POSITIVE STEADY-STATE VARIETIES OF 2-REACTION CHEMICAL REACTION NETWORKS

A THESIS

Presented to

The Faculty of the Department of Mathematics and Computer Science

Colorado College

In Partial Fulfillment of the Requirements for the Degree

Bachelor of Arts

By

Galileo Fries

April 2024

1 Introduction

Chemical reaction network theory is utilized in many areas of science including systems biology, epidemiology, and ecology to model systems where objects interact to form new products. For example, the authors of [8] study reaction networks occurring in yeast which exhibit switch-like behavior, finding the smallest networks with the property of bistability. Many biological networks are bistable, meaning they have two distinct stable equilibrium points. Understanding networks' equilibrium points, or steady-states, has been a major focus of mathematical chemical reaction network theory. When viewing networks as systems which change over time, these steady-states are conditions which result in no net change in species' concentrations over time. Following the assumption of mass-action kinetics, a chemical reaction network can be translated into a system of polynomial differential equations whose solution set is an algebraic variety.

While many different areas of mathematics can be used to analyze chemical reaction networks to understand their various properties–including graph theory, combinatorics, and dynamical systems–algebraic geometry provides valuable tools to study the variety produced by networks' corresponding systems of equations. Traditional modeling approaches require numerical data that is often difficult or impossible to determine in complex biological systems; algebraic geometry provides a more flexible approach that analyzes networks' mathematical properties independent of any specific numerical values, concisely explained in [5]. For a more detailed discussion of algebraic geometry's use in chemical reaction network theory, the types of properties it can illuminate, and the types of biological networks it can model, see [4].

The solution set of a network's corresponding equations describe all its equilibrium points, but the biologically relevant portions of the variety are those components which intersect the interior of the real positive orthant, as they correspond to steady-states with positive concentrations of all reacting species. While mathematical solutions with zero or negative concentrations may exist, we focus on nontrivial and biologically feasible equilibrium points of a network. This set is known as the positive steady-state locus, which is not necessarily an algebraic variety but rather is a semi-algebraic set. In this thesis, we instead examine the components of the steady-state variety that nontrivially intersect the positive orthant, called the positive steady-state variety. The analysis of this variety requires techniques from real algebraic geometry that, in some restricted cases, may give a full description of the defining ideal. However, this computation continues to pose a significant challenge in general.

In this thesis, we focus on small networks: those with two reactions and at-most-bimolecular complexes. Stemming from an initial systematic study of 1-, 2-, and 3-species small networks, we identify which of these are capable of supporting positive steady-states (Theorem 3.1). When studying networks with two reactions, the corresponding equations are binomial, giving rise to binomial ideals and toric steady-state varieties. Other researchers have focused on networks with toric steady-states, notably in [6]; the conditions provided here are simplified significantly to better fit the 2-reaction case, and were developed independently. While most biological networks contain many more than two reactions, toric varieties do appear in biological contexts, including multi-site phosphorylation networks as studied in [1].

The classification theorems in this thesis rely on observations of the network's reactant complexes and stoichiometric matrix, which is simple to construct directly from the network. These conditions eliminate the need for potentially computationally expensive calculations of polynomial systems' solution sets or the more involved matrix calculations used in [6]. Additionally, beyond identifying small networks where a positive steady-state variety exists, we classify 2- and 3-species networks by the type of variety produced by giving additional conditions on the networks' reactant complexes (Sections 5 and 6); give a systematic analysis of 1-species, at-most-bimolecular networks (Section 4); and begin to build techniques to extend these classifications to networks with complexes of higher molecularity (Section 8).

2 Background

To understand the mathematical definition of chemical reaction networks, it is useful to follow an example to see how a chemical reaction network gives rise to a polynomial dynamical system. Consider a closed system containing two types of objects, or *species*, labeled A and B whose interactions are represented diagrammatically as follows:

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

This pictorial representation gives that within this closed system, one copy of A produces two copies of B, and two copies of B react to produce one copy of A. The *complexes* are A and $2B$; these are the nonnegative integer combinations of the species. The *reactions* are represented as labeled arrows between complexes. The labels κ_i are positive real numbers called the *reaction rate constants*, which can be thought of as governing the speed at which each reaction occurs. With the goal of understanding how much of each species is present in the system over time–in other words, measuring the *concentration* of each species–we examine the net change of each species in each reaction. For example, in the reaction $A \xrightarrow{\kappa_1} 2B$, consider species A. There is one copy of A at the tail of the reaction arrow, in the *reactant complex*, and zero copies of A at the tip of the arrow, in the *product complex*. So, A experiences a net change in concentration of -1 when this reaction occurs once. Species B, however, experiences a net change in concentration of 2. These integer values, called the *stoichiometric coefficients*, can be arranged into a matrix:

$$N = \begin{array}{c} \\ A \\ B \end{array} \begin{array}{c} \begin{array}{cc} A \to 2B & 2B \to A \end{array} \\ \begin{bmatrix} -1 & 1 \\ 2 & -2 \end{bmatrix} \end{array}$$

This is called the *stoichiometric matrix* of the network. As indicated above, each row represents the net change of a given species over all reactions, and each column represents the net change of all species over a given reaction.

To understand how species' concentrations change over time within a network, we combine the stoichiometric matrix with the assumption of mass-action kinetics to generate a system of polynomial differential equations. Under the assumption of mass-action kinetics, a reaction's output rate is proportional to the product of the concentrations of the reacting species. Each species' change in concentration over time is then a function of the species' concentrations, where each reaction contributes a term. Let $x_A(t)$ and $x_B(t)$ denote the concentrations of A and B respectively at time t. The forward reaction $A \xrightarrow{\kappa_1} 2B$ contributes the term $-\kappa_1 x_A$ to the change in concentration of A, and the term $2\kappa_1 x_A$ to the change in concentration of B. These are monomial terms constructed by multiplying the stoichiometric coefficient, the reaction rate, and the reacting species' concentration variables. Examining the entire network, mass-action kinetics give rise to the following system of polynomial ordinary differential equations:

$$\frac{dx_A}{dt} = f_A(x) := -\kappa_1 x_A + \kappa_2 x_B^2$$
$$\frac{dx_B}{dt} = f_B(x) := 2\kappa_1 x_A - 2\kappa_2 x_B^2$$

In general, a *chemical reaction network* $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ is made up of three finite sets. First, a set of species $\mathcal{S} = \{A_1, A_2, \ldots, A_s\}$; second, a set of complexes $\mathcal{C} = \{y_1, y_2, \ldots, y_p\}$, where each y_i is a formal sum of the species with nonnegative integer coefficients; and finally a set of reactions $\mathcal{R} \subseteq (\mathcal{C} \times \mathcal{C}) \setminus \{(y, y) | y \in \mathcal{C}\}$, which are non-diagonal ordered pairs of complexes such that (y_i, y_j) corresponds to the reaction graphically represented as $y_i \to y_j$. Each reaction also has a reaction rate κ_i, a positive real parameter. As in the above example, we construct the polynomial steady-state equations, one for each species, where each reaction contributes one monomial term.

To build the steady-state equations in general, we require some additional definitions and notation. Writing out the i^{th} complex gives $y_i := \alpha_{1i} A_1 + \alpha_{2i} A_2 + \ldots + \alpha_{si} A_s$, where the α_{ki} are the coefficients on the k^{th} species in the i^{th} complex. We collect information from these coefficients in the stoichiometric matrix N, whose rows correspond to species and whose columns correspond to reactions. The entry in the row corresponding to the k^{th} species and the column corresponding to the reaction (y_i, y_j) is the difference $\alpha_{kj} - \alpha_{ki}$, which is the net change in species A_k. Next, we construct a diagonal matrix of the reaction rates $\text{diag}(\kappa)$ whose nonzero entries are the reaction rates κ_i. Finally, we construct a vector x^B of monomials in the species' concentrations. Here B is the *reactant matrix*, where again each column corresponds to a reaction and each row corresponds to a species; the column corresponding to the reaction (y_i, y_j) lists the

α_{ki} coefficients of the reactant complex y_i for $k = 1, \ldots, s$. If $b_i = (\alpha_{1i}, \ldots, \alpha_{si})$ is the i^{th} column of B, then $x^{b_i} = x_{A_1}^{\alpha_{1i}} \cdots x_{A_s}^{\alpha_{si}}$ is the i^{th} entry of x^B. Then, the steady-state equations are the entries of the product

$$\frac{dx}{dt} = f(x) := N \cdot \text{diag}(\kappa) \cdot x^B$$

Using the steady-state equations, we can find equilibrium points of the network, called its *steady-states*.

Definition 2.1. *A **steady-state** of a chemical reaction network is a tuple of species concentrations $x = (x_{A_1}, x_{A_2}, \ldots, x_{A_s})$ for which all the steady-state equations equal zero.*

Intuitively, these are inputs that will result in no change in the species' concentrations over time. The solution set of the system of steady-state equations forms a complex algebraic variety called the *steady-state variety*. In the opening example network of Section 2, the two steady-state equations are constant multiples of each other, and so their individual zero sets are identical. Setting either equation equal to zero gives that the steady-state variety is the curve $x_A = \frac{\kappa_2}{\kappa_1} x_B^2$, which is a parabola opening in the positive x_A direction. Of particular interest is the *positive steady-state variety*, which is the Zariski closure of the intersection of the steady-state variety with the interior of the positive orthant of $\mathbb{R}^s$. This variety represents all biologically relevant steady-states. In our example, the positive steady-state variety is the same as the steady-state variety. However, in general, the computation of the positive real portion of the steady-state variety is a subtle and challenging task. For example, consider the univariate polynomial $f = x^2(x^2-1)(x^2+1)$ which appears in [4]. The defining ideal of the variety $V(f)$ is generated by $x(x^2-1)(x^2+1)$; over the real numbers the defining ideal is generated by $x(x^2-1)$; and, finally, the positive real part of this variety is given by the polynomial $x - 1$. Due to the difficulty of a more general approach, we restrict our focus to a specific category of small chemical reaction networks.

Networks can be grouped together based on their size. One notion of size is the number of species and the number of reactions in the network. In this thesis we consider networks with 2 reactions, and with 1, 2, and 3 species. Additionally, we require that all species appear at least once among the network's complexes. Networks with this extra property are defined as *genuine* networks. To capture a different notion of size, we consider the network's complexes:

Definition 2.2. *The **molecularity** of a complex $y_i = \alpha_{i1}A_1 + \alpha_{i2}A_2 + \ldots + \alpha_{is}A_s$ is the sum $\sum_{j=1}^{s} \alpha_{ij}$.*

Intuitively, this measures the number of total objects appearing in a complex. Small networks can then be characterized by a fixed upper bound on the molecularity of its complexes, called *at-most-n-molecular* networks, which then give rise to steady-state polynomials whose degree is bounded above by n. For example, the above network of $A \underset{\kappa_2}{\overset{\kappa_1}{\rightleftarrows}} 2B$ is *at-most-bimolecular*, since the largest complex $2B$ contains two objects and the degree of its steady-state polynomials is at most two. This thesis concerns networks of any molecularity, but focuses on the at-most-bimolecular case. As such, a genuine, at-most-bimolecular, 2-reaction chemical reaction network will henceforth be referred to as a "small network," with different numbers of species and any different molecularity bounds specifically indicated as necessary.

