

Spring 2019

# Investigations and Analysis of Dynamical and Steady State Properties of Chemical Reaction Systems

Diego Ortega Hernandez  
*San Jose State University*

Follow this and additional works at: [https://scholarworks.sjsu.edu/etd\\_theses](https://scholarworks.sjsu.edu/etd_theses)

---

## Recommended Citation

Hernandez, Diego Ortega, "Investigations and Analysis of Dynamical and Steady State Properties of Chemical Reaction Systems" (2019). *Master's Theses*. 5002.

DOI: <https://doi.org/10.31979/etd.8mas-gnhe>  
[https://scholarworks.sjsu.edu/etd\\_theses/5002](https://scholarworks.sjsu.edu/etd_theses/5002)

This Thesis is brought to you for free and open access by the Master's Theses and Graduate Research at SJSU ScholarWorks. It has been accepted for inclusion in Master's Theses by an authorized administrator of SJSU ScholarWorks. For more information, please contact [scholarworks@sjsu.edu](mailto:scholarworks@sjsu.edu).

INVESTIGATIONS AND ANALYSIS OF DYNAMICAL AND STEADY STATE  
PROPERTIES OF CHEMICAL REACTION SYSTEMS

A Thesis

Presented to

The Faculty of the Department of Mathematics & Statistics

San José State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Diego O. Hernandez

May 2019

© 2019

Diego O. Hernandez

ALL RIGHTS RESERVED

The Designated Thesis Committee Approves the Thesis Titled

INVESTIGATIONS AND ANALYSIS OF DYNAMICAL AND STEADY STATE  
PROPERTIES OF CHEMICAL REACTION SYSTEMS

by

Diego O. Hernandez

APPROVED FOR THE DEPARTMENT OF MATHEMATICS & STATISTICS

SAN JOSÉ STATE UNIVERSITY

May 2019

Matthew D. Johnston, Ph.D.	Department of Mathematics & Statistics
Plamen S. Koev, Ph.D.	Department of Mathematics & Statistics
Dashiell E.A. Fryer, Ph.D.	Department of Mathematics & Statistics
Liam G. Stanton, Ph.D.	Department of Mathematics & Statistics

## ABSTRACT

### INVESTIGATIONS AND ANALYSIS OF DYNAMICAL AND STEADY STATE PROPERTIES OF CHEMICAL REACTION SYSTEMS

by Diego O. Hernandez

In this paper, we investigate results from chemical reaction network theory and a list of techniques to test for the reaction-coordinates dynamical system to have a partial order induced by a positive orthant cone. A successful result from one of these tests guarantees mono-stationarity (and indeed convergence). We also investigate a recently published algorithmic and computational approach to determine whether a reaction network establishes mono- or multi-stationarity. We test new reactions that have not been previously introduced in the literature for mono- or multi-stationarity using this approach. This includes the two-site phosphorylation reaction network and a modified double phosphorylation reaction network that more accurately models the action of the enzymes of two distinct sites. We also use the enzymatic futile cycle as a running example to illustrate these results. We conclude the two-site phosphorylation reaction network is multi-stationary; while the original double phosphorylation reaction network is also multi-stationary, our modified version is mono-stationary.

## DEDICATION

To Fernando and Mary-Lou Ortega.

## ACKNOWLEDGMENTS

I would like to thank my advisor Professor Matthew D. Johnston for putting up with my ambitions despite wanting me to do a project instead of a thesis. I give thanks to all of my professors and the friends I made in these last two years including Jordan Schettler, Wasin So, Daniel Brinkman, Slobodan Simic, Plamen Koev, Dashiell Fryer, Steven Crunk, Sharon Stranahan, Yan Zhang, Guanliang Chen, Benjamin Godkin, Khai Le, Le Phan, Tuan Nguyen, Jarrett Jimeno, and many others. I would also like to thank my beautiful Diana, my parents Jorge and Veronica, and her parents Marco and Angelina for their continual support.

## TABLE OF CONTENTS

List of Tables .....	ix
List of Figures .....	x
1 Introduction.....	1
1.1 Cross-Assumptions and Adopted Conventions .....	2
1.2 Deficiency Zero and One Theorems .....	3
1.3 Monotone Systems Theory .....	4
1.4 Graphical Tests for Monotonicity .....	5
1.5 Using an Algorithm to Establish Multi-Stationarity .....	6
1.6 Conclusion .....	7
2 Preliminaries .....	8
2.1 Notation and Definitions .....	8
2.2 Glossary .....	9
2.2.1 Pre-Ordered and Partially Ordered Cones .....	9
2.2.2 Monotone Systems .....	11
3 Introduction to Reaction Modeling .....	13
3.1 Reactions and Reaction Rates.....	13
3.2 Dynamical Systems and Modeling Chemical Reaction Networks .....	15
3.3 Limit Cycles, Persistence, and Consistence .....	18
4 The Angeli-Sontag Theorem .....	22
4.1 Stoichiometric Subspace and the Stoichiometric Compatibility Class .....	22
4.2 Projection Mappings.....	25
4.3 Statement of the Angeli-Sontag Theorem .....	27
4.4 Satisfying the Angeli-Sontag Theorem.....	28
4.4.1 Drawing Conclusions of Dynamics Between Species-Space and Reaction-Space.....	28
4.4.2 Independence of Reaction Modeling and Bounded-ness of Solutions.....	29
4.5 Using the Angeli-Sontag Theorem with Chemical Reaction Dynamical Systems.....	32
4.6 Finding an Equilibrium of the Enzymatic Futile Cycle .....	36
5 Graphs of Chemical Reaction Networks.....	41
5.1 Jacobian Graph .....	43
5.1.1 Construction .....	43
5.2 Species Reaction Graph .....	43



5.2.1	Construction .....	43
5.3	Reaction Graph .....	44
5.3.1	Construction .....	44
5.4	Species Graph .....	45
5.5	Directed Species Reaction Graph .....	45
5.6	Tests for Monotonicity and the Positive Loop Property .....	46
5.6.1	Positive Loop Property .....	47
5.6.2	Graphical Tests for Monotonicity in Reaction-Coordinates Dynamical Systems .....	48
5.7	Finding an Equilibrium of the Enzymatic Futile Cycle .....	51
6	An Algorithm for Establishing Multi-Stationarity .....	55
6.1	Dissipative and Conservative Reaction Networks .....	56
6.2	Siphons and Equilibria .....	59
6.3	Reduction System of Equations .....	61
6.4	Theorem for Establishing Multi-Stationarity .....	63
6.5	Steps of Procedure for the Algorithm .....	64
6.6	Performing the Algorithm in Maple (2018 version) and Python 2.2+ ..	66
6.7	Establishing Mono-Stationarity in the Enzymatic Futile Cycle .....	70
7	Main Examples .....	73
7.1	Example 1: Two-Component Signaling System (Histidine Kinase) ...	73
7.1.1	Positive Loop Property .....	73
7.1.2	Testing for Multi-Stationarity .....	76
7.2	Example 2: Double Phosphorylation Cycle .....	78
7.2.1	Positive Loop Property .....	80
7.2.2	Testing for Multi-Stationarity .....	80
7.2.3	Additional comments .....	84
7.3	Example 3: Two Site Phosphorylation Network with Distinct Sites ...	88
7.3.1	Positive Loop Property .....	90
7.3.2	Testing for Multi-Stationarity .....	90
8	Conclusion .....	96
	Literature Cited .....	97

LIST OF TABLES

Table 1.	Some Terms of the Numerator of $J = DM(S(t))$ Corresponding to Extreme Vertices .....	84
Table 2.	Some Terms of the Numerator of $J = DM(S(t))$ Corresponding to Extreme Vertices With Reaction Rate Constants $k_3 = s$ and All Other $k_i = 1$ .....	95

## LIST OF FIGURES

Fig. 1.1.	Organization of the literature review with respect to developmental contributions in CRNT.....	2
Fig. 4.1.	The stoichiometric subspace of the reaction in Example 23. ....	23
Fig. 4.2.	The stoichiometric compatibility class of Example 23. ....	24
Fig. 4.3.	Shifting the extent of a reaction by a kernel vector. ....	29
Fig. 5.1.	A positive edge in the R-graph does not imply uniqueness of species in the SR-graph. ....	45
Fig. 5.2.	Special graphs of Example 18. ....	52
Fig. 7.1.	Special graphs of the XY reaction. ....	74
Fig. 7.2.	Special graphs of the Double Phosphorylation reaction. ....	79
Fig. 7.3.	SR-graph of Example 2 with $S_3, E_2, F_2$ included. ....	85
Fig. 7.4.	Special graphs of the Two-Site Phosphorylation network. ....	89

## 1 INTRODUCTION

A chemical reaction is a relationship between species elements (e.g., hydrogen and oxygen) and the reaction that forms products (e.g., water). Some reactions are reversible (two-way), and others are irreversible (one-way). It is useful to know whether a chemical reaction will reach equilibrium, but this is not easy to determine. One commonly has to experimentally run the reaction multiple times and obtain empirical information about the reaction rates, which can be expensive and time-consuming. Fortunately, there are several mathematical frameworks for addressing these problems by providing insight in a cheaper and less time-consuming manner.

Chemical reaction network theory (CRNT) is a recent branch of applied mathematics that uses graph theory and dynamical systems techniques to study the existence and properties of reaction equilibria, e.g., uniqueness, isolation, etc. The study of chemical reactions is not exclusive to chemists, as mathematicians have taken a significant interest in studying reaction networks and analyzing general tools. The field is expanding to computer science in such areas as Petri-net theory [1] and the biological studies of population dynamics [2]. Chemical reaction network theory has sourced from different branches of mathematics such as linear algebra, combinatorics, analysis, and graph theory. Chemical reaction networks model real life situations, so we have to make assumptions that fit certain physics-based conditions, e.g., time always moves forward, and one cannot have negative values for concentrations. This means the study of dynamical systems has taken precedence for CRNT in recent years because of its ability to study behavior of solutions as time moves forward. There is a discussion about how to (potentially) automate these approaches in reference [3].

This literature review is organized by the approaches used to draw conclusions about the equilibria of chemical reaction networks and key contributions researchers have made to adapt them. We will source from published books, lecture notes, conference

proceedings, and academic articles from Math Biology, AMS, Computational Physics, etc. We will follow how authors from the literature have used their contributions to answer certain questions from the 1980s to the present outlined in Figure 1.1. Namely, what assumptions do we make on the species of a reaction? How can we construct a dynamical system from a chemical reaction network? When is this dynamical system solvable? Does the system have any asymptotic behavior? Can the system be monotone, cooperative, or even strongly monotone? Is it possible to implement an algorithmic approach? Finally, we will close with a discussion of relevant topics for future endeavors.

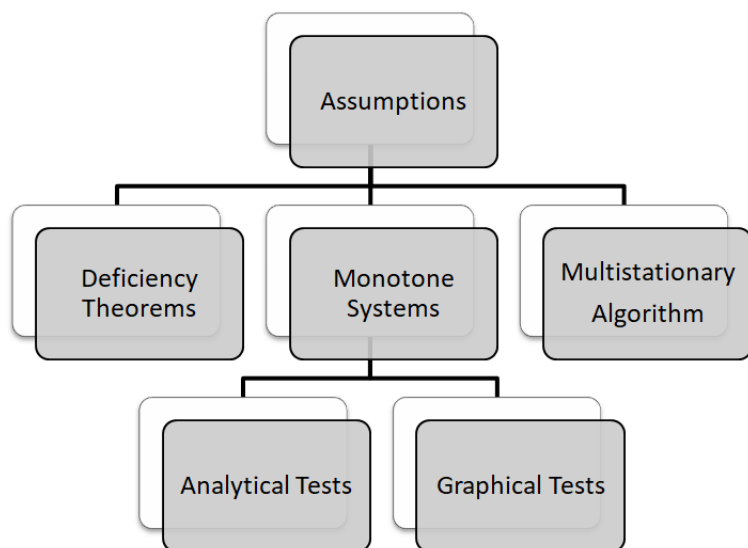


Fig. 1.1: Organization of the literature review with respect to developmental contributions in CRNT.

## 1.1 Cross-Assumptions and Adopted Conventions

Although CRNT is a relatively recent field, there are some established assumptions and conventions that some researchers adopt as seen in references [1]–[5], etc. For example, monotone systems theory fixes reactants and products for reversible reactions and consider the forward and backward components of a reversible reaction only once; this is not adopted in deficiency theory. There are physical limitations that a reaction rate

function must satisfy if it is to remain relevant to model real-life situations: the rate function is non-negative, it is zero if any consistent species is absent, and it is monotonically increasing in all of the consistent species. Every reaction rate depends monotonically on the amount of species present in the reaction. Since a larger concentration of species implies the reactions must go faster, the opposite is also true. Thus, if species are present, reactions occur; if species are not present, reactions stop.

There is a trade-off to having general assumptions about the reaction rates and species that will influence the size of the class of reaction networks that satisfy the result. A reaction rate is a function that measures the non-negative speed of the reaction, and a set of reaction rates is called kinetics. Some results are only computationally possible with specific kinetics [3], while the results of Angeli, Leenheer and Sontag in papers [1], [5], [6] are independent of kinetics and are more visual-based. For example, Conradi, Feliu, Mincheva, and Wiuf assume mass-action, Michaelis-Menten, or Hill kinetics to construct an algorithm to establish multi-stationarity for reaction networks in the paper [3], but this result is computationally infeasible for large networks with 20 or more species [3], [7]. The papers [5], [6] assume species are present on only one side of the reaction to ensure the sign of a reaction rate does not change between positive (increasing) and negative (decreasing), but this fails in practice.

## **1.2 Deficiency Zero and One Theorems**

A chemical reaction network induces a nonlinear system of differential equations, with some linear components, which is a list of equations that measure the dynamics of the change in species with respect to time. Martin Feinberg's lectures [8], [9] are at the heart of this, as he used vector analysis to construct what are now called the species-space, reaction-space, and the stoichiometric compatibility class. Using linear algebra and assumptions about how species react, one is able to directly see how the conservation laws hold. Feinberg's major contributions were the Deficiency Zero and One Theorems.

The former guarantees uniqueness and stability of steady states. The latter guarantees every compatibility class has the same number of steady states with no conclusion on stability; if the reaction network's structure is also weakly reversible, then there is a unique steady state. Unfortunately, these Deficiency theorems do not easily hold in practice. Johnston and Tonello recently introduced a technique in the paper [10] that shifts a network by (usually an enzymatic) species to get an equivalent network that satisfies the assumptions of the Deficiency theorems in a more general framework. Banaji and Craciun explored other graphical criteria for unique equilibria in the paper [11].

### **1.3 Monotone Systems Theory**

Determining if a chemical reaction system is monotone can be very valuable in analyzing models of real world reactions. Monotone systems are useful for analytic and computational purposes because there is no asymptotic behavior in which solutions grow to infinity or eventually vanish. While monotonicity is not common in practice according to references [12], [13], one can say more about the global stability of solutions if a dynamical system is strongly monotone. One wants all solutions to be bounded. This makes sense in the real world, as chemicals do not simply vanish or appear spontaneously because there are finite resources such as energy and space to consider when running experiments. To see an example of this type of behavior in non-monotone systems, Errami, Eiswirth, Grigoriev, Seiler, Sturm, and Weber presented chemical reaction systems that have Hopf Bifurcations in the papers [12], [13].

Determining if a dynamical system is monotone is a difficult question to answer in general. The system can be tested for monotonicity with respect to a positive orthant cone using the following analytic strategy mentioned in Hirsch's Monotone Systems Theory [14] that was adopted in references [1], [2], [4]–[7], [15], [16]: calculate the Jacobian matrix and inspect its off-diagonal entries. The matrix needs to have non-negative off-diagonal entries for this test to work. Sadly, this can be infeasible as a

Jacobian is difficult to compute in practice. Fortunately, the paper [5] developed a graphical test to verify monotonicity.

#### 1.4 Graphical Tests for Monotonicity

Recall that chemical reaction networks are a model constructed from vertices and edges, and one can often use graph theory to study a reaction's underlying structure to draw specific conclusions such as those presented in the paper [17]. For example, Species Reaction (SR) graphs can be used to identify a class of monotone reaction systems. One can assign a positive (or negative edge) between species and reaction vertices if the former contributes to the reaction. This idea is not new. Volpert et al. used group theory [18] to study signs of these edges, whereas Banaji in reference [15] determined the classes of cones and partial orders that make a reaction monotone using the same positive and negative strategy. Feinberg also mentioned the DSR-graph in the paper [19], but this was not in the context of monotone systems according to reference [5].

The main contribution from Angeli, De Leenheer and Sontag in their paper [5] was the construction of a graphical check called the positive loop property. The authors presented necessary and sufficient conditions for a reaction system to be monotone that utilized the positive loop property and additional assumptions on the reactions. If the SR-graph satisfied the positive loop property, then reaction-coordinates dynamical system is monotone with respect to a partial order. The positive loop property was useful because it was an exact and simple graphical check. One does not need to perform computationally expensive operations or lengthy algebra. All one has to do is construct the SR-graph, and calculate the product of positive and negative signs.

The use of cooperativity (reactions contributing products that are used to perform other reactions) to study monotonic reactions is not new, but the concept has been studied in different contexts to get similar results. For example, Smith in reference [16] used an irreducible Jacobian matrix to make inferences and draw conclusions about the



asymptotic behavior for a dynamical system. Angeli and Sontag also used the Jacobian of the reaction-coordinates system of a chemical reaction network to draw the same conclusions about its asymptotic behavior in the paper [4]. Smith also used a form of a graph constructed from the Jacobian matrix to determine if a dynamical system is monotone in reference [16]. See reference [20] for the most recent developments that expand the graphical tests.

### **1.5 Using an Algorithm to Establish Multi-Stationarity**

Conradi, Feliu, Mincheva, and Wiuf constructed a general procedure to determine if a species-coordinates dynamical system will admit exactly one or multiple equilibria (multi-stationarity) in paper [3]. The authors generalize results by Conradi and Mincheva in papers [21], [22] to give sufficient conditions for a chemical reaction network to have multiple positive equilibria based on the signs of a computed polynomial. If the network has a positive parametrization, then they check for multi-stationarity using their algorithm. A parametrization is a lower dimensional change of variables, so one can (possibly) rewrite a non-linear system with fewer variables using computer software. Most symbolic computations fail with more than 20 variables on common laptops [7]. See the paper [7] for some techniques to expand the determinant along non-symbolic rows (conservation relations).

If a reaction network has a conservation relation between using a set of species that are necessary for a reaction to occur (called a siphon), then one can determine the existence of boundary equilibria by checking the persistence condition outlined in reference [1]. Determining these siphons is often performed by inspection in which one can work backwards from the reactions systematically, but this is a non-trivial and tedious task for large networks with many reactions. Shiu and Sturmfels managed to introduce a group-theory framework for computing the minimal siphons of a CRN in the paper [23]. Unfortunately, even if one could find the minimal siphons, there are no necessary and

sufficient conditions to characterize persistence in the presence of boundary equilibria because the system may still be persistent if there are boundary equilibria, e.g., they could be unstable.

## **1.6 Conclusion**

Chemical reaction networks play a significant role in science, but calculating equilibria can be an inefficient and almost impossible task. Fortunately, Chemical Reaction Network Theory has developed alongside monotone systems theory to handle large and small classes of reaction networks. Although analytically testing for monotonicity is usually a daunting task that required heavy mathematical machinery, this is no longer the case as there are graphical tests that can be performed conveniently.

Monotone systems theory and the graphical tests are not without their limitations. Most real-world reactions do not satisfy monotonicity in reaction- or species-coordinates, and some actually exhibit unstable behavior. The analytical method can still handle a much larger class of reaction networks despite being harder to implement in practice. In addition, the SR-graph having the positive loop property does not guarantee a positive orthant cone induces the partial order, as that would automatically imply the system is cooperative, which is a stronger result. While there is an algorithm to handle this process, it is still in its infancy since automating the steps has its own computational issues.

## 2 PRELIMINARIES

In this chapter, the reader will find the notation and glossary of terms used throughout the paper for convenience. This is not a comprehensive list since other terms will be defined as needed in context.

### 2.1 Notation and Definitions

From time to time, we will interchange chemical reaction network (CRN), chemical reaction system, reaction network, and network system. However, the term *reaction* alone will not refer to a CRN since has its own meaning (and to avoid confusion).

- $\mathbb{R}^n$  is Euclidean space, or the set of real numbers in  $n$  dimensions.
- $x, y, z$  are vectors in  $\mathbb{R}^n$ .
- $a, b, c$  are scalars in  $\mathbb{R}$ .
- Matrices (operators) and Vector spaces are capitalized.
- Matrix entries are denoted  $[A]_{ij}$  for some matrix  $A$ .
- $\xi$  is an initial condition to a differential equation.
- $\dot{x}$  indicates the time derivative of  $x$ .
- $K$  denotes a positive orthant cone or a compact set.
- $im(A)$  denotes the image of a matrix  $A$ .
- $\ker(A)$  is the right kernel of a matrix  $A$ .
- $\ker(A^T)$  is the left kernel of a matrix  $A$ .
- $n_s$  is the number of species in a reaction network.
- $n_r$  is the number of reactions in a reaction network (with reversible reactions being clear from context).
- $\Gamma$  is the stoichiometric matrix of a reaction network, such that  $\Gamma \in \mathbb{R}^{n \times m}$  in which  $n$  is the number of species and  $m$  is the number of reactions.
- $(v_i, v_j)$  denote that there is an edge between vertices  $v_i$  and  $v_j$ .
- $v_i \not\sim v_j$  denotes that there is no edge between vertices  $v_i$  and  $v_j$ .

- $v_i \rightarrow v_j$  denotes that there is a directed edge from vertex  $v_i$  to vertex  $v_j$ .
- $X_\sigma, C_{S_0}, P_c$  all denote the stoichiometric compatibility class.
- $\mathbb{Q}[A]$  is the set of rational polynomials adjoined with elements of a set  $A$ .
- $P_c^+$  denotes the positive stoichiometric compatibility class.
- $V_S$  denotes the vertex set of species and corresponds to the vector of species such that  $V_S \in \mathbb{R}^{n_s}$ .
- $Z$  denotes the siphon of a reaction.

## 2.2 Glossary

This glossary of terms is split up by subjects for the reader's convenience.

### 2.2.1 Pre-Ordered and Partially Ordered Cones

The terms we use in this subsection are commonly replicated, but they can also be found in reference [24, Chapter 2.4].

**Definition 1.** A *pre-ordered vector space*, denoted  $(V, \leq)$ , is a vector space equipped with a pre-order, in which  $\leq$  satisfies

- (i)  $x + z \leq y + z$
- (ii)  $y \leq x$  implies  $\lambda y \leq \lambda x$ .

**Definition 2.** The *positive cone* of a pre-ordered vector space  $(V, \leq)$  is the subspace

$$V^+ = \{x \in V : x \geq 0\} \subset V.$$

This is also known as a *convex cone* since it satisfies  $ax + by \in V^+$  for all  $x, y \in V^+$ . In other words, a *positive cone* is a closed subset  $K$  of a Banach space  $B$  such that

- (i)  $K + K \subset K$
- (ii)  $K \subset aK$  for all  $a \geq 0$
- (iii)  $K \cap (-K) = \{0\}$ .

**Definition 3.** A positive *orthant* cone is a positive cone  $(V, \leq)$  where  $V$  is a Euclidean space.

**Definition 4.** A *well-ordered vector space*, denoted  $(V, \leq)$ , is a vector space equipped with a partial order, in which  $\leq$  satisfies a trichotomy for all  $x, y, z$ :

$$x \geq x$$

$$x \geq y, y \geq z \text{ implies } x \geq z$$

$$x \geq y, y \geq x \text{ implies } x = y.$$

**Definition 5.** A *proper cone* of a well-ordered vector space  $(V, \leq)$  is a positive cone  $V^+ < V$  such that  $V^+ \cap (-V^+) = \{0\}$ .

**Lemma 6.** If  $(V, \mathbb{R})$  is a real vector space and  $C$  is a proper convex cone in  $V$ , then there exists a unique partial order  $\leq$  on  $V$  such that  $V$  is ordered and  $V^+ = C$  in which  $x \leq y$  if and only if  $x - y \in C = V^+$ .

*Proof.* Existence is clear. Let  $\leq$  such that  $x \leq y$  if and only if  $x - y \in C$ . Now, we will show  $V$  is partial-ordered to show sufficiency. Since  $C$  is convex,  $ax + by \in C$  for all  $x, y \in V$  and  $a, b \in \mathbb{R}$ . Observe that  $(ax + by) - (ax + by) = 0 \in C$  since  $C < V$  implies  $ax + by \leq ax + by$  for all  $x, y \in C$ . Let  $z \in C$ ,  $c \in \mathbb{R}$  such that  $ax - cz \in C$  and  $cz - by \in C$ . Finally,  $ax \leq cz$  and  $cz \leq by$  implies

$$ax + cz \leq cz + cz \leq cz + by$$

as desired. □

**Definition 7.** If  $(V, \leq)$  is a well-ordered vector space in which the partial order  $\leq$  is induced by a positive cone  $K$  (In other words,  $x \leq y$  if and only if  $x - y \in K$ , in which  $\text{int}(K) = \bar{K} - K'$  is non-empty), then we can define a *strict partial ordering*  $<$  in the usual sense:  $x < y$  if  $x \leq y$  and  $x \neq y$ .

