient for multistationarity. Theorem 3.11 is constructive in the sense that given such a pair \( \mu, z \) one can construct rate constants \( k \) and a corresponding pair of steady states \( a \) and \( b \).

**Theorem 3.11** If \( V^+ \) admits a monomial parametrization with exponent matrix \( A \in \mathbb{Q}^{(n-p) \times n} \) and there are \( \mu \in \text{rowspace}(A) \) and \( z \in \text{im}(S) \) such that \( \text{sign}(\mu) = \text{sign}(z) \), then \( \mathcal{N} \) admits multistationarity. Specifically, for arbitrary \( \bar{a}_i \in \mathbb{R}_{>0}, i \in [n] \), let \( a \in \mathbb{R}_{>0}^n \) denote the vector with entries

\[
a_i = \begin{cases} 
\frac{z_i}{e^{z_i} - 1} & \text{if } z_i \neq 0, \\
\bar{a}_i & \text{else},
\end{cases}
\]

and let \( b = a^*e^\mu \).

Then, for any \( \lambda \in \Lambda(E) \), setting

\[
k = \phi(a^{-1})^*E\lambda,
\]

\( \square \) Springer
$N$ admits multistationarity as

$$(k, a) \in V^+, \ (k, b) \in V^+, \text{ and } (b - a) \in \text{im}(S).$$

**Proof** The vector $b$ is positive whenever $a$ is positive, and the vector $a$ is positive, whenever $\text{sign}(\mu) = \text{sign}(z)$. By definition, $(b - a) = z \in \text{im}(S)$. Then, Theorem 2.13 shows $(k, a) \in V^+$ and Lemmas 3.4 and 3.5 also show $(k, b) \in V^+$.  

**Theorem 3.12** Assume $V^+$ admits a monomial parametrization with exponent matrix $A \in \mathbb{Q}^{(n-p) \times n}$ and let $k \in \mathbb{K}_+^n$ and $a, b \in \mathbb{R}^n_{\geq 0}$, $a \neq b$, be such that $(k, a) \in V^+$, $(k, b) \in V^+$, and $(b - a) \in \text{im}(S)$. Let $z = b - a$ and $\mu = \ln b - \ln a$. Then,

(i) $z \in \text{im}(S)$, $\mu \in \text{rowspace}(A)$, $\text{sign}(z) = \text{sign}(\mu)$,

(ii) $k, a, \text{ and } b$ together with $z$ and $\mu$ satisfy (14a)–(14c).

**Proof** For item (i), $z \in \text{im}(S)$ by assumption. As $V^+$ admits a monomial parametrization, by Lemma 3.5, there are $\kappa_1$ and $\kappa_2 \in \mathbb{R}^{n-p}$ such that $a = \psi(k) \ast (e^{\kappa_1} A)$ and $b = \psi(k) \ast (e^{\kappa_2} A)$. Hence, $\mu = (\kappa_2 - \kappa_1) A$ and, consequently, $\mu \in \text{rowspace}(A)$. By construction, $b = e^\mu \ast a$, and thus, $z = (e^\mu - 1) \ast a$. As $a$ is positive, $\text{sign}(e^\mu - 1) = \text{sign}(z)$ must hold. As $\text{sign}(e^\mu - 1) = \text{sign}(\mu)$, $\text{sign}(\mu) = \text{sign}(z)$. For item (ii), (14b) holds by construction and (14a) follows from the equation $z = (e^\mu - 1) \ast a$. Now, $(k, a) \in V^+$ implies that $k \ast \phi(a) = E \lambda$ for some $\lambda \in \Lambda(E)$ by Theorem 2.13; hence, (14c) also holds.

The following Theorem 3.14 rests on the set of all sign patterns associated with a linear subspace: Let $U \subseteq \mathbb{R}^n$ be a linear subspace, then $\text{sign}(U)$ is the set of all sign patterns of all its elements:

$$\text{sign}(U) = \{ \delta \in \{-1, 0, 1\}^n | \exists u \in U \text{ with } \text{sign}(u) = \delta \}$$

(15)

**Example 3.13** Let $U = \text{im} \left( \begin{bmatrix} -5 \\ 2 \end{bmatrix} \right)$ and observe that for every vector $u \in U$ one has either

$$\text{sign}(u) = \begin{pmatrix} -1 \\ 1 \end{pmatrix} \text{ or } \text{sign}(u) = \begin{pmatrix} 0 \\ 0 \end{pmatrix} \text{ or } \text{sign}(u) = \begin{pmatrix} 1 \\ -1 \end{pmatrix}. $$

Consequently

$$\text{sign}(U) = \left\{ \begin{pmatrix} -1 \\ 1 \end{pmatrix}, \begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} 1 \\ -1 \end{pmatrix} \right\}. $$

Theorem 3.14 below is similar to Müller et al. (2016, Proposition 3.9 and Corollary 3.11). All of them employ analysis of the aforementioned sign patterns to decide the existence of two positive real solutions $a$ and $b$ to the parametrized family of polynomials (6) such that both are elements of the affine space $\{ x | Zx = Za = Zb \}$. For a detailed discussion on how to verify sign conditions, see Müller et al. (2016, Section 4).
Theorem 3.14 If $V^+$ admits a monomial parametrization with exponent matrix $A$, then there are $k \in K^+_\gamma$, $a \neq b \in \mathbb{R}^n_{>0}$ such that $(k, a) \in V^+$, $(k, b) \in V^+$, and $Z(b - a) = 0$ if and only if
\[
\text{sign(rowspace}(A)) \cap \text{sign}(\text{im}(S)) \neq \{0\}.
\]

Proof This is the combination of Theorems 3.11 and 3.12. \qed

3.2 Multistationarity in the Space of Total Concentrations

In this section, we study multistationarity in the space of total concentrations under the assumption that $V^+$ admits a monomial parametrization. The first result translates some of the results of the previous section into the space of total concentrations.

Theorem 3.15 Assume $V^+$ admits a monomial parametrization with exponent matrix $A \in \mathbb{Q}^{(n-p) \times n}$. Let $c$ be an element of $\text{im}_+(Z)$, then the following are equivalent:

(i) $\exists k \in K^+_\gamma$ and $a \neq b \in \mathbb{R}^n_{>0}$ such that $(k, a), (k, b) \in V^+$, and $c = Za = Zb$,
(ii) $\exists a \in \mathbb{R}^n_{>0}$ and $\xi \neq 1 \in \mathbb{R}^n_{>0}$, such that $Z(a \cdot \xi^A - a) = 0$ and $c = Za = Zb$.

Proof (i)⇒(ii): Let $k \in K^+_\gamma$ and $a \neq b \in \mathbb{R}^n_{>0}$ as in (i). By Lemma 3.5, there are $\xi_1, \xi_2 \in \mathbb{R}^n_{>0}$ such that $a = \psi(k) \cdot \xi_1^A$ and $b = \psi(k) \cdot \xi_2^A$ and $a \neq b$ implies $\xi_1 \neq \xi_2$. Since $c = Za = Zb$, the equation $Z(\psi(k) \cdot \xi^A) = c$ has at least the two positive solutions $\xi_1$ and $\xi_2$.

(ii)⇒(iii): Let $k \in K^+_\gamma$ and $\xi_1 \neq \xi_2 \in \mathbb{R}^n_{>0}$ as in (ii). For $a = \psi(k) \cdot \xi_1^A$ and $b = \psi(k) \cdot \xi_2^A \neq a \cdot (\xi_2/\xi_1)^A$, one has $Za = Zb = c$ by assumption. Hence, $Z(a \cdot \xi^A - a) = 0$ has the positive solution $a = \psi(k) \cdot \xi_1^A$ and $\xi = \xi_2/\xi_1$. Further $\xi_1 \neq \xi_2$ implies $\xi \neq 1$.

(iii)⇒(i): Let $a \in \mathbb{R}^n_{>0}$ and $\xi \neq 1 \in \mathbb{R}^n_{>0}$ be as in (iii). Choose $\lambda \in \Lambda(E)$ and let $k = \phi(a^{-1}) \cdot E \lambda$. By Theorem 2.13 $(k, a) \in V^+$. Let $b = a \cdot \xi^A$. By Lemma 3.4 also $(k, b) \in V^+$. As $Za = Zb = c$, we have $a, b \in \mathcal{P}_c$. \qed

Theorem 3.15 item (iii) is independent of the rate constants. That is, given any $c \in \text{im}_+(Z)$ one can decide about multistationarity within the corresponding $\mathcal{P}_c$, even if all or some of the rate constants are unknown. In fact, as the following two corollaries show, arbitrary semi-algebraic constraints in the total concentrations $c$ can be added to the description of the multistationarity locus and a variant of Theorem 3.15 still holds. Already the case of linear inequalities is interesting (see Sect. 4).