The general forms of an s-species small network, its steady-state equations, and its stoichiometric matrix are as follows, respectively:

$$\begin{aligned} \alpha_{10}A_1 + \alpha_{20}A_2 + \ldots + \alpha_{s0}A_s &\xrightarrow{\kappa_1} \alpha_{11}A_1 + \alpha_{21}A_2 + \ldots + \alpha_{s1}A_s \\ \alpha_{12}A_1 + \alpha_{22}A_2 + \ldots + \alpha_{s2}A_s &\xrightarrow{\kappa_2} \alpha_{13}A_1 + \alpha_{23}A_2 + \ldots + \alpha_{s3}A_s \end{aligned} \tag{1}$$

$$f(x) = \begin{cases} f_{A_1}(x) &= (\alpha_{11} - \alpha_{10})\kappa_1 x_{A_1}^{\alpha_{10}} x_{A_2}^{\alpha_{20}} \cdots x_{A_s}^{\alpha_{s0}} + (\alpha_{13} - \alpha_{12})\kappa_2 x_{A_1}^{\alpha_{12}} x_{A_2}^{\alpha_{22}} \cdots x_{A_s}^{\alpha_{s2}} \\ f_{A_2}(x) &= (\alpha_{21} - \alpha_{20})\kappa_1 x_{A_1}^{\alpha_{10}} x_{A_2}^{\alpha_{20}} \cdots x_{A_s}^{\alpha_{s0}} + (\alpha_{s3} - \alpha_{s2})\kappa_2 x_{A_1}^{\alpha_{12}} x_{A_2}^{\alpha_{22}} \cdots x_{A_s}^{\alpha_{s2}} \\ &\vdots \\ f_{A_s}(x) &= (\alpha_{s1} - \alpha_{s0})\kappa_1 x_{A_1}^{\alpha_{10}} x_{A_2}^{\alpha_{20}} \cdots x_{A_s}^{\alpha_{s0}} + (\alpha_{s3} - \alpha_{s2})\kappa_2 x_{A_1}^{\alpha_{12}} x_{A_2}^{\alpha_{22}} \cdots x_{A_s}^{\alpha_{s2}} \end{cases} \tag{2}$$

$$N = \begin{bmatrix} \alpha_{11} - \alpha_{10} & \alpha_{13} - \alpha_{12} \\ \alpha_{21} - \alpha_{20} & \alpha_{23} - \alpha_{22} \\ \vdots & \vdots \\ \alpha_{s1} - \alpha_{s0} & \alpha_{s3} - \alpha_{s2} \end{bmatrix} \tag{3}$$

In the 2- and 3-species cases, the species will be labeled A, B, and C with corresponding stoichiometric coefficients α_i, β_i, and γ_i and corresponding variables x, y, and z for simplicity of notation.

3 Conditions for a Nonempty Positive Steady-State Variety

When examining 2- and 3-species small networks, all networks that support a nonempty positive steady-state variety share a common property in their stoichiometric matrix: the columns are negative multiples of each other. Indeed, this property is shared by all 2-reaction networks with nonempty positive steady-state varieties. In the following proof, $x_{A_i} := x_i$ for simplicity of notation, and the terms "positive" and "negative" denote values that are strictly greater or less than zero, respectively.

Theorem 3.1. *A two-reaction network with any number of species and with complexes of any molecularity produces a nonempty positive steady-state variety if and only if the columns of its stoichiometric matrix are (nonzero) negative constant multiples of each other, and its reactant complexes are not identical.*

Proof. ($\Rightarrow$) We give a proof by contrapositive, showing that if either condition does not hold then the positive steady-state variety is empty. First, suppose that the two reactant complexes are identical and are $\alpha_1 A_1 + \alpha_2 A_2 + \cdots + \alpha_s A_s$. Then, after factoring, all s steady-state equations take the form $c x_1^{\alpha_1} x_2^{\alpha_2} \dots x_s^{\alpha_s} = 0$ for some (possibly different) real constants c. This clearly only has solutions when at least one variable equals zero, corresponding to coordinate hyperplane solutions. Recall that the steady-state variety is the intersection of the individual zero sets of the steady-state equations, meaning the variety is therefore also restricted to the coordinate hyperplanes, and the positive steady-state variety is empty. So, if a network has a nonempty positive steady-state variety, its reactant complexes must be non-identical.

Now, suppose that the columns of the stoichiometric matrix are not negative multiples of each other. There are two cases to consider, as the columns being multiples of each other by zero implies that one of the reactions is trivial. First, consider the case where the columns are positive multiples of each other. This means the rank of the stoichiometric matrix is one, and so the rows must all be multiples of each other as well. The variable and rate constant appearing in each monomial term are fixed by the reaction, so the s steady-state equations differ only by a constant real factor and we need only consider one equation when examining the steady-state variety. Without loss of generality, suppose that f_{A_1} is nonzero and consider its general form

$$\begin{aligned} f_{A_1} &= (\alpha_{11} - \alpha_{10})\kappa_1 x_1^{\alpha_{10}} x_2^{\alpha_{20}} \cdots x_s^{\alpha_{s0}} + (\alpha_{13} - \alpha_{12})\kappa_2 x_1^{\alpha_{12}} x_2^{\alpha_{22}} \cdots x_s^{\alpha_{s2}} \\ &= c_0 x_1^{\alpha_{10}} x_2^{\alpha_{20}} \cdots x_s^{\alpha_{s0}} + c_2 x_1^{\alpha_{12}} x_2^{\alpha_{22}} \cdots x_s^{\alpha_{s2}} \end{aligned}$$

where the (nonzero) real constants c_0 and c_2 encode the reaction rates and the stoichiometric coefficients. The steady-state variety is then given by

$$-x_1^{\alpha_{10}} x_2^{\alpha_{20}} \cdots x_s^{\alpha_{s0}} = \frac{c_2}{c_0} x_1^{\alpha_{12}} x_2^{\alpha_{22}} \cdots x_s^{\alpha_{s2}}.$$

The reaction rates are always positive and the stoichiometric coefficients have the same sign by assumption, so c_0 and c_2 must have the same sign; thus the fraction $\frac{c_2}{c_0}$ is positive. We wish to show that the x_i cannot all be simultaneously positive. Suppose without loss of generality that $x_1 ... x_{s-1}$ are positive. Then, the sign of both sides is determined entirely by the value of the powers of x_s. For equality to hold, the two sides must first have the same sign, which can clearly only happen if x_s is negative or zero. Thus, no positive steady-states can exist.

Now, consider the case where the columns are not multiples of each other. Then, the rank of the matrix is two, giving that there exists at least one pair of nonzero rows that are linearly independent and therefore not multiples of each other. If any steady-state equation is a monomial, the positive steady-state variety is empty. However, both steady-state equations corresponding to a pair of linearly independent rows could be binomials. Suppose without loss of generality that the first two rows are not multiples of each other, and consider the general form steady-state equations for A_1 and A_2:

$$\begin{aligned} f_{A_1} &= (\alpha_{11} - \alpha_{10})\kappa_1 x_1^{\alpha_{10}} x_2^{\alpha_{20}} \cdots x_s^{\alpha_{s0}} + (\alpha_{13} - \alpha_{12})\kappa_2 x_1^{\alpha_{12}} x_2^{\alpha_{22}} \cdots x_s^{\alpha_{s2}} \\ f_{A_2} &= (\alpha_{21} - \alpha_{20})\kappa_1 x_1^{\alpha_{10}} x_2^{\alpha_{20}} \cdots x_s^{\alpha_{s0}} + (\alpha_{23} - \alpha_{22})\kappa_2 x_1^{\alpha_{12}} x_2^{\alpha_{22}} \cdots x_s^{\alpha_{s2}} \end{aligned}$$

where none of the stoichiometric coefficients are zero. Set equal to zero, we can rewrite f_A and f_B respectively as

$$-x_1^{\alpha_{10}}x_2^{\alpha_{20}}\cdots x_s^{\alpha_{s0}} = \frac{\kappa_2(\alpha_{13}-\alpha_{12})}{\kappa_1(\alpha_{11}-\alpha_{10})}x_1^{\alpha_{12}}x_2^{\alpha_{22}}\cdots x_s^{\alpha_{s2}}$$
$$\text{and } -x_1^{\alpha_{10}}x_2^{\alpha_{20}}\cdots x_s^{\alpha_{s0}} = \frac{\kappa_2(\alpha_{23}-\alpha_{22})}{\kappa_1(\alpha_{21}-\alpha_{20})}x_1^{\alpha_{12}}x_2^{\alpha_{22}}\cdots x_s^{\alpha_{s2}}.$$

The fractions are real number scaling factors, affecting a stretch or shrink on the curve. The values of κ_1 and κ_2 are fixed, so the relative steepness of the two zero sets is determined by the ratios of the stoichiometric coefficients. If

$$\frac{\alpha_{13}-\alpha_{12}}{\alpha_{11}-\alpha_{10}} = \frac{\alpha_{23}-\alpha_{22}}{\alpha_{21}-\alpha_{20}},$$

then the zero sets overlap completely. Consider the 2×2 submatrix of the stoichiometric matrix corresponding to these two species:

$$\begin{bmatrix}\alpha_{11}-\alpha_{10} & \alpha_{13}-\alpha_{12}\\ \alpha_{21}-\alpha_{20} & \alpha_{23}-\alpha_{22}\end{bmatrix}$$

By assumption, these two rows of the stoichiometric matrix are linearly independent, so this submatrix has rank two. But, the above equality implies that the columns are multiples of each other, giving a rank of one, meaning that the zero sets of f_{A_1} and f_{A_2} must have different scaling factors. A solution to the system with all $x_i \neq 0$ would require a nonzero left-hand side of the equation to simultaneously equal two distinct nonzero values, which is impossible. So, these two zero sets can only intersect at the origin and/or in coordinate hyperplanes. The steady-state variety of the network is the intersection of the zero sets of all s steady-state equations, and so is also restricted to the coordinate hyperplanes, resulting in an empty positive steady-state variety. Thus, if a network has a nonempty positive steady-state variety, the columns of its stoichiometric matrix are negative multiples of each other.