**Definition 8.** A vector  $x$  satisfies  $x \gg 0$  if  $x_i > 0$  for all elements in  $x$ .

### 2.2.2 Monotone Systems

**Definition 9.** Let  $\mu$  be a measure on  $\mathbb{R}^n$  equipped with the Borel  $\sigma$ -algebra  $\mathcal{B}(\mathbb{R}^n)$ . If for all  $x \in \mathbb{R}^n$ , and for all  $B \in \mathcal{B}(\mathbb{R}^n)$ , then  $\mu$  is *translation invariant* if  $\mu(x + B) = \mu(B)$  in which  $x + B = \{x + b : b \in B\}$ .

**Definition 10.** A dynamical system is *monotone* if its mapping is well-ordered preserving

In other words, a dynamical system is monotone if two initial states  $x_1 \geq x_2$  for  $t \geq 0$  implies their solutions satisfy  $x(t, x_1) \geq x(t, x_2)$  for all  $x_1, x_2, t \in X \subset \mathbb{R}^n$ .

**Definition 11.** A dynamical system  $\dot{x} = f(x)$  is *cooperative* if  $\frac{\partial f_i}{\partial x_j} \geq 0$  for all  $x$  and for all  $i \neq j$ , and *competitive* (or not cooperative) if  $\frac{\partial f_i}{\partial x_j} \leq 0$ .

**Definition 12.** If the partial order is induced by a positive orthant, then the dynamical system is cooperative.

**Definition 13.** A *Z-matrix* is a matrix  $Z$  such that its off-diagonal entries are less than or equal to zero, i.e.,  $[Z]_{ij} \leq 0$  for all  $i \neq j$ .

**Proposition 14.** If  $Df(x)$  is the Jacobian of a dynamical system  $\dot{x} = f(x)$  such that  $-[Df(x)]_{ij} \leq 0$  for all  $i \neq j$ , then the system is cooperative. Otherwise, if  $Df(x)$  satisfies  $[Df(x)]_{ij} \leq 0$  for all  $i \neq j$  (a Z-matrix), then the system is competitive.

*Proof.* Observe that  $-[Df(x)]_{ij} \leq 0$  for all  $i \neq j$  implies  $[Df(x)]_{ij} \geq 0$  for all  $i \neq j$ . Thus, the system is cooperative. Otherwise,  $[Df(x)]_{ij} \leq 0$  is competitive by definition.  $\square$

**Definition 15.** Given  $X \subseteq \mathbb{R}^n$  a Hilbert space, an operator  $f : X \rightarrow X^*$  is *strongly monotone* if there exists a constant  $c > 0$  such that

$$\langle f(x_1) - f(x_2), x_1 - x_2 \rangle \geq c \|x_1 - x_2\|^2$$

for all  $x_1, x_2 \in X$ , i.e., if two inputs satisfy  $x_1 > x_2$ , then their outputs satisfy  $f(x_1) \gg f(x_2)$ .

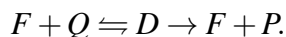
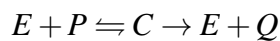
**Definition 16.** A dynamical system is strongly monotone if  $x_1 > x_2$  implies  $x(t, x_1) \gg x(t, x_2)$  for all  $t > 0$ .

**Definition 17.** In a dynamical system, a *state space* is an  $n$ -dimensional space where  $n$  is the number of degrees of freedom (aka number of parameters) in the system.

### 3 INTRODUCTION TO REACTION MODELING

A chemical reaction is a relationship in which reactants form products. Irreversible reactions are of the form  $Reactant \rightarrow Product$ , and reversible reactions are of the form  $Reactant \rightleftharpoons Product$ . A chemical  $S$  is called a *species*, and a linear combination of species is called a *complex*.

**Example 18.** We will work with this running example called the Enzymatic Futile Cycle (which underlies many metabolic pathways) with species  $E, P, C, Q, F, D$ .  $E, F$  are enzymes and  $P, Q, C, D$  are protein substrates.  $P$  binds and unbinds with an enzyme  $E$  to form a substrate  $C$ , and  $C$  unbinds to form a substrate  $Q$  with enzyme  $E$ , and similarly for enzyme  $F$  and substrates  $Q, D$ , and  $P$



The *complexes* in this case are  $E + P$ ,  $C$ ,  $E + Q$ ,  $F + Q$ ,  $D$ , and  $F + P$ . Notice that this chemical reaction network has two reversible reactions and two forward reactions.

#### 3.1 Reactions and Reaction Rates

A *reaction rate* measures the speed of the reaction, and a set of reaction rates is called *kinetics*. Let  $S = \langle S_1, \dots, S_{n_s} \rangle \in \mathbb{R}^{n_s}$  be a vector of species. The  $i$ th reaction  $R_i$  of a chemical reaction network is of the form

$$R_i : \sum_{i=1}^{n_s} a_{ij} S_i \rightarrow \sum_{i=1}^{n_s} b_{ij} S_i$$

where  $a_{ij}, b_{ij}$  are scalars and  $S_i$  are species, i.e., *reactants*  $\rightarrow$  *products*. In this context,  $R_i$  are forward reactions with reactant coefficients  $a_{ij}$  and product coefficients  $b_{ij}$  (these are also known as stoichiometry coefficients). Any reaction network is composed of forward or reversible reactions. Reactions have the following constraints:



**A1**  $R_i \geq 0$  for all  $i$  (reactions are always happening) as long as species are present, time is moving forward. This can be summarized as follows:  $R_i(S) = 0$  if and only if  $S_i = 0$  for some  $a_{ij} > 0$  [3].

**A2** Reactions occur monotonically with respect to concentrations of species.

Analogously, this means more fuel in your car means you can drive longer.

If reactant species  $a_{ij}$  exist, then reactions occur because  $\frac{\partial R_i(S)}{\partial S_j}$ , the partial derivative of the reaction rate on the species vector with respect to a given species  $S_j \in S$ , is greater than or equal to 0. Otherwise, if species are gone, then reactions stop.

**A3**  $R_i$  are convex functions on  $\mathbb{R}^{n_s}$  (which implies they are locally Lipschitz)

a) The paper [4] assumes locally Lipschitz

b) The papers [3], [5] assume  $R_i \in C^1$

**A4** Avoid auto-catalytic reactions: a type of reaction in which a species appears as both a reactant and a product.

Observe that  $S_1 + S_2 \rightarrow S_1$  is not allowed even though they are different complexes. So no species is allowed to be present as both a reactant and a product for the same reaction.

**A5**  $\frac{\partial R_i(S)}{\partial S_j} \geq 0$  and it cannot change signs.

**A6** If a reaction is reversible, then for all species vectors  $S \in \text{int}(\mathbb{R}_{\geq 0}^{n_s})$ , the paper [5] defines the relationship between the forward and backward components of a reaction as,

$$R_i(S) := R_{i,\text{forward}}(S) - R_{i,\text{backward}}(S).$$

Some examples of kinetics are

- mass-action:  $k_i \prod_{j=1}^n S_j^{D_{ij}}$  (multiplying reactant concentrations)
- Michaelis-Menten:  $m_i \prod_{j=1}^n \frac{S_j}{S_j + a_j}$  (multiplying ratios of reactant concentrations)
- Hill:  $M_i \prod_{j=1}^n \frac{S_j^{n_j}}{S_j^{n_j} + b_j}$  (multiplying ratios of product concentrations)

### 3.2 Dynamical Systems and Modeling Chemical Reaction Networks

We can view a chemical reaction dynamical system as

$$\dot{S} = \Gamma R(S)$$

where  $S$  is an  $n_s$ -dimensional Euclidean vector such that each entry is the concentration of a chemical species,  $\Gamma \in \mathbb{R}^{n_s \times n_r}$  is the *stoichiometry matrix*, and  $R: \mathbb{R}_{\geq 0}^{n_s} \rightarrow \mathbb{R}^{n_r}$  is a function that takes a vector of concentrations in  $S$  and outputs a vector of reaction-rates.

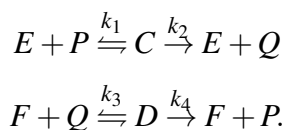
To summarize the sizes:

$$n_s \times 1 = [n_s \times n_r] \cdot [n_r \times 1].$$

We will demonstrate how to construct the species dynamical system  $\dot{S} = \Gamma R(S)$  using Example 18 on page 13. Let the species have the same letters as the concentrations. Therefore our species vector is also a concentration vector, i.e.,

$$S = \langle P, Q, E, F, C, D \rangle^T \in \mathbb{R}^6.$$

If we treat reversible reactions as one reaction and label the reaction network with reaction modeling labels  $\langle k_1, k_2, k_3, k_4 \rangle$  like so:

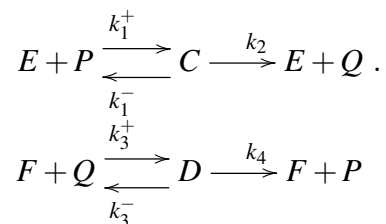


Thus, the stoichiometry matrix is

$$\Gamma = \begin{bmatrix} -1 & 0 & 0 & 1 \\ 0 & 1 & -1 & 0 \\ -1 & 1 & 0 & 0 \\ 0 & 0 & -1 & 1 \\ 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix},$$

in which the rows of  $\Gamma$  encode the stoichiometric change of each individual species across the given reaction and the columns span reaction-space, such that  $k_1$  and  $k_3$  have forward and backward reactions. For example, we lose a molecule of  $E$  to reaction  $k_1$  and gain a molecule of  $E$  from  $k_4$ .

The stoichiometry matrix  $\Gamma$  has entries  $b_{ij} - a_{ij}$  or the difference in product and amount of reactants, and reaction rates are assigned to each reaction. We split the label for these reversible reactions as  $k_1^+, k_1^-$  for  $k_1$  and similarly  $k_3^+, k_3^-$  for  $k_3$ . We will later prove there is more than one such labeling outside of permutation. Let us relabel our reactions with these  $k_i$ :



To construct the vector  $R(S) \in \mathbb{R}^{n_r}$ , we only consider the reactants of each reaction  $k_i$ , not the product. We adopt the convention that the sign of the reaction is based on whether the species contributes (positive) or is produced (negative) by the reaction.

$$R(S) = \begin{bmatrix} k_1EP - k_{-1}C \\ k_2C \\ k_3FQ - k_{-3}D \\ k_4D \end{bmatrix}$$

Thus,  $\dot{S} = \Gamma R(S)$  is

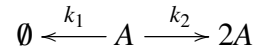
$$\begin{bmatrix} \dot{P} \\ \dot{Q} \\ \dot{E} \\ \dot{F} \\ \dot{C} \\ \dot{D} \end{bmatrix} = \begin{bmatrix} -1 & 0 & 0 & 1 \\ 0 & 1 & -1 & 0 \\ -1 & 1 & 0 & 0 \\ 0 & 0 & -1 & 1 \\ 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix} \begin{bmatrix} k_1EP - k_{-1}C \\ k_2C \\ k_3FQ - k_{-3}D \\ k_4D \end{bmatrix}.$$

A necessary condition for having fixed points (steady states) of the species dynamical system  $\dot{S} = \Gamma R(S)$  is the following lemma.

**Lemma 19.** *If some vector  $x$  satisfies  $\Gamma x \neq 0$ , then  $\Gamma R(S) \neq 0$ .*

Note: this result is not sufficient due to the counterexample below.

**Example 20.** Consider the following chemical reaction network, which describes a net gain of nothing.<sup>1</sup>



Observe that  $\Gamma = [-1, 1]$ , so if reactions 1 and 2 have mass-action reaction coefficients  $k_1, k_2$  respectively, then the dynamical system for this reaction network is

$$\dot{A} = [-1, 1] \begin{bmatrix} k_1 A \\ k_2 A \end{bmatrix} = (k_2 - k_1)A$$

and  $v = [1, 1] \in \ker(\Gamma)$ , so  $\Gamma v = 0$ , but  $\dot{A} = (k_2 - k_1)A = \Gamma R(S) \neq 0$  unless  $k_1 = k_2$ .

The left and right kernels of  $\Gamma$  also play an important role in how we understand the significance of certain species. Since  $\Gamma \in \mathbb{R}^{n_s \times n_r}$  encodes the stoichiometric change of species, the left kernel of  $\Gamma$ ,

$$\ker(\Gamma^T) = \left\{ w \in \mathbb{R}^{n_s} : \sum_i w_i \dot{S}_i(t) = 0 \right\} = \left\{ w \in \mathbb{R}^{n_s}, c \in \mathbb{R} : \sum_i w_i S_i(t) = c \right\}$$

gives us the (positive) linear combination of species that will stay constant for all  $t \geq 0$ , i.e., a *conservation law*. On the other hand, the right kernel of  $\Gamma$

$$\ker(\Gamma) = \left\{ v \in \mathbb{R}^{n_r} : \sum_i v_i R_i(t) = 0 \right\}$$

gives us the linear combination of reactions that results in a net-change in the production of species.

1. Depending on the reaction rates, there may be an increase in A or a decrease in A (or, rarely, no change in A at all).

We claim  $R_i(S) = 0 \iff S_i = 0$  for some  $i$  with  $a_{ij} > 0$  implies  $\mathbb{R}_{\geq 0}^{n_s}$  and  $\mathbb{R}_{> 0}^{n_s}$  are forward invariant under  $\Gamma R(S)$ . We will only provide a brief sketch of the proof presented in the paper [25]. Each row  $\Gamma R_j(S)$  only has negative coefficients for terms involving  $S_i$  if  $S_i$  is consumed in reaction  $R_j$ . Since  $R_j(S) = 0 \iff S_i = 0$  for some  $i$  with  $a_{ij} > 0$ , these corresponding rows of  $\Gamma$  will be greater than or equal to 0. The other rows of  $\Gamma$  will also be positive because  $a_{ij} > 0$  for  $R_i$ . Then,  $\dot{S}$  can be split into two a sum of components that satisfy the two previously mentioned relations, and can be solved with an exponential integrating factor. Thus, the solution will contain only positive components, hence  $\mathbb{R}_{\geq 0}^{n_s}$  and  $\mathbb{R}_{> 0}^{n_s}$  are forward invariant under  $\Gamma R(S)$ .

### 3.3 Limit Cycles, Persistence, and Consistence

We wish to discuss the minimal requirements to classify trajectories of solutions for the monotone dynamical systems of a chemical reaction network system. Recall that if we have a dynamical system with no fixed points, then the limit of the trajectory is a limit cycle. Limit cycles are isolated periodic orbits by definition, and there exist dynamical systems such as the undamped harmonic oscillator with no limit cycles but many periodic orbits [26]. If there are no limit points and no limit cycle, then we have a chaotic strange attractor. This is not an exhaustive list of the possibilities for dynamical systems as many other types of behavior, e.g. quasi-periodic motion, space filling curves, ergodic theory, are possible. However, the paper [6] demonstrates monotone systems is a satisfactory condition for determining if a state space achieves an equilibrium.

Recall that if we have a bounded sequence in Euclidean space, then the Bolzano-Weierstrass Theorem implies there is a convergent sub-sequence. The same is also true if our sequence is in a compact set, as compact sets are closed and bounded by the Heine-Borel Theorem. These convergent sub-sequences are dependent on initial conditions, so we will adopt the notation from reference [1] to define the set of

sub-sequences from the trajectory that converge,

$$\omega(x_0) = \{x \in \mathbb{R}^n : x(t_n) \rightarrow x, t_n \rightarrow \infty \text{ as } n \rightarrow \infty\}$$

where  $x(t) = \varphi(t, x_0)$  depends on time and an initial condition. We need this set to be able to characterize the trajectories of a chemical reaction network. For a species dynamical system  $\dot{S} = \Gamma R(S)$  with initial condition  $S(0) =: S_0$ , we claim sub-sequential limit points do not approach the boundary under certain conditions, which implies  $\omega(S_0)$  is set of sub-sequential limit points by definition. Fixed points also satisfy this definition. In fact, they are the most common objects that satisfy this definition. If  $\omega(x_0) \cap \partial \mathbb{R}_{\geq 0}^{n_s} = \emptyset$ , then the associated chemical reaction network is known as *persistent*. Next, we introduce a necessary but not sufficient condition for a reaction network to have an equilibrium.

**Definition 21.** A chemical reaction network is called *consistent* if there exists a vector  $v \in \mathbb{R}_{>0}^{n_s}$  such that  $v \gg 0$  (i.e., every component of the vector satisfies  $v_i > 0$ ) and  $\Gamma v = 0$ .

Observe that this vector  $v$  is not hard to find in general because we simply need a right kernel vector with all strictly positive entries for our reaction network to be labeled consistent. This theorem from reference [1] provides the necessary conditions for a CRN to be consistent, based on persistence. The proof presented below is the same as the one in the paper with a few added details.

**Theorem 22** ([1, Theorem 1]). *If  $\dot{S} = \Gamma R(S)$  with any initial condition  $S_0 \in \text{int}(\mathbb{R}_{\geq 0}^{n_s})$  where  $R$  satisfies A3(b),  $w \in \ker(\Gamma^T)$  satisfies  $w \gg 0$ , and  $\omega(S_0) \cap \partial \mathbb{R}_{\geq 0}^{n_s} = \emptyset$ , then the associated chemical reaction network is consistent.*

*Proof.* Given any initial condition  $S_0 \in \text{int}(\mathbb{R}_{\geq 0}^{n_s})$ , taking  $w \in \mathbb{R}^{n_s}$  on both sides of the dynamical system yields

$$w^T \dot{S} = w^T \Gamma R(S) \implies w^T \dot{S} = 0 \implies w^T S(t) = w^T S_0.$$

Thus, the solution is bounded because the right-hand side is a constant and  $w$  has all positive entries, and this implies  $\omega(S_0)$  is a compact set. Then, given any  $\tilde{S}_0 \in \omega(S_0)$ ,  $\omega(S_0) \cap \partial \mathbb{R}_{\geq 0}^{n_s} = \emptyset$  implies  $R(\tilde{S}_0) \gg 0$ . Also, since  $R \in C^1$ , then  $R(\omega(S_0))$  is also compact, so there is a vector  $v \in \mathbb{R}_{\geq 0}^{n_s}$  such that

$$R(\tilde{S}_0) \geq v \gg 0$$

for all  $\tilde{S}_0 \in \omega(S_0)$ . Define the bounded solution of  $\dot{S} = \Gamma R(S)$  to be  $S(t) := \varphi(t, \tilde{S}_0)$ .

Observe that

$$S(T) - S_0 = \int_0^T \Gamma R(S(\tau)) d\tau \implies S(T) = \tilde{S}_0 + \Gamma \int_0^T R(S(\tau)) d\tau.$$

Thus, by the invariance of the positive limit set,  $R(S(t)) \geq v \gg 0$  for all  $t \in \mathbb{R}$ . Therefore,

$$\int_0^T R(S(\tau)) d\tau \geq v \gg 0$$

for all  $T > 0$ . Since  $S(t)$  is bounded, then the asymptotic time average of the system goes to 0, i.e.,

$$\lim_{T \rightarrow \infty^+} \frac{\int_0^T \Gamma R(S(\tau)) d\tau}{T} = \lim_{T \rightarrow \infty^+} \frac{\Gamma \int_0^T R(S(\tau)) d\tau}{T} \geq \lim_{T \rightarrow \infty^+} \frac{\Gamma v}{T} \rightarrow 0.$$

Finally, Bolzano-Weierstrass implies there is a convergent sub-sequence for  $T_n \rightarrow \infty$ , then

$$\lim_{T_n \rightarrow \infty^+} \frac{\int_0^{T_n} \Gamma R(S(\tau)) d\tau}{T_n} = \Gamma \bar{v} \geq \Gamma v \gg 0.$$

Hence, there exists a vector  $\bar{v} \gg 0$  such that  $\Gamma \bar{v} = 0$ , so the reaction network is consistent by definition as desired.  $\square$

It is important to give context for why we need a reaction to be consistent. Recall that  $\Gamma$  is the stoichiometric matrix, which means its columns span the stoichiometric subspace

of reaction-space. In other words,

$$\Gamma = [\dots |y_i - y_j| \dots]$$

in which reaction  $|y_i - y_j|$  is of the form  $|\text{products} - \text{consumed}|$ . A network is consistent if, roughly speaking, some combination of all reactions implies there is a balance in the production of species. A right kernel vector with all positive entries guarantees every reaction must be accounted for and cannot be ignored, which means  $v$  has to strictly live in the interior of the positive orthant and not on the boundary. In other words, there is no net change in the amount of species present when those reactions occur. The enzymatic futile in Example 18 on page 13 is consistent since the vector

$$[1 \ 1 \ 1 \ 1]^T \in \ker(\Gamma),$$

which means we need all four reactions  $k_1, \dots, k_4$  to happen an equal number of times for there to be no net change in the amount of species present.



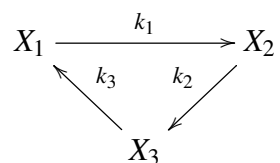
## 4 THE ANGELI-SONTAG THEOREM

In this chapter, we formally introduce the stoichiometric subspace and the stoichiometric compatibility class. We introduce a new dynamical system based on  $\dot{S} = \Gamma R(S)$ , known as the reaction-coordinates dynamical system, and we also discuss important results from papers [4], [6] that we can use to draw conclusions about species-space. It is important to remark that our monotone dynamical system is translation invariant with respect to a positive vector, and equilibria are never unique.

### 4.1 Stoichiometric Subspace and the Stoichiometric Compatibility Class

The *stoichiometric subspace* is defined to be the span of reaction-space, i.e., the columns of the stoichiometric matrix  $\Gamma \in \mathbb{R}^{n_s \times n_r}$  where  $n_s$  is the number of species and  $n_r$  is the number of reactions. Formally, if reactions are of the form  $y_i \rightarrow y'_i$  for all  $i = 1, \dots, n_r$ , then the stoichiometric subspace  $G = \text{span} \{y_i - y'_i\} = \text{im}(\Gamma)$ . Since any linear subspace is a convex set, the stoichiometric subspace is also convex.

**Example 23.** Consider the simple reaction network



with the following stoichiometric matrix

$$\Gamma = \begin{bmatrix} -1 & 0 & 1 \\ 1 & -1 & 0 \\ 0 & 1 & -1 \end{bmatrix}.$$

Observe that

$$\text{im}(\Gamma) = \text{span} \left\{ \begin{bmatrix} -1 \\ 1 \\ 0 \end{bmatrix}, \begin{bmatrix} 0 \\ -1 \\ 1 \end{bmatrix} \right\},$$

since the last column is a negative of the sum of the first two column vectors in  $\Gamma$ . From this example, we can see a cycle in the reaction model means we lose one degree of

freedom in the state space. To understand why this is the case, imagine  $k_3$  being the vector sum of  $k_1$  and  $k_2$ , in which one adds vectors head to tail. In this situation, the stoichiometric subspace is the plane spanned by two vectors that slice through  $\mathbb{R}^3$ . We plot this in Figure 4.1.

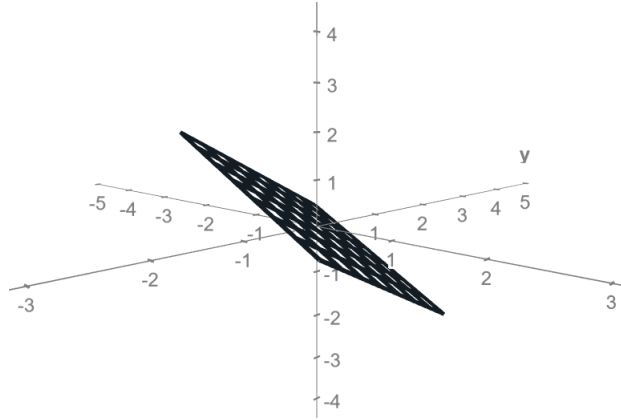


Fig. 4.1: The stoichiometric subspace of the reaction in Example 23.

Assume the reaction function  $R : \mathbb{R}_{\geq 0}^{n_s} \rightarrow \mathbb{R}^{n_r}$  is a locally Lipschitz function ( $R_i(S)$  satisfies assumption A3(a) for all  $i$ ) so the solution of the dynamical system  $\dot{S} = \Gamma R(S)$  is unique [4]. Let  $\mathbb{R}_{\geq 0}^{n_s}$  be invariant and forward complete so all solutions are defined for  $t \geq 0$ . We can change the system from species-coordinates to reaction-coordinates for every fixed initial condition  $\sigma \in \mathbb{R}_{\geq 0}^{n_s}$ :

$$\dot{x} = f_\sigma(x) = R(\sigma + \Gamma x)$$

such that  $f_\sigma : X_\sigma \rightarrow \mathbb{R}^{n_r}$  where the state space,

$$X_\sigma = \{x \in \mathbb{R}^{n_r} : \sigma + \Gamma x \geq 0\}$$

is called the *stoichiometric compatibility class* for reaction coordinates. Similarly, we define the state space of the species-coordinates dynamical system.

