Algebraic Methods for the Study of Multistationarity in Mass-Action Networks

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Summary

The subject of this thesis is Algebraic Systems Biology with focus on detecting multistationarity in mass-action networks. The main contributions of this thesis are divided into three parts. First, in Chapter 2 we develop the theory of multistationarity for mass-action networks with toric positive steady states and in Chapter 3 we apply this theory to sequential and distributive phosphorylation networks. Second, in Chapter 4 we prove that dynamical systems with the isolation property have toric positive steady states. Finally, in Chapter 5 we introduce Sturm discriminants.

Zusammenfassung

Das Thema dieser Dissertation ist Algebraische Systembiologie mit dem Fokus auf die Erkennung von Multistationarität in Massenwirkungsnetzwerken. Die Hauptbeiträge dieser Arbeit sind in drei Teile gegliedert. Zuerst entwickeln wir in Kapitel 2 die Theorie der Multistationarität für Massenwirkungsnetzwerke mit torische positive stationären Zuständen, und in Kapitel 3 wenden wir diese Theorie auf sequentielle und distributive Phosphorylierungsnetzwerke an. Zweitens beweisen wir in Kapitel 4, dass dynamische Systeme mit der Isolationseigenschaft torische positive stationären Zustände haben. Schließlich führen wir in Kapitel 5 Sturm Diskriminanten ein.

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Contents

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Introduction

Many mechanisms in Systems Biology can be modelled by chemical reaction networks. The Theory of Chemical Reaction Networks started to develop early in the 1970s, mainly by works of Feinberg, Horn, Jackson, and their collaborators (see, for example, Dickenstein's survey article [14]). When the dynamics of such a network is of mass-action form it is called a *mass-action network* and it is described by systems of ordinary differential equations (ODEs) with polynomial right hand side. Often one does not try to solve these polynomial ODEs, but rather pick a more modest objective. For example, the steady states of these polynomial ODEs contain information about long time behaviour and equilibria points, and they give clues about different modi operandi. In particular, one is frequently interested in the existence of multiple steady states (that is, multistationarity) [7, 8]. As steady states are described by polynomial equations, algebra plays a central role in the study of multistationarity. In recent years, the Algebraic Systems Biology Community presented many results on multistationarity. Particularly interesting is the classification with respect to multistationarity of small chemical reaction networks done in 2016 by Joshi and Shiu [29]. A similar classification with respect to nondegenerate multistationarity was done in 2018 by Shiu and de Wolff [44].

Chemical reaction networks originating in Biology are usually large and measurement data is often noisy. Hence this data can be encoded in a set of parameters. As polynomial systems originating in biochemical reaction networks are sparse, the study of their Newton polytopes may give insight about steady states. In [4], Bihan, Dickenstein, and Giaroli use regular subdivisions of Newton polytopes to find parameter values which lead to multiple positive roots and apply this method to biochemical reaction networks. A more general, but computationally more laborious, approach is through the discriminant variety. The discriminant variety of a system of polynomial equations and inequalities is a hypersurface which divides the parameter space into connected regions with constant number of solutions. Discriminants of parametric systems have been implemented in the `Maple` package `RootFinding[Parametric]`, based on algorithms introduced in 2007 by Lazard and Rouillier [31], [34], and [23].

Realistic models tend to be large and even numerically determining which parameter values lead to multistationarity can be challenging. However, biochemical reaction networks usually have nice combinatorial properties. For instance, in 2012, Conradi and Flockerzi defined the *isolation and the bridging properties* [8, Definition 3], and introduced linear systems that test for *multistationarity* in chemical reaction networks with these properties [8, Lemma 4 and Theorem 2].

Along the same lines, in 2016, Millán and Dickenstein [38] defined *MESSI biological systems* as a general framework for *Modifications of type Enzyme-Substrate or Swap with Intermediates*. A notable property of many MESSI systems is that they have *toric steady states*, in which case they describe combinatorial certificates for *monostationarity* [38, Theorems 28, 32]. Such mass-action networks with toric steady states were first studied in full generality in 2011 by Millán, Dickenstein, Shiu, and Conradi [33] and they are of particular interest because their *positive steady states* admit monomial parameterizations which can be used as a complexity reduction step in larger computations [10]. For example, in Chapter 3 we computationally prove that in the space of total concentrations of the two-site phosphorylation network there is a full-dimensional convex cone in which multistationarity is not attainable. The proof, which uses quantifier elimination, was only feasible because this system has toric steady states. The algebraic framework for systems with toric steady states is *Binomial Ideals*. Binomial ideals were studied by Eisenbud and Surmfels in 1996 [17].

This thesis is divided into five chapters. In Chapter 1 we introduce the Theory of Chemical Reaction Networks putting special emphasis on multistationarity. In Chapter 2 we develop the theory of multistationarity for mass-action networks with *toric positive steady states* (that is, mass-action networks whose positive steady states admit monomial parameterizations). Systems with toric positive steady states are generalizations of systems with toric steady states. This is of particular interest as in many applications only the positive steady states are relevant. For these systems we show that, in the space of total concentrations, multistationarity is scale invariant (Theorems 2.18 and 2.19). Moreover, for these systems we give semialgebraic conditions for multistationarity in terms of only the total concentrations (Theorem 2.15 and Corollary 2.17). In Chapter 3 we apply the results from Chapter 2 to the well-known sequential and distributive phosphorylation of a protein. For the two-site phosphorylation we prove a relation between multistationarity and the chamber decomposition of the cone of total concentrations (Theorem 3.5 and Corollary 3.7). In Chapter 4 we analyze dynamical systems with the isolation property. In particular, in Theorem 4.17 we show that dynamical systems with the isolation property have toric positive steady states. In general testing for toric positive steady states is a hard problem. As the isolation property is easier to test, it can be used as a certificate for toric positive steady states. Finally, in Chapter 5 we introduce Sturm discriminants. Sturm discriminants use Sturm sequences for the computation of discriminants. While these discriminants are in general not minimal, they are easily implementable in open source computer algebra systems like Macaulay2.

Notation

If S is a finite multiset, then $|S|$ denotes the cardinality of S. If G is a graph, then $V(G)$ and $E(G)$ denote the set of vertices and the set of edges of G, respectively. If a is a sequence of real numbers, then $\mathrm{Var}(a)$ denotes the number of sign changes in a after removing the 0 entries. For example $\mathrm{Var}(1,0,-1) = \mathrm{Var}(1,-1) = 1$.

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

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

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

$$\begin{aligned} \ln x^A &= (\ln x)A, && \text{if } x \text{ is a row vector,} \\ &\text{and} \\ \ln x^A &= A^T \ln x, && \text{if } x \text{ is a column vector.} \end{aligned}$$

If A_1 and A_2 are two matrices with the same number of rows, then the matrix whose columns are the columns of A_1 followed by the columns of A_2 is denoted by $(A_1|A_2)$. A vector which has 1 in every entry is denoted by $\mathbf{1}$ and a matrix which has 0 in every entry is denoted by 0. Let A be a matrix with columns $A_1, \ldots, A_n$ and let $I = \{i_1, \ldots, i_p\}$ be a multiset of elements of $[n]$; then $A_I = (A_{i_1}| \ldots |A_{i_p})$.

Let R be an integral domain and let k be a field. By $K(R)$ we denote the field of fractions of R and by $\overline{k}$ we denote the algebraic closure of k. If $p \in R[x]$, the derivative of p with respect to x is denoted by p'. Let $q \in k[x_1, \ldots, x_n]$ and let **order** be a monomial ordering on the set of monomials of $k[x_1, \ldots, x_n]$. The leading term and leading coefficient of q with respect to **order** are denoted by $\mathrm{LT}_{\mathbf{order}}(q)$ and $\mathrm{LC}_{\mathbf{order}}(q)$, respectively, and the constant term of q is denoted by $\mathrm{CT}(q)$. The Zariski closure of an affine set S is denoted by $\overline{S}$. If I is a polynomial ideal, its variety is denoted by $\mathbb{V}(I)$. The set of polynomials vanishing on an affine set S is denoted by $\mathbb{I}(S)$. If I is a polynomial ideal that has only one generator g, then the unique generator of $\mathrm{Rad}(I)$ is denoted by g_{red}.

Chapter 1

Chemical reaction networks

This chapter is based on Section 2 of the paper "Multistationarity in the space of total concentrations for systems that admit a monomial parametrization" [10], joint work with Carsten Conradi and Thomas Kahle.

A *chemical reaction network* is a finite directed graph whose vertices are labelled by chemical complexes and whose edges are labelled either by positive real numbers, or by parameters, called *rate constants*. The network $\mathcal{N}_{\text{ph}}$ of Example 1.1 is a typical biochemical reaction network. The digraph is denoted by $\mathcal{N} = ([m], E)$, with vertex set $[m]$ and edge set E. Each complex $i \in [m]$ has the form $\sum_{j=1}^{n}(y_i)_j X_j$ for some $y_i \in \mathbb{Z}^n_{\geq 0}$, 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 dissertation the integers n, m, and r, unless otherwise stated, denote the number of species, complexes, and reactions, respectively. 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 the educt and product complex for several reactions. For each reaction network one has two matrices Y_e and Y_p whose columns are the complex-species incidence vectors corresponding to all educt and product complexes, respectively. That is

$$Y_e = (\widetilde{y}_1, \ldots, \widetilde{y}_r), \text{ where } \widetilde{y}_i = y_k \text{ when reaction } i \text{ has educt complex } k,$$
$$Y_p = (\widetilde{y}_1, \ldots, \widetilde{y}_r), \text{ where } \widetilde{y}_i = y_k \text{ when reaction } i \text{ has product complex } k,$$

Example 1.1. The following reaction network is the *1-site phosphorylation network* and it models the phosphorylation of a protein at one site:

$$\begin{aligned} X_1 + X_2 &\underset{k_2}{\overset{k_1}{\rightleftarrows}} X_3 \xrightarrow{k_3} X_1 + X_4 \\ X_4 + X_5 &\underset{k_5}{\overset{k_4}{\rightleftarrows}} X_6 \xrightarrow{k_6} X_2 + X_5. \end{aligned} \tag{$\mathcal{N}_{\text{ph}}$}$$

The chemical species are X_1, X_2, X_3, X_4, X_5, and X_6 and the 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. Here X_1 and X_2 play the roles of unphosphorylated and phosphorylated protein. The network has 6 reactions, each one labelled by a rate constant k_1, k_2, k_3, k_4, k_5, or k_6. The matrices Y_e and Y_p of this network are

$$Y_e = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 \end{pmatrix} \text{ and } Y_p = \begin{pmatrix} 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 \\ 0 & 0 & 0 & 1 & 0 & 0 \end{pmatrix}.$$

1.1 Dynamical systems defined by mass-action networks

Every chemical reaction network defines a dynamical system of the form

$$\dot{x} = S\nu(k, x), \tag{1.1}$$

where $S = Y_p - Y_e$ is the *stoichiometric matrix* and $\nu(k, x)$ is the *vector of reaction rates*. The vector space $\mathcal{L}_{\text{stoi}}$ spanned by the columns of S is called the *stoichiometric space*. Here the vector of reaction rates is a function of the concentrations x and of the vector of rate constants k. In this thesis 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,

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

where $\phi(x) = x^{Y_e}$, and $k = (k_1, \ldots, k_r)^T$ is the vector of rate constants. More general kinetics exist (e.g. [37] and the references therein) and they have been recently analyzed, partly motivated by the *Global Atractor Conjecture* [13].

Example 1.2. The stoichiometric matrix of the network $\mathcal{N}_{\text{ph}}$ is

$$S = \begin{pmatrix} -1 & 1 & 1 & 0 & 0 & 0 \\ -1 & 1 & 0 & 0 & 0 & 1 \\ 1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 1 & 0 \\ 0 & 0 & 0 & -1 & 1 & 1 \\ 0 & 0 & 0 & 1 & -1 & -1 \end{pmatrix}.$$

As a mass-action network, $\mathcal{N}_{\text{ph}}$ has the following reaction rates:

$$\nu_1 = k_1x_1x_2,\ \nu_2 = k_2x_3,\ \nu_3 = k_3x_3,\ \nu_4 = k_4x_4x_5,\ \nu_5 = k_5x_6,\ \text{and}\ \nu_6 = k_6x_6.$$

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

$$\begin{aligned} \dot{x}_1 &= -k_1x_1x_2 + (k_2+k_3)x_3, & \dot{x}_4 &= k_3x_3 - k_4x_4x_5 + k_5x_6, \\ \dot{x}_2 &= -k_1x_1x_2 + k_2x_3 + k_6x_6, & \dot{x}_5 &= -k_4x_4x_5 + (k_5+k_6)x_6, \\ \dot{x}_3 &= k_1x_1x_2 - (k_2+k_3)x_3, & \dot{x}_6 &= k_4x_4x_5 - (k_5+k_6)x_6. \end{aligned}$$

A natural question that arises is 'Which polynomial ODEs describe the dynamics of a mass-action network?'. This question was answered in [25] by Hárs and Tóth:

Lemma 1.3 ([25])**.** *An ODE system $\dot{x}_1 = f_1, \ldots, \dot{x}_n = f_n$ describes the dynamics of a mass-action network on n species whose rate constants are fixed if and only if $f_i \in \mathbb{R}[x_1, \ldots, x_n]$ and each negative term of f_i is divisible by x_i.*

1.2 Steady states

If the rate constants $k_1, \ldots, k_r$ and the concentrations $x_1, \ldots, x_n$ are such that

$$S\nu(k, x) = 0, \tag{1.2}$$

then x is a *steady state*. As x is a vector of concentrations of chemical species, only nonnegative x are meaningful. Consequently steady states are nonnegative real solutions of equations (1.2). A steady state is *positive* if all its coordinates are positive real numbers. It is a *boundary steady state* if all its coordinates are nonnegative but it is not positive. The *steady state ideal* I is the polynomial ideal generated by the entries of $S\nu(k, x)$. This ideal can be considered in different polynomial rings. Thus, the parameters k can be part of the indeterminates, i.e. $I \subset \mathbb{R}[k, x]$, or appear as variables in the rational functions that serve as coefficients. In the second case $I \subset \mathbb{R}(k)[x]$. In Chapter 4 yet another approach is considered: rate constants are considered to be fixed positive real numbers and whenever there is given a collection of them a family of ideals is considered. In all these cases the *steady state variety* is the zero locus of the steady state ideal.

Example 1.4. The equations $\dot{x}_i = 0$ define the steady state ideal of $\mathcal{N}_{\text{ph}}$:

$$\begin{aligned} I &= \langle -k_1x_1x_2 + (k_2+k_3)x_3, -k_1x_1x_2 + k_2x_3 + k_6x_6, k_1x_1x_2 - (k_2+k_3)x_3, \\ &\qquad k_3x_3 - k_4x_4x_5 + k_5x_6, -k_4x_4x_5 + (k_5+k_6)x_6, k_4x_4x_5 - (k_5+k_6)x_6\rangle \\ &= \langle k_1x_1x_2 - (k_2+k_3)x_3, k_3x_3 - k_6x_6, k_4x_4x_5 - (k_5+k_6)x_6\rangle. \end{aligned}$$

The second equality results from elementary simplification and omitting redundant generators. While such simplifications are useful to understand the geometry of steady states, any interpretation of the resulting polynomials is lost.

Definition 1.5. The *positive steady state variety* of $\mathcal{N}$ is

$$V^+ = \{(k,x) \in \mathbb{R}^r_{>0} \times \mathbb{R}^n_{>0} |\ S\nu(k,x) = 0\}\,.$$

Example 1.6. According to Example 1.4, the steady state ideal of $\mathcal{N}_{\text{ph}}$ is generated by 3 polynomials. Since we are only interested in positive x_i, the equations that describe the positive steady state variety can be rearranged as

$$\frac{x_3}{x_6} = \frac{k_6}{k_3},\ \frac{x_1x_2}{x_3} = \frac{k_2+k_3}{k_1},\ \frac{x_4x_5}{x_6} = \frac{k_5+k_6}{k_4}. \tag{1.3}$$

These equations can be solved as

$$x_3 = \frac{k_1}{k_2+k_3}x_1x_2,\ x_4 = \frac{k_1k_3(k_5+k_6)}{(k_2+k_3)k_4k_6}\frac{x_1x_2}{x_5},\ x_6 = \frac{k_1k_3}{(k_2+k_3)k_6}x_1x_2. \tag{1.4}$$

This shows that the positive steady state variety of $\mathcal{N}_{\text{ph}}$ can be parameterized by x_1, x_2, and x_5 together with $k_1, \ldots, k_6$. This parameterization uses only products (and divisions) of the x_i, but no sums. This *monomial parameterization* is crucial for the developments of Chapter 2.

As we saw in Lemma 1.3, ODE systems describing the dynamics of mass-action networks are quite special. Hence it is natural to ask whether steady state ideals are also that special. This question was answered by Dickenstein in 2016:

Proposition 1.7 ([14])**.** *If $I \subseteq \mathbb{R}[x_1, \ldots, x_n]$ is an ideal generated by at most n polynomials, then there exists a mass-action network whose steady state ideal J has the following property:*

$$\mathbb{V}(I) \cap (\mathbb{R}^*)^n = \mathbb{V}(J) \cap (\mathbb{R}^*)^n.$$

Corollary 1.8. *If $L \subset \mathbb{Z}^n$ is a lattice and $\rho : L \to \mathbb{R}^*$ is a partial character, then there is a mass-action network with binomial steady state ideal J such that*

$$\mathbb{V}(I(\rho)) = \mathbb{V}(J) \cap (\mathbb{R}^*)^n\,.$$

Proof. Let $\{m_1, \ldots, m_s\}$ be a basis of L. It follows from [17, Theorem 2.1.b] that

$$I(\rho) = \langle x^{m_1} - \rho(m_1), \ldots, x^{m_s} - \rho(m_s) \rangle.$$

Let $f_i = x_1 \ldots x_n \left(x^{m_i^+} - \rho(m_i) x^{m_i^-} \right)$, $i \in [s]$ and choose $f_{s+1}, \ldots, f_n$ randomly among $f_1, \ldots, f_s$. Let $J = \langle f_1, \ldots, f_s \rangle$. Then

$$\mathbb{V}(I(\rho)) = \mathbb{V}(J) \cap (\mathbb{R}^*)^n .$$

As, for each $i \in [n]$, the polynomial f_i is divisible by the monomial $x_1 \ldots x_n$, by Lemma 1.3, the ODE system $\dot{x}_1 = f_1, \ldots, \dot{x}_n = f_n$ describes the dynamics of some mass-action network on n species. □

Remark 1.9. Proposition 1.7 cannot be generalized to the case when I is an ideal of $\mathbb{R}[k][x]$. The main obstacle lies in choosing rate constants, as in most applications rate constants, when considered as parameters, are algebraically independent and, moreover, in mass-action networks, each parameter has a unique monomial associated to it. For example, the ideal $\langle kx - ky \rangle \subseteq \mathbb{R}[k][x, y]$ is the steady state ideal of some network $\mathcal{N}$ if and only if $\mathcal{N}$ has two reactions with rate constants k_1 and k_2 and $k_1 = k_2 = k$.

Remark 1.10. While Proposition 1.7 shows that ideals arising as steady state ideals of chemical networks are quite general, biochemical networks tend to have special combinatorial properties. For example, in 2016, Millán and Dickenstein [38] introduced *MESSI biological systems* as a general framework for *Modifications of type Enzyme-Substrate or Swap with Intermediates*. A notable property of many MESSI systems is that they have toric steady states [38].

1.3 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*. By (1.1), 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

$$c = Zx. \tag{1.5}$$

As the coordinates of x denote concentrations of chemical species, x is a nonnegative vector. Thus, only values of c corresponding to nonnegative x have chemical meaning. The set of such c is denoted by $\mathrm{im}_+(Z)$:

$$\mathrm{im}_+(Z) = \{c \in \mathbb{R}^{n-s} | \exists x \in \mathbb{R}^n_{\geq 0} \text{ such that } Zx = c\}.$$

If $x(0) \in \mathbb{R}^n_{>0}$ is a vector of initial concentrations, then, under mass-action kinetics, the trajectory $\{x(t) |\ t > 0\}$ is constrained to the polyhedron

$$\mathcal{P}_c = \{x \in \mathbb{R}^n_{\geq 0} |\ Zx = c\}. \tag{1.6}$$

The set $\mathcal{P}_c$ is known as the *invariant polyhedron* with respect to $x(0)$ [43, Section 1.3.3], or the *stoichiometric compatibility class* of $x(0)$ [19, 20].