($\Leftarrow$) Now, suppose that a network satisfies the above conditions. The relationship between the columns means the stoichiometric matrix has rank one, meaning the rows are all multiples of each other. Therefore, all of the steady-state equations are constant multiples of each other, since the other components of each monomial term are fixed by the reaction. All of the nonzero equations thus define the same zero set, and we need only consider one equation to fully understand the steady-state variety. Without loss of generality, suppose that f_{A_1} is nonzero. In general form,

$$\begin{aligned} f_{A_1} &= (\alpha_{11}-\alpha_{10})\kappa_1 x_1^{\alpha_{10}}x_2^{\alpha_{20}}\cdots x_s^{\alpha_{s0}} + (\alpha_{13}-\alpha_{12})\kappa_2 x_1^{\alpha_{12}}x_2^{\alpha_{22}}\cdots x_s^{\alpha_{s2}} \\ &= c_0 x_1^{\alpha_{10}}x_2^{\alpha_{20}}\cdots x_s^{\alpha_{s0}} + c_2 x_1^{\alpha_{12}}x_2^{\alpha_{22}}\cdots x_s^{\alpha_{s2}}, \end{aligned}$$

where the (nonzero) c_i encode the rate constants and stoichiometric coefficients. We wish to verify the existence of at least one solution where all of the x_i are simultaneously positive. Setting equal to zero, the steady-state variety is defined by

$$x_1^{\alpha_{10}}x_2^{\alpha_{20}}\cdots x_s^{\alpha_{s0}} = \frac{-c_2}{c_0}x_1^{\alpha_{12}}x_2^{\alpha_{22}}\cdots x_s^{\alpha_{s2}}.$$

The value of $\frac{-c_2}{c_0}$ is some positive real constant because the c_i have opposite signs by assumption. To understand the possible values of the exponents, consider the stoichiometric matrix

$$N = \begin{bmatrix}\alpha_{11}-\alpha_{10} & \alpha_{13}-\alpha_{12}\\ \alpha_{21}-\alpha_{20} & \alpha_{23}-\alpha_{22}\\ \vdots & \vdots \\ \alpha_{s1}-\alpha_{s0} & \alpha_{s3}-\alpha_{s2}\end{bmatrix}$$

The α_{ij} are all nonnegative integers. The values where j is even correspond to our exponents and cannot all be zero, as this would result in an entirely nonnegative stoichiometric matrix, contradicting our

assumption. Additionally, if $\alpha_{10} = 0$, then $\alpha_{12} \neq 0$ because $\alpha_{13} - \alpha_{12}$ must be negative by assumption. Similarly, if $\alpha_{12} = 0$ then $\alpha_{10} \neq 0$. Without loss of generality, suppose that $\alpha_{10} \neq 0$. Returning to the steady-state equation, we can group the variables:

$$x_1^{\alpha_{10}-\alpha_{12}} = \frac{-c_2}{c_0} x_2^{\alpha_{22}-\alpha_{20}} \cdots x_s^{\alpha_{s2}-\alpha_{s0}}$$

This division has the potential to eliminate shared copies of a given variable from both monomial terms of the steady-state equation, which corresponds to removing coordinate hyperplane solutions which are never positive, and therefore is appropriate. By our assumption that the reactant complexes are not identical, these new exponents cannot all be simultaneously zero, so at least one of the x_i will always have a nonzero exponent. Suppose without loss of generality that $\alpha_{10} - \alpha_{12} \neq 0$, and consider some point in $\mathbb{R}^s$ where $x_i = b_i$ for some positive constant b_i for all $i \neq 1$. Then, the equation becomes

$$x_1^{\alpha_{10}-\alpha_{12}} = \frac{-c_2}{c_0} b_2^{\alpha_{22}-\alpha_{20}} \cdots b_s^{\alpha_{s0}-\alpha_{s2}} \Rightarrow x_1 = \left(\frac{-c_2}{c_0} b_2^{\alpha_{22}-\alpha_{20}} \cdots b_s^{\alpha_{s0}-\alpha_{s2}} \right)^{\frac{1}{\alpha_{10}-\alpha_{12}}}$$

The right hand side of the equation is a positive real number, as it consists only of positive numbers raised to different powers. So, a solution exists to the equation defining the steady-state variety such that all variables are simultaneously positive, meaning that the steady-state variety intersects the interior of the positive orthant and thus the positive steady-state variety is nonempty. □

Remark 3.1. *Note that the first criterion, phrased here in terms of the stoichiometric matrix, can also be described with the notion of T-alternating subnetworks described in [7]. Through this lens, we instead require that, when viewing the behavior of each species individually, the network is 1-alternating.*

As an additional note, the steady-state ideals of all 2-reaction networks are binomial ideals, since the corresponding steady-state equations are binomials; so, the steady-state varieties are toric. Networks with toric nonempty positive steady-state varieties have been studied previously, most notably in [6]. The authors give three conditions to classify networks with toric nonempty positive steady-state varieties. Their condition 3.1 is unnecessary in the 2-reaction case, as the varieties are necessarily toric. Furthermore, 2-reaction networks trivially satisfy condition 3.6, as the matrix Δ from [6] has full column rank; this condition also encapsulates the second condition above by removing solutions contained in the coordinate hyperplanes. Finally, their condition 3.4 is equivalent to having the columns of the stoichiometric matrix be (nonzero) negative multiples of each other, the first condition above. Then, 2-reaction networks can be considered a special case of the toric networks discussed in [6], and the above theorem an independently derived simplification of their conditions for small networks to have nonempty positive steady-state varieties.

3.1 Reaction Rate Independence

In some reaction networks, the specific values of the rate constants do not significantly impact the shape of the positive steady-state variety. They may alter the slope of a line or the circularity of a cone, but the overall form and existence remains the same. However, this is not always the case, as seen in the following 1-species, 3-reaction network:

Example 3.1. *Consider the one-species network in Figure 1. The steady-state variety is defined by the polynomial* $f_A = \kappa_1 x_A - \kappa_2 x_A - \kappa_3 x_A^2$. *Factoring and setting equal to zero to find the steady-state variety, we see that* $0 = x_A(\kappa_1 - \kappa_2 - \kappa_3 x_A)$. *The steady-state variety consists of the point* $x_A = 0$ *and the point* $x_A = \frac{\kappa_1 - \kappa_2}{\kappa_3}$. *The three rate constants are always positive, meaning that the nonzero point in the variety will be positive when* $\kappa_1 > \kappa_2$, *and negative otherwise.*

In other words, the existence of a nonempty positive steady-state variety depends on the specific values of the rate constants. This problem of rate-dependence greatly complicates the study of positive steady-state varieties; fortunately in the case of 2-reaction networks, rate-dependence is not a concern.

$$A \xrightarrow{\kappa_1} 2A$$
$$A \xrightarrow{\kappa_2} 0$$
$$2A \xrightarrow{\kappa_3} A$$

Figure 1: 1-species, 3-reaction network exhibiting rate dependence

Corollary 3.1. *The existence of a nonempty positive steady-state variety for a small network with complexes of any molecularity is independent of the specific choices of reaction rates.*

Proof. Consider some small network that has a nonempty positive steady-state variety for some specific values of the rate constants. Theorem 3.1 gives that this network has a stoichiometric matrix whose columns are negative multiples of each other, and has non-identical reactant complexes. Following the logic from the backwards direction of the proof of Theorem 3.1, the steady-state variety is defined by

$$x_1^{\alpha_{10}} x_2^{\alpha_{20}} \cdots x_s^{\alpha_{s0}} = \frac{-\kappa_2(\alpha_{23} - \alpha_{22})}{\kappa_1(\alpha_{21} - \alpha_{20})} x_1^{\alpha_{12}} x_2^{\alpha_{22}} \cdots x_s^{\alpha_{s2}}.$$

Since the κ_i are always positive and the stoichiometric coefficients have opposite signs, the coefficient on the right hand side is positive regardless of the exact values of the rate constants. Call the value of this fraction c. We wish to show that a positive solution exists. To this end, we can divide the like variable terms:

$$x_1^{\alpha_0 - \alpha_2} = c x_2^{\alpha_{22} - \alpha_{20}} \cdots x_s^{\alpha_{s2} - \alpha_{s0}}$$

The logic is identical to that presented at the end of Theorem 3.1; a positive steady-state exists, and its existence did not depend on the particular values of the rate constants. □

4 The 1-Species Case

We now narrow our focus to at-most-bimolecular networks; with the molecularity of the complexes bounded there are finitely many networks to consider, which can be listed using a graph theoretical approach. The Macaulay2 code used to extract the lists of small networks from the information available at [2] is in the appendix. We begin with a comprehensive examination of 1-species networks. There are only six possible at-most-bimolecular 1-species reactions:

$$0 \longrightarrow A \ ; \ 0 \longrightarrow 2A$$
$$A \longrightarrow 0 \ ; \ A \longrightarrow 2A$$
$$2A \longrightarrow 0 \ ; \ 2A \longrightarrow A$$

These six reactions comprise a total of 63 networks of one to six reactions. All six 1-reaction networks have empty positive steady-state varieties: for these networks the steady-state equation is a monomial, whose only possible solutions are $x = 0$. The 2-reaction case is already covered by Theorem 3.1, but the 3-, 4-, 5-, and 6-reaction cases require additional analysis. As seen in Figure 3.1, the positive steady-state varieties produced by 1-species networks with more than two reactions can exhibit rate dependence. In this section, we examine when the positive steady-state variety is empty, nonempty, or rate-dependent for 1-species at-most-bimolecular networks.

To begin, note that a 1-species network will have only one steady-state equation. In the at-most-bimolecular case, this equation is a quadratic in one variable. Consider the general form:

$$f_A = ax^2 + bx + c$$

where $a, b, c \in \mathbb{R}$ are not all zero. The values of the coefficients are determined by the rate constants and the stoichiometric coefficients. Of particular interest are the possible signs of each coefficient; since the rate constants are always positive, the sign is determined by the stoichiometric coefficients.

Consider first the possible values of a. As the coefficient on the x^2 term, the sign is determined by the stoichiometric coefficient from reactions with $2A$ as the reactant complex. In an at-most-bimolecular network, this coefficient is always negative. So, any nonzero value of a is negative. Consider next the possible values of c. The sign is determined by the stoichiometric coefficient from reactions with 0 as a reactant complex, which is always positive. So, any nonzero value of c is positive.

Finally, consider the possible values of b. The sign is determined by the stoichiometric coefficient from reactions with A as a reactant complex. Depending on the product complex, this value could be positive, negative, or rate-dependent. If the only relevant reaction in the network is $A \longrightarrow 2A$, then b is positive. On the other hand, if the only relevant reaction in the network is $A \longrightarrow 0$, then b is negative. If both reactions are present in the network, then the coefficient on x will take the form $(k_i - k_j)$ where $A \longrightarrow 2A$ is the i^{th} reaction and $A \longrightarrow 0$ is the j^{th} reaction. Then, b is negative when $k_i < k_j$, positive when $k_i > k_j$, and zero when $k_i = k_j$.

For 1-species networks with three or more reactions, at least two distinct reactant complexes must appear, as there are only two reactions with each reactant complex. There are four possible cases to consider.

