

Algebraic methods for biochemical reaction network theory

by

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Abstract

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This dissertation develops the algebraic study of chemical reaction networks endowed with mass-action kinetics. These form a class of dynamical systems that have a wide range of applications in the physical and biological sciences. Early results in chemical reaction network theory relied on techniques from linear algebra, dynamical systems, and graph theory. More recently, motivated by problems in systems biology, other areas of mathematics have contributed to this subject. These branches of mathematics include control theory, homotopy theory, and matroid theory. As a complement to these varied perspectives, the approach of this dissertation is algebraic.

Chapter 2 develops the basic theory of toric dynamical systems, which are those chemical reaction systems that have the property that for any chemical complex (a product or reactant), the amount produced of that complex at steady state is equal to the amount consumed by reactions. Toric dynamical systems are known as complex-balancing mass-action systems in the mathematical chemistry literature, where many of their properties have been established. Special cases of toric dynamical systems include all zero deficiency systems and all detailed-balancing systems. One feature is that the steady state locus of a toric dynamical system is a toric variety. Furthermore, this variety intersects the interior of each invariant polyhedron (a polyhedron in which a trajectory of the dynamical system is confined) in a unique point. For any chemical reaction network, there is an associated moduli space that consists of those vectors of reaction rate constants for which the resulting dynamical system is a toric dynamical system. The main result states that this moduli space is a toric variety whose combinatorial structure we can characterize.

To determine the steady states on the boundaries of invariant polyhedra, the concept of a siphon is important. Siphons in a chemical reaction system are subsets of the chemical species that have the potential of being absent in a steady state. The main result of Chapter 3 characterizes minimal siphons in terms of primary decomposition of binomial ideals. Further, we explore the underlying geometry, and we demonstrate the effective computation of siphons using computer algebra software. This leads to a new method for determining whether given initial concentrations allow for various boundary steady states; this classification arises from a chamber decomposition.

Siphons determine which faces of an invariant polyhedron contain steady states, and a relevant question is whether any trajectories of a chemical reaction system approach such a boundary

steady state. The global attractor conjecture, which is the subject of Chapter 4, implies that no interior trajectories approach boundary steady states in the case of toric dynamical systems. Our main result states that this conjecture holds when all of the siphons correspond to facets (codimension-one faces), vertices (zero-dimensional faces), or empty faces of the invariant polyhedron. As a corollary, the conjecture holds when the associated invariant polyhedra are two-dimensional.

Chapter 5 pertains to the topic of multistationarity, which refers to the capacity of a biochemical reaction system to exhibit multiple steady states in one invariant polyhedron. Known results from chemical reaction network theory provide sufficient conditions for the existence of bistability, and on the other hand can rule out the possibility of multiple steady states. Understanding small networks is important because the existence of multiple steady states in a subnetwork of a biochemical model sometimes can be lifted to establish multistationarity in the larger network. The main result establishes the smallest reversible, mass-preserving network that admits bistability and determines the semi-algebraic set of parameters for which more than one steady state exists.

Chapter 6 focuses on mathematical methods for predicting gene expression from regulatory sequence. The chemical reactions that underlie transcriptional regulation include the bindings of transcription factors to cis-regulatory sequences of genes. For each such sequence, many possible combinations of transcription factors can bind to the sequence. Accordingly, thermodynamic models give quantitative predictions of gene expression levels that are weighted averages over the set of all possible binding configurations. This chapter describes the implementation of such a model in the context of early embryonic development in *Drosophila melanogaster*.

To
my family.

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Chapter 1

Introduction

1.1 From molecular biology to systems biology

This dissertation provides a mathematical bridge between a part of molecular biology and a part of systems biology. In particular, we analyze biochemical reactions such as binding-dissociating reactions, which are fundamental at the molecular level (for example, in gene regulation) as well as at the systems level (for example, in metabolic processes). As increasingly complex biological data are being collected, a rigorous study of systems biology models is imperative. This dissertation focuses on a class of such models that arise from chemical reaction networks. Moreover, our approach is mathematical, which allows us to prove theorems instead of relying on simulations.

The main objects of study in this dissertation are chemical reaction networks endowed with mass-action kinetics. Mass-action kinetics has a wide range of applications in the physical sciences, and now it plays a role in systems biology [ET89, §1.5]. Examples of reaction systems include pharmacological models of drug interaction [GSG⁺07], T-cell signal transduction models [Cha03, McK95, Son01], and enzymatic mechanisms [SM00]. Pioneered by Feinberg, Horn, and Jackson in the 1970s [Fei79, Fei87, HJ72], chemical reaction network theory is the mathematical study of mass-action kinetics; we will highlight many of their results in this dissertation. Early results relied on techniques from linear algebra, dynamical systems, and graph theory. More recently, motivated by problems in systems biology, other areas of mathematics have contributed to this subject. These branches of mathematics include control theory [ADS07b, AS06, Cha03], homotopy theory [CHW08], and matroid theory [BBCQ04, CFR08]. Convex geometry and differential geometry have also played a role in chemical reactor design [Fei00a, Fei00b, FH97]. As a complement to these varied perspectives, the approach of this dissertation is algebraic. We will reinterpret some known results from the point of view of computational algebra and algebraic geometry, and in addition we will prove new theorems. We remark that related work of Craciun, Pantea, and Rempala [CPR09a, CPR09b], Dickenstein and Pérez Millán [DM10], Gatermann and Huber [GH02], Manrai, Thomson, and Gunawardena [MG08, TG09b], and Wang and Xia [WX05] also take an algebraic viewpoint. In fact, computed algebraic invariants of biochemical systems have begun to be used for experimental data analysis [TG09a, §4].

The remainder of this Introduction provides an overview of the results contained in this

dissertation (Section 1.2), establishes the notation of chemical reaction network theory (Section 1.3), and illustrates these concepts through several examples (Section 1.4).

1.2 Overview of main results in this dissertation

Among all chemical reaction systems, toric dynamical systems are the best understood. Chapter 2 develops the basic theory of toric dynamical systems, which are those chemical reaction systems that have the property that for any chemical complex (a product or reactant), the amount produced of that complex at steady state is equal to the amount consumed by reactions. Toric dynamical systems are known as complex-balancing mass-action systems in the mathematical chemistry literature. Special cases of toric dynamical systems include all zero deficiency systems and all detailed-balancing systems. One feature is that the steady state locus of a toric dynamical system is a toric variety. Furthermore, this variety intersects the interior of each invariant polyhedron (a polyhedron in which a trajectory of the dynamical system is confined) in a unique point. For any chemical reaction network, there is an associated moduli space that consists of those vectors of reaction rate constants for which the resulting dynamical system is a toric dynamical system. Our main result states that this moduli space is a toric variety, whose combinatorial structure we can characterize.

To determine the steady states on the boundaries of invariant polyhedra, the concept of a siphon is important. Siphons in a chemical reaction system are subsets of the chemical species that have the potential of being absent in a steady state. The main result of Chapter 3 characterizes minimal siphons in terms of primary decomposition of binomial ideals. Further, we explore the underlying geometry, and we demonstrate the effective computation of siphons using computer algebra software. This leads to a new method for determining whether given initial concentrations allow for various boundary steady states; this classification arises from a chamber decomposition.

Siphons determine which faces of an invariant polyhedron contain steady states, and a relevant question is whether any trajectories of a chemical reaction system approach such a boundary steady state. The Global Attractor Conjecture, which is the subject of Chapter 4, implies that no interior trajectories approach boundary steady states in the case of toric dynamical systems. Our main results state that this conjecture holds when all of the siphons correspond to facets (codimension-one faces), vertices (zero-dimensional faces), or empty faces of the invariant polyhedron. As a corollary, the conjecture holds when the associated invariant polyhedra are two-dimensional.

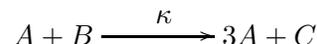
Chapter 5 pertains to the topic of multistationarity, which refers to the capacity of a biochemical reaction system to exhibit multiple steady states in one invariant polyhedron. Known results from chemical reaction network theory provide sufficient conditions for the existence of bistability, and on the other hand can rule out the possibility of multiple steady states. Understanding small networks is important because the existence of multiple steady states in a subnetwork of a biochemical model sometimes can be lifted to establish multistationarity in the larger network. The main result establishes the smallest reversible, mass-preserving network that admits bistability and determines the semi-algebraic set of parameters for which more than one steady state exists.

Chapter 6 examines a model for predicting gene expression levels from regulatory sequence.

The chemical reactions that underlie transcriptional regulation include the bindings of transcription factors to cis-regulatory sequences of genes. For each such sequence, many possible combinations of transcription factors can bind to the sequence. Accordingly, thermodynamic models give quantitative predictions of gene expression levels that are weighted averages over the set of all possible binding configurations. This chapter describes the implementation of such a model in the context of early embryonic development in *Drosophila melanogaster*. Finally, Chapter 7 contains a list of open problems in chemical reaction network theory.

1.3 Chemical reaction network theory

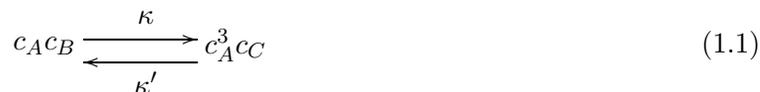
This section is an introduction to chemical reaction network theory. Before providing precise definitions, we present an intuitive example that illustrates how a chemical reaction network gives rise to a dynamical system. An example of a *chemical reaction*, as it usually appears in the literature, is the following:



In this reaction, one unit of chemical *species* A and one of B react (at reaction rate κ) to form three units of A and one of C . The concentrations of these three species, denoted by c_A , c_B , and c_C , will change in time as the reaction occurs. Under the assumption of *mass-action kinetics*, species A and B react at a rate proportional to the product of their concentrations, where the proportionality constant is the rate constant κ . Noting that the reaction yields a net change of two units in the amount of A , we obtain the first differential equation in the following system:

$$\begin{aligned} \frac{d}{dt}c_A &= 2\kappa c_A c_B, \\ \frac{d}{dt}c_B &= -\kappa c_A c_B, \\ \frac{d}{dt}c_C &= \kappa c_A c_B. \end{aligned}$$

The other two equations arise similarly. Next we include the reverse reaction and switch from additive to multiplicative notation to highlight the monomials that appear in our differential equations; most *chemical reaction networks* in this dissertation will appear with the following notation:



This network defines differential equations that are each a sum of the monomial contribution from the reactant of each chemical reaction in the network:

$$\begin{aligned} \frac{d}{dt}c_A &= 2\kappa c_A c_B - 2\kappa' c_A^3 c_C, \\ \frac{d}{dt}c_B &= -\kappa c_A c_B + \kappa' c_A^3 c_C, \\ \frac{d}{dt}c_C &= \kappa c_A c_B - \kappa' c_A^3 c_C. \end{aligned} \tag{1.2}$$

The recipe for obtaining these differential equations from any reaction network generalizes from this example as we will see in Section 1.3.2. However, in order to display the linearity hidden in these non-linear equations, the equations will appear next in a different but equivalent form in equation (1.3) below.

1.3.1 Chemical reaction systems

We now establish the notation for this dissertation. A *chemical reaction network* is a finite directed graph whose vertices are labeled by distinct monomials and whose edges are labeled by parameters. Specifically, the digraph is denoted $G = (V, E)$, with vertex set $V = \{1, 2, \dots, n\}$ and edge set $E \subseteq \{(i, j) \in V \times V : i \neq j\}$. This digraph is also called a *reaction diagram*. Throughout this dissertation, the integer unknowns n and s denote the number of complexes and the number of species, respectively. The vertex i of G represents the i -th chemical complex, and it is labeled by the monomial

$$c^{y_i} = c_1^{y_{i1}} c_2^{y_{i2}} \dots c_s^{y_{is}} .$$

This yields $Y = (y_{ij})$, an $n \times s$ -matrix of non-negative integers. The unknowns c_1, c_2, \dots, c_s represent the concentrations of the s species in the network, and we regard them as functions $c_i(t)$ of time t . The monomial labels form the entries in the following row vector:

$$\Psi(c) = (c^{y_1}, c^{y_2}, \dots, c^{y_n}) .$$

A directed edge $(i, j) \in E$ represents a reaction from the i -th chemical complex to the j -th chemical complex, and it is labeled by a positive parameter κ_{ij} which represents the rate constant of the reaction. It will often be fruitful to treat the rate constants κ_{ij} as unknowns. A network is *reversible* if the graph G is undirected, in which case each undirected edge has two labels κ_{ij} and κ_{ji} . Let A_κ denote the negative of the *Laplacian* of the digraph G . In other words, A_κ is the $n \times n$ -matrix whose off-diagonal entries are the κ_{ij} and whose row sums are zero. *Mass-action kinetics* specified by the digraph G is the dynamical system defined by the following ordinary differential equations:

$$\frac{dc}{dt} = \Psi(c) \cdot A_\kappa \cdot Y . \tag{1.3}$$

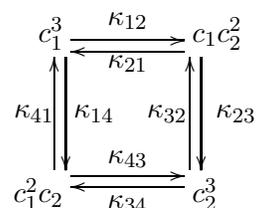
By decomposing the mass-action equations in this way, we see that they are linear in the κ_{ij} by way of the matrix A_κ . In addition, the right-hand side of each differential equation dc_i/dt is a polynomial in the polynomial ring $\mathbb{R}[\kappa_{ij}, c_i]$. A *chemical reaction system* refers to the dynamical system (1.3) arising from a specific chemical reaction network G and a choice of rate parameters $(\kappa_{ij}^0) \in \mathbb{R}_{>0}^{|E|}$. Solutions of these systems will be called *trajectories*. This dissertation is devoted to the study of chemical reaction systems. Although the complexes c^y of actual reaction networks typically have small exponents, the framework of mass-action kinetics allows us to study more general networks. For contexts other than that of modeling chemical kinetics in which these dynamical systems are relevant, see the discussion in Érdi and Tóth [ET89, §1.5].

Looking back at the differential equations (1.2), we see that any monomials having a negative coefficient that appear in $\frac{d}{dt}c_i$ must be divisible by c_i . Chemically, this corresponds to the fact that any reaction that depletes a species must have that species as a reactant. In fact, this property is the defining characteristic of our dynamical systems, as stated in the following lemma.

Lemma 1.3.1. *Let $f : \mathbb{R}^s \rightarrow \mathbb{R}^s$ be a polynomial function, that is, assume that $f_i \in \mathbb{R}[c_1, c_2, \dots, c_s]$ for $i = 1, 2, \dots, s$. Then f can arise as the right-hand side of the differential equations (1.3) if and only if for all $i = 1, 2, \dots, s$, any negative monomial in f_i is divisible by the unknown c_i .*

Note that Lemma 1.3.1 answers the question of which polynomial differential equation systems may arise as chemical reaction systems. For a proof of this lemma, we refer the reader to the textbook of Érdi and Tóth [ET89, §4.7.1.1]. The discussion there also notes that two networks G and G' may give rise to the same differential equations (1.3). For a mathematical analysis on when this is possible, see the recent paper of Craciun and Pantea [CP08].

Example 1.3.2. The following chemical reaction network will be called the *Square*:



In the horizontal reactions, two units of species one are transformed into two of species two (or vice-versa), while a third unit remains unchanged by the reaction. In the vertical reactions, only one is transformed. The matrices whose product defines the dynamical system (1.3) follow:

$$\begin{aligned}
 \Psi(c) &= (c_1^3, c_1 c_2^2, c_2^3, c_1^2 c_2), \\
 A_\kappa &= \begin{pmatrix} -\kappa_{12} - \kappa_{14} & \kappa_{12} & 0 & \kappa_{14} \\ \kappa_{21} & -\kappa_{21} - \kappa_{23} & \kappa_{23} & 0 \\ 0 & \kappa_{32} & -\kappa_{32} - \kappa_{34} & \kappa_{34} \\ \kappa_{41} & 0 & \kappa_{43} & -\kappa_{41} - \kappa_{43} \end{pmatrix}, \quad \text{and} \\
 Y &= \begin{pmatrix} 3 & 0 \\ 1 & 2 \\ 0 & 3 \\ 2 & 1 \end{pmatrix}.
 \end{aligned}$$

The Square appeared in non-reversible form as network 7-3 in [HJ72] and network 4.2 in [Fei80]. This network will be our main object of study in Chapter 5. \square

Remark 1.3.3. The chemical reaction system (1.3) was shown by Kurtz to be a valid mathematical model for chemical kinetics in the setting of *continuous-flow stirred-tank reactors*, that is, when chemical reactions take place in a large tank whose contents are well-stirred [Kur72, Kur81]. (See also an informal discussion about these results in the textbook of Érdi and Tóth [ET89, §5.9.3].) In particular, our mass-action systems are deterministic and have continuous time and continuous species concentrations. In other settings, it may be appropriate to model chemical systems in a different manner. Four common modifications are the following: assuming other choices of kinetics, modeling the system stochastically, treating time as a discrete variable, and treating each species

concentration as a discrete variable [ET89, Chapter 2]. For instance, see the recent work of Gómez-Uribe and Verghese on a model of chemical systems as a continuous-time discrete-state Markov process [GUV07]. For an analysis of trajectories that arise from classical reactors besides those of continuous-flow stirred-tank reactors, for example, plug-flow reactors and distributed sidestream reactors, see the work of Feinberg and Hildebrandt on the attainable region problem [Fei00a, Fei00b, FH97].

1.3.2 Rate formation functions

An equivalent way to define the differential equations (1.3) is by way of a rate function for each reaction. This point of view will be used in Chapter 4. For each reaction $c^{y_i} \rightarrow c^{y_j}$, we now define the following *rate formation function*:

$$R_{ij}(c) = \kappa_{ij} c_1^{y_{i1}} c_2^{y_{i2}} \cdots c_s^{y_{is}} = \kappa_{ij} c^{y_i}. \quad (1.4)$$

The dynamics of the system are given by the following differential equations:

$$\frac{dc}{dt} = \sum_{(i,j) \in E} R_{ij}(c(t))(y_j - y_i) =: f(c(t)). \quad (1.5)$$

It can be seen that these equations (1.5) are equal to those (1.3) defined above.

This setup via rate formation functions fits into a more general framework, which we now discuss. Namely, we could allow for more general kinetics than those of mass-action (which is the most common choice): for a reaction $c^{y_i} \rightarrow c^{y_j}$ we suppose the existence of a continuously differentiable function $R_{ij}(\cdot) = R_{y_i \rightarrow y_j}(\cdot)$ that satisfies the following assumption:

Assumption 1.3.1. *For all reactions $(i, j) \in E$, the function $R_{ij}(\cdot) = R_{y_i \rightarrow y_j}(\cdot) : \mathbb{R}_{\geq 0}^s \rightarrow \mathbb{R}$ satisfies:*

1. $R_{y_i \rightarrow y_j}(\cdot)$ depends explicitly upon x_ℓ only if $y_{k\ell} \neq 0$.
2. $\frac{\partial}{\partial x_\ell} R_{y_k \rightarrow y_k'}(c) \geq 0$ for those c_i for which $y_{i\ell} \neq 0$, and equality can hold only if some coordinate of c is zero.
3. $R_{y_i \rightarrow y_j}(c) = 0$ if $c_i = 0$ for some ℓ with $y_{i\ell} \neq 0$.
4. If $1 \leq y_{ki} < y_{li}$, then $\lim_{c_i \rightarrow 0} \frac{R_{\ell u}(c)}{R_{kv}(c)} = 0$, where all other $c_j > 0$ are held fixed in the limit.

The final assumption simply states that if the reaction $c^{y_\ell} \rightarrow c^{y_v}$ requires strictly more molecules of species i as inputs than does the reaction $c^{y_k} \rightarrow c^{y_u}$, then the rate of the first reaction decreases to zero faster than the second reaction, as the concentration of species i goes to zero. The functions R_k are called the *kinetics* of the system, and the dynamics are given by equation (1.5). The most common kinetics is that of mass-action kinetics, which is the focus of this dissertation. (As noted in Remark 1.3.3, other mathematical models of chemical reactions are analyzed in the textbook of Érdi and Tóth [ET89].) It is easily verified that each rate function R_{ij} defined via (1.4) satisfies Assumption 1.3.1.

1.3.3 Steady states and invariant polyhedra

A *steady state* of a chemical reaction system is a non-negative concentration vector $c \in \mathbb{R}_{\geq 0}^s$ at which the equations (1.3) vanish. Steady states are sometimes called *equilibria* or *rest points* in the literature. We distinguish between positive steady states (or interior steady states) $c \in \mathbb{R}_{> 0}^s$ and *boundary steady states* $c \in (\mathbb{R}_{\geq 0}^s \setminus \mathbb{R}_{> 0}^s)$. By construction, the steady states of a chemical reaction system form the non-negative part of a real algebraic variety, which hints at why algebraic techniques will feature prominently in this dissertation. In fact, in certain cases this variety has a rational parametrization; for instance, this is true for multisite post-translational modification systems as analyzed by Thomson and Gunawardena [TG09a].

The *stoichiometric subspace* is the vector subspace spanned by the *reaction vectors* $y_j - y_i$ (where (i, j) is an edge of G), and we will denote this space by S and its dimension by σ :

$$S := \mathbb{R}\{y_j - y_i \mid (i, j) \in E\} .$$

In the earlier example (1.1), we have $y_1 - y_2 = (-2, -1, 1)$, which means that with the occurrence of each reaction, two units of A and one of B are consumed, while one unit of C is produced (or vice-versa). This vector $(-2, -1, 1)$ spans the stoichiometric subspace S for the network (1.1). We see that integrating (1.5) yields

$$c(t) = c^0 + \sum_{(i,j) \in E} \left(\int_0^t R_{ij}(c(s)) ds \right) (y_j - y_i) ,$$

so the differential equations (1.3) remain in S for all time $t \geq 0$. Therefore, a trajectory $c(t)$ beginning at a positive vector $c(0) = c^0 \in \mathbb{R}_{> 0}^s$ remains in the *invariant polyhedron* (also called the “stoichiometric compatibility class” or “reaction simplex”), which we denote by

$$\mathcal{P} := (c^0 + S) \cap \mathbb{R}_{\geq 0}^s , \tag{1.6}$$

for all positive time; in other words, this set is forward-invariant with respect to the dynamics (1.3). In fact, the relative interior, which we denote by $\text{int}(\mathcal{P})$, is also forward-invariant. We remark that \mathcal{P} clearly depends upon a choice of $c^0 \in \mathbb{R}_{> 0}^s$. Throughout this dissertation, a reference to \mathcal{P} assumes the existence of a positive initial condition $c^0 \in \mathbb{R}_{> 0}^s$ for which \mathcal{P} is defined by (1.6). It follows that any invariant polyhedron of a network has the same dimension as the stoichiometric subspace. Two points in the same invariant polyhedron \mathcal{P} are said to be *stoichiometrically compatible*. An introduction to polyhedral geometry appears in Section 1.3.6.

Multistationarity refers to the existence of an invariant polyhedron \mathcal{P} with two or more steady states in its relative interior. A chemical reaction network may admit multistationarity for all, some, or no choices of positive parameters κ_{ij} , as we will see in Chapter 5 for a family of networks related to the Square network from Example 1.3.2.

1.3.4 The deficiency of a chemical reaction network

The deficiency of a chemical reaction network is an important invariant. Its definition and related results, notably the Deficiency Zero and Deficiency One Theorems, are due to work of

Feinberg. For a chemical reaction network, let n denote the number of complexes, l the number of linkage classes, and σ the dimension of the stoichiometric subspace, S . The *deficiency* of the reaction network is the integer

$$\delta := n - l - \sigma .$$

The deficiency of a reaction network is non-negative because it can be interpreted as either the dimension of a certain linear subspace [Fei79] or the codimension of a certain ideal, as we will see in Chapter 2. Note that the deficiency depends only on the reaction network and not on the specific values of the rate constants.

1.3.5 Ideals, varieties, and primary decomposition

Concepts from algebraic geometry and polyhedral geometry will feature prominently in this dissertation. For background information concerning ideals, varieties, primary decomposition, and additional topics from algebra, we refer the reader to the introductory textbooks of Cox, Little, and O’Shea [CLO07], Miller and Sturmfels [MS05], and Sturmfels [Stu02]. We now end this section with a presentation of the concepts of polyhedral geometry that will be used in this dissertation.

1.3.6 Connection to polyhedral geometry

We begin with some basic terminology. For additional background, we recommend the textbook of Ziegler [Zie95].

Definition 1.3.1. The *half-space* in \mathbb{R}^m defined by a vector $v \in \mathbb{R}^m$ and a constant $c \in \mathbb{R}$ is the set of all points lying on one side of the hyperplane defined by $\langle v, - \rangle = c$, that is,

$$H_{v,c} := \{x \in \mathbb{R}^m \mid \langle v, x \rangle \geq c\} . \quad (1.7)$$

A (convex) *polyhedron* in \mathbb{R}^m is an intersection of finitely many half-spaces.

For example, the non-negative orthant $\mathbb{R}_{\geq 0}^m$ is a polyhedron, as it can be written as the intersection of the m half-spaces $H_{e_i,0}$, where the e_i ’s are the canonical unit vectors of \mathbb{R}^m . We now give three elementary facts about polyhedra from which we will deduce the fact that invariant polyhedra \mathcal{P} are indeed polyhedra. First, any linear space of \mathbb{R}^m is a polyhedron. Second, any translation $x + Q$ of a polyhedron Q by a vector $x \in \mathbb{R}^m$ is again a polyhedron. Third, the intersection of two polyhedra is a polyhedron. Therefore, as any translate $(x^0 + S)$ and the orthant $\mathbb{R}_{\geq 0}^s$ are both polyhedra, it follows that any invariant polyhedron \mathcal{P} defined by (1.6) is indeed a polyhedron. The next definitions will allow us later to discuss boundary steady states.

Definition 1.3.2. Let Q be a polyhedron in \mathbb{R}^m . The *interior* of Q , $\text{int}(Q)$, is the largest relatively open subset of Q . The *dimension* of Q , $\dim(Q)$, is the dimension of the span of any translate of Q that contains the origin.

For example, the interior of an invariant polyhedron \mathcal{P} is

$$\text{int}(\mathcal{P}) = (c^0 + S) \cap \mathbb{R}_{> 0}^s .$$

Also, the dimension of \mathcal{P} equals the dimension of the stoichiometric subspace S : $\dim(\mathcal{P}) = \dim(S)$. We now define the faces of a polyhedron.

Definition 1.3.3. Let Q be a polyhedron in \mathbb{R}^m . For a vector $v \in \mathbb{R}^m$, the *face* of Q that it defines is the (possibly empty) set of points of Q that minimize the linear functional $\langle v, \cdot \rangle : \mathbb{R}^m \rightarrow \mathbb{R}$.

If the minimum in Definition 1.3.3 (denoted by c_{\min}) is attained, then we can write the face as $F = Q \cap H_{v, c_{\min}} \cap H_{-v, c_{\min}}$. Therefore any face of a polyhedron is itself a polyhedron, so we may speak of its dimension or its interior.

Definition 1.3.4. Let Q be a polyhedron in \mathbb{R}^m . A *facet* of Q is a face whose dimension is one less than that of Q . A *vertex* is a nonempty zero-dimensional face (thus, it is a point).

We make some remarks. First, note that what we call the “interior” is sometimes defined as the “relative interior” [Zie95]. Second, vertices are called “extreme points” in [And08], whose work we build on in Chapter 4. (In fact vertices and extreme points are equivalent concepts in the case of polyhedra.) Third, the interior of a vertex is seen to be the vertex itself. Fourth, the boundary of Q is the disjoint union of the interiors of the proper faces of Q .

We now return to the invariant polyhedra \mathcal{P} of our system of interest, defined via (1.6). For a subset of the set of species $W \subset [s]$, let $Z_W \subset \mathbb{R}^s$ denote its *zero set*:

$$Z_W = \{x \in \mathbb{R}^s : x_i = 0 \text{ if } i \in W\} .$$

It can be seen that for any face F of an invariant polyhedron \mathcal{P} , there exists some (possibly non-unique) subset $W \subset [s]$ such that

$$F = F_W := \mathcal{P} \cap Z_W . \tag{1.8}$$

In other words, each face of \mathcal{P} consists of all points of \mathcal{P} whose sets of zero-coordinates contain a certain subset $W \subset [s]$. However, it is important to note that for some subsets W , the face is empty: $F_W = \emptyset$. In this case we say that the set Z_W is *stoichiometrically unattainable*. In Chapter 3, such a set W will be called *non-relevant* for the invariant polyhedron \mathcal{P} . We see also that $F_W = \mathcal{P}$ if and only if W is empty. For definiteness, if there exist subsets $W_1 \subsetneq W_2 \subset [s]$ for which $F_{W_1} = F_{W_2}$, we denote the face by F_{W_2} . Under this convention, it can be seen that the interior of a face F_W is

$$\text{int}(F_W) = \{x \in \mathcal{P} \mid x_i = 0 \text{ if and only if } i \in W\} . \tag{1.9}$$

We remark that Anderson [And08] and Angeli *et al.* [ADS07a, ADS09] denoted $\text{int}(F_W)$ by $L_W \cap \mathcal{P}$.

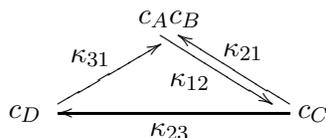
1.4 Examples of reaction networks

We now give examples of chemical reaction networks with an eye toward biology.

Example 1.4.1. We revisit the Square network of Example 1.3.2. The stoichiometric subspace S is spanned by the reaction vector $(1, -1)$, so each invariant polyhedron \mathcal{P} is a line segment whose two vertices are $(0, T)$ and $(T, 0)$ for some positive constant T . \square

In the previous example, the invariant polyhedra are one-dimensional. The next example features two-dimensional polyhedra.

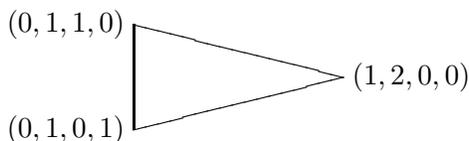
Example 1.4.2. We consider the T-cell signal transduction model of McKeithan [McK95]. Its mathematical analysis appears in work of Sontag [Son01] and in the dissertation of Chavez [Cha03, §7.1]. The chemical reaction network is a triangle:



The biochemical species are as follows: A represents a T-cell receptor, which is bound by the MHC of an antigen-presenting cell B , and when these two bind, they form an intermediate C , and D represents the activated form of C . The stoichiometric subspace is spanned by two reaction vectors $(1, 1, -1, 0)$ and $(0, 0, -1, 1)$. One invariant polyhedron \mathcal{P} is the set

$$\mathcal{P} = \{(c_A, c_B, c_C, c_D) \in \mathbb{R}_{\geq 0}^4 \mid c_A + c_C + c_D = 1, c_B + c_C + c_D = 2\},$$

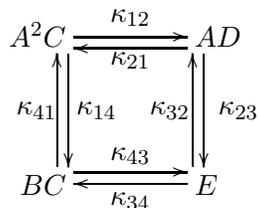
which is depicted here (although not to scale):



The polyhedron is a triangle in the positive orthant $\mathbb{R}_{\geq 0}^4$. The three facets (edges) are one-dimensional line segments $F_{\{A\}}$, $F_{\{C\}}$, and $F_{\{D\}}$, and the three vertices are $F_{\{A,D\}} = \{(0, 1, 1, 0)\}$, $F_{\{C,D\}} = \{(1, 2, 0, 0)\}$, and $F_{\{A,C\}} = \{(0, 1, 0, 1)\}$. All other non-empty subsets of $\{A, B, C, D\}$, such as $\{A, B\}$, yield empty faces of \mathcal{P} : for example, $F_{\{A,B\}} = \emptyset$. \square

Our final example has three-dimensional invariant polyhedra.

Example 1.4.3. We consider a receptor-ligand dimer model, which is analyzed by Chavez in her dissertation [Cha03, §7.2] and by Anderson [And08, Example 4.1]:



The biochemical species are as follows: A denotes a receptor, B denotes a “dimer” state of A (two receptors joined together), and C denotes a ligand that can bind either to A (to form D) or to B (to form E). The stoichiometric subspace is spanned by the reaction vectors $(1, 0, 1, -1, 0)$, $(1, 0, 0, 1, -1)$, and $(0, 1, 1, 0, -1)$. Depictions of three of the five combinatorial types of the invariant polyhedra of this network will be displayed in Figure 3.1 of Chapter 3. \square

For many more examples of reaction networks, see the 17 networks listed in Table I of [Fei80] or the 9 networks listed in Table 1 of [CTF06]. We also refer the reader to the textbook of Beard and Qian [BQ08]. We end the chapter by noting that in practice the set of reactions comprising a network may not be fully known. For details on the topic of network inference and estimation of reaction rate constants, see the work of Craciun, Pantea, and Rempala and the references contained therein [CPR09a, CPR09b]. Related work of Kuepfer, Sauer, and Parrilo gives a semidefinite programming approach to the problems of parameter estimation and model discrimination [KSP07a].

Chapter 2

Toric dynamical systems

The material in this chapter is based on the paper “Toric dynamical systems” [CDSS09], which was authored jointly with Gheorghe Craciun, Alicia Dickenstein, and Bernd Sturmfels. In addition, we include proofs of Proposition 2.2.3 and Corollary 2.2.4, a reference to related work on stochastic systems at the end of Section 2.3, and a reference to the connection to “formal balancing” at the end of Section 2.6.

2.1 Introduction to toric dynamical systems

Toric dynamical systems are mass-action kinetics systems with complex-balancing states. These systems have been studied extensively in mathematical chemistry, starting with the pioneering work of Feinberg, Horn, and Jackson [Fei72, HJ72, Hor72, Hor73b], and continuing with Feinberg’s deficiency theory [Fei79, Fei87, Fei89, Fei95]. Important special cases of these dynamical systems include detailed-balancing systems, recombination equations in population genetics [Aki79], and quadratic dynamical systems in computer science [RSW92].

Karin Gatermann introduced the connection between mass-action kinetics and computational algebra. Our work drew inspiration both from her publications [Gat01, GH02, GW05] and from her unpublished research notes on toric dynamical systems. We wholeheartedly agree with her view that “*the advantages of toric varieties are well-known*” [Gat01, page 5].

We now review the set-up given in Section 1.3 of the Introduction. Let $G = (V, E)$ denote a chemical reaction network, with vertex set $V = \{1, 2, \dots, n\}$ and edge set $E \subseteq \{(i, j) \in V \times V : i \neq j\}$. The node i of G is labeled with the monomial $c^{y_i} = c_1^{y_{i1}} c_2^{y_{i2}} \cdots c_s^{y_{is}}$, and this gives rise to the $n \times s$ -matrix $Y = (y_{ij})$. The monomial labels are the entries in the row vector $\Psi(c) := (c^{y_1}, c^{y_2}, \dots, c^{y_n})$. Each directed edge $(i, j) \in E$ is labeled by a positive parameter κ_{ij} which is the rate constant in the corresponding reaction. We let A_κ denote the negative of the *Laplacian* of the digraph G (so A_κ is the $n \times n$ -matrix whose off-diagonal entries are the κ_{ij} and whose row sums are zero). Mass-action kinetics specified by the digraph G is the following dynamical system (1.3):

$$\frac{dc}{dt} = \Psi(c) \cdot A_\kappa \cdot Y .$$

A *toric dynamical system* is a dynamical system (1.3) for which the algebraic equations $\Psi(c) \cdot A_\kappa = 0$ admit a strictly positive solution $c^* \in \mathbb{R}_{>0}^s$. Note that such a solution c^* is a *steady state* of the system, i.e., the s coordinates of $\Psi(c^*) \cdot A_\kappa \cdot Y$ vanish. The requirement that all n coordinates of $\Psi(c^*) \cdot A_\kappa$ be zero is stronger. An equivalent definition of a toric dynamical system is a chemical reaction system that has the property that for any chemical complex (a product or reactant), the amount produced of that complex at some steady state is equal to the amount consumed by reactions. Later we will see that in fact this property holds at all steady states of a toric dynamical system.

The first to study toric dynamical systems, Horn and Jackson called these systems *complex-balancing mass-action systems* and called c^* a *complex-balancing steady state* [HJ72]. The condition for a system (1.3) to be complex-balancing (i.e., toric) depends on both the digraph G and the rate constants κ_{ij} . The aim of Horn and Jackson in [HJ72] was to define a class of systems that generalize detailed-balancing systems and that remain consistent with “extended thermodynamics conditions.” These systems are shown to satisfy the “quasi-thermodynamic” (QTS) and “quasi-thermodynamic (QTD) conditions, which roughly mean that a Lyapunov function of a certain form exists, for a unique interior steady state in each invariant polyhedron \mathcal{P} . We will see this Lyapunov function in Proposition 2.3.2.

Example 2.1.1. Let $s = 2$, $n = 3$, and let G be the complete bidirected graph on three nodes labeled by c_1^2 , c_1c_2 , and c_2^2 . Here the mass-action kinetics system (1.3) equals

$$\frac{d}{dt}(c_1, c_2) = (c_1^2 \quad c_1c_2 \quad c_2^2) \cdot A_\kappa \cdot \begin{pmatrix} 2 & 0 \\ 1 & 1 \\ 0 & 2 \end{pmatrix}, \quad (2.1)$$

where A_κ is the following matrix:

$$\begin{pmatrix} -\kappa_{12} - \kappa_{13} & \kappa_{12} & \kappa_{13} \\ \kappa_{21} & -\kappa_{21} - \kappa_{23} & \kappa_{23} \\ \kappa_{31} & \kappa_{32} & -\kappa_{31} - \kappa_{32} \end{pmatrix}.$$

This is a toric dynamical system if and only if the following algebraic identity holds:

$$(\kappa_{21}\kappa_{31} + \kappa_{32}\kappa_{21} + \kappa_{23}\kappa_{31})(\kappa_{13}\kappa_{23} + \kappa_{21}\kappa_{13} + \kappa_{12}\kappa_{23}) = (\kappa_{12}\kappa_{32} + \kappa_{13}\kappa_{32} + \kappa_{31}\kappa_{12})^2. \quad (2.2)$$

The equation (2.2) appears in [Hor73b, Equation (3.12)] where it is derived from the necessary and sufficient conditions for complex-balancing in mass-action kinetics given by [Hor72]. Our results in Section 2.2 provide a refinement of these conditions.

Let us now replace G by the digraph with four edges $(1, 3)$, $(2, 1)$, $(2, 3)$, and $(3, 1)$. This corresponds to setting $\kappa_{12} = \kappa_{32} = 0$ in (2.2). We can check that, for this new G , the system (1.3) is not toric for any positive rate constants. Note that G is not strongly connected. \square

Among all chemical reaction networks, toric dynamical systems have remarkable properties. Some of these properties are explained in [Fei79], starting with Proposition 5.3; see also [Gun03, Theorem 6.4]. We shall review them in detail in Sections 2.2 and 2.3. From our point

of view, the foremost among these remarkable properties is that the set Z of all steady states is a toric variety [Gat01, §3]. Recall from the Introduction that each trajectory of (1.3) is confined to a certain *invariant polyhedron*, which is also known as the *stoichiometric compatibility class*. It is known that each invariant polyhedron intersects the toric variety Z in precisely one point c^* . In order to highlight the parallels between toric dynamical systems and *toric models* in algebraic statistics [PS05, §1.2] (which are also known as *log-linear models* [DSS09, §1.1]), we shall refer to the steady state c^* as the *Birch point*; for a statement of Birch’s Theorem, see [Stu96, Theorem 8.20]. In Example 2.1.1, the steady state variety Z is a line through the origin, and the Birch point equals

$$c^* = \text{constant} \cdot (\kappa_{12}\kappa_{32} + \kappa_{13}\kappa_{32} + \kappa_{31}\kappa_{12}, \kappa_{13}\kappa_{23} + \kappa_{21}\kappa_{13} + \kappa_{12}\kappa_{23}) .$$

Here the constant is determined because $c_1 + c_2$ is conserved along trajectories of (2.1).

This chapter is organized as follows. In Section 2.2 we develop the basic theory of toric dynamical systems within the context of computational algebraic geometry. For each directed graph G we introduce the moduli space of toric dynamical systems on G . This space parametrizes all rate constants κ for which (1.3) is toric. In Example 2.1.1 this space is the hypersurface (2.2). Our first main result, Theorem 2.2.7, states that this moduli space is itself a toric variety in a suitable system of coordinates. These coordinates are the maximal non-zero minors of the Laplacian of G , and their explicit form as positive polynomials in the κ_{ij} is given by the *Matrix-Tree Theorem*. Our results in Section 2.2 furnish a two-fold justification for attaching the adjective “toric” to chemical reaction networks with complex-balancing, namely, both the steady state variety and the moduli space are toric. In addition, the subvariety of reaction networks with detailed-balancing is toric.