**Definition 24.** Given the dynamical species-coordinates system,  $\dot{S} = \Gamma R(S)$  such that  $R(S) : \mathbb{R}_{\geq 0}^{n_s} \rightarrow \mathbb{R}^{n_r}$  with initial condition  $S_0 := S(0)$ , the state space

$$C_{S_0} = \mathbb{R}^{n_s} \cap (S_0 + im(\Gamma))$$

is called the stoichiometric compatibility class.

It is easier to understand where this state space  $X_\sigma$  comes from if we pay attention to the dimensions:  $\sigma \in \mathbb{R}_{\geq 0}^{n_s}$ ,  $\Gamma \in \mathbb{R}^{n_s \times n_r}$ , and  $R : \mathbb{R}_{\geq 0}^{n_s} \rightarrow \mathbb{R}^{n_r}$ , so  $\sigma + \Gamma x \in \mathbb{R}_{\geq 0}^{n_r}$ , which means we need a vector  $x \in \mathbb{R}^{n_r}$  such that

$$n_s \times 1 + (n_s \times n_r) \cdot (n_r \times 1) \geq 0.$$

**Definition 25.** The  $x_i$  component of  $x \in X_\sigma$  is called the *extent* of the reaction, which is a function that records how much of a reaction has occurred up to a given time  $t$ .

The stoichiometric compatibility class  $X_\sigma$  is the shift of the stoichiometric subspace into the positive orthant since  $im(\Gamma)$  may not be positive. In order to assist with modeling real-world reaction systems, it is appropriate to choose an initial condition vector  $\sigma \in \mathbb{R}_{\geq 0}^{n_r}$  to ensure  $\sigma + im(\Gamma) \geq 0$ . One can see an example of such a shift in Figure 4.2.

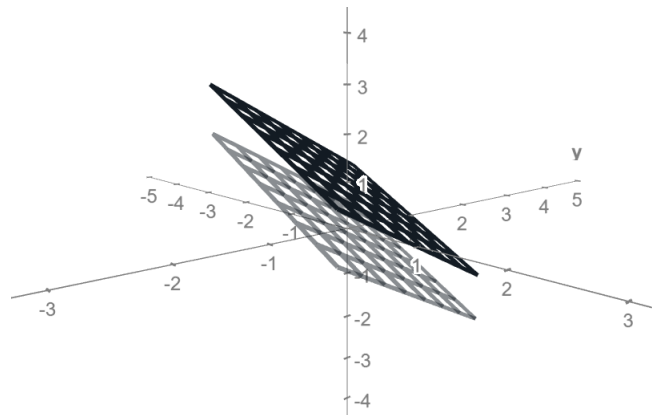


Fig. 4.2: The stoichiometric compatibility class of Example 23.

If the extent of the reaction  $x(t)$  is a trajectory in  $X_\sigma$  and if the chemical reaction network is in equilibrium, then  $\Gamma x = 0$  so there is no change in reaction-space. The converse is not true:  $x$  could change so long as  $x \in \ker(\Gamma)$  and there would be no change in species-space. The amount of species is not changing, but the reactions are always occurring due to the dynamics. This is an important distinction between species-space and reaction-space of a CRN at equilibrium: there is no net change in the concentration of species in species-space because equilibrium implies their resultant rate of change is zero, but reactions continue to grow with changing species-space.

## 4.2 Projection Mappings

Given any unit vector  $v$ , the paper [4] introduces the linear mapping:

$$\pi_v : \mathbb{R}^n \rightarrow \mathbb{R}^n \text{ such that } \pi_v(x) = x - (v^T x) v.$$

This map subtracts an orthogonal projection onto  $v^T$ , or the component, along the vector  $v$ . Since  $v^T v = 1$ , we can see  $\pi_v(v) = v - (v^T v) v = 0$ .

**Definition 26** ([4]). Let  $\xi \in X$  be an initial condition vector with solution  $\varphi_t(\xi)$ . We say  $\varphi_t(\xi)$  is *bounded modulo (distance)  $v$*  if  $\pi_v(\varphi_t(\xi))$  is bounded as a function of  $t$ , for  $t \geq 0$ .

We will assume the projection  $\overline{\pi_v(\varphi_t(\xi))}$  is compact, and  $\pi_v(\varphi_t(\xi))$  will be a trajectory of the projected system  $\dot{\tilde{x}} = (I - vv^T) f(\tilde{x})$ .

**Proposition 27** ([4]). *The solution  $\varphi_t(\xi)$  is bounded mod  $v$  if and only if there exists some scalar function  $\beta(\xi, t) : X \times [0, +\infty) \rightarrow \mathbb{R}$  such that  $\varphi_t(\xi) - \beta(\xi, t)v$  is bounded mod  $v$ . In other words, the solution is bounded mod  $v$  if and only if there is another function from  $X \times [0, \infty) \rightarrow \mathbb{R}$  such that the difference between the solution and the scalar function is bounded mod  $v$ .*

Notice that the difference  $\varphi_t(\xi) - \beta(\xi, t)v$  is also an element in  $X$  because  $X$  is translation invariant under all multiples of  $v$ . In other words, a vector  $y \in X$  is of the form  $y = x - \lambda v$  in which  $x = \varphi_t(\xi) \in X$ ,  $\beta(\xi, t) = \lambda$ , and  $v \in X$ .

*Proof.* Assume  $\varphi_t(\xi)$  is bounded modulo  $v$ . By definition,  $\pi_v(\varphi_t(\xi))$  is bounded as a function of  $t$ , for  $t \geq 0$ . Then, by definition of  $\pi_v(x)$ ,

$$\pi_v(\varphi_t(\xi)) = \varphi_t(\xi) - (v^T \varphi_t(\xi))v.$$

Let  $\beta(\xi, t) : X \times [0, \infty) \rightarrow \mathbb{R}$  such that  $\beta(\xi, t) = v^T \varphi_t(\xi)$ . Thus, since  $X$  is translation invariant,  $y = \varphi_t(\xi) - \beta(\xi, t)v$  is a member of  $X$ , and is bounded mod  $v$  by definition since  $\pi_v(y)$  is bounded.

Conversely, let there be some scalar function  $\beta(\xi, t) : X \times [0, \infty) \rightarrow \mathbb{R}$  such that  $\varphi_t(\xi) - \beta(\xi, t)v$  is bounded mod  $v$ .

First, observe that

$$v^T \beta(\xi, t)v = \beta(\xi, t)v^T v = \beta(\xi, t) \cdot 1 = \beta(\xi, t)$$

since  $v$  is a unit vector. Then,

$$\pi_v(\beta(\xi, t)v) = \beta(\xi, t)v - (v^T \beta(\xi, t)v)v = \beta(\xi, t)v - (\beta(\xi, t))v = 0.$$

Since  $X$  is a closed space and  $\varphi_t(\xi) - \beta(\xi, t)v$  is bounded mod  $v$  by assumption, then the set of positive bounded differences,

$$\{\varphi_t(\xi) - \beta(\xi, t)v \geq 0\},$$

is a bounded subset of  $X$ . Observe that its closure

$$\overline{\{\varphi_t(\xi) - \beta(\xi, t)v \geq 0\}}$$

is a compact set by the Heine-Borel Theorem. Next, since projections are continuous, and continuous functions preserve compactness, then since  $\pi_v(\beta(\xi, t)v) = 0$  and because linear,

$$\pi_v\left(\overline{\{\varphi_t(\xi) - \beta(\xi, t)v \geq 0\}}\right) = \pi_v(\{\varphi_t(\xi) \geq 0\})$$

is compact, and thus closed and bounded. Hence,  $\pi_v(\varphi_t(\xi))$  being bounded implies  $\varphi_t(\xi)$  is bounded mod  $v$  by definition.  $\square$

### 4.3 Statement of the Angeli-Sontag Theorem

Recall that  $\mathbb{R}^n$  is a finite dimensional Banach space.

**Theorem 28** (Angeli-Sontag Theorem [4, Theorem 1]). *Let  $f : X \subseteq K \subset \mathbb{R}^n \rightarrow \mathbb{R}^n$  be a locally Lipschitz function in which  $X$  is the closure of its interior and  $K$  is a closed positive orthant cone with non-empty interior and partial orders  $\leq, <, \ll$ . Let  $\dot{x} = f(x)$  be a forward-complete nonlinear dynamical system with states  $x \in X$ , initial conditions  $\xi \in X$ , solutions of the form  $\varphi_t(\xi)$ , and collectively satisfies the following:*

(i) *Strong monotonicity:  $\xi_1 > \xi_2$  implies  $\varphi_t(\xi_1) \gg \varphi_t(\xi_2)$  for all  $t \geq 0$  and all  $\xi_i \in X$ .*

(ii) *Solutions are translation invariant:  $\varphi_t(\xi + \lambda v) = \varphi_t(\xi) + \lambda v$  for all  $\lambda \in \mathbb{R}$  and  $v \in \text{int}(K)$  such that  $\|v\| = 1$  without loss of generality.*

(iii) *The state space  $X$  is translation invariant with respect to  $v \in \text{int}(K)$ .*

*Then, the bounded projection  $\pi_v(\varphi_t(\xi))$  for every solution bounded modulo  $v$  converges to a unique equilibrium.*

*Remark 29.* Observe that the partial order  $\leq$  also satisfies local finite-ness since we are not on a real line; this is also known as the *causal ordering*  $\preceq$ .

*Remark 30.* We assume  $\varphi_t(\xi)$  is uniquely maximally defined as an element of  $X$  that contains  $[0, \infty)$  in its interior for  $t \in I_\xi$ , in which  $I_\xi$  is an interval in  $\mathbb{R}$  [4]. In other words, the system is assumed to be forward—but not necessarily backward—complete, which means we cannot guarantee results are the same for negative time.

*Remark 31.* We will assume the state space  $X$  is invariant with respect to translation by  $v$  because of the translation invariant property, namely:  $x \in X$  implies  $x + \lambda v \in X$  for all  $\lambda \in \mathbb{R}$ .

*Remark 32.* Every solution bounded modulo  $v$  converges monotonically to a unique equilibrium [4].

#### 4.4 Satisfying the Angeli-Sontag Theorem

We want our construction of reaction coordinate systems  $\dot{x} = f_\sigma(x) = R(\sigma + \Gamma x)$  to satisfy the conditions of Theorem 28 on the preceding page for  $x \in X_\sigma$ . In order to use the result as an application to chemical reaction networks, this means we need a locally Lipschitz function on a state space that is the closure of its interior and is translation-invariant with respect to a vector in a non-empty interior of a closed positive cone. We previously assumed  $R$  is locally Lipschitz, the stoichiometric compatibility class  $X_\sigma$  is a closed set that is the closure of its interior, and the positive orthant  $\mathbb{R}_{\geq 0}^{n_s}$  is a closed cone with interior  $\ker(\Gamma)$ . In fact, we need  $\ker(\Gamma)$  to have strictly positive components since it is in the interior of the positive orthant. Also, observe that  $X_\sigma$  is translation invariant for any  $v \in \ker(\Gamma)$  [4]. Given  $x \in X_\sigma$ ,  $\lambda \in \mathbb{R}$ , then

$$\begin{aligned} \sigma + \Gamma(x + \lambda v) &= \sigma + \Gamma x + \Gamma(\lambda v) \\ &= \sigma + \Gamma x + \lambda \Gamma v \\ &= \sigma + \Gamma x \geq 0. \end{aligned}$$

Hence,  $x \in X_\sigma$  implies  $x + \lambda v \in X_\sigma$  for all  $\lambda \in \mathbb{R}$ . This implies  $X_\sigma$  is convex, which means it is also a polytope [24].

##### 4.4.1 Drawing Conclusions of Dynamics Between Species-Space and Reaction-Space

Let  $x(t)$  be the extent of the reaction. The resultant vector  $\tilde{x} := x + \lambda v$  captures the same information about the dynamics in reaction-space since  $X_\sigma$  is translation invariant

(and thus forward invariant), which means we can shift the extent of the reaction without changing the corresponding species vector in species-space. Theorem 28 on page 27 allows us to draw conclusions about equilibria in species-space by associating each point in species-space to a point in the projected reaction-space. In Figure 4.3, there are multiple points in reaction-space that are mapped onto the same point in projected reaction-space. The pre-image under  $\Gamma$  of species-space is exactly the projected reaction-space, so there is a one-to-one correspondence between the two state spaces.

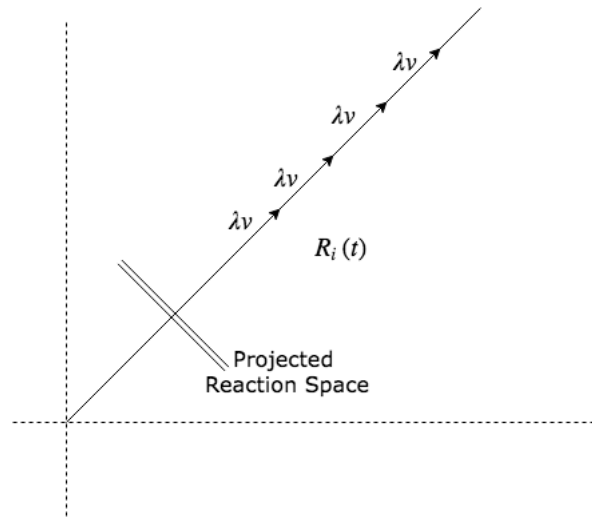


Fig. 4.3: Shifting the extent of a reaction by a kernel vector.

#### 4.4.2 Independence of Reaction Modeling and Bounded-ness of Solutions

The following lemma from reference [4] describes when a solution of a reaction-coordinates dynamical system is bounded. In the context of chemical reaction systems, this result depends on a stoichiometric matrix whose reaction labeling guarantees a one-dimensional kernel.

**Lemma 33** ([4, Lemma 3.2]). *Suppose a matrix  $\Gamma \in M_{n \times m}(\mathbb{Z})$  has rank  $m - 1$  whose right kernel is spanned by some positive vector  $v$ . Let  $x(t)$  be a solution to the reaction*



coordinate system  $\dot{x} = f_\sigma(x)$  with initial condition  $x_0$  for all  $t \geq 0$ . Then,  $\Gamma x(t)$  is bounded if and only if  $\pi_{v,x}(t)$  is bounded.

*Proof.* Suppose  $\pi_{v,x}(t)$  be bounded. Observe that

$$\Gamma \pi_{v,x}(t) = \Gamma(x - (v^T x)v) = \Gamma x - \Gamma(v^T x)v = \Gamma x - \Gamma v(v^T x) = \Gamma x,$$

in which the last equality follows from  $\ker(\Gamma) = \{v\}$  by assumption.

Conversely, let  $W := \{v^\perp \in \mathbb{R}^m : \langle v^\perp, v \rangle = 0\}$  and let  $M = \Gamma|_W$ , the restriction of  $\Gamma$  to  $W$ . Observe that  $\Gamma x = \Gamma \pi_{v,x}(x)$ , and we will prove  $M$  is injective: if there is an  $x \in v^\perp$  such that  $Mx = 0$ , then  $\Gamma x = 0$  since they have the same image. Then,

$$x \in \ker(\Gamma) = \ker(M) \implies \langle x, v^\perp \rangle = 0,$$

so  $x \in \text{span}(v)$ . Thus,  $x = 0$  since it is in  $\text{span}(v) \cap v^\perp$ . Now, we can define the inverse of  $M$ ,  $M^{-1} : \text{im}(\Gamma) \rightarrow v^\perp$  and perform algebra. If  $\Gamma x(t)$  is bounded, observe that

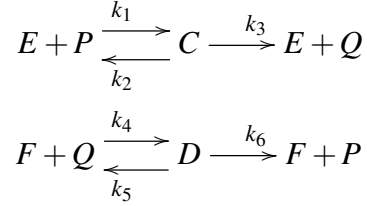
$$M^{-1}\Gamma x(t) = M^{-1}\Gamma \pi_{v,x}(t) = M^{-1}M \pi_{v,x}(t) = \pi_{v,x}(t)$$

is bounded. □

This is useful because it guarantees any projection of the solution  $x(t)$  to the reaction-coordinates system implies  $\Gamma x$  is bounded, because  $x(t)$  could easily grow to infinity. However, the positive or negative changes in reaction-coordinates does not imply the same change of signs occurs in species-coordinates because reactions can continue to occur while the CRN is in equilibrium, and the extent of the reaction matters because  $x(t) \neq 0$  for all  $t$ .

The previous result introduced in [4] assumed the matrix  $\Gamma$  has a one-dimensional kernel. Unfortunately this restricted the amount of labeling choices for the reaction model up to permutation. This is a problem, as there is no adopted convention for labeling

bio-chemical reaction models with mass-action coordinates. For example, if we decided to remodel the futile cycle by treating every reversible reaction as its own reaction as in the following:



then the species vector  $S = \langle P, Q, E, F, C, D \rangle^T$  with reaction vector  $R \in \mathbb{R}^{n_r} = \mathbb{R}^6$  yields a square  $6 \times 6$  stoichiometry matrix  $\Gamma$

$$\begin{bmatrix}
 -1 & 1 & 0 & 0 & 0 & 1 \\
 0 & 0 & 1 & -1 & 1 & 0 \\
 -1 & 1 & 1 & 0 & 0 & 0 \\
 0 & 0 & 0 & -1 & 1 & 1 \\
 1 & -1 & -1 & 0 & 0 & 0 \\
 0 & 0 & 0 & 1 & -1 & -1
 \end{bmatrix}$$

where the rows are with respect to the species model in  $S$  and the rows are the reactions  $\langle k_1, k_2, k_3, k_4, k_5, k_6 \rangle$ . Observe that this matrix has a right kernel of 3 dimensions.

We propose the following updated version of Lemma 3.2 from reference [4] as a reprise to include a larger dimensional kernel.

**Lemma 34** ([4, Lemma 3.2] (Reprise)). *Suppose a matrix  $\Gamma \in M_{n \times m}(\mathbb{Z})$  has rank exactly  $m - k$  whose right kernel is spanned by positive vectors  $v_i \in V$  such that  $\dim(V) = k$ . Let  $x(t)$  be a solution to the system  $\dot{x} = f_\sigma(x)$  with initial condition  $x_0$  for all  $t \geq 0$ . Then,  $\Gamma x(t)$  is bounded if and only if  $\pi_V x(t)$  is bounded where  $V = \sum v_i$ .*

*Proof.* If  $\pi_V x(t)$  is bounded, then we have the same proof as the 1-dimensional kernel result since projection mappings are linear.

Conversely, consider the following vector space,

$$V = \{v_i \in \mathbb{R}^n : \Gamma v_i = 0\},$$

and recall that  $\text{rank}(\Gamma) = m - \dim(V)$ . Let

$$W := \left\{ v^\perp \in \mathbb{R}^m : \langle v^\perp, V \rangle = 0 \right\},$$

and let  $M = \Gamma|_W$ , the restriction of  $\Gamma$  to  $W$ . Observe that  $\Gamma x = \Gamma \pi_V(x)$ , and we will prove  $M$  is injective: if  $x \in V^\perp$  such that  $Mx = 0$ , then  $\Gamma x = 0$  since they have the same image.

Then,

$$x \in \ker(\Gamma) = \ker(M) \implies \langle x, V^\perp \rangle = 0,$$

so  $x \in \text{span}(V)$ . Thus,  $x \in \text{span}(V) \cap V^\perp$  implies  $x = 0$ . Just as before, we can define the inverse of  $M$ ,  $M^{-1} : \text{im}(\Gamma) \rightarrow V^\perp$  and perform algebra. If  $\Gamma x(t)$  is bounded, observe that

$$M^{-1}\Gamma x(t) = M^{-1}\Gamma \pi_V x(t) = M^{-1}M \pi_V x(t) = \pi_V x(t),$$

is bounded. □

#### 4.5 Using the Angeli-Sontag Theorem with Chemical Reaction Dynamical Systems

Before we prove a direct application of Theorem 28 on page 27 to chemical reaction systems, we need the following lemma.

**Lemma 35** ([4, Lemma 3.1]). *The system  $\dot{x} = f_\sigma(x) = R(\sigma + \Gamma x)$  is forward complete, meaning every solution is defined for all  $t \geq 0$  and they remain in  $X_\sigma = \{x \in \mathbb{R}^m : \sigma + \Gamma x \geq 0\}$ . Furthermore, if every solution of  $\dot{S} = \Gamma R(S)$  is bounded, then  $\Gamma x(t)$  is bounded for every solution  $x(t)$  of  $f_\sigma(x)$ .*

*Proof.* Let  $x_0$  be any initial condition in  $X_\sigma$ . Consider the solution  $S(t)$  of  $\dot{S} = \Gamma R(S)$  with initial condition  $S(0) = S_0 := \sigma + \Gamma x_0 \in \mathbb{R}_{\geq 0}^n$ . This solution is well-defined and forward complete since it satisfies  $S(t) \geq 0$  for all  $t \geq 0$ . We will use this to construct a forward complete solution for  $\dot{x} = f_\sigma(x) = R(\sigma + \Gamma x)$ .

Define for  $t \geq 0$

$$x(t) := x_0 + \int_0^t R(S(\tau)) d\tau.$$

Notice that  $\dot{x}(t) = R(S(t))$  for all  $t$  and  $x(0) = x_0 \in X_\sigma$  by assumption.

We claim  $x \in X_\sigma$  and is forward complete, which means  $x(t)$  is a solution to the system  $\dot{x} = f_\sigma(x)$  with initial condition  $x_0$  for all  $t \geq 0$ .

Take  $P(t) := \sigma + \Gamma x(t)$  as another solution in  $X_\sigma$ , with initial condition  $P(0) = \sigma + \Gamma x_0$ . Observe that for all  $t \geq 0$ ,

$$\begin{aligned} \dot{P}(t) &= \frac{d\sigma}{dt} + \frac{d}{dt}(\Gamma x(t)) \\ &= 0 + \frac{d\Gamma}{dt}x(t) + \Gamma \frac{dx(t)}{dt} \\ &= \Gamma \dot{x} \\ &= \Gamma(R(S(t))) \\ &= \dot{S}(t). \end{aligned}$$

Then,  $\dot{P} - \dot{S} = 0$  implies  $P - S$  is a constant. Also,

$$P(0) - S(0) = \sigma + \Gamma x_0 - \sigma - \Gamma x_0 = 0$$

implies  $P = S$ , which means our solution to  $\dot{S} = \Gamma R(S)$  is explicitly  $S(t) = \sigma + \Gamma x(t)$ . We also have a unique solution since  $f_\sigma$  is locally Lipschitz.

Thus, for  $t \geq 0$

$$\dot{x}(t) = R(S(t)) = R(\sigma + \Gamma x(t)) = f_\sigma(x(t))$$

and  $x(t)$  is a solution to  $f_\sigma$ .

Finally, assume every solution of  $\dot{S} = \Gamma R(S)$  is bounded, so by extension,  $S(t) = \sigma + \Gamma x(t)$  is bounded implies  $\Gamma x(t) = S(t) - \sigma$  is bounded for every solution  $x(t)$  of  $f_\sigma(x)$ .  $\square$

**Corollary 36** ([4, Corollary 3.3]). *Suppose*

(i)  $\Gamma$  has rank  $m - k$  whose left kernel is  $k$ -dimensional.

(ii) Every solution of  $\dot{S} = \Gamma R(S)$  is bounded, so  $\Gamma x(t)$  is bounded for every solution  $x(t)$  of  $f_\sigma(x)$  by Lemma 35 on page 32.

(iii) There is a vector  $\sigma \in \mathbb{R}_{\geq 0}^n$  such that the system  $\dot{x} = f_\sigma(x)$  is strongly monotone.

Then, there is an initial condition vector  $\xi_\sigma \in \mathbb{R}_{\geq 0}^n$  with this property: for each  $\rho \in \mathbb{R}_{\geq 0}^n$  such that  $\rho - \sigma \in \text{im}(\Gamma)$ , the solution  $S(t) = S_0 + \Gamma x(t)$  to  $\dot{S} = \Gamma R(S)$  in which  $S_0 = \rho$ , implies  $S(t) \rightarrow \xi_\sigma$  as  $t \rightarrow \infty$ .

We extended this result to include a  $k$ -dimensional kernel. The proof below is roughly the same as the one presented in [4] with a few more details, but their introduction of the function  $z(t)$  did not seem justified. Below is an alternate proof that motivates where  $z(t)$  comes from.

