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Multiestacionariedad en redes de reacciones bioquímicas y soluciones positivas de sistemas polinomiales ralos

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Multistationarity in biochemical reaction networks and positive solutions of sparse polynomial systems

Abstract

The motivation of this work is to apply and develop algebraic and geometric tools for the study of multistationarity in biochemical networks and, more generally, positive solutions of sparse polynomial systems.

We start by presenting a general framework to find coefficients for which a real sparse polynomial system has more than one positive solution, based on the recent article by Bihan, Santos, and Spaenlehauer [7]. We apply this approach to find *reaction rate constants* and *total conservation constants* in biochemical reaction networks for which the associated dynamical system is multistationary. Moreover, we propose a mixed approach, considering different supports for each polynomial. We exemplify our theoretical results in different biochemical networks of interest of arbitrary size and number of variables. In particular, our results are the key tools to identify multistationarity regions for enzymatic cascades of Goldbeter–Koshland loops with an arbitrary number of layers, when a same phosphatase catalyzes the transfer of phosphate groups at two different layers.

We also use this method to study the distributive n-site phosphorylation system. We give joint conditions on the reaction rate constants and the total conservation constants that ensure $n+1$ positive steady states for n even (and n steady states for n odd), only assuming in the modeling that $\frac{1}{4}$ of the intermediate complexes occur in the reaction mechanism. In this framework of elimination of intermediates, we obtain general conditions built on results from [42], to extend nondegenerate steady states of the reduced network to the original network, under certain conditions in the reaction rate constants.

Finally, for certain sparse polynomial systems, we give conditions on the support and coefficients that guarantee the existence of at least one positive real root, based on degree theory and Gale duality.

Keywords: chemical reaction networks; multistationarity; sparse polynomial system; positive solutions.

Contents

Introduction

The objective of this thesis is to apply and develop algebraic and geometric tools for the study of multistationarity in biochemical reaction networks and, more generally, positive solutions of sparse polynomial systems.

Chemical Reaction Network Theory (CRNT) has been development over the last five decades, through the seminal works of Horn and Jackson [64, 65, 66], Feinberg [30, 31, 33, 34, 35], and Vol'pert [106]. These systems have a wide range of applications in the physical sciences and play an important role in systems biology.

We can approach these biochemical reaction networks by a continuous dynamic modeling. In general, the nonlinearities present in molecular networks prevent mathematical analysis of network behavior, which has been traditionally studied by numerical simulation. In general, this entails the difficulty (or impossibility) of estimating the parameters. However, molecular networks with mass-action kinetics give rise to polynomial dynamical systems, whose steady states are, therefore, zeros of a polynomial system. These equations may be analyzed by algebraic methods, in which parameters are treated as symbolic expressions whose numerical values do not have to be known in advance.

Karin Gatermann introduced the connection between mass-action kinetics and computational algebra between 2001 and 2005 [49, 50, 51]. Gunawardena and collaborators also started approaching results from CRNT with algebraic tools over the last years [59, 60, 74, 102, 103]. In [18], Craciun et al. studied toric dynamical systems, with an algebro-geometric perspective. Since then, more algebraic tools have been introduced by different authors, see for example [22, 26, 41, 43, 61, 69, 87, 84, 91].

For a better understanding of the outline of this work, we introduce basic concepts on chemical reaction networks in the following paragraphs, that we will develop in detail in Chapter 1.

A reaction network G on a given set of s chemical species is a finite directed graph whose edges $\mathscr{R}$ represent the reactions and are labeled by parameters $\kappa \in \mathbb{R}_{>0}^{|\mathscr{R}|}$, known as *reaction rate constants*, and whose vertices are labeled by complexes, usually represented as nonnegative integer linear combinations of species. After numbering the species, a complex can be identified with a vector in $\mathbb{Z}_{\geq 0}^{s}$. Under mass-action kinetics, the network G defines the following autonomous system of ordinary differential equations in the concentrations $x_1, x_2, \ldots, x_s$ of the species as

functions of time t:

$$\dot{x} = \left(\frac{dx_1}{dt}, \frac{dx_2}{dt}, \ldots, \frac{dx_s}{dt}\right) = f(x) := \sum_{y \to y' \in \mathscr{R}} \kappa_{yy'}\, x^y\, (y' - y),$$

where $x = (x_1, x_2, \ldots, x_s)$, $f = (f_1, \ldots, f_s)$, $x^y = x_1^{y_1} x_2^{y_2} \ldots x_s^{y_s}$ and $y \to y' \in \mathscr{R}$ indicates that the complex y reacts to the complex y', that is, $(y, y') \in \mathscr{R}$ (here $\kappa_{yy'}$ is the label of the reaction $y \to y'$). The steady states of the system correspond to constant trajectories, that is, to the common zero set of the polynomials $f_1, \ldots, f_s \in \mathbb{R}[x_1, \ldots, x_s]$. As the vector $\dot{x}(t)$ lies for all time t in the linear subspace $\mathcal{S}$ spanned by the reaction vectors $\{y' - y : y \to y' \in \mathscr{R}\}$ (which is known as the *stoichiometric subspace*), it follows that any trajectory $x(t)$ lies in a translate of $\mathcal{S}$. Moreover, if $x(0) = x^0 \in \mathbb{R}^s_{>0}$, then $x(t)$ lies for any t (in the domain of definition) in the *stoichiometric compatibility class* $(x^0 + \mathcal{S}) \cap \mathbb{R}^s_{\geqslant 0}$. The linear equations of $x^0 + \mathcal{S}$ give *conservation laws*. If $x^0 \in \mathbb{R}^s_{>0}$, we can also write the linear variety $x^0 + \mathcal{S}$ in the form: $\{x \in \mathbb{R}^s : \ell_1(x) = c_1, \ldots, \ell_d(x) = c_d\}$, where $\ell_1, \ldots, \ell_d$ are linear forms defining a basis of the subspace orthogonal to $\mathcal{S}$ and $c = (c_1, \ldots, c_d) \in \mathbb{R}^d_{\geq 0}$. These constant values are called *total conservation constants*.

The network G is said to *have the capacity for multistationarity* if there exists a choice of reaction rate constants κ and total conservation constants c such that there are two or more steady states of system in the stoichiometric compatibility class determined by c. In the first part of this work, we focus on the study of multistationarity.

Multistationarity is a key property of biochemical reaction networks, because it provides a mechanism for switching between different response states. This enables multiple outcomes for cellular-decision making in cell signaling systems. Questions about steady states in biochemical reaction networks under mass-action kinetics are fundamentally questions about nonnegative real solutions to parametrized polynomial ideals.

Starting with [19, 21], several papers studied the capacity for multistationarity from the structure of the directed graph of reactions [2, 40, 42, 47, 67, 80, 86]. Once the capacity for multistationarity is determined, the following difficult step is to find values of multistationary parameters as exhaustively and explicitly as possible. This is a question of quantifier elimination in real algebraic geometry, which is effective, but the inherent high complexity does not allow to treat interesting networks with standard general tools. Several articles in the literature addressed this question, with different approaches based on degree theory [12, 16], on algebraic and analytic computations at steady state including perturbation techniques [108], on sign conditions [63], on reduction to univariate polynomials [70] and on the study of sparse real polynomials via Viro's deformation techniques [51].

The first chapters of this thesis deal with the question of finding parameters that give rise to multistationarity. We propose a general method to find open parameter regions for networks which admit multiple steady states, based on the article by Bihan, Santos, and Spaenlehauer [7]. We apply this method to study several networks

of biological relevance, such as sequential distributive multisite phosphorylation systems and enzymatic cascades. Some of the previous results that we mentioned above allow to determine reaction rate constants where multistationarity occurs, but it is not possible to directly give conditions on the total concentration constants. In our results, we give conditions in both type of parameters: if the total concentrations constants satisfy certain inequalities (with coefficients depending on some reaction rate constants), then, we prove that there exist a choice on the remaining reaction rate constants such that the network is multistationarity. This is a desirable condition, since the total concentrations constants are experimentally more accessible than the reaction rate constants. Also, in some cases we can give with this method lower bounds on the number of positive steady states, and not only decide if the network has more than one steady state in a same stoichiometric compatibility class.

In the last chapter, we focus on generalized polynomial systems (we allow real exponents). In the context of chemical reaction networks, lower bounds of positive roots guarantee the existence of positive steady states and there are several results in the literature. However, there are few results on lower bounds of the number of real or positive roots of polynomial systems (see [7, 93, 94, 107]). There are some techniques used in the study of chemical reaction networks such as degree theory [12] or sign conditions [80], that are used to decide if a network is monostationarity or multistationarity. In Chapter 5 we apply some of these techniques and Gale duality, to give sign conditions on the support and coefficients of a generalized polynomial system that guarantee the existence of a positive real root.

Outline and contributions

In Chapter 1, we start by presenting some preliminaries on Chemical Reaction Network Theory. We collect definitions from the literature and prepare the setting for our results in the subsequent chapters. We present an important example of chemical reaction network: the distributive multisite phosphorylation system, which is widely studied and we will use as an example for our applications in the next chapters. We introduce the notion of intermediate complexes in the framework of [42]. We also present a class of biological systems that describe Modifications of type Enzyme-Substrate or Swap with Intermediates. These systems are called MESSI systems and were introduced in [86].

Motivated by the problem of finding multistationary parameters, in Chapter 2 we present a general framework to find coefficients for which a real sparse polynomial system has more than one positive solution. As we said before, our approach is based on the article by Bihan, Santos, and Spaenlehauer [7]. The basic idea we develop is to detect in the convex hull of the support of the monomials that define the equations, at least two simplices *positively decorated* (see Definition 2.2.10) that form part of a regular subdivision. With this regularity condition we can extend the positive real solutions of the corresponding subsystems to the total system. We also present a mixed approach to the previous results, considering different supports for

each polynomial. Our main results of this chapter are Theorems 2.2.13 and 2.3.3, in which we describe open multistationarity regions in the space of all parameters. We apply this method to find reaction rate constants and total conservation constants in biochemical reaction networks of interest, for which the associated dynamical system is multistationary, for example, the sequential distributive multisite phosphorylation systems. These networks are a particular example of MESSI systems, introduced in Chapter 1. We prove in Theorem 2.5.2 a general result for MESSI networks, that is the key to apply the framework of Theorem 2.2.13 to describe multistationarity regions for all these biological systems.

Based on the method developed in Chapter 2, we consider in Chapter 3 cascades of enzymatic Goldbeter-Koshland loops [56] with any number n of layers, for which there exist two layers involving the same phosphatase. We find regions in reaction rate constant and total conservation constant space for which the associated mass-action kinetics dynamical system is multistationary. Our main results of this chapter are Theorems 3.2.1 and 3.2.3, in which we give conditions on the parameters to ensure multistationarity in this general case. Here, the associated polynomial systems have positive dimensions growing linearly with n. The number of conservation laws also grows linearly with n, and it is at least four if $n \geq 2$.

In Chapter 4, we focus on the study of multistationarity in networks while assuming in the modeling the removal of some of the intermediates complexes; more precisely we apply our results to the distributive sequential n-site phosphorylation system. The removal of intermediates was introduced in [42], where the main properties are established. More specifically, the emergence of multistationarity of the n-site phosphorylation system with less intermediates was studied in [90]. It is known that the n-site phosphorylation network without any intermediates complexes has only one steady state for any choice of parameters. In [90], the authors showed which are the minimal sets of intermediates that give rise to a multistationarity system, but they do not give information about how many positive steady states can occur, and also, they do not describe the parameter regions for which these subnetworks are multistationary. Wang and Sontag [108] showed that for certain choices of the reaction rate constants and total conservation constants, the system can have $2[\frac{n}{2}]+1$ positive steady states. In this chapter, again based on the results of Chapter 2, we give open parameter regions in the space of reaction rate constants and total conservation constants that ensure these number of positive steady states, while assuming in the modeling that roughly only $\frac{1}{4}$ of the intermediates occur in the reaction mechanism. In this framework of elimination of intermediates, we obtain a general result built on results from [42], that allows to extend steady states of the reduced network to the original network, under certain conditions in the reaction rate constants.

Finally, in Chapter 5 we give conditions on the coefficients and the support of a generalized polynomial system that guarantee the existence of at least one positive real root. We obtain these conditions using degree theory and Gale duality. In Theorem 5.2.7, we give a result from the Gale duality side. Then, we work with

mixed dominating matrices (see [45, 46]) to obtain in Theorem 5.3.6 a sign result which holds for certain type of supports. We also give geometric conditions on the point configuration of the support and coefficients, in Theorem 5.4.8. When the support does not satisfy the previous conditions, we work with the particular case when the codimension is equal to 2. In the case that the exponents are integers, we related the previous results with algebraic conditions studied in the literature, such complete intersection lattice ideals.

Publications

The work presented in this thesis is based on articles done in collaboration with various authors.

Chapter 2 is based on

Lower bounds for positive roots and regions of multistationarity in chemical reaction networks. F. Bihan, A. Dickenstein, and M. Giaroli, 2018. Submitted. [6].

Chapter 3 is based on

Regions of multistationarity in cascades of Goldbeter-Koshland loops. M. Giaroli, F. Bihan, and A. Dickenstein. Journal of Mathematical Biology, 78(4), 1115-1145, 2019. [54].

Chapter 4 is based on

Parameter regions that give rise to $2[\frac{n}{2}]+1$ *positive steady states in the* n*-site phosphorylation system.* M. Giaroli, R. Rischter, M. Pérez Millán, and A. Dickenstein, 2019. Submitted. [55]

Also, there are some results of this chapter that are part of the article in preparation *Detecting the Multistationarity Structure in Enzymatic Networks*, which is joint work with M. Pérez Millán, R. Rischter, and A. Dickenstein.

Chapter 5 is based on

Sign conditions for the existence of at least one positive solution of a sparse polynomial system. F. Bihan, A. Dickenstein, and M. Giaroli. In preparation.

Chapter 1

Background on Chemical Reaction Network Theory

In this chapter, we give a brief introduction on Chemical Reaction Network Theory (CRNT). We present the basic concepts and the notation that we will use in the following chapters.

We refer the reader to the seminal works of Feinberg and Horn and Jackson [32, 66], and to the work of Gunawerdena [58] for further references on CRNT.

1.1 Chemical Reaction Networks

First, we present a basic example of how a chemical reaction network gives rise to a dynamical system.

$$A + B \to C \tag{1.1.1}$$

This is an example of a *chemical reaction*. In this reaction, *species* A and B react to form species C. The *reactant* $A + B$ and the *product* C are called *complexes*.

When reaction (1.1.1) takes place, a molecule of A and a molecule of B are transformed into a molecule of C. If we denote by n_A, n_B, and n_C the number of molecules of species A, B and C respectively before the reaction occurs, then the number of molecules after the reaction are $n_A - 1$, $n_B - 1$, and $n_C + 1$. More reactions involving the same species can appear. A set of reactions involving certain species is a *chemical reaction network*. For example, consider the chemical reaction network with reaction (1.1.1) and the additional reaction $2C \to B$:

$$A + B \xrightarrow{\kappa_1} C, \qquad 2C \xrightarrow{\kappa_2} B \tag{1.1.2}$$

Here the reactions are represented together with a label. The label of a reaction indicates how fast or often the reaction occurs and is called *reaction rate constant*. It is always a positive value.

If the number of molecules of the species are large enough, is reasonable use concentrations, that is, number of molecules divided by the volume, as measure of species abundance. The concentrations of the three species of network (1.1.2),

denoted by x_A, x_B, and x_C, will change in time as the reactions of the network occurs. Under the assumption of *mass-action kinetics*, in each reaction species react at a rate proportional to the product of their concentrations, where the proportionality constant is the reaction rate constant. From the network (1.1.2), we obtain the following differential equations:

$$\begin{aligned}\frac{d}{dt}x_A &= \dot{x_A} = -\kappa_1 x_A x_B,\\ \frac{d}{dt}x_B &= \dot{x_B} = -\kappa_1 x_A x_B + \kappa_2 x_C^2,\\ \frac{d}{dt}x_C &= \dot{x_C} = \kappa_1 x_A x_B - 2\kappa_2 x_C^2.\end{aligned}$$

In this example, we observe that $\dot{x_A} - 2\dot{x_B} - \dot{x_C} = 0$. Then, $x_A - 2x_B - x_C = c_1$, where c_1 is a constant which depends on the initial conditions ($c_1 = x_A(0) - 2x_B(0) - x_C(0)$). That is, the concentration vector $(x_A(t), x_B(t), x_C(t))$ is contained in the affine variety $L = \{(x, y, z) \in \mathbb{R}^3 : x - 2y - z = c_1\}$. The subspace $\mathcal{S} = \{(x, y, z) \in \mathbb{R}^3 : x - 2y - z = 0\}$ is called the *stoichiometric subspace* of the network.

A *chemical reaction network* G on a given set $\mathscr{S}$ of chemical species is a finite directed graph whose edges $\mathscr{R}$ represent the reactions and whose vertices represent the complexes, usually represented as nonnegative integer linear combinations of species. After numbering the species, a complex can be identified with a vector in $\mathbb{Z}^s_{\geq 0}$, where s denotes the cardinality of $\mathscr{S}$.

The vertex i of G represents the i-th complex, and we associated to it the monomial

$$x^{y_i} = x_1^{(y_i)_1} x_2^{(y_i)_2} \cdots x_s^{(y_i)_s}.$$

In other words, if the i-th complex is of the form $(y_i)_1 A + (y_i)_2 B + \cdots$, the corresponding monomial is $x^{y_i} = x_A^{(y_i)_1} x_B^{(y_i)_2} \cdots$.

For example, in the reaction (1.1.1), to the complex $A + B$ we associated the monomial $x_A x_B$, which determines the vector $y_1 = (1, 1, 0)$ and to the complex C we associated the monomial x_C, which determines the vector $y_2 = (0, 0, 1)$. We will refer $y_1, \ldots, y_n$ as the complexes in the network.

A chemical reaction network, then, consists of three sets:

- A finite set of *species* $\mathscr{S} = \{X_1, X_2, \ldots, X_s\}$.
- A finite set of vectors $\mathscr{C} = \{y_1, y_2, \ldots, y_n\}$, with $y_i \in \mathbb{Z}^s_{\geq 0}$, which represent the complexes of the network. They must satisfy that for each species $X_i \in \mathscr{S}$, there exists a complex $y \in \mathscr{C}$ in which the species X_i "appears", that is, there are no superfluous species in $\mathscr{S}$.
- A set of reactions $\mathscr{R} \subset \mathscr{C} \times \mathscr{C}$, which satisfy:
  - $(y, y) \notin \mathscr{R}$ for all $y \in \mathscr{C}$, that is, no complex reacts to itself.
  - For each complex $y \in \mathscr{C}$, there exists $y' \in \mathscr{C}$ such that $(y, y') \in \mathscr{R}$ or $(y', y) \in \mathscr{R}$, that is, there exists a reaction in $\mathscr{R}$ such that y is the reactant or the product complex.

Here $(y, y') \in \mathscr{R}$ indicates that the complex y reacts to the complex y'; in general we will write $y \to y'$.

We denote $G = \{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ to a chemical reaction network G with species set $\mathscr{S}$, complex set $\mathscr{C}$ and reaction set $\mathscr{R}$.

1.1.1 Chemical reaction systems

The concentration vector

$$x = x(t) = (x_1(t), x_2(t), \ldots, x_s(t)),$$

represents the concentration $x_i(t)$ of the specie X_i at time t. As we saw at the beginning of this chapter, a chemical reaction network defines a dynamical system that arises from a *rate formation function* for each reaction. That is, to each reaction $y \to y'$ we assign a nonnegative real valued continuous function, $\mathcal{K}_{yy'}(.) = \mathcal{K}_{y \to y'}(.)$, where $\mathcal{K}_{yy'}(x)$ represents the instantaneous occurrence rate of reaction $y \to y'$ when the instantaneous concentration of the species is given by the vector x.

The support of a vector $v \in \mathbb{R}^m$ is defined as the set of its nonzero coordinates, and we denote it by $\text{supp}(v)$.

Definition 1.1.1. *A kinetics $\mathcal{K}$ for a chemical reaction network $G = \{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ is an assignment to each reaction $y \to y' \in \mathscr{R}$ of a continuous rate function $\mathcal{K}_{yy'}(.) \colon \mathbb{R}^s_{\geq 0} \to \mathbb{R}_{\geq 0}$, such that*

$$\mathcal{K}_{yy'}(x) > 0 \text{ if and only if } \text{supp}(y) \subseteq \text{supp}(x).$$

Now, we introduce the definition of a chemical reaction system.

Definition 1.1.2. *A chemical reaction system $G = \{\mathscr{S}, \mathscr{C}, \mathscr{R}, \mathcal{K}\}$ is a chemical reaction network $G = \{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ endowed with a kinetics $\mathcal{K}$.*

Given a chemical reaction system $G = \{\mathscr{S}, \mathscr{C}, \mathscr{R}, \mathcal{K}\}$, we have the following associated dynamical system:

$$\dot{x}(t) = f(x(t)) \coloneqq \sum_{y \to y' \in \mathcal{R}} \mathcal{K}_{yy'}(x(t))(y' - y). \tag{1.1.3}$$

The function f is called *the species formation rate function.* We observe that for each species X_i, $f_i(x)$ gives the instantaneous rate of generation of the species X_i due to the simultaneous occurrence of all reactions in $\mathscr{R}$. We have that

$$\dot{x}_i(t) = f_i(x(t)) = \sum_{y \to y' \in \mathscr{R}} \mathcal{K}_{yy'}(x(t))((y')_i - (y)_i),$$

then $f_i(x)$ is obtained by adding all the rate formation functions, each one multiplied by the net number of molecules of X_i produced in the corresponding reaction.

1.1.2 Mass-action kinetics systems

In this thesis, we will study chemical reaction networks endowed with mass-action kinetics, an important example of kinetics. The Law of Mass Action was proposed by two Norwegians: Peter Waage (1833-1900), a chemist, and Cato Guldberg (1836-1902), a mathematician, in an article published in Norwegian in 1864. Mass-action kinetics is based on the idea that the rate of a reaction is proportional to the product of the concentrations of the species in the reactant complexes, if the molecules are homogeneously distributed and in abundance. The precise definition is the following:

Definition 1.1.3. *The kinetics of a chemical reaction system is mass-action if all the rate formation functions $\mathcal{K}_{yy'}$ are of the form:*

$$\mathcal{K}_{yy'}(x) \coloneqq \kappa_{yy'} x^y = \kappa_{yy'} x_1^{y_1} x_2^{y_2} \dots x_s^{y_s},$$

for a certain positive vector of reaction rate constants $\kappa = (\kappa_{yy'}) \in \mathbb{R}_{>0}^{|\mathscr{R}|}$, with the convention $0^0 = 1$.

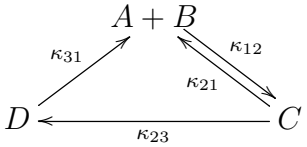
By (1.1.3), the species formation rate function of a chemical reaction system with mass-action kinetics is the following:

$$f(x(t)) := \dot{x}(t) = \sum_{y \to y \in \mathscr{R}} \kappa_{yy'} x(t)^y (y' - y). \tag{1.1.4}$$

Note that $f_1, \dots, f_s \in \mathbb{R}[x_1, \dots, x_s]$.

The non-negative orthant $\mathbb{R}^s_{\geq 0}$ is forward invariant with respect to the mass-action system (1.1.4) (see [92]), that is, if the initial condition $x(0)$ belongs to $\mathbb{R}^s_{\geq 0}$, then so does the trajectory $x(t)$ for any positive time t. The same holds for the positive orthant $\mathbb{R}^s_{>0}$. Then, mass-action systems behave as expected in the chemical reaction networks modeling: no coordinate of the concentration vector will never become negative.

Example 1.1.4. Consider the T-cell signal transduction model proposed by the immunologist McKeithan [78]. T-cell receptors bind to both self-antigens and foreign antigens and this model give a possible explanation of how T-cells can recognize self versus foreign antigens. A mathematical analysis of this network was done by Sontag in [92]. In its simplest case, the reaction network is the following:



A denotes the T-cell receptor protein, B denotes the Major Histocompatibility protein Complex (MHC) of antigen-presenting cell, C denotes the species A bound to species B, and D denotes an activated form of C. The binding of A and B forms C, which undergoes a modification into its activated form D before transmitting a

signal. The general mechanism proposed by McKeithan includes several activated forms of C, until a final active form that triggers the attack to the foreign antigen is obtained.

In this example, the network has 4 reactions, 4 species: A, B, C, and D, and 3 complexes: $A+B$, C, and D. The differential equations for the concentrations of the species in the network endowed with mass-action kinetics is the following:

$$\frac{dx}{dt} = \begin{pmatrix} \frac{dx_A}{dt} \\ \vdots \\ \frac{dx_D}{dt} \end{pmatrix} = \kappa_{12} x_A x_B \begin{pmatrix} -1 \\ -1 \\ 1 \\ 0 \end{pmatrix} + \kappa_{21} x_C \begin{pmatrix} 1 \\ 1 \\ -1 \\ 0 \end{pmatrix} + \kappa_{23} x_C \begin{pmatrix} 0 \\ 0 \\ -1 \\ 1 \end{pmatrix} + \kappa_{31} x_D \begin{pmatrix} 1 \\ 1 \\ 0 \\ -1 \end{pmatrix}.$$

That is,

$$\begin{aligned} \frac{dx_A}{dt} &= -\kappa_{12} x_A x_B + \kappa_{21} x_C + \kappa_{31} x_D, \\ \frac{dx_B}{dt} &= -\kappa_{12} x_A x_B + \kappa_{21} x_C + \kappa_{31} x_D, \\ \frac{dx_C}{dt} &= \kappa_{12} x_A x_B - \kappa_{21} x_C - \kappa_{23} x_C, \\ \frac{dx_D}{dt} &= \kappa_{23} x_C - \kappa_{31} x_D. \end{aligned}$$

1.1.3 Stoichiometric compatibility class

The fundamental idea here is that, regardless of the kinetics, the structure of the reaction alone imposes restrictions on the trajectories. In particular, a trajectory that passes through $x \in \mathbb{R}^s_{\geq 0}$ can eventually reach $x' \in \mathbb{R}^s_{\geq 0}$ only if x and x' are compatible with certain "stoichiometrical" conditions the reaction network imposes. For example, for the network (1.1.2), we observed that the trajectories $(x_A(t), x_B(t), x_C(t))$ were contained in an affine variety.

Definition 1.1.5. *The stoichiometric subspace of the chemical reaction network $G = \{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ is the linear subspace spanned by the reaction vectors $y' - y$ if $y \to y' \in \mathscr{R}$. We denote this subspace by $\mathcal{S}$:*

$$\mathcal{S} \coloneqq \langle y' - y \,:\, y \to y' \in \mathscr{R} \rangle \subset \mathbb{R}^s.$$

We compute the stoichiometric subspace $\mathcal{S}$ for the network of Example 1.1.4. In this case, the complexes are: $y_1 = (1,1,0,0)$, $y_2 = (0,0,1,0)$, $y_3 = (0,0,0,1)$. The reactions that occur are: $y_1 \to y_2$, $y_2 \to y_1$, $y_2 \to y_3$, $y_3 \to y_1$. Then:

$$\mathcal{S} = \langle (-1,-1,1,0), (1,1,-1,0), (0,0,-1,1), (1,1,0,-1) \rangle.$$

So, we obtain: $\mathcal{S} = \langle (1,1,-1,0), (0,0,-1,1) \rangle$.

In the network (1.1.2), we observed that the trajectory $x(t) = (x_A(t), x_B(t), x_C(t))$, which starts in $x(0) = (x_A(0), x_B(0), x_C(0)) \in \mathbb{R}^3_{>0}$, remains in $\mathcal{S} + x(0)$. In fact, we see that integrating (1.1.3) yields:

$$x(t) = x(0) + \sum_{y \to y' \in \mathscr{R}} \left(\int_0^t \mathcal{K}_{yy'}(x(s)) ds \right) (y' - y).$$

Therefore, a trajectory $x(t)$, beginning at a positive vector $x(0) = x^0 \in \mathbb{R}^s_{>0}$, remains in $\mathcal{S} + x^0$ for all $t \geq 0$. Recall also that the non-negative orthant $\mathbb{R}^s_{\geq 0}$ is forward-invariant with respect to the dynamics (1.1.4). This facts leads to the following definition:

Definition 1.1.6. *Let $G = \{\mathscr{S}, \mathscr{C}, \mathscr{R}\}$ be a chemical reaction network and let $\mathcal{S}$ be its stoichiometric subspace. For each $x^0 \in \mathbb{R}^s_{>0}$ we define a stoichiometric compatibility class:*

$$\mathcal{S}_{x^0} \coloneqq (x^0 + \mathcal{S}) \cap \mathbb{R}^s_{\geqslant 0}$$

We say that $x, x' \in \mathbb{R}^s_{\geq 0}$ are stoichiometrically compatible if they are in the same stoichiometric compatibility class, that is, if $x - x' \in \mathcal{S}$.

With mass-action kinetics, any stoichiometric compatibility class is also forward-invariant with respect to the system (1.1.4).

If $d = s - \dim(\mathcal{S})$ and $x^0 \in \mathbb{R}^s_{>0}$, we can also write $\mathcal{S}_{x^0}$ in the form:

$$\mathcal{S}_{x^0} = \{x \in \mathbb{R}^s \,:\, \ell_1(x) = c_1, \ldots, \ell_d(x) = c_d\},$$

where $\ell_1, \ldots, \ell_d$ are linear forms defining a basis of the subspace orthogonal to $\mathcal{S}$ and $c = (c_1, \ldots, c_d) \in \mathbb{R}^d$. These constant values are called *total conservation constants*. The linear equations that define $x(0)+S$ are called *conservation laws* or *conservation relations*.

A *conservation-law matrix* of G, denoted by W, is any row-reduced $d \times s$-matrix whose rows form a basis of $\mathcal{S}^\perp$. Note that $W\dot{x} = Wf(x) = 0$, with f the rate formation function. Sometimes we will use the notation $\mathcal{S}_c$ to refer the compatibility class with respect to the total conservation vector $c = Wx^0$, that is,

$$\mathcal{S}_c \coloneqq \{x \in \mathbb{R}^s_{\geq 0} \,:\, Wx = Wx^0 = c\} = \mathcal{S}_{x^0}.$$

In Example 1.1.4, $x, x' \in \mathbb{R}^4_{\geq 0}$ are stoichiometrically compatible if $x - x' \in \mathcal{S} = \langle (1, 1, -1, 0), (0, 0, -1, 1) \rangle$. Moreover, if we have an initial condition $x(0) = x^0$, then the trajectory $x(t)$ is contained in $\mathcal{S} + x^0$.

Example 1.1.7. Consider the simple network

$$2A \underset{\kappa_2}{\overset{\kappa_1}{\rightleftarrows}} B \tag{1.1.5}$$

The stoichiometric subspace is $\mathcal{S} = \langle (2, -1) \rangle$, and the stoichiometric compatibility classes are of the form $(\langle (2, -1) \rangle + x^0) \cap \mathbb{R}^2_{\geq 0}$ and are depicted in Figure 1.1. The equation $x_A + 2x_B = c$ is a conservation law for the network.

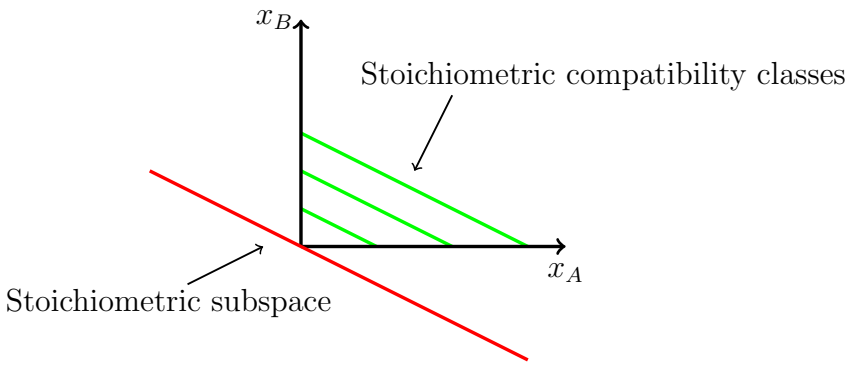


Figure 1.1: Stoichiometric compatibility classes for network (1.1.5).

1.1.4 Steady states and multistationarity

A *steady state* of a chemical reaction system is a nonnegative concentration vector at which the species formation rate function takes the value zero. Formally:

Definition 1.1.8. *A concentration vector $\bar{x} \in \mathbb{R}^s_{\geq 0}$ is a steady state of a chemical reaction system if $f(\bar{x}) = 0$, with f the species formation rate function* (1.1.3)*, and $\bar{x}$ is a positive steady state if is a steady state and $\bar{x} \in \mathbb{R}^s_{>0}$.*

For chemical reaction networks with mass-action kinetics, the steady states of the system (1.1.4) are the nonnegative real zeros of $f_1, \ldots, f_s \in \mathbb{R}[x_1, \ldots, x_s]$, that is, the elements of the *steady state variety*:

$$V(f) = \{x \in \mathbb{R}^s_{\geq 0} \,:\, f_1(x) = \cdots = f_s(x) = 0\}.$$

Some reaction systems, taken with mass-action kinetics, admit no positive steady states for some or even for any choices of the rate constants. For example, in network (1.1.2), from the differential equation corresponding to the concentration x_A, any steady state satisfy:

$$\kappa_1 x_A x_B = 0.$$

Then, the network does not admit positive steady states for any positive choice of the constant κ_1. In this case, the steady states are characterized by the extinction of some species.

On the other hand, the network (1.1.5), with mass-action kinetics, admits one positive steady state in each positive stoichiometric compatibility class, regardless of the values that the reaction rate constants take.

The existence or nonexistence of positive steady states can be easy to decide for simple networks like the previous one, but this is not true for complicated networks. One have to deal with a large system of polynomial equations in many variables (species concentrations) in which many parameters (reaction rate constants) appear.

A question of interest is if a chemical reaction system with mass-action kinetics admit multiple steady states in a fixed stoichiometric compatibility class.

Definition 1.1.9. *Let G chemical reaction system with mass-action kinetics, and fixed reaction rate constants κ. We say that the system exhibits multistationarity if there are at least two positive steady states in some stoichiometrically compatibility class. In case there is a single positive steady state in each stoichiometrically compatibility class, we say instead that the system is monostationary.*

We say that a chemical reaction network G is multistationary (or that it has the capacity for multistationarity) if there exists a choice of reaction rate constants such that the mass-action system exhibits multistationarity.

Figure 1.2 illustrates the intersection of the steady state variety $V(f)$ for certain chemical reaction system, with different stoichiometric compatibility classes. In one of them there are 3 different positive steady states $x^{(1)}, x^{(2)}$ and $x^{(3)}$. Then, the system admits multistationarity.

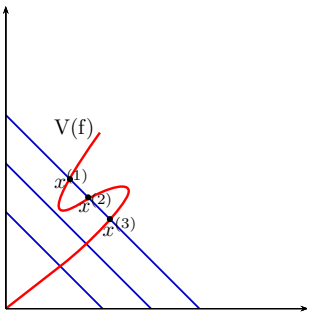


Figure 1.2: An example of a steady state variety cut out by different stoichiometrically compatibility classes.

A unique positive (stable) steady state can underlie robustness in the corresponding biological system; on the other hand, the existence of multiple positive (stable) steady states can explain switching behavior in the system. Multistationarity is linked to cellular decision making [72, 83, 109] and there is evidence suggesting that different steady states of a cell represent different cell types [44, 101].

There are several methods for deciding whether a chemical reaction network taken with mass-action kinetics have the capacity for multistationarity, that can either preclude or guarantee multistationarity for certain classes of networks. For instance, the are criteria based on injectivity [1, 19, 37, 80] and deficiency results [29, 33, 36]. These last criteria based on deficiency (which is an important invariant of the graph structure of the network) are implemented in a free software called CRNToolbox [28]. There are also criteria to preclude or guarantee multistationarity when the positive steady states can be described by binomials, for example [87, 86]. Another tool for studying multistationarity are the results for assessing multistationarity of one network from another, in general these results "lift" steady states of a small subnetwork to a bigger one [2, 14, 20, 42, 67]. For more details about methods for deciding the capacity for multistationarity of a network see [68].

Once we know that a network is multistationary, the next step is to finding parameter region where the network has more than one positive steady state. This is a

problem of quantifier elimination in real algebraic geometry. For instance, a decomposition of the parameter space into regions with different numbers of steady states could be done using Cylindrical Algebraic Decomposition [10], but this method is very limited, since the models have usually a large number of variables and parameters. As we mention in the Introduction, there are other approaches based on degree theory [12, 16], on perturbation techniques [108], on sign conditions [63], on reduction to univariate polynomials [70] and on the study of sparse real polynomials via Viro's deformation techniques [51]. In the first chapters of this thesis we will deal with this question.

1.2 The distributive multisite phosphorylation system

In this section, we introduce an important example: the distributive multisite phosphorylation system, which is widely studied in the literature and we will use as an example for our applications in the next chapters.

Phosphorylation/dephosphorylation are post-translational modification of proteins mediated by enzymes, particular proteins that add or take off a phosphate group at a specific site, inducing a conformational change that allows/prevents the protein to perform its function. The standard building block in cell signaling is the following enzyme mechanism, which is called a Michaelis-Menten mechanism.

$$S_0 + E \underset{k_{\text{off}}}{\overset{k_{\text{on}}}{\rightleftarrows}} ES_0 \overset{k_{\text{cat}}}{\to} S_1 + E \tag{1.2.1}$$

This basic network involves four species: the substrate S_0, the phosphorylated substrate S_1, the enzyme E, called kinase, and the intermediate species ES_0, and 3 reactions, with reaction constants called $k_{\text{on}}, k_{\text{off}}, k_{\text{cat}}$. The enzyme E is not consumed after the whole mechanism, which is assumed to be with mass-action kinetics. The concentration of the donor of the phosphate group is considered to be constant, thus hidden in the reaction constants and ignored. This mechanism with 4 species, 3 complexes, and 3 reactions is usually represented by the scheme depicted in Figure 1.3.

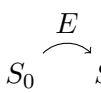


Figure 1.3: Shorthand for the network (1.2.1).

The addition of phosphate groups to multiple sites of a single molecule, may be distributive or processive. Distributive systems require an enzyme and substrate to bind several times in order to add/remove multiple phosphate groups. Processive systems require only one binding to add/remove all phosphate groups and it was shown in [17] that such systems cannot admit more than one steady state in each

stoichiometric compatibility class. The distibutive multisite phosphorylation system describes the n-site phosphorylation of a protein by a kinase/phosphatase pair in a sequential and distributive mechanism and it is known that it has the capacity of multistationarity for any $n \geq 2$ [108].

The reaction mechanism for the sequential distributive mechanism for the n-site network is a sequence of reactions as in (1.2.1), where we append n subgraphs of the form:

$$S_i + E \underset{k_{\mathrm{off_i}}}{\overset{k_{\mathrm{on_i}}}{\rightleftarrows}} ES_i \overset{k_{\mathrm{cat_i}}}{\to} S_{i+1} + E, \quad i = 0, \ldots, n-1,$$

and, on the other side, n subgraphs of the form:

$$S_i + F \underset{\ell_{\mathrm{off_{i-1}}}}{\overset{\ell_{\mathrm{on_{i-1}}}}{\rightleftarrows}} FS_i \overset{\ell_{\mathrm{cat_{i-1}}}}{\to} S_{i-1} + F, \quad i = 1, \ldots, n,$$

where F denotes another enzyme called phosphatase, to obtain the network:

$$\begin{aligned} &S_0 + E \underset{k_{\mathrm{off_0}}}{\overset{k_{\mathrm{on_0}}}{\rightleftarrows}} ES_0 \overset{k_{\mathrm{cat_0}}}{\to} S_1 + E \ \cdots \ \to S_{n-1} + E \underset{k_{\mathrm{off_{n-1}}}}{\overset{k_{\mathrm{on_{n-1}}}}{\rightleftarrows}} ES_{n-1} \overset{k_{\mathrm{cat_{n-1}}}}{\to} S_n + E \\ &S_n + F \underset{\ell_{\mathrm{off_{n-1}}}}{\overset{\ell_{\mathrm{on_{n-1}}}}{\rightleftarrows}} FS_n \overset{\ell_{\mathrm{cat_{n-1}}}}{\to} S_{n-1} + F \ \cdots \ \to S_1 + F \underset{\ell_{\mathrm{off_0}}}{\overset{\ell_{\mathrm{on_0}}}{\rightleftarrows}} FS_1 \overset{\ell_{\mathrm{cat_0}}}{\to} S_0 + F \end{aligned} \tag{1.2.2}$$

It represents one substrate that can sequentially acquire up to n phosphate groups, via the action of the kinase E, and which can be sequentially released via the action of the phosphatase F, in both cases via an intermediate species formed by the interaction of the substrate and the enzyme. The kinetics of this network is deduced by applying the law of mass action to this explicit labeled digraph. There are $3n+3$ species: the substrate species S_0, S_1,...,S_n, the enzyme species E and F, and the intermediate species ES_0, ES_1, ..., ES_{n-1}, FS_1, FS_2, ..., FS_n. We denote by s_0, $s_1, \ldots, s_n$, e, f, y_0, y_1, ..., y_{n-1}, u_0, u_1, ..., u_{n-1} the concentration of the species S_0, S_1,...,S_n, E, F, ES_0, ES_1, ..., ES_{n-1}, FS_1, FS_2, ..., FS_n respectively. The associated dynamical system that arises under mass-action kinetics equals:

$$\begin{aligned} \frac{ds_0}{dt} &= -k_{\mathrm{on_0}} s_0 e + k_{\mathrm{off_0}} y_0 + \ell_{\mathrm{cat_0}} u_0, \\ \frac{ds_i}{dt} &= k_{\mathrm{cat_{i-1}}} y_{i-1} - k_{\mathrm{on_i}} s_i e + k_{\mathrm{off_i}} y_i + \ell_{\mathrm{cat_i}} u_i - \ell_{\mathrm{on_{i-1}}} s_i f + \ell_{\mathrm{off_{i-1}}} u_{i-1}, \quad i = 1, \ldots, n-1, \\ \frac{ds_n}{dt} &= k_{\mathrm{cat_{n-1}}} y_{n-1} - \ell_{\mathrm{on_{n-1}}} s_n f + \ell_{\mathrm{off_{n-1}}} u_{n-1}, \\ \frac{dy_i}{dt} &= k_{\mathrm{on_i}} s_i e - (k_{\mathrm{off_i}} + k_{\mathrm{cat_i}}) y_i, \quad i = 0, \ldots, n-1, \\ \frac{du_i}{dt} &= \ell_{\mathrm{on_i}} s_{i+1} f - (\ell_{\mathrm{off_i}} + \ell_{\mathrm{cat_i}}) u_i, \quad i = 0, \ldots, n-1, \\ \frac{de}{dt} &= -\sum_{i=0}^{n-1} \frac{dy_i}{dt}, \quad \frac{df}{dt} = -\sum_{i=0}^{n-1} \frac{du_i}{dt}. \end{aligned} \tag{1.2.3}$$

There are three linearly independent conservation laws for any value of n (and no more):

$$\sum_{i=0}^{n} s_i + \sum_{i=0}^{n-1} y_i + \sum_{i=0}^{n-1} u_i = S_{tot}, \quad e + \sum_{i=0}^{n-1} y_i = E_{tot}, \quad f + \sum_{i=0}^{n-1} u_i = F_{tot}, \tag{1.2.4}$$

where clearly the total amounts $S_{tot}, E_{tot}, F_{tot}$ are positive for any trajectory of the dynamical system starting in the positive orthant.

In Section 2.4 and in Chapter 4 we will study these systems.

1.3 Intermediates

Typically, intermediate species are complexes such as a enzyme-substrate complex. Intermediate species in biochemical reaction networks are often ignored in the modeling, for simplicity or because of lack of knowledge. For instance, the model of the multisite phosphorylation systems presented in the previous section vary considerably in terms of intermediates. In the network (1.2.2) we assume in the modeling that there are $2n$ intermediates, but there are models with less intermediates. The n-site phosphorylation system without any intermediates species has only one steady state for any choice of parameters, however there exists parameters such that the network (1.2.2) admits multiple steady states. So, it is important to compare the dynamical properties of models that differ in the intermediates that are included. In this section, we present the framework introduced by Feliu and Wiuf in [42] to study the removal of intermediates in chemical reaction networks, and the main properties.

Let G be a chemical reaction network with set of species $\mathscr{S}_G$, of cardinality s. Consider a fixed subset of *intermediate* species $\mathcal{I} = \{U_1, U_2, \ldots, U_p\} \subset \mathscr{S}$, and we denote $\mathscr{S}_G \setminus \mathcal{I} = \{X_1, \ldots, X_n\}$. The intermediate species of $\mathcal{I}$ fulfill:

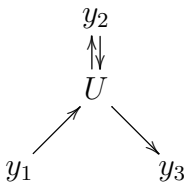
- For every $U_i \in \mathcal{I}$, the only complex that involves U_i is U_i (intermediate complex).

We say that complex y reacts to complex y' *via intermediates* of $\mathcal{I}$ if either $y \to y'$ or there exists a path of reactions from y to y' only through intermediate complexes of $\mathcal{I}$. This is denoted by $y \to_\circ y'$. Intermediate complexes must also fulfill:

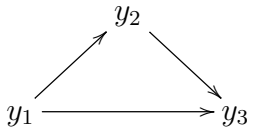
- For every $U_i \in \mathcal{I}$, there is a sequence of reactions $y \to_\circ U_i \to_\circ y'$, with y, y' complexes that only involve species in $\mathscr{S}_G \setminus \mathcal{I}$.

We consider now the network G' which is obtained from G by removing the intermediate species in $\mathcal{I}$. The set of species of G' is $\mathscr{S}_{G'} = \mathscr{S}_G \setminus \mathcal{I}$; the complexes of G', $\mathscr{C}_{G'}$, are the complexes of G which are not an intermediate complex U_i, for $i = 1, \ldots, p$; and the set of reactions of G', $\mathscr{R}_{G'}$, is obtained from the set of reactions of G collapsing the sequences $y \to_\circ y'$, to the reaction $y \to y'$, where y, y' are complexes only involving species in $\mathscr{S}_{G'}$.

Example 1.3.1. Consider the network G, with nonintermediate complexes y_1,y_2 and y_3, and with U the only intermediate complex:



Then, the following network:



is obtained from G removing the intermediate species U.

We recall some results and properties from [42]. In what follows, let G a chemical reaction network with set of species $\mathscr{S}_G = \{X_1, \ldots, X_n, U_1, \ldots, U_p\}$ and a fixed subset of intermediates species $\mathcal{I} = \{U_1, \ldots, U_p\}$. Consider G' the network obtained from G by removing the intermediate species in $\mathcal{I}$.

Conservation laws (Theorem 1 in [42]): The conservation laws in G' are in one-to-one correspondence with the conservation laws in G.

We show, following Lemma 1 in the ESM of [42], more explicitly this correspondence. We first note that the definition of the intermediate complexes impose that the network G' obtained from a network G by removing the intermediate complexes in $\mathcal{I}$, have both the same number of connected components. Let $\mathcal{S}$ and $\mathcal{S}'$ be the stoichiometric subspaces of G and G', respectively, and J be the number of connected components of G and G'. Let $w \in \mathcal{S}'^{\perp}$ and, for each connected component of G' choose a complex y^j in that connected component. Define $a_j = \langle w, y^j \rangle$, $j = 1, \ldots, J$ and the vector $\bar{w} \in \mathbb{R}^{n+p}$ as follows: $\bar{w}_i = w_i$ for $i = 1, \ldots, n$, and $\bar{w}_{n+k} = a_j$ if U_k is in the j-th component for $k = 1, \ldots, p$, where $\langle , \rangle$ denotes the canonical inner product of $\mathbb{R}^n$.

Then, if $\{w^1, \ldots, w^d\}$ is a basis of $\mathcal{S}'^{\perp}$, the set $\{\bar{w}^1, \ldots, \bar{w}^d\}$ is a basis of $\mathcal{S}^{\perp}$. This is, if the conservation laws of G' are

$$\ell_i(x) = \langle w^i, x \rangle = c_i, \tag{1.3.1}$$

where $c_i \in \mathbb{R}$, for $1 \leq i \leq d$, then the conservations laws for G are

$$\bar{\ell}_i(x, u) = \ell_i(x) + \sum_{j=1}^{J} \sum_{\substack{U_k \text{ in the} \\ j-th \text{ conn.comp.}}} \langle w^i, y^j \rangle u_k = \bar{c}_i, \tag{1.3.2}$$

with $\bar{c}_i \in \mathbb{R}$, for $1 \leq i \leq d$.

Consider G with mass-action kinetics and reaction rate constants κ. The following result gives an expression of the concentration of the intermediates at the steady state in terms of the reaction rate constants κ and the concentration of the species in $\mathcal{S}_G \setminus \mathcal{I}$.

Concentration of intermediates at steady state (Theorem 2 in [42]): The system of differential equations $\dot{u_i} = 0$, for all intermediates U_i, $i = 1, \ldots, p$, is linear on the $u's$, and the concentration u_i at steady state has a unique solution, given by

$$u_i = \sum_{y \in \mathscr{C}_{G'}} \mu_{i,y}(\kappa) x^y, \tag{1.3.3}$$

where $\mu_{i,y}(\kappa)$ is a nonnegative rational function on the reaction rate constants κ. Further $\mu_{i,y} \neq 0$ if and only if $y \to_\circ U_i$. In this case, the numerator and the denominator of $\mu_{i,y}$ are homogeneous polynomials in κ of degree p; the denominator involves only rate constants for reactions whose source is an intermediate of $\mathcal{I}$ and the numerator also involves rate constants for reactions whose source is an intermediate of $\mathcal{I}$, except for one factor in each summand, which comes from a reaction whose source is the complex y, with target one intermediate complex of $\mathcal{I}$.

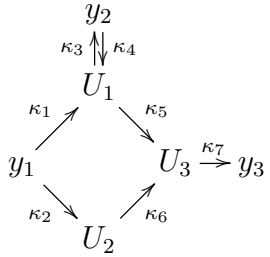
The next result says that if we substitute the previous expressions of the concentration of the intermediates in $\mathcal{I}$ into the differential equations of the mass action system associated to G, we obtain a mass-action system for the reduced network G'.

Mass-action system for G' (Theorem 3 in [42]): After replacing the expressions $u_i = \sum_{y \in \mathscr{C}_{G'}} \mu_{i,y}(\kappa) x^y$ into the differential equations $\dot{x}_i$ of G, we obtain a dynamical system associated to the network G' with mass-action kinetics, with reaction rate constants $\tau(\kappa) = (\tau_{yy'}(\kappa))$ depending on the reaction rate constants κ of G. In particular, the reaction constants $\tau_{yy'}(\kappa)$ are given by rational functions of κ, with positive coefficients:

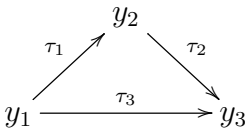
$$\tau_{yy'}(\kappa) = \kappa_{yy'} + \sum_{j=1}^{p} \kappa_{U_j y'} \mu_{j,y}(\kappa), \tag{1.3.4}$$

where $\kappa_{yy'} \geq 0$ is positive when $y \xrightarrow{\kappa_{yy'}} y'$ in G (and $\kappa_{yy'} = 0$ otherwise), and $\kappa_{U_j y'}$ is positive if $U_j \xrightarrow{\kappa_{U_j y'}} y'$ in G (and $\kappa_{U_j y'}$ otherwise), where $\mu_{j,y}$ is in (1.3.3).

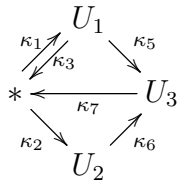
Example 1.3.2. Consider the reaction network G given by the following digraph:



Then, the network G' obtained by removing the intermediates from the set $\mathcal{I} = \{U_1, U_2, U_3\}$ is the following reaction network:



Using (1.3.4), we can express the reaction rate constants τ in terms of the reaction rate constants κ. For example, we have that $\tau_1 = \kappa_3 \mu_{1,y_1}(\kappa)$. In the proof of Theorem 2 of [42] it is shown how to obtain μ_{1,y_1} from a graphical procedure, using the Matrix-Tree theorem (see [79, 104]). Here we explain how to do it in this example. Consider the digraph with node set $\mathcal{I} \cup \{*\}$ and labeled edges as follows:



Recall that a spanning tree of a digraph is a subgraph that contains all the vertices, is connected and acyclic as an undirected graph. An i-tree of a graph is a spanning tree where the vertex i is its unique sink (that is, the only node with outdegree zero). Then, $\mu_{1,y_1} = \frac{\rho_1}{\rho_*}$, where

$$\rho_1 = \sum_{T \text{ an } U_1- \text{ tree}} \pi(T), \quad \text{and } \rho_* = \sum_{T \text{ an } *- \text{ tree}} \pi(T),$$

where $\pi(T)$ is the product of the labels of all the edges of T. From the graph we can check that: $\mu_{1,y_1}(\kappa) = \frac{\rho_1}{\rho_*} = \frac{\kappa_1\kappa_6\kappa_7}{\kappa_3\kappa_6\kappa_7+\kappa_5\kappa_6\kappa_7} = \frac{\kappa_1}{\kappa_3+\kappa_5}$.