In Section 2.3 we introduce the *Global Attractor Conjecture* which states that the Birch point is a global attractor for any toric dynamical system. More precisely, we conjecture that all trajectories beginning at strictly positive vectors c^0 will converge to the Birch point c^* in the invariant polyhedron of c^0 . The conjecture is currently open, even for *zero deficiency systems* (cf. Theorem 2.2.9). The conjecture will be the topic of Chapter 4 of this dissertation. Section 2.5 illustrates the main results by way of several examples, and Section 2.6 discusses the special case of detailed-balancing systems.

2.2 Ideals, varieties, and chemistry

This section concerns the connection between chemical reaction network theory and toric geometry. We use the language of ideals and varieties as in the textbook of Cox, Little, and O’Shea [CLO07]. Our reference on toric geometry and its relations with computational algebra is the textbook of Sturmfels [Stu96]. With regard to the dynamical system (1.3), we use the notation from [Fei79, §5] and [Gun03, §3] which has the virtue of separating the roles played by the concentrations c_i , the monomials c^{y_i} , and the rate constants κ_{ij} .

To study the dynamical system (1.3) algebraically, we work in the polynomial ring

$$\mathbb{Q}[c, \kappa] = \mathbb{Q}[\{c_1, c_2, \dots, c_s\} \cup \{\kappa_{ij} : (i, j) \in E\}] ,$$

and we introduce various ideals in this polynomial ring. First, there is the *steady state ideal*

$$\langle \Psi(c) \cdot A_\kappa \cdot Y \rangle$$

which is generated by the s entries of the row vector on the right-hand side of the differential equations (1.3). Second, we consider the ideal

$$\langle \Psi(c) \cdot A_\kappa \rangle$$

which is generated by the n entries of the row vector $\Psi(c) \cdot A_\kappa$. The generators of both ideals are linear in the κ_{ij} , but they are usually non-linear in the c_i . Next, we define the *complex-balancing ideal* of G to be the following ideal quotient whose generators are usually non-linear in the κ_{ij} :

$$C_G := \left(\langle \Psi(c) \cdot A_\kappa \rangle : (c_1 c_2 \cdots c_s)^\infty \right).$$

We have thus introduced three ideals in $\mathbb{Q}[c, \kappa]$. They are related by the inclusions

$$\langle \Psi(c) \cdot A_\kappa \cdot Y \rangle \subseteq \langle \Psi(c) \cdot A_\kappa \rangle \subseteq C_G.$$

If I is any polynomial ideal, then we write $V(I)$ for its complex variety. Likewise, we define two varieties over the real numbers: the positive variety $V_{>0}(I)$ and the non-negative variety $V_{\geq 0}(I)$. They consist of all points in $V(I)$ whose coordinates are real and positive or, respectively, non-negative. Our algebraic approach to chemical reaction network theory focuses on the study of these varieties. The inclusions of ideals above imply the following inclusions of varieties:

$$V(C_G) \subseteq V(\langle \Psi(c) \cdot A_\kappa \rangle) \subseteq V(\langle \Psi(c) \cdot A_\kappa \cdot Y \rangle). \quad (2.3)$$

The definition of C_G by means of saturation implies that the left-hand inclusion becomes equality when we restrict to the points with all coordinates non-zero. In particular,

$$V_{>0}(C_G) = V_{>0}(\langle \Psi(c) \cdot A_\kappa \rangle). \quad (2.4)$$

Recall from [Stu96] that a *toric ideal* is a prime ideal which is generated by binomials. We soon will replace C_G by a subideal T_G which is toric. This is possible by Proposition 5.3 (ii,iv) in [Fei79] or Theorem 6.4 (3) in [Gun03], which essentially state that $V_{>0}(C_G)$ is a positive toric variety. But let us first examine the case when C_G is already a toric ideal.

Example 2.2.1. Suppose that each chemical complex appears in only one reaction, and each reaction is bi-directional. Hence the number of complexes $n = 2m$ is even and, after relabeling, we have the edge set $E = \{(1, 2), (2, 1), (3, 4), (4, 3), \dots, (n-1, n), (n, n-1)\}$. We start with the binomial ideal

$$\langle \Psi(c) \cdot A_\kappa \rangle = \left\langle \kappa_{12}c^{y_1} - \kappa_{21}c^{y_2}, \kappa_{34}c^{y_3} - \kappa_{43}c^{y_4}, \dots, \kappa_{n-1,n}c^{y_{n-1}} - \kappa_{n,n-1}c^{y_n} \right\rangle.$$

The complex-balancing ideal C_G is a saturation of $\langle \Psi(c) \cdot A_\kappa \rangle$, and it coincides with the toric ideal of the extended Cayley matrix which will appear in the proof of Theorem 2.2.7. There are many programs for computing toric ideals. For instance, the methods in [Stu96, §12.A] are available in `Maple` under the command `ToricIdealBasis`. Explicitly, the complex-balancing ideal C_G is generated by all binomials $\kappa^{u+}c^{v+} - \kappa^{u-}c^{v-}$ where

$$\begin{aligned} \sum_{i=1}^m u_{2i-1,2i}(y_{2i-1} - y_{2i}) &= v \quad \text{and} \\ u_{2i-1,2i} + u_{2i,2i-1} &= 0 \quad \text{for } i = 1, 2, \dots, m. \end{aligned} \quad (2.5)$$

Eliminating the unknowns c_1, \dots, c_s from C_G , we obtain the ideal of all binomials $\kappa^{u+} - \kappa^{u-}$ where $u \in \mathbb{N}^E$ satisfies (2.5) with $v = 0$. This is the moduli ideal M_G to be featured in Theorems 2.2.7 and 2.2.9 below. It is a prime binomial ideal of Lawrence type [Stu96, §7]. \square

2.2.1 The Matrix-Tree Theorem and the toric balancing ideal

Let us next assume that $G = (V, E)$ is an arbitrary digraph with n nodes which is *strongly connected*. This means that, for any two nodes i and j , there exists a directed path from i to j . In this case the matrix A_κ has rank $n - 1$, and all of its minors of size $(n - 1) \times (n - 1)$ are non-zero. The next result gives a formula for these comaximal minors.

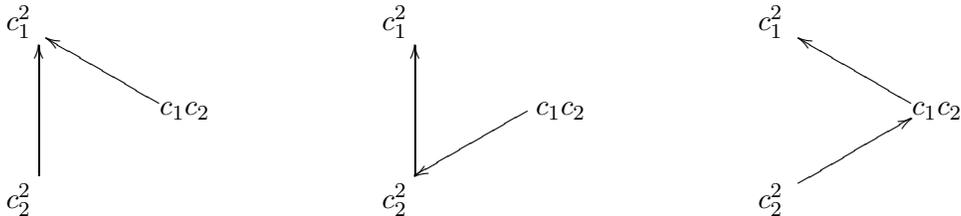
Consider any directed subgraph T of G whose underlying graph is a *spanning tree* of G , that is, T has $n - 1$ edges and contains no cycle. We write κ^T for the product of all edge labels of the edges in T :

$$\kappa^T := \prod_{(i,j) \in E(T)} \kappa_{ij} .$$

This is a squarefree monomial in $\mathbb{Q}[\kappa]$. Let i be one of the nodes of G . The directed tree T is called an *i -tree* if the node i is its unique sink, i.e., all edges are directed towards node i . We introduce the following polynomial of degree $n - 1$:

$$K_i = \sum_{T \text{ an } i\text{-tree}} \kappa^T . \quad (2.6)$$

Example 2.2.2. We return to Example 2.2.1 above. For the first vertex of G ($i = 1$), which corresponds to the complex c_1^2 , there are three 1-trees T_1, T_2 , and T_3 which we depict here:



Each of these three 1-trees gives rise to a polynomial κ^T , so that we obtain the polynomial $K_1 = \kappa_{21}\kappa_{31} + \kappa_{23}\kappa_{31} + \kappa_{21}\kappa_{32}$, as defined in equation (2.6). \square

The next result is a straightforward generalization of the *Matrix-Tree Theorem* which appears in the textbook of Stanley [Sta99, §5.6]. This theorem first appeared in work of Tutte [Tut48, §3.6]. For another application of this theorem and a description of how it implies the well-known King-Altman method for computing the reaction rate function of an enzyme [KA56], see the recent paper of Thomson and Gunawardena [TG09a, §2]. The King-Altman method is illustrated in detail in [BQ08, §4.4.2]. We also make note of the closely related Markov Chain Tree Theorem. This theorem states that if the Laplacian matrix A_κ is viewed as the infinitesimal generator of a continuous-time Markov chain, then the normalized polynomials K_i are the steady state values of the Markov chain [AT89].

Proposition 2.2.3. *Consider a submatrix of A_κ obtained by deleting the i^{th} row and any one of the columns. The signed determinant of this $(n-1) \times (n-1)$ -matrix equals $(-1)^{n-1}K_i$.*

We denote by $M(i, j)$ the $(n-1) \times (n-1)$ -matrix obtained from A_κ by removing row i and column j . The “signed determinant” in the proposition is then equal to $(-1)^{i+j} \det M(i, j)$. We now prove the proposition, following the proof in Stanley [Sta99].

Proof. We first verify that the minor is independent of the choice of which column to remove. In other words, we now check that the signed determinants of $M(i, j)$ and $M(i, 1)$ coincide:

$$(-1)^{i+j} \det M(i, j) = (-1)^{i+1} \det M(i, 1) . \quad (2.7)$$

This follows from the fact that the row sums of A_κ are 0. Namely, performing the following column operations on the matrix $M(i, j)$ yields the matrix $M(i, 1)$: first add columns 2 through $n-1$ to column 1, next negate column 1, and then switch columns 1 and 2, then 2 and 3, and so on through columns $j-2$ and $j-1$. The determinant of the two matrices then differ by a multiplicative factor of $(-1)^{1+(j-2)}$, so equation (2.7) holds.

We next show that we may assume that $i = 1$ as well. Consider the graph G^0 which simply relabels nodes 1 and i by each other. The Laplacian of G^0 is obtained from that of G by switching rows 1 and i and columns 1 and i . So, letting M_{G^0} denote a submatrix of the Laplacian of G^0 , we obtain the first equality below:

$$\begin{aligned} \det M(i, 1) &= \det M_{G^0}(1, i) \\ &= (-1)^{i+1} \det M_{G^0}(1, 1) . \end{aligned}$$

The second equality follows from equation (2.7).

We now need only verify that the result holds for $i = j = 1$, i.e. that

$$\det M(1, 1) = (-1)^{n-1}K_1 . \quad (2.8)$$

Define G' to be the graph obtained from G by removing all edges $(1, k)$ that are directed from node 1 to any node k . We proceed by induction on the number of directed edges of G' . This number, which we denote by q , is equal to the number of nonzero κ_{ij} with $i \neq 1$. Our base case is when $q = n-1$, that is, when G' is a 1-tree (recall that we have assumed that G is strongly connected). Each node k (where $k \neq 1$) has a unique out-neighbor which we denote by $out(k)$; this is the unique node $k' \in [n]$ such that (k, k') is an edge of G . Now reorder the vertices of G so that $1 < k < out(k)$ for $k \neq 1$ which is possible because G' is a tree. The submatrix of interest is then $M_{G'}(1, 1)$, obtained by removing the first row and first column of the Laplacian of G' . The matrix $M_{G'}(1, 1)$ has all zero entries below the diagonal and entries equal to $-\kappa_{k, out(k)}$ along the diagonal. Therefore its determinant is

$$\begin{aligned} \det M_{G'}(1, 1) &= (-1)^{n-1} \prod_{k=1}^{n-1} \kappa_{k, out(k)} \\ &= (-1)^{n-1}K_1 , \end{aligned}$$

where the second equality follows because G' is a 1-tree. Comparing this expression to our desired equality (2.8), we see that it remains only to show that $\det M_{G'}(1, 1) = \det M(1, 1)$. This follows because rows two through n and columns two through n are rearranged by the same permutation to obtain the new Laplacian; this does not affect the determinant of the submatrix obtained by removing the first row and first column. This completes the $q = n - 1$ case.

Now assume that $q > n - 1$. Then there exists a vertex $u \neq i$ with outdegree at least 2 in G . Let e be one edge directed from u . Denote by D_1 the graph G with e removed, and by D_2 the graph G with all other edges $e' \neq e$ directed from u removed. Note that D_2 is a proper subgraph of G by the outdegree assumption on u . Now by induction, we have

$$\begin{aligned} (-1)^{n-1} K_1(D_1) &= \det M_{D_1}(i, 1) , \quad \text{and} \\ (-1)^{n-1} K_1(D_2) &= \det M_{D_2}(i, 1) . \end{aligned} \tag{2.9}$$

We also have

$$K_1(G) = K_1(D_1) + K_1(D_2) , \tag{2.10}$$

because a 1-tree contains some edge directed from u : either edge e or some edge e' . From equations (2.9) and (2.10), it remains only to show that

$$\det M_G(1, 1) = \det M_{D_1}(1, 1) + \det M_{D_2}(1, 1) .$$

This equality follows from the multilinearity of the determinant: row u of $M_G(1, 1)$ is the sum of row u of $M_{D_1}(1, 1)$ and row u of $M_{D_2}(1, 1)$, while all other corresponding rows are identical among the three $(n - 1) \times (n - 1)$ -matrices. \square

It then follows from this proposition that all $(n - 1) \times (n - 1)$ -minors of the matrix A_κ are nonzero, and thus the rank of A_κ is $n - 1$. Combining Proposition 2.2.3 with a little linear algebra leads to the following corollary:

Corollary 2.2.4. *The complex-balancing ideal C_G contains the polynomials $K_i c^{y_j} - K_j c^{y_i}$.*

Proof. It is enough to prove that $K_1 c^{y_2} - K_2 c^{y_1}$ is in the ideal $\langle \psi(c) \cdot A_\kappa \rangle$, because we can reorder the complexes so that i and j are the first and second vertices. We introduce the notation $M(I, J)$ to denote the submatrix of A_κ obtained by removing rows I and columns J . Let V denote the $(n \times 1)$ -matrix (column vector) whose n -th entry is zero, and whose other entries are $(-1)^k \det M(12, kn)$ for $k = 1, 2, \dots, n - 1$. We now claim that

$$A_\kappa \cdot V = \pm(K_1 e_2 - K_2 e_1) , \tag{2.11}$$

where the e_i denote the canonical basis vectors. To see this, first note that the k -th coordinate of $A_\kappa \cdot V$ takes the form of row-expansion along the first row of the following matrix:

$$B(k) := \begin{pmatrix} a_{k1} & a_{k2} & \cdots & a_{k,n-1} \\ a_{31} & a_{32} & \cdots & a_{3,n-1} \\ a_{41} & a_{42} & \cdots & a_{4,n-1} \\ \vdots & \vdots & \ddots & \vdots \\ a_{n1} & a_{n2} & \cdots & a_{n,n-1} \end{pmatrix} ,$$

where $a_{ij} := (A_\kappa)_{ij}$ for ease of notation. In other words,

$$(A_\kappa \cdot V)_k = \det B(k) .$$

So if $k \neq 1, 2$, then $(A_\kappa \cdot V)_k = 0$ because rows 1 and $k - 1$ of the matrix $B(k)$ are identical. Now if $k = 1$, then

$$(A_\kappa \cdot V)_1 = \det M(2, n) = (-1)^{2+n} K_2 ,$$

where the second equality follows from Proposition 2.2.3. Similarly, for $k = 2$ we have

$$(A_\kappa \cdot V)_2 = \det M(1, n) = (-1)^{1+n} K_1 .$$

Therefore, our claim in (2.11) holds. Finally by definition, the ideal $\langle \psi(c) \cdot A_\kappa \rangle$ contains the column space of A_κ with respect to the basis $c^{y_1}, c^{y_2}, \dots, c^{y_n}$; hence the equation (2.11) implies that $K_1 c^{y_2} - K_2 c^{y_1}$ is in $\langle \psi(c) \cdot A_\kappa \rangle$. This completes the proof. \square

We now form the ideal generated by the $\binom{n}{2}$ polynomials in the previous result, and we again saturate with respect to the product $c_1 c_2 \cdots c_s$. The resulting ideal T_G will be called the *toric balancing ideal*:

$$T_G := \left(\langle K_i c^{y_j} - K_j c^{y_i} : 1 \leq i < j \leq n \rangle : (c_1 c_2 \cdots c_s)^\infty \right) .$$

It is natural to consider T_G as an ideal in the polynomial subring

$$\mathbb{Q}[c, K] = \mathbb{Q}[c_1, \dots, c_s, K_1, \dots, K_n] \subset \mathbb{Q}[c, \kappa] .$$

The following lemma states that this subring is itself a polynomial ring.

Lemma 2.2.5. *The polynomials $K_1, \dots, K_n \in \mathbb{Q}[\kappa]$ are algebraically independent over \mathbb{Q} .*

Proof. Let $K'_i \in \mathbb{Q}[\kappa_1, \kappa_2, \dots, \kappa_n]$ denote the polynomial obtained from K_i by substituting the new unknown κ_i for all κ_{ij} . We need only verify that the new polynomials K'_i are algebraically independent, because an algebraic relation among the K_i would be satisfied by the new polynomials K'_i as well. Our polynomials are

$$K'_i = (\text{number of } i\text{-trees in } G) \cdot \prod_{t \neq i} \kappa_t .$$

The n squarefree monomials $\prod_{t \neq i} \kappa_t$ (for $i = 1, 2, \dots, n$) are algebraically independent because an algebraic dependence among these monomials would specify a dependence among $1/\kappa_1, 1/\kappa_2, \dots, 1/\kappa_n$. Hence, the polynomials K'_1, K'_2, \dots, K'_n are algebraically independent. \square

The following result concerns the toric balancing ideal T_G .

Proposition 2.2.6. *The toric balancing ideal T_G is a toric ideal in $\mathbb{Q}[c, K]$. Moreover, the ideal T_G is generated by the binomials*

$$K^{u_+} \cdot c^{(uY)_-} - K^{u_-} \cdot c^{(uY)_+} ,$$

where u is any row vector in \mathbb{Z}^n whose coordinate sum $u_1 + u_2 + \cdots + u_n$ is equal to zero.

Proof. Let Δ denote the edge-node incidence matrix of the complete directed graph on n nodes. Thus Δ is the $\binom{n}{2} \times n$ -matrix whose rows are $e_i - e_j$ for $1 \leq i < j \leq n$. We also consider the $n \times (n+s)$ -matrix $(-Y \ \mathbf{I}_n)$. The binomials $K_i c^{y_j} - K_j c^{y_i}$ which define the ideal T_G correspond to the rows of the $\binom{n}{2} \times (n+s)$ -matrix $\Delta \cdot (-Y \ \mathbf{I}_n)$, and the binomial $K^{u_+} \cdot c^{(uY)_-} - K^{u_-} \cdot c^{(uY)_+}$ corresponds to the row vector $U \cdot \Delta \cdot (-Y \ \mathbf{I}_n)$, where U is any row vector of length $\binom{n}{2}$ such that $u = U \cdot \Delta$. The binomial $K^{u_+} \cdot c^{(uY)_-} - K^{u_-} \cdot c^{(uY)_+}$ is a $\mathbb{Q}[c_1^{\pm 1}, \dots, c_s^{\pm 1}, K_1, \dots, K_n]$ -linear combination of the binomials $K_i c^{y_j} - K_j c^{y_i}$. This shows that T_G is the *lattice ideal* in $\mathbb{Q}[c, K]$ associated with the lattice spanned by the rows of $\Delta \cdot (-Y \ \mathbf{I}_n)$, i.e., there are no monomial zero-divisors modulo T_G . To see that T_G is actually a toric ideal, i.e., T_G is prime, it suffices to note that \mathbb{Z}^{n+s} modulo the lattice spanned by the rows of $\Delta \cdot (-Y \ \mathbf{I}_n)$ is free abelian of rank $s+1$. Indeed, the latter matrix has rank $n-1$, and its $(n-1) \times (n-1)$ -minors span the unit ideal in the ring of integers \mathbb{Z} , because each $(n-1) \times (n-1)$ -minor of Δ is either $+1$ or -1 . \square

The variety of T_G is a toric variety in $\text{Spec } \mathbb{Q}[c, K]$, but we continue to regard it as a subvariety of $\mathbb{C}^s \times \mathbb{C}^E$ (or of $\text{Spec } \mathbb{Q}[c, \kappa]$). In this interpretation we have

$$V_{>0}(T_G) = V_{>0}(C_G) = V_{>0}(\langle \Psi(c) \cdot A_\kappa \rangle). \quad (2.12)$$

Thus T_G still correctly describes the steady state locus of the toric dynamical system. The equation (2.12) holds because the matrix A_κ has rank $n-1$ over the rational function field $\mathbb{Q}(\kappa)$, and the vector (K_1, K_2, \dots, K_n) spans its kernel under left multiplication.

2.2.2 Main results

The following elimination ideal is called the *moduli ideal* of the digraph G :

$$M_G := T_G \cap \mathbb{Q}[\kappa]. \quad (2.13)$$

Here $\mathbb{Q}[\kappa]$ is the polynomial ring in only the edge unknowns κ_{ij} . The generators of M_G are obtained from the generators of C_G by eliminating the unknown concentrations c_i . For instance, if G is the complete bidirected graph on c_1^2 , $c_1 c_2$, and c_2^2 as in Example 2.1.1, then the moduli ideal M_G is the principal ideal generated by $K_1 K_3 - K_2^2$. This coincides with condition (2.2) because $K_1 = \kappa_{21} \kappa_{31} + \kappa_{32} \kappa_{21} + \kappa_{23} \kappa_{31}$, and similarly for K_2 and K_3 .

Suppose now that G is an arbitrary directed graph. Throughout this chapter, we will let l denote the number of connected components of G ; these components are also called the *linkage classes*. If one of the components G_i fails to be strongly connected, then $V_{>0}(C_{G_i})$ is empty and hence $V_{>0}(C_G)$ is empty, by [Fei79, Remark 5.2] or [SM00, Theorem 2.16]. In other words, a complex-balanced system must have strongly connected components (this property is sometimes called *weakly-reversibility*), so in the case that some G_i is not strongly connected, we define T_G and M_G to be the ideal generated by 1. If each connected component G_i of G is strongly connected, then we define the toric steady state ideal as

$$T_G := ((T_{G_1} + T_{G_2} + \dots + T_{G_l}) : (c_1 c_2 \dots c_s)^\infty).$$

The moduli ideal M_G is defined as before in (2.13). The equality in (2.12) still holds and this positive variety is in fact non-empty. Here is the first main result of this chapter:

Theorem 2.2.7. *The equations (1.3) specify a toric dynamical system if and only if the positive vector of rate constants κ_{ij} lies in the toric variety $V(M_G)$. In this case, the set of steady states c^* of (1.3) with all $c_i^* > 0$ equals the set of positive points on the toric variety $V(T_G)$.*

Proof. The positive variety $V_{>0}(T_G)$ consists of all pairs (c, κ) where κ is a strictly positive vector of rate constants and c is a strictly positive solution of the complex balancing equations $\Psi(c) \cdot A_\kappa = 0$. The elimination in (2.13) corresponds to the map of toric varieties $V(T_G) \rightarrow V(M_G)$ given by $(c, \kappa) \mapsto \kappa$. This map is a dominant morphism (by definition of M_G), so its image is Zariski dense in $V(M_G)$. The restriction to real positive points, $V_{>0}(T_G) \rightarrow V_{>0}(M_G)$, is a homomorphism of abelian groups $(\mathbb{R}_{>0})^*$ whose image is dense, so it is the monomial map specified by a matrix with maximal row rank. It follows that this restriction is surjective, and this proves our first assertion. The second assertion follows from [Fei79, Proposition 5.3]. \square

We now justify calling $V(M_G)$ a toric variety by writing the moduli ideal M_G explicitly as a toric ideal in $\mathbb{Q}[K]$. As before, G is a directed graph with n nodes labeled by monomials c^{y_1}, \dots, c^{y_n} . We assume that each connected component G_1, G_2, \dots, G_l of G is strongly connected, for otherwise $M_G = \langle 1 \rangle$. Let Y_i denote the matrix with s rows whose columns are the vectors y_j where j runs over the nodes of the component G_i . We define the *Cayley matrix*

$$\text{Cay}_G(Y) = \begin{pmatrix} Y_1 & Y_2 & \cdots & Y_l \\ \mathbf{1} & \mathbf{0} & \cdots & \mathbf{0} \\ \mathbf{0} & \mathbf{1} & \cdots & \mathbf{0} \\ \vdots & \vdots & \ddots & \vdots \\ \mathbf{0} & \mathbf{0} & \cdots & \mathbf{1} \end{pmatrix}.$$

This is an $(s + l) \times n$ -matrix. Here $\mathbf{1}$ and $\mathbf{0}$ are appropriate row vectors with all entries 1 and 0 respectively. The term ‘‘Cayley matrix’’ comes from geometric combinatorics, and it refers to the Cayley trick in elimination theory [HRS00].

Recall from the Introduction that the *stoichiometric subspace* S is the linear subspace of \mathbb{R}^s which is spanned by the *reaction vectors* $y_j - y_i$ where $(i, j) \in E$. We write $\sigma = \dim(S)$ for its dimension. Recalling that l denotes the number of connected components of G , the quantity $\delta := n - l - \sigma$ is known as the *deficiency* of the chemical reaction network G . For instance, $\delta = 3 - 1 - 1 = 1$ in Example 2.1.1.

Remark 2.2.8. The rank of the Cayley matrix $\text{Cay}_G(Y)$ equals $\sigma + l$. Hence the deficiency δ of the reaction network coincides with the dimension of the kernel of the Cayley matrix.

The following theorem is the second main result in this chapter:

Theorem 2.2.9. *The moduli ideal M_G equals the toric ideal of the Cayley matrix $\text{Cay}_G(Y)$, i.e., M_G is the ideal in $\mathbb{Q}[K]$ generated by all binomials $K^u - K^v$ where $u, v \in \mathbb{N}^n$ satisfy $\text{Cay}_G(Y) \cdot (u - v) = 0$. The codimension of this toric ideal equals the deficiency δ .*

Proof. Let \mathbf{Id}_s denote the $s \times s$ identity matrix and consider the extended Cayley matrix

$$\begin{pmatrix} -\mathbf{Id}_s & Y_1 & Y_2 & \cdots & Y_l \\ \mathbf{0} & \mathbf{1} & \mathbf{0} & \cdots & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{1} & \cdots & \mathbf{0} \\ \mathbf{0} & \vdots & \vdots & \ddots & \vdots \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \cdots & \mathbf{1} \end{pmatrix}.$$

The toric ideal of this matrix is precisely the toric balancing ideal T_G , where the unknowns c_1, c_2, \dots, c_s correspond to the first s columns. Deleting these s columns corresponds to forming the elimination ideal M_G as in (2.13). This shows that M_G is the toric ideal of the matrix $\text{Cay}_G(Y)$. The dimension of the affine toric variety $V(M_G)$ in \mathbb{C}^n is equal to $\sigma + l = \text{rank}(\text{Cay}_G(Y))$, and hence its codimension equals the deficiency $\delta = n - \sigma - l$. \square

We conclude that $V_{>0}(M_G)$ is a positive toric variety of codimension δ in $\mathbb{R}_{>0}^n$. The moment map of toric geometry establishes a natural bijection between $V_{>0}(M_G)$ and the interior of the *Cayley polytope*, which is the convex hull of the columns of $\text{Cay}_G(Y)$.

In summary, given any chemical reaction network whose components are strongly connected, we have shown that the positive toric variety of the Cayley polytope equals the moduli space $V_{>0}(M_G)$ of toric dynamical systems on G . The deficiency δ is precisely the codimension of this moduli space. In particular, if the deficiency is zero then the Cayley polytope is a simplex and the dynamical system (1.3) is toric for all rate constants κ_{ij} . Moreover, the positive steady states of a toric dynamical system form a positive toric variety $V_{>0}(T_G)$.

2.3 The Global Attractor Conjecture

This section establishes the existence and uniqueness of the Birch point for toric dynamical systems, and introduces the Global Attractor Conjecture. However, we postpone a discussion of partial results for the conjecture until Chapter 4. In this section, we consider a fixed toric dynamical system or, equivalently, a chemical reaction network G and a choice of positive rate constants κ^0 in the moduli space $V_{>0}(M_G)$. As usual, the network $G = (V, E)$ is taken to have n nodes which are labeled by monomials $c^{y_1}, c^{y_2}, \dots, c^{y_n}$. We also fix a strictly positive vector $c^0 \in \mathbb{R}_{>0}^s$ which represents the initial concentrations of the s species. The polynomial differential equations (1.3) describe the evolution of these concentrations over time. We seek to understand the long-term behavior of the trajectory which starts at c^0 , that is, $c(0) = c^0$.

Let $T_G(\kappa^0)$ denote the lattice ideal in $\mathbb{R}[c]$ obtained from T_G by substituting the specific rate constants $\kappa_{ij}^0 \in \mathbb{R}_{>0}$ for the unknowns κ_{ij} . Then the positive variety $V_{>0}(T_G(\kappa^0))$ coincides with the set of all positive steady states of the toric dynamical system (1.3). The following result is well-known:

Proposition 2.3.1. [Existence and Uniqueness of the Birch Point] *The affine subspace $c^0 + S$ of \mathbb{R}^s intersects the positive toric variety $V_{>0}(T_G(\kappa^0))$ in precisely one point c^* .*

For a proof and references in the chemistry literature see [HJ72]; a different proof can be found in [Fei79, Proposition 5.3] or [Gun03, Proposition 6.4]. We remark that variants of Proposition 2.3.1 are ubiquitous across the mathematical sciences, and the result has been rediscovered many times. In statistics, this result is known as *Birch's Theorem*; see [PS05, Theorem 1.10] or [DSS09, §2.1]. To emphasize the link with toric models in algebraic statistics we call c^* the *Birch point* of the toric dynamical system (1.3) with starting point c^0 .

Recall from Section 1.3 of the Introduction that the right-hand side of (1.3) is always a vector in the stoichiometric subspace $S = \mathbb{R}\{y_j - y_i : (i, j) \in E\}$, so the trajectory stays in the invariant polyhedron $\mathcal{P} := (c^0 + S) \cap \mathbb{R}_{\geq 0}^s$. Proposition 2.3.1 says that in each invariant polyhedron \mathcal{P} of a toric dynamical system, there is a unique steady state in its interior and this steady state is a complex-balancing state. The following result concerns the trajectories.

Proposition 2.3.2. *The Birch point c^* is the unique point in the invariant polyhedron \mathcal{P} for which the transformed entropy function*

$$E(c) = \sum_{i=1}^s (c_i \cdot \log(c_i) - c_i \cdot \log(c_i^*) - c_i + c_i^*) \quad (2.14)$$

is a strict Lyapunov function of the toric dynamical system (1.3). This means the following:

- (a) For all $c \in \mathcal{P}$ we have $E(c) \geq 0$ and equality holds if and only if $c = c^*$,
- (b) we have $dE(c)/dt \leq 0$ along any trajectory $c(t)$ in \mathcal{P} , and
- (c) equality in (b) holds at a point t^* of any trajectory $c(t)$ in $\text{int}(\mathcal{P})$ if and only if $c(t^*) = c^*$.

This proposition was proved by [HJ72]. A different proof can be found in [Fei79]; see especially Proposition 5.3 and its corollaries. See also [Gun03, Theorem 6.4] and the preceding paragraph, as well as the proof of [PS05, Theorem 1.10].

By the existence of the Lyapunov function in Proposition 2.3.2, aberrant behavior (such as multistationarity, oscillations, and unbounded trajectories) of toric dynamical systems is forbidden. Further, *local asymptotic stability* of the Birch point (relative to the invariant polyhedron) is guaranteed. In other words, there exists a neighborhood of the Birch point (within the polyhedron) such that any trajectory beginning in the neighborhood will converge to the Birch point. The question of *global asymptotic stability*, which asks whether this neighborhood can be taken to be the entire interior of the invariant polyhedron, is the content of the following conjecture. This conjecture was formulated first by Horn in 1974 [Hor74], and was given the name ‘‘Global Attractor Conjecture’’ by Craciun *et al.* [CDSS09]. It is stated to be the main open question in the area of chemical reaction network theory by Adleman *et al.* [AGH⁺08]. In fact, Feinberg states the more general conjecture that all weakly-reversible systems are persistent [Fei79, §6.1]. A system is *persistent* if no trajectories beginning in the interior of an invariant polyhedron \mathcal{P} has an accumulation point on the boundary \mathcal{P} (see Definition 4.2.1). A steady state x in \mathcal{P}° is called a *global attractor* if any trajectory that begins in \mathcal{P}° converges to x .

Global Attractor Conjecture. *For any toric dynamical system (1.3) and any starting point c^0 , the Birch point c^* is the global attractor of the invariant set $\mathcal{P}^\circ = (c^0 + S) \cap \mathbb{R}_{>0}^s$.*

An important subclass of toric dynamical systems consists of the chemical reaction networks of deficiency zero. If the deficiency $\delta = n - l - \sigma$ is zero then the moduli ideal M_G is the zero ideal by Theorem 2.2.9, and the chemical system (1.3) is toric for all choices of rate constants. As remarked earlier, the Global Attractor Conjecture is open even for zero deficiency systems. Chapter 4 is devoted to partial results on the conjecture.

2.4 Connections to deficiency theory

The results in this chapter show that any weakly-reversible dynamical system (1.3) whose deficiency is zero is complex-balancing, and that this fact is independent of the choice of rate constants κ_{ij} ; see also [Fei79]. In fact, this property of being complex-balancing regardless of rate parameters defines the space of deficiency zero systems. On the other hand, a reaction diagram with a deficiency that is positive may give rise to a system that is both complex- and detailed-balancing, complex- but not detailed-balancing, or neither, depending on the values of the rate constants κ_{ij} [Fei72, Fei89, Hor72]; see also Section 2.6.

2.4.1 Deficiency theorems

Propositions 2.3.1 and 2.3.2 are typically known as Feinberg's **Deficiency Zero Theorem**. We also make note of the following proposition whose statement is sometimes included in the Deficiency Zero Theorem.

Proposition 2.4.1. *Consider a chemical system (1.3) whose underlying network has zero deficiency and is not weakly-reversible. Then the system has no strictly positive steady states.*

Recall that a *weakly-reversible* network is one in which all linkage classes are strongly connected. Proposition 2.4.1 essentially follows from the fact that a positive steady state of such a system would have to be complex-balancing, but a toric dynamical system is necessarily weakly-reversible. For a full proof, see the thesis of Guberman [Gub03, Theorem 9.3].

For completeness, we also state the Deficiency One Theorem, which concerns networks whose components have deficiencies at most one. These two deficiency theorems form the basis of Feinberg's Deficiency Theory [Fei79]. We also refer the reader to the expository work on this subject by Guberman [Gub03] and by Gunawardena [Gun03].

We first record some relevant definitions. A connected component of a reaction network is also called a *linkage class*. A *terminal strong linkage class* is a strongly connected component of a network such that no reaction points out of the component (that is, there does not exist a reaction $c^{y_i} \rightarrow c^{y_j}$, where c^{y_i} is in the component, while c^{y_j} is not).

Theorem 2.4.2 (Deficiency One Theorem [Fei87]). *Let G be a weakly-reversible chemical reaction network, and let L_1, \dots, L_l denote its linkage classes. Let δ and $\delta_1, \dots, \delta_l$ denote the deficiencies of G and the linkage classes, respectively. Assume that the classes satisfy the following conditions:*

1. each class L_i contains a unique terminal strong linkage class,
2. $\delta_i \leq 1$ for all $i = 1, \dots, l$, and

$$3. \sum_{i=1}^l \delta_i = \delta.$$

Then for any choice of positive rate constants for the reactions of G , each invariant polyhedron \mathcal{P} contains in its interior a unique steady state of the chemical reaction system (1.3).

Finally, we also make note of the related Deficiency One Algorithm and Advanced Deficiency Algorithm, which were implemented by Ellison and Feinberg in the Chemical Reaction Network Theory Toolbox software [Ell98, EF, Fei95].

2.4.2 Stochastic complex-balancing chemical systems

We end this section by making note of related work on stochastic models of chemical systems. Recall that complex-balancing is a property of a network and a choice of rate parameters. The results in this section state that under the dynamics of mass-action, each invariant polyhedron has a unique steady state. When such a reaction system is modeled stochastically, typically the state vector is discrete, recording the numbers of each chemical species that are present. Also, the integer points of invariant polyhedra further decompose into closed, irreducible subsets which are finite or countable. The stochastic analogue of a steady state is a *stationary distribution*, which describes the long-term behavior of a stochastic process. The following recent result is due to Anderson, Craciun, and Kurtz.

Theorem 2.4.3. [Theorem 4.1 of [ACK08]] *Let G be a chemical reaction network, and let κ_{ij} be a choice of complex-balancing rate constants for G . Let c^* be a Birch point of some invariant polyhedron arising from G . Now consider the system modeled according to stochastic mass-action with parameters κ_{ij} . Then for any closed, irreducible subset Γ of the state space, the stochastic system has a product-form stationary distribution*

$$\pi(x) = M \frac{(c^*)^x}{x!} = M \prod_{i=1}^s \frac{(c_i^*)^{x_i}}{x_i!},$$

which holds for all $x \in \Gamma$, and where M denotes a normalizing constant.

For further details, see the work of Anderson, Craciun, and Kurtz [ACK08].

2.5 Biological applications

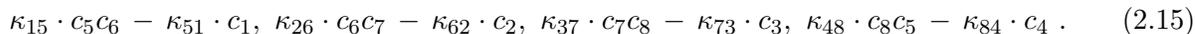
The biological examples in this section illustrate the concepts developed so far.

Example 2.5.1. [Networks with trivial moduli] We expect that our toric approach will be useful for parametric analyses of chemical reaction networks in systems biology. Analyses of this kind include [CFR08, GSG⁺07, KSP07b, Son01]. Many of the explicit examples in the literature have trivial toric moduli in the sense that either M_G is the unit ideal or M_G is the zero ideal.

If $M_G = \langle 1 \rangle$, then (1.3) is **never** a toric dynamical system regardless of what values the κ_{ij} take. As discussed earlier, this happens when at least one connected component of G is not strongly connected (in other words, the network is said to be not weakly-reversible). Examples

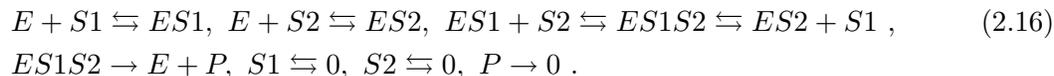
include *Michaelis-Menten kinetics* and the *covalent modification cycle* in [Gun03, §5]. If $M_G = \{0\}$, then the network has deficiency zero and (1.3) is **always** a toric dynamical system, regardless of what the κ_{ij} are. The converse holds as well. Examples include the cycle in [KSP07b, Equation (9)], the monotone networks in [DAS07], and the following network which is taken from [GSG⁺07].