Example 1.11. The conservation space $\mathcal{L}_{\text{cons}}$ of $\mathcal{N}_{\text{ph}}$ is spanned by the row vectors

$$(1,0,1,0,0,0),\ (0,0,0,0,1,1),\ \text{and}\ (0,1,1,1,0,1).$$

Consequently, the network $\mathcal{N}_{\text{ph}}$ has three linearly independent conservation relations and three total concentrations c_1, c_2, and c_3:

$$\begin{aligned} x_1 + x_3 &= c_1, \\ x_5 + x_6 &= c_2, \\ x_2 + x_3 + x_4 + x_6 &= c_3. \end{aligned} \tag{1.7}$$

The quantities c_1, c_2, and c_3 can be interpreted as total amount of kinase, phosphatase and substrate, respectively.

Remark 1.12. If $\mathcal{L}_{\text{cons}}$ contains at least one positive point, then it is always possible to choose a basis in which Z has positive entries. Mass-action networks with this property are called *conservative*, and their stoichiometric compatibility classes are bounded. Consequently, the trajectories of conservative mass-action networks are also bounded.

1.4 Chamber decomposition of $\mathcal{P}_c$

We now introduce the *chamber decomposition*, a natural subdivision of the set of total concentrations c. In Chapter 3 we use it to partition the space of total concentrations into chambers and test for multistationarity within these chambers. The chamber decomposition requires that Z in (1.6) is a $d \times n$ matrix of full rank and c is the parameter indexing the family. The decomposition is for cone(Z), the cone of nonnegative combinations of columns of Z, because $\mathcal{P}_c$ is empty for

all $c \notin \operatorname{cone}(Z)$. To give the precise definition one more notion is necessary: a *basis of* Z is a subset B of the columns of Z that is a basis of $\mathbb{R}^d$. Each basis B defines a *basic cone* $\operatorname{cone}(B)$ consisting of nonnegative linear combinations of the columns in B.

Definition 1.13. 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

$$c_1 \in \operatorname{cone}(B) \Leftrightarrow c_2 \in \operatorname{cone}(B) \qquad \text{for all bases } B \text{ of } Z.$$

Example 1.14. The chamber complex corresponding to the sequential and distributive multisite phosphorylation of a protein is examined in Section 3.4.

1.5 Multistationarity

Multistationarity refers to the capacity of a chemical reaction network to admit multiple positive steady states in some stoichiometric compatibility class [8]:

Definition 1.15. A system *admits multistationarity* if there are $k \in \mathbb{R}^r_{>0}$ and $a \neq b \in \mathbb{R}^n_{>0}$ such that $(k, a) \in V^+$, $(k, b) \in V^+$, and $(b - a) \in \operatorname{im}(S)$.

Example 1.16. It is well known that $\mathcal{N}_{\text{ph}}$ has one steady state for each $k \in \mathbb{R}^6_{>0}$ and $c \in \mathbb{R}^3_{>0}$ (see for example [26] and [21] and the references therein).

Example 1.17. Let $\mathcal{G}$ denote the following chemical reaction network:

$$3X_1 \underset{k_2}{\overset{k_1}{\rightleftarrows}} 2X_1 + X_2 \qquad X_1 + X_2 \xrightarrow{k_3} 2X_2 \qquad X_2 \xrightarrow{k_4} X_1 \qquad (\mathcal{G})$$

The dynamics of this network is given by the following system of ODEs:

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

Consequently, the steady state ideal of $\mathcal{G}$ is

$$I = \langle -k_1 x_1^3 + k_2 x_1^2 x_2 - k_3 x_1 x_2 + k_4 x_2 \rangle \subseteq \mathbb{R}[k_1, k_2, k_3, k_4][x_1, x_2].$$

There is also a conservation relation: $x_1 + x_2 = c$. Hence the study of the positive steady states of $\mathcal{G}$ reduces to the study of the positive solutions of the system

$$\begin{aligned} k_1 x_1^3 - k_2 x_1^2 x_2 + k_3 x_1 x_2 - k_4 x_2 &= 0 \\ x_1 + x_2 &= c, \end{aligned} \qquad (1.8)$$

for positive parameters k_1, k_2, k_3, k_4, and c. If one eliminates the variable x_2 from (1.8), then one gets the following equation in the variable x_1:

$$(k_1+k_2)x_1^3-(k_2c+k_3)x_1^2+(k_3c+k_4)x_1-k_4c=0. \tag{1.9}$$

The left hand side of this equation is a cubic polynomial in $\mathbb{R}[k_1,\ldots,k_4,c][x_1]$. As for all positive values of the parameters one has

$$\mathrm{Var}(k_1+k_2,-k_2c-k_3,k_3c+k_4,-k_4c)=3,$$

by Descartes' Law of signs [1, Theorem 2.33], (1.9) has either one or three positive solutions counted with multiplicity. The values of the parameters for which (1.9) has a double solution are given by the vanishing of its discriminant:

$$\begin{aligned}&k_2^2k_3^2c^4-4k_2^3k_4c^4-4k_1k_3^3c^3-2k_2k_3^3c^3+18k_1k_2k_3k_4c^3+8k_2^2k_3k_4c^3+\\&k_3^4c^2+6k_1k_3^2k_4c^2-2k_2k_3^2k_4c^2-27k_1^2k_4^2c^2-36k_1k_2k_4^2c^2-8k_2^2k_4^2c^2-\\&2k_3^3k_4c+6k_1k_3k_4^2c+8k_2k_3k_4^2c+k_3^2k_4^2-4k_1k_4^3-4k_2k_4^3=0\end{aligned} \tag{1.10}$$

In Figure 1.1 we fix the rate constants and we represent the intersection between the positive steady state variety of $\mathcal{G}$ and several invariant polyhedra; in particular this picture shows that network $\mathcal{G}$ admits multistationarity.

Remark 1.18. Definition 1.15 could in principle be extended to include boundary steady states. However techniques from toric geometry cannot be directly applied there, and these cases need to be dealt with separately. Consequently, in this thesis multistationarity refers to the existence of multiple positive steady states.

1.6 Internal cycles

An *internal cycle* [45], of a chemical reaction networks is a minimal multiset $\mathfrak{C}$ of elements of $[r]$ such that the two monomials arising as the product of the educt complexes indexed by $\mathfrak{C}$ and the product of the product complexes indexed by $\mathfrak{C}$ are equal. That is,

$$x^{(Y_e)_{\mathfrak{C}}\mathbf{1}}=x^{(Y_p)_{\mathfrak{C}}\mathbf{1}},$$

where $\mathbf{1}=\{1\}^{|\mathfrak{C}|}$ is a column vector. We recall that if $\mathfrak{C}=\{i_1,\ldots,i_s\}$ is a multiset of elements of $[r]$ with $i_1\leq i_2\leq\ldots,\leq i_s$, then $(Y_\bullet)_{\mathfrak{C}}$ denotes a matrix whose j^{th} column is the i_j^{th} columns of $Y_\bullet$, where $\bullet$ stands for e or p (see the Notation).

Internal cycles are encoded in the rays of the nonnegative kernel of the stoichiometric matrix [45]. Let $E_1,\ldots,E_e$ denote the generators of the cone $\ker(S)\cap\mathbb{R}^r_{\geq 0}$. The *cone generator matrix* of $\mathcal{N}$ is $E=(E_1|\ldots|E_e)$ and the *coefficients cone* is $\Lambda(E)=\{\lambda\in\mathbb{R}^e_{\geq 0}|\ E\lambda>0\}$ [8, Section 3.1]. We observe that,

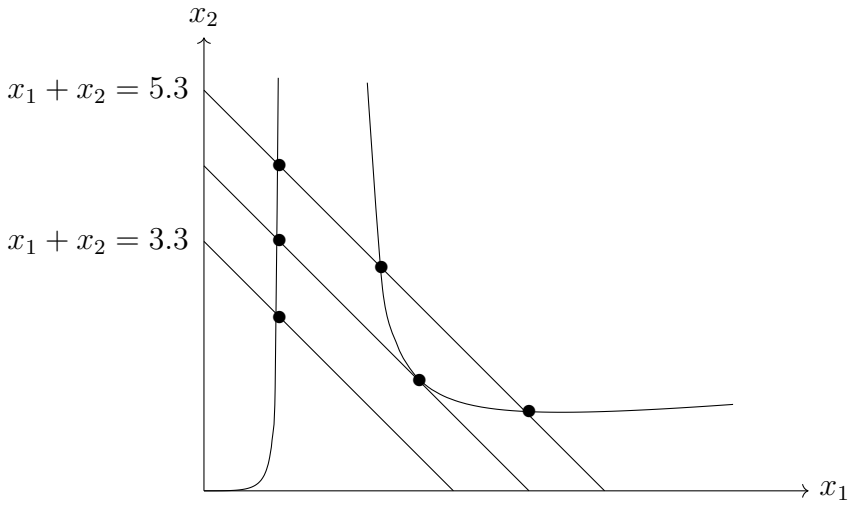


Figure 1.1: The intersection of the positive steady state variety of $\mathcal{G}$ and several invariant polyhedra. For this picture we fixed the rate constants to the following values: $k_1 = 0.1$, $k_2 = 1$, $k_3 = 3$ and $k_4 = 2$. By substituting these values in (1.10), one gets up to multiplying by a rational number that the discriminant of (1.9) is $25c^4 - 150c^3 + 208c^2 - 120c + 20$. This discriminant has two real roots. The first one (approximately 0.3) corresponds to a negative double solution of (1.9) and the second one (approximately 4.3) corresponds to a positive double solution of (1.9). In particular, if the value of the total concentration $x_1 + x_2$ is larger than the second value, then multistationarity is attained in $\mathcal{G}$.

if E has a zero row, then V^+ is empty. In Theorem 2.7, for fixed x, we show how to compute values of k for which $(k, x) \in V^+$. Note that the matrix E can always be chosen so that its columns span $\ker(S) \cap \mathbb{Z}^r_{\geq 0}$. Proposition 1.19 below relates internal cycles to the matrix E and it was first stated in [42, Theorem 4.1].

Proposition 1.19 ([42])**.** *Columns of the cone generator matrix E and internal cycles of $\mathcal{N}$ are in one to one correspondence. The i^{th} column of E corresponds to the internal cycle in which the multiplicity of every $j \in [r]$ is E_{ji}.*

Proof. Equation $x^{(Y_e)_{\mathfrak{C}}\mathbf{1}} = x^{(Y_p)_{\mathfrak{C}}\mathbf{1}}$ is equivalent to $x^{(S)_{\mathfrak{C}}\mathbf{1}} = 1$, which is equivalent to $S_{\mathfrak{C}}\mathbf{1} = 0$. Let $v \in \mathbb{Z}^r_{\geq 0}$ be a column vector such that v_i is equal to the multiplicity of i in $\mathfrak{C}$. Then $S_{\mathfrak{C}}\mathbf{1} = 0$ if and only if $Sv = 0$. Now, $\mathfrak{C}$ is minimal if and only if the only $\mathfrak{C}'$ and $\mathfrak{C}''$ such that $\mathfrak{C} = \mathfrak{C}' + \mathfrak{C}''$, $S_{\mathfrak{C}'}\mathbf{1} = 0$, and $S_{\mathfrak{C}''}\mathbf{1} = 0$ are either $\emptyset$ and $\mathfrak{C}$ or $\mathfrak{C}$ and $\emptyset$. So $\mathfrak{C}$ is minimal if and only if the only $v', v'' \in \mathbb{Z}^r_{\geq 0}$ such that $v = v' + v''$, $Sv' = 0$, and $Sv'' = 0$ are either v and 0 or 0 and v. Hence $\mathfrak{C}$ is minimal if and only if v is an extremal ray of $\ker(S) \cap \mathbb{Z}^r_{\geq 0}$. □

Remark 1.20. In the language of [42], Proposition 1.19 says that internal cycles are *nonnegative elementary flux modes*.

Remark 1.21. As a consequence of Proposition 1.19, the cone generator matrix can be computed by analyzing the reaction network. While for big networks this might be computationally infeasible, for small examples this proposition offers the possibility of fast computations by hand.

Example 1.22. Consider the following network

$$2X_1 \underset{k_2}{\overset{k_1}{\rightleftarrows}} X_1 + X_2 \xrightarrow{k_3} 2X_2 \ . \tag{1.11}$$

The matrices Y_e and Y_p of this network are

$$Y_e = \begin{pmatrix} 2 & 1 & 1 \\ 0 & 1 & 1 \end{pmatrix} \text{ and } Y_e = \begin{pmatrix} 1 & 2 & 0 \\ 1 & 0 & 2 \end{pmatrix}.$$

Hence the stoichiometric and the cone generator matrices of this network are

$$S = \begin{pmatrix} -1 & 1 & -1 \\ 1 & -1 & 1 \end{pmatrix} \text{ and } E = \begin{pmatrix} 1 & 0 \\ 1 & 1 \\ 0 & 1 \end{pmatrix},$$

respectively, and its internal cycles are $\{1, 2\}$ and $\{2, 3\}$.

Chapter 2

Multistationarity in the space of total concentrations for systems that admit a monomial parameterization

The first 5 sections of this chapter are based on Section 3 of the paper "Multistationarity in the space of total concentrations for systems that admit a monomial parametrization" [10], joint work with Carsten Conradi and Thomas Kahle. Section 2.6 is joint work with Thomas Kahle and Ruilong Zhuang.

2.1 Introduction

As total concentrations are experimentally more accessible than rate constants, conditions directly including total concentrations are useful. In this chapter we study such conditions with focus on systems whose positive steady states admit a monomial parameterization (Definition 2.1). These systems are closely related to systems with toric steady states described in [33], that is to systems whose steady state ideal is binomial. For such systems that admit a monomial parameterization we show that in the space of total concentrations multistationarity is scale invariant, i.e. there is multistationarity for some value c of the total concentrations if and only if, for any $\alpha > 0$, there is multistationarity for αc (Theorems 2.18 and 2.19). In Theorem 2.15 and Corollary 2.16 we provide semialgebraic conditions 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.

2.2 Monomial parameterization of the positive steady states

In this chapter $\mathcal{N}$ denotes a mass-action network on n species and r reactions, with $\dim \mathcal{L}_{\text{cons}} \neq 0$, and S and Z denote the stoichiometric and a conservation matrix of $\mathcal{N}$, respectively. The following definition was already used in [35]:

Definition 2.1. The positive steady state variety V^+ of $\mathcal{N}$ *admits a monomial parameterization* if there are $M \in \mathbb{Z}^{n\times d}$ of rank $p < n$ and a rational function

$$\begin{array}{rccc} \gamma: & \mathcal{K}_\gamma^+ & \to & \mathbb{R}^d \\ & k & \mapsto & \gamma(k), \end{array}$$

such that

$$(k,x) \in V^+ \Leftrightarrow x^M = \gamma(k) \quad \forall (k,x) \in \mathcal{K}_\gamma^+ \times \mathbb{R}^n_{>0},$$

where

$$\mathcal{K}_\gamma^+ = \left\{ k \in \mathbb{R}^r_{>0} |\ \gamma_i^+(k) \cdot \gamma_i^-(k) > 0,\ i \in [d] \right\}$$

and $\gamma_i^\pm(k) \in \mathbb{R}[k]$ are such that

$$\gamma^\pm(k) = \left(\gamma_1^\pm(k), \ldots, \gamma_d^\pm(k)\right), \text{ where } \gamma_i(k) = \frac{\gamma_i^-(k)}{\gamma_i^+(k)} \text{ for } i \in [d].$$

Throughout this chapter M denotes the matrix of the monomial parameterization from Definition 2.1.

Example 2.2. According to (1.3), the positive steady state variety of the network $\mathcal{N}_{\text{ph}}$ admits a monomial parameterization.

If $M^\pm \in \mathbb{Z}_{\geq 0}$ denote the positive and negative part of M, then the system $x^M = \gamma(k)$ of Definition 2.1 is equivalent to the following binomial system:

$$\gamma^+(k) \star x^{M^+} - \gamma^-(k) \star x^{M^-} = 0. \tag{2.1}$$

The polynomials $\gamma^\pm(k)$ need not be monomials. Therefore, in general, the system (2.1) is binomial only in the variables x. More generally, $\mathbb{I}(V^+) \subseteq \mathbb{R}(k)[x]$ is a binomial ideal. By [17, Theorem 2.1], the ideal

$$\langle \left(x^M\right)_i - \gamma_i(k) |\ \ i \in [d] \rangle \subset \mathbb{R}(k)[x^\pm] \tag{2.2}$$

is a complete intersection and, consequently, it has a generating set for which M has full rank, i.e. there is a γ such that $M \in \mathbb{Z}^{n\times p}$. Subsequently, unless otherwise stated, we assume that M and $\gamma(k)$ are of this form. Observe that, as the ideal from Equation (2.2) has several generating sets of binomials, neither the polynomials $\gamma^\pm(k)$ nor the matrix M need be unique. In the next lemmata we explicitly study the consequences of a monomial parameterization for V^+.

Lemma 2.3. *If V^+ admits a monomial parameterization and there exists a matrix $A \in \mathbb{Q}^{q\times n}$ of rank $q < n$ such that $AM = 0$, then the following are equivalent:*

a) $(k, x) \in V^+$,

b) $(k, x \star \xi^A) \in V^+ \ \forall \xi \in \mathbb{R}^q_{>0}$,

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

Proof. As V^+ admits a monomial parameterization, a) is equivalent to $x^M = \gamma(k)$ and b) is equivalent to $(x \star \xi^A)^M = \gamma(k)$. As $AM = 0$, we deduce that $\left(x \star \xi^A\right)^M = x^M$. Hence a) is equivalent to b). By replacing ξ with e^κ, we deduce that b) is equivalent to c). □

Lemma 2.4. *If V^+ admits a monomial parameterization, then there are $A \in \mathbb{Q}^{(n-p)\times n}$ of rank $n - p$ with $AM = 0$, a function $\psi : \mathcal{K}^+_\gamma \to \mathbb{R}^n$, and an exponent $\eta \in \mathbb{Z}_{>0}$, such that ψ^η is a rational function and the following are equivalent:*

a) $(k, x) \in V^+$,

b) $k \in \mathcal{K}^+_\gamma$ *and there exist* $\xi \in \mathbb{R}^{n-p}_{>0}$ *such that* $x = \psi(k) \star \xi^A$.

Proof. According to Definition 2.1, $(k, x) \in V^+$ if and only if $x^M = \gamma(k)$ and $k \in \mathcal{K}^+_\gamma$. As $k \in \mathcal{K}^+_\gamma$ implies that $\gamma(k) > 0$, we can take logarithms on both sides of the previous equation: $M^T \cdot (\ln x) = \ln \gamma(k)$. By [17, Theorem 2.1], the ideal $\langle x^M - \gamma(k)\rangle \subset \mathbb{R}(k)[x^\pm]$ is a complete intersection. Hence it has a generating set in which M has full rank and format $n \times p$ for a suitable γ. Assume that the chemical species are ordered such that the first p rows of M are linearly independent. We deduce that there is an invertible matrix $U \in \mathbb{Q}^{p\times p}$ such that, up to a permutations of rows,

$$MU = \begin{bmatrix} I_p \\ -W \end{bmatrix},$$

where $W \in \mathbb{Q}^{(n-p)\times p}$. Hence, for $k \in \mathcal{K}^+_\gamma$,

$$(k, x) \in V^+ \Leftrightarrow U^T M^T (\ln x) = U^T (\ln \gamma(k)).$$

Decomposing x into $x' = (x_1, \ldots, x_p)^T$ and $\xi = (x_{p+1}, \ldots, x_n)^T$, we deduce, for $k \in \mathcal{K}^+_\gamma$, that

$$(k, x) \in V^+ \Leftrightarrow \ln x' - W^T \cdot (\ln \xi) = U^T \cdot (\ln \gamma(k)) \Leftrightarrow x' = \gamma(k)^U \star \xi^W.$$

Let A denote the matrix $(W|I_{n-p})$ and let $\psi(k) = \gamma(k)^{(U|0_{p\times n-p})}$. As $AMU = 0$ and U is invertible, we deduce that $AM = 0$. Hence $x = \psi(k) \star \xi^A$. Let η denote

the least common multiple of the denominators in U. As $\gamma(k)$ is rational, the coordinate-wise power $\psi^\eta(k)$ is also rational. As there exist $x \in \mathbb{R}^n_{>0}$ such that $(k,x) \in V^+$ only if $k \in \mathcal{K}^+_\gamma$, we only need to consider $k \in \mathcal{K}^+_\gamma$. We conclude that $\psi(k)$ is well defined on $\mathcal{K}^+_\gamma$. □

The matrix A from Lemma 2.4 is called the *exponent matrix* of the parameterization. Next we exemplify the steps taken in the proof of Lemma 2.4.