1.4 MESSI systems

In [86], Dickenstein and Pérez Millán introduced a general framework for biological systems, called *MESSI systems*, that describe Modifications of type Enzyme-Substrate or Swap with Intermediates. Distributive multisite phosphorylation systems and enzymatic cascades with any number of layers which occur in cell signaling pathways, and we will study in detail in Chapter 4, are examples of MESSI systems of biological significance. In particular they are examples of s-toric MESSI systems, an important subclass of MESSI systems. The authors proved in [86] that any s-toric MESSI system is toric, that is, the positive steady states can be described with binomials and under certain hypotheses, they can choose explicit binomials with coefficients in $\mathbb{Q}(\kappa)$ which describe the positive steady states. Moreover, under certain combinatorial conditions, they describe a basis of conservation laws for these systems.

In this section, we briefly introduce the basic definitions of MESSI systems. For a more detailed explanation, see [86].

A MESSI network is a chemical reaction network, for which exists a partition of the set of species $\mathscr{S}$ into disjoint subsets:

$$\mathscr{S} = \mathscr{S}^{(0)} \bigsqcup \mathscr{S}^{(1)} \bigsqcup \mathscr{S}^{(2)} \bigsqcup \cdots \bigsqcup \mathscr{S}^{(m)}, \tag{1.4.1}$$

where $m \geq 1$ and $\bigsqcup$ denotes disjoint union. Species in $\mathscr{S}^{(0)}$ are called *intermediate* and species in $\mathscr{S}_1 \coloneqq \mathscr{S} \setminus \mathscr{S}^{(0)}$ are called *core*, with $\#\mathscr{S}^{(0)} = p$ and $\#\mathscr{S}_1 = s - p > 0$. As before, we denote the species with upper letters and the concentration of the species with small letters, for example x_j denotes the concentration of the species X_j.

There are two types of complexes allowed in a MESSI network: intermediate complexes and core complexes. The intermediate complexes are complexes that consist of a unique intermediate species that only appears in that complex. The core complexes are mono or bimolecular and consist of either one or two core species. When a core complex consists of two species X_i, X_j, they must belong to different sets $\mathscr{S}^{(\alpha)}, \mathscr{S}^{(\beta)}$ with $\alpha \neq \beta$, $\alpha, \beta \geq 1$.

Recall from the previous section, that a complex y reacts to a complex y' via intermediates (and we note it $y \to_\circ y'$) if either $y \to y'$ or there exists a path of reactions from y to y' only through intermediate complexes. Another condition in the intermediate complexes is that for every intermediate complex y, there must exist core complexes y_1 and y_2 such that $y_1 \to_\circ y$ and $y \to_\circ y_2$. The reactions in a MESSI network satisfy the following rules: if three species are related by $X_i + X_j \to_\circ X_k$ or $X_k \to_\circ X_i + X_j$, then X_k is an intermediate species. If two monomolecular complexes consisting of a single core species X_i, X_j are related by $X_i \to_\circ X_j$, then there exists $\alpha \geq 1$ such that both belong to $\mathscr{S}^{(\alpha)}$. And if $X_i + X_j \to_\circ X_k + X_\ell$ then, there exist $\alpha \neq \beta$ such that $X_i, X_k \in \mathscr{S}^{(\alpha)}$, $X_j, X_\ell \in \mathscr{S}^{(\beta)}$ or $X_i, X_\ell \in \mathscr{S}^{(\alpha)}$, $X_j, X_k \in \mathscr{S}^{(\beta)}$.

A partition in the set of species that satisfies all the previous conditions in the complexes and reactions defines a MESSI *structure*.

Remark 1.4.1. Note that in the framework of [42] presented in Section 1.3, we can consider subsets of intermediates and nonintermediate species that do not satisfy the rules of the MESSI systems. For instance, in the n-site phosporylation system (1.2.2), a subset $\mathcal{I}$ of intermediates may include or not the species ES_0. But by the rules that satisfy the reactions in a MESSI system, any partition in the set of species that define a MESSI structure of network (1.2.2) must satisfy $ES_0 \in \mathscr{S}^{(0)}$.

There can be many possible partitions that define a MESSI structure of a fixed network. If we have two partitions $\mathscr{S} = \mathscr{S}^{(0)} \sqcup \mathscr{S}^{(1)} \sqcup \mathscr{S}^{(2)} \sqcup \cdots \sqcup \mathscr{S}^{(m)}$ and $\mathscr{S} = \mathscr{S}'^{(0)} \sqcup \mathscr{S}'^{(1)} \sqcup \mathscr{S}'^{(2)} \sqcup \cdots \sqcup \mathscr{S}'^{(m')}$, we say that the first partition refines the second one if and only if $\mathscr{S}^{(0)} \supseteq \mathscr{S}'^{(0)}$ and for any $\alpha \geq 1$, there exists $\alpha' \geq 1$ such that $\mathscr{S}^{(\alpha)} \subseteq \mathscr{S}'^{(\alpha')}$. This defines a partial order in the set of all possible partitions, and in particular we have the notion of a *minimal* partition.

Example 1.4.2. In Section 1.3 we presented the distributive multisite phosphorylation systems. The following network is an example of a mixed phosphorylation mechanism (partially distributive, partially processive) studied in [98]. The reaction network is as follows:

$$\begin{aligned} S_0 + E &\underset{k_2}{\overset{k_1}{\rightleftarrows}} ES_0 \overset{k_3}{\to} S_1 + E \underset{k_5}{\overset{k_4}{\rightleftarrows}} ES_1 \overset{k_6}{\to} S_2 + E \\ S_2 + F &\underset{k_8}{\overset{k_7}{\rightleftarrows}} FS_2 \overset{k_9}{\to} FS_1 \overset{k_{10}}{\to} S_0 + F \end{aligned}$$

A MESSI structure of the network is given by the following minimal partition of the species: $\mathscr{S}^{(0)} = \{ES_0, ES_1, FS_1, FS_2\}$ (the intermediate species), $\mathscr{S}^{(1)} = \{E\}$, $\mathscr{S}^{(2)} = \{F\}$, and $\mathscr{S}^{(3)} = \{S_0, S_1, S_2\}$. We will use this partition in the next examples featuring this network. Another example of a partition giving a MESSI structure, which is not minimal, is the following: $\mathscr{S}'^{(0)} = \{ES_0, ES_1, FS_1, FS_2\}$, $\mathscr{S}'^{(1)} = \{E, F\}$, and $\mathscr{S}'^{(2)} = \{S_0, S_1, S_2\}$.

We now present three digraphs associated to a MESSI network with digraph G. First, we introduce the associated digraph G_1, where the intermediate species are eliminated, that is, with set of species $\mathscr{S}_1$. We associated to this set the inherited partition

$$\mathscr{S}_1 = \mathscr{S}^{(1)} \bigsqcup \mathscr{S}^{(2)} \bigsqcup \cdots \bigsqcup \mathscr{S}^{(m)}. \tag{1.4.2}$$

The vertex set of G_1 consists of all the core complexes. An edge $y \to y'$, with y, y' core complexes, belongs to the edge set of G_1 if and only if $y \to_\circ y'$ in G. If the edge labels of G are the reaction rate constants κ, then the labels of G_1, called τ, are rational functions in the reaction rate constans κ. We already described these explicit rate constants τ of the edges of G_1 in (1.3.4) (here we take $\mathcal{I} = \mathscr{S}^{(0)}$ and G_1 is equal to G').

We next introduce a labeled associated multidigraph G_2 where we "hide" the concentrations of some of the species in the labels. We keep all monomolecular reactions $X_i \to X_j$ in G_1 and for each reaction $X_i + X_\ell \xrightarrow{\tau} X_j + X_m$ in G_1, with $X_i, X_j \in \mathscr{S}^{(\alpha)}$, $X_\ell, X_m \in \mathscr{S}^{(\beta)}$, we consider two reactions $X_i \xrightarrow{\tau x_\ell} X_j$ and $X_\ell \xrightarrow{\tau x_i} X_m$. In principle this multidigraph MG_2 might contain loops or parallel edges between any pair of nodes. We obtain the digraph G_2 by collapsing into one edge all parallel edges of MG_2. The label of an edge in G_2 is the sum of the labels of the parallel edges in the multidigraph. By the rules of the reactions in a MESSI network, G_2 is a linear graph (each node is indicated by a single variable) and the labels on the edges depend on the rate constants but might also depend on the concentrations of some species. We call G_2° the digraph obtained from the deletion of loops and isolated nodes of G_2. It can be shown (see Lemma 18 of [86]) that if the partition associated to a MESSI system is minimal, the connected components of the associated digraph G_2 are in bijection with the subsets $\mathscr{S}^{(\alpha)}$ corresponding to a core species and the set of nodes of the corresponding component equals $\mathscr{S}^{(\alpha)}$.

Finally, given a MESSI system with a minimal partition of the set of species, we define the associated digraph G_E, whose vertices are the sets $\mathscr{S}^{(\alpha)}$ for $\alpha \geq 1$, and

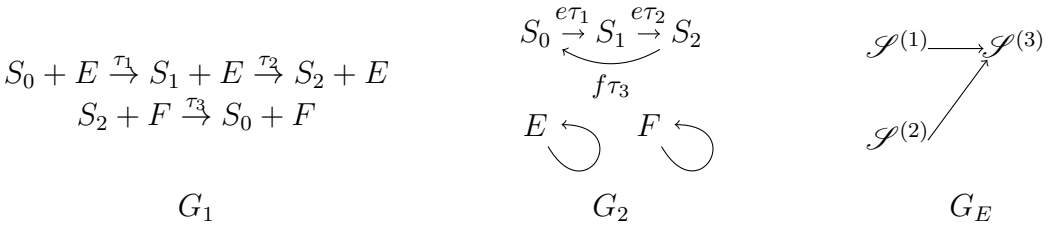


Figure 1.4: The digraphs G_1, G_2, and G_E of the network in Example 1.4.2.

there is an edge from $\mathscr{S}^{(\alpha)}$ to $\mathscr{S}^{(\beta)}$ if there is a species in $\mathscr{S}^{(\alpha)}$ in a label of an edge in G_2° between species of $\mathscr{S}^{(\beta)}$.

Example 1.4.3 (Example 1.4.2, continued)**.** The digraphs G_1, G_2, and G_E associated to the network of Example 1.4.2 are depicted in Figure 1.4.

Now, we give the definition of an s-toric MESSI. Recall that an i-tree of a graph is a spanning tree where the i-th vertex is its unique sink. Given an i-tree T, we call c^T the product of the labels of all the edges of T. An s-toric MESSI system is a MESSI system that also satisfies the following conditions: i) for any intermediate complex y, there exists a unique core complex y_1 such that $y_1 \to_\circ y$, ii) the associated multidigraph MG_2 does not have parallel edges and the digraph G_2 is weakly reversible (i.e., for any pair of nodes in the same connected component there is a directed path joining them), iii) for each vertex i of G_2° and any choice of i-trees T, T' of G_2°, the quotient $c^T/c^{T'}$ only depends on the rate constants τ. It is interesting to note that even if this definition is restrictive, many of the common enzymatic networks in the literature satisfy these conditions.

Chapter 2

Lower bounds for positive roots and regions of multistationarity

In this chapter, we develop tools from real algebraic geometry based on the paper [7] by Bihan, Santos, and Spaenlehauer, to find coefficients for which a given real sparse polynomial system has more than one positive solution. The basic idea we develop in this chapter is to detect in the convex hull of the support of the monomials that define the equations, (at least two) simplices *positively decorated* (see Definition 2.2.10) that form part of a regular subdivision. This ensures the extension of the positive real solutions of the corresponding subsystems to the total system. We apply this general framework to analyze systems biology models. In particular, we use it to describe *multistationarity regions* in parameter space, that is, to find parameters for which multistationarity occurs.

We exemplify our theoretical results in different biochemical networks of interest of arbitrary size and number of variables. For this, we need to adapt the theoretical results to make them amenable to effective computations in a variety of specific instances in the modeling of biochemical systems. Our developments are also based on the existence of explicit parametrizations of the corresponding steady state varieties, as described in Theorems 4.1 and 4.8 in [86].

We give two complementary approaches. On one side, we show how to deform a given choice of reaction rate constants and total concentration constants in order to produce multistationarity. On the other side, we describe open multistationarity regions in the space of all these constants. We derive inequalities in the reaction constants and in the total conservation constants whose validity implies the presence of multistationarity.

First, to illustrate our approach, we present in Section 2.1 our results for a two component system. In Sections 2.2 and 2.3 we state and explain the theoretical setting, which is of general interest for the search of positive solutions of sparse real polynomial systems beyond the applications we consider. In Section 2.2 we work with the same support for all the polynomials of the system. In Section 2.3 we present a mixed approach to the results of Section 2.2, considering different supports for each polynomial. We refer the reader to [24, 53] for the definitions and main

properties of the combinatorial objects we deal with.

Our main results in these sections are Theorems 2.2.13 and 2.3.3. In the following sections, we apply these results for a class of biochemical reaction networks under mass-action kinetics. This application is not straightforward and requires both known and new results on the structure of their steady states.

In Section 2.4, we study the sequential distributive multisite phosphorylation systems with any number n of phosphorylation sites, introduced in Section 1.2 of Chapter 1. Such systems were studied by many authors, starting with Wang and Sontag [108]. They gave bounds and conditions on the total conservation constants for which there exists rate constants ensuring monostationarity or multistationarity, with an interesting treatment ad hoc, which also allowed them to find improved lower bounds (see also [63]). In [16], Conradi and Mincheva showed using degree theory and computations with the aid of a computer algebra system, that catalytic constants determine the capacity for multistationarity in the dual phosphorylation mechanism. They also indicate in the case $n = 2$ how to find values of the total concentrations such that multistationarity occurs. The more general interesting approach in [12] is also based on degree theory. The authors show how to find conditions on the reaction rates to guarantee mono or multistationarity, but they do not describe the particular total concentration constants for which there are multiple equilibria. With our approach, we obtain for any n a system of three polynomial equations in three variables that describes the steady states, in the framework of [7]. We give conditions both on some of the reaction constants and on the total concentration constants, so that there are at least two positively decorated simplices in a regular subdivision of the convex hull of our support and by rescaling the rest of the parameters, we guarantee the existence of at least two nondegenerate positive steady states (see Theorem 2.4.1).

These systems, as well as the two-component system that we will introduce in Section 1.1, are examples of MESSI systems, presented in Section 1.4 of Chapter 1. In Section 2.5, we focus on s-toric MESSI systems, which includes the sequential phosphorylation systems, for which explicit monomial parametrizations of the steady states are given in [86]. We prove general results for s-toric MESSI systems, that in particular explain our computations in Section 2.4. Theorem 2.5.2 is the key to apply the framework of Theorem 2.2.13 to describe multistationarity regions for all these biological systems.

2.1 Our results for a two-component system

We showcase our results in a simple meaningful example. The following chemical reaction network is a *two-component system* [95] with *hybrid* histidine kinase (hybrid HK) whose multistationarity was studied in [12, 70]. Two-component signal transduction systems enable bacteria to sense, respond, and adapt to a wide range of environments, stressors, and growth conditions. This network has six species $X_1, \ldots, X_6$, ten complexes, and six reactions, with labels given by positive reaction

rate constants $k_1, \ldots, k_6$:

$$\begin{aligned} X_1 &\xrightarrow{k_1} X_2 \xrightarrow{k_2} X_3 \xrightarrow{k_3} X_4 \\ X_3 + X_5 &\xrightarrow{k_4} X_1 + X_6 \\ X_4 + X_5 &\xrightarrow{k_5} X_2 + X_6 \\ X_6 &\xrightarrow{k_6} X_5 \end{aligned} \tag{2.1.1}$$

This labeled digraph represents the following biological mechanism. Two component signaling relies on phosphotransfer reactions between histidine and aspartate residues on histidine kinases (HKs) and response regulator (RR) proteins. The hybrid HK consists of two phosphorylable domains. We denote the phosphorylation state of each site by p if the site is phosphorylated and 0 if it is not; the four possible states of HK are denoted by HK_{00}, HK_{p0}, HK_{0p}, and HK_{pp}. We let RR be the unphosphorylated response regulator protein, and RR_p the phosphorylated form. Upon receiving a signal, the HK can auto-phosphorylate. Whenever the second phosphorylation site is occupied, the phosphate group can be transferred to RR. In (2.1.1), we displayed the corresponding network of reactions denoting by $X_1, \ldots, X_6$ the chemical species $HK_{00}, HK_{p0}, HK_{0p}, HK_{pp}, RR, RR_p$, respectively.

In what follows, we denote the concentration of the chemical species $X_1, \ldots, X_6$ by lower-case letters $x_1, \ldots, x_6$. Under mass-action kinetics, these concentrations are assumed to be functions which evolve in time t, according to the following polynomial autonomous dynamical system:

$$\begin{aligned} \frac{dx_1}{dt} &= f_1(x) = -k_1x_1 + k_4x_3x_5, & \frac{dx_2}{dt} &= f_2(x) = k_1x_1 - k_2x_2 + k_5x_4x_5, \\ \frac{dx_3}{dt} &= f_3(x) = k_2x_2 - k_3x_3 - k_4x_3x_5, & \frac{dx_4}{dt} &= f_4(x) = k_3x_3 - k_5x_4x_5, \\ \frac{dx_5}{dt} &= f_5(x) = -k_4x_3x_5 - k_5x_4x_5 + k_6x_6, & \frac{dx_6}{dt} &= f_6(x) = k_4x_3x_5 + k_5x_4x_5 - k_6x_6. \end{aligned}$$

It is straightforward to check that there are two linearly independent relations: $f_1 + f_2 + f_3 + f_4 = f_5 + f_6 = 0$, which imply the existence of two constants T_1, T_2 such that for any value of time t:

$$\begin{aligned} \ell_1(x) = x_1 + x_2 + x_3 + x_4 &= T_1, \\ \ell_2(x) = x_5 + x_6 &= T_2. \end{aligned} \tag{2.1.2}$$

We assume that the linear variety cut out by these equations intersects the positive orthant, so T_1, T_2 are also positive parameters. These parameters T_1, T_2 are the total conservation constants and the linear equations ℓ_1 and ℓ_2 are the conservation laws.

We now explain our strategy in the previous network (2.1.1). Our problem is to determine values of $(k_1, \ldots, k_6, T_1, T_2)$ in $\mathbb{R}^8_{>0}$ for which the polynomial system

$$f_1(x) = \cdots = f_6(x) = \ell_1(x) - T_1 = \ell_2(x) - T_2 = 0,$$

has *more than one positive solution* $x \in \mathbb{R}^6_{>0}$. We have, using the framework of the main Theorems 2.2.11 and 2.2.13:

Theorem 2.1.1. *With the notation of (2.1.1) and (2.1.2), assume that a fixed choice of reaction rate constants satisfies the condition* $k_3 > k_1$. *Then,* $k_6\left(\frac{1}{k_2}+\frac{1}{k_3}\right) < k_6\left(\frac{1}{k_1}+\frac{1}{k_2}\right)$ *and for any choice of total concentration constants verifying the inequalities*

$$k_6\left(\frac{1}{k_2}+\frac{1}{k_3}\right) < \frac{T_1}{T_2} < k_6\left(\frac{1}{k_1}+\frac{1}{k_2}\right), \tag{2.1.3}$$

there exist positive constants N_1, N_2 *such that for any values of* β_4 *and* β_5 *satisfying* $\beta_4 > N_1$ *and* $\frac{\beta_5}{\beta_4} > N_2$, *the system has at least three positive steady states after modifying only the parameters* k_4, k_5 *via the rescaling* $\overline{k_4} = \beta_4\, k_4$, $\overline{k_5} = \beta_5\, k_5$.

In [12], the authors present necessary and sufficient conditions for the multistationarity of the network. They prove that the region of the reaction rate constant space for which multistationarity exists is completely characterized by the inequality $k_3 > k_1$, but they do not describe the particular stoichiometric compatibility classes, determined by the total conservation constants, for which there are multistationarity. In contrast, we go further by giving *joint* conditions on the total conservation and the reaction rate constants for the ocurrence of multistationarity.

In [70] necessary and sufficient conditions on all the parameters of this same network for bistability are provided, with an ad-hoc treatment using Sturm's Theorem. Our approach is systematic and can be used in a great variety of biological networks.

2.2 Positive solutions of sparse polynomial systems

Along this section, we fix a finite point configuration

$$\mathcal{A} = \{a_1, \ldots, a_n\} \subset \mathbb{Z}^d,\ n \geq d+2,$$

and we assume that the convex hull of $\mathcal{A}$ is a full-dimensional polytope. A subset of $\mathcal{A}$ consisting of affinely independent points will be called a simplex; we will also say that it is a d-simplex when the dimension of its convex hull is d.

2.2.1 Regular subdivisions

A *regular subdivision of* $\mathcal{A}$ is induced by a height function $h : \mathcal{A} \to \mathbb{R}$, also identified with the vector $h = (h(a_1), \ldots, h(a_n))$, as follows. Consider the *lower convex hull* of the lifted configuration

$$\mathcal{A}^h = \{(a_1, h(a_1)), \ldots, (a_n, h(a_n))\} \subset \mathbb{R}^{d+1},$$

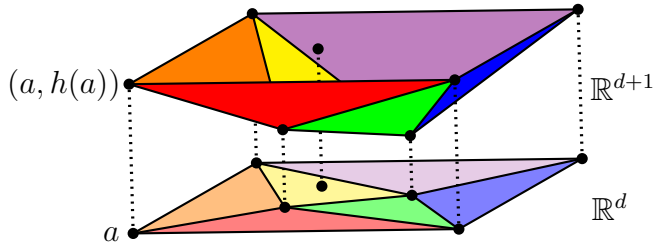


Figure 2.1: Regular triangulation.

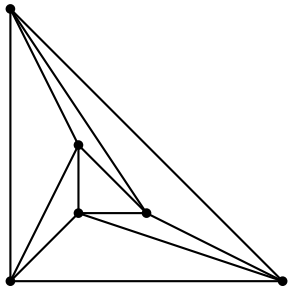


Figure 2.2: Nonregular triangulation.

which is the union of the faces of the convex hull of $\mathcal{A}^h$ for which the inner normal directions have positive last coordinate. The associated regular subdivision Γ_h is the collection of the subsets $\mathcal{A}_F = \{a_i : (a_i, h(a_i)) \in F\}$ of $\mathcal{A}$, which are the projections back to $\mathcal{A}$ of lifted points in a face F of this lower convex hull.

It is useful to have a more geometric picture of this subdivision, like the one depicted in Figure 2.1, but it is important to note that these subsets $\mathcal{A}_F$ cannot be identified in general with their convex hulls, which are convex polytopes with integer vertices, but with their *marked* convex hulls containing all the points $a_j \in \mathcal{A}$ for which the affine linear function which interpolates the values of h at the vertices, takes the value $h(a_j)$ at a_j (so, other points in $\mathcal{A}$ besides the vertices of the polytopes can occur in the subsets $\mathcal{A}_F$).

A regular subdivision is called a *regular triangulation* of $\mathcal{A}$ if the only points in each subset of the subdivision are the vertices of their convex hull and these vertices are affinely independent. Figure 2.2 depicts a triangulation into simplices which is not regular, that is, which cannot be induced by any height function h.

The set of all height vectors inducing a regular subdivision Γ of $\mathcal{A}$ is defined by a finite number of linear inequalities. Thus, this set is a finitely generated convex cone $\mathcal{C}_\Gamma$ in $\mathbb{R}^n$ with apex at the origin. When Γ is a triangulation, the cone $\mathcal{C}_\Gamma$ is full dimensional (cut out by strict inequalities). All these facts and many more are described in Chapter 7 in [53].

We will denote by $A \in \mathbb{Z}^{(d+1)\times n}$ the integer matrix:

$$A = \begin{pmatrix} 1 & \dots & 1 \\ a_1 & \dots & a_n \end{pmatrix}, \tag{2.2.1}$$

and by $A_h \in \mathbb{R}^{(d+2)\times n}$ the matrix:

$$A_h = \begin{pmatrix} 1 & \dots & 1 \\ a_1 & \dots & a_n \\ h_1 & \dots & h_n \end{pmatrix}. \tag{2.2.2}$$

Note that our assumption that the convex hull of $\mathcal{A}$ has dimension d is equivalent to assuming that the rank of A is equal to $d+1$.

Let $\Delta = \{a_{i_1}, \ldots, a_{i_{d+1}}\}$ be a d-simplex with vertices in $\mathcal{A}$. Let $I = \{i_1, \ldots, i_{d+1}\}$ and assume that $i_1 < \cdots < i_{d+1}$. Denote by d_I the determinant of the $(d+1) \times (d+1)$ submatrix of A with columns indicated by I, which is nonzero because we are assuming that Δ is a simplex. Also, for any index $i \notin I$, denote by $d_{I\cup\{i\}}(h)$ the determinant of the $(d+2) \times (d+2)$ submatrix of A_h with columns indicated by $I \cup \{i\}$, multiplied by the sign of the permutation that sends the set of indices in $I \cup \{i\}$ ordered by $<$ to $(i_1, \ldots, i_{d+1}, i)$ with i as the last index. Using the Laplace expansion of the determinant along the last row, we see that $d_{I\cup\{i\}}(h)$ is an affine linear function of h. Thus, there exists an integer vector m_i^I with support included in $I \cup \{i\}$ and with nonzero i-th coordinate d_I such that

$$d_{I\cup\{i\}}(h) \,=\, \langle m_i^I, h\rangle.$$

Moreover, m_i^I belongs to the kernel of A. This follows from the well known fact that for any $k \times (k+1)$ matrix M, the vector with coordinates $(-1)^j \operatorname{minor}(M, j)$, $j = 1, \ldots, k+1$, belongs to the kernel of M (here $\operatorname{minor}(M, j)$ is the determinant of the square matrix obtained by removing the j-th column, compare with Definition 2.2.8).

Consider the cone $\mathcal{C}_\Delta$ of all height vectors inducing a regular subdivision of $\mathcal{A}$ that contains Δ. Observe that $\mathcal{C}_\Delta$ is nonempty; for instance, any vector $h \in \mathbb{R}^n$ with $h_i = 0$ for any $i \in I$ and $h_i > 0$ for any index $i \notin I$, belongs to $\mathcal{C}_\Delta$. Moreover, C_Δ is an open rational polyhedral cone, described as follows:

Lemma 2.2.1. *With the previous notations, we have:*

$$\mathcal{C}_\Delta = \{h \in \mathbb{R}^n \,:\, d_I \cdot d_{I\cup\{i\}}(h) > 0 \text{ for all } i \notin I\}$$

and the $n - (d+1)$ vectors $d_I \cdot m_i^I, i \notin I$, are a basis of the kernel of A.

The proof of Lemma 2.2.1 is straightforward. Note that the coefficient of h_i in the linear function $d_I \cdot d_{I\cup\{i\}}(h) = \langle d_I \cdot m_i^I, h\rangle$ is $d_I^2 > 0$. Hence, for any any vector $h \in \mathbb{R}^n$ with $h_i = 0$ for any $i \in I$ and $h_i > 0$ for any index $i \notin I$ we have $\langle d_I \cdot m_i^I, h\rangle > 0$ as wanted.

Let $p \geq 1$ and consider $\Delta_1, \ldots, \Delta_p$ d-simplices in $\mathcal{A}$. We denote by $\mathcal{C}_{\Delta_1,\ldots,\Delta_p}$ the cone of all height vectors h defining a regular subdivision of $\mathcal{A}$ that contains $\Delta_1, \ldots, \Delta_p$. We deduce from Lemma 2.2.1 the following description.

Lemma 2.2.2. *Let $\Delta_1, \ldots, \Delta_p$ be simplices in $\mathcal{A}$ which occur in a regular subdivision of A. Letting I_k denote the index set of the vertices of Δ_k for any $k = 1, \ldots, p$, the nonempty open polyhedral cone $\mathcal{C}_{\Delta_1,\ldots,\Delta_p}$ is defined by the linear inequalities*

$$\mathcal{C}_{\Delta_1,\ldots,\Delta_p} \,=\, \{h \in \mathbb{R}^n \,:\, d_{I_k} \cdot d_{I_k\cup\{i\}}(h) > 0 \quad \forall k = 1, \ldots, p, \text{ and } \forall i \notin I_k\}, \quad (2.2.3)$$

and the vectors $d_{I_k} \cdot m_i^{I_k}$, with $k = 1, \ldots, p$ and $i \notin I_k$, generate the kernel of A.

Remark 2.2.3. An equivalent way to define the cone $\mathcal{C}_{\Delta_1,\ldots,\Delta_p}$ is as follows. Given a d-simplex Δ with vertices in $\mathcal{A}$, we consider height vectors $h \in \mathbb{R}^n$, where each coordinate h_j of h gives the value of a lifting function on the point a_j of $\mathcal{A}$. Denote

by $\varphi_{\Delta,h}$ the unique affine function that agrees with h on the points of Δ, that is, $\varphi_{\Delta,h}(a_j) = h_j$ for all $a_j \in \Delta$. We associate with Δ the following cone:

$$\mathcal{C}_\Delta = \{h = (h_1, \ldots, h_n) \in \mathbb{R}^n \,:\, \varphi_{\Delta,h}(a_j) < h_j \text{ for all } a_j \notin \Delta\}.$$

Then, $\mathcal{C}_{\Delta_1,\ldots,\Delta_p} = \cap_{i=1}^p \mathcal{C}_{\Delta_i}$.

We introduce the following notation.

Definition 2.2.4. *We will say that two d-simplices $\Delta_1, \Delta_2 \subset \mathcal{A}$ share a facet if the intersection of their convex hulls is a facet of both, that is, a face of codimension one. See Figure 2.3.*

Figure 2.3: Examples of 2-simplices Δ_1 and Δ_2, which share a facet.

We will need the following observation:

Remark 2.2.5. A point configuration $\mathcal{B} = \{b_1, \ldots, b_{d+2}\}$ with $d+2$ points which span $\mathbb{R}^d$ and such that any proper subset is affinely independent, is called a *circuit*. Any circuit $\mathcal{B}$ has exactly two triangulations $\Gamma_\pm$, which are furthermore regular. They can be described in the following way (see Proposition 1.2, Chapter 7 in [53]). Consider any nonzero vector $\lambda \in \mathbb{R}^{d+2}$ such that $\sum_{i=1}^{d+2} \lambda_i = 0$ and $\sum_{i=1}^{d+2} \lambda_i b_i = 0$ (in other words, any nontrivial affine relation on $\mathcal{B}$). Note that all coordinates of λ are nonzero. Write $[d+2] = \{1, \ldots, d+2\}$ as the disjoint union $N_+ \sqcup N_-$, with $N_+ = \{i \in [d+2] \,:\, \lambda_i > 0\}$ and $N_- = \{i \in [d+2] \,:\, \lambda_i < 0\}$. The d-simplices of Γ_+ are the sets $[d+2] \setminus \{i\}$ for $i \in N_+$. Similarly, the d-simplices of Γ_- are the sets $[d+2] \setminus \{i\}$ for $i \in N_-$.

We are ready to prove the following proposition, that we will need in our applications.

Proposition 2.2.6. *Let Δ_1, Δ_2 be two d-simplices in $\mathcal{A}$ which share a facet. Then, there exists a regular subdivision of $\mathcal{A}$ containing Δ_1 and Δ_2, and so the cone $\mathcal{C}_{\Delta_1,\Delta_2}$ is nonempty. Moreover, there exists a regular triangulation containing both simplices.*

Proof. The configuration $\mathcal{B} = \Delta_1 \cup \Delta_2$ has cardinality $d+2$ and it is a circuit. By Remark 2.2.5, $\mathcal{B}$ has exactly two regular triangulations $\Gamma_\pm$. Without loss of generality, assume $\mathcal{B} = \{a_1, \ldots, a_{d+2}\}$, with $F = \{a_1, \ldots, a_d\}$ the common facet of Δ_1 and Δ_2. Let $\lambda \in \mathbb{Z}^{d+2}$ be a nontrivial affine relation on $\mathcal{B}$. As a_{d+1} and a_{d+2} lie in opposite sides of the hyperplane passing through F, it holds that λ_{d+2} and λ_{d+1}

have the same sign. Therefore Δ_1 and Δ_2 belong to the same regular triangulation, say Γ_+.

Let $h : \mathcal{B} \to \mathbb{R}$ be a height function inducing Γ_+. Let $\varphi_1, \ldots, \varphi_\ell$ be the affine linear functions which interpolate the values of h at each of the d-simplices of Γ_+ and set $\varphi = \max\{\varphi_1, \ldots, \varphi_\ell\}$. For any choice of generic positive values $h_{d+3}, \ldots, h_n$ verifying $h_j > \varphi(a_j)$ for any $j = d+3, \ldots, n$, the height function $h' : \mathcal{A} \to \mathbb{R}$ that extends h by defining $h'(a_j) = h_j, j = d+3, \ldots, n$, induces a regular triangulation of $\mathcal{A}$ extending Γ_+, and so in particular, it contains Δ_1 and Δ_2. □

Remark 2.2.7. Under the notations of Lemma 2.2.2 with $p = 2$, if Δ_1 and Δ_2 share a common facet with vertices a_i with $i \in I$, then the inequality corresponding to $k = 1$ and $i \in I_2 \setminus I$ coincides with the one corresponding to $k = 2$ and $i \in I_1 \setminus I$, so that we can forget one of these inequalities in (2.2.3) to get $2(n-d-1)-1 = 2n-2d-3$ inequalities defining $\mathcal{C}_{\Delta_1,\Delta_2}$. This generalizes the circuit case where any of the two regular triangulations is determined by one of its simplices.

2.2.2 Decorated simplices and lower bounds for the number of positive solutions

Consider a sparse polynomial system in d variables $x = (x_1, \ldots, x_d)$ with support included in $\mathcal{A}$ and coefficient matrix $C = (c_{ij}) \in \mathbb{R}^{d \times n}$:

$$f_1(x) = \cdots = f_d(x) = 0, \tag{2.2.4}$$

with

$$f_i(x) = \sum_{j=1}^{n} c_{ij}\, x^{a_j} \in \mathbb{R}[x_1, \ldots, x_d],\ i = 1, \ldots, d.$$

A solution of (2.2.4) is nondegenerate when it is not a zero of the Jacobian of $f_1, \ldots, f_d$. We recall some definitions from Section 3 in [7].

Definition 2.2.8. *A $d \times (d+1)$ matrix M with real entries is called positively spanning if all the values $(-1)^i \operatorname{minor}(M, i)$ are nonzero and have the same sign, where $\operatorname{minor}(M, i)$ is the determinant of the square matrix obtained by removing the i-th column.*

Equivalently, a matrix is positively spanning if all the coordinates of any nonzero vector in the kernel of the matrix are nonzero and have the same sign.

Proposition 3.3 in [7] says that if the support $\mathcal{A}$ of the system (2.2.4) is a d-simplex, then it has one nondegenerate positive solution if and only if the matrix of coefficients C is positively spanning.

Proposition 2.2.9 (Proposition 3.3 in [7])**.** *Consider $\mathcal{A} = \{a_1, \ldots, a_{d+1}\} \subset \mathbb{Z}^d$ a d-simplex and a matrix $C = (c_{ij}) \in \mathbb{R}^{d \times (d+1)}$. The system with support $\mathcal{A}$ and coefficient matrix C as in* (2.2.4) *has at most one nondegenerate positive solution, and it has one nondegenerate positive solution if and only if the matrix C is positively spanning.*

Proof. Multiplying the system by $x^{-a_{d+1}}$ (which does not change the set of positive solutions), we can assume without loss of generality that $a_{d+1} = 0$. Consider the monomial map

$$\begin{array}{rcl} \phi\colon \mathbb{R}^d_{>0} & \to & \mathbb{R}^d_{>0}, \\ x & \mapsto & (x^{a_1}, \ldots, x^{a_d}). \end{array}$$

This map is a bijection since $\mathcal{A}$ is affinely independent. Let $\ell_1(x_1, \ldots, x_d) = \cdots = \ell_d(x_1, \ldots, x_d) = 0$ be the linear system defined by:

$$\ell_i(x) = \sum_{j=1}^{d} c_{ij} x_j, \ i = 1, \ldots, d.$$

Since $(\ell_i \circ \phi)(x_1, \ldots, x_d) = f_i(x)$ for each $i = 1, \ldots, d$, the positive solutions of $f_1(x) = \cdots = f_d(x) = 0$ are in bijection with the positive solutions of $\ell_1(x_1, \ldots, x_d) = \cdots = \ell_d(x_1 \ldots, x_d) = 0$. And this linear system has a unique positive solution if and only if the matrix C is positively spanning. □

Let $\mathcal{A} = \{a_1, \ldots, a_n\} \subset \mathbb{Z}^d$ and coefficient matrix $C = (c_{ij}) \in \mathbb{R}^{d \times n}$. Let Γ be a regular subdivision of $\mathcal{A}$ and $h \in \mathcal{C}_\Gamma$. Consider the following family of polynomial systems parametrized by a positive real number t:

$$f_{1,t}(x) = \cdots = f_{d,t}(x) = 0, \tag{2.2.5}$$

where

$$f_{i,t}(x) = \sum_{j=1}^{n} c_{ij}\, t^{h(a_j)}\, x^{a_j} \in \mathbb{R}[x_1, \ldots, x_d], \ i = 1, \ldots, d, \ t > 0.$$

For each positive real value of t, this system has again support included in $\mathcal{A}$.

Definition 2.2.10. *Let C be a $d \times n$ matrix with real entries. We say that a d-simplex $\Delta = \{a_{i_1}, \ldots, a_{i_{d+1}}\}$ in Γ is positively decorated by C if the $d \times (d+1)$ submatrix of C with columns indicated by $\{i_1, \ldots, i_{d+1}\}$ is positively spanning.*

The following result is a slight generalization of Theorem 3.4 in [7]. This last theorem is a version of Viro's method ([105]) which was used in [96] to construct sparse polynomial systems such that all their solutions are real.

Theorem 2.2.11. *Let $\mathcal{A} = \{a_1, \ldots, a_n\} \subset \mathbb{Z}^d$ be a finite point configuration. Let $\Delta_1, \ldots, \Delta_p$ be distinct d-simplices which occur in a regular subdivision Γ of $\mathcal{A}$, and which are positively decorated by a matrix $C \in \mathbb{R}^{d \times n}$. Let h be a height function that defines Γ. Then, there exists $t_0 \in \mathbb{R}_{>0}$ such that for all $0 < t < t_0$, the number of (nondegenerate) solutions of (2.2.5) contained in the positive orthant is at least p. Moreover, if there are two d-simplices with vertices in $\mathcal{A}$ sharing a facet and which are both positively decorated by C, then, there exists $t_0 \in \mathbb{R}_{>0}$ such that for all $0 < t < t_0$ the number of positive solutions of (2.2.5) is at least two.*

The first part of Theorem 2.2.11 is a slight extension of Theorem 3.4 in [7] in that we don't assume that Γ is a triangulation. Clearly, the proof of Theorem 3.4 in [7] works identically when Γ is any regular subdivision and thus gives a proof of the first part of Theorem 2.2.11. The idea of the proof is to observe that the system obtained by considering only the monomials in a positively decorated d-simplex has exactly one nondegenerate positive solution by Proposition 2.2.9. Then, as a consequence of the fact that the subdivision is regular, we can jointly extend the positive solutions of the p restricted systems to p positive solutions of system (2.2.5), for small values of $t > 0$. We present the complete proof below.

Proof of Theorem 2.2.11. For each $\ell = 1, \ldots, p$, let φ_ℓ be the affine function that agrees with h for all $a_j \in \Delta_\ell$ and $\varphi_\ell(a_j) < h_j$ for $a_j \notin \Delta_\ell$. Let $\alpha_\ell = (\alpha_{1\ell}, \ldots, \alpha_{d\ell}) \in \mathbb{R}^d$ and $\beta_\ell \in \mathbb{R}$ such that $\varphi_\ell(x) = \langle \alpha_\ell, x\rangle + \beta_\ell$.

Set $xt^{-\alpha_\ell} = (x_1 t^{-\alpha_{1\ell}}, \ldots, x_d t^{-\alpha_{d\ell}})$. We have that

$$\frac{f_{i,t}(xt^{-\alpha_\ell})}{t^{\beta_\ell}} = f_i^{(\ell)}(x) + r_{i,t}(x), \tag{2.2.6}$$

where $f_i^{(\ell)}(x) = \sum_{a_j \in \Delta_\ell} c_{ij} x^{a_j}$ and $r_{i,t}(x)$ is a polynomial, each of whose coefficients is equal to a positive power of t multiplied by a coefficient of C. Since Δ_ℓ is positively decorated by C, by Proposition 2.2.9, the system $f_1^{(\ell)}(x) = \cdots = f_d^{(\ell)}(x) = 0$ has one nondegenerate positive solution z_ℓ. It follows that for t small enough the system $f_1^{(\ell)}(x) + r_{1,t}(x) = \cdots = f_d^{(\ell)}(x) + r_{d,t}(x) = 0$ has one nondegenerate positive solution close to z_ℓ. More precisely, for all $\varepsilon > 0$, there exists $t_{\varepsilon,\ell} > 0$ such that for all $0 < t < t_{\varepsilon,\ell}$, there exist a nondegenerate solution $z_{\ell,t}$ of $f_1^{(\ell)}(x) + r_{1,t}(x) = \cdots = f_d^{(\ell)}(x) + r_{d,t}(x) = 0$ such that $|z_{\ell,t} - z_\ell| < \varepsilon$. Then, from (2.2.6), $z_{\ell,t} t^{-\alpha_\ell}$ is a solution of system (2.2.5). Choose ε small enough such that the balls of radius ε centered at z_ℓ, $\ell = 1, \ldots, p$ are contained in a compact set $K \subset \mathbb{R}^d_{>0}$. Since the vectors α_ℓ are distinct, there exists $t' > 0$ such that for all $0 < t < t'$, the sets $K \cdot t^{-\alpha_\ell} = \{(x_1 t^{-\alpha_{1\ell}}, \ldots, x_d t^{-\alpha_{d\ell}}) : (x_1, \ldots, x_d) \in K\}$ for $\ell = 1, \ldots, p$ are pairwise disjoint. Take $t_0 = \min\{t', t_{\varepsilon,1}, \ldots, t_{\varepsilon,p}\}$. Then, for $0 < t < t_0$, each set $K \cdot t^{-\alpha_\ell}$ contains a nondegenerate positive solution $z_{\ell,t} t^{-\alpha_\ell}$ of the system (2.2.5). □

We will give a similar result in Theorem 2.2.13 below, but our focus is to describe a subset with nonempty interior in the space of coefficients where we can bound from below the number of positive solutions of the associated system. We start with a general result about convex polyhedral cones.

Proposition 2.2.12. *Let L be a linear subspace of $\mathbb{R}^n$ of dimension ℓ_1 together with a basis $\{v_1, \ldots, v_{\ell_1}\}$. Let $m_1, \ldots, m_\ell$ be a system of generators of $L^\perp$ such that the open polyhedral cone*

$$\mathcal{C} = \{h \in \mathbb{R}^n : \langle m_r, h\rangle > 0, \quad r = 1, \ldots, \ell\}$$

is nonempty. For any $\varepsilon \in \mathbb{R}^\ell_{>0}$, denote by $\mathcal{C}_\varepsilon$ the n-dimensional convex polyhedral cone

$$\mathcal{C}_\varepsilon = \{h \in \mathbb{R}^n : \langle m_r, h\rangle > \varepsilon_r, \quad r = 1, \ldots, \ell\}. \tag{2.2.7}$$

Consider the map $\varphi : \mathbb{R}^{\ell_1}_{>0} \times \mathbb{R}_{>0} \times \mathbb{R}^n \to \mathbb{R}^n_{>0}$*:*

$$\varphi(\alpha, t, h) \;=\; (t^{h_1} \prod_{j=1}^{\ell_1} \alpha_j^{v_{j1}}, \ldots, t^{h_n} \prod_{j=1}^{\ell_1} \alpha_j^{v_{jn}}).$$

Then, we have:

$$\varphi(\mathbb{R}^{\ell_1}_{>0} \times (0, t_0) \times \mathcal{C}_\varepsilon) \;=\; \{\gamma \in \mathbb{R}^n_{>0} \,:\, \gamma^{m_r} < t_0^{\varepsilon_r},\, r = 1 \ldots, \ell\} \text{ and} \quad (2.2.8)$$

$$\varphi(\mathbb{R}^{\ell_1}_{>0} \times (0, t_0] \times \bar{\mathcal{C}}_\varepsilon) \;=\; \{\gamma \in \mathbb{R}^n_{>0} \,:\, \gamma^{m_r} \leq t_0^{\varepsilon_r},\, r = 1 \ldots, \ell\}, \quad (2.2.9)$$

where $\bar{\mathcal{C}}_\varepsilon$ *denotes the closure of* $\mathcal{C}_\varepsilon$*.*

Proof. We first prove that a positive vector γ is of the form $\gamma = \varphi(\alpha, t, h)$ if and only if

$$\gamma^{m_r} = t^{\langle m_r, h\rangle}, \quad r = 1, \ldots, \ell.$$

The only if part is straightforward, taking into account that we are assuming that for any r, j it holds that $\langle m_r, v_j\rangle = 0$:

$$\varphi(\alpha, t, h)^{m_r} \;=\; t^{\langle m_r, h\rangle} \prod_{j=1}^{\ell_1} \alpha_j^{\langle m_r, v_j\rangle} \;=\; t^{\langle m_r, h\rangle}.$$

On the other side, if $\gamma^{m_r} = t^{\langle m_r, h\rangle}$ for any $r = 1, \ldots, \ell$, then the vector

$$\gamma_{t,h} = (\gamma_1\, t^{-h_1}, \ldots, \gamma_n\, t^{-h_n})$$

verifies that $\gamma_{t,h}^m \;=\; 1$, for any $m \in L^\perp$. Thus, taking coordinatewise logarithms, we get that

$$\langle m, \log(\gamma_{t,h})\rangle \;=\; 0 \text{ for any } m \in L^\perp,$$

which means that $\log(\gamma_{t,h}) \in L$. Then, there exist real constants $\lambda_1, \ldots, \lambda_\ell$ such that $\log(\gamma_{t,h}) = \sum_{j=1}^{\ell} \lambda_j\, v_j$. Calling $\alpha \in \mathbb{R}^\ell_{>0}$ the vector with coordinates $\alpha_j = e^{\lambda_j}$ we get that $\gamma = \varphi(\alpha, t, h)$, as wanted.

Now, assuming $0 < t < t_0 < 1$ and $\langle m_r, h\rangle > \varepsilon_r$ for all $r = 1 \ldots, \ell$, we have that $t^{\langle m_r, h\rangle} < t_0^{\varepsilon_r}$ and moreover $(0, t_0^{\varepsilon_r}) = \{t^{\langle m_r, h\rangle} \,:\, 0 < t < t_0,\, h \in \mathcal{C}_\varepsilon\}$, which proves both containments. The other equality follows immediately. □

We now present the main result of this section.

Theorem 2.2.13. *Consider a set* $\mathcal{A} = \{a_1, \ldots, a_n\}$ *of* n *points in* $\mathbb{Z}^d$ *and a matrix* $C = (c_{ij}) \in \mathbb{R}^{d \times n}$*. Assume there are distinct* d*-simplices* $\Delta_1, \ldots, \Delta_p$ *contained in* $\mathcal{A}$*, which are part of a regular subdivision of* $\mathcal{A}$ *and are positively decorated by the matrix* C*.*

Let $m_1 \ldots, m_\ell \in \mathbb{R}^n$ *be vectors that define a presentation of the cone* $\mathcal{C}_{\Delta_1, \ldots, \Delta_p}$ *of all height vectors* $h \in \mathbb{R}^n$ *that induce a regular subdivision of* $\mathcal{A}$ *containing* $\Delta_1, \ldots, \Delta_p$*:*

$$\mathcal{C}_{\Delta_1, \ldots, \Delta_p} \;=\; \{h \in \mathbb{R}^n \,:\, \langle m_r, h\rangle > 0,\; r = 1, \ldots, \ell\}. \quad (2.2.10)$$

Then, for any $\varepsilon \in (0,1)^\ell$ there exists $t_0(\varepsilon) > 0$ such that for any γ in the open set

$$U \,=\, \cup_{\varepsilon\in(0,1)^\ell} \{\gamma \in \mathbb{R}^n_{>0} \,:\, \gamma^{m_r} < t_0(\varepsilon)^{\varepsilon_r},\, r = 1\ldots,\ell\},$$

the system

$$\sum_{j=1}^{n} c_{ij}\,\gamma_j\,x^{a_j} = 0, \quad i = 1,\ldots,d, \tag{2.2.11}$$

has at least p nondegenerate solutions in the positive orthant. In particular, given two d-simplices contained in $\mathcal{A}$ that share a facet, the system (2.2.11) *has at least* 2 *nondegenerate positive solutions for any γ in U.*

Proof. Let L be the linear subspace generated by the rows of the matrix A, and let $v_1,\ldots,v_{d+1}$ denote its row vectors, which are a basis of L because we are assuming that A has rank $d+1$. With this choice, we can apply Proposition 2.2.12 to the cone $\mathcal{C} = \mathcal{C}_{\Delta_1,\ldots,\Delta_p}$, by Lemma 2.2.2. Note that the map $\varphi : \mathbb{R}^{d+1}_{>0} \times \mathbb{R}_{>0} \times \mathbb{R}^n \to \mathbb{R}^n_{>0}$ equals in this case:

$$\varphi(\alpha,t,h) \,=\, (\alpha^{(1,\,a_1)}\,t^{h_1},\ldots,\alpha^{(1,\,a_n)}\,t^{h_n}).$$

We denote by $\overline{\mathcal{C}}_\varepsilon$ the closure of the cone $\mathcal{C}_\varepsilon$ defined in (2.2.7). Let B denote the closed unit ball in $\mathbb{R}^n$. In the proof of Theorem 3.4 in [7], and thus in the proof of Theorem 2.2.11, one can see that given any h, it is possible to find a positive number t_0 for which the conclusion of Theorem 2.2.11 holds for any h' close to h. As $B \cap \overline{\mathcal{C}}_\varepsilon$ is compact, there exists $t_1(\varepsilon) \in (0,1)$ such that the conclusion holds for any $t \in (0, t_1(\varepsilon))$ and any $h \in B \cap \overline{\mathcal{C}}_\varepsilon$. But if $h \in \overline{\mathcal{C}}_\varepsilon$ satisfies $||h|| > 1$, we can write it as $h = ||h||h'$, with $h' \in B \cap \overline{\mathcal{C}}_\varepsilon$. Then, for any $t \in (0,1)$ and for $i = 1,\ldots,n$ we have $t^{h_i} = (t^{||h||})^{h'_i}$ with $0 < t^{||h||} < t$ and so the conclusion of Theorem 2.2.11 holds for any $h \in \overline{\mathcal{C}}_\varepsilon$ provided $t \in (0, t_1(\varepsilon)]$. By Proposition 2.2.12, the image by φ of $\mathbb{R}^{d+1}_{>0} \times (0, t_1(\varepsilon)] \times \overline{\mathcal{C}}_\varepsilon$ equals $\{\gamma \in \mathbb{R}^n_{>0} \,:\, \gamma^{m_r} \le t_1(\varepsilon)^{\varepsilon_r},\, r = 1\ldots,\ell\}$. Note also that $\mathcal{C}_{\Delta_1,\ldots,\Delta_p} = \cup_{\varepsilon\in(0,1)^\ell}\,\overline{\mathcal{C}}_\varepsilon$.

Observe that if $\gamma = \varphi(\alpha,t,h)$, then for any $j = 1,\ldots,n$,

$$\gamma_j x^{a_j} = \alpha^{(1,\,a_j)} x^{a_j} = \alpha_1 t^{h_j} y^{a_j},$$

where $y_i = \alpha_{i+1} x_i$ for any $i = 1,\ldots,d$. As all $\alpha_i > 0$, system (2.2.11) has the same number of positive solutions as

$$\sum_{j=1}^{n} c_{ij}\,t^{h_j}\,y^{a_j} = 0, \quad i = 1,\ldots,d, \tag{2.2.12}$$

and this number is at least p for $t \in (0, t_1(\varepsilon)]$. □

Remark 2.2.14. Theorem 2.2.13 says that if we choose $\varepsilon \in (0,1)^\ell$, then there exists positive real numbers $M_1 = M_1(\varepsilon),\ldots,M_r = M_r(\varepsilon)$, such that system (2.2.11) has at least p nondegenerate solutions in the positive orthant for any vector γ in $\mathbb{R}^n_{>0}$ satisfying

$$\gamma^{m_r} < M_r \quad \text{for } r = 1,\ldots,\ell. \tag{2.2.13}$$

We have to remark that the choice of the positive constants $M_1, \ldots, M_r$ is *not* algorithmic, but our result makes clear that there is an open set in coefficient space for which many positive solutions can be found, and inequalities (2.2.13) indicate "in which directions" the coefficients have to be scaled in order to get at least as many positive solutions as the number of decorated simplices.

As a first application of Theorems 2.2.11 and 2.2.13, we give a proof of Theorem 2.1.1, corresponding to the example of the two component system with Hybrid Histidine Kinase (2.1.1) in Section 2.1. The systematic procedure to determine a multistationarity region using our approach is the following. We first replace a parametrization of the steady states into the conservation relations and we obtain a new sparse polynomial system to which we apply Theorems 2.2.11 and 2.2.13. After that, we show that the rescaling of the coefficients in this new system can be achieved by rescaling some of the original parameters.