Corollary 3.16 Assume $V^+$ admits a monomial parametrization with exponent matrix $A \in \mathbb{Q}^{(n-p) \times n}$. Let $g_1, \ldots, g_l \in \mathbb{R}[c], \square \in \{>, \ge\}^l$, and $\mathcal{F}(g(c) \square 0)$ be any logical combination of the inequalities $g(c) \square 0$. Then, there are $k \in K^+_\gamma$ and $c \in \text{im}_+(Z)$ such that
\[
Z(\psi(k) \cdot \xi^A) = c, \quad \mathcal{F}(g(c) \square 0)
\]
has at least two positive solutions $\xi_1 \neq \xi_2$, if and only if the system

$$Z(a \star A^A - a) = 0, \ F(g(Za) \square 0)$$  \hspace{1cm} (17)

has a solution $a \in \mathbb{R}_{>0}^n$ and $\xi \in \mathbb{R}_{>0}^{(n-p)}$ with $\xi \neq 1$.

**Proof** This is Theorem 3.15 (ii) and (iii) together with $c = Za$. \hfill $\square$

As a consequence of Theorem 3.14, multistationarity is possible if and only if the sign condition (16) holds. Frequently, one first asks whether multistationarity is possible at all (by checking condition (16)) before asking whether it is possible under some conditions on the total concentrations. Hence, one often computes the intersection (16) before employing Corollary 3.16. In this case, one can add the information contained in the sign pattern (16) to the system (17) of Corollary 3.16. To this end, let $\Delta$ be the set of sign patterns satisfying condition (16) and recall that, by Theorem 3.12 (a), the sign patterns satisfying (16) to the system (17) of Corollary 3.16. Further recall that, under the standing assumption that $V^+$ admits a monomial parametrization, we have $b = a \star A^A$ by Lemma 3.8. Let $\delta \in \Delta$, then

$$\text{sign}(b - a) = \delta \iff \text{sign}(\ln b - \ln a) = \delta \iff \text{sign}(\xi^A - 1) = \delta. \hspace{1cm} (18)$$

Now, we can ask whether multistationarity is possible for a given sign pattern $\delta$ and some semi-algebraic constraint on the total concentrations:

**Corollary 3.17** Assume $V^+$ admits a monomial parametrization with exponent matrix $A \in \mathbb{Q}^{(n-p) \times n}$, and that $E$ does not contain a zero row. Let $\Delta$ be the set of sign patterns from (16) with $\Delta \neq \emptyset$. Pick $\delta \in \Delta$ and let $g_1, \ldots, g_l \in \mathbb{R}[c]$, $\square \in \{>, \geq\}$, and $F(g(c) \square 0)$ be any logical combination of the inequalities $g(c) \square 0$. Then, there are $k \in K_+^+, c \in \text{im}_+(Z)$ and $a, b \in \mathbb{R}_{>0}^n$ with $a \neq b$ such that

$$(k, a) \in V^+, (k, b) \in V^+, \quad \text{sign}(b - a) = \delta \quad \text{and} \quad F(g(c) \square 0)$$

if and only if there are $a \in \mathbb{R}_{>0}^n$ and $\xi \in \mathbb{R}_{>0}^{(n-p)}$ such that

$$Z((\xi^A - 1) \star a) = 0, \quad \text{sign}(\xi^A - 1) = \delta, \quad F(g(Za) \square 0).$$

**Proof** This is Corollary 3.16 with $b - a = (\xi^A - 1) \star a$ and (18). \hfill $\square$

The next theorem shows that if there is multistationarity for some value of the total concentrations $c$, then there is also multistationarity for any rescaled $\alpha c$ ($\alpha > 0$), albeit $k$ needs to be adjusted in a nonlinear way.

**Theorem 3.18** Assume $V^+$ admits a monomial parametrization with exponent matrix $A \in \mathbb{Q}^{(n-p) \times n}$. Let $c \in \text{im}_+(Z)$. Then, the following are equivalent:

(i) There exist $a \neq b \in \mathbb{R}_{>0}^n$ and $k \in \mathbb{R}_{>0}^r$ such that $(k, a) \in V^+, (k, b) \in V^+$ and $a, b \in \mathcal{P}_c$. 

$\Box$ Springer
(ii) For every $\alpha > 0$, there exists $a(\alpha) \neq b(\alpha) \in \mathbb{R}^n_\geq$ and $k(\alpha) \in \mathbb{R}^r_\geq$ such that $(k(\alpha), a(\alpha)) \in V^+$, $(k(\alpha), b(\alpha)) \in V^+$ and $a(\alpha), b(\alpha) \in \mathcal{P}_c$.

**Proof** Let $a \neq b \in \mathbb{R}^n_\geq$ and $k \in \mathbb{R}^r_\geq$ as in (i). By Theorem 3.15, item (i) $\Rightarrow$ (iii) there exists $a \in \mathbb{R}^n_\geq$ and $\xi \neq 1 \in \mathbb{R}^{n-p}_\geq$ such that $Z(a \star \xi^A) = Za = c$. Pick $\alpha > 0$ and $\lambda \in \Lambda(E)$ and define

$$a(\alpha) = \alpha a, \quad b(\alpha) = a(\alpha) \star \xi^A \quad \text{and} \quad k(\alpha) = \Phi(a(\alpha)^{-1}) \star E\lambda.$$ 

Then $(k(\alpha), a(\alpha)) \in V^+$ by construction of $k(\alpha)$. By Lemma 3.4, this implies $(k(\alpha), b(\alpha)) \in V^+$ as well. By construction, $Za(\alpha) = Zb(\alpha) = \alpha c$. Pick $\alpha > 0$ and $\lambda \in \Lambda(E)$ and define

$$a(\alpha) = \alpha a, \quad b(\alpha) = a(\alpha) \star \xi^A \quad \text{and} \quad k(\alpha) = \Phi(a(\alpha)^{-1}) \star E\lambda.$$ 

Thus, $(k(\alpha), a(\alpha)) \in V^+$ by construction of $k(\alpha)$. By Lemma 3.4, this implies $(k(\alpha), b(\alpha)) \in V^+$ as well. By construction, $Z(a(\alpha) \star \xi^A) = Za = c$. Pick $\alpha > 0$ and $\lambda \in \Lambda(E)$ and define

$$a(\alpha) = \alpha a, \quad b(\alpha) = a(\alpha) \star \xi^A \quad \text{and} \quad k(\alpha) = \Phi(a(\alpha)^{-1}) \star E\lambda.$$ 

Then $(k(\alpha), a(\alpha)) \in V^+$ by construction of $k(\alpha)$. By Lemma 3.4, this implies $(k(\alpha), b(\alpha)) \in V^+$ as well. By construction, $Za(\alpha) = Zb(\alpha) = \alpha c$, that is, $a(\alpha), b(\alpha) \in \mathcal{P}_c$.

Vice versa, assume $a(\alpha) \neq b(\alpha) \in \mathbb{R}^n_\geq$ and $k \in \mathbb{R}^r_\geq$ are as in (ii). Then, $\alpha = 1$ yields the desired result.  

Theorem 3.18 states that a network $\mathcal{N}$ for which $V^+$ admits a monomial parametrization (and $E$ does not contain a zero row) admits multistationarity for some value $c \in \text{im}_+(Z)$, if and only if it admits multistationarity for all $\alpha c$ with $\alpha > 0$.

The next result can be used to preclude multistationarity on entire rays in the space of total concentrations.

**Corollary 3.19** Assume $V^+$ admits a monomial parametrization with exponent matrix $A \in \mathbb{Q}^{(n-p) \times n}$ and let $c \in \text{im}_+(Z)$. If the system

$$Z(a \star \xi^A) = c \quad (19)$$

does not have a solution $a \in \mathbb{R}^n_\geq$, $\xi \neq 1 \in \mathbb{R}^{n-p}_\geq$, then there do not exist $k \in K_\gamma^+$ and $\alpha \in \mathbb{R}_\geq$ such that the system

$$Z(\psi(k) \star \xi^A) = \alpha c \quad (20)$$

has at least two solutions $\xi_1 \neq \xi_2 \in \mathbb{R}^p_\geq$.