Example 2.5. The monomial parameterization from (1.3) can be expressed as

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

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

The numerators and denominators of $\gamma(k)$ are positive polynomials for all $k \in \mathbb{R}^6_{>0}$. Hence $\mathcal{K}^+_\gamma = \mathbb{R}^6_{>0}$. Now, for the matrix

$$U = \begin{pmatrix} 0 & -1 & -1 \\ -1 & -1 & -1 \\ 0 & 0 & 1 \end{pmatrix}, \text{ we get } MU = \begin{pmatrix} -1 & -1 & -1 \\ -1 & -1 & -1 \\ 1 & 0 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 1 \\ 0 & 1 & 0 \end{pmatrix},$$

which, for the ordering x_3, x_6, x_4, x_1, x_2, x_5, is equivalent to

$$\begin{pmatrix} I_3 \\ -W \end{pmatrix} \text{ with } W = \begin{pmatrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 0 & 0 & -1 \end{pmatrix}.$$

If $\psi(k) = \gamma(k)^{(U \,|\, 0_{3\times 3})} = \left(\frac{k_1}{k_2+k_3}, \frac{k_3}{k_6}\frac{k_1}{k_2+k_3}, \frac{k_3}{k_6}\frac{k_1}{k_2+k_3}\frac{k_5+k_6}{k_4}, 1, 1, 1 \right)^T$, then, for $\xi = (\xi_1, \xi_2, \xi_3)^T$, we deduce that

$$\begin{aligned} (x_3, x_6, x_4, x_1, x_2, x_5)^T &= \psi(k) \star \xi^{(W|I_3)} = \psi(k) \star \xi^A \\ &= \left(\frac{k_1}{k_2+k_3}\xi_1\xi_2,\ \frac{k_1k_3}{(k_2+k_3)k_6}\xi_1\xi_2,\ \frac{k_1k_3(k_5+k_6)}{(k_2+k_3)k_4k_6}\frac{\xi_1\xi_2}{\xi_3},\ \xi_1,\ \xi_2,\ \xi_3 \right)^T. \end{aligned}$$

Lemma 2.6. *Assume V^+ admits a monomial parameterization with exponent matrix $A \in \mathbb{Q}^{(n-p)\times n}$ and let $k \in \mathcal{K}^+_\gamma$ and $a \neq b \in \mathbb{R}^n_{>0}$ be such that $(k,a) \in V^+$ and $(k,b) \in V^+$. Then*

a) there exists $\xi \in \mathbb{R}^{n-p}_{>0} \setminus \{\mathbf{1}\}$ *such that* $b = a \star \xi^A$,

b) there exists $\mu \in \text{rowspace}(A) \setminus \{0\}$ *such that* $b = a \star e^{\mu}$.

Proof. For a) note that, by Lemma 2.4, there are $\xi_1, \xi_2 \in \mathbb{R}^{n-p}_{>0}$ such that $a = \psi(k) \star \xi_1^A$ and $b = \psi(k) \star \xi_2^A$. Hence $\psi(k) = a \star \xi_1^{-A}$ and $b = a \star \xi_1^{-A} \star \xi_2^A = a \star \xi^A$ with $\xi = \frac{\xi_2}{\xi_1}$. For b) replace ξ^A with $(e^{\ln(\xi)})^A$ in a). □

Next Theorem shows, for a fixed value of x, how to compute values of k for which $(k, x) \in V^+$.

Theorem 2.7. *Let* $x \in \mathbb{R}^n_{>0}$ *and* $k \in \mathbb{R}^r_{>0}$. *If* E *does not have any zero row, then the following are equivalent:*

a) $(k, x) \in V^+$,

b) $\exists \lambda \in \Lambda(E)$ *such that* $k = \phi\left(x^{-1}\right) \star E\lambda$.

Proof. By Definition 1.5, $(k, x) \in V^+$ if and only if $S(k \star \phi(x)) = 0$.
a) ⇒ b) Every element of $\ker(S) \cap \mathbb{R}^r_{>0}$ is of the form $E\lambda$ for some $\lambda \in \Lambda(E)$. Then, if $Sk \star \phi(x) = 0$, there is a $\lambda \in \Lambda(E)$ such that $k \star \phi\left(x\right) = E\lambda$. Hence $k = \phi\left(x^{-1}\right) \star E\lambda$.
b) ⇒ a) If $k = \phi\left(x^{-1}\right) \star E\lambda$ for some $\lambda \in \Lambda(E)$, then $k \star \phi\left(x\right) = E\lambda$. As $\forall \lambda \in \Lambda(E)$, $E\lambda \in \ker(S) \cap \mathbb{R}^r_{>0}$, $Sk \star \phi(x) = 0$. Hence $(k, x) \in V^+$. □

Next Corollary is very similar to Lemma [39, Lemma 2.5].

Corollary 2.8. *If* E *does not have any zero row then, for every* $x \in \mathbb{R}^n_{>0}$, *there is a* $k \in \mathcal{K}^+_\gamma$ *such that* $(k, x) \in V^+$.

Proof. If E does not have a zero row, then $V^+ \neq \emptyset$. Let $\lambda \in \Lambda(E)$ and $k = \phi(x^{-1}) \star E\lambda$. We have $k \star \phi(x) = E\lambda$ which is equivalent to $\nu(k, x) \in \ker(S) \cap \mathbb{R}^r_{>0}$. Hence $(k, x) \in V^+$. □

This final corollary summarizes the development so far.

Corollary 2.9. *If* V^+ *admits a monomial parameterization with exponent matrix* $A \in \mathbb{Q}^{(n-p)\times n}$, *then for every positive* $x \in \mathbb{R}^n_{>0}$ *there exists a vector* $k \in \mathbb{R}^r_{>0}$ *such that the following equivalent conditions hold:*

a) $(k, x) \in V^+$,

b) $x^M = \gamma(k)$ *and* $k \in \mathcal{K}^+_\gamma$,

c) $\exists \xi \in \mathbb{R}^{n-p}_{>0}$ *such that* $x = \psi(k) \star \xi^A$ *and* $k \in \mathcal{K}^+_\gamma$.

2.3 Multistationarity

This section collects results concerning multistationarity under the assumption that V^+ admits a monomial parameterization. Some conditions involve sign patterns similar to [8] and [35]. For a scalar x we use $\operatorname{sign}(x)$ to denote its sign, for a vector $v \in \mathbb{R}^n$ we use $\operatorname{sign}(v) = (\operatorname{sign}(v_1), \ldots, \operatorname{sign}(v_n))$ to denote its sign pattern. Theorem 2.13 appeared in a different formulation in [35].

Lemma 2.10. *If V^+ admits a monomial parameterization with exponent matrix A and cone generator matrix E, then the following are equivalent:*

a) $\mathcal{N}$ admits multistationarity,

b) $\exists\ x \in \mathbb{R}^n_{>0}$ and $\xi \in \mathbb{R}^{n-p}_{>0} \setminus \{\mathbf{1}\}$, such that $Z(x - x \star \xi^A) = 0$,

c) $\exists\ x \in \mathbb{R}^n_{>0}$ and $\kappa \in \mathbb{R}^{n-p} \setminus \{0\}$, such that $Z(x - x \star (e^\kappa)^A) = 0$.

Proof. Items b) and c) are equivalent as for any $\xi \in \mathbb{R}^{n-p}_{>0}$ there is a $\kappa \in \mathbb{R}^{n-p}$ such that $\xi = e^\kappa$. Now assume b) holds for some x and ξ. We prove that a) holds. By Lemma 2.4, there exists a $k \in \mathcal{K}^+_\gamma$ such that $(k, x) \in V^+$ and there is a $\xi' \in \mathbb{R}^{n-p}_{>0}$ such that $x = \psi(k) \star (\xi')^A$. Hadamard multiplying that last equation with ξ^A yields that $x \star \xi^A = \psi(k) \star (\xi' \star \xi)^A$ and thus $(k, x \star \xi^A) \in V^+$, again by Lemma 2.4. Since $(x - x \star \xi^A) \in \ker(Z) = \operatorname{im}(S)$, $\mathcal{N}$ admits multistationarity. When a) 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 2.6 implies $x' = x \star \xi^A$ and thus b). □

Theorem 2.11. *If V^+ admits a monomial parameterization with exponent matrix $A \in \mathbb{Q}^{(n-p)\times n}$ and there are $\mu \in \operatorname{rowspace}(A)$ and $z \in \operatorname{im}(S)$ such that $\operatorname{sign}(\mu) = \operatorname{sign}(z)$, then $\mathcal{N}$ admits multistationarity. Specifically, for arbitrary $\bar{a}_i \in \mathbb{R}_{>0}$, $i \in [n]$, let $a \in \mathbb{R}^n_{>0}$ denote the vector with entries*

$$a_i = \begin{cases} \frac{z_i}{e^{\mu_i} - 1} & \text{if } z_i \neq 0, \\ \bar{a}_i & \text{else,} \end{cases} \tag{2.3a}$$

and let

$$b = a \star e^\mu. \tag{2.3b}$$

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

$$k = \phi(a^{-1}) \star E\lambda, \tag{2.3c}$$

$\mathcal{N}$ admits multistationarity as

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

Proof. The vector b is positive, whenever a is positive and the vector a is positive, whenever $\operatorname{sign}(\mu) = \operatorname{sign}(z)$. By definition, $(b-a) = z \in \operatorname{im}(S)$. Then Theorem 2.7 shows $(k, a) \in V^+$ and Lemmata 2.3 and 2.4 also $(k, b) \in V^+$. □

Theorem 2.12. *Assume V^+ admits a monomial parameterization with exponent matrix $A \in \mathbb{Q}^{(n-p)\times n}$ and let $k \in \mathcal{K}_\gamma^+$ and $a, b \in \mathbb{R}^n_{>0}$, with $a \neq b$, be such that $(k, a) \in V^+$, $(k, b) \in V^+$, and $(b - a) \in \operatorname{im}(S)$. Let $z = b - a$ and $\mu = \ln b - \ln a$. Then*

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

b) k, a, and b together with z and μ satisfy (2.3a) – (2.3c).

Proof. For item a), $z \in \operatorname{im}(S)$ by assumption. As V^+ admits a monomial parameterization, by Lemma 2.4, there are κ_1 and $\kappa_2 \in \mathbb{R}^{n-p}$ such that $a = \psi(k) \star (e^{\kappa_1})^A$ and $b = \psi(k) \star (e^{\kappa_2})^A$. Hence $\mu = (\kappa_2 - \kappa_1)A$ and, consequently, $\mu \in \operatorname{rowspace}(A)$. By construction $b = e^\mu \star a$, and thus $z = (e^\mu - \mathbf{1}) \star a$. As a is positive, $\operatorname{sign}(e^\mu - \mathbf{1}) = \operatorname{sign}(z)$ must hold. As $\operatorname{sign}(e^\mu - \mathbf{1}) = \operatorname{sign}(\mu)$, $\operatorname{sign}(\mu) = \operatorname{sign}(z)$. For item b), (2.3b) holds by construction and (2.3a) follows from the equation $z = (e^\mu - \mathbf{1}) \star a$. Now, $(k, a) \in V^+$ implies that $k \star \phi(a) = E\lambda$ for some $\lambda \in \Lambda(E)$; hence (2.3c) also holds. □

The following Theorem is similar to [35, Proposition 3.9 and Corollary 3.11] and it employs sign patterns analysis to decide the existence of two positive real solutions to the parameterized family of polynomials (1.2) such that both are elements of the affine space $\{x \mid Zx = Za\}$.

Theorem 2.13. *If V^+ admits a monomial parameterization with exponent matrix A, then there are $k \in \mathcal{K}_\gamma^+$ and $a \neq b \in \mathbb{R}^n_{>0}$ such that $(k, a) \in V^+$, $(k, b) \in V^+$, and $Z(b - a) = 0$ if and only if*

$$\operatorname{sign}(\operatorname{rowspace}(A)) \cap \operatorname{sign}(\operatorname{im}(S)) \neq \{0\}. \tag{2.4}$$

Proof. This is the combination of Theorems 2.11 and 2.12. □

We conclude this section with a result about conservative mass-action networks that admit a monomial parameterization with only one parameter. Recall that a mass-action network is *conservative* if the conservation space $\mathcal{L}_{\text{cons}}$ contains at least one positive point (see Remark 1.12).

Theorem 2.14. *Assume that $\mathcal{N}$ is a conservative mass-action network that admits a monomial parameterization with only one parameter and $\dim(\mathcal{L}_{cons}) = 1$. Then:*

a) For any $k \in \mathcal{K}_\gamma^+$, the network $\mathcal{N}$ admits at most two positive steady states in each stoichiometric compatibility class.

b) If the exponent matrix A is nonnegative, then $\mathcal{N}$ is not multistationary.

Proof. As the positive steady state variety V^+ admits a monomial parameterization with only one parameter $t \in \mathbb{R}_{>0}$, we have $A \in \mathbb{Z}^{1\times n}$.
a) By substituting the parameterization of V^+ in the unique (up to rescaling) conservation relation of $\mathcal{N}$, say $z_1x_1 + \ldots z_nx_n - c = 0$, one obtains a univariate polynomial $P(t) \in \mathbb{R}(k)[t^{\pm}]$ with constant term $-c$. If α denotes the smallest entry of A, then $t^{|\alpha|}P(t)$ is a polynomial of the ring $\mathbb{R}(k)[t]$. As $\mathcal{N}$ is conservative, the coefficients $z_1, \ldots, z_n$ are positive. Hence, the coefficients of $t^{|\alpha|}P(t)$, when ordered decreasingly by the degree of $t^{|\alpha|}P(t)$, change sign at most twice. By Descartes' Law of Signs [1, Theorem 2.33], $t^{|\alpha|}P(t)$ has at most two positive roots for each $k \in \mathcal{K}_\gamma^+$, and so $P(t)$ does. Hence, for any $k \in \mathcal{K}_\gamma^+$, there are at most two distinct $a_1, a_2 \in \mathbb{R}^n_{>0}$ such that $(k, a_1) \in V^+$ and $(k, a_2) \in V^+$.
b) As A is nonnegative, by substituting the parameterization of V^+ in the unique (up to rescaling) conservation relation of $\mathcal{N}$, $z_1x_1 + \ldots z_nx_n - c = 0$, one obtains a univariate polynomial $P(t) \in \mathbb{R}(k)[t]$ with constant term $-c$. As $\mathcal{N}$ is conservative, the coefficients $z_1, \ldots, z_n$ are positive. Hence, the coefficients of $P(t)$, when ordered decreasingly by the degree of $P(t)$, change sign exactly once. By Descartes' Law of Signs [1, Theorem 2.33], $P(t)$ has at most one positive root for each $k \in \mathcal{K}_\gamma^+$. Hence, for $k \in \mathcal{K}_\gamma^+$, there is at most one $a \in \mathbb{R}^n_{>0}$ such that $(k, a) \in V^+$. □

2.4 Multistationarity in the space of total concentrations

In this section we study multistationarity in the space of total concentrations.

Theorem 2.15. *If V^+ admits a monomial parameterization with exponent matrix $A \in \mathbb{Q}^{(n-p)\times n}$, then the following are equivalent:*

a) $\exists k \in \mathcal{K}_\gamma^+$ and $a \neq b \in \mathbb{R}^n_{>0}$ such that $(k, a), (k, b) \in V^+$, and $(b - a) \in \mathrm{im}(S)$,

b) $\exists k \in \mathcal{K}_\gamma^+$ and $c \in \mathrm{im}_+(Z)$ such that $Z(\psi(k) \star \xi^A) = c$ has at least two solutions $\xi_1 \neq \xi_2$,

c) $\exists a \in \mathbb{R}^n_{>0}$ and $\xi \in \mathbb{R}^{n-p}_{>0}$, $\xi \neq \mathbf{1}$, such that $Z(a \star \xi^A - a) = 0$.

Proof. a)⇒b): By Lemma 2.4, there are $\xi_1, \xi_2 \in \mathbb{R}^{n-p}_{>0}$ such that $a = \psi(k) \star \xi_1^A$ and $b = \psi(k) \star \xi_2^A$; as $a \neq b$, also $\xi_1 \neq \xi_2$. Since $(b - a) \in \mathrm{im}(S)$, $Zb = Za$. Therefore, for $c = Za$, the equation $Z(\psi(k) \star \xi^A) = c$ has at least the two positive solutions ξ_1 and ξ_2.
b)⇒c): For $a = \psi(k) \star \xi_1^A$ and $b = \psi(k) \star \xi_2^A = a \star \left(\frac{\xi_2}{\xi_1}\right)^A$ one has $Za = Zb$. Hence

$Z(a \star \xi^A - a) = 0$ has the positive solution $a = \psi(k) \star \xi_1^A$ and $\xi = \frac{\xi_2}{\xi_1}$.
c)⇒a): Let $\lambda \in \Lambda(E)$ and let $k = \phi(a^{-1}) \star E\lambda$. By Theorem 2.7 $(k, a) \in V^+$. Let $b = a \star \xi^A$. By Lemma 2.3 also $(k, b) \in V^+$. As $Za = Zb$, we have $(b - a) \in \mathrm{im}(S)$. □

The following two corollaries show that arbitrary semialgebraic constraints in the total concentrations c can be added to the description of the multistationarity locus and a variant of Theorem 2.15 still holds. A *semialgebraic set* is the set of real solutions of a logical combination of finitely many polynomial equations and inequalities in which some of the variables might be quantified by the quantifiers ∃ and ∀. For more details on semialgebraic sets we refer to [1, Sections 2.4 and 2.5].

Corollary 2.16. *Assume V^+ admits a monomial parameterization with exponent matrix $A \in \mathbb{Q}^{(n-p)\times n}$, let $g_1, \ldots, g_l \in \mathbb{R}[c]$, $\square \in \{>, \geq\}^l$, and $\mathcal{F}(g(c) \square 0)$ be any logical combination of the inequalities $g(c) \square 0$. Then there are $k \in \mathcal{K}_\gamma^+$ and $c \in \mathrm{im}_+(Z)$ such that*

$$Z(\psi(k) \star \xi^A) = c, \ \mathcal{F}(g(c) \square 0)$$

has at least two positive solutions $\xi_1 \neq \xi_2$, if and only if the system

$$Z(a \star \xi^A - a) = 0, \ \mathcal{F}(g(Za) \square 0) \tag{2.5}$$

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

Proof. This is Theorem 2.15 b) and c) together with $c = Za$. □

Now suppose that Δ denotes the set of sign patterns satisfying condition (2.4). By Theorem 2.12, there are $a, b \in \mathbb{R}_{>0}^n$ with $a \neq b$ and $k \in \mathcal{K}_\gamma^+$ with $(k, a) \in V^+$, $(k, b) \in V^+$, and $(b - a) \in S$. Hence, $\mathrm{sign}(b - a) \in \Delta$. Consequently, if V^+ admits a monomial parameterization, then $b = \xi^A \star a$. Moreover, if $\delta \in \Delta$, then

$$\mathrm{sign}(b - a) = \delta \Leftrightarrow \mathrm{sign}(\ln b - \ln a) = \delta \Leftrightarrow \mathrm{sign}(\xi^A - \mathbf{1}) = \delta. \tag{2.6}$$

The following is a natural question: 'Is multistationarity possible for a given sign pattern δ and some semialgebraic constraint on the total concentrations?' The following Corollary provides a way for answering this question.

Corollary 2.17. *Assume V^+ admits a monomial parameterization with exponent matrix $A \in \mathbb{Q}^{(n-p)\times n}$, let δ be a sign pattern, let $g_1, \ldots, g_l \in \mathbb{R}[c]$, $\square \in \{>, \geq\}^l$, and $\mathcal{F}(g(c) \square 0)$ be any logical combination of the inequalities $g(c) \square 0$. Then there are $k \in \mathcal{K}_\gamma^+$, $c \in \mathrm{im}_+(Z)$, and $a, b \in \mathbb{R}_{>0}^n$ with $a \neq b$ such that*

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

if and only if there are $a \in \mathbb{R}_{>0}^n$ and $\xi \neq 1 \in \mathbb{R}_{>0}^{n-p}$ such that

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

Proof. This is Corollary 2.16 with $b - a = (\xi^A - \mathbf{1}) \star a$ and (2.6). □

The next theorems show that there is multistationarity for some value of the total concentrations c if and only if there is multistationarity for any rescaled αc.

Theorem 2.18. *Assume V^+ is nonempty and admits a monomial parameterization with exponent matrix $A \in \mathbb{Q}^{(n-p)\times n}$. For fixed $(k, a) \in V^+$ let*

$$\begin{array}{rccc} k_a: & \mathbb{R}_{>0} \times \mathbb{R}^e_{>0} & \to & \mathbb{R}^r_{>0} \\ & (\alpha, \lambda) & \mapsto & \phi\left((\alpha a)^{-1}\right) \star E\lambda \end{array} \tag{2.7}$$

Then, for fixed $c \in \mathrm{im}_+(Z)$,

$$Z(\psi(k) \star \xi^A) = c \tag{2.8}$$

has at least two positive solutions $\xi_1 \neq \xi_2$ if and only if $\exists \lambda \in \Lambda(E)$ such that

$$Z(\psi\left(k_a(\alpha, \lambda)\right) \star \xi^A) = \alpha c \tag{2.9}$$

has at least two positive solutions $\xi'_1 \neq \xi'_2$.