The *ligand-receptor-antagonist-trap network* has $s = 8$ species and $n = 8$ complexes. This network G has four reversible reactions which we write in binomial notation:



Here the number of components is $l = 4$ and the dimension of the stoichiometric subspace is $\sigma = 4$, so the deficiency is $\delta = 0$. In the algebraic notation of Section 2.2, the toric ideal T_G equals the complex-balancing ideal $\langle \Psi(c) \cdot A_\kappa \rangle$ and is generated by the four binomials in (2.15). Eliminating c_1, c_2, \dots, c_8 as prescribed by equation (2.13) yields the zero ideal $M_G = \{0\}$. \square

Example 2.5.2. [DHFR catalysis] Here are some examples from systems biology which show more complicated dynamical behavior. For an example of a network with several positive equilibria for certain values of the reaction rate parameters, we refer to reader to the DHFR catalysis network in the work of Craciun, Tang, and Feinberg [CTF06, Figure 5]; bistability is illustrated in [CTF06, Figure 7]). One subset of these reactions (called “group A” in their work) has almost the same structure as mechanism 6 in [CTF06, Table 1], shown below in the usual additive notation for chemical reactions:



This reaction network allows for inflow and outflow of some chemical species; in the language of deficiency theory, we say that one of the complexes of this reaction network is the zero complex (see [Fei79]), i.e., one of the vectors y_i is the zero vector. Like the more complicated DHFR catalysis network, the network (2.16) also has several positive steady states (within a single invariant polyhedron) for some values of the reaction rate parameters. It is easy to compute the deficiency of this simpler mechanism: the number of complexes is $n = 12$ (including the zero complex), the number of linkage classes is $l = 4$ (including the linkage class that contains the inflow and outflow reactions for the substrates $S1$, $S2$, and the product P), and the dimension of its stoichiometric subspace is $\sigma = 6$. Therefore the deficiency of the network (2.16) is $\delta = 12 - 4 - 6 = 2$. This network cannot be toric for any choice of the constant rates because it is not weakly-reversible. If we make all reactions reversible in (2.16), then the complexes, the linkage classes, and the stoichiometric subspace remain the same, and thus the deficiency of the reversible version of (2.16) is also two. \square

Example 2.5.3. [Recombination on the 3-cube] In *population genetics* [Aki79, Aki82], the evolution of a population is modeled by a dynamical system whose left-hand side is the sum of three terms, corresponding to *mutation*, *selection* and *recombination*. The contribution made by recombination alone is a quadratic dynamical system [RSW92] which can be written in the form of our chemical systems (1.3). In our view, toric dynamical systems are particularly well-suited

to model recombination. Here we consider a population of three-locus diploids, so the underlying *genotype* of the haploid gametes is the standard three-dimensional cube [BPS07, Example 3.9]. The eight vertices of the cube are the genotypes. They now play the role of the species in chemistry:

$$s = 8 \quad \begin{array}{l} \text{genotypes} \\ \text{frequencies} \end{array} \quad \begin{array}{cccccccc} [000] & [001] & [010] & [011] & [100] & [101] & [110] & [111] \\ c_1 & c_2 & c_3 & c_4 & c_5 & c_6 & c_7 & c_8 \end{array}$$

The *recombination network* G has $n = 16$ nodes which correspond to the pairs of genotypes which are not adjacent on the cube. There are twelve bidirectional edges, representing interactions, and we label them using the notation of [BPS07, Example 3.9]. Six of the interactions correspond to *conditional epistasis*, which are displayed by additive notation and by their monomials:

$$\begin{array}{lll} [000] + [110] \rightleftharpoons [010] + [100] & \kappa_{1,2} \cdot c_1 c_7 - \kappa_{2,1} \cdot c_3 c_5 & K_1 = \kappa_{2,1} \text{ and } K_2 = \kappa_{1,2} \\ [001] + [111] \rightleftharpoons [011] + [101] & \kappa_{3,4} \cdot c_2 c_8 - \kappa_{4,3} \cdot c_4 c_6 & K_3 = \kappa_{4,3} \text{ and } K_4 = \kappa_{3,4} \\ [000] + [101] \rightleftharpoons [001] + [100] & \kappa_{5,6} \cdot c_1 c_6 - \kappa_{6,5} \cdot c_2 c_5 & K_5 = \kappa_{6,5} \text{ and } K_6 = \kappa_{5,6} \\ [010] + [111] \rightleftharpoons [011] + [110] & \kappa_{7,8} \cdot c_3 c_8 - \kappa_{8,7} \cdot c_4 c_7 & K_7 = \kappa_{8,7} \text{ and } K_8 = \kappa_{7,8} \\ [000] + [011] \rightleftharpoons [001] + [010] & \kappa_{9,10} \cdot c_1 c_4 - \kappa_{10,9} \cdot c_2 c_3 & K_9 = \kappa_{10,9} \text{ and } K_{10} = \kappa_{9,10} \\ [100] + [111] \rightleftharpoons [101] + [110] & \kappa_{11,12} \cdot c_5 c_8 - \kappa_{12,11} \cdot c_6 c_7 & K_{11} = \kappa_{12,11} \text{ and } K_{12} = \kappa_{11,12} . \end{array}$$

Second, we have *marginal epistasis*, giving rise to the six pairwise interactions among

$$\begin{array}{lllll} \text{four complexes} & [000] + [111] & [001] + [110] & [010] + [101] & [100] + [011] \\ \text{four monomials} & K_{13} \cdot c_1 c_8 & K_{14} \cdot c_2 c_7 & K_{15} \cdot c_3 c_6 & K_{16} \cdot c_4 c_5 . \end{array}$$

Here $K_{13}, K_{14}, K_{15}, K_{16}$ are cubic polynomials with 16 terms indexed by trees as in (2.6). By Proposition 2.2.3, they are the 3×3 minors of the Laplacian of the complete graph \mathbf{K}_4 :

$$\left(\begin{array}{cccc} \kappa_{13,14} + \kappa_{13,15} + \kappa_{13,16} & -\kappa_{13,14} & -\kappa_{13,15} & -\kappa_{13,16} \\ -\kappa_{14,13} & \kappa_{14,13} + \kappa_{14,15} + \kappa_{14,16} & -\kappa_{14,15} & -\kappa_{14,16} \\ -\kappa_{15,13} & -\kappa_{15,14} & \kappa_{15,13} + \kappa_{15,14} + \kappa_{15,16} & -\kappa_{15,16} \\ -\kappa_{16,13} & -\kappa_{16,14} & -\kappa_{16,15} & \kappa_{16,13} + \kappa_{16,14} + \kappa_{16,15} \end{array} \right) .$$

The recombination network G has $l = 7$ connected components and its deficiency is $\delta = 5$, as there are $n = 16$ complexes, and the stoichiometric subspace S has dimension $\sigma = 4$. The moduli ideal M_G is minimally generated by 18 binomials. Twelve of them are cubics:

$$\begin{array}{lll} K_8 K_{11} K_{15} - K_7 K_{12} K_{16} & K_6 K_9 K_{15} - K_5 K_{10} K_{16} & K_4 K_{11} K_{14} - K_3 K_{12} K_{16} \\ K_2 K_9 K_{14} - K_1 K_{10} K_{16} & K_4 K_7 K_{14} - K_3 K_8 K_{15} & K_2 K_5 K_{14} - K_1 K_6 K_{15} \\ K_6 K_{12} K_{13} - K_5 K_{11} K_{14} & K_2 K_{12} K_{13} - K_1 K_{11} K_{15} & K_8 K_{10} K_{13} - K_7 K_9 K_{14} \\ K_4 K_{10} K_{13} - K_3 K_9 K_{15} & K_2 K_8 K_{13} - K_1 K_7 K_{16} & K_4 K_6 K_{13} - K_3 K_5 K_{16} . \end{array}$$

The remaining six generators of M_G are quartics:

$$\begin{array}{ll} K_9 K_{11} K_{14} K_{15} - K_{10} K_{12} K_{13} K_{16} & K_6 K_8 K_{13} K_{15} - K_5 K_7 K_{14} K_{16} \\ K_2 K_4 K_{13} K_{14} - K_1 K_3 K_{15} K_{16} & K_5 K_8 K_{10} K_{11} - K_6 K_7 K_9 K_{12} \\ K_1 K_4 K_{10} K_{11} - K_2 K_3 K_9 K_{12} & K_1 K_4 K_6 K_7 - K_2 K_3 K_5 K_8 . \end{array}$$

The moduli space (of toric dynamical systems on G) is the toric variety $V(M_G)$ defined by these 18 binomials. It has codimension 5 and degree 56. For any recombination rates $\kappa^0 \in V_{>0}(M_G)$ and any starting point c^0 in the *population simplex* Δ_7 , the trajectory of the toric dynamical system (1.3) stays in the 4-dimensional polytope $\mathcal{P} = (c^0 + S) \cap \Delta_7$ and is conjectured to converge to the Birch point c^* . Akin calls c^* the *Wright point* [Aki79]. The point generalizes the classical *Hardy-Weinberg equilibrium* of the two-locus system. \square

2.6 Detailed-balancing systems

In this section we discuss an important subclass of toric dynamical systems called detailed-balancing systems. Here, every edge of the digraph G exists in both directions; that is, each chemical reaction is reversible. We can thus identify $G = (V, E)$ with the underlying undirected graph $\tilde{G} = (V, \tilde{E})$, where $\tilde{E} = \{\{i, j\} : (i, j) \in E\}$. For each undirected edge $\{i, j\} \in \tilde{E}$ of the graph \tilde{G} we define an $n \times n$ -matrix $A_\kappa^{\{i,j\}}$ as follows. In rows i, j and columns i, j the matrix $A_\kappa^{\{i,j\}}$ equals

$$\begin{pmatrix} -\kappa_{ij} & \kappa_{ij} \\ \kappa_{ji} & -\kappa_{ji} \end{pmatrix},$$

and all other entries of the matrix $A_\kappa^{\{i,j\}}$ are 0. Then the Laplacian of G decomposes as the sum

$$A_\kappa = \sum_{\{i,j\} \in \tilde{E}} A_\kappa^{\{i,j\}}. \quad (2.17)$$

A *detailed-balancing system* is a dynamical system (1.3) for which the algebraic equations $\Psi(c) \cdot A_\kappa^{\{i,j\}} = 0$ for $\{i, j\} \in \tilde{E}$ admit a strictly positive solution $c^* \in \mathbb{R}_{>0}^s$. In light of the decomposition (2.17), every detailed-balancing system is a toric dynamical system, so the positive solution c^* (in a fixed invariant polyhedron) is unique and coincides with the Birch point. As it is for toric dynamical systems, the condition of being detailed-balancing depends on both the graph \tilde{G} and the rate constants κ_{ij} . Properties of detailed-balancing systems are described by Feinberg in [Fei89] and by Vol'pert and Khud'iaev in [VK85, §12.3.3].

We rewrite the condition for being detailed-balancing in terms of binomials in the polynomial ring $\mathbb{Q}[c, \kappa]$. The two non-zero entries of the row vector $\Psi(c) \cdot A_\kappa^{\{i,j\}}$ are $\kappa_{ij}c^{y_i} - \kappa_{ji}c^{y_j}$ and its negative. Moreover, we find that

$$\Psi(c) \cdot A_\kappa^{\{i,j\}} \cdot Y = (\kappa_{ij}c^{y_i} - \kappa_{ji}c^{y_j}) \cdot (y_j - y_i),$$

and hence the right-hand side of the dynamical system (1.3) can be rewritten as follows:

$$\Psi(c) \cdot A_\kappa \cdot Y = \sum_{\{i,j\} \in \tilde{E}} \Psi(c) \cdot A_\kappa^{\{i,j\}} \cdot Y = \sum_{\{i,j\} \in \tilde{E}} (\kappa_{ij}c^{y_i} - \kappa_{ji}c^{y_j}) \cdot (y_j - y_i). \quad (2.18)$$

For a detailed-balancing system, each summand in (2.18) vanishes at the Birch point c^* .

Example 2.6.1. We revisit Example 2.1.1. Let $s = 2$, $n = 3$, and let \tilde{G} be the complete graph on three nodes labeled by c_1^2 , c_1c_2 , and c_2^2 . The dynamical system (2.1) is now written as

$$\frac{d}{dt}(c_1, c_2) = (\kappa_{12}c_1^2 - \kappa_{21}c_1c_2) \cdot (-1, 1) + (\kappa_{13}c_1^2 - \kappa_{31}c_2^2) \cdot (-2, 2) + (\kappa_{23}c_1c_2 - \kappa_{32}c_2^2) \cdot (-1, 1) .$$

This is a detailed-balancing system if and only if the following algebraic identities hold:

$$\kappa_{12}^2\kappa_{31} - \kappa_{21}^2\kappa_{13} = \kappa_{23}^2\kappa_{31} - \kappa_{32}^2\kappa_{13} = \kappa_{12}\kappa_{32} - \kappa_{21}\kappa_{23} = 0 . \quad (2.19)$$

This defines a toric variety of codimension two which lies in the hypersurface (2.2). \square

To fit our discussion into the algebraic framework of Section 2.2, we now propose the following definitions. The *detailed-balancing ideal* is the following toric ideal in $\mathbb{Q}[\kappa, c]$:

$$\tilde{T}_G := (\langle \kappa_{ij}c^{y_i} - \kappa_{ji}c^{y_j} \mid \{i, j\} \in \tilde{E} \rangle : (c_1c_2 \cdots c_s)^\infty) . \quad (2.20)$$

The corresponding elimination ideal in $\mathbb{Q}[\kappa]$ will be called the *detailed moduli ideal*:

$$\tilde{M}_G := \tilde{T}_G \cap \mathbb{Q}[\kappa] .$$

The ideal \tilde{T}_G is toric, by the same reasoning as in Proposition 2.2.6. The detailed moduli ideal \tilde{M}_G is a toric ideal of Lawrence type, as was the ideal in Example 2.2.1. Note, however, that the ideals \tilde{T}_G and \tilde{M}_G are toric in the original coordinates κ_{ij} . Here, we did not need the transformation to the new coordinates K_1, \dots, K_n defined in (2.6).

From the inclusion of polynomial rings $\mathbb{Q}[K, c] \subset \mathbb{Q}[\kappa, c]$, we obtain the following:

$$T_G \subseteq \tilde{T}_G \quad \text{and} \quad M_G \subseteq \tilde{M}_G .$$

An instance for which equality holds is Example 2.2.1, in which each chemical complex appears in only one reaction and each reaction is reversible. In fact, Feinberg proved that for any reversible network that is a forest (has no cycles), the conditions for detailed-balancing and complex-balancing are the same [Fei89, Remark 3.2]. A generalization of this result, that a condition called “formal balancing” implies the equivalence of detailed- and complex-balancing, appears in recent work of Dickenstein and Pérez Millán [DM10, Theorem 1.1]. Formal balancing is also known as Feinberg’s “circuit conditions” or Wegscheider’s condition: for any cycle of a network G , the product of the rate constants along one orientation of the cycle must equal the product in the opposite orientation [Fei89]. Clearly, these conditions are vacuous for a cycle-free network. Further details on conditions for complex- and detailed-balancing appear in work of Feinberg [Fei72, Fei89] and Horn [Hor72].

In general, as seen in Example 2.6.1 above, the corresponding inclusion of moduli spaces will be strict:

$$V_{>0}(\tilde{M}_G) \subset V_{>0}(M_G) .$$

In words: every detailed-balancing system is a toric dynamical system but not vice-versa. Convergence properties of detailed-balancing systems will be analyzed in Section 4.5 of Chapter 4.

Chapter 3

Siphons and primary decomposition

The material in this chapter is based on the paper “Siphons in chemical reaction systems” [SS09], which was authored jointly with Bernd Sturmfels.

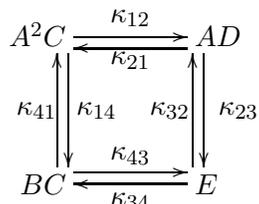
3.1 Introduction to siphons

In systems biology, a population model or chemical reaction system is said to be “persistent” if none of its species can become extinct if all species are present at the initial time. Those subsets of the species that can be absent in steady state are called “siphons.” Angeli *et al.* [ADS07a] suggested the concept of siphons to study the long-term behavior of dynamical systems that model chemical reactions. In terms of the dynamics, a siphon is the index set of a forward-invariant face of the positive orthant. Any boundary steady state must lie in the interior of such a face. Hence, to investigate the trajectories, it is useful to list all minimal siphons. The present chapter offers an algebraic characterization of siphons, and it shows how this translates into a practical tool for computing siphons.

Recall from Section 1.3 of the Introduction that a chemical reaction network is a directed graph G whose nodes are labeled by monomials and whose edges correspond to reactions. A *siphon* of G is a non-empty subset Z of the variables such that, for every directed edge $m \rightarrow m'$ in G , whenever one of the variables in the monomial m' lies in Z then so does at least one of the variables in m . In Section 3.2 we relate this definition to the description of siphons given in [ADS07a, CFP05], we review the underlying dynamics, and we discuss its meaning in terms of polyhedral geometry. Our algebraic approach is presented in Section 3.3. Theorem 3.3.1 expresses the minimal siphons of G in terms of the primary decomposition of a binomial ideal associated to G . If the directed graph G is strongly connected then the ideal encoding the minimal siphons is generated by squarefree monomials. In Theorem 3.3.2 and Algorithm 3.3.6, we explain how to compute the relevant (that is, stoichiometrically compatible) siphons for any set of initial conditions. In particular, a chemical reaction system without relevant siphons has no boundary steady states, and this property is sufficient for proving persistence of many systems [ADS07a, SM00]. In Section 3.4, we demonstrate that the relevant computations can be performed effectively using computer algebra software, such as Macaulay 2 [GS02].

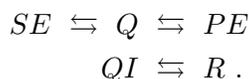
In the remainder of this section, we present three examples from the systems biology literature, with the aim of illustrating our algebraic representation of chemical reaction networks and the computation of siphons.

Example 3.1.1. We return to the receptor-ligand dimer model of Example 1.4.3:



There are three minimal siphons, $\{A, B, E\}$, $\{A, C, E\}$, and $\{C, D, E\}$, which correspond to the minimal primes of the monomial ideal of the complexes $\langle A^2C, AD, E, BC \rangle$. By “minimal” we mean with respect to inclusion. We will revisit this example in Section 3.4. \square

Example 3.1.2. The following enzymatic mechanism was analyzed by Siegel and MacLean [SM00], and also by Chavez [Cha03, Example 4.6.1]:

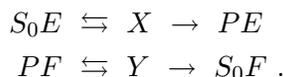


The species are S (a substrate), E (an enzyme), P (a product), I (an uncompetitive inhibitor), and intermediate complexes Q and R . Here the graph consists of two strong components, and we encode it in the binomial ideal $\langle SE - Q, Q - PE, QI - R \rangle + \langle EPQRS \rangle$. The radical of this ideal equals

$$\langle E, Q, R \rangle \cap \langle I, R, ES - Q, P - S \rangle \cap \langle P, Q, R, S \rangle .$$

By Theorem 3.3.1, the minimal siphons are the variables in these prime ideals. Thus the minimal siphons are $\{E, Q, R\}$, $\{I, R\}$, and $\{P, Q, R, S\}$. \square

Example 3.1.3. Here is the network for a basic one-step conversion reaction:



The enzyme E helps convert a substrate S_0 into a product P , and a second enzyme F reverts the product P back into the original enzyme S_0 ; these are also called “futile cycles” [AS06, MG08, SPA05]. Such reactions include phosphorylation and de-phosphorylation events, and they take place in MAPK cascades. This network has three minimal siphons: $\{E, X\}$, $\{F, Y\}$, and $\{P, S_0, X, Y\}$. To see this algebraically, we form the binomial ideal

$$\langle ES_0 - X, X(EP - X), FP - Y, Y(FS_0 - Y), EFPS_0XY \rangle .$$

This ideal corresponds to \mathfrak{T}_G in Theorem 3.3.1, and it has six minimal primes:

$$\begin{aligned}
 &\langle E, X, FS_0 - Y, P - S_0 \rangle, \langle F, Y, P - S_0, S_0E - X \rangle, \langle P, S_0, X, Y \rangle, \\
 &\langle E, X, Y, F \rangle, \langle E, X, P, Y \rangle, \text{ and } \langle F, S_0, X, Y \rangle .
 \end{aligned}$$

The three minimal siphons arise from the first three of these six primes. \square

3.2 Reaction networks, siphons, and steady states

We now review the basic setup from Section 1.3 of the Introduction. A *chemical reaction network* is defined by a finite labeled directed graph G with n vertices. The i -th vertex of G is labeled with a monomial $c^{y_i} = c_1^{y_{i1}} c_2^{y_{i2}} \cdots c_s^{y_{is}}$ in s unknowns c_1, \dots, c_s , and an edge (i, j) is labeled by a positive parameter κ_{ij} . This graph defines the ordinary differential equations

$$\frac{dc}{dt} = \Psi(c) \cdot A_\kappa \cdot Y,$$

where $\Psi(c) = (c^{y_1}, c^{y_2}, \dots, c^{y_n})$ is the row vector of the monomials, $Y = (y_{ij})$ is the $n \times s$ -matrix of exponent vectors of the n monomials, and A_κ is the $n \times n$ -matrix whose off-diagonal entries are the κ_{ij} and whose row sums are zero (i.e. minus the Laplacian of G). The equations (1.3) are those of *mass-action kinetics*, although the concept of a siphon is independent of the choice of kinetics. In order for each chemical *complex* c^{y_i} to be a reactant or product of at least one reaction, we assume that G has no isolated points. For a complex c^{y_i} and for $a \in [s]$, we write $c_a | c^{y_i}$ (“ c_a divides c^{y_i} ”) if $y_{ia} > 0$; in other words the i -th complex contains species a . If the i -th complex does not contain species a , then we write $c_a \nmid c^{y_i}$.

A non-empty subset Z of the index set $[s] := \{1, 2, \dots, s\}$ is a *siphon* if for all $z \in Z$ and all reactions $c^{y_i} \rightarrow c^{y_j}$ with $c_z | c^{y_j}$, there exists $a \in Z$ such that $c_a | c^{y_i}$. Siphons were called “semilocking sets” in [And08, AS10]. Note that the set of siphons of G does not depend on the choice of parameters κ_{ij} .

With any non-empty subset $Z \subset [s]$ we associate the prime ideal

$$\mathfrak{P}_Z := \langle c_a : a \in Z \rangle$$

in the polynomial ring $\mathbb{Q}[c_1, c_2, \dots, c_s]$. Recall (e.g. from [CLO07]) that the *variety* of \mathfrak{P}_Z , denoted by $V(\mathfrak{P}_Z)$, is the set of points $x \in \mathbb{R}^s$ such that $f(x) = 0$ for all polynomials $f \in \mathfrak{P}_Z$. Thus, the non-negative variety $V_{\geq 0}(\mathfrak{P}_Z)$ is the face of the positive orthant $\mathbb{R}_{\geq 0}^s$ defined by all Z -coordinates being zero.

Proposition 3.2.1. *A non-empty subset Z of $[s]$ is a siphon if and only if $V_{\geq 0}(\mathfrak{P}_Z)$ is forward-invariant with respect to the dynamical system (1.3).*

Proof. This is the content of Proposition 2 in Angeli *et al.* [ADS07a]. □

Related results that concern the “reachability” of species include Theorems 1 and 2 in the textbook of Vol’pert and Khudíáev [VK85, §12.2.3]. See also the recent paper of Gnacadja concerning reachability and persistence that builds on the work of Vol’pert [Gna09a, §3].

In Example 3.1.1, the dynamical system (1.3) takes the explicit form

$$\begin{aligned} dA/dt &= -(\kappa_{12} + 2\kappa_{14})A^2C + (\kappa_{21} - \kappa_{23})AD + \kappa_{32}E + 2\kappa_{41}BC \\ dB/dt &= \kappa_{14}A^2C - (\kappa_{41} + \kappa_{43})BC + \kappa_{34}E \\ dC/dt &= -\kappa_{12}A^2C + \kappa_{21}AD - \kappa_{43}BC + \kappa_{34}E \\ dD/dt &= \kappa_{12}A^2C - (\kappa_{21} + \kappa_{23})AD + \kappa_{32}E \\ dE/dt &= \kappa_{23}AD + \kappa_{43}BC - (\kappa_{32} + \kappa_{34})E. \end{aligned}$$

This is a dynamical system on $\mathbb{R}_{\geq 0}^5$. Each of the three minimal siphons $\{A, B, E\}$, $\{A, C, E\}$, and $\{C, D, E\}$ defines a two-dimensional face of $\mathbb{R}_{\geq 0}^5$. For example, $V_{\geq 0}(\mathfrak{P}_{\{A, B, E\}})$ is the face in which the coordinates A , B , and E are zero and C and D are non-negative. The minimality of the three siphons implies that no face of dimension three or four is forward-invariant.

We next collect some results relating siphons to *boundary steady states*, that is, non-negative steady states of (1.3) having at least one zero-coordinate. These connections are behind our interest in computing siphons. In addition, we refer the reader to Chapter 4, which focuses on how siphons are related to questions of *persistence* (the property that positive trajectories of (1.3) have no accumulation points on the boundary of the orthant $\mathbb{R}_{\geq 0}^s$). We first show that a boundary steady state necessarily lies in the relative interior of a face $V_{\geq 0}(\mathfrak{P}_Z)$ indexed by a siphon Z .

Lemma 3.2.2. *Fix a reaction network G , and let γ be a point on the boundary of the positive orthant $\mathbb{R}_{\geq 0}^s$ with zero coordinate set $Z := \{i \in [s] : \gamma_i = 0\}$. If γ is a boundary steady state of (1.3), then the index set Z is a siphon.*

Proof. Assume that $c_z | c^{y_j}$ for some species $z \in Z$ and some complex c^{y_j} of G . Let \mathcal{I} index complexes that react to c^{y_j} but do not contain the species z :

$$\mathcal{I} := \{i \in [n] : c^{y_i} \longrightarrow c^{y_j} \text{ is a reaction of } G \text{ and } c_z \not\prec c^{y_i}\}.$$

Then we have

$$\left. \frac{dc_z}{dt} \right|_{c=\gamma} = \sum_{i \in \mathcal{I}} \kappa_{ij} y_j z \gamma^{y_i} = 0, \quad (3.1)$$

where the second equality holds because γ is a steady state. The summands of (3.1) are non-negative, so we have $\gamma^{y_i} = 0$ for all $i \in \mathcal{I}$. Thus if $i \in \mathcal{I}$ there exists $a_i \in [s]$ with $\gamma_{a_i} = 0$ (so, $a_i \in Z$) and hence $c_{a_i} | c^{y_i}$. \square

A similar result holds for boundary ω -limit points (accumulation points) of a trajectory; see [ADS07a, And08] or [AS10, Theorem 2.13]. As usual, the following *invariant polyhedron* is the forward-invariant set arising from some initial condition $c^{(0)} \in \mathbb{R}_{> 0}^s$:

$$\mathcal{P}_{c^{(0)}} := (c^{(0)} + L_{\text{stoi}}) \cap \mathbb{R}_{\geq 0}^s.$$

Here $L_{\text{stoi}} := \text{span}\{y_j - y_i : c^{y_i} \rightarrow c^{y_j} \text{ is a reaction}\}$ is the *stoichiometric subspace* in \mathbb{R}^s . (This chapter deviates from the usual notation S for this space.) For any index set $W \subset [s]$, we have the corresponding (possibly empty) face of $\mathcal{P}_{c^{(0)}}$:

$$F_W := \{x \in \mathcal{P}_{c^{(0)}} : x_i = 0 \text{ if } i \in W\} = V_{\geq 0}(\mathfrak{P}_W) \cap \mathcal{P}_{c^{(0)}}.$$

As we saw in Section 1.3.6, all faces of $\mathcal{P}_{c^{(0)}}$ have this form. Lemma 3.2.2 implies the following: *Given an invariant polyhedron $\mathcal{P}_{c^{(0)}}$, if all siphons Z yield empty faces, $F_Z = \emptyset$, then $\mathcal{P}_{c^{(0)}}$ contains no boundary steady states.* In Theorem 3.3.5 we shall present an algebraic method for deciding when this happens.

We now examine the case when the chemical reaction network is *strongly connected*, which means that between any two complexes c^{y_i} and c^{y_j} , there is a directed sequence of reactions from c^{y_i} to c^{y_j} .

Lemma 3.2.3. *Assume that G is strongly connected. Then a point $\gamma \in \mathbb{R}_{\geq 0}^s$ is a boundary steady state if and only if its zero set $Z = \{i \in [s] : \gamma_i = 0\}$ is a siphon.*

Proof. The forward implication is Lemma 3.2.2. Now let γ be a boundary point whose zero-coordinate set Z is a siphon. Because G is strongly connected, all complexes c^{y_i} evaluated at γ are zero ($\gamma^{y_i} = 0$), and hence each monomial that appears on the right-hand side of (1.3) vanishes at $c = \gamma$. \square

From a polyhedral geometry point of view, Lemma 3.2.3 states the following: *For strongly connected reaction networks G , any face of an invariant polyhedron $\mathcal{P}_{c^{(0)}}$ either has no steady states in its interior or the entire face consists of steady states.* We shall see now that a similar result holds for toric dynamical systems. Recall from Chapter 2 that (1.3) is a *toric dynamical system* if the parameters κ_{ij} are such that $\Psi(c) \cdot A_\kappa = 0$ has a positive solution $c \in \mathbb{R}_{> 0}^s$ (which is called a *complex-balancing steady state*). The following result concerns the faces of invariant polyhedra of toric dynamical systems.

Lemma 3.2.4. *Let $c^{(0)} \in \mathbb{R}_{> 0}^s$ be a positive initial condition of a toric dynamical system. Then a face F_Z of the invariant polyhedron $\mathcal{P}_{c^{(0)}}$ contains a steady state in its interior if and only if Z is a siphon.*

Proof. This result is a straightforward generalization of Theorem 2.5 of [And08], and its proof is similar. \square

We end this section by noting that the results mentioned above can be reformulated in terms of the complements of siphons, which are the “reach-closed” sets; see the recent work of Gnacadja [Gna09a, §3].

3.3 Binomial ideals and monomial ideals

In what follows we characterize the minimal siphons of a chemical reaction network in the language of combinatorial commutative algebra [MS05]. Namely, they arise as components in primary decompositions. For any initial conditions $c^{(0)}$, we characterize those siphons that define non-empty faces of the invariant polyhedron $\mathcal{P}_{c^{(0)}}$. In the next section we shall see that these results translate into a practical new method for enumerating siphons.

Throughout this section we fix the ring $R = \mathbb{Q}[c_1, \dots, c_s] / \langle c_1 c_2 \dots c_s \rangle$. This is the ring of polynomial functions with \mathbb{Q} -coefficients on the union of the coordinate hyperplanes in \mathbb{R}^s . All of our ideals will live in this ring.

With a given network G we associate the following three ideals in R :

$$\begin{aligned} \mathfrak{I}_G &= \langle c^{y_i} \cdot (c^{y_j} - c^{y_i}) : c^{y_i} \rightarrow c^{y_j} \text{ is a reaction of } G \rangle, \\ \mathfrak{J}_G &= \langle c^{y_j} - c^{y_i} : c^{y_i} \rightarrow c^{y_j} \text{ is a reaction of } G \rangle, \\ \mathfrak{M}_G &= \langle \Psi(c) \rangle = \langle c^{y_1}, c^{y_2}, \dots, c^{y_n} \rangle. \end{aligned}$$

Thus \mathfrak{I}_G encodes the directed edges, and \mathfrak{J}_G encodes the underlying undirected graph. These are pure difference binomial ideals [DMM10, ES96], while \mathfrak{M}_G is the monomial ideal of the complexes. The following is the main result of this chapter.

Theorem 3.3.1. *The minimal siphons of a chemical reaction network G are the inclusion-minimal sets $\{i \in [s] : c_i \in \mathfrak{P}\}$ where \mathfrak{P} runs over the minimal primes of \mathfrak{T}_G . If each connected component of G is strongly connected then \mathfrak{T}_G can be replaced in this formula by the simpler ideal \mathfrak{J}_G . Moreover, if G is strongly connected then \mathfrak{T}_G can be replaced by the monomial ideal \mathfrak{M}_G .*

Proof. The complex variety $V_{\mathbb{C}}(\mathfrak{T}_G)$ consists of all points $\gamma \in \mathbb{C}^s$ having at least one zero coordinate and satisfying $\gamma^{y_i} \cdot (\gamma^{y_j} - \gamma^{y_i}) = 0$ for all reactions. We first claim that our assertion is equivalent to the statement that the minimal siphons are the inclusion-minimal sets of the form $\{i \in [s] : \gamma_i = 0\}$ where γ runs over $V_{\mathbb{C}}(\mathfrak{T}_G)$. Indeed if \mathfrak{P} is a minimal associated prime of \mathfrak{T}_G , let $\gamma \in \{0, 1\}^s$ be defined by $\gamma_i = 1$ if and only if $c_i \notin \mathfrak{P}$. It follows that $\{i \in [s] : c_i \in \mathfrak{P}\} = \{i \in [s] : \gamma_i = 0\}$ and $\gamma \in V_{\mathbb{C}}(\mathfrak{P}) \subset V_{\mathbb{C}}(\mathfrak{T}_G)$. Conversely, if $\gamma \in V_{\mathbb{C}}(\mathfrak{T}_G)$, then $\gamma \in V_{\mathbb{C}}(\mathfrak{P})$ for some minimal associated prime \mathfrak{P} , and so we have the containment $\{i \in [s] : c_i \in \mathfrak{P}\} \subset \{i \in [s] : \gamma_i = 0\}$. If, furthermore, the set $\{i \in [s] : \gamma_i = 0\}$ is minimal among those defined by $\gamma' \in V_{\mathbb{C}}(\mathfrak{T}_G)$, then by above it must follow that the containment is in fact equality.

Next, if γ is in $V_{\mathbb{C}}(\mathfrak{T}_G)$, then we can replace γ by the 0 – 1 vector δ defined by $\delta_i = 0$ if $\gamma_i = 0$ and $\delta_i = 1$ if $\gamma_i \neq 0$. This non-negative real vector has the same support as γ and lies in the variety of \mathfrak{T}_G . Hence our claim is that the minimal siphons are the inclusion-minimal sets of the form $\{i \in [s] : \delta_i = 0\}$ where δ runs over $V_{\{0,1\}}(\mathfrak{T}_G)$. But this is obvious because $\delta^{y_i} \cdot (\delta^{y_j} - \delta^{y_i}) = 0$ if and only if $\delta^{y_j} = 0$ implies $\delta^{y_i} = 0$.

Now, the minimal associated primes of \mathfrak{T}_G depend only on the radical of \mathfrak{T}_G , so we can replace \mathfrak{T}_G by any other ideal that has the same radical. If the components of G are strongly connected then the complex c^{y_i} can produce c^{y_j} if and only if c^{y_j} can produce c^{y_i} , and in this case both $c^{y_i} \cdot (c^{y_j} - c^{y_i})$ and $c^{y_j} \cdot (c^{y_i} - c^{y_j})$ are in \mathfrak{T}_G . Hence the radical of \mathfrak{T}_G contains the binomial $c^{y_i} - c^{y_j}$, and we conclude that \mathfrak{T}_G and \mathfrak{J}_G have the same radical.

Finally, \mathfrak{M}_G is a monomial ideal, and associated primes of a monomial ideal are of the form \mathfrak{P}_Z for some subset $Z \subset [s]$. It is straightforward to see that if G is strongly connected, \mathfrak{P}_Z contains \mathfrak{M}_G if and only if Z is a siphon. \square

When analyzing a concrete chemical reaction network G , one often is given an initial vector $c^{(0)} \in \mathbb{R}_{>0}^s$ for the dynamical system (1.3), or at least a subset Ω of $\mathbb{R}_{>0}^s$ that contains $c^{(0)}$. A siphon $Z \subset [s]$ of G is called *$c^{(0)}$ -relevant* if the face F_Z of the invariant polyhedron $\mathcal{P}_{c^{(0)}}$ is non-empty. In other words, if Z is $c^{(0)}$ -relevant, then there exists a boundary point that is stoichiometrically compatible with $c^{(0)}$ and has zero-coordinate set containing Z . For any subset Ω of $\mathbb{R}_{>0}^s$, we say that Z is *Ω -relevant* if it is $c^{(0)}$ -relevant for at least one point $c^{(0)}$ in Ω . Finally we call a siphon *relevant* if it is $\mathbb{R}_{>0}^s$ -relevant. Relevant siphons are also called “critical” siphons [ADS07a, ADS09], and non-relevant siphons are also called “stoichiometrically infeasible” siphons [AS10] and “structurally non-emptiable” siphons [ADS07a]. In [Ang08], the main theorem of Angeli gives a class of networks for which all siphons are non-relevant; these are the so-called “state machine decomposable networks” that have a “tree structure” and satisfy a certain “overlap compatibility” condition. We next explain how to enlarge the ideals \mathfrak{T}_G , \mathfrak{J}_G , and \mathfrak{M}_G so that their minimal primes encode only the siphons that are relevant.

We recall that the *stoichiometric subspace* L_{stoi} of \mathbb{R}^s is spanned by all vectors $y_j - y_i$ where $c^{y_i} \rightarrow c^{y_j}$ is a reaction in G . (Note that we deviate in this chapter from our usual notation, S , for

this space.) Its orthogonal complement $L_{\text{cons}} := (L_{\text{stoi}})^\perp$ is the space of *conservation relations*. Let \mathcal{Q} denote the image of the non-negative orthant $\mathbb{R}_{\geq 0}^s$ in the quotient space $\mathbb{R}^s/L_{\text{stoi}} \simeq L_{\text{cons}}$. Thus \mathcal{Q} is a convex polyhedral cone and its interior points are in bijection with the invariant polyhedra $\mathcal{P}_{c^{(0)}}$. Further, \mathcal{Q} is isomorphic to the cone spanned by the columns of any matrix \mathbf{A} whose rows form a basis for L_{cons} . This isomorphism is given by the map

$$\phi_{\mathbf{A}} : \mathcal{Q} \rightarrow \left\{ \sum_{i=1}^s \alpha_i a_i : \alpha_1, \alpha_2, \dots, \alpha_s \geq 0 \right\} \quad , \quad \bar{q} \mapsto \sum_{i=1}^s q_i a_i \quad ,$$

where $q = (q_1, q_2, \dots, q_s) \in \mathbb{R}_{\geq 0}^s$ and a_1, a_2, \dots, a_s are the columns of the matrix \mathbf{A} . For simplicity, we identify the cone \mathcal{Q} with the image of $\phi_{\mathbf{A}}$. A subset F of $[s] = \{1, 2, \dots, s\}$ is called a *facet* of \mathcal{Q} if the corresponding columns of \mathbf{A} are precisely the rays lying on a maximal proper face of \mathcal{Q} . Any maximal proper face of \mathcal{Q} also is called a facet. The list of all facets of \mathcal{Q} can be computed using polyhedral software such as `polymake` [JG00].

We represent the facets of \mathcal{Q} by the following squarefree monomial ideal:

$$\mathfrak{B} = \bigcap_{F \text{ facet of } \mathcal{Q}} \langle c_i : i \notin F \rangle =: \bigcap_{F \text{ facet of } \mathcal{Q}} \mathfrak{B}_{F^c} \quad .$$

Each vertex of an invariant polyhedron $\mathcal{P}_{c^{(0)}}$ is encoded uniquely by its support V , which is a subset of $[s]$. Consider the squarefree monomial ideal

$$\mathfrak{B}_{c^{(0)}} = \left\langle \prod_{i \in V} c_i : V \text{ encodes a vertex of } \mathcal{P}_{c^{(0)}} \right\rangle \quad .$$

The distinct combinatorial types of the polyhedra $\mathcal{P}_{c^{(0)}}$ determine a natural *chamber decomposition* of the cone \mathcal{Q} into finitely many smaller cones: if two polyhedra $\mathcal{P}_{c^{(0)}}$ and $\mathcal{P}_{d^{(0)}}$ correspond to points in such a chamber of the decomposition, then the polyhedra have the same set of supports V of their vertices. For an example, see Figure 3.1. In general the face of \mathcal{P} defined by the vanishing of some subset $W \subset [s]$ may have different dimensions in different chambers. In the context of chemical reaction networks, such a chamber decomposition appeared in recent work of Craciun, Pantea, and Rempala [CPR09b]. Specifically, its chambers were denoted S_i in [CPR09b, §2.1].

The ideal $\mathfrak{B}_{c^{(0)}}$ depends only on the chamber that contains the image of $c^{(0)}$. For any subset $\Omega \subset \mathbb{R}_{> 0}^s$, we take the sum of the ideals corresponding to all chambers that intersect the image of Ω in \mathcal{Q} . That sum is the ideal

$$\mathfrak{B}_\Omega = \left\langle \prod_{i \in V} c_i : V \text{ encodes a vertex of } \mathcal{P}_{c^{(0)}} \text{ for some } c^{(0)} \in \Omega \right\rangle \quad .$$

The above ideals are considered either in the polynomial ring $\mathbb{Q}[c_1, \dots, c_s]$ or in its quotient $R = \mathbb{Q}[c_1, \dots, c_s]/\langle c_1 c_2 \cdots c_s \rangle$, depending on the context.

Let \mathfrak{I}_1 and \mathfrak{I}_2 be two arbitrary ideals in R . Recall (e.g. from [CLO07]) that the *saturation* of \mathfrak{I}_1 with respect to \mathfrak{I}_2 is a new ideal that contains \mathfrak{I}_1 , namely,

$$\text{Sat}(\mathfrak{I}_1, \mathfrak{I}_2) = (\mathfrak{I}_1 : \mathfrak{I}_2^\infty) = \{f \in R : f \cdot (\mathfrak{I}_2)^m \subseteq \mathfrak{I}_1 \text{ for some } m \in \mathbb{Z}_{> 0}\} \quad .$$

Here, we shall be interested in the following nine saturation ideals:

$$\begin{array}{lll}
\text{Sat}(\mathfrak{T}_G, \mathfrak{B}), & \text{Sat}(\mathfrak{T}_G, \mathfrak{B}_{c^{(0)}}), & \text{Sat}(\mathfrak{T}_G, \mathfrak{B}_\Omega), \\
\text{Sat}(\mathfrak{J}_G, \mathfrak{B}), & \text{Sat}(\mathfrak{J}_G, \mathfrak{B}_{c^{(0)}}), & \text{Sat}(\mathfrak{J}_G, \mathfrak{B}_\Omega), \\
\text{Sat}(\mathfrak{M}_G, \mathfrak{B}), & \text{Sat}(\mathfrak{M}_G, \mathfrak{B}_{c^{(0)}}), & \text{Sat}(\mathfrak{M}_G, \mathfrak{B}_\Omega).
\end{array} \tag{3.2}$$

The following theorem is a refinement of Theorem 3.3.1.