Proof of Theorem 2.1.1. From $f_2 = f_3 = f_4 = f_5 = 0$ we get:

$$x_1 = \frac{k_4k_5x_4x_5^2}{k_1k_3},\ x_2 = \frac{k_4k_5x_4x_5^2}{k_2k_3} + \frac{k_5x_4x_5}{k_2},\ x_3 = \frac{k_5x_4x_5}{k_3},\ x_6 = \frac{k_4k_5x_4x_5^2}{k_3k_6} + \frac{k_5x_4x_5}{k_6}.$$

Then, at steady state, the concentrations of the species can be obtained from the values of x_4 and x_5. If we replace these expressions into the conservation laws (2.1.2), we get the equations:

$$\begin{aligned}\frac{k_4k_5x_4x_5^2}{k_1k_3} + \frac{k_4k_5x_4x_5^2}{k_2k_3} + \frac{k_5x_4x_5}{k_2} + \frac{k_5x_4x_5}{k_3} + x_4 - T_1 &= 0,\\ x_5 + \frac{k_4k_5x_4x_5^2}{k_3k_6} + \frac{k_5x_4x_5}{k_6} - T_2 &= 0.\end{aligned}$$

We can write this system in matricial form:

$$C\begin{pmatrix} x_4 & x_5 & x_4x_5 & x_4x_5^2 & 1\end{pmatrix}^t = 0,$$

where $C \in \mathbb{R}^{2\times 5}$ is the coefficient matrix:

$$C = \begin{pmatrix} 1 & 0 & C_{13} & C_{14} & -T_1\\ 0 & 1 & C_{23} & C_{24} & -T_2\end{pmatrix},$$

and $C_{13} = k_5\left(\frac{1}{k_2} + \frac{1}{k_3}\right)$, $C_{14} = \frac{k_4k_5}{k_3}\left(\frac{1}{k_1} + \frac{1}{k_2}\right)$, $C_{23} = \frac{k_5}{k_6}$ and $C_{24} = \frac{k_4k_5}{k_3k_6}$. If we order the variables (x_4, x_5) the support of this system is:

$$\mathcal{A} = \{(1,0),(0,1),(1,1),(1,2),(0,0)\}.$$

The 2-simplices $\Delta_1 = \{(1,0),(1,1),(0,0)\}$, $\Delta_2 = \{(1,1),(1,2),(0,0)\}$, and $\Delta_3 = \{(0,1),(1,2),(0,0)\}$ form a regular triangulation Γ of $\mathcal{A}$, associated for instance with any height function $h : \mathcal{A} \to \mathbb{R}$ satisfying $h(1,0) = h_1, h(0,1) = h_2$, $h(1,1) = 0$, $h(1,2) = 0$, and $h(0,0) = 0$, with $h_1, h_2 > 0$. We depict this triangulation in Figure 2.4.

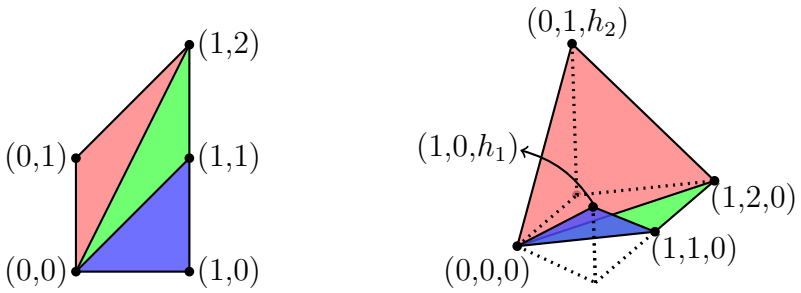


Figure 2.4: A regular triangulation Γ of $\mathcal{A}$.

The simplex Δ_1 is positively decorated by C if and only if

$$T_1\, k_2\, k_3 - T_2\, k_2\, k_6 - T_2\, k_3\, k_6 > 0, \tag{2.2.14}$$

and the simplex Δ_3 is positively decorated by C if and only if

$$T_1\, k_1\, k_2 - T_2\, k_1\, k_6 - T_2\, k_2\, k_6 < 0. \tag{2.2.15}$$

If conditions (2.2.14) and (2.2.15) hold, then the simplex Δ_2 is also positively decorated by C if and only if $k_1 < k_3$. So, the three simplices are positively decorated by C precisely when (2.1.3) holds:

$$k_6\left(\frac{1}{k_2}+\frac{1}{k_3}\right) < \frac{T_1}{T_2} < k_6\left(\frac{1}{k_1}+\frac{1}{k_2}\right).$$

Assume that both inequalities in (2.1.3) hold. In this case, using the height function described above, Theorem 2.2.11 says that there exists $t_0 \in \mathbb{R}_{>0}$ such that for all $0 < t < t_0$, the system

$$\begin{aligned} t^{h_1}x_4 + C_{13}\, x_4x_5 + C_{14}\, x_4x_5^2 - T_1 &= 0,\\ t^{h_2}x_5 + C_{23}\, x_4x_5 + C_{24}\, x_4x_5^2 - T_2 &= 0, \end{aligned} \tag{2.2.16}$$

has at least three positive nondegenerate solutions.

If we make the change of variables: $\bar{x}_4 = t^{h_1}\, x_4$, $\bar{x}_5 = t^{h_2}\, x_5$ we have:

$$\begin{aligned} \bar{x}_4 + t^{-(h_1+h_2)}\, C_{13}\, \bar{x}_4\bar{x}_5 + t^{-(h_1+2h_2)}\, C_{14}\, \bar{x}_4\bar{x}_5^2 - T_1 &= 0,\\ \bar{x}_5 + t^{-(h_1+h_2)}\, C_{23}\, \bar{x}_4\bar{x}_5 + t^{-(h_1+2h_2)}\, C_{24}\, \bar{x}_4\bar{x}_5^2 - T_2 &= 0. \end{aligned} \tag{2.2.17}$$

If we consider the rescalings:

$$\overline{k_4} = t^{-h_2}\, k_4, \qquad \overline{k_5} = t^{-(h_1+h_2)}\, k_5,$$

and we keep fixed the values of the remaining constants k_1, k_2, k_3, k_6 and the total concentrations T_1, T_2, then the steady states of the dynamical system associated with the network with these rate and total conservation constants are the solutions of the polynomial system (2.2.17). And then, for these constants the network has at least three positive steady states. If we take $N_1 = t_0^{-h_2}$ and $N_2 = t_0^{-h_1}$ and we

consider any positive β_4, β_5 satisfying $\beta_4 > N_1$ and $\frac{\beta_5}{\beta_4} > N_2$, there exist $0 < t < t_0$ such that $\beta_4 = t^{-h_1}$ and $\beta_5 = t^{-(h_1+h_2)}$ and we are done.

Another way to finish the proof of Theorem 2.1.1 is using Theorem 2.2.13. The inequalities that define the cone $\mathcal{C}_\Gamma$ are: $\langle m_1, h\rangle > 0$, $\langle m_2, h\rangle > 0$, where $m_1 = (1, 0, -2, 1, 0)$ and $m_2 = (0, 1, 1, -1, -1)$ (they can be computed using Lemma 2.2.2). Fix $\varepsilon \in (0, 1)^2$. As (2.1.3) holds, Theorem 2.2.13 says that there exist $M_1 = M_1(\varepsilon), M_2 = M_2(\varepsilon) > 0$ such that the polynomial system

$$\begin{aligned} \gamma_1\, x_4 + \gamma_3\, C_{13}\, x_4 x_5 + \gamma_4\, C_{14}\, x_4 x_5^2 - \gamma_5\, T_1 = \ 0, \\ \gamma_2\, x_5 + \gamma_3\, C_{23}\, x_4 x_5 + \gamma_4\, C_{24}\, x_4 x_5^2 - \gamma_5\, T_2 = \ 0, \end{aligned} \tag{2.2.18}$$

has at least three nondegenerate positive solutions for any vector $\gamma \in (\mathbb{R}_{>0})^5$ satisfying $\gamma^{m_1} < M_1$ and $\gamma^{m_2} < M_2$. In particular, this holds if we take $\gamma_1 = \gamma_2 = \gamma_5 = 1$, and γ_3 and γ_4 satisfy:

$$\gamma_3^{-2}\gamma_4 < M_1, \qquad \gamma_3\gamma_4^{-1} < M_2. \tag{2.2.19}$$

If we call $\beta_4 = \frac{\gamma_4}{\gamma_5}$, $\beta_5 = \gamma_5$, $N_1 = \frac{1}{M_1}$, $N_2 = \frac{1}{M_2}$, the inequalities in (2.2.19) are equivalent to $\beta_4 > N_1$ and $\frac{\beta_5}{\beta_4} > N_2$. Then, if β_4 and β_5 satisfy these bounds, rescaling of the given parameters k_4, k_5 by $\overline{k_4} = \beta_4\, k_4, \overline{k_5} = \beta_5\, k_5$, gives rise to a multistationary dynamical system, as we claimed. □

2.3 The mixed approach

In this section, we present a similar but different approach to Theorems 2.2.11 and 2.2.13. As the polynomials $f_1, \ldots, f_d$ might have different supports $\mathcal{A}_1, \ldots, \mathcal{A}_d \subset \mathbb{Z}^d$, one usually takes the union of the supports $\mathcal{A} = \cup_{i=1}^d \mathcal{A}_i$. That is, we can write the polynomial system

$$f_i(x) = \sum_{a_j \in \mathcal{A}_i} c_{ij}\, x^{a_j} \in \mathbb{R}[x_1, \ldots, x_d],\ i = 1, \ldots, d \tag{2.3.1}$$

in the form

$$f_i(x) = \sum_{a_j \in \mathcal{A}} c_{ij}\, x^{a_j} \in \mathbb{R}[x_1, \ldots, x_d],\ i = 1, \ldots, d,$$

where $c_{ij} = 0$ in case $a_j \notin \mathcal{A}_i$.

If one considers the approach in Section 2.2, the coefficient matrix C might have many zero minors, which could prevent to find decorated simplices. We now allow different height functions $h^{(i)} : \mathcal{A}_i \to \mathbb{R}$, $i = 1, \ldots, d$. Instead of considering regular subdivisions of $\mathcal{A}$, we will consider *regular mixed subdivisions* of the Minkowski sum $\mathcal{M} = \sum_{i=1}^d \mathcal{A}_i$ defined by height functions $h^{(i)} : \mathcal{A}_i \to \mathbb{R}$, $i = 1, \ldots, d$. The projection of the lifted points in each of the faces of the lower convex hull of the Minkowski sum $\sum_{i=1}^d \mathcal{A}^{h^{(i)}}$ of the lifted point sets $\mathcal{A}^{h^{(i)}} \subset \mathbb{R}^{d+1}$ defines the associated regular mixed subdivision S_h of $\mathcal{M}$. The convex hull of the cells in S_h do not intersect or the intersection is a common face.

Regular mixed subdivisions of $\mathcal{M}$ are in bijection with regular subdivisions of the associated *Cayley configuration* $C(\mathcal{A}_1, \ldots, \mathcal{A}_d)$. This is the lattice configuration in $\mathbb{Z}^d \times \mathbb{Z}^d$ defined by

$$C(\mathcal{A}_1, \ldots, \mathcal{A}_d) = (\mathcal{A}_1 \times \{e_1\}) \cup \cdots (\mathcal{A}_{d-1} \times \{e_{d-1}\}) \cup (\mathcal{A}_d \times \{e_d\}), \tag{2.3.2}$$

where $e_1, \ldots, e_d$ denotes the canonical basis in $\mathbb{Z}^d$. This is the support of the Cayley polynomial:

$$F(x, y) = \sum_{i=1}^{d} y_i f_i(x),$$

in variables $(x_1, \ldots, x_d, y_1, \ldots, y_d)$, associated with polynomials $f_i(x)$ with support in $\mathcal{A}_i$, $i = 1, \ldots, d$.

Note that the sum of the last d coordinates of any point in $C(\mathcal{A}_1, \ldots, \mathcal{A}_d)$ equals 1, so the maximal dimension of a simplex in the Cayley configuration is $2d-1$ and then this simplex consists of $2d$ points. We will assume that $C(\mathcal{A}_1, \ldots, \mathcal{A}_d)$ contains a $(2d-1)$-simplex.

A tuple of height functions $(h^{(1)}, \ldots, h^{(d)})$ as above can be identified with a height function $h : C(\mathcal{A}_1, \ldots, \mathcal{A}_d) \to \mathbb{R}$, defining $h(a_j, e_i) = h^{(i)}(a_j), i = 1, \ldots, d$. In case Δ is a $(2d-1)$-simplex in the associated regular subdivision Γ_h of $C(\mathcal{A}_1, \ldots, \mathcal{A}_d)$, necessarily Δ contains at least one point (a_j, e_i) in each $\mathcal{A}_i$. The corresponding maximal cell in the associated regular subdivision S_h of $\mathcal{M}$ consists of all points of the form $b_1 + \cdots + b_d$ with (b_i, e_i) in Δ. For more details about the translation between regular subdivisions of $C(\mathcal{A}_1, \ldots, \mathcal{A}_d)$ and regular mixed subdivisions of $\mathcal{M}$, we refer to Section 9.2 in [24]. We show this correspondence in Example 2.3.4 below.

We introduce the concept of positively decorated mixed simplex, which is related to Definition 2.2.10. The connection between these definitions is that in both cases the system obtained by considering only the monomials in a positively decorated simplex, has exactly one nondegenerate positive solution.

Definition 2.3.1. *A $(2d-1)$-simplex Δ in the Cayley configuration $C(\mathcal{A}_1, \ldots, \mathcal{A}_d)$ is said to be mixed if it consists of two points $(a_{j_1}, e_i), (a_{j_2}, e_i)$ for each $i = 1, \ldots, d$, with $a_{j_1}, a_{j_2} \in \mathcal{A}_i$. A mixed simplex Δ is said to be positively decorated by C if for each $i = 1, \ldots, d$, the coefficients of the polynomial f_i as in (2.3.1) corresponding to the monomials a_{j_1} and a_{j_2} have opposite signs, that is, if $c_{ij_1} c_{ij_2} < 0$.*

Let Γ be a regular subdivision of the Cayley configuration $C(\mathcal{A}_1, \ldots, \mathcal{A}_d)$. Let h be a height vector that induces Γ and denote by $h^{(1)}, \ldots, h^{(d)}$ the real vectors of size equal to the cardinality of $\mathcal{A}_i$, such that $h^{(i)}(a_j) = h(a_j, e_i)$, for $i = 1, \ldots, d$, and $a_j \in \mathcal{A}_i$. Consider the family of polynomial systems parametrized by a positive real number t:

$$f_{i,t}(x) = \sum_{a_j \in \mathcal{A}_i} c_{ij}\, t^{h^{(i)}(a_j)}\, x^{a_j} \in \mathbb{R}[x_1, \ldots, x_d],\ i = 1, \ldots, d,\ t > 0. \tag{2.3.3}$$

We then have:

Theorem 2.3.2. *Let $\mathcal{A}_1, \ldots, \mathcal{A}_d$ be finite sets in $\mathbb{Z}^d$. Assume there are p mixed $(2d-1)$-simplices $\Delta_1, \ldots, \Delta_p$ which occur in a regular subdivision Γ of $C(\mathcal{A}_1, \ldots, \mathcal{A}_d)$ and which are positively decorated by a matrix $C \in \mathbb{R}^{d\times n}$. Let h be a height function inducing Γ and $h^{(i)}, i = 1, \ldots, d$, defined as before. Then, there exists $t_0 \in \mathbb{R}_{>0}$ such that for all $0 < t < t_0$, the number of (nondegenerate) solutions of (2.3.3) contained in the positive orthant is at least p. In particular, if there are two mixed $(2d-1)$-simplices of $C(\mathcal{A}_1, \ldots, \mathcal{A}_d)$ sharing a facet and which are both positively decorated by C, there exists $t_0 \in \mathbb{R}_{>0}$ such that for all $0 < t < t_0$ the number of positive solutions of (2.3.3) is at least two.*

Proof. Let Δ be a mixed $(2d-1)$-simplex in Γ. Then it consists of $2d$ points: two points $(a_{j_1}, e_i), (a_{j_2}, e_i)$ for each $i = 1, \ldots, d$, with $a_{j_1}, a_{j_2} \in \mathcal{A}_i$. Consider the system (2.3.1) restricted to the binomials with exponents a_{j_1}, a_{j_2} in each f_i. When Δ is positively decorated by C, we get a binomial system of equations equal to zero with coefficients of opposite signs:

$$c_{ij_1}\, x^{a_{j_1}} + c_{ij_2}\, x^{a_{j_2}} \;=\; 0, \quad i = 1, \ldots, d.$$

The positive solutions of this binomial system are in correspondence with the solutions of a system of a form:

$$x^M = \beta,$$

where $M \in \mathbb{R}^{d\times d}$ is the matrix with i-th row equal to $a_{j_1} - a_{j_2}$ and $\beta_i = -\frac{c_{ij_2}}{c_{ij_1}} \in \mathbb{R}_{>0}$, for each $i = 1, \ldots, d$. Taking logarithms, we obtain the equivalent linear system:

$$M^t \log(x) = \log(\beta), \tag{2.3.4}$$

where $\log(x) = (\log(x_1), \ldots, \log(x_d))$. As Δ is a maximal dimensional simplex, the matrix M is invertible. Then, the linear system (2.3.4) has a solution, and thus the binomial system has a positive solution. Therefore, for each positively decorated simplex Δ with vertices $(a_{j_1}, e_i), (a_{j_2}, e_i)$, for each i, system (2.3.1) restricted to the monomials with exponents a_{j_1},a_{j_2} in each f_i has a solution in $\mathbb{R}^d_{>0}$. The rest of the proof follows from the arguments in the proof of Theorem 2.2.11. □

The following result is an adaptation to the mixed setting of Theorem 2.2.13. Again, instead of working with particular height functions, we use the cone of all height functions inducing a regular subdivision of the associated Cayley configuration containing a given set of p positively decorated simplices. This allows us to describe open conditions to scale the coefficients in order to ensure at least p positive solutions of the system.

Theorem 2.3.3. *Let $\mathcal{A}_1, \ldots, \mathcal{A}_d$ be finite sets in $\mathbb{Z}^d$. Assume there exist p mixed $(2d-1)$-simplices $\Delta_1, \ldots, \Delta_p$ in $C(\mathcal{A}_1, \ldots, \mathcal{A}_d)$, which are part of a regular subdivision (for instance, when $p = 2$ and the two simplices share a facet) and are positively decorated by C. Set $N = |\mathcal{A}_1| + \ldots |\mathcal{A}_d|$. Assume that the cone $\mathcal{C}_{\Delta_1,\ldots,\Delta_p}$ of all height vectors h inducing regular subdivisions of $C(\mathcal{A}_1, \ldots, \mathcal{A}_d)$ containing $\Delta_1, \ldots, \Delta_p$ is defined by*

$$\mathcal{C}_{\Delta_1,\ldots,\Delta_p} \;=\; \{h \in \mathbb{R}^N \,:\, \langle m_r, h\rangle > 0,\; r = 1, \ldots, \ell\}, \tag{2.3.5}$$

where $m_r = (m_{r,1}, \ldots, m_{r,N}) \in \mathbb{R}^N$.

Then, for any $\varepsilon \in (0,1)^\ell$ *there exists* $t_0(\varepsilon) > 0$ *such that for any* γ *in the set*

$$U = \cup_{\varepsilon \in (0,1)^\ell} \{\gamma = (\gamma^1, \ldots, \gamma^d) \in \mathbb{R}^N_{>0} : \gamma^{m_r} \leq t_0(\varepsilon)^{\varepsilon_r},\ r = 1 \ldots, \ell\},$$

the system

$$\sum_{a_j \in \mathcal{A}_i} c_{ij} \gamma^i_j \, x^{a_j} = 0, \quad i = 1, \ldots, d,$$

has at least p *nondegenerate solutions in the positive orthant, where* γ^i *is a vector of size* $|\mathcal{A}_i|$ *with coordinates* γ^i_j*, with* $a_j \in \mathcal{A}_i$.

As we said, Theorem 2.3.3 is an adaptation of Theorem 2.2.13, with a similar proof (using Theorem 2.3.2 instead of Theorem 2.2.11), but with heavier notation, so we omit the proof.

We now present an application of the mixed approach in Theorem 2.3.3 to the previous example of the two component system with Hybrid Histidine Kinase (2.1.1).

Example 2.3.4. Recall that we are looking for positive solutions of the system:

$$C \begin{pmatrix} x_4 & x_5 & x_4x_5 & x_4x_5^2 & 1 \end{pmatrix}^t = 0,$$

where $C \in \mathbb{R}^{2\times 5}$ is the coefficient matrix:

$$C = \begin{pmatrix} 1 & 0 & C_{13} & C_{14} & -T_1 \\ 0 & 1 & C_{23} & C_{24} & -T_2 \end{pmatrix},$$

with $C_{13} = k_5\left(\frac{1}{k_2} + \frac{1}{k_3}\right)$, $C_{14} = \frac{k_4k_5}{k_3}\left(\frac{1}{k_1} + \frac{1}{k_2}\right)$, $C_{23} = \frac{k_5}{k_6}$ and $C_{24} = \frac{k_4k_5}{k_3k_6}$.

The support of the first polynomial is $\mathcal{A}_1 = \{(1,0),(1,1),(1,2),(0,0)\}$ and the support of the second polynomial is $\mathcal{A}_2 = \{(0,1),(1,1),(1,2),(0,0)\}$. We want to find mixed positively decorated mixed 3-simplices of the Cayley configuration $C(\mathcal{A}_1, \mathcal{A}_2)$ occuring in a regular subdivision. As we mentioned, these mixed 3-simplices correspond to maximal dimension 2 mixed cells of the associated mixed subdivision of the Minkowski sum $\mathcal{A}_1 + \mathcal{A}_2$ (see Figure 2.5).

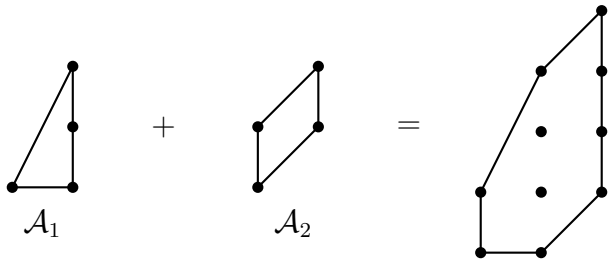


Figure 2.5: The Minkowski sum $\mathcal{M} = \mathcal{A}_1 + \mathcal{A}_2$.

We can choose the following mixed 3-simplices with vertices in $C(\mathcal{A}_1, \mathcal{A}_2)$:

$$\Delta_1 = \{(0,0,e_1),(1,2,e_1),(0,0,e_2),(0,1,e_2)\},$$

$$\Delta_2 = \{(0,0,e_1),(1,2,e_1),(0,0,e_2),(1,1,e_2)\},$$
$$\Delta_3 = \{(0,0,e_1),(1,0,e_1),(0,0,e_2),(1,1,e_2)\},$$

which are positively decorated by C. The simplices Δ_1, Δ_2, and Δ_3 are in correspondence, respectively, with the mixed cells $\sigma_1 = \{(0,0) = (0,0)+(0,0), (1,2) = (1,2)+(0,0), (0,1) = (0,0)+(0,1), (1,3) = (1,2)+(0,1)\}$, $\sigma_2 = \{(0,0),(1,2),(1,1),(2,3)\}$, and $\sigma_3 = \{(0,0),(1,0),(1,1),(2,1)\}$, depicted in Figure 2.6.

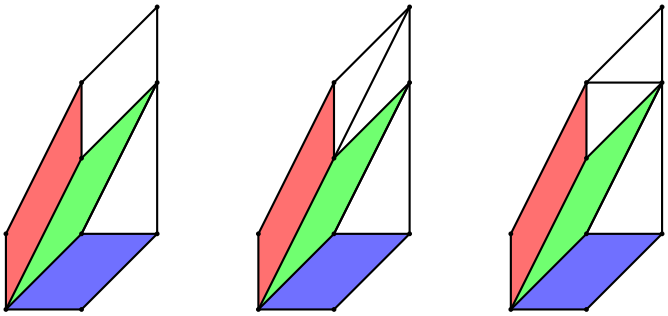


Figure 2.6: Three regular mixed subdivisions of $\mathcal{M}$ that contain the mixed cells σ_1, σ_2, and σ_3.

The cone $\mathcal{C}_{\Delta_1,\Delta_2,\Delta_3}$ of height vectors $h = (h_1,\ldots,h_8) \in \mathbb{R}^8$ inducing regular subdivisions of $C(\mathcal{A}_1,\mathcal{A}_2)$ containing Δ_1, Δ_2, and Δ_3 is defined by the inequalities $\langle m_i, h\rangle > 0$, $i = 1,\ldots,8$, where

$$m_1 = (1,0,-1,0,2,0,0,-2),\ m_2 = (0,1,-1,0,1,0,0,-1),\ m_3 = (0,0,-1,1,0,0,1,-1),$$
$$m_4 = (0,0,-1,1,1,1,0,-2),\ m_5 = (1,0,1,-2,0,-2,0,2),\ m_6 = (0,1,0,-1,0,-1,0,1),$$
$$m_7 = (1,0,0,-1,1,-1,0,0),\ m_8 = (1,0,0,-1,0,-2,1,1),$$

with $h_1 = h(1,0,e_1)$, $h_2 = h(1,1,e_1)$, $h_3 = h(1,2,e_1)$, $h_4 = h(0,0,e_1)$, $h_5 = h(0,1,e_2)$, $h_6 = h(1,1,e_2)$, $h_7 = h(1,2,e_2)$, and $h_8 = h(0,0,e_2)$.

Fix $\varepsilon \in (0,1)^8$. Theorem 2.3.3 says that there exist positive constants $M_i = M_i(\varepsilon)$, $i = 1,\ldots,8$ such that the number of positive nondegenerate solutions of the polynomial system

$$\begin{aligned} \gamma_1^1\, x_4 + \gamma_2^1\, C_{13}\, x_4 x_5 + \gamma_3^1\, C_{14}\, x_4 x_5^2 - \gamma_4^1\, T_1 = 0,\\ \gamma_1^2\, x_5 + \gamma_2^2\, C_{23}\, x_4 x_5 + \gamma_3^2\, C_{24}\, x_4 x_5^2 - \gamma_4^2\, T_2 = 0, \end{aligned} \tag{2.3.6}$$

is at least the number of mixed positively decorated simplices, in this case 3, for any vector $\gamma = (\gamma_1^1,\gamma_2^1,\gamma_3^1,\gamma_4^1,\gamma_1^2,\gamma_2^2,\gamma_3^2,\gamma_4^2) \in \mathbb{R}^8_{>0}$ that satisfies $\gamma^{m_i} < M_i$, for each $i = 1,\ldots,8$.

In particular we have the following result:

Proposition 2.3.5. *Given positive reactions constants $k_1,\ldots,k_6$ and positive total conservations constants T_1 and T_2, there exist positive constants N_1, N_2, N_3, and N_4 such that for any $\beta_1,\beta_2 > 0$ satisfying*

$$N_1 < \beta_1, \quad N_2 < \beta_2, \quad \frac{\beta_2}{\beta_1} < N_3, \quad \frac{\beta_1}{(\beta_2)^2} < N_4,$$

the dynamical system corresponding to the Hybrid Histidine Kinase network (2.1.1) has at least 3 *positive steady states, after replacing* k_1 *by* $\bar{k}_1 = (\beta_1(\frac{1}{k_1+k_2}) - \frac{1}{k_2})^{-1}$ *and rescaling* $\bar{k}_6 = (\beta_2)^{-1} k_6$*, without altering the value of the other reaction and total conservation constants.*

Proof. Take any positive vector $\gamma = (\gamma_1^1, \gamma_2^1, \gamma_3^1, \gamma_4^1, \gamma_1^2, \gamma_2^2, \gamma_3^2, \gamma_4^2)$ satisfying $\gamma_1^1 = \gamma_2^1 = \gamma_4^1, = \gamma_1^2 = \gamma_4^2 = 1$, and $\gamma_2^2 = \gamma_3^2$. Call $\beta_1 = \gamma_3^1$ and $\beta_2 = \gamma_2^2$. Then if β_1, β_2 satisfy

$$N_1 < \beta_1, \quad N_2 < \beta_2, \quad \frac{\beta_2}{\beta_1} < N_3, \quad \frac{\beta_1}{(\beta_2)^2} < N_4,$$

where $N_1 = (\min\{M_1, M_2, \frac{k_2}{k_2+k_1}\})^{-1}$, $N_2 = (\min\{M_6, M_7, M_8\})^{-1}$, $N_3 = \min\{M_3, M_4\}$, and $N_4 = M_5$, the system

$$\begin{aligned} x_4 + C_{13}\, x_4 x_5 + \beta_1\, C_{14}\, x_4 x_5^2 - T_1 = \ & 0, \\ x_5 + \beta_2\, C_{23}\, x_4 x_5 + \beta_2\, C_{24}\, x_4 x_5^2 - T_2 = \ & 0, \end{aligned} \tag{2.3.7}$$

has at least 3 positive solutions. Returning to the original constants, if we keep fixed k_2, k_3, k_4, k_5, T_1, T_2 and we replace k_1, k_6 by $\bar{k}_1 = (\beta_1(\frac{1}{k_1+k_2}) - \frac{1}{k_2})^{-1}$, and $\bar{k}_6 = (\beta_2)^{-1} k_6$, the positive steady states arising from the network with these constants are the positive solutions of the polynomial system (2.3.7), and so it is multistationary because there are at least 3 positive steady states in a fixed stoichiometric compatibility class. Observe that k_1 is positive because of the choice of N_1. □

Notice that if the value of β_1 in the proof of Proposition 2.3.5 is large enough, $\bar{k}_1$ is smaller than k_3, the necessary and sufficient condition to guarantee multistationarity that appears in [12].

2.4 Application to n-site phosphorylation systems

In this section, we apply our results to the distributive n-site phosphorylation network (1.2.2) presented in Section 1.3. Recall that the reaction mechanism for the sequential distributive n-site phosphorylation network is given by the following digraph:

$$S_0 + E \underset{k_{\mathrm{off}_0}}{\overset{k_{\mathrm{on}_0}}{\rightleftarrows}} ES_0 \overset{k_{\mathrm{cat}_0}}{\to} S_1 + E \ \cdots \to S_{n-1} + E \underset{k_{\mathrm{off}_{n-1}}}{\overset{k_{\mathrm{on}_{n-1}}}{\rightleftarrows}} ES_{n-1} \overset{k_{\mathrm{cat}_{n-1}}}{\to} S_n + E$$

$$S_n + F \underset{\ell_{\mathrm{off}_{n-1}}}{\overset{\ell_{\mathrm{on}_{n-1}}}{\rightleftarrows}} FS_n \overset{\ell_{\mathrm{cat}_{n-1}}}{\to} S_{n-1} + F \ \cdots \to S_1 + F \underset{\ell_{\mathrm{off}_0}}{\overset{\ell_{\mathrm{on}_0}}{\rightleftarrows}} FS_1 \overset{\ell_{\mathrm{cat}_0}}{\to} S_0 + F$$

Also, recall that we denote by $s_0, \ldots, s_n$, e, f, $y_0, \ldots, y_{n-1}$, $u_0, \ldots, u_{n-1}$ the concentration of the species $S_0, \ldots, S_n$, E, F, $ES_0, \ldots, ES_{n-1}$, $FS_1, \ldots, FS_n$ respectively. The associated dynamical system that arises under mass-action kinetics is equal to (1.2.3).

In Section 1.3, we also see that there are three linearly independent conservation laws for any value of n:

$$\sum_{i=0}^{n} s_i + \sum_{i=0}^{n-1} y_i + \sum_{i=0}^{n-1} u_i = S_{tot}, \quad e + \sum_{i=0}^{n-1} y_i = E_{tot}, \quad f + \sum_{i=0}^{n-1} u_i = F_{tot}, \tag{2.4.1}$$

where the total amounts $S_{tot}, E_{tot}, F_{tot}$ are positive for any trajectory of the dynamical system starting in the positive orthant. It is straightforward to see from the differential equations (1.2.3) that the concentrations of the intermediates species at steady state satisfy the following binomial equations:

$$y_i - K_i\, e s_i = 0,\ i = 0, \ldots, n-1, \qquad u_i - L_i\, f s_{i+1} = 0,\ i = 0, \ldots, n-1, \tag{2.4.2}$$

where $K_i = \frac{k_{\mathrm{on_i}}}{k_{\mathrm{off_i}} + k_{\mathrm{cat_i}}}$ and $L_i = \frac{\ell_{\mathrm{on_i}}}{\ell_{\mathrm{off_i}} + \ell_{\mathrm{cat_i}}}$ for each $i = 0, \ldots, n-1$ (K_i^{-1} and L_i^{-1} are usually called *Michaelis-Menten constants*, $i = 0, \ldots, n-1$).

Sequential phosphorylation mechanisms are an example of s-toric MESSI networks, defined in [86] and presented in Section 1.4 of Chapter 1. In particular, by Theorem 4.8 in [86] we can find the following binomial equations that describe the steady states. The whole steady state variety can be cut out in the positive orthant by adding to the binomials in (2.4.2), the binomial equations:

$$\tau_i s_i e - \nu_i s_{i+1} f = 0,$$

where $\tau_i = k_{\mathrm{cat_i}} K_i$ and $\nu_i = \ell_{\mathrm{cat_i}} L_i$, for each $i = 0, \ldots, n-1$. Using these binomial equations, we can parametrize the positive steady states by monomials. For instance, we can write the concentration at steady state of all species in terms of the species s_0, e, f:

$$\begin{array}{rcrl} s_i & = & T_{i-1}\, \frac{s_0 e^i}{f^i}, & i = 1, \ldots, n, \\ y_i & = & K_i\, T_{i-1}\, \frac{s_0 e^{i+1}}{f^i}, & i = 0, \ldots, n-1, \\ u_i & = & L_i\, T_i\, \frac{s_0 e^{i+1}}{f^i}, & i = 0, \ldots, n-1, \end{array} \tag{2.4.3}$$

where $T_i = \prod_{j=0}^{i} \frac{\tau_j}{\nu_j}$ for $i = 0, \ldots, n-1$, and $T_{-1} = 1$.

We will use this parametrization in order to apply Theorems 2.2.11 and 2.2.13 to the sequential phosphorylation mechanisms for any n:

Theorem 2.4.1. *With the previous notation, assume*

$$S_{tot} > F_{tot}. \tag{2.4.4}$$

Then, there is a choice of rate constants for which the distributive n-site phosphorylation system is multistationary. More explicitly, for any choice of positive real numbers $k_{\mathrm{cat_1}}, \ell_{\mathrm{cat_1}}$ satisfying

$$\frac{k_{\mathrm{cat_1}}}{\ell_{\mathrm{cat_1}}} > \max\left\{ \frac{F_{tot}}{S_{tot} - F_{tot}}, \frac{F_{tot}}{E_{tot}} \right\}, \tag{2.4.5}$$

fix any value of the remaining rate constants and positive numbers h_i, for $i = 4, \ldots, 2n+3$ such that $i\, h_{n+5} < h_{i+3}$ for $i = 1, \ldots, n$, and $(i-1)\, h_{n+5} < h_{n+i+3}$ for

$i = 1, 3, \ldots, n$. *Then, there exists* $t_0 > 0$ *such that for any value of* $t \in (0, t_0)$ *the system is multistationary after the rescalings* $t^{h_{n+4}}\, k_{\mathrm{on}_0}$, $t^{h_{n+4+i}-h_{i+3}}\, k_{\mathrm{on}_i}, i = 1, \ldots, n-1$, $t^{h_{n+4+i}-h_{i+4}}\, \ell_{\mathrm{on}_i}, i = 0, \ldots, n-1$.

Similarly, for any fixed choice of reaction rate constants and total conservation constants satysfying (2.4.4) and (2.4.5), there exist positive constants M_i, $i = 1, \ldots, 4n-2$ *such that for any positive values of* γ_i, $i = 1, \ldots, 2n$ *verifying*

$$\gamma_i < M_i, i = 1, \ldots, 2n, \qquad \frac{\gamma_i}{\gamma_{n+2}^i} < M_{2n+i}, i = 1, \ldots, n, \tag{2.4.6}$$
$$\frac{\gamma_{n+i}}{\gamma_{n+2}^{i-1}} < M_{3n-2+i}, i = 3, \ldots, n,$$

the rescaling of the given parameters k_{on_1}, k_{on_i}, $i = 2, \ldots, n-1$, ℓ_{on_i}, $i = 1, \ldots, n-1$ *by*

$$\gamma_{n+1}\, k_{on_1},\ \frac{\gamma_{n+1+i}}{\gamma_i}\, k_{on_i}, i = 2, \ldots, n-1,\ \frac{\gamma_{n+1+i}}{\gamma_{i+1}}\, \ell_{on_i}, i = 1, \ldots, n-1, \tag{2.4.7}$$

respectively, gives rise to a multistationary system.

Proof. Previously in this section, we showed that we can write the concentration at steady state of all species in terms of the species (s_0, e, f), as in (2.4.3). We substitute this monomial parametrization of the steady states into the linear conservation relations (2.4.1). We have a system of three equations and we write it in matricial form:

$$C \begin{pmatrix} s_0 & e & f & s_0 e f^{-1} & \ldots & s_0 e^n f^{-n} & s_0 e f^0 & \ldots & s_0 e^n f^{-(n-1)} & 1 \end{pmatrix}^t = 0,$$

where the matrix $C \in \mathbb{R}^{3\times(2n+4)}$ is the matrix of coefficients:

$$C = \begin{pmatrix} 1 & 0 & 0 & T_0 & \ldots & T_{n-1} & K_0 + L_0 T_0 & \ldots & K_{n-1}T_{n-2} + L_{n-1}T_{n-1} & -S_{tot} \\ 0 & 1 & 0 & 0 & \ldots & 0 & K_0 & \ldots & K_{n-1}T_{n-2} & -E_{tot} \\ 0 & 0 & 1 & 0 & \ldots & 0 & L_0 T_0 & \ldots & L_{n-1}T_{n-1} & -F_{tot} \end{pmatrix}.$$

If we order the variables in this way: s_0, e, f, the support of the system is:

$$\mathcal{A} = \{(1,0,0), (0,1,0), (0,0,1), (1,1,-1), (1,2,-2), \ldots, (1,n,-n), \\ (1,1,0), (1,2,-1), \ldots, (1,n,-(n-1)), (0,0,0)\}.$$

We want to find two positively decorated 3-simplices with vertices in $\mathcal{A}$ which share a facet. For example we take the simplices

$$\Delta_1 = \{(1,0,0), (0,1,0), (0,0,1), (0,0,0)\},$$
$$\Delta_2 = \{(1,0,0), (0,1,0), (1,2,-1), (0,0,0)\}.$$

They are shown in Figure 2.7, made with `Polymake` [52], which is a very useful tool to visualize and to do computations with polytopes and triangulations.

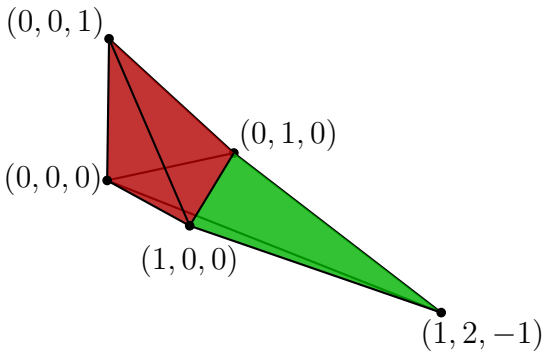


Figure 2.7: The simplices Δ_1 and Δ_2.

The simplex Δ_1 is automatically positively decorated by C. The simplex Δ_2 is positively decorated by C if and only if:

$$E_{tot} - \frac{K_1 T_0 F_{tot}}{L_1 T_1} > 0, \quad \text{and } S_{tot} - \frac{(K_1 T_0 + L_1 T_1) F_{tot}}{L_1 T_1} > 0.$$

Getting back to the original constants, we can write the previous conditions in the following form:

$$\begin{aligned} S_{tot} &> F_{tot}, \\ \frac{k_{\text{cat}_1}}{\ell_{\text{cat}_1}} &> \max\left\{ \frac{F_{tot}}{S_{tot} - F_{tot}}, \frac{F_{tot}}{E_{tot}} \right\}. \end{aligned} \tag{2.4.8}$$

Suppose that conditions (2.4.8) hold. Then the simplices Δ_1 and Δ_2 are positively decorated. Proposition 2.2.6 says that exists a regular triangulation of Γ of the convex hull of $\mathcal{A}$, such that the two simplices Δ_1 and Δ_2 are part of that triangulation. Given any height function h inducing such a Γ, by Theorem 2.2.11 there exists $t_0 \in \mathbb{R}_{>0}$ such that for all $0 < t < t_0$, the number of positive nondegenerate solutions of the scaled system:

$$\begin{aligned} t^{h_1} s_0 + \sum_{i=1}^{n} T_{i-1} t^{h_{i+3}} \frac{s_0 e^i}{f^i} + \sum_{i=0}^{n-1} (K_i T_{i-1} + L_i T_i) t^{h_{n+4+i}} \frac{s_0 e^{i+1}}{f^i} - S_{tot} t^{h_{2n+4}} &= 0, \\ t^{h_2} e + \sum_{i=0}^{n-1} K_i T_{i-1} t^{h_{n+4+i}} \frac{s_0 e^{i+1}}{f^i} - E_{tot} t^{h_{2n+4}} &= 0, \\ t^{h_3} f + \sum_{i=0}^{n-1} L_i T_i t^{h_{n+4+i}} \frac{s_0 e^{i+1}}{f^i} - F_{tot} t^{h_{2n+4}} &= 0, \end{aligned} \tag{2.4.9}$$

is at least two, where $h_1 = h(1,0,0)$, $h_2 = h(0,1,0)$, $h_3 = h(0,0,1)$, $h_{i+3} = h(1,i,-i)$, for $i = 1,\ldots,n$, $h_{n+3+i} = h(1,i,-(i-1))$, for $i = 1,\ldots,n$, and $h_{2n+4} = h(0,0,0)$.

We can suppose without loss of generality that $h_1 = h_2 = h_3 = h_{2n+4} = 0$, and $h(1,2,-1) = h_{n+5} > 0$. Let φ_1 and φ_2 be the affine linear functions $\varphi_1(x,y,z) = 0$ and $\varphi_2(x,y,z) = -h_{n+5}\, z$ which agree with h on the simplices Δ_1 and Δ_2 respectively. Then,

$$\begin{array}{rcccccll} 0 & < & h_{i+3}, & \varphi_2(1,i,-i) & = & h_{n+5}\, i & < & h_{i+3} \quad \text{for } i=1,\dots,n, \\ 0 & < & h_{n+3+i}, & \varphi_2(1,i,-(i-1)) & = & h_{n+5}(i-1) & < & h_{n+3+i} \quad \text{for } i=1,3\dots,n,\ i\neq 2. \end{array}$$

Any such choice defines a regular subdivision containing both simplices (and if the heights are generic the subdivision is a regular triangulation).

If we rescale the following constants:

$$\begin{aligned} & t^{h_{n+4}}\, K_0, \quad t^{h_{n+4+i}-h_{i+3}}\, K_i,\ i=1,\dots,n-1, \\ & t^{h_{n+4+i}-h_{i+4}}\, L_i,\ i=0,\dots,n-1, \end{aligned} \tag{2.4.10}$$

and we keep fixed the values of the constants k_{cat_1} and ℓ_{cat_1} and the total values E_{tot}, F_{tot}, and S_{tot} (such that (2.4.8) holds), the dynamical system obtained from the network with these constants is the system (2.4.9). And then, for these constants the network has at least two positive steady states. Moreover, it is straightforward to check that it is enough to rescale the following original constants as indicated in the statement:

$$\begin{aligned} & t^{h_{n+4}}\, k_{\mathrm{on}_0}, \quad t^{h_{n+4+i}-h_{i+3}}\, k_{\mathrm{on}_i},\ i=1,\dots,n-1, \\ & t^{h_{n+4+i}-h_{i+4}}\, \ell_{\mathrm{on}_i},\ i=0,\dots,n-1, \end{aligned} \tag{2.4.11}$$

to get the equalities (2.4.10).

The last part of the statement follows with similar arguments via Proposition 2.2.12 and Theorem 2.2.13. □

Remark 2.4.2. Using a parametrization of the concentrations of the species at steady state in terms of other variables (or with another choice of the simplices) we can obtain other regions in the parameters space that guarantee multistationarity .

2.5 Results for MESSI Systems

In order to apply our method in Section 2.2 to determine a multistationarity region for the network (2.1.1) or in Section 2.4 for the distributive multisite phosphorylation systems, we proposed and replaced a rational parametrization of the steady state variety into a basis of the conservation relations, and we then had to rescale some of original parameters at the end of the procedure (as in (2.4.11), (2.4.7)). Our main result in this section is Theorem 2.5.2, which guarantees that the rescaling of the parameters can be done for any s-toric MESSI system, together with Proposition 2.5.1 which ensures and describes the existence of a rational parametrization of the steady state variety.

2.5.1 Existence of rescalings

The following proposition summarizes some results of [86] and describes the conservation laws as well as the existence of a positive parametrization of the positive

steady states. By a positive parametrization of the variety V of positive steady states we mean a $\mathcal{C}^1$ and bijective function

$$\phi\colon \mathbb{R}^m_{>0} \to V \cap \mathbb{R}^s_{>0},$$

$$\bar{x} = (\bar{x}_1, \ldots, \bar{x}_m) \mapsto (\phi_1(\bar{x}), \ldots, \phi_s(\bar{x})),$$

for some $m < s$. Proposition 2.5.1 also features the form of the system when we replace the concentration at steady state of the species by its parametrization into the conservation laws, which is our procedure when we apply our results to the question of determining regions of multistationarity of biochemical reaction networks.

Proposition 2.5.1. *Let G be the underlying digraph of a MESSI system with fixed reaction rate constants κ. Consider a minimal partition of the set of species as in* (1.4.1) *and the associated digraphs G_2 and G_E defined in Section 1.4 of Chapter 1. Suppose that the system is s-toric, G_E has no directed cycles and assume that any pair of nodes in the same connected component of G_2 are connected by a unique simple path.*[1]

Choose m species $X_{i_1}, \ldots, X_{i_m}$, such that $X_{i_\alpha} \in \mathscr{S}^{(\alpha)}$ for $\alpha = 1, \ldots, m$. Then, there exists an explicit basis of m conservation laws with coefficients $0, 1$ and a positive monomial parametrization of the positive steady states in terms of the m concentration variables $x_{i_1}, \ldots, x_{i_m}$. Moreover, if we replace the concentrations of the species by its parametrization in these conservations laws we obtain a system of the form:

$$\ell_\alpha(x, \kappa) := \sum_{j=1}^{n} \varphi_{\alpha,j}(\kappa) x^{a_j} = T_\alpha, \quad \alpha = 1, \ldots, m, \tag{2.5.1}$$

where $x = (x_{i_1}, \ldots, x_{i_m})$, with $a_j \in \mathbb{Z}^m - \{0\}$ for each $j = 1, \ldots, n$, for some constants T_α which are positive for any trajectory that starts in the positive orthant for each $\alpha = 1, \ldots, m$. Here $\varphi_{\alpha,j}(\kappa)$ is a positive rational function in the reaction rate constants for each $\alpha = 1, \ldots, m$ and $j = 1, \ldots, n$.

Now, we state the main result of this section, which guarantees that the rescaling of the original parameters κ can always be done in our setting. Its proof can be implemented as an algorithm.

Theorem 2.5.2. *Let G be the underlying digraph of a MESSI system. Consider a minimal partition of the set of species, and the associated digraphs G_2 and G_E defined as before. Suppose that the system is s-toric, G_E has no directed cycles and assume that any pair of nodes in the same connected component of G_2 are connected by a unique simple path. Fix m species $X_{i_1}, \ldots, X_{i_m}$, such that $X_{i_\alpha} \in \mathscr{S}^{(\alpha)}$ for $\alpha = 1, \ldots, m$ and consider the parametrization and the system* (2.5.1) *obtained in Proposition 2.5.1.*

[1] A simple path is a path that visits each vertex exactly once.

Given $\gamma \in \mathbb{R}^{n+1}_{>0}$, reaction rate constants κ and total conservation constants $T_\alpha > 0$, there exists a choice of positive reaction rate constants $\bar{\kappa}$ such that the positive solutions of the system

$$\sum_{j=1}^{n} \gamma_j \varphi_{\alpha,j}(\kappa) x^{a_j} - \gamma_{n+1} T_\alpha = 0, \quad \alpha = 1, \ldots, m, \tag{2.5.2}$$

are in bijection with the positive solutions of

$$\ell_\alpha(x, \bar{\kappa}) - T_\alpha = \sum_{j=1}^{n} \varphi_{\alpha,j}(\bar{\kappa}) x^{a_j} - T_\alpha = 0, \quad \alpha = 1, \ldots, m. \tag{2.5.3}$$

Moreover, the reaction rate constants $\bar{\kappa}$ can be obtained from the original constants κ by scaling only the rate constants of those reactions coming out from a core complex.

The proofs of these two results are given below. First, we show in the network of Example 1.4.2 in Chapter 1, which parameters we rescale in the proof of Theorem 2.5.2.

Example 2.5.3 (Example 1.4.2, continued)**.** It is easy to check that the network of Example 1.4.2 with the MESSI structure defined before is an s-toric MESSI system and satisfies the hypotheses of Theorem 2.5.2. In the proof of this theorem, we show that it is sufficient to rescale the parameters k_1, k_4, and k_7, which are the rate constants of reactions coming out from the core complexes $S_0 + E$, $S_1 + E$, and $S_2 + F$ respectively.

We need to introduce the following sets, defined in the proof of Theorem 3.15 of [86].

Definition 2.5.4. *Let G be the underlying digraph of a MESSI system. Consider a minimal partition of the set of species as in* (1.4.1) *and the associated digraph G_E. We define the following subsets of indices:*

$L_0 = \{\beta \geq 1 : \textit{indegree of } \mathscr{S}^{(\beta)} \textit{ is } 0\}$, *and for* $k \geq 1$:

$$L_k = \{\beta \geq 1 : \textit{for any edge } \mathscr{S}^{(\gamma)} \to \mathscr{S}^{(\beta)} \textit{ in } G_E \textit{ it holds that } \gamma \in L_t, \textit{ with } t < k\} \backslash \bigcup_{t=0}^{k-1} L_t.$$

Example 2.5.5 (Example 1.4.2, continued)**.** The subsets L_k, $k \geq 0$ of Definition 2.5.4 for the network of Example 1.4.2 with the MESSI structure defined before are: $L_0 = \{1, 2\}$ and $L_1 = \{3\}$ (see the corresponding digraph G_E at Figure 1.4).

2.5.2 The proofs

We will need a series of remarks and technical lemmas in order to prove our main result Theorem 2.5.2 that ensures that the general method developed in Section 2.3 can be applied to the determination of regions of multistatinarity for *any* s-toric

MESSI system. We introduce new ideas, but unluckily our results lie heavily on the machinery developed in [86] and they require the reader to consult that paper. We will need some combinatorial definitions that we will recall succintly.

We first give the proof of Proposition 2.5.1.

Proof of Proposition 2.5.1 . Suppose that we have a minimal partition of the set of species as in (1.4.1). We will use the following notation for the intermediate species: $\mathscr{S}^{(0)} = \{U_1, \dots, U_p\}$. With our assumptions, the hypotheses of Theorem 3.2 in [86] are satisfied, then there exist m conservation laws of the form

$$\ell_\alpha(u, \boldsymbol{x}) \,=\, T_\alpha, \text{ where } \ell_\alpha(u, \boldsymbol{x}) = \sum_{X_j \in \mathscr{S}^{(\alpha)}} x_j + \sum_{k \in \mathrm{Int}(\alpha)} u_k, \quad \alpha = 1, \dots, m, \quad (2.5.4)$$

for some constants T_α, which are positive if the trajectory intersects the positive orthant for each $\alpha = 1, \dots, m$, where $\boldsymbol{x}$ and u denote the vector of variables corresponding to the concentration of core species and intermediate species respectively, and where $\mathrm{Int}(\alpha) \subset \{1, \dots, p\}$ is the following set of indices:

$$\mathrm{Int}(\alpha) = \{k \,:\, \exists\, y \to_\circ U_k, \text{ with } y \text{ core complex with one species belonging to } \mathscr{S}^{(\alpha)}\}.$$

Because the system is an s-toric MESSI system, by Proposition 4.7 of [86], we can obtain the concentration of the intermediate species at steady state in terms of the concentrations of the core species. That proposition states that there are (explicit) rational functions $\mu_k(\kappa) \in \mathbb{Q}(\kappa), 1 \leq k \leq p$ (as in (2.5.11)) such that at steady state:

$$u_k(\boldsymbol{x}) \,=\, \mu_k(\kappa)\, \boldsymbol{x}^y, \quad k = 1, \dots, p, \quad (2.5.5)$$

where y is the unique core complex reacting through intermediates to U_k (here we identify the complex y with the corresponding vector in $\mathbb{Z}^s_{\geq 0}$). Note that the expressions $\mu_k(\kappa)$ are the same as in (1.3.3), introduced in Theorem 2 in [42], and that we presented in Section 1.3. Also, as G_2 is weakly reversible and G_E has no direct cycles, we can apply Theorem 4.1 of [86] to obtain a rational parametrization of the concentration of the core species. Consider the subsets L_k, $k \geq 0$ as in Definition 2.5.4. Observe that the set L_0 is not empty because G_E has no direct cycles. Fix $x_{i_\alpha} \in \mathscr{S}^{(\alpha)}$ for each $\alpha = 1, \dots, m$. In the proof of Theorem 4.1 of [86] it is shown that we can then parametrize all the species of $\mathscr{S}^{(\alpha)}$ for $\alpha \in L_k$ in terms of x_{i_α}, species corresponding to core subsets in L_t with $t < k$ and the rate constants τ (defined in (1.3.4)), which are rational functions of the reaction rate constants κ. Under the assumption that any pair of nodes in the same connected component of G_2 is connected by a single simple path, we can show that this parametrization is a positive monomial parametrization, using Theorem 4.8 of [86].

