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Analyzing Multistationarity in a Model of a Dual Phosphorylation Network

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ABSTRACT

ANALYZING MULTISTATIONARITY IN A MODEL OF A DUAL PHOSPHORYLATION NETWORK

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Otero-Muras, Banga, and Alonso in [18] state biological reaction systems exhibit multistationarity, a system with at least two positive steady states. According to Otero-Muras, Banga, and Alonso in [18], initial conditions within the system, however, dictate the number of stable states in that system. Furthermore, multistationarity is an important component present in biological reaction systems that manage cellular responses [18]. Signal pathways regulating cell maturation, cell replication, and cell death rely on such systems [18]. Given the importance of multistationarity in biological reaction systems, it is necessary to construct a mathematical model, one in which multistationarity will be configured. More importantly, inequalities will be obtained to determine multistationarity region in parameter space.
ANALYZING MULTISTATIONARITY IN A MODEL OF A DUAL PHOSPHORYLATION NETWORK

BY

KILLIAN ANDERSON
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A THESIS SUBMITTED TO THE GRADUATE SCHOOL IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE MASTER OF SCIENCE

DEPARTMENT OF MATHEMATICAL SCIENCES

Thesis Director:
Maya Mincheva
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DEDICATION

The completion of my master’s studies would not have been possible without the support of my family and friends. My mother, Ms. Julie Anderson, who kept me focused throughout my entire period of study. Without their kindness and warm words, it would not have been possible for me to complete my goal of finishing the degree.
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CHAPTER 1
PRELIMINARIES

An introduction and discussion of concepts that are important for developing a mathematical model representative of a dual phosphorylation/dephosphorylation event in a signaling pathway follows in the subsequent sections.

1.1 Introduction

Many biological processes in the body rely on a series of chemical reactions. The chemical reactions examined in intracellular signaling are of great importance. Specifically, this network of reactions rely on enzymes to ensure that the signal is amplified and continues to move along in the signaling pathway to its effector. Two enzymes tasked with propagating of intracellular signaling are protein kinase and protein phosphatase. These two enzymes are unique in that they manage the transfer of phosphate groups to and from the protein substrate. Depending on the protein substrate, adding or removing the phosphate group activates or deactivates the activity. Proving the blueprint for how, when, and how many of these enzymes are active in each reaction in a network can be challenging. In this paper, we build a model based on knowledge that transferring phosphate groups, adding as in phosphorylation or removing as in dephosphorylation, to the protein substrate requires two enzymes [11]. Further, the two enzymes will act in succession [11]. When two enzymes are involved and they are said to act in succession, the reaction is referred to as dual phosphorylation/dephosphorylation. This paper goes further and examines the plausibility that
within dual phosphorylation/dephosphorylation multistationarity is exhibited. In Chapter 1 of this paper, principles for modeling the reaction network are defined and the framework for the final model is provided. In Chapter 2 of this paper, a brief overview of the biology and chemical processes of phosphorylation/dephosphorylation. Finally, in Chapter 3 a qualifying model representative of dual phosphorylation/dephosphorylation that exhibits multistationarity is presented. The final model adopts principles including convex parameters, Jacobian, and degree theory to show the dual phosphorylation/dephosphorylation networks exhibits multistationarity from Conradi and Mincheva [11].

1.2 Preliminary Chemical Reaction Network Modeling

Chemical reaction network models can provide an explanation for biological processes such as intracellular signal transduction. Such models rely on dynamic ordinary differential equation systems to explain behaviors of the network over time [1]. Furthermore, mass-action kinetics, the stoichiometric matrix, and the law of mass conservation are applied to construct the model. The following pages provide an explanation of the mathematical theory necessary to develop a chemical reaction network mathematical model.

1.2.1 The Example Chemical Reaction Network

A chemical reaction network diagram for a non-specific biological process is represented below. A set of reactions between a set of species and their complexes is characteristic of a chemical reaction network. The example reaction network model includes

\[ A_1 + A_3 \xrightleftharpoons[k_2]{k_1} A_2 \]  \hspace{1cm} (1.1)
\[ A_2 \xrightarrow{k_3} A_1 + A_4. \] 

(1.2)

The example network diagram composed of (1.1) and (1.2) consists of a set of species identified as \(A_1, A_2, A_3,\) and \(A_4\) for which a set of three elementary reactions is shown. In (1.1) and (1.2), the direction of the arrow(s) indicate which species are the reactants and which are the products. An arrow direction also identifies whether the chemical reaction is reversible or irreversible. A reversible chemical reaction shown in (1.1), is indicated by the presence of two arrows showing both a forward and backward reaction. This means the forward, or first, reaction can be reversed by the backward, or second, reaction [1]. In contrast, an irreversible reaction as shown in (1.2) is indicated by a single directional arrow [1]. This type of reaction is not reversible and therefore, the reactants of species \(A_1\) and \(A_4\) yield species \(A_2,\) which is the product.