Proof. It suffices to show one direction as by Theorem 2.7, if $(k, a) \in V^+$, then there exists $\lambda \in \Lambda(E)$ such that $k = k_a(1, \lambda)$. Assume (2.8) has two distinct solutions. By Theorem 2.15, there are a, $b \in \mathbb{R}^n_{>0}$ such that $(k, a) \in V^+$, $(k, b) \in V^+$, and $(b - a) \in \mathrm{im}(S)$. By Theorem 2.12, the vectors a, b, k, $z = b - a$, and $\mu = \ln b - \ln a$ satisfy (2.3a)–(2.3c) for some $\lambda \in \Lambda(E)$. In particular, $b = a \star e^\mu$ with $\mu \in \mathrm{rowspace}(A)$. Let $a' = \alpha a$ and $b' = a' \star e^\mu = \alpha b$. Then, for $c' = Za' = \alpha c$ and $Zb' = c'$, we have that $(b' - a') \in \mathrm{im}(S)$. It holds that $k_a(\alpha, \lambda) \star \phi(a') = E\lambda$. Hence $(k_a(\alpha, \lambda), a') \in V^+$. Therefore also $k_a(\alpha, \lambda) \in \mathcal{K}^+_\gamma$, by Corollary 2.9. By Theorem 2.12, $\mu \in \mathrm{rowspace}(A)$, and Lemma 2.3 then yields $(k_a(\alpha, \lambda), b') \in V^+$. By Lemma 2.4, there are ξ'_1, $\xi'_2 \in \mathbb{R}^{(n-p)}_{>0}$ such that $a' = \psi(k_a(\alpha, \lambda)) \star {\xi'_1}^A$, $b' = \psi(k_a(\alpha, \lambda)) \star {\xi'_2}^A$. Using Theorem 2.15 again it follows that (2.9) has two distinct positive solutions. □

Theorem 2.19. *Assume V^+ admits a monomial parameterization with exponent matrix $A \in \mathbb{Q}^{(n-p)\times n}$ and let $c \in \mathrm{im}_+(Z)$. If the system*

$$Z(a \star \xi^A) = c \tag{2.10}$$

does not have a solution $a \in \mathbb{R}^n_{>0}$, $\xi \neq 1 \in \mathbb{R}^{(n-p)}_{>0}$, then there do not exist $k \in \mathcal{K}^+_\gamma$ and $\alpha \in \mathbb{R}_{>0}$ such that the system

$$Z(\psi(k) \star \xi^A) = \alpha c \tag{2.11}$$

has at least two solutions $\xi_1 \neq \xi_2 \in \mathbb{R}^p_{>0}$.

Proof. We prove the negation of the theorem. Equations (2.10) are equivalent to $Z(a \star \xi^A - a) = 0$, $Za = c$. Assume there are $k \in \mathcal{K}_\gamma^+$ and $\alpha \in \mathbb{R}_{>0}$ such that (2.11) has two distinct positive solutions. By Theorem 2.18, $Z(\psi(k(\alpha^{-1})) \star \xi^A) = c$ has two distinct positive solutions and by the implication b)⇒c) in Theorem 2.15, (2.10) has a solution $a \in \mathbb{R}^n_{>0}$, $\xi \in \mathbb{R}^{n-p}_{>0}$. □

The scaling invariance in the previous results can be reformulated in terms of cones. For this, let $s = \dim \mathcal{L}_{\text{stoi}}$ and denote by $\mathbb{S}^{n-s-1} \subset \mathbb{R}^{n-s}$ the unit sphere.

$$\text{Let } \mathcal{C} = \{c \in \text{im}_+(Z) \,|\, \exists k \in \mathcal{K}_\gamma^+ \text{ and } a \neq b \in \mathbb{R}^n_{>0} \text{ such that } (k,a),\ (k,b) \in V^+, \text{ and } Za = Zb = c\}.$$

By the Tarski–Seidenberg Theorem [12, Theorem 2.3], $\mathcal{C}$ is a semialgebraic set (cf. Remark 2.21). We have shown that (except the missing origin) it is a cone:

Corollary 2.20. *If V^+ admits a monomial parameterization, then $\mathcal{C}$ is a cone with the origin removed, i.e.*

$$\mathcal{C} = \left(\mathcal{C} \cap \mathbb{S}^{n-s-1}\right) \times \mathbb{R}_{>0}.$$

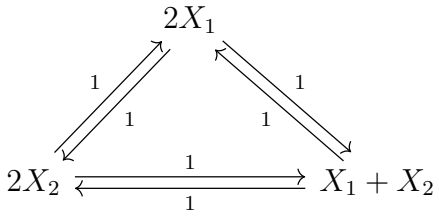
Proof. According to Theorem 2.18, given arbitrary $k \in \mathcal{K}_\gamma^+$, $c \in \text{im}_+(Z)$, and $\alpha \in \mathbb{R}_{>0}$, $Z(\psi(k) \star \xi^A) = c$ has at least two solutions $\xi_1 \neq \xi_2$ if and only if $Z(\psi(k(\alpha)) \star \xi^A) = \alpha c$ has at least two solutions $\xi_1' \neq \xi_2'$. By Theorem 2.18 $\mathcal{C}$ is a cone missing the origin. □

Remark 2.21. By Tarski–Seidenberg Theorem [12, Theorem 2.3], semialgebraic sets are closed under projections (note that this is in general not true for algebraic varieties). Another interesting feature of semialgebraic sets is that they can always be described by a logical combination of finitely many polynomial equations and inequalities without quantifiers [1, Theorem 2.77]. The process of converting a formula with quantifiers into a quantifier free formula is called *quantifier elimination*. One of the methods used for quantifier elimination is called *cylindrical algebraic decomposition* [1, Definitions 5.1 and 5.5 and Algorithm 11.16].

2.5 Toric versus positive toric steady states

By [17, Corollary 1.2], a binomial Gröbner basis of the steady state ideal is a certificate for toric steady states and thus, by Proposition 2.24, for monomial parameterizations of the positive steady states. However, as the steady state ideal may possess primary components that are irrelevant to the positive real part, a binomial steady state ideal, is not necessary for a monomial parameterization of the steady states. Next examples illustrate this circumstance.

Example 2.22. Let $\mathcal{N}_1$ be the following triangular network [33, Example 2.3]:



($\mathcal{N}_1$)

Let x_i denote the concentration of X_i. The steady state ideal of $\mathcal{N}_1$ is

$$I_1 = \langle x_1^2 - x_2^2 \rangle = \langle x_1 - x_2 \rangle \cap \langle x_1 + x_2 \rangle.$$

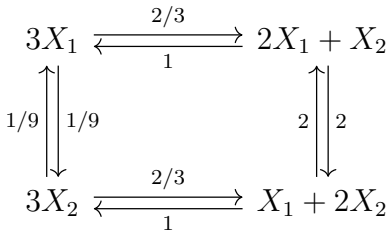
The Zariski closure of the positive steady states $\overline{V_{\mathcal{N}_1}^+} = \mathbb{V}(x_1 - x_2)$ has exactly one irreducible component defined by one binomial and is thus a toric variety. It has a monomial parameterization $x_1 = x_2 = s$, for $s \in \mathbb{R}$. Restricting this monomial parameterization to the interior of the positive orthant yields a parameterization for V_1^+ (see Fig. 2.1a). Let

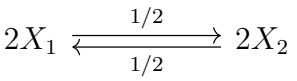
$$\begin{aligned} I_2 &= I_1 \cap \langle x_1 + x_2 + 1 \rangle = \langle x_1 - x_2 \rangle \cap \langle x_1 + x_2 \rangle \cap \langle x_1 + x_2 + 1 \rangle \\ &= \langle -x_1^3 - x_1^2 x_2 + x_1 x_2^2 + x_2^3 - x_1^2 + x_2^2 \rangle. \end{aligned} \tag{2.12}$$

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. 2.1b. Only the component $\mathbb{V}(x_1 - x_2)$ of $\mathbb{V}(I_2)$ intersects the interior of the positive orthant. Still, I_2 can be the steady state ideal of some mass-action network. According to [18, 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:

$$\dot{x}_1 = -\dot{x}_2 = -x_1^3 - x_1^2 x_2 + x_1 x_2^2 + x_2^3 - x_1^2 + x_2^2.$$

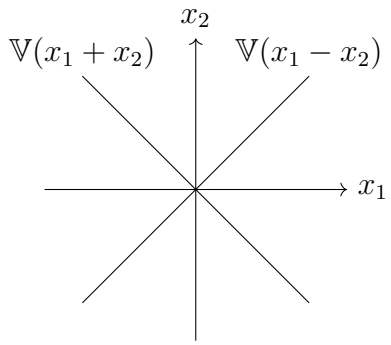
One network whose state ideal is equal to I_2 is $\mathcal{N}_2$:



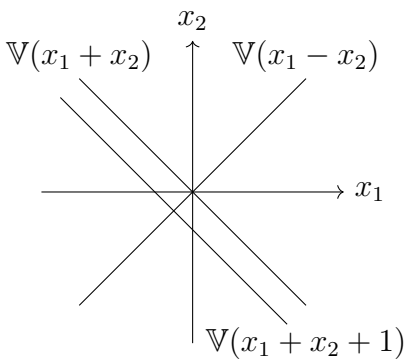


($\mathcal{N}_2$)

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



(a) The variety $\mathbb{V}(x_1^2 - x_2^2) = \mathbb{V}(x_1 - x_2) \cup \mathbb{V}(x_1 + x_2)$ of $\mathcal{N}_1$ from Example 2.22. $\mathcal{N}_1$ has toric steady states as its steady state ideal is binomial and $\overline{V_1^+}$ is nonempty and irreducible (see [33, Definition 2.2]). V_1^+ is parameterized by $s \mapsto (s, s)$, for $s \in \mathbb{R}_{>0}$.



(b) The variety $\mathbb{V}((x_1^2 - x_2^2)(x_1 + x_2 + 1)) = \mathbb{V}(x_1 - x_2) \cup \mathbb{V}(x_1 + x_2) \cup \mathbb{V}(x_1 + x_2 + 1)$ of $\mathcal{N}_2$ from Example 2.22. $\mathcal{N}_2$ does not have toric steady states according to [33, Definition 2.2] because I_2 is not binomial. Still, $\mathbb{V}(I_2) \cap \mathbb{R}^n_{>0}$ is toric and parameterized by $s \mapsto (s, s)$, for $s \in \mathbb{R}_{>0}$.

Figure 2.1: The positive steady state varieties of $\mathcal{N}_1$ and $\mathcal{N}_2$ are equal. $\mathcal{N}_1$ has binomial toric steady states while $\mathcal{N}_2$ has non-binomial toric steady states.

Example 2.23. Consider the following binomial ideal:

$$I = \langle k_1 x - k_2 x \rangle \in \mathbb{R}[k_1, k_2, k_3, k_4][x, y].$$

If we intersect I with $\langle k_3 x + k_4 y \rangle$, then we get the non-binomial ideal

$$J = \langle (k_1 x - k_2 y)(k_3 x + k_4 y) \rangle = \langle k_1 k_3 x^2 + (k_1 k_4 - k_2 k_3) xy - k_2 k_4 y^2 \rangle.$$

For all specializations $k \in \mathbb{R}^4_{>0}$, the corresponding specialization of $\mathbb{V}(J) \cap \mathbb{R}^2_{>0}$ admits a monomial parameterization. Yet, in many applications, rate constants are unknown, or they are known with big uncertainties, and the coefficients $k_1 k_3$, $k_1 k_4$, $k_2 k_3$, and $k_2 k_4$ cannot play the role of rate constants because they are not algebraically independent. One could argue that $k_1 k_3$, $k_1 k_4 - k_2 k_3$, and $k_2 k_4$ should play the role of rate constants. However, if this approach is taken, special attention has to be paid, as the sign of $k_1 k_4 - k_2 k_3$ is not constant in $\mathbb{R}^4_{>0}$.

Next proposition is a reformulation of results from [17, Section 2] and it shows why the term *toric* was used to describe systems with binomial steady state ideal.

Proposition 2.24. *If $I \subseteq \mathbb{R}[x]$ is a binomial ideal, then at most one of the irreducible components of its variety intersects $\mathbb{R}^n_{>0}$.*

In order to prove Proposition 2.24 we need to introduce a few notions. Let k be a field, let k^* denote the multiplicative group of k, and let $k[x^{\pm}]$ denote the ring of Laurent polynomials in the variables $x_1, \ldots, x_n$ with coefficients in k:

$$k[x^{\pm}] = k[x_1, \ldots, x_n, x_1^{-1}, \ldots, x_n^{-1}].$$

The ring $k[x^{\pm}]$ can be expressed as the following quotient ring:

$$k[x_1, \ldots, x_n, \widetilde{x}_1, \ldots, \widetilde{x}_n] / \langle x_i \widetilde{x}_i - 1 |\ i \in [n] \rangle.$$

A *Laurent binomial ideal* is a proper binomial ideal of $k[x^{\pm}]$. For a detailed study of binomial ideals see [17]. Every Laurent binomial ideal is of the form

$$I(\rho) = \langle x^m - \rho(m) |\ m \in L_\rho \rangle,$$

for some *partial character* ρ of $\mathbb{Z}^n$ and, if $I(\rho_1) = I(\rho_2)$, then $\rho_1 = \rho_2$ [17, Theorem 2.1.a]. A partial character ρ of $\mathbb{Z}^n$ is a homomorphism from a sublattice of $\mathbb{Z}^n$ to k^*; the domain of ρ is denoted by L_ρ. By [17, Theorem 2.1.b], if $m_1, \ldots, m_s$ form a basis for L_ρ, then

$$I(\rho) = \langle x^{m_i} - \rho(m_i) |\ i \in [s] \rangle.$$

Proof of Proposition 2.24. Without loss of generality, we can assume that $I = I : (x_1 \dots x_n)^\infty$ and $I = I\mathbb{R}[x^\pm] \cap \mathbb{R}[x]$ as all other components are contained in coordinate hyperplanes. By [17, 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 [17, 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 $\{\rho_1, \dots, \rho_g\}$ is the set of extensions of ρ to the saturation $\mathrm{Sat}(L)$ of L and g is the order of the group $\mathrm{Sat}(L)/L$. A variety $\mathbb{V}(I_+(\rho_k))$ has positive points if and only if ρ_k takes only positive real values. Fixing $b_1, \dots, b_r$ to be a basis of $\mathrm{Sat}(L_\rho)$, any basis $c_1, \dots, c_r$ 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 ρ_k be any of the extensions of ρ; since $\rho = \rho_k|_L$, we have

$$\rho(c_i) = \rho_k \left(\sum_j a_{ij} b_j \right) = \prod_j \rho_k(b_j)^{a_{ij}}. \tag{2.13}$$

These equations in the unknowns $\rho_k(b_j)$ determine the extensions of ρ 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 $\mathbb{V}(I_+(\rho_k)) \cap \mathbb{R}^n_{>0} = \emptyset$. 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 (2.13):

$$\log(\rho(c_i)) = \sum_j a_{ij} \log(\rho_k(b_j)). \tag{2.14}$$

The result is a linear equation for $\log(\rho_k(b_j))$ whose solutions yield characters ρ_k such that $\mathbb{V}(I_+(\rho_k))$ has positive points. The matrix A can be inverted over $\mathbb{Q}$. Write $\log \rho_k(b) = (\log \rho_k(b_1), \dots, \log \rho_k(b_r))$ and similarly $\log \rho(c) = (\log \rho(c_1), \dots, \log \rho(c_r))$. So (2.14) has a unique solution: $\log \rho_k(b) = A^{-1} \log \rho(c)$. Hence there is a unique saturation $\rho^* : \mathrm{Sat}(L) \to \mathbb{R}^*$ of ρ such that $\rho^*(b_i) > 0$. □

2.6 An algorithm for computing the monomial parameterization of a toric variety

This section is joint work with Thomas Kahle and Ruilong Zhuang. In particular, Algorithm 2.28 was implemented in the Macaulay2 package, Binomials [30].

In this section we present an algorithm for finding the monomial parameterization of a toric variety as the one from Example 2.5.

Let $I(\rho)$ be as in the paragraph preceding the proof of Proposition 2.24. For each $i \in [s]$, we introduce an auxiliary variable t_i and we consider the ring

$$k[y^{\pm}] = k[y_1, \ldots, y_{n+s}, y_1^{-1}, \ldots, y_{n+s}^{-1}],$$

where $y = (x_1, \ldots, x_n, t_1, \ldots, t_s)$. Similarly as for $k[x^{\pm}]$, the ring $k[y^{\pm}]$ can be seen as the following quotient ring

$$k[y_1, \ldots, y_{n+s}, \widetilde{y}_1, \ldots, \widetilde{y}_{n+s}]/\langle y_i\widetilde{y}_i - 1 | i \in [n+s]\rangle.$$

Let $M = (m_1|\ldots|m_s)$ and let $M' = (M^T| - I_s)^T$. By L' we denote the sublattice of $\mathbb{Z}^{n+s}$ spanned by the columns of M' and by $1_{L'}$ we denote the identiy element of $\mathrm{Hom}(L', k^*)$. We have obtained the following ideal of $k[x^{\pm}]$:

$$I(1_{L'}) = \langle y^m - 1_{L'}(m) |\ m \in L'\rangle = \langle x^{m_i}/t_i - 1 |\ i \in [s]\rangle.$$

Proposition 2.27 below shows that if k is algebraically closed and $I(\rho)$ is prime, in order to obtain a monomial parameterization for $\mathbb{V}(I(\rho))$ it is enough to compute a monomial parameterization for $\mathbb{V}(I(1_{L'}))$ and then specialize it to $t_i = \rho(m_i)$. Lemma 2.26 shows that in this case $\mathbb{V}(I(\rho))$ admits a monomial parameterization for which this substitution is well defined. As $1_{L'}$ is the unity of $\mathrm{Hom}(L', k^*)$, the variety $\mathbb{V}(I(1_{L'}))$ contains $\mathbf{1}$. Hence, to compute a monomial parameterization for $\mathbb{V}(I(1_{L'}))$ it is enough to find the orthogonal complement of L'.

Remark 2.25. If $I(\widetilde{\rho}) \subseteq k[x^{\pm}]$ is a geometrically prime Laurent binomial ideal and a is a point on $\mathbb{V}(I(\widetilde{\rho}))$, then $\mathbb{V}(I(\widetilde{\rho}))$ is parameterized by the monomial map

$$\begin{array}{ccc} (k^*)^r & \to & (k^*)^n \\ \xi & \mapsto & a \star \xi^A, \end{array}$$

where A is a matrix of maximal rank whose rows span the saturated lattice orthogonal to $L_{\widetilde{\rho}}$. The matrix A is easy to compute. Proposition 2.27 shows how to find an $a \in \mathbb{V}(I(\widetilde{\rho}))$.

Lemma 2.26. *Let k be an algebraically closed field. If $I(\rho) \subseteq k[x^{\pm}]$ is prime, then there exists a rank n matrix $C \in \mathbb{Z}^{n\times n}$ such that the variety $\mathbb{V}(I(1_{L'}))$ is parameterized by*

$$\phi: \begin{array}{ccc} (k^*)^n & \to & (k^*)^{n+s} \\ \xi & \mapsto & \xi^{(C|(I_s|0)^T)}, \end{array}$$

where $I_s \in \mathbb{Z}^{s\times s}$ is the identity matrix.

Proof. As $1_{L'}$ is the unity of $\mathrm{Hom}(L', k^*)$, the variety $\mathbb{V}(I(1_{L'}))$ contains $\mathbf{1}$, and we only need to prove that there exists a rank n matrix $C \in \mathbb{Z}^{n\times n}$ such that the rows of $(C|(I_s|0)^T)$ span the left kernel of M'. As k is algebraically closed and $I(\rho)$ is prime, by [17, Theorem 2.1.c] there exists $W \in \mathbb{Z}^{n\times(n-s)}$ such that the columns of $(M|W)$ span $\mathbb{Z}^n$. Hence $(M|W)$ has an inverse B in $\mathbb{Z}^{n\times n}$ and, if D denotes the submatrix corresponding to the first s rows of B, then $DM = I_s$. As $\mathrm{rank}(M) = s$, there is a rank $n-s$ matrix $E \in \mathbb{Z}^{(n-s)\times n}$ such that $EM = 0$. Then

$$\begin{pmatrix} D & I_s \\ E & 0 \end{pmatrix} \begin{pmatrix} M \\ -I_s \end{pmatrix} = 0. \tag{2.15}$$

As $\mathrm{rank}(D^T|E^T) = n$, the matrix C can be chosen to be $C = \begin{pmatrix} D \\ E \end{pmatrix}$. □

Proposition 2.27. *Let ϕ be as in Lemma 2.26 and let ψ and π denote*

$$\begin{array}{rccc} \psi: & (k^*)^{n-s} & \to & (k^*)^n \\ & \chi & \mapsto & (\rho(m_1),\ldots,\rho(m_s),\chi_1,\ldots,\chi_{n-s}) \end{array}$$

and

$$\begin{array}{rccc} \pi: & (k^*)^{n+s} & \to & (k^*)^n \\ & \zeta & \mapsto & (\zeta_1,\ldots,\zeta_n). \end{array}$$

If $I(\rho)$ is geometrically prime, then $\pi\circ\phi\circ\psi$ parameterizes $\mathbb{V}(I(\rho))$.