**Proof** This is Theorem 3.18 together with 3.15.  

**Remark 3.20** The scaling invariance in the previous results can be reformulated in terms of cones. For this, let $s = \text{dim}(\text{im}(S))$ and denote by $S^{n-s-1} \subset \mathbb{R}^{n-s}$ the unit sphere. Define the set of all total concentrations $c \in \text{im}_+(Z)$ for which the network admits multistationarity (for some value of the rate constants $k$):

$$\mathcal{C} = \{ c \in \text{im}_+(Z) \mid \exists k \in K_\gamma^+ \quad \text{and} \quad a \neq b \in \mathbb{R}^n_\geq \text{s.t.} (k, a), (k, b) \in V^+, \text{ and } a, b \in \mathcal{P}_c \}. $$

By the Tarski–Seidenberg Theorem (Coste 2002, Theorem 2.3), $\mathcal{C}$ is a semi-algebraic set. We have shown that (except the missing origin) it is a cone: if $c \in \mathcal{C}$, then by Theorem 3.18 $\alpha c \in \mathcal{C}$, $\forall \alpha > 0$, i.e.,

$$\mathcal{C} = \left( \mathcal{C} \cap S^{n-s-1} \right) \times \mathbb{R}_\geq.$$
Remark 3.21 As a consequence of Theorem 3.18, for systems that admit a monomial parametrization, if there exist rate constants such that multistationarity occurs for some value \( c \) of the total concentrations, then there exist rate constants, such that it occurs for arbitrarily small values \( \alpha c, \alpha \ll 1 \). That is, multistationarity persists for arbitrarily small total concentrations, as long as the ratios \( \frac{c_i}{c_j} \) remain constant.

4 Multistationarity Conditions on the Total Concentrations for Sequential and Distributive Phosphorylation

In this section, we apply the results of Sect. 3 to networks describing the sequential and distributive phosphorylation of a protein. Our results complement recent results of Bihan et al. (2018). Their Theorem 4.1 states that, for any \( n \geq 2 \), if the total concentration of substrates is greater than the sum of the concentrations of phosphatase and intermediate products with phosphatase, then there is a choice of rate constants for which multistationarity is attained. Our results are also on the total concentrations and motivate the inequalities using the chamber decomposition as a natural, intrinsic subdivision of the cone of values of the total concentrations.

4.1 Sequential Distributive Phosphorylation of a Protein

Phosphorylation processes are frequently encountered in the modeling of biochemical processes; see, for example, Conradi and Shiu (2018) and the references therein. The following network models the phosphorylation of a protein \( A \) at \( n \) binding sites in a sequential and distributive way:

\[
\begin{align*}
A + K & \xleftrightarrow[k_2]{k_1} AK & \xrightarrow[k_3]{k_2} A_p + K \\
A_p + P & \xleftrightarrow[l_2]{l_1} A_P & \xrightarrow[l_3]{l_2} A + P \\
\vdots & & \\
A_{p^{(n-1)}} + K & \xleftrightarrow[k_3n-1]{k_3n-2} A_{p^{(n-1)}} & \xrightarrow[k_3n]{k_3n} A_{p^{(n)}} + K \\
A_{p^{(n)}} + P & \xleftrightarrow[l_3n-1]{l_3n-2} A_{p^{(n)}} & \xrightarrow[l_3n]{l_3n} A_{p^{(n-1)}} + P
\end{align*}
\]

\[ (N_n) \]

The first two connected components of network \( N_n \) form network \( (N_1) \) from Example 2.1 (after the change in variables \( X_1 = K, X_2 = A, X_3 = AK, X_4 = A_p, X_5 = P, X_6 = A_{pP} \)). In this sense, \( N_n \) extends \( N_1 \) to \( n \) phosphorylation steps. Due to their biochemical importance, such networks have been extensively studied in mathematical biology. For example, it is known that \( N_n \) is multistationary if and only if \( n \geq 2 \) (Holstein et al. 2013). For \( n = 2 \), there are known sufficient conditions on the rate constants for the presence or absence of multistationarity and it is known that
the number of positive steady states is 1, 2, or 3 (Conradi and Mincheva 2014). For \( n > 2 \), there are bounds on the maximum number of positive steady states that can be attained (Wang and Sontag 2008; Flockerzi et al. 2014).

The aim of this section is to describe the multistationarity locus in the space of total concentrations. The strongest results are available for the \( n = 2 \) case which we consider first:

\[
\begin{align*}
A + K & \underset{k_1}{\overset{k_2}{\longrightarrow}} AK \underset{k_3}{\rightarrow} A_p + K \\
A_{pp} + P & \underset{k_7}{\overset{k_8}{\longrightarrow}} A_{pp} P \underset{k_9}{\rightarrow} A_p + P \\
A_{pp} & \underset{k_6}{\overset{k_5}{\rightarrow}} A_{pp} K \underset{k_{10}}{\overset{k_{11}}{\rightarrow}} A_{pp} P \underset{k_{12}}{\rightarrow} A + P
\end{align*}
\]

(A2)

If all reactions of (A2) are of mass-action form, we obtain the following set of ODEs. Here, \( x_1 \) denotes the concentration of \( A \), \( x_2 \) of \( K \), \( x_3 \) of \( AK \), \( x_4 \) of \( A_p \), \( x_5 \) of \( A_p K \), \( x_6 \) of \( A_{pp} \), \( x_7 \) of \( P \), \( x_8 \) of \( A_{pp} P \) and \( x_9 \) of \( A_p P \).

\[
\begin{align*}
\dot{x}_1 &= f_1(x_1, \ldots, x_9) = -k_1 x_1 x_2 + k_2 x_3 + k_12 x_9 \\
\dot{x}_2 &= f_2(x_1, \ldots, x_9) = -k_1 x_1 x_2 + (k_2 + k_3) x_3 - k_4 x_2 x_4 + (k_5 + k_6) x_5 \\
\dot{x}_3 &= f_3(x_1, \ldots, x_9) = k_1 x_1 x_2 - (k_2 + k_3) x_3 \\
\dot{x}_4 &= f_4(x_1, \ldots, x_9) = k_3 x_3 - k_4 x_2 x_4 + k_5 x_5 + k_9 x_8 - k_{10} x_4 x_7 + k_{11} x_9 \\
\dot{x}_5 &= f_5(x_1, \ldots, x_9) = k_4 x_2 x_4 - (k_5 + k_6) x_5 \\
\dot{x}_6 &= f_6(x_1, \ldots, x_9) = k_6 x_5 - k_7 x_6 x_7 + k_8 x_8 \\
\dot{x}_7 &= f_7(x_1, \ldots, x_9) = -k_7 x_6 x_7 + (k_8 + k_9) x_8 - k_{10} x_4 x_7 + (k_{11} + k_{12}) x_9 \\
\dot{x}_8 &= f_8(x_1, \ldots, x_9) = k_7 x_6 x_7 - (k_8 + k_9) x_8 \\
\dot{x}_9 &= f_9(x_1, \ldots, x_9) = k_{10} x_4 x_7 - (k_{11} + k_{12}) x_9.
\end{align*}
\]

There are three independent linear relations among the polynomials \( f_1, \ldots, f_9 \) and thus three linearly independent conserved quantities under the dynamics of the network:

\[
\begin{align*}
x_2 + x_3 + x_5 &= c_1, \\
x_7 + x_8 + x_9 &= c_2, \\
x_1 + x_3 + x_4 + x_5 + x_6 + x_8 + x_9 &= c_3.
\end{align*}
\]

(21)

In (21) above, \( c_1 \) represents the total amount of kinase \( K \), \( c_2 \) the total amount of phosphatase \( P \) and \( c_3 \) the total amount of protein \( A \), the substrate. Relations (21) are the rays of the cone of conservation relations. According to (21), we can choose the conservation matrix as

\[
Z = \begin{bmatrix}
0 & 1 & 1 & 0 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 \\
1 & 0 & 1 & 1 & 1 & 0 & 1 & 1 & 1
\end{bmatrix}.
\]

(22)
In Pérez-Millán et al. (2012), it has been shown that the positive steady-state variety $V^+$ of $(\mathcal{N}_2)$ admits a monomial parametrization of the form

$$x = \psi(k) \star \xi^A$$

with $k \in \mathbb{R}_{>0}^{12}$ and $\xi \in \mathbb{R}_{>0}^3$ free,

where

$$\psi(k) = \frac{(k_2 + k_3) k_4 k_6 (k_{11} + k_{12}) k_{12}}{k_1 k_3 (k_5 + k_6) k_9 k_{10}} \cdot \frac{(k_5 + k_6) k_9 k_{10}}{k_3} \cdot \frac{k_{11} + k_{12}}{k_{10}} \cdot \frac{k_9}{k_6} \cdot \frac{k_8 + k_9}{k_7} \cdot 1, 1, 1 \right)^T$$

and

$$A = \begin{bmatrix} 2 & -1 & 1 & 1 & 0 & 0 & 0 & 1 \\ -1 & 1 & 0 & 0 & 1 & 1 & 0 & 1 \\ -1 & 1 & 0 & -1 & 0 & -1 & 1 & 0 \end{bmatrix}. \tag{23}$$

### 4.2 A Numerical Study of Multistationarity in the Space of Total Concentrations

We did a numerical study of multistationarity in the space of total concentrations which is depicted in Fig. 1a. For this computation, the rate constants have been numerically

![Numerical computation with Paramotopy](image)

**Fig. 1** (Color figure online) Representation of regions of multistationarity in the space of total concentrations for $\mathcal{N}_2$. For both figures, all rate constants have been fixed to the values given in Conradi and Mincheva (2014, Fig. 3)
fixed to the values in Conradi and Mincheva (2014, Fig. 3). The computation was done using Paramotopy (Brake and Niemberg 2016) which builds on Bertini (Bates et al. (2006)) and allows one to efficiently analyze the solutions of a polynomial systems with unknown coefficients (a parametric polynomial system). We computed the isolated solutions for each point in the grid $[0, 1000]^3 \cap (10\mathbb{Z})^3$ and plotted those which yield multistationarity. An alternative approach is through the discriminant which in this case can be found with Maple (Maplesoft 2017). A discriminant of a parametric semi-algebraic system is a polynomial which vanishes in those points of the parameter space where the solution behavior can change. For an extensive discussion of discriminants with a special emphasis on computation, we refer to Lazard and Rouillier (2007). Discriminants for multistationarity have also appeared in Gross et al. (2016, Section 4).