*Proof.* Let  $\ker(\Gamma) = \{v_i \in \mathbb{R}^m : \Gamma v_i = 0\}$  and  $V = \sum v_i$ . Since every solution of  $\dot{S} = \Gamma R(S)$  is bounded, then by Lemmas 34 on page 31 and 35 on page 32,  $\Gamma x(t)$  is bounded for every solution  $x(t)$  of  $f_\sigma(x)$  if and only if  $\pi_V x(t)$  is bounded. Additionally,  $X_\sigma$  is translation invariant with respect to  $V$ . Also, since  $\dot{x} = f_\sigma(x)$  is strongly monotone for some  $\sigma \in \mathbb{R}_{\geq 0}^n$  by assumption, we can appeal to the Angeli-Sontag Theorem 28 on page 27. Thus, the bounded projection  $\pi_V(x)$  for every solution

$$x(t) := x_0 + \int_0^t R(S(\tau)) d\tau$$

bounded modulo  $V$  converges to a unique equilibrium. In other words, there is a unique equilibrium  $\xi \in \mathbb{R}_{\geq 0}^n$  of the projected system  $\tilde{x} = (I - v v^T) f(\tilde{x})$  such that every solution

$x(t)$  satisfies  $\pi_V(x) \rightarrow \xi$  as  $t \rightarrow \infty$ . Fix  $\rho \in \mathbb{R}_{\geq 0}^n$  such that  $\rho - \sigma \in \text{im}(\Gamma)$ . Since  $\Gamma \in \mathbb{R}^{n \times m}$ , let  $a \in \mathbb{R}^m$  and take  $\Gamma a \in \text{im}(\Gamma)$ . Thus,  $\rho - \sigma = \Gamma a$ .

Let  $S(t) = S_0 + \Gamma x(t)$  be a solution to  $\dot{S} = \Gamma R(S)$  in which  $S_0 = \rho$ . Since  $R$  is locally Lipschitz, we have uniqueness of solutions and thus

$$S(t) = \rho + \Gamma x(t)$$

where

$$\dot{x} = R(S(t)) = R(\rho + \Gamma x(t))$$

such that  $x_0 = 0$ . Since  $\Gamma(x) = \Gamma \pi_V(x)$ , then

$$S(t) = \rho + \Gamma x(t) = \rho + \Gamma \pi_V(x(t)) \rightarrow \rho + \Gamma \xi$$

as  $t \rightarrow \infty$ . However, we want our initial condition to depend on  $\sigma$  and not  $\rho$ .

Recall that  $\rho - \sigma = \Gamma a$  implies  $\rho = \sigma + \Gamma a \geq 0$ , which means to finish the proof, we need to show  $a \in X_\sigma$ , where  $a$  is the initial condition of some solution to the reaction-coordinates system. Observe that

$$\dot{x} = R(S) = R(\rho + \Gamma x(t)) = R(\sigma + \Gamma a + \Gamma x) = R(\sigma + \Gamma(a + x(t))).$$

Since  $x(t) + a$  satisfies this system with  $x(0) + a = a \in X_\sigma$ , it also conveniently satisfies the system  $\dot{x} = R(\sigma + \Gamma x(t))$  on  $X_\sigma$ . Thus, by Theorem 28 on page 27 again,

$$\pi_V(x(t) + a) \rightarrow \xi$$

$$\pi_V x(t) + \pi_V a \rightarrow \xi$$

$$\pi_V x(t) \rightarrow \xi - \pi_V a$$

as  $t \rightarrow \infty$ . So finally,

$$\begin{aligned}
S(t) = \rho + \Gamma x(t) &= \rho + \Gamma \pi_V(x(t)) \rightarrow \rho + \Gamma \xi - \Gamma \pi_V a \\
&\rightarrow \rho + \Gamma \xi - \Gamma a \\
&\rightarrow \rho + \Gamma \xi + \sigma - \rho \\
&\rightarrow \sigma + \Gamma \xi
\end{aligned}$$

as  $t \rightarrow \infty$ . Define  $\xi_\sigma := \sigma + \Gamma \xi$ . □

This result is useful because we get a unique equilibrium in each stoichiometry class  $X_\sigma$  of  $\sigma + im(\Gamma)$ , which is important because this implies there is a direct connection between reaction-space and species-space. Any initial condition vector  $\sigma \in \mathbb{R}_{\geq 0}^n$  fixes the stoichiometric compatibility class, so the solution to the species system  $\dot{S} = \Gamma R(S)$ ,

$$S(t) = S(0) + \Gamma \int_0^t R(S(\tau)) d\tau$$

implies  $S \in S(0) + im(\Gamma)$ , and the result states there exists a global attractor  $\xi_\sigma$  in which  $S(0) = \rho \rightarrow \xi_\sigma$  for all  $\rho$ . This is great since we can take  $\rho - \sigma \in im(\Gamma)$  in the projected system and draw conclusions about equilibria in species-space from reaction-space.

#### 4.6 Finding an Equilibrium of the Enzymatic Futile Cycle

We will finish this chapter with an example to illuminate how to use Corollary 36 on page 34 by reproducing the results of [4] with Example 18 on page 13. Recall that the species-coordinates dynamical system  $\dot{S} = \Gamma R(S)$  for this CRN is

$$\begin{bmatrix} \dot{P} \\ \dot{Q} \\ \dot{E} \\ \dot{F} \\ \dot{C} \\ \dot{D} \end{bmatrix} = \begin{bmatrix} -1 & 0 & 0 & 1 \\ 0 & 1 & -1 & 0 \\ -1 & 1 & 0 & 0 \\ 0 & 0 & -1 & 1 \\ 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix} \begin{bmatrix} k_1 EP - k_{-1} C \\ k_2 C \\ k_3 F Q - k_{-3} D \\ k_4 D \end{bmatrix}.$$

Observe that

$$\ker(\Gamma^T) = \text{span} \left\{ \begin{bmatrix} 1 \\ 1 \\ 0 \\ 0 \\ 1 \\ 1 \end{bmatrix}, \begin{bmatrix} 0 \\ 0 \\ 1 \\ 1 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 1 \end{bmatrix} \right\}$$

yields the conservation laws

$$E(t) + C(t) = c_1$$

$$F(t) + D(t) = c_2$$

$$P(t) + Q(t) + C(t) + D(t) = c_3$$

for some constants  $c_1, c_2, c_3 \in \mathbb{R}$ . So the enzymes and substrates are constant (and bounded) for all  $t \geq 0$ . Since

$$\ker(\Gamma) = \text{span} \left\{ \begin{bmatrix} 1 & 1 & 1 & 1 \end{bmatrix} \right\},$$

let us normalize  $x \in \ker(\Gamma)$  by the sup norm to define our unit vector  $v$  as

$$v := \frac{x}{\|x\|_{\text{sup}}} = \frac{\begin{bmatrix} 1 & 1 & 1 & 1 \end{bmatrix}}{4} = \begin{bmatrix} \frac{1}{4} & \frac{1}{4} & \frac{1}{4} & \frac{1}{4} \end{bmatrix}.$$

Consider this simplex that lies in the interior of the positive orthant:

$$\Sigma = \left\{ \sigma = \begin{bmatrix} P & Q & E & F & C & D \end{bmatrix} \in \mathbb{R}_{\geq 0}^{n_s} : E(t) + C(t) > 0, F(t) + D(t) > 0 \right\}$$

where those sums are from the linear dependence of rows in  $\Gamma$  from our bounded enzyme functions from above. We are now ready to show Example 18 on page 13 attains an equilibrium.

Recall our summary from Section 4.4 on page 28: Assume  $R$  satisfies A3(b). Let  $\Sigma \subset \mathbb{R}_{\geq 0}^{n_s}$  be invariant and forward complete, so all solutions are defined for  $t \geq 0$ . For



every fixed  $\sigma \in \Sigma$ , we have the following reaction-coordinates system:

$$\dot{x} = f_\sigma(x) = R(\sigma + \Gamma x) = \begin{bmatrix} k_1(\sigma_3 - x_1 + x_2)(\sigma_1 - x_1 + x_4) - k_{-1}(\sigma_5 + x_1 - x_2) \\ k_2(\sigma_5 + x_1 - x_2) \\ k_3(\sigma_4 - x_3 + x_4)(\sigma_2 + x_2 - x_3) - k_{-3}(\sigma_6 + x_3 - x_4) \\ k_4(\sigma_6 + x_3 - x_4) \end{bmatrix}$$

such that  $f_\sigma : \Sigma \rightarrow \Sigma$  with state space

$$\Sigma \subset X_\sigma = \{x \in \mathbb{R}^{n_r} : \sigma + \Gamma x \geq 0\}$$

where  $x \in X_\sigma$  is called the extent of the reaction. We assumed  $R$  (and  $f$  by extension) is locally Lipschitz,  $\Sigma$  is a closed set that is the closure of its interior and is translation invariant for any  $v \in \ker(\Gamma)$ , and the positive orthant  $\mathbb{R}_{\geq 0}^{n_s}$  is a closed cone with interior  $\ker(\Gamma)$ . To satisfy the conditions of Corollary 36 on page 34 to the theorem, we need to show the reaction-coordinates system  $\dot{x} = f_\sigma(x)$  for Example 18 on page 13 is strongly monotone for  $\sigma \in \Sigma$ . To do this, we need to show the Jacobian matrix of  $R(\sigma + \Gamma x)$ ,  $Df_\sigma(x)$ , is a cooperative and irreducible matrix.

**Theorem 37.** *The reaction-coordinates dynamical system  $\dot{x} = f_\sigma(x)$  for Example 18 on page 13 is strongly monotone independent of reaction kinetics.*

*Proof.* Let us compute  $Df_\sigma(x)$ .

$$DR(\sigma + \Gamma x) = \begin{bmatrix} D_{11} & k_1(\sigma_1 - x_1 + x_4) + k_{-1} & 0 & k_1(\sigma_3 - x_1 + x_2) \\ k_2 & -k_2 & 0 & 0 \\ 0 & k_3(\sigma_4 - x_3 + x_4) & D_{33} & k_3(\sigma_2 + x_2 - x_3) + k_{-3} \\ 0 & 0 & k_4 & -k_4 \end{bmatrix}$$

where

$$D_{11} = -k_1(\sigma_1 - x_1 + x_4) - k_1(\sigma_3 - x_1 + x_2) - k_{-1}$$

$$D_{33} = -k_3(\sigma_2 + x_2 - x_3) - k_3(\sigma_4 - x_3 + x_4) - k_{-3}.$$

We can also express the Jacobian in  $\Sigma$  (or  $S$ ) species-coordinates:

$$DR(\Sigma) = \begin{bmatrix} -k_1P - k_1E - k_{-1} & k_1P + k_{-1} & 0 & k_1E \\ k_2 & -k_2 & 0 & 0 \\ 0 & k_3F & -k_3Q - k_3F - k_{-3} & k_3Q + k_{-3} \\ 0 & 0 & k_4 & -k_4 \end{bmatrix}.$$

Notice that the diagonal has all negative entries, and both upper and lower diagonals have non-negative entries. By Proposition 14 on page 11, this matrix is cooperative. If  $DR(\sigma + \Gamma x)$  is irreducible almost everywhere along trajectories, then the system is strongly monotone [4], [14]. This amounts to proving  $E, F \neq 0$  along any solution since  $f \in C^1$ .

Take

$$\Sigma = \{ \sigma = [ P \ Q \ E \ F \ C \ D ] \in \mathbb{R}_{\geq 0}^{n_s} : E(t) + C(t) > 0, F(t) + D(t) > 0 \}$$

and let  $\sigma \in \Sigma$  where

$$\sigma = [ P \ Q \ E \ F \ C \ D ] = [ \sigma_1 \ \sigma_2 \ \sigma_3 \ \sigma_4 \ \sigma_5 \ \sigma_6 ]$$

is a species vector. Thus,  $\sigma_3 + \sigma_5 > 0$  and  $\sigma_4 + \sigma_6 > 0$  implies  $\sigma_3 + \sigma_5 \neq 0$  and  $\sigma_4 + \sigma_6 \neq 0$ . Seeking a contradiction, suppose  $E = k_1(\sigma_3 - x_1 + x_2) = 0$  where  $k_1 \neq 0$ , which implies  $\sigma_3 - x_1 + x_2 = 0$  and  $\sigma_3 = x_1 - x_2$ . Then, taking the time derivative of both sides yields  $\dot{x}_1 - \dot{x}_2 = 0$ , which implies

$$0 = k_1(\sigma_3 - x_1 + x_2)(\sigma_1 - x_1 + x_4) - k_{-1}(\sigma_5 + x_1 - x_2) - k_2(\sigma_5 + x_1 - x_2)$$

$$0 = k_1 \cdot 0 \cdot (\sigma_1 - x_1 + x_4) - (k_{-1} + k_2)(\sigma_5 + x_1 - x_2)$$

$$0 = -(k_{-1} + k_2)(\sigma_5 + x_1 - x_2)$$

$$0 = -(k_{-1} + k_2)(\sigma_5 + \sigma_3)$$

and thus,  $(\sigma_5 + \sigma_3) = 0$  since  $k_{-1}, k_2 \neq 0$ . This is a contradiction because  $\sigma_3 + \sigma_5 > 0$ , and in fact,  $\sigma_3 + \sigma_5 \neq 0$ . Thus,  $E \neq 0$ , and similarly,  $F \neq 0$  as well. Hence,  $f_\sigma$  is strongly monotone due to Theorem 3.9 from reference [4], [14, Theorem 3.9].  $\square$

Finally, by Corollary 36 on page 34, then there is an initial condition vector  $\xi_\sigma \in \Sigma$  with this property: for each  $\rho \in \Sigma$  such that  $\rho - \sigma \in \text{im}(\Gamma)$ , the solution  $S(t) = S_0 + \Gamma x(t)$  to  $\dot{S} = \Gamma R(S)$  where  $S_0 = \rho$  implies  $S(t) \rightarrow \xi_\sigma$  as  $t \rightarrow \infty$ . In other words,  $\xi_\sigma$  converges monotonically to an equilibrium, and there is a unique equilibrium in each stoichiometric compatibility class  $X_\sigma$ .

## 5 GRAPHS OF CHEMICAL REACTION NETWORKS

Recall that reaction models are simply represented by graphs. A *graph* is an object made of vertices and edges; *directed graphs* have arrows; *signed graphs* have weighted edges. A directed graph is *strongly connected* if there is a path from a vertex to any other vertex. One question we wish to answer is the following: is there a correlation between a reaction network's structure and its graphical properties? This question was answered by Feinberg in references [8], [9] with the Deficiency Zero Theorem, which gave sufficient conditions for a strongly connected chemical reaction network to have one unique and stable equilibrium within each stoichiometric compatibility class in species-coordinates. However, it is not satisfied for many examples, because most CRNs are not strongly connected.

The paper [4] introduced conditions to determine when the reaction-coordinates dynamical system  $\dot{x} = f_{\sigma}(x)$  is strongly monotone by showing that the associated Jacobian matrix is cooperative and irreducible in order to use the Angeli-Sontag theorem and conclude the existence of a globally attracting equilibria in each stoichiometric compatibility class. We would prefer that there exists a way to graphically model chemical reaction networks and keep track of the relationships between reactions and species to draw the same conclusions and avoid computationally tedious algebra. Indeed, the same authors from the paper [4] constructed a graphical framework in their paper [5] by adopting the same conventions of treating reversible reactions as one (which effectively restricts our reaction labeling) and constructing the reaction vector  $R(S) \in \mathbb{R}^r$  by considering the species that contribute or are produced by a reaction.

In this chapter, we will always fix reactants and products in reversible reactions as in reference [5]. For example, if  $A + B \rightleftharpoons C$ , we fix  $A + B$  as the reactant side and  $C$  as the product side. In order to use the graphical checks and results to verify monotonicity from reference [5], we will have to adopt assumptions A1, A2, A3(b), A4, and A5. We want all

solutions of  $\dot{S} = \Gamma R(S)$ , a species-coordinates dynamical system with an associated stoichiometric compatibility class (as defined in Definition 24 on page 23)

$$C_{S_0} = \mathbb{R}_{\geq 0}^{n_s} \cap \{S_0 + im(\Gamma)\}$$

to be bounded, so we will also assume all stoichiometric compatibility classes are compact sets. The latter is only true if there is a strictly positive vector in  $\ker(\Gamma^T)$ . Indeed, if we take a left kernel vector  $w \gg 0$  on both sides of the dynamical system, then

$$w^T \dot{S} = w^T \Gamma R(S) \implies w^T \dot{S} = 0 \implies w^T S(t) = w^T S_0.$$

Hence, the solution is bounded because the right-hand side is a constant and all coordinates in  $w$  are positive. This fact depends on the sign of  $w$ 's components. For example, if we had the vector

$$w = [ 1 \quad -1 ],$$

then we would have

$$-S_1(t) + S_2(t) = -S_1(0) + S_2(0)$$

which is not a bounded set because there are infinitely many solutions to this equation.

We only need reaction rates to satisfy A1, A2, A3, A4, A5, and A6 for our graphical tests to work according to reference [5]. However, in certain reaction kinetic assumptions such as mass-action, Hill, or Michaelis-Menten, we will omit A4 and not require all stoichiometric compatibility classes to be compact [5]. We remark that A4 is necessary for the partial derivatives of the reactions to remain monotone and not change signs. In the next sections, we summarize the definitions and properties of the special graphs of a chemical reaction network introduced in reference [5].

## 5.1 Jacobian Graph

The Jacobian or J-graph  $(V, E_+, E_-)$  of reference [5] for a state space  $X$  is an undirected weighted graph of order  $|V| = \dim(X)$  where the vertex set is the elements of the Jacobian matrix  $Df(x)$ , and the edge sets  $E_+, E_-$  consist of positive (+) edges and negative (−) labeled edges respectively.

### 5.1.1 Construction

There is an edge  $(v_i, v_j)$  from vertices  $v_i, v_j \in V$  if  $[Df(x)]_{ji} \geq 0 \in X$  and  $[Df(x^*)]_{ji} > 0 \in X$  for at least one  $x^*$ . Alternatively, for the negative case, there exist an edge  $[Df(x)]_{ji} \leq 0 \in X$  and  $[Df(x^*)]_{ji} < 0 \in X$ . Namely, for some  $x^*$  in each case,

- $(v_i, v_j) \in E_+$  if  $[Df(x)]_{ji} \geq 0 \in X$  and  $[Df(x^*)]_{ji} > 0 \in X$ .
- $(v_i, v_j) \in E_-$  if  $[Df(x)]_{ji} \leq 0 \in X$  and  $[Df(x^*)]_{ji} < 0 \in X$ .
- $v_i \not\sim v_j$  if  $[Df(x)]_{ji} = 0$  for all  $x \in X$ .

The J-graph is an undirected weighted graph, which implies  $v_i \sim v_j$  has three cases: one edge of the same sign, two edges of the same sign, two edges of mixed sign. The positive (+) and negative (−) signs of its edges correspond to the signs of elements in the Jacobian matrix.

## 5.2 Species Reaction Graph

The Species-Reaction graph or SR-Graph  $(V_S, V_R, E_+, E_-)$  of [5] is an undirected, bipartite, labeled graph with  $\{+, -\}$  labels where  $V_S$  is the vertex set of species,  $V_R$  is the vertex set of reactions, and the edge sets  $E_+, E_- \subseteq V_S \times V_R$ . We can use SR-graphs to identify a class of monotone reaction systems.

### 5.2.1 Construction

There exists an edge  $(S_i, R_j)$  between  $S_i \in V_S$  and  $R_j \in V_R$  if the species is a product or reactant of the reaction. We assign the labels as follows for  $S_i \in V_S, R_i, R_j \in V_R$ :

- $(S_i, R_j) \in E_+$  if  $a_{ij} > 0$

- $(R_i, S_j) \in E_-$  if  $b_{ij} > 0$
- $v_i \not\sim v_j$  otherwise.

To summarize, there is a positive (+) edge between  $(S_j, R_i)$  if  $S_j$  contributes to the reaction, and a negative (−) edge between  $(R_j, S_i)$  if  $S_i$  is produced by the reaction. If a species is the reactant or product of only one reaction, i.e., if  $(S_i, R_j) \in E_+ \cup E_-$ , then there does not exist an  $R_k \in V_R$  such that  $(R_k, S_i)$  or  $(S_i, R_k)$ .

Since the SR-graph is bipartite, the vertex set of species and the vertex set of reactions are disjoint, i.e.,  $V_S \cap V_R = \emptyset$ . Also, since it is an undirected weighted graph, we have three cases for any weighted labels of edges: one edge of the same sign, two edges of the same sign, two edges of mixed sign

### 5.3 Reaction Graph

The Reaction Graph or R-graph  $(V_R, \tilde{E}_+, \tilde{E}_-)$  of reference [5] is a weighted induced sub-graph of the SR-graph, which means it is an undirected multi-graph and the positive (+) and negative (−) signs of its edges depend strictly on the signs in the SR-graph. We label the edges in the R-graph with  $\sigma$ , in which  $\sigma$  is the negative (−) product of signs between two reactions along the path of order 2 in the SR-graph.

#### 5.3.1 Construction

There exists an edge  $(R_i, R_j)$  between  $R_i, R_j \in V_R$  if there exists a species  $S_j \in V_S$  such that  $R_i \sim S_k$  and  $S_k \sim R_j$  in the SR-graph. In graph theory terminology, this means there is a path of order 2 in the SR-graph with  $R_i, R_j \in V_R$  at the endpoints. In other words, there is a (signed) edge in the R-graph if there is a species that connects two reactions in the R-graph. Notice that this species is not unique since more than one path of order 2 can occur between two reactions in an SR-graph, which implies there are at most two edges between  $R_i, R_j \in V_R$  in the R-graph (see Figure 5.1). If these two edges have the same sign, then we can simply use one – otherwise, we keep both edges if they have different signs.

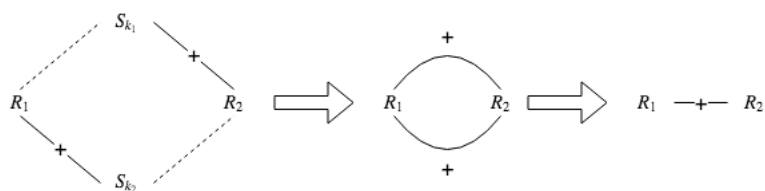


Fig. 5.1: A positive edge in the R-graph does not imply uniqueness of species in the SR-graph.

#### 5.4 Species Graph

The Species graph or S-graph  $(V_S, \tilde{E}_+, \tilde{E}_-)$  of reference [5] is another induced sub-graph of the SR-graph in which the vertices are species. It is constructed the same way as the R-graph with signs, except there has to exist a reaction between two species. Just like the R-graph, the path between two species is not unique.

#### 5.5 Directed Species Reaction Graph

The Directed Species Reaction graph, or DSR-graph  $(V_S, V_r, V_i, E)$  of [5] is a bipartite, directed, and labeled version of the SR-graph such that

- $V_S$  is the set of vertices corresponding to species
- $V_r$  is the set of vertices corresponding to reversible (two-way) reactions
- $V_i$  is the set of vertices corresponding to irreversible (one-way) reactions
- $E$  is the set of positive and negatively labeled edges

Forming the DSR-graph is almost exactly the same as the SR-graph, except now we have to account for the directed edges that follow the same reaction sequence of the original CRN.

$$\begin{cases} S \rightarrow R_k \in V_r & S \text{ contributes to reaction } R_k : S \in V_S, R_k \in V_r \cup V_i \\ R_k \in V_r \rightarrow S & S \text{ is produced by reaction } R_k : S \in V_S, R_k \in V_r \cup V_i \\ S \rightarrow R_k \in V_i & S \text{ contributes to reaction } R_k : S \in V_S, R_k \in V_r \cup V_i \end{cases}$$



This amounts to saying there is an edge between a species and a reaction if the former contributes to, or is produced by the former. We say a *DSR-graph* is *R-strongly connected* if there is a path between any reactions  $R_i$  and  $R_j$  in  $V_r$ .

## 5.6 Tests for Monotonicity and the Positive Loop Property

A dynamical system  $\dot{x} = f(x, t)$  can be tested for monotonicity with respect to a positive orthant cone using the following analytic or graphical strategies. Analytically, if the matrix  $\Sigma Df(x) \Sigma$  has non-negative diagonal entries for all  $x \in X$ , such that  $Df(x)$  is the Jacobian, and  $\Sigma$  is a diagonal matrix with  $\pm 1$  along the diagonal, then the system is monotone [14], [16]. The matrix  $\Sigma$  also canonically defines the orthant [5]. Graphically, one can also calculate the Jacobian of the system and inspect the off-diagonal entries via results from references [4], [14], then construct the J-graph or Jacobian graph according to the paper [5]. It is important that these signs never change in order to use the graphical test; they must always remain greater than or equal to zero, or less than equal to zero. To ensure this, we will keep in mind the assumptions we made in Section 3.1 on page 13.

**Definition 38.** Closure property: if  $x_n \geq y_n$  for all  $n \in \mathbb{N}$  and  $y_n \rightarrow y$ ,  $x_n \rightarrow x$  as  $n \rightarrow \infty$ , then  $x \geq y$ .

One can induce a partial order from a closed pointed convex cone  $K \subset \mathbb{R}^n$  that satisfies the closure property: if  $K$  is the set of positive vectors, then  $x \leq y$  if and only if  $y - x \in K$  from Lemma 6 on page 10. We will use the partial order  $\leq$  induced by a positive orthant cone  $K = \mathbb{R}_{\geq 0}^{n_s}$ , which amounts to the vector satisfying  $y - x \in K$  as the trajectories for vectors  $x$  and  $y$  move throughout the state space. Since  $y - x$  is in the positive orthant  $K$ , then  $x$  must be greater than  $y$  component-wise.