Theorem 3.3.2. *The relevant minimal siphons of G are the inclusion-minimal sets $\{i \in [s] : c_i \in \mathfrak{P}\}$ where \mathfrak{P} runs over minimal primes from the ideals (3.2). The ideals in the first, second, and third columns yield relevant siphons, $c^{(0)}$ -relevant siphons, and Ω -relevant siphons, respectively. The ideals in the first row are for all networks G , those in the third row for strongly connected networks, and those in the middle row for networks with strongly connected components.*

Proof. The variety of the ideal $\text{Sat}(\mathfrak{T}_1, \mathfrak{T}_2)$ is the union of all irreducible components of the variety $V(\mathfrak{T}_1)$ that do not lie in $V(\mathfrak{T}_2)$. The result now follows from Theorem 3.3.1 and the following observations. The non-negative variety $V_{\geq 0}(\mathfrak{B})$ consists of all points in $\mathbb{R}_{\geq 0}^s$ whose image modulo L_{stoi} lies in the boundary of the cone \mathcal{Q} . Thus, for a minimal siphon Z , the image of the variety $V_{\geq 0}(\mathfrak{P}_Z)$ is in the boundary of \mathcal{Q} if and only if Z is not relevant. More precisely, the image of $V_{\geq 0}(\mathfrak{P}_Z)$ is in the interior of the subcone spanned by $\{a_i : i \notin Z\}$, so there exists a facet of \mathcal{Q} that contains the subcone if and only if Z is not relevant. Therefore, any irreducible component of $V(\mathfrak{J}_G)$ (or $V(\mathfrak{T}_G)$ or $V(\mathfrak{M}_G)$) defines a non-relevant siphon Z if and only if it lies in $V(\mathfrak{P}_{F^c})$ for some facet F of \mathcal{Q} , which is equivalent to lying in $V(\mathfrak{B})$.

Next, the variety $V_{\geq 0}(\mathfrak{B}_{c^{(0)}})$ is the union of all faces of the orthant $\mathbb{R}_{\geq 0}^s$ that are disjoint from the invariant polyhedron $\mathcal{P}_{c^{(0)}}$. So, for a minimal siphon Z , the ideal \mathfrak{P}_Z does not contain $\mathfrak{B}_{c^{(0)}}$ if and only if there exists a vertex of $\mathcal{P}_{c^{(0)}}$ whose zero-coordinate set contains Z , which is equivalent to the condition that the face F_Z of the polyhedron is non-empty. Hence, any component of the variety $V(\mathfrak{J}_G)$ (or $V(\mathfrak{T}_G)$ or $V(\mathfrak{M}_G)$) that defines a minimal siphon Z lies in $V(\mathfrak{B}_{c^{(0)}})$ if and only if Z is not relevant. Finally, the variety $V_{\geq 0}(\mathfrak{B}_\Omega)$ is the intersection of the varieties $V_{\geq 0}(\mathfrak{B}_{c^{(0)}})$ as $c^{(0)}$ runs over Ω . \square

Example 3.3.3. In Examples 3.1.2 and 3.1.3, \mathcal{Q} is the cone over a triangle, and the three minimal siphons are precisely the facets of that triangular cone. Thus, there are no relevant siphons at all. This is seen algebraically by verifying the identities $\text{Sat}(\mathfrak{J}_G, \mathfrak{B}) = \langle 1 \rangle$ and $\text{Sat}(\mathfrak{T}_G, \mathfrak{B}) = \langle 1 \rangle$. \square

We now discuss the case when a network has no relevant siphons, by making the connection to work of Angeli *et al.* [ADS07a], which focuses on chemical reaction networks whose siphons Z all satisfy the following condition:

- (\star) there exists a non-negative conservation relation $l \in L_{\text{cons}} \cap \mathbb{R}_{\geq 0}^s$ whose support $\text{supp}(l) = \{i \in [s] : l_i > 0\}$ is a subset of Z .

Recall that Angeli *et al.* call siphons satisfying this property “structurally non-emptiable” or “stoichiometrically constrained” [ADS09, §3]. Note that the property (\star) needs only to be checked for minimal siphons in order for all siphons to satisfy the property [ADS07a, §8]. For some chemical

reaction systems, such as toric dynamical systems (including Examples 3.1.1 and 3.1.2), this property is sufficient for proving persistence [ADS07a, And08, AS10, SM00], and what was offered in this section are elegant and efficient algebraic tools for deriving such proofs.

Lemma 3.3.4. *For a chemical reaction network G , a siphon Z satisfies property (\star) if and only if Z is not relevant (which is equivalent to the containment $\mathfrak{B} \subseteq \mathfrak{P}_Z$).*

Proof. The “only if” direction is clear. For the “if” direction, let Z be a non-relevant siphon. As usual, for $\sigma := \dim L_{\text{stoic}}$, we fix a matrix $\mathbf{A} \in \mathbb{R}^{(s-\sigma) \times s}$ whose rows span L_{cons} , and we identify \mathcal{Q} with the cone spanned by the columns a_i of \mathbf{A} . Let F be a facet of \mathcal{Q} that contains the image of $V_{\geq 0}(\mathfrak{P}_Z)$, and let $v \in \mathbb{R}^{s-\sigma}$ be a vector such that the linear functional $\langle v, - \rangle$ is zero on F and is positive on points of \mathcal{Q} outside of F . The vector $l := v\mathbf{A}$ is in L_{cons} , and we claim that this is a non-negative vector as in (\star) . Indeed, $l_i = \langle v, a_i \rangle$ is zero if $i \in F$ and is positive if $i \notin F$, and thus, $\text{supp}(l) = F^c \subseteq Z$. \square

The following result extends Theorem 2 in Angeli *et al.* [ADS07a].

Theorem 3.3.5. *None of the siphons of the network G are relevant if and only if $\text{Sat}(\mathfrak{T}_G, \mathfrak{B}) = \langle 1 \rangle$ if and only if all siphons satisfy property (\star) . In this case, none of the invariant polyhedra $\mathcal{P}_{c^{(0)}}$ has a boundary steady state.*

Proof. The first claim follows from Lemma 3.3.4 above. The second claim follows from the definition of relevant siphons and Lemma 3.2.2. \square

We next present a characterization of the ideals \mathfrak{B} and $\mathfrak{B}_{c^{(0)}}$ in terms of combinatorial commutative algebra. This allows us to compute these ideals entirely within a computer algebra system (such as Macaulay 2), without having to make any calls to polyhedral software (such as `polymake`). We assume a subroutine that computes the largest monomial ideal contained in a given binomial ideal in the polynomial ring $\mathbb{R}[c_1, \dots, c_s]$. Let $\mathfrak{T}_{\text{stoi}}$ and $\mathfrak{T}_{\text{cons}}$ denote the lattice ideals associated with the subspaces L_{stoi} and L_{cons} . These ideals are generated by the binomials $c^{u_+} - c^{u_-}$ where $u = u_+ - u_-$ runs over all vectors in \mathbb{Z}^s that lie in the respective subspace. Here, $u_+ \in \mathbb{Z}_{\geq 0}^s$ and $u_- \in \mathbb{Z}_{\geq 0}^s$ denote the positive and negative parts of a vector u in \mathbb{Z}^s .

Algorithm 3.3.6. *The ideals \mathfrak{B} and $\mathfrak{B}_{c^{(0)}}$ can be computed as follows:*

1. *The squarefree monomial ideal \mathfrak{B} is the radical of the largest monomial ideal contained in $\mathfrak{T}_{\text{stoi}} + \langle c_1 c_2 \cdots c_s \rangle$.*
2. *The squarefree monomial ideal $\mathfrak{B}_{c^{(0)}}$ is Alexander dual to the radical of the largest monomial ideal contained in the initial ideal $\text{in}_{c^{(0)}}(\mathfrak{T}_{\text{cons}})$.*
3. *If $c^{(0)}$ is generic (i.e. the polyhedron $\mathcal{P}_{c^{(0)}}$ is simple) then the radical of $\text{in}_{c^{(0)}}(\mathfrak{T}_{\text{cons}})$ is a monomial ideal, and its Alexander dual equals $\mathfrak{B}_{c^{(0)}}$.*

The correctness of part 1 rests on the fact that the zero set of the lattice ideal $\mathfrak{T}_{\text{stoi}}$ is precisely the affine toric variety associated with the cone \mathcal{Q} . Adding the principal ideal $\langle c_1 c_2 \cdots c_s \rangle$

to $\mathfrak{T}_{\text{stoi}}$ is equivalent to taking the image of $\mathfrak{T}_{\text{stoi}}$ in R . The nonnegative variety of the resulting ideal is the union of all faces of $\mathbb{R}_{\geq 0}^s$ whose image modulo L_{stoi} is in the boundary of \mathcal{Q} .

For parts 2 and 3 we are using concepts and results from the textbook [MS05]. The key idea is to use the initial concentration vector $c^{(0)}$ as a partial term order. Initial ideals of lattice ideals are discussed in [MS05, §7.4]. Alexander duality of squarefree monomial ideals is introduced in [MS05, §5.1]. The correctness of part 3 is an immediate corollary to [MS05, Theorem 7.33], and part 2 is derived from part 3 by a perturbation argument. In the next section, we demonstrate how to compute all these ideals in Macaulay 2.

3.4 Computing siphons in practice

We start with a network that has both relevant and non-relevant siphons. This example serves to illustrate the various results in the previous section.

Example 3.4.1. We return to the chemical reaction network in Example 3.1.1. The sums $C+D+E$ and $A+2B+D+2E$ are both constant along trajectories. Chemically, this says that both the total amount of free and bound forms of the ligand and the total amount of the free and bound forms of the receptor remain constant. Thus, the matrix \mathbf{A} can be taken to be

$$\mathbf{A} = (a_A, a_B, a_C, a_D, a_E) = \begin{pmatrix} 0 & 0 & 1 & 1 & 1 \\ 1 & 2 & 0 & 1 & 2 \end{pmatrix}. \quad (3.3)$$

The two rows of \mathbf{A} form a basis of the vector space of conservation relations L_{cons} . The cone \mathcal{Q} is spanned by the columns of \mathbf{A} . The chamber decomposition of \mathcal{Q} is depicted in Figure 3.1. We see that the two facets of \mathcal{Q} define the following ideal of $\mathbb{Q}[A, B, C, D, E]$:

$$\mathfrak{B} = \langle C, D, E \rangle \cap \langle A, B, D, E \rangle = \langle AC, BC, D, E \rangle.$$

The relevant siphons are derived from $\mathfrak{M}_G = \langle A^2C, AD, E, BC \rangle$ as follows:

$$\text{Sat}(\mathfrak{M}_G, \mathfrak{B}) = \langle A, BC, E \rangle = \langle A, B, E \rangle \cap \langle A, C, E \rangle.$$

Thus two of three minimal siphons in Example 3.1.1 are relevant. The third siphon is not relevant as its ideal $\langle C, D, E \rangle$ contains the ideal \mathfrak{B} . This corresponds to the fact, seen in Figure 3.1, that the vectors a_A and a_B span a facet of \mathcal{Q} .

The chamber decomposition of \mathcal{Q} consists of three open chambers $\Omega(1)$, $\Omega(2)$, and $\Omega(3)$, and two rays $\Omega(12)$ and $\Omega(23)$ between the three chambers. These five chambers are encoded in the following ideals, whose generators can be read off from the vertex labels of the polyhedra \mathcal{P}_Ω in Figure 3.1:

$$\begin{aligned} \mathfrak{B}_{\Omega(1)} &= \langle CD, CE, AC, BC \rangle, \\ \mathfrak{B}_{\Omega(12)} &= \langle D, CE, AC, BC \rangle, \\ \mathfrak{B}_{\Omega(2)} &= \langle AD, BD, DE, CE, AC, BC \rangle, \\ \mathfrak{B}_{\Omega(23)} &= \langle AD, BD, E, AC, BC \rangle, \\ \mathfrak{B}_{\Omega(3)} &= \langle AD, BD, AE, BE, AC, BC \rangle. \end{aligned}$$

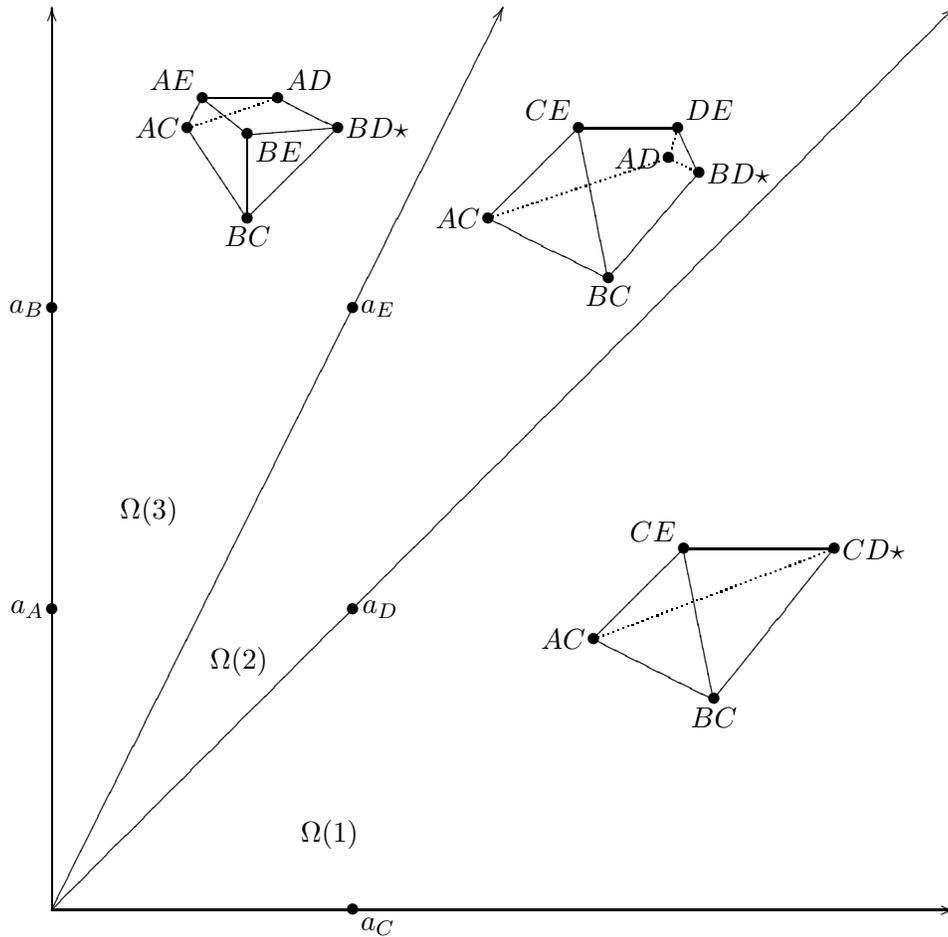


Figure 3.1: The chamber decomposition of the cone \mathcal{Q} for the network in Example 3.1.1. The cone is spanned by the columns of the matrix \mathbf{A} in (3.3). Each of the three maximal chambers $\Omega(1)$, $\Omega(2)$, and $\Omega(3)$ contains a picture of the corresponding 3-dimensional polyhedron $\mathcal{P}_{c^{(0)}}$. The vertices of each polyhedron are labeled by their supports. The star “ \star ” indicates the unique vertex steady state, which arises from one of the two relevant siphons $\{A, B, E\}$ or $\{A, C, E\}$. For example, a vertex labeled by BD^* denotes the unique point on the invariant polyhedron \mathcal{P} for which the coordinates A , C , and E are all equal to zero.

For each chamber Ω , the ideal $\text{Sat}(\mathfrak{M}_G, \mathfrak{B}_\Omega)$ reveals the Ω -relevant siphons. We find that $\langle A, B, E \rangle$ is $\Omega(1)$ - and $\Omega(12)$ -relevant, and that $\langle A, C, E \rangle$ is $\Omega(12)$ -, $\Omega(2)$ -, $\Omega(23)$ -, and $\Omega(3)$ -relevant. These two siphons define a unique vertex steady state on each invariant polyhedron $\mathcal{P}_{c^{(0)}}$. Note that the vertices $F_{\{A,B,E\}}$ and $F_{\{A,C,E\}}$ coincide for polyhedra along the ray $\Omega(12)$. \square

The need for efficient algorithms for computing minimal siphons has been emphasized by Angeli *et al.* [ADS07a], who argued that such an algorithm would allow quick verification of the hypotheses of Theorem 3.3.5. Indeed, the problem of computing siphons is well-studied; for an overview, see Chapter 5 in the textbook of Karatkevich [Kar07]. Cordone *et al.* introduced one algorithm for computing minimal siphons in [CFP05]. We advocate Theorem 3.3.1 as a new method for computing all minimal siphons, and Algorithm 3.3.6 as a direct method for identifying relevant siphons. Rather than implementing any such algorithm from scratch, it is convenient to harness existing tools for monomial and binomial primary decomposition [DMM10, ES96]. We recommend the widely-used computer algebra system `Macaulay 2` [GS02], and the implementations developed by Kahle [Kah10] and Roune [Rou09].

In what follows we show some snippets of `Macaulay 2` code, and we discuss how they are used to compute (relevant) minimal siphons of small networks. Thereafter we examine two larger examples, which illustrate the efficiency and speed of monomial and binomial primary decomposition. These examples support our view that the algebraic methods of Section 3 are competitive for networks whose size is relevant for research in systems biology.

Example 3.4.2. The following `Macaulay 2` input uses the command `decompose` to output the minimal primes for the three examples in the Introduction.

```
-- Example 1.1
R1 = QQ[A,B,C,D,E];
M = ideal(A^2*C, A*D, E, B*C);
decompose(M)

-- Example 1.2
R2 = QQ[e,i,p,q,r,s];
I = ideal(s*e-q, q-p*e, q*i-r);
decompose (I + ideal product gens R2)

-- Example 1.3
R3 = QQ[E,F,P,S_0,X,Y];
J = ideal(E*S_0-X, X*(E*P-X), F*P-Y, Y*(F*S_0-Y));
decompose (J + ideal product gens R3)
```

By Theorem 3.3.1, the minimal siphons can be read off from the primes. \square

Example 3.4.3. We return to the chemical reaction network of Examples 3.1.1 and 3.4.1. The following `Macaulay 2` code utilizes item 2 in Algorithm 3.3.6.

```
-- Example 1.1: c0-relevant siphons
```

```

c0 = {0,0,1,1,0};
R = QQ[A,B,C,D,E, Weights => c0];
IG = ideal(A^2*C-A*D, A*D-E, E-B*C, A*B*C*D*E);
ICons = ideal(C*D*E-1, A*B^2*D*E^2-1);
Bc0 = dual radical monomialIdeal leadTerm ICons;
decompose saturate(IG,Bc0)

```

In the first line, the vector $c^{(0)}$ was chosen to represent a point in the chamber $\Omega(1)$, so the output is the unique $\Omega(1)$ -relevant minimal siphon. \square

The next example is of a large strongly-connected chemical reaction, and the computation shows the power of monomial primary decomposition. For another application of monomial primary decomposition to biology, see Jarrah *et al.* [JLSS07].

Example 3.4.4. Consider the following strongly connected network which is comprised of s species, $s - 1$ complexes, and $s - 2$ reversible reactions:



The number of minimal siphons satisfies the recursion $N(s) = N(s-2) + N(s-3)$, where $N(2) = 2$, $N(3) = 2$, and $N(4) = 3$. For $s = 50$ species we obtain $N(50) = 1,221,537$. The following Macaulay 2 code verifies this:

```

s = 50
R = QQ[c_1..c_s];
M = monomialIdeal apply(1..s-1,i->c_i*c_(i+1));
time betti gens dual M

```

We now explain the commands that are used above. First, M denotes the monomial ideal \mathfrak{M}_G generated by the complexes, and `dual` outputs its Alexander dual [MS05], which is the monomial ideal whose generators are the products of the species-variables in any minimal siphon. Secondly, `betti` applied to `gens dual M` outputs the degrees of all the generators of `dual M`; these degrees are exactly the sizes of all minimal siphons. The command `time` allows us to see that the computation of the minimal siphons takes only a few seconds. Displayed below is a portion the output of the last command above; the list tells the number of minimal siphons of each possible size.

```

          0          1
o5 = total: 1 1221537
          0: 1          .
          1: .          .
          2: .          .
          ...
         23: .          .
         24: .          26
         25: .          2300
         26: .          42504

```

27: .	245157
28: .	497420
29: .	352716
30: .	77520
31: .	3876
32: .	18

The current version of `dual` in `Macaulay 2` uses Roune's implementation of his Slice Algorithm [Rou09]. For background on the relation of Alexander duality and primary decomposition of monomial ideals, see the textbook [MS05]. \square

Our final example aims to illustrate the computation of minimal siphons for a larger network with multiple strongly connected components.

Example 3.4.5. Here we consider a chemical reaction network G with $s = 25$ species, 16 bidirectional reactions, and $n = 32$ complexes. The binomials representing the 16 reactions are the adjacent 2×2 -minors of a 5×5 -matrix (c_{ij}) , and \mathfrak{J}_G is the ideal generated by these 16 minors $c_{i,j}c_{i+1,j+1} - c_{i,j+1}c_{i+1,j}$. For this network, L_{stoi} is the 16-dimensional space consisting of all matrices whose row sums and column sums are zero, and \mathcal{Q} is a 9-dimensional convex polyhedral cone, namely the cone over the product of simplices $\Delta_4 \times \Delta_4$.

What follows is an extension of the results for adjacent minors of a 4×4 -matrix in [DES98, §4]. The ideal \mathfrak{J}_G is not radical. Using Kahle's software [Kah10], we found that it has 103 minimal primes, of which precisely 26 contribute minimal siphons that are relevant. Up to symmetry, these 26 siphons fall into four symmetry classes, with representatives given by the following:

$$\begin{aligned} Z_1 &= \{c_{14}, c_{21}, c_{22}, c_{23}, c_{24}, c_{32}, c_{34}, c_{42}, c_{43}, c_{44}, c_{45}, c_{52}\}, \\ Z_2 &= \{c_{14}, c_{21}, c_{22}, c_{23}, c_{24}, c_{33}, c_{34}, c_{35}, c_{41}, c_{42}, c_{43}, c_{53}\}, \\ Z_3 &= \{c_{14}, c_{24}, c_{31}, c_{32}, c_{33}, c_{34}, c_{42}, c_{43}, c_{44}, c_{45}, c_{52}\}, \\ Z_4 &= \{c_{14}, c_{24}, c_{31}, c_{32}, c_{33}, c_{34}, c_{43}, c_{44}, c_{45}, c_{53}\}. \end{aligned}$$

Under the group D_8 of reflections and rotations of the matrix (c_{ij}) , the orbit of Z_1 consists of two siphons, and the orbits of Z_2 , Z_3 , and Z_4 each are comprised of eight siphons. The corresponding four types of minimal primes have codimensions 13, 12, 12, and 12, and degrees 1, 2, 3, and 6.

By randomly generating chambers, we found that, for every integer r between 0 and 26, other than 23 and 25, there is a point $c^{(0)}$ in \mathcal{Q} such that the number of $c^{(0)}$ -relevant siphons is precisely r . We briefly discuss this for three initial conditions. First, let $c^{(0)}$ be the all-ones matrix. Then $\mathcal{P}_c^{(0)}$ is the *Birkhoff polytope* which consists of all non-negative 5×5 -matrices with row and column sums equal to five. In this case, all 26 minimal siphons are $c^{(0)}$ -relevant: Z_1 defines a vertex, Z_2 and Z_3 define edges, and Z_4 defines a three-dimensional face of $\mathcal{P}_{c^{(0)}}$. Next, consider the following initial conditions:

$$d^{(0)} = \begin{pmatrix} 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 - \epsilon & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 \end{pmatrix} \quad \text{and} \quad e^{(0)} = \begin{pmatrix} 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 + \epsilon & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 \end{pmatrix},$$

where $\epsilon > 0$. Again, all 26 minimal siphons are $d^{(0)}$ -relevant, and F_{Z_1} is a vertex, F_{Z_2} and F_{Z_3} are edges of $\mathcal{P}_{d^{(0)}}$, but now F_{Z_4} is a five-dimensional face. Finally, for initial condition $e^{(0)}$, only two minimal siphons are $e^{(0)}$ -relevant, both in the class of Z_1 , and they define vertices. The results of Chapter 4 will allow us to conclude that the system (1.3) is persistent for $e^{(0)}$. \square

3.5 Siphons and persistence

In the present chapter, we gave a method that computes siphons and determines which of them are relevant. To our knowledge, this is the first automatic procedure for checking the relevance of a siphon. As noted by Angeli *et al.* [ADS07a], such a procedure is desirable for verifying whether large biochemical reaction systems are persistent. Persistence is the property that no species concentration tends to zero. In practice, this corresponds to the observed behavior that a substrate that is present at the beginning of an experiment will also be present in some amount for all time. For a toric dynamical system, the non-relevance of all siphons is a sufficient condition for such a system to be persistent, so our procedure can be used to verify quickly that a large network is persistent. Mathematically, the claim that toric dynamical systems are persistent is the content of the global attractor conjecture, and we speculate that an algebraic approach to understanding siphons may be a step toward the conjecture.

Chapter 4

The global attractor conjecture

The material in this chapter is based partly on Sections 3–5 from the paper “Toric dynamical systems” [CDSS09], which was authored jointly with Gheorghe Craciun, Alicia Dickenstein, and Bernd Sturmfels, and also from the paper “The dynamics of weakly-reversible population processes near facets” [AS10], which was joint with David F. Anderson. Additionally, Section 4.6.3 appears in extended form: Lemma 4.6.8 simplifies the definition of “dynamically non-emptiable” which subsequently shortens the proof of Corollary 4.6.9. Further, a discussion concerning the dynamic non-emptiability of vertex-defining siphons is included.

4.1 Introduction

Chapter 3 concerned the computation of siphons. The present chapter relates siphons to the global attractor conjecture. Namely, the relevant siphons determine which faces of an invariant polyhedron contain steady states, and to prove the conjecture it is sufficient to verify that no positive trajectory approaches such a steady state. This is due to two results: Theorem 4.2.2 in this chapter, which says that only faces that arise from siphons can admit ω -limit points (accumulation points of a trajectory), and a known result, which states that positive trajectories converge to the set of steady states (see Corollary 2.6.4 of [Cha03] or Theorem 1 of [Son01]). The two main results of this chapter state that if a siphon defines a vertex or a facet, then the corresponding steady states have repelling neighborhoods of the toric dynamical system. Moreover, we prove that those siphons that define facets are so-called “dynamically non-emptiable.” In particular, this gives the following corollary: *the global attractor conjecture holds for systems for which the invariant polyhedra are two-dimensional.* We remark that the results in this section apply more generally to deterministic population processes that satisfy basic assumptions as stated in Section 1.3.2 of the Introduction. A discussion concerning population processes in this context appears in [AS10, §1].

We now describe the layout of the chapter. In Section 4.2, we discuss the connection between siphons and persistence. Section 4.3 recalls the statement of the conjecture and reviews the known partial results. Section 4.4 provides some preliminary results, and Section 4.5 pertains to the special case of detailed-balancing systems. Our main results are then stated and proven in Section 4.6. We also show that siphons associated with facets are “dynamically non-emptiable”

in the terminology of Angeli, De Leenheer, and Sontag [ADS07a], thereby providing a large class of dynamically non-emptiable semilocking sets. Applications of this work to the global attractor conjecture is the topic of Section 4.7. Finally, Section 4.8 provides examples that illustrate how our results fit within the context of related results.

4.2 Boundary steady states, persistence, and siphons

In this section, we introduce the concept of persistence for chemical reaction systems and connect this idea to the results concerning siphons in the previous chapter. Let us first recall the notation that will be used throughout this chapter. Our chemical reaction systems are defined by mass-action equations (1.3), and in this chapter we deviate from the usual notation, and write the differential equations as in (1.5), but we deviate slightly by indexing reactions by $k \in [R]$ rather than by the edges (i, j) of the reaction graph:

$$\frac{dc}{dt} = \sum_{k=1}^R R_k(c(t))(y'_k - y_k) =: f(c(t)) ,$$

where the rate formation function for the reaction $y_k \rightarrow y'_k$ is:

$$R_k(c) = \kappa_k c_1^{y_{k1}} c_2^{y_{k2}} \dots c_s^{y_{ks}} = \kappa_k c^{y_k} .$$

In addition, we will denote a *chemical reaction network* by a triple $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$, where $\mathcal{S} = \{c_i\}$, $\mathcal{C} = \{c^y\}$, and $\mathcal{R} = \{c^y \rightarrow c^{y'}\}$ are the sets of species, complexes, and reactions, respectively. The *reaction diagram* is the usual directed graph G , whose nodes are the complexes, \mathcal{C} , and whose edges correspond to reactions. Each connected component of the resulting graph is termed a *linkage class* of the graph. A chemical reaction network is said to be *weakly-reversible* if each linkage class of the corresponding reaction diagram is strongly connected. In other words, if $y \rightarrow y' \in \mathcal{R}$, then there exists a sequence of directed reactions $y' \rightarrow \dots \rightarrow y$ in \mathcal{R} . A network is said to be *reversible* if $y' \rightarrow y \in \mathcal{R}$ whenever $y \rightarrow y' \in \mathcal{R}$.

It will be convenient to view the set of species \mathcal{S} as interchangeable with the set $[s]$, where σ denotes the number of species. Therefore, a subset of the species, $W \subset \mathcal{S}$, is also a subset of $[s]$, and we will refer to the *W-coordinates* of a concentration vector $c \in \mathbb{R}^s$, meaning the concentrations c_i for species i in W . Further, we will write $i \in W$ or $i \in [s]$ to represent $c_i \in W$ or $c_i \in \mathcal{S}$, respectively. Similarly, we sometimes will consider subsets of the set of reactions \mathcal{R} as subsets of the set $[R]$, where R denotes the number of reactions. The *zero-coordinates* of a vector $x \in \mathbb{R}^s$ are the indices i for which $x_i = 0$. The *support* of x is the set of indices for which $x_i \neq 0$. Based upon these definitions and the preceding remarks, both the set of zero-coordinates and the support of a vector x can, and will, be viewed as subsets of the species.

Recalling that trajectories remain in their invariant polyhedra \mathcal{P} for all time, we see that it is appropriate to ask about the existence and stability of steady states of system (1.3) within and relative to an invariant polyhedron \mathcal{P} . We will take this viewpoint in Section 4.3. We are now prepared to define persistence. Let $c(t)$ be a solution to (1.3) with strictly positive initial condition $c^0 \in \mathbb{R}_{>0}^s$. The set of *ω -limit points* for this trajectory is the set of accumulation points:

$$\omega(c^0) := \{x \in \mathbb{R}_{\geq 0}^s \mid c(t_n) \rightarrow x \text{ for some sequence } t_n \rightarrow \infty\} . \quad (4.1)$$

Definition 4.2.1. A trajectory with initial condition c^0 is said to be *persistent* if $\omega(c^0) \cap \partial\mathbb{R}_{\geq 0}^s = \emptyset$. A dynamical system is *persistent* if each trajectory with strictly positive initial condition is persistent.

The concept of persistence is important in the ecology and population biology literature; for example, see [BS09]. We now make note of two variations on the concept of persistence, although these will not be treated in this dissertation. A stronger version of persistence, called “vacuous persistence,” which allows for trajectories beginning on the boundary of an invariant polyhedron as well as in the interior, is considered by Gnacadja in [Gna09a]. A weaker version of persistence, called “bounded-persistence,” which allows for only those interior trajectories that are bounded, is considered by Angeli *et al.* in [ADS09, §4].

In order to show that a system is persistent, we must understand which points on the boundary of an invariant polyhedron are capable of being ω -limit points. To this end, we recall the definition of a siphon: a nonempty subset W of the set of species is called a *siphon* if for each reaction in which there is an element of W in the product complex, there is an element of W in the reactant complex.

The intuition behind siphons lies in the next proposition, which is due to Angeli *et al.* [ADS07a]. Related results that concern the “reachability” of species include Theorems 1 and 2 in the textbook of Vol’pert and Khudiaev [VK85, §12.2.3].

By Assumption 1.3.1, the rate of the reaction $y \rightarrow y'$ at a concentration c^0 is zero if and only if at least one species in the support of y has zero concentration at c^0 . Therefore $W \subset \mathcal{S}$ is a siphon if it satisfies the following property: if all the W -coordinates of a species concentration vector c^0 are zero, then $i \in W$ implies that $x_i(t) = 0$ for all $t > 0$ where $c(t)$ is the trajectory with initial condition c^0 . In words, this means that if no species of W is present at time zero, then this remains the case for all positive times. This was the content of Proposition 3.2.1, which we repeat here; it is Proposition 2 in Angeli *et al.* [ADS07a].

Proposition 4.2.1 ([ADS07a]). *Let $W \subset \mathcal{S}$ be non-empty. Then W is a siphon if and only if the face $\{x \in \mathbb{R}^s \mid x_i = 0 \text{ if } i \in W\}$ of the positive orthant is forward-invariant for the dynamics (1.5).*

If in addition the reaction network is weakly-reversible (that is, each connected component is strongly connected), then it is straightforward to conclude the following: if a linkage class has a complex whose support contains an element of W , then the rates of all reactions within that linkage class will be zero for all positive time. In other words, certain linkage classes are “shut off.”

Recall from Section 1.3.6 that any face of \mathcal{P} has the form

$$F_W := \{c \in \mathcal{P} \mid c_i = 0 \text{ if } i \in W\},$$

where $W \subseteq \{1, \dots, s\}$, and the boundary ∂P of \mathcal{P} is the union of all faces F_W where W is a proper subset of $\{1, \dots, s\}$. This includes $F_\emptyset = \mathcal{P}$. In light of the characterization of the interior of a face F_W given in equation (1.9), the following theorem is proven in [And08, ADS07a]; it states that the siphons are the possible sets of zero-coordinates of boundary ω -limit points.

Theorem 4.2.2 ([ADS07a, And08]). *Let $W \subset \mathcal{S}$ be a nonempty subset of the set of species. Let $c^0 \in \mathbb{R}_{>0}^s$ be a strictly positive initial condition for the system (1.3), and let $\mathcal{P} = (c^0 + S) \cap \mathbb{R}_{\geq 0}^s$*

denote the corresponding invariant polyhedron. If there exists an $\omega(c^0)$ -limit point, $z \in \omega(c^0)$, and a subset of the species, W , such that z is contained within the interior of the face F_W of \mathcal{P} , then W is a siphon.

This theorem will be used in conjunction with results in this chapter to prove the persistence of the following class of weakly-reversible systems: those for which each siphon W satisfies the property that the face F_W is a facet or empty; see Theorem 4.6.7.

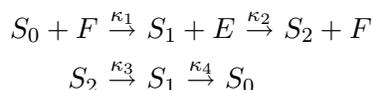
4.3 History of the conjecture

In this section, we recall the statement of the global attractor conjecture and discuss its known results. The conjecture concerns the limiting behavior of toric dynamical systems. We begin by recalling some properties of toric dynamical systems from Chapter 2. A steady state $c^* \in \mathbb{R}_{\geq 0}^s$ of (1.3) is said to be *complex-balancing* if $\Psi(c^*) \cdot A_\kappa = 0$. A *toric dynamical system* is a dynamical system (1.3) that admits a strictly positive complex-balancing steady state. The algebraic structure of toric dynamical systems was highlighted in Chapter 2. In the interior of each invariant polyhedron \mathcal{P} , there exists a unique steady state c^* , with strictly positive components, and this steady state is complex-balancing. This point, c^* , is called the *Birch point* due to the connection to Birch's Theorem in algebraic statistics (see Theorem 1.10 of [PS05]). Note that a system was defined to be a toric dynamical system if at least one such steady state exists; we have seen that so long as at least one \mathcal{P} contains a complex-balancing steady state, then they all do (Proposition 2.3.1). As for the stability of the steady state within the interior of the corresponding \mathcal{P} , a strict Lyapunov function exists for each such point (Proposition 2.3.2). Hence local asymptotic stability relative to \mathcal{P} is guaranteed; see Theorem 6A of [HJ72] and the Deficiency Zero Theorem of [Fei79]. The global attractor conjecture states that this steady state of \mathcal{P} is globally asymptotically stable relative to the interior of \mathcal{P} [CDSS09]. In the following statement, a *global attractor* for a set V is a point $v^* \in V$ such that any trajectory $v(t)$ with initial condition $v^0 \in V$ converges to v^* , in other words, $\lim_{t \rightarrow \infty} v(t) = v^*$.

The global attractor conjecture *For any toric dynamical system and any strictly positive initial condition c^0 , the Birch point $c^* \in \mathcal{P} := (c^0 + S) \cap \mathbb{R}_{\geq 0}^s$ is the global attractor of the interior of the invariant polyhedron, $\text{int}(\mathcal{P})$.*

This conjecture first appeared in a paper of Horn [Hor74], and was given the name “Global Attractor Conjecture” by Craciun *et al.* [CDSS09]. It is stated to be the main open question in the area of chemical reaction network theory by Adleman *et al.* [AGH⁺08]. In fact, Feinberg states the more general conjecture that all weakly-reversible systems are persistent; see [Fei79, §6.1]. (A reaction system (1.3) is weakly-reversible or reversible, if its underlying network is.) Note that non-weakly-reversible systems can fail to be persistent; for example, consider the dynamics of the reaction $A \rightarrow B$. A less trivial example is the following, which is due to Angeli:

Example 4.3.1. Consider the following network:



We write a concentration vector as $c = (c_{S_0}, c_{S_1}, c_{S_2}, c_E, c_F)$. In [Ang08, §IV.A], Angeli shows that if $\kappa_2 < \kappa_4$, then the boundary steady state $c^* := (1, 0, 0, 1, 0)$ is locally asymptotically stable relative to its invariant polyhedron. Hence this non-weakly-reversible network is not persistent. \square

There are some persistence results for certain classes of systems. Gnacadja proves that the class of networks of “pre-complete reversible binding reactions” are persistent; these systems include non-toric dynamical systems [Gna09b]. Another class of systems that is known to be persistent is the class of translation-invariant strongly monotone dynamical systems; this result is due to Angeli and Sontag [AS06, Theorem 1]. It is important to note that persistence of a network may depend on its specific values of reaction rate constants. We refer the reader to an apoptosis regulation network analyzed by Angeli *et al.* whose persistence depends on the sign of the determinant of a certain Jacobian matrix [ADS09, §10]. Similarly, a MAPK cascade model with inhibitory feedback which was shown by Arcak and Sontag to be persistent when a certain parametrized 5×5 -matrix is positive definite [AS08, Example 2].

We now describe known partial results regarding the global attractor conjecture. By an *interior trajectory* we shall mean a solution $c(t)$ to the dynamical system (1.3) that begins at a strictly positive initial condition $c^0 \in \mathbb{R}_{>0}^s$. First, trajectories are known to be bounded [SM00, Lemma 3.5]. Next, it is known that the set of ω -limit points of such systems is contained within the set of steady states; Corollary 2.6.4 due to Chavez [Cha03], Proposition 3.8 due to Gnacadja [Gna09a], and Theorem 1 due to Sontag [Son01] are special cases of this fact, and the general result follows by similar arguments. Hence, the conjecture is equivalent to the following statement: *for a toric dynamical system, any boundary steady state is not an ω -limit point of an interior trajectory.* (See also the related results in this chapter, Proposition 4.4.1 and Lemma 4.4.2.) It clearly follows that if an invariant polyhedron \mathcal{P} has no boundary steady states, then the conjecture holds for this \mathcal{P} ; see the work of Siegel and MacLean [SM00, Theorem 4.1]. Thus, sufficient conditions for the non-existence of boundary steady states are conditions under which the global attractor conjecture holds (see Theorem 2.9 of [And08]); a result of this type is Theorem 6.1 of Adleman *et al.* [AGH⁺08]. Recall that, by Theorem 4.2.2, we know that the only faces F_W of an invariant polyhedron \mathcal{P} that may contain ω -limit points in their interiors are those for which W is a siphon. In particular, if the set Z_W is stoichiometrically unattainable for all siphons W , then \mathcal{P} has no boundary steady states, and hence, the global attractor conjecture holds for this \mathcal{P} ; see the main theorem of Angeli *et al.* [ADS07a]. Biological models in which the non-existence of boundary steady states implies global convergence include the ligand-receptor-antagonist-trap model of Gnacadja *et al.* [GSG⁺07], the enzymatic mechanism of Siegel and MacLean [SM00], and McKeithan’s T-cell signal transduction model [McK95] (the mathematical analysis appears in the work of Sontag [Son01] and in the dissertation of Chavez [Cha03, §7.1]). We remark that this type of argument first appeared in the work of Feinberg [Fei87, §6.1].