Two relevant irreducible components of the discriminant of the parametric system are visualized in Fig. 1b. The algebraic boundary of the region from Fig. 1a is a subvariety of the discriminant from Fig. 1b. Specifically, the cone shaped region in Fig. 1a is also visible in the top center of Fig. 1b. Both figures indicate that, for the values of the rate constants chosen in Conradi and Mincheva (2014, Fig. 3), multistationarity does not occur for all values of the total concentrations. In the next section, we employ the results of Sect. 3.2 to elucidate conditions on the total concentrations for the presence or absence of multistationarity.

4.3 The Chamber Decomposition of $\text{im}_+(Z)$ for $\mathcal{N}_2$ and $\mathcal{N}_n$

Using the sets $\text{im}_+(Z)$ and $\mathcal{P}_c$ from (4) and (5), we now introduce the chamber decomposition of $\text{im}_+(Z)$ induced by the columns of the conservation matrix $Z$. We assume that $Z$ is of full row rank $n - s$ and call a subset of $n - s$ linear independent columns a basis of $\text{im}_+(Z)$. Each basis $B$ defines a basic cone $\text{cone}(B)$ consisting of nonnegative linear combinations of the columns in $B$.

**Definition 4.1** The chamber complex of a matrix $Z$ is the common refinement of the basic cones of all its bases. More precisely, $c_1$ and $c_2$ are in the same chamber of the chamber complex if and only if

$$c_1 \in \text{cone}(B) \Leftrightarrow c_2 \in \text{cone}(B)$$

for all bases $B$ of $Z$.

**Remark 4.2** The chamber complex is important in linear programming as it classifies the different combinatorial types that the polyhedron $\mathcal{P}_c$ can take for any $c$: Within one chamber, all polyhedra $\mathcal{P}_c$ are combinatorially equal, that is, their face lattices are the same. See (De Loera et al. 2009, Section 2.1) for an interpretation of the vertices of $\mathcal{P}_c$ in this context. Chamber complexes can be computed with polyhedral geometry software such as POLYMAKE (Gawrilow and Joswig 2000) or TOPCOM (Rambau 2002). Chambers are also related to siphons of chemical reactions (Shiu and Sturmfels 2010). A chamber complex of a slightly different type appears in Craciun et al. (2009), where every basic cone encodes a possible reaction network among a given finite set of experimentally indistinguishable networks. We believe that the chamber complex is an interesting structure to study for different chemical reaction networks.
The polyhedron $\mathcal{P}_c$ is defined by the matrix $Z$ from (22). The cone generated by the columns of $Z$ is $\mathbb{R}^3 \geq 0$. There are eight basic cones generated by the following sets of columns of $Z$:

$$
\{1, 2, 7\}, \{1, 2, 8\}, \{1, 3, 7\}, \{1, 3, 8\}, \{2, 3, 7\}, \{2, 3, 8\}, \{2, 7, 8\}, \{3, 7, 8\}.
$$

Any of the basic cones is the intersection of three linear half-spaces of $\mathbb{R}^3$, and each of these half-spaces is spanned by exactly two of the three columns (see Ziegler 2012, Section 1.1 for more details on polyhedra). For example, the cone generated by the columns of $\{1, 2, 7\}$ of $Z$ is $\mathbb{R}^3 \geq 0$ and equals the intersection of the half-spaces $c_1 \geq 0$, $c_2 \geq 0$, and $c_3 \geq 0$. There are six distinct planes occurring among the defining hyperplanes of all cones: $c_1 = 0$, $c_2 = 0$, $c_3 = 0$, $c_1 = c_3$, $c_2 = c_3$, and $c_1 + c_2 = c_3$. These planes divide $\mathbb{R}^3 \geq 0$ into five full-dimensional cones. The interiors of these cones are the full-dimensional chambers of $\mathbb{R}^3 \geq 0$. See Fig. 2 for a two-dimensional representation of this chamber decomposition. There are also smaller dimensional chambers: the interiors of the faces of the full-dimensional chambers.

For $n \geq 2$, the chamber complex does not change:

**Theorem 4.3** The cone of conservation relations of $\mathcal{N}_n$ is $\mathbb{R}^3 \geq 0$, and it has five full-dimensional chambers:

\[
\begin{align*}
\Omega(1) : & \begin{cases} c_3 > 0 \\
c_2 > c_3 \\
c_1 > c_3, 
\end{cases} & \Omega(2) : & \begin{cases} c_1 > 0 \\
c_2 > c_3 \\
c_1 < c_3, 
\end{cases} & \Omega(3) : & \begin{cases} c_2 > 0 \\
c_2 < c_3 \\
c_1 > c_3, 
\end{cases} \\
\Omega(4) : & \begin{cases} c_2 < c_3 \\
c_1 + c_2 > c_3, 
\end{cases} & \Omega(5) : & \begin{cases} c_2 > 0 \\
c_1 + c_2 < c_3. 
\end{cases}
\end{align*}
\]

**Proof** As described in Holstein et al. (2013, Section 3), the conservation matrix of $\mathcal{N}_n$, for the ordering of the concentrations defined in Holstein et al. (2013, Table 1), has the form $Z^{(n)} = (Z_0|Z_1|\ldots|Z_1) \in \mathbb{R}^{3 \times (3n+3)}$, where $Z_1$ is repeated $n$ times and
\[ Z_0 = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 0 & 1 \\ 0 & 1 & 0 \end{bmatrix}, \quad Z_1 = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 0 & 1 \\ 1 & 1 & 1 \end{bmatrix}. \]

As \( Z^{(n)} \) has the same set of columns for every \( n \geq 1 \), it follows that all chamber decompositions of all \( N_n \) are equal. \( \square \)

**Remark 4.4** Although the ordering of variables defined in Holstein et al. (2013, Table 1) is different from the one we use with \( N_2 \), a reordering of the variables corresponds to a reordering of the columns of \( Z^{(n)} \), and thus, it leaves the chamber decomposition invariant.

**Remark 4.5** Although \( N_n \) has the same chamber complex for each \( n \), the constants \( c \) express nonnegative linear combinations of the concentrations specific to each network.

### 4.4 Multistationarity Conditions in the Space of Total Concentrations

Now, we turn to \( n = 2 \) and \( N_n \) and employ Corollary 3.17 to decide whether multistationarity is possible for total concentrations in the chambers \( \Omega(i) \). The linear inequality conditions \( c \in \Omega(i) \) become the conditions \( F(\bullet) \) in Corollary 3.17. We also integrate the information in the sign patterns in the intersection (16). These have been computed in Conradi et al. (2008) and are encoded as rows of the following matrix \( \Delta \) (or their negatives):

\[ \Delta = \begin{bmatrix} -1 & -1 & -1 & 1 & 1 & 1 & -1 & 1 & -1 \\ 1 & 0 & 1 & -1 & -1 & -1 & 1 & -1 & 1 \\ 1 & -1 & 1 & 1 & -1 & -1 & 1 & -1 & 1 \\ 1 & -1 & 1 & 1 & -1 & -1 & 0 & -1 & 1 \\ 1 & -1 & 1 & 1 & -1 & -1 & -1 & -1 & 1 \\ 1 & -1 & 1 & 0 & -1 & -1 & 1 & -1 & 1 \\ 1 & -1 & 1 & -1 & -1 & -1 & 1 & -1 & 1 \end{bmatrix}. \] (24)

The rows \( \delta_i \) of \( \Delta \) define the conditions \( \text{sign}(\xi^A - 1) = \delta_i, i = 1, \ldots, 7 \) of Corollary 3.17. Using the matrix \( A \) from (23), this condition reads

\[ \text{sign}\left(\frac{\xi_1^2}{\xi_2 \xi_3} - 1, \frac{\xi_2 \xi_3}{\xi_1} - 1, \xi_1 - 1, \frac{\xi_1}{\xi_3} - 1, \xi_2 - 1, \frac{\xi_2}{\xi_3} - 1, \xi_3 - 1, \xi_2 - 1, \xi_1 - 1\right) = \delta_i. \] (25)

To check multistationarity for \( c \) in all of the chambers \( \Omega(i) \), we use Mathematica (Wolfram Research, Inc 2017). For each chamber and each row \( \delta_i \), we set up the conditions of Corollary 3.17 and use the command \texttt{Reduce} to decide the existence of solutions. In the following example, we show how to set up the Mathematica code to check multistationarity.