### 5.6.1 Positive Loop Property

An important characteristic of a chemical reaction network's graphical modeling is the ability to identify the relationship between the contribution and production of species and reactions. This characteristic is known as the positive loop property.

**Definition 39.** A *simple loop* (or cycle) is a path of at least two edges from a vertex to itself with no repeated vertices in between.

**Definition 40.** Positive Loop Property: A simple loop (or cycle) has the *positive loop property* if it has an even number of negative ( $-$ ) edges.

Let  $L$  be a cycle in an SR-graph.  $L$  has an even number of edges by construction since we only have edges for species-reaction relationships. Define  $\lambda := \lfloor \frac{L}{2} \rfloor \in \mathbb{Z}$  and let  $\sigma$  be the product of signs of all edges in  $L$ . Then, we can define the following characteristics of  $L$ .

**Definition 41.** If  $(-1)^\lambda = \sigma \in \{\pm 1\}$ , then  $L$  is called an *even-loop* (e-loop). Otherwise,  $(-1)^\lambda \neq \sigma$  and  $L$  is called an *odd-loop* (o-loop).

An important question to ask ourselves is what kind of situations arise if  $L$  is an e or o-loop and what are the implications for the SR-graph to have such a loop? The following proposition answers this question and states necessary and sufficient conditions for the positive loop property's relation to e-loops.

**Proposition 42** ([5, Proposition 4.5]). *The R-graph has the positive loop property if and only if*

- 1) *All cycles in the SR-graph are e-loops*
- 2) *Each  $S \in V_S$  in the SR-graph is linked to at most two  $R$ 's  $\in V_R$ .*

*The result also holds similarly for the S-graph.*

### 5.6.2 Graphical Tests for Monotonicity in Reaction-Coordinates Dynamical Systems

Given a species-coordinates dynamical system and an associated stoichiometric compatibility class,

$$\dot{S} = \Gamma R(S), \quad C_{S_0} = \mathbb{R}^{n_s} \cap (S_0 + \text{im}(\Gamma))$$

then we can project to reaction-coordinates from  $C_{S_0}$

$$\dot{x}(t) = R(S_0 + \Gamma x(t)), \quad x \in X_0 = \{x \in \mathbb{R}^{n_r} : x_0 + \Gamma x \geq 0\}.$$

**Proposition 43.** *Let  $x(t)$  be a solution of the reaction-coordinates dynamical system, then*

$$S(t) = S_0 + \Gamma x(t)$$

*solves the species-coordinates dynamical system. Conversely, if  $S(t)$  is a solution in species-coordinates, then there exists a solution  $x(t)$  (not unique) in reaction-coordinates such that*

$$S(t) = S_0 + \Gamma x(t).$$

This result is related to Lemma 35 on page 32 from the paper [4] by the same authors, except we are strictly speaking about the existence of solutions between species-space and reaction-space and not bounded-ness.

*Proof.* If  $x(t)$  is a solution to  $\dot{x}(t) = R(S_0 + \Gamma x(t))$ , then

$$\dot{S}(t) = \frac{d(S_0 + \Gamma x(t))}{dt} = \Gamma \dot{x}(t) = \Gamma R(S_0 + \Gamma x(t)).$$

Conversely, if  $S(t)$  is a solution in species-coordinates, then there exists an  $x_0$  such that  $S(0) = S_0 + \Gamma x_0$  for an initial condition  $S_0 \in C_{S_0}$ . We propose

$$x(t) = x_0 + \int_0^t R(S(\tau)) d\tau$$

is a solution to the reaction-coordinates system. Indeed,  $x(0) = x_0$ , so  $S(0) = S_0 + \Gamma x_0$ .

Also, observe that

$$\frac{d(S_0 + \Gamma x(t))}{dt} = \Gamma \dot{x}(t) = \Gamma R(S(t)) = \dot{S}(t)$$

for all  $t$ . Since we previously assumed  $R$  satisfies A3(b), then solutions to the species dynamical system are unique. Hence,  $S(t) = S_0 + \Gamma x(t)$  holds for all  $t$ , and  $x(t)$  is a solution of the reaction-coordinates system.  $\square$

This result implies that we can make inferences about the stability of solutions in species-coordinates by studying solutions of the projected reaction-coordinates system. We need the following results from reference [5], which describe necessary and sufficient conditions for a dynamical system to be monotone on a partial order induce by a positive orthant cone.

**Theorem 44** ([5, Theorem 1]). *Pick any  $S_0 \in \mathbb{R}^{n_s}$ . The reaction-coordinates dynamical system is monotone with respect to a partial order induced by an orthant cone if and only if the R-graph has the positive loop property.*

The R-graph having the positive loop property does not state that the partial order is induced by a positive orthant cone, because that would automatically imply the system is cooperative, which is a stronger result.

**Proposition 45** ([5, Proposition 5.3]). *Assume the R-graph has the positive loop property. The Jacobian matrix of the reaction-coordinates system is irreducible in*

$$\text{int}(X_0) = \{x \in \mathbb{R}_{\geq 0}^{n_r} : x_0 + \Gamma x \gg 0\}$$

*if and only if the DSR-graph is R-strongly connected.*

Notice that this result depends on assumptions A3 and A5. Also, if either of the previous results were to imply cooperativity, then they would successfully replace Theorem 28 on page 27.

**Corollary 46** ([5, Corollary 1]). *If the R-graph has the positive loop property and the DSR-graph is R-strongly connected, then the reaction-coordinates system is monotone on*

$$X_0 = \{x \in \mathbb{R}^{n_r} : S_0 + \Gamma x \geq 0\}$$

*with respect to the order induced by an orthant cone, and strongly monotone on*

$$\text{int}(X_0) = \{x \in \mathbb{R}_{\geq 0}^{n_r} : S_0 + \Gamma x \gg 0\}$$

*with respect to the same order.*

The following lemma implies the relationship for an orthant cone  $K$

$$\{0\} \neq \ker(\Gamma) \cap K \subset \partial K$$

is impossible under certain conditions.

**Lemma 47** ([5, Lemma 6.1]). *Assume the R-graph has the positive loop property, and the DSR-graph is R-strongly connected. Let  $K$  be an orthant cone that induces the partial order from Theorem 1. Then, either one of*

$$\ker(\Gamma) \cap \text{int}(K) \neq \emptyset$$

*or*

$$\ker(\Gamma) \cap K = \{0\}$$

*holds.*

**Theorem 48** ([5, Theorem 2]). *Choose any  $S_0 \in \text{int}(\mathbb{R}^{n_s})$ . Assume the species system satisfies  $\omega(x_0) \cap \partial\mathbb{R}_{\geq 0}^{n_s} = \emptyset$  (persistent), the R-graph has the positive loop property, and the DSR-graph is R-strongly connected. Let  $K$  be an orthant cone.*

*If  $\ker(\Gamma) \cap \text{int}(K) \neq \emptyset$ , then all solutions of the species system in  $\text{int}(\mathbb{R}_{\geq 0}^{n_s})$  converges to a unique equilibrium within each stoichiometric compatibility class  $C_{S_0}$ .*

*If  $\ker(\Gamma) \cap K = \{0\}$ , then almost all solutions of the species system in  $\text{int}(\mathbb{R}_{\geq 0}^{n_s})$  converges to the set of equilibria. The set of non-converging initial conditions has measure 0.*

These results are useful because they provide exact and simple graphical checks for whether there exists an orthant cone that induces a partial order without performing computationally expensive operations or lengthy algebra – effectively replacing Theorem 28 on page 27. If there is such a cone  $K$ , then the system must be monotone (or strongly monotone if the system is cooperative) with respect to that partial order and have a convergent equilibrium in each stoichiometric compatibility class. All one has to do is construct the DSR- and R-graphs, and calculate the product of positive and negative signs, then conclude the absence of boundary equilibria. We will demonstrate how to quickly check this via persistence in Chapter 6 on page 55.

## 5.7 Finding an Equilibrium of the Enzymatic Futile Cycle

We will finish this chapter with an example to illuminate how to use Theorem 44 on page 49, Corollary 46 on the previous page, and Theorem 48 on the preceding page by reproducing the results found in the paper [4] with Example 18 on page 13. We present the R-graph, SR-graph, and DSR-graph in Figure 5.2.

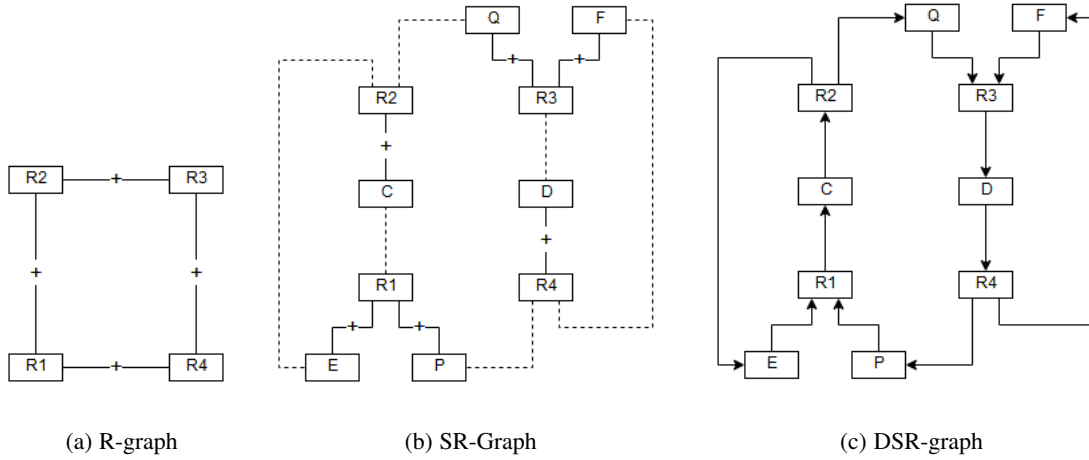


Fig. 5.2: Special graphs of Example 18.

Observe that the R-graph has the positive loop property by Definition 40 on page 47 since there is only one cycle and there are zero negative ( $-$ ) edges. Indeed, by Proposition 42 on page 47, since each species in the SR-graph is linked to at most two reactions and the product of signs in every cycle (in the SR-graph) is positive ( $+$ ). Next, we need to show the DSR-graph is R-strongly connected by demonstrating we can travel from one reaction vertex  $R_i$  to any reaction vertex  $R_j$ . Indeed we can, since  $R_1$  can get to  $R_2, R_3, R_4$ , and back to itself via path

$$(R_1, C, R_2, Q, R_3, D, R_4, P, R_1).$$

Recall that from our discussion in Section 4.6 on page 36, we have the following conservation laws for some constants  $c_1, c_2, c_3 \in \mathbb{R}$ ,

$$E + C = c_1$$

$$F + D = c_2$$

$$P + Q + C + D = c_3.$$

The first two of these conservation laws canonically define the following simplex (in species-coordinates)

$$\Sigma = \left\{ \sigma = [ P \ Q \ E \ F \ C \ D ] \in \mathbb{R}_{\geq 0}^6 : E + C > 0, F + D > 0 \right\}.$$

By Corollary 46 on page 50 from the paper [5, Corollary 1], the reaction-coordinates system is monotone on

$$X_0 = \{x \in \mathbb{R}^{n_r} = \mathbb{R}^4 : S_0 + \Gamma x \geq 0\}$$

with respect to the order induced by an orthant cone, and strongly monotone on

$$\text{int}(X_0) = \{x \in \mathbb{R}_{\geq 0}^{n_r} = \mathbb{R}^4 : S_0 + \Gamma x \gg 0\}$$

with respect to the same order. The positive orthant cone that preserves this order (in reaction-coordinates) is  $K = \mathbb{R}_{\geq 0}^{n_r} = \mathbb{R}_{\geq 0}^4$ . The paper [1] showed Example 18 on page 13 satisfies

$$\omega(x_0) \cap \partial \mathbb{R}_{\geq 0}^6 = \emptyset$$

and thus is persistent. We will demonstrate why in Chapter 6 on page 55. Next, we will determine which of the following two situations are satisfied in order to use Theorem 48 on page 50 from reference [5, Theorem 2]

$$\ker(\Gamma) \cap \text{int}(\mathbb{R}_{\geq 0}^4) \neq \emptyset$$

or

$$\ker(\Gamma) \cap \mathbb{R}_{\geq 0}^4 = \{0\}$$

where  $\ker(\Gamma)$  is the right kernel of  $\Gamma$ . Recall that  $\ker(\Gamma)$  for Example 18 on page 13 from Section 4.6 on page 36 is spanned by a single vector  $[1, 1, 1, 1]'$ . Thus, we are in the



situation  $\ker(\Gamma) \cap \text{int}(\mathbb{R}_{\geq 0}^4) \neq \emptyset$ . Hence, by Theorem 48 on page 50 from reference [5, Theorem 2], all solutions of the species system in  $\text{int}(\mathbb{R}_{\geq 0}^{n_s}) = \text{int}(\mathbb{R}_{\geq 0}^6)$  converges to a unique equilibrium within each stoichiometric compatibility class

$$C_{S_0} = \mathbb{R}^6 \cap (S_0 + \text{im}(\Gamma)).$$

## 6 AN ALGORITHM FOR ESTABLISHING MULTI-STATIONARITY

In this chapter, we explore the algorithm of authors Conradi, Feliu, Mincheva, and Wiuf presented in the paper [3], which gives sufficient conditions for a reaction network to have multiple positive equilibria in each stoichiometric compatibility class. The success of the algorithm is also fairly dependent (for large systems) on finding a parametrization of the steady state set, but we will not go into too much detail regarding this point. If the network has a positive parametrization, then one can rewrite a non-linear system with fewer variables by hand or with symbolic computer software in order to make computations easier. A positive parametrization of a set of positive equilibria exists for systems with toric steady states and for some post-translational modification systems [27].

Given an ODE system with a parametrized equilibrium (steady state) set, we will compute a single polynomial supported by the *Newton Polytope* in species-space. This polynomial is a function of reaction coefficients and species concentrations, and its sign will tell us if there is a single or multiple equilibria in each stoichiometric compatibility class. In general, the result gives a rational function of polynomials in  $\mathbb{Q}[V_S]$ , although the denominator is typically always positive and thus we only need to consider the polynomial in the numerator. One needs all criteria to succeed or the algorithm fails.

For now, we will assume our reaction rates  $R_j : \mathbb{R}_{\geq 0}^n \rightarrow \mathbb{R}_{\geq 0}$  satisfy A1, A2, and A3(b). To ensure we get a polynomial or a rational function, we will assume the reaction's kinetics are mass-action, Michaelis-Menten, or Hill. We will only need to explore the number of equilibria in the species-coordinates dynamical system, as the theory presented in this chapter does not require the usage of the reaction-coordinates dynamical system.

## 6.1 Dissipative and Conservative Reaction Networks

The trajectories of the ordinary differential system of equations are a stoichiometric compatibility class, which we will redefine as

$$P_c := \{S \in \mathbb{R}_{\geq 0}^{n_s} : WS = c\}$$

where  $W$  is a full rank  $d$  left kernel matrix of  $\Gamma$ , and  $n_s$  is the number of species. In this context,  $W \in \mathbb{R}^{d \times n_s} = \mathbb{R}^{(n_s - \text{rank}(\Gamma)) \times n_s}$  and its rows compose a basis of  $\ker(\Gamma^T)$ . Observe that if  $d = n_s - \text{rank}(\Gamma) = 0$ , then  $W$  is zero dimensional. We will show  $P_c$  is equivalent to Definition 24 on page 23.

**Proposition 49.** *Given a species differential system  $\dot{S} = \Gamma R(S)$  for a chemical reaction network with stoichiometric matrix  $\Gamma$  and species vector  $S$ , the associated stoichiometric compatibility class*

$$C_{S_0} = \mathbb{R}_{\geq 0}^{n_s} \cap (S_0 + \text{im}(\Gamma))$$

where  $n_s$  is the number of species, and  $S_0 \in \mathbb{R}^{n_s}$  is equivalent to

$$P_c = \{S \in \mathbb{R}_{\geq 0}^{n_s} : WS = c\}$$

where  $W$  is a full rank  $d = n_s - \text{rank}(\Gamma)$  matrix whose rows form a basis of  $\ker(\Gamma^T)$ .

*Proof.* Given  $\dot{S} = \Gamma R(S)$ , then observe that

$$W\dot{S} = W\Gamma R(S) = 0.$$

Integrating both sides of this equation yields  $WS(t) = c$  for some constant vector  $c$ . Given a solution to  $\dot{S} = \Gamma R(S)$

$$S(t) = S_0 + \Gamma \int_0^t R(S(\tau)) d\tau,$$

recall that  $\int_0^t R(S(\tau)) d\tau$  is the extent of the reaction  $x(t)$ . Then,  $\Gamma x(t) \in \text{im}(\Gamma)$ . Taking  $W$  on both sides yields

$$WS(t) = W(S_0 + \text{im}(\Gamma)) = c$$

for some constant vector  $c$ . Thus,  $C_{S_0} \subseteq P_c$ .

Conversely, take  $S_0$  satisfying  $WS_0 = c$ , which is a linear system that has (generally) an infinite number of solutions given by  $WS = c$ , such that

$$S = S_0 + v$$

for  $v \in \ker(W)$ . By duality,  $\ker(W) = \text{im}(\Gamma)$ , so  $v \in \text{im}(\Gamma)$ . Thus, any solution of  $WS = c$  is given by  $S = S_0 + v$ , in which the vector  $v$  is in the stoichiometric subspace.

Hence,  $P_c \subseteq C_{S_0}$ . □

Recall that the stoichiometric subspace is the span of reaction-space. The dimension of the state space for any chemical reaction network is  $n_s$ , the number of species, and recall any vector that satisfies  $w^T \Gamma = 0$  will yield a conservation law. Since  $W$  has rank  $d = n_s - \text{rank}(\Gamma)$  whose rows form a basis for  $\ker(\Gamma^T)$ , the Rank-Nullity Theorem implies

$$\dim(\text{Stoichiometric Subspace}) = \dim(\text{State space}) - \# \text{ of Conservation Laws}$$

or

$$\ker(\Gamma^T) + \text{rank}(\Gamma) = n_s.$$

The difference in representation of the stoichiometric compatibility classes is just a matter of representing the set of points in implicit or explicit form. Observe that  $WS = c$  is in implicit form whereas  $P_c$  is a simplex containing vectors related to  $\ker(\Gamma^T)$ . We could also represent  $P_c$  by taking an arbitrary point on  $WS$  and then parametrizing in  $d$

directions; we could explicitly write  $S = S_0 + im(\Gamma)$ , then  $C_{S_0}$  is a subspace containing vectors related to  $rank(\Gamma)$ .

If we take  $P_c \cap \mathbb{R}_{>0}^{n_s}$ , we define the *positive stoichiometric compatibility class* as

$$P_c^+ := \{S \in \mathbb{R}_{>0}^{n_s} : WS = c\}.$$

Since the stoichiometric compatibility class is convex and forward invariant, then so is  $P_c^+$ , especially since  $WS$  is conserved over time and determined by an initial condition in  $\mathbb{R}^{n_s}$ . Also,  $WS = c$  for a system of conservation equations.

**Definition 50.** A chemical reaction network is *conservative* if there exists a conservation relation with only positive coefficients. So given a stoichiometric matrix  $\Gamma$ , there exist a vector  $w \in \ker(\Gamma)$  with positive entries such that  $w^T \Gamma = 0$ .

A necessary and sufficient condition to have a conservation law is to have support on all species. If a network is conservative, then there is a strictly positive vector in the left kernel of  $\Gamma$ .

**Definition 51** ([3]). Consider  $\dot{S} = \Gamma R(S)$  for some reaction network with an associated stoichiometric compatibility class  $P_c$ . Then, this CRN is *dissipative* if for all  $S_0 \in \mathbb{R}_{>0}^{n_s}$ , there is a constant  $T > 0$  and a compact set  $K_c$ , such that  $S(t) \in K_c \subseteq P_c$  for all  $t \geq T$ .

A dissipative reaction network is a chemical reaction network in which all trajectories of all stoichiometric compatibility classes  $P_c$  eventually reach and stay in a compact set – even if they were originally outside of the compact set. There is no necessary and sufficient condition for dissipativity. However, we have a sufficient condition.

**Proposition 52.** *If a chemical reaction network is conservative, then it is dissipative.*

*Proof.* Seeking a contradiction, suppose a conservative reaction network has at least one trajectory  $S(t)$  in Euclidean space that does not eventually reach a compact set. By definition, if this chemical reaction network system with associated species dynamical system

$$\dot{S}(t) = \Gamma R(S(t))$$

obeys a conservation law, then  $\ker(\Gamma^T) \neq \emptyset$ . So there exists a vector  $w \in \ker(\Gamma)$  with positive entries such that  $w^T \Gamma = 0$ . Then, taking  $w^T$  on both sides implies

$$w^T \dot{S}(t) = w^T \Gamma R(S(t)) = 0 \cdot R(S(t)) = 0.$$

Thus,  $w^T \dot{S}(t) = 0$ , which implies  $w^T S(t)$  is a constant for all  $t$  since integrating both sides yields  $w^T S(t) = w^T S_0$  for some  $S_0 = S(0)$ . This is a contradiction since  $S(t) \rightarrow \infty$  implies  $w^T S(t) \rightarrow \infty$ . Hence, the network is dissipative.  $\square$

## 6.2 Siphons and Equilibria

We have a sufficient condition to determine the absence of boundary equilibria in a CRN. If there is no boundary equilibria, then the reaction network is persistent [28]. In other words, all trajectories of the limit cycle converge in the interior, which is the same as saying the trajectories of the stoichiometric compatibility class converge in a compact set. There is no necessary and sufficient condition to characterize persistence in the presence of bounded equilibria; however, we can characterize persistence via the siphons of a reaction network.

**Definition 53** ([1], [3]). A *siphon* is a subset of species  $Z \subseteq \{S_i\}_1^n$  with the closure property: if  $S_i \in Z$  and some reaction  $R_j$  produces  $S_i$  such that  $R_j \rightarrow S_i$ , then there exists a species  $S_k \in Z$  such that  $S_k \rightarrow R_j \rightarrow S_i$ . A siphon is *minimal* if it does not properly contain any other siphon.

Notice that  $Z_i \not\subseteq Z_j$  and vice versa is not enough to guarantee the two siphons are minimal. For instance, there are some reaction networks such that  $\{X_1, X_2\}$  and  $\{X_3, X_4\}$  are siphons for arbitrary species, but  $\{X_1, X_2, X_3\}$  and  $\{X_2, X_3, X_4\}$  can also be siphons. We have that neither of these sets is contained in the other, but neither is a minimal siphon either. A siphon  $Z$  is minimal if  $Z$  is no longer a siphon after removing any single species. If one can compute the minimal siphons and the conservation laws of a CRN, then a result from reference [1, Theorem 2] states if the reaction network is conservative and each siphon contains the support of any row vector  $w > 0$  such that  $w^T \Gamma = 0$ , then the reaction network is persistent. The paper [3] reworded this result in the context of chemical reaction networks. But first, we need to define a few sets. Let

$$V = \{S \in \mathbb{R}_{\geq 0}^{n_s} : \Gamma R(S) = 0\}$$

be the set of non-negative equilibria. The set we are most interested in is the set of points that simultaneously solve the equations  $\Gamma R(S) = 0, WS = c$ , or the set of positive equilibria

$$V \cap P_c^+ = \{S \in \mathbb{R}_{> 0}^{n_s} : \Gamma R(S) = 0, WS = c\}.$$

In general, these points may not be isolated, but they are the stoichiometrically relevant equilibria. In other words, these equilibria are the ones that satisfy both the equilibrium condition and satisfy the relevant conservation laws. Finally, we introduce the set of boundary equilibria for some  $c \in \mathbb{R}^d$  as

$$\partial(V \cap P_c^+) := (V \cap P_c) - (V \cap P_c^+).$$

**Proposition 54** ([3, Proposition 2]). *If every minimal siphon has a subset*

*$\{S_{i_1}, \dots, S_{i_k}\} \subseteq Z$  and a conservation law  $\sum_{j=1}^k \lambda_j S_{i_j} = c$  for some positive  $\{\lambda_j\}_1^k$ , then the reaction network of  $Z$  has no boundary equilibria in any  $P_c$  with  $P_c^+ \neq \emptyset$ .*

In other words, if each minimal siphon contains the support of a positive conservation law, then there are no boundary equilibria in any  $P_c$  with  $P_c^+ \neq \emptyset$ . Multiple equilibria occurs if there is a vector  $c \in \mathbb{R}^d$  such that  $|V \cap P_c^+| \geq 2$ .

### 6.3 Reduction System of Equations

There are exactly as many independent linear relations as rows of  $W$  by construction, which means the number of linearly independent conservation laws is the same as the number of equations we can eliminate.