The remaining case of the global attractor conjecture, in which steady states exist on the boundary of \mathcal{P} , is still open. The two main results of this chapter concern this case. The first result states that vertices of \mathcal{P} can not be ω -limit points even if they are steady states; see also Theorem 3.7 in the work of Anderson [And08]. The second result states that ω -limit points in the interior of facets of \mathcal{P} have repelling neighborhoods. For two other classes of systems for which the global attractor conjecture holds despite the presence of boundary steady states, see

Proposition 7.2.1 of the work of Chavez [Cha03] and Theorem 4 due to Angeli *et al.* [ADS07a]. The hypotheses of Chavez's result are that the set of boundary steady states in \mathcal{P} is discrete, that each boundary steady state is hyperbolic with respect to \mathcal{P} , and that a third, more technical condition holds.

4.4 Preliminary results

This section collects some general results on trajectories of toric dynamical systems, which are interesting in their own right. Consider a fixed toric dynamical system (1.3) with strictly positive starting point $c(0) = c^0 \in \mathbb{R}_{>0}^s$. Recall that the trajectory $c(t)$ remains in the invariant polyhedron $\mathcal{P} = (c^0 + S) \cap \mathbb{R}_{\geq 0}^s$. For positive ε , the ε -neighborhood in \mathcal{P} of the boundary of \mathcal{P} will be denoted by $V_\varepsilon(\partial P)$ in this section and the next.

We note that the transformed entropy function (2.14) from Chapter 2 can be extended continuously to the boundary of \mathcal{P} , because $c_i \log c_i \rightarrow 0$ as $c_i \rightarrow 0^+$. Equivalent formulations of the following result are well-known. For instance, see [SC94, Son01].

Proposition 4.4.1. *For a toric dynamical system, suppose that the invariant polyhedron \mathcal{P} is bounded and the distance between the boundary of \mathcal{P} and the set $\{c(t) \in \mathcal{P} \mid t > 0\}$ is strictly positive. Then the global attractor conjecture holds for this system.*

Proof. We assume that $c(t)$ does not converge to c^* . Let $\varepsilon > 0$ be such that $c(t) \notin V_\varepsilon(\partial P)$ for all $t > t_0$. The strict Lyapunov function (2.14) ensures that there exists a neighborhood $V_{\varepsilon'}(c^*)$ of the Birch point c^* such that all trajectories that visit $V_{\varepsilon'}(c^*)$ converge to c^* . Then $c(t) \notin V_{\varepsilon'}(c^*)$ for all $t > t_0$. Denote the complement of the two open neighborhoods by $\mathcal{P}_0 := \mathcal{P} \setminus (V_\varepsilon(\partial P) \cup V_{\varepsilon'}(c^*))$. Then the non-positive and continuous function $c \mapsto (\nabla E \cdot \frac{dc}{dt})(c)$ does not vanish on \mathcal{P}_0 by Proposition 2.3.2, so it is bounded above by some $-\delta < 0$ on \mathcal{P}_0 . Therefore, the value of $E(c(t))$ decreases at a rate of at least δ for all $t > t_0$, which implies that E is unbounded on \mathcal{P}_0 . This is a contradiction. \square

Proposition 4.4.1 says that if the trajectory $c(t)$ does not have any ω -limit points on the boundary of \mathcal{P} , then it must converge to the Birch point c^* . Thus, as we noted in the previous section, in order to prove the global attractor conjecture, it would suffice to show that no boundary point of \mathcal{P} is an ω -limit point. We will rule out the vertices and interior points of facets of \mathcal{P} in Section 4.6. The final result of this section states that a non-persistent trajectory approaches the boundary, when the invariant polyhedron is bounded. The results in this section will be applied in the next section to analyze detailed-balancing systems.

Lemma 4.4.2. *Suppose that \mathcal{P} is bounded and that the trajectory $c(t)$ has an ω -limit point on the boundary of \mathcal{P} . Then for any $\varepsilon > 0$ there exists a positive number $t_\varepsilon > 0$ such that $c(t)$ belongs to $V_\varepsilon(\partial P)$ for all $t > t_\varepsilon$. In other words, the trajectory approaches the boundary.*

Proof. Suppose that for some $\varepsilon > 0$ there exists a sequence $t_n \rightarrow \infty$ such that $c(t_n) \notin V_\varepsilon(\partial P)$ for all $n \in \mathbb{Z}_{>0}$. As \mathcal{P} is bounded, the trajectory $c(t)$ has an ω -limit point $p \in \mathcal{P} \setminus V_\varepsilon(\partial P)$. On the other hand, $c(t)$ also has an ω -limit point on the boundary of \mathcal{P} . Consider a ball $B_{2\delta}(p)$ of radius 2δ around p , whose closure lies fully in the relative interior of \mathcal{P} . The trajectory $c(t)$ enters and exits

the neighborhood $B_\delta(p)$ of p infinitely many times, and also enters and exits the neighborhood $\mathcal{P} \setminus B_{2\delta}(p)$ of the boundary infinitely many times. Hence, the trajectory $c(t)$ travels repeatedly between these two sets which are at distance δ from each other. Note that $|dc/dt|$ is bounded above, and $\nabla E \cdot dc/dt$ is bounded away from zero on the annulus $B_{2\delta}(p) \setminus B_\delta(p)$. Then, as in the proof of Proposition 4.4.1, each traversal between the neighborhoods decreases the value of $E(c(t))$ by a positive amount that is bounded away from zero. This contradicts the fact that the function E is bounded on \mathcal{P} . \square

4.5 Results for detailed-balancing systems

In this section, the global attractor conjecture is proved for detailed-balancing systems whose invariant polyhedron is bounded and of dimension two. Recall that detailed-balancing systems, which were introduced in Section 2.6, are those systems whose steady states have the property that the forward reaction rate is equal to the backward reaction rate for any reaction. In particular, the reaction diagram of a detailed-balancing system is reversible, so we may identify the network with its underlying undirected graph, which is denoted by $\tilde{G} = (V, \tilde{E})$. Further, a detailed-balancing steady state is a solution c^* to the equations $\kappa_{ij}c^{y_i} - \kappa_{ji}c^{y_j}$. The following characterization of detailed-balancing systems will be used in the next section. If L is any vector in \mathbb{R}^s and c the unknown concentration vector, then we write

$$L * c := (L_1c_1, L_2c_2, \dots, L_sc_s) .$$

Lemma 4.5.1. *A toric dynamical system is detailed-balancing if and only if all the binomials $\kappa_{ij}c^{y_i} - \kappa_{ji}c^{y_j}$ have the form $\beta_{ij}((L * c)^{y_i} - (L * c)^{y_j})$, for some positive vector $L \in \mathbb{R}_{>0}^s$ and positive constants $\beta_{ij} \in \mathbb{R}_{>0}$. Thus, a detailed-balancing system is a toric dynamical system of the special form*

$$\frac{dc}{dt} = \sum_{\{i,j\} \in \tilde{E}} \beta_{ij}((L * c)^{y_i} - (L * c)^{y_j}) \cdot (y_j - y_i) . \quad (4.2)$$

Proof. The if-direction is easy: if our binomials have the special form $\beta_{ij}((L * c)^{y_i} - (L * c)^{y_j})$ then $c^* = (1/L_1, 1/L_2, \dots, 1/L_s)$ is a positive solution to the equations $\Psi(c) \cdot A_\kappa^{\{i,j\}} = 0$. For the only-if direction, we first define L as the reciprocal of the Birch point c^* in some invariant polyhedron: $L = (1/c_1^*, 1/c_2^*, \dots, 1/c_s^*)$. Therefore it follows that $\kappa_{ij}(c^*)^{y_i} = \kappa_{ji}(c^*)^{y_j}$ for all $(i, j) \in \tilde{E}$, so we may denote this value as $\beta_{ij} := \kappa_{ij}(c^*)^{y_i} = \kappa_{ji}(c^*)^{y_j}$. Then the binomial of interest is

$$\begin{aligned} \kappa_{ij}c^{y_i} - \kappa_{ji}c^{y_j} &= \kappa_{ij} \left(c^{y_i} - \frac{\kappa_{ji}}{\kappa_{ij}} c^{y_j} \right) \\ &= \kappa_{ij} \left(c^{y_i} - (c^*)^{y_i - y_j} c^{y_j} \right) \\ &= \kappa_{ij}(c^*)^{y_i} \left(\frac{c^{y_i}}{(c^*)^{y_i}} - \frac{c^{y_j}}{(c^*)^{y_j}} \right) \\ &= \beta_{ij}((L * c)^{y_i} - (L * c)^{y_j}) , \end{aligned}$$

which completes the proof. \square

We now fix a detailed-balancing system (4.2) with a particular starting point $c(0)$. Then the trajectory $c(t)$ evolves inside the invariant polyhedron $\mathcal{P} = (c(0) + S) \cap \mathbb{R}_{\geq 0}^s$. Consider any *acyclic orientation* $E' \subset \tilde{E}$ of the undirected graph \tilde{G} . This means that E' contains one from each pair of directed edges (i, j) and (j, i) in E , in such a way that the resulting directed subgraph of G has no directed cycles. The acyclic orientation E' specifies a *stratum* \mathfrak{S} in the interior of the invariant polyhedron, $\text{int}(\mathcal{P}) = (c(0) + S) \cap \mathbb{R}_{> 0}^s$, as follows:

$$\mathfrak{S} := \{ c \in \mathcal{P} \mid (L * c)^{y_i} > (L * c)^{y_j} \text{ for all } (i, j) \text{ in } E' \}.$$

The invariant polyhedron \mathcal{P} is partitioned into such strata and their boundaries. We are interested in how the strata meet the boundary of \mathcal{P} .

Lemma 4.5.2. *Consider a detailed-balancing system (4.2), and fix an acyclic orientation E' of the graph \tilde{G} . If the closure of the stratum \mathfrak{S} corresponding to E' intersects the relative interior of a face F_I of the invariant polyhedron \mathcal{P} , then there exists a strictly positive vector $\alpha \in \mathbb{R}_{> 0}^I$ such that $\sum_{k \in I} (y_{jk} - y_{ik}) \cdot \alpha_k \geq 0$ for all directed edges (i, j) in E' .*

Proof. We proceed by contradiction: assume that the inequalities $\sum_{k \in I} (y_{jk} - y_{ik}) \alpha_k \geq 0$ have no strictly positive solution $\alpha \in \mathbb{R}_{> 0}^I$. By Linear Programming Duality (Farkas' Lemma), there is a non-negative linear combination $v = \sum_{(i, j) \in E'} \lambda_{ij} (y_j - y_i)$ such that the following two conditions on v hold: (a) $\text{supp}(v_+) \cap I = \emptyset$, and (b) $\text{supp}(v_-)$ contains some $j_0 \in I$. We shall prove the following two claims, which give the desired contradiction:

Claim One: *If c is a point in the relative interior of F_I , then $(L * c)^{v_+} > (L * c)^{v_-}$.*

Since $(L * c)_i = 0$ if and only if $i \in I$, and $(L * c)_j > 0$ for all $j \notin I$, (a) implies that $(L * c)^{v_+}$ is strictly positive, while (b) implies that $(L * c)^{v_-} = 0$, and we are done.

Claim Two: *If c is a point in the closure of the stratum \mathfrak{S} , then $(L * c)^{v_+} \leq (L * c)^{v_-}$.*

Consider any point $\sigma \in \mathfrak{S}$. By the construction of v , the following equation holds:

$$(L * \sigma)^v = (L * \sigma)^{\sum_{(i, j) \in E'} \lambda_{ij} (y_j - y_i)} = \prod_{(i, j) \in E'} ((L * \sigma)^{y_j - y_i})^{\lambda_{ij}}. \quad (4.3)$$

Recall that $(L * \sigma)^{y_j - y_i} \leq 1$ for each oriented edge $(i, j) \in E'$. Also, each λ_{ij} is non-negative, so $((L * \sigma)^{y_j - y_i})^{\lambda_{ij}} \leq 1$. Using the equation (4.3), this implies that $(L * \sigma)^v \leq 1$, and therefore $(L * \sigma)^{v_+} \leq (L * \sigma)^{v_-}$. By continuity we can replace σ by any point c in the closure of \mathfrak{S} in this last inequality. \square

The vector $\alpha \in \mathbb{R}_{> 0}^I$ in Lemma 4.5.2 will play a special role in the proof of Theorem 4.5.4. In Corollary 4.5.3 below we regard α as a vector in $\mathbb{R}_{\geq 0}^s$ by setting $\alpha_j = 0$ for all $j \in \{1, \dots, \sigma\} \setminus I$.

Corollary 4.5.3. *Let $c(t)$ be a trajectory of a detailed-balancing system (4.2) on the invariant polyhedron \mathcal{P} , and suppose that a point $c(t_0)$ on this trajectory lies both in the closure of a stratum \mathfrak{S} and in the relative interior of a face F_I of \mathcal{P} . Let $\alpha \in \mathbb{R}_{\geq 0}^s$ be the vector obtained as in Lemma 4.5.2. Then, the inner product $\langle \alpha, \frac{dc}{dt}(t_0) \rangle$ is non-negative.*

Proof. Let E' denote the orientation which specifies \mathfrak{S} . The velocity vector $\frac{dc}{dt}(t_0)$ equals

$$\sum_{(i,j) \in E'} \beta_{ij} ((L * c(t_0))^{y_i} - (L * c(t_0))^{y_j}) \cdot (y_j - y_i).$$

Since $c(t_0)$ is in the closure of the stratum \mathfrak{S} , we have $(L * c(t_0))^{y_i} - (L * c(t_0))^{y_j} \geq 0$. We also have $\langle \alpha, y_j - y_i \rangle \geq 0$ because α comes from Lemma 4.5.2. This implies

$$\langle \alpha, \frac{dc}{dt}(t_0) \rangle = \sum_{(i,j) \in E'} \beta_{ij} ((L * c(t_0))^{y_i} - (L * c(t_0))^{y_j}) \cdot \langle \alpha, y_j - y_i \rangle \geq 0.$$

This is the claimed inequality. It will be used in the proof of Theorem 4.5.4. \square

The following result concerns detailed-balancing systems; it will be generalized by Theorem 4.7.2 in a later section.

Theorem 4.5.4. *Consider a detailed-balancing system (4.2) whose stoichiometric subspace $S = \mathbb{R}\{y_j - y_i \mid (i, j) \in \tilde{E}\}$ is two-dimensional, and assume that the invariant polygon $\mathcal{P} = (c^0 + S) \cap \mathbb{R}_{\geq 0}^s$ is bounded. Then the global attractor conjecture holds for this system.*

Proof. By Proposition 4.4.1, we need only rule out the possibility that the trajectory $c(t)$ has an ω -limit point on the boundary of \mathcal{P} . Proposition 4.6.1 gives the existence of open neighborhoods of the vertices such that no trajectory $c(t)$ that starts outside them can visit them. Let V denote the union of these neighborhoods. Suppose now that $c(t)$ has an ω -limit point on ∂P . That limit point lies in the relative interior of some edge F of \mathcal{P} . Let F_ε denote the set of points in \mathcal{P} which have distance at most ε from the edge F .

We claim that there exists $\varepsilon > 0$ and $t_\varepsilon > 0$, such that the trajectory $c(t)$ remains in the subset $F_\varepsilon \setminus V$ for all $t > t_\varepsilon$. This is true because $c(t)$ belongs to the neighborhood $V_\varepsilon(\partial P)$ of the boundary for $t \gg 0$, by Lemma 4.4.2, and hence $c(t)$ belongs to $V_\varepsilon(\partial P) \setminus V$ for $t \gg 0$. But this implies that $c(t)$ belongs to $F_\varepsilon \setminus V$ for $t \gg 0$ because $F_\varepsilon \setminus V$ is a connected component of $V_\varepsilon(\partial P) \setminus V$ for ε sufficiently small. This uses the dimension-two assumption.

Consider the closures of all strata \mathfrak{S} that intersect the relative interior of F . After decreasing ε if necessary, we may assume that the union of these closures contains the set $F_\varepsilon \setminus V$, which contains the trajectory $c(t)$ for $t > t_\varepsilon$. To complete the proof, we will show that the distance from $c(t)$ to the edge F never decreases after $c(t)$ enters $F_\varepsilon \setminus V$.

Any stratum \mathfrak{S} whose closure intersects the relative interior of F contributes a vector $\alpha = \alpha(\mathfrak{S})$ which satisfies the statement of Lemma 4.5.2 for $F = F_I$. The orthogonal projection of $\alpha(\mathfrak{S})$ into the two-dimensional stoichiometric subspace is a positive multiple of the unit inner normal $\alpha_0 \in S$ to F in \mathcal{P} . By Corollary 4.5.3 we have $\langle \alpha(S), \frac{dc}{dt}(t) \rangle \geq 0$ and hence $\langle \alpha_0, \frac{dc}{dt}(t) \rangle \geq 0$ for $t > t_\varepsilon$. Therefore the distance from $c(t)$ to F cannot decrease. This is a contradiction to the assumption that F contains an ω -limit point. \square

4.6 Main results

Our results concern the case when a face F_W of an invariant polyhedron \mathcal{P} defined by a siphon W is a vertex or a facet. In addition, we connect our results to the concept of “dynamically non-emptiable” siphons. Recall that if there exist subsets $W_1 \subsetneq W_2 \subset \mathcal{S}$ for which $F_{W_1} = F_{W_2}$, then we denote the face by F_{W_2} . Under this convention, we saw in (1.9) that the interior of a face F_W is $\text{int}(F_W) = \{x \in P \mid x_i = 0 \text{ if and only if } i \in W\}$.

4.6.1 Result concerning vertices of invariant polyhedra

Theorem 4.6.1. *Consider a toric dynamical system with invariant polyhedron \mathcal{P} . Let r be a vertex of \mathcal{P} and consider any $\varepsilon > 0$. Then, there exists a neighborhood W of r such that any trajectory $c(t)$ with starting point $c(0) = c^0$ satisfying $\text{dist}(c^0, r) > \varepsilon$ does not visit W for any $t > 0$.*

Proof. The following set is the intersection of a closed cone with a sphere of radius one:

$$\mathcal{V} := \left\{ \frac{v}{\|v\|} \mid v \in S \setminus \{0\} \text{ and } r + v \text{ lies in } \mathcal{P} \right\}.$$

Hence \mathcal{V} is compact. We set $I = \{j \in \{1, \dots, \sigma\} : r_j = 0\}$. For each $v \in \mathcal{V}$, the ray $\gamma_v(t) := r + tv$ extends from the vertex $\gamma_v(0) = r$ into the polyhedron \mathcal{P} for small $t > 0$. We consider how the transformed entropy function defined in (2.14) changes along such a ray:

$$\begin{aligned} \frac{d}{dt} E(\gamma_v(t)) &= \sum_{j \in I} v_j (\log(0 + tv_j)) + \sum_{j \notin I} v_j \log(r_j + tv_j) - \sum_{i=1}^s \log(c_i^* v_j) \\ &= (\sum_{j \in I} v_j) \cdot \log(t) + w(t), \end{aligned}$$

where the function $w(t)$ admits a universal upper bound for t close to 0 and $v \in \mathcal{V}$. For each $j \in I$ we have $v_j \geq 0$ because $r_j = 0$ and $r + tv \in \mathcal{P}$ for small $t > 0$. Also, since v points into \mathcal{P} , there exists $j \in I$ with $v_j > 0$. Thus, the function $\sum_{j \in I} v_j$ has a positive minimum over \mathcal{V} . It follows that $\frac{d}{dt} E(\gamma_v(t))$ tends to $-\infty$ for $t \rightarrow 0$. There exists $t_0 < \varepsilon$ such that for all $v \in \mathcal{V}$ the function $t \mapsto E(r + tv)$ decreases for $0 < t \leq t_0$. So, $E(r) > \mu := \max_{v \in \mathcal{V}} E(r + t_0 v)$. On the other hand, E is continuous, so there is a neighborhood W of the vertex r (contained in $\{r + tv \mid t < t_0, v \in \mathcal{V}\}$) such that $E(c) > (E(r) + \mu)/2$ for all $c \in W$. Since E decreases along trajectories, we conclude that no trajectory $c(t)$ that starts at distance $\geq \varepsilon$ from the vertex r can enter W . \square

We note that this proposition provides an alternate proof of Theorem 3.7 due to Anderson [And08]. Namely, his result says that for a toric dynamical system, *if the only possible boundary steady states are vertices of \mathcal{P} , then the Birch point is a global attractor*. Our proposition above proves that vertices are never ω -limit points, so Theorem 3.7 of [And08] follows from the fact that when there are no boundary steady states, then the Birch point is globally asymptotically stable (see [Son01, Theorem 2] or [And08, Corollary 2.10]).

Remark 4.6.2. Chemical reaction networks for which \mathcal{P} is bounded are called *conservative*. For conservative networks, there exists a positive mass assignment m_i for each species i that is conserved

by all reactions; in other words, S^\perp meets the positive orthant [Fei79]. On the other hand, if $0 \in \mathcal{P}$, then the reaction network is not conservative. Thus Proposition 4.6.1 ensures that, for a toric dynamical system, complete depletion of all the concentrations c_1, c_2, \dots, c_s is impossible.

4.6.2 Result concerning facets of invariant polyhedra

In order to state Theorem 4.6.4, we need the following definition.

Definition 4.6.1. Let $Q \subset \mathcal{P}$ be an open set relative to \mathcal{P} , for which $\emptyset \neq Q \cap \partial\mathcal{P} \subset F_W$, for some face F_W of \mathcal{P} . Then the face F_W is *repelling in the neighborhood* $Q \cap \text{int}(\mathcal{P})$ with respect to the dynamics (1.5) if

$$\sum_{i \in W} x_i f_i(x) \geq 0 \quad (4.4)$$

for all $x \in Q \cap \text{int}(\mathcal{P})$, where the $f_i := \left(\frac{d}{dt}c\right)_i$ are the functions given in (1.5).

Remark 4.6.3. Note that F_W is repelling in the neighborhood $Q \cap \text{int}(\mathcal{P})$ with respect to the dynamics (1.5) if and only if $\frac{d}{dt} \text{dist}(c(t), F_W) \geq 0$ whenever $c(t) \in Q \cap \text{int}(\mathcal{P})$. Thus, F_W is repelling in a neighborhood if any trajectory in the neighborhood can not get closer to the face F_W while remaining in the neighborhood.

We note also that the main theorem concerning vertices, Theorem 4.6.1, states that for toric dynamical systems, vertices of invariant polyhedra are repelling. Theorem 4.6.4 states the analogous result for points in the interiors of facets that arise from siphons.

Theorem 4.6.4. Let $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ be a weakly-reversible chemical reaction network with dynamics governed by mass-action kinetics (1.5). Let $W \subset \mathcal{S}$ be such that F_W is a facet of \mathcal{P} , and take z to be in the interior of F_W . Then there exists a $\delta > 0$ for which the facet F_W is repelling in the neighborhood $B_\delta(z) \cap \text{int}(\mathcal{P})$, where $B_\delta(z)$ is the open ball of radius δ centered at the point z .

Proof. First we will assume that there is only one linkage class in the reaction diagram. The proof of the more general case is similar and will be discussed at the end. Now, let c^0, z, W , and F_W be as in the statement of the theorem. The set of zero-coordinates of z is W , and by Theorem 4.2.2, W must be a siphon. Without loss of generality, assume that $W = \{1, \dots, m\}$ for some $m \leq s$.

Letting $\sigma := \dim(S) = \dim(\mathcal{P})$, the facet F_W has dimension $\sigma - 1$, which, by definition, means that $Z_W \cap S$ is an $(\sigma - 1)$ -dimensional subspace of S . Let $\pi : \mathbb{R}^s \rightarrow \mathbb{R}^s$ be the projection onto the first m coordinates. That is, it is given by $\pi(x_1, x_2, \dots, x_m, x_{m+1}, \dots, x_s) := (x_1, x_2, \dots, x_m, 0, \dots, 0)$. As a shorthand we will also write $x|_W$ for $\pi(x)$. Because $Z_W \cap S = \ker(\pi|_S)$ has dimension $(\sigma - 1)$, it follows that the image $\pi(S)$ is one-dimensional. Therefore, we may let $v \in S$ be a vector such that $v|_W$ spans the projection $\pi(S)$. We also let $\{w_2, \dots, w_\sigma\}$ span the subspace $Z_W \cap S$ so that $\{v, w_2, \dots, w_\sigma\}$ is a basis for the subspace S . We note for future reference that by construction we have

$$y'|_W - y|_W \in \text{span}(v|_W), \quad (4.5)$$

for each reaction $y \rightarrow y' \in \mathcal{R}$. Finally, for $x \in \mathbb{R}^s$ we define $x|_{W^c}$ similarly to $x|_W$: the vector $x|_{W^c}$ is the projection of x onto the final $s - m$ coordinates.

We may assume that all coordinates of $v|_W$ are non-zero, for otherwise the concentrations of certain species $j \in W$ would remain unchanged under the action of each reaction (note that we necessarily have $w_{ki} = 0$ for $k \in \{2, \dots, \sigma\}$ and $i \in \{1, \dots, m\}$). In such a case, the concentrations of those species j would remain constant in time, so we could simply remove them from the system by incorporating their concentrations into the rate constants appropriately.

We will show that $v|_W$ has coordinates all of one sign and will use this fact to guarantee the existence of a “minimal complex” (with respect to the elements of W). We will then show that this minimal complex corresponds with a dominating monomial that appears as a positive term in each of the first m components of (1.5).

Suppose, in order to find a contradiction, that $v|_W$ has coordinates of both positive and negative sign; that is, assume that $v_i < 0 < v_j$ for some indices $i, j \leq m$. Let $u := v_j e_i - v_i e_j \in \mathbb{R}_{\geq 0}^s$ (where e_k denotes the k -th canonical basis vector). It follows that $u \in S^\perp$ because (i) $\langle u, v \rangle = 0$ by construction, and (ii) $\langle u, w_k \rangle = 0$ for all $k \in \{2, \dots, \sigma\}$ because these vectors have non-overlapping support. Note also that $\langle u, z \rangle = 0$ because the support of u is a subset of W whereas the support of z is W^c . Let $c^0 \in \mathbb{R}_{> 0}^s \cap \mathcal{P}$; such a point always exists by the definition given in (1.6). As z and c^0 both lie in \mathcal{P} , there exist constants $\alpha_k \in \mathbb{R}$ for $k \in [\sigma]$, such that

$$z = c^0 + \alpha_1 v + \sum_{k=2}^{\sigma} \alpha_k w_k .$$

Combining all of the above, we conclude that

$$0 = \langle u, z \rangle = \langle u, c^0 \rangle + \alpha_1 \langle u, v \rangle + \sum_{k=2}^{\sigma} \alpha_k \langle u, w_k \rangle = \langle u, c^0 \rangle > 0 ,$$

where the final inequality holds because u is non-negative and nonzero and c^0 has strictly positive components. This is a contradiction, so we conclude that $v|_W$ does not have both positive and negative coordinates, and, without loss of generality, we now assume that all coordinates of $v|_W$ are positive.

We recall from (4.5) that $y'|_W - y|_W \in \text{span}(v|_W)$ for each reaction $y \rightarrow y' \in \mathcal{R}$. For concreteness, we let $y'_k|_W - y_k|_W = \gamma_k v|_W$ for some $\gamma_k \in \mathbb{R}$ where $k \in \mathcal{R}$. Combining this with the fact that $v_i > 0$ for each $i \in \{1, \dots, m\}$ shows that each reaction yields either (i) a net gain of all species of W , (ii) a net loss of all species of W , or (iii) no change in any species of W . We also see that there exists a $\tilde{y} \in \mathcal{C}$ such that $\tilde{y}|_W \leq y|_W$ for all $y \in \mathcal{C}$, where we say $x \leq y$ for $x, y \in \mathbb{R}^N$ if $x_i \leq y_i$ for each i . Note that it is the sign of γ_k that determines whether a given reaction accounts for an increase or a decrease in the abundances of the elements of W .

We now find a neighborhood of positive radius δ around z , denoted by $B_\delta(z)$, for which the facet F_W is repelling in the neighborhood $B_\delta(z) \cap \text{int}(\mathcal{P})$. The first condition we impose on δ is that it must be less than the distance between z and any proper face of \mathcal{P} that is not F_W , which can be done because z is in the interior of the facet. Also, this condition ensures that for any point $x \in B_\delta(z) \cap \text{int}(\mathcal{P})$, the coordinates x_i , for $i > m$, are uniformly bounded both above and below. Therefore, there exist constants D_{\min} and D_{\max} such that for all $x \in B_\delta(z) \cap \text{int}(\mathcal{P})$ and all

complexes y , we have the inequalities

$$0 < D_{\min} < x|_{W^c}^{y|_{W^c}} < D_{\max} . \quad (4.6)$$

The monomial $x|_W^{\tilde{y}|_W}$ will dominate all other monomials for $x \in B_\delta(z) \cap \text{int}(\mathcal{P})$ for sufficiently small δ , and this will force trajectories away from the facet. To make this idea precise, let \mathcal{R}_+ denote those reactions that result in a net gain of the species in W and \mathcal{R}_- those that result in a net loss. We now have that for $i \in [m]$ and $x \in B_\delta(z) \cap \text{int}(\mathcal{P})$ and for sufficiently small $\delta > 0$,

$$\begin{aligned} f_i(x) &= v_i \sum_{y_k \rightarrow y'_k \in \mathcal{R}_+} \gamma_k \kappa_k x|_W^{y_k|_W} x|_{W^c}^{y_k|_{W^c}} - v_i \sum_{y_k \rightarrow y'_k \in \mathcal{R}_-} |\gamma_k| \kappa_k x|_W^{y_k|_W} x|_{W^c}^{y_k|_{W^c}} \\ &\geq v_i D_{\min} \sum_{y_k \rightarrow y'_k \in \mathcal{R}_+} \gamma_k \kappa_k x|_W^{y_k|_W} - v_i D_{\max} \sum_{y_k \rightarrow y'_k \in \mathcal{R}_-} |\gamma_k| \kappa_k x|_W^{y_k|_W} . \end{aligned} \quad (4.7)$$

Finally, by weak-reversibility (and possibly after choosing a different \tilde{y} that still satisfies the minimality condition), there is a reaction $\tilde{y} \rightarrow y' \in \mathcal{R}$ with $\tilde{y}_i < y'_i$ for all $i \in \{1, \dots, m\}$ (i.e. the γ_k associated with this reaction is strictly positive). This reaction, which belongs to \mathcal{R}_+ , has a monomial, $x|_W^{\tilde{y}|_W}$, that necessarily dominates all monomials associated with reactions in \mathcal{R}_- (which necessarily have source complexes that contain a *higher* number of each element of W than \tilde{y} does). Combining this fact with the inequality (4.7) shows that $f_i(x) \geq 0$ for $i \in [m]$ and for $x \in B_\delta(z) \cap \text{int}(\mathcal{P})$, and therefore, the facet F_W is repelling in the neighborhood $B_\delta(z) \cap \text{int}(\mathcal{P})$.

In the case of more than one linkage class, each linkage class will have its own minimal complex that will dominate all other monomials associated with that linkage class. Thus the desired result follows. \square

Remark 4.6.5. Note that weak-reversibility was used in the previous proof only to guarantee the existence of the reaction $\tilde{y} \rightarrow y'$, where $\tilde{y}|_W$ is minimal and $\tilde{y}_i < y'_i$ for all $i \in \{1, \dots, m\}$, and was *not* needed to prove the existence of such a complex \tilde{y} . If the network were not weakly-reversible, but such a reaction nevertheless existed, then the same proof would proceed unchanged.

The previous theorem implies that a trajectory with an ω -limit point in the interior of a facet of \mathcal{P} must enter and exit a repelling neighborhood infinitely often. Furthermore, the set of “entering” points of the trajectory with respect to this neighborhood must itself have a subsequence whose distance to the facet approaches zero. This fact will be exploited in the proofs of the following results, which assert the persistence of a class of chemical reaction systems.

Corollary 4.6.6. *Let $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ be a weakly-reversible chemical reaction network with dynamics governed by mass-action kinetics (1.5) such that all trajectories are bounded. Suppose there exist a subset $W \subset \mathcal{S}$, a positive initial condition $c^0 \in \mathbb{R}_{>0}^s$, and a point $z \in \omega(c^0) \cap F_W$ such that F_W is a facet of \mathcal{P} . Then $\omega(c^0) \cap \partial F_W \neq \emptyset$.*

Proof. Suppose not. That is, suppose that $\omega(c^0) \cap F_W \subset \text{int}(F_W)$ holds. Let $\mathcal{Y} := \omega(c^0) \cap F_W$. We claim that \mathcal{Y} is a compact set; indeed, the trajectory $c(t)$ is bounded so \mathcal{Y} is as well, and \mathcal{Y} is the intersection of two closed sets, and therefore is itself closed.

Combining the compactness of $\mathcal{Y} \subset \text{int}(F_W)$ with Theorem 4.6.4, we obtain that there exists an open covering of \mathcal{Y} consisting of a finite number of balls $B_{\delta_i}(z_i)$ of positive radius δ_i , each centered around an element z_i of \mathcal{Y} , such that (i) each δ_i is sufficiently small so that $B_{\delta_i}(z_i) \cap \partial F_W = \emptyset$, and (ii) for $Q := \cup_i B_{\delta_i}(z_i)$, the facet F_W is repelling in $Q \cap \text{int}(\mathcal{P})$. Combining these facts with the existence of $z \in \omega(c^0) \cap \text{int}(F_W) \cap Q$ shows the existence of a point $w \in \omega(c^0) \cap \text{int}(F_W) \cap \partial Q$. However, this is impossible because $w \in \omega(c^0) \cap \text{int}(F_W) = \mathcal{Y}$ necessitates that $w \in \mathcal{Y} \subset \text{int}(Q)$. Thus, the claim is shown. \square

We now present the main result of this subsection.

Theorem 4.6.7. *Let $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ be a weakly-reversible chemical reaction network with dynamics governed by mass-action kinetics (1.5) such that all trajectories are bounded. Suppose that for each siphon W , the corresponding face F_W either is a facet or is empty. Then the system is persistent.*

Proof. This is an immediate consequence of Theorem 4.2.2 and Corollary 4.6.6. \square

4.6.3 Connection to dynamically non-emptiable siphons

In [ADS07a, §9], Angeli *et al.* introduced the notion of a “dynamically non-emptiable” siphon, which we now recall. For a given chemical reaction network and a subset $W \subset [s]$ of the species, we define the following closed cones in the positive orthant:

$$C(W) := \left\{ \alpha \in \mathbb{R}_{\geq 0}^R \mid w = \sum_{k=1}^R \alpha_k (y'_k - y_k) \text{ satisfies } w|_W \leq 0 \right\} \text{ and}$$

$$\mathcal{F}_\epsilon(W) := \left\{ \alpha \in \mathbb{R}_{\geq 0}^R \mid \alpha_j \leq \epsilon \alpha_i, \forall i, j \in [R] \text{ such that } y_i|_W \preceq y_j|_W \right\},$$

where $\epsilon > 0$. We say that a siphon W is *dynamically non-emptiable* if $C(W) \cap \mathcal{F}_\epsilon(W) = \{\bar{0}\}$ for some $\epsilon > 0$. (Recall that R denotes the number of reactions.) Here the notation $z \leq z'$ means that all coordinates satisfy the inequality $z_i \leq z'_i$, and $z \preceq z'$ means that furthermore at least one inequality is strict. Intuitively, this condition guarantees that all the concentrations of species in a siphon can not simultaneously decrease while preserving the necessary monomial dominance. Angeli *et al.* proved that if every siphon is dynamically non-emptiable and if another condition holds, then the system is persistent; see [ADS07a, Theorem 4] for details.

Note that the family of closed cones $\mathcal{F}_\epsilon(W)$ is order-preserving: if $0 < \epsilon(1) < \epsilon(2)$, then $\mathcal{F}_{\epsilon(1)}(W) \subset \mathcal{F}_{\epsilon(2)}(W)$. Hence, the intersection of this family, which we denote by $\mathcal{F}_0(W)$, is also a closed cone:

$$\begin{aligned} \mathcal{F}_0(W) &:= \bigcap_{\epsilon > 0} \mathcal{F}_\epsilon(W) \\ &= \left\{ \alpha \in \mathbb{R}_{\geq 0}^R \mid \alpha_j = 0 \text{ if there exists } i \in [R] \text{ such that } y_i|_W \preceq y_j|_W \right\}. \end{aligned}$$

Thus, $\mathcal{F}_0(W)$ is the suborthant of $\mathbb{R}_{\geq 0}^R$ for which the coordinates corresponding to reactions that have dominating sources are zero. We have the following lemma.

Lemma 4.6.8. 1. For a chemical reaction network, a siphon W is dynamically non-emptiable if and only if

$$C(W) \cap \mathcal{F}_0(W) = \{\bar{0}\} .$$

2. For two siphons that satisfy $W \subset W'$, if W is dynamically non-emptiable, then W' is dynamically non-emptiable.

Proof. If a siphon W is dynamically non-emptiable, then the desired equality holds due to the order-preserving property of the cones $\mathcal{F}_\epsilon(W)$. Conversely, if $C(W) \cap \mathcal{F}_\epsilon(W) \not\supseteq \{\bar{0}\}$ for all $\epsilon > 0$, then let $x_{1/n} \in C(W) \cap \mathcal{F}_{\frac{1}{n}}(W)$ be a point in the intersection that has norm one: $|x_{1/n}| = 1$. As these points lie on the unit sphere, there exists a convergent subsequence with limit which we now denote by x_0 . Clearly, this point x_0 is a non-zero point in the intersection $C(W) \cap \mathcal{F}_0(W)$. Finally, part 2 of this lemma follows from the fact that the containments $C(W') \subset C(W)$ and $\mathcal{F}_0(W') \subset \mathcal{F}_0(W)$ hold whenever $W \subset W'$. \square

Note that the intersection $C(W) \cap \mathcal{F}_0(W)$ can be computed using polyhedral software such as `polymake` [JG00]. We next prove that equation (4.7) and a slight variant of the surrounding argument can be used to show that any siphon W associated with a facet of a weakly-reversible system is dynamically non-emptiable. We therefore have provided a large set of examples of dynamically non-emptiable siphons.

Corollary 4.6.9. Let $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ be a weakly-reversible chemical reaction network with dynamics governed by mass-action kinetics (1.5). If a siphon W defines a facet for some invariant polyhedron \mathcal{P} , then W is dynamically non-emptiable.

Proof. As in the proof of Theorem 4.6.4, we may assume that there is one linkage class. The case of more than one linkage class is similar. Let W be a siphon for which F_W is a facet; we may assume that $W = \{1, 2, \dots, m\}$. Let $v \in \mathbb{R}_{\geq 0}^s$, which satisfies $v_i > 0$ if $i \in [m]$, be as in the proof of Theorem 4.6.4. That is, $y'|_W - y|_W \in \text{span}(v|_W)$ for each reaction $y \rightarrow y' \in \mathcal{R}$, and $y'_k|_W - y_k|_W = \gamma_k v|_W$ for some $\gamma_k \in \mathbb{R}$ where $k \in [R]$ indexes a reaction. Now consider \mathcal{R}_+ and $\mathcal{R}_- \subset \mathcal{R}$ defined similarly as in the proof of Theorem 4.6.4. Hence, $y_k|_W \geq y'_k|_W$ for all reactions $y_k \rightarrow y'_k$ for $k \in \mathcal{R}_-$, so $\alpha \in \mathcal{F}_0(W)$ satisfies $\alpha_k = 0$ for $k \in \mathcal{R}_-$. Thus, any vector $\alpha \in C(W) \cap \mathcal{F}_0(W)$, the vector w as in the definition of $C(W)$ satisfies

$$\begin{aligned} 0 \geq w|_W &= v|_W \sum_{y_k \rightarrow y'_k \in \mathcal{R}_+} \alpha_k \gamma_k - v|_W \sum_{y_k \rightarrow y'_k \in \mathcal{R}_-} \alpha_k |\gamma_k| \\ &= v|_W \sum_{y_k \rightarrow y'_k \in \mathcal{R}_+} \alpha_k \gamma_k - 0 , \end{aligned}$$

which implies that $\alpha_k = 0$ for $k \in \mathcal{R}_+$ as well; hence $\alpha = \bar{0}$, and the result is shown. \square

We remark that a siphon that defines a facet of some invariant polyhedron \mathcal{P} may in fact define a lower-dimensional face of another invariant polyhedron \mathcal{P}' . Hence, the statement of the previous corollary specifies only the existence of an invariant polyhedron of the first type. As

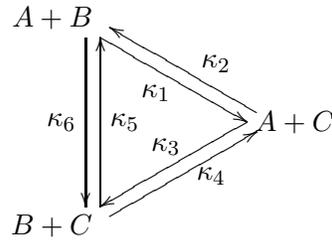
an example, consider any strongly connected network comprised of the three complexes $2A + B$, $A + D$, and $A + C$. The set $\{A\}$ is a siphon. Each invariant polyhedron \mathcal{P} is defined by two positive constants T_1 and T_2 :

$$\mathcal{P} = \{(x_a, x_b, x_c, x_d) \mid x_a + x_c + x + d = T_1 \text{ and } x_b + x_c + x + d = T_2\} .$$

If $T_1 = 1$ and $T_2 = 2$, then the invariant polyhedron is a triangle with vertices $(0, 1, 1, 0)$, $(0, 1, 0, 1)$, and $(1, 2, 0, 0)$, and the face defined by $\{A\}$ is a facet (an edge). For the invariant polyhedron defined by $T_1 = 2$ and $T_2 = 1$, the siphon $\{A\}$ defines the empty face.