**Example 4.6** We check multistationarity via Corollary 3.17 for \( \Omega(1) \) and \( \delta = (1, 1, 1, -1, -1, -1, 1, -1, 1) \) (the negative of the first row of \( \Delta \) from (24)). First, we formulate...
the three conditions \( Z((\xi^A - 1)\ast a) = 0 \), \( \text{sign}(\xi^A - 1) = \delta \) and \( F(g(Za)) \uplus 0 \) of the corollary:

- \( \text{sign}(\xi^A - 1) = \delta \): After adding the constraints \( \xi_1 > 0 \), \( \xi_2 > 0 \), \( \xi_3 > 0 \) and removing redundant inequalities, (25) reduces to

\[
1 < \xi_1 < \xi_2 \xi_3 < \xi_1^2 \text{ and } 0 < \xi_2 < 1.
\]  

(26)

- \( F(g(Za) \uplus 0) \): We want to encode \( c \in \Omega(1) \). By Theorem 4.3, this is equivalent to \( c_3 > 0 \), \( c_1 > c_3 \) and \( c_2 > c_3 \). By (21), \( c_1 = a_2 + a_3 + a_5 \), \( c_2 = a_7 + a_8 + a_9 \) and \( c_3 = a_1 + a_3 + a_4 + a_5 + a_6 + a_8 + a_9 \). The condition \( a_i > 0 \) then implies \( c_3 > 0 \) and we only need to account for the remaining inequalities:

\[
\begin{align*}
& a_1 - a_2 + a_4 + a_6 + a_8 + a_9 < 0 \text{ (for } c_1 > c_3) \text{ and } \\
& a_1 + a_3 + a_4 + a_5 + a_6 - a_7 < 0 \text{ (for } c_2 > c_3).
\end{align*}
\]  

(27)

- In the condition \( Z((\xi^A - 1)\ast a) = 0 \), we use the matrix

\[
Z' = \begin{bmatrix}
0 & 1 & 1 & 0 & 1 & 0 & 0 & 0 \\
1 & 0 & 1 & 1 & 1 & -1 & 0 & 0 \\
1 & -1 & 0 & 1 & 0 & 1 & 1
\end{bmatrix},
\]  

(28)

obtained from (22) by elementary row operations (compared to \( Z \) from (22), we found that computation times are significantly shorter when \( Z' \) is used, cf. Remark 4.9). To obtain polynomial conditions (\( \xi^A \) is rational), we write this condition as \( Z'(\xi^A \ast a) = Z'a \) and clear denominators:

\[
\begin{align*}
\xi_2 \xi_3 a_2 + \xi_1^2 a_3 + \xi_1 \xi_2 a_5 &= \xi_1 (a_2 + a_3 + a_5) \\
\xi_1^3 a_1 + \xi_1 \xi_2 \xi_3 a_3 + \xi_1 \xi_2 a_4 + \xi_2^2 \xi_3 a_5 + \xi_2^2 a_6 - \xi_2^2 \xi a_7 \\
&= \xi_2 \xi_3 (a_1 + a_3 + a_4 + a_5 + a_6 - a_7) \\
\xi_1^3 a_1 - \xi_2^2 \xi_3 a_2 + \xi_2^2 \xi_2 a_4 + \xi_3^2 a_6 + \xi_1 \xi_2 \xi_3 a_8 + \xi_1^2 \xi_2 a_9 \\
&= \xi_1 \xi_2 \xi_3 (a_1 - a_2 + a_4 + a_6 + a_8 + a_9)
\end{align*}
\]  

(29)

The following Mathematica code can be used to decide the existence of \( \xi_i \) and \( a_i \) satisfying the conditions (26), (27) and (29) together with the condition \( a_i > 0 \) (\( x_1, x_2, x_3 \) are shorthand for the variables \( \xi_1, \xi_2, \xi_3 \)):

\begin{verbatim}
Reduce[Exists[{a1,a2,a3,a4,a5,a6,a7,a8,a9},
a1>0 && a2>0 && a3>0 && a4>0 && a5>0 && a6>0 && a7>0 && a8>0 && a9>0 &&
x2*x3*a2 + x1^2*a3 + x1*x2*a5 == x1*(a2+a3+a5) &&
x1^2*a1 + x1*x2*x3*a3 + x1*x2*a4 + x2^2*a3*a5 + x2^2*a6 - x2*x3^2*a7 == x2*x3*(a1+a3+a4+a5+a6-a7) &&
x1^3*a1 - x2^2*x3^2*a2 + x1^2*x2*a4 + x1*x2^2*a6 + x1*x2^2*x3*a8 + x1^2*x2*x3*a9 == x1*x2*x3*
\end{verbatim}
Table 1  The chamber-signs incidence table of \((N_n)\). In particular, multistationarity is not possible in \(\Omega(1)\)

| \(\delta_1\) | \(\delta_2\) | \(\delta_3\) | \(\delta_4\) | \(\delta_5\) | \(\delta_6\) | \(\delta_7\) |
|-----------|------------|------------|------------|------------|------------|------------|
| \(\Omega(1)\) | – | – | – | – | – | – |
| \(\Omega(2)\) | – | – | + | + | ++ | + | ++ |
| \(\Omega(3)\) | ++ | + | ++ | – | – | + | + |
| \(\Omega(4)\) | ++ | + | + | + | ++ | |
| \(\Omega(5)\) | ++ | + | + | + | + | + | + |

\((a_1-a_2+a_4+a_6+a_8+a_9) \&\& a_1+a_3+a_4+a_5+a_6-a_7<0 \&\& a_1-a_2+a_4+a_6+a_8+a_9<0 \&\& x_2*x_3<x_1^2 \&\& x_1<x_2*x_3 \&\& 1<x_1 \&\& 1>x_2 \&\& x_2>0 \;\)\]

The computation takes a few hours, but then the result is ‘False’, that is, there do not exist \(a_1, \ldots, a_9\) satisfying the constraints, no matter what the values of \(\xi_1, \xi_2, \xi_3\) are. Consequently, in the chamber \(\Omega(1)\) there is no multistationarity coming from the first row of \(\Delta\). Theorem 4.7 below shows that there is no multistationarity in \(\Omega(1)\) at all.

Theorem 4.7 spells out for which chambers and which sign patterns there is multistationarity. For a pair \((\Omega(i), \delta_j)\), we write + if there is multistationarity in \(\Omega(i)\) for all values of \(\xi\) compatible with (25). We write ++ if there is multistationarity in \(\Omega(i)\) with extra conditions for \(\xi\) stronger than (25). We write – if there is no multistationarity. If the computation does not finish in reasonable time, we leave the cell empty.

Theorem 4.7  Up to the three empty cells, the chambers-signs incidence table of \((N_n)\) is Table 1. For the ++ entries, the following additional constraints are derived:

\((\Omega(2), \delta_7)\) : \(0 < \xi_3 < \xi_1 < 1 \land \xi_2 > \xi_1^2 / \xi_3^2\),
\((\Omega(2), \delta_5)\) : \(\xi_3 > 1 \land 0 < \xi_1 < 1 \land \xi_2 > \xi_1^2 / \xi_3^2\),
\((\Omega(4), \delta_5)\) : \(\xi_3 > 1 \land 0 < \xi_1 < 1 \land \xi_2 > \xi_1^2 / \xi_3^2\),
\((\Omega(3), \delta_3)\) : \(\xi_1^2 < \xi_3 < 1 \land \xi_2 > 1\),
\((\Omega(3), \delta_1)\) : \(\xi_3 > 1\)
\(\land \left( \left( 1 < \xi_1 < \xi_3^{2/3} \land \xi_1 / \xi_3 < \xi_2 < \xi_1^3 / \xi_3^3 \right) \lor \left( \xi_3^{2/3} < \xi_1 < \xi_3 \land \xi_1 / \xi_3 < \xi_2 < 1 \right) \right) \),
\((\Omega(4), \delta_1)\) : \(\xi_3 > 1\)
\(\land \left( \left( 1 < \xi_1 < \xi_3^{2/3} \land \xi_1 / \xi_3 < \xi_2 < \xi_1^3 / \xi_3^3 \right) \lor \left( \xi_3^{2/3} < \xi_1 < \xi_3 \land \xi_1 / \xi_3 < \xi_2 < 1 \right) \right) \),
\((\Omega(5), \delta_1)\) : \(\xi_3 > 1\)
\(\land \left( \left( 1 < \xi_1 < \xi_3^{1/2} \land \xi_1 / \xi_3 < \xi_2 < \xi_1^2 / \xi_3^2 \right) \lor \left( \xi_3^{1/2} < \xi_1 < \xi_3 \land \xi_1 / \xi_3 < \xi_2 < 1 \right) \right) \).
Computational Proof The quantifier elimination problems were set up similarly to Example 4.6 and solved using Mathematica.