Then, the concentration of a core species at steady state can be written as a monomial in terms of the variables x_{i_α}, for $\alpha = 1, \dots, m$ and the rate constants κ, and using this and (2.5.5), the same holds for any intermediate species. We denote by $\{a_1, \dots, a_n\}$ the different monomials that appear in this monomial parametrization. We replace this parametrization in the conservations laws (2.5.4) and we get a system

as in (2.5.1), where $\varphi_{\alpha,j}(\kappa)$ is the sum of the coefficients in the parametrization of the species that appear in the α-th conservation law and have the monomial x^{a_j}, that is, $\varphi_{\alpha,j}(\kappa)$ is a positive rational function depending on the reaction rate constants κ. □

In order to prove Theorem 2.5.2 we need some lemmas. The following lemma shows how the values of $\tau(\kappa)$ and $\mu_k(\kappa)$ for $k = 1, \ldots, p$, depending on the reaction rate constants κ, are modified if we consider new rate constants $\bar{\kappa}$ obtained from κ after scaling by a positive number all constants in a reaction coming out from a core complex.

Lemma 2.5.6. *Let G be the underlying digraph of an s-toric MESSI system, with reaction rate constants κ, $\mu_k(\kappa)$ as in (2.5.5). Fix $\ell_y \in \mathbb{R}_{>0}$ for each y core complex. Consider the following reaction rate constants $\bar{\kappa}$ obtained from the rate constants κ:*

$$\bar{\kappa}_{yy'} = \begin{cases} \ell_y \kappa_{yy'} & \text{if } y \text{ is a core complex,} \\ \kappa_{yy'} & \text{if } y \text{ is not a core complex.} \end{cases} \tag{2.5.6}$$

That is, we multiply the reactions rate constants coming out from a core complex (we multiply by ℓ_y if the core complex is y) and we keep fixed the other rate constants (the constants coming out from an intermediate complex). Then, for each $k = 1, \ldots, p$ we have

$$\mu_k(\bar{\kappa}) = \ell_y \mu_k(\kappa) \quad \text{if } y \text{ is the unique complex core such that } y \to_\circ U_k. \tag{2.5.7}$$

Consequently, if $y \xrightarrow{\tau} y'$ is in G_1, then

$$\tau(\bar{\kappa}) = \ell_y \tau(\kappa). \tag{2.5.8}$$

Proof. Following the proofs of Proposition 4.7 in [86] and Theorem 2 in [42], we recall how to obtain the constants $\mu_k(\kappa)$ for fixed reaction rate constants κ. They build a new linear labeled directed graph $\widehat{G}$ with node set $\mathscr{S}^{(0)} \cup \{*\}$, which consists of collapsing all core complexes into the vertex $*$, and labeled directed edges that are obtained from hiding the core complexes in the labels. For example, $X_i + X_j \xrightarrow{\kappa} U_k$ becomes $* \xrightarrow{\kappa x_i x_j} U_k$ and $U_k \xrightarrow{\kappa'} X_i + X_j$ becomes $U_k \xrightarrow{\kappa'} *$.

They show, using the Laplacian of a graph and the Matrix-tree Theorem (see [79, 104]), that

$$\mu_k(\kappa) = \rho_k / \rho,$$

for any $k = 1, \ldots, p$, where

$$\rho_k = \sum_{T \text{ an } U_k\text{−tree}} c^T, \quad \rho = \sum_{T \text{ an } *\text{−tree}} c^T.$$

It is easy to check that every $*$-tree involves labels in $\mathbb{Q}[\kappa]$, and only labels from edges coming out from an intermediate complex. As the system is s-toric, for every intermediate complex formed with the intermediate species U_k, there is a unique

core complex y such that $y \to_\circ U_k$. Then, every U_k-tree involves labels in terms of κ and the concentrations of the species that form y. Moreover, as there must be a path from $*$ to U_k in each U_k-tree, then, a label from an edge coming out from y necessarily appears in each tree (and is the unique label from an edge coming out from a core complex). Then, if we consider the constants $\bar{\kappa}$, each label from an edge coming out from y is multiplied by ℓ_y and then $\mu_k(\bar{\kappa}) = \ell_y \mu_k(\kappa)$ if $y \to_\circ U_k$, as wanted. The expression of the constants $\tau(\bar{\kappa})$ follows from (1.3.4). □

In the following lemma we give in more detail the form of the positive parametrization given in Proposition 2.5.1.

Lemma 2.5.7. *With the hypotheses of Theorem 2.5.2, fix $X_{i_1}, \ldots, X_{i_m}$ species as in Proposition 2.5.1, with $X_{i_\alpha} \in \mathscr{S}^{(\alpha)}$, for each $\alpha = 1, \ldots, m$. Take any other species $X_i \in \mathscr{S}^{(\alpha)}$ with $\alpha \in L_k$, $X_i \neq X_{i_\alpha}$, with L_k as in Definition 2.5.4. Then, the concentration at steady state of X_i in terms of $x_{i_1}, \ldots, x_{i_m}$ can be expressed in the form:*

$$x_i = \phi(\tau)\, x_{i_\alpha}\, \underline{x}^a, \tag{2.5.9}$$

for some $\phi(\tau) \in \mathbb{Q}(\tau)$, where $\underline{x}^a$ is a monomial that depends only on variables x_{i_β} with $\beta \in L_t$, with $t < k$. Moreover, $\phi(\tau)$ has the form

$$\phi(\tau) = \left(\prod_{j=1}^{q} \frac{\tau_{j,1}}{\tau_{j,2}} \right) g(\tau') \tag{2.5.10}$$

for some $q \geq 1$, where $g(\tau')$ is a rational function of the constants τ', with τ' the label of edges of connected components of G_2 corresponding to $\mathscr{S}^{(\beta)}$, with $\beta \in L_t$, with $t < k$, and $\tau_{j,1}, \tau_{j,2}$ label of edges of the connected component of G_2 corresponding to $\mathscr{S}^{(\alpha)}$, for each $j = 1, \ldots, q$.

Proof. We have that any pair of nodes in the component of G_2 corresponding to $\mathscr{S}^{(\alpha)}$ are connected by a unique simple path. Then, two different (simple) cycles can only share a node in common (if there are two nodes in common, there will be more than a single path connecting one of the nodes to the other, a contradiction). For each species $X_j \in \mathscr{S}^{(\alpha)}$, we consider the set of cycles in the subgraph G_2 that have X_j as a node, that is:

$$\mathcal{C}(X_j) = \{C \,:\, C \text{ is a (simple) cycle with } X_j \text{ a node of } C\}.$$

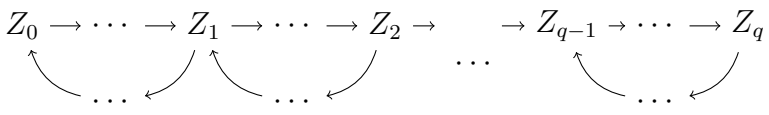
Observe that these sets are nonempty because G_2 is weakly reversible by hypothesis. Now, we define the following subsets of $\mathscr{S}^{(\alpha)}$.

$$N_0 = \{X_{i_\alpha}\},$$

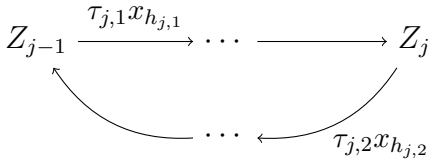
$$N_q = \{X_j \in \mathscr{S}^{(\alpha)} \,:\, X_j \in C, \text{ for some } C \in \mathcal{C}(X_{j'}), \text{ with } X_{j'} \in N_{q-1}\} \backslash \bigcup_{t=0}^{q-1} N_t, \; q \geq 1.$$

Suppose that $X_i \in N_q$, for some $q \geq 1$. By hypothesis, there is a unique simple path between two nodes species in $\mathscr{S}^{(\alpha)}$, so there exist unique species $Z_0 = X_{i_\alpha}, Z_1, \ldots, Z_q = X_i$, such that $Z_j \in \mathcal{C}(Z_{j-1})$, for $j = 1, \ldots, q$.

Then, there exist q cycles in G_2:



each one of the form:



where $x_{h_j,1}$, $x_{h_j,2}$ are the concentrations of species in core subsets belonging to L_t for $t < k$ or are equal to 1. Following the proof of Theorem 4.8 of [86], we have that at steady state:

$$\tau_{j,1} x_{h_{j,1}} z_{j-1} = \tau_{j,2} x_{h_{j,2}} z_j,$$

for each $j = 1, \ldots, q$. From all these equations, we have that:

$$x_i = z_q = \left(\prod_{j=1}^{q} \frac{\tau_{j,1}}{\tau_{j,2}} \right) \left(\prod_{j=1}^{q} \frac{x_{h_{j,1}}}{x_{h_{j,2}}} \right) x_{i_\alpha}.$$

Using a recursive argument for the variables $x_{h_j,1}$, $x_{h_j,2}$, we obtain what we wanted. □

In the proof of Theorem 2.5.2 we will show how to modify the rate constants coming out from core complexes. If the digraph G_E has no directed cycles, we can consider the sets L_k, $k \geq 0$ as in Definition 2.5.4. Given $k \geq 1$ and $\alpha \in L_k$, we denote by $\mathcal{Y}_\alpha$ the set of reactant[2] core complexes which consist only of one species of $\mathscr{S}^{(\alpha)}$ or which consist of one species of $\mathscr{S}^{(\alpha)}$ and one species in a core subset with index in L_t with $t < k$.

If G_2 is weakly reversible, for each $y \in \mathcal{Y}_\alpha$, there exist at least one simple cycle C in G_2 that contains an outgoing edge of the form $X_i \xrightarrow{\tau x_j}$ if $y = X_i + X_j$ or an edge of the form $X_i \xrightarrow{\tau}$ if $y = X_i$, where $X_i \in \mathscr{S}^{(\alpha)}$. In this case, we say that the complex y *appears* in the simple cycle C. We define the following subsets of $\mathcal{Y}_\alpha$.

Definition 2.5.8. *Assume G is the underlying digraph of a MESSI system satisfying the hypotheses of Theorem 2.5.2; in particular, we fix $X_{i_\alpha} \in \mathscr{S}^{(\alpha)}$ for each $\alpha = 1, \ldots, m$. Let N_q and $\mathcal{C}(X_j)$ defined as in the proof of Lemma 2.5.7. For any $k \geq 1$*

[2]Recall that a reactant complex y is a complex for which exists a reaction $y \to y'$.

and $\alpha \in L_k$, we define the following subsets of $\mathcal{Y}_\alpha$:

$M_0 = \{y \in \mathcal{Y}_\alpha : \textit{one species of } y \textit{ is } X_{i_\alpha}\}$,
$M_0' = \{y \in \mathcal{Y}_\alpha : y \textit{ appears in } C \textit{ with } C \in \mathcal{C}(X_{i_\alpha})\} \backslash M_0$, *and for* $q \geq 1$:

$$M_q = \{y \in \mathcal{Y}_\alpha : \textit{one species of } y \textit{ belongs to } N_q\} \backslash \bigcup_{t=0}^{q-1} (M_t \cup M_t'),$$

$$M_q' = \{y \in \mathcal{Y}_\alpha : y \textit{ appears in } C \textit{ with } C \in \mathcal{C}(Z), \textit{for some } Z \in N_q\}) \backslash (\bigcup_{t=0}^{q-1} (M_t \cup M_t') \cup M_q).$$

We clarify in our example the previous definitions.

Example 2.5.9 (Example 1.4.2, continued)**.** Consider the network and its MESSI structure of Example 1.4.2. Choose the species $S_0 \in \mathscr{S}^{(3)}$. Looking at the connected component corresponding to $\mathscr{S}^{(3)}$ in the digraph G_2 in Figure 1.4, the sets N_q that appear in the proof of Lemma 2.5.7 are: $N_0 = \{S_0\}$, $N_1 = \{S_1, S_2\}$. The set $\mathcal{C}(S_0)$ consists only of the simple cycle:

$$S_0 \overset{e\tau_1}{\rightarrow} S_1 \overset{e\tau_2}{\rightarrow} S_2 \underset{f\tau_3}{\curvearrowleft}$$

The set $\mathcal{Y}_3$ is $\{S_0 + E, S_1 + E, S_2 + F\}$. The sets M_q of Definition 2.5.8 are: $M_0 = \{S_0 + E\}$, $M_0' = \{S_1 + E, S_2 + F\}$ (the complexes $S_1 + E$ and $S_2 + F$ appear in the the previous cycle of $\mathcal{C}(S_0)$).

Now we are ready to present the proof of Theorem 2.5.2.

Proof of Theorem 2.5.2. We can suppose without loss of generality that the coefficient γ_{n+1} in the system (2.5.2) is equal to 1; if not, we divide each equation by γ_{n+1} and we obtain new values of γ for each monomial. Note that x_{i_α} is one of the monomials that appears in the system (2.2.11) for all $\alpha = 1, \ldots, m$. We can suppose that the corresponding multiplier γ_α of x_{i_α} in system (2.2.11) is equal to 1 for all α. Otherwise, we change the variables

$$\gamma_\alpha x_{i_\alpha} = \bar{x}_{i_\alpha}.$$

In this case, we get a system with new values of the vector γ, in which the positive solutions are in bijection with the positive solutions of system (2.5.2).

With these assumptions, we assert that we can transform system (2.5.2) into system (2.5.3), just rescaling the rate constants of reactions coming out from a core complex, in a certain order, multiplying each one by an appropriate constant. We consider the sets L_k, as in Definition 2.5.4. Recall that L_0 is no empty because G_E has no direct cycles. Because the partition is minimal the subsets of core species $\mathscr{S}^{(\alpha)}$ are in bijection with the connected components of G_2 and the set of nodes of the corresponding component equals $\mathscr{S}^{(\alpha)}$.

Let $\mathscr{S}^{(\alpha)} \in L_0$. We showed in Lemma 2.5.7 that all the core species in $\mathscr{S}^{(\alpha)}$ can be written in terms of the monomial x_{i_α}, reaction rate constants and no other variables. If an intermediate complex has its unique core complex reacting to it via intermediates formed with species only in $\mathscr{S}^{(\alpha)}$, then the concentration of the corresponding intermediate species also depends only on x_{i_α} and reaction rate constants. That is, all the concentrations of these species have x_{i_α} as the corresponding monomial in the parametrization. We supposed that in system (2.5.2) the monomial x_{i_α} is multiplied by $\gamma_\alpha = 1$, then, there is nothing to rescale.

Now we proceed recursively. Fix $k \geq 1$. Suppose that we have already rescaled properly the reaction rate constants of edges coming out from core complexes whose parametrizations depends only on variables x_{i_β} with $\beta \in L_t$, with $t < k$. Fix one core subset $\mathscr{S}^{(\alpha)}$, with $\alpha \in L_k$. We will show how to rescale the rate constants of reactions coming out from complexes in the set $\mathcal{Y}_\alpha$, defined above.

The digraph G_2 is weakly reversible, then we can consider the sets M_q, M_q', $q \geq 0$, as in Definition 2.5.8. We are going to rescale the rate constants of reactions coming out from a complex in M_0, then in M_0', then in M_1 and so on, in that order. First, we show how to modify the constants of reactions coming out from a complex in M_0. Because the system is s-toric, each intermediate complex has a unique core complex reacting through intermediates to it. We consider the intermediates complexes such the unique core complex reacting through intermediates to it is in M_0 or in M_0' (if there is no one, we don't rescale anything). Suppose then that there is one intermediate complex formed by an intermediate species U_ℓ such that $y \to_\circ U_\ell$, with $y \in M_0$ or $y \in M_0'$. If the core complex $y \in M_0$, then $y = X_{i_\alpha}$ or $y = X_{i_\alpha} + X_j$, with X_j in a core subset belonging to L_t, with $t < k$. If $y = X_i$, the concentration of U_k is $u_\ell = \mu_\ell(\kappa) x_{i_\alpha}$, with μ_ℓ as in (2.5.11), and we are assuming that the monomial x_{i_α} is multiplied by $\gamma_\alpha = 1$. If $y = X_{i_\alpha} + X_j$, then we can write:

$$u_\ell = \mu_\ell(\kappa) x_{i_\alpha} x_j.$$

Now, x_j is a concentration of a core species and its parametrization can be written in terms of core species of subsets in the partition with indices in L_t, with $t < k$ and reaction rate constants $\tau'(\kappa)$, with $\tau'(\kappa)$ labels of edges of connected components of G_2, corresponding to core subsets with indices in L_t, with $t < k$. We write $x_j = g(\tau'(\kappa))\underline{x}^a$, with $\underline{x}^a$ a monomial in these other species and g a rational function, and we get:

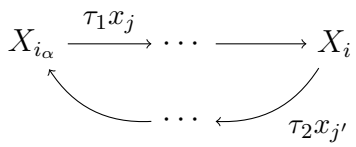
$$u_\ell = \mu_\ell(\kappa) g(\tau'(\kappa)) \underline{x}^a x_{i_\alpha}.$$

Suppose that the monomial $\underline{x}^a x_{i_\alpha}$ appears in system (2.5.2) multiplied by γ. Then, we want new reaction rate constants $\bar{\kappa}$ such that:

$$\gamma \mu_\ell(\kappa) g(\tau'(\kappa)) = \mu_\ell(\bar{\kappa}) g(\tau'(\bar{\kappa})). \tag{2.5.11}$$

To ease the notation, we denote $\mu_\ell = \mu_\ell(\kappa)$, $\tau' = \tau'(\kappa)$, $\bar{\mu}_\ell = \mu_\ell(\bar{\kappa})$, $\bar{\tau}' = \tau'(\bar{\kappa})$ (and we will denote with a bar the constants depending on $\bar{\kappa}$ and without a bar, the constants depending on κ). The constants τ' have been modified previously by hypothesis (note that a constant τ can only appear in one edge of G_2, because of the

condition that G_E has no cycles) and replaced by the constants $\bar{\tau}'$. It is clear that we can do the rescaling: it is enough to multiply each reaction constant of reactions coming out from the core complex y by the constant: $\gamma\frac{g(\tau')}{g(\bar{\tau}')}$. Then, by Lemma 2.5.6, we obtain the equality (2.5.11). Now, if $y \in M_0'$, y appears in C with $C \in \mathcal{C}(X_{i_\alpha})$. Then, $y = X_i$ or $y = X_i + X_{j'}$, with C of the form



where $x_{j'}$ is the concentration of $X_{j'}$ or is equal to 1 (if $y = X_i$), and similarly for x_j. Then, we have at steady state:

$$u_\ell = \mu_\ell\, x_i\, x_{j'} = \mu_\ell \frac{\tau_1}{\tau_2}\, x_{i_\alpha}\, x_j.$$

That is, u_ℓ depends on the concentrations of the species of the complex $X_{i_\alpha} + X_j$, which belongs to M_0. We then modify the reaction rate constants coming out of $X_{i_\alpha} + X_j$ multiplying it by an appropiate constant in a similar way as we did in the previous case, looking at the value of γ that appears in the corresponding monomial (note that if we modified these constants before, the previous rescaling also works for this case). Note that when later we modify the constants of the complex $y = X_i + X_{j'}$ which belongs to M_0' (we will see how to do this), the rescaling will be coherent. That is, if we multiply the constants of each reaction coming out from $X_{i_\alpha} + X_j$ by ν_1, and the constants coming out from y by ν_2 the rescaling will be coherent if we have:

$$\bar{\mu}_\ell \frac{\bar{\tau}_1}{\bar{\tau}_2} = \nu_1\, \mu_\ell \frac{\tau_1}{\tau_2},$$

but this holds by Lemma 2.5.6:

$$\bar{\mu}_\ell \frac{\bar{\tau}_1}{\bar{\tau}_2} = \nu_2\, \mu_\ell \frac{\nu_1\, \tau_1}{\nu_2\, \tau_2} = \nu_1\, \mu_\ell \frac{\tau_1}{\tau_2},$$

where $\bar{\mu}_\ell$, $\bar{\tau}_1$, $\bar{\tau}_2$ denotes the values of the functions μ_ℓ, τ_1, τ_2 corresponding to the new constants $\bar{\kappa}$. We modify all the reactions rate constants coming out of complexes y belonging to M_0 in this way: looking at intermediates complexes U_ℓ such that $y \to_\circ U_\ell$ or $y' \to_\circ U_\ell$, with $y' \in M_0'$ and such that in the parametrization of the intermediate species appears the monomial corresponding to the complex y. If there is no such intermediate complex we multiply the constants by 1. Also, we observe that with this rescaling, we modified all the constants τ that label an edge in G_2 of the form $X_{i_\alpha} \xrightarrow{\tau x_j}$.

Now, we show how to rescale the constants of complexes in M_0'. Let $y \in M_0'$, then $y = X_i$ or $y = X_i + X_{j'}$ and we have a cycle as we showed previously in this proof. By Lemma 2.5.7, the concentration x_i is of the form

$$x_i = \frac{\tau_1}{\tau_2} g(\tau')\underline{x}^a x_{i_\alpha},$$

where τ' are labels of edges of connected components of G_2, corresponding to core subsets belong to L_t, with $t < k$, $g(\tau)$ a rational form and $\underline{x}^a$ a monomial in variables in core subsets belong to L_t, with $t < k$. The constant τ_1 and the constants τ' have been already modified by the constants $\bar{\tau}_1$ and $\bar{\tau}'$ respectively. It is clear that we can do the rescaling if we modify τ_2. If γ is the constant that multiplies the monomial $\underline{x}^a x_{i_\alpha}$ in system (2.5.2), we want

$$\gamma \frac{\tau_1}{\tau_2} g(\tau') = \frac{\bar{\tau}_1}{\bar{\tau}_2} g(\bar{\tau}'),$$

and we get this equality if we multiply each rate constant of a reaction coming out from y by the constant $\frac{\bar{\tau}_1 g(\bar{\tau}')}{\gamma \tau_1 g(\tau')}$ and we apply Lemma 2.5.6. We do this for all complexes in M_0'.

We proceed recursively rescaling the remaining constants of reactions coming out from complexes in M_q, and then from M_q', for each q. We first modify the constants of complexes in M_q, by looking at the parametrization of intermediate species as we did when we showed how to rescale the constants of reactions coming out from complexes in M_0. After that, we modify the constants of complexes in M_q' by looking the concentration of the core species in $\mathscr{S}^{(\alpha)}$ that appear in the complex, as we did for the complexes in M_0'. Then, we can rescale all the complexes of $\mathcal{Y}_\alpha$, for all $\alpha \in L_k$. We can proceed for all k, $k \geq 1$, in order, and then we are done. □

Example 2.5.10. For the distributive multisite phosphorylation systems showed in Section 2.4 the hypotheses in Theorem 2.5.2 are satisfied. A MESSI structure of the network for the double phosporylation ($n = 2$) is given by this minimal partition of the species:

$\mathscr{S}^{(0)} = \{ES_0, ES_1, FS_1, FS_2\}$ (the intermediate species), $\mathscr{S}^{(1)} = \{E\}$, $\mathscr{S}^{(2)} = \{F\}$, and $\mathscr{S}^{(3)} = \{S_0, S_1, S_2\}$.

The digraphs G_1, G_2, and G_E are depicted in Figure 2.8. It is easy to check the conditions of Theorem 2.5.2 in this case. Following the proof of this theorem, we can show which parameters are sufficient to rescale. For this case is sufficient to modify k_{on_0}, k_{on_1}, ℓ_{on_0}, and ℓ_{on_1}, the rate constants of reactions coming out of core complexes.

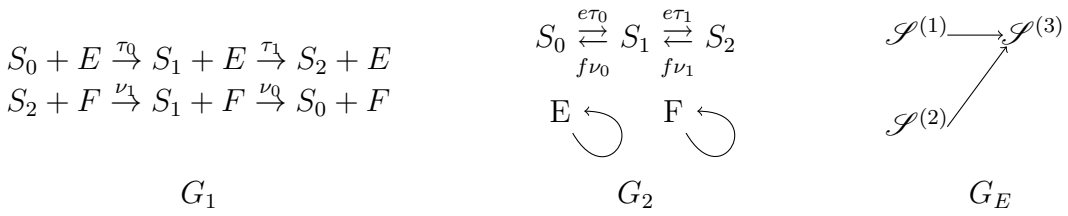


Figure 2.8: The digraphs G_1, G_2, and G_E of the double phosphorylation network.

Chapter 3

Regions of multistationarity in cascades of Goldbeter-Koshland loops

Signal transduction is the process through which cells communicate with the external environment, interpret stimuli and respond to them. This mechanism is controlled by signaling cascades. Classical signaling pathways typically contain a cascade of phosphorylation cycles where the activated protein in one layer acts as the modifier enzyme in the next layer. An example of signaling cascades is the Ras cascade (see Figure 3.1, as it is usually depicted in the biochemistry literature), which is an important signaling pathway in mitogen-activated protein kinases (MAPKs). This cascade reaction activates transcription factors and regulates gene expression. The Ras signaling pathway has a significant role in the occurrence and development of diseases such as cancer [73] or developmental defects [57]. One key property is the occurrence of multistability, which triggers different crucial cellular events. A basic condition for these different cellular responses is the emergence of multistationarity.

In this chapter, we use tools from real algebraic geometry based on the results of Chapter 2, to analyze multistationarity in cascades of enzymatic Goldbeter-Koshland loops. A second important ingredient is the observation that enzymatic cascades have the structure of MESSI systems introduced in [86], and presented in Section 1.4, from which an explicit parametrization of the steady states can be obtained, even in presence of multistationarity. We show how to deform a given set of parameters of the model to produce multistationarity, including both the reaction rate constants and the total concentration constants. Moreover, we identify open sets where multistationarity occurs in the space of all these parameters.

In Section 3.1 we apply our method to an enzymatic cascade with two layers and in Section 3.2 we work with the general case of n layers and present our main results of this chapter (Theorems 3.2.1 and 3.2.3.) In this case, the associated polynomial systems have positive dimensions growing linearly with n. The number of conservation relations (and then of total conservation constants) also grows linearly with n, and it is at least four if $n \geq 2$. Such systems were studied in [11, 38] when

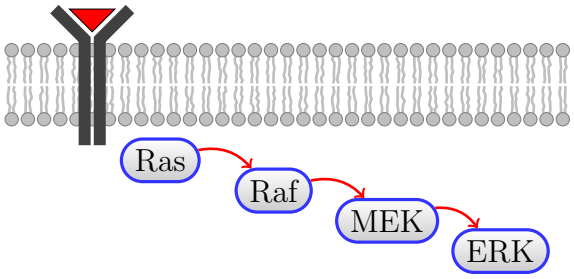


Figure 3.1: The Ras pathway.

all the enzymes are different, in which case there cannot be more than one positive steady state. This fact is proved in [38] and also is a particular case of a more general result in [3], in which the authors work with a more general structure: tree networks of Goldbeter-Koshland loops. In the case of two layers (see Figure 3.2), it was shown in [40] that if the same phosphatase is acting at both layers, then the network has the capacity for multistationarity. It can be deduced from the results in [2], that if there are any number of layers, and the last two share a phosphatase, multistationarity parameters for the case $n = 2$ can be extended to produce multistationarity parameters in the general case.

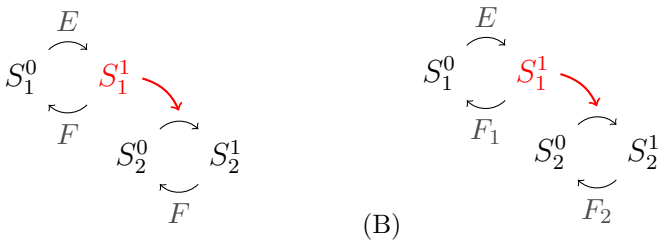


Figure 3.2: Same and different phosphatases in a 2-layer cascade of GK-loops.

Our results can be generalized to describe multistationarity regions for other architectures of cascades which define MESSI systems. For this purpose, in Section 3.3 we present some general results that are the basis for some of our arguments. We state and prove the extension Theorem 3.3.3, that abstracts some of our computations in Section 3.2, which could be combined with the general results in Section 2.5 of Chapter 2. For example, in the case of the Ras cascade in Figure 3.1, previous papers studied rate constant multistationarity parameters (see e.g. [13, 88]). Our methods yield multistationarity regions for this signaling pathway in terms of rate constants and total concentration parameters. We omitted these computations, because they are similar to the ones we detail in Sections 3.1 and 3.2.

We expect that our methods can be applied to study other chemical reaction networks of interest, not only to find multistationarity regions but also to find regions of parameters that ensure several positive steady states in a same stoichiometric compatibility class.

3.1 Enzymatic cascades with two layers

In this section we work with the case of an enzymatic cascade with two layers, and then in Section 3.2 we will work with the general case. The network involves two phosphorylation cycles. We call S_1 and S_2 the substrate proteins in the first and second layers respectively. The upper index can be interpreted as the absence (0) or the presence (1) of a phosphate group. The phosphorylation in the first layer is catalyzed by the enzyme E. The activated protein S_1^1 in the first layer acts as the modifier enzyme in the second layer, which is depicted in (A) in Figure 3.2. Note that the dephosphorylation is carried out by the same phosphatase F, which as we pointed out before, gives the capacity for multistationarity to the network by [40]. The kinetics is deduced by applying the law of mass-action to the following chemical reaction network:

$$\begin{array}{ll} S_1^0 + E \underset{k_{\text{off}_1}}{\overset{k_{\text{on}_1}}{\rightleftarrows}} Y_1^0 \overset{k_{\text{cat}_1}}{\to} S_1^1 + E & S_2^0 + S_1^1 \underset{k_{\text{off}_2}}{\overset{k_{\text{on}_2}}{\rightleftarrows}} Y_2^0 \overset{k_{\text{cat}_2}}{\to} S_2^1 + S_1^1 \\ S_1^1 + F \underset{\ell_{\text{off}_1}}{\overset{\ell_{\text{on}_1}}{\rightleftarrows}} Y_1^1 \overset{\ell_{\text{cat}_1}}{\to} S_1^0 + F & S_2^1 + F \underset{\ell_{\text{off}_2}}{\overset{\ell_{\text{on}_2}}{\rightleftarrows}} Y_2^1 \overset{\ell_{\text{cat}_2}}{\to} S_2^0 + F. \end{array} \tag{3.1.1}$$

We denote by Y_1^0, Y_2^0, Y_1^1, Y_2^1 the intermediate complexes, which consist of a single chemical species formed by the union of the substrate with the enzyme. The concentrations of the species will be denoted with small letters, for example s_1^0 will denote the concentration of S_1^0. The associated dynamical system that arises under mass-action kinetics equals:

$$\begin{aligned} \frac{ds_1^0}{dt} =& -k_{\text{on}_1} s_1^0 e + k_{\text{off}_1} y_1^0 + \ell_{\text{cat}_1} y_1^1, & \frac{dy_1^1}{dt} =& \ell_{\text{on}_1} s_1^1 f - (\ell_{\text{off}_1} + \ell_{\text{cat}_1}) y_1^1, \\ \frac{ds_1^1}{dt} =& k_{\text{cat}_1} y_1^0 - \ell_{\text{on}_1} s_1^1 f + \ell_{\text{off}_1} y_1^1 & \frac{dy_2^0}{dt} =& k_{\text{on}_2} s_2^0 s_1^1 - (k_{\text{off}_2} + k_{\text{cat}_2}) y_2^0, \\ & + k_{\text{on}_2} s_2^0 s_1^1 + (k_{\text{off}_2} + k_{\text{cat}_2}) y_2^0, & \frac{dy_2^1}{dt} =& \ell_{\text{on}_2} s_2^1 f - (\ell_{\text{off}_2} + \ell_{\text{cat}_2}) y_2^1, \\ \frac{ds_2^0}{dt} =& -k_{\text{on}_2} s_2^0 s_1^1 + k_{\text{off}_2} y_2^0 + \ell_{\text{cat}_2} y_2^1, & \frac{de}{dt} =& -k_{\text{on}_1} s_1^0 e + (k_{\text{off}_1} + k_{\text{cat}_1}) y_1^0, \\ \frac{ds_2^1}{dt} =& k_{\text{cat}_2} y_2^0 - \ell_{\text{on}_2} s_2^1 f + \ell_{\text{off}_2} y_2^1, & \frac{df}{dt} =& -\ell_{\text{on}_1} s_1^1 f + (\ell_{\text{off}_1} + \ell_{\text{cat}_1}) y_1^1 \\ \frac{dy_1^0}{dt} =& k_{\text{on}_1} s_1^0 e - (k_{\text{off}_1} + k_{\text{cat}_1}) y_1^0, & & - \ell_{\text{on}_2} s_2^1 f + (\ell_{\text{off}_2} + \ell_{\text{cat}_2}) y_2^1. \end{aligned}$$

In this case, there is a basis of the conservation laws given by the four linear equations:

$$\begin{aligned} e + y_1^0 =& E_{tot}, \\ f + y_1^1 + y_2^1 =& F_{tot}, \\ s_1^0 + s_1^1 + y_1^0 + y_1^1 + y_2^0 =& S_{1,tot}, \\ s_2^0 + s_2^1 + y_2^0 + y_2^1 =& S_{2,tot}. \end{aligned} \tag{3.1.2}$$

Enzymatic cascades are an example of s-toric MESSI networks. By Theorem 4.8 in [86] we can find binomial equations that describe the steady states. This is a general procedure, that in this case is easily obtained by manipulating the differential equations. First, the concentrations of the intermediates species $y_1^0, y_1^1, y_2^0, y_2^1$ at steady state satisfy the following binomial equations:

$$\begin{aligned} y_1^0 - K_1\, e s_1^0 = 0, \qquad y_1^1 - L_1\, f s_1^1 = 0, \\ y_2^0 - K_2\, s_1^1 s_2^0 = 0, \qquad y_2^1 - L_2\, f s_2^1 = 0, \end{aligned} \tag{3.1.3}$$

where $K_1 = \frac{k_{\mathrm{on}_1}}{k_{\mathrm{off}_1}+k_{\mathrm{cat}_1}}$, $K_2 = \frac{k_{\mathrm{on}_2}}{k_{\mathrm{off}_2}+k_{\mathrm{cat}_2}}$, $L_1 = \frac{\ell_{\mathrm{on}_1}}{\ell_{\mathrm{off}_1}+\ell_{\mathrm{cat}_1}}$ and $L_2 = \frac{\ell_{\mathrm{on}_2}}{\ell_{\mathrm{off}_2}+\ell_{\mathrm{cat}_2}}$. The whole steady state variety can be cut out in the positive orthant by adding to the binomials in (3.1.3), the following binomial equations:

$$\tau_1\, s_1^0\, e - \nu_1\, s_1^1\, f = 0, \quad \tau_2\, s_2^0\, s_1^1 - \nu_2\, s_2^1\, f = 0,$$

where $\tau_1 = k_{\mathrm{cat}_1} K_1$, $\tau_2 = k_{\mathrm{cat}_2} K_2$, $\nu_1 = \ell_{\mathrm{cat}_1} L_1$ and $\nu_2 = \ell_{\mathrm{cat}_2} L_2$.

Therefore, we can parametrize the positive steady states by monomials. For instance, we can write the concentration at steady state of S_1^0, S_2^0 and the intermediate species, in terms of the concentration of the species E, F, S_1^1, S_2^1:

$$\begin{aligned} s_1^0 = G_1\, \frac{s_1^1\, f}{e}, \qquad y_1^0 = K_1\, G_1\, s_1^1\, f, \qquad y_1^1 = L_1\, s_1^1\, f, \\ s_2^0 = G_2\, \frac{s_2^1\, f}{s_1^1}, \qquad y_2^0 = K_2\, G_2\, s_2^1\, f, \qquad y_2^1 = L_2\, s_2^1\, f, \end{aligned} \tag{3.1.4}$$

where $G_1 = \frac{\nu_1}{\tau_1}$ and $G_2 = \frac{\nu_2}{\tau_2}$.

Now, we apply our results to this case. Denote by

$$A_1 = \frac{\ell_{\mathrm{cat}_1}}{k_{\mathrm{cat}_1}}, \quad A_2 = \frac{\ell_{\mathrm{cat}_2}}{k_{\mathrm{cat}_2}}, \tag{3.1.5}$$

and assume that $S_{1,tot}, S_{2,tot}, E_{tot}, F_{tot} > 0$. Consider the following rational functions $\alpha_1, \alpha_2, \alpha_3, \alpha_4$ depending on the catalytic reaction rate constants and total concentration constants:

$$\begin{aligned} \alpha_1 =& \frac{S_{1,tot}}{F_{tot}} - A_2, \\ \alpha_2 =& (A_1 + 1) - \frac{S_{1,tot}}{F_{tot}}, \\ \alpha_3 =& \frac{A_1 + 1 - A_2}{A_1}\, \frac{E_{tot}}{F_{tot}} - \left(\frac{S_{1,tot}}{F_{tot}} - A_2 \right), \\ \alpha_4 =& \frac{A_1 + 1 - A_2}{A_2 + 1}\, \frac{S_{2,tot}}{F_{tot}} - \left(A_1 + 1 - \frac{S_{1,tot}}{F_{tot}} \right). \end{aligned}$$

We then have:

Theorem 3.1.1. *Consider the enzymatic cascade with two layers with digraph as in* (3.1.1) *and let* A_1, A_2 *as in* (3.1.5). *Assume that the reaction rate constants*

satisfy $A_1 + 1 > A_2$ *and the total concentration constants satisfy the inequalities* $\alpha_1, \alpha_2, \alpha_3, \alpha_4 > 0$, *that is:*

$$A_1 + 1 > \frac{S_{1,tot}}{F_{tot}} > A_2, \quad \frac{E_{tot}}{F_{tot}} > \left(\frac{S_{1,tot}}{F_{tot}} - A_2\right) \frac{A_1}{A_1 + 1 - A_2},$$

$$\frac{S_{2,tot}}{F_{tot}} > \left(A_1 + 1 - \frac{S_{1,tot}}{F_{tot}}\right) \frac{A_2 + 1}{A_1 + 1 - A_2},$$

or instead, that $A_1 + 1 < A_2$ *and* $\alpha_1, \alpha_2, \alpha_3, \alpha_4 < 0$.

Fix generic positive numbers h_2, h_3, h_7, h_8 *such that* $h_8 < h_2$. *Then, there exists* $t_0 > 0$ *such that for any value of* $t \in (0, t_0)$ *the system has at least two positive steady states after modifying the coefficients* $k_{\mathrm{on}_1}, k_{\mathrm{on}_2}, \ell_{\mathrm{on}_1}, \ell_{\mathrm{on}_2}$ *via the rescaling* $t^{-h_7} k_{\mathrm{on}_1}$, $t^{-h_3-h_8} k_{\mathrm{on}_2}$, $t^{-h_2-h_3} \ell_{\mathrm{on}_1}$ *and* $t^{-h_2} \ell_{\mathrm{on}_2}$.

Also, for any fixed choice of reaction rate constants and total concentration constants lying in the open set defined by one of the previous set of inequalities, there exist positive constants $M_1, \ldots, M_6$ *such that for any values of* $\beta_1, \beta_2, \eta_1, \eta_2$ *satisfying*

$$\frac{1}{\eta_2} < M_1, \quad \frac{\eta_2}{\eta_1} < M_2, \quad \frac{1}{\beta_1} < M_3, \quad \frac{\eta_1}{\eta_2 \beta_2} < M_4, \quad \frac{\beta_2}{\eta_1} < M_5, \quad \frac{1}{\beta_2} < M_6, \tag{3.1.6}$$

the rescaling of the given parameters k_{on_0}, k_{on_1}, ℓ_{on_0} *and* ℓ_{on_1} *by* $\beta_1 k_{\mathrm{on}_1}$, $\beta_2 k_{\mathrm{on}_2}$, $\eta_1 \ell_{\mathrm{on}_1}$ *and* $\eta_2 \ell_{\mathrm{on}_2}$ *respectively, gives rise to a multistationary system.*

Proof. We substitute the monomial parametrization of the steady states in terms of the concentrations e, f, s_1^1, s_2^1 (3.1.4) into the linear conservation relations (3.1.2). We write this system in matricial form:

$$C \begin{pmatrix} e & f & s_1^1 & s_2^1 & s_1^1 f & s_2^1 f & s_1^1 f e^{-1} & s_2^1 f (s_1^1)^{-1} & 1 \end{pmatrix}^t = 0,$$

where the matrix of coefficients $C \in \mathbb{R}^{4\times 9}$ equals:

$$C = \begin{pmatrix} 1 & 0 & 0 & 0 & K_1 G_1 & 0 & 0 & 0 & -E_{tot} \\ 0 & 1 & 0 & 0 & L_1 & L_2 & 0 & 0 & -F_{tot} \\ 0 & 0 & 1 & 0 & K_1 G_1 + L_1 & K_2 G_2 & G_1 & 0 & -S_{1,tot} \\ 0 & 0 & 0 & 1 & 0 & K_2 G_2 + L_2 & 0 & G_2 & -S_{2,tot} \end{pmatrix}. \tag{3.1.7}$$

If we order the variables as before, e, f, s_1^1, s_2^1 , the support of this system is:

$$\begin{aligned} \mathcal{A} = \{&(1,0,0,0), (0,1,0,0), (0,0,1,0), (0,0,0,1), (0,1,1,0), \\ &(0,1,0,1), (-1,1,1,0), (0,1,-1,1), (0,0,0,0)\}. \end{aligned}$$

We want to find two positively decorated 4-simplices with vertices in $\mathcal{A}$ which share one facet. For example we take the simplices

$$\begin{aligned} \Delta_1 &= \{(1,0,0,0), (0,0,0,1), (0,1,1,0), (0,1,0,1), (0,0,0,0)\}, \\ \Delta_2 &= \{(1,0,0,0), (0,1,1,0), (0,1,0,1), (0,1,-1,1), (0,0,0,0)\}. \end{aligned}$$

It is straightforward to check that both simplices are positively decorated by C if either $A_1+1>A_2$ and $\alpha_1,\alpha_2,\alpha_3,\alpha_4>0$, or $A_1+1<A_2$ and $\alpha_1,\alpha_2,\alpha_3,\alpha_4<0$, as in the statement.

Given $h\in\mathcal{C}_{\Delta_1,\Delta_2}$, by Theorem 2.2.11, there exists $t_0\in\mathbb{R}_+$ such that for all $0<t<t_0$, the number of positive (nondegenerate) solutions of the scaled system:

$$\begin{aligned} t^{h_1}\,e+t^{h_5}\,K_1G_1s_1^1f-t^{h_9}\,E_{tot}&=\ 0,\\ t^{h_2}\,f+t^{h_5}\,L_1fs_1^1+t^{h_6}\,L_2fs_2^1-t^{h_9}\,F_{tot}&=\ 0,\\ t^{h_3}\,s_1^1+t^{h_7}\,G_1\frac{s_1^1f}{e}+t^{h_5}\,(K_1G_1+L_1)s_1^1f+t^{h_6}\,K_2G_2s_2^1f-t^{h_9}\,S_{1,tot}&=\ 0,\\ t^{h_4}\,s_2^1+t^{h_8}\,G_2\frac{s_2^1f}{s_1^1}+t^{h_6}\,(K_2G_2+L_2)s_2^1f-t^{h_9}\,S_{2,tot}&=\ 0, \end{aligned}\tag{3.1.8}$$

is at least two. If we think of the vector h as a function $\mathcal{A}\to\mathbb{R}$ (defined by $h(a_j)=h_j$), then $h_1=h(1,0,0,0)$, $h_2=h(0,1,0,0)$, $h_3=h(0,0,1,0)$, $h_4=h(0,0,0,1)$, $h_5=h(0,1,1,0)$, $h_6=h(0,1,0,1)$, $h_7=h(-1,1,1,0)$, $h_8=h(0,1,-1,1)$ and $h_9=h(0,0,0,0)$. Let φ_1 and φ_2 be the affine linear functions which agree with h on the simplices Δ_1 and Δ_2 respectively. We can take $h_1=h_4=h_5=h_6=h_9=0$. Then $\varphi_1=0$, $h_8>0$ and φ_2 is defined by $\varphi_2(x,y,z,w)=h_8y-h_8z-h_8w$. Moreover,

$$\begin{array}{lcccl} 0<h_2, & \varphi_2(0,1,0,0) & = & h_8 & <\ h_2,\\ 0<h_3, & \varphi_2(0,0,1,0) & = & -h_8 & <\ h_3,\\ 0<h_7, & \varphi_2(-1,1,1,0) & = & 0 & <\ h_7, \end{array}$$

where we could take h_2,h_3 and h_7 generic.

If we change the variables $\bar f=t^{h_2}f$, $\bar s_1^1=t^{h_3}s_1^1$, we get the following (Laurent) polynomial equations:

$$\begin{aligned} e+t^{-h_2-h_3}\,K_1G_1\,\bar s_1^1\bar f-E_{tot}&=\ 0,\\ \bar f+t^{-h_2-h_3}\,L_1\,\bar f\bar s_1^1+t^{-h_2}\,L_2\,\bar fs_2^1-F_{tot}&=\ 0,\\ \bar s_1^1+t^{h_7-h_2-h_3}\,G_1\,\frac{\bar s_1^1\bar f}{e}+t^{-h_2-h_3}\,(K_1G_1+L_1)\,\bar s_1^1\bar f+t^{-h_2}\,K_2G_2\,s_2^1\bar f-S_{1,tot}&=\ 0,\\ s_2^1+t^{h_8+h_3-h_2}\,G_2\frac{s_2^1\bar f}{\bar s_1^1}+t^{-h_2}\,(K_2G_2+L_2)\,s_2^1\bar f-S_{2,tot}&=\ 0. \end{aligned}\tag{3.1.9}$$

It is straightforward to verify that if we scale the constants:

$$t^{-h_7}K_1,\ t^{-h_3-h_8}K_2,\ t^{-h_2-h_3}L_1,\ t^{-h_2}L_2,\tag{3.1.10}$$

and we keep fixed the values of k_{cat_1}, k_{cat_2}, ℓ_{cat_1} and ℓ_{cat_2} and the total values E_{tot}, F_{tot}, $S_{1,tot}$ and $S_{2,tot}$, the intersection of the steady state variety and the linear varieties of fixed total concentrations of the dynamical system associated with the corresponding network, is described by system (3.1.9).

It is easy to check that to get the scaling in (3.1.10), it is enough to rescale the original constants as follows: $t^{-h_7}k_{\mathrm{on}_1}$, $t^{-h_3-h_8}k_{\mathrm{on}_2}$, $t^{-h_2-h_3}\ell_{\mathrm{on}_1}$ and $t^{-h_2}\ell_{\mathrm{on}_2}$. Then, for these choices of constants the system has at least two positive steady states. The last part of the statement follows from the previous rescaling or from the inequalities

that define the cone $\mathcal{C}_{\Delta_1,\Delta_2}$ of heights inducing regular subdivisions of the convex hull of $\mathcal{A}$ that contain Δ_1 and Δ_2 and Theorem 2.2.13. For instance, we can check that $\mathcal{C}_{\Delta_1,\Delta_2}$ is defined by 6 inequalities. We can write:

$$\mathcal{C}_{\Delta_1,\Delta_2} = \{h = (h_1, \ldots, h_8) \in \mathbb{R}^8 \,:\, \langle m_r, h\rangle > 0,\ r = 1, \ldots, 6\},$$

where

$$\begin{aligned} m_1 &= (0,1,0,1,0,-1,0,0,-1),\ m_2 = (0,0,1,-1,-1,1,0,0,0),\\ m_3 &= (1,0,0,0,-1,0,1,0,-1),\ m_4 = (0,0,0,1,1,-2,0,1,-1),\\ m_5 &= (0,1,0,0,-1,1,0,-1,0),\ m_6 = (0,0,1,0,0,-1,0,1,-1). \end{aligned}$$

By Theorem 2.2.13, there exist $M_1, \ldots, M_6 > 0$ such that for any $\gamma = (\gamma_1, \ldots, \gamma_9)$ in the open set

$$U = \{\gamma \in \mathbb{R}^9_{>0} \,:\, \gamma^{m_r} < M_r,\ r = 1 \ldots, 6\},$$

the system

$$\begin{aligned} \gamma_1\, e + \gamma_5\, K_1G_1\, s_1^1 f - \gamma_9\, E_{tot} &= 0,\\ \gamma_2\, f + \gamma_5\, L_1\, f s_1^1 + \gamma_6\, L_2\, f s_2^1 - \gamma_9\, F_{tot} &= 0,\\ \gamma_3\, s_1^1 + \gamma_7\, G_1 \frac{s_1^1 f}{e} + \gamma_5\, (K_1G_1 + L_1)\, s_1^1 f + \gamma_6\, K_2G_2\, s_2^1 f - \gamma_9\, S_{1,tot} &= 0,\\ \gamma_4\, s_2^1 + \gamma_8\, G_2 \frac{s_2^1 f}{s_1^1} + \gamma_6\, (K_2G_2 + L_2)\, s_2^1 f - \gamma_9\, S_{2,tot} &= 0, \end{aligned} \tag{3.1.11}$$

has at least two positive solutions. If we take $\gamma_1 = \gamma_2 = \gamma_3 = \gamma_4 = \gamma_9 = 1$, and we denote $\beta_1 = \frac{\gamma_5}{\gamma_7}$, $\beta_2 = \frac{\gamma_6}{\gamma_8}$, $\eta_1 = \gamma_5$ and $\eta_2 = \gamma_6$, the conditions such that γ belongs to U are equivalent to the conditions (3.1.6), and it is easy to check that the steady state equations of the network after the rescaling of the given parameters k_{on_0}, k_{on_1}, ℓ_{on_0} and ℓ_{on_1} by $\beta_1\, k_{\mathrm{on}_1}$, $\beta_2\, k_{\mathrm{on}_2}$, $\eta_1\, \ell_{\mathrm{on}_1}$ and $\eta_2\, \ell_{\mathrm{on}_2}$ give system (3.1.11). □

Example 3.1.2. Note that the inequalities in the statement of Theorem 3.1.1 are clearly compatible. For example, the inequalities are satisfied if we take in the first case $\frac{\ell_{\mathrm{cat}_1}}{k_{\mathrm{cat}_1}} = 1$, $\frac{\ell_{\mathrm{cat}_2}}{k_{\mathrm{cat}_2}} = 1$, $E_{tot} = F_{tot} = 20$, $S_{1,tot} = S_{2,tot} = 30$. We can obtain in this case a value of t such that the system (3.1.9) has two or more positive solutions, using Singular [25], with the library "signcond.lib" implemented by E. Tobis.

Fix for example $h_2 = 2$, $h_3 = 1$, $h_7 = 1$, $h_8 = 1$, $K_1 = 1$, $K_2 = 1$, $L_1 = 1$ and $L_2 = 1$. We have then that $G_1 = 1$ and $G_2 = 1$. If we take $t = \frac{1}{24}$, we have that the system has 3 positive solutions, checked with the following code:

```
>LIB "signcond.lib";
>ring r=(0,t), (x,y,z,w), dp;
>poly f1=x+t^3*y*z-20;
>poly f2=y+t^3*y*z+t^2*y*w-20;
>poly f3=x*z+t^2*z*y+t^3*2*y*z*x+t^2*y*w*x-30*x;
>poly f4=z*w+y*w+t^2*y*z*w-30*z;
>poly g1=subst(f1,t,24);
```

```
>poly g2=subst(f2,t,24);
>poly g3=subst(f3,t,24);
>poly g4=subst(f4,t,24);
>ideal i=g1,g2,g3,g4;
>ideal j=std(i);
>firstoct(j);
3
```

Here $x = e$, $y = \bar{f}$, $z = \bar{s}_1^1$ and $w = s_2^1$. It can be checked that if we take a slightly higher value $t = \frac{1}{23}$, the corresponding system has only one positive solution. This procedure is symbolic and thus certified, as opposed to numeric algorithms to compute the roots which can be affected by numerical instability. It is based on the algorithms described in [4].

3.2 Enzymatic cascades with n layers

We now present our results to the general case of an enzymatic cascade of n layers, where we have n phosphorylation cycles, as in Figure 3.3, under the assumption that there are (at least) two layers which share a phosphatase. We separate our study into two cases: the case of the occurrence of the same phosphatase in two consecutive layers (Theorem 3.2.1) and the case where the layers which share the phosphatase are not consecutive (Theorem 3.2.3). The difficulty to deal with these networks is that the simplified polynomials that we get to describe the steady states in a given stoichiometric compatibility class depend on a number of variables that grows linearly with n and the corresponding coefficient matrix does not have generic entries. We are nevertheless able to detect two simplices in these high dimensional spaces which share a facet, which are positively decorated by the (huge) coefficient matrix.

We first set the notation.

3.2.1 Our setting

Using the notation in Figure 3.3, we call S_i^0, S_i^1 the substrate proteins in the i-th layer, for $i = 1, \ldots, n$. As before, the upper index can be interpreted as the absence (0) or the presence (1) of a phosphate group in the substrate. The phosphorylation in the first layer is catalyzed by the enzyme S_0^1. The activated protein S_i^1 in the i-th layer acts as the modifier enzyme in the $(i+1)$-th layer. The dephosphorylation in the i-th layer is carried out by a phosphatase F_i. Some of the F_i can be the same species, that is, the same phosphatase can react at different layers.