To begin, suppose that all three reactant complexes appear in the network; that is, the set of reactant complexes is $\{0, A, 2A\}$. Then, a and c are certainly nonzero and b has the potential to be zero or nonzero. In this case, the steady-state variety is best calculated using the quadratic formula. Let positive constants $c_1 = -4ac$ and $c_2 = -2a$. Then,

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} = \frac{-b \pm \sqrt{b^2 + c_1}}{-c_2} = \frac{b \pm \sqrt{b^2 + c_1}}{c_2}$$

The denominator is positive, so the sign is determined entirely by the numerator. The square root function is strictly increasing and $c_1 \neq 0$ by assumption, so

$$\sqrt{b^2 + c_1} > \sqrt{b^2} \Longrightarrow \left|\sqrt{b^2 + c_1}\right| > \left|\sqrt{b^2}\right| = |b|$$

If b is negative, then $b + \sqrt{b^2 + c_1}$ is positive while $b - \sqrt{b^2 + c_1}$ is negative; if b is positive, then $b + \sqrt{b^2 + c_1}$ is positive while $b - \sqrt{b^2 + c_1}$ is negative. If $b = 0$, then the equation defining the steady-state variety becomes

$$\frac{\pm\sqrt{c_1}}{c_2}$$

In all cases, the solution set consists of one positive and one negative value, resulting in a nonempty, one-point positive steady-state variety. Note that in 5- and 6-reaction networks, this is the only possible case.

Next, suppose the set of reactant complexes is $\{0, 2A\}$. Then, the steady-state equation has the following form and solutions:

$$f_A = ax^2 + c \Longrightarrow x = \pm\sqrt{\frac{-c}{a}}$$

We know that a is always negative, so the radicand is positive and the steady-state variety consists of two points, one positive and one negative. Therefore, the positive steady-state variety is nonempty and consists of one point.

Now, suppose the set of reactant complexes is $\{0, A\}$. Then, the steady-state equation has the following form and solutions:

$$f_A = bx + c \Longrightarrow x = \frac{-c}{b}$$

We know that c is always positive. Therefore, the sign of the solution depends entirely on the value of b. If b is negative the steady-state variety consists of one positive point; if b is positive the steady-state variety consists of one negative point; and if $b = 0$ then the steady-state variety is empty. Thus, the positive steady-state variety is rate-dependent if both $A \longrightarrow 0$ and $A \longrightarrow 2A$ are present in the network.

Finally, suppose the set of reactant complexes is $\{A, 2A\}$. Then, the steady-state equation has the following form and solutions:

$$f_A = ax^2 + bx \Longrightarrow x = 0 \ , \ x = \frac{-b}{a}$$

We know that a is always negative, so the sign of the nonzero solution depends on the value of b. If b is negative the steady-state variety is 0 and a negative point; if b is positive the steady-state variety is 0 and a

positive point; and if $b = 0$ the steady-state variety is the point 0. Thus, the positive steady-state variety is rate-dependent if both $A \longrightarrow 0$ and $A \longrightarrow 2A$ are present in the network.

As mentioned above, the first case is the only possibility for 5- and 6-reaction networks, while 3- and 4-reaction networks might have any of the four combinations of reactant complexes. The above discussion is summarized in the following propositions:

Proposition 4.1. *The positive steady-state variety of an at-most-bimolecular, 1-species network with three or more reactions will be nonempty and a single point if and only if one of the following holds:*

1. *The set of reactant complexes is* $\{0, A, 2A\}$ *or* $\{0, 2A\}$,
2. *The set of reactant complexes is* $\{0, A\}$ *and* $A \longrightarrow 2A$ *does not appear in the network,*
3. *The set of reactant complexes is* $\{A, 2A\}$ *and* $A \longrightarrow 0$ *does not appear in the network.*

Corollary 4.1. *An at-most-bimolecular, 1-species network with 5 or 6 reactions has a nonempty positive steady-state variety.*

Proposition 4.2. *The positive steady-state variety of an at-most-bimolecular, 1-species network with three or more reactions will be rate-dependent if and only if the set of reactant complexes is either* $\{A, 0\}$ *or* $\{A, 2A\}$ *and both* $A \longrightarrow 0$ *and* $A \longrightarrow 2A$ *appear in the network.*

5 The 2-Species Case

Focusing now on small networks with two species, there are 210 non-isomorphic networks, extracted from data available at [2]. Before classifying the positive steady-state varieties produced by these networks, we require one additional piece of vocabulary:

Definition 5.1. *The* ***support*** *of a complex is the set of all species which appear within it.*

Example 5.1. *The support of the complex* $2A + B$ *is the set* $\{A, B\}$. *The support of the complex* $3B$ *is* $\{B\}$

Among the 2-species small networks, four classes of nonempty positive steady-state varieties arose following a systematic calculation of every steady-state variety: non-axis horizontal or vertical lines, slanted lines through the origin, parabolas, and hyperbolas. Of the 210 non-isomorphic 2-species small networks, three networks have a horizontal or vertical line as their positive steady-state variety; seven networks have slanted lines; five networks have parabolas; and and three networks have hyperbolas. The following theorems characterize all small networks that produce each class of variety; in this section, "small network" refers to the two-species case.

Theorem 5.1. *Given a small network satisfying the assumptions of Theorem 3.1, the positive steady-state variety will be a non-axis horizontal or vertical line if and only if one reactant complex is* $A + B$ *and the other is unimolecular.*

Proof. ($\Rightarrow$) Suppose we have an arbitrary small network whose positive steady-state variety is a non-axis horizontal or vertical line. Up to relabeling species, the simplified positive steady-state variety is $V(x - c)$ for some positive real number c. Since the positive steady-state variety is a component of the steady-state variety, the equation defining the steady-state variety must have $x - c$ as a factor, but may also contain a term whose degree is limited by the network's bimolecularity:

$$(x - c) \cdot (\text{Factor of degree 1 or 0})$$

Note that for all small networks, the corresponding equations will have at most two terms, limiting the degree-one factors to be monomials or $(x + c)$, and there are four potential equations defining the steady-state variety. We also have that $V(x - c) = V(f_A) \cap V(f_b)$, so the same logic applies to the individual steady-state equations. As in Theorem 3.1 the two steady-state equations are multiples of each other and take on the same form. So, up to multiplication by constants, the steady-state equations take on one of these four forms:

$$f_i = x - c \ ; \ f_i = x^2 - c^2 \ ; \ f_i = x^2 - cx \ ; \ f_i = xy - cy$$

By Theorem 3.1, the columns of the network's stoichiometric matrix are negative multiples of each other. This restriction necessitates that the first three forms of equations correspond to non-genuine networks: $A \longleftrightarrow 0$, $2A \longleftrightarrow 0$, and $2A \longleftrightarrow A$ respectively, as any copies of B in the network must be in the product complexes which can only result in positive entries of the stoichiometric matrix. Thus, the steady-state equations only take the following form:

$$f_A = xy - cy \ , \ f_B = t(xy - cy)$$

for some $t \in \mathbb{R}$. The monomial terms must therefore have variable parts of xy and y, corresponding to reactant complexes of $A+B$ and B or, with arbitrary labeling of species, $A+B$ and a unimolecular complex.

($\Leftarrow$) Suppose we have a small network where one reactant complex is $A + B$ while the other is, without loss of generality, A. By the assumptions of Theorem 3.1, we need only consider one steady-state equation; without loss of generality consider a nonzero f_A.

$$\begin{aligned} f_A &= (\alpha_1 - 1)\kappa_1 xy + (1 - \alpha_1)\kappa_2 x \\ &= x[(\alpha_1 - 1)\kappa_1 y + (\alpha_3 - 1)\kappa_2] \end{aligned}$$

Setting equal to zero and solving yields the following:

$$\begin{aligned} 0 &= x[(\alpha_1 - 1)\kappa_1 y + (\alpha_3 - 1)\kappa_2] \\ \Rightarrow x = 0,\ y &= \frac{-(\alpha_3 - 1)\kappa_2}{(1 - \alpha_1)\kappa_1} \end{aligned}$$

By assumption the stoichiometric coefficients have opposite signs, so the fixed value of y is positive and therefore defines a non-axis horizontal line, which is the desired positive steady-state variety. □

Theorem 5.2. *Given a small network satisfying the assumptions of Theorem 3.1, the positive steady-state variety will be a line through the origin if and only if the following hold:*

1. *The two reactant complexes have the same molecularity,*
2. *The supports of the reactant complexes are nonempty and distinct (not necessarily disjoint).*

Proof. ($\Rightarrow$) Suppose we have a small network whose positive steady-state variety is a line through the origin. Up to relabeling species, the simplified positive steady-state variety is $V(y - cx)$ for some positive $c \in \mathbb{R}$. Since the positive steady-state variety is a component of the steady-state variety, the equation defining the steady-state variety must have $y - cx$ as a factor, but may also contain a factor term whose degree is limited by the network's bimolecularity:

$$(y - cx) \cdot (\text{Factor of degree 1 or 0})$$

Since the network has two reactions the corresponding equations have at most two terms, limiting the degree-one factors to be monomials or $(y + cx)$ and there are four potential equations defining the steady-state variety. In addition, $V(y-cx) = V(f_A) \cap V(f_B)$, so the same logic applies to the individual steady-state equations. By Theorem 3.1 the two steady-state equations are constant multiples of each other and take on the same form. So, up to multiplication by constants, the steady-state equations take on one of the following forms:

$$f_i = y - cx \ ; \ f_i = y^2 - c^2x^2 \ ; \ f_i = y^2 - cxy \ ; \ f_i = xy - cx^2$$

In all cases, the degree of all monomial terms is the same and at least one variable appears in exactly one monomial term. By the relationship between the monomial terms and the reactant complexes, this corresponds to reactant complexes of the same molecularity which have distinct supports.