$W\Gamma = 0$  since the rank of  $W$  corresponds to the size of the left kernel of  $\Gamma$ . For example, in a  $10 \times 10$  system with four conservation laws, we have  $6 \times 10 + 4 \times 10$  in which the conservation laws are there to set full rank.

Define a restriction to non-redundant equations

$$\varphi_c(S)_i = \begin{cases} \Gamma R_i(S) & i \notin \{i_1, \dots, i_d\} \\ (WS - c)_i & i \in \{i_1, \dots, i_d\} \end{cases}$$

where  $d = n_s - \text{rank}(\Gamma)$  and  $\{i_1, \dots, i_d\}$  is the indexed set of conservation laws. These are the rows that have been removed from the system by the linear relationships from  $WS$ . If we rearrange indices across  $S$ , coordinates of  $f$ , and rows of  $W$ , then we can have  $\{i_1, \dots, i_d\} = \{1, \dots, d\}$  without loss of generality [3]. Now, we define the set of equilibria as

$$V \cap P_c^+ := \{S \in \mathbb{R}_{\geq 0}^n : \Gamma R(S) = 0, WS = c\} = \{S \in \mathbb{R}_{\geq 0}^n : \varphi_c = 0\}.$$

This set is the collection of species vectors that satisfy the linearly independent set of the left kernel. In the following example, we will demonstrate how to construct the system of non-redundant equations.



**Example 55.** Let

$$\Gamma = \begin{bmatrix} -1 & 0 & 1 \\ 1 & -1 & 0 \\ 0 & 1 & -1 \end{bmatrix}$$

from the simple reaction in Example 23 on page 22 and

$$W = [ 1 \quad 1 \quad 1 ]$$

which satisfies  $W\Gamma = 0$ , then

$$WS = X_1 + X_2 + X_3 = c \in \mathbb{R}^1.$$

Observe that

$$\Gamma R(S) = \begin{bmatrix} -X_1k_1 + X_3k_3 \\ X_1k_1 - X_2k_2 \\ X_2k_2 - X_3k_3 \end{bmatrix}.$$

Now,  $WS - c$  implies  $X_1 + X_2 + X_3 - c = 0$ , so we can eliminate either equation of the linear dependency between rows 1, 2, or 3 of  $\Gamma R(S)$  because of the relationship

$$\Gamma R_3(S) = -\Gamma R_1(S) - \Gamma R_2(S).$$

Also, the relationship  $X_1 + X_2 + X_3 = c$  implies  $X_3$  is dependent on  $X_1, X_2$  and vice versa, so we only need one of these three equations to determine the value of the other. Thus, if we set

$$X_1 + X_2 + X_3 - c = (WS - c)_3,$$

then

$$\varphi_c = \begin{bmatrix} -X_1k_1 + X_3k_3 \\ X_1k_1 - X_2k_2 \\ (WS - c)_3 \end{bmatrix} = \begin{bmatrix} -X_1k_1 + X_3k_3 \\ X_1k_1 - X_2k_2 \\ X_1 + X_2 + X_3 - c \end{bmatrix}.$$

This example highlights that the construction of  $\varphi_c$  does not state the equation which substitutes the old one is equivalent, i.e.,  $\Gamma R_3(S) \neq (WS - c)_3$ . They are not equivalent because original system is not linearly independent (since all three species linearly

depend on one another by the conservation law), so that the determinant of the Jacobian would be zero. In fact, replacing either of the dependent equations by the conservation law itself guarantees linear independence and therefore we have a nonzero determinant for the Jacobian matrix  $D\varphi_c(S)$ .

**Definition 56** ([3]). A chemical reaction network is *multi-stationary* if there are two or more positive solutions in each stoichiometric compatibility class  $P_c$  for some  $c \in \mathbb{R}^d$ . In other words, multiple equilibria occurs if  $\varphi_c = 0$ .

We define the Jacobian matrix of the reduction system as

$$M(S(t)) \in \mathbb{R}^{n_s \times n_s} := D\varphi_c(S(t)).$$

This Jacobian matrix does not depend on  $c$  because  $c$  is a constant and we are taking partial derivatives with respect to  $t$ . We note that this definition holds even if the original system is parametrized.

**Definition 57.** An equilibrium  $S^* \in V \cap P_c$  is *non-degenerate* if  $\det(M(S^*)) \neq 0$ , i.e.,  $M(S)$  is non-singular at  $S^*$ .

#### 6.4 Theorem for Establishing Multi-Stationarity

**Theorem 58** (Unique and multiple equilibria [3, Theorem 1]). *Let  $S^* \in V \cap P_c$  denote an equilibrium of a chemical reaction network.*

*Assume:*

*(i) Reactions of a dissipative chemical reaction network satisfy A1.*

*(ii) All equilibria belong to  $V \cap P_c^+$  for  $P_c^+ \neq \emptyset$  and for  $c \in \mathbb{R}^d$  (There are no boundary equilibria in  $P_c$ ).*

*Then, one exactly of the three holds:*

*(A) Unique equilibria: if  $\text{sign}(\det(M(S^*))) = (-1)^s$  for all positive equilibria in  $V \cap P_c^+$ , then there is exactly one  $S^* \in P_c$  and it is non-degenerate.*

(B) *Multiple equilibria: if  $\text{sign}(\det(M(S^*))) = (-1)^{s+1}$  for some positive equilibria  $S \in V \cap P_c^+$ , then there are at least two  $S^* \in P_c$  such that at least one is non-degenerate. (if all are non-degenerate, then there are always an odd number at least 3).*

*If  $\text{sign}(\det(M(S^*))) = 0$  for some  $x$ , but never  $(-1)^{s+1}$ , then the result is inconclusive.*

**Corollary 59** (Unique equilibria [3, Corollary 1]). *If  $\text{sign}(\det(M(S^*))) = (-1)^s$  for all  $S \in \mathbb{R}_{\geq 0}^{n_s}$ , then there is one non-degenerate  $S^*$  in each  $P_c$ .*

A chemical reaction network that has a constant  $\text{sign}(\det(M(S^*)))$  is also called an *injective network* [3]. Non-degenerative equilibria are always isolated from each other in a given  $P_c$  since  $\det(M(S^*)) \neq 0$  implies  $M(S^*)$  is locally invertible by the Inverse Function Theorem.

## 6.5 Steps of Procedure for the Algorithm

The original 7 steps of the algorithm to establish multi-stationarity are listed in the paper [3] are elaborated in the following steps.

Given a chemical reaction network with stoichiometric matrix  $\Gamma$  and rate function  $R$  equipped with kinetics (e.g mass-action, Michaelis-Menten, or Hill):

- 1) Calculate the matrix  $W$  spanned by the left kernel of  $\Gamma$ .
- 2) Check if the reaction network is dissipative using Proposition 52 on page 59.
  - a) If it is not conservative, check Proposition 60 on the next page from reference [3].
- 3) Check for the absence of bounded equilibria using Proposition 54 on page 60.
  - a) If it is not persistent, for each  $i$ , assume  $S_i = 0$  and get a contradiction about the conservation laws.
- 4) Construct  $\varphi_c, M(S)$ , and  $\det(M(S))$ .
- 5) Determine if the sign of  $\det(M(S))$  is a constant.

- a) If it is, then conclude the reaction network establishes mono-stationarity from Corollary 59 on the previous page.
  - b) If not, continue to step 6.
- 6) Compute a positive parametrization of  $M(S)$  from a subset of species, and re-perform steps 4 and 5.
- a) If step 5 fails again, compute the isolated numerator of  $\det(M(S))$ .
  - b) If this is computationally expensive, we can restrict to a subset of the parameter space by fixing certain reaction rate constants to numerical values.
- 7) Compute the convex hull from the exponents of terms from the isolated numerator, which has support in a Newton polytope.
- 8) Match the convex hull with terms from the isolated numerator, and determine if their signs are constant.
- a) If it is, then conclude the reaction network establishes mono-stationarity from Corollary 59 on the preceding page.
  - b) If at least two terms have mixed signs, then conclude the reaction network establishes multi-stationarity from Theorem 58 on page 63.

**Proposition 60** ([3, Proposition 1]). *Let  $(\mathbb{R}^n, \|\cdot\|)$  be a normed vector space. Assume  $P_c^+ \neq \emptyset$ , there exists  $w_c \in \mathbb{R}_{\geq 0}^n$ , and there exists an  $N > 0$ . If  $w_c \Gamma R(S) < 0$  for all  $S \in P_c$  satisfies  $\|S\| > N$  for each  $c$ , then the associated chemical reaction network for  $\Gamma R(S)$  is dissipative.*

*In other words, one needs to look for  $w_c$  with all positive coordinates such that  $w_c \Gamma R(S) < 0$  for large values.*

Determining the sign of  $\det(M(S))$  will be the most difficult step. If the reaction is equipped with mass-action, Michaelis-Menten, or Hill kinetics, then  $\det(M(S))$  will be a rational function in  $S$  with a strictly positive denominator, which means we only have to

consider the numerator. Indeed, the set of reaction rate functions is

$$R_j \in \mathbb{Q}^+[S] = \left\{ \frac{p_j(S)}{q_j(S)} : p_j(S) \geq 0, q_j(S) > 0, \forall S \in \mathbb{R}_{>0}^n \right\}$$

for  $j = 1, \dots, l$ . Then,

$$\det(M(S)) = \frac{p(S)}{q(S)}$$

in which the definition of  $M(S)$  and differentiation of  $R_j$  for  $j = 1, \dots, l$  yields

$$q(S) = \prod_{j=1}^l q_j(S)^2 > 0.$$

by the quotient rule.

## 6.6 Performing the Algorithm in Maple (2018 version) and Python 2.2+

We believe it is best to give an explicit sequence of steps to follow, especially if the reader is not familiar with the Maple software.

- 1) Define a stoichiometric matrix  $A$  from the chemical reaction network that is the same as  $\Gamma$  using  $:=$ .
- 2) Define a symbolic vector  $R$  from the chemical reaction network that is the same as  $R(S)$ .
- 3) Define  $M := A.R$ , in which  $(\cdot)$  is matrix multiplication.
- 4) Solve the system of equations using  $solve(\{M(i) = 0\}_{i=1}^n)$ , where  $n$  is the number of rows in  $M$ , and  $M(i)$  corresponds to each element in  $V_S$ .
- 5) Simplify with respect to each conservation law using  $simplify(\sum M(i))$ , where  $\sum M(i)$  is the conservation law and that corresponds to  $\dot{S}_i = \Gamma R(S_i)$  for each  $i$ .
  - a) Note: This step is completely optional, and simply serves as a check for the user that the conservation laws have been calculated correctly.
- 6) Define each conservation law explicitly as  $g_j := \sum S_i$  where  $\sum S_i$  is a conservation law.

- 7) Import the linear algebra and vector calculus commands using *with(LinearAlgebra)* and *with(VectorCalculus)*.
- 8) Define a Jacobian matrix  $J := \text{Jacobian}([\{g_j\}, \{M(i)\}_{i=1}^n], [V_S])$  with each entry separated by a comma.
  - a) Note: If  $J$  is not square, then that means  $M$  has two or more linearly dependent rows. If  $M(k)$  and  $M(r)$  are linearly dependent, remove either one from  $\{M(i)\}_{i=1}^n$ .
- 9) Calculate the determinant of  $J$  using  $\text{det}J := \text{Determinant}(J)$ .
  - a) If symbolic exceeds computation time, restrict to a subset of the parameter space by fixing reaction coefficients  $k_j$  to (mostly) numerical values by defining a list of substitutions  $SUBS := \{k_j = 1\}_{j=1}^m$  where  $m$  is the total number of reactions, then re-defining  $M := \text{subs}(SUBS, M)$  using the substitution command.

If one cannot immediately determine the sign of the determinant, then they must determine the extreme vertices of the Newton polytope and continue the algorithm from reference [3, Step 7, page 12].

- (10) Get the list of vertices from the species powers in the determinant of the Jacobian using the following commands (in order):<sup>2</sup>

```
Exponents := proc(p, x :: list) local L, S, i, j;
    for i to nops(p)
        do
            L := [op(L), [seq(degree(op(i), p), j), j in x]]
        end do;
    L := subsop(1=NULL, L)
end proc;
```

2. Courtesy of Nida Obatake at Texas A&M University

```

FindTerm := proc(p,v::list,x::list) local i,j;
    for i to nops(p)
        do
            if [seq(degree(op(i,p),j),j in x) = v
                then print(op(i,p),j)
            end if
        end do
    end proc;

```

- (11) Import the Polyhedral commands using *with(PolyhedralSets)*.
- (12) Define the isolated numerator polynomial with support in the Newton polytope using
 
$$J2 := \text{expand}(\text{simplify}(\det J * \text{denom}(\det J))).$$
- (13) Define a new list  $J3$ , which is the list of exponents of the terms of  $\det J$  using
 
$$J3 := \text{Exponents}(J2, [V_S]).$$
- (14) Remove duplicates from this calculated list by defining another list  $J4$  using
 
$$J4 := \text{ListTools} : -\text{MakeUnique}(J3).$$

We think it is important to note that Maple (or even Mathematica) is computationally slow at calculating the convex hull of set of points in dimensions greater than three even with i7 (4th gen) laptop processors, which is why we included this next step for Python 2.2+ using the `scipy.spatial.ConvexHull` function. This script runs reasonably fast on even a comparably slower i3 (2nd gen) desktop processor.

- (15) Copy this list  $J4$  as points into the Python 2.2 code to get the list of extreme points (the convex hull) using SciPy's `ConvexHull` class function.<sup>3</sup>

3. Courtesy of Matthew D. Johnston at San José State University

- a) If this produces an error stemming from the high dimension of the points, the set of extreme points might be made simpler by using a parametrization. This can be calculated in Maple in Step 16.
- b) If this does not produce an error, proceed to Step 21.

```

from scipy.spatial import ConvexHull
import numpy as np

points = J4
hull = ConvexHull(points)
print hull.vertices
vertices = []
for i in hull.vertices:
    vertices.append(points[i])
print vertices

```

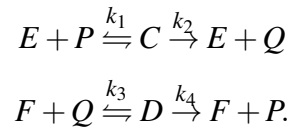
- (16) Define a positive parametrization  $P$  by re-performing Step 4 and excluding a subset of the species set  $V_Z \subseteq V_S$  with  $solve(\{M(i) = 0\}_{i=1}^n, \{V_S - V_Z\})$ .
  - a) Note: All the signs of this parametrization must be positive
  - b) We advise (first) choosing  $V_Z = Z$ , a siphon who has support in a conservation law  $g_i$ , but this is not always guaranteed to produce a positive parametrization.
- (17) Re-define the Jacobian matrix  $J$  from Step 8 by substituting the parametrization  $P$  into  $J$  using  $J := subs(P, Jacobian([\{g_j\}, \{M(i)\}_{i=1}^n], [V_S]))$
- (18) Re-perform Steps 9-12.
- (19) Re-define the list of exponents  $J3$  of the terms of  $detJ$  with coefficients in the siphon  $Z$  from Step 16 using  $J3 := Exponents(J2, [Z])$
- (20) Re-perform Steps 14 and 15



- (21) Define a new list  $J5$  in Maple using the calculated vertices from Step 15.
- (22) Generate the list of terms in the numerator of the determinant considered  $J3$ , which corresponds to extreme vertices in  $J5$  using **for  $i$  to  $\text{numelems}(J5)$  do**  
*FindTerm*( $J2, J5[i], [Z]$ ) **end do**.

## 6.7 Establishing Mono-Stationarity in the Enzymatic Futile Cycle

We finish off this chapter with an implementation of the algorithm for Example 18 on page 13, shown here for the reader's convenience assuming mass-action kinetics,



Since we have conservation laws from Section 4.4 on page 28, then the reaction network is dissipative by Proposition 52 on page 59. We will now determine the existence of boundary equilibria by determining the minimal siphons of this reaction. If we work backwards from the reactions:

- To produce  $Q$ , we need  $C$ . To produce  $C$ , we need  $P$  or  $E$ . If we choose  $P$ , then we need to produce it from  $D$ . To produce  $D$ , we also need  $Q$ . Thus,

$$Z_1 = \{Q, C, P, D\}$$

is a siphon.

- To produce  $E$ , we need  $C$ . To produce  $C$ , we need  $P$  or  $E$ . If we choose  $E$ , then

$$Z_2 = \{E, C\}$$

is a siphon.

- Similarly,

$$Z_3 = \{F, D\}$$

is also a siphon.

Observe that these siphons are minimal since they are not contained in each other and removing any single species from any  $Z_i$  would no longer make them a siphon. Also, since  $\text{supp}(Z_i)$  are in conservation laws

$$E + C = c_1$$

$$F + D = c_2$$

$$P + Q + C + D = c_3$$

for all  $i$  and for some constants  $c_1, c_2, c_3 \in \mathbb{R}$ , then the reaction network is persistent and by Proposition 54 on page 60, Example 18 on page 13 has no boundary equilibria.

Next, we construct  $\Gamma R(S)$  with  $k_1, k_{-1}, k_2, k_3, \dots$  relabeled as  $k_1, k_2, k_3, \dots, k_6$

$$\Gamma R(S) = \begin{bmatrix} -k_1EP + k_2C + k_6D \\ k_3C - k_4FQ + k_5D \\ -k_1EP + k_2C + k_3C \\ -k_4FQ + k_5D + k_6D \\ k_1EP - k_2C - k_3C \\ k_4FQ - k_5D - k_6D \end{bmatrix}.$$

Observe that we have the following equations,

$$\Gamma R_1(S) = -\Gamma R_2(S) - \Gamma R_5(S) - \Gamma R_6(S)$$

$$\Gamma R_3(S) = -\Gamma R_5(S)$$

$$\Gamma R_4(S) = -\Gamma R_6(S).$$

Thus, we can replace exactly two rows of  $\Gamma R(S)$  with exactly two conservation laws.

$$\varphi_c(S) = \begin{bmatrix} P + Q + C + D - c_3 \\ k_3C - k_4FQ + k_5D \\ E + C - c_1 \\ -k_4FQ + k_5D + k_6D \\ k_1EP - k_2C - k_3C \\ F + D - c_2 \end{bmatrix}.$$

Next, we need to calculate  $\det(M(S))$  and check the signs of its terms. If we calculate  $M(S)$  and reorder the rows in which the first ones correspond to our conservation laws, then

$$M(S) = \begin{bmatrix} 1 & 1 & 0 & 0 & 1 & 1 \\ 0 & 0 & 1 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 & 0 & 1 \\ 0 & -k_4F & 0 & -Qk_4 & k_3 & k_5 \\ 0 & -k_4F & 0 & -Qk_4 & 0 & k_5 + k_6 \\ k_1E & 0 & Pk_1 & 0 & -k_2 - k_3 & 0 \end{bmatrix}.$$

This Jacobian matrix has determinant

$$-EFk_1k_4(k_3 + k_6) - EQk_1k_3k_4 - FPk_1k_4k_6 - Ek_1k_3(k_5 + k_6) - Fk_4k_6(k_2 + k_3)$$

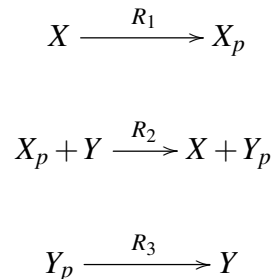
Finally, since all terms of the determinant have the same sign, we can conclude this reaction network establishes mono-stationarity by Corollary 59 on page 64.

## 7 MAIN EXAMPLES

In this chapter, we will systematically check each chemical reaction network for equilibria via the Deficiency Theorems, the positive-loop property, and test for multi-stationarity. We will construct the special graphs needed to check the positive loop property, assume mass-action coordinates and construct the species dynamical system, and then calculate  $\ker(\Gamma^T)$  to determine if there are conservation laws. When testing for multi-stationarity, we will test for dissipativity (from the existence of conservation laws), existence of boundary equilibria, find a positive parametrization (if necessary) using Maple, and determine the sign of  $\det(M(x))$ . In our pseudo-code from Section 6.6 on page 66, we set  $V_S(i) = M(i)$  for each  $i$ .

### 7.1 Example 1: Two-Component Signaling System (Histidine Kinase)

Consider the following reaction network found in reference [3]:



with the R-graph, DSR-graph, and S-graph presented in Figure 7.1 in which

$$V_S = \{X, X_p, Y, Y_p\} \text{ and } V_R = \{R_i\}_{i=1}^3.$$

#### 7.1.1 Positive Loop Property

This reaction is simple enough that we can easily inspect the positive loop property. Observe that both the DSR-graph and the R-graph have the positive loop property by definition since there are an even number of edges in all cycles, but the S graph does not. This does not affect the monotonicity of the reaction. In fact, the authors of reference [5] make a remark of this by saying, “analysis in species-coordinates does not allow [us] to

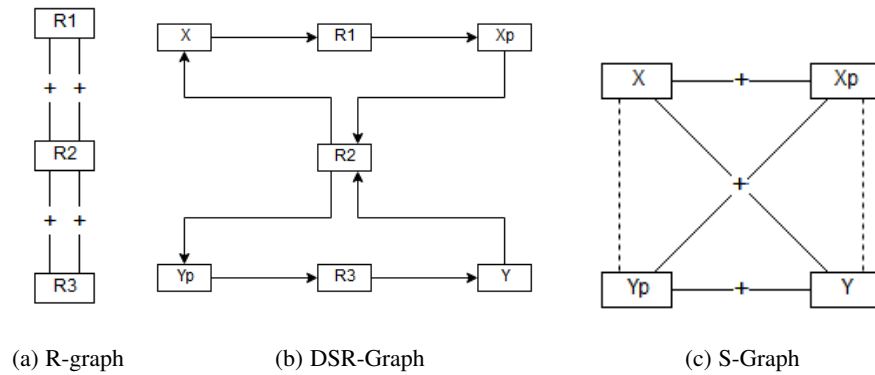


Fig. 7.1: Special graphs of the XY reaction.

derive similar conclusions,” which means we must use the dynamics of reaction-coordinates in order to substantiate any characteristics that verify global convergence.

Next, we need to verify the DSR-graph is R-strongly connected, which means we can travel from one reaction vertex  $R_i$  to any reaction vertex  $R_j$ . Indeed we can, since

$R_1$  can get to  $R_2$  and  $R_3$  via path

$$(R_1, X_p, R_2, Y_p, R_3)$$

$R_2$  can get to  $R_1$  and  $R_3$  via paths

$$(R_2, X, R_1), (R_2, Y_p, R_3)$$

$R_3$  can get to  $R_1$  and  $R_2$  via path

$$(R_3, Y, R_2, X, R_1).$$

We construct the species dynamical system equipped with mass-action kinetics

$$\begin{bmatrix} \dot{X} \\ \dot{X}_p \\ \dot{Y} \\ \dot{Y}_p \end{bmatrix} = \begin{bmatrix} -1 & 1 & 0 \\ 1 & -1 & 0 \\ 0 & -1 & 1 \\ 0 & 1 & -1 \end{bmatrix} \cdot \begin{bmatrix} k_1 X \\ k_2 X_p Y \\ k_3 Y_p \end{bmatrix} = \begin{bmatrix} -k_1 X + k_2 X_p Y \\ k_1 X - k_2 X_p Y \\ -k_2 X_p Y + k_3 Y_p \\ k_2 X_p Y - k_3 Y_p \end{bmatrix}.$$

The matrix  $\Gamma$  has a column-space with rank 2. By the rank-nullity theorem, the dimension of  $\ker(\Gamma)$  is 1. We also have the following solutions to  $w^T \Gamma = 0$  using the MATLAB command `null(A, 'r')`,

$$w \in \text{span} \left\{ \begin{bmatrix} 1 \\ 1 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} 0 \\ 0 \\ 1 \\ 1 \end{bmatrix} \right\},$$

which yields the following conservation laws

$$X + X_p = c_1$$

$$Y + Y_p = c_2$$

for some constants  $c_1, c_2$ . These conservation laws canonically define the following simplex (in species-coordinates):

$$\Sigma = \{ \sigma \in [X \ X_p \ Y \ Y_p] \subset \mathbb{R}_{\geq 0}^4 : X + X_p > 0, Y + Y_p > 0 \}.$$

By Corollary 46 on page 50 from reference [5, Corollary 1], the reaction-coordinates system is monotone on

$$X_0 = \{ x \in \mathbb{R}^{n_r} = \mathbb{R}^3 : S_0 + \Gamma x \geq 0 \}$$

with respect to the order induced by an orthant cone, and strongly monotone on

$$\text{int}(X_0) = \{ x \in \mathbb{R}_{\geq 0}^{n_r} = \mathbb{R}^3 : S_0 + \Gamma x \gg 0 \}$$

with respect to the same order. The positive orthant cone that preserves this order (in reaction-coordinates) is  $K = \mathbb{R}_{\geq 0}^{n_r} = \mathbb{R}_{\geq 0}^3$ . The paper [3] showed this reaction network satisfies

$$\omega(x_0) \cap \partial \mathbb{R}_{\geq 0}^4 = \emptyset$$

to conclude it is persistent. We will demonstrate why in the next subsection when we test this for multi-stationarity by calculating the minimal siphons. Next, we will determine which of the following two situations are satisfied in order to use Theorem 48 on page 50 from reference [5, Theorem 2],

$$\ker(\Gamma) \cap \text{int}(\mathbb{R}_{\geq 0}^3) \neq \emptyset$$

or

$$\ker(\Gamma) \cap \mathbb{R}_{\geq 0}^3 = \{0\}$$

where  $\ker(\Gamma)$  is the right kernel of  $\Gamma$ . Using MATLAB again, we see  $\ker(\Gamma)$  is spanned by a single vector  $[1, 1, 1]'$ . Thus, we are in the situation  $\ker(\Gamma) \cap \text{int}(\mathbb{R}_{\geq 0}^3) \neq \emptyset$ . Hence, by Theorem 48 on page 50 from reference [5, Theorem 2], all solutions of the species system in  $\text{int}(\mathbb{R}_{\geq 0}^{n_s}) = \text{int}(\mathbb{R}_{\geq 0}^4)$  converges to a unique equilibrium within each stoichiometric compatibility class

$$C_{S_0} = \mathbb{R}^4 \cap (S_0 + \text{im}(\Gamma)).$$

### 7.1.2 Testing for Multi-Stationarity

Although testing for multi-stationarity is not necessary for this example, it is enlightening to see how the algorithm works for small reactions. Assuming the reaction has mass-action kinetics, the reaction is dissipative because  $\ker(\Gamma^T) \cap \mathbb{R}_{> 0}^{n_r} \neq \emptyset$  (i.e., we need a strictly positive vector to show there is a conservation law).