We assume the following reaction scheme:

$$S_i^0 + S_{i-1}^1 \underset{k_{\mathrm{off_i}}}{\overset{k_{\mathrm{on_i}}}{\rightleftarrows}} Y_i^0 \overset{k_{\mathrm{cat_i}}}{\to} S_i^1 + S_{i-1}^1, \qquad i = 1 \ldots, n,$$

$$S_i^1 + F_i \underset{\ell_{\mathrm{off_i}}}{\overset{\ell_{\mathrm{on_i}}}{\rightleftarrows}} Y_i^1 \overset{\ell_{\mathrm{cat_i}}}{\to} S_i^0 + F_i, \qquad i = 1, \ldots, n.$$

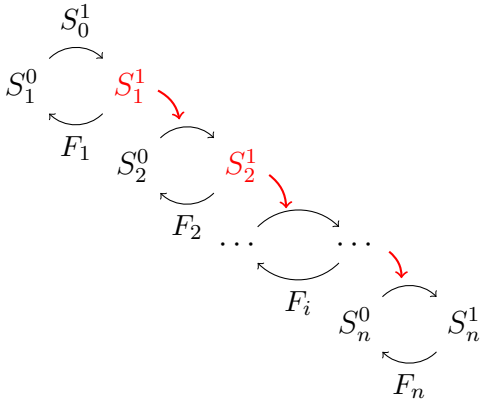


Figure 3.3: Enzymatic cascade with n layers.

We denote by $\mathcal{F} = \{P_1, \ldots, P_r\}$ the set of phosphatases that appear in the network. In this case we have $4n+r+1$ chemical species: S_0^1, S_1^0, S_1^1, S_2^0, S_2^1, $\ldots$,S_n^0, S_n^1,, P_1, $P_2 \ldots P_r$, Y_1^0, Y_1^1, Y_2^0, Y_2^1, $\ldots$, Y_n^0, Y_n^1. We denote the concentration of the species with small letters. For each $j = 1, \ldots, r$, we call $\Lambda_j = \{i \in \{1, \ldots, n\} : F_i = P_j\}$ and we consider the function $j\colon \{1, \ldots, n\} \to \{1, \ldots, r\}$, defined by $j(i) = j$ if $F_i = P_j$.

The associated dynamical system that arises under mass-action kinetics is equal to:

$$
\begin{aligned}
\frac{ds_i^0}{dt} &= -k_{\mathrm{on_i}} s_i^0 s_{i-1}^1 + k_{\mathrm{off_i}} y_i^0 + \ell_{\mathrm{cat_i}} y_i^1, \quad i = 1, \ldots, n,\\
\frac{ds_i^1}{dt} &= k_{\mathrm{cat_i}} y_i^0 - \ell_{\mathrm{on_i}} s_i^1 p_{j(i)} + \ell_{\mathrm{off_1}} y_i^1 - k_{\mathrm{on_{i+1}}} s_{i+1}^0 s_i^1 + (k_{\mathrm{off_{i+1}}} + k_{\mathrm{cat_{i+1}}}) y_{i+1}^0, \quad i = 1, \ldots, n-1,\\
\frac{ds_n^1}{dt} &= k_{\mathrm{cat_n}} y_n^0 - \ell_{\mathrm{on_n}} s_n^1 p_{j(n)} + \ell_{\mathrm{off_n}} y_n^1,\\
\frac{dy_i^0}{dt} &= k_{\mathrm{on_i}} s_i^0 s_{i-1}^1 - (k_{\mathrm{off_i}} + k_{\mathrm{cat_i}}) y_i^0, \quad i = 1, \ldots, n,\\
\frac{dy_i^1}{dt} &= \ell_{\mathrm{on_i}} s_i^1 p_{j(i)} - (\ell_{\mathrm{off_i}} + \ell_{\mathrm{cat_i}}) y_i^1, \quad i = 1, \ldots, n,\\
\frac{ds_0^1}{dt} &= -\frac{dy_1^0}{dt}, \qquad \frac{dp_j}{dt} = -\sum_{i \in \Lambda_j} \frac{dy_i^1}{dt}, \quad j = 1, \ldots, r.
\end{aligned}
$$

The space of linear forms yielding conservation laws has dimension $n + r + 1$, and we consider the following $n + r + 1$ linearly independent conservation relations:

$$
\begin{aligned}
s_0^1 + y_1^0 &= S_{0,tot},\\
s_i^0 + s_i^1 + y_i^0 + y_i^1 + y_{i+1}^0 &= S_{i,tot}, \quad i = 1, \ldots, n-1,\\
s_n^0 + s_n^1 + y_n^0 + y_n^1 &= S_{n,tot},\\
p_j + \sum_{i \in \Lambda_j} y_i^1 &= P_{j,tot}, \quad j = 1, \ldots, r.
\end{aligned}
\tag{3.2.1}
$$

Again, following the general procedure described in [86], we can find binomial equations that describe the concentration of the species at steady state. The con-

centration of the intermediate species satisfy these binomial equations:

$$y_i^0 - K_i\, s_{i-1}^1 s_i^0 = 0, \quad i = 1\ldots, n, \qquad y_i^1 - L_i\, p_{j(i)} s_i^1 = 0, \quad i = 1\ldots, n,$$

where $K_i = \frac{k_{\mathrm{on_i}}}{k_{\mathrm{off_i}}+k_{\mathrm{cat_i}}}$, $i = 1,\ldots,n$, $L_i = \frac{\ell_{\mathrm{on_i}}}{\ell_{\mathrm{off_i}}+\ell_{\mathrm{cat_i}}}$, $i = 1,\ldots,n$. The remaining binomials can be (algorithmically) chosen to be:

$$\tau_i\, s_i^0 s_{i-1}^1 - \nu_i\, s_i^1 p_{j(i)} = 0,\ i = 1,\ldots,n,$$

where $\tau_i = k_{\mathrm{cat_i}} K_i$, $\nu_i = \ell_{\mathrm{cat_i}} L_i$, $i = 1,\ldots,n$.

As in the previous case of two layers, we can parametrize the positive steady states by monomials. For instance, we can write the concentrations of all species in terms of s_i^1, for $i = 0, 1, \ldots, n$ and $p_1, \ldots, p_r$:

$$\begin{array}{rcrl} s_i^0 &=& G_i \frac{s_i^1 p_{j(i)}}{s_{i-1}^1}, & i = 1,\ldots,n,\\ y_i^0 &=& K_i G_i\, s_i^1 p_{j(i)}, & i = 1,\ldots,n,\\ y_i^1 &=& L_i\, s_i^1 p_{j(i)}, & i = 1,\ldots,n, \end{array}$$

where $G_i = \frac{\nu_i}{\tau_i}$ for all $i = 1,\ldots,n$.

3.2.2 Statement of our main results

Suppose first that there are two consecutive layers i_0, i_0+1, $1 \le i_0 \le n-1$, with the same phosphatase F, that is, $P_{j(i_0)} = P_{j(i_0+1)}$, and with no restriction in the other layers. As in (3.1.5), we will denote for any $j = 1,\ldots,n$:

$$A_j = \frac{\ell_{\mathrm{cat_j}}}{k_{\mathrm{cat_j}}}. \tag{3.2.2}$$

Let $\alpha_{1,i_0}, \alpha_{2,i_0}, \alpha_{3,i_0}$ and α_{4,i_0} be as in the case $n = 2$, but these constants correspond to the restriction to the two layers i_0 and i_0+1. That is:

$$\begin{aligned} \alpha_{1,i_0} =& \frac{S_{i_0,tot}}{F_{tot}} - A_{i_0+1},\\ \alpha_{2,i_0} =& (A_{i_0}+1) - \frac{S_{i_0,tot}}{F_{tot}},\\ \alpha_{3,i_0} =& \frac{A_{i_0}+1-A_{i_0+1}}{A_{i_0}}\,\frac{S_{i_0-1,tot}}{F_{tot}} - \left(\frac{S_{i_0,tot}}{F_{tot}} - A_{i_0+1}\right),\\ \alpha_{4,i_0} =& \frac{A_{i_0}+1-A_{i_0+1}}{A_{i_0+1}+1}\,\frac{S_{i_0+1,tot}}{F_{tot}} - \left(A_{i_0}+1-\frac{S_{i_0,tot}}{F_{tot}}\right), \end{aligned}$$

where the value of E_{tot} in the case $n = 2$ now corresponds to the value $S_{i_0-1,tot}$ and $F_{tot} = P_{j(i_0),tot} = P_{j(i_0+1),tot}$. We have the following result:

Theorem 3.2.1. *Suppose $n \ge 3$, and suppose that there are two consecutive layers i_0, i_0+1, with $1 \le i_0 \le n-1$, with the same phosphatase and with no restriction*

in the other layers. Let A_{i_0}, A_{i_0+1} be as in (3.2.2)*. Assume that the reaction rate constants satisfy*

$$A_{i_0} + 1 > A_{i_0+1} \tag{3.2.3}$$

and the total concentration constants satisfy the inequalities $\alpha_{1,i_0}, \alpha_{2,i_0}, \alpha_{3,i_0}, \alpha_{4,i_0} > 0$, that is:

$$A_{i_0} + 1 > \frac{S_{i_0,tot}}{F_{tot}} > A_{i_0+1}, \quad \frac{S_{i_0-1,tot}}{F_{tot}} > \left(\frac{S_{i_0,tot}}{F_{tot}} - A_{i_0+1}\right) \frac{A_{i_0}}{A_{i_0} + 1 - A_{i_0+1}},$$

$$\frac{S_{i_0+1,tot}}{F_{tot}} > \left(A_{i_0} + 1 - \frac{S_{i_0,tot}}{F_{tot}}\right) \frac{A_{i_0+1} + 1}{A_{i_0} + 1 - A_{i_0+1}},$$

or

$$A_{i_0} + 1 < A_{i_0+1}, \ \alpha_{1,i_0}, \alpha_{2,i_0}, \alpha_{3,i_0}, \alpha_{4,i_0} < 0.$$

Then, there exists a rescaling in the constants $k_{on_i}, i = 1, \ldots, n$ and ℓ_{on_i}, $i = 1, \ldots, n$, such that the system has at least two positive steady states.

We will give an explicit rescaling in the proof.

Remark 3.2.2. In the statement of Theorem 3.2.1 we have conditions which are similar to those in the case $n = 2$, but depending on the reaction rate constants corresponding to the layers i_0 and $i_0 + 1$ and total conservation constants. Again, the two sets of inequalities in the statement of Theorem 3.2.1 are clearly compatible.

For $n \geq 3$, there is not only an increase in the number of variables but also in the number of conservation laws. The idea of the proof of Theorem 3.2.1 is to extend the simplices that appear in the proof of Theorem 3.1.1 to simplices in the higher dimensional space, showing that in fact the conditions of the new simplices to be positively decorated are basically the same.

The other case is when the layers which share a phosphatase are not consecutive. Assume $i_1 < i_2$ are two non-consecutive layers sharing the same phosphatase. Assume also that there are no other layers with a common phosphatase between them (otherwise, we would be in the hyphothesis of the previous case or we could choose the indexes of these other layers). That is, there exists i_1, i_2, with $1 \leq i_1 < i_1 + 1 < i_2 \leq n$, such that $P_{j(i_1)} = P_{j(i_2)} = F$, and $P_{j(i)}$ for $i = i_1 + 1, \ldots, i_2 - 1$ are all distinct and different from F. We impose no restrictions on the phosphatases of the remaining layers layers $1, \ldots, i_1 - 1, i_2 + 1, \ldots, n$.

Consider the following rational functions β_{1,i_1,i_2}, β_{2,i_1,i_2}, β_{3,i_1,i_2} and β_{4,i_1,i_2} depending on the catalytic reaction rate constant and total concentration constants:

$$\begin{aligned}
\beta_{1,i_1,i_2} =& \frac{S_{i_1-1,tot}}{S_{i_1,tot}} - \frac{A_{i_1}}{A_{i_1+1}}, \\
\beta_{2,i_1,i_2} =& (A_{i_1} + 1) - \frac{S_{i_1,tot}}{F_{tot}}, \\
\beta_{3,i_1,i_2} =& \frac{S_{i_2-1,tot}}{F_{tot}} - (A_{i_1} + 1) \frac{S_{i_2,tot}}{F_{tot}}, \\
\beta_{4,i_1,i_2} =& \frac{S_{i_1,tot}}{F_{tot}} - \left(\frac{A_{i_1} + 1}{A_{i_2} + 1}\right) \left(A_{i_2} + 1 - \frac{S_{i_2,tot}}{F_{tot}}\right),
\end{aligned}$$

where $F_{tot} = P_{j(i_1),tot} = P_{j(i_2),tot}$.

We then have:

Theorem 3.2.3. *Suppose $n \geq 3$, and suppose there exists layers i_1, i_2, with $1 \leq i_1 < i_1 + 1 < i_2 \leq n$, such that $P_{j(i_1)} = P_{j(i_2)} = F$, $P_{j(i)}$ for $i = i_1 + 1, \ldots, i_2 - 1$ are all distinct and different from F, and with no restriction in the phosphatases of layers $1, \ldots, i_1 - 1, i_2 + 1, \ldots, n$. Assume the reaction rate constants and the total concentration constants satisfy*

$$\beta_{1,i_1,i_2},\ \beta_{2,i_1,i_2},\ \beta_{3,i_1,i_2},\ \beta_{4,i_1,i_2} > 0.$$

Then, there exists a rescaling in the constants $k_{on_i}, i = 1, \ldots, n$ and ℓ_{on_i}, $i = 1, \ldots, n$, such that the system has at least two positive steady states.

Again, we will give an explicit rescaling in the proof.

Remark 3.2.4. The inequalities in the statement of Theorem 3.2.3 are compatible. They have a similar flavour, but they are different from the conditions defining the regions of multistationarity in Theorems 3.1.1 and 3.2.1.

3.2.3 The proof of Theorem 3.2.1

Proof of Theorem 3.2.1. Without loss of generality we suppose that the phosphatase in the layers i_0 and $i_0 + 1$ is the phosphatase P_1, that we call F. We showed in (3.2.1) that we can parametrize the steady states in terms of the concentrations s_i^1, for $i = 0, \ldots, n$, f (we use f instead of p_1) and p_i, for $i = 2, \ldots, r$. To avoid unnecessary notation, in this proof we call $s_i = s_i^1$ for all $i = 0, \ldots, n$.

Consider the following set of monomials:

$$\mathcal{M} = \{s_{i_0-1}, f, s_{i_0}, s_{i_0+1}, s_{i_0}f, s_{i_0+1}f, s_{i_0}f(s_{i_0-1})^{-1}, s_{i_0+1}f(s_{i_0})^{-1}, 1\}.$$

These monomials appear in the parametrization of the concentration at steady state of the species in layers i_0 and $i_0 + 1$. Now, consider the set

$$\mathcal{M}' = \mathcal{M} \cup \{s_0, s_1, \ldots, s_{i_0-2}, s_{i_0+2}, \ldots, s_n, p_2, \ldots, p_r\}.$$

And consider also the set of all the monomials that appear in the parametrization:

$$\begin{aligned}\mathcal{M}'' = \mathcal{M}' \cup \{&s_1 p_{j(1)}, \ldots, s_{i_0-1}p_{j(i_0-1)}, s_{i_0+2}p_{j(i_0+2)}, \ldots, s_n p_{j(n)}, s_1 p_{j(1)}(s_0)^{-1}, \ldots \\ &\ldots, s_{i_0-1}p_{j(i_0-1)}(s_{i_0-2})^{-1}, s_{i_0+2}p_{j(i_0+2)}(s_{i_0+1})^{-1}, \ldots, s_n p_{j(n)}(s_{n-1})^{-1}\}.\end{aligned}$$

We have $n + r + 1$ variables: $s_0, s_1, \ldots, s_n, f, p_2, \ldots, p_r$. Consider the variables with this last order. Let $\mathcal{A}, \mathcal{A}', \mathcal{A}'' \subset \mathbb{R}^{n+r+1}$ be the subsets corresponding to the supports of the sets $\mathcal{M}, \mathcal{M}', \mathcal{M}''$ respectively, that is, the exponents of the monomials in each set.

We consider an order in $\mathcal{A}''$ given by the order in which we construct $\mathcal{M}''$: first the exponents corresponding to monomials in $\mathcal{M}$ (in that order), then the exponents

corresponding to monomials that we add to obtain $\mathcal{M}'$ (in that order), and then the rest of the exponents, in the same order as enumerated above. We have $3n + r + 2$ monomials.

As in the case $n = 2$, we replace the monomial parametrization into the conservation laws (3.2.1) and we write this system in a matricial form. We call $C \in \mathbb{R}^{(n+r+1)\times(3n+r+2)}$ the matrix of coefficients of the resulting polynomial system.

We want to find two simplices with vertices in $\mathcal{A}''$ which share a facet. Inspired by the 4-simplices that we chooses for the case $n = 2$, we take the following $(n+r+1)$-simplices:

$$\Delta_1 = \{e_{i_0},\, e_{i_0+1} + e_{n+2},\, e_{i_0+2} + e_{n+2},\, e_{i_0+2},\, 0\} \cup (\mathcal{A}' \setminus \mathcal{A}),$$
$$\Delta_2 = \{e_{i_0},\, e_{i_0+1} + e_{n+2},\, e_{i_0+2} + e_{n+2},\, e_{i_0+2} + e_{n+2} - e_{i_0+1},\, 0\} \cup (\mathcal{A}' \setminus \mathcal{A}).$$

where e_i denotes the i-th canonical vector of $\mathbb{R}^{n+r+1}$. Note that the points e_{i_0}, e_{i_0+2}, $e_{i_0+1} + e_{n+2}$, $e_{i_0+2} + e_{n+2}$, 0 correspond to the monomials s_{i_0-1}, s_{i_0+1}, $s_{i_0}f$, $s_{i_0+1}f$, 1, and the points e_{i_0}, $e_{i_0+1} + e_{n+2}$,$e_{i_0+2} + e_{n+2}$, $e_{i_0+2} + e_{n+2} - e_{i_0+1}$, 0, correspond to the monomials s_{i_0-1}, $s_{i_0}f$, $s_{i_0+1}f$, $s_{i_0+1}f(s_{i_0})^{-1}$, 1 which are in correspondence with the points of the simplices in the proof of Theorem 3.1.1.

We consider first the equations corresponding to the conservation laws with total conservation constants $S_{i_0-1,tot}$, F_{tot}, $S_{i_0,tot}$, $S_{i_0+1,tot}$ and then the equations corresponding to the conservation constants $S_{0,tot}$, $\ldots$, $S_{i_0-2,tot,}$, $S_{i_0+2,tot},\ldots$, $S_{n,tot}$, $P_{2,tot}$, $\ldots$, $P_{n,tot}$. The submatrices of C restricted to the columns corresponding to the simplex Δ_j, for $j = 1, 2$, are equal to:

$$C_{\Delta_j} = \left(\begin{array}{cccc|c} & C_j & & & 0 \\ \hline 0 & \ldots & 0 & -S_{0,tot} & \\ \vdots & \ddots & \vdots & \vdots & \\ 0 & \ldots & 0 & -S_{i_0-2,tot} & \\ 0 & \ldots & 0 & -S_{i_0+2,tot} & \\ \vdots & \ddots & \vdots & \vdots & \mathrm{Id}_{n+r-3} \\ 0 & \ldots & 0 & -S_{n,tot} & \\ 0 & \ldots & 0 & -P_{2,tot} & \\ \vdots & \ddots & \vdots & \vdots & \\ 0 & \ldots & 0 & -P_{r,tot} & \end{array}\right),$$

where C_1 is the submatrix corresponding to columns of the exponents $\{e_{i_0}, e_{i_0+1} + e_{n+2}, e_{i_0+2} + e_{n+2}, e_{i_0+2}, 0\}$ and C_2 is the submatrix corresponding to the columns of the exponents $\{e_{i_0}, e_{i_0+1} + e_{n+2}, e_{i_0+2} + e_{n+2}, e_{i_0+2} + e_{n+2} - e_{i_0+1}, 0\}$, that is:

$$C_j = \begin{pmatrix} 1 & K_{i_0}G_{i_0} & 0 & 0 & -S_{i_0-1,tot} \\ 0 & L_{i_0} & L_{i_0+1} & 0 & -F_{tot} \\ 0 & K_{i_0}G_{i_0} + L_{i_0} & K_{i_0+1}G_{i_0+1} & 0 & -S_{i_0,tot} \\ 0 & 0 & K_{i_0+1}G_{i_0+1} + L_{i_0+1} & (C_j)_{44} & -S_{i_0+1,tot} \end{pmatrix},$$

where $(C_1)_{44} = 1$ and $(C_2)_{44} = G_{i_0+1}$.

Note that the matrix C_{Δ_j} is positively spanning if and only if C_j is positively spanning, for $j = 1, 2$. Moreover, C_1 and C_2 are positively spanning if and only if the

condition of the statement holds, that is, $A_{i_0}+1 > A_{i_0+1}$ and $\alpha_{1,i_0}, \alpha_{2,i_0}, \alpha_{3,i_0}, \alpha_{4,i_0} > 0$; or $A_{i_0}+1 < A_{i_0+1}$ and $\alpha_{1,i_0}, \alpha_{2,i_0}, \alpha_{3,i_0}, \alpha_{4,i_0} < 0$.

Given $h \in \mathcal{C}_{\Delta_1,\Delta_2}$, by Theorem 2.2.11 there exists $t_0 \in \mathbb{R}_{>0}$ such that for all $0 < t < t_0$, the number of positive (nondegenerate) solutions of the scaled system, that is, the system with the same support $\mathcal{A}''$ and matrix of coefficients C_t with $(C_t)_{ij} = t^{h(\alpha_j)}c_{ij}$, with $\alpha_j \in \mathcal{A}''$ and $C = (c_{ij})$, is at least two. This system has the following form. Call $x = (s_0, s_1, \ldots, s_n, f, p_2, \ldots, p_r)$ and note that each coefficient c_{ij} is a rational function of the vector of reaction rate constants that we call $\kappa = (k_{\mathrm{on}_1}, \ell_{\mathrm{on}_1}, \ldots,)$. To emphasize this, we write $c_{ij} = c_{ij}(\kappa)$. Moreover, setting $\gamma_j = t^{h(\alpha_j)}$ for any j, we have a Laurent polynomial system of $n+r+1$ equations in $n+r+1$ variables:

$$\sum_j c_{ij}(\kappa)\,\gamma_j\,x^{\alpha_j} \;=\; 0,\; i = 1, \ldots, n+r+1. \tag{3.2.4}$$

Now, the reaction network we are considering satisfies the hypotheses of Theorem 2.5.2. Then, there exists a vector of reaction rate constants $\bar{\kappa}$ such that the number of positive solutions of system (3.2.4) coincides with the number of positive solutions of the following system:

$$\sum_j c_{ij}(\bar{\kappa})\,x^{\alpha_j} \;=\; 0,\; i = 1, \ldots, n+r+1. \tag{3.2.5}$$

We now describe the associated vector $\bar{\kappa}$ in an explicit form. We first describe the cone $\mathcal{C}_{\Delta_1,\Delta_2}$. We denote by h_j the height corresponding to $\alpha_j \in \mathcal{A}''$, for $j = 1, \ldots, 3n+r+2$ (in the order corresponding to the construction of $\mathcal{M}''$ that we described before). Let φ_1 and φ_2 be the affine linear functions which agree with h on the simplices Δ_1 and Δ_2 respectively. We can take the heights of the points of Δ_1 as zero, that is, $h_1 = h_4 = h_5 = h_6 = h_9 = 0$ and $h_j = 0$ for $j = 10, \ldots, n+r+1$, and $h_8 > 0$ (the height of the remaining point of Δ_2 which is not in Δ_1). Then, $\varphi_1(x_1, \ldots, x_{n+r+1}) = 0$ and $\varphi_2(x_1, \ldots, x_{n+r+1}) = -h_8\,x_{i_0+1} - h_8\,x_{i_0+2} + h_8\,x_{n+2}$. Moreover, h satisfies $0 = \varphi_1(\alpha) < h(\alpha)$, for all $\alpha \notin \Delta_1$ and $\varphi_2(\alpha) < h(\alpha)$, for all $\alpha \notin \Delta_2$. Then, we have:

$$\begin{aligned}
&h_8 < h_2, \quad 0 < h_3, \quad 0 < h_7,\\
&h_j > 0, \text{ for } j = n+r+7, \ldots, 3n+r+2,\\
&h_8 < h_{n+r+6+j}, \text{ for } j \in \Lambda_1, \text{with } j = 1, \ldots, i_0 - 1,\\
&h_8 < h_{n+r+4+j}, \text{ for } j \in \Lambda_1, \text{with } j = i_0+2, \ldots, n,\\
&h_8 < h_{2n+r+4+j} \text{ for } j \in \Lambda_1, \text{with } j = 1, \ldots, i_0 - 1,\\
&2h_8 < h_{2n+r+4+i}, \text{ if } i_0+2 \in \Lambda_1, \text{and } \; h_8 < h_{2n+r+2+j} \text{ for } j \in \Lambda_1, \text{with } j = i_0+3, \ldots, n.
\end{aligned}$$

Any such choice of h defines a regular subdivision that contains Δ_1 and Δ_2.

If we change the variables $\bar{f} = t^{h_2} f$, $\bar{s}_{i_0} = t^{h_3} s_{i_0}$, we consider the constants:

$$\begin{aligned}
&\overline{K_{i_0}} = t^{-h_7} K_{i_0}, \quad \overline{K_{i_0+1}} = t^{-h_3-h_8} K_{i_0+1}, \quad \overline{L_{i_0}} = t^{-h_2-h_3} L_{i_0}, \quad \overline{L_{i_0+1}} = t^{-h_2} L_{i_0+1},\\
&\overline{K_i} = t^{h_{n+r+6+i}-h_{2n+r+4+i}} K_i, i = 1, \dots, i_0 - 1,\\
&\overline{K_i} = t^{h_{n+r+4+i}-h_{2n+r+2+i}} K_i, i = i_0 + 2, \dots, n,\\
&\overline{L_i} = t^{h_{n+r+6+i}-h_2} L_i, \text{ if } i \in \Lambda_1, \ \overline{L_i} = t^{h_{n+r+6+i}} L_i, \text{ if } i \notin \Lambda_1, \text{ for } i = 1, \dots, i_0 - 1,\\
&\overline{L_i} = t^{h_{n+r+4+i}-h_2} L_i, \text{ if } i \in \Lambda_1, \ \overline{L_i} = t^{h_{n+r+4+i}} L_i, \text{ if } i \notin \Lambda_1, \text{ for } i = i_0 + 2, \dots, n,
\end{aligned} \tag{3.2.6}$$

and we keep fixed the values of the constants $k_{\mathrm{cat_{i_0}}}$, $k_{\mathrm{cat_{i_0+1}}}$, $\ell_{\mathrm{cat_{i_0}}}$ and $\ell_{\mathrm{cat_{i_0+1}}}$ and the total conservation constants $S_{i_0-1,tot}$, F_{tot}, $S_{i_0,tot}$ and $S_{i_0+1,tot}$, then the dynamical system associated with the network with these constants is system (3.2.5) with coefficients depending on the scaled reaction constants. Therefore, the cascade we are considering has at least two positive steady states for these constants.

To get the scalings in (3.2.6) it can be checked that it is enough to rescale the original constants as follows:

$$\begin{aligned}
&\overline{k_{\mathrm{on_{i_0}}}} = t^{-h_7} k_{\mathrm{on_{i_0}}}, \quad \overline{k_{\mathrm{on_{i_0+1}}}} = t^{-h_3-h_8} k_{\mathrm{on_{i_0+1}}}, \quad \overline{\ell_{\mathrm{on_{i_0}}}} = t^{-h_2-h_3} \ell_{\mathrm{on_{i_0}}}, \quad \overline{\ell_{\mathrm{on_{i_0+1}}}} = t^{-h_2} \ell_{\mathrm{on_{i_0+1}}},\\
&\overline{k_{\mathrm{on_i}}} = t^{h_{n+r+6+i}-h_{2n+r+4+i}} k_{\mathrm{on_i}}, i = 1, \dots, i_0 - 1,\\
&\overline{k_{\mathrm{on_i}}} = t^{h_{n+r+4+i}-h_{2n+r+2+i}} k_{\mathrm{on_i}}, i = i_0 + 2, \dots, n,\\
&\overline{\ell_{\mathrm{on_i}}} = t^{h_{n+r+6+i}-h_2} \ell_{\mathrm{on_i}}, \text{ if } i \in \Lambda_1, \ \overline{\ell_{\mathrm{on_i}}} = t^{h_{n+r+6+i}} \ell_{\mathrm{on_i}}, \text{ if } i \notin \Lambda_1, \text{ for } i = 1, \dots, i_0 - 1,\\
&\overline{\ell_{\mathrm{on_i}}} = t^{h_{n+r+4+i}-h_2} \ell_{\mathrm{on_i}}, \text{ if } i \in \Lambda_1, \ \overline{\ell_{\mathrm{on_i}}} = t^{h_{n+r+4+i}} \ell_{\mathrm{on_i}}, \text{ if } i \notin \Lambda_1, \text{ for } i = i_0 + 2, \dots, n.
\end{aligned}$$

□

3.2.4 The proof of Theorem 3.2.3

For simplicity and to fix ideas, we only present a proof in the case when the first and the last layer have the same phosphatase (that is, $i_1 = 1, i_2 = n$), and the other layers have all different phosphatases. We also present an explicit rescaling for this case. The general case is similar, but with a heavier notation.

Proof of Theorem 3.2.3. We call f the concentration of the phosphatase F, the phosphatase that appear in the layers 1 and n, and we call $f_i = p_{j(i)}$ for $i = 2, \dots, n-1$. By assumption the variables f_i are all distinct and different from f. We showed in (3.2.1) that we can parametrize the steady states in terms of the concentrations s_i^1, for $i = 0, \dots, n$, f and f_i, for $i = 2, \dots, n-1$. To avoid unnecessary notation, in this proof we call again $s_i = s_i^1$ for all $i = 0, \dots, n$.

We have $2n$ variables, and we consider these $2n$ variables with the following order: $s_0, s_1, \dots, s_n, f, f_2, \dots, f_{n-1}$. In the monomial parametrization there are $4n+1$ different monomials, and we consider these monomials with this order: $s_0, s_1, \dots, s_n, f, f_2, \dots, f_{n-1}, s_1 f, s_2 f_2, \dots, s_{n-1} f_{n-1}, s_n f, s_1 f (s_0)^{-1}, s_2 f_2 (s_1)^{-1}, \dots, s_{n-1} f_{n-1} (s_{n-2})^{-1}, s_n f (s_{n-1})^{-1}, 1$.

As in the previous cases, we replace the monomial parametrization into the conservation laws and we write this system in matricial form. Let $C \in \mathbb{R}^{(2n)\times(4n+1)}$

be the matrix of coefficients of this polynomial system. We call $\mathcal{A}$ the support of the system.

We want to find two simplices with vertices in $\mathcal{A}$ which share a facet. We denote $\mathcal{B} \subset \mathcal{A}$ the set of the exponents corresponding to the monomials: $s_2 f_2 (s_1)^{-1}, \ldots, s_{n-2} f_{n-2} (s_{n-3})^{-1}$, $f_2, \ldots, f_{n-1}$. We consider the two following simplices: Δ_1 given by the exponents corresponding to the monomials s_0, $s_1 f$, $s_n f$, $s_{n-1} f_{n-1} (s_{n-2})^{-1}$, s_n, 1, and the points in $\mathcal{B}$, and Δ_2 given by the exponents corresponding to the monomials s_0, $s_1 f$, $s_n f$, $s_{n-1} f_{n-1} (s_{n-2})^{-1}$, $s_n f (s_{n-1})^{-1}$, 1 and the points in $\mathcal{B}$. That is:

$$\Delta_1 = \{e_1, e_2 + e_{n+2}, e_{n+1} + e_{n+2}, e_n + e_{2n} - e_{n-1}, e_{n+1}, 0\} \cup \mathcal{B},$$
$$\Delta_2 = \{e_1, e_2 + e_{n+2}, e_{n+1} + e_{n+2}, e_n + e_{2n} - e_{n-1}, e_{n+1} + e_{n+2} - e_n, 0\} \cup \mathcal{B},$$

where e_i denotes the i-th canonical vector of $\mathbb{R}^{2n}$.

If we consider first the equations corresponding to the conservation laws with total conservation constants $S_{0,tot}$, F_{tot}, $S_{1,tot}$, $S_{n-1,tot}$, $S_{n,tot}$ and then the equations corresponding to the conservation constants $S_{2,tot}, \ldots, S_{n-2,tot}$, $F_{2,tot}, \ldots, F_{n-1,tot}$, the submatrices of C restricted to the columns corresponding to the simplices Δ_j for $j = 1, 2$ are as follows. We change the order of the columns following the order of the monomials in each simplex; the property of being positively spanning remains invariant:

$$C_{\Delta_j} = \left(\begin{array}{cccc|c|c} \multicolumn{4}{c|}{C_j} & 0 & 0 \\ \hline 0 & \ldots & 0 & -S_{2,tot} & & \\ \vdots & \ddots & \vdots & \vdots & G & 0 \\ 0 & \ldots & 0 & -S_{n-2,tot} & & \\ \hline 0 & \ldots & 0 & -F_{2,tot} & & \\ \vdots & \ddots & \vdots & \vdots & 0 & \mathrm{Id}_{n-2} \\ 0 & \ldots & 0 & -F_{n-1,tot} & & \end{array}\right),$$

where $G \in \mathbb{R}^{(n-3)\times(n-3)}$ is the diagonal matrix with $G_{ii} = G_{i+1}$, for $i = 1, \ldots, n-3$, C_1 is the submatrix corresponding to columns of the exponents $\{e_1, e_2 + e_{n+2}, e_{n+1} + e_{n+2}, e_n + e_{2n} - e_{n-1}, e_{n+1}, 0\}$ and C_2 is the submatrix corresponding to the columns of the exponents $\{e_1, e_2 + e_{n+2}, e_{n+1} + e_{n+2}, e_n + e_{2n} - e_{n-1}, e_{n+1} + e_{n+2} - e_n, 0\}$:

$$C_j = \begin{pmatrix} 1 & K_1 G_1 & 0 & 0 & 0 & -S_{0,tot} \\ 0 & L_1 & L_n & 0 & 0 & -F_{tot} \\ 0 & K_1 G_1 + L_1 & 0 & 0 & 0 & -S_{1,tot} \\ 0 & 0 & K_n G_n & G_{n-1} & 0 & -S_{n-1,tot} \\ 0 & 0 & K_n G_n + L_n & 0 & (C_j)_{55} & -S_{n,tot} \end{pmatrix}, \tag{3.2.7}$$

with $(C_1)_{55} = 1$ and $(C_2)_{55} = G_n$.

We observe that the matrix C_{Δ_j} if positively spanning if and only if C_j is positively spanning, for $i = 1, 2$. It is straightforward to check that the conditions under which C_1 and C_2 are positively spanning are equivalent to the conditions of the statement: $\beta_{1,1,n}$, $\beta_{2,1,n}$, $\beta_{3,1,n}$, $\beta_{4,1,n} > 0$.

Given $h \in \mathcal{C}_{\Delta_1,\Delta_2}$, by Theorem 2.2.11 there exists $t_0 \in \mathbb{R}_{>0}$ such that for all $0 < t < t_0$, the number of positive (nondegenerate) solutions of the scaled system,

i.e. the system with support $\mathcal{A}$ and matrix of coefficients C_t, with $(C_t)_{ij} = t^{h(\alpha_j)}c_{ij}$, (with $\alpha_j \in \mathcal{A}$, $C = (c_{ij})$) is at least two.

We now argue as in the proof of Theorem 3.2.1. We can write our system in the form (3.2.4), and since any cascade of Goldbeter-Koshland loops satisfies the hypotheses of Theorem 2.5.2, we again deduce the existence of a vector of rate constants $\bar{\kappa}$ such that the number of positive solutions of this system coincides with the number of positive solutions of the corresponding system of the form (3.2.5). In what follows, we also explicitly describe the rescaling of the parameters.

We denote by h_j the height corresponding to $\alpha_j \in \mathcal{A}$, for $j = 1, \ldots, 4n+1$, with the order of the monomials as we described before. Let φ_1 and φ_2 be the affine linear functions which agree with h on the simplices Δ_1 and Δ_2 respectively.

We can take zero heights at the points of Δ_1, that is, $h_1 = h_{2n+1} = h_{3n} = h_{4n-1} = h_{n+1} = h_{4n+1} = 0$, $h_j = 0$ for $j = n+3, \ldots, 2n$, $h_j = 0$, for $j = 3n+2, \ldots, 4n-2 = 0$ if $n > 3$ (note that if $n = 3$, $h_{3n+2} = h_{4n-1}$, already defined) and $h_{4n} > 0$ (the height of the other point of Δ_2). Then, $\varphi_1(x_1, \ldots, x_{2n}) = 0$ and $\varphi_2(x_1, \ldots, x_{2n}) = -h_{4n}\sum_{i=2}^{n+1} x_i + h_{4n}\, x_{n+2}$.

As $h \in \mathcal{C}_{\Delta_1,\Delta_2}$, we have that h satisfies $0 = \varphi_1(\alpha) < h(\alpha)$, for all $\alpha \notin \Delta_1$ and $\varphi_2(\alpha) < h(\alpha)$, for all $\alpha \notin \Delta_2$. Then, we have these conditions:

$$h_{4n} < h_{n+2}, \qquad h_j > 0, \text{ for } j = 2, \ldots, n, 2n+2, \ldots, 3n-1, 3n+1.$$

If we change the variables $\bar{s}_i = t^{h_{i+1}}s_i$, for $i = 1, \ldots, n-1$ and $\bar{f} = t^{h_{n+2}}f$, we consider the constants:

$$\begin{array}{rclcrcll} \overline{K_1} & = & t^{-h_{3n+1}}K_1, & & \overline{L_1} & = & t^{-h_2-h_{n+2}}L_1, & \\ \overline{K_i} & = & t^{h_{2n+i}-h_i}K_i, & & \overline{L_i} & = & t^{h_{2n+i}-h_{i+1}}L_i, & \text{for } i = 2, \ldots, n-1, \\ \overline{K_n} & = & t^{-h_n-h_{4n}}K_n, & & \overline{L_n} & = & t^{-h_{n+2}}L_n, & \end{array} \tag{3.2.8}$$

without altering the values of the constants k_{cat_1}, k_{cat_n}, ℓ_{cat_1}, ℓ_{cat_n} and the total conservation values, then the dynamical system associated with the network with these constants is the scaled system. Therefore, the network has at least two positive steady states for this choice of constants.

It is straightforward to check that to get the scalings in (3.2.8) it is enough to rescale the original constants as follows:

$$\begin{array}{rclcrcll} \overline{k_{\text{on}_1}} & = & t^{-h_{3n+1}}k_{\text{on}_1}, & & \overline{\ell_{\text{on}_1}} & = & t^{-h_2-h_{n+2}}\ell_{\text{on}_1}, & \\ \overline{k_{\text{on}_i}} & = & t^{h_{2n+i}-h_i}k_{\text{on}_i}, & & \overline{\ell_{\text{on}_i}} & = & t^{h_{2n+i}-h_{i+1}}\ell_{\text{on}_i}, & \text{for } i = 2, \ldots, n-1, \\ \overline{k_{\text{on}_n}} & = & t^{-h_n-h_{4n}}k_{\text{on}_n}, & & \overline{\ell_{\text{on}_n}} & = & t^{-h_{n+2}}\ell_{\text{on}_n}. & \end{array}$$

□

3.3 General statements behind our results

In the proof of Theorem 3.2.1 we extrapolated the multistationarity behaviour and the description of a region of multistationarity of a subnetwork (described in 3.1.1) to the whole network, even if it has more linearly independent conservation relations.

For this, we developed some ideas that we now abstract in Theorem 3.3.3 and that can be used in the study of other cascade mechanisms. As we remarked at the beginning of this chapter, they can be applied to describe a multistationarity region for the Ras cascade in Figure 3.1 (see [88] for details about different models), extrapolating our results about a single layer with two sequential phosphorylation cycles (see Theorem 2.4.1).

We start with a couple of lemmas. Given a lattice configuration $\mathcal{A}$, we will denote by $\mathrm{Aff}(\mathcal{A})$ the affine span of $\mathcal{A}$ consisting of of all points $\sum_{a\in\mathcal{A}} \lambda_a \cdot a$ with $\lambda_a \in \mathbb{Z}$ for all $a \in \mathcal{A}$ and $\sum_{a\in\mathcal{A}} \lambda_a = 0$.

Lemma 3.3.1. *Let $\mathcal{A} \subset \mathcal{A}' \subset \mathbb{Z}^d$ be finite point configurations, with $\mathrm{Aff}(\mathcal{A}) = \mathrm{Aff}(\mathcal{A}') = \mathbb{Z}^d$. Suppose that τ is a regular subdivision of $\mathcal{A}$. Then, there exists a regular subdivision τ' of $\mathcal{A}'$ such that $\tau \subset \tau'$. Moreover, we can choose a lifting function h' inducing τ' such that $h := h'|_{\mathcal{A}}$ induces τ.*

Proof. Let $h_\tau : \mathcal{A} \to \mathbb{R}$ be any lifting function inducing the subdivision τ. Let $h_{\mathcal{A},\mathcal{A}'} : \mathcal{A}' \to \mathbb{R}$ be any lifting function which is zero on $\mathcal{A}$ and positive otherwise. Extending h_τ by zero outside $\mathcal{A}$, we get that for $\varepsilon > 0$ small enough the function $h' := h_{\mathcal{A},\mathcal{A}'} + \varepsilon \cdot h_\tau$ induces a regular subdivision τ' of $\mathcal{A}'$ containing the cells in τ and $h \coloneqq h'|_{\mathcal{A}} = \varepsilon \cdot h_\tau$ induces τ. □

Lemma 3.3.2. *Let $\mathcal{A} \subset \mathcal{A}' \subset \mathbb{Z}^{d'}$ be finite point configurations, with $\mathrm{rkAff}(\mathcal{A}) = d < \mathrm{rkAff}(\mathcal{A}') = d'$. Assume moreover that $\mathcal{A}' \setminus \mathcal{A}$ has cardinality $d' - d$. Suppose that τ is a regular subdivision (triangulation) of $\mathcal{A}$. For each $\sigma \in \tau$ define $\sigma' = \sigma \cup (\mathcal{A}' \setminus \mathcal{A})$. Then, the collection $\tau' \coloneqq \{\sigma', \sigma \in \tau\}$ defines a regular subdivision (triangulation) of $\mathcal{A}'$. Moreover, τ' can be induced by a lifting function h' whose restriction to $\mathcal{A}$ induces τ.*

Proof. Consider a point $a \in \mathcal{A}' \setminus \mathcal{A}$. Then a is outside the hyperplane spanned by $\mathcal{A}$, that is, $a \cup \mathcal{A}$ is a pyramid over $\mathcal{A}$. It is known (see Observation 4.2.3 in [24]) that the collection $\{\sigma \cup a, \sigma \in \tau\}$ is a subdivision of $a \cup \mathcal{A}$, and it is regular if and only if τ is regular. Then, we can see $\mathcal{A}'$ as an iterated pyramid over $\mathcal{A}$ and the lemma follows by applying successively the previous fact. □

Given a matrix $D \in \mathbb{R}^{d_D \times n_D}$ and $I \subset \{1, \dots, n_D\}$, we will denote by D_I the submatrix of D corresponding to the columns indexed by I. For $i \in \{1, \dots, n_D\}$, $D(i)$ will denote the matrix obtained by removing the i-th column of D, and for $j \in \{1, \dots, d_D\}$, D_j will denote the j-th row of D.

Theorem 3.3.3. *Let $d, d' \in \mathbb{N}$ with $d \leq d'$. Let $\mathcal{A} \subset \mathcal{A}'' \subset \mathbb{Z}^{d'}$ be finite point configurations, with $\mathrm{rkAff}(\mathcal{A}) = d$, $\mathrm{rkAff}(\mathcal{A}'') = d'$. Write $\mathcal{A} = \{a_1, \dots, a_n\}$, $\mathcal{A}'' = \mathcal{A} \cup \{a_{n+1}, \dots, a_m\}$, with $m \geq d' > n$. Set $\mathcal{A}' = \mathcal{A} \cup \{a_{n+1}, \dots, a_{n+d'-d}\}$ and assume that $\mathrm{rkAff}\mathcal{A}' = d'$. Let τ be a regular subdivision of $\mathcal{A}$ induced by a lifting function h, τ' the regular subdivision of $\mathcal{A}'$ obtained as in Lemma 3.3.2, and τ'' any regular subdivision of $\mathcal{A}''$ such that $\tau' \subset \tau''$, induced by a lifting function h'', such that h'' restricted to $\mathcal{A}$ induces τ.*

Let $f_1, \dots, f_d$ be polynomials with support in $\mathcal{A}$ and coefficient matrix C of rank d. Let $f''_1, \dots, f''_d, \dots, f''_{d'}$ be polynomials with support $\mathcal{A}''$ and coefficient matrix C'' of rank d' of the form

$$\begin{pmatrix} C & 0 & D_1 \\ M & B & D_2 \end{pmatrix},$$

with $B \in \mathbb{R}^{(d'-d)\times(d'-d)}$ invertible. Assume τ has p d-simplices positively decorated by C and the determinants of the submatrices

$$\begin{pmatrix} C_I \\ (B^{-1}M_I)_j \end{pmatrix},$$

have all the same sign as $(-1)^{d+i}\det(C_I(i))$, for each $i = 1, \dots, d+1$, for each $j = 1, \dots, d'-d$, and for each subset $I \subset \{1, \dots, n\}$ which indexes a positively decorated simplex. Then, there exists $t_0 > 0$, such that for $0 < t < t_0$, the deformed system $f''_{1,t} = \cdots = f''_{d',t} = 0$, where

$$f''_{i,t}(x) = \sum_{j=1}^{m} c''_{i,j} t^{h''(a_j)} x^{a_j},$$

has at least p positive nondegenerate real roots.

Proof. The subdivision τ'' can be obtained by Lemma 3.3.1. Note that the columns of B correspond to the points in $\mathcal{A}' \setminus \mathcal{A}$.

Suppose that $\Delta \in \tau$ is a d-simplex positively decorated by C. Then $\Delta' = \Delta \cup \{a_{n+1}, \dots, a_{n+d'-d}\}$ is a d'-simplex of $\tau' \subset \tau''$. We will show that Δ' is positively decorated by C''.

Suppose that Δ is indexed by the set $I = \{i_1, \dots, i_{d+1}\}$ of $\{1, \dots, n\}$. We have to prove that the submatrix $C''_{I'}$ of C'' is positively spanning, with $I' = I \cup \{n+1, \dots, n+d'-d\}$. This is equivalent to prove that the matrix

$$G = \begin{pmatrix} \mathrm{Id_d} & 0 \\ 0 & B^{-1} \end{pmatrix} \begin{pmatrix} C_I & 0 \\ M_I & B \end{pmatrix} = \begin{pmatrix} C_I & 0 \\ B^{-1}M_I & \mathrm{Id}_{d'-d} \end{pmatrix},$$

is positively spanning, as the property of being positively spanning remains invariant under multiplication by invertible matrices.

We compute $(-1)^i \det(G(i))$ for $i = 1, \dots, d'+1$. For $i = 1, \dots, d+1$, we have:

$$(-1)^i \det(G(i)) = (-1)^i \det \begin{pmatrix} C_I(i) & 0 \\ B^{-1}M_I(i) & \mathrm{Id_{d'-d}} \end{pmatrix} = (-1)^i C_I(i),$$

which have all the same sign, because Δ is positively decorated by C. Take now $i > d+1$. Let $j \in 1, \dots, d'-d$ such that $d+1+j = i$. Moving the i-th row of G

to the row $d+2$ in $j-1$ interchanges of consecutive rows, we have:

$$\begin{aligned}(-1)^i \det(G(i)) =&(-1)^i \det \begin{pmatrix} C_I & 0 \\ B^{-1}M_I & \mathrm{Id_{d'-d}(j)} \end{pmatrix} \\ =&(-1)^{d+1+j}(-1)^{j-1} \det \begin{pmatrix} C_I & 0 \\ (B^{-1}M_I)_j & 0 \\ (B^{-1}M_I)[j] & \mathrm{Id_{d'-d-1}(j)} \end{pmatrix} \\ =&(-1)^d \det \begin{pmatrix} C_I \\ (B^{-1}M_I)_j \end{pmatrix},\end{aligned}$$

where $(B^{-1}M_I)[j]$ denotes the submatrix of $B^{-1}M_I$ obtained by removing its j-th row. For each $j = 1, \ldots, d'-d$, this determinant has the same sign as $(-1)^i C_I(i)$, for each $j = 1, \ldots, d'-d$, by hypothesis. Then, the simplex Δ' is positively decorated by C''.

We deduce that if τ has p d-simplices positively decorated by C, then τ'' has p d'-simplices positively decorated by C''. Then, by Theorem 2.2.11, there exists $t_0 > 0$, such that for $0 < t < t_0$, the system $f''_{1,t} = \cdots = f''_{d',t} = 0$, has at least p positive nondegenerate real roots. □

Remark 3.3.4. The conditions that guarantee that the p d'-simplices of τ'' are positively decorated by C'' include the conditions such that the p d-simplices of τ are positively decorated by C, plus other conditions. In the cases of cascades of Goldbeter-Koshland loops that we studied in Section 3.2, these other conditions are automatically fulfilled.

Chapter 4

Intermediates and parameter regions that give rise to $2[\frac{n}{2}]+1$ positive steady states in the n-site phosphorylation system

We introduced in Section 1.3 of Chapter 1, the distributive sequential n-site phosphorylation/dephosphorylation system, which is an important building block in networks of chemical reactions arising in molecular biology.

In [108] it is shown that for certain choices of the reaction rate constants and total conservation constants, the distributive n-site phosphorylation system can have n (resp. $n+1$) positive steady states for n odd (resp. even); that is, the system can have $2[\frac{n}{2}]+1$ positive steady states, where [.] denotes integer part. In this chapter we give open parameter regions in the space of reaction rate constants and total conservation constants that ensure these number of positive steady states, while assuming in the modeling that roughly only $\frac{1}{4}$ of the intermediates occur in the reaction mechanism. This result is based on the general framework developed in Chapter 2.

The possible number of positive steady states of the n-site phosphorylation system (for fixed total conservation constants) has been studied in several articles. For $n=2$, it is a well known fact that the number of nondegenerate positive steady states is one or three [76, 108]. The existence of bistability is proved in [62]. In [12] and [16], the authors give conditions on the reaction rate constants to guarantee the existence of three positive steady states based on tools from degree theory. This approach does not describe explicit conditions on the total conservation constants for which there is multistationarity, but the authors show how to find values of the total concentrations such that multistationarity occurs. For an arbitrary number n of phosphorylation sites, it was shown in [108] that the system has at most $2n-1$ positive steady states. In the same article, the authors showed that there exist reaction rate constants and total conservation constants such that the network has $2[\frac{n}{2}]+1$ positive steady states for any value of n.

In [48] (see also [63]) the authors showed parameter values such that for $n = 3$ the system has five positive steady states, and for $n = 4$ the system has seven positive steady states, obtaining the upper bound given in [108]. In the recent article [15] the authors show that if the n-site phosphorylation system is multistationary for a choice of rate constants and total conservation constants $(S_{tot}, E_{tot}, F_{tot})$ then for any positive real number c there are rate constants for which the system is multistationary when the total conservation constants are scaled by c. Concerning the stability, in [103] it is shown evidence that the system can have $2[\frac{n}{2}]+1$ positive steady states with $[\frac{n}{2}]+1$ of them being stable. Recently, a proof of this unlimited multistability was presented in [39], where the authors find a choice of parameters that gives the result for a smaller system, and then extend this result using techniques from singular perturbation theory.

In Section 2.4 of Chapter 2, we gave parameter regions for the occurrence of multistationarity for the n-site sequential phosphorylation system, but no more than three positive steady states are ensured. These conditions are based on a general framework to obtain multistationary regions jointly in the reaction rate constants and the total conservation constants. Our approach in this chapter uses this systematic technique.

In [42] was introduced a mathematical framework to study the dynamical properties of models that differ in how intermediates are included, see Section 1.3 of Chapter 1. More specifically, the emergence of multistationarity of the n-site phosphorylation system with less intermediates was studied in [90]. Recall hat the n-site phosphorylation network without any intermediates complexes has only one steady state for any choice of parameters. In [90], the authors proposed a criterion to characterize which subsets of intermediate complexes are responsible for multistationarity, when the network obtained by removing intermediates has a binomial steady state ideal. In particular, they show which are the minimal sets of intermediates that give rise to a multistationarity system in the n-site phosphorylation system, but they do not give information about the possible number of positive steady states, and they do not describe the parameter regions for which these subnetworks are multistationary.