Proof. Let D and E be as in the proof of Lemma 2.26, let $\rho(m) = (\rho(m_1),\ldots,\rho(m_s))$, and let $\chi \in (k^*)^{n-s}$. Then

$$\pi\circ\phi\circ\psi(\chi) = \rho(m)^D \star \chi^E.$$

As, by Lemma 2.26, the rows of E span the left kernel of M, we only need to prove that

$$\pi\circ\phi\circ\psi(\mathbf{1}) \in \mathbb{V}(I(\rho)).$$

From (2.15), we deduce that $(Dm_i)_j = \delta_{ij}$. Hence
$(\pi\circ\phi\circ\psi(\mathbf{1}))^{m_k} - \rho(m_k) = \rho(m)^{Dm_k} - \rho(m_k) = \rho(m_k) - \rho(m_k) = 0.$ □

Proposition 2.27 motivates the following algorithm.

Algorithm 2.28.
Input: *A geometrically prime Laurent binomial ideal $I(\rho) \subseteq k[x^{\pm}]$.*
Output: *A monomial parameterization for $\mathbb{V}(I(\rho))$.*
1) *Let:*

- *$\{m_1,\ldots,m_s\}$ be a basis for L_ρ*

- $I(1_{L'}) = \langle x^{m_i}/t_i - 1 |\ i \in [s] \rangle \subseteq k[x']$.
- $M = (m_1 | \dots | m_s)$ *and* $M' = (M^T | -I_s)^T$.

2) *Compute a rank* n *matrix* $A \in \mathbb{Z}^{n \times (n+s)}$ *such that* $AM' = 0$.
3) *Find invertible matrices* $C, G \in \mathbb{Z}^{n \times n}$ *such that* $GA = (C|(I_s|0)^T)$ *and define*

$$\begin{array}{rccc} \phi: & (k^*)^n & \to & (k^*)^{n+s} \\ & \xi & \mapsto & \xi^{GA} \end{array}, \qquad \begin{array}{rccc} \psi: & (k^*)^{n-s} & \to & (k^*)^n \\ & \chi & \mapsto & (\rho(m_1), \dots, \rho(m_s), \chi_1, \dots, \chi_{n-s}) \end{array}$$

$$\text{and} \qquad \begin{array}{rccc} \pi: & (k^*)^{n+s} & \to & (k^*)^n \\ & \zeta & \mapsto & (\zeta_1, \dots, \zeta_n). \end{array}$$

4) *Output* $\pi \circ \phi \circ \psi$.

Proof of correctness. By Lemma 2.26 there is a rank n matrix $C \in \mathbb{Z}^{n \times n}$ such that the rows of $(C|(I_s|0)^T)$ span the left kernel of M'. If A is another matrix whose rows span the left kernel of M', there exists an invertible matrix $G \in \mathbb{Z}^{n \times n}$ such that $GA = (C|(I_s|0)^T)$. Hence Step 3 is correct. The correctness of Step 4 follows from Proposition 2.27. □

Remark 2.29. In the Macaulay2 implementation of Algorithm 2.28 special care was taken in order to avoid working in Laurent rings. This is because in Macaulay2 functions as "kernel" are not implemented for modules over Laurent rings.

Example 2.30. The Veronese surface.
The Veronese surface is defined as the image of the Veronese map ν_2 of degree 2 (see for example [24, Examples 2.4–2.7]):

$$\begin{array}{rccc} \nu_2 & \mathbb{P}^2_k & \to & \mathbb{P}^5_k \\ & (x:y:z) & \mapsto & (x^2:y^2:z^2:xy:xz:yz). \end{array} \tag{2.16}$$

This surface can be seen as the set of points $s \in \mathbb{P}^5_k$ such that the following matrix has rank 1:

$$Y_2 = \begin{pmatrix} s_0 & s_3 & s_4 \\ s_3 & s_1 & s_5 \\ s_4 & s_5 & s_2 \end{pmatrix}.$$

This rank condition can be described as the ideal generated by the minors of Y_2. The following Macaulay2 code implements the computation of a monomial Parameterization for the Veronese surface:

```
i1 : R = QQ[s_0..s_5];

i2 : Y_2 = matrix{{s_0,s_3,s_4},{s_3,s_1,s_5},{s_4,s_5,s_2}};
```

```
i3 : m = monomialParameterization minors (2,Y_2)

             QQ[tt , tt , tt , ti , ti , ti ]
                 1    2    3    1    2    3
o3 = map(-----------------------------------, R,
          (tt ti  - 1, tt ti  - 1, tt ti  - 1)
            1  1        2  2        3  3

     {tt tt ti , tt tt ti , tt tt ti , tt , tt , tt })
       1  2  3    1  3  2    2  3  1    1    2    3

                QQ[tt , tt , tt , ti , ti , ti ]
                    1    2    3    1    2    3
o3 : RingMap -----------------------------------  <--- R
              (tt ti  - 1, tt ti  - 1, tt ti  - 1)
                1  1        2  2        3  3
```

The output o3 is given as a map from the ring R of I to the ring of parameters, which we interpret as a map $\phi:\ (k^*)^3 \to (k^*)^6$: Here tt are interpreted as the parameters of the monomial parameterization while ti are interpreted as auxiliary variables such that $tt_k ti_k - 1 = 0$ (i.e. ti_k is the inverse of tt_k). Hence, if tt is denoted by t, the map ϕ can be interpreted as the following map:

$$\phi:\ \begin{array}{ccc}(k^*)^3 & \to & (k^*)^6 \\ t & \mapsto & t^A\end{array}, \qquad \text{for } A = \begin{pmatrix} 1 & 1 & -1 & 1 & 0 & 0 \\ 1 & -1 & 1 & 0 & 1 & 0 \\ -1 & 1 & 1 & 0 & 0 & 1 \end{pmatrix}.$$

Finally, for

$$G = \begin{pmatrix} 1 & 1 & 0 \\ 1 & 0 & 1 \\ 0 & 1 & 1 \end{pmatrix},$$

the following map is the Veronese map of degree 2 from (2.16):

$$\phi:\ \begin{array}{ccc}\mathbb{P}^2 & \to & \mathbb{P}^5 \\ t & \mapsto & t^{GA}\end{array}.$$

Example 2.31. In this example we compute a monomial parameterization for the positive state variety of network $\mathcal{N}_{\text{ph}}$ from Example 1.4. The following Macaulay2 code encodes this computation:

```
i1 : R = frac(QQ[k_1..k_6])[x_1..x_6];

i2 : I = ideal(k_1*x_1*x_2 - (k_2 + k_3)*x_3, k_3*x_3 - k_6*x_6,
          k_4*x_4*x_5 - (k_5 + k_6)*x_6);

i3 : m = monomialParameterization I

o3 =  map(

frac QQ[k , k , k , k , k , k ][tt , tt , tt , ti , ti , ti ]
        1   2   3   4   5   6    1    2    3    1    2    3
-------------------------------------------------------------,
           (tt ti  - 1, tt ti  - 1, tt ti  - 1)
              1  1         2  2         3  3

   k k  + k k              k      k  + k
    2 6    3 6              6      5    6
R,{-----------tt ti , tt , --tt , -------tt ti , tt , tt ,
       k k      3  1    1  k   3     k     3  2    2    3
        1 3                3         4

 k , k , k , k , k , k })
  1   2   3   4   5   6

              frac QQ[k , k , k , k , k , k ][tt , tt , tt , ti ,
                      1   2   3   4   5   6    1    2    3    1
o3 : RingMap ----------------------------------------------------
                           (tt ti  - 1, tt ti  - 1, tt ti  - 1)
                              1  1         2  2         3  3

 ti , ti ]
   2    3
---------   <--- R
```

In the output o3, the rate constants $k_1 \ldots k_6$ are treated as parameters which are mapped to themselves. To chek that this parameterization is the correct one can do the following:

```
i4 : kernel monomialParameterization I == I

o4 = true
```

The output of this computation is just `true`, which means that the kernel of the monomial parameterization is the ideal I.

Chapter 3

Multistationarity conditions on the total concentrations for sequential and distributive phosphorylations

This chapter is based on Section 4 of the paper "Multistationarity in the space of total concentrations for systems that admit a monomial parametrization" [10], joint work with Carsten Conradi and Thomas Kahle.

3.1 Introduction

In this chapter we apply the results from Chapter 2 to the well-known sequential distributive phosphorylation of a protein at two binding sites. Phosphorylations are among the best studied systems when it comes to multistationarity (e.g. [9], [26], and [21]). Applying Corollary 2.16, in Corollaries 3.6 and 3.7 we show that multistationarity is possible only if the total concentration of the substrate is larger than either the concentration of the kinase or the phosphatase. To arrive at this condition we make use of the chamber decomposition of the cone of total concentrations. Thus, in Theorem 3.1 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 3.5 we show that for two sites, multistationarity is only possible in four of these chambers.

3.2 Sequential distributive phosphorylation of a protein

The following chemical reaction network models the phosphorylation of a protein at n binding sites in a sequential and distributive way:

$$
\begin{aligned}
A + E_1 &\underset{k_2}{\overset{k_1}{\rightleftarrows}} AE_1 \xrightarrow{k_3} A_p + E_1 \\
A_p + E_2 &\underset{l_2}{\overset{l_1}{\rightleftarrows}} A_pE_2 \xrightarrow{l_3} A + E_2 \\
&\vdots \\
A_{p^{(n-1)}} + E_1 &\underset{k_{3n-1}}{\overset{k_{3n-2}}{\rightleftarrows}} A_{p^{(n-1)}}E_1 \xrightarrow{k_{3n}} A_{p^{(n)}} + E_1 \\
A_{p^{(n)}} + E_2 &\underset{l_{3n-1}}{\overset{l_{3n-2}}{\rightleftarrows}} A_{p^{(n)}}E_2 \xrightarrow{l_{3n}} A_{p^{(n-1)}} + E_2
\end{aligned}
\qquad (\mathcal{N}_{\mathrm{ph}^{(n)}})
$$

It is known that $\mathcal{N}_{\mathrm{ph}^{(n)}}$ is multistationary if and only if $n \geq 2$ [21, 26]. 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 [11]. For $n > 2$ there are bounds on the maximum number of positive steady states that can be attained [21, 26, 46]. The aim of this chapter 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:

$$
\begin{aligned}
X_1 + X_2 &\underset{k_2}{\overset{k_1}{\rightleftarrows}} X_3 \xrightarrow{k_3} X_4 + X_2 \underset{k_5}{\overset{k_4}{\rightleftarrows}} X_5 \xrightarrow{k_6} X_6 + X_2 \\
X_6 + X_7 &\underset{k_8}{\overset{k_7}{\rightleftarrows}} X_8 \xrightarrow{k_9} X_4 + X_7 \underset{k_{11}}{\overset{k_{10}}{\rightleftarrows}} X_9 \xrightarrow{k_{12}} X_1 + X_7
\end{aligned}
\qquad (\mathcal{N}_{\mathrm{ph}^{(2)}})
$$

If all reactions of $\mathcal{N}_{\mathrm{ph}^{(2)}}$ are of mass-action form, we obtain the following ODEs:

$$
\begin{aligned}
\dot{x}_1 &= f_1(x_1, \ldots, x_9) = -k_1x_1x_2 + k_2x_3 + k_{12}x_9 \\
\dot{x}_2 &= f_2(x_1, \ldots, x_9) = -k_1x_1x_2 + (k_2 + k_3)x_3 - k_4x_2x_4 + (k_5 + k_6)x_5 \\
\dot{x}_3 &= f_3(x_1, \ldots, x_9) = k_1x_1x_2 - (k_2 + k_3)x_3 \\
\dot{x}_4 &= f_4(x_1, \ldots, x_9) = k_3x_3 - k_4x_2x_4 + k_5x_5 + k_9x_8 - k_{10}x_4x_7 + k_{11}x_9 \\
\dot{x}_5 &= f_5(x_1, \ldots, x_9) = k_4x_2x_4 - (k_5 + k_6)x_5 \\
\dot{x}_6 &= f_6(x_1, \ldots, x_9) = k_6x_5 - k_7x_6x_7 + k_8x_8 \\
\dot{x}_7 &= f_7(x_1, \ldots, x_9) = -k_7x_6x_7 + (k_8 + k_9)x_8 - k_{10}x_4x_7 + (k_{11} + k_{12})x_9
\end{aligned}
$$

$$\dot{x}_8 = f_8(x_1, \ldots, x_9) = k_7 x_6 x_7 - (k_8 + k_9) x_8$$
$$\dot{x}_9 = f_9(x_1, \ldots, x_9) = k_{10} x_4 x_7 - (k_{11} + k_{12}) x_9.$$

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

$$\begin{aligned} x_2 + x_3 + x_5 &= c_1, \\ x_7 + x_8 + x_9 &= c_2, \\ x_1 + x_3 + x_4 + x_5 + x_6 + x_8 + x_9 &= c_3. \end{aligned} \tag{3.1}$$

Relations (3.1) are the rays of the *cone of conservation relations*. According to (3.1), we can choose the conservation matrix as

$$Z = \begin{pmatrix} 0 & 1 & 1 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 \\ 1 & 0 & 1 & 1 & 1 & 1 & 0 & 1 & 1 \end{pmatrix}. \tag{3.2}$$

The equations $\dot{x}_i = 0$ define the steady state ideal of $\mathcal{N}_{\mathrm{ph}^{(2)}}$:

$$\begin{aligned} I = \; & \langle -k_1 x_1 x_2 + k_2 x_3 + k_{12} x_9, -k_1 x_1 x_2 + (k_2 + k_3) x_3 - k_4 x_2 x_4 + (k_5 + k_6) x_5, \\ & k_1 x_1 x_2 - (k_2 + k_3) x_3, k_3 x_3 - k_4 x_2 x_4 + k_5 x_5 + k_9 x_8 - k_{10} x_4 x_7 + k_{11} x_9, \\ & k_4 x_2 x_4 - (k_5 + k_6) x_5, k_6 x_5 - k_7 x_6 x_7 + k_8 x_8, -k_7 x_6 x_7 + (k_8 + k_9) x_8 \\ & -k_{10} x_4 x_7 + (k_{11} + k_{12}) x_9, k_7 x_6 x_7 - (k_8 + k_9) x_8, k_{10} x_4 x_7 - (k_{11} + k_{12}) x_9 \rangle. \end{aligned}$$

The steady state ideal $I = \langle f_1, \ldots, f_9 \rangle$ is binomial as it can be seen from the following Gröbner basis (Gröbner bases can be computed in Macaulay2):

$$\begin{aligned} & \Big\{ x_5 - \frac{k_9}{k_6} x_8, x_3 - \frac{k_{12}}{k_3} x_9, x_7 x_8 - \frac{k_4 k_6 (k_{11} + k_{12})}{(k_5 + k_6) k_9 k_{10}} x_2 x_9, x_4 x_8 - \frac{k_7 (k_{11} + k_{12})}{(k_8 + k_9) k_{10}} x_6 x_9, \\ & x_1 x_8 - \frac{(k_2 + k_3) k_4 k_6 k_{12}}{k_1 k_3 (k_5 + k_6) k_9} x_4 x_9, x_6 x_7 - \frac{k_8 + k_9}{k_7} x_8, x_4 x_7 - \frac{k_{11} + k_{12}}{k_{10}} x_9, \\ & x_2 x_4 - \frac{(k_5 + k_6) k_9}{k_4 k_6} x_8, x_1 x_2 - \frac{(k_2 + k_3) k_{12}}{k_1 k_3} x_9, x_2 x_6 x_9 - \frac{(k_5 + k_6)(k_8 + k_9) k_9 k_{10}}{k_4 k_6 k_7 (k_{11} + k_{12})} x_8^2, \\ & \left(x_4^2 - \frac{k_1 k_3 (k_5 + k_6) k_7 k_9 (k_{11} + k_{12})}{(k_2 + k_3) k_4 k_6 (k_8 + k_9) k_{10} k_{12}} x_1 x_6 \right) x_9 \Big\}. \end{aligned}$$

By setting the polynomials of this Gröbner basis to zero, rearranging terms such that the x_i are on one side and the k_i on the other we obtain

$$
\begin{aligned}
\frac{x_5}{x_8} &= \frac{k_9}{k_6},\\
\frac{x_3}{x_9} &= \frac{k_{12}}{k_3},\\
\frac{x_7x_8}{x_2x_9} &= \frac{k_4k_6(k_{11}+k_{12})}{(k_5+k_6)k_9k_{10}},\\
\frac{x_4x_8}{x_6x_9} &= \frac{k_7(k_{11}+k_{12})}{(k_8+k_9)k_{10}},\\
\frac{x_1x_8}{x_4x_9} &= \frac{(k_2+k_3)k_4k_6k_{12}}{k_1k_3(k_5+k_6)k_9},\\
\frac{x_6x_7}{x_8} &= \frac{k_8+k_9}{k_7},\\
\frac{x_4x_7}{x_9} &= \frac{k_{11}+k_{12}}{k_{10}},\\
\frac{x_2x_4}{x_8} &= \frac{(k_5+k_6)k_9}{k_4k_6},\\
\frac{x_1x_2}{x_9} &= \frac{(k_2+k_3)k_{12}}{k_1k_3},\\
\frac{x_2x_6x_9}{x_8^2} &= \frac{(k_5+k_6)(k_8+k_9)k_9k_{10}}{k_4k_6k_7(k_{11}+k_{12})},\\
\frac{x_4^2}{x_1x_6} &= \frac{k_1k_3(k_5+k_6)k_7k_9(k_{11}+k_{12})}{(k_2+k_3)k_4k_6(k_8+k_9)k_{10}k_{12}}.
\end{aligned}
$$

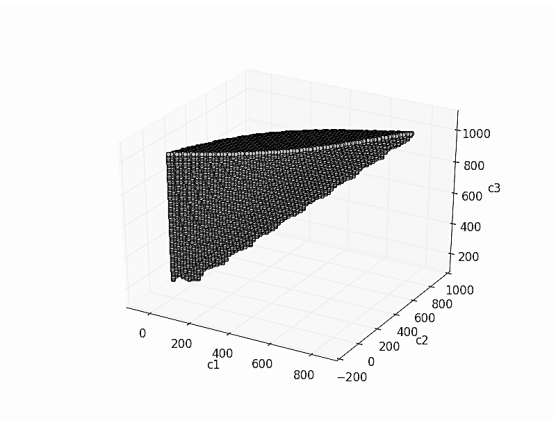
After removing redundant relations and rearranging terms one can show that the positive steady state variety V^+ admits a monomial parameterization of the form

$$x = \psi(k) \star \xi^A \text{ with } k \in \mathbb{R}^{12}_{>0} \text{ and } \xi \in \mathbb{R}^3_{>0} \text{ free, where}$$

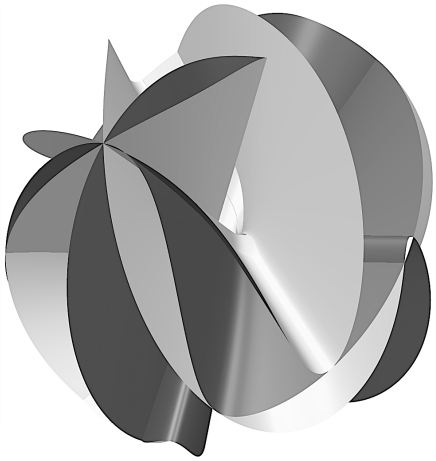
$$
\begin{aligned}
\psi(k) = \Bigg(&\frac{(k_2+k_3)k_4k_6(k_{11}+k_{12})k_{12}}{k_1k_3(k_5+k_6)k_9k_{10}},\\
&\frac{(k_5+k_6)k_9k_{10}}{k_4k_6(k_{11}+k_{12})}, \frac{k_{12}}{k_3}, \frac{k_{11}+k_{12}}{k_{10}}, \frac{k_9}{k_6}, \frac{k_8+k_9}{k_7}, 1, 1, 1\Bigg)^T
\end{aligned}
$$

and

$$
A = \begin{pmatrix}
2 & -1 & 1 & 1 & 0 & 0 & 0 & 0 & 1\\
-1 & 1 & 0 & 0 & 1 & 1 & 0 & 1 & 0\\
-1 & 1 & 0 & -1 & 0 & -1 & 1 & 0 & 0
\end{pmatrix}. \tag{3.3}
$$



(a) Numerical computation with Paramotopy. We consider a grid of 10^6 points in the space of total concentrations and represent every point which leads to multistationarity. The boundary of the corresponding multistationarity region is represented in grey and the interior in black. This cone shaped region is semialgebraic and its boundary is part of the discriminant in Fig. 3.1b.