We end this section by showing that the analogous result to Corollary 4.6.9 for vertices does not hold. In fact, the following examples demonstrate that some vertex-defining siphons are dynamically non-emptiable, while others are not.

Example 4.6.10. Consider the following reaction network:



The invariant polyhedra are triangles $\mathcal{P} = \{(a, b, c) \in \mathbb{R}_{\geq 0}^3 \mid a + b + c = T\}$ for positive total concentrations $T > 0$. The minimal siphons $\{A, B\}$, $\{A, B\}$, and $\{A, B\}$ each define a vertex of \mathcal{P} , and we claim that they are in fact dynamically non-emptiable. It is enough to consider the siphon $W = \{A, B\}$. By Lemma 4.6.8, the cones of interest are

$$C(W) = \left\{ \alpha \in \mathbb{R}_{\geq 0}^6 \mid (\alpha_1 - \alpha_2) \begin{pmatrix} 0 \\ 1 \end{pmatrix} + (\alpha_3 - \alpha_4) \begin{pmatrix} -1 \\ 1 \end{pmatrix} + (\alpha_5 - \alpha_6) \begin{pmatrix} 1 \\ 0 \end{pmatrix} \leq 0 \right\}$$

$$\mathcal{F}_0(W) = \{ \alpha \in \mathbb{R}_{\geq 0}^6 \mid \alpha_1 = \alpha_6 = 0 \} .$$

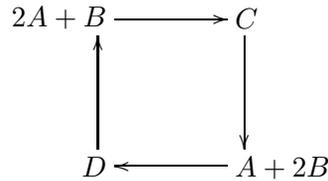
Hence the intersection of these two cones is

$$C(W) \cap \mathcal{F}_0(W) = \{ \alpha \in \mathbb{R}_{\geq 0}^6 \mid \alpha_1 = \alpha_6 = 0, \quad -(\alpha_3 - \alpha_4) + \alpha_5 \leq 0, \quad \alpha_2 + (\alpha_3 - \alpha_4) \leq 0 \}$$

$$= \{ \bar{0} \} .$$

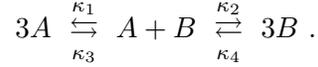
Hence the siphon $\{A, B\}$ is dynamically non-emptiable. \square

Other networks for which the vertex-defining siphons are dynamically non-emptiable include the receptor-ligand-dimer network (analyzed in Examples 1.4.3 and 3.1.1), the network in Example 4.3.1 due to Angeli [Ang08], and the network depicted below, which is analyzed by Angeli *et al.* in [ADS07a, §10]:



We now present an example of a network that has a vertex-defining siphon that is not dynamically non-emptiable.

Example 4.6.11. Consider the following network:



The unique invariant polyhedron is the entire positive orthant $\mathcal{P} = \mathbb{R}_{\geq 0}^2$, and the unique minimal siphon $W = \{A, B\}$ defines the origin, which is the unique vertex of \mathcal{P} . We compute the cones of Lemma 4.6.8 to be

$$\begin{aligned} C(W) &= \left\{ \alpha \in \mathbb{R}_{\geq 0}^4 \mid (\alpha_1 - \alpha_3) \begin{pmatrix} 2 \\ -1 \end{pmatrix} + (\alpha_2 - \alpha_4) \begin{pmatrix} -1 \\ 2 \end{pmatrix} \leq 0 \right\} \\ \mathcal{F}_0(W) &= \mathbb{R}_{\geq 0}^4 . \end{aligned}$$

Their intersection is

$$C(W) \cap \mathcal{F}_0(W) = \left\{ \alpha \in \mathbb{R}_{\geq 0}^4 \mid \frac{1}{2}(\alpha_3 - \alpha_1) \leq (\alpha_4 - \alpha_2) \leq 2(\alpha_3 - \alpha_1) \right\} ,$$

and one non-zero vector in the intersection is $\alpha = (1, 1, 1, 1)$. Hence the siphon W is not dynamically non-emptiable. \square

4.7 Applications to the global attractor conjecture

In this section, we use the results of the previous section to resolve some special cases of the global attractor conjecture. In particular, the main result of this section, Theorem 4.7.1, establishes that the conjecture holds if all boundary steady states are confined to facets and vertices of an invariant polyhedron. It is our main contribution to the global attractor conjecture.

Theorem 4.7.1. *The global attractor conjecture holds for any toric dynamical system (and in particular, any detailed-balancing or weakly-reversible zero deficiency chemical reaction system) whose boundary steady states are confined to facet-interior points or vertices of the invariant polyhedra. Equivalently, if a face F_W is a facet, a vertex, or an empty face whenever W is a siphon, then the global attractor conjecture holds.*

Proof. The equivalence of the two statements in the theorem follows from the fact that a face F_W contains a steady state in its interior if and only if W is a siphon; see Lemma 4.7.3 for further clarification. As noted in the previous section, persistence is a necessary and sufficient condition for the global attractor conjecture to hold. Further, by Proposition 4.6.1, vertices may not be ω -limit points. The remainder of the proof is similar to that of Theorem 4.6.7 and is omitted. \square

We remark again that Proposition 4.6.1 and Theorem 4.7.1 provide repelling neighborhoods for ω -limit points of toric dynamical systems that reside within the interiors of facets or of vertices of \mathcal{P} . The following corollary resolves the global attractor conjecture for systems of dimension two; note that the one-dimensional case is straightforward.

Corollary 4.7.2 (The global attractor conjecture for two-dimensional \mathcal{P}). *The global attractor conjecture holds for all toric dynamical systems (and in particular, detailed-balancing or weakly-reversible zero deficiency chemical reaction networks) whose invariant polyhedra are two-dimensional.*

Proof. This follows immediately from Theorem 4.7.1 as each face of a two-dimensional polyhedron (that is, a polygon) must be either a facet or a vertex. \square

We end this section with a lemma which classifies faces of \mathcal{P} that contain steady states. This is related to the following definition.

Definition 4.7.1. Suppose that $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is a weakly-reversible chemical reaction network, endowed with mass-action kinetics, and $W \subset \mathcal{S}$ is a siphon. Then, the W -reduced system is the chemical reaction network, endowed with the same rate constants as the original system, composed of only those linkage classes in which all complexes are comprised of species outside of the set W .

As noted in comments following Definition 4.2.1, for a weakly-reversible system and any siphon W , either each complex in a given linkage class contains an element of W or each complex in that linkage class does not contain an element of W . Therefore, W -reduced systems are themselves weakly-reversible. Furthermore, it is easy to check that for a toric dynamical system, any W -reduced system is itself a toric dynamical system. Indeed, boundary steady states of such systems are also complex-balancing; see Corollary 3.7 of [SM00]. We now repeat Lemma 3.2.4.

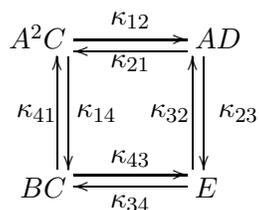
Lemma 4.7.3 (Classification of faces containing steady states). *Consider a toric dynamical system. A face $F_{W'}$ of a invariant polyhedron \mathcal{P} contains a steady state in its interior if and only if W' is a siphon.*

In the next section, we provide examples that illustrate our results, as well as a three-dimensional example for which our results do not apply.

4.8 Examples

As discussed earlier, the global attractor conjecture previously has been shown to hold if the invariant polyhedra \mathcal{P} have no boundary steady states. So, the examples in this section feature boundary steady states.

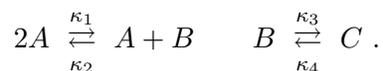
Example 4.8.1. We return to the receptor-ligand dimer model in Example 1.4.3:



As we saw in Example 3.1.1, there are three minimal siphons, $\{A, B, E\}$, $\{A, C, E\}$, and $\{C, D, E\}$. From Figure 3.1, we see that the corresponding non-empty faces are vertices of the invariant polyhedra; namely, each polyhedron contains a unique vertex steady state. Therefore, by Theorem 4.6.1, the global attractor conjecture holds for this system. \square

The next example contains non-vertex boundary steady states.

Example 4.8.2. Consider the network given by the following reactions:



The corresponding invariant polyhedra are two-dimensional simplices (triangles) of the form

$$\mathcal{P} = \left\{ (x_a, x_b, x_c) \in \mathbb{R}_{\geq 0}^3 \mid x_a + x_b + x_c = T \right\} , \quad (4.8)$$

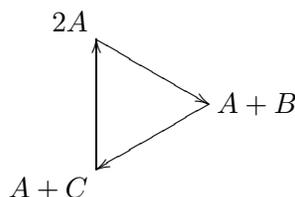
where $T > 0$. It is straightforward to check that \mathcal{P} has a unique boundary steady state given by

$$z = \left(0, \frac{\kappa_4}{\kappa_3 + \kappa_4} T, \frac{\kappa_3}{\kappa_3 + \kappa_4} T \right) ,$$

and that this point lies in the interior of the facet $F_{\{A\}}$. (Note that this boundary steady state is the Birch point of the reversible zero deficiency subnetwork $B \rightleftharpoons C$.) Therefore, both Theorem 4.7.1 and Corollary 4.7.2 allow us to conclude that in spite of the existence of the boundary steady state z , the Birch point in the interior of \mathcal{P} is globally asymptotically stable. \square

We remark that Theorem 4 of Angeli *et al.* [ADS07a] also applies to the previous example. However, for the following example, no previously known results apply.

Example 4.8.3. Consider the reaction network depicted here:



The invariant polyhedra are the same triangles as in the previous example. For each \mathcal{P} , the set of boundary steady states is the entire face $F_{\{A\}}$ (one of the three edges of \mathcal{P}), which includes the two vertices $F_{\{A,B\}}$ and $F_{\{A,C\}}$. Hence the results of [And08, Cha03] do not apply. This is a weakly-reversible zero deficiency network. Both Theorem 4.7.1 and Corollary 4.7.2 imply that the global attractor conjecture holds for all choices of rate constants and for all invariant polyhedra \mathcal{P} defined by this network, despite the presence of boundary steady states. \square

In the next example, the invariant polyhedra are three-dimensional.

Example 4.8.4. The following zero deficiency network is obtained from Example 4.8.2 by adding a reversible reaction:



For this network, the invariant polyhedra are three-dimensional simplices (tetrahedra)

$$\mathcal{P} = \left\{ (x_a, x_b, x_c, x_d) \in \mathbb{R}_{\geq 0}^4 \mid x_a + x_b + x_c + x_d = T \right\} ,$$

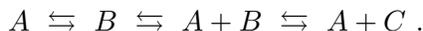
for positive total concentration $T > 0$. The unique boundary steady state in \mathcal{P} is the Birch point of the zero deficiency subnetwork $B \rightleftharpoons C \rightleftharpoons D$, and it lies in the facet $F_{\{A\}}$. In other words, the point is $z = (0, x_b, x_c, x_d)$ where (x_b, x_c, x_d) is the Birch point for the system defined by the subnetwork



We see that z lies in the interior of the facet $F_{\{A\}}$, so by Theorem 4.7.1 the global attractor conjecture holds for all \mathcal{P} and all choices of rate constants for this network. \square

As in the previous example, the invariant polyhedra in our final example are three-dimensional. However neither previously known results [And08, Cha03] nor those in this chapter can resolve the question of global asymptotic stability.

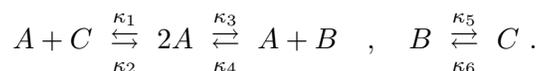
Example 4.8.5. The following zero deficiency network consists of three reversible reactions:



As there are no conservation relations, the unique positive invariant polyhedron is the entire non-negative orthant: $\mathcal{P} = \mathbb{R}_{\geq 0}^3$. The set of boundary steady states is the one-dimensional face (ray) $F_{\{A,B\}}$, which includes the origin $F_{\{A,B,C\}}$. Therefore non-vertex, non-facet boundary steady states exist, so the results in this chapter do not apply. \square

In our final example, the results of Section 4.6.2 apply but those of Section 4.7 do not.

Example 4.8.6. The following reversible network is obtained from Example 4.8.2 by adding another reversible reaction:



The positive stoichiometric compatibility classes are again the two-dimensional triangles given by (4.8). One can check that the network has a deficiency of one, so there exist rate constants for which the system is not complex-balancing (for example, $\kappa_1 = \kappa_3$, $\kappa_5 = \kappa_6$, and $\kappa_2 \neq \kappa_4$). Thus the results of Section 4.7 do not apply. It is also easy to verify that $\{A\}$ and $\{A, B, C\}$ are the only siphons and that $F_{\{A\}}$ is a facet and $F_{\{A,B,C\}}$ is empty. Therefore, Theorem 4.6.7 applies and we conclude that, independent of the choice of rate constants, the system is persistent. \square

Chapter 5

The smallest multistationary mass-preserving chemical reaction network

This chapter is based on the paper “The smallest multistationary mass-preserving chemical reaction network” [Shi08]. Also, we include a proof of Proposition 5.2.1, and provide additional references for related work in Section 5.1 and for real root counting and classification in Section 5.2.

5.1 Introduction to multistationarity

Bistable biochemical models often are presented as the possible underpinnings of chemical switches [CA00, LK99], which play an important role during development [MSG⁺09] and other biological activities. As we have seen in this dissertation, systematic study of mass-action kinetics models—which *a priori* may or may not admit multiple positive steady states—constitutes chemical reaction network theory (CRNT). Recalling the results in Chapter 2, certain classes of networks, including toric dynamical systems, do not exhibit multistationarity. Such results go back to the work of Feinberg, Horn, Jackson, and Vol’pert [Fei79, HJ72, VK85]. The thesis of Lipson contains an overview of recent results concerning multistationarity in chemical reaction networks [Lip07], and includes a discussion of the work of Craciun and Feinberg which gives graphical conditions that rule out multistationarity [CF05, CF06, CF10].

On the other hand, there are conditions that are sufficient for establishing whether a network supports multiple steady states. The CRNT Toolbox, which was developed by Feinberg and improved by Ellison, implements the Deficiency One and Advanced Deficiency Algorithms [Ell98, Fei95]; this software is available online [EF]. For a large class of systems, the CRNT Toolbox either provides a witness for multistationarity or concludes that it is impossible. For another implementation, see the software ERNEST for Matlab [SA09]. For systems for which the CRNT Toolbox is inconclusive, we refer the reader to the approach of Conradi *et al.* [CFRS07].

Another article of Conradi *et al.* includes an algebraic approach that determines the full set of parameters for which a system is multistationary; a necessary and sufficient condition for

multistationarity is the existence of a non-trivial sign vector in the intersection of two subsets of Euclidean space [CFR08]. This method is then applied to one level of a MAPK cascade model. For details, see the recent dissertation of Conradi [Con08]. One aim of this chapter is to perform a similar analysis for a small network. Related work includes the analysis of multistationarity of multisite phosphorylation systems by Wang and Sontag in [WS08] and by Gunawardena, Manrai, and Thomson in [MG08, TG09a, TG09b]. In particular, Wang and Sontag reduce the problem of computing the number of steady states to that of counting the number of positive roots (that lie in certain intervals) of a parametrized family of univariate polynomials. From this formulation, they prove bounds on the number of steady states and characterize certain regions of parameter space as giving rise to systems with one steady state and other regions as having many steady states. Thomson and Gunawardena prove that a similar reduction to a system of two polynomials in two unknowns holds in general for post-translational modification systems [TG09a, TG09b]. Similarly, Gatermann and Xia fully characterize the parameter regions that give rise to three positive steady states for three chemical reaction systems [GX03].

To model biological processes, one typically reverse-engineers a system of non-linear differential equations that exhibits specific dynamical behavior, such as bistability or oscillations, observed experimentally [BQ08, §5.2]. For example, Segel proposes a small immune network consisting only two cell types, which has three stable steady states, corresponding to “normal,” “vaccinated,” and “diseased” states [Seg98]. Similarly, the Brusselator is a mass-action kinetics network with a stable limit cycle [AH, Fei80].

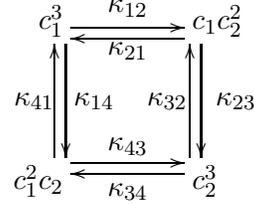
This chapter focuses on the smallest chemical reaction networks that admit multiple positive steady states. Therefore, the aim is similar to work of Wilhelm and Heinrich which identified and analyzed the smallest chemical reaction network that has a Hopf bifurcation [WH95, WH96]. Moreover, a recent article of Wilhelm answers the same question as this chapter when the condition of reversibility is relaxed; Table 1 which appears there gives the smallest examples of chemical systems with oscillations, limit cycles, and so on [Wil09]. For a further discussion on the smallest networks capable of admitting periodic solutions, see the textbook of Érdi and Tóth [ET89, §4.5.3]. In Section 5.2, a special network called the *Square* is shown to be a smallest reversible multistationary chemical reaction network. Sections 5.3 and 5.4 determine precisely which parameters of the Square give rise to multiple steady states. Finally, Section 5.5 discusses the possible connection of the Square to known biochemical networks.

5.2 The smallest multistationary network

Horn initiated the investigation of small chemical reaction networks by enumerating networks comprised of “short complexes,” those whose corresponding monomials c^y have degree at most two [Hor73a, Hor73b]. Networks that consist of at most three short complexes do not permit multiple steady states.

The present section establishes that the *Square* of Example 1.3.2 is a smallest reversible multistationary chemical reaction network. The Square appeared in non-reversible form as net-

work 7-3 in [HJ72] and network 4.2 in [Fei80]. We depict the network again here:



For completeness, here are the matrices whose product defines the dynamical system (1.3):

$$\begin{aligned}
 \Psi(c) &= (c_1^3, c_1 c_2^2, c_2^3, c_1^2 c_2), \\
 A_\kappa &= \begin{pmatrix} -\kappa_{12} - \kappa_{14} & \kappa_{12} & 0 & \kappa_{14} \\ \kappa_{21} & -\kappa_{21} - \kappa_{23} & \kappa_{23} & 0 \\ 0 & \kappa_{32} & -\kappa_{32} - \kappa_{34} & \kappa_{34} \\ \kappa_{41} & 0 & \kappa_{43} & -\kappa_{41} - \kappa_{43} \end{pmatrix}, \quad \text{and} \\
 Y &= \begin{pmatrix} 3 & 0 \\ 1 & 2 \\ 0 & 3 \\ 2 & 1 \end{pmatrix}. \tag{5.1}
 \end{aligned}$$

The differential equations (1.3) are given by the composition

$$\frac{dc}{dt} = \Psi(c) \cdot A_\kappa \cdot Y.$$

Recall that a *steady state* is a non-negative concentration vector $c \in \mathbb{R}_{\geq 0}^s$ at which the equations (1.3) vanish. In this chapter, all steady states refer to positive steady states $c \in \mathbb{R}_{> 0}^s$, unless they are specified as *boundary steady states*, i.e., those steady states $c \in (\mathbb{R}_{\geq 0}^s \setminus \mathbb{R}_{> 0}^s)$. We are interested in counting the number of steady states within a single invariant polyhedron \mathcal{P} ; Example 5.2.2 in the next section provides a choice of positive rate constants κ_{ij} that give rise to three steady states. Sections 5.3 and 5.4 determine precisely which parameters give rise to two steady states and which yield three. Moreover, we compute this semi-algebraic parametrization for all networks on the same four vertices as the Square, in other words, networks whose complexes are c_1^3 , $c_1 c_2^2$, c_2^3 , and $c_1^2 c_2$. The parametrization is captured in Table 5.1 and can be computed “by hand,” but larger systems may require techniques of computational real algebraic geometry [BPR06]. For example, our problem of classifying parameters according to the number of steady states is labeled as Problem P2 by Wang and Xia in [WX05], where it is addressed with computer algebra methods. We also refer the reader to recent work of Lazard and Rouillier [LR07], Liang and Jeffrey [LJ09], and Yang [Yan99] for an overview of recent advances in real root counting and classification. Such algebraic techniques are harnessed by Gatermann and Xia to identify the locus

of parameters that give rise to three steady states for each of three chemical reaction networks [GX03].

Following equation (2.6) of Chapter 2, the Matrix-Tree Theorem defines the following four polynomials in the rate constants of the Square:

$$\begin{aligned} K_1 &= \kappa_{23}\kappa_{34}\kappa_{41} + \kappa_{21}\kappa_{34}\kappa_{41} + \kappa_{21}\kappa_{32}\kappa_{41} + \kappa_{21}\kappa_{32}\kappa_{43} , \\ K_2 &= \kappa_{14}\kappa_{32}\kappa_{43} + \kappa_{12}\kappa_{34}\kappa_{41} + \kappa_{12}\kappa_{32}\kappa_{41} + \kappa_{12}\kappa_{32}\kappa_{43} , \\ K_3 &= \kappa_{14}\kappa_{23}\kappa_{43} + \kappa_{14}\kappa_{21}\kappa_{43} + \kappa_{12}\kappa_{23}\kappa_{41} + \kappa_{12}\kappa_{23}\kappa_{43} , \\ K_4 &= \kappa_{14}\kappa_{23}\kappa_{34} + \kappa_{14}\kappa_{21}\kappa_{34} + \kappa_{14}\kappa_{21}\kappa_{32} + \kappa_{12}\kappa_{23}\kappa_{34} . \end{aligned}$$

Recall that Theorem 2.2.7 of Chapter 2 provides an ideal M_G that is toric in these K_i coordinates, and the variety of M_G is the moduli space of toric dynamical systems on the Square. In this case, the ideal M_G is the *twisted cubic curve* in the K_i coordinates, generated by the 2×2 -minors of the following matrix:

$$\begin{pmatrix} K_1 & K_2 & K_4 \\ K_4 & K_3 & K_2 \end{pmatrix} . \quad (5.2)$$

Moreover, Theorem 2.2.7 states that for a given choice of positive rate constants κ_{ij} , the equations (1.3) define a toric dynamical system if and only if the minors of the matrix (5.2) vanish. In general the codimension of M_G is the *deficiency* of a network; see Theorem 2.2.9 of Chapter 2. Here the deficiency is two. Recall that a *toric dynamical system* is a dynamical system (1.3) for which the algebraic equations $\Psi(c) \cdot A_\kappa = 0$ admit a strictly positive solution $c^* \in \mathbb{R}_{>0}^s$, and in this case there is a unique steady state in the interior of each invariant polyhedron \mathcal{P} , so multistationarity is precluded.

It is no coincidence that the original monomials of the Square, namely c_1^3 , $c_1c_2^2$, c_2^3 , and $c_1^2c_2$, parametrize the twisted cubic curve. In fact, the following general result follows from Theorem 2.2.9 in Chapter 2. The result concerns *mass-preserving* networks, those in which all monomials c^{y_i} have the same total degree. These networks are a special type of *conservative* networks, as defined in Remark 4.6.2.

Proposition 5.2.1. *Assume that a chemical reaction network G is strongly connected and all of its monomials c^{y_i} have the same total degree. Then the toric variety parametrized by the monomial map $\Psi(c)$ coincides with the variety of M_G .*

Proof. By Theorem 2.2.9 in Chapter 2, M_G is generated by $K^u - K^v$ where $u, v \in \mathbb{N}^n$ satisfy $\text{Cay}_G(Y) \cdot (u - v) = 0$, where $\text{Cay}_G(Y)$ in this case is the $((s+1) \times n)$ -matrix obtained by adding an all-one row at the bottom of the matrix Y^T . The columns of Y^T are the exponent vectors y_i , so the sum of the first s rows of $\text{Cay}_G(Y)$ is the vector (d, d, \dots, d) of length n , where d is the shared total degree of the monomials c^{y_i} . So the last row of $\text{Cay}_G(Y)$ is in the span of the other rows; this implies that

$$\ker \text{Cay}_G(Y) = \ker Y^T .$$

Therefore it remains only to show that the ideal of relations that are satisfied by vectors $\Psi(c) = (c^{y_1}, c^{y_2}, \dots, c^{y_n})$ is generated by $K^u - K^v$ where $(u - v) \in \ker Y^T$. This is an easy property of

monomial maps. If $(u - v) \in \ker Y^T$, then

$$\begin{aligned}\Psi(c)^u &= c^{Y^T u} \\ &= c^{Y^T(u-v)} c^{Y^T v} \\ &= 1 \cdot c^{Y^T v} \\ &= \Psi(c)^v .\end{aligned}$$

Finally, any polynomial relation over the complex numbers easily can be rewritten as a \mathbb{C} -linear combination of binomials $K^u - K^v$ with $(u - v) \in \ker Y^T$. Therefore, there is no loss when considering real versus complex varieties. In other words, the equality $\overline{\Psi(c)} = V(M_G)$ holds when taken over \mathbb{C} (see the Closure Theorem of [CLO07, §3.2] and the discussion in [PS05, §3.2]), but the fact that M_G is generated by polynomials over \mathbb{R} allows us to restrict our attention to the real variety. \square

For the Square, each one-dimensional invariant polyhedron \mathcal{P} is defined by some positive concentration total $T = c_1 + c_2$. The positive steady states in \mathcal{P} correspond precisely to the positive roots of the following cubic polynomial:

$$p_S(x) = (-2\kappa_{12} - \kappa_{14})x^3 + (\kappa_{41} - 2\kappa_{43})x^2 + (2\kappa_{21} - \kappa_{23})x + (\kappa_{32} + 2\kappa_{34}) ;$$

this polynomial arises by substituting $x := c_1/c_2$ into the equation $dc_1/dt = -dc_2/dt$. From this point of view, we reach some immediate conclusions. First, the *algebraic degree* of this system is three, which bounds the number of steady states. Second, the number of steady states and their stability depend only on the rate parameters κ_{ij} , and not on the invariant polyhedron \mathcal{P} or equivalently the choice of total concentration T . Also, by noting that $p_S(x)$ is positive at $x = 0$ and is negative for large x , we see that the Square admits at least one positive steady state for any choice of rate constants. Recall that the discriminant of a univariate polynomial f is a polynomial that vanishes precisely when f has a multiple root over the complex numbers [Stu02]. The software Maple computes the discriminant of p_S to be the following polynomial:

$$\begin{aligned}& -108\kappa_{12}^2\kappa_{32}^2 - 432\kappa_{12}^2\kappa_{32}\kappa_{34} - 432\kappa_{12}^2\kappa_{34}^2 - 108\kappa_{12}\kappa_{14}\kappa_{32}^2 \\ & - 432\kappa_{12}\kappa_{14}\kappa_{32}\kappa_{34} - 432\kappa_{12}\kappa_{14}\kappa_{34}^2 + 64\kappa_{12}\kappa_{21}^3 - 96\kappa_{12}\kappa_{21}^2\kappa_{23} + 48\kappa_{12}\kappa_{21}\kappa_{23}^2 \\ & - 72\kappa_{12}\kappa_{21}\kappa_{32}\kappa_{41} + 144\kappa_{12}\kappa_{21}\kappa_{32}\kappa_{43} - 144\kappa_{12}\kappa_{21}\kappa_{34}\kappa_{41} + 288\kappa_{12}\kappa_{21}\kappa_{34}\kappa_{43} \\ & - 8\kappa_{12}\kappa_{23}^3 + 36\kappa_{12}\kappa_{23}\kappa_{32}\kappa_{41} - 72\kappa_{12}\kappa_{23}\kappa_{32}\kappa_{43} + 72\kappa_{12}\kappa_{23}\kappa_{34}\kappa_{41} \\ & - 144\kappa_{12}\kappa_{23}\kappa_{34}\kappa_{43} - 27\kappa_{14}^2\kappa_{32}^2 - 108\kappa_{14}^2\kappa_{32}\kappa_{34} - 108\kappa_{14}^2\kappa_{34}^2 + 32\kappa_{14}\kappa_{21}^3 \\ & - 48\kappa_{14}\kappa_{21}^2\kappa_{23} + 24\kappa_{14}\kappa_{21}\kappa_{23}^2 - 36\kappa_{14}\kappa_{21}\kappa_{32}\kappa_{41} + 72\kappa_{14}\kappa_{21}\kappa_{32}\kappa_{43} \\ & - 72\kappa_{14}\kappa_{21}\kappa_{34}\kappa_{41} + 144\kappa_{14}\kappa_{21}\kappa_{34}\kappa_{43} - 4\kappa_{14}\kappa_{23}^3 + 18\kappa_{14}\kappa_{23}\kappa_{32}\kappa_{41} \\ & - 36\kappa_{14}\kappa_{23}\kappa_{32}\kappa_{43} + 36\kappa_{14}\kappa_{23}\kappa_{34}\kappa_{41} - 72\kappa_{14}\kappa_{23}\kappa_{34}\kappa_{43} + 4\kappa_{21}^2\kappa_{41}^2 \\ & - 16\kappa_{21}^2\kappa_{41}\kappa_{43} + 16\kappa_{21}^2\kappa_{43}^2 - 4\kappa_{21}\kappa_{23}\kappa_{41}^2 + 16\kappa_{21}\kappa_{23}\kappa_{41}\kappa_{43} - 16\kappa_{21}\kappa_{23}\kappa_{43}^2 \\ & + \kappa_{23}^2\kappa_{41}^2 - 4\kappa_{23}^2\kappa_{41}\kappa_{43} + 4\kappa_{23}^2\kappa_{43}^2 - 4\kappa_{32}\kappa_{41}^3 + 24\kappa_{32}\kappa_{41}^2\kappa_{43} - 48\kappa_{32}\kappa_{41}\kappa_{43}^2 \\ & + 32\kappa_{32}\kappa_{43}^3 - 8\kappa_{34}\kappa_{41}^3 + 48\kappa_{34}\kappa_{41}^2\kappa_{43} - 96\kappa_{34}\kappa_{41}\kappa_{43}^2 + 64\kappa_{34}\kappa_{43}^3 .\end{aligned}$$

As p_S is cubic and has at least one positive root, its discriminant is negative if and only if p_S has one real root and one pair of complex conjugate roots; in this case, the Square has precisely one positive steady state. When the discriminant of p_S is non-negative, the system may admit one, two, or three positive steady states; we analyze this case fully in the next section.

Example 5.2.2. Consider the following rate constants for the Square:

$$(\kappa_{12}, \kappa_{14}, \kappa_{21}, \kappa_{23}, \kappa_{32}, \kappa_{34}, \kappa_{41}, \kappa_{43}) = (1/4, 1/2, 1, 13, 1, 2, 8, 1).$$

This yields the polynomial $p_S(x) = -x^3 + 6x^2 - 11x + 6$, which has three positive roots: $x = 1, 2$, and 3 . This is an instance of bistability; it is easy to determine that $x = 1$ and $x = 3$ correspond to *stable* steady states, while the third is unstable. In the next section we determine the conditions for an arbitrary vector of rate constants to admit one, two, or three steady states. \square

Recalling the definitions given in Section 1.3 of the Introduction, the Square has the following properties: the number of complexes is $n = 4$, the number of connected components of the network is $l = 1$, the number of species is $s = 2$, and the dimension of any invariant polyhedron is $\sigma = 1$. The main result of this section states that this network is minimal with respect to each of these four parameters.

Theorem 5.2.3. *The Square is a smallest multistationary, mass-preserving, reversible chemical reaction network with respect to each of the following parameters: the number of complexes, the number of connected components, the number of species, and the dimension of an invariant polyhedron.*

Proof. First $l = 1$ and $\sigma = 1$ are clearly minimal. Next any mass-preserving system with $n \leq 2$ or $s = 1$ has no reactions or has deficiency zero. Finally, an $n = 3$ system has deficiency zero or one; in the deficiency one case, the Deficiency One Theorem of Feinberg rules out the possibility of multistationarity [Fei95]. \square

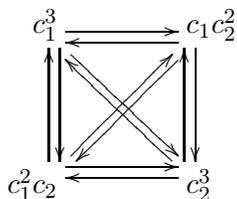
Among all mass-preserving multistationary networks that share these four minimal parameters, the Square is distinguished because its monomials are of minimal degree. A connected network of lower degree would consist of at most three of Horn’s “short” complexes [Hor73a].

The remainder of this chapter focuses on the Square and more generally, the networks that share the same complexes as the Square. In the following section, we shall determine which of these are bistable. The one with the fewest edges is the only one with two connected components rather than one, and is featured in the last section.

5.3 Parametrizing multistationarity

The aim of this section is similar to that of Conradi *et al.* [CFR08], which determined the full set of parameters that give rise to multistationarity for a biochemical model describing a single layer of a MAPK cascade. However we additionally determine the precise number of steady states: zero, one, two, or three, and determine their stability. The family of networks we consider are those

that have the same four complexes as the Square. In other words, we classify subnetworks of the complete network depicted here:



Again the four complexes are labeled by the entries of

$$\Psi(c) = (c^{y_1}, c^{y_2}, c^{y_3}, c^{y_4}) = (c_1^3, c_1c_2^2, c_2^3, c_1^2c_2),$$

and we let κ_{ij} denote the reaction rate constant for the reaction from c^{y_i} to c^{y_j} . Each of these twelve rate constants κ_{ij} is permitted to be zero, which defines the parameter space $\mathbb{R}_{\geq 0}^{12}$ of dynamical systems. The main result of this section is summarized in Table 5.1, which is the semi-algebraic decomposition of the twelve-dimensional parameter space according to the number of steady states of the dynamical system. We note that this table is a refinement of classical “root classification tables” introduced by Arnon [Arn88, §4] for our polynomial of interest; these are also called “complete root classifications” by Liang and Jeffrey [LJ09]. See also the work of Lazard and Rouillier on solving parametrized polynomial systems [LR07].

The conditions listed in the table (which form a “complete discrimination system” in the language of Yang [Yan99]) make use of certain polynomials in the rate constants, including the following signed coefficients of the polynomial p :

$$\begin{aligned} S_0 &= 2\kappa_{12} + 3\kappa_{13} + \kappa_{14}, \\ S_1 &= \kappa_{41} - \kappa_{42} - 2\kappa_{43}, \\ S_2 &= -2\kappa_{21} + \kappa_{23} - \kappa_{24}, \\ S_3 &= 3\kappa_{31} + \kappa_{32} + 2\kappa_{34}, \end{aligned}$$

where p generalizes the polynomial p_S from the Square:

$$p(x) = -S_0x^3 + S_1x^2 - S_2x + S_3. \quad (5.3)$$

We now derive the entries of Table 5.1 for those networks without boundary steady states (this includes the case of the Square). These cases are precisely the ones in which $S_0 > 0$ and $S_3 > 0$. Our approach is simply to determine the conditions on the coefficients of p for the polynomial to have one, two, or three positive roots, as we have seen that this is equivalent to our task at hand. Recall that we already have determined that p always has at least one positive root.

In this twelve-parameter case, the discriminant of p is a homogeneous degree-four polynomial with 113 terms. For the same reason as that for the Square, there is one positive steady state when the discriminant is negative. Now assume that the discriminant is non-negative. Then p has three real roots, counting multiplicity; recall that the positive ones correspond to the positive steady states of the chemical reaction network. Now the constant term of a monic cubic polynomial is the

Condition	Steady states	Stable states
$D < 0$ and $S_0 S_3 = 0$	0	0
$D < 0$ and <i>else</i>	1	1
$D > 0$ and $S_0, S_1, S_2, S_3 > 0$	3	2
$D > 0$ and $S_0, S_1, S_2 > 0$ and $S_3 = 0$	2	1
$D > 0$ and $S_1, S_2, S_3 > 0$ and $S_0 = 0$	2	1
$D > 0$ and $S_0 = S_3 = 0$ and $S_1 S_2 < 0$	0	0
$D > 0$ and <i>else</i>	1	1
$D = 0$ and $S_0, S_1, S_2, S_3 > 0$ and triple root condition	1	1
$D = 0$ and $S_0, S_1, S_2, S_3 > 0$, no triple root condition	2	1
$D = 0$ and $S_1 \leq S_0 = 0 \leq S_2$ and $S_3 > 0$	0	0
$D = 0$ and $S_1 \leq S_3 = 0 \leq S_2$ and $S_0 > 0$	0	0
$D = 0$ and <i>else</i>	2	1

Table 5.1: Classification of dynamical systems arising from non-trivial (having at least one reaction) networks with complexes c_1^3 , $c_1 c_2^2$, c_2^3 , and $c_1^2 c_2$. Listed are the number of positive steady states and the number of those steady states that are stable. The discriminant of p is denoted by D . The signed coefficients of the polynomial p (5.3) are denoted by S_0 , S_1 , S_2 , and S_3 . The triple root condition consists of the two equations (5.4).

negative of the product of its roots, so by examining the sign of the constant term of p , we conclude that p has either one positive root and two negative roots, or three positive roots. Continuing the sign analysis with the other coefficients of p , we conclude that there are three positive roots if and only if $S_1 > 0$ and $S_2 > 0$. We proceed by distinguishing between the cases when the discriminant is positive or zero. If the discriminant is positive, then we have derived criteria for having one or three positive steady states; this is because the roots of p are distinct. If the discriminant is zero, then in the case of one positive root, the two negative roots come together (one steady state). In the case of discriminant zero and three positive roots, then at least two roots come together (at most two steady states); a triple root occurs if and only if the following *triple root condition* holds:

$$3S_0 S_2 = S_1^2 \quad \text{and} \quad 27S_0^2 S_3 = S_1^3. \quad (5.4)$$

These equations are precisely what must hold in order for p to have the form $p(x) = -(x - \alpha)^3$. Finally, stability analysis in this one-dimensional system is easy, and this completes the analysis for the networks without boundary steady states. The remaining cases can be classified similarly, and therefore we can complete the entries of Table 5.1. To parametrize the behavior of the Square, we can simply reduce to the case when each of its parameters κ_{12} , κ_{14} , κ_{21} , κ_{23} , κ_{32} , κ_{34} , κ_{41} , and κ_{43} are positive and all others are zero.

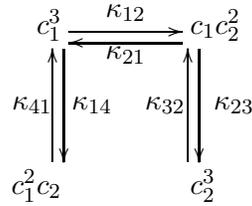
Remark 5.3.1. By determining which sign vectors in $(0, +)^{12}$ can be realized by a vector of parameters that yields multistationarity, we find a necessary and sufficient condition for a directed graph on the four complexes of the Square to admit multistationarity. This condition for a network to be multistationarity is that *the graph must include the edges labeled by κ_{23} and κ_{41} and at least one*

edge directed from the vertex c_1^3 or c_2^3 . In this case, for appropriate rate parameters arising from Table 5.1, the dynamical system has multiple positive steady states. Therefore, we can enumerate the reversible networks on the four complexes that admit multistationarity: there is one network with all six (bi-directional) edges, four with five edges, six (including the Square) with four edges, four with three edges, and one with two edges. These sixteen networks comprise the family of “smallest” multistationary networks. The multistationary subnetworks of the Square network are analyzed in the next section, and in particular the decomposition from Table 5.1 for the two-edge network is depicted in Figure 5.1.

5.4 Multistationary subnetworks of the Square network

Subnetworks of the Square are obtained by removing edges. From the parametrization in the previous section, we know that up to symmetry between c_1 and c_2 , only two reversible subnetworks of the Square exhibit multiple positive steady states. We now examine both of them.