In this chapter, we work with subnetworks of the sequential n-site phosphorylation system that only have intermediates in the E component (that is, in the connected component of the network where the kinase E reacts), see Definition 4.1.1. In case of the full mechanism on the E component or if we only assume that there are intermediate species that are formed between the phosphorylated substrates with *parity equal to* n (that is, roughly only $\frac{1}{4}$ of the intermediates of the n sequential phosphorylation cycle), we obtain conditions on the parameters to ensure as many positive steady states as possible. Indeed, we show in Proposition 4.1.7 that the maximum number of complex solutions to the steady state equations intersected with the linear conservation relations is always $n+1$, the maximum number of real roots is also $n+1$, that could be all positive when n is even, while only n of them can be positive when n is odd, so the maximum number of positive steady states equals $2[\frac{n}{2}]+1$ for any n. In Theorem 4.1.2 and Corollary 4.1.5, which follows

from Theorem 4.1.4, we give conditions on the parameters so that the associated phosphorylation/dephosphorylation system admits these number of positive steady states.

The Chapter is organized as follows. In Section 4.1 we state present our results for subnetworks of the n-site phosphorylation system. The main results of this section are Theorem 4.1.2 and Theorem 4.1.4. We give upper bounds in Proposition 4.1.7 and then we give the proofs of the theorems. Also, in Proposition 4.1.9 we show that a single intermediate is enough to ensure $2[\frac{n}{2}]+1$ positive steady states. This result has been independently found by Elisenda Feliu (personal communication). In Section 4.2 we present a general lifting result, Theorem 4.2.1, which is built on Theorem 4 in [42]. We apply this result to the subnetworks G_J. In Corollary 4.2.2 we give precise conditions on the reaction rate constants to lift the regions of multistationarity for the subnetworks G_J to regions of multitationarity with $2[\frac{n}{2}]+1$ positive steady states of the full n-sequential phosphorylation system. Finally, we present an Appendix in which we explain how to implement the computational approach to find new regions of multistationarity. The implementations and computations in this Appendix were made by Rick Rischter.

4.1 Results for n-site phosphorylation subnetworks

In order to state Theorem 4.1.2 and Theorem 4.1.4, the main results of this section, we need to introduce some notations.

Definition 4.1.1. *For any natural number n, we write $I_n = \{0, \dots, n-1\}$. Given $n \geq 1$, and a subset $J \subset I_n$, we denote by G_J the network whose only intermediate complexes are Y_j for $j \in J$, and none of the U_i. It is given by the following reactions:*

$$\begin{aligned} S_j + E \underset{k_{\mathrm{off_j}}}{\overset{k_{\mathrm{on_j}}}{\rightleftarrows}} Y_j \overset{k_{\mathrm{cat_j}}}{\to} & \quad S_{j+1} + E, && \text{if } j \in J, \\ S_j + E \overset{\tau_j}{\to} & \quad S_{j+1} + E, && \text{if } j \notin J, \\ S_{j+1} + F \overset{\nu_j}{\to} & \quad S_j + F, && 0 \leq j \leq n-1, \end{aligned} \tag{4.1.1}$$

where the labels of the arrows are positive numbers. We will also denote by G_J the associated differential system with mass-action kinetics.

For all these systems G_J, there are always three linearly independent conservation laws for any value of n:

$$\sum_{i=0}^{n} s_i + \sum_{j \in J} y_j = S_{tot}, \quad e + \sum_{j \in J} y_j = E_{tot}, \quad f = F_{tot}, \tag{4.1.2}$$

where the total conservation constants $S_{tot}, E_{tot}, F_{tot}$ are positive for any trajectory of the differential system which intersects the positive orthant. Note that the concentration of the phosphatase F is constant, equal to F_{tot}.

To get lower bounds on the number of positive steady states with fixed positive total conservation constants, we first consider the network G_{I_n}, that is, when all the intermediates in the E component appear. It has the following associated digraph:

$$\begin{array}{l} S_0+E \underset{k_{\mathrm{off}_0}}{\overset{k_{\mathrm{on}_0}}{\rightleftarrows}} Y_0 \overset{k_{\mathrm{cat}_0}}{\to} S_1+E \;\cdots\; \to S_{n-1}+E \underset{k_{\mathrm{off}_{n-1}}}{\overset{k_{\mathrm{on}_{n-1}}}{\rightleftarrows}} Y_{n-1} \overset{k_{\mathrm{cat}_{n-1}}}{\to} S_n+E \\ S_n+F \overset{\nu_{n-1}}{\to} S_{n-1}+F \;\cdots\; \to S_1+F \overset{\nu_0}{\to} S_0+F. \end{array} \tag{4.1.3}$$

We have the following result:

Theorem 4.1.2. *Let $n \geq 1$. With the previous notation, consider the network G_{I_n} in* (4.1.3)*, and suppose that the reaction rate constants $k_{\mathrm{cat_i}}$ and ν_i, $i = 0, \ldots, n-1$, satisfy the inequality*

$$\max_{i\ even}\left\{\frac{k_{\mathrm{cat_i}}}{\nu_i}\right\} < \min_{i\ odd}\left\{\frac{k_{\mathrm{cat_i}}}{\nu_i}\right\}.$$

For any positive values S_{tot}, E_{tot} and F_{tot} of the total conservation constants with

$$S_{tot} > E_{tot},$$

verifying the inequalities:

$$\max_{i\ even}\left\{\frac{k_{\mathrm{cat_i}}}{\nu_i}\right\} < \left(\frac{S_{tot}}{E_{tot}}-1\right)F_{tot} < \min_{i\ odd}\left\{\frac{k_{\mathrm{cat_i}}}{\nu_i}\right\}, \tag{4.1.4}$$

there exist positive constants $B_1, \ldots, B_n$ such that for any choice of positive constants $\lambda_0, \ldots, \lambda_{n-1}$ satisfying

$$\frac{\lambda_j}{\lambda_{j-1}} < B_j \quad \text{for } j = 1, \ldots, n-1, \quad \frac{1}{\lambda_{n-1}} < B_n, \tag{4.1.5}$$

rescaling of the given parameters $k_{\mathrm{on_j}}$ by $\lambda_j\, k_{\mathrm{on_j}}$, for each $j = 0, \ldots, n-1$, gives rise to a system with exactly $2[\frac{n}{2}]+1$ nondegenerate positive steady states.

Remark 4.1.3. We will also show in the proof of Theorem 4.1.2, that for any reaction rate constants and total conservation constants satisfying (4.1.4), there exist $t_0 > 0$ such that for any value of $t \in (0, t_0)$, the system G_{I_n} has exactly $2[\frac{n}{2}]+1$ nondegenerate positive steady states after modifying the constants $k_{\mathrm{on_j}}$ by $t^{j-n}k_{\mathrm{on_j}}$ for each $j = 0, \ldots, n-1$.

We now consider subnetworks G_J, with $J \subset J_n$ where

$$J_n := \{i \in I_n \,:\, (-1)^{i+n} = 1\}, \text{ for } n \geq 1, \tag{4.1.6}$$

that is, subsets J with indexes that have the *same parity as* n.

Theorem 4.1.4. *Let $n \geq 1$, and consider a subset $J \subset J_n$. Let G_J be its associated system as in* (4.1.1)*. Assume moreover that*

$$S_{tot} > E_{tot}.$$

A multistationarity region in the space of parameters for which the system G_J admits at least $1+2|J|$ positive steady states can be described as follows. Given any positive value of F_{tot}, choose any positive real numbers $k_{\mathrm{cat_j}}, \nu_j$, with $j \in J$ satisfying

$$\max_{j\in J}\left\{\frac{k_{\mathrm{cat_j}}}{\nu_j}\right\} < \left(\frac{S_{tot}}{E_{tot}} - 1\right) F_{tot}. \tag{4.1.7}$$

Then, there exist positive constants $B_1, \ldots, B_n$ such that for any choice of positive constants $\lambda_0, \ldots, \lambda_{n-1}$ satisfying

$$\frac{\lambda_j}{\lambda_{j-1}} < B_j \ \text{ for } j = 1, \ldots, n-1, \quad \frac{1}{\lambda_{n-1}} < B_n, \tag{4.1.8}$$

rescaling of the given parameters $k_{\mathrm{on_j}}$ by $\lambda_j\, k_{\mathrm{on_j}}$, for $j \in J$ and τ_j by $\lambda_j \tau_j$ if $j \notin J$ gives rise to a system with at least $1 + 2|J|$ positive steady states.

The following immediate Corollary of Theorem 4.1.4 implies that we can obtain a region in parameters space with $[\frac{n}{2}]$ intermediates, where the associated system has $2[\frac{n}{2}] + 1$ positive steady states.

Corollary 4.1.5. *Let $n \geq 1$, and consider the network G_{J_n} as in* (4.1.1)*, with J_n as in* (4.1.6)*. Assume moreover that*

$$S_{tot} > E_{tot}.$$

Then, there is a multistationarity region in the space of parameters for which the network G_{J_n} admits $2[\frac{n}{2}] + 1$ steady states (with fixed total conservation constants corresponding to the coordinates of a vector of parameters in this region), described in the statement of Theorem 4.1.4.

We chose to assume that the subnetworks we consider have intermediate species only in the E component, but of course there is a symmetry in the network interchanging E with F, each S_i with S_{n-i}, the corresponding intermediates and rate constants, and completely similar results hold if we assume that there are only intermediates in the F component.

In Subsection 4.1.3 we present the proofs of Theorems 4.1.2 and 4.1.4.

4.1.1 Parametrizing the steady states

The following lemma gives a positive parametrization of the concentration of the species at steady state for the systems G_J, in terms of the concentrations of the unphosphorylated substrate S_0 and the kinase E. It is a direct application of the general procedure presented in Theorem 4.8 in [86], and generalizes the parametrization (2.4.3) given in Section 2.4 in Chapter 2.

Lemma 4.1.6. *Given $n \geq 1$ and a subset $J \subset I_n$, consider the system G_J as in Definition 4.1.1. Denote for each $j \in J$*

$$K_j = \frac{k_{\mathrm{on_j}}}{k_{\mathrm{off_j}} + k_{\mathrm{cat_j}}}, \quad \tau_j = k_{\mathrm{cat_j}} K_j, \tag{4.1.9}$$

set $T_{-1} = 1$, and for any $i = 0, \ldots, n-1$:

$$T_i = \prod_{j=0}^{i} \frac{\tau_j}{\nu_j}. \tag{4.1.10}$$

Then, the parametrization of the concentrations of the species at a steady state in terms of s_0 and e is equal to:

$$s_i = \frac{T_{i-1}}{(F_{tot})^i}\, s_0 e^i,\ i = 1, \ldots, n, \qquad y_j = \frac{K_j\, T_{j-1}}{(F_{tot})^j}\, s_0 e^{j+1},\ j \in J, \tag{4.1.11}$$

Let $n \geq 1$ and a subset $J \subset I_n$. If we substitute the monomial parametrization of the concentration of the species at steady state (4.1.11) into the conservation laws, we obtain a system of two equations in two variables s_0 and e. We have:

$$\begin{aligned} s_0 + \sum_{j \in J} \left(\frac{T_j}{(F_{tot})^{j+1}} + \frac{K_j\, T_{j-1}}{(F_{tot})^j} \right) s_0 e^{j+1} + \sum_{j \notin J} \frac{T_j}{(F_{tot})^{j+1}}\, s_0 e^{j+1} - S_{tot} = 0, \\ e + \sum_{j \in J} \frac{K_j\, T_{j-1}}{(F_{tot})^j}\, s_0 e^{j+1} - E_{tot} = 0. \end{aligned} \tag{4.1.12}$$

We can write system (4.1.12) in matricial form:

$$C \begin{pmatrix} s_0 & e & s_0 e & s_0 e^2 & \ldots & s_0 e^n & 1 \end{pmatrix}^t = 0, \tag{4.1.13}$$

where $C \in \mathbb{R}^{2 \times (n+3)}$ is the matrix of coefficients:

$$C = \begin{pmatrix} 1 & 0 & \frac{T_0}{F_{tot}} + \beta_0 & \frac{T_1}{(F_{tot})^2} + \beta_1 & \ldots & \frac{T_{n-1}}{(F_{tot})^n} + \beta_{n-1} & -S_{tot} \\ 0 & 1 & \beta_0 & \beta_1 & \ldots & \beta_{n-1} & -E_{tot} \end{pmatrix}, \tag{4.1.14}$$

with

$$\beta_j = \frac{K_j\, T_{j-1}}{(F_{tot})^j} \text{ for } j \in J, \text{ and } \beta_j = 0 \text{ if } j \notin J. \tag{4.1.15}$$

Note that if we order the variables s_0, e, the support of the system (that is, the exponents of the monomials that occur) is the following set $\mathcal{A}$:

$$\mathcal{A} = \{(1,0), (0,1), (1,1), (1,2), \ldots, (1,n), (0,0)\} \subset \mathbb{Z}^2, \tag{4.1.16}$$

independently of the choice of $J \subset I_n$.

4.1.2 Upper bounds on the number of positive steady states

We first recall Kushnirenko Theorem, a fundamental result about sparse systems of polynomial equations, which gives a bound on the number of complex solutions with nonzero coordinates. Given a finite point configuration $\mathcal{A} \subset \mathbb{Z}^d$, denote by $\text{chull}(\mathcal{A})$ the convex hull of $\mathcal{A}$. We write vol to denote Euclidean volume, and set $\mathbb{C}^* = \mathbb{C} \setminus \{0\}$.

Kushnirenko Theorem [71]: Given a finite point configuration $\mathcal{A} \subset \mathbb{Z}^d$, a sparse system of d Laurent polynomials in d variables with support $\mathcal{A}$ has at most $d!\,\text{vol}(\text{chull}(\mathcal{A}))$ isolated solutions in $(\mathbb{C}^*)^d$ (and exactly this number if the polynomials have *generic* coefficients.)

We also recall the classical Descartes rule of signs.

Descartes rule of signs: Let $p(x) = c_0 + c_1 x + \cdots + c_m x^m$ be a nonzero univariate real polynomial with r positive real roots counted with multiplicity. Denote by s the number of sign variations in the ordered sequence of the signs $\text{sign}(c_0), \ldots, \text{sign}(c_m)$ of the coefficients, i.e., discard the 0's in this sequence and then count the number of times two consecutive signs differ. Then $r \leq s$ and r and s have the same parity, which is even if $c_0 c_m > 0$ and odd if $c_0 c_m < 0$.

We then have that $2[\frac{n}{2}] + 1$ is an upper bound for the number of positive real solutions of the system of equations defining the steady states of any system G_J in Definition 4.1.1:

Proposition 4.1.7. *For any choice of rate constants and total conservation constants, the dynamical system G_J associated with any subset $J \subset I_n$ has at most $2[\frac{n}{2}] + 1$ isolated positive steady states. In fact, the polynomial system of equations defining the steady states of G_J can have at most $n+1$ isolated solutions in $(\mathbb{C}^*)^d$.*

Proof. The number of positive steady states of the subnetwork G_J is the number of positive solutions of the sparse system (4.1.12) of two equations and two variables. The support of the system is (4.1.16) whose convex hull has Euclidean volume equal to $\frac{n+1}{2}$. By Kushnirenko Theorem, the number of isolated solutions in $(\mathbb{C}^*)^2$ is at most $2!\frac{(n+1)}{2} = n+1$. In particular, the number of isolated positive solutions is at most $n+1$.

Moreover, when all positive solutions are nondegenerate, their number is necessarily odd by Corollary 2 in [12], which is based on the notion of Brouwer's degree. Indeed, in our case, we can bypass the condition of nondegeneracy because we can use Descartes rule of signs in one variable. In fact, from the first equation of system (4.1.12), we can write:

$$s_0 = \frac{S_{tot}}{p(e)}, \tag{4.1.17}$$

where $p(e)$ is the following polynomial of degree n on the variable e:

$$p(e) := 1 + \sum_{i=0}^{n-1} (\alpha_i + \beta_i) e^{i+1}, \tag{4.1.18}$$

with

$$\alpha_i = \frac{T_i}{(F_{tot})^{i+1}}, \quad i = 0, \dots, n-1, \tag{4.1.19}$$

and $\beta_i = \frac{K_j T_{j-1}}{(F_{tot})^j}$ if $j \in J$ or $\beta_j = 0$ if $j \notin J$ were defined in (4.1.15). Note that for any $e > 0$ it holds that $p(e) > 0$, and so $s_0 > 0$. If we replace (4.1.17) in the second equation of (4.1.12), we have:

$$e + \sum_{i=0}^{n-1} \beta_i \frac{S_{tot}}{p(e)} e^{i+1} - E_{tot} = 0. \tag{4.1.20}$$

The number of positive solutions of the equation (4.1.20) is the same if we multiply by $p(e)$:

$$q(e) \coloneqq e\, p(e) + \sum_{i=0}^{n-1} \beta_i S_{tot} e^{i+1} - E_{tot}\, p(e) = 0. \tag{4.1.21}$$

This last polynomial q has degree $n+1$, with leading coefficient equal to $\alpha_{n-1} + \beta_{n-1} > 0$ and constant coefficient equal to $-E_{tot} < 0$. The sign variation of the coefficients of q has the same parity as the sign variation of the leading coefficient and the constant coefficient, which is one. So, by Descartes rule of signs, as the sign variation is odd, the number of positive solutions is also odd. □

4.1.3 Proofs of Theorems 4.1.2 and 4.1.4

We start this section with a lemma.

Lemma 4.1.8. *Consider* $\mathcal{A} = \{(1,0),(0,1),(1,1),(1,2),\dots,(1,n),(0,0)\} \subset \mathbb{Z}^2$. *The triangulation* Γ *of* $\mathcal{A}$ *with the following* 2*-simplices:*

$$\{\{(1,j),(1,j+1),(0,0)\}_{j=0,\dots,n-1}, \{(0,1),(1,n),(0,0)\}\}$$

is regular (see Figure 4.1).

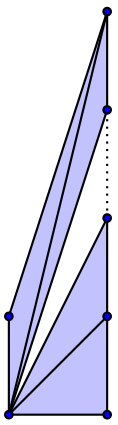


Figure 4.1: Triangulation Γ of $\mathcal{A}$.

Proof. We can take $h\colon \mathcal{A} \to \mathbb{R}$, with $h(0,0) = 0$, $h(0,1) = n$ and $h(1,j) = \frac{j(j-1)}{2}$, for $j = 0, \ldots, n-1$. It is easy to check h defines a regular triangulation that is equal to Γ.

□

The idea in the proofs of Theorem 4.1.2 and Theorem 4.1.4 is to detect positively decorated simplices in the regular triangulation Γ.

Proof of Theorem 4.1.2. By Proposition 4.1.7, the number of positive solutions of the system G_{I_n} is at most $2[\frac{n}{2}] + 1$. So, it is enough to prove that this number is also a lower bound.

The number of positive steady states of the system G_{I_n} is the number of positive solutions of the system (4.1.12). As we saw before, the support of this last system is

$$\mathcal{A} = \{(1,0), (0,1), (1,1), (1,2), \ldots, (1,n), (0,0)\} \subset \mathbb{Z}^2,$$

with coefficient matrix C (4.1.14). Note that if one multiplies a column of C by a positive number, then a simplex is positively decorated by C if and only if it is positively decorated by the new matrix. After multiplying the columns by convenient positive numbers, we obtain the following matrix from C:

$$Csimple = \begin{pmatrix} 1 & 0 & M_0 & \ldots & M_{n-1} & -S_{tot} \\ 0 & 1 & 1 & \ldots & 1 & -E_{tot} \end{pmatrix},$$

where $M_i = \frac{k_{\mathrm{cat_i}}}{\nu_i F_{tot}} + 1$, for each $i = 0, \ldots, n-1$. We will work with this new matrix $Csimple$.

We consider the regular triangulation Γ in Lemma 4.1.8.

The simplex $\{(1,0), (1,1), (0,0)\}$ of Γ is positively decorated by $Csimple$ if and only if $E_{tot}M_0 - S_{tot} < 0$. The simplex $\{(1,j), (1,j+1), (0,0)\}$, for $j = 1, \ldots, n-1$, corresponds to the submatrix

$$Csimple_j = \begin{pmatrix} M_{j-1} & M_j & -S_{tot} \\ 1 & 1 & -E_{tot} \end{pmatrix},$$

and it is positively decorated by $Csimple$ if and only if $E_{tot}M_{j-1} - S_{tot}$ and $E_{tot}M_j - S_{tot}$ have opposite signs. The last simplex $\{(0,1), (1,n), (0,0)\}$ is positively decorated by $Csimple$ if and only if $E_{tot}M_{n-1} - S_{tot} > 0$.

Therefore we always have at least n positively decorated simplices using all simplices of Γ but the last one, just by imposing

$$(E_{tot}M_i - S_{tot})(-1)^i < 0, \quad \text{for } i = 0, \ldots, n-1. \tag{4.1.22}$$

We can include the last simplex if and only if n is even (because otherwise the inequalities are not compatible), and in this case we have at least $n+1$ positively decorated simplices. We can obtain $2[\frac{n}{2}] + 1$ positively decorated simplices if the inequalities (4.1.22) are satisfied. These inequalities are equivalent to the inequalities (4.1.4) in the statement.

Assume (4.1.4) holds. Given any height function h inducing the triangulation Γ, by Theorem 2.2.11 there exists t_0 in $\mathbb{R}_{>0}$ such that for all $0 < t < t_0$, the number of positive nondegenerate solutions of the deformed system as in (2.2.5) with support $\mathcal{A}$ and coefficient matrix C_t, with $(C_t)_{ij} = t^{h(\alpha_j)}c_{ij}$ (with $\alpha_j \in \mathcal{A}$, $C = (c_{ij})$), is at least $2[\frac{n}{2}] + 1$. In particular if we choose h as in the proof of Lemma 4.1.8, there exists t_0 in $\mathbb{R}_{>0}$, such that for all $0 < t < t_0$, the system

$$\begin{aligned} s_0 + \sum_{j=0}^{n-1} \left(\frac{T_j}{(F_{tot})^{j+1}} + \frac{K_j\, T_{j-1}}{(F_{tot})^j} \right) t^{\frac{j(j+1)}{2}} s_0 e^{j+1} - S_{tot} = 0, \qquad (4.1.23) \\ t^n e + \sum_{j=0}^{n-1} \frac{K_j\, T_{j-1}}{(F_{tot})^j}\, t^{\frac{j(j+1)}{2}} s_0 e^{j+1} - E_{tot} = 0, \end{aligned}$$

has at least $2[\frac{n}{2}]+1$ positive solutions. If we change the variable $\bar{e} = t^n e$, we get the following system:

$$\begin{aligned} s_0 + \sum_{j=0}^{n-1} \left(\frac{T_j}{(F_{tot})^{j+1}} + \frac{K_j\, T_{j-1}}{(F_{tot})^j} \right) t^{(j+1)(\frac{j}{2}-n)} s_0 \bar{e}^{j+1} - S_{tot} = 0, \qquad (4.1.24) \\ \bar{e} + \sum_{j=0}^{n-1} \frac{K_j\, T_{j-1}}{(F_{tot})^j}\, t^{(j+1)(\frac{j}{2}-n)} s_0 \bar{e}^{j+1} - E_{tot} = 0. \end{aligned}$$

It is straightforward to check that if we scale the constants K_j by

$$t^{j-n} K_j, \quad j = 0, \ldots, n-1, \qquad (4.1.25)$$

while keeping fixed the values of the constants $k_{\mathrm{cat_j}}$, ν_j for $j = 0, \ldots, n-1$ and the values of the total conservation constants E_{tot}, F_{tot} and S_{tot} (assuming that condition (4.1.4) holds), the intersection of the steady state variety and the total conservation equations of the corresponding network is described by system (4.1.24). It is easy to check that in order to get the scaling in (4.1.25), it is sufficient to rescale only the original constants $k_{\mathrm{on_j}}$ as follows: $t^{j-n}k_{\mathrm{on_j}}$, for $j = 0, \ldots, n-1$. Then, for these choices of constants, the system has at least $2[\frac{n}{2}] + 1$ positive steady states.

Now, we will show how to obtain the more general rescaling in the statement. The existence of the positive constants $B_1, \ldots, B_n$ follows from the inequalities that define the cone $\mathcal{C}_\Gamma$ of heights inducing the regular triangulation Γ and Theorem 2.2.13. For instance, we can check that $\mathcal{C}_\Gamma$ is defined by n inequalities:

$$\mathcal{C}_\Gamma = \{h = (h_1, \ldots, h_{n+3}) \in \mathbb{R}^{n+3} \,:\, \langle m_j, h\rangle > 0,\, j = 1, \ldots, n\},$$

where $m_1 = e_1 - 2e_3 + e_4$, $m_j = e_{j+1} - 2e_{j+2} + e_{j+3}$, for $j = 2, \ldots, n-1$ and $m_n = e_2 + e_{n+1} - e_{n+2} - e_{n+3}$, where e_i denotes the i-th canonical vector of $\mathbb{R}^{n+3}$. Fix $\varepsilon \in (0,1)^{n+3}$. As (4.1.4) holds, Theorem 2.2.13 says that there exist positive numbers B_j for $j = 1, \ldots, n$ (depending on ε), such that the system

$$\begin{aligned} \gamma_1 s_0 + \sum_{j=0}^{n-1} \left(\frac{T_j}{(F_{tot})^{j+1}} + \frac{K_j\, T_{j-1}}{(F_{tot})^j} \right) \gamma_{j+3} s_0 e^{j+1} - \gamma_{n+3} S_{tot} = 0, \qquad (4.1.26) \\ \gamma_2 e + \sum_{j=0}^{n-1} \frac{K_j\, T_{j-1}}{(F_{tot})^j}\, \gamma_{j+3} s_0 e^{j+1} - \gamma_{n+3} E_{tot} = 0, \end{aligned}$$

has at least $2[\frac{n}{2}]+1$ nondegenerate positive solutions, for any vector $\gamma \in \mathbb{R}^{n+3}$ satisfying $\gamma^{m_j} < B_j$, for all $j = 1, \ldots, n$. In particular, this holds if we take $\gamma_1 = \gamma_2 = \gamma_{n+3} = 1$ and

$$\gamma_3^{-2}\gamma_4 < B_1, \quad \gamma_{j+1}\gamma_{j+2}^{-2}\gamma_{j+3} < B_j, \text{ for } j = 2, \ldots, n-1, \quad \gamma_{n+1}\gamma_{n+2}^{-1} < B_n. \quad (4.1.27)$$

If we call $\lambda_0 = \gamma_3$ and $\lambda_j = \frac{\gamma_{j+3}}{\gamma_{j+2}}$ for $j = 1, \ldots, n-1$, the inequalities in (4.1.27) are equivalent to the conditions (4.1.5). Then, if λ_j, $j = 0, \ldots, n-1$, satisfy these inequalities, the rescaling of the given parameters $k_{\mathrm{on_j}}$ by $\lambda_j k_{\mathrm{on_j}}$ for $j = 0, \ldots, n-1$, gives rise to a system with exactly $2[\frac{n}{2}]+1$ positive steady states. □

The proof of Theorem 4.1.4 is similar to the previous one.

Proof of Theorem 4.1.4. Again, the number of positive steady states of our system is equal to the number of positive solutions of the system (4.1.12). Recall that the support of the system is

$$\mathcal{A} = \{(1,0), (0,1), (1,1), (1,2), \ldots, (1,n), (0,0)\} \subset \mathbb{Z}^2.$$

In this case, the coefficient matrix C (4.1.14) is equal, after multiplying the columns by convenient positive numbers, to the matrix

$$Csimple = \begin{pmatrix} 1 & 0 & M_0 & \ldots & M_{n-1} & -S_{tot} \\ 0 & 1 & D_0 & \ldots & D_{n-1} & -E_{tot} \end{pmatrix},$$

where $M_i = \frac{k_{\mathrm{cat_i}}}{\nu_i F_{tot}} + 1$ and $D_i = 1$, for each $i \in J$, and $M_i = 1$ and $D_i = 0$, for each $i \notin J$.

We consider again the regular triangulation Γ in Lemma 4.1.8. Recall that $J \subset J_n$, see (4.1.6), and therefore each $j \in J$ has the same parity as n, in particular $0 \leq j \leq n-2$. For each $j \in J$, consider the simplices $\Delta_j = \{(1,j), (1,j+1), (0,0)\}$ and $\Delta_{j+1} = \{(1,j+1), (1,j+2), (0,0)\}$. Note that if $j \neq j'$ then $\{\Delta_j, \Delta_{j+1}\}$ and $\{\Delta_{j'}, \Delta_{j'+1}\}$ are disjoint since j, j' and n have the same parity.

The simplices are positively decorated by $Csimple$ (and then by C) if and only if the submatrices

$$Csimple_j = \begin{pmatrix} 1 & M_j & -S_{tot} \\ 0 & 1 & -E_{tot} \end{pmatrix}, \quad Csimple_{j+1} = \begin{pmatrix} M_j & 1 & -S_{tot} \\ 1 & 0 & -E_{tot} \end{pmatrix},$$

are positively spanning, and this happens if and only if $E_{tot}M_j - S_{tot} < 0$, where $M_j = \frac{k_{\mathrm{cat_j}}}{\nu_j F_{tot}} + 1$, since $j \in J$. The simplex $\Delta_n = \{(0,1), (1,n), (0,0)\}$ is trivially positively decorated by $Csimple$. Then, by imposing the inequalities $E_{tot}M_j - S_{tot} < 0$ for $j \in J$, which are equivalent to the ones in the statement (4.1.7), we can obtain $2|J|+1$ positively decorated simplices.

Assume (4.1.7) holds. Given any height function h inducing the triangulation Γ, by Theorem 2.2.11 there exists t_0 in $\mathbb{R}_{>0}$, such that for all $0 < t < t_0$, the number of positive nondegenerate solutions of the deformed system with support $\mathcal{A}$ and coefficient matrix C_t, with $(C_t)_{ij} = t^{h(\alpha_j)}c_{ij}$ (with $\alpha_j \in \mathcal{A}$, $C = (c_{ij})$) is at least

$2|J|+1$. In particular if we choose h as in the proof of Lemma 4.1.8, there exists t_0 in $\mathbb{R}_{>0}$, such that for all $0<t<t_0$, the system

$$s_0+\sum_{j\in J}\left(\frac{T_j}{(F_{tot})^{j+1}}+\frac{K_j\,T_{j-1}}{(F_{tot})^{j}}\right)t^{\frac{j(j+1)}{2}}s_0e^{j+1}+\sum_{j\notin J}\frac{T_j}{(F_{tot})^{j+1}}\,t^{\frac{j(j+1)}{2}}s_0e^{j+1}-S_{tot}=0, \quad (4.1.28)$$

$$t^ne+\sum_{j\in J}\frac{K_j\,T_{j-1}}{(F_{tot})^{j}}\,t^{\frac{j(j+1)}{2}}s_0e^{j+1}-E_{tot}=0,$$

has at least $2|J|+1$ positive solutions. If we change the variable $\bar{e}=t^ne$, we get the following system:

$$s_0+\sum_{j\in J}\left(\frac{T_j}{(F_{tot})^{j+1}}+\frac{K_j\,T_{j-1}}{(F_{tot})^{j}}\right)t^{(j+1)(\frac{j}{2}-n)}s_0\bar{e}^{j+1}+$$

$$+\sum_{j\notin J}\frac{T_j}{(F_{tot})^{j+1}}\,t^{(j+1)(\frac{j}{2}-n)}s_0\bar{e}^{j+1}-S_{tot}=0, \quad (4.1.29)$$

$$\bar{e}+\sum_{j\in J}\frac{K_j\,T_{j-1}}{(F_{tot})^{j}}\,t^{(j+1)(\frac{j}{2}-n)}s_0\bar{e}^{j+1}-E_{tot}=0.$$

Similarly as we did in the previous proof, if we scale the original parameters $k_{\mathrm{on_j}}$, for $j\in J$, and τ_j if $j\notin J$ by

$$t^{j-n}k_{\mathrm{on_j}} \text{ if } j\in J, \quad t^{j-n}\tau_j \text{ if } j\notin J,$$

respectively, and if we keep fixed the values of the remaining rate constants and the values of the total conservation constants E_{tot}, F_{tot} and S_{tot}, the intersection of the steady state variety and the linear conservation relations is described by system (4.1.29). Then, for these choices of constants the system G_J has at least $2|J|+1$ positive steady states. The general rescaling that appears in the statement can be obtained in a similar way as we did in the proof of Theorem 4.1.2. □

4.1.4 One intermediate is enough in order to obtain $2[\frac{n}{2}]+1$ positive steady states in the n-site phosphorylation system

Proposition 4.1.9 shows that having a single intermediate is enough to get $2[\frac{n}{2}]+1$ positive steady states, for particular choices of the reaction rate constants. This says that while Corollary 4.1.5 is optimal, the regions obtained for any subset J with indexes of the same parity of n in Theorem 4.1.4 properly contained in J_n, only ensure $2|J|+1$ positive steady states. However, note that we are able to describe open regions in parameter space and Proposition 4.1.9 only allows us to get choices of parameters. This computation by reduction to the univariate case is not systematic as the general approach that we use to describe multistationarity regions in Theorems 4.1.2 and 4.1.4, which can be applied to study other quite different mechanisms. As we mentioned before, the following result has been independently found by Elisenda Feliu (personal communication).

Proposition 4.1.9. *If $J=\{0\}$, then there exists parameter values such that the system G_J admits $2[\frac{n}{2}]+1$ positive steady states.*

Proof. Assume n is even, then $n+1$ is odd. As we did in the proof of Proposition 4.1.7, the positive solutions of the system (4.1.12) are in bijection with the positive solutions of the polynomial $q(e)$ in (4.1.21). Here $\beta_0 = K_0$ and $\beta_i = 0$ for $i \neq 0$. We will consider the polynomial $\tilde{q}(e) := \frac{q(e)}{E_{tot}}$, with constant coefficient equal to -1.

Consider any polynomial of degree $n+1$

$$c_{n+1}e^{n+1} + c_n e^n + \cdots + c_1 e - 1, \tag{4.1.30}$$

with $n+1$ distinct positive roots, and with constant term equal to -1. Then, we have that $c_i(-1)^{i+1} > 0$, and in particular, $c_{n+1} > 0$.

Our goal is to find reaction rate constants and total conservation constants such that the polynomial (4.1.30) coincides with the polynomial $\tilde{q}(e)$. Comparing the coefficient of e^i, for $i = 1, \ldots, n+1$ in both polynomials, we need to have:

$$\begin{aligned} \frac{\alpha_{n-1}}{E_{tot}} &= c_{n+1}, \\ \frac{\alpha_{i-2}}{E_{tot}} - \alpha_{i-1} &= c_i, \text{ for } i = 3, \ldots, n, \\ \frac{\alpha_0 + K_0}{E_{tot}} - \alpha_1 &= c_2, \\ \frac{1 + S_{tot}K_0}{E_{tot}} - (\alpha_0 + K_0) &= c_1. \end{aligned} \tag{4.1.31}$$

Keep in mind that the values of c_i are given. We may solve (4.1.31) in terms of E_{tot} and of the $c_i, i = 1, \ldots, n+1$:

$$\begin{aligned} \alpha_{n-1-k} &= E_{tot} \sum_{i=0}^{k} c_{n+1-i} \, (E_{tot})^{k-i}, \text{ for each } k = 0, 1, \ldots, n-2, \\ \alpha_0 + K_0 &= E_{tot} \sum_{i=0}^{n-1} c_{n+1-i} \, (E_{tot})^{n-1-i}, \\ 1 + S_{tot}K_0 &= E_{tot} \sum_{i=0}^{n} c_{n+1-i} \, (E_{tot})^{n-i}. \end{aligned} \tag{4.1.32}$$

Note that if we take any value for $E_{tot} > 0$, then the values of α_i for $i = 1, \ldots, n-1$, $\alpha_0 + K_0$ and $S_{tot}K_0$ are completely determined. So, we find an appropriate value of E_{tot} such that the previous values α_i, K_0 and S_{tot} are all positive. For that, we choose $K_0 = 1$, and we take E_{tot} large enough such that

$$\begin{aligned} \sum_{i=0}^{k} c_{n+1-i} \, (E_{tot})^{k-i} &> 0, \text{ for each } k \in \{0, 1, \ldots, n-2\} \text{ with } k \text{ odd}, \\ E_{tot} \sum_{i=0}^{n-1} c_{n+1-i} \, (E_{tot})^{n-1-i} &> 1, \quad E_{tot} \sum_{i=0}^{n-1} c_{n+1-i} \, (E_{tot})^{n-i} > 1. \end{aligned}$$

This is possible since $c_{n+1} > 0$ and that imposing the first condition just on k odd is enough to ensure that it holds for all $k \in I_{n-1}$ as well because of the signs of the

c_i. With these conditions, and using the equations (4.1.32), the values of α_i for each $i = 0, \ldots, n-1$ and S_{tot} are determined and are all positive.

Now, it remains to show that we can choose reaction rate constants such that the values of $\alpha_i, i = 0, \ldots, n-1$ are the given ones. Recall that $\alpha_i = \frac{T_i}{(F_{tot})^{i+1}}$, where $T_i = \prod_{j=0}^{i} \frac{\tau_j}{\nu_j}$ for $i = 0, \ldots, n-1$ and $T_{-1} = 1$, where $\tau_0 = k_{\text{cat}_0} K_0 = k_{\text{cat}_0}$ (we have chosen $K_0 = 1$). Take for example $F_{tot} = 1$, $k_{\text{on}_0} = 2$, $k_{\text{off}_0} = 1$ and $k_{\text{cat}_0} = 1$ (to obtain $K_0 = 1$). Then, $\tau_0 = 1$, so we take $\nu_0 = \frac{1}{\alpha_0}$. As $\alpha_{i+1} = \alpha_i \frac{\tau_{i+1}}{\nu_{i+1}}$, for $i = 0, \ldots, n-2$, we can choose any positive values of τ_{i+1}, ν_{i+1} such that $\frac{\tau_{i+1}}{\nu_{i+1}} = \frac{\alpha_{i+1}}{\alpha i}$, and we are done.

When n is odd, with a similar argument, we can find reaction rate constants and total conservation constants such that the polynomial $\tilde{q}(e)$ gives a polynomial like (4.1.30) (but with n distinct positive roots and one negative root). □

4.2 Lifting regions of multistationarity

The main result in this section is Theorem 4.2.1, which is built on Theorem 4 in [42]. In this last paper, the authors proved that if a network has m non-degenerate stoichiometrically compatible steady states, then any extended network that realizes the rate constants has at least m non-degenerate stoichiometrically compatible steady states. Here we present basically the same result, but also we describe more precisely regions in the space of parameters for which we can lift the steady states of the reduced network to the original network. We apply this result to the subnetworks G_J. In Theorem 4.2.2 we give precise conditions on the rate constants to lift the regions of multistationarity for the subnetworks G_J to regions of multitationarity with $2[\frac{n}{2}] + 1$ positive steady states (with fixed total conservation constants) of the complete n-sequential phosphorylation cycle.

4.2.1 A result on lifting multistationarity

Before present the statement of Theorem 4.2.1, we introduce the following notation. Consider a chemical reaction network G with mass-action kinetics. In order to analyze steady states within a stoichiometric compatibility class, in this subsection we use the conservation laws in place of redundant steady-state equations, as follows. Recall that a conservation-law matrix W of G is row-reduced. Let $I = \{i_1, \ldots, i_d\}$ be the indices of the first nonzero coordinate of the rows of W, and assume that $i_1 < i_2 < \cdots < i_d$. Given $c \in \mathbb{R}^d$ and reaction rate constants κ, define the function $f_{c,\kappa} : \mathbb{R}^s_{\geq 0} \to \mathbb{R}^s$ by

$$f_{c,\kappa,i} = f_{c,\kappa}(x)_i = \begin{cases} f_{\kappa,i}(x) & \text{if } i \notin I, \\ (Wx - c)_k & \text{if } i = i_k \in I, \end{cases} \tag{4.2.1}$$

where f_κ is the species formation rate function of G as in (1.1.4), with reaction rate constants κ, that is:

$$f_\kappa(x) = \sum_{y \to y' \in \mathscr{R}_G} \kappa_{yy'} x^y (y' - y). \tag{4.2.2}$$

We refer to system (4.2.1) as the system (4.2.2) *augmented by conservation laws*.

We present Theorem 4.2.1. Here we use the notation introduced in Section 1.3.

Theorem 4.2.1. *Let G be a chemical reaction network, with set of species $\mathscr{S}_G$ and reaction rate constants $\kappa^0 = (\kappa^0_{yy'})$. Fix a subset of intermediate species $\mathcal{I} \subset \mathscr{S}_G$. Let G' be the network obtained from G removing the intermediate species from $\mathcal{I}$, with reaction rate constants $\tau^0 = (\tau_{yy'}(\kappa^0))$ as in (1.3.4). Consider the fiber $\mathcal{F}_{\tau_0} = \{\kappa > 0 : \tau(\kappa) = \tau^0\}$ and the open set $\mathcal{W}_\varepsilon = \{\kappa > 0 : \mu_{i,y}(\kappa) < \varepsilon \ \forall y \in \mathscr{C}_{G'}, i = 1, \dots, p\}$, for any $\varepsilon > 0$, with $\mu_{i,y}$ as in (1.3.3). Fix $c_1, \dots, c_d \in \mathbb{R}$ and consider the stoichiometric compatibility class $\mathcal{S}_c$ defined by the equations $\ell_1(x) = c_1, \dots, \ell_d(x) = c_d$, where $\ell_1(x), \dots, \ell_d(x)$ is a basis of conservations laws of the system associated with G'.*

Then,

1. *$\mathcal{F}_{\tau_0,\varepsilon} := \mathcal{F}_{\tau_0} \cap \mathcal{W}_\varepsilon$ is a nonempty open set of $\mathcal{F}_{\tau_0}$ for all $\varepsilon > 0$.*
2. *If G' has m nondegenerate positive steady states in $\mathcal{S}_c$, there exists $\varepsilon_0 > 0$ such that for all $\kappa \in \mathcal{F}_{\tau_0,\varepsilon}$, with $0 < \varepsilon < \varepsilon_0$, there are at least m non degenerate positive steady states of G with reaction rate constants κ in the stoichiometric class of the system associated with G defined by $\bar{\ell}_1(x, u) = c_1 \dots, \bar{\ell}_d(x, u) = c_d$, where $\bar{\ell}_1, \dots, \bar{\ell}_d$ is a basis of conservation laws of the system associated with G obtained from $\ell_1(x), \dots, \ell_d(x)$ as in (1.3.2).*

Proof. The proof is heavily based on the proof of Theorem 4 in [42]. Suppose that the subset $\mathcal{I}$ has p intermediate species $U_1, \dots, U_p$, and the set of species $\mathscr{S}_G$ is ordered as: $X_1, \dots, X_n, U_1, \dots, U_p$. Then the set of species of G' is equal to $\mathscr{S}_{G'} = \{X_1, \dots, X_n\}$.

The first part of the statement follows from a construction of reaction rate constants given in the proof of Theorem 4 in [42]. That construction is the following. Given the reaction rate constants $\kappa^0 = (\kappa^0_{yy'})$, for $\theta \in \mathbb{R}_{>0}$ we define a new set of rate constants $\kappa^\theta = (\kappa^\theta_{yy'})$ by $\kappa^\theta_{yy'} = \kappa^0_{yy'}/\theta$ if y is an intermediate species of $\mathcal{I}$ and $\kappa^\theta_{yy'} = \kappa^0_{yy'}$ otherwise. In this case, by Theorem 2 and 3 in [42] (presented in Section 1.3 in Chapter 1), and using the expressions of $\mu_{i,y}$ and $\tau_{yy'}$ (1.3.4), we have:

$$\mu^\theta_{i,y} = \theta \mu_{i,y}, \ \tau^\theta_{yy'} = \tau^0_{yy'},$$

and so, $\kappa^\theta \in \mathcal{F}_{\tau_0}$. If we take θ small enough, $\kappa^\theta \in \mathcal{W}_\varepsilon$ for given $\varepsilon > 0$, and then, $\mathcal{F}_{\tau_0,\varepsilon}$ is a nonempty open set of $\mathcal{F}_{\tau_0}$ for all $\varepsilon > 0$.

For the other part of the statement, first take W' a row reduced conservation-law matrix of G'. We can assume that the set of indices of the first nonzero coordinates of the rows of W' is $I = \{1, \dots, d\}$. This can be always be done, potentially by reordering the set of species of G'. Then, a basis of conservation laws for G' is

$\ell_1(x) = w_1 \cdot x, \ldots, \ell_d(x) = w_d \cdot x$, where $w_1, \ldots, w_d$ are the rows of W'. By (1.3.2), we have the following conservation laws for G: $\bar{\ell}_1(x,u) = \ell_1(x) + a_1^1 u_1 + \cdots + a_p^1 u_p, \ldots, \bar{\ell}_d(x,u) = \ell_d(x) + a_1^d u_1 + \cdots + a_p^d u_p$, for certain values of a_j^i, for $i = 1, \ldots, d$, $j = 1, \ldots, p$.

Consider now the system augmented by conservation laws as in (4.2.1) corresponding to the network G, with reaction rate constants κ and total conservation constants $c_1, \ldots, c_d$:

$$f_{c,\kappa}(x,u) = (\bar{\ell}_1(x,u) - c_1, \ldots, \bar{\ell}_d(x,u) - c_d, f_{\kappa,d+1}(x,u), \ldots, f_{\kappa,n+p}(x,u)), \tag{4.2.3}$$

where $f_{\kappa,i}(x,u)$ is the i-th coordinate of the function $f_\kappa(x,u)$ as in (4.2.2), that is

$$f_\kappa(x,u) = \sum_{y \to y' \in \mathscr{R}_G} \kappa_{yy'}(x,u)^y (y' - y).$$

Then, a vector (x,u) is a steady state of G for the reaction rate constants κ and for the stoichiometric compatibility class defined by $c_1, \ldots, c_d$, if and only if $f_{c,\kappa}(x,u) = 0$.

Analogously, we consider the system augmented by conservation laws as in (4.2.1) corresponding to the network G'. A vector x is a steady state of G' for reaction rate constants τ in the stoichiometric compatibility class defined by $c_1, \ldots, c_d$ if and only if x is zero of the following function:

$$g_{c,\tau}(x) = (\ell_1(x) - c_1, \ldots, \ell_d(x) - c_d, g_{\tau,d+1}(x), \ldots, g_{\tau,n}(x)), \tag{4.2.4}$$

where

$$g_\tau(x) = \sum_{y \to y' \in \mathscr{R}_{G'}} \tau_{yy'} x^y (y' - y).$$

We rewrite the function $f_{c,\kappa}(x,u)$ in an equivalent form. By Theorem 2 in [42], $f_{\kappa,n+1}(x,u) = 0, \ldots, f_{\kappa,n+p}(x,u) = 0$ if and only if $u_i = \sum_{y \in \mathscr{C}_{G'}} \mu_{i,y} x^y$ for each $i = 1, \ldots, p$. Then, we replace the expression $f_{\kappa,n+i}(x,u)$ by $u_i - \sum_{y \in \mathscr{C}_{G'}} \mu_{i,y} x^y$, for $i = 1, \ldots, p$ and replace the variables u_j by the expression $\sum_{y \in \mathscr{C}_{G'}} \mu_{j,y} x^y$ in the functions $f_{c,\kappa,i}(x,u)$ for $i \leq n$. We obtain the function $F_{c,\kappa}(x,u)$, where

$$F_{c,\kappa,i}(x,u) = \begin{cases} f_{c,\kappa,i}(x, \sum_{y \in \mathscr{C}_{G'}} \mu_{1,y} x^y, \ldots, \sum_{y \in \mathcal{C}_{G'}} \mu_{p,y} x^y), & i = 1, \ldots, n, \\ u_i - \sum_{y \in \mathscr{C}_{G'}} \mu_{i,y} x^y, & i = n+1, \ldots, n+p. \end{cases} \tag{4.2.5}$$

The zeros of system (4.2.3) are in one-to-one correspondence with the zeros of system (4.2.5). Furthermore, in the proof of Theorem 4 in [42], it is shown that the Jacobian matrix of $f_{c,\kappa}$ evaluated at (x,u) is nonsingular if and only if the Jacobian matrix of $F_{c,\kappa}$ evaluated at (x,u) is nonsingular. Then, the nondegenerate steady states of G are the solutions of $F_{c,\kappa}(x,u) = 0$, for which the Jacobian matrix is nonsingular.

By construction we have:

$$F_{c,\kappa,i}(x,u) = \begin{cases} g_{c,\tau,i}(x) + \sum_{j=1}^p a_{n+j}^i \sum_y \mu_{j,y} x^y, & i = 1, \ldots, d, \\ g_{c,\tau,i}(x), & i = d+1, \ldots, n, \\ u_i - \sum_{y \in \mathscr{C}_{G'}} \mu_{i,y} x^y, & i = n+1, \ldots, n+p. \end{cases}$$

Assume now that G' has m nondegenerate positive steady states $x^{(i)} \in \mathbb{R}^n_{>0}$, $i = 1, \ldots, m$ in the stoichiometric compatibility class defined by the total amounts $c_1, \ldots, c_d$, and for the reaction rate constants $\tau(\kappa^0) = \tau^0$.

We consider all the nonzero coefficients $\mu_{i,y}$, for all $i = 1, \ldots, p$ and all complexes $y \in G'$, and let N be the number of nonzero $\mu_{i,y}$ coefficients. Let $\mu \in \mathbb{R}^N_{>0}$ be the vector which coordinates are the $\mu_{i,y}$, in some order.

For fixed $c_1, \ldots, c_d$, we can think the function $F_{c,\kappa}(x,u)$ depending only on the parameters τ and μ, and not in κ. With τ^0 fixed, if we choose values of κ such that $\kappa \in \mathcal{F}_{\tau^0}$, we can consider the function $F_{c,\kappa}(x,u)$ depending only on μ. In that case, we can rewrite $F_{c,\kappa}(x,u) = F_\mu(x,u)$.

That is, for each μ we consider the function $F_\mu(x,u)$:

$$F_{\mu,i}(x,u) = \begin{cases} g_{c,\tau^0,i}(x) + \sum_{j=1}^p a^i_{n+j} \sum_y \mu_{j,y} x^y, & i = 1, \ldots, d, \\ g_{c,\tau^0,i}(x), & i = d+1, \ldots, n, \\ u_i - \sum_{y \in \mathscr{C}_{G'}} \mu_{i,y} x^y, & i = n+1, \ldots, n+p. \end{cases}$$

We observe that the Jacobian matrix of F_μ in (x,u) is of the form:

$$J_{(x,u)}(F_\mu) = \begin{pmatrix} J_x(g_{c,\tau^0}) + A & 0 \\ B & I_p \end{pmatrix},$$

where A and B are matrices that are zero when are evaluated at $\mu = 0$.

By continuity, the function F_μ is well defined for all $\mu \in \mathbb{R}^N$ and is differentiable, so we can consider the following map:

$$\begin{array}{rcl} F\colon \mathbb{R}^n \times \mathbb{R}^p \times \mathbb{R}^N & \to & \mathbb{R}^{n+p} \\ (x,u,\mu) & \mapsto & F(x,u,\mu) := F_\mu(x,u). \end{array}$$

For $\mu = 0$, $F(x^{(i)}, 0, 0) = 0$, because $g_{c,\tau^0}(x^{(i)}) = 0$ for all $i = 1, \ldots, m$. And the Jacobian matrix of F_0 has the form:

$$J_{(x,u)}(F_0) = \begin{pmatrix} J_x(g_{c,\tau^0}) & 0 \\ 0 & I_p \end{pmatrix}.$$

Since the steady states $x^{(i)}$ are nondegenerate, $J_x(g_{c,\tau^0})$ evaluated at $x^{(i)}$ is nonsingular for each $i = 1, \ldots, m$. Then, $J_{(x,u)}(F_0)$ evaluated at $(x^{(i)}, 0)$ is nonsingular for each $i = 1, \ldots, m$. By the Implicit Function Theorem applied to F at $(x^{(i)}, 0, 0)$, there exists an open set $\mathcal{U}_i \in \mathbb{R}^N$, with $0 \in \mathcal{U}_i$, and an open set $\mathcal{V}_i \in \mathbb{R}^n \times \mathbb{R}^p$, with $(x^{(i)}, 0) \in \mathcal{V}_i$ such that for all $\mu \in \mathcal{U}_i$, there is a steady state $(x^{(i)}(\mu), u^{(i)}(\mu)) \in \mathcal{V}_i$ in the stoichiometric compatibility class defined by $c_1, \ldots, c_d$, with $(x^{(i)}(0), u^{(i)}(0)) = (x^{(i)}, 0)$.

Because $x^{(i)} > 0$ and $x^{(i)}$ is a nondegenerate steady state, we can take the open set $\mathcal{U}_i$ such that $x^{(i)}(\mu) > 0$ and $J_{(x^{(i)}(\mu), u^{(i)}(\mu))}(F_\mu)$ is nonsingular for all $\mu \in \mathcal{U}_i$.

We take $\mathcal{U}_i^+ = \mathcal{U}_i \cap \mathbb{R}^N_{>0}$. Since $x^{(i)}(\mu) > 0$, it follows that $u^{(i)}(\mu) > 0$ for all $\mu \in \mathcal{U}_i^+$, by construction. Then $(x^{(i)}(\mu), u^{(i)}(\mu))$ is a nondegenerate positive steady state of G in the stoichiometric class defined by $c_1, \ldots, c_d$. Because the $x^{(i)}$ are distinct, we can choose the open sets $\mathcal{U}_i$ (smaller if needed, contained in the original $\mathcal{U}_i$) such that $\cap_{i=1}^m \mathcal{V}_i = \emptyset$.