To determine the existence of boundary equilibria, let us calculate the minimum siphons of this reaction, recalling that siphons are sets of species that are necessary for the reaction network to occur. In order to produce  $X_p$ , we need  $X$ , so  $Z_1 = \{X_p, X\}$  is a siphon. In order to produce  $Y_p$ , we only need  $Y$ , so  $Z_2 = \{Y_p, Y\}$  is another siphon. Observe that both  $Z_1$  and  $Z_2$  are minimum siphons because  $Z_1 \not\subset Z_2$  and vice versa, and also since removing either any species would not make it a siphon. Also observe that  $\text{supp}(Z_1)$  and  $\text{supp}(Z_2)$  is in the conservation laws from above. Thus, by Proposition 54 on page 60 from reference [3], the reaction network has no boundary equilibria in any stoichiometric compatibility class with a non-empty positive interior.

We will perform the steps of the pseudo-code from Section 6.6 on page 66 exactly as described, in which  $M = A \cdot R$  is a  $4 \times 1$  vector. We do not have any reversible reactions, so  $k_i$  are exactly as in the original reaction.

$$M = \begin{bmatrix} X_p Y k_2 - X k_1 \\ -X_p Y k_2 + X k_1 \\ -X_p Y k_2 + Y_p k_3 \\ X_p Y k_2 - Y_p k_3 \end{bmatrix}$$

Observe that  $M(1) = -M(2)$  and  $M(3) = -M(4)$ , which means we can replace either one of these linearly dependent rows with the conservation laws to construct  $\varphi_c(M(S))$ ,

$$\varphi_c = \begin{bmatrix} X_p Y k_2 - X k_1 \\ X + X_p - c_1 \\ -X_p Y k_2 + Y_p k_3 \\ Y + Y_p - c_2 \end{bmatrix}.$$

We only need  $M(2), M(3)$  to construct the Jacobian matrix  $J$  as described in Step 8a with reordered rows:

$$J = \begin{bmatrix} 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & 1 \\ k_1 & -Y k_2 & -X_p k_2 & 0 \\ 0 & -Y k_2 & -X_p k_2 & k_3 \end{bmatrix},$$



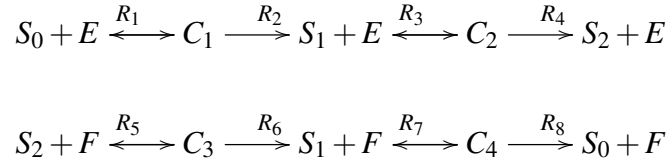
where the first two rows of  $J$  correspond to the conservation laws. Finally,

$$\det J := X_p k_1 k_2 + Y k_2 k_3 + k_1 k_3$$

where all terms have the same sign. Hence, by Corollary 59 on page 64 from reference [3], there is exactly one unique and globally attracting equilibria in each stoichiometric compatibility class.

## 7.2 Example 2: Double Phosphorylation Cycle

Consider the reaction network, which is an extension of the enzymatic futile cycle:



with the SR-graph, R-graph, and S-graph presented in Figure 7.2 in which

$$V_S = \{S_0, S_1, S_2, C_1, C_2, C_3, C_4, E, F\}$$

and  $V_R = \{R_i\}_{i=1}^8$ .

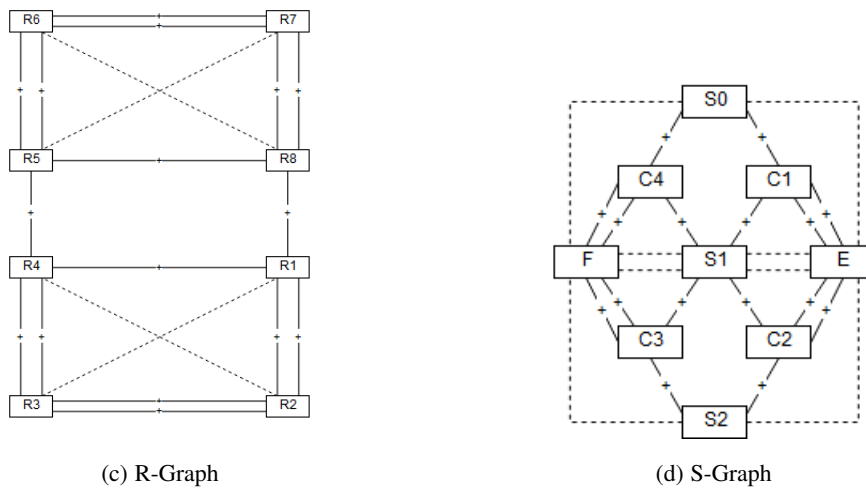
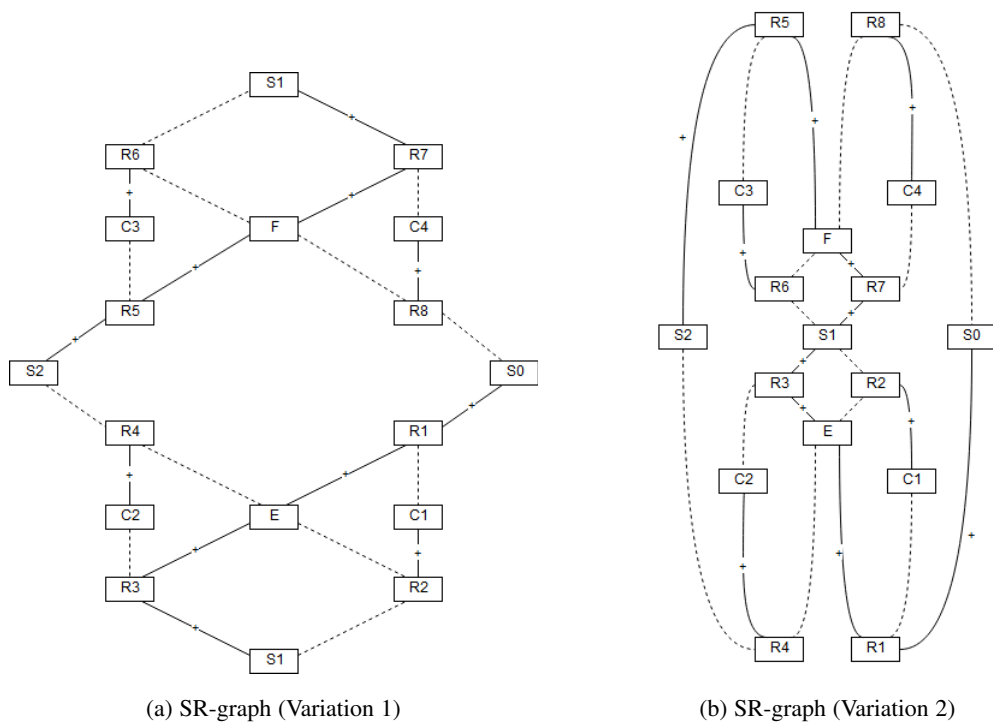


Fig. 7.2: Special graphs of the Double Phosphorylation reaction.

### 7.2.1 Positive Loop Property

Next, observe that this reaction network's  $R$ -graph does not satisfy the positive loop property by Proposition 42 on page 47 as  $S_1, E, F \in V_S$  are linked to four vertices in  $V_R$ . Thus, by Theorem 44 on page 49, the reaction-coordinates dynamical system is not monotone with respect to a partial order induced by an orthant cone for any initial condition vector in  $\mathbb{R}$  – independent of the reaction kinetics.

### 7.2.2 Testing for Multi-Stationarity

We will determine if this reaction network establishes multi-stationarity according to the algorithm. Assuming the reaction network has mass-action kinetics, we will test for dissipativity, existence of boundary equilibria, find a positive parametrization using Maple (if necessary), and determine the sign of  $\det(M(x))$ . We can construct the species-coordinates dynamical system equipped with mass-action kinetics,

$$\begin{bmatrix} \dot{S}_0 \\ S_1 \\ S_2 \\ C_1 \\ C_2 \\ C_3 \\ C_4 \\ E \\ F \end{bmatrix} = \begin{bmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 1 & -1 & 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 \\ -1 & 1 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & 1 & -1 & 1 \end{bmatrix} \cdot \begin{bmatrix} k_1 S_0 E - k_{-1} C_1 \\ k_2 C_1 \\ k_3 S_1 E - k_{-3} C_2 \\ k_4 C_2 \\ k_5 S_2 F - k_{-5} C_3 \\ k_6 C_3 \\ k_7 S_1 F - k_{-7} C_4 \\ k_8 C_4 \end{bmatrix},$$

where  $\Gamma$  is a  $9 \times 8$  matrix and  $R(S)$  is an  $8 \times 1$  vector. To test for dissipativity, we check if the reaction network is conservative by solving  $w^T \Gamma = 0$ . One can observe that

$$w \in \text{span} \left\{ \begin{bmatrix} 1 \\ 1 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ -1 \\ -1 \end{bmatrix}, \begin{bmatrix} 0 \\ 0 \\ 0 \\ 1 \\ 1 \\ 0 \\ 0 \\ 1 \\ 0 \end{bmatrix}, \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 1 \\ 0 \\ 1 \end{bmatrix} \right\} = \text{span} \left\{ \begin{bmatrix} 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} 0 \\ 0 \\ 0 \\ 1 \\ 1 \\ 0 \\ 1 \\ 1 \\ 0 \end{bmatrix}, \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 1 \\ 0 \\ 1 \end{bmatrix} \right\}$$

by hand or use MATLAB's `null(A, 'r')` and `colspace(sym())` commands with the proper matrix  $A$  to get rational coefficients. We prefer to choose kernel vectors with all positive entries as we are modeling real world chemical systems. Also, since our left kernel is non-empty, we have a conservation law and our reaction satisfies dissipativity. We have the following conservation laws for some constants  $c_1, c_2, c_3 \in \mathbb{R}$ ,

$$C_1 + C_2 + E = c_1$$

$$C_3 + C_4 + F = c_2$$

$$\sum_{i=0}^2 S_i + \sum_{i=1}^4 C_i = c_3.$$

Next, we check for the existence of boundary equilibria. To do this, we will calculate the minimal siphons of the reaction and check if they have support in one of the conservation laws. Let us work backwards in each reaction, seeing what minimal reactants are necessary to produce species.

- To produce  $E$  in  $R_4$ , we need  $C_2$ . To produce  $C_2$ , we need  $E$  or  $S_1$  from  $R_3$ . If we choose  $E$ , then we need  $C_1$  to produce it from  $R_2$ , which also requires  $E$  from  $R_1$ . Thus,  $Z_1 = \{E, C_1, C_2\}$  is a siphon.
- If we work backwards from reactions  $R_8$  to  $R_5$  in the same way as we did before, then  $Z_2 = \{F, C_3, C_4\}$  is another siphon for this reaction.
- To produce  $S_2$  in  $R_4$ , we need  $C_2$ . To produce  $C_2$ , we choose  $S_1$ . To produce  $S_1$  in  $R_1$ , we need  $S_0$ . Since some of these species are present in other reactions, we need to continue. To produce  $S_0$  in  $R_8$ , we need  $C_4$ . To produce  $C_4$ , we choose  $S_1$ . To produce  $S_1$  in  $R_5$ , we need  $S_2$ . We observe that  $Z_3 = \{S_0, S_1, S_2, C_1, C_2, C_3, C_4\}$  is a siphon.

Observe that all of these siphons are minimal by definition because  $Z_i \not\subseteq Z_j$  for any  $i \neq j \in \{1, 2, 3\}$ , and removing any single species from the set(s) would not longer make it a siphon. Also observe that  $\text{supp}(Z_i)$  is in the conservation laws from above for all

$i = 1, 2, 3$ . Thus, by Proposition 54 on page 60 from reference [3], the reaction network has no boundary equilibria in any stoichiometric compatibility class with a non-empty positive interior.

Using the symbolic software Maple, we proceed with the steps from the pseudo-code from Section 6.6 on page 66. Since this CRN has 12 (including reversible) reactions, we will relabel

$$k_1, k_{-1}, k_2, k_3, \dots \text{ as } k_1, k_2, \dots, k_{12}$$

in Maple. Calculating  $M = A \cdot R$  yields

$$M = \begin{bmatrix} -ES_0k_1 + C_1k_2 + C_4k_{12} \\ -ES_1k_4 - FS_1k_{10} + C_1k_3 + C_2k_5 + C_3k_9 + C_4k_{11} \\ -FS_2k_7 + C_2k_6 + C_3k_8 \\ ES_0k_1 - C_1k_2 - C_1k_3 \\ ES_1k_4 - C_2k_5 - C_2k_6 \\ FS_2k_7 - C_3k_8 - C_3k_9 \\ FS_1k_{10} - C_4k_{11} - C_4k_{12} \\ -ES_0k_1 - ES_1k_4 + C_1k_2 + C_1k_3 + C_2k_5 + C_2k_6 \\ -FS_1k_{10} - FS_2k_7 + C_3k_8 + C_3k_9 + C_4k_{11} + C_4k_{12} \end{bmatrix}.$$

Observe that we have the following equations,

$$M(7) = -\sum_{i=1}^6 M(i)$$

$$M(8) = -M(4) - M(5)$$

$$M(9) = -M(6) - M(7)$$

which means we only need  $M(i)$  for  $i = 1, \dots, 6$  to construct the Jacobian matrix  $J$  (not shown). Indeed, we can also replace exactly three rows of  $M$  with three conservation laws.

Thus,

$$\varphi_c = \begin{bmatrix} -ES_0k_1 + C_1k_2 + C_4k_{12} \\ -ES_1k_4 - FS_1k_{10} + C_1k_3 + C_2k_5 + C_3k_9 + C_4k_{11} \\ -FS_2k_7 + C_2k_6 + C_3k_8 \\ ES_0k_1 - C_1k_2 - C_1k_3 \\ ES_1k_4 - C_2k_5 - C_2k_6 \\ FS_2k_7 - C_3k_8 - C_3k_9 \\ \sum_{i=0}^2 S_i + \sum_{i=1}^4 C_i - c_3 \\ C_1 + C_2 + E - c_1 \\ C_3 + C_4 + F - c_2 \end{bmatrix}.$$

Our computer can compute the symbolic determinant of the Jacobian matrix, but it is impossible to determine the sign at a glance. However, we will evaluate the Jacobian at multiple points, calculate the convex hull of the Newton Polytope, then use those extreme points to determine the sign of the determinant. If we follow the steps of the algorithm described in Section 6.6 on page 66, the computed set of unique extreme points from Step 14 produces

$$J4 = [[0, 1, 0, 0, 0, 0, 0, 2, 1], [0, 1, 0, 0, 0, 0, 0, 1, 2], [1, 1, 0, 0, 0, 0, 0, 1, 1], \dots],$$

which also produces an error (possibly) as a result of having 9-dimensional points. To proceed, we calculate the positive parametrization  $P$  by excluding siphon

$Z_3 = \{F, C_3, C_4\}$ , which has support in the conservation law

$$C_3 + C_4 + F = c_2.$$

This produces a determinant (not shown) and a new list based on the exponents of elements in  $Z_3$ ,

$$J4 = [[3, 0, 3], [4, 0, 1], [3, 1, 1], [3, 0, 2], [1, 4, 0], [0, 4, 1], [3, 1, 2], \dots]$$

with the following unique vertices in its convex hull (Step 15),

$$J5 = [[3, 0, 3], [4, 0, 1], [3, 0, 2], [1, 4, 0], [0, 4, 1], [3, 1, 2], [2, 1, 3], [3, 2, 0], [1, 2, 2], [2, 2, 2]].$$

Next, we match the vertices to their corresponding terms in  $J2$ . We display some representative terms in Table 1 to emphasize that there are mixed signs in the convex hull.

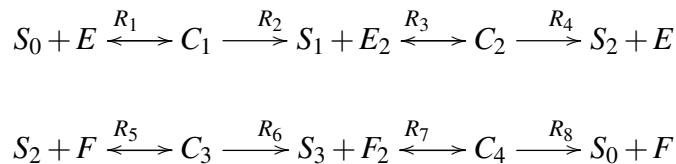
TABLE 1: Some Terms of the Numerator of  $J = DM(S(t))$  Corresponding to Extreme Vertices

$-C_3C_4^4k_{11}^2k_{12}^2k_3k_4^2k_6^3k_7$	$2C_3^3F^2k_1k_{10}^2k_3^2k_5k_6^2k_9^3$	$-C_3C_4^4k_{11}^2k_{12}^2k_2k_4^2k_6^3k_7$
$-2C_3C_4^4k_{11}k_{12}^3k_2k_4^2k_6^3k_7$	$C_3^3F^2k_1k_{10}^2k_3^2k_6^3k_8k_9^2$	$-C_3C_4^4k_{11}^2k_{12}^2k_3k_4^2k_6^3k_7$
$C_3^4Fk_1k_{10}^2k_3^2k_6^3k_8k_9^2$	$-C_3C_4^4k_{12}^4k_2k_4^2k_6^3k_7$	$C_3C_4^4k_{11}^2k_{12}^2k_3^2k_4^2k_6^2k_7k_9$
$C_3^3F^2k_1k_{10}^2k_3^2k_6^3k_9^3$	$-C_3C_4^4k_{12}^4k_3k_4^2k_6^3k_7$	$2C_3C_4^4k_{11}k_{12}^2k_3^2k_4^2k_6^2k_7k_9$
$C_3^3F^2k_1k_{10}^2k_3^2k_5k_6k_8k_9^2$	$2C_3^3F^2k_1k_{10}^2k_3^2k_5k_6^2k_8k_9^2$	$C_3C_4^4k_{12}^3k_2k_3k_4^2k_6^2k_7k_9$

Finally, since there are mixed signs in at least two of the terms in the numerator of the terms corresponding to the extreme points of the convex hull of the Newton Polytope, then the determinant  $\det J$  necessarily changes sign and we conclude the reaction network establishes multi-stationarity by Theorem 58 on page 63.

### 7.2.3 Additional comments

We mentioned earlier that  $S_1, E, F \in V_S$  are linked to more than two reactions.  $S_1$  is linked to reactions  $R_2, R_3, R_6, R_7$ ,  $E$  is linked to reactions  $R_1, R_2, R_3, R_4$ , and  $F$  is linked to reactions  $R_5, R_6, R_7, R_8$ . It is worth noting if we replace the second instance of  $S_1$  with an additional species  $S_3$  between reactions  $R_6, R_7$ , then  $S_1$  is linked to only two reactions. If we perform the same action and introduce new enzymes  $E_2$  and  $F_2$  to link reaction pairs  $R_2, R_3$  and  $R_6, R_7$  respectively, then we have a new reaction network model for the double phosphorylation reaction network:



This new reaction network satisfies the positive loop property as shown in Figure 7.3. The directed variation of this SR-graph is also strongly connected, which means we could

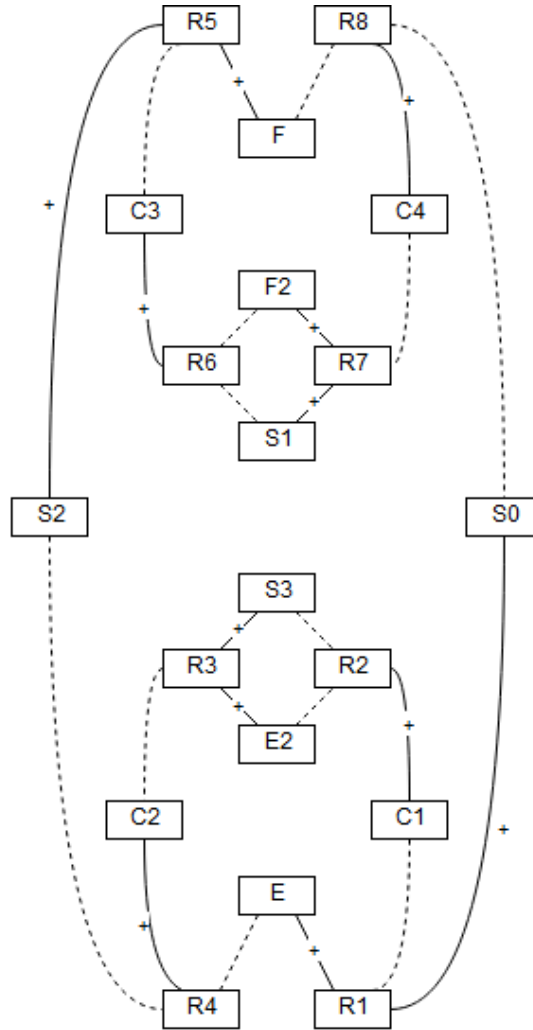


Fig. 7.3: SR-graph of Example 2 with  $S_3, E_2, F_2$  included.

apply Corollary 46 on page 50 to conclude this reaction is strongly monotone on

$$\text{int}(X_0) = \{x \in \mathbb{R}_{\geq 0}^{n_r} = \mathbb{R}^8 : S_0 + \Gamma x \gg 0\}.$$

We will also show this CRN is persistent after we calculate the minimal siphons, which means we can appeal to Theorem 48 on page 50 from reference [5, Theorem 2] to



conclude all solutions of the species system in  $\text{int}(\mathbb{R}_{\geq 0}^{n_s}) = \text{int}(\mathbb{R}_{\geq 0}^8)$  converges to a unique equilibrium within each stoichiometric compatibility class

$$C_{S_0} = \mathbb{R}^{12} \cap (S_0 + \text{im}(\Gamma)).$$

If we reconstruct the species dynamical system with a new  $\Gamma R(S)$  reflect the introduction of  $S_3, E_2, F_2$ , assume the same kinetics, and perform the algorithm again, then we get the following conservation laws, siphons, and  $M$  with  $k_1, k_{-1}, k_2, k_3, \dots$  relabeled  $k_1, k_2, \dots, k_{12}$  as before:

Conservation laws,

$$S_0 + S_2 + C_1 + C_2 + C_3 + C_4 + E_2 + F_2 = c_1$$

$$S_1 + C_1 + C_2 + E = c_2$$

$$S_3 + C_3 + C_4 + F = c_3$$

$$C_1 + C_2 + E + E_2 = c_4$$

$$C_3 + C_4 + F + F_2 = c_5$$

Siphons,

$$Z_1 = \{S_0, S_1, S_2, S_3, C_1, C_2, C_4, C_3\}$$

$$Z_2 = \{E, E_2, C_1, C_2\}$$

$$Z_3 = \{F, F_2, C_3, C_4\}$$

$$Z_4 = \{E, C_2, S_1, C_1\}$$

$$Z_5 = \{F, C_4, S_3, C_3\}$$

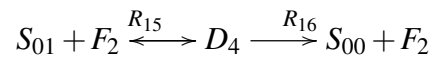
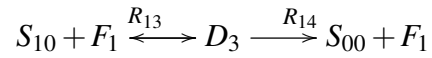
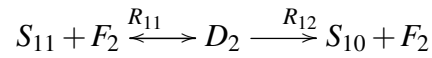
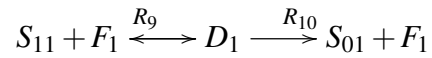
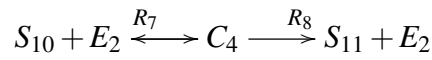
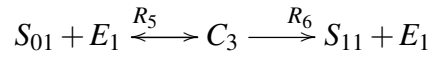
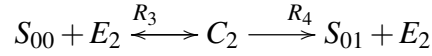
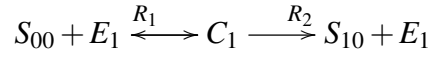
$$M = A \cdot R,$$

$$M = \begin{bmatrix} -ES_0k_1 + C_1k_2 + C_4k_{12} \\ -E_2S_1k_4 + C_1k_3 + C_2k_5 \\ -FS_2k_7 + C_2k_6 + C_3k_8 \\ -F_2S_3k_{10} + C_3k_9 + C_4k_{11} \\ ES_0k_1 - C_1k_2 - C_1k_3 \\ E_2S_1k_4 - C_2k_5 - C_2k_6 \\ FS_2k_7 - C_3k_8 - C_3k_9 \\ F_2S_3k_{10} - C_4k_{11} - C_4k_{12} \\ -ES_0k_1 + C_1k_2 + C_2k_6 \\ -E_2S_1k_4 + C_1k_3 + C_2k_5 \\ -FS_2k_7 + C_3k_8 + C_4k_{12} \\ -F_2S_3k_{10} + C_3k_9 + C_4k_{11} \end{bmatrix}.$$

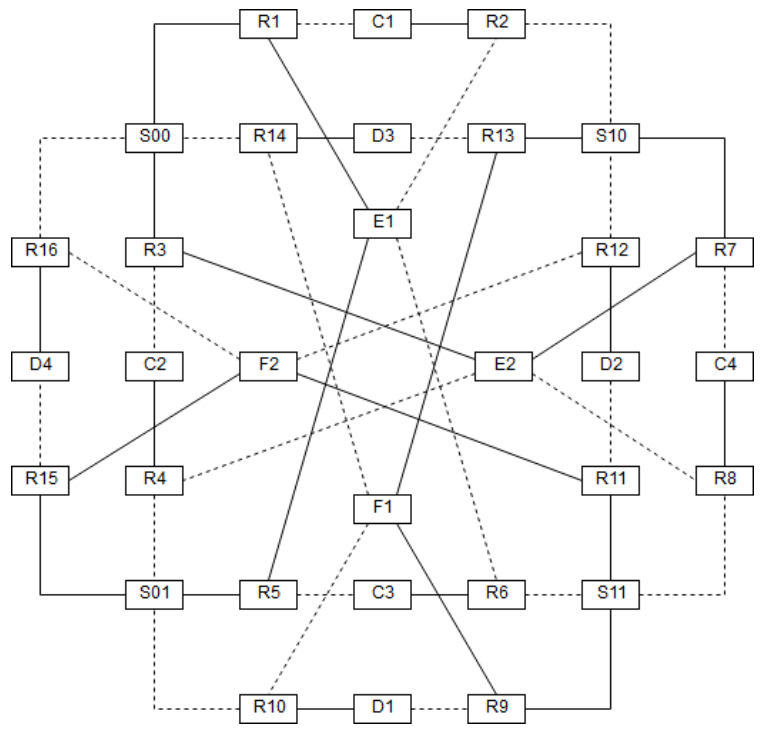
Notice that  $M(2)$  is significantly different from the one presented for the original reaction network, because  $M(2)$  originally contained reaction coefficients from linearly independent reactions. Observe that  $\text{supp}(Z_i)$  is in a conservation law for all  $i$ , so we can use Proposition 54 on page 60 to conclude the absence of boundary equilibria, and thus conclude this CRN is persistent. We can immediately see  $\det(M)$  is negative in Step 9 of Section 6.6 on page 66 without the need to construct a positive parametrization, so this reaction network is mono-stationary by Corollary 59 on page 64 from reference [3].