($\Leftarrow$) Now, suppose we have a nontrivial small network satisfying the above conditions. As a consequence of Theorem 3.1 the steady-state equations are constant multiples of each other and we need only examine

one equation to understand the steady-state variety. Without loss of generality suppose that f_B is nonzero and consider its general form:

$$f_B = \kappa_1(\beta_1 - \beta_0)x^{\alpha_0}y^{\beta_0} + \kappa_2(\beta_3 - \beta_2)x^{\alpha_2}y^{\beta_2}.$$

By (*2*) at least one reactant appears in exactly one reactant complex, meaning that at least one of the reactant coefficients α_0, β_0, α_2, and β_2 is 0. Suppose without loss of generality that $\beta_0 = 0$. Then, $\beta_1 - \beta_0 = \beta_1$ is positive and nonzero and, following the assumptions of Theorem 3.1, $\beta_3 - \beta_2$ is negative. We set f_B equal to zero and rewrite it as

$$\kappa_1\beta_1 x^{\alpha_0} = \kappa_2(\beta_2 - \beta_3)x^{\alpha_2}y^{\beta_2},$$

where all coefficients are positive. Since $\beta_0 = 0$ we have $\alpha_0 \neq 0$, as there is no reactant complex with an empty support. By (*2*), $\beta_2 \neq 0$; if it were, the reactant complex's supports would not be distinct. So, the quantity $\beta_2 - \beta_0 = \beta_2$ is positive. Furthermore, (*1*) gives that $\alpha_0 + \beta_0 = \alpha_2 + \beta_2$, meaning $\alpha_0 - \alpha_2 = \beta_2 - \beta_0 = \beta_2$. Therefore, $\alpha_0 - \alpha_2$ is positive. Armed with this knowledge, we return to our equation, and divide:

$$\kappa_1\beta_1\frac{x^{\alpha_0}}{x^{\alpha_2}} = \kappa_2(\beta_2 - \beta_3)y^{\beta_2}.$$

In general, it is inadvisable to divide by variables; however, in this case, canceling a common factor of x corresponds to eliminating a coordinate axis from the steady-state variety. These axes are not a part of the positive steady-state variety and are therefore irrelevant. Simplifying gives

$$y^{\beta_2} = \frac{\kappa_1\beta_1}{\kappa_2(\beta_2 - \beta_3)}x^{\alpha_0 - \alpha_2}.$$

Note that $\beta_2 - \beta_3$ is nonzero, since we assumed $f_B \neq 0$. So, all coefficients and exponents in this equation are positive. We also have that $2 \geq \alpha_0 - \alpha_2 = \beta_2 > 0$ by (*1*) and by bimolecularity, so the exponents are equal and either 1 or 2. In both cases, the portion of the variety that intersects the interior of the positive orthant is a line through the origin with positive slope, which is the desired positive steady-state variety. □

Unlike the other classes of positive steady-state variety discussed in this section, the slanted line case can arise from multiple distinct types of pairs of reactant complexes. These result in distinct types of steady-state varieties even when the positive portion remains the same. This flexibility is due to the difference in degree between the positive steady-state variety and the steady-state variety; if we were to examine similar networks with complexes of higher molecularity, there would be more classes of steady-state variety per class of positive steady-state variety, even for the degree-two varieties discussed below.

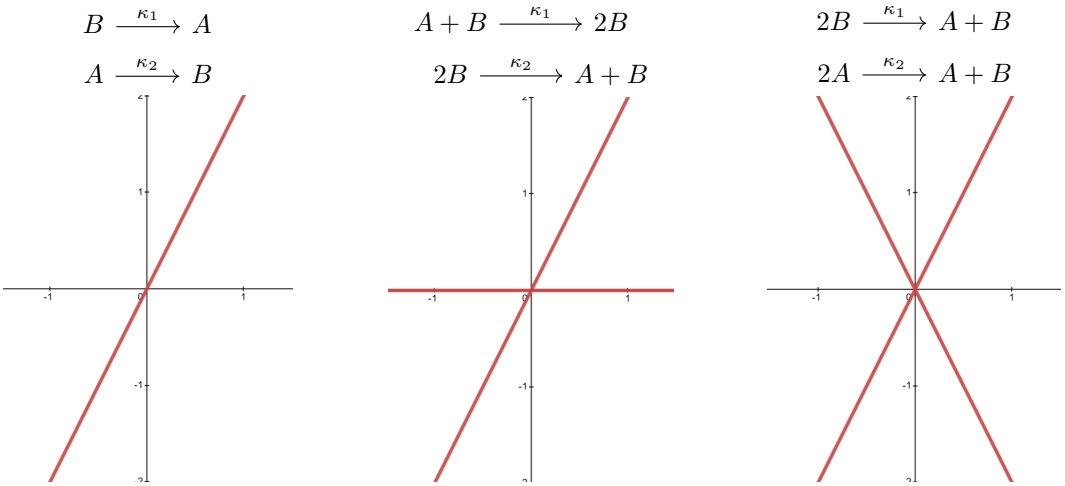

Figure 2: Different slanted line steady-state varieties

Example 5.2. *Consider the networks in Figure 2 and their corresponding steady-state varieties. While their positive steady-state varieties are identical, their steady-state varieties are not. The first variety is of the form $V(y - cx)$; the second is $V(y^2 - cxy)$; and the third is $V(y^2 - c^2x^2)$ where $c = 2$ in all cases.*

Theorem 5.3. *Given a small network satisfying the assumptions of Theorem 3.1, the positive steady-state variety will be a parabola if and only if the following hold:*

1. *One reactant complex is bimolecular and the other is unimolecular,*
2. *The supports of the reactant complexes are disjoint.*

Proof. ($\Rightarrow$) Given a small network whose positive steady-state variety is a parabola, the variety is $V(y-cx^2)$ for some positive $c \in \mathbb{R}$ up to relabeling species. We also have $V(y - cx^2) = V(f_A) \cap V(f_B)$ and know the network is at-most-bimolecular, implying both $\deg(f_A)$ and $\deg(f_B)$ cannot be greater than 2. Then, we have

$$f_A = y - cx^2$$
$$f_B = t(y - cx^2)$$

where t is some nonzero real constant as per Theorem 3.1. By definition of the steady-state equations, the variable components of the monomial terms of f_A and f_B correspond to the network's reactant complexes. Since these are exactly y and x^2, the reactant complexes are B and $2A$. With arbitrary labeling of species, the network has a bimolecular and a unimolecular reactant complex which have disjoint supports, proving both conditions.

($\Leftarrow$) Suppose that the reactant complexes have disjoint supports with one bimolecular and the other unimolecular. By Theorem 3.1 the steady-state equations are constant multiples of each other and we need only consider one equation; without loss of generality suppose that the reactant complexes are A and $2B$ and consider a nonzero f_A:

$$f_A = \kappa_1(\alpha_1 - 1)x + \kappa_2(\alpha_3)y^2$$

Setting equal to zero and solving gives

$$x = \frac{-\kappa_2(\alpha_3)}{\kappa_1(\alpha_1 - 1)}y^2$$

The stoichiometric coefficients have opposite signs by assumption meaning the coefficient on y^2 is positive and the equation defines a parabola crossing into the first quadrant; the positive steady-state variety is a parabola, as desired.

□

Theorem 5.4. *Given a small network satisfying the assumptions of Theorem 3.1, the positive steady-state variety will be a hyperbola if and only if the reactant complexes are $A + B$ and 0.*

Proof. ($\Rightarrow$) Given a small network whose positive steady-state variety is a hyperbola, the variety is

$$V(xy - c) = V(f_A, f_B) = V(f_A) \cap V(f_B)$$

for some positive $c \in \mathbb{R}$. Note that $(xy - c)$ must be a shared factor of both steady-state equations f_A and f_B. The network is at-most-bimolecular, meaning each steady-state equation has degree at most two, so $xy - c$ is not a factor of a higher degree reducible polynomial. Additionally, the first assumption of Theorem 3.1 necessitates that the steady-state equations are constant multiples of each other, taking the form

$$f_A = xy - c$$
$$f_B = t(xy - c)$$

for some real constant t. By the relationship between the reactant complexes and the monomial terms, the reactant complexes are $A + B$ and 0.

($\Leftarrow$) Suppose a small network has reactant complexes of $A+B$ and 0. Since the network satisfies the conditions of Theorem 3.1, the steady-state equations are multiples of each other, so we need only consider one equation to understand the steady-state variety. Without loss of generality consider a nonzero f_B

$$f_B = \kappa_1(\beta_1 - 1)xy + \kappa_2(\beta_3)$$

Setting equal to zero and solving gives

$$xy = \frac{-\kappa_2(\beta_3)}{\kappa_1(\beta_1 - 1)}$$

The stoichiometric coefficients have opposite signs by assumption meaning the constant value of xy is positive and this equation defines a hyperbola in the first and third quadrants; the positive steady-state variety is a hyperbola, as desired.

□

5.1 At-Most-Trimolecular Networks

When expanding our focus to include at-most-trimolecular 2-species small networks, two new class of positive steady-state variety arise. Unlike the at-most-bimolecular case, the following theorems do not arise from a systematic analysis of all possible networks so the exact number of networks with each class of variety is unknown. The shape of the first class is formally known as a semicubical parabola. Informally, it looks like a cartoon bird flying through the 2D plane:

Example 5.3. *Consider the network and corresponding steady-state equations and variety in Figure 3. Here, $\kappa_1 = \kappa_2 = 1$ with x_A as the horizontal axis and x_B as the vertical axis. The steady-state variety is a semicubial parabola.*

$$3B \xrightarrow{\kappa_1} A + 2B$$
$$2A \xrightarrow{\kappa_2} A + B$$
$$f_A = \kappa_1 x_B^3 - \kappa_2 x_A^2$$
$$f_B = -\kappa_1 x_B^3 + \kappa_2 x_A^2$$

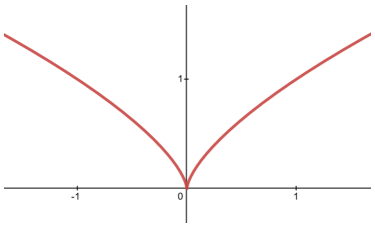

Figure 3: Semicubical parabola positive steady-state variety

The following theorem classifies all at-most-trimolecular small networks with this type of positive steady-state variety.

Theorem 5.5. *Given an at-most-trimolecular small network satisfying the assumptions of Theorem 3.1, the positive steady-state variety will be a semicubical parabola if and only if the following hold:*

1. *One reactant complex is bimolecular and the other reactant complex is trimolecular,*
2. *The supports of the reactant complexes are disjoint.*

Proof. ($\Rightarrow$) Given a small network whose positive steady-state variety is a semicubical parabola, the variety is $V(x^3 - cy^2)$ where c is a positive constant, up to relabeling species. Furthermore, $V(x^3 - cy^2) = V(f_A) \cap V(f_B)$ since it is a 2-species network. The network is at-most-trimolecular, so the degrees of our steady-state equations cannot be greater than three. Then, as a consequence of Theorem 3.1 we have

$$f_A = x^3 - cy^2$$
$$f_B = t\left(x^3 - cy^2\right)$$

for some nonzero $t \in \mathbb{R}$. By the relationship between the monomial terms and the reactant complexes, the reactant complexes are $3A$ and $2B$. With arbitrary labeling of species, one reactant complex is bimolecular

while the other is trimolecular, proving (*1*). Additionally, this also implies that the supports of the reactant complexes are disjoint, proving (*2*).