Now we take $\mathcal{U} = \cap_{i=1}^{m} \mathcal{U}_i^+$. Then, for all $\mu \in \mathcal{U}$, the original network G has m nondegenerate positive steady states $(x^{(i)}(\mu), u^{(i)}(\mu))$, $i = 1, \ldots, m$ in the stoichiometric compatibility class defined by $c_1, \ldots, c_d$, for all the constants $\kappa \in \mathcal{F}_{\tau^0}$ with $|\mu_{i,y}(\kappa)| << 1$ (small enough such that $\mu(\kappa) \in \mathcal{U}$). □

4.2.2 Application to the n-site phosphorylation system

Multistationarity of any of the subsystems G_J can be extended to the full n-site phosphorylation system for instance, by Theorem 4 in [42]. We give a precise statement of this result in Corollary 4.2.2, using Theorem 4.2.1.

Consider the full n-site phosphorylation network (1.2.2), with a given vector of reaction rate constants $\kappa \in \mathbb{R}^{6n}$:

$$\kappa = (k_{\mathrm{on}_0}, k_{\mathrm{off}_0}, k_{\mathrm{cat}_0}, \ldots, k_{\mathrm{on}_{n-1}}, k_{\mathrm{off}_{n-1}}, k_{\mathrm{cat}_{n-1}}, \ell_{\mathrm{on}_0}, \ell_{\mathrm{off}_0}, \ell_{\mathrm{cat}_0}, \ldots, \ell_{\mathrm{on}_{n-1}}, \ell_{\mathrm{off}_{n-1}}, \ell_{\mathrm{cat}_{n-1}}).$$

We define the following rational functions of κ (as in (1.3.4)):

$$\tau_j(\kappa) = k_{\mathrm{cat_j}}\, \mu_j(\kappa) \text{ if } j \notin J \text{ and } \nu_j(\kappa) = \ell_{\mathrm{cat_j}}\, \eta_j(\kappa) \text{ for } j = 0, \ldots, n-1, \tag{4.2.6}$$

where $\mu_j(\kappa)$ and $\eta_j(\kappa)$ are in turn the following rational functions:

$$\mu_j(\kappa) = \frac{k_{\mathrm{on_j}}}{k_{\mathrm{off_j}} + k_{\mathrm{cat_j}}} \text{ if } j \notin J \text{ and } \eta_j(\kappa) = \frac{\ell_{\mathrm{on_j}}}{\ell_{\mathrm{off_j}} + \ell_{\mathrm{cat_j}}} \text{ for } j = 0, \ldots, n-1. \tag{4.2.7}$$

Note that these last rational functions are the functions $\mu_{i,y}(\kappa)$ as in Section 1.3 in Chapter 1. We denote by $\varphi\colon \mathbb{R}^{6n}_{>0} \to \mathbb{R}^{2n+2|J|}_{>0}$ the function that takes κ and gives a vector of (positive) reaction rate constants with the following order: first, the constants $k_{\mathrm{on_j}}, k_{\mathrm{off_j}}, k_{\mathrm{cat_j}}, j \in J$, then $\tau(\kappa), j \notin J$, and then $\nu_j(\kappa), j = 0, \ldots, n-1$.

Given a subset $J \subset I_n$ and a vector of reaction rate constants $\kappa \in \mathbb{R}^{6n}_{>0}$, we consider the subnetwork $G_J^{\varphi(\kappa)}$ as in Definition 4.1.1, with rate constants $\varphi(\kappa)$:

$$\begin{aligned} S_j + E &\underset{k_{\mathrm{off_j}}}{\overset{k_{\mathrm{on_j}}}{\rightleftarrows}} Y_j \overset{k_{\mathrm{cat_j}}}{\to} S_{j+1} + E, \quad \text{if } j \in J \\ S_j + E &\overset{\tau_j(\kappa)}{\to} S_{j+1} + E, \quad \text{if } j \notin J \\ S_{j+1} + F &\overset{\nu_j(\kappa)}{\to} S_j + F, \quad 0 \le j \le n-1. \end{aligned} \tag{4.2.8}$$

As a consequence of Theorem 4.2.1 we get the following lifting result.

Corollary 4.2.2. *Consider the full n-site phosphorylation network* (1.2.2) *with fixed reaction rate constants κ^0 and the network $G_J^{\varphi(\kappa^0)}$, both with total conservation amounts S_{tot}, E_{tot}, $F_{tot} > 0$. Suppose that system $G_J^{\varphi(\kappa^0)}$ admits m nondegenerate positive steady states.*

Then, there exists $\varepsilon_0 > 0$ such that for any choice of rate constants κ such that $\varphi(\kappa) = \varphi(\kappa^0)$ and

$$\max_{j \notin J} \mu_j(\kappa), \quad \max_{j \in I_n} \eta_j(\kappa) \,<\, \varepsilon_0, \tag{4.2.9}$$

the n-site sequential phosphorylation system admits m positive nondegenerate steady states in the stoichiometric compatibility class defined by S_{tot}, E_{tot} and $F_{tot} > 0$. Moreover, the set of rate constants κ verifying $\varphi(\kappa) = \varphi(\kappa^0)$ and (4.2.9) is nonempty.

Note that the function φ is surjective, that is, any vector of reaction rate constants for the reduced network G_J can be obtained as $\varphi(\kappa)$, for some vector κ of reaction rate constants of the full n-site phosphorylation network. For instance, given $\tau_j \in \mathbb{R}_{>0}$, we can take $k_{\mathrm{on_j}} = 2\tau_j, k_{\mathrm{off_j}} = k_{\mathrm{cat_j}} = 1$, and then $\tau_j(\kappa) = \tau_j$. Similarly, we can do this for the other reaction rate constants of G_J. Then, Corollary 4.2.2 allows us to obtain multistationary regions for the complete n-site phosphorylation system, combining the conditions on the parameters given in Theorem 4.1.2 and Theorem 4.1.4, with conditions (4.2.9) of Corollary 4.2.2. In particular, let $J_n \subset I_n$ as in (4.1.6). By lifting a multistationarity region for the system G_{J_n} in Corollary 4.1.5, we get a multistationarity region of parameters of the n-site phosphorylation cycle with $2[\frac{n}{2}] + 1$ positive steady states in the same stoichiometric compatibility class.

Appendix: Computer aided results

The algorithms and computations that give rise to the computed aided results presented in this appendix, were implemented by Rick Rischter. The files with the computations are available at: `http://mate.dm.uba.ar/~alidick/DGRPMFiles/`.

In this appendix we explore a computational approach to the multistationarity problem, more precisely we find new regions of multistationarity, using the method develop in Chapter 2. We first give the idea and then apply it for the n-site phosphorylation system for $n = 2, 3, 4$, and 5, where we have successfully found several regions of multistationarity. This approach can be, in principle, applied to other systems if they satisfy certain hypotheses (see Theorem 2.5.2 in Chapter 2), and are sufficiently small in order for the computations to be done in a reasonable amount of time.

The strategy is the following. Given a polynomial system with support $\mathcal{A}$ and matrix of coefficients C, one first computes all possible regular triangulations of $\mathcal{A}$ with the aid of a computer. The number of such triangulations can be very large depending on the size of $\mathcal{A}$, thus the next step is to discard in each such triangulation the simplices that obviously will not be positively decorated by C. With the reduced number of triangulations one can now search through all of them for the ones giving the biggest number of simultaneously positively decorated simplices. Each set of k simultaneously positively decorated simplices gives a candidate for a region of multistationarity with k positive steady states. If one finds m of such sets, then it is possible to have up to m such regions. Have in mind, however, that among these regions can be repetitions.

Next we apply this to the n-site phosphorylation system with all intermediates and explain more concretely this procedure in this case.

Consider G of the n-site phosphorylation system with all possible intermediates,

as in (1.2.2). Consider the parametrization (2.4.3) of the concentration at steady state of all species in terms of the species s_0, e, f.

We replace this parametrization into the conservation laws (1.2.4), as we did in Section 2.4, and we consider that system. The support $\mathcal{A}$ of this system, which has $2n+4$ elements, ordering the variables as s_0, e, f, is given by the columns of the following matrix:

$$A = \begin{pmatrix} 1 & 0 & 0 & 1 & \dots & 1 & 1 & 1 & \dots & 1 & 0 \\ 0 & 1 & 0 & 1 & \dots & n & 1 & 2 & \dots & n & 0 \\ 0 & 0 & 1 & -1 & \dots & -n & 0 & -1 & \dots & 1-n & 0 \end{pmatrix},$$

and the corresponding matrix of coefficients for the system is:

$$C = \begin{pmatrix} 1 & 0 & 0 & T_0 & \dots & T_{n-1} & K_0 + L_0T_0 & \dots & K_{n-1}T_{n-2} + L_{n-1}T_{n-1} & -S_{tot} \\ 0 & 1 & 0 & 0 & \dots & 0 & K_0 & \dots & K_{n-1}T_{n-2} & -E_{tot} \\ 0 & 0 & 1 & 0 & \dots & 0 & L_0T_0 & \dots & L_{n-1}T_{n-1} & -F_{tot} \end{pmatrix}.$$

Recall that if one multiplies a column of a matrix C by a positive number, then a simplex is positively decorated by C if and only if it is positively decorated by the modified matrix. So, in order to test whether a simplex with vertices in $\mathcal{A}$ is positively decorated by C is enough to test if it is positively decorated by the following matrix

$$Csimple = \begin{pmatrix} 1 & 0 & 0 & 1 & \dots & 1 & 1 & 1 & \dots & 1 & -S_{tot} \\ 0 & 1 & 0 & 0 & \dots & 0 & N_0 & N_1 & \dots & N_{n-1} & -E_{tot} \\ 0 & 0 & 1 & 0 & \dots & 0 & 1-N_0 & 1-N_1 & \dots & 1-N_{n-1} & -F_{tot} \end{pmatrix},$$

where $0 < N_i = \dfrac{K_iT_{i-1}}{K_iT_{i-1} + L_iT_i} = \left(1 + \dfrac{k_{\text{cat}_\text{i}}}{l_{\text{cat}_\text{i}}}\right)^{-1} < 1$ for $i = 0, 1, \dots, n-1$. Here the matrix $Csimple$ is obtained by dividing the fourth until the last column by its first entry.

Now we compute all possible regular triangulations of $\mathcal{A}$ and search through them looking for the ones with the maximal possible number of simplices simultaneously positively decorated by $Csimple$. Since the number of such triangulations grows very fast with n we approach it with the following strategy:

Algorithm 4.2.3. (1) Compute $L_1 := \{\text{all possible regular triangulations of } \mathcal{A}\}$.[1]

(2) With L_1 compute L_2 by discarding all simplices which do not have the last vertex $(0, 0, 0)$. In fact we only need these simplices since a simplex not containing the last vertex cannot be positively decorated, because the corresponding coefficients of $Csimple$ will be all positive.

(3) Compute L_3 from L_2 by removing all simplices with the corresponding 3×4 submatrix of $Csimple$ having a zero 3×3 minor. The reason for this is clear, such simplices will never be positively decorated by $Csimple$.

[1] We are calling $L_1, \dots, L_7$ the sets defined in Algorithm 4.2.3. They are completely unrelated to the rational functions of the rate constants denoted with the same letters.

(4) Compute L_4 from L_3 using the symmetry of $Csimple$. More precisely, change any index $4, 5, \ldots, n+3$ on the simplex to 1 because on $Csimple$ they yield the same column. Here we are using the easy-to-check fact that changing the order of indexes does not change the conditions for a simplex to be positively decorated.

(5) Compute L_5 from L_4 removing all $T \in L_4$ such that there is another $T' \in L_4$ with $T \subset T'$.

(6) For each $T \in L_5$, check for each set $S \subset T$ of simplices if there is viable $N_0, \ldots, N_{n-1}$ such that all $\Delta \in S$ is positively decorated by $Csimple$ at the same time, call L_6 the list of such S's.

(7) If the maximum size of a element in L_6 is k, set $L_7 \coloneqq \{T \in L_6 : \#T = k\}$. This k is the number of positive steady states and $m \coloneqq \#L_7$ is the number of candidates for regions of multistationarity.

Step (1) can be done with the package TOPCOM inside SAGE [100], the other steps are quite simple to implement, for instance in MAPLE [75]. We show in the table below the number of elements in some of the lists and an approximation of the computation time for small values of n.

n	$\#L_1$	$\#L_2$	$\#L_3$	$\#L_4$	$\#L_5$	$\#L_7$	k	regions of multistationarity	computation time
2	44	25	15	7	6	1	3	1	negligible
3	649	260	100	21	18	6	3	6	about 1 sec
4	9094	2728	682	62	53	5	5	4	about 2 min
5	122835	28044	4560	177	149	23	5	15	about 3 hours

The most computationally expensive part is to compute all regular triangulations, taking at least 90% of the time. These computations were done in a Linux virtual machine with 4MB of RAM and with 4 cores of 3.2GHz of processing. With a faster computer or more time one probably can do $n = 6$ or even $n = 7$ but probably not much more than this. For $n = 5$ just the file for the raw list L_1 of regular triangulations already has 10Mb.

An alternative path to Steps (6) and (7) is to set a number k and look for sets $T \in L_5$ and $S \subset T$ with $\#S \geq k$ such that there is viable $N_0, \ldots, N_{n-1}$ such that all $\Delta \in S$ are positively decorated by $Csimple$ at the same time. We actually used this with $k = 2[\frac{n}{2}] + 1$. This other route depends upon a good guess one may previously have at how many positive steady states to expect.

After Step (7) one has to determine if there are any repetitions among the candidates for regions of multistationarity in L_7 and also if there are any superfluous candidates of regions, that is conditions C_1 and C_2 such that C_1 implies C_2. In our case we did it by hand since the $\#L_7$ was quite small.

Once Step (7) is done, one has a list of inequalities for each element S of L_7. These come from the conditions imposing that the simplices in S are positively decorated by $Csimple$. We are going to use these conditions to describe the regions of

mulstistationarity. Because of the uniformity of $Csimple$ the only kind of conditions that appear are

$(\mathrm{I})_{\mathrm{i,j}}$ $N_i - N_j > 0$

$(\mathrm{II})_{\mathrm{i}}$ $S_{tot}N_i - E_{tot} > 0$

$(\mathrm{III})_{\mathrm{i}}$ $E_{tot}N_i + F_{tot}N_i - E_{tot} > 0$

$(\mathrm{IV})_{\mathrm{i}}$ $-S_{tot}N_i - F_{tot} + S_{tot} > 0$

(V) $S_{tot} > E_{tot} + F_{tot}$,

or the opposite inequalities, and these translate from the N_i to the $k_{\mathrm{cat_i}}, \ell_{\mathrm{cat_i}}$ as follows

$(\mathrm{I})'_{\mathrm{i,j}}$ $\frac{k_{\mathrm{cat_j}}}{\ell_{\mathrm{cat_j}}} > \frac{k_{\mathrm{cat_i}}}{\ell_{\mathrm{cat_i}}}$

$(\mathrm{II})'_{\mathrm{i}}$ $\frac{S_{tot}-E_{tot}}{E_{tot}} > \frac{k_{\mathrm{cat_i}}}{\ell_{\mathrm{cat_i}}}$

$(\mathrm{III})'_{\mathrm{i}}$ $\frac{F_{tot}}{E_{tot}} > \frac{k_{\mathrm{cat_i}}}{\ell_{\mathrm{cat_i}}}$

$(\mathrm{IV})'_{\mathrm{i}}$ $\frac{F_{tot}}{S_{tot}-F_{tot}} < \frac{k_{\mathrm{cat_i}}}{\ell_{\mathrm{cat_i}}}$.

Note that

- conditions $(\mathrm{III})_{\mathrm{i}}$ and (V) together imply $(\mathrm{II})_{\mathrm{i}}$;
- the opposite of condition $(\mathrm{II})_{\mathrm{i}}$ together with condition (V) imply the opposite of $(\mathrm{III})_{\mathrm{i}}$;
- the opposite of condition $(\mathrm{III})_{\mathrm{i}}$ together with condition (V) imply $(\mathrm{IV})_{\mathrm{i}}$;
- the opposite of condition $(\mathrm{IV})_{\mathrm{i}}$ together with condition (V) imply $(\mathrm{III})_{\mathrm{i}}$;
- condition $(\mathrm{III})_{\mathrm{i}}$ and the opposite of $(\mathrm{III})_{\mathrm{j}}$ together imply $(\mathrm{I})_{\mathrm{i,j}}$.

Using these properties it is easy to describe in a nice manner the regions of multistationarity and discard the repeated and superfluous ones. We sum up our findings on the following results which are proved in the same fashion as Theorems 4.1.2 and 4.1.4, once you have the regular triangulation obtained with the computer script. In the following propositions we describe the regions of multistationarity for $n = 2, 3, 4$ and 5. Here we used that the rescaling of some of parameters can be done, using Theorem 2.5.2.

Proposition 4.2.4. *Let $n = 2$. Assume that $S_{tot} > E_{tot}+F_{tot}$. Then there is a choice of reaction rate constants for which the distributive sequential 2-site phosphorylation system admits 3 positive steady states. More explicitly, given rate constants and total concentrations such that*

$$\frac{k_{\mathrm{cat_0}}}{\ell_{\mathrm{cat_0}}} < \frac{F_{tot}}{E_{tot}} < \frac{k_{\mathrm{cat_1}}}{\ell_{\mathrm{cat_1}}},$$

after rescaling of the k_{on}'s and ℓ_{on}'s the distributive sequential 2-site phosphorylation system has 3 positive steady states.

Proposition 4.2.5. *Let $n = 3$. Assume that $S_{tot} > E_{tot} + F_{tot}$. Then, there is a choice of rate constants for which the distributive sequential 3-site phosphorylation system admits at least 3 positive steady states. More explicitly, if the rate constants and total concentrations are in one of the regions described below*

$$(\mathrm{R}_{3.1})\quad \frac{k_{\mathrm{cat}_0}}{\ell_{\mathrm{cat}_0}} < \frac{F_{tot}}{E_{tot}} < \frac{k_{\mathrm{cat}_1}}{\ell_{\mathrm{cat}_1}},$$

$$(\mathrm{R}_{3.2})\quad \frac{k_{\mathrm{cat}_0}}{\ell_{\mathrm{cat}_0}} < \frac{F_{tot}}{E_{tot}} < \frac{k_{\mathrm{cat}_2}}{\ell_{\mathrm{cat}_2}},$$

$$(\mathrm{R}_{3.3})\quad \frac{k_{\mathrm{cat}_1}}{\ell_{\mathrm{cat}_1}} < \frac{F_{tot}}{E_{tot}} < \frac{k_{\mathrm{cat}_2}}{\ell_{\mathrm{cat}_2}},$$

then after rescaling of the k_{on}'s and ℓ_{on}'s the distributive sequential 3-site phosphorylation system has at least 3 positive steady states.

Proposition 4.2.6. *Let $n = 3$. If the rate constants and total concentrations are in one of the regions described below*

$$(\mathrm{R}_{3.4})\quad \max\left\{\frac{F_{tot}}{E_{tot}}, \frac{F_{tot}}{S_{tot}-F_{tot}}\right\} < \min\left\{\frac{k_{\mathrm{cat}_0}}{\ell_{\mathrm{cat}_0}}, \frac{k_{\mathrm{cat}_2}}{\ell_{\mathrm{cat}_2}}\right\},\ S_{tot} > F_{tot},$$

$$(\mathrm{R}_{3.5})\quad \max\left\{\frac{F_{tot}}{E_{tot}}, \frac{F_{tot}}{S_{tot}-F_{tot}}\right\} < \min\left\{\frac{k_{\mathrm{cat}_1}}{\ell_{\mathrm{cat}_1}}, \frac{k_{\mathrm{cat}_2}}{\ell_{\mathrm{cat}_2}}\right\},\ S_{tot} > F_{tot},$$

$$(\mathrm{R}_{3.6})\quad \min\left\{\frac{F_{tot}}{E_{tot}}, \frac{S_{tot}-E_{tot}}{E_{tot}}\right\} > \max\left\{\frac{k_{\mathrm{cat}_1}}{\ell_{\mathrm{cat}_1}}, \frac{k_{\mathrm{cat}_2}}{\ell_{\mathrm{cat}_2}}\right\},\ S_{tot} > E_{tot},$$

then after rescaling of the k_{on}'s and ℓ_{on}'s the distributive sequential 3-site phosphorylation system has at least 3 positive steady states.

Proposition 4.2.7. *Let $n = 4$. Assume that $S_{tot} > E_{tot} + F_{tot}$. Then, there is a choice of rate constants for which the distributive sequential 4-site phosphorylation system has at least 5 steady states. More explicitly, if the rate constants and total concentrations are in one of the regions described below*

$$(\mathrm{R}_{4.1})\quad \frac{k_{\mathrm{cat}_2}}{\ell_{\mathrm{cat}_2}} < \frac{F_{tot}}{E_{tot}} < \min\left\{\frac{k_{\mathrm{cat}_1}}{\ell_{\mathrm{cat}_1}}, \frac{k_{\mathrm{cat}_3}}{\ell_{\mathrm{cat}_3}}\right\},$$

$$(\mathrm{R}_{4.2})\quad \frac{k_{\mathrm{cat}_0}}{\ell_{\mathrm{cat}_0}} < \frac{F_{tot}}{E_{tot}} < \min\left\{\frac{k_{\mathrm{cat}_1}}{\ell_{\mathrm{cat}_1}}, \frac{k_{\mathrm{cat}_3}}{\ell_{\mathrm{cat}_3}}\right\},$$

$$(\mathrm{R}_{4.3})\quad \max\left\{\frac{k_{\mathrm{cat}_0}}{\ell_{\mathrm{cat}_0}}, \frac{k_{\mathrm{cat}_2}}{\ell_{\mathrm{cat}_2}}\right\} < \frac{F_{tot}}{E_{tot}} < \frac{k_{\mathrm{cat}_3}}{\ell_{\mathrm{cat}_3}},$$

$$(\mathrm{R}_{4.4})\quad \max\left\{\frac{k_{\mathrm{cat}_0}}{\ell_{\mathrm{cat}_0}}, \frac{k_{\mathrm{cat}_2}}{\ell_{\mathrm{cat}_2}}\right\} < \frac{F_{tot}}{E_{tot}} < \frac{k_{\mathrm{cat}_1}}{\ell_{\mathrm{cat}_1}},$$

then after rescaling of the k_{on}'s and ℓ_{on}'s the distributive sequential 4-site phosphorylation system has at least 5 steady states.

Proposition 4.2.8. *Let $n = 5$. Assume that $S_{tot} > E_{tot} + F_{tot}$. Then, there is a choice of rate constants for which the distributive sequential 5-site phosphorylation system has at least 5 steady states. More explicitly, if the rate constants and total concentrations are in one of the 13 regions described below*

$$(\mathrm{R}_{5.(I,J)}) \qquad \max_{i\in I}\left\{\frac{k_{\mathrm{cat_i}}}{\ell_{\mathrm{cat_i}}}\right\} < \frac{F_{tot}}{E_{tot}} < \min_{j\in J}\left\{\frac{k_{\mathrm{cat_j}}}{\ell_{\mathrm{cat_j}}}\right\},$$

with (I, J) in the following list (where we write e.g. 14 instead of $\{1, 4\}$):

$$(0,14),(0,24),(1,24),(2,13),(2,14),(3,14),(3,024),(02,3),(02,4),(03,1),(03,2),(13,2),(13,4),$$

then after rescaling of the k_{on}'s and ℓ_{on}'s the distributive sequential 5-site phosphorylation system has at least 5 steady states.

Proposition 4.2.9. *Let $n = 5$. If the rate constants and total concentrations are in one of the regions described below*

$$(\mathrm{R}_{5.1}) \ \max\left\{\frac{F_{tot}}{E_{tot}}, \frac{F_{tot}}{S_{tot}-F_{tot}}\right\} < \min\left\{\frac{k_{\mathrm{cat_1}}}{\ell_{\mathrm{cat_1}}}, \frac{k_{\mathrm{cat_2}}}{\ell_{\mathrm{cat_2}}}, \frac{k_{\mathrm{cat_4}}}{\ell_{\mathrm{cat_4}}}\right\}, \ S_{tot} > F_{tot},$$

$$(\mathrm{R}_{5.2}) \ \min\left\{\frac{F_{tot}}{E_{tot}}, \frac{S_{tot}-E_{tot}}{E_{tot}}\right\} > \max\left\{\frac{k_{\mathrm{cat_0}}}{\ell_{\mathrm{cat_0}}}, \frac{k_{\mathrm{cat_2}}}{\ell_{\mathrm{cat_2}}}, \frac{k_{\mathrm{cat_3}}}{\ell_{\mathrm{cat_3}}}\right\}, \ S_{tot} > E_{tot},$$

then after rescaling of the k_{on}'s and ℓ_{on}'s the distributive sequential 5-site phosphorylation system has at least 5 positive steady states.

Note that the conditions in this section describe different regions from the ones described by the inequalities in Theorem 4.1.2 and Theorem 4.1.4. For instance, in Propositions 4.2.4, 4.2.5, 4.2.7, 4.2.8 the inequalities between the reaction rate constants and total conservations constants do not involve the value of S_{tot}. In Propositions 4.2.6 and 4.2.9, the conditions onse the total conservation constants are also different (e.g. on $\frac{F_{tot}}{E_{tot}}$ and $\frac{S_{tot}}{E_{tot}} - 1$ instead of the product $F_{tot}(\frac{S_{tot}}{E_{tot}} - 1)$). The inequalities in Theorem 4.1.2 and Theorem 4.1.4 hold for reactions rate constants of a reduced system G_J, but if we use Theorem 4.2.2 to extrapolate these conditions to the full n-site phosphorylation network, the regions are different as well.

Chapter 5

Sign conditions for the existence of at least one positive solution of a sparse polynomial system

Deciding whether a real polynomial system has a positive solution is a basic question, that is decidable via effective elimination of quantifiers [4]. There are few results on lower bounds of the number of real or positive roots of polynomial systems (see e.g. [7, 93, 94, 107]). In this chapter, we give sign conditions on the support and coefficients of a sparse system of d generalized polynomials (that is, polynomials with real exponents, for which the positive solutions are well defined) in d variables, that guarantee the existence of at least one positive real root, based on degree theory and Gale duality.

We fix an exponent set $\mathcal{A} = \{a_1, \ldots, a_n\} \subset \mathbb{R}^d$ of cardinality n and for any given real matrix $C = (c_{ij}) \in \mathbb{R}^{d\times n}$ we consider the associated sparse generalized multivariate polynomial system in d variables $x = (x_1, \ldots, x_d)$ with support $\mathcal{A}$:

$$f_i(x) = \sum_{j=1}^{n} c_{ij} x^{a_j} = 0\,, \quad i = 1, \ldots, d. \tag{5.0.1}$$

We will be interested by the existence of the positive solutions of (5.0.1) in $\mathbb{R}^d_{>0}$. Denoting by $n_{\mathcal{A}}(C)$ the (possibly infinite) number of positive real solutions of the system (5.0.1), our main goal is to give sufficient conditions on the exponent set $\mathcal{A}$ and the coefficient matrix C that ensure that $n_{\mathcal{A}}(C) > 0$. When $\mathcal{A} \subset \mathbb{Z}^d$ we will consider the existence of solutions in the real torus $(\mathbb{R}^*)^d$ of points in $\mathbb{R}^d$ with nonzero coordinates, and we will relate our conditions to well-studied algebraic properties of lattice ideals associated with the configuration $\mathcal{A}$.

In applications, for example, in the context of chemical reaction networks, lower bounds of positive roots of polynomial systems guarantee the existence of (stoichiometrically compatible) positive steady states. In [80], sign conditions are used to decide if a family of polynomial systems associated with a given reaction network cannot admit more than one positive solution for any choice of the parameters and,

in this case, conditions for the existence of one positive solution are given as a corollary of a result from [82], based on degree theory. Our point of view of searching for conditions on the exponent and the coefficient matrices of the system comes from this paper. As we do not assume injectivity (at most one root), we cannot use tools from these papers or the more recent article [81], as Hadamard's theorem.

In [12] the authors use degree theory in the study of chemical reaction networks to describe parameters for which there is a single positive solution or for which the network is multistationary. We apply some of these techniques in a Gale duality setting, more precisely, based on Theorem 5.2.1, which is version of a particular case of Theorem 2 in the Supplementary Information of [12].

We can use different convex sets to apply Theorem 5.2.1. The first one which comes in mind is the positive orthant, which is not bounded. Another natural idea is to consider the Newton polytope of the polynomials in the system, or some dilates of it. This is reasonable since it is completely determined by the *monomials* appearing in the system. In this chapter, we use another convex polytope which seems natural since it is determined by *the coefficients* of the system. This polytope is obtained using the Gale duality trick for polynomial systems that was studied by Bihan and Sottile in [8], see also [9]. We can think of this polytope as a "shadow" of the positive orthant via Gale duality, which has the advantage that it can be chosen to be bounded.

The chapter is organized as follows. In Section 5.1 we recall the notion of Gale duality and the basic duality of solutions (see Theorem 5.1.5), and we introduce useful notation as well as the necessary condition (5.1.3). In Section 5.2, we recall the basic concepts of degree theory and we present our main result Theorem 5.2.7, which gives conditions on the Gale duality side to guarantee the existence of positive solutions.

In the following sections we give sufficient conditions on the support and the matrix of coefficients that ensure that Theorem 5.2.7 can be applied. In Section 5.3, we consider the notion of mixed dominating matrices from [46] to get Theorem 5.3.6. In Section 5.4, we give geometric conditions on $\mathcal{A}$ and C that guarantee that the hypotheses of Theorem 5.2.7 are satisfied, see Theorem 5.4.8. In Section 5.5, we concentrate our study on integer configurations $\mathcal{A}$; we relate the dominance conditions to algebraic conditions that emerged in the study of toric ideals, and we naturally extend in this case our study to the existence of solutions in the real torus $(\mathbb{R}^*)^d$.

5.1 Gale duality for positive solutions of polynomial systems

We first present basic definitions and results on Gale duality. Given a matrix $M \in \mathbb{R}^{r\times s}$ of maximal rank r, a *Gale dual matrix* of M is any matrix $N \in \mathbb{R}^{s\times(s-r)}$ of maximal rank whose columns vectors are a basis of the kernel of M. Clearly a Gale dual matrix is not unique as it corresponds to a choice of a basis: it is unique up to right multiplication by an invertible $(s-r)\times(s-r)$ matrix. We will also

say that the s row vectors of N define a Gale dual configuration in $\mathbb{R}^{s-r}$ to the configuration in $\mathbb{R}^r$ defined by the s column vectors of M. We will introduce a Gale dual system (5.1.8) and polyhedra Δ_P (5.1.7), depending on the choice of a Gale dual matrix to the coefficient matrix C. We will then recall Theorem 5.1.5, which gives a fundamental link between the positive real roots of system (5.0.1) and the solutions in Δ_P of the Gale dual system (5.1.8).

5.1.1 The matrix A

Let $\mathcal{A} = \{a_1, \ldots, a_n\}$ be a finite subset of $\mathbb{R}^d$ of cardinality n and $C = (c_{ij}) \in \mathbb{R}^{d\times n}$. As we mentioned at the beginning of the chapter, we are interested in the solvability of the associated sparse generalized multivariate polynomial system (5.0.1) in d variables $x = (x_1, \ldots, x_d)$ with support $\mathcal{A}$ and coefficient matrix C.

Note that if we multiply each equation of system (5.0.1) by a monomial (i.e, we translate the configuration $\mathcal{A}$), the number of positive real solutions does not change, and then $n_{\mathcal{A}}(C)$ is an affine invariant of the point configuration $\mathcal{A}$. It is then natural to consider the matrix $A \in \mathbb{R}^{(d+1)\times n}$ with columns $(1, a_1), (1, a_2), \ldots, (1, a_n) \in \mathbb{R}^{d+1}$:

$$A = \begin{pmatrix} 1 & \ldots & 1 \\ a_1 & \ldots & a_n \end{pmatrix}. \tag{5.1.1}$$

We will refer to the matrix A as the corresponding matrix of the point configuration $\mathcal{A}$.

We will always assume that C is of maximal rank d and A is of maximal rank $d+1$. Then, we need to have $n \geq d+1$. If equality holds, it is easy to see that system (5.0.1) has a positive solution if and only if the necessary condition (5.1.3) holds. So we will suppose that $n \geq d+2$.

We denote by $k = n - d - 1$ the codimension of A (and of $\mathcal{A}$). Note that the codimension of C equals $k+1$. Let $B = (b_{ij}) \in \mathbb{R}^{n\times k}$ be a matrix which is Gale dual to A, and let $D = (d_{ij}) \in \mathbb{R}^{n\times(k+1)}$ be any a matrix which is Gale dual to C. We will number the *columns* of B from 1 to k and the *columns* of D from 0 to k and denote by $P_1, \ldots, P_n \in \mathbb{R}^{k+1}$ the *row* vectors of D, that is, the Gale dual configuration to the columns of C.

5.1.2 A necessary condition

There is a basic necessary condition for $n_{\mathcal{A}}(C)$ to be positive. Denote by $C_1, \ldots, C_n \in \mathbb{R}^d$ the *column* vectors of the coefficient matrix C and call

$$\mathcal{C}^\circ = \mathbb{R}_{>0}C_1 + \cdots + \mathbb{R}_{>0}C_n, \tag{5.1.2}$$

the positive cone generated by them. Given a solution $x \in \mathbb{R}^d_{>0}$ of system (5.0.1), the vector $(x^{a_1}, \ldots, x^{a_n})$ is positive and so the origin $\mathbf{0} \in \mathbb{R}^d$ belongs to $\mathcal{C}^\circ$. Then, necessarily

$$\mathbf{0} \in \mathcal{C}^\circ. \tag{5.1.3}$$

It is a well-known result that Condition (5.1.3) holds if and only if the vectors $P_1, \ldots, P_n$ lie in an open halfspace through the origin. Note that Condition (5.1.3), together with the hypothesis that C is of maximal rank d, is equivalent to $\mathcal{C}^\circ = \mathbb{R}^d$.

5.1.3 Defining cones and polytopes in Gale dual space

We also define other cones that we will use. Denote by

$$\mathcal{C}_P = \mathbb{R}_{>0}P_1 + \cdots + \mathbb{R}_{>0}P_n, \tag{5.1.4}$$

the positive cone generated by the rows of a Gale dual matrix D and let

$$\mathcal{C}_P^\nu = \{y \in \mathbb{R}^{k+1} \,:\, \langle P_i, y\rangle > 0, i = 1, \ldots, n\}, \tag{5.1.5}$$

be its dual open cone. Note that if C has maximal rank d and Condition (5.1.3) holds, the cone $\mathcal{C}_P$ is strictly convex. Therefore, its dual open cone $\mathcal{C}_P^\nu$ is a nonempty full dimensional open convex cone. We will also consider the closed cone

$$\overline{\mathcal{C}}_P = \mathbb{R}_{\geq 0}P_1 + \cdots + \mathbb{R}_{\geq 0}P_n. \tag{5.1.6}$$

The following Lemma is straightforward.

Lemma 5.1.1. *Assume that C has maximal rank d and that $\mathbf{0} \in \mathcal{C}^\circ$. Then for any nonzero $u \in \overline{\mathcal{C}}_P$ and any $c \in \mathbb{R}_{>0}$, the polytope $\mathcal{C}_P^\nu \cap \{y \in \mathbb{R}^{k+1} \,:\, \langle u, y\rangle = c\}$ has dimension k. Moreover, this polytope is bounded if and only $u \in \mathcal{C}_P$.*

Define

$$\Delta_P = \mathcal{C}_P^\nu \cap \{y \in \mathbb{R}^{k+1} \,:\, y_0 = 1\}. \tag{5.1.7}$$

Corollary 5.1.2. *Assume that C has maximal rank and that $\mathbf{0} \in \mathcal{C}^\circ$ and let D is a Gale dual matrix of C, then $(1, 0, \ldots, 0) \in \mathcal{C}_P$ if and only if Δ_P has dimension k and is bounded.*

We next show that we can always find a Gale matrix D such that Δ_P is nonempty and bounded.

Lemma 5.1.3. *Assume that C has maximal rank. Then there is a Gale dual matrix D of C such that $(1, 0, \ldots, 0) \in \mathcal{C}_P$.*

Proof. Start with any Gale dual matrix D of C and pick any vector $u \in \mathcal{C}_P$. Then there is an invertible matrix $R \in \mathbb{R}^{(k+1)\times(k+1)}$ such that $u \cdot R = (1, 0, \ldots, 0)$, where u is written as a row vector. Consider the matrix $D' = DR$ and denote by $P_1', \ldots, P_n'$ its row vectors. Then D' is Gale dual to C, and $(1, 0, .., 0) \in \mathcal{C}_{P'} = \mathbb{R}_{>0}P_1' + \cdots + \mathbb{R}_{>0}P_n'$. □

To any choice of Gale dual matrices B and D of A and C respectively, we associate the following system with unknowns $y = (y_0, \ldots, y_k)$:

$$G_j(y) \;=\; \prod_{i=1}^{n} \langle P_i, y\rangle^{b_{ij}} = 1,\; j = 1, \ldots, k, \tag{5.1.8}$$

which is called a *Gale dual system* of (5.0.1). Another choice D' of a Gale dual matrix for C corresponds to another choice y' of linear coordinates for $\mathbb{R}^{k+1}$: if $D' = DR$ with $R \in \mathbb{R}^{(k+1)\times(k+1)}$ invertible, then setting $y' = R^{-1}(y)$ we get $D'y' = Dy$, where y and y' as considered as column vectors. Another choice B' of a Gale dual matrix for A gives an equivalent Gale system $H_1, \ldots, H_k$, where for each j there exists exponent $(\mu_1, \ldots, \mu_k)$ such that $H_j = G_1^{\mu_1} \ldots G_k^{\mu_k}$.

Note that (5.1.8) is homogeneous of degree zero since the columns of B sum up to zero. For any cone $\mathcal{C} \in \mathbb{R}^n$ with apex the origin, its projectivization $\mathbb{P}\mathcal{C}$ is the quotient space $\mathcal{C}/\sim$ under the equivalence relation $\sim$ defined by: for all $y, y' \in \mathcal{C}$, we have $y \sim y'$ if and only if there exists $\alpha > 0$ such that $y = \alpha y'$.

We will often use the following observation.

Remark 5.1.4. If $(1, 0, \ldots, 0) \in \overline{\mathcal{C}}_P$, then $\mathcal{C}_P^\nu$ is contained in the open halfspace defined by $y_0 > 0$ and thus the map $(y_0, y_1, \ldots, y_k) \mapsto (1, y_1/y_0, \ldots, y_k/y_0)$ induces a bijection between $\mathbb{P}\mathcal{C}_P^\nu$ and Δ_P.

5.1.4 The equivalence of solutions

Here is a slight variation of Theorem 2.2 in [8].

Theorem 5.1.5. *There is a bijection between the positive solutions of the initial system* (5.0.1) *and the solutions of the Gale dual system* (5.1.8) *in* $\mathbb{P}\mathcal{C}_P^\nu$*, which induces a bijection between the positive solutions of* (5.0.1) *and the solutions of* (5.1.8) *in* Δ_P *when* $(1, 0, \ldots, 0) \in \overline{\mathcal{C}}_P$.

Proof. If $x \in \mathbb{R}^d_{>0}$ is a solution of the system (5.0.1), then $(x^{a_1}, \ldots, x^{a_n})$ belongs to $\ker(C) \cap \mathbb{R}^n_{>0}$. Thus, there exists $y \in \mathbb{R}^{k+1}$ (which is unique since D has maximal rank) such that $x^{a_i} = \langle P_i, y\rangle$ for $i = 1, \ldots, n$. Then, $y \in \mathcal{C}_P^\nu$ and y is a solution of the Gale dual system (5.1.8). If furthermore $(1, 0, \ldots, 0) \in \overline{\mathcal{C}}_P$, then dividing by y_0 if necessary, a solution $y \in \mathcal{C}_P^\nu$ of (5.1.8) gives a solution of the same system in Δ_P because it is homogeneous of degree zero.

We showed in Remark 5.1.4 that the previous map is bijective by giving explicitly its inverse map. Now, let $y \in \mathcal{C}_P^\nu$ be a solution of (5.1.8). Let $(e_1, \ldots, e_d)$ be the canonical basis of $\mathbb{R}^d$. Since A has maximal rank, there exists $\alpha_j = (\alpha_{1j}, \ldots, \alpha_{nj}) \in \mathbb{R}^n$, for $j = 1, \ldots, d$, such that $e_j = \sum_{i=1}^n \alpha_{ij} a_i$. To any column vector $z \in \mathbb{R}^{k+1}$, we associate the vector $D \cdot z$ with coordinates $\langle P_i, z\rangle$, $i = 1, \ldots, n$. Consider now the map

$$\begin{aligned} \varphi\colon \mathbb{R}^{k+1} &\to \mathbb{R}^d \\ z &\mapsto ((D\cdot z)^{\alpha_1}, \ldots, (D\cdot z)^{\alpha_d}), \end{aligned}$$

where $(D \cdot z)^{\alpha_j} = \prod_{i=1}^n \langle P_i, z\rangle^{\alpha_{ij}}$. Let $x = \varphi(y)$. Then, $x^{a_i} = \langle P_i, y\rangle$ for $i = 1, \ldots, n$, which gives $(x^{a_1}, \ldots, x^{a_n}) \in \ker(C)$. Moreover, since $y \in \mathcal{C}_P^\nu$, we have that $x \in \mathbb{R}^d_{>0}$, and then x is a positive solution of system (5.0.1). □

Remark 5.1.6. Theorem 2.2 in [8] is a particular case of Theorem 5.1.5 taking a Gale dual matrix D with the identity matrix I_{k+1} at the top (in which case the condition that $(1, 0, \ldots, 0) \in \overline{\mathcal{C}}_P$ is trivially satisfied).

5.2 Existence of positive solutions via Gale duality and degree theory

In this section, we present Theorem 5.2.7, which gives conditions on the Gale dual matrices B and D that guarantee the existence of at least one positive solution of the system (5.0.1). As we mentioned before, our results are based on degree theory. Assume from now on that the matrix C is *uniform*, that is, that no maximal minor of C vanish, and the necessary condition (5.1.3) is satisfied.

Given an open set $U \subset \mathbb{R}^k$, a function $h \in \mathcal{C}^0(\overline{U}, \mathbb{R}^k)$ and $y \in \mathbb{R}^k \setminus h(\partial U)$, the symbol $\deg(h, U, y)$ denotes the Brouwer degree (which belongs to $\mathbb{Z}$) of h with respect to (U, y). A main result in degree theory is that if $\deg(h, U, y) \neq 0$, then there exists at least one $x \in U$ such that $y = h(x)$. For background and the main properties about Brouwer degree, we refer to Section 2 in the Supplementary Information of [12] and Section 14.2 in [99].

We present the version of the Brouwer's theorem that we will use. This version is a particular case of Theorem 2 in the Supplementary Information of [12] (here we take W empty), and also appears in the proof of Lemma 2 of [23]. Recall that a vector $v \in \mathbb{R}^k$ *points inwards* a $U \subset \mathbb{R}^k$ U at a boundary point $x \in \partial U$, if for small $\varepsilon > 0$ it holds that $x + \varepsilon v \in \overline{U}$.

Theorem 5.2.1 ([12, 23])**.** *Let $h : \mathbb{R}^k \to \mathbb{R}^k$ be a $\mathcal{C}^1$-function. Let U be an open, nonempty, bounded and convex subset of $\mathbb{R}^k$ such that*

i) $h(x) \neq 0$ for any $x \in \partial U$.

ii) for every $x \in \partial U$, the vector $h(x)$ points inwards U at x.

Then,

$$\deg(h, U, 0) = (-1)^k.$$

In particular, there exists a point x in U such that $h(x) = 0$. Moreover, assuming the zeros are nondegenerate, if there exists a zero $x^* \in U$ where the sign of the Jacobian at x^* is $(-1)^{k+1}$, then there are at least three zeros and always an odd number.

Define the sign of any real number r by $\operatorname{sign}(r) = +1, -1, 0$ according as $r > 0$, $r < 0$ or $r = 0$ respectively. The sign of any vector $r = (r_1, \ldots, r_k) \in \mathbb{R}^k$ is then defined by $\operatorname{sign}(r) = (\operatorname{sign}(r_1), \ldots, \operatorname{sign}(r_k))$.

In view of Theorem 5.1.5, we look for the solutions of (5.1.8) in Δ_P. Plugging $y_0 = 1$ in (5.1.8) and clearing the denominators, we get a generalized polynomial system in Δ_P on variables $y = (y_1, \ldots, y_k)$:

$$g_j(y) = 0, \quad j = 1, \ldots, k, \quad \text{with } g_j(y) = \prod_{b_{ij}>0} p_i(y)^{b_{ij}} - \prod_{b_{ij}<0} p_i(y)^{-b_{ij}}, \tag{5.2.1}$$

where

$$p_i(y) = \langle P_i, (1, y) \rangle. \tag{5.2.2}$$

We denote by g the Gale map:

$$g = (g_1, \ldots, g_k) \colon \mathbb{R}^k \to \mathbb{R}^k. \tag{5.2.3}$$

Definition 5.2.2. *Given $C \in \mathbb{R}^{d\times n}$ uniform, we denote by $I_C \subset \{1,\dots,n\}$ the set of indexes corresponding to the minimal set of generators $\{P_i, i \in I_C\}$ of the polyhedral cone $\mathcal{C}_P$.*

Note that the set I_C is unique since C is uniform and satisfies Condition (5.1.3), which implies that $P_1,\dots,P_n$ lie in an open halfspace through the origin. The facets of $\mathcal{C}_P^\nu$ are supported on the orthogonal hyperplanes $P_i^\perp$ for $i \in I_C$. Note that for any $i \in I_C$ the vector P_i is an inward normal vector of $\mathcal{C}_P^\nu$ at any point in the relative interior of the facet supported on $P_i^\perp$. It follows that the facets of the polytope Δ_P are supported on the hyperplanes $p_i(y) = 0$ for $i \in I_C$, and that $(d_{i1},\dots,d_{ik})$ is an inward normal vector of Δ_P at any point in the relative interior of the facet supported on $p_i(y) = 0$. Note also that I_C depends on C and is independent of the choice of a Gale dual matrix D. In fact, it can be characterized by the following property: for any $(z_1,...,z_n)$ in the kernel of C, we have $z_i > 0$ for $i = 1,...,n$ if and only if $z_i > 0$ for all $i \in I_C$.

Definition 5.2.3. *For any $i \in I_C$ denote by F_i the facet of Δ_P supported on $p_i(y) = 0$, and set*

$$F_L = \cap_{i\in L} F_i, \text{ for any } L \subset I_C.$$

Here by a face of Δ_P we mean a face of the closure of Δ_P. We denote by F_L° the relative interior of F_L. We set

$$\mathcal{F}(\Delta_P) = \{L \subset I_C \,:\, F_L \text{ is a face of } \Delta_P\}.$$

We want to compute the number of zeros of the Gale map g in (5.2.3) inside Δ_P. The sign of g along the boundary of Δ_P can sometimes be determined as follows.

Lemma 5.2.4. *Let $A \in \mathbb{R}^{(d+1)\times n}$ as in (5.1.1), $C \in \mathbb{R}^{d\times n}$, and $B \in \mathbb{R}^{n\times k}$ and $D \in \mathbb{R}^{n\times(k+1)}$ Gale dual matrices of A and C respectively. Let $g = (g_1,\dots,g_k)$ the Gale map as in (5.2.3). Let $j \in \{1,\dots,k\}$.*

(1) Let F_i be any facet of Δ_P and let $x \in F_i^\circ$. If $b_{ij} \neq 0$, then $\operatorname{sign}(g_j(x)) = -\operatorname{sign}(b_{ij})$.

(2) Let $L \in \mathcal{F}(\Delta_P)$ and $x \in F_L$. Assume that $\{b_{\ell j} \,:\, \ell \in L\} \neq \{0\}$.

(i) If there exists $\ell_0, \ell_1 \in L$ such that $b_{\ell_0 j} \cdot b_{\ell_1 j} < 0$, then $g_j(x) = 0$.

(ii) If $b_{\ell j} \geq 0$ for all $\ell \in L$ then $\operatorname{sign}(g_j(x)) = -1$, and if $b_{\ell j} \leq 0$ for all $\ell \in L$ then $\operatorname{sign}(g_j(x)) = +1$.

Corollary 5.2.5. *Let $A \in \mathbb{R}^{(d+1)\times n}$ as in (5.1.1), $C \in \mathbb{R}^{d\times n}$, and $B \in \mathbb{R}^{n\times k}$ and $D \in \mathbb{R}^{n\times(k+1)}$ Gale dual matrices of A and C respectively. Let g be the Gale map (5.2.3) associated to B and D. If $g(x) = 0$ and $x \in F_L$ (so $L \in \mathcal{F}(\Delta_P)$), then for $j = 1,\dots,k$, either $\{b_{\ell j} \,:\, \ell \in L\} = \{0\}$, or $\{b_{\ell j} \,:\, \ell \in L\}$ contains a positive and a negative element.*

In particular, if g vanishes in the relative interior of a facet F_ℓ then the ℓ-th row of B contains only zero entries.

Definition 5.2.6. *We say that a matrix M is* weakly mixed *if any column of M either has only zero entries, or contains a (strictly) positive and a (strictly) negative element.*

Otherwise said, a matrix M is not weakly mixed if and only if it has a non zero column whose entries are all either nonpositive, or nonnegative.

Given $B \in \mathbb{R}^{n\times k}$ and $L \subset \{1,\dots,n\}$, we denote by $B_L \in \mathbb{R}^{|L|\times k}$ the submatrix of B given by the rows with indexes in L. We now present the main result of this section.

Theorem 5.2.7. *Let $\mathcal{A} = \{a_1,\dots,a_n\} \subset \mathbb{R}^d$ and $C \in \mathbb{R}^{d\times n}$ uniform. Let $A \in \mathbb{R}^{(d+1)\times n}$ as in* (5.1.1)*, and $B \in \mathbb{R}^{n\times k}$ and $D \in \mathbb{R}^{n\times(k+1)}$ Gale dual matrices of A and C respectively. Assume that $\mathbf{0} \in \mathcal{C}^\circ$ and that Δ_P is a full dimensional bounded polytope.*

Assume furthermore that the following conditions hold:

(1) For any $L \in \mathcal{F}(\Delta_P)$ the submatrix $B_L \in \mathbb{R}^{|L|\times k}$ is not weakly mixed.

(2) For any $i \in I_C$ the following holds:

- *$b_{ij} \cdot d_{ij} \geq 0$ for $j = 1,\dots,k$,*
- *there exists $j \in \{1,\dots,k\}$ such that $b_{ij} \cdot d_{ij} > 0$,*
- *for all $j \in \{1,\dots,k\}$, if $b_{ij} = 0$ then $d_{ij} = 0$.*

Then $n_{\mathcal{A}}(C) > 0$.

Proof. Since Δ_P is a full dimensional and bounded, $(1,0,\dots,0) \in \mathcal{C}_P$. By Theorem 5.1.5 it is sufficient to show that the Gale system (5.2.1) has at least one solution in Δ_P. First note that a vector $v \in \mathbb{R}^k$ points inwards Δ_P at a point y contained in the relative interior of a facet F_i ($i \in I_C$) if and only if $\langle (d_{i1},\dots,d_{ik}), v\rangle \geq 0$. More generally $v \in \mathbb{R}^k$ points inwards Δ_P at a point y in the relative interior of a face F_L ($L \in \mathcal{F}(\Delta_P)$) if and only if $\langle (d_{\ell 1},\dots,d_{\ell k}), v\rangle \geq 0$ for any $\ell \in L$, by a classical result of convex geometry. The assumption (1) ensures that g does not vanish at $\partial\Delta_P$, by Corollary 5.2.5. Condition (2) ensures that $-g$ points inwards Δ_P at each point x in the relative interior of any facet F_i. Then $-g$ also point inwards Δ_P at any point x in the relative interior of a face F_L. The result follows now from Theorem 5.2.1, taking $U = \Delta_P$ and $h = -g$. □

Example 5.2.8. Consider the codimension one case $k = 1$ (which is treated carefully in [5]). Then $B \in \mathbb{R}^{(d+2)\times 1}$ is a column matrix and its entries are the coefficients $\lambda_1,\dots,\lambda_{d+2}$ of a nontrivial affine relation on $\mathcal{A}$. Assume that A is uniform (equivalently, assume that $\mathcal{A}$ is a circuit [1]). Then, B has no zero entry. Assume moreover that C is uniform and that $\mathbf{0} \in \mathcal{C}^\circ$. Then, there exists a Gale dual matrix D such that Δ_P is a bounded interval of $\mathbb{R}$. Moreover, there exists a vector $\delta \in \mathbb{R}^2$ such

[1] Recall that a point configuration $\mathcal{A}$ of $d+2$ points is a *circuit* if any subset of $d+1$ points of $\mathcal{A}$ is affinely independent.

that $\langle P_i, \delta\rangle > 0$ for $i = 1, \ldots, d+2$, where $P_1, \ldots, P_{d+2} \in \mathbb{R}^2$ are the row vectors of D. Let $\alpha : \{1, \ldots, d+2\} \to \{1, \ldots, d+2\}$ be the bijection such that all the determinants $\det(P_{\alpha_i}, P_{\alpha_{i+1}})$ for $i = 1, \ldots, d+1$ are positive. Then, by Theorem 2.9 in [5], we have $n_{\mathcal{A}}(C) \leq \text{signvar}(\lambda_{\alpha_1}, \lambda_{\alpha_2}, \ldots, \lambda_{\alpha_{d+2}})$ and moreover the difference is an even integer number (see Proposition 2.12 in [5]). The endpoints of the interval Δ_P are the roots of the two extremal polynomials p_{α_1} and $p_{\alpha_{d+2}}$, equivalently, $I_C = \{\alpha_1, \alpha_{d+2}\}$. Now the Gale polynomial $g = g_1 : \mathbb{R} \to \mathbb{R}$ points inwards Δ_P at its vertices if and only if $\lambda_{\alpha_1} \cdot \lambda_{\alpha_{d+2}} < 0$, which is equivalent to $\text{signvar}(\lambda_{\alpha_1}, \lambda_{\alpha_{d+2}}) = 1$. But, $\text{signvar}(\lambda_{\alpha_1}, \lambda_{\alpha_2}, \ldots, \lambda_{\alpha_{d+2}})$ and $\text{signvar}(\lambda_{\alpha_1}, \lambda_{\alpha_{d+2}})$ have the same parity. Thus $g : \mathbb{R} \to \mathbb{R}$ points inwards Δ_P at its vertices if and only if $n_{\mathcal{A}}(C)$ is odd by Proposition 2.12 in [5]. Therefore, in the circuit case the sufficient condition to have $n_{\mathcal{A}}(C) > 0$ which is given by Theorem 5.2.7 is equivalent to $n_{\mathcal{A}}(C)$ being odd. Now, for any integer $d \geq 2$, it is not difficult to get examples of circuits $\mathcal{A} \subset \mathbb{R}^d$ and matrices C such that $n_{\mathcal{A}}(C)$ is odd and is different from 1. This shows that our sufficient condition does not imply $n_{\mathcal{A}}(C) = 1$ in general, and thus is not equivalent to the condition given in [80] ensuring that $n_{\mathcal{A}}(C) = 1$.