Remark 4.8 To obtain Table 1, some of the computations were made indirectly. For example, we checked that for $\delta_1$ multistationarity does not take place in $\Omega(1)$ but we could not check directly that it does not take place in $\Omega(2)$, as the computations did not finish within one to five days. We therefore checked that it does not take place in $\Omega(1) \cup \Omega(2) \cup \Omega(1, 2)$, where $\Omega(1, 2)$ denotes the boundary between $\Omega(1)$ and $\Omega(2)$. This computation was feasible. It is an interesting computational challenge to classify all boundaries between chambers.

Remark 4.9 The quantifier elimination problems arising from the analysis of multistationarity have additional structure that should be exploited. In particular, the run times of our computations seem to be sensitive to the formulation of the input. We experimented with different equivalent semi-algebraic systems in Mathematica. One knob to turn is the system $Z((\xi A - 1) \bullet a) = 0$ in Corollary 3.17. Different bases for the row space of $Z$ lead to different run times. Consider the pair $(\Omega(4), \delta_4)$ and let $R_1, R_2, and R_3$ be the rows of $Z$ from eq. (22). Let $Z_1 = [(R_1 + R_2 + R_3)^T | (R_2 + R_3)^T | R_3^T]^T$, $Z_2 = [R_1^T | R_2^T | (R_3 - R_1 - R_2)^T]^T$, and $Z_3 = [R_1^T | (R_3 - R_2)^T | (R_3 - R_1)^T]^T$. Using in Corollary 3.17 the matrix $Z$ from (22), the computation takes about seven seconds while with either of $Z_1, Z_2, and Z_3$ the computation did not finish within 24 hours. It is tempting to think that the computations with the matrix $Z$ are faster because it is in row echelon form; however, this is not the case: For the pair $(\Omega(1), \delta_1)$, the computation with $Z$ did not finish in several days while the computation with $Z_3$ finished within a few hours.

Remark 4.10 Since in Corollary 3.17 we are only interested in the positive solutions of the system $Z((\xi A - 1) \bullet x) = 0$, clearing denominators does not add any new solutions. Let $\varsigma(Z, \xi A, \delta, x)$ denote the system obtained from $Z((\xi A - 1) \bullet x) = 0$ and $\delta$, by clearing denominators. If $Z'$ and $A'$ are matrices obtained by performing elementary row operations on $Z$ and $A$, respectively, then $\varsigma(Z, \xi A, \delta, x)$ and $\varsigma(Z', \xi A', \delta, x)$ have the same set of positive solutions (they are equivalent systems), yet they are not linearly equivalent systems.

Remark 4.11 Throughout, we found Mathematica to have the fastest implementation of quantifier elimination. It would be nice to implement heuristics for pre-simplification, e.g., along the lines of Brown and Strzeboński (2010), in open-source systems such as qepcadB (Brown 2003) or REDLOG (Dolzmann and Sturm 1997). The performance of quantifier elimination on systems from biology has been explored in Bradford et al. (2017).

The first row of Table 1 shows that multistationarity is only possible if $c \notin \Omega(1)$:

Corollary 4.12 For $(N_2)$, if $c \in \Omega(1)$, then there is no $k \in \mathbb{R}_{>0}^{12}$ such that the equations

$$S(v(k, x) = 0, \quad Zx = c$$

have at least two positive solutions.
Together with results of Bihan, Dickenstein, and Giaroli, we almost obtain a characterization of multistationarity for 2-site phosphorylation in the total concentration coordinates. The regions of multistationarity are polyhedral, and the only unresolved cases are when \( c_2 = c_3, c_1 = c_3, \) or both.

**Corollary 4.13** In the 2-site phosphorylation network, multistationarity is impossible if \( c_3 < c_2 \) and \( c_3 < c_1 \), and possible if \( c_3 > c_2 \) or \( c_3 > c_1 \). If \( S_{tot} \) is the total concentration of substrate, \( F_{tot} \) that of phosphatase, and \( E_{tot} \) that of kinase, then multistationarity is impossible if \( S_{tot} < E_{tot} \) and \( S_{tot} < F_{tot} \) and possible if \( S_{tot} > E_{tot} \) or \( S_{tot} > F_{tot} \).

**Proof** Theorem 4.1 in Bihan et al. (2018) says (in our notation) that if \( c_3 > c_2 \), then there is a choice of rate constants \( k \) for which there is multistationarity. There is an inherent symmetry of the system, exchanging \( x_2, x_3, x_5 \) with, respectively, \( x_7, x_8, x_9 \). Under this symmetry, the mathematical properties are unchanged, but \( c_1 \) and \( c_2 \) change roles. Therefore, there is multistationarity also if just \( c_3 > c_1 \). Theorem 4.7 shows that if both \( c_1 < c_3 \) and \( c_2 < c_3 \), then multistationarity is impossible. \( \square \)

To characterize multistationarity for \((N_2)\), the following boundary cases remain
- \( c_3 = c_2 \) and \( c_1 < c_3 \),
- \( c_3 < c_2 \) and \( c_1 = c_3 \),
- \( c_3 = c_2 \) and \( c_1 = c_3 \).

We attempted this classification using computations as in Example 4.6. For several combinations of signs and items above, we could rule out multistationarity, but no conclusions were possible. One region we could not rule out was sign \( \delta_7 \) combined with \( c_1 = c_2 = c_3 \). For this instance, we employed the numerical solver \texttt{scip} (Gleixner et al. 2018). It could not find a solution to the corresponding inequality system, and the run on the computation indicates that multistationarity is impossible in this region too. From these computational experiences, we conjecture that multistationarity is impossible in the boundary regions.

### 5 On the Existence of Monomial Parametrizations for \( V^+ \)

Definition 3.1 uses the strictly positive steady states. This differs from the definition of toric steady states, which uses the steady-state ideal and thus poses restrictions on all complex solutions of the steady-state equations. Example 5.1 demonstrates the, maybe unsurprising, fact that the positive real part can have a monomial parametrization while the whole steady-state variety does not. We discuss these phenomena in the context of decompositions of binomial ideals (Eisenbud and Sturmfels 1996; Kahle and Miller 2014).

It follows from Eisenbud and Sturmfels (1996, Corollary 1.2) that a binomial Gröbner basis of the steady-state ideal is sufficient for toric steady states and thus a monomial parametrization of the positive steady states (by Proposition 5.2). A binomial steady-state ideal, however, is not necessary for this. The steady-state ideal may possess primary components that are irrelevant to the positive real part. We first illustrate this fact with an example.
Example 5.1 Let $T$ be the following triangular network (Pérez-Millán et al. 2012, Example 2.3):

\[
\begin{align*}
\text{2X}_1 \xrightarrow{1} & \text{X}_1 + \text{X}_2 \\
\text{2X}_2 \xleftarrow{1} & \text{X}_1 + \text{X}_2
\end{align*}
\]

Let $x_i$ denote the concentration of $X_i$. The steady-state ideal of network $T$ is $I_1 = \langle x_1^2 - x_2^2 \rangle = \langle x_1 - x_2 \rangle \cap \langle x_1 + x_2 \rangle$. The Zariski closure of the positive steady-state variety $\bar{V^+}_{x_1} = \mathbb{V}(x_1 - x_2)$ has exactly one irreducible component defined by one binomial and is thus a toric variety. It has a monomial parametrization $x_1 = x_2 = s$, for $s \in \mathbb{R}$. Restricting this monomial parametrization to the interior of the positive orthant yields a parametrization for $V^+_1$ (see Fig. 3a). Let

\[
I_2 = I_1 \cap (x_1 + x_2 + 1) = \langle x_1 - x_2 \rangle \cap (x_1 + x_2) \cap (x_1 + x_2 + 1)
\]

\[
= \langle -x_1^3 - x_1^2 x_2 + x_1 x_2^3 + x_2^3 - x_1^2 + x_2^2 \rangle.
\]