The first network is obtained by removing the bottom edge of the Square:



In other words, A_κ is replaced by

$$A_\kappa = \begin{pmatrix} -\kappa_{12} - \kappa_{14} & \kappa_{12} & 0 & \kappa_{14} \\ \kappa_{21} & -\kappa_{21} - \kappa_{23} & \kappa_{23} & 0 \\ 0 & \kappa_{32} & -\kappa_{32} & 0 \\ \kappa_{41} & 0 & 0 & -\kappa_{41} \end{pmatrix}.$$

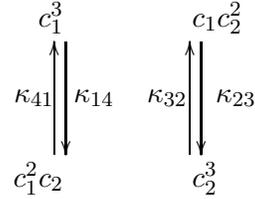
The other two matrices $\Psi(c)$ and Y given in equation (5.1) remain the same.

In this subnetwork, the four parameters of Theorem 5.2.3 are the same as those of the Square. The system is a toric dynamical system if and only if the following four binomial generators of M_G vanish:

$$\begin{aligned}
 & \kappa_{14} \kappa_{32} - \kappa_{23} \kappa_{41} , \\
 & \kappa_{12} \kappa_{32} \kappa_{41} - \kappa_{14} \kappa_{21} \kappa_{23} , \\
 & \kappa_{14}^2 \kappa_{21} - \kappa_{12} \kappa_{41}^2 , \\
 & \kappa_{12} \kappa_{32}^2 - \kappa_{21} \kappa_{23}^2 .
 \end{aligned}$$

We note that both κ_{23} times the third binomial and κ_{14} times the fourth binomial are in the ideal generated by the first two binomials. Therefore, an assignment of positive parameters for this network defines a toric dynamical system if and only if the following two equations hold: $\kappa_{14} \kappa_{32} = \kappa_{23} \kappa_{41}$ and $\kappa_{12} \kappa_{32} \kappa_{41} = \kappa_{14} \kappa_{21} \kappa_{23}$.

The second subnetwork of the Square is obtained by removing one additional edge, the one between the vertices labeled by c_1^3 and $c_1c_2^2$; we depict it here:



The new matrix A_κ is

$$A_\kappa = \begin{pmatrix} -\kappa_{14} & 0 & 0 & \kappa_{14} \\ 0 & -\kappa_{23} & \kappa_{23} & 0 \\ 0 & \kappa_{32} & -\kappa_{32} & 0 \\ \kappa_{41} & 0 & 0 & -\kappa_{41} \end{pmatrix}.$$

The network graph G is now disconnected, and the polynomial p from (5.3) reduces to

$$p(x) = -\kappa_{14}x^3 + \kappa_{41}x^2 - \kappa_{23}x + \kappa_{32}.$$

The discriminant of p is

$$D = -27\kappa_{14}^2\kappa_{32}^2 - 4\kappa_{14}\kappa_{23}^3 + 18\kappa_{14}\kappa_{23}\kappa_{32}\kappa_{41} + \kappa_{23}^2\kappa_{41}^2 - 4\kappa_{32}\kappa_{41}^3.$$

Further, the toric condition reduces to the single equation

$$\kappa_{23}\kappa_{41} = \kappa_{14}\kappa_{32},$$

which defines the *Segre variety*. A single equation suffices to define the space of toric dynamical systems; this corresponds to the fact that this subnetwork has deficiency one, while the previous subnetwork has deficiency two. The semi-algebraic decomposition of the previous section for this four-parameter network can be depicted in three dimensions by setting one parameter equal to one, in other words, by scaling the equations (1.3); this is displayed in Figure 5.1.

We remark that Horn and Jackson performed the same parametrization for the following special rate constants:

$$(\kappa_{12}, \kappa_{14}, \kappa_{21}, \kappa_{23}, \kappa_{32}, \kappa_{34}, \kappa_{41}, \kappa_{43}) = (\epsilon, 0, 1, 0, \epsilon, 0, 1, 0),$$

where $\epsilon > 0$. Their results are summarized as Table 1 in [HJ72]. Their analysis notes that any instance of three positive steady states can be lifted to establish the same in the (reversible) Square. In other words, in a small neighborhood in $\mathbb{R}_{\geq 0}^8$ of a vector of parameters that yields three positive steady states of the directed Square, there is a vector of parameters for the bi-directional Square that also exhibits multistationarity. The precise criterion for when lifting of this form is possible appears in Theorem 2 of Conradi *et al.* [CFRS07]. As this approach is widely applicable, further analysis of small networks may be fruitful for illuminating the dynamics of larger biochemical networks.

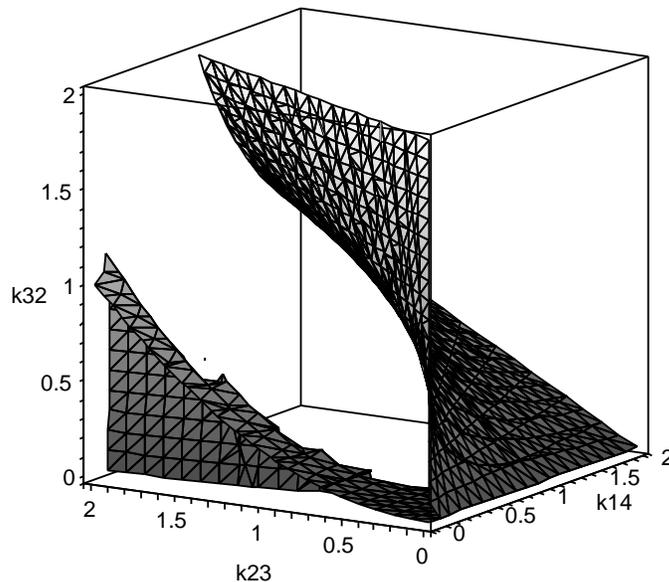
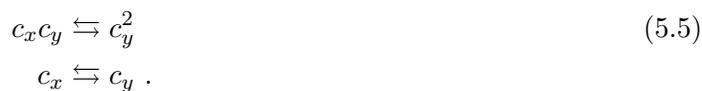


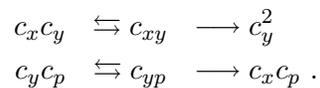
Figure 5.1: This depicts the semi-algebraic decomposition of Section 5.3 for the subnetwork of the Square in which only the vertical edges remain and $\kappa_{41} = 1$. At the left is the discriminant-zero locus. Parameter vectors lying below this surface give rise to dynamical systems with three positive steady states. Those above the surface yield one positive steady state; these include parameters of the toric dynamical systems, which are the points on the Segre variety which appears on the right. Parameters on the discriminant-zero locus correspond to systems with either one (if and only if $3\kappa_{14}\kappa_{32} = \kappa_{23}$) or two positive steady states. This figure was created using the software `Maple`.

5.5 Connection to known biochemical networks

In this final section, we comment on the possible connection of the Square network to known networks in systems biology. We first compare it to the following simple network:



Network (5.5) is a modified version of the following molecular switch mechanism proposed by Lisman [Lis85]:



Here x denotes a kinase in an inactive state, y is the active version, and p is a phosphatase. In the first reactions, y catalyzes the phosphorylation of x , turning x into y ; the second reactions correspond to dephosphorylation. By skipping the binding steps, making all reactions reversible, and noting that removing p effectively scales the second reaction rate constant, we obtain the network (5.5). The reactions of (5.5) are similar to



which are reactions in the generalization of the Square network examined in the next section; this suggests the possible biological relevance of the reactions of the Square. For example $c_1^2 c_2 \rightarrow c_2^3$ can be viewed as a reaction in which species two catalyzes the reaction $c_1^2 \rightarrow c_2^2$. Such a positive feedback loop—in which a high amount of some species y encourages the further production of the same species—occurs in biological settings. For example, the recent work of Dentin *et al.* finds that high glucose levels in diabetic mice promote further glucose production in the liver, which is triggered by the binding of glucose (which we may view as species y) to the transcription factor CREB (species x) [DHX⁺08].

We end by mentioning a possible “design principle” that exists in the Square network. We have seen that the family of Square networks is the smallest class of bistable mass-action kinetics networks. Whether nature has implemented one of these networks (perhaps with additional components to provide robustness) in a biological setting is as yet unknown, but it is remarkable that these networks exhibit a simple switch mechanism, which we now explain. Consider the case of three steady states. The corresponding positive roots $x_1 < x_2 < x_3$ of the polynomial p , which is defined in equation (5.3), are the steady states for the ratio of concentrations c_1/c_2 . To switch from the low stable equilibrium x_1 to the high stable equilibrium x_3 is easy: simply increase the concentration ratio c_1/c_2 above x_2 , and the resulting dynamics will do the rest.

Chapter 6

Predicting gene expression from regulatory sequence

This chapter is joint work with Lester Mackey and Lior Pachter.

6.1 Introduction

An important problem in systems biology is to understand how the biochemical processes underlying transcriptional regulation give rise to precise patterns in space and time. Key players in these processes are *transcription factors*, proteins that enhance or repress transcription by binding to specific DNA sequences. The chemical reactions that underlie transcriptional regulation include the bindings of transcription factors to *cis-regulatory modules* (CRMs) of genes. Such modules consist of subsequences that are usually (but not always) located upstream (5') to genes and that control transcription via the binding of transcription factors. Recent high-throughput sequencing technologies have allowed for the prediction and experimental verification of CRMs, and of the transcription factors that bind to them.

At first glance, the underlying mechanisms of transcriptional regulation appear to be extremely complex. Even in well-understood model systems, such as *Drosophila*, there are thousands of transcription factors regulating thousands of genes, even in very early stages of development. Nevertheless, it is becoming increasingly apparent that there are high levels of organization of the transcription machinery, resulting in modularity of the system. This can be exploited in order to understand transcriptional regulation. For each such sequence, many possible combinations of transcription factors can bind to the sequence. Such *thermodynamic models* give quantitative predictions of gene expression levels that are weighted averages over the set of all possible binding configurations.

One aspect of this line of research is the development of models of transcription factor binding that are sufficiently detailed to enable prediction of gene expression. Two recently proposed models for these processes are the following: (1) a (spatiotemporal) ODE model of the Reinitz lab which predicts gene expression levels based on concentrations of transcription factors and other genes' expression levels [MSG⁺09], and (2) a thermodynamic model (for a fixed time point) of the

Segal lab which predicts gene expression level based on concentrations of transcription factors and the gene regulatory sequence [SRSS⁺08]. The aim of this chapter is to describe an implementation and mathematical analysis of the Segal *et al.* model. For a machine learning approach to predicting expression, see the recent paper of Zinzen *et al.* [ZGG⁺09].

This chapter is organized in the following way. Section 6.2 defines the concept of a gene expression function and describes the gene expression function introduced by Segal *et al.* Section 6.3 explains how to implement this model by means of dynamic programming. Section 6.4 provides a recursion for computing the number of configurations.

6.2 The gene expression function of Segal *et al.*

Our goal is to predict the expression level of a gene G from its CRM, denoted by S_G , and the concentrations, denoted by R_i , of various transcription factors present. Accordingly, we define a *gene expression function* to be a map whose inputs are a CRM and a vector of m transcription factor concentrations and whose output is a predicted expression level (normalized to be a real number between 0 and 1):

$$\begin{aligned} \phi : \mathcal{S} \times \mathbb{R}_+^m &\rightarrow [0, 1] \\ (S_G, R_1, \dots, R_m) &\mapsto P(E); \end{aligned}$$

here, \mathcal{S} is the set of finite sequences over the nucleotide alphabet, $\{A, C, G, T\}$. We note that for fixed concentration levels, the map ϕ is then a *fitness landscape* [BPS07].

In this section, we describe the specific gene expression function that was introduced by Segal *et al.* In particular, any specification of parameters defines the gene expression function detailed in the Supplementary Information of [SRSS⁺08], which predicts the expression level of a gene based on its regulatory sequence and the concentration levels of $m = 8$ transcription factors:

Parameters: For each transcription factor $a = 1, 2, \dots, m$, position-specific scoring matrix (PSSM) M_a , concentration scaling parameter α_a , binding cooperativity strength λ_a , and (positive or negative) expression contribution w_a . For the gene G of interest, basal expression rate w_0 .

Gene expression function: The function takes as input, the *cis*-regulatory sequence S_G of a gene G of interest and (experimentally measured) concentrations R_a of m transcription factors.

During *Drosophila* embryogenesis, the precursors to the body segments are established; related genes are expressed in stripes, delineating the segments to be formed along the anterior-posterior spatial axis. At 100 points along this axis, concentrations of the eight transcription factors have been measured experimentally. Then for a fixed gene of interest, the gene expression function predicts its expression profile along the spatial axis by evaluating the gene expression function 100 times, at each of the different concentration levels of transcription factors. In [SRSS⁺08], this is repeated for each of 44 genes and compared to experimental data. Further, they estimate some of the parameters.

6.2.1 Configuration models

We now describe a more general framework, of which this model will be seen to be a special case. Each transcription factor $a = 1, 2, \dots, m$ binds to sequences of a specified length,

which we denote by $L(a)$. For a fixed gene G , there is a CRM S_G , the (predicted) region in which transcription factors bind. So, S_G is a sequence of length $|S_G|$ over the nucleotide alphabet; in [SRSS⁺08], we have $500 \leq |S_G| \leq 2500$.

To define the gene expression function, we consider all possible ways for transcription factors to bind to the regulatory sequence of a gene; we call possible combinations “configurations.”

Definition 6.2.1. For a sequence S , a *pre-configuration* c that involves $k := k(c)$ transcription factors is a choice of k transcription factors $f(1), f(2), \dots, f(k) \in \{1, 2, \dots, m\}$ and k positions $1 \leq p(1) < p(2) < \dots < p(k) \leq |S|$ of binding such that the *binding sites*

$$(p(i), p(i) + 1, \dots, p(i) + L(f(i)) - 1)$$

of adjacent transcription factors do not overlap. We say that a pre-configuration c is a *configuration* if the last binding site does not extend past the end of the sequence S , in other words, if $p(k) + L(f(k)) - 1 \leq |S|$.

Letting \mathcal{C} denote the set of all possible configurations (for a fixed CRM S_G), the gene expression function computes the gene expression level $P(E) := P(S_G)(E)$ in the following probabilistic way:

$$P(E) := \sum_{c \in \mathcal{C}} P(E|c)P(c) . \quad (6.1)$$

We define *configuration models* to be the family of all gene expression functions that take the form given in (6.1), where $P(c)$ is the probability (under some distribution) that the configuration of transcription factors that bind to the CRM is c , and $P(E|c)$ is a function that predicts the level of gene expression given that the configuration is c . In [SRSS⁺08], the probability of the configuration c that consists of transcription factors $f(i)$ at positions $p(i)$ takes the following form:

$$P(c) = \prod_i (\text{concentration of } f(i)) \cdot (\text{probability } f(i) \text{ binds at } p(i)) \cdot (\text{binding cooperativity terms}).$$

We note that the probability that the transcription factor $f(i)$ binds at position $p(i)$ along the CRM should come from the (experimentally derived) PSSM of $f(i)$. The “binding cooperativity terms” should depend on the distance between adjacent transcription factors; an oddity of the model in [SRSS⁺08] is that binding cooperativity terms contribute only for adjacent transcription factors that are identical, for example, two bicoids binding in adjacent positions. We note also that their choice of $P(E|c)$ incorporates a baseline level of expression, the length of the CRM, and which transcription factors are in the configuration c ; however, it does not weight the contribution of binding sites nearest the promotor any differently from those far from the promotor.

6.2.2 Configuration probabilities and expression level prediction

We now define for the Segal *et al.* model, the probability of having a certain configuration c of transcription factors there: $P(c) := P_G(c)$, and at the end of this section, define the predicted

amount of expression of gene G under the assumption of having the configuration c : $P(E|c) := P_G(E|c)$. These two definitions will specify the configuration model as defined above in (6.1). How to compute $P(E)$ in practice is discussed in Section 6.3; see equation (6.14).

To describe the probability $P(c)$ that a configuration c occurs, the model incorporates the binding specificities of each transcription factor. This takes the form of a PSSM (position-specific scoring matrix, also called a position-weight matrix) for each transcription factor a ; in other words, for each position $j = 1 \dots L(a)$, we have a probability distribution on the set of nucleotides:

$$M_a^j(-) = \text{probability the } j\text{th nucleotide of the binding site of } a \text{ is } -.$$

Generally these PSSMs are estimated from experimental data.

From the PSSMs, we will define the probability function P_a that a transcription factor a binds to a sequence of length $L(a)$ (on either strand). First, recall that DNA is a double helix, whose two strands are called the Watson and Crick strands. Now, P_a^W denotes the probability of binding to the Watson strand:

$$P_a^W(S_{p(i)}, S_{p(i)+1}, \dots, S_{p(i)+L(i)-1}) := \prod_{j=0}^{L(a)-1} M_a^{j+1}(S_{p(i)+j}). \quad (6.2)$$

The probability P_a^C of binding to the complementary Crick strand is computed similarly:

$$P_a^C(S_{p(i)}, S_{p(i)+1}, \dots, S_{p(i)+L(i)-1}) := \prod_{j=0}^{L(a)-1} M_a^{j+1}(S'_{p(i)+j}), \quad (6.3)$$

where $S'_{p(i)+j}$ denotes the base complementary to the base $S_{p(i)+j}$. Then to allow for binding on both strands, we define the probability function P_a as the mean of the two probabilities P_a^W and P_a^C :

$$\begin{aligned} P_a(S_{p(i)}, \dots, S_{p(i)+L(i)-1}) &:= \frac{1}{2} P_a^W(S_{p(i)}, \dots, S_{p(i)+L(i)-1}) \\ &\quad + \frac{1}{2} P_a^C(S_{p(i)}, \dots, S_{p(i)+L(i)-1}). \end{aligned} \quad (6.4)$$

A more natural definition for P_a might be extend the definition of a configuration so that a binding event takes place between a transcription factor, a position along the sequence, and one of the strands, although this would be more difficult to compute. The baseline binding probability function P_B is computed as follows:

$$P_B(\text{a sequence of length } L) := \frac{1}{4^L}. \quad (6.5)$$

A second term that appears in $P(c)$ takes into consideration the concentrations of transcription factors in the configuration c ; the *absolute concentration* of transcription factor a is given by

$$\tau_a = \alpha_a R_a,$$

recalling that α_a is the *concentration scaling parameter* and R_a is the measured concentration.

The remaining term is the binding cooperativity term, for which we define for transcription factors a and b which bind to regions $T_1^a, \dots, T_{L(a)}^a$ and $T_1^b, \dots, T_{L(b)}^b$ that are some positive distance $\delta = T_1^b - T_{L(a)}^a$ apart,

$$\gamma(a, b, \delta) := \begin{cases} \exp(\lambda_a \cdot \text{Gaussian}_{\mu=0, \sigma=50}(\delta)) & \text{if } a = b \\ 1 & \text{if } a \neq b, \end{cases} \quad (6.6)$$

where $\text{Gaussian}_{\mu=0, \sigma=50}(\delta)$ denotes the density function of a normal distribution with mean 0 and standard deviation 50 which is evaluated at δ , and λ_a is the parameter that represents the binding cooperativity strength. The binding cooperativity term will take the form of a product of terms:

$$\gamma(f(i), f(i+1), p(i+1) - p(i) - L(a)) ,$$

which corresponds to adjacent transcription factors a and a' binding at positions $p(i)$ and $p(i+1)$ in the configuration c . Note that

$$\delta = p(i+1) - p(i) - L(a) \quad (6.7)$$

is the number of free bases between the two bases. We view the binding cooperativity term as introducing *epistasis* (transcription factor interaction) into the model.

We define $W(c)$ to be the “statistical weight” of the configuration c :

$$W(c) := \left(\prod_{i=1}^k \tau_{f(i)} \frac{P_{f(i)}(S_{p(i)}, \dots, S_{p(i)+L(f(i))-1})}{P_B(S_{p(i)}, \dots, S_{p(i)+L(f(i))-1})} \right) \cdot \prod_{i=1}^{k-1} \gamma(f(i), f(i+1), p(i+1) - p(i) - L(f(i))) .$$

We are now equipped to define

$$P(c) := \frac{W(c)}{\sum_{c' \in \mathcal{C}} W(c')} . \quad (6.8)$$

Note that by construction this defines a probability distribution on the set \mathcal{C} of configurations.

Finally, the last ingredient for the definition of $P(E)$ given in equation (6.1), is the following: for the predicted gene expression under a configuration c that involves k transcription factors, we have

$$\begin{aligned} P(E|c) &:= \sigma \left(w_0 + \frac{1000}{|S|} \sum_{i=1}^k w_{f(i)} \right) \\ &= \sigma \left(w_0 + \frac{1000}{|S|} \sum_{a=1}^m z_a(c) w_a \right) \end{aligned} \quad (6.9)$$

where $z_a(c)$ is the number of times transcription factor a is bound in configuration c , and $\sigma()$ is the logistic function $\left(\sigma(t) := \frac{1}{1+\exp(-t)} \right)$. In the second equality, we see that to calculate $P(E|c)$, the dependence on the configuration c is only up to the multiset over $\{1, 2, \dots, m\}$ that its transcription factors define.

6.2.3 Parameters

In this section, we list the specific parameters used in [SRSS⁺08], as reported in Figure 2 of the Supplementary Information and obtained by email from Tali Raveh-Sadka. Each transcription factor $a = 1, 2, \dots, m$ has a position-specific scoring matrix (PSSM) M_a , which is available from the Supplementary Information website¹. As an example, the matrix for bicoid is listed here where the columns correspond to the bases A, C, G, T :

```
0.022007100 5.15419e-01 0.263775000 1.98799e-01
0.000313491 5.26394e-01 0.181190000 2.92100e-01
0.012125800 3.67034e-04 0.005086510 9.82421e-01
0.546444000 4.44226e-01 0.006383100 2.94640e-03
0.999180000 4.68222e-05 0.000745253 2.75202e-05
0.000255355 2.22943e-02 0.418216000 5.59234e-01
0.019107700 9.28256e-01 0.000817427 5.18188e-02
0.029907400 4.40646e-01 0.293858000 2.35588e-01
0.013844600 9.14434e-01 0.006140290 6.55808e-02
0.000288269 2.29073e-01 0.466637000 3.04002e-01
0.082691400 4.52687e-01 0.393126000 7.14960e-02
```

Transcription factor	α_a	λ_a	w_a
bicoid	0.003	5.73	1.85
caudal	0.001	4.20	1.03
giant	0.01	6.68	-1.98
hunchback	0.001	6.23	-0.05
knirps	0.09	1.03	-1.16
kruppel	0.004	4.45	-0.22
tailless	0.17	1.46	-0.36
torRE	0.008	7.44	2.03

Table 6.1: Parameters of the eight transcription factors in the Segal *et al.* model.

For each transcription factor, its concentration scaling parameters α_a , binding cooperativity strength λ_a , and (positive or negative) expression contribution w_a are listed in Table 6.1. Note that those transcription factors with positive expression contribution $w_a > 0$ are considered “enhancers”; all others are “repressors.” For each gene G of interest, the basal expression rate w_0 is listed in Table 6.2. Note that Segal *et al.* fit these parameters *after* computing the gene expression function.

6.2.4 Clarifying notes on the Segal *et al.* gene expression function

The Segal *et al.* gene expression function is defined in the Supplementary Information of [SRSS⁺08], but a few details need to be clarified, which were provided by the authors of the paper.

¹<http://genie.weizmann.ac.il/pubs/segnet08/>.

Gene	Basal expression rate w_0
Kr_CD2.ru	-1.82464637868443
cnc_(+5)	-3.5790481144348
gt_(-6)	-5.30754149756432
hb_anterior_actv	-4.13371297543959
hkb_ventral_elem	-3.74318844640332
kni_(-5)	-4.87080629961396
oc_(+7)	-2.907000925884
knrl_(+8)	-3.36136713429632
Kr_AD2.ru	-4.6158
kni_(+1)	-3.32013676583045
oc_otd_early	-2.6049558325662
tll_P2	-4.12622284224731
slp2_(-3)	-3.94259094977981
gt_(-10)	-3.55129310232093
gt_(-1)	-3.34633255113909
btd_head	-2.78749093059981
h_15.ru	-2.63368499359713
prd_+4	-4.30336855597686
eve_1.ru	-5.58092128934552
run_stripe1	-6.2158
ftz +3	-1.86577033144262
odd_(-5)	-0.635179773902596
D_(+4)	-2.11216906913894
eve_stripe2	-9.4158
hb_centr_&_post	-0.954515917746382
Kr_CD1.ru	-2.21843242859067
h_stripe34_rev	-0.585860196667668
eve_37ext.ru	-3.60101997673599
run_-9	-4.02326700480848
run_stripe3	-3.8158
odd_(-3)	-5.00187783049153
pdm2_(+1)	-4.88402834083858
nub_(-2)	-4.71017748776955
eve_stripe4_6	-4.6158
kni_83.ru	-4.00411145489719
run_-17	-4.14253008497171
eve_stripe5	-5.87745912409451
run_stripe5	-3.2158
gt_(-3)	-3.88095644802846
(continued on next page)	

(continued from previous page)

Gene	Basal expression rate w_0
h_6_ru	-4.77716880076935
h_stripe7_rev	-3.8158
cad_(+14)	-2.05702215586585
tll_K2	-3.2158
fkh_(-2)	-4.6158

Table 6.2: Basal expression rate of each gene in the Segal *et al.* model.

First, the definition of the probability function P_a in (6.4) as the sum of the bindings on both strands does not appear in the Supplementary Information. Next, the definition of the distance δ in (6.7) between two adjacent binding sites is slightly different in the Supplementary Information. Additionally, the factor of 1000 in the definition of $P(E|c)$ defined in (6.9) is missing from its definition in the Supplementary Information. Finally, four of the parameter values appearing in Table 6.1 deviate slightly from the values reported in the Supplementary Information.

6.3 Computing the gene expression function

This section describes how to compute $P(E)$. First, the terms $W(c)$ and $P(E|c)$ are straightforward to compute. To compute the term $P(c)$ defined in (6.8), we must compute its denominator $\sum_{c' \in \mathcal{C}} W(c')$. This is performed using dynamic programming, and from this setup, $P(E)$ will be approximated by sampling from the probability distribution $P(c)$ which we will see in the estimation formula (6.14). For further details on dynamic programming in this context, see the article of Cowley and Pachter [CP03]. In Section 6.4, we will see that the number of configurations is prohibitively large, so the estimation of $P(E)$ is necessary.

6.3.1 Dynamic programming

The dynamic programming approach of Section 3.1 in the Supplementary Information of [SRSS⁺08] suffices only if there are no binding cooperativity terms (6.6). To handle binding cooperativity, we must generalize in a manner which we now describe. We view configurations c as walks in a Markov chain \mathcal{M} which we now define. The states of \mathcal{M} are given by locations (a, b, d) of the following form:

- **Pre-transcription factor states** denoted by $(0, 0, d)$, which is the state at time d of a walk that has never visited a transcription factor, in other words, no transcription factors bind to the first d nucleotides of the CRM,
- **Within-transcription factor states** denoted by $(a, 1, d)$, which is the state of being at position $1 \leq d \leq L(a)$ within the transcription factor $1 \leq a \leq m$, and
- **Post-transcription factor states** denoted by $(a, 2, d)$, which is the state of being $d \geq 1$ bases past after the transcription factor $1 \leq a \leq m$.

Note that in the location (a, b, d) , a denotes a transcription factor (or the empty one), $b = 0, 1$, or 2 indicates pre-, within-, or post-transcription factor, respectively, and d indicates the position along the CRM. For example $(a, b, d) = (1, 2, 5)$ is the state in which the previous transcription factor in the configuration is bicoid, and after that binding site there are five unbound bases. We note that there is a distinguished *empty configuration* whose walk consists entirely of pre-transcription factor states. The edges (transitions) of \mathcal{M} are the following:

- **New transcription factor** edges are of the form

$$\begin{aligned} (0, 0, d) &\longrightarrow (a, 1, 1), \\ (a', 1, L(a')) &\longrightarrow (a, 1, 1), \text{ or} \\ (a', 2, d') &\longrightarrow (a, 1, 1), \end{aligned}$$

for $1 \leq d$ and $1 \leq a, a' \leq m$, and these edges are labeled with the following weights (respectively),

$$\begin{aligned} \tau_a \frac{P_a}{P_B}, \\ \tau_a \frac{P_a}{P_B} \cdot \gamma(a', a, 0), \text{ or} \\ \tau_a \frac{P_a}{P_B} \cdot \gamma(a', a, d'). \end{aligned}$$

- All **remaining edges** are of the form

$$(a, b, d) \longrightarrow (a, b, d + 1),$$

and have the label “1.”

The resulting graph will be called the “transition diagram” (or “transition graph”). For some sequence S , consider a walk in the transition diagram of length $|S| - 1$ that begins at a state $(0, 0, 1)$ or some beginning binding site $(a, 1, 1)$. It follows that walks of this form in the transition graph are in one-to-one correspondence with configurations $c \in \mathcal{C}$, and in the remainder of this section, we explain how we can sample a walk such that the resulting configuration c is chosen according to the probability distribution $P(c)$. The edge weights along a walk are the factors whose product is $W(c)$, where c denotes the corresponding configuration². An example of a transition diagram for a short sequence is shown in Figure 6.1.

For any state (a, b, d) in the Markov chain such that $b = 1$ implies that $|S| - i \geq L(a) - d$, and for an integer $1 \leq i \leq |S|$, we define

$$f_{a,b,d}(S, i) := \sum_{c \in \tilde{\mathcal{C}}_{i,(a,b,d)}} W(c),$$

²More precisely, the edge weights are labeled by functions of CRMs S and time i (the position along S), whose evaluation is the factor that appears in $W(c)$. More precisely, if $(a, 1, 1)$ is the state at time i , then the probability functions P_a and P_B should be evaluated at the subsequence $S_i, \dots, S_{i+L(a)-1}$. That is, the random process taking states (a, b, d, i) is a Markov chain.

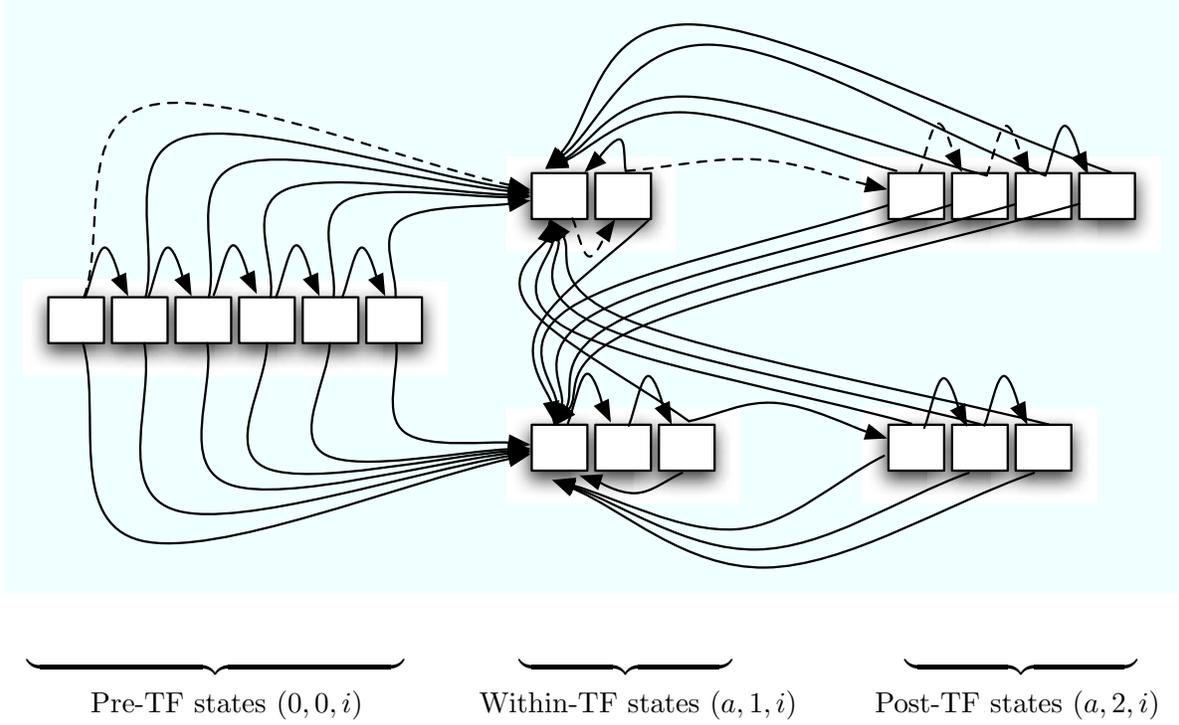


Figure 6.1: This depicts the transition diagram for the Markov chain \mathcal{M} in the case of $m = 2$ transcription factors, which bind to sequences of length $L(1) = 2$ and $L(2) = 3$, and the sequence S has length 6. A configuration is given by a walk of length 5 that begins at state $(0, 0, 1)$, $(1, 1, 1)$, or $(2, 1, 1)$. The walk labeled by the dashed arrows corresponds to the configuration which consists of the first transcription factor binding at the second position of the sequence.

where the sum is taken over the set $\tilde{\mathcal{C}}_{i,(a,b,d)}$ of pre-configurations on the subsequence

$$S_1, S_2, \dots, S_i$$

whose corresponding walks in the transition diagram end precisely at location (a, b, d) . Note that the dependence on the regulatory sequence S is implicit in the definition of $W(c)$, and this definition extends to pre-configurations c in $\tilde{\mathcal{C}}_{i,(a,b,d)}$ by construction: if the state (a, b, d) is a within-transcription factor state, then the inequality $|S| - i \geq L(a) - d$ ensures that the last binding site does not extend past the end of S . With this notation, the term in the denominator in the definition of $P(c)$ in (6.8) is

$$\sum_{c \in \mathcal{C}} W(c) = 1 + \sum_{a=1}^m f_{a,1,L(a)}(S, |S|) + \sum_{a=1}^m \sum_{d=1}^{|S|-L(a)} f_{a,2,d}(S, |S|). \quad (6.10)$$

Note that the summand “1” corresponds to the empty configuration that ends at state $(0, 0, |S|)$.

Now it remains only to show how to compute $f_{a,b,d}(S,i)$ for all $i \leq |S|$. This is the aim of the remainder of this subsection. These computations will be used in the next section to sample configurations from the probability distribution $P(c)$. For $i = 1$, we have

$$\begin{aligned} f_{0,0,1}(S,1) &= 1, \text{ and} \\ f_{a,1,1}(S,1) &= \tau_a \frac{P_a(1, \dots, L(a))}{P_B(1, \dots, L(a))}, \end{aligned}$$

for $1 \leq a \leq m$, and all others are 0. For $i > 1$, we will give a recursion. Note that any configuration whose last transcription factor a begins binding at the i -th position is obtained by adding the binding site of a to the end of a shorter configuration. Therefore if $f_{a',b',d'}(S,i')$ has been computed for all $0 \leq a' \leq m$, b', d' , and $1 \leq i' < i$, then $f_{a,b,d}(S,i)$ can be computed. That we need only two update rules derives from the fact that in the transition diagram, only the states that correspond to the beginnings of a transcription factor binding site, namely those of the form $(a, 1, 1)$, have more than one incoming arrow. (As an illustration, see Figure 6.1.) These states we will later call “head states”. All other states have a unique incoming arrow (labeled by the multiplicative factor “1”), so for $(b, d) \neq (1, 1)$, we have for $i \leq |S| - L(a) + 1$:

$$f_{a,b,d}(S,i) = f_{a,b,d-1}(S,i-1).$$

For those states $(a, 1, 1)$ that are the beginning of transcription factor binding sites, we have:

$$\begin{aligned} f_{a,1,1}(S,i) &= \tau_a \frac{P_a(S_i, \dots, S_{i+L(a)-1})}{P_B(S_i, \dots, S_{i+L(a)-1})} \cdot \\ &\left(1 + \sum_{a'=1}^m f_{a',1,L(a')}(S,i-1) \gamma(a, a', 0) \right. \\ &\quad \left. + \sum_{a'=1}^m \sum_{l=1}^{i-L(a')-1} f_{a',2,l}(S,i-1) \gamma(a, a', l) \right). \end{aligned} \tag{6.11}$$

The correctness of this update rule follows from writing $f_{a,1,1}(S,i)$ as a sum over incoming arrows to the state $(a, 1, 1)$ in the transition diagram:

$$f_{a,1,1}(S,i) = \sum_{(a',b',d') \rightarrow (a,1,1)} (\psi_{(a',b',d') \rightarrow (a,1,1)}(S_i, \dots, S_{i+L(a)-1})) \cdot f_{a',b',d'}(S,i-1).$$

Here $\psi_{(a',b',d') \rightarrow (a,1,1)}$ denotes the function that labels the edge $(a', b', d') \rightarrow (a, 1, 1)$ in the transition diagram, which we evaluate at the subsequence $S_i, \dots, S_{i+L(a)-1}$. In the update rule, the summand “1” corresponds to the configuration that consists of only the transcription factor a that binds at positions $i, \dots, i + L(a) - 1$, and the two sums correspond to the end-of-transcription factor states $(a', 1, L(a'))$ and the post-transcription factor states $(a', 2, l)$, respectively.

6.3.2 Head state Markov chain

We now describe how to sample a configuration c from the distribution $P(c)$ given in (6.8), using the efficient computation of the terms $f_{a,b,d}(S, i)$ described in the previous subsection. Following the backtrack algorithm, we perform a backward walk which we denote by $(X_{|S|}, X_{|S|-1}, \dots, X_1)$ in the transition diagram. That is, the walk is a path along backward edges in the transition diagram such as the one depicted in Figure 6.1. By sampling backward steps appropriately, the resulting configuration c then will be sampled from the distribution $P(c)$. As a shortcut, we will keep track of only the beginning positions of binding, which we call the *head states*'. Note that these head states $(a, 1, 1)$ fully determine a backward walk on a given sequence S , so we can encode a configuration by writing all head states as $(a, 1, 1, i)$, where a is the transcription factor and i is the initial binding position along S .

We let $Y_n = (a_n, 1, 1, i_n)$ denote the n -th head state in the *backward* chain, so that Y_1 is the head state of the last transcription factor that is bound to the sequence in the forward walk, and the positions i_n of binding are decreasing in n . In order to specify how to sample a walk (Y_1, Y_2, \dots) of head states, we now give the transition probabilities of this backward Markov chain. For convenience, we introduce two additional dummy states, START and END, which represent the state after all bound transcription factors and the state before any bound transcription factors, respectively. Letting $Y_0 = \text{START}$, we have the following initial distribution for the first head state Y_1 :

$$\begin{aligned} P(Y_1 = \text{END} \mid Y_0 = \text{START}) &\propto 1 \\ P(Y_1 = (a, 1, 1, i) \mid Y_0 = \text{START}) &\propto f_{a,1,1}(S, i), \end{aligned}$$

where the proportionality constant is the same for all $1 \leq a \leq m$ and $1 \leq i < |S| - L(a) + 1$. For all other transitions, the update rule is given by:

$$\begin{aligned} P(Y_n = \text{END} \mid Y_{n-1} = (a, 1, 1, i)) &\propto 1 \\ P(Y_n = (a', 1, 1, i') \mid Y_{n-1} = (a, 1, 1, i)) &\propto \\ &\begin{cases} f_{a',1,1}(S, i') \cdot \gamma(a, a', i - i' - L(a')) & \text{if } i' + L(a') \leq i \\ 0 & \text{else,} \end{cases} \end{aligned}$$

for $1 \leq a' \leq m$. For any given past head state $Y_{n-1} = y$, the proportionality constant is the same across all transition terms $P(Y_n = - \mid Y_{n-1} = y)$, so the unnormalized transition weights above can be used to sample the backward walk. (Recall that the previous section explained how to compute the values of $f_{a,b,d}(S, i)$ efficiently.) We now prove that a walk obtained in this manner is equivalent to sampling from a configuration from the probability distribution $P(c)$.