### 7.3 Example 3: Two Site Phosphorylation Network with Distinct Sites

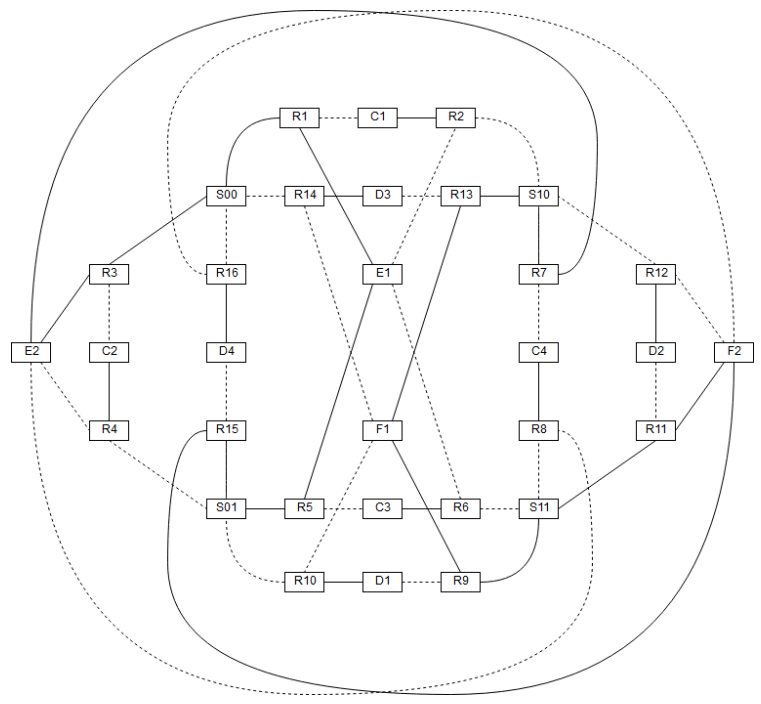
Consider the following reaction that has not been previously considered in the literature.



Observe that there are two sites, and two enzymes for each site in which order matters, i.e.,  $S_{01} \neq S_{10}$ , with variations of the SR-graphs presented in Figure 7.4. Also, this network has 16 species and 16 reactions – eight of whom are reversible.



(a) SR-Graph (Variation 1)



(b) SR-Graph (Variation 2)

Fig. 7.4: Special graphs of the Two-Site Phosphorylation network.

### 7.3.1 Positive Loop Property

This reaction network's  $R$  graph (not shown) does not satisfy the positive loop property by Proposition 42 on page 47 because each of the species in the set

$$\{S_{00}, S_{01}, S_{10}, S_{11}, E_1, E_2, F_1, F_2\}$$

is linked to four reactions in the DSR-graph. Thus, the reaction-coordinates dynamical system is not monotone with respect to a partial order induced by an orthant cone for any  $S_0 \in \mathbb{R}^{n_s} = \mathbb{R}^{16}$  by Theorem 44 on page 49.

### 7.3.2 Testing for Multi-Stationarity

We will now check whether this reaction system establishes multi-stationarity. We construct the system  $\dot{S} = \Gamma R(S)$  in mass-action coordinates.

$$\begin{bmatrix} S_{00} \\ S_{10} \\ S_{01} \\ S_{11} \\ C_1 \\ C_2 \\ C_3 \\ C_4 \\ D_1 \\ D_2 \\ D_3 \\ D_4 \\ E_1 \\ E_2 \\ F_1 \\ F_2 \end{bmatrix} = \begin{bmatrix} -1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 \\ 0 & 1 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 1 & -1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\ -1 & 1 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 1 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & -1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & -1 & 1 \end{bmatrix} \cdot \begin{bmatrix} E_1 S_{00} k_1 - C_1 k_{-1} \\ C_1 k_2 \\ E_2 S_{00} k_3 - C_2 k_{-3} \\ C_2 k_4 \\ E_1 S_{01} k_5 - C_3 k_{-5} \\ C_3 k_6 \\ E_2 S_{10} k_7 - C_4 k_{-7} \\ C_4 k_8 \\ F_1 S_{11} k_9 - D_1 k_{-9} \\ D_1 k_{10} \\ F_2 S_{11} k_{11} - D_2 k_{-11} \\ D_2 k_{12} \\ F_1 S_{10} k_{13} - D_3 k_{-13} \\ D_3 k_{14} \\ F_2 S_{01} k_{15} - D_4 k_{-15} \\ D_4 k_{16} \end{bmatrix}$$

Next, we will test for dissipativity by solving for strictly positive vectors  $w$  such that  $w^T \Gamma = 0$ . Using the MATLAB commands `null(A, 'r')` and `colspace(sym())` with the proper

matrix  $A$  yields such a  $w$ .

$$w \in \text{span} \left\{ \begin{bmatrix} 1 \\ 1 \\ 1 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ -1 \\ -1 \\ -1 \\ -1 \end{bmatrix}, \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \\ 1 \end{bmatrix} \right\} = \text{span} \left\{ \begin{bmatrix} 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1 \\ 0 \\ 1 \\ 0 \\ 0 \\ 0 \\ 1 \end{bmatrix} \right\}$$

Remember, we prefer to use positive vectors because they yield conservation laws. It does not make much sense to refer to the negative amount of a species in this context.

Nonetheless, we have the following conservation laws for some constants

$$c_1, c_2, c_3, c_4, c_5 \in \mathbb{R},$$

$$C_1 + C_3 + E_1 = c_1$$

$$C_2 + C_4 + E_2 = c_2$$

$$D_1 + D_3 + F_1 = c_3$$

$$D_2 + D_4 + F_2 = c_4$$

$$S_{00} + S_{10} + S_{01} + S_{11} + \sum_{i=1}^4 (C_i + D_i) = c_5.$$

Next we check for the existence of boundary equilibria. To do this, we will calculate the minimal siphons of the reaction and check if they have support in one of the conservation laws. Let us work backwards in each connected component. If we work backwards from all reactions,

- To produce  $S_{10}$  in each reaction, we need  $C_1, D_2, S_{00}, S_{11}$ . To produce  $S_{00}$  in each reaction, we need  $D_3, D_4, S_{01}, S_{10}$ . We already have  $S_{10}$ . To produce  $S_{01}$  in each reaction, we need  $C_2, D_1, S_{00}, S_{11}$ . We already have  $S_{00}$ . To produce  $S_{11}$  in each reaction, we need  $C_3, C_4, S_{01}, S_{10}$ . We already have  $S_{01}$  and  $S_{10}$ . If we remove all overlapping species, we have the minimal siphon

$$Z_1 = \{S_{10}, C_1, D_2, S_{00}, D_3, D_4, S_{01}, C_2, D_1, S_{11}, C_3, C_4\}.$$

- To produce the enzymes  $E_i$  present in each reaction, we need

$$Z_2 = \{E_1, C_1, C_3\}$$

$$Z_3 = \{E_2, C_2, C_4\}.$$

- To produce the enzymes  $F_i$  present in each reaction, we need

$$Z_4 = \{F_1, D_1, D_3\}$$

$$Z_5 = \{F_2, D_2, D_4\}.$$

Observe that all of these siphons are minimal by definition since  $Z_i \not\subseteq Z_j$  for any  $i \neq j \in \{1, 2, 3, 4, 5\}$ , and also because taking any species away would not make it a siphon. Also observe that  $\text{supp}(Z_i)$  is in the conservation laws from above for all  $i = 1, 2, 3, 4, 5$ . Thus, by Proposition 54 on page 60 from reference [3], the reaction network has no boundary equilibria in any stoichiometric compatibility class with a non-empty positive interior.

We will proceed with the steps from the pseudo-code from Section 6.6 on page 66 using Maple. Since this CRN has 16 (including reversible) reactions, we will relabel

$$k_1, k_{-1}, k_2, k_3, \dots \text{ as } k_1, k_2, \dots, k_{16}$$

in Maple. Calculating  $M = A \cdot R$  yields

$$M = \begin{bmatrix} -E_1S_{00}k_1 - E_2S_{00}k_4 + C_1k_2 + C_2k_5 + D_3k_{21} + D_4k_{24} \\ -E_2S_{10}k_{10} - F_1S_{10}k_{19} + C_1k_3 + C_4k_{11} + D_2k_{18} + D_3k_{20} \\ -E_1S_{01}k_7 - F_2S_{01}k_{22} + C_2k_6 + C_3k_8 + D_1k_{15} + D_4k_{23} \\ -F_1S_{11}k_{13} - F_2S_{11}k_{16} + C_3k_9 + C_4k_{12} + D_1k_{14} + D_2k_{17} \\ E_1S_{00}k_1 - C_1k_2 - C_1k_3 \\ E_2S_{00}k_4 - C_2k_5 - C_2k_6 \\ E_1S_{01}k_7 - C_3k_8 - C_3k_9 \\ E_2S_{10}k_{10} - C_4k_{11} - C_4k_{12} \\ F_1S_{11}k_{13} - D_1k_{14} - D_1k_{15} \\ F_2S_{11}k_{16} - D_2k_{17} - D_2k_{18} \\ F_1S_{10}k_{19} - D_3k_{20} - D_3k_{21} \\ F_2S_{01}k_{22} - D_4k_{23} - D_4k_{24} \\ -E_1S_{00}k_1 - E_1S_{01}k_7 + C_1k_2 + C_1k_3 + C_3k_8 + C_3k_9 \\ -E_2S_{00}k_4 - E_2S_{10}k_{10} + C_2k_5 + C_2k_6 + C_4k_{11} + C_4k_{12} \\ -F_1S_{10}k_{19} - F_1S_{11}k_{13} + D_1k_{14} + D_1k_{15} + D_3k_{20} + D_3k_{21} \\ -F_2S_{01}k_{22} - F_2S_{11}k_{16} + D_2k_{17} + D_2k_{18} + D_4k_{23} + D_4k_{24} \end{bmatrix}$$

Observe that we have the following equations,

$$M(12) = - \sum_{i=1}^{11} M(i)$$

$$M(13) = -M(7) - M(5)$$

$$M(14) = -M(8) - M(6)$$

$$M(15) = -M(11) - M(9)$$

$$M(16) = -M(12) - M(10)$$



which means we can replace five of these linearly dependent rows with exactly five conservation laws to construct  $\varphi_c(M(S))$

$$\varphi_c = \begin{bmatrix} -E_1S_{00}k_1 - E_2S_{00}k_4 + C_1k_2 + C_2k_5 + D_3k_{21} + D_4k_{24} \\ -E_2S_{10}k_{10} - F_1S_{10}k_{19} + C_1k_3 + C_4k_{11} + D_2k_{18} + D_3k_{20} \\ -E_1S_{01}k_7 - F_2S_{01}k_{22} + C_2k_6 + C_3k_8 + D_1k_{15} + D_4k_{23} \\ -F_1S_{11}k_{13} - F_2S_{11}k_{16} + C_3k_9 + C_4k_{12} + D_1k_{14} + D_2k_{17} \\ E_1S_{00}k_1 - C_1k_2 - C_1k_3 \\ E_2S_{00}k_4 - C_2k_5 - C_2k_6 \\ E_1S_{01}k_7 - C_3k_8 - C_3k_9 \\ E_2S_{10}k_{10} - C_4k_{11} - C_4k_{12} \\ F_1S_{11}k_{13} - D_1k_{14} - D_1k_{15} \\ F_2S_{11}k_{16} - D_2k_{17} - D_2k_{18} \\ F_1S_{10}k_{19} - D_3k_{20} - D_3k_{21} \\ S_{00} + S_{10} + S_{01} + S_{11} + \sum_{i=1}^4 (C_i + D_i) - c_5 \\ C_1 + C_3 + E_1 - c_1 \\ C_2 + C_4 + E_2 - c_2 \\ D_1 + D_3 + F_1 - c_3 \\ D_2 + D_4 + F_2 - c_4 \end{bmatrix}.$$

We only need  $M(i)$  for  $i = 1, \dots, 11$  to construct the Jacobian matrix  $J$  (not shown). We skip straight to step 16 of Section 6.6 on page 66 and calculate the positive parametrization  $P$  by excluding catalytic species  $H = \{S_{11}, E_1, E_2, F_1, F_2\}$ . Computing the determinant symbolically exceeded Maple's standard computing time, which means we will have to restrict to a subset of the parameter space by fixing our 24 reaction coefficients to numerical values via step 9(a) of Section 6.6 on page 66. Namely, we fix  $k_i = 1$  for all  $i = \{1, \dots, 24\}$ , then let  $k_j = s$  for some  $j \in \{1, \dots, 24\}$ .

If  $k_3 = s$ , then this produces a determinant (not shown as the length of the output contains over  $10^6$  characters) and a list based on the exponents of elements in  $H$

$$J4 = [[2, 7, 2, 1, 1], [2, 7, 1, 1, 2], [2, 6, 3, 1, 1], [2, 6, 2, 2, 1], [2, 6, 2, 1, 2], [2, 6, 1, 2, 2], \dots]$$

with the following unique vertices in its convex hull (Step 15),

$$J5 = [[2, 7, 2, 1, 1], [2, 7, 1, 1, 2], [2, 6, 3, 1, 1], [2, 6, 2, 2, 1], [2, 6, 2, 1, 2], [2, 6, 1, 2, 2], \dots].$$

Next, we match the vertices to their corresponding terms in  $J2$ . We display some representative terms in Table 2 to emphasize that there are mixed signs in the convex hull.

TABLE 2: Some Terms of the Numerator of  $J = DM(S(t))$  Corresponding to Extreme Vertices With Reaction Rate Constants  $k_3 = s$  and All Other  $k_i = 1$

$4E_1^3 E_2^6 F_1 F_2 S_{11}^2 s^4$	$16E_1^5 F_1^2 F_2^3 S_{11}^3 s^2$	$-32E_1^5 E_2^2 F_1^2 F_2 S_{11}^3 s^3$
$16E_1^5 F_1 F_2^4 S_{11}^3 s^2$	$-32E_1^5 F_1^2 F_2^3 S_{11}^3 s^3$	$-8E_1^3 E_2^6 F_1 F_2 S_{11}^2 s$
$-32E_1^5 F_1 F_2^4 S_{11}^3 s^3$	$16E_1^5 F_1^2 F_2^3 S_{11}^3 s^4$	$-4E_1^3 E_2^6 F_1 F_2 S_{11}^2$
$16E_1^5 F_1 F_2^4 S_{11}^3 s^4$	$16E_1^5 E_2^2 F_1^2 F_2 S_{11}^3 s^2$	$4E_1^3 E_2^5 F_1 F_2 S_{11}^3 s^4$
$16E_1^5 E_2^3 F_1 F_2 S_{11}^3 s^4$	$32E_1^7 E_2 F_1 F_2^2 S_{11}^3 s^3$	$-16E_1^3 E_2 F_1 F_2^2 S_{11}^3 s^4$

Finally, since there are mixed signs in at least two of the terms in the numerator of the terms corresponding to the extreme points of the convex hull of the Newton Polytope, then the determinant  $\det J$  necessarily changes sign and we conclude the reaction network establishes multi-stationarity by Theorem 58 on page 63.

## 8 CONCLUSION

We looked at the results of existing monotone systems theory and tested several reaction networks for the positive loop property to demonstrate how quickly and efficiently one can verify if a reaction coordinate system is monotone with respect to a positive orthant cone. A dynamical system that is monotone with respect to a positive orthant cone guarantees the system is mono-stationary and its steady states are convergent. We also tested the two-site phosphorylation reaction network and a slightly modified double phosphorylation network with the algorithm and proved that they establish multi-stationarity and mono-stationarity respectively.

Computing the sign of the determinant of the two-site phosphorylation reaction network was computationally expensive, so we needed to use a positive parametrization to generate points for the convex hull in a reasonable number of dimensions. We also had to resort to a systematic exploration through parameter space by substituting our reaction coefficients with numerical values. Since we ran into computational issues with the two-site phosphorylation reaction network, we propose as future work to establish alternative tests for multi-stationarity in a more efficient manner. Additionally, we wish to determine an upper bound for the number of equilibria in a multi-stationary reaction. We would also like to test the stability of these equilibria in parameter space.

## Literature Cited

- [1] D. Angeli, P. De Leenheer, and E. Sontag, “A Petri net approach to persistence analysis in chemical reaction networks,” in *Biology and Control Theory: Current Challenges*, pp. 181–216, Springer, 2007.
- [2] G. A. Enciso, “Fixed points and convergence in monotone systems under positive or negative feedback,” *International Journal of Control*, vol. 87, no. 2, pp. 301–311, 2014.
- [3] C. Conradi, E. Feliu, M. Mincheva, and C. Wiuf, “Identifying parameter regions for multistationarity,” *PLoS Computational Biology*, vol. 13, no. 10, p. e1005751, 2017.
- [4] D. Angeli and E. D. Sontag, “Translation-invariant monotone systems, and a global convergence result for enzymatic futile cycles,” *Nonlinear Analysis: Real World Applications*, vol. 9, no. 1, pp. 128–140, 2008.
- [5] D. Angeli, P. De Leenheer, and E. Sontag, “Graph-theoretic characterizations of monotonicity of chemical networks in reaction coordinates,” *Journal of Mathematical Biology*, vol. 61, no. 4, pp. 581–616, 2010.
- [6] P. De Leenheer, D. Angeli, and E. D. Sontag, “A tutorial on monotone systems-with an application to chemical reaction networks,” in *Proc. 16th Int. Symp. Mathematical Theory of Networks and Systems (MTNS)*, pp. 2965–2970, Citeseer, 2004.
- [7] E. Feliu and C. Wiuf, “A computational method to preclude multistationarity in networks of interacting species,” *Bioinformatics*, vol. 29, no. 18, pp. 2327–2334, 2013.
- [8] M. Feinberg, “Chemical reaction network structure and the stability of complex isothermal reactors–I. The deficiency zero and deficiency one theorems,” *Chemical Engineering Science*, vol. 42, no. 10, pp. 2229–2268, 1987.
- [9] M. Feinberg, “Chemical reaction network structure and the stability of complex isothermal reactors–II. Multiple steady states for networks of deficiency one,” *Chemical Engineering Science*, vol. 43, no. 1, pp. 1–25, 1988.
- [10] E. Tonello and M. D. Johnston, “Network Translation and Steady-State Properties of Chemical Reaction Systems,” *Bulletin of Mathematical Biology*, vol. 80, no. 9, pp. 2306–2337, 2018.

- [11] M. Banaji and G. Craciun, “Graph-theoretic criteria for injectivity and unique equilibria in general chemical reaction systems,” *Advances in Applied Mathematics*, vol. 44, no. 2, pp. 168–184, 2010.
- [12] H. Errami, W. M. Seiler, M. Eiswirth, and A. Weber, “Computing Hopf bifurcations in chemical reaction networks using reaction coordinates,” in *International Workshop on Computer Algebra in Scientific Computing*, pp. 84–97, Springer, 2012.
- [13] H. Errami, M. Eiswirth, D. Grigoriev, W. M. Seiler, T. Sturm, and A. Weber, “Detection of Hopf bifurcations in chemical reaction networks using convex coordinates,” *Journal of Computational Physics*, vol. 291, pp. 279–302, 2015.
- [14] M. W. Hirsch, “Stability and convergence in strongly monotone dynamical systems,” *J. Reine Angew. Math*, vol. 383, no. 1, p. 53, 1988.
- [15] M. Banaji, “Monotonicity in chemical reaction systems,” *Dynamical Systems*, vol. 24, no. 1, pp. 1–30, 2009.
- [16] H. L. Smith, *Monotone dynamical systems: an introduction to the theory of competitive and cooperative systems*. American Mathematical Soc., 2008.
- [17] M. Mincheva and M. R. Roussel, “Graph-theoretic methods for the analysis of chemical and biochemical networks. i. multistability and oscillations in ordinary differential equation models,” *Journal of Mathematical Biology*, vol. 55, no. 1, pp. 61–86, 2007.
- [18] A. I. Volpert, V. A. Volpert, and V. A. Volpert, *Traveling wave solutions of parabolic systems*, vol. 140. American Mathematical Soc., 1994.
- [19] G. Craciun and M. Feinberg, “Multiple equilibria in complex chemical reaction networks: Ii. the species-reaction graph,” *SIAM Journal on Applied Mathematics*, vol. 66, no. 4, pp. 1321–1338, 2006.
- [20] M. A. Al-Radhawi and D. Angeli, “Piecewise linear in rates Lyapunov functions for complex reaction networks,” in *52nd IEEE Conference on Decision and Control*, pp. 4595–4600, IEEE, 2013.
- [21] C. Conradi and M. Mincheva, “Catalytic constants enable the emergence of bistability in dual phosphorylation,” *Journal of The Royal Society Interface*, vol. 11, no. 95, p. 20140158, 2014.

- [22] C. Conradi and M. Mincheva, “Graph-theoretic analysis of multistationarity using degree theory,” *Mathematics and Computers in Simulation*, vol. 133, pp. 76–90, 2017.
- [23] A. Shiu and B. Sturmfels, “Siphons in chemical reaction networks,” *Bulletin of Mathematical Biology*, vol. 72, no. 6, pp. 1448–1463, 2010.
- [24] S. Boyd and L. Vandenberghe, *Convex optimization*. Cambridge University Press, 2004.
- [25] M. D. Johnston, *Topics in Chemical Reaction Network Theory*. Ph.D. dissertation, University of Waterloo, 2012.
- [26] A. Fuchs, “Dynamical systems in one and two dimensions: A geometrical approach,” in *Nonlinear Dynamics in Human Behavior*, pp. 1–33, Springer, 2010.
- [27] M. P. Millán, A. Dickenstein, A. Shiu, and C. Conradi, “Chemical reaction systems with toric steady states,” *Bulletin of Mathematical Biology*, vol. 74, no. 5, pp. 1027–1065, 2012.
- [28] D. Siegel and D. MacLean, “Global stability of complex balanced mechanisms,” *Journal of Mathematical Chemistry*, vol. 27, no. 1-2, pp. 89–110, 2000.