($\Leftarrow$) Suppose the network has one bimolecular and one trimolecular reactant complex with disjoint supports. The steady-state equations are constant multiples of each other by the assumptions of Theorem 3.1 and so we need only consider one equation to understand the steady-state variety; without loss of generality suppose the reactant complexes are $3A$ and $2B$ and consider a nonzero f_A:

$$f_A = \kappa_1(\alpha_1 - 3)x^3 + \kappa_2(\alpha_3)y^2$$

Setting equal to zero gives

$$x^3 = \frac{-\kappa_2(\alpha_3)}{\kappa_1(\alpha_1 - 3)}y^2$$

The stoichiometric coefficients have opposite signs by assumption meaning the coefficient on y^2 is positive, and the equation defines a semicubical parabola crossing into the first quadrant. The positive steady-state variety is therefore a semicubical parabola, as desired.

□

This theorem deals strictly with at-most-trimolecular small networks, where the semicubical parabola is the only expected variety with this general shape. Expanding to complexes with higher molecularities, one can expect to find additional classes of positive steady-state variety with a similar appearance; informally, "bird-type" varieties. Mathematically, these would have steady-state equations with term-by-term degree ratios of 5:2, 7:2, 9:2, etc, whereas our example has a 3:2 ratio. It seems appropriate to group these classes together due to their qualitative similarities when considering 2-species small networks with any molecularity bound, detailed below in Section 8.4. The second additional positive steady-state variety class is a cubic; the following theorem classifies all at-most-trimolecular small networks with this type of variety.

Theorem 5.6. *Given a small network satisfying the assumptions of Theorem 3.1, the positive steady-state variety will be a cubic if and only if the following hold:*

1. *One reactant complex is trimolecular and the other is unimolecular,*
2. *The supports of the reactant complexes are disjoint.*

Proof. ($\Rightarrow$) Given a small network whose positive steady-state variety is a cubic, the variety is $V(y - cx^3)$ for some positive $c \in \mathbb{R}$ up to relabeling species. We also have $V(y - cx^3) = V(f_A) \cap V(f_B)$ and know the network is at-most-trimolecular, implying both $\deg(f_A)$ and $\deg(f_B)$ cannot be greater than 3. Then, we have

$$f_A = y - cx^3$$
$$f_B = t(y - cx^3)$$

where t is some nonzero real constant as per Theorem 3.1. By definition of the steady-state equations, the variable components of the monomial terms of f_A and f_B correspond to the network's reactant complexes. Since these are exactly y and x^3, the reactant complexes are B and $3A$. With arbitrary labeling of species, the network has a trimolecular and a unimolecular reactant complex which have disjoint supports, proving both conditions.

($\Leftarrow$) Suppose that the reactant complexes have disjoint supports with one trimolecular and the other unimolecular. By Theorem 3.1 the steady-state equations are constant multiples of each other and we need only consider one equation; without loss of generality suppose that the reactant complexes are A and $3B$ and consider a nonzero f_A:

$$f_A = \kappa_1(\alpha_1 - 1)x + \kappa_2(\alpha_3)y^3$$

Setting equal to zero and solving gives

$$x = \frac{-\kappa_2(\alpha_3)}{\kappa_1(\alpha_1 - 1)}y^3$$

The stoichiometric coefficients have opposite signs by assumption meaning the coefficient on y^3 is positive and the equation defines a cubic crossing into the first quadrant; the positive steady-state variety is a cubic, as desired. □

While the degree-three varieties are new in the at-most-trimolecular case, the other four classes of positive steady-state variety discussed above can certainly be produced here as well. However, the additional flexibility provided by having equations with degree at most three complicates the necessary conditions. The relevant theorems above are therefore not sufficient for the trimolecular case; their potential generalization is discussed in Section 8.2.

6 The 3-Species Case

Moving from 2- to 3-species small networks, we shift from studying curves in $\mathbb{R}^2$ to surfaces in $\mathbb{R}^3$, shown in the following example.

Example 6.1. *Consider the 3-species small network and corresponding steady-state equations and variety in Figure 4. While the 2-species case generates two steady-state equations the 3-species case generates three, and the relevant binomials are now in three rather than two variables. This network's positive steady-state variety is a saddle shape, one of three variety classes for 3-species small networks.*

$$A + B \underset{\kappa_2}{\overset{\kappa_1}{\rightleftarrows}} C$$

$$\begin{aligned} f_A &= -\kappa_1 x_A x_B + \kappa_2 x_C \\ f_B &= -\kappa_1 x_A x_B + \kappa_2 x_C \\ f_C &= \kappa_1 x_A x_B - \kappa_2 x_C \end{aligned}$$

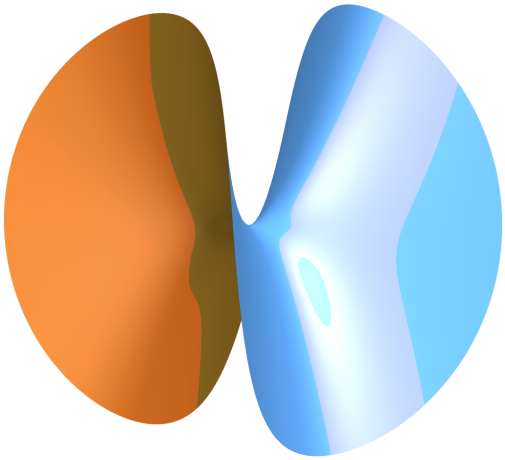

Figure 4: 3-species small network with saddle variety

Among the 495 non-isomorphic 3-species small networks, three classes of positive steady-state variety appear: slanted plane, cone, and saddle. Two networks have slanted planes as their positive steady-state variety; three networks have cones; and three networks have saddles. The following theorems characterize all small networks that produce each class of variety; in this section, "small network" refers to the 3-species case.

Theorem 6.1. *Given a small network satisfying the assumptions of Theorem 3.1, the positive steady-state variety will be a slanted plane if and only if the following hold:*

1. *Both reactant complexes are dual-species,*
2. *The supports of the reactant complexes are distinct (not necessarily disjoint).*

Proof. ($\Rightarrow$) Given a small network whose positive steady-state variety is a slanted plane, up to relabeling species the variety is $V(y - cx)$ for some positive $c \in \mathbb{R}$. The positive steady-state variety is a component of the steady-state variety, meaning that the equation defining the steady-state variety must have $(y - cx)$ as a factor, but may also contain another factor whose degree is limited by the complexes' molecularities:

$$(y - cx) \cdot (\text{Factor of degree 1 or 0})$$

As a two-reaction network, the equation defining the steady-state variety is at most a binomial, limiting the degree-one factors to be monomials or $(y + cx)$; there are therefore five potential equations defining the

steady-state variety. Also, $V(y-cx) = V(f_A) \cap V(f_B) \cap V(f_C)$, so the same logic applies to the individual steady-state equations; from Theorem 3.1 we have that the steady-state equations are multiples of each other and take on the same form. So, up to multiplication by constants, the steady-state equations take on one of these five forms:

$$f_i = y - cx \ ; \ f_i = y^2 - c^2x^2 \ ; \ f_i = y^2 - cxy \ ; \ f_i = xy - cx^2 \ ; \ f_i = yz - cxz$$

By the relationship between the reactant complexes and the steady-state equations' monomial terms, the first four forms correspond to networks with reactant complexes containing only A and B. In order to be genuine, the third species C must appear somewhere in the network; if C can only appear in the product complexes, the stoichiometric matrix cannot meet the assumptions of Theorem 3.1 as the row for C will be nonzero and entirely nonnegative. Thus, the steady-state equations can only take on the following form:

$$f_i = yz - cxz$$

This form corresponds to reactant complexes of $B + C$ and $A + C$; with arbitrary labeling of species, the network has dual-species reactant complexes whose supports are distinct.

($\Leftarrow$) Suppose now that a small network has dual-species reactant complexes with distinct supports. By the assumptions of Theorem 3.1 the steady-state equations are constant multiples of each other and we need only consider one equation; without loss of generality suppose the reactant complexes are $A+B$ and $B+C$ and consider a nonzero f_A:

$$f_A = \kappa_1(\alpha_1 - 1)xy + \kappa_2(\alpha_3)yz$$

Setting equal to zero and solving gives

$$\begin{aligned} 0 &= y\left[\kappa_1(\alpha_1 - 1)x + \kappa_2(\alpha_3)z\right] \\ \Rightarrow y = 0 \ , \ z &= \frac{-\kappa_1(\alpha_1 - 1)}{\kappa_2(\alpha_3)}x \end{aligned}$$

By assumption the stoichiometric coefficients have opposite signs, so the coefficient on z is positive and therefore the equation defines a slanted plane crossing into the positive orthant, which is the desired positive steady-state variety.

□

Theorem 6.2. *Given a small network satisfying the assumptions of Theorem 3.1, the positive steady-state variety will be a cone if and only if the following hold:*

1. *One reactant complex is dual-species while the other is bimolecular and single-species,*
2. *The supports of the reactant complexes are disjoint.*

Proof. ($\Rightarrow$) Suppose we have a small network whose positive steady-state variety is a cone. Up to relabeling species, the variety is $V(x^2 - cyz)$ for some positive real constant c. Additionally, $V(x^2 - cyz) = V(f_A) \cap V(f_B) \cap V(f_C)$ and the network is at-most-bimolecular, meaning the degrees of the steady-state equations are at most two and must have the degree-two expression $x^2 - cyz$ as a factor. Then, as in Theorem 3.1, the steady-state equations are of the form

$$\begin{aligned} f_A &= x^2 - cyz \\ f_B &= s(x^2 - cyz) \\ f_C &= t(x^2 - cyz) \end{aligned}$$

where $s, t \in \mathbb{R}$ are not both zero. The monomial terms must have variable components of x^2 and yz, corresponding to reactant complexes of $2A$ and $B+C$. With arbitrary labeling of species, these are precisely a bimolecular single-species complex and a dual-species complex which have disjoint supports.

($\Leftarrow$) Suppose we have a small network satisfying the above conditions and, without loss of generality, the reactant complexes are $2C$ and $A+B$. As in Theorem 3.1 the steady-state equations are constant multiples

of each other and we need only examine one to understand the variety; without loss of generality consider a nonzero f_B:

$$f_B = \kappa_1(\beta_1)z^2 + \kappa_2(\beta_3 - 1)xy$$

Setting equal to zero and solving for z^2 gives

$$z^2 = \frac{-\kappa_2(\beta_3 - 1)}{\kappa_1(\beta_1)}xy$$

The stoichiometric coefficients have opposite signs by assumption, so the coefficient on xy is positive and the equation defines a cone crossing into the positive orthant; the positive steady-state variety is a cone, as desired.