Proposition 6.3.1. *For a fixed sequence S , let $Y = (Y_0 = \text{START}, Y_1, Y_2, \dots, \text{END})$ denote a random walk obtained from the update rules defined above. Then the corresponding configuration c_Y is sampled according to the probability distribution $P(c)$ given in (6.8).*

Proof. We begin by computing the proportionality constants in the two update rules for (Y_n) . First for the initial distribution, concerning the terms $P(Y_1 = - \mid Y_0 = \text{START})$, the proportionality

constant is

$$\begin{aligned}
& 1 + \sum_{a=1}^m \sum_{i=1}^{|S|-L(a)+1} f_{a,1,1}(S, i) \\
&= 1 + \sum_{a=1}^m \sum_{i=1}^{|S|-L(a)} f_{a,1,1}(S, i) + \sum_{a=1}^m f_{a,1,1}(S, |S| - L(a) + 1) \\
&= 1 + \sum_{a=1}^m \sum_{l=1}^{|S|-L(a)} f_{a,2,l}(S, |S|) + \sum_{a=1}^m f_{a,1,L(a)}(S, |S|) \\
&= \sum_{c \in \mathcal{C}} W(c).
\end{aligned}$$

We remark that the second equality above is due to the “1” factors on the relevant arrows in the transition diagram, and the third equality is simply equation (6.10). Note also that this gives an equation for $\sum_{c \in \mathcal{C}} W(c)$. Using similar techniques, we compute the proportionality constant for the update rule concerning $P(Y_n = \cdot \mid Y_{n-1} = (a, 1, 1, i))$:

$$\begin{aligned}
& 1 + \sum_{a'=1}^m \sum_{i'=1}^{i-L(a')} f_{a',1,1}(S, i') \cdot \gamma(a, a', i - i' - L(a')) \\
&= 1 + \sum_{a'=1}^m \sum_{i'=1}^{i-L(a')-1} f_{a',1,1}(S, i') \cdot \gamma(a, a', i - i' - L(a')) \\
&\quad + \sum_{a'=1}^m f_{a',1,1}(S, i - L(a)) \cdot \gamma(a, a', 0) \\
&= 1 + \sum_{a'=1}^m \sum_{l=1}^{i-L(a')-1} f_{a',2,l}(S, i - 1) \cdot \gamma(a, a', l) \\
&\quad + \sum_{a'=1}^m f_{a',1,L(a')}(S, i - 1) \cdot \gamma(a, a', 0) \\
&= f_{a,1,1}(S, i) \left(\tau_a \frac{P_a(S_i, \dots, S_{i+L(a)-1})}{P_B(S_i, \dots, S_{i+L(a)-1})} \right)^{-1}.
\end{aligned}$$

The final equality is equation (6.11).

Now fix a backward walk, which we denote by

$$Y = (Y_0 = \text{START}, Y_1 = (a_1, 1, 1, i_1), Y_2 = (a_2, 1, 1, i_2), \dots, Y_{k+1} = \text{END}),$$

and let c_Y be the corresponding configuration (so, it consists of transcription factors a_n with beginning binding positions i_n). First, consider the case c_Y is the empty configuration: $Y = (Y_0 = \text{START}, Y_1 = \text{END})$. Having computed the proportionality constant in the first update rule above,

the probability of obtaining the trivial walk is

$$P(Y_0 = \text{START}, Y_1 = \text{END}) = \frac{1}{\sum_{c \in \mathcal{C}} W(c)},$$

which agrees with the probability of the empty configuration given in (6.8).

In the remaining case, the configuration c_Y contains at least one transcription factor, so $k \geq 1$. Then we have:

$$\begin{aligned} P(Y) &= P(Y_1 = (a_1, 1, 1, i_1) \mid Y_0 = \text{START}) \cdot \\ &\quad P(Y_2 = (a_2, 1, 1, i_2) \mid Y_1 = (a_1, 1, 1, i_1)) \cdot \\ &\quad \dots \cdot P(Y_{k+1} = \text{END} \mid Y_k = (a_k, 1, 1, i_k)) \\ &= \frac{f_{a_1,1,1}(S, i_1)}{\sum_{c \in \mathcal{C}} W(c)} \cdot \frac{f_{a_2,1,1}(S, i_2) \cdot \gamma(a_1, a_2, i_1 - i_2 - L(a_2))}{f_{a_1,1,1}(S, i_1) \left(\tau_{a_1} \frac{P_{a_1}(S_{i_1}, \dots, S_{i_1+L(a_1)-1})}{P_B(S_{i_1}, \dots, S_{i_1+L(a_1)-1})} \right)^{-1}} \cdot \\ &\quad \dots \cdot \frac{f_{a_k,1,1}(S, i_k) \cdot \gamma(a_{k-1}, a_k, i_{k-1} - i_k - L(a_k))}{f_{a_{k-1},1,1}(S, i_{k-1}) \left(\tau_{a_{k-1}} \frac{P_{a_{k-1}}(S_{i_{k-1}}, \dots, S_{i_{k-1}+L(a_{k-1})-1})}{P_B(S_{i_{k-1}}, \dots, S_{i_{k-1}+L(a_{k-1})-1})} \right)^{-1}} \cdot \\ &\quad \cdot \frac{1}{f_{a_k,1,1}(S, i_k) \left(\tau_{a_k} \frac{P_{a_k}(S_{i_k}, \dots, S_{i_k+L(a_k)-1})}{P_B(S_{i_k}, \dots, S_{i_k+L(a_k)-1})} \right)^{-1}} \\ &= \frac{W(c_Y)}{\sum_{c \in \mathcal{C}} W(c)}. \end{aligned}$$

The second equality comes from the proportionality constants computed earlier, and the third equality comes from canceling terms and applying the definition of $W(c)$. Therefore, we conclude that $P(Y)$ equals the probability $P(c_Y)$ as given in (6.8). \square

Notice that the update rules of our backward walk require evaluations of $f_{a,b,d}(S, i)$ at only head states (i.e., where $(b, d) = (1, 1)$). By computing and storing the values of f at only head states, we can reduce our storage requirements from $O(|S|^2)$ to $O(|S|)$. The following formula demonstrates how to compute $f_{a,1,1}(S, i)$ using only terms of the form $f_{a',1,1}(S, i')$ for positions $i' < i$:

$$f_{a,1,1}(S, i) = \tau_a \frac{P_a(S_i, \dots, S_{i+L(a)-1})}{P_B(S_i, \dots, S_{i+L(a)-1})}. \quad (6.12)$$

$$\left(1 + \sum_{a'=1}^m \sum_{i'=1}^{i-L(a')} f_{a',1,1}(S, i') \gamma(a, a', i - i' - L(a')) \right). \quad (6.13)$$

The correctness of this formula comes from recalling that $f_{a',1,1}(S, i') = f_{a',1,l}(S, i' + l - 1)$ for all $1 \leq l \leq L(a)$, and $f_{a',1,1}(S, i') = f_{a',2,l}(S, i' + L(a') + l - 1)$ for all $1 \leq l$; by making these substitutions, it then follows that this recursion is equivalent to the one given earlier in (6.11). Therefore, once these values of $f_{a,1,1}(S, i)$ are stored for all $i \leq |S| - L(a) + 1$, the backward walk

($Y_0 = \text{START}, Y_1, Y_2, \dots, \text{END}$) can be sampled easily, and this therefore establishes how to sample from the distribution $P(c)$ by sampling walks. We can now approximate $P(E)$ in the following way:

$$P(E) \approx \frac{1}{M} \sum_{i=1}^M P(E|c_i), \quad (6.14)$$

where M is the number of samples, and the c_i denote configurations sampled independently according to $P(c)$. Therefore, we have shown how to estimate the gene expression function.

6.3.3 Log coordinates

For numerical reasons, we perform all computations in log coordinates; for details, see the book of Durbin *et al.* [DEKM98]. In this section, we record the relevant formulas. First, $\log W(c)$ now takes the following form:

$$\begin{aligned} \log W(c) = & \left(\sum_{i=1}^k \log \tau_{f(i)} \right) \\ & + \left(\sum_{i=1}^k \sum_{j=0}^{L(f(i))-1} \log M_{f(i)}^{j+1}(S_{p(i)+j}) \right) \\ & + \left(\sum_{i=1}^k L(f(i)) \cdot \log 4 \right) \\ & + \left(\sum_{i=1}^{k-1} \log \gamma (f(i), f(i+1), p(i+1) - p(i) - L(f(i))) \right). \end{aligned}$$

Recall that when a transcription factor a binds to a sequence S beginning at position i , this introduces the multiplicative factor $\tau_a \frac{P_a(S_i, \dots, S_{i+L(a)-1})}{P_B(S_i, \dots, S_{i+L(a)-1})}$ into $W(c)$. The log version of this factor we write as follows:

$$\psi(a, S, i) := \log \tau_a + \sum_{j=0}^{L(a)-1} (\log M_a^{j+1}(S_{i+j}) + \log 4) .$$

Therefore, the log version of the $f_{a,1,1,i}(S, i)$ update rule (6.12) is:

$$\log f_{a,1,1}(S, i) = \psi(a, S, i) + \log \left(1 + \sum_{a'=1}^m \sum_{i'=1}^{i-L(a')} f_{a',1,1}(S, i') \gamma(a, a', i - i' - L(a')) \right) .$$

The log of a sum should be computed recursively according to the rule $\log(x+y) = \log(x) + \log(1 + \exp(\log(y) - \log(x)))$ for $x > y$. Similarly, $\log(\sum_{c \in \mathcal{C}} W(c))$ should be computed according to this rule.

Next is the formula for $\log P(c)$:

$$\log P(c) = \log W(c) - \log \left(\sum_{c' \in \mathcal{C}} W(c') \right).$$

The update rule given before Proposition 6.3.1 becomes the following:

$$P(Y_n = (a', 1, 1, i') \mid Y_{n-1} = (a, 1, 1, i)) \propto \begin{cases} \exp(\log f_{a',1,1}(S, i') + \log \gamma(a, a', i - i' - L(a'))) & \text{if } i' + L(a') \leq i \\ 0 & \text{else,} \end{cases}$$

From these equations, a backward walk can be sampled, and $P(E)$ can be estimated according to equation (6.14).

6.3.4 Maximum-weight configurations

We are interested in the expression levels computed from the highest-weight configurations. Here we describe how these maximum-weight configurations are computed. We work in log-coordinates. We for transcription factors a and positions i for which $|S| - i \geq L(a) - 1$,

$$g(a, S, i) := \max_{c \in \tilde{\mathcal{C}}_{i,(a,1,1)}} (\log W(c)).$$

Therefore, the log of the highest weight of a configuration is equal to

$$\max_{c \in \mathcal{C}} (\log W(c)) = \max \left(0, \max_{\substack{1 \leq a \leq m \\ 1 \leq i \leq |S| - L(a) + 1}} (g(a, S, i)) \right).$$

Note that the “0” is the log-weight of the empty configuration. Recall that any configuration $c \in \tilde{\mathcal{C}}_{i,(a,1,1)}$ is obtained by adjoining the final binding site to either a configuration $c' \in \tilde{\mathcal{C}}_{i',(a',1,1)}$ for some transcription factor a' and $1 \leq i' \leq i - L(a') + 1$, or to the empty configuration. Hence, the terms $g(a, S, i)$ can be computed according to the following recursion:

$$g(a, S, i) = \psi(a, S, i) + \max \left(0, \max_{\substack{1 \leq a' \leq m \\ 1 \leq i' \leq |S| - L(a') + 1}} (g(a', S, i') + \log \gamma(a, a', i - L(a') - i')) \right).$$

6.4 Computing the number of configurations

This section gives a recursion to compute the number of configurations on a sequence of some given length. Our setup consists of transcription factors $a = 1, 2, \dots, m$, which bind to subsequences of length $L(1), L(2), \dots, L(m)$, respectively; for the Segal *et al.* model, we have $m = 8$ with binding lengths that range between 9 and 15. Next, note that the set of configurations

C depends only on the length of the sequence of interest. So, we define for sequence lengths i the following expression:

$$\begin{aligned} C(i) &:= \text{number of configurations on a sequence of length } i \\ &= \text{number of configurations that end with binding of the transcription factor } a \text{ at} \\ &\quad \text{position } i + 1 \text{ on a sequence of length } > i + L(a) . \end{aligned} \tag{6.15}$$

Note that the second equality above holds for all $a = 1, 2, \dots, m$, because a configuration that ends with binding at position $i + 1$ is obtained by adjoining that final binding to a configuration on the subsequence of length i . Also, we use the convention $C(i) = 0$ for $i < 1$. Recall that the sum in the definition of $P(E)$ in (6.1) is over $C(|S|)$ configurations; the sequence lengths that Segal *et al.* consider satisfy $500 \leq |S| \leq 2500$. In order to show how to compute $C(-)$, we define for $i \in \mathbb{Z}_{>0}$ and $a = 1, 2, \dots, m$

$$\begin{aligned} B(i, a) &:= \sum_{i'=1}^{i-L(a)} C(i') \\ &= \text{number of configurations on a sequence of length } i \\ &\quad \text{that end with binding of the transcription factor } a . \end{aligned} \tag{6.16}$$

The second equality (6.16) follows from (6.15) above. The next proposition gives the relationship between the terms $C(i)$ and $B(i, a)$ and a recursion for computing them.

Proposition 6.4.1. *The sequences $C(i)$ and $B(i, a)$ defined above satisfy the following relations:*

$$\begin{aligned} C(i) &= 1 + \sum_{a=1}^m B(i, a) \quad \text{for } i \geq 1 \\ B(1, a) &= \begin{cases} 0 & \text{if } L(a) > 1 \\ 1 & \text{if } L(a) = 1 \end{cases} \\ B(i, a) &= B(i - 1, a) + C(i - L(a)) \quad \text{for } i > 1 . \end{aligned}$$

These can be used to compute the values of $C(i)$ and $B(i, a)$.

Proof. The equation for $C(i)$ follows from equation (6.15) and the fact that a configuration is either empty or ends with the binding of some transcription factor. Next, the equation for $B(1, a)$ is straightforward. As for the equation for $B(i, a)$, recall that this term counts the configurations on a sequence of length i that end with binding of the transcription factor a . These configurations have two types: (i) those configurations with at least two bindings in which the last two binding sites are directly next to each other (with no unbound bases between them), and (ii) those configurations in which the base one position before the beginning binding position of the last transcription factor is unbound. For $i > 1$, we see that $C(i - L(a))$ counts configurations of type (i). Also, $B(i - 1, a)$

counts those of type (ii), via the following bijection:

$$\begin{aligned} \{\text{configurations counted by } B(i-1, a)\} &\rightarrow \{\text{configurations of type (ii)}\} \\ c &\mapsto \text{configuration obtained from } c \text{ by changing} \\ &\quad \text{the last binding site of the transcription factor } a \\ &\quad \text{at some position } i' \text{ to bind instead at } i' + 1 . \end{aligned}$$

This completes the proof. □

Example 6.4.2. The shortest sequence considered by Segal *et al.* has length 500. Under their regime (with $m = 8$ transcription factors of binding lengths $L(i) = 9, 9, 11, 11, 12, 12, 14, 15$), the number of configurations computed according to Proposition 6.4.1 equals

$$C(500) \approx 2.3 \times 10^{66} .$$

We see that dynamic programming as described in Section 6.3 is necessary for estimating $P(E)$ as enumeration of all configurations is not feasible.

Chapter 7

Further directions

In this chapter, we highlight some open problems in chemical reaction network theory.

7.1 Basic chemical reaction network theory questions

The following four open problems were posed by Feinberg [Fei80, §4], and they concern chemical reaction networks taken with mass-action kinetics.

1. Describe the class of chemical reaction networks such that, regardless of the values of the rate constants, their resulting differential equations admit *at least* one positive steady state in each invariant polyhedron.
2. Describe the class of chemical reaction networks such that, regardless of the values of the rate constants, their resulting differential equations admit *precisely* one positive steady state in each invariant polyhedron.
3. Describe the class of chemical reaction networks such that, regardless of the values of the rate constants, every positive steady state is locally asymptotically stable with respect to the corresponding invariant polyhedron.
4. Describe the class of chemical reaction networks such that, regardless of the values of the rate constants, there are no periodic solutions.

Question (1) above has a partial answer, which is discussed in the following section.

7.2 Existence of positive steady states

Consider the following questions: *Does there exist a reversible chemical reaction network without a positive steady state in some invariant polyhedron?*

The answer is apparently “no,” according to unpublished work of Deng *et al.* [DFJN], and in fact, this result holds even for weakly-reversible networks, i.e., those in which each connected component is strongly connected. Is there a proof of a more algebraic flavor?

7.3 Homotopy methods for multiple steady states

In recent work of Craciun, Helton, and Williams, homotopy methods are used to prove the existence and uniqueness of positive steady states for certain chemical reaction systems [CHW08]. This applies to proving the existence of multiple positive steady states as well. What is the simplest example of this?

7.4 Thermodynamic feasibility of toric dynamical systems

Conradi asked the following question: are toric dynamical systems automatically “thermodynamically feasible” in the sense of Beard *et al.* [BBCQ04]?

7.5 Boundary steady states without repelling neighborhoods

In Chapter 4, it is proven that boundary steady states that are vertices or facet-interior points of an invariant polyhedron have repelling neighborhoods. Does there exist a non-vertex, non-facet-interior point of an invariant polyhedron that does not have a repelling neighborhood?

Bibliography

- [ACK08] David F. Anderson, Gheorghe Craciun, and Thomas G. Kurtz. Product-form stationary distributions for deficiency zero chemical reaction networks. *To appear in Bull. Math. Biol.* Available at [arXiv:0803.3042](https://arxiv.org/abs/0803.3042), 2008.
- [ADS07a] David Angeli, Patrick De Leenheer, and Eduardo Sontag. *A Petri net approach to persistence analysis in chemical reaction networks*, pages 181–216. Springer-Verlag, Berlin, 2007.
- [ADS07b] David Angeli, Patrick De Leenheer, and Eduardo D. Sontag. A Petri net approach to the study of persistence in chemical reaction networks. *Math. Biosci.*, 210(2):598–618, 2007.
- [ADS09] David Angeli, Patrick De Leenheer, and Eduardo Sontag. On persistence and cascade decompositions of chemical reaction networks. Available at [arxiv:0905.1332](https://arxiv.org/abs/0905.1332), 2009.
- [AGH⁺08] Leonard Adleman, Manoj Gopalkrishnan, Ming-Deh Huang, Pablo Moisset, and Dustin Reishus. On the mathematics of the law of mass action. Available at [arXiv:0810.1108](https://arxiv.org/abs/0810.1108), 2008.
- [AH] Shaun Ault and Erik Holmgren. Dynamics of the Brusselator. Available at <http://www-dimat.unipv.it/boffi/teaching/download/Brusselator.pdf>.
- [Aki79] Ethan Akin. *The geometry of population genetics*. Springer-Verlag, New York, 1979.
- [Aki82] Ethan Akin. Cycling in simple genetic systems. *J. Math. Biol.*, 13(3):305–324, 1982.
- [And08] David F. Anderson. Global asymptotic stability for a class of nonlinear chemical equations. *SIAM J. Appl. Math.*, 68(5):1464–1476, 2008.
- [Ang08] David Angeli. On modularity and persistence of chemical reaction networks. In *Decision and Control, 2008. CDC 2008. 47th IEEE Conference on*, pages 2650–2655, Dec. 2008.
- [Arn88] Dennis Arnon. Geometric reasoning with logic and algebra. *Artif. Intell.*, 37(1-3):37–60, 1988.
- [AS06] David Angeli and Eduardo D. Sontag. Translation-invariant monotone systems, and a global convergence result for enzymatic futile cycles. *Nonlinear Anal.: Real World Appl.*, 9(1):128–140, 2006.

- [AS08] Murat Arcak and Eduardo Sontag. A passivity-based stability criterion for a class of biochemical reaction networks. *Math. Biosci. and Eng.*, 5(1):1, 2008.
- [AS10] David F. Anderson and Anne Shiu. The dynamics of weakly reversible population processes near facets. *SIAM J. Appl. Math.*, 70(6):1840–1858, 2010.
- [AT89] Venkat Anantharam and Pantelis Tsoucas. A proof of the Markov chain tree theorem. *Statist. Probab. Lett.*, 8(2):189–192, 1989.
- [BBCQ04] Daniel Beard, Eric Babson, Edward Curtis, and Hong Qian. Thermodynamic constraints for biochemical networks. *J. Theoret. Biol.*, 228(3):327–333, 2004.
- [BPR06] Saugata Basu, Richard Pollack, and Marie-Françoise Roy. *Algorithms in real algebraic geometry. 2nd ed.* Algorithms and Computation in Mathematics 10. Berlin: Springer, 2006.
- [BPS07] Niko Beerenwinkel, Lior Pachter, and Bernd Sturmfels. Epistasis and shapes of fitness landscapes. *Statist. Sinica*, 17(4):1317–1342, 2007.
- [BQ08] Daniel Beard and Hong Qian. *Chemical biophysics: quantitative analysis of cellular systems.* Cambridge University Press, 2008.
- [BS09] Michel Benaïm and Sebastian J. Schreiber. Persistence of structured populations in random environments. *Theor. popul. biol.*, 76:19–34, 2009.
- [CA00] Joshua L. Cherry and Frederick R. Adler. How to make a biological switch. *J. Theoret. Biol.*, 203(2):117–133, 2000.
- [CDSS09] Gheorghe Craciun, Alicia Dickenstein, Anne Shiu, and Bernd Sturmfels. Toric dynamical systems. *J. Symbolic Comput.*, 44(11):1551–1565, 2009.
- [CF05] Gheorghe Craciun and Martin Feinberg. Multiple equilibria in complex chemical reaction networks. I. The injectivity property. *SIAM J. Appl. Math.*, 65(5):1526–1546, 2005.
- [CF06] Gheorghe Craciun and Martin Feinberg. Multiple equilibria in complex chemical reaction networks. II. The species-reaction graph. *SIAM J. Appl. Math.*, 66(4):1321–1338, 2006.
- [CF10] Gheorghe Craciun and Martin Feinberg. Multiple equilibria in complex chemical reaction networks: Semiopen mass action systems. *SIAM J. Appl. Math.*, 70(6):1859–1877, 2010.
- [CFP05] Roberto Cordone, Luca Ferrarini, and Luigi Piroddi. Enumeration algorithms for minimal siphons in Petri nets based on place constraints. *IEEE T. Syst. Man. Cy. A*, 35(6):844–854, 2005.

- [CFR08] Carsten Conradi, Dietrich Flockerzi, and Jörg Raisch. Multistationarity in the activation of a MAPK: Parametrizing the relevant region in parameter space. *Math. Biosci.*, 211(1):105–131, 2008.
- [CFRS07] Carsten Conradi, Dietrich Flockerzi, Jörg Raisch, and Jörg Stelling. Subnetwork analysis reveals dynamic features of complex (bio)chemical networks. *Proc. Natl. Acad. Sci. USA*, 104(49):19175–19180, 2007.
- [Cha03] Madalena Chavez. *Observer design for a class of nonlinear systems, with applications to chemical and biological networks*. PhD thesis, Rutgers University, New Brunswick, NJ, 2003.
- [CHW08] Gheorghe Craciun, J. William Helton, and Ruth J. Williams. Homotopy methods for counting reaction network equilibria. *Math. Biosci.*, 216(2):140–149, 2008.
- [CLO07] David Cox, Jon Little, and Donal O’Shea. *Ideals, varieties, and algorithms: an introduction to computational algebraic geometry and commutative algebra*. Springer-Verlag, 2007.
- [Con08] Carsten Conradi. *Multistationarity in (bio)chemical reaction networks with mass action kinetics: model discrimination, robustness and beyond*. PhD thesis, Technische Universität Berlin, 2008.
- [CP03] Simon Cawley and Lior Pachter. HMM sampling and applications to gene finding and alternative splicing. *Bioinformatics*, 19(Supp. 2):ii36–ii41, 2003.
- [CP08] Gheorghe Craciun and Casian Pantea. Identifiability of chemical reaction networks. *J. Math. Chem.*, 44(1):244–259, 2008.
- [CPR09a] Gheorghe Craciun, Casian Pantea, and Grzegorz A. Rempala. A Dimension Reduction Method for Inferring Biochemical Networks. *Available at arXiv:0902.4417*, 2009.
- [CPR09b] Gheorghe Craciun, Casian Pantea, and Grzegorz A. Rempala. Algebraic methods for inferring biochemical networks: A maximum likelihood approach. *Comput. Biol. Chem.*, 33(5):361–367, 2009.
- [CTF06] Gheorghe Craciun, Yangzhong Tang, and Martin Feinberg. Understanding bistability in complex enzyme-driven reaction networks. *Proc. Natl. Acad. Sci. USA*, 103(23):8697–8702, 2006.
- [DAS07] Patrick De Leenheer, David Angeli, and Eduardo D. Sontag. Monotone chemical reaction networks. *J. Math. Chem.*, 41(3):295–314, 2007.
- [DEKM98] Richard Durbin, Sean Eddy, Anders Krogh, and Graeme Mitchison. *Biological sequence analysis: Probabilistic models of proteins and nucleic acids*. Cambridge University Press, 1998.

- [DES98] Persi Diaconis, David Eisenbud, and Bernd Sturmfels. Lattice walks and primary decomposition. In *Mathematical essays in honor of Gian-Carlo Rota (Cambridge, MA, 1996)*, volume 161 of *Progr. Math.*, pages 173–193. Birkhäuser, Boston, MA, 1998.
- [DFJN] Jian Deng, Martin Feinberg, Chris Jones, and Adrian Nachman. On the steady states of weakly reversible chemical reaction networks. Preprint, 2008.
- [DHX⁺08] Renaud Dentin, Susan Hedrick, Jianxin Xie, John Yates III, and Marc Montminy. Hepatic Glucose Sensing via the CREB Coactivator CRT2. *Science*, 319(5868):1402–1405, 2008.
- [DM10] Alicia Dickenstein and Mercedes Pérez Millán. How far is complex balancing from detailed balancing? Available at [arXiv:1001.0947](https://arxiv.org/abs/1001.0947), 2010.
- [DMM10] Alicia Dickenstein, Laura F. Matusevich, and Ezra Miller. Combinatorics of binomial primary decomposition. *Math. Z.*, 264(4):745–763, 2010.
- [DSS09] Mathias Drton, Bernd Sturmfels, and Seth Sullivant. *Lectures on Algebraic Statistics, Oberwolfach Seminars vol. 39*. Springer, 2009.
- [EF] Phillipp Ellison and Martin Feinberg. CRNT Toolbox. Available at <http://www.che.eng.ohio-state.edu/~feinberg/crnt/>.
- [Ell98] Phillipp Ellison. *The advanced deficiency algorithm and its applications to mechanism discrimination*. PhD thesis, University of Rochester, 1998.
- [ES96] David Eisenbud and Bernd Sturmfels. Binomial ideals. *Duke Math. J.*, 84(1):1–45, 1996.
- [ET89] Péter Érdi and János Tóth. *Mathematical models of chemical reactions – Theory and applications of deterministic and stochastic models*. Manchester University Press, Manchester, U.K., 1989.
- [Fei72] Martin Feinberg. Complex balancing in general kinetic systems. *Arch. Ration. Mech. Anal.*, 49(3):187–194, 1972.
- [Fei79] Martin Feinberg. Lectures on chemical reaction networks. Notes of lectures given at the Mathematics Research Center of the University of Wisconsin in 1979, available at <http://www.che.eng.ohio-state.edu/~feinberg/LecturesOnReactionNetworks>, 1979.
- [Fei80] Martin Feinberg. *Chemical oscillations, multiple equilibria, and reaction network structure*, pages 59–130. Academic Press, 1980.
- [Fei87] Martin Feinberg. Chemical reaction network structure and the stability of complex isothermal reactors I. The deficiency zero and deficiency one theorems. *Chem. Eng. Sci.*, 42(10):2229–2268, 1987.

- [Fei89] Martin Feinberg. Necessary and sufficient conditions for detailed balancing in mass action systems of arbitrary complexity. *Chem. Eng. Sci.*, 44(9):1819–1827, 1989.
- [Fei95] Martin Feinberg. The existence and uniqueness of steady states for a class of chemical reaction networks. *Arch. Ration. Mech. Anal.*, 132(4):311–370, 1995.
- [Fei00a] Martin Feinberg. Optimal reactor design from a geometric viewpoint—III. Critical CFSTRs. *Chem. Eng. Sci.*, 55(17):3553–3565, 2000.
- [Fei00b] Martin Feinberg. Optimal reactor design from a geometric viewpoint. Part II. Critical sidestream reactors. *Chem. Eng. Sci.*, 55(13):2455–2479, 2000.
- [FH97] Martin Feinberg and Diane Hildebrandt. Optimal reactor design from a geometric viewpoint—I. Universal properties of the attainable region. *Chem. Eng. Sci.*, 52(10):1637–1665, 1997.
- [Gat01] Karin Gatermann. Counting stable solutions of sparse polynomial systems in chemistry. In *Symbolic computation: solving equations in algebra, geometry, and engineering (South Hadley, MA, 2000)*, volume 286 of *Contemp. Math.*, pages 53–69. Amer. Math. Soc., Providence, RI, 2001.
- [GH02] Karin Gatermann and Birkett Huber. A family of sparse polynomial systems arising in chemical reaction systems. *J. Symbolic Comput.*, 33(3):275–305, 2002.
- [Gna09a] Gilles Gnacadja. Reachability, persistence, and constructive chemical reaction networks. Preprint, available at <http://math.gillesgnacadja.info/files/ConstructiveCRNT.html>, 2009.
- [Gna09b] Gilles Gnacadja. Univalent positive polynomial maps and the equilibrium state of chemical networks of reversible binding reactions. *Adv. in Appl. Math.*, 43(4):394–414, 2009.
- [GS02] Daniel Grayson and Michael Stillman. Macaulay 2, a software system for research in algebraic geometry. Available at <http://www.math.uiuc.edu/Macaulay2/>, 2002.
- [GSG⁺07] Gilles Gnacadja, Alex Shoshitaishvili, Michael J. Gresser, Brian Varnum, David Balaban, Mark Durst, Chris Vezina, and Yu Li. Monotonicity of interleukin-1 receptor–ligand binding with respect to antagonist in the presence of decoy receptor. *J. Theoret. Biol.*, 244(3):478–488, 2007.
- [Gub03] Jonathan Guberman. Mass action reaction networks and the deficiency zero theorem. Undergraduate thesis, Harvard University, 2003.
- [Gun03] Jeremy Gunawardena. Chemical reaction network theory for in-silico biologists. Technical report, available at <http://vcp.med.harvard.edu/papers/crnt.pdf>, 2003.

- [GUV07] Carlos A. Gomez-Urbe and George C. Verghese. Mass fluctuation kinetics: Capturing stochastic effects in systems of chemical reactions through coupled mean-variance computations. *The Journal of Chemical Physics*, 126(2):024109, 2007.
- [GW05] Karin Gatermann and Matthias Wolfrum. Bernstein’s second theorem and Viro’s method for sparse polynomial systems in chemistry. *Adv. in Appl. Math.*, 34(2):252–294, 2005.
- [GX03] Karin Gatermann and Bican Xia. Existence of 3 Positive Solutions of Systems from Chemistry. Available at www.orcca.on.ca/gatermann/webpage/publi.html, 2003.
- [HJ72] Fritz Horn and Roy Jackson. General mass action kinetics. *Arch. Ration. Mech. Anal.*, 47(2):81–116, 1972.
- [Hor72] Fritz Horn. Necessary and sufficient conditions for complex balancing in chemical kinetics. *Arch. Ration. Mech. Anal.*, 49(3):172–186, 1972.
- [Hor73a] Fritz Horn. Dynamics of open reaction systems II. Stability and the complex graph. *Proc. Roy. Soc. Lond. Ser. A*, 334:313–330, 1973.
- [Hor73b] Fritz Horn. Stability and complex balancing in mass-action systems with three short complexes. *Proc. Roy. Soc. Lond. Ser. A*, 334:331–342, 1973.
- [Hor74] Fritz Horn. The dynamics of open reaction systems. In *Mathematical aspects of chemical and biochemical problems and quantum chemistry (Proc. SIAM-AMS Sympos. Appl. Math., New York, 1974)*, pages 125–137. SIAM–AMS Proceedings, Vol. VIII. Amer. Math. Soc., Providence, R.I., 1974.
- [HRS00] Birkett Huber, Jörg Rambau, and Francisco Santos. The Cayley trick, lifting subdivisions and the Bohne-Dress theorem on zonotopal tilings. *J. Eur. Math. Soc. (JEMS)*, 2(2):179–198, 2000.
- [JG00] Michael Joswig and Evgenij Gawrilow. Polymake: a framework for analyzing convex polytopes. In Gil Kalai and Günter Ziegler, editors, *Polytopes — Combinatorics and Computation*, pages 43–74. Birkhäuser, 2000.
- [JLSS07] Abdul Salam Jarrah, Reinhard Laubenbacher, Brandilyn Stigler, and Michael Stillman. Reverse-engineering of polynomial dynamical systems. *Adv. in Appl. Math.*, 39(4):477–489, 2007.
- [KA56] E.L. King and C. Altman. A schematic method of deriving the rate laws for enzyme-catalyzed reactions. *J. Phys. Chem.*, 60:1375–1378, 1956.
- [Kah10] Thomas Kahle. Decompositions of binomial ideals. *To appear in special issue “Algebraic Methods in Computational Statistics” of Annals of the Institute of Statistical Mathematics (AISM)*, 2010.

- [Kar07] Andrei Karatkevich. *Dynamic analysis of Petri net-based discrete systems*. Springer, 2007.
- [KSP07a] Lars Kuepfer, Uwe Sauer, and Pablo Parrilo. Efficient classification of complete parameter regions based on semidefinite programming. *BMC Bioinf.*, 8(1):12, 2007.
- [KSP07b] Lars Kuepfer, Uwe Sauer, and Pablo Parrilo. Efficient classification of complete parameter regions based on semidefinite programming. *BMC Bioinf.*, 8(1):12, 2007.
- [Kur72] Thomas G. Kurtz. The relationship between stochastic and deterministic models for chemical reactions. *J. Chem. Phys.*, 57:2976–2978, 1972.
- [Kur81] Thomas G. Kurtz. *Approximation of population processes*, volume 36 of *CBMS-NSF Regional Conference Series in Applied Mathematics*. Society for Industrial and Applied Mathematics (SIAM), Philadelphia, Pa., 1981.
- [Lip07] Mark Lipson. Differential and graphical approaches to multistability in chemical reaction networks. Undergraduate thesis, Harvard University. Available at [arXiv:0709.0125](https://arxiv.org/abs/0709.0125), 2007.
- [Lis85] John E. Lisman. A mechanism for memory storage insensitive to molecular turnover: a bistable autophosphorylating kinase. *Proc. Natl. Acad. Sci. USA*, 82(9):3055–3057, 1985.
- [LJ09] Songxin Liang and David Jeffrey. Automatic computation of the complete root classification for a parametric polynomial. *J. Symbolic Comput.*, 44(10):1487–1501, 2009.
- [LK99] Michel Laurent and Nicolas Kellershohn. Multistability: a major means of differentiation and evolution in biological systems. *Trends Biochem. Sci.*, 24(11):418–422, 1999.
- [LR07] Daniel Lazard and Fabrice Rouillier. Solving parametric polynomial systems. *J. Symbolic Comput.*, 42(6):636–667, 2007.
- [McK95] Timothy McKeithan. Kinetic proofreading in T-cell receptor signal transduction. *Proc. Natl. Acad. Sci. USA*, 92(11):5042–5046, 1995.
- [MG08] Arjun K. Manrai and Jeremy Gunawardena. The geometry of multisite phosphorylation. *Biophys. J.*, 95(12):5533–5543, 2008.
- [MS05] Ezra Miller and Bernd Sturmfels. *Combinatorial commutative algebra*, volume 227 of *Graduate Texts in Mathematics*. Springer-Verlag, New York, 2005.
- [MSG⁺09] S.S. Manu, A.V. Spirov, V.V. Gursky, H. Janssens, A.R. Kim, O. Radulescu, C.E. Vanario-Alonso, D.H. Sharp, M. Samsonova, and J. Reinitz. Canalization of gene expression and domain shifts in the *Drosophila* blastoderm by dynamical attractors. *PLoS Comput. Biol.*, 5(3), 2009.

- [PS05] Lior Pachter and Bernd Sturmfels. *Algebraic statistics for computational biology*. Cambridge University Press, 2005.
- [Rou09] Bjarke Roune. The Slice Algorithm for irreducible decomposition of monomial ideals. *J. Symbolic Comput.*, 44(4):358–381, 2009.
- [RSW92] Yuri Rabinovich, Alistair Sinclair, and Avi Wigderson. Quadratic dynamical systems. In *Quadratic dynamical systems, Proc. 33rd Annual Symposium on Foundations of Computer Science (FOCS)*, pages 304–313, 1992.
- [SA09] Nicola Soranzo and Claudio Altafini. ERNEST: a toolbox for chemical reaction network theory. *Bioinformatics*, 25(21):2853–2854, 2009.
- [SC94] David Siegel and Yu-Fang Chen. Global stability of deficiency zero chemical networks. *Canadian Appl. Math Quarterly*, 2:413–434, 1994.
- [Seg98] Lee A. Segel. Multiple attractors in immunology: theory and experiment. *Biophys. J.*, 72(1-2):223–230, 1998.
- [Shi08] Anne Shiu. The smallest multistationary mass-preserving chemical reaction network. *Lect. Notes Comput. Sc.*, 5147:172–184, 2008.
- [SM00] David Siegel and Debbie MacLean. Global stability of complex balanced mechanisms. *J. Math. Chem.*, 27(1-2):89–110, 2000.
- [Son01] Eduardo D. Sontag. Structure and stability of certain chemical networks and applications to the kinetic proofreading model of T-cell receptor signal transduction. *IEEE Trans. on Automat. Control*, 46(7):1028–1047, 2001.
- [SPA05] Michael Samoilov, Sergey Plyasunov, and Adam Arkin. Stochastic amplification and signaling in enzymatic futile cycles through noise-induced bistability with oscillations. *Proc. Natl. Acad. Sci. USA*, 102(7):2310, 2005.
- [SRSS⁺08] Eran Segal, Tali Raveh-Sadka, Mark Schroeder, Ulrich Unnerstall, and Ulrike Gaul. Predicting expression patterns from regulatory sequence in drosophila segmentation. *Nature*, 451(7178):535–540, 01 2008.
- [SS09] Anne Shiu and Bernd Sturmfels. Siphons in chemical reaction networks. *To appear in Bull. Math. Biol.* Available at [arxiv:0904.4529](https://arxiv.org/abs/0904.4529), 2009.
- [Sta99] Richard P. Stanley. *Enumerative combinatorics. Vol. 2*, volume 62 of *Cambridge Studies in Advanced Mathematics*. Cambridge University Press, Cambridge, 1999.
- [Stu96] Bernd Sturmfels. *Gröbner bases and convex polytopes*, volume 8 of *University Lecture Series*. American Mathematical Society, Providence, RI, 1996.
- [Stu02] Bernd Sturmfels. *Solving systems of polynomial equations*, volume 97 of *CBMS Regional Conference Series in Mathematics*. Published for the Conference Board of the Mathematical Sciences, Washington, DC, 2002.

- [TG09a] Matthew Thomson and Jeremy Gunawardena. The rational parameterisation theorem for multisite post-translational modification systems. *J. Theoret. Biol.*, 261(4):626–636, 2009.
- [TG09b] Matthew Thomson and Jeremy Gunawardena. Unlimited multistability in multisite phosphorylation systems. *Nature*, 460(7252):274–277, 2009.
- [Tut48] William T. Tutte. The dissection of equilateral triangles into equilateral triangles. *Proc. Cambridge Philos. Soc.*, 44:463–482, 1948.
- [VK85] Aĭzik Vol’pert and Sergeĭ Khudĭaev. *Analysis in Classes of Discontinuous Functions and Equations of Mathematical Physics*. Springer, Dordrecht, 1985.
- [WH95] Thomas Wilhelm and Reinhart Heinrich. Smallest chemical reaction system with Hopf bifurcation. *J. Math. Chem.*, 17(1):1–14, 1995.
- [WH96] Thomas Wilhelm and Reinhart Heinrich. Mathematical analysis of the smallest chemical reaction system with Hopf bifurcation. *J. Math. Chem.*, 19(2):111–130, 1996.
- [Wil09] Thomas Wilhelm. The smallest chemical reaction system with bistability. *BMC Syst. Biol.*, 3:90, 2009.
- [WS08] Liming Wang and Eduardo Sontag. On the number of steady states in a multiple futile cycle. *J. Math. Biol.*, 57(1):29–52, 2008.
- [WX05] Dongming Wang and Bican Xia. Stability analysis of biological systems with real solution classification. In *ISSAC’05*, pages 354–361 (electronic). ACM, New York, 2005.
- [Yan99] Lu Yang. Recent advances on determining the number of real roots of parametric polynomials. *J. Symbolic Comput.*, 28(1-2):225–242, 1999.
- [ZGG⁺09] R.P. Zinzen, C. Girardot, J. Gagneur, M. Braun, and E.E.M. Furlong. Combinatorial binding predicts spatio-temporal cis-regulatory activity. *Nature*, 462(7269):65–70, 2009.
- [Zie95] Günter Ziegler. *Lectures on polytopes*, volume 152 of *Graduate Texts in Mathematics*. Springer-Verlag, New York, 1995.