Clearly, $I_2$ is not binomial; $I_2$ is the intersection of two prime binomial ideals and a prime trinomial ideal. Geometrically, the intersection of ideals corresponds to taking the union of the corresponding varieties as in Fig. 3b. Only the component $\mathbb{V}(x_1 - x_2)$ of $\bar{V}(I_2)$ intersects the interior of the positive orthant. Still, $I_2$ can be the steady-state ideal of some mass-action network. According to Érdei and Tóth (1989, Section 4.7.1.1), a mass-action network is described by a system of ODEs of the form $\dot{x} = f$, where $f \in \mathbb{R}[x]^n$, if and only if every negative term in $f_i$ is divisible by the variable $x_i$. This condition is fulfilled by the following system of ODEs:

\[
\begin{align*}
\dot{x}_1 = -\dot{x}_2 = -x_1^3 - x_1^2 x_2 + x_1 x_2^3 + x_2^3 - x_1^2 + x_2^2.
\end{align*}
\]

One network whose steady-state ideal is equal to $I_2$ is $S$:

\[
\begin{align*}
3X_1 \xleftarrow{2/3} & 2X_1 + X_2 \\
1/9 \xleftarrow{} & 1/9 \\
2 \xrightarrow{} & 2 \\
3X_2 \xleftarrow{2/3} & X_1 + 2X_2
\end{align*}
\]

Summarizing, the steady-state variety $\bar{V}(I_2)$ has three irreducible components, but only $\mathbb{V}(x_1 - x_2)$ intersects the interior of the positive orthant. Since $V^+_1 = V^+_2$, the positive steady-state varieties of $T$ and $S$ share the parametrization $x_1 = x_2 = s$, for $s \in \mathbb{R}_{>0}$.

The following proposition uses (Eisenbud and Sturmfels 1996, Section 2) to show why the name toric steady states is justified. We include it, as it seems to have never appeared explicitly in the literature. Proposition 5.2 below shows that if the steady state
The variety $V(x_1^2 - x_2^2) = V(x_1 - x_2) \cup V(x_1 + x_2)$ of $T$ from Example 5.1. $S$ has toric steady states as its steady state ideal is binomial and $V_1^+$ is nonempty and irreducible (see [43, Definition 2.2]). $V_1^+$ is parametrized by $s \mapsto (s, s)$, for $s \in \mathbb{R}^>0$.

**Proposition 5.2** If $I \subseteq \mathbb{R}[x]$ is a binomial ideal, then at most one of the irreducible components of its variety intersects $\mathbb{R}^n_{>0}$.

**Proof** Without loss of generality, we can assume that $I = I : (x_1 \ldots x_n)^\infty$ and $I = I\mathbb{R}[x^\pm] \cap \mathbb{R}[x]$ as all other components are contained in coordinate hyperplanes. By Eisenbud and Sturmfels (1996, Corollary 2.5),

$$I = I_+(\rho) = \langle x^{m^+} - \rho(m)x^{m^-} : m \in L_\rho \rangle$$

for a unique lattice $L \subset \mathbb{Z}^n$ and partial character $\rho : L \to \mathbb{R}^*$. By Eisenbud and Sturmfels (1996, Corollary 2.2), $I_+(\rho)$, seen as an ideal of $\mathbb{C}[x]$, is radical and it has a decomposition into prime ideals as

$$I_+(\rho) = \cap_{j=1}^g I_+(\rho_j),$$

where $\{\rho_1, \ldots, \rho_g\}$ is the set of extensions of $\rho$ to the saturation $\text{Sat}(L)$ of $L$ and $g$ is the order of the group $\text{Sat}(L)/L$. A variety $\forall(I_+(\rho_k))$ has positive points if and only if $\rho_k$ takes only positive real values. Fixing $b_1, \ldots, b_r$ to be a basis of $\text{Sat}(L_\rho)$, any basis $c_1, \ldots, c_r$ of $L$ can be expressed in terms of the $b_i$ as $c_i = \sum_j a_{ij} b_j$ where $A = (a_{ij}) \in \mathbb{Z}^{r \times r}$ has determinant $g$. Let $\rho_k$ be any of the extensions of $\rho$; since

![Fig. 3](Image)

The positive steady-state varieties of $T$ and $S$ are equal. $T$ has a binomial steady-state ideal, while $S$ has not. In both cases, the equations that describe only the positive steady states are binomial.
\[
\rho = \rho_k|_L, \text{ we have}
\]
\[
\rho(c_i) = \rho_k \left( \sum_j a_{ij} b_j \right) = \prod_j \rho_k(b_j)^{a_{ij}}. \quad (31)
\]

These equations in the unknowns \( \rho_k(b_j) \) determine the extensions of \( \rho \) and thus the irreducible components of \( \mathbb{V}(I) \). If \( \rho_k(b_j) \) is not positive and real for some \( k \) and \( j \in [r] \), then \( \mathbb{V}(I_+(\rho_k)) \cap \mathbb{R}^*_+ = \emptyset \). We only need to consider components for which \( \rho_k(b_j) > 0 \) for all \( j \in [r] \). In this case, we can take logarithms on both sides of (31):
\[
\log(\rho(c_i)) = \sum_j a_{ij} \log(\rho_k(b_j)). \quad (32)
\]

The result is a linear equation for \( \log(\rho_k(b_j)) \) whose solutions yield characters \( \rho_k \) such that \( \mathbb{V}(I_+(\rho_k)) \) has positive points. The matrix \( A \) can be inverted over \( \mathbb{Q} \). Write \( \log \rho_k(b) = (\log \rho_k(b_1), \ldots, \log \rho_k(b_r)) \) and similarly \( \log \rho(c) = (\log \rho(c_1), \ldots, \log \rho(c_r)) \). Then, (32) has the unique solution \( \log \rho_k(b) = A^{-1} \log \rho(c) \).

Consequently, there is a unique saturation \( \rho^* : \text{Sat}(L) \to \mathbb{R}^*_+ \) of \( \rho \) such that \( \rho^*(b_i) > 0 \).

With Example 5.1 and Proposition 5.2 in mind, one would like to analyze the primary decomposition of any steady-state ideal that one encounters. If the original steady-state ideal was not binomial, then maybe the primary decomposition reveals that at least all components whose varieties intersect the positive orthant are binomial. In this case, one has a monomial parametrization for each such component. Deciding whether a non-binomial variety contains positive real points is very hard, though. Only in the binomial case, it is easy using the analysis of characters as in the proof of Proposition 5.2.

**Remark 5.3** If the steady-state equations in variables \((k, x)\) are binomials in \( x \), then Proposition 5.2 holds locally. In particular, for any specialization of the \( k \) to positive real numbers, one has a binomial ideal, as specialization of the \( k \) could only reduce the number of terms. For a careful analysis of the consequences of specialization on two different generating sets of the same steady-state ideal, see (Sadeghimanesh and Feliu 2019b, Section 2). It remains an interesting problem to systematically analyze primary decompositions of steady-state ideals in \( \mathbb{R}(k)[x] \).

### 6 Discussion

The results in this paper show that multistationarity of mass-action systems is a semi-algebraic condition. Polynomial inequalities are used to describe where in parameter space multistationarity can occur. On the 2-site phosphorylation network, the result is particularly satisfying as Corollary 4.13 complements the results of Bihan et al. and shows—in biologically meaningful terms—exactly where multistationarity is possible. The chamber decomposition is an interesting structure because it is inherent to
the biological system. It would be interesting to apply our methods to other systems where the chamber decomposition is explicitly known, e.g., the Wnt pathway from Gross et al. (2016). For us, this shows that it is worthwhile for biologists to interact with real algebraic geometry. Mass-action systems whose steady-state varieties admit a monomial parametrization appear as a natural hunting ground. Here, the techniques of this paper can be applied and combined with ever more powerful exact computational methods from logic. As an immediate goal, it would be very interesting to prove or disprove Corollary 4.13 for 3-site or n-site phosphorylation.

Acknowledgements  This project is funded by the Deutsche Forschungsgemeinschaft, 284057449. Alexandru Iosif and Thomas Kahle are also partially supported by the DFG-RTG “MathCore,” 314838170. We thank the anonymous reviewers for their valuable comments. One reviewer helped to improve the paper by providing a simpler proof of Lemma 3.10, clarifying the statements of Theorems 3.15 and 3.18, and pointing us to Frédéric et al. (2018, Theorem 4.1) which yields Corollary 4.13.