(b) The discriminant has seven $\mathbb{Q}$-irreducible components which can be found with `Maple`. Three of them are coordinate hyperplanes and two others are sums of squares. We show only the two components which intersect the interior of the positive orthant. The boundary of the numerical approximation of the multistationarity region from Fig. 3.1a is a subset of this discriminant surface.

Figure 3.1: Representation of multistationarity in the space of total concentrations for $\mathcal{N}_{\mathrm{ph}^{(2)}}$. For both figures all rate constants have been fixed to the values given in [11, Figure 3].

3.3 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 Figure 3.1a. For this computation the rate constants have been numerically fixed to the values in [11, Figure 3]. The computation was done using Paramotopy [6] which builds on Bertini [2] and allows to efficiently analyze the solutions of 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` [32]. A discriminant of a parametric semialgebraic system Σ is a polynomial which vanishes in those points of the parameter space where the solution behaviour of Σ can change. For an extensive discussion of discriminants with a special emphasis on computation we refer to [31]. Two relevant irreducible components of the discriminant of the parametric system are visualized in Figure 3.1b. The algebraic boundary of the region from Fig. 3.1a is a subvariety of the discriminant from Fig. 3.1b. Specifically, the cone shaped region in Fig. 3.1a is also visible in the top centre of Fig. 3.1b. Both figures indicate that, for the values of the rate constants chosen in [11, Figure 3], multistationarity does not occur for all values of the total concentrations. In the next section we employ the results of Section 2.4 together with the chamber decomposition of Section 1.3 to elucidate conditions on the total concentrations for the presence or absence of multistationarity.

3.4 The chamber decomposition for $\mathcal{N}_{\mathbf{ph}^{(n)}}$

The polyhedron $\mathcal{P}_c$ corresponding to Network $\mathcal{N}_{\mathrm{ph}^{(2)}}$ is defined by the matrix Z from (3.2). The cone over the columns of Z is $\mathbb{R}^3_{\geq 0}$. There are eight basic cones generated by the following eight 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 these half-spaces are spanned by exactly two of the three columns (see [47, 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 Figure 3.2 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. As it turns out, the whole above analysis extends beyond $\mathcal{N}_{\text{ph}^{(2)}}$ and is valid for any $\mathcal{N}_{\text{ph}^{(n)}}$.

Theorem 3.1. *The cone of conservation relations of $\mathcal{N}_{ph^{(n)}}$ is $\mathbb{R}^3_{\geq 0}$ and it has five full-dimensional chambers:*

$$\Omega(1): \begin{cases} c_3 > 0 \\ c_2 > c_3 \\ c_1 > c_3, \end{cases} \quad \Omega(2): \begin{cases} c_1 > 0 \\ c_2 > c_3 \\ c_1 < c_3, \end{cases} \quad \Omega(3): \begin{cases} c_2 > 0 \\ c_2 < c_3 \\ c_1 > c_3, \end{cases}$$

$$\Omega(4): \begin{cases} c_1 < c_3 \\ c_2 < c_3 \\ c_1 + c_2 > c_3, \end{cases} \quad \Omega(5): \begin{cases} c_1 > 0 \\ c_2 > 0 \\ c_1 + c_2 < c_3. \end{cases}$$

Proof. As described in [26, Section 3], the conservation matrix of $\mathcal{N}_{\text{ph}^{(n)}}$, for the ordering of the concentrations defined in [26, 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

$$Z_0 = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 0 & 1 \\ 0 & 1 & 0 \end{pmatrix}, \quad Z_1 = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 0 & 1 \\ 1 & 1 & 1 \end{pmatrix}.$$

As $Z^{(n)}$ has the same set of columns for every $n \geq 1$, it follows that all chamber decomposition of all $\mathcal{N}_{\text{ph}^{(n)}}$ are equal. □

Remark 3.2. Although the ordering of variables defined in [26, Table 1] is different from the ordering of variables we use with $\mathcal{N}_{\text{ph}^{(2)}}$, a reordering of the variables corresponds to a reordering of the columns of $Z^{(n)}$ and thus, it leaves the chamber decomposition invariant.

Remark 3.3. Although $\mathcal{N}_{\text{ph}^{(n)}}$ has the same chamber complex for each n, the constants c express nonnegative linear combinations of the concentrations specific to each network.

3.5 Multistationarity conditions in the space of total concentrations

Now we employ Corollary 2.17 to decide whether multistationarity is possible for total concentrations in the chambers $\Omega(i)$. The linear inequality conditions $c \in \Omega(i)$

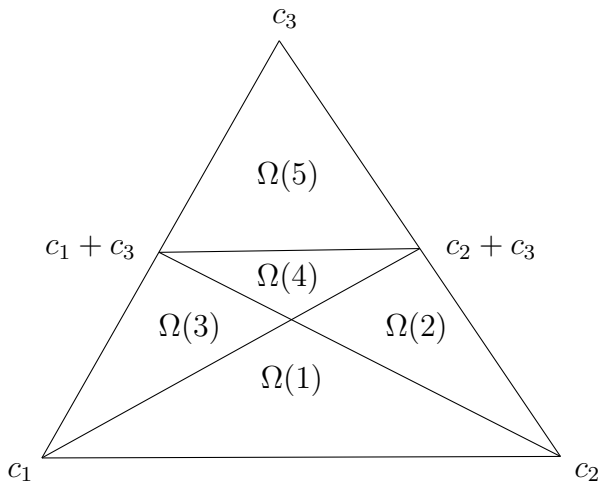


Figure 3.2: The intersection of the chamber complex associated to $\mathcal{N}_{\mathrm{ph}^{(n)}}$ with the plane $c_1 + c_2 + c_3 = 1$. Labelled vertices correspond to different columns in $Z^{(n)}$.

become the conditions $\mathcal{F}(\bullet)$ in Corollary 2.17. We also integrate the information in the sign patterns in the intersection (2.4). These have been computed in [9] and are encoded as rows of the following matrix Δ (or their negatives):

$$\Delta = \begin{pmatrix} -1 & -1 & -1 & 1 & 1 & 1 & -1 & 1 & -1 \\ 1 & 0 & 1 & -1 & -1 & -1 & 1 & -1 & 1 \\ 1 & -1 & 1 & 1 & -1 & -1 & 1 & -1 & 1 \\ 1 & -1 & 1 & 1 & -1 & -1 & 0 & -1 & 1 \\ 1 & -1 & 1 & 1 & -1 & -1 & -1 & -1 & 1 \\ 1 & -1 & 1 & 0 & -1 & -1 & 1 & -1 & 1 \\ 1 & -1 & 1 & -1 & -1 & -1 & 1 & -1 & 1 \end{pmatrix}. \tag{3.4}$$

The rows δ_i of Δ define the conditions $\operatorname{sign}(\xi^A - \mathbf{1}) = \delta_i$, $i = 1, \ldots, 7$ of Corollary 2.17. Using the matrix A from (3.3), this condition reads

$$\operatorname{sign}\left(\frac{\xi_1^2}{\xi_2\xi_3} - 1, \frac{\xi_2\xi_3}{\xi_1} - 1, \xi_1 - 1, \frac{\xi_1}{\xi_3} - 1, \xi_2 - 1, \frac{\xi_2}{\xi_3} - 1, \xi_3 - 1, \xi_2 - 1, \xi_1 - 1\right) = \delta \tag{3.5}$$

To check multistationarity for c in all of the chambers $\Omega(i)$ we use MATHEMATICA [27]. For each chamber and each row δ_i we set up the conditions of

Corollary 2.17 and use the command `Reduce` to decide the existence of solutions. In the following example we show how to set up the MATHEMATICA code to check multistationarity in $\Omega(1)$ for the sign pattern $-\delta_1$.

Example 3.4. After adding the constraints $\xi_1 > 0$, $\xi_2 > 0$, $\xi_3 > 0$ and removing redundant inequalities, (3.5) reduces to $1 < \xi_1 < \xi_2\xi_3 < \xi_1^2$ and $0 < \xi_2 < 1$. We combine this with the linear description of $\Omega(1)$ in Theorem 3.1. In the condition $Z((\xi^A - \mathbf{1}) \star a) = 0$ we use the matrix

$$Z' = \begin{pmatrix} 0 & 1 & 1 & 0 & 1 & 0 & 0 & 0 & 0 \\ 1 & 0 & 1 & 1 & 1 & 1 & -1 & 0 & 0 \\ 1 & -1 & 0 & 1 & 0 & 1 & 0 & 1 & 1 \end{pmatrix}, \tag{3.6}$$

obtained from (3.2) by elementary row operations. To obtain polynomial conditions (ξ^A is rational) we write this condition as $Z(\xi^A \star a) = Za$ and clear denominators. The following MATHEMATICA code implements this (after renaming the variables ξ_1, ξ_2, ξ_3 to `x1,x2,x3`):

```
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*x3*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*(a1-a2+a4+a6+a8+a9) &&
a1+a3+a4+a5+a6-a7<0 &&
a1-a2+a4+a6+a8+a9<0 &&
x2*x3<x1^2 && x1<x2*x3 &&
1<x1 && 1>x2 && x2>0]]
```

The result of this computation is `False`. This means that there do not exist $a_1, \ldots, a_9$ satisfying the constraints, no matter what the values of ξ_1, ξ_2, ξ_3 are and, consequently, in the chamber $\Omega(1)$ there is no multistationarity coming from $-\delta_1$. Theorem 3.5 below shows that there is no multistationarity in $\Omega(1)$ at all.

Theorem 3.5 spells out for which chambers and which signs there is multistationarity. For a pair $(\Omega(i), \delta_j)$, we write $+$ if there is multistationarity in $\Omega(i)$ for all values of ξ compatible with (3.5). We write $++$ if there is multistationarity in $\Omega(i)$ with extra conditions for ξ stronger than (3.5). We write $-$ if there is no multistationarity. If we have no conclusion, we leave the cell empty.

	δ_1	δ_2	δ_3	δ_4	δ_5	δ_6	δ_7
$\Omega(1)$	$-$	$-$	$-$	$-$	$-$	$-$	$-$
$\Omega(2)$	$-$	$-$	$+$	$+$	$++$	$+$	$++$
$\Omega(3)$	$++$	$+$	$++$	$-$	$-$	$+$	$+$
$\Omega(4)$	$++$	$+$		$+$	$++$		
$\Omega(5)$	$++$	$+$	$+$	$+$	$+$	$+$	$+$

Table 3.1: The chamber-signs incidence table of $\mathcal{N}_{\mathrm{ph}^{(2)}}$. In particular, multistationarity is not possible in $\Omega(1)$.

Theorem 3.5. *Up to the three empty cells, the chambers-signs incidence table of $\mathcal{N}_{ph^{(2)}}$ is Table 3.1. For the ++ entries the following additional constraints on the variables ξ_1, ξ_2, and ξ_3 are derived:*

$$(\Omega(2),\delta_7): 0<\xi_3<\xi_1<1 \ \wedge \ \xi_2>\frac{\xi_1^2}{\xi_3^2},$$
$$(\Omega(2),\delta_5): \xi_3>1 \ \wedge \ 0<\xi_1<1 \ \wedge \ \xi_2>\xi_3^2,$$
$$(\Omega(4),\delta_5): \xi_3>1 \ \wedge \ 0<\xi_1<1 \ \wedge \ \xi_2>\xi_3^2,$$
$$(\Omega(3),\delta_3): \xi_3^2<\xi_1<\xi_3<1 \ \wedge \xi_2>1,$$
$$(\Omega(3),\delta_1): \xi_3>1 \wedge \left(\left(1<\xi_1<\xi_3^{2/3} \ \wedge \ \frac{\xi_1}{\xi_3}<\xi_2<\frac{\xi_1^{3/2}}{\xi_3}\right)\vee\left(\xi_3^{2/3}<\xi_1<\xi_3 \ \wedge \ \frac{\xi_1}{\xi_3}<\xi_2<1\right)\right),$$
$$(\Omega(4),\delta_1): \xi_3>1 \wedge \left(\left(1<\xi_1<\xi_3^{2/3} \ \wedge \ \frac{\xi_1}{\xi_3}<\xi_2<\frac{\xi_1^{3/2}}{\xi_3}\right)\vee\left(\xi_3^{2/3}<\xi_1<\xi_3 \ \wedge \ \frac{\xi_1}{\xi_3}<\xi_2<1\right)\right),$$
$$(\Omega(5),\delta_1): \xi_3>1 \wedge \left(\left(1<\xi_1<\xi_3^{1/2} \ \wedge \ \frac{\xi_1}{\xi_3}<\xi_2<\frac{\xi_1^{2}}{\xi_3}\right)\vee\left(\xi_3^{1/2}<\xi_1<\xi_3 \ \wedge \ \frac{\xi_1}{\xi_3}<\xi_2<1\right)\right).$$

Computational Proof. The quantifier elimination problems were set up similarly to Example 3.4 and solved using MATHEMATICA. □

Table 3.1 shows that multistationarity is only possible if $c \notin \Omega(1)$:

Corollary 3.6. *For $\mathcal{N}_{ph^{(2)}}$, if $c \in \Omega(1)$, then there is no $k \in \mathbb{R}^{12}_{>0}$ such that the following equations have at least two positive solutions:*

$$Sv(k,x)=0, \ Zx=c.$$

Corollary 3.7. *For $\mathcal{N}_{ph^{(2)}}$, multistationarity can only occur if the total concentration of substrate is bigger than the total concentration of either kinase or phosphatase, i.e. $c_3 > c_2$ or $c_3 > c_1$.*

3.6 Challenging QE problems from mass-action networks

To obtain Table 3.1, some of the computations were made indirectly. For example, we checked that for δ_1 multistationarity doesn't take place in $\Omega(1)$ but we couldn't check directly that it doesn't take place in $\Omega(2)$, so we checked that it does not take place in $\Omega(1) \cup \Omega(2) \cup \Omega(1,2)$. Here $\Omega(1,2)$ denotes the boundary between $\Omega(1)$ and $\Omega(2)$. This computation was feasible. The run times of our computations seem to be sensitive to the formulation of the input. We experimented with different equivalent semialgebraic systems in MATHEMATICA. One knob to turn is the system $Z((\xi^A - \mathbf{1}) \star a) = 0$ in Corollary 2.17. As in Corollary 2.17 we are only interested in the positive solutions of the system $Z((\xi^A - \mathbf{1}) \star x) = 0$, clearing denominators does not add any new solutions. Let $\varsigma(Z, \xi^A, \delta, x)$ denote the system obtained from $Z((\xi^A - \mathbf{1}) \star x) = 0$ and δ, 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. 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 (3.2). Let $Z_1 = \left((R_1 + R_2 + R_3)^T | (R_2 + R_3)^T | R_3^T\right)^T$, $Z_2 = \left(R_1^T | R_2^T | (R_3 - R_1 - R_2)^T\right)^T$, and $Z_3 = \left(R_1^T | (R_3 - R_2)^T | (R_3 - R_1)^T\right)^T$. Using in Corollary 2.17 the matrix Z from (3.2), 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, but 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 in a few hours.

Chapter 4

Dynamical systems with the isolation property

This chapter is joint work with with Carsten Conradi and Thomas Kahle.

4.1 Introduction

Chemical reaction networks originating in Biology are usually large, but they tend to have nice combinatorial properties. In 2012 Conradi and Flockerzi [8] defined the *isolation and the bridging properties*, and found linear systems that test multistationarity in mass-action networks with these properties. In Theorem 4.17 we show that systems with the isolation property have toric positive steady states (that is their positive steady state variety admits a monomial parameterization). This theorem should be regarded in the context of Chapter 2, where the theory of multistationarity for systems with toric positive steady states is developed.

4.2 The isolation property

In this chapter chemical complexes are denoted by their corresponding monomials, rate constants are fixed, and the positive steady state variety is defined as

$$V^+ = \{x \in \mathbb{R}^n_{>0} |\ S\nu(k,x) = 0\}\,.$$

This is a local picture of the positive steady state variety defined in Chapter 2. There is also a local version of the property "admits a monomial parameterization" (cf. Definition 2.1 and Lemma 2.4). Thus, in this chapter, V^+ *admits a monomial parameterization* when there exist $A \in \mathbb{Q}^{d\times n}$, $\operatorname{rank}(A) = d < n$, and $\gamma \in \mathbb{R}^n_{>0}$,

such that V^+ is the image of the following map

$$\begin{array}{ccc} \mathbb{R}^d_{>0} & \to & \mathbb{R}^n_{>0} \\ t & \mapsto & \gamma \star t^A. \end{array}$$

If $i, j \in [r]$ are such that $i \neq j$ and k_i and k_j have the same source, then $\{i, j\}$ is called a *doubling set* (see Example 4.1). Let D denote the set of all doubling sets. The *doubling graph* is $\mathfrak{G} = ([r], D)$. Let $e_1, \ldots, e_r$ denote the canonical basis of $\mathbb{R}^r$ and let U^{doub} be a matrix which has one column $e_j - e_i \in \mathbb{R}^r$ for each doubling set $\{i, j\}$, with $i < j$. Let $\widetilde{U}$ be a matrix such that $\ker(Y_e)$ is spanned by the columns of $U = (U^{\text{doub}}|\widetilde{U})$ and let E be the cone generator matrix. Rows of E are denoted by $n_1, \ldots, n_r$.

Example 4.1. Let $k_1, k_2, k_3 \in \mathbb{R}_{>0}$, with $k_2 > k_3$, and let $\mathcal{N}_3$ denote the network

$$x^2 \underset{k_2}{\overset{k_1}{\rightleftarrows}} xy \xrightarrow{k_3} y^2 \ , \qquad (\mathcal{N}_3)$$

The only doubling set of this network is $\{2, 3\}$, so $D = \{\{2, 3\}\}$, $\mathfrak{G} = ([3], \{\{2, 3\}\})$, and $U^{\text{doub}} = (0, -1, 1)^T$. The steady state ideal of this network is $\langle k_1 x^2 - (k_2 - k_3)xy \rangle$ and the stoichiometric and the E matrices are

$$S = \begin{pmatrix} -1 & 1 & -1 \\ 1 & -1 & 1 \end{pmatrix} \text{ and } E = \begin{pmatrix} 1 & 0 \\ 1 & 1 \\ 0 & 1 \end{pmatrix}, \text{ respectively.}$$

Now suppose that E has p columns and let $\Lambda(E) = \left\{\lambda \in \mathbb{R}^p_{\geq 0} |\ E\lambda > 0\right\}$. As $V^+ \neq \emptyset$, by [8, Section 3.1] the set $\Lambda(E)$ is nonempty. Let $\Lambda_D = \left\{\lambda \in \Lambda(E) |\ \frac{E\lambda}{k} \in \ker\left(U^{\text{doub}}\right)^T\right\}$, let $\{i, j\} \in D$, and fix $\nu, \lambda \in \Lambda_D$ and $0 \neq n \in \text{span}(n_i, n_j)$. Then

$$\frac{n_i \nu}{n_i \lambda} = \frac{n_j \nu}{n_j \lambda} = \frac{n\nu}{n\lambda}.$$

Example 4.2. For $\mathcal{N}_3$ we have $\Lambda(E) = \mathbb{R}^2_{>0}$. Hence

$$\Lambda_D(E) = \{\lambda \in \mathbb{R}^2_{>0} |\ (0, -1, 1)\frac{E\lambda}{k} = 0\} = \{\lambda \in \mathbb{R}^2_{>0} |\ \frac{\lambda_1 + \lambda_2}{k_2} = \frac{\lambda_2}{k_3}\}.$$

Definition 4.3. The *preclustering graph* $\mathfrak{P}$ is the output of Algorithm 4.4. A precluster is a connected component of $\mathfrak{P}$.

The following algorithm is very similar to [8, Algorithm 1].