Complexes include the sum of species used and generated in each reaction and are on either side of the arrow [1]. Complexes in the reaction network model are easily identified. In (1.1), there are two complexes in the reaction, \(A_1 + A_3\) and \(A_2\) in (1.2) there are two, \(A_2\) and \(A_1 + A_4.\)

Let the species \(A_1, A_2, A_3,\) and \(A_4\) from (1.1) and (1.2) have their concentrations, i.e. molecular amount, be represented by \([A_1], [A_2], [A_3],\) and \([A_4].\) Also, each chemical concentration is written as a function of time for the reaction network described by (1.1) and (1.2) in the form \(x_i(t) = [A_i]\) for each \(i = 1, 2, 3, 4.\) Rate constants establish the constant change at which species concentration/volume is flowing in a chemical reaction [1]. The example network diagram also shows the rate constants for each reaction, \(k_i\) where \(i = 1, 2, 3.\) The rate constants are always positive and can be considered as parameters in the mathematical model.
A chemical reaction can be expressed in general by the following formula:

$$
\sum_{i=1}^{n} \alpha_{ij} A_i \xrightarrow{k_j} \sum_{i=1}^{n} \beta_{ij} A_i
$$

(1.3)

Here, $\alpha_{ij} > 0$ and $\beta_{ij} > 0$ are stoichiometric coefficients and represents the molecularity, i.e. number of species. The species, $A_i$, for each $i = 1, \ldots, n$ participate in each reaction. An elementary reaction, for each $j = 1, \ldots, m$, takes place at a constant rate, $k_j > 0$. The overall reaction in (1.3) is a chemical reaction network.

The equations (1.1) and (1.2) and their respective corresponding stoichiometric coefficients are

$$
\alpha_{11} A_1 + \alpha_{31} A_3 \xrightarrow{k_1} \beta_{21} A_2
$$

(1.4)

$$
\alpha_{22} A_2 \xrightarrow{k_2} \beta_{12} A_1 + \beta_{32} A_3
$$

(1.5)

$$
\alpha_{23} A_2 \xrightarrow{k_3} \beta_{13} A_1 + \beta_{43} A_4
$$

(1.6)

By comparison, (1.4), (1.5), and (1.6) yields

$$
\alpha_{11} = \alpha_{31} = \beta_{21} = 1
$$

$$
\alpha_{22} = \beta_{12} = \beta_{32} = 1
$$

$$
\alpha_{23} = \beta_{13} = \beta_{43} = 1
$$

Any stoichiometric coefficients for each $i, j$ not listed above is zero.

Moreover, each reaction is described mathematically by a rate law, which is explained in the next section.
1.2.2 The Rate Law

Reaction kinetics is based on a relationship between concentration and rate [1]. In the reaction network model, the law of mass action, a type of rate law can be supported. The law of mass action is defined as the rate at which reactants are generated or consumed. When applying mass action kinetics to reactions in the network the rate constant will be positive [1]. Recall, in (1.1) and (1.2) \( k_i \) for each \( i = 1, 2, 3 \) represents the rate constants in the reaction network.

Therefore, using the law of mass action the rate functions for each reaction in the network given in (1.1) and (1.2) are

\[
\begin{align*}
    r_1 &= k_1 x_1 x_3 \\
    r_2 &= k_2 x_2 \\
    r_3 &= k_3 x_2
\end{align*}
\]

Each rate function or law is best generalized mathematically as

\[
r_j = k_j \prod_{i}^{n} x_i^{\alpha_{ij}}
\]

where the species concentration, \( x_i \), is raised to \( \alpha_{ij} \), the molecularity, and where the constant rate of change \( k_j > 0 \) for the \( j \)-th reaction [13]. The reader should take note that a kinetic order matrix, \( Y = [\alpha_{ij}] \), where \( Y \in \mathbb{R}^{m \times m} \), can be obtained from (1.10) [13]. By examining
(1.7), (1.8), and (1.9) and utilizing (1.10) each non-zero entry of $Y$ from the respective rate function is

$$\alpha_{11} = \alpha_{13} = 1 \quad (1.11)$$

$$\alpha_{22} = 1 \quad (1.12)$$

$$\alpha_{32} = 1 \quad (1.13)$$

Therefore, the kinetic order matrix determining the functional relationship between concentration and rate in the given chemical reaction network is

$$Y = \begin{bmatrix}
1 & 0 & 0 \\
0 & 1 & 1 \\
1 & 0 & 0 \\
0 & 0 & 0
\end{bmatrix} \quad (1.14)$$

### 1.2.3 The Stoichiometric Matrix

The stoichiometric matrix is formulated based on the number of species and elementary reactions involved in a chemical reaction network [1]. Coefficients of each species from the reaction network are the basis for generating the stoichiometric matrix. Stoichiometric coefficients are important because they provide a tool for quantifying the relationship between reactants involved in the reaction and the product. A stoichiometric matrix for a chemical network contains $n$ rows corresponding to $n$ species, $A_i$, and $m$ columns corresponding to $m$ reactions, $R_j$. The net production of species $x_i$ in reaction $R_j$, labeled $N_{ij}$, is called the stoichiometry. The $N_{ij}$ for each $i = 1, 2, ..., n$ and $j = 1, 2, ..., m$ form an $n \times m$ stoichiometric
matrix that we shall refer to as $N$. Each entry of $N$, $N_{ij}$, is calculated by using the difference of stoichiometry coefficients between products and reactants in (1.3) in Section 1.2.1

$$N_{ij} = \beta_{ij} - \alpha_{ij} \quad (1.15)$$

where the stoichiometric coefficients, $\alpha_{ij}$ and $\beta_{ij}$, are non-negative integers from (1.3).