We now present an example with $k = d = 2$ to illustrate Theorem 5.2.7.

Example 5.2.9. Let $\mathcal{A} \subset \mathbb{Z}^2$ be the set of points $a_1 = (0, 4)$, $a_2 = (5, 4)$, $a_3 = (2, 8)$, $a_4 = (3, 0)$ and $a_5 = (3, 5)$. Consider the matrix of coefficients

$$C = \begin{pmatrix} -1 & -1 & 1 & 1 & 0 \\ -(3c+8) & -c & 2c+8 & 0 & 2 \end{pmatrix},$$

where $c \in \mathbb{R}$ is a parameter. The polynomial system of two polynomial equations and two variables x, y:

$$\begin{aligned} -y^4 - x^5y^4 + x^2y^8 + x^3 &= 0, \\ -(3c+8)y^4 - cx^5y^4 + (2c+8)x^2y^8 + 2x^3y^5 &= 0, \end{aligned}$$

has support $\mathcal{A}$ and coefficient matrix C. Let A as in (5.1.1). Choose the following Gale dual matrices of A and C :

$$B = \begin{pmatrix} 1 & 0 \\ 2 & 1 \\ 1 & 2 \\ 0 & 1 \\ -4 & -4 \end{pmatrix} \qquad D = \left(\begin{array}{c|cc} 1 & 1 & 0 \\ 1 & 1 & 2 \\ 1 & 2 & 1 \\ 1 & 0 & 1 \\ c & -4 & -4 \end{array}\right)$$

Then $p_1(y) = 1 + y_1$, $p_2(y) = 1 + y_1 + 2y_2$, $p_3(y) = 1 + 2y_1 + y_2$, $p_4(y) = 1 + y_2$ and $p_5(y) = c - 4y_1 - 4y_2$. If $c > 0$, the convex polytope Δ_P is nonempty, bounded and has all the possible facets. Moreover, if $c > 0$, then the assumptions of Theorem 5.2.7 are satisfied and thus $n_{\mathcal{A}}(C) > 0$.

We use Singular [25] to check what happens when we vary the value of the parameter $c > 0$.

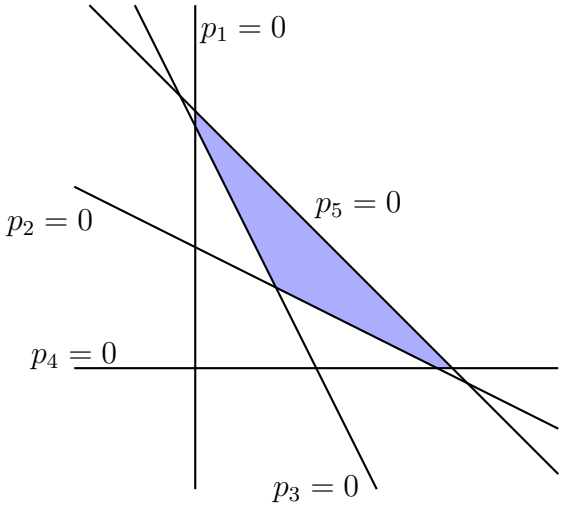


Figure 5.1: The polytope Δ_P of Example 5.2.9, with $c > 0$.

```
LIB "signcond.lib";
ring r=0, (c,x,y,t), dp;
poly f1=-y^4-x^5*y^4+x^2*y^8+x^3;
poly f2=-(3*c+8)*y^4-c*x^5*y^4+(2*c+8)*x^2*y^8+2*x^3*y^5;
ideal i=f1,f2, diff(f1,x)*diff(f2,y)-diff(f1,y)*diff(f2,x),x*y*t-1;
ideal j=std(i);
ideal k =eliminate(j, x*y*t);
k;
k[1]=48c12+1280c11+12288c10+49152c9+65467c8-2560c7-24576c6-
98304c5-131078c4+1280c3+12288c2+49152c+65563
```

The roots of this last polynomial in c correspond to systems with a degenerate solution, and we can check that the only positive root of f_1, f_2 and their jacobian is 1. We check, again using Singular [25], with the library "signcond.lib" (implemented by E. Tobis, based on the algorithms described in [4]) that if we take for example $c = \frac{1}{2}$ ($c < 1$), the system has 3 positive solutions, and if we take $c = \frac{8}{7}$ ($c > 1$), the system has only 1 positive solution. We use the command `firstoct`, that computes the number of roots of a system in the first octant, that is, the positive roots.

```
LIB "signcond.lib";
ring r=0, (x,y), dp;
poly f1=-y^4-x^5*y^4+x^2*y^8+x^3;
poly f2=-(3*(1/2)+8)*y^4-(1/2)*x^5*y^4+(2*(1/2)+8)*x^2*y^8+2*x^3*y^5;
poly f3=-(3*(8/7)+8)*y^4-(8/7)*x^5*y^4+(2*(8/7)+8)*x^2*y^8+2*x^3*y^5;
ideal i2 = f1,f2;
ideal j2 = std(i2);
firstoct(j2);
3
ideal i3 = f1,f3;
ideal j3 = std(i3);
```

```
firstoct(j3);
1
```

For $c = \frac{1}{2}$, the condition in [80] to ensure exactly one positive solution is trivially not satisfied (as expected since the system has 3 positive solutions).

This computation of the number of positive solutions with the command `firstoct` works for moderately sized polynomial systems with coefficients in $\mathbb{Q}$ or an algebraic extension of it. Our results are particularly useful to study families of polynomials.

5.3 Dominating matrices

In this section, we present some conditions on A and C that guarantee that the hypotheses of Theorem 5.2.7 are satisfied. Our main result is Theorem 5.3.6.

We first present conditions that guarantee that a matrix A admits a choice of a Gale dual matrix B, which satisfies condition (1) of Theorem 5.2.7, for *any* uniform matrix of coefficients C satisfying (5.1.3) (which means that it does not depend on I_C). When $A \in \mathbb{Z}^{(d+1)\times n}$ we will relate these conditions with complete intersection lattice ideals in Section 5.5.

We recall some definitions from [46], with the difference that we replace rows by columns and allow matrices with real entries.

Definition 5.3.1. *A vector is said to be mixed if contains a strictly positive and a strictly negative coordinate. More generally, a real matrix is called mixed if every column contains a strictly positive and a strictly negative entry. A real matrix is called dominating if it contains no square mixed submatrix. An empty matrix is mixed and also dominating.*

Observe that since a matrix A as in (5.1.1) has a row of ones, the columns of any Gale dual matrix B add up to zero, and thus B is always mixed. Also note that a mixed matrix is weakly mixed (see Definition 5.2.6), but the converse is not true in general as a weakly mixed matrix can also contain a column with only zero entries.

Lemma 5.3.2. *Assume that $A \in \mathbb{R}^{(d+1)\times n}$ is a uniform matrix. If $B \in \mathbb{R}^{n\times k}$ is a Gale dual matrix of A which is dominating, then condition (1) of Theorem 5.2.7 is satisfied for all $C \in \mathbb{R}^{d\times n}$ uniform satisfying $\mathbf{0} \in \mathcal{C}^\circ$.*

Proof. Let $C \in \mathbb{R}^{d\times n}$ uniform, and take any Gale dual matrix $D \in \mathbb{R}^{n\times(k+1)}$ of C such that Δ_P is nonempty and bounded (which exists due to Lemma 5.1.3 and Corollary 5.1.2).

If $L = \{\ell\} \in \mathcal{F}(\Delta_P)$, then B_L weakly mixed means that it has only zeros, which forces the matrix A minus the ℓ-th column to have rank $< d+1$. Consider $L \in \mathcal{F}(\Delta_P)$ such that $|L| \geq 2$. Note that $|L| \leq k$ since C is uniform. If B_L is weakly mixed then at least $k - |L| + 1$ columns of B_L contain only zero entries, for otherwise we would get a square submatrix of size $|L| \times |L|$ containing a positive and a negative coefficient in each column. But if $k - |L| + 1$ columns of B_L contain only zeros, then we get $k - |L| + 1$ linearly independent vectors in the kernel of the

matrix $A_{\setminus L}$ obtained from A by removing the columns indexed by L. This forces the matrix $(A_{\setminus L})$ to have rank less than $d+1$, which is a contradiction, since A is uniform. □

The following results will be useful. The following propositions are only stated for matrices with integer matrices in [46], but clearly the proofs given in that paper also work for real matrices.

Proposition 5.3.3 ([46], Corollary 2.7 and 2.8)**.** *If a real matrix is mixed dominating, then any nonzero linear combination of its columns is a mixed vector. In particular, its columns are linearly independent.*

Proposition 5.3.4 ([45], Proposition 4.1)**.** *The left kernel of any mixed dominating real matrix contains a positive vector.*

We will also need the following Lemma:

Lemma 5.3.5. *Assume that $C \in \mathbb{R}^{d\times n}$ has maximal rank d and that $\mathbf{0} \in \mathcal{C}^\circ$. Let $\tilde{D} \in \mathbb{R}^{n\times k}$ be any matrix of maximal rank k such that $C\tilde{D} = 0$. Assume that*

$$\mathbf{0} \in \mathbb{R}_{>0}\tilde{P}_1 + \cdots + \mathbb{R}_{>0}\tilde{P}_n, \tag{5.3.1}$$

where $\tilde{P}_1, \ldots, \tilde{P}_n$ are the row vectors of $\tilde{D}$. Then, there exists a positive vector D_0 in the kernel of C which does not belong to the linear span of the column vectors of $\tilde{D}$, and the matrix $D \in \mathbb{R}^{n\times(k+1)}$ obtained from $\tilde{D}$ by adding D_0 as a first column vector is Gale dual to C and satisfies $(1, 0, \ldots, 0) \in \mathcal{C}_P$.

Proof. By (5.3.1) there exists a positive vector in the left kernel of $\tilde{D}$, in other words, a row vector λ with positive coordinates such that $\lambda \cdot \tilde{D} = (0, \ldots, 0)$. Since $\mathbf{0} \in \mathcal{C}^\circ$ we have $\ker(C) \cap \mathbb{R}^n_{>0} \neq \emptyset$. Then, as $\ker(C)$ has dimension $k+1$ and $\tilde{D}$ has rank k, there exists a vector $D_0 \in \ker(C) \cap \mathbb{R}^n_{>0}$ which does not belong to the linear span of the column vectors of $\tilde{D}$. The matrix $D \in \mathbb{R}^{n\times k}$ obtained from $\tilde{D}$ by adding D_0 as a first column vector is Gale dual to C. Moreover, we have $\lambda \cdot D = (\lambda \cdot D_0, 0, \ldots, 0)$ and thus $(1, 0, \ldots, 0) \in \mathcal{C}_P$ since $\lambda \cdot D_0 > 0$ (here λ is a row vector, D_0 is a column vector so that $\lambda \cdot D_0$ is a real number, which is positive since λ and D_0 are positive vectors). □

If $S \subset \mathbb{R}^n$ if a subspace, we denote $\operatorname{sign}(S) = \{\operatorname{sign}(v) : v \in S\}$. Recall that we denote the column vectors of a matrix B by $B_1, \ldots, B_k$.

Theorem 5.3.6. *Let $\mathcal{A} = \{a_1, \ldots, a_n\} \subset \mathbb{R}^d$. Assume $A \in \mathbb{R}^{(d+1)\times n}$ as in (5.1.1), and $C \in \mathbb{R}^{d\times n}$ are uniform matrices. Suppose there exist $B \in \mathbb{R}^{n\times k}$ Gale dual matrix of A such that B is dominating. Assume $\mathbf{0} \in \mathcal{C}^\circ$ and $\operatorname{sign}(B_j) \in \operatorname{sign}(\ker(C))$ for each $j = 1, \ldots, k$. Then, $n_{\mathcal{A}}(C) > 0$.*

Proof. As B is dominating and A, C are uniform, condition (1) of Theorem 5.2.7 is satisfied by Lemma 5.3.2. As $\operatorname{sign}(B_j) \in \operatorname{sign}(\ker(C))$ for $j = 1, \ldots, k$, there exist vectors $D_1, \ldots, D_k$ in $\ker(C)$ such that $\operatorname{sign}(D_j) = \operatorname{sign}(B_j)$ for each $j =$

$1, \ldots, k$. Consider the matrix $\tilde{D}$ with column vectors $D_1, \ldots, D_k$. Since B is mixed dominating (it is mixed since A contains a row of ones) the matrix $\tilde{D}$ is mixed dominating and furthermore $\tilde{D}$ has rank k by Proposition 5.3.3. Moreover, by Proposition 5.3.4, there is a positive vector in the left kernel of $\tilde{D}$. Then, condition (5.3.1) is satisfied, and thus by Lemma 5.3.5 and Corollary 5.1.2, there is a positive vector D_0 such that the matrix D with column vectors $D_0, \ldots, D_k$ is Gale dual to C and the associated polytope Δ_P is nonempty and bounded. By construction, condition (2) of Theorem 5.2.7 is also satisfied, and thus $n_{\mathcal{A}}(C) > 0$. □

Recall that the support of a vector $v \in \mathbb{R}^n$ is defined as the set of its nonzero coordinates, and we denote it by $\operatorname{supp}(v)$. Given a subspace $S \subset \mathbb{R}^n$, a circuit of S is a nonzero element $s \in S$ with minimal support (with respect to inclusion). Given a vector v, a circuit $s = (s_1, \ldots, s_n)$ is said to be conformal to $v = (v_1, \ldots, v_n)$ if for any index i in $\operatorname{supp}(s)$, $\operatorname{sign}(s_i) = \operatorname{sign}(v_i)$. The next lemma shows that if A admits a Gale dual mixed dominating matrix, then there exist a choice of Gale mixed dominating matrix of A whose columns are circuits of $\ker(A)$. Note that all the circuits of $\ker(A)$ can be described in terms of vectors of maximal minors of A, and so they only depend on the *associated oriented matroid* of A.

Lemma 5.3.7. *Assume $A \in \mathbb{R}^{(d+1)\times n}$ as in* (5.1.1)*. Suppose there exist a dominating Gale dual matrix $B \in \mathbb{R}^{n\times k}$ of A. Then, there exists $B' \in \mathbb{R}^{n\times k}$ a Gale dual matrix of A such that B' is dominating and every column of B' is a circuit of* $\ker(A)$.

Proof. It is a known result that every vector in $\ker(A)$ can be written as a nonnegative sum of circuits conformal to it (see [89]). In particular, for every vector in $\ker(A)$, there exists a circuit conformal to it. For each column B_i of B, $i = 1, \ldots, k$, take a circuit B'_i of $\ker(A)$ such that B'_i is conformal to B_i. Now, we take B' the matrix with columns $B'_1, \ldots, B'_k$. Every column of B' is a circuit of $\ker(A)$, B' is mixed since A has a row of ones, and is dominating because B'_i is conformal to B_i for each $i = 1, \ldots, k$ and the matrix B is dominating. Since B' is mixed dominating, the columns of B' are linearly independent by Proposition 5.3.3, and then B' is a Gale dual matrix of A. □

5.4 Geometric conditions on A and C

The main result of this section is Theorem 5.4.8, where we give geometric conditions on A and C that guarantee that the hypotheses of Theorem 5.2.7 are satisfied.

A characterization of matrices A admitting a mixed dominating Gale dual matrix B can be found in [45]. Recall that our definition of mixed dominating matrix differs from the one in [45] by replacing rows by columns. Here we present this result with our notation. We denote the convex hull of a point configuration $\mathcal{A}$ by $\operatorname{chull}(\mathcal{A})$. Recall also that we assume $n \geq d + 2$, so that $\mathcal{A}$ cannot be the set of vertices of a d-dimensional simplex.

Theorem 5.4.1 ([45], Theorem 5.6). *Let $\mathcal{A} = \{a_1, \ldots, a_n\} \subset \mathbb{R}^d$, with $n \geq d+2$ and $A \in \mathbb{R}^{(d+1)\times n}$ as in* (5.1.1)*. Then A admits a mixed dominating Gale dual matrix B if and only if $\mathcal{A}$ can be written as a disjoint union $\mathcal{A} = \mathcal{A}_1 \sqcup \mathcal{A}_2$ such that*

(1) the polytopes $\operatorname{chull}(\mathcal{A}_1)$ *and* $\operatorname{chull}(\mathcal{A}_2)$ *intersect in exactly one point,*

(2) the corresponding matrices A_1 and A_2 as in (5.1.1) *of $\mathcal{A}_1$ and $\mathcal{A}_2$ respectively, admit mixed dominating Gale dual matrices, and*

(3) $\dim \operatorname{chull}(\mathcal{A}) = \dim \operatorname{chull}(\mathcal{A}_1) + \dim \operatorname{chull}(\mathcal{A}_2)$.

Moreover, we have:

Lemma 5.4.2 ([45], Corollary 5.7)**.** *If A admits a mixed dominating Gale dual matrix B then* $\operatorname{chull}(\mathcal{A})$ *has at most $2d$ vertices.*

In particular, by Lemma 5.3.2, we have:

Corollary 5.4.3. *Let $\mathcal{A} = \{a_1, \ldots, a_n\} \subset \mathbb{R}^d$. Assume that A as in* (5.1.1) *is uniform and that $\mathcal{A} \subset \mathbb{R}^d$ can be decomposed as a disjoint union $\mathcal{A} = \mathcal{A}_1 \sqcup \mathcal{A}_2$ such that conditions (1), (2) and (3) of Theorem 5.4.1 hold. Then, there exists a Gale dual matrix $B \in \mathbb{Z}^{n\times k}$ of A such that condition (1) of Theorem 5.2.7 is satisfied.*

The following observation says that if we have a point configuration $\mathcal{A}_v \subset \mathbb{R}^d$ such that the corresponding matrix A_v admits a Gale dual mixed dominating matrix, then, for any other point configuration $\mathcal{A} \subset \mathbb{R}^d$ that contains $\mathcal{A}_v$ and their convex hulls $\operatorname{chull}(\mathcal{A}), \operatorname{chull}(\mathcal{A}_v)$ coincide (that is, $\mathcal{A}$ can be obtained from $\mathcal{A}_v$ adding points inside the convex hull), the corresponding matrix A also admits a Gale dual mixed dominating matrix.

Lemma 5.4.4. *Let $\mathcal{A} = \{a_1, \ldots, a_n\}$, $\mathcal{A}_v \subset \mathbb{R}^d$ be two point configurations such that $\mathcal{A}_v \subset \mathcal{A}$. Assume that the corresponding matrix $A \in \mathbb{R}^{(d+1)\times n}$ is uniform and that the following conditions hold:*

(1) $\operatorname{chull}(\mathcal{A}) = \operatorname{chull}(\mathcal{A}_v)$,

(2) the corresponding matrix $A_v \in \mathbb{R}^{(d+1)\times|A_v|}$ has a Gale dual matrix B_v which is dominating.

Then, there exists a a mixed dominating Gale dual matrix $B \in \mathbb{Z}^{n\times k}$ of A and thus condition (1) of Theorem 5.2.7 is satisfied.

Note that Lemma 5.4.4 follows from applying several times Theorem 5.4.1 (taking one point from $\mathcal{A}_v$ as $\mathcal{A}_2$), but we present a constructive proof.

Proof of Lemma 5.4.4. Without loss of generality, assume that $\mathcal{A}_v = \{a_1, \ldots, a_s\}$, with $s \geq d$. For $i = s+1, \ldots, n$, there exists a subset $\mathcal{A}_i$ of $\mathcal{A}_v$ such that $\mathcal{A}_i$ is the set of vertices of a d-simplex and a_i is contained in the interior of $\operatorname{chull}(\mathcal{A}_i)$. Then there exists an affine relation on $\mathcal{A}_i \cup \{a_i\}$ where the coefficient of a_i is equal to one

and the coefficients of the points of $\mathcal{A}_i$ are all negative. Using the affine relations on $\mathcal{A}_v$ given by the column vectors of B_v, we get k linearly independent vectors in the kernel of A which are the column vectors of a upper triangular block matrix B Gale dual to A of the following form:

$$B = \begin{pmatrix} B_v & R \\ 0 & I_{n-s} \end{pmatrix},$$

where R has only nonpositive entries (and at least two negative entries in each column) and I_{n-s} is the identity matrix of size $n-s$. Clearly, if B_v is dominating then B is dominating and thus by Lemma 5.3.2 the first item of Theorem 5.2.7 is satisfied. □

We have the following corollary.

Corollary 5.4.5. *Let $\mathcal{A} = \{a_1, \ldots, a_n\}$, $\mathcal{A}_v \subset \mathbb{R}^d$ be two point configurations such that $\mathcal{A}_v \subset \mathcal{A}$ and* $\operatorname{chull}(\mathcal{A}) = \operatorname{chull}(\mathcal{A}_v)$. *Assume that the corresponding matrix A is uniform and that $\mathcal{A}_v$ is either the set of vertices of d-simplex, or a circuit in $\mathbb{R}^d$. Then, there exists a Gale dual matrix $B \in \mathbb{Z}^{n\times k}$ of A such that condition (1) of Theorem 5.2.7 is satisfied.*

Consider $\mathcal{A}$ and the point configuration $\mathscr{C} = \{C_1, \ldots, C_n\}$ given by the columns of the coefficient matrix C. Consider the $(d+1) \times n$-matrix

$$\bar{C} = \begin{pmatrix} 1 & \cdots & 1 \\ & C & \end{pmatrix}$$

and assume that A and $\bar{C}$ are uniform. Given a subset $J \subset \{1, \ldots, n\}$, we denote $\mathcal{A}_J = \{a_j : j \in J\}$.

Definition 5.4.6. *Given a subset $I \subset \{1, \ldots, n\}$, we say that A and C are I-*compatible *if the following conditions hold:*

(1) the corresponding matrices A_I and $\overline{C}_I$ admit Gale dual matrices which are mixed dominating and have the same sign pattern,

(2) $\operatorname{chull}(\mathcal{A}_I) = \operatorname{chull}(\mathcal{A})$ *and* $\operatorname{chull}(\mathscr{C}_I) = \operatorname{chull}(\mathscr{C})$,

(3) for each $j \notin I$, there exist $J \subset I$, with $|J| = d+1$, such that $a_j \in \operatorname{chull}(\mathcal{A}_J)$ and $C_j \in \operatorname{chull}(\mathscr{C}_J)$.

The condition that A and C are *I-compatible* can be translated in terms of signs of maximal minors of A and $\bar{C}$. Also note that not all the maximal minors need to have the same sign, that is, the configurations may have different matroids. The following Example 5.4.7 shows two I-compatible configurations with different matroids.

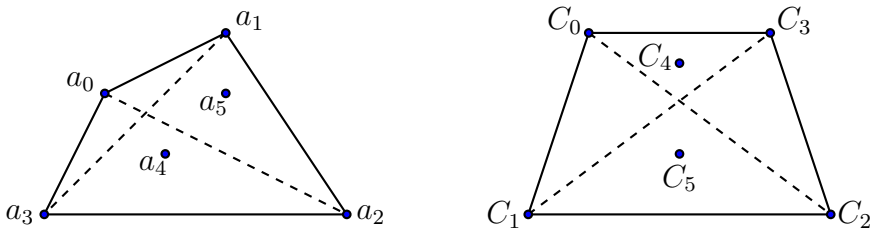


Figure 5.2: Example of A and C I-compatible.

Example 5.4.7. *We show in Figure 5.4 an example of two point configurations,* $\mathcal{A} = \{a_1, \ldots, a_6\}$ *and* $\mathscr{C} = \{C_1, \ldots, C_6\}$ *with* $d = 2$ *and* $k = 3$*, which are* I*-compatible, for* $I = \{1, 2, 3, 4\}$*. In this case* $a_4 \in ch(\mathcal{A}_{I_4})$*,* $C_4 \in ch(\mathscr{C}_{I_4})$ *for* $I_4 = \{0, 2, 3\}$ *and* $a_5 \in ch(\mathcal{A}_{I_5})$*,* $C_5 \in ch(\mathscr{C}_{I_5})$ *for* $I_5 = \{0, 1, 2\}$*.*

We have the following result:

Theorem 5.4.8. *Assume that* A*,* C *and* $\bar{C}$ *are uniform. Suppose* $\mathbf{0} \in \mathcal{C}^\circ$*, and there exists* $I \subset \{1, \ldots, n\}$ *such that* A *and* C *are* I*-compatible. Then,* $n_{\mathcal{A}}(C) > 0$*.*

Proof. Let B_I be a Gale dual matrix of A_I as in Condition (1) of Definition 5.4.6. As $a_j \in \text{chull}(\mathcal{A}_I)$ for each $j \notin I$, we can use Lemma 5.4.4. We construct a dominating matrix B, using the matrix B_I and using for each a_j, $j \notin I$, the affine relation given by the circuit $a_j \cup \mathcal{A}_J$, with J as in Condition (3) of Definition 5.4.6, to obtain a vector in the kernel of A as in the proof of Lemma 5.4.4. Conditions (1) and (3) of the definition of being I-compatible, mean that there exist k vectors in the kernel of $\bar{C}$ with the same sign pattern as the columns of the constructed B, and these k vectors are linearly independent because they form a mixed dominating matrix. We have that $\ker(\bar{C}) \subset \ker(C)$, and $\text{sign}(B_1), \ldots, \text{sign}(B_k) \in \text{sign}(\ker(C))$. We can apply Theorem 5.3.6 and then $n_{\mathcal{A}}(C) > 0$. □

Remark 5.4.9. When $|I| = d+2$, condition (1) in Definition 5.4.6 can be translated in terms of signatures of circuits. Given a circuit $\mathcal{U} = \{u_1, \ldots, u_{d+2}\} \subset \mathbb{R}^d$, and a nonzero affine relation $\lambda \in \mathbb{R}^{d+2}$ among the u_i, we call $\Lambda_+ = \{i \in \{1, \ldots, d+2\} : \lambda_i > 0\}$ and $\Lambda_- = \{i \in \{1, \ldots, d+2\} : \lambda_i < 0\}$. The pair (Λ_+, Λ_-) is usually call a *signature* of $\mathcal{U}$. As $\mathcal{U}$ is a circuit, the pairs (Λ_+, Λ_-) and (Λ_-, Λ_+) are the two possible signatures. Then, we consider the (unordered) signature partition $\mathcal{S}(\mathcal{U}) = \{\Lambda_+, \Lambda_-\}$. Given a subset $I \subset \{1, \ldots, n\}$, with $|I| = d + 2$, and A and C uniform, condition (1) in Definition 5.4.6 is equivalent to the following condition:

(1') $\mathcal{S}(\mathcal{A}_I) = \mathcal{S}(\mathscr{C}_I)$

In this case, condition (3) in Definition 5.4.6 implies that $\{a_j\} \cup \mathcal{A}_J$ and $\{C_j\} \cup \mathscr{C}_J$ have the same signature partition, which is $(d + 1, 1)$.

5.4.1 The case $k = 2$

The point configurations such that the corresponding matrix admits a Gale dual which is dominating are limited. So, if we are not in this case, checking condition (1) of Theorem 5.2.7 involves knowing the incidences of the facets of the polytope Δ_P. However, we now show that in case A has codimension $k = 2$, there always exists a choice of Gale dual matrix B such that we can conclude that $n_{\mathcal{A}}(C) > 0$ with the help of Theorem 5.2.7 without checking Condition (1) as it becomes a consequence of the other conditions.

Lemma 5.4.10. *Assume that A and C are uniform matrices and $k = 2$. Suppose that $\mathbf{0} \in \mathcal{C}^\circ$. Then there exists a matrix B Gale dual to A such that for any matrix D Gale dual to C for which Δ_P is nonempty, bounded and the condition (2) of Theorem 5.2.7 is satisfied, condition (1) of Theorem 5.2.7 is satisfied, and thus $n_{\mathcal{A}}(C) > 0$.*

Proof. Let B be any Gale dual matrix of A with row vectors $b_1, \ldots, b_n$. Choose any $i_2 \in I_C$. Then there exist $i_1 \in I_C$ such that the cone $\mathbb{R}_{>0}b_{i_1} + \mathbb{R}_{<0}b_{i_2}$ does not contain vectors b_i with $i \in I_C$. Note that the latter cone has dimension two since A is uniform (which implies that B is uniform as well). There exists a matrix R of rank two, with integer entries if A in an integer matrix, such that $B_{\{i_1,i_2\}} \cdot R = \mathrm{I}_2$ (if we assume that A, B have integer entries, then there exists a matrix R of rank two such that $B_{\{i_1,i_2\}} \cdot R = a \cdot \mathrm{I}_2$ where $a = |\det(B_{\{i,j\}})|$). Consider the matrix $B' = B \cdot R$, with row vectors $b'_1, \ldots, b'_n$. Then B' is a Gale dual matrix to A such that $b'_{i_1} = (1, 0)$, $b'_{i_2} = (0, 1)$ and the open quadrant $\mathbb{R}_{>0} \times \mathbb{R}_{<0}$ does not contain any vector b'_i with $i \in I_C$. Note also that if $i \in I_C$ and $i \neq i_1, i_2$ then both coordinates of b'_i are nonzero for otherwise this would give a vanishing maximal minor of B'. In particular, we get $b'_i \neq 0$, and thus b'_i is not weakly mixed, for all $i \in I_C$. Suppose now that there are two distinct vectors b'_i and b'_j with $i, j \in I_C$ such that the submatrix $B_{\{i,j\}}$ is weakly mixed. Then these row vectors lie in opposite quadrants of $\mathbb{R}^2$ and these quadrants should be $\mathbb{R}^2_{>0}$ and $\mathbb{R}^2_{<0}$. But then the cone $\mathbb{R}_{>0}b'_i + \mathbb{R}_{>0}b'_j$ contains either $b'_{i_1} = (1, 0)$ or $b'_{i_2} = (0, 1)$, and thus $\{i, j\} \notin F_L$. □

Given a vector $v \in \mathbb{R}^n$ and $I \subset \{1, \ldots, n\}$ we denote by $v_I \in \mathbb{R}^{|I|}$ the vector obtained from v after removing the coordinates with indexes that do not belong to I. Given a set $S \subset \mathbb{R}^n$, we denote $S_I = \{v_I : v \in S\}$. In case that there exists a Gale dual matrix B with rows in each of the quadrants, we have the following result.

Lemma 5.4.11. *Given $A \in \mathbb{R}^{(d+1)\times(d+3)}$ uniform, let $B \in \mathbb{R}^{(d+3)\times 2}$ be a Gale dual matrix of A. Suppose there exists rows of B, b_{i_j}, with $1 \leq j \leq 4$, such that b_{i_j} lies in the j-th open quadrant of $\mathbb{R}^2$. Let $C \in \mathbb{R}^{d\times n}$ uniform. Suppose that $\mathbf{0} \in \mathcal{C}^\circ$, and suppose that the index i_j corresponds to a facet of any associated polytope Δ_P, for $1 \leq j \leq 4$. If $\operatorname{sign}((B_j)_{I_C}) \in \operatorname{sign}((\ker(C))_{I_C})$ for $j = 1, 2$, then $n_{\mathcal{A}}(C) > 0$.*

Proof. As $\operatorname{sign}((B_j)_{I_C}) \in \operatorname{sign}((\ker(C))_{I_C})$ for $j = 1, 2$, there are vectors $D_1, D_2 \in \ker(C)$ such that $\operatorname{sign}((D_j)_{I_C}) = \operatorname{sign}((B_j)_{I_C})$ for each $j = 1, 2$. We can assume that D_1 and D_2 are linearly independent. If not, the zero coordinates of D_1 and D_2

(which are at most two, since C is uniform) have to be the same. That is, $(D_1)_j = 0$ if and only if $(D_2)_j = 0$ (otherwise, they can not be linearly dependent). Suppose that $(D_1)_j = (D_2)_j = 0$ for certain j. If $j \in I_C$, then $(B_1)_j = (B_2)_j = 0$, but since A is uniform, B_1 and B_2 have at most one zero coordinate, and then, B_1 and B_2 are scalar multiples of each other, a contradiction. Then if $(D_1)_j = (D_2)_j = 0$, $j \notin I_C$. We take a vector v in $\ker(C)$ such that D_1 and v are linearly independent. Then we can take $D_2' = D_2 + \lambda v$, with λ small enough such that $\text{sign}((D_2)_{I_C}) = \text{sign}((B_2)_{I_C})$.

So, we can suppose that D_1 and D_2 are linearly independent. Consider the matrix $\tilde{D}$ with column vectors D_1 and D_2. We have that $\mathbf{0}$ belongs to the open cone generated by the rows of $\tilde{D}$, because the i_j-th row of $\tilde{D}$ belongs to the j-th open quadrant, then Condition 5.3.1 of Lemma 5.3.5 is satisfied. As $\mathbf{0} \in \mathcal{C}^\circ$, by Lemma 5.3.5 and Corollary 5.1.1, there exists a positive vector D_0 such that the matrix obtained from $\tilde{D}$ by adding D_0 as a first column vector is Gale dual to C and the associated polytope Δ_P is nonempty and bounded. Also note that Δ_P has a facet for each row vector i_j of $\tilde{D}$, each one in the j-quadrant of $\mathbb{R}^2$, for $j = 1, \ldots, 4$. Then, if we have a 2×2 mixed submatrix of B, it does not correspond to a submatrix B_L, with $L \in \mathcal{F}(\Delta_P)$ (and any row of $\tilde{D}$ corresponding to $i \in I_C$ is not equal to zero). Then, all the conditions of Theorem 5.2.7 are satisfied and $n_{\mathcal{A}}(C) > 0$.

□

5.5 Algebraic conditions and real solutions of integer configurations

In this section we will consider integer configurations $\mathcal{A}$ and thus, integer matrices A. Interestingly, in Corollary 5.5.2 we will relate Lemma 5.3.2 with known algebraic results in the study of toric ideals [97, Ch.4]. Indeed, we summarize in § 5.5.1 some known algebraic results that show the existence of a mixed dominating Gale dual matrix is equivalent to the fact that there is a full dimensional sublattice of the integer kernel $\ker_{\mathbb{Z}}(A)$ whose associated lattice ideal (5.5.1) is a complete intersection. This means that it can be generated by as many polynomials as the codimension of its zero set. In the opposite spectrum, an ideal is not Cohen-Macaulay when its homological behavior is complicated (see for instance [27]). When $k = 2$, we also consider lattice ideals which are not Cohen-Macaulay. Proposition 5.5.4 shows how to deal with this bad algebraic case. Also, in § 5.5.2 we naturally extend the search for positive solutions to the search for real solutions with nonzero coordinates.

5.5.1 Algebraic conditions

A polynomial ideal is called *binomial* if it can be generated with polynomials with at most two terms. A subgroup $\mathcal{L} \subset \mathbb{Z}^n$ is called a *lattice*. We associate to a lattice $\mathcal{L}$ the following binomial ideal:

$$I_{\mathcal{L}} = \langle x^{u^+} - x^{u^-} \,:\, u \in \mathcal{L} \rangle \subset \mathbb{R}[x_1, \ldots, x_n], \tag{5.5.1}$$

where $u = u^+ - u^-$ is the decomposition in positive and negative components. For example, if $u = (1, -2, 1, 0) \in \mathbb{Z}^4$, then $x^{u^+} - x^{u^-} = x_1x_3 - x_2^2$.

Given a configuration $\mathcal{A} = \{a_1, \ldots, a_n\} \subset \mathbb{Z}^d$ of integral points, and the associated matrix $A \in \mathbb{Z}^{(d+1)\times n}$, let $B \in \mathbb{Z}^{n\times k}$ a Gale dual matrix of A, and denote by $B_1, \ldots, B_k$ the columns vectors of B. Note that $\{B_1, \ldots, B_k\}$ is a $\mathbb{Q}$-basis of $\ker_{\mathbb{Z}}(A)$, but it is not necessarily a $\mathbb{Z}$-basis unless the greatest common divisor of the maximal minors of B is equal to 1. When this is the case, we will say that B is a $\mathbb{Z}$-Gale dual of A. We associate to any choice of Gale dual B of A the following lattice:

$$\mathcal{L}_B = \mathbb{Z}B = \mathbb{Z}B_1 \oplus \cdots \oplus \mathbb{Z}B_k \subset \mathbb{Z}^n,$$

and its corresponding lattice ideal $I_{\mathcal{L}_B}$. In particular, when $\mathcal{L}_B = \ker_{\mathbb{Z}}(A)$, then the lattice ideal $I_{\mathcal{L}_B}$ is the so called *toric ideal* I_A. We have the following known result from [46]. See also Theorem 2.1 of [77], where the notation is similar to the notation of this paper.

Theorem 5.5.1 ([46], Theorem 2.9)**.** *The lattice ideal $I_{\mathcal{L}_B}$ is a complete intersection if and only if $\mathcal{L}_B = \mathcal{L}_{B'}$ for some dominating matrix $B' \in \mathbb{Z}^{n\times k}$. In this case, $I_{\mathcal{L}_B} = \langle x^{u^+} - x^{u^-} : u \text{ is a column of } B' \rangle$.*

The following result is a direct consequence of Lemma 5.3.2 and Theorem 5.5.1.

Corollary 5.5.2. *If $A \in \mathbb{Z}^{(d+1)\times n}$ as in* (5.1.1) *and $C \in \mathbb{R}^{d\times n}$ are uniform matrices and $B \in \mathbb{Z}^{n\times k}$ is a Gale dual matrix of A such that the lattice ideal $I_{\mathcal{L}_B}$ is a complete intersection, then there exists a Gale dual matrix $B' \in \mathbb{Z}^{n\times k}$ of A which satisfies the condition (1) of Theorem 5.2.7.*

Given A, let $B \in \mathbb{Z}^{n\times k}$ a Gale dual matrix of A, and consider the lattice $\mathcal{L}_B = \mathbb{Z}B$. The set of rows of B, $\{b_1, \ldots, b_n\} \subset \mathbb{Z}^k$ is called a Gale diagram of $\mathcal{L}_B$. Any other $\mathbb{Z}$-basis for $\mathcal{L}_B$ yields a Gale diagram, which means that Gale diagrams are unique up to an invertible matrix with integer coefficients. The following proposition from [85], relates Gale diagrams with algebraic properties of the lattice ideal $\mathcal{L}_B$ when $k = 2$:

Proposition 5.5.3 ([85], Proposition 4.1)**.** *Given $A \in \mathbb{Z}^{(d+1)\times(d+3)}$, let $B \in \mathbb{Z}^{n\times 2}$ be a Gale dual matrix of A. The lattice ideal $I_{\mathcal{L}_B}$ is not Cohen-Macaulay if and only if it has a Gale diagram which intersects all the four open quadrants of $\mathbb{R}^2$.*

The following result follows from Proposition 5.5.3 and Lemma 5.4.11.

Proposition 5.5.4. *Given $A \in \mathbb{Z}^{(d+1)\times(d+3)}$ uniform, let $B \in \mathbb{Z}^{n\times 2}$ be a Gale dual matrix of A. Suppose that the lattice ideal $I_{\mathcal{L}_B}$ is not Cohen-Macaulay and let B' be any other Gale Dual matrix of A such that the columns of B' form a $\mathbb{Z}$-basis of $\mathcal{L}_B$ and such that the corresponding Gale diagram $\{b'_1, \ldots, b'_n\}$ intersects all the four open quadrants of $\mathbb{R}^2$. Let b'_{i_j}, with $1 \leq j \leq 4$, the rows of B' such that b'_{i_j} lies in the j-th open quadrant of $\mathbb{R}^2$. Let $C \in \mathbb{R}^{d\times n}$ uniform. Suppose that $\mathbf{0} \in \mathcal{C}^\circ$, and suppose that i_j corresponds to a facet of any polytope Δ_P associated. If $\operatorname{sign}((B'_j)_{I_C}) \in \operatorname{sign}((\ker(C))_{I_C})$ for $j = 1, 2$, then $n_{\mathcal{A}}(C) > 0$.*

5.5.2 Real solutions

When A has integer entries, (5.0.1) is a system of Laurent polynomials with real coefficients, which are defined over the real torus $(\mathbb{R}^*)^d$. In this subsection, we are interested on the existence of real solutions of (5.0.1) with nonzero coordinates for integer matrices A of exponents. Our main result is Theorem 5.5.10. We will only consider matrices B which are $\mathbb{Z}$-Gale dual to A, whose columns generate $\ker_{\mathbb{Z}}(A)$ over $\mathbb{Z}$.

Given any $s = (s_1, \ldots, s_d) \in \mathbb{Z}^d$, denote by $\mathbb{R}^d_s$ the orthant

$$\mathbb{R}^d_s = \{x \in \mathbb{R}^d : (-1)^{s_i} x_i > 0, i = 1, \ldots, d\}.$$

In particular, $\mathbb{R}^d_s = \mathbb{R}^d_{>0}$ if $s \in 2\mathbb{Z}^d$. Let $x \in (\mathbb{R}^*)^d$ be a solution of (5.0.1). Then $x \in \mathbb{R}^d_s$ for some $s \in \mathbb{Z}^d$ (which is unique up to adding a vector in $2\mathbb{Z}^d$). Setting $z_i = (-1)^{s_i} x_i$, we get that $z = (z_1, \ldots, z_d)$ is a positive solution of the system with exponent matrix A and coefficient matrix C_s defined by $(C_s)_{ij} = (-1)^{\langle s, a_j \rangle} c_{ij}$. Moreover, if D is a matrix Gale dual to C, then the matrix D_s defined by $(D_s)_{ij} = (-1)^{\langle s, a_i \rangle} d_{ij}$ is Gale dual to C_s. Denote by $P_{i,s}$ the i-th row vector of D_s. Thus $P_{i,0} = P_i$ (i-th row of D) and $P_{i,s} = (-1)^{\langle s, a_i \rangle} P_i$, $i = 1, \ldots, n$. Denote by $\mathcal{C}_{P_s}$ the positive cone generated by $P_{i,s}$ for $i = 1, \ldots, n$.

Let $\mathcal{M}_P$ denote the complement in $\mathbb{R}^{k+1}$ of the hyperplane arrangement given by the hyperplanes $\{y \in \mathbb{R}^{k+1} : \langle P_i, y \rangle = 0\}$, $i = 1, \ldots, n$. For any $\varepsilon \in \mathbb{Z}^n$ denote by $\mathcal{C}^\nu_\varepsilon$ the connected component of $\mathcal{M}_P$ defined by

$$\mathcal{C}^\nu_\varepsilon = \{y \in \mathbb{R}^{k+1} : (-1)^{\varepsilon_i} \langle P_i, y \rangle > 0, \, i = 1, \ldots, n\}.$$

Note that $\mathcal{C}^\nu_0 = \mathcal{C}^\nu_P$.

Write A' for the matrix with column vectors $a_1, \ldots, a_n$ (A' is obtained by removing the first row of A). It is convenient to introduce the map $\psi : \mathbb{Z}^d \to \mathbb{Z}^{1 \times n}$ defined by $\psi(s) = s \cdot A'$ (here we see $s \in \mathbb{Z}^d$ as a row vector, i.e. as an element of $\mathbb{Z}^{1 \times d}$). Then, for any integer vector $b \in \ker(A)$ we have:

$$\prod_{i=1}^n \langle P_{i,s}, y \rangle^{b_i} = (-1)^{\langle s, A'b \rangle} \prod_{i=1}^n \langle P_i, y \rangle^{b_i} = \prod_{i=1}^n \langle P_i, y \rangle^{b_i}.$$

Thus, applying Theorem 5.1.5 to the system with coefficient matrix C_s and exponent matrix A, we obtain that the real solutions of (5.0.1) contained in the orthant $\mathbb{R}^d_s$ are in bijection with the solutions of (5.1.8) in the quotient $\mathbb{P}\mathcal{C}^\nu_{\psi(s)}$ of the open cone $\mathcal{C}^\nu_{\psi(s)}$ by the equivalence relation $\sim$ ($y \sim y'$ if and only if there exists $\alpha > 0$ such that $y = \alpha y'$), defined in Section 5.1.

We have proved the following result:

Proposition 5.5.5. *For any $s \in \mathbb{Z}^d$, there is a bijection between the real solutions of (5.0.1) contained in $\mathbb{R}^d_s$ and the solutions of (5.1.8) in $\mathbb{P}\mathcal{C}^\nu_{\psi(s)}$, which induces a bijection between the solutions of (5.0.1) in $\mathbb{R}^d_s$ and the solutions of (5.1.8) in $\Delta_{P_s} = \mathcal{C}^\nu_{\psi(s)} \cap \{y_0 = 1\}$ when $(1, 0, \ldots, 0)$ lies in the closure of the cone $\mathcal{C}_{P_s}$.*

If M is any matrix or vector with integer entries, we denote by $[M]_2$ the matrix or vector with coefficients in the field $\mathbb{Z}/2\mathbb{Z}$ obtained by taking the image of each entry by the quotient map $\mathbb{Z} \to \mathbb{Z}/2\mathbb{Z}$. Note that the following relation between the ranks holds: $\mathrm{rk}([A]_2) = \mathrm{rk}([A']_2)$ if $[(1,1,\dots,1)]_2$ belongs to the row span of $[A']_2$ and $\mathrm{rk}([A]_2) = \mathrm{rk}([A']_2) + 1$ otherwise. The following result is straightforward.

Lemma 5.5.6. *For any $s, s' \in \mathbb{Z}^d$, we have $\mathcal{C}^\nu_{\psi(s)} = \mathcal{C}^\nu_{\psi(s')}$ if and only if $[s'-s]_2$ belongs to the left kernel of $[A']_2$. For each $s \in \mathbb{Z}^d$, there are $2^{d-\mathrm{rk}([A']_2)}$ distinct orthants $\mathbb{R}^d_{s'}$ such that $\mathcal{C}^\nu_{\psi(s)} = \mathcal{C}^\nu_{\psi(s')}$. Finally, the image of $\mathbb{Z}^d$ via the map $s \mapsto \mathcal{C}^\nu_{\psi(s)}$ consists of $2^{\mathrm{rk}([A']_2)}$ connected components of $\mathcal{M}_P$.*

Recall that since A contains a row of ones, each polynomial in (5.1.8) is homogeneous of degree 0, which implies the following fact.

Lemma 5.5.7. *For any $\varepsilon \in \mathbb{Z}^n$, the map $y \mapsto -y$ induces a bijection between the solutions of (5.1.8) in $\mathcal{C}^\nu_\varepsilon$ and the solutions of (5.1.8) in $\mathcal{C}^\nu_{\varepsilon+(1,1,\dots,1)}$.*

Choose a $\mathbb{Z}$-Gale dual $B \in \mathbb{Z}^{n\times k}$ of A and consider the Gale dual system (5.1.8) it defines for a given Gale dual matrix D of a full rank matrix C.

Proposition 5.5.8. *1. Assume $\mathrm{rk}([A]_2) = \mathrm{rk}([A']_2)$ and let $\varepsilon \in \mathbb{Z}^n$. If (5.1.8) has a solution in $\mathcal{C}^\nu_\varepsilon$ then there exists $s \in \mathbb{Z}^d$ such that $[\varepsilon]_2 = [\psi(s)]_2$.*

2. Assume now $\mathrm{rk}([A]_2) > \mathrm{rk}([A'])_2$ and let $\varepsilon \in \mathbb{Z}^n$. If (5.1.8) has a solution in $\mathcal{C}^\nu_\varepsilon$ then either there exists $s \in \mathbb{Z}^d$ such that $[\varepsilon]_2 = [\psi(s)]_2$, or there exists $s \in \mathbb{Z}^d$ such that $[\varepsilon]_2 + [(1,1,\dots,1)]_2 = [\psi(s)]_2$. Moreover, there do not exist $s, s' \in \mathbb{Z}^d$ such that $[\psi(s')]_2 = [(1,1,\dots,1)]_2 + [\psi(s)]_2$, so that only one of the two previous cases occurs.

Proof. Let y be a solution of (5.1.8) in $\mathcal{C}^\nu_\varepsilon$ and let b be any element of $\ker(A) \cap \mathbb{Z}^n$. Writing b as an integer linear combination of the column vectors of B and using (5.1.8), we get $\prod_{i=1}^n \langle P_i, y\rangle^{b_i} = 1$. Then, using $y \in \mathcal{C}^\nu_\varepsilon$ we obtain that $\sum_{i=1}^n \varepsilon_i b_i$ is an even integer number. The fact that the column vectors of B form a basis of $\ker(A)\cap\mathbb{Z}^n$ implies that the column vectors of $[B]_2$ form a basis of $\ker([A]_2)$ (in other words $[B]_2$ is Gale dual to $[A]_2$). Then, $\sum_{i=1}^n \varepsilon_i b_i \in 2\mathbb{Z}$ for any $b \in \ker(A) \cap \mathbb{Z}^n$ is equivalent to the fact that $[\varepsilon]_2$ belongs to the left kernel of $[B]$. This left kernel is the image of the map $\mathbb{Z}^{d+1} \to \mathbb{Z}^{1\times n}$ sending $(s_0, s_1, \dots, s_d)$ to $[(s_0, s_1, \dots, s_d)]_2 \cdot [A]_2 = [s_0(1,1,\dots,1)]_2 + [\psi(s)]_2$, where $s = (s_1, \dots, s_d)$. The image of this map coincides with the image of the map $s \mapsto [\psi(s)]_2$ precisely when $\mathrm{rk}([A]_2) = \mathrm{rk}([A']_2)$, which proves item 1). To finish it remains to see that if $\mathrm{rk}([A]_2) > \mathrm{rk}([A']_2)$ there do not exist distinct $s, s' \in \mathbb{Z}^{1\times(d)}$ such that $[\psi(s')]_2 = [(1,1,\dots,1) + \psi(s)]_2$ for otherwise $[(1,\dots,1)]_2$ would belong to the row span of $[A']_2$. □

Example 5.5.9. If $a_1, \dots, a_n \in 2\mathbb{Z}^d$, then $\mathrm{rk}([A']_2) = 0$ and $\mathrm{rk}([A]_2) = 1$. Moreover, the number of real solutions of (5.0.1) is 2^d times its number of positive solutions, the latter number being equal to the number of solutions of (5.1.8) in $\mathcal{C}^\nu_0 = \mathcal{C}^\nu_P$ by Theorem 5.1.5.

As a direct consequence of Proposition 5.5.5, Proposition 5.5.8, and Lemma 5.5.6, we get the following result.

Theorem 5.5.10. *There exists a solution of* (5.0.1) *in* $(\mathbb{R}^*)^d$ *if and only if there exists a solution of* (5.1.8) *in the complement* $\mathcal{M}_P$ *of the hyperplane arrangement defined by* $P_1, \ldots, P_n$*. Moreover,*

(1) For any $s \in \mathbb{Z}^d$ *there is a bijection between the solutions of* (5.0.1) *in* $\mathbb{R}^d_s$ *and the solutions of* (5.1.8) *in* $\mathbb{P}\mathcal{C}^\nu_\varepsilon$*, with* $[\varepsilon]_2 = [\psi(s)]_2$*.*

(2) There are at most $2^{\mathrm{rk}[A]_2}$ *connected components* $\mathcal{C}^\nu_\varepsilon$ *of* $\mathcal{M}_P$ *where* (5.1.8) *has a solution.*

Given A and C and a choice of Gale dual matrices B, D, we saw in the proof of Theorem 5.2.7, that under the hypotheses of the theorem, it follows from Theorem 5.1.5 that $n_{\mathcal{A}}(C) > 0$ is indeed equivalent to the existence of a solution to (5.2.1) in Δ_P. In the previous sections, we have given different sufficient conditions on D and B such that system (5.2.1) has at least one solution in Δ_P. When A has integer entries it is then enough to check if these sufficient conditions are satisfied by B and any matrix D_ε obtained by multipliying the i-th row of D by $(-1)^{\varepsilon_i}$ for some $\varepsilon \in \mathbb{Z}^n$. In this case, (5.0.1) has at least one solution in $(\mathbb{R}^*)^d$ by Theorem 5.5.10.

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