Algorithm 4.4.
Input: *The doubling graph* $\mathfrak{G}$.
Output: *A graph.*
1) *Let* $\mathfrak{P} = \mathfrak{G}$.
2) *While* $\exists\ \{i,j\} \in E(\mathfrak{P})$ *and* $\exists\ s \in [r]$, *with* s *not in the same connected component as* i *and* j, *such that* $n_s \in span\{n_i, n_j\}$, *adjoin* $\{i,s\}$ *and* $\{j,s\}$ *to* $E(\mathfrak{P})$.
3) *Output* $\mathfrak{P}$.

Example 4.5. For $\mathcal{N}_3$ we have $n_1 \in \text{span}(n_2, n_3)$, so the indices 1, 2, 3 are contained in the same precluster. As there are no other reaction indices, $\mathcal{N}_3$ has exactly one precluster, given by [3]. Hence

$$\mathfrak{P} = ([3], \{\{1,2\}, \{1,3\}, \{2,3\}\}).$$

Definition 4.6. We call $\mathfrak{J} = ([r], E(\mathfrak{J}))$ the *clustering graph*, where

$$E(\mathfrak{J}) = \{\{i,j\} |\ \frac{n_i\nu}{n_i\lambda} = \frac{n_j\nu}{n_j\lambda}\ \forall \nu, \lambda \in \Lambda_D\}.$$

A *cluster* is a connected component of $\mathfrak{J}$.

Example 4.7. The network $\mathcal{N}_3$ has only 3 reaction indices and [3] is a precluster, so [3] is a cluster. Hence

$$\mathfrak{J} = \mathfrak{P} = ([3], \{\{1,2\}, \{1,3\}, \{2,3\}\}).$$

Suppose that $\mathcal{N}$ has γ clusters $J_1, \ldots, J_\gamma$ and let ψ denote

$$\begin{array}{rccc} \psi: & \Lambda_D^2 & \to & \mathbb{R}^\gamma \\ & (\nu, \lambda) & \mapsto & \big(\psi_{J_1}(\nu,\lambda), \ldots, \psi_{J_\gamma}(\nu,\lambda)\big)^T, \end{array}$$

where $\psi_{J_i}(\nu,\lambda) = \ln \frac{n_j\nu}{n_j\lambda}$ for all $j \in J_i$ and $i \in [\gamma]$. Let $\Pi \in \{0,1\}^{r\times\gamma}$ be such that $\text{supp}(\Pi_i) = J_i$. Throughout this chapter $\gamma \in \mathbb{Z}_{\geq 0}$ denotes the number of clusters. Let us now define the main property of this chapter:

Definition 4.8. A mass-action network has the *isolation property* if $V^+ \neq \emptyset$ and any two rows of E indexed by different clusters have disjoint supports.

Remark 4.9. The isolation property was first defined by Conradi and Flockerzi in 2011 for parametric families of mass-action networks [8]. Our definition differs from the one of Conradi and Flockerzi in that we numerically fixed rate constants.

Example 4.10. The positive steady state variety of $\mathcal{N}_3$ is nonempty if and only if $k_2 > k_3$. Hence $\mathcal{N}_3$ has the isolation property if and only if $k_2 > k_3$.

Not all clusters are preclusters (see Example 4.12). However, by definition any precluster is contained in a cluster. This motivates the following definition:

Definition 4.11. A mass-action network has the *strong isolation property* if $V^+ \neq \emptyset$, its preclusters partition $[r]$, and any two rows of E indexed by different preclusters have disjoint supports.

The following example shows that the isolation and the strong isolation property are not equivalent properties.

Example 4.12. Consider the following chemical reaction network:

$$x^2 \underset{k_2}{\overset{k_1}{\rightleftarrows}} xy$$

As this network does not have doubling sets, according to Definition 4.3 it has two preclusters: $\{1\}$ and $\{2\}$. As its stoichiometric and E matrices are

$$S = \begin{pmatrix} -1 & 1 \\ 1 & -1 \end{pmatrix} \text{ and } E = \begin{pmatrix} 1 \\ 1 \end{pmatrix}, \text{ respectively,}$$

this network does not have the strong isolation property. However, by Definition 4.8 this network has only one cluster: $\{1, 2\}$. Hence it has the isolation property.

Remark 4.13. If a mass-action network has the strong isolation property then it has the isolation property.

Example 4.14. Clusters and preclusters are identical for $\mathcal{N}_3$, so this network has the strong isolation property if and only if $k_2 > k_3$.

Remark 4.15. Preclusters need not be computed in order to decide the isolation property. However, clusters are defined by bilinear equations in $\nu, \lambda \in \Lambda_D(E)$ (see Definition 4.6), which might be hard to solve. As preclusters are purely combinatorial objects, they might simplify this problem. In particular, knowledge about the preclusters is enough for systems with the strong isolation property.

Remark 4.16. In biochemical reaction networks rate constants are usually either not known, or they are known with big uncertainties. In such situations it is convenient to treat them as parameters. If $\{\mathcal{N}(k) |\ k \in \mathbb{R}^r_{\geq 0}\}$ denotes a parametric family of mass-action networks, then for each $k \in \mathbb{R}^r_{>0}$, the symbol $V^+(k)$ denotes the positive steady state variety of $\mathcal{N}(k)$. As the matrix E, doubling sets, and preclusters do not depend on k, and the only object which could change with k is $V^+(k)$, if there is a $k \in \mathbb{R}^r_{>0}$ such that $\mathcal{N}(k)$ has the strong isolation property, then for all $k \in \mathbb{R}^r_{>0}$ such that $V^+(k) \neq \emptyset$, the network $\mathcal{N}(k)$ has the strong isolation property. Note that the same might not be true for the isolation property, as clusters depend on Λ_D, which depends on k. This justifies the adjective *strong* in *strong isolation property*.

Now we have all ingredients to state the main result:

Theorem 4.17. *If $\mathcal{N}$ has the isolation property, then the positive steady state variety V^+ of $\mathcal{N}$ admits a monomial parameterization.*

We split the proof of Theorem 4.17 into the following six lemmata.

Lemma 4.18. *For any $a \in \mathbb{R}^n_{>0}$, one has $a \in V^+$ if and only if there exists $\lambda \in \Lambda_D$ such that $k \star a^{Y_e} = E\lambda$.*

Proof. Fix $a \in \mathbb{R}^n_{>0}$. Then $a \in V^+$ if and only if $k \star a^{Y_e} \in \ker(S) \cap \mathbb{R}^n_{>0}$ if and only if there exists $\lambda \in \Lambda(E)$ such that $k \star a^{Y_e} = E\lambda$. As $a^{Y_e} \in \ker((U^{\mathrm{doub}})^T)$, we conclude that $\lambda \in \Lambda_D$. □

Lemma 4.19. *If the clusters partition $[r]$ and $a, b \in V^+$, then there exists $\kappa \in \operatorname{im}\psi \cap \ker(U^T\Pi)$ such that $a^{Y_e} = e^{(\Pi\kappa)^T} \star b^{Y_e}$.*

Proof. If $a, b \in V^+$ then, by Lemma 4.18, there exist $\lambda, \nu \in \Lambda_D$ such that $k \star a^{Y_e} = E\lambda$ and $k \star b^{Y_e} = E\nu$. Hence $\frac{E\nu}{E\lambda} = \left(\frac{b}{a}\right)^{Y_e}$, and $\ln \frac{E\nu}{E\lambda} = Y_e^T \frac{a}{b}$. As $Y_e U = 0$, we deduce that $U^T \ln \frac{E\nu}{E\lambda} = 0$. As the clusters partition $[r]$, we deduce that $\ln \frac{E\nu}{E\lambda} = \Pi\psi(\nu, \lambda)$. Hence $\psi(\nu, \lambda) \in \ker(U^T\Pi)$. Consequently, for $\kappa = \psi(\nu, \lambda)$, we have $a^{Y_e} = e^{(\Pi\kappa)^T} \star b^{Y_e}$. □

Lemma 4.20. *If the clusters partition $[r]$ and $\kappa \in \operatorname{im}\psi \cap \ker(U^T\Pi)$, then there exists $t \in \mathbb{R}^n_{>0}$ such that $t^{Y_e} = e^{(\Pi\kappa)^T}$.*

Proof. If $\kappa \in \operatorname{im}\psi$, then there exist $\nu, \lambda \in \Lambda_D$ such that $\Pi\kappa = \ln \frac{E\nu}{E\lambda}$. If additionally $\kappa \in \ker(U^T\Pi)$, then we also have $U^T\Pi\kappa = 0$, and we deduce that $\left(\frac{E\nu}{E\lambda}\right)^U = 1$. As the rows of Y_e span the left kernel of U, there exists $t \in \mathbb{R}^n_{>0}$ such that $t^{Y_e} = \frac{E\nu}{E\lambda} = e^{(\Pi\kappa)^T}$. □

Lemma 4.21. *If $\mathcal{N}$ has the isolation property, $\kappa \in \operatorname{im}\psi$, and $v \in \ker(S) \cap \mathbb{R}^r_{>0}$, then $e^{(\Pi\kappa)^T} \star v \in \ker(S) \cap \mathbb{R}^r_{>0}$.*

Proof. Fix $\kappa \in \operatorname{im}\psi$ and $v \in \ker(S) \cap \mathbb{R}^r_{>0}$. Then there exists $\lambda \in \Lambda(E)$ such that $v = E\lambda$. Let $\nu = (e^{\kappa_1}\lambda^T_{\mathrm{supp}(J_1)}| \ldots |e^{\kappa_\gamma}\lambda^T_{\mathrm{supp}(J_\gamma)})^T$. As the clusters partition $[r]$, we deduce that $e^{(\Pi\kappa)^T} \star (E\lambda) = E\nu$. As $\operatorname{sign}(\nu) = \operatorname{sign}(\lambda)$, we conclude that $\nu \in \Lambda(E)$ [8, Remark 4.(3)]. Hence $E\nu \in \ker(S) \cap \mathbb{R}^r_{>0}$. □

Lemma 4.22. *If $\mathcal{N}$ has the isolation property, $a \in V^+$, $\kappa \in \operatorname{im}\psi \cap \ker(U^T\Pi)$, and $t \in \mathbb{R}^n_{>0}$ is such that $t^{Y_e} = e^{(\Pi\kappa)^T}$, then $a \star t \in V^+$.*

Proof. By Lemma 4.18 there is a $\lambda \in \Lambda_D$ such that $k \star a^{Y_e} = E\lambda$. By Lemma 4.20 there is a $t \in \mathbb{R}^n_{>0}$ such that $t^{Y_e} = e^{(\Pi\kappa)^T}$. After multiplying $k \star a^{Y_e} = E\lambda$ with $t^{Y_e} = e^{(\Pi\kappa)^T}$ we deduce that $k \star (a \star t)^{Y_e} = e^{(\Pi\kappa)^T} \star (E\lambda)$. By Lemma 4.21 $e^{(\Pi\kappa)^T} \star (E\lambda) \in \ker(S) \cap \mathbb{R}^r_{>0}$. Hence $k \star (a \star t)^{Y_e} \in \ker(S)$, and $a \star t \in V^+$. □

The following Lemma was first stated in [8].

Lemma 4.23. *If $\mathcal{N}$ has the isolation property, then* $\operatorname{im}\psi = \mathbb{R}^\gamma$.

Proof. Fix $\kappa \in \mathbb{R}^\gamma$ and $\lambda \in \Lambda_D$ and let $\nu = (e^{\kappa_1}\lambda^T_{\operatorname{supp}(J_1)}|\dots|e^{\kappa_\gamma}\lambda^T_{\operatorname{supp}(J_\gamma)})^T$. As the clusters partition $[r]$ and $\operatorname{sign}(\lambda) = \operatorname{sign}(\nu)$, by [8, Remark 4.(3)], we have $\nu \in \Lambda(E)$. As $\frac{n_i\nu}{\nu_i\lambda} = \frac{n_j\nu}{\nu_j\lambda}$ whenever i, j are elements of the same cluster, we conclude that $\nu \in \Lambda_D$. Hence $\kappa \in \operatorname{im}\psi$. □

Proof of Theorem 4.17. By Lemma 4.23 $\operatorname{im}\psi = \mathbb{R}^\gamma$. By Lemma 4.19 there is a $\kappa \in \mathbb{R}^\gamma \cap \ker(U^T\Pi)$ such that $a^{Y_e} = e^{(\Pi\kappa)^T} \star b^{Y_e}$ and, by Lemma 4.20, e^κ can be chosen to partially parameterize the solution set of this equation. Hence there are $d \in \mathbb{Z}_{\geq 0}$ and $A \in \mathbb{Z}^{d\times n}$ such that, for arbitrary $a, b \in V^+$, there exists $s \in \mathbb{R}^d_{>0}$ with $b = s^A \star a$. Finally, by Lemma 4.22, for arbitrary $a \in V^+$ and $s \in \mathbb{R}^d_{>0}$, $a \star s^A \in V^+$. □

Corollary 4.24. *If $\mathcal{N}$ has the strong isolation property, then the positive steady state variety V^+ of $\mathcal{N}$ admits a monomial parameterization.*

Example 4.25. The set of positive steady states of $\mathcal{N}_3$ can be parameterized as

$$\begin{array}{ccc} \mathbb{R}_{>0} & \to & \mathbb{R}^2_{>0} \\ s & \mapsto & (s, \frac{k_1}{k_2-k_3}s). \end{array}$$

The following example shows that there exist distinct mass-action networks with the same dynamics, one of which has the strong isolation property, while the other one does not.

Example 4.26. Let $k_1, k_2, k_3 \in \mathbb{R}_{>0}$, with $k_2 > k_3$, and let $\mathcal{N}_4$ denote

$$x^2 \underset{k_2-k_3}{\overset{k_1}{\rightleftarrows}} xy \ . \tag{$\mathcal{N}_4$}$$

The dynamics of both networks $\mathcal{N}_3$ and $\mathcal{N}_4$ are defined by the same system of ODEs. However, $\mathcal{N}_4$ has no doubling sets, so it doesn't have the strong isolation property. In particular, this example shows that a binomial steady state does not imply the strong isolation property.

Remark 4.27. In [8] the isolation property was defined under the assumption that there is at least one doubling set [8, Assumption 2]. For the purpose of this chapter, this requirement is not necessary. However, if we add this assumption to the definition of isolation property, network $\mathcal{N}_4$ not only does not have the strong isolation property, but it also doesn't have the isolation property.

Remark 4.28. In general it was very difficult to find reaction networks with the isolation property starting from a fixed E matrix. We couldn't find examples of systems with the isolation property without binomial steady state ideal.

Question 4.29. *Given a mass-action network $\mathcal{N}$ without the (strong) isolation property, is there an algorithm which decides whether the dynamical systems defined by $\mathcal{N}$ can be represented by another mass-action network with the (strong) isolation property?*

Question 4.30. *Do all mass-action networks with the isolation or the strong isolation property have binomial steady state ideals?*

Remark 4.31. Another class of nice chemical reaction networks is formed by mass-action networks with the *bridging property*. The bridging property is defined similarly to the isolation property [8, Definition 3]. In [8, Theorem 2] the authors give necessary and sufficient conditions for the existence of multistationarity in systems with the isolation or the bridging properties, very similar to those given for systems with the isolation property [8, Lemma 4]. However, it is not clear that Theorem 4.17 can be generalized to systems with the bridging property. The main difficulty is that Lemmata 4.21, 4.22, and 4.23 fail to be true for systems with the bridging property, as for these systems rows of E indexed by different clusters do not have disjoint supports.

Chapter 5

Sturm discriminants

5.1 Introduction

Up to date, discriminants of parametric systems of semialgebraic systems have only been implemented in the `RootFinding[Parametric]` subpackage of `Maple` and this implementation is based on algorithms introduced in 2007 by Lazard and Rouillier [31], [34], and [23]. While at the present moment these algorithms seem to be the most efficient ones, they are not easily implementable in free computer algebra systems as Macaulay2. In this chapter we offer an easier alternative, via Sturm sequences, for the computation of discriminants of systems with positive roots. Discriminants computed this way are in general not minimal.

Given a univariate polynomial p, its Sturm sequence $s(p)$ is a list of at most degree of p other polynomials containing information about the real roots of p. In many applications, only the positive roots of a polynomials are meaningful (e.g. when variables denote concentrations of chemical species). The number of distinct positive roots of p is encoded in the signs of the leading coefficients and the constant terms of the elements of $s(p)$. Sturm sequences can also be associated to zero dimensional ideals: if $I \subseteq \mathbb{R}[x_1, \ldots x_n]$ defines a zero dimensional variety then, for $i \in [n]$, a Sturm sequence can be assigned to the unique generator of the elimination ideal $I \cap \mathbb{R}[x_i]$. In this chapter we compute Sturm sequences of certain parametric families of zero dimensional ideals and we show that they can be used to compute discriminants. For a more detailed treatment of Sturm sequences, we refer to [1, Chapters 1 and 2] and [3, Chapter 1].

5.2 Sturm sequences and discriminants

Let $R = \mathbb{R}[t_1, \ldots, t_m]$ and $\mathbb{K} = \mathbb{R}(t_1, \ldots, t_m)$. If $p \in R[x]$ is any univariate polynomial, then the *Sturm sequence* $s(p)$ of p is the sequence of signed remainders of p and p', i.e.

$$s(p) = (s_0(p), \ldots, s_r(p)) \in \mathbb{K}^{r+1},$$

where $s_0(p) = p$, $s_1(p) = p'$, $s_i = -\operatorname{Rem}(s_{i-2}(p), s_{i-1}(p))$ for $2 \leq i \leq r$, and r is such that $s_r(p) \neq 0$ and $\operatorname{Rem}(s_{r-1}(p), s_r(p)) = 0$.

Definition 5.1. The *Sturm discriminant* $\Delta_S(p)$ of $p \in R[x]$ is the polynomial obtained by multiplying the numerators and denominators of the leading coefficients and nonzero constant terms of elements of $s(p)$. The *reduced Sturm discriminant* is $(\Delta_S(p))_{\text{red}}$. Connected components of $\mathbb{R}^m \setminus \mathbb{V}(\Delta_S(p))$ or $\mathbb{R}^m \setminus \mathbb{V}((\Delta_S(p))_{\text{red}})$ are called cells of the discriminant.

Remark 5.2. There might be values of the parameters t for which the corresponding specialization of the Sturm sequence is not well defined. However, a specialization of the Sturm sequence is always well defined for values of the parameters lying in $\mathbb{R}^m \setminus \mathbb{V}((\Delta_S(p))_{\text{red}})$.

In order to have nice formulas for $\Delta_S(p)$, the polynomial p should be generic enough. The following definition makes this precise in our setting.

Definition 5.3. A degree d polynomial $p \in R[x]$ is *Sturm generic* if its Sturm sequence has $d+1$ elements and, for $i \in \{0, \ldots, d\}$, $s_i(p)$ has $d - i + 1$ terms.

Remark 5.4. The discriminant $\Delta(p)$ of the univariate polynomial p is a polynomial in the coefficients of p which vanishes whenever p has a double root in $\mathbb{C}$ [22, Equation (1.23)]. If p is Sturm generic, then, by [1, Corollary 8.33], $\Delta(p)$ is not identically zero. The converse of this fact fails to be true. For example, the discriminant of the polynomial $x^2 - 1$ has nonzero discriminant, yet it is not Sturm generic. For a review of classical results about discriminants of univariate polynomials we refer to [22, Chapter 12].

Lemma 5.5. *Sturm generic polynomials exist in any degree.*

Proof. Let $\epsilon_1, \epsilon_2 \in \mathbb{R}_{>0}$, with $\epsilon_1 > 1$, and let $d \in \mathbb{Z}_{>0}$. Let $p_0 = 1$, $p_1 = x + 1$, and for $2 \leq i \leq d-1$, let $p_i = (x + 1 + \epsilon_1)p_{i-1} - p_{i-2}$. If p_d denotes a polynomial with constant term ϵ_2 such that $p_d' = p_{d-1}$, then p_d is Sturm generic with Sturm sequence $(p_d, \ldots, p_0)$. □

The computation of Sturm sequences is in general slow, so ready to use formulas are always useful. Proposition 5.6 below shows that this is possible whenever the coefficients of p are algebraically independent.