By utilizing (1.15) and referencing the stoichiometric coefficients for the given example network that includes 4 species reactants and 3 elementary reactions we write the stoichiometric matrix. The stoichiometric matrix is represented by $N = [N_{ij}]$ for each $i = 1, ..., 4$, $j = 1, ..., 3$.

The first row of the stoichiometric matrix $N$ is computed using (1.15)

$$N_{11} = \beta_{11} - \alpha_{11} = 0 - 1 = -1$$
$$N_{12} = \beta_{12} - \alpha_{12} = 1 - 0 = 1$$
$$N_{13} = \beta_{13} - \alpha_{13} = 1 - 0 = 1$$

Similarly, the entries of $N$ for the remaining rows can be calculated and the stoichiometric matrix for the example reaction network is:

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

### 1.2.4 The Ordinary Differential Equation System

An ordinary differential equation (ODE) system, also referred to as a dynamical system, is important for establishing a mathematical model of species concentrations that depend
on time. More precisely, the dynamical system or differential equation system is a means of describing global behavior of a structure over the course of time [2]. In the system, the ordinary differential equation is an equation that contains some function, or a dependent variable along with its derivatives with respect to an independent variable. For the example, \( \frac{dx}{dt} = \dot{x}(t) \), is a derivative of a function \( x(t) \), the dependent variable is \( x \), with respect to \( t \), the independent variable. Each derivative represents a rate of change, while the differential equation represents the continuously varying change in quantity with respect to change in another quantity. In the case of chemical reaction networks, \( x(t) \) is a species concentration at time \( t \geq 0 \).

An ODE for one of the reactions in the example network from Section (1.2.1) is described considering the constant change of the first concentrate over time, \( x_1(t) = [A_1] \). The rate of change of \( x_1(t) \) can be inferred by referencing (1.1) and (1.2) with its corresponding rate functions (1.4), (1.5), and (1.6) to obtain

\[
\frac{dx_1(t)}{dt} = \dot{x}_1(t) = -k_1 x_1 x_3 + k_2 x_2 + k_3 x_2 = -r_1 + r_2 + r_3 \quad (1.17)
\]

The differential equation summarizes the rate functions of the species in a reaction network over its entire life cycle. The sign of the rate functions is based on whether the species is a reactant, a minus (-), or a product, a plus (+).

A system of differential equations that models the species concentrations of the reaction network described in (1.1) and (1.2) is

\[
\dot{x}_1(t) = -k_1 x_1 x_3 + k_2 x_2 + k_3 x_2 \quad (1.18)
\]

\[
\dot{x}_2(t) = -k_2 x_2 + k_1 x_1 x_3 - k_3 x_2 \quad (1.19)
\]

\[
\dot{x}_3(t) = -k_1 x_1 x_3 + k_2 x_2 \quad (1.20)
\]
\[ \dot{x}_4(t) = k_3 x_2 \] (1.21)

The derivative is represented by a vector \( \dot{x} = [\dot{x}_1, \dot{x}_2, \ldots, \dot{x}_n] \) for each \( i = 1, 2, \ldots, n \). This vector is a product of the stoichiometric matrix \( N \) and the reaction rate vector \( r(k, x) \), 
\[ \dot{x} = N \cdot r(k, x), \] from Conradi, Feliu, and Mincheva in [13].

### 1.2.5 Mass Conservation

The mass of each of the reactants in the chemical reaction network obeys the law of mass conservation [3]. According to the law, species representing the reactants will have a combined mass such that the mass of the product in the reaction will be equal to the mass of those reactants. Basically, since there is a rearrangement of mass in the reaction, nothing is lost. For example, in the reaction of burning wood, as described by Antoine Lavoisier in 1789, the mass of soot ash and gases that result in that action is equal to the mass of wood and oxygen.

To determine mass conservation from the reaction network including (1.1) and (1.2). The process can be best summarized as the following from Conradi, Feliu, and Mincheva in [13]. The mass conservation relations for species concentrations in a chemical reaction network are determined by a transposed matrix \( W^T \) such that

\[ W^T \cdot N = 0. \] (1.22)

Since \( W^T \dot{x} = W^T \cdot N \cdot r(k, x) = 0 \cdot r(k, x) = 0 \) it follows that \( W^T x = c \) is a constant. The conservation relation matrix \( W^T \) must meet these requirements: \( W \in R^n \times R^{n-s} \) where \( s = \text{rank}