□

Theorem 6.3. *Given a small network satisfying the assumptions of Theorem 3.1, the positive steady-state variety will be a saddle if and only if the following hold:*

1. *One reactant complex is dual-species while the other is unimolecular and single-species,*
2. *The supports of the reactant complexes are disjoint.*

Proof. ($\Rightarrow$) Suppose we have a small network whose positive steady-state variety is a cone. Up to relabeling species, the variety is $V(x-cyz)$ for some positive $c \in \mathbb{R}$. Furthermore, $V(x-cyz) = V(f_A) \cap V(f_B) \cap V(f_C)$ and the network is at-most-bimolecular, so the degrees of the steady-state equations are at most two and must have $x - cyz$ as a factor. From the assumptions of Theorem 3.1 the steady-state equations are of the form

$$\begin{aligned} f_A &= x - cyz \\ f_B &= s(x - cyz) \\ f_C &= t(x - cyz) \end{aligned}$$

where $s, t \in \mathbb{R}$ are not both zero. The monomial terms therefore have variable components of x and yz, which correspond to reactant complexes of A and $B + C$. With arbitrary labeling of species, these are a unimolecular single-species complex and a dual-species complex whose supports are disjoint.

($\Leftarrow$) Suppose we have a small network satisfying the above conditions and, without loss of generality, the reactant complexes are B and $A + C$. By the assumptions of Theorem 3.1 the steady-state equations are multiples of each other so we need only consider one to understand the variety; without loss of generality consider a nonzero f_C:

$$f_C = \kappa_1(\gamma_1)y + \kappa_2(\gamma_3 - 1)xz$$

Setting equal to zero and solving for y gives

$$y = \frac{-\kappa_2(\gamma_3 - 1)}{\kappa_1(\gamma_1)}xz$$

By assumption, the stoichiometric coefficients have opposite signs, so the coefficient on xz is positive and the equation defines a saddle crossing into the positive orthant, which is the desired positive steady-state variety. □

Remark 6.1. *Since the classification theorems in this and the previous section only directly impose conditions upon the reactant complexes, they also classify the positive steady-state varieties of networks with product complexes of higher molecularities, provided that they satisfy the assumptions of Theorem 3.1. For example, the positive steady-state variety of the network comprised of* $2A \to 3A + B$ *and* $A + B \to 0$ *is a line through the origin by Theorem 5.2.*

7 Conditions for an Empty Positive Steady-State Variety

The theorems in Sections 5 and 6 completely classify the nonempty positive steady-state varieties produced by 2- and 3-species small networks; however, it is also worth discussing network properties that can be used to identify networks–small and otherwise–with empty positive steady-state varieties. First, consider 3-species small networks. We know that a network failing either assumption of Theorem 3.1 will have an empty positive steady-state variety. However, for 3-species small networks in particular, the steady-state variety will be empty if either reactant complex is empty, as seen in the following example:

Example 7.1. *Consider the 3-species network and its steady-state equations in Figure 5. The first two equations require $x_A x_B$ to equal some positive number, while the third equation requires $x_A x_B$ to equal zero; therefore, the steady-state variety is empty.*

$$\begin{aligned} 0 &\xrightarrow{\kappa_1} A+B \\ A+B &\xrightarrow{\kappa_2} 2C \end{aligned} \qquad \begin{aligned} f_A &= \kappa_1 - \kappa_2 x_A x_B \\ f_B &= \kappa_1 - \kappa_2 x_A x_B \\ f_C &= 2\kappa_2 x_A x_B \end{aligned}$$

Figure 5: 3-Species, 2-Reaction Network with Empty Steady-State Variety

Due to the condition of bimolecularity, the reaction with the empty complex will contain at most two species. This means there will be one species whose corresponding steady-state variety is a monomial. We know the positive steady-state variety will be empty directly from this, as the presence of any monomial steady-state equation restricts the potential steady-state variety to coordinate planes; however, there is a stronger result here. First, consider the case where there is a monomial steady-state equation whose term is generated from the reaction with the empty reactant complex. Then, the equation will be of the form $f_i = \kappa_i$, which has an empty zero set, so the steady-state variety is empty. But, even if there is no such monomial, the same result occurs. As in Example 7.1, any binomial steady-state equation will require the relevant variables to equal some nonzero constant, and if there are no monomials generated from the empty-complex reaction there must be a monomial generated from the other complex. This monomial will require the same variables to simultaneously equal zero; there are never any solutions to such a three-equation system and the steady-state variety is empty. This discussion is summarized in the following proposition:

Proposition 7.1. *If a 3-species small network has an empty reactant complex, then its steady-state variety will be empty.*

This result is particular to the at-most-bimolecular case. If we alter the network in Figure 5 so that the second network is $A+B+2C \xrightarrow{\kappa_2} 2C$, then $f_C = 0$ and the steady-state variety is defined by $x_A x_B x_C^2 = \frac{\kappa_1}{\kappa_2}$, which is not only nonempty, but also has a nonempty positive steady-state variety.

Now, consider networks of more than two reactions. We know from Theorem 3.1 that if a small network has identical reactant complexes, then its positive steady-state variety will be empty. This holds for networks with more than two reactions as well; consider a network with n reactions and s species where all n reactant complexes are identical. Each f_{A_i} will be as follows, where α_i is the coefficient on A_i in the reactant complex and α_{ij} is the coefficient on A_i in the j^{th} product complex:

$$\begin{aligned} f_{A_i} &= (\alpha_{11} - \alpha_1)\kappa_1 x_1^{\alpha_1} \cdots x_s^{\alpha_s} + \ldots + (\alpha_{1n} - \alpha_1)\kappa_n x_1^{\alpha_1} \cdots x_s^{\alpha_s} \\ &= x_1^{\alpha_1} \cdots x_s^{\alpha_s} \left[(\alpha_{11} - \alpha_1)\kappa_1 + \ldots + (\alpha_{1n} - \alpha_1)\kappa_n\right] \\ &= x_1^{\alpha_1} \cdots x_s^{\alpha_s} \cdot c_i \end{aligned}$$

where c_i is some real constant. The steady-state equations are clearly all monomials, whose solution sets consist only of coordinate hyperplanes. However, identical reactant complexes are not the only way for a network to have monomial steady-state equations. Indeed, if any steady-state equation is a monomial, the

steady-state variety is restricted to coordinate hyperplanes, since the steady-state variety is the intersection of all varieties produced by the individual steady-state equations. The number of nonzero terms in a steady-state equation is equal to the number of nonzero entries in the corresponding row of the stoichiometric matrix. So, if any row of the stoichiometric matrix has exactly one nonzero entry, that species' steady-state equation will be a monomial and the network's steady-state variety is contained within some collection of coordinate hyperplanes. These observations are summarized in the following proposition:

Proposition 7.2. *If a network of any size has identical reactant complexes or if any row of its stoichiometric matrix has exactly one nonzero entry, then its positive steady-state variety will be empty.*

8 Generalizing to Larger Networks

The theorems in Sections 5 and 6 rely heavily on the molecularity bounds on the complexes to classify the positive steady-state varieties. While most reactions appearing in nature are indeed bimolecular, the mathematical study of reaction networks is not limited by the same constraints. With the goal of generalizing the 2-species classification theorems to small networks with complexes of higher molecularity, we return to some more general properties of networks.

8.1 Reactant Complex Overlap

A direct result of the assumption of mass action kinetics is the connection between a network's reactant complexes and its corresponding steady-state equations' monomial terms; this relationship is fundamental to all previous proofs in this thesis. Consequently, shared species within a small network's reactant complexes corresponds to coordinate hyperplane components of the variety.

Proposition 8.1. *A network of any size will have shared species within its reactant complexes if and only if its variety has coordinate hyperplane components whose order corresponds to the number of shared elements.*

Proof. Suppose all reactant complexes of a network with s species and n reactions share $\alpha_i A_i$, so that $\alpha_{ij} \geq \alpha_i > 0$ for all j. For the m^{th} term of the j^{th} steady-state equation, the stoichiometric coefficient will be $(\alpha_{j(2m-1)} - \alpha_{j(2m-2)})$ and the exponent on each x_k will be $\alpha_{k(2m-2)}$. Combining this with the general form steady-state equations yields the following after factoring for any A_j:

$$f_{A_j} = x_i^{\alpha_i}\big[(\alpha_{j1} - \alpha_{j0})\kappa_1 x_1^{\alpha_{10}} \cdots x_i^{\alpha_{i0}-\alpha_i} \cdots x_s^{\alpha_{s0}} + \ldots \\ \ldots + (\alpha_{j(2n-1)} - \alpha_{j(2n-2)})\kappa_n x_1^{\alpha_{1(2n-2)}} \cdots x_i^{\alpha_{i(2n-2)}-\alpha_i} \cdots x_s^{\alpha_{s(2n-2)}}\big]$$

Defining the n-term polynomial within the brackets for f_{A_j} as $P_j(x)$, the steady-state variety then becomes

$$[V(x^{\alpha_i}) \cup V(P_1(x))] \cap \cdots \cap [V(x^{\alpha_i}) \cup V(P_s(x))] = V(x^{\alpha_i}) \cup [V(P_1(x)) \cap \cdots \cap V(P_s(x))]$$

This variety is composed of a coordinate hyperplane solution when $x_i = 0$ of order α_i, along with the intersection of the zero sets of the remaining polynomials.

Now, suppose a network has a coordinate hyperplane of order m as a component its steady-state variety, and suppose the component is $V(x_i^m)$. Then, $x_i^m = 0$ must be a solution to the steady-state system, and therefore must be a shared factor among all monomial terms. Each monomial term is built from the reactant complexes, and so every reactant complex must contain mA_i. □

Example 8.1. *Consider the network in Figure 6 along with its steady-state equations and variety, here with $\kappa_1 = \kappa_2 = 1$ and the x_B axis as the vertical axis. The shared elements of the reactant complexes, here $2A + B$, result in both coordinate axes as components of the steady-state variety.*

It follows immediately that two distinct networks have the potential to produce the same non-coordinate-hyperplane steady-state variety if the non-shared components of their reactant complexes are the same; however, differences in the product complexes could alter the variety by changing the stoichiometric coefficients. To identify some cases where distinct networks can produce the same variety, we consider actions under

$$3A + B \underset{\kappa_2}{\overset{\kappa_1}{\rightleftarrows}} 2A + 3B$$

$$f_A = -\kappa_1 x_A^3 x_B + \kappa_2 x_A^2 x_B^3 = x_A^2 x_B(-\kappa_1 x_A + \kappa_2 x_B^2)$$
$$f_B = \kappa_1 x_A^3 x_B - \kappa_2 x_A^2 x_B^3 = x_A^2 x_B(\kappa_1 x_A - \kappa_2 x_B^2)$$

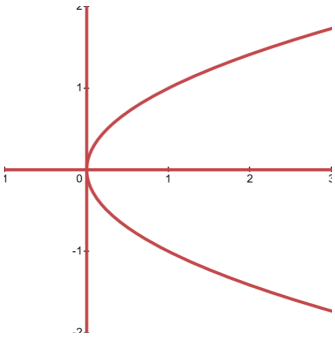

Figure 6: Reactant complex overlap and axis components

which a network's non-coordinate-hyperplane steady-state variety is invariant. Observe that the network in Figure 6 has the same non-axis steady-state variety as the example network in Section 2, and is indeed the same network with $2A+B$ added to both reactant complexes. The following property allows the construction of higher-molecularity networks within a given variety family by building off of a smaller network.