Proposition 5.6. *If the coefficients of p are all nonzero and algebraically independent, then p is a Sturm generic polynomial.*

Proof. Suppose that there is a $p \in R[x]$ for which the proposition is false and let $d = \deg(p)$. Then there are natural numbers i, j such that the j^{th} coefficient of $s_i(p)$ is zero. As the coefficients of p are algebraically independent, there is no degree d polynomial $q \in \mathbb{R}[x]$ such that the j^{th} coefficient of $s_i(q)$ is nonzero. But this is in contradiction with Lemma 5.5. □

Example 5.7. The Sturm sequence of $p_1 = s_1 + s_2x + s_3x^2 + s_4x^3 \in \mathbb{R}[s_1, s_2, s_3, s_4][x]$ consists of 4 polynomials, the last of which is

$$s_3(p_1) = \frac{9s_4(s_2^2s_3^2 - 4s_1s_3^3 - 4s_2^3s_4 + 18s_1s_2s_3s_4 - 27s_1^2s_4^2)}{4(s_3^2 - 3s_2s_4)^2}.$$

As the zero locus of the denominator of $s_4(p_1)$ is the image of

$$\begin{array}{ccc} \mathbb{R}^3 & \to & \mathbb{R}^4 \\ (\xi_1, \xi_2, \xi_3) & \mapsto & (\xi_1, \xi_2, \xi_2\xi_3, \frac{1}{3}\xi_2\xi_3^2), \end{array}$$

the Sturm sequence of the polynomial $p_2 = \xi_1 + \xi_2x + \xi_2\xi_3x^2 + \frac{1}{3}\xi_2\xi_3^2x^3 \in \mathbb{R}[\xi_1, \xi_2, \xi_3][x]$ cannot be obtained by substituting s_1 with ξ_1, s_2 with ξ_2, s_3 with $\xi_2\xi_3$, and s_4 with $\frac{1}{3}\xi_2\xi_3^2$ in $s(p_1)$.

For $a \in \mathbb{R}^m$, let p_a denote the specialization of p to $t = a$.

Theorem 5.8. *If $p(0) \neq 0$ and a and b are contained in a common cell of $\mathbb{V}(\Delta_S(p))$, then p_a and p_b have the same number of distinct positive roots.*

Proof. By Sturm's Theorem [1, Theorem 2.62], for any specialization $a \in \mathbb{R}^m \setminus \mathbb{V}(\Delta_s(p))$, the number of distinct positive roots of p_a is

$$\operatorname{Var}(s_0(p_a(0)), \ldots, s_d(p_a(0))) - \operatorname{Var}(s_0(p_a(\infty)), \ldots, s_d(p_a(\infty))).$$

As sign $s_i(p_a(0)) =$ sign $\operatorname{CT}(s_i(p_a))$ and sign $s_i(p_a(\infty)) =$ sign $LC(s_i(p_a))$, the number of positive roots of p_a is equal to

$$\operatorname{Var}(\operatorname{CT}(s_0(p_a)), \ldots, \operatorname{CT}(s_d(p_a))) - \operatorname{Var}(\operatorname{LC}(s_0(p_a)), \ldots, \operatorname{LC}(s_d(p_a))).$$

But the signs of $\operatorname{CT}(s_i(p_a))$ and $\operatorname{LC}(s_j(p_a))$ can change at most when a crosses the variety $\mathbb{V}(\Delta_S(p))$. □

Example 5.9. The Sturm discriminant of $p_1 = t_1 + t_2x + t_3x^2 \in \mathbb{R}[t_1, t_2, t_3][x]$ is

$$(\Delta_S(p_1))_{\text{red}} = t_1t_2t_3\Delta(p_1) = t_1t_2t_3(t_2^2 - 4t_1t_3).$$

Let $p_2 = s_1s_2 + s_3x + s_4x^2 \in \mathbb{R}[s_1, s_2, s_3, s_4][x]$. As both p_1 and p_2 are Sturm generic of the same degree, $\Delta_S(p_2)$ can be computed by substituting t_1 with s_1s_2, t_2 with s_3, and t_3 with s_4 in $\Delta_S(p_1)$:

$$(\Delta_S(p_2))_{\text{red}} = s_1s_2s_3s_4\Delta(p_2) = s_1s_2s_3s_4(s_3^2 - 4s_1s_2s_4).$$

The polynomial $p_3 = s_1 + s_3x^2$ is not Sturm generic, and if one substitutes its coefficients in $(\Delta_S(p_1))_{\text{red}}$, then one gets 0. This is due to the fact that in p_3 the coefficient of x is 0 and, consequently, the constant term of p_3' is zero. However, according to Definition 5.1, zero constant terms don't play any role in $\Delta_S(p_3)$. Hence, for p_3 a new Sturm sequence has to be computed in order to get its Sturm discriminant. By doing so, one gets that

$$(\Delta_S(p_3))_{\text{red}} = s_1s_3.$$

Positive or negative factors of $(\Delta_S(p))_{\text{red}}$ do not play any role in the discriminant as they cannot vanish. The following definition takes care of this situation.

Definition 5.10. The *minimal Sturm discriminant* $(\Delta_S(p))_{\text{min}}$ of p is the polynomial obtained by removing from the reduced Sturm discriminant $(\Delta_S(p))_{\text{red}}$ its positive and its negative irreducible components.

Example 5.11. The Sturm discriminant of $p = t_1^2 + t_2^2 + 1 + t_2x + t_3x^2$ is

$$(\Delta_S(p))_{\text{red}} = t_2t_3(t_1^2 + t_2^2 + 1)(t_2^2 - 4(t_1^2 + t_2^2 + 1)t_3).$$

As the only positive or negative factor of $(\Delta_s(p))_{\text{red}}$ is $t_1^2 + t_2^2 + 1$, we get

$$(\Delta_S(p))_{\text{min}} = t_2t_3(t_2^2 - 4(t_1^2 + t_2^2 + 1)t_3).$$

Remark 5.12. A polynomial is positive if it can be written as the sum of a nonnegative polynomial and a positive constant. The classical method for certifying the nonnegativity of a multivariate polynomial is writing it as a sum of squares. However, in 1888 Hilbert proved that every nonnegative degree d polynomial $P \in \mathbb{R}[y_1, \ldots, y_n]$ is a sum of squares only if $n = 1$, or $n = 2$ and $d = 4$, or $d = 2$ (e.g. [5] and the references therein). Another certificate of nonnegativity, based on *circuit polynomials*, was introduced in [15] and [16]. Recently, the authors of [36] introduced yet another certificate of nonnegativity, based on signomials. Moreover, in [36, Theorem 20], the authors provide a computationally tractable formulation for the corresponding cone of nonnegative polynomials.

5.3 Zero dimensional ideals

If Σ is a parametric family of multivariate zero dimensional systems of equations, a Sturm sequence can be associated to the univariate polynomial resulting from eliminating all variables of Σ except one, whenever there is a unique such polynomial. Let $I \subseteq R[x_1, \ldots, x_n]$ denote the ideal generated by the polynomials of Σ and suppose that I is such that, for all $i \in [n]$, the elimination ideal $I_i = I \cap R[x_i]$ is principal and nonzero (see Section 5.4 for an instance of this phenomenon). Let $p_i \in R[x_i]$ denote the generator of I_i.

Definition 5.13. The ideal I is called *Sturm generic* if all p_i are Sturm generic.

For any $g \in R$, let

$$\Delta_S(I, g) = (g \prod_{i=1}^{m} \Delta_S(p_i))_{\text{red}}.$$

Remark 5.14. For all $i \in [n]$, p_i is zero if and only if all its coefficients are zero. Hence there is an algebraic variety $V \neq \mathbb{R}^m$ such that, for all $a \in \mathbb{R}^m \setminus V$, $\dim I_a = 0$. Here I_a denotes the specialization of I at $t = a$. Then there exists $0 \neq h \in R$ such that for all $a \in \mathbb{R}^m \setminus \mathbb{V}(h)$, we have $\dim I_a = 0$.

Definition 5.15. The *Sturm discriminant* of I is $\Delta_S(I) = \Delta_S(I, 1)$ and the *Sturm discriminant variety* of I is $\mathbb{V}(\Delta_S(I))$. Connected components of $\mathbb{R}^m \setminus \mathbb{V}(\Delta_S(I))$ are called *cells* of the discriminant.

Theorem 5.16. *If, for all $i \in [n]$, $p_i(0) \neq 0$ and a and b are contained in a common cell of $\mathbb{V}(\Delta_S(I))$, then the sets $\mathbb{V}(I_a) \cap \mathbb{R}^m_{>0}$ and $\mathbb{V}(I_b) \cap \mathbb{R}^m_{>0}$ have the same number of points.*

Proof. This is a consequence of Proposition 5.8. □

5.4 The discriminant of the 1-site phosphorylation

After substituting the parameterization (1.4) in the conservation laws (1.7) we get the following parametric family of semialgebraic systems:

$$\begin{cases} x_1 + a_1 x_1 x_2 - c_1 = 0, \\ x_5 + a_3 x_1 x_2 - c_2 = 0, \\ x_2 x_5 + a_1 x_1 x_2 x_5 + a_2 x_1 x_2 + a_3 x_1 x_2 x_5 - c_3 x_5 = 0, \\ x_1 > 0,\ x_2 > 0,\ x_3 > 0, \end{cases} \tag{5.1}$$

where

$$a_1 = \frac{k_1}{k_2 + k_3},\ a_2 = \frac{k_1 k_3 (k_5 + k_6)}{(k_2 + k_3) k_4 k_6},\ \text{and}\ \ a_3 = \frac{k_1 k_2}{(k_2 + k_3) k_6}.$$

The discriminant can be computed with `RootFinding[Parametric]`, which is a `Maple` package. The following code implements this computation for (5.1):

```
with(RootFinding[Parametric]):
DiscriminantVariety([x1+a1*x1*x2-c1=0, x5+a3*x1*x2-c2=0,
x2*x5+a1*x1*x2*x5+a2*x1*x2+a3*x1*x2*x5-c3*x5=0,
a1>0,a2>0,a3>0,c1>0,c2>0,c3>0,x1>0,x2>0,x5>0],[x1,x2,x5]);
```

The result of this computation is a list of 9 polynomials in the variables a and c with degrees between 1 and 12 (cf. Table 5.1). They represent $\mathbb{Q}$-irreducible components of the discriminant. As it turns out, the previous computation can be done via Sturm sequences, albeit the resulting discriminant is not minimal. We wrote a Macaulay2 package which has one main function called SturmDiscriminants [28]. The following Macaulay2 code computes the Sturm discriminant of (5.1):

```
R = QQ[a1,a2,a3,c1,c2,c3][x1,x2,x5];
SturmDiscriminant ideal(x1+a1*x1*x2-c1, x5+a3*x1*x2-c2,
x2*x5+a1*x1*x2*x5+a2*x1*x2+a3*x1*x2*x5-c3*x5)
```

The result of this computation is a list of 17 polynomials in the variables a and c with degrees between 1 and 12. In Table 5.1 we compare the degrees of the $\mathbb{Q}$-irreducible components of the discriminants computed with Maple and Macaulay2. In particular, the highest degree of any irreducible component of both the Sturm and the `Maple` discriminant is 12.

Degree	1	2	3	4	6	7	8	12	Total degree
`Maple` discriminant	7	1	0	0	0	0	0	1	21
$(\Delta_S)_{\text{red}}$	7	1	1	2	1	3	1	1	67

Table 5.1: The component-degrees incidence table. The first row represents the possible degrees of the $\mathbb{Q}$-irreducible components of the discriminants and the other rows represent the number of components in each degree that the `Maple` and the Sturm discriminant have.

Remark 5.17. It is well known that the 1-site phosphorylation is monostationary for all values of the parameters [26]. We used it nevertheless because it has the right size to compare the `Maple` and the Sturm discriminants.

Appendix 5.1. Discriminants as analytic covers

In [31], the authors propose an efficient algorithm for the computation of discriminant varieties of basic parametric constructible sets of the form

$$\mathcal{C} = \{(t, x) \in \mathbb{C}^{m+n} |\ f_i \neq 0, g_j = 0, i \in [l], j \in [s]\},$$

where $f_i, g_j \in \mathbb{Q}[t, x]$ and t are interpreted as parameters. If π denotes the canonical projection onto the parameter space, then the minimal discriminant of $\mathcal{C}$ is defined as the complement in $\overline{\pi(\mathcal{C})}$ of the union of all open sets $\mathcal{U} \subseteq \overline{\pi(\mathcal{C})}$ for which $\pi : \mathcal{C} \to \mathcal{U}$ is an analytic cover. In [31, Proposition 6] the authors show that, whenever the discriminant of $\mathcal{C}$ is not equal to $\overline{\pi(\mathcal{C})}$, it can be turned into a real discriminant for the basic parametric semialgebraic set

$$\mathcal{S} = \{(t, x) \in \mathbb{R}^{m+n} |\ f_i > 0, g_j = 0, i \in [l], j \in [s]\}.$$

The complement of the real discriminant variety consists, in general, of more than one connected component [31]. For algorithms to compute one point in each connected component of a semialgebraic set, we refer to [40] and [41].

Bibliography

[1] Saugata Basu, Richard Pollack, and Marie-Francoise Roy. *Algorithms in real algebraic geometry*, volume 10. Springer, second edition, 2006.

[2] Daniel J. Bates, Jonathan D. Hauenstein, Andrew J. Sommese, and Charles W. Wampler. Bertini: Software for Numerical Algebraic Geometry. Available at bertini.nd.edu with permanent doi: dx.doi.org/10.7274/R0H41PB5.

[3] Riccardo Benedetti and Jean-Jacques Risler. *Real algebraic and semialgebraic sets*. Hermann, 1990.

[4] Frédéric Bihan, Alicia Dickenstein, and Magalí Giaroli. Lower bounds for positive roots and regions of multistationarity in chemical reaction networks. *preprint, arXiv:1807.05157*, 2018.

[5] Grigoriy Blekherman. Nonnegative polynomials and sums of squares. *Journal of the American Mathematical Society*, 25(3):617–635, 2012.

[6] Dani Brake and Matt Niemberg. Paramotopy. available at http://paramotopy.com, 2016.

[7] Carsten Conradi, Elisenda Feliu, Maya Mincheva, and Carsten Wiuf. Identifying parameter regions for multistationarity. *PLOS Computational Biology*, 13(10):1–25, 10 2017.

[8] Carsten Conradi and Dietrich Flockerzi. Multistationarity in mass action networks with applications to ERK activation. *Journal of mathematical biology*, 65(1):107–156, 2012.

[9] Carsten Conradi, Dietrich Flockerzi, and Jörg Raisch. Multistationarity in the activation of a MAPK: parametrizing the relevant region in parameter space. *Mathematical biosciences*, 211(1):105–131, 2008.

[10] Carsten Conradi, Alexandru Iosif, and Thomas Kahle. Multistationarity in the space of total concentrations for systems that admit a monomial parametrization. *preprint, arXiv:1810.08152*, 2018.

[11] Carsten Conradi and Maya Mincheva. Catalytic constants enable the emergence of bistability in dual phosphorylation. *Journal of The Royal Society Interface*, 11(95):20140158, 2014.

[12] Michel Coste. An introduction to semialgebraic geometry. *RAAG network school*, 145:30, 2002.

[13] Gheorghe Craciun, Stefan Müller, Casian Pantea, and Polly Y Yu. A generalization of Birch's theorem and vertex-balanced steady states for generalized mass-action systems. *preprint, arXiv:1802.06919*, 2018.

[14] Alicia Dickenstein. Biochemical reaction networks: An invitation for algebraic geometers. In *Mathematical Congress of the Americas*, volume 656, pages 65–83. American Mathematical Soc., 2016.

[15] Mareike Dressler, Sadik Iliman, and Timo de Wolff. A positivstellensatz for sums of nonnegative circuit polynomials. *SIAM Journal on Applied Algebra and Geometry*, 1(1):536–555, 2017.

[16] Mareike Dressler, Sadik Iliman, and Timo De Wolff. An approach to constrained polynomial optimization via nonnegative circuit polynomials and geometric programming. *Journal of Symbolic Computation*, 91:149–172, 2019.

[17] David Eisenbud and Bernd Sturmfels. Binomial Ideals. *Duke Mathematical Journal*, 84(1):1–45, 1996.

[18] Péter Érdi and János Tóth. *Mathematical models of chemical reactions: theory and applications of deterministic and stochastic models*. Manchester University Press, 1989.

[19] Martin Feinberg. The existence and uniqueness of steady states for a class of chemical reaction networks. *Archive for Rational Mechanics and Analysis*, 132(4):311–370, 1995.

[20] Martin Feinberg. Multiple steady states for chemical reaction networks of Deficiency One. *Archive for Rational Mechanics and Analysis*, 132(4):371–406, 1995.

[21] Dietrich Flockerzi, Katharina Holstein, and Carsten Conradi. N-site Phosphorylation Systems with 2N-1 Steady States. *Bulletin of Mathematical Biology*, 76(8):1892–1916, Aug 2014.

[22] Israel M Gelfand, Mikhail Kapranov, and Andrei Zelevinsky. *Discriminants, resultants, and multidimensional determinants*. Springer Science & Business Media, 2008.

[23] Jürgen Gerhard, DJ Jeffrey, and Guillaume Moroz. A package for solving parametric polynomial systems. *ACM Communications in Computer Algebra*, 43(3/4):61–72, 2010.

[24] Joe Harris. Algebraic geometry, GTM 133, 1992.

[25] Vera Hárs and János Tóth. On the inverse problem of reaction kinetics. *Qualitative Theory of Differential Equations*, 30:363–379, 1981.

[26] Katharina Holstein, Dietrich Flockerzi, and Carsten Conradi. Multistationarity in sequential distributed multisite phosphorylation networks. *Bulletin of mathematical biology*, 75(11):2028–2058, 2013.

[27] Wolfram Research, Inc. Mathematica, Version 11.2. Champaign, IL, 2017.

[28] Alexandru Iosif. SturmDiscriminants, a Macaulay2 package, Version 0.1. https://github.com/alexandru-iosif/SturmDiscriminants.

[29] Badal Joshi and Anne Shiu. Which small reaction networks are multistationary? *SIAM Journal on Applied Dynamical Systems*, 16(2):802–833, 2017.

[30] Thomas Kahle. Decompositions of binomial ideals. *Journal of Software for Algebra and Geometry*, 4(1):1–5, 2012.

[31] Daniel Lazard and Fabrice Rouillier. Solving parametric polynomial systems. *Journal of Symbolic Computation*, 42(6):636 – 667, 2007.

[32] Maplesoft, a division of Waterloo Maple Inc., Waterloo, Ontario. Maple 2017.

[33] Mercedes Pérez Millán, Alicia Dickenstein, Anne Shiu, and Carsten Conradi. Chemical reaction systems with toric steady states. *Bulletin of mathematical biology*, 74(5):1027–1065, 2012.

[34] Guillaume Moroz. *Sur la décomposition réelle et algébrique des systemes dépendant de parametres*. PhD thesis, Université Pierre et Marie Curie-Paris VI, 2008.

[35] Stefan Müller, Elisenda Feliu, Georg Regensburger, Carsten Conradi, Anne Shiu, and Alicia Dickenstein. Sign conditions for injectivity of generalized polynomial maps with applications to chemical reaction networks and real algebraic geometry. *Foundations of Computational Mathematics*, 16(1):69–97, 2016.

[36] Riley Murray, Venkat Chandrasekaran, and Adam Wierman. Newton polytopes and relative entropy optimization. *preprint, arXiv:1810.01614*, 2018.

[37] Stefan Müller and Georg Regensburger. Generalized mass action systems: Complex balancing equilibria and sign vectors of the stoichiometric and kinetic-order subspaces. *SIAM Journal on Applied Mathematics*, 72(6):1926–1947, 2012.

[38] Mercedes Pérez Millán and Alicia Dickenstein. The structure of MESSI biological systems. *SIAM Journal on Applied Dynamical Systems*, 17(2):1650–1682, 2018.

[39] AmirHosein Sadeghimanesh and Elisenda Feliu. The multistationarity structure of networks with intermediates and a binomial core network. *preprint, arXiv:1808.07548*, 2018.

[40] Mohab Safey El Din and Éric Schost. Polar varieties and computation of one point in each connected component of a smooth real algebraic set. In *Proceedings of the 2003 international symposium on Symbolic and algebraic computation*, pages 224–231. ACM, 2003.

[41] Mohab Safey El Din and Éric Schost. Properness defects of projections and computation of at least one point in each connected component of a real algebraic set. *Discrete & Comp. Geom.*, 32(3):417–430, 2004.

[42] Stefan Schuster and Claus Hilgetag. On elementary flux modes in biochemical reaction systems at steady state. *Journal of Biological Systems*, 2(02):165–182, 1994.

[43] Anne Shiu. *Algebraic methods for biochemical reaction network theory.* PhD thesis, University of California, Berkeley, 2010.

[44] Anne Shiu and Timo de Wolff. Nondegenerate multistationarity in small reaction networks. *preprint, arXiv:1802.00306*, 2018.

[45] Artur Wachtel, Riccardo Rao, and Massimiliano Esposito. Thermodynamically consistent coarse graining of biocatalysts beyond Michaelis–Menten. *preprint, arXiv:1709.06045*, 2017.

[46] Liming Wang and Eduardo Sontag. On the number of steady states in a multiple futile cycle. *Journal of Mathematical Biology*, 57:29–52, 2008.

[47] Günter M. Ziegler. *Lectures on polytopes*, volume 152. Springer Science & Business Media, 2012.

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