References

Banaji M, Craciun G (2009) Graph-theoretic approaches to injectivity and multiple equilibria in systems of interacting elements. Commun Math Sci 7(4):867–900
Banaji M, Craciun G (2010) Graph-theoretic criteria for injectivity and unique equilibria in general chemical reaction systems. Adv Appl Math 44(2):168–184
Bates DJ, Hauenstein JD, Sommese AJ, Wampler CW (2006) Bertini: Software for Numerical Algebraic Geometry. https://bertini.nd.edu with permanent https://doi.org/10.7274/R0H41PB5
Becker E, Neuhaus R (1993) Computation of real radicals of polynomial ideals. Computational algebraic geometry. Springer, Berlin, pp 1–20
Bihan F, Dickenstein A, Giaroli M (2018) Lower bounds for positive roots and regions of multistationarity in chemical reaction networks. preprint, arXiv:1807.05157
Bradford R, Davenport J, England M, Errami H, Gerdt VP, Grigoriev D, Hoyt C, Kosta M, Radulescu O, Sturm T, Weber A (2017) A case study on the parametric occurrence of multiple steady states. In: Proceedings of the 42nd international symposium on symbolic and algebraic computation (ISSAC ’17), ACM, pp 45–52
Brake D, Niemberg M (2016) Paramotopy. http://paramotopy.com
Brown CW (2003) QEPCAD B: a program for computing with semi-algebraic sets using CADs. ACM SIGSAM Bull 37(4):97–108
Brown CW, Strzeboński A (2010) Black-box/white-box simplification and applications to quantifier elimination. In: Proceedings of the 2010 international symposium on symbolic and algebraic computation. ACM, pp 69–76
Conradi C, Flockerzi D (2012) Multistationarity in mass action networks with applications to ERK activation. J Math Biol 65(1):107–156
Conradi C, Mincheva M (2014) Catalytic constants enable the emergence of bistability in dual phosphorylation. J R Soc Interface 11(95):20140158
Conradi C, Shiu A (2018) Dynamics of posttranslational modification systems: recent progress and future directions. Biophys J 114(3):507–515
Conradi C, Pantea C (2019) Chapter 9–multistationarity in biochemical networks: results, analysis, and examples. In: Robeva R, Macauley M (eds) Algebraic and combinatorial computational biology. Academic Press, Cambridge, pp 279–317
Conradi C, Saez-Rodriguez J, Gilles E-D, Raisch J (2005) Using chemical reaction network theory to discard a kinetic mechanism hypothesis, systems biology. IEEE Proc (now IET Syst Biol) 152(4):243–248
Conradi C, Flockerzi D, Raisch J (2008) Multistationarity in the activation of a MAPK: parametrizing the relevant region in parameter space. Math Biosci 211(1):105–131
Conradi C, Felici E, Mincheva M, Wiu C (2017) Identifying parameter regions for multistationarity. PLOS Comput Biol 13(10):1–25
Coste M (2002) An introduction to semialgebraic geometry. RAAG Netw Sch 145:30
Cox DA, Little JB, O'Shea D (1996) Ideals, varieties, and algorithms, 2nd edn. Springer, New York
Craciun G, Dickenstein A, Shiu A, Sturmfels B (2009) Toric dynamical systems. J Symb Comput 44(11):1551–1565 Ornder: Gatermann
Craciun G, Pantea C, Rempla GA (2009) Algebraic methods for inferring biochemical networks: a maximum likelihood approach. Comput Biol Chem 33(5):361–367
De Loera JA, Kim ED, Onn S, Santos F (2009) Graphs of transportation polytopes. J Comb Theory Ser A 116(8):1306–1325
Dickenstein A (2016) Biochemical reaction networks: an invitation for algebraic geometers, vol 656. Mathematical congress of the Americas. American Mathematical Society, Providence, pp 65–83
Dickenstein A, Pérez-Millán M, Shiu A, Tang X (2019) Multistationarity in structured reaction networks. Bull Math Biol 81(5):1527–1581
Dolzmann A, Sturm T (1997) REDLOG: computer algebra meets computer logic. SIGSAM Bull 31(2):2–9
Eisenbud D, Sturmfels B (1996) Binomial ideals. Duke Math J 84(1):1–45
Ellison PR (1998) The Advanced Deficiency Algorithm and its applications to mechanism discrimination, Ph.D. thesis, The University of Rochester
Ellison P, Feinberg M (2000) How catalytic mechanisms reveal themselves in multiple steady-state data: I. Basic principles. J Mol Catal A Chem 154(1–2):155–167
Ellison P, Feinberg M, Yueb M-H, Saltsburg H (2000) How catalytic mechanisms reveal themselves in multiple steady-state data: II. An ethylene hydrogenation example. J Mol Catal A Chem 154(1–2):169–184
Érdi P, Tóth J (1989) Mathematical models of chemical reactions: theory and applications of deterministic and stochastic models. Manchester University Press, Manchester
Feinberg M (1995a) The existence and uniqueness of steady states for a class of chemical reaction networks. Arch Ration Mech Anal 132(4):311–370
Feinberg M (1995b) Multiple steady states for chemical reaction networks of deficiency one. Arch Ration Mech Anal 132(4):371–406
Feliu E, Wiuf C (2012) Preclusion of switch behavior in networks with mass-action kinetics. Appl Math Comput 219(4):1449–1467
Flockerzi D, Holstein K, Conradi C (2014) N-site phosphorylation systems with 2n–1 steady states. Bull Math Biol 76:1–25
Gawrilow E, Joswig M (2000) polymake: a framework for analyzing convex polytopes, polytopes–combinatorics and computation, vol 29. Birkhäuser, Basel, pp 43–47
Gleixner A, Bastubbe M, Eifler L, Gally T, Gamrath G, Gottwald RL, Hendel G, Hojny C, Koch T, Lübbecke ME, Maher SJ, Miltenberger M, Müller B, Pfetsch ME, Puchert C, Reiβfeldt D, Schlösser F, Schubert C, Serrano F, Shinano Y, Viernickel JM, Walter M, Wegscheider F, Witt JT, Witzig J (2018) The SCIP optimization suite 6.0, Technical report, Optimization Online
Gross E, Harrington HA, Rosen Z, Sturmfels B (2016) Algebraic systems biology: a case study for the wnt pathway. Bull Math Biol 78(1):21–51
Holstein K, Flockerzi D, Conradi C (2013) Multistationarity in sequential distributed multisite phosphorylation networks. Bull Math Biol 75(11):2028–2058
Kahle T, Miller E (2014) Decompositions of commutative monoid congruences and binomial ideals. Algebra Number Theory 8(6):1297–1364
Lazard D, Rouillier F (2007) Solving parametric polynomial systems. J Symb Comput 42(6):636–667
Maplesoft (2017) a division of Waterloo Maple Inc., Waterloo, Ontario, Maple
Markevich NI, Hoek JB, Kholodenko BN (2004) Signaling switches and bistability arising from multisite phosphorylation in protein kinase cascades. J Cell Biology 164(3):353–359
Müller S, Feliu E, Regensburger G, Conradi C, Shiu A, Dickenstein A (2016) Sign conditions for injectivity of generalized polynomial maps with applications to chemical reactions and real algebraic geometry. Found Comput Math 16(1):69–97
Neuhaus R (1998) Computation of real radicals of polynomial ideals–II. J Pure Appl Algebra 124(1–3):261–280
Pérez-Millán M, Dickenstein A (2018) The structure of MESSI biological systems. SIAM J Appl Dyn Syst 17(2):1650–1682
Pérez-Millán M, Dickenstein A, Shiu A, Conradi C (2012) Chemical reaction systems with toric steady states. Bull Math Biol 74(5):1027–1065
Rambau J (2002) TOPCOM: triangulations of point configurations and oriented matroids. In: Arjeh MC, Xiao-Shan G, Nobuki T (eds) Mathematical software–ICMS 2002. World Scientific, Singapore, pp 330–340

Rockafellar RT (1970) Convex analysis. Princeton University Press, Princeton

Sadeghimanesh AH, Feliu E (2019a) Gröbner bases of reaction networks with intermediate species. Adv Appl Math 107:74–101

Sadeghimanesh AH, Feliu E (2019b) The multistationarity structure of networks with intermediates and a binomial core network. Bull Math Biol 81:2428–2462

Schlosser PM, Feinberg M (1994) A theory of multiple steady states in isothermal homogeneous CFSTRs with many reactions. Chem Eng Sci 49(11):1749–1767

Shinar G, Feinberg M (2012) Concordant chemical reaction networks. Math Biosci 240(2):92–113

Shinar G, Feinberg M (2013) Concordant chemical reaction networks and the species-reaction graph. Math Biosci 241(1):1–23

Shiu A (2010) Algebraic methods for biochemical reaction network theory, Ph.D. thesis, University of California, Berkeley

Shiu A, Sturmfels B (2010) Siphons in chemical reaction networks. Bull Math Biol 72(6):1448–1463

Ordner: Gatermann

Wang L, Sontag E (2008) On the number of steady states in a multiple futile cycle. J Math Biol 57:29–52

Wiuf C, Feliu E (2013) Power-law kinetics and determinant criteria for the preclusion of multistationarity in networks of interacting species. SIAM J Appl Dyn Syst 12(4):1685–1721

Wolfram Research, Inc., Mathematica, Version 11.2, Champaign, IL (2017)

Ziegler Günter M (2012) Lectures on polytopes, GTM, vol 152. Springer, Berlin

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.