Corollary 8.1. *A network's non-coordinate-hyperplane steady-state variety is invariant under the operation of adding a linear combination of species to all complexes.*

Proof. Consider the transformation of adding $m_i A_i$ to all complexes of a network, where the m_i are non-negative integers and the following is the i^{th} reaction:

$$(\alpha_{1(2i-2)} + m_1)A_1 + \ldots + (\alpha_{s(2i-2)} + m_s)A_s \xrightarrow{\kappa_i} (\alpha_{1(2i-1)} + m_1)A_1 + \ldots + (\alpha_{s(2i-1)} + m_s)A_s$$

The corresponding steady-state equation for each A_i after simplification is

$$f_{A_i} = x_1^{m_1} \cdots x_s^{m_s} \left[(\alpha_{i1} - \alpha_{i0})\kappa_1 x_1^{\alpha_{10}} \cdots x_s^{\alpha_{s0}} + \ldots + (\alpha_{i(2n-1)} - \alpha_{i(2n-2)})\kappa_n x_1^{\alpha_{1(2n-2)}} \cdots x_s^{\alpha_{s(2n-2)}}\right]$$

The added species are shared by the reactant complexes, so by Proposition 8.1 these steady-state equations formulate the same non-axis variety as the network before alteration, as the additional species only add coordinate hyperplane solutions and the $P_j(x)$ are unaltered. □

8.2 Generalizing the 2-species Case

While the classification theorems in Section 5 fully capture the at-most-bimolecular case, those same four classes of varieties can certainly occur in small networks with higher molecularity bounds. Using the ideas introduced in Section 8.1, we can consider the *reduced reactant complexes* of a network, which are the reactant complexes with all shared species removed. By Proposition 8.1, these contain all necessary information about the non-axis components of the steady-state variety, which are precisely the components with the potential to be positive. The following four conjectures classify the positive steady-state variety classes seen in Section 5 for 2-reaction networks with complexes of any molecularity.

Conjecture 8.1. *Given a network satisfying the conditions of Theorem 3.1, the positive steady-state variety will be a non-axis horizontal or vertical line if and only if the two reduced reactant complexes are 0 and a single-species complex.*

Conjecture 8.2. *Given a network satisfying the conditions of Theorem 3.1, the positive steady-state variety will be a line through the origin if and only if the two reduced reactant complexes are are nonempty, disjoint, and have the same molecularity.*

Conjecture 8.3. *Given a network satisfying the conditions of Theorem 3.1, the positive steady-state variety will be a parabola if and only if the two reduced reactant complexes are nonempty, disjoint, and the molecularity of one is twice that of the other.*

Conjecture 8.4. *Given a network satisfying the conditions of Theorem 3.1, the positive steady-state variety will be a hyperbola if and only if the two reduced reactant complexes are 0 and a dual-species complex.*

Theorem 3.1 tells us when a nonempty positive steady-state variety exists, but gives no further information about the identity or geometrical properties of that variety. As mentioned at the end of Section 5.1, extending to arbitrary molecularities allows for infinitely many different term-by-term degree ratios; but, they share the common "bird-like" shape. Indeed, there are several similar large families of positive steady-state varieties, and a network's membership in a given family is tied to the relative size and parity of its reduced reactant complexes' molecularities, and it seems that there are eight total families of networks. Three of them are fully described by conjectures 8.1, 8.2, and 8.4. The remaining families are as follows:

8.3 Even Power Functions

Just as Conjecture 8.3 describes networks whose positive steady-state varieties are parabolas, a network can also have a quartic, sixth power, or any higher even-power curve.

Conjecture 8.5. *The positive steady-state variety of a network satisfying the conditions of Theorem 3.1 is an even-power curve if and only if the molecularities of the (nonempty and disjoint) reduced reactant complexes are n and $(2k)n$ for any natural numbers n and k.*

In particular, the variety will be given by $y = x^{2k}$, and note that if $k = 1$ we have the parabola case.

8.4 "Bird-Type"

Generalizing the semicubical case, there are other term-by-term degree ratios that result in a similar "bird-like" shape, where there is a cusp at the origin and the curve bends away from the axis of symmetry.

Conjecture 8.6. *The positive steady-state variety of a network satisfying the conditions of Theorem 3.1 is bird-type if and only if the ratio in lowest terms of the molecularities of the reduced reactant complexes is even:odd with the odd number larger.*

Note that if the ratio is 2:3 in particular, we have the semicubical parabola case.

8.5 "Claw-Type"

Similar to the bird-type form is a "claw-like" shape, like a parabola with a cusp at the vertex.

Conjecture 8.7. *The positive steady-state variety of a network satisfying the conditions of Theorem 3.1 is claw-type if and only if the ratio in lowest terms of the molecularities of the reduced reactant complexes is even:odd with the even number larger.*

8.6 Odd Power Functions

A network can also have a positive steady-state variety in the shape of an odd power function; the criteria are similar to that of Conjecture 8.3.

Conjecture 8.8. *The positive steady-state variety of a network satisfying the conditions of Theorem 3.1 is an odd-power curve if and only if the molecularities of the (nonempty and disjoint) reduced reactant complexes are n and $(2k+1)n$ for any natural numbers n and k.*

In particular, the variety will be given by $y = x^{2k+1}$.

8.7 "Snake-Type"

Finally, just as the claw-type networks are similar to parabolas but with a cusp at the vertex, a network can have a "snake-like" shape, similar to an odd power function but with a cusp at the origin.

Conjecture 8.9. *The positive steady-state variety of a network satisfying the conditions of Theorem 3.1 is snake-type if and only if the ratio in lowest terms of the molecularities of the reduced reactant complexes is odd:odd.*

9 Conclusion

As mentioned in the introduction, calculating the positive steady-state variety is difficult in general and existing tools are limited in their scope. With Theorem 3.1, the case of 2-reaction networks is fully described. This thesis also classified 2- and 3-species at-most-bimolecular networks by the shape of their positive steady-state variety (Sections 5 and 6), and gave a comprehensive analysis of at-most-bimolecular 1-species networks (4). The reactant complex conditions are verifiable by inspection and the calculation of the stoichiometric matrix is straightforward, providing significantly simpler conditions than those given in [6] to identify networks with toric positive steady-state varieties. Finally, we gave some potential tools and conjectures to expand the classification theorems of Sections 5 and 6 to networks with complexes of higher molecularity (Section 8).

In chemistry, most valid reactions involve bimolecular reactant complexes (and, rarely, trimolecular ones), since the likelihood of more than two components coming into contact simultaneously, in the proper orientation and with sufficient energy, is very low. So, the conjectures of Section 8 would describe few biologically relevant networks. However, as mentioned in Remark 6.1, the theorems of Sections 5 and 6 apply to 2-reaction networks with product complexes of higher molecularity, provided that the reactant complexes are bimolecular. The authors of [3] studied 3-reaction so-called "bimolecular-sourced" networks and their oscillatory behavior. Generalizing the systematic approach and findings of this thesis to larger networks–for example, to 3-reaction networks–could lend valuable insight into biologically relevant bimolecular-sourced networks.

Acknowledgements

Thank you to my thesis advisor, Dr. Luis David Garcia Puente. A portion of this research (the 2-species case) was completed as part of the 2023 Pomona Research in Mathematics Experience, supported by Pomona College and the NSF (DMS-1560394). Work at PRiME was done in collaboration with Elise Farr (Boston University), Julian Hutchins (Morehouse College), and Vuong Nguyen Hoang (Wingate University), with Maize Curiel (University of Hawai'i at Mānoa) and Dr. Luis David Garcia Puente as research mentors.

References

[1] Michael F. Adamer and Martin Helmer. Families of toric chemical reaction networks. *Journal of Mathematical Chemistry*, 58:2061–2093, oct 2020.

[2] Murad Banaji. Chemical reaction network enumeration. `https://reaction-networks.net/networks/`.

[3] Murad Banaji, Balázs Boros, and Josef Hofbauer. Oscillations in three-reaction quadratic mass-action systems. *Studies in Applied Mathematics*, 152(1):249–278, 2024.

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

[5] Alicia Dickenstein. Algebraic geometry tools in systems biology. *Notices of the American Mathematical Society*, 67:1706–1715, 2020.

[6] Mercedes Pérez Millán, Alicia Dickenstein, Anne Shiu, and Carsten Conradi. Chemical reaction systems with toric steady states. *Bulletin of Mathematical Biology*, 74:1027–1065, may 2012.

[7] Nida Obatake, Anne Shiu, and Dilruba Sofia. Mixed volume of small reaction networks. *Involve, a Journal of Mathematics*, 13(5):845–860, dec 2020.

[8] Dan Siegal-Gaskins, Maria Katherine Mejia-Guerra, Gregory D. Smith, and Erich Grotewold. Emergence of switch-like behavior in a large family of simple biochemical networks. *PLOS Computational Biology*, 7:1–12, 05 2011.

Appendix: Network Translation Code

For the lists of networks given at [2], we created a function that would read the database text files and translate them into lists of networks usable by the Macaulay2 ReactionNetworks package. The translation code is as follows.

```
    --loads necessary packages:
loadPackage "ReactionNetworks"
loadPackage "ReflexivePolytopesDB"

--calling text file:
file = "file name.txt"
directory = "~/folder where file is located"
    --use your file name and location here
fn3 = concatenate(directory,"/",file)
get fn3;

--converting database number lists into ordered pairs representing reaction edges:
codes = apply(lines get fn3, s-> flatten entries matrixFromString s)
edges = for c in codes list(t = drop(c, 2); while #t > 0 list (t_0, t_1) do t = drop(t,2))

--setting numbers of species and reactions:
m = (codes_0)_0
n = (codes_0)_1

--formatting list of reaction edges into matrices of species coefficients:
R2 = apply (edges, f -> matrix table(n,m, (i,j) -> number(f, e -> e == (j,i+m)) ));
L2 = apply (edges, f -> matrix table(n,m, (i,j) -> number(f, e -> e == (i+m,j)) ));

--translating from matrices to networks:
crnRing = QQ[A, B]
    --include all necessary species here.
varMatrix = vars crnRing
makeCRN = (m, LHS, RHS) -> (
    myList = apply (m, i -> concatenate(toString((flatten entries LHS_0)_i), " --> ",
    toString((flatten entries RHS_0)_i)) );
        --joins left- and right-hand sides with reaction arrows
    myList2 = apply (m, i -> concatenate separate("[*]", myList_i));
        --removes multiplication symbol to get proper network formatting
    myCRN = reactionNetwork apply(myList2, s -> replace( "0", "0A", s ))
        --changes 0 into 0 times a variable for empty complexes
    )

--applying to the first i+1 networks in the file:

Section1 = for g from 0 to i list makeCRN(m, varMatrix*(L2_{g}), varMatrix*(R2_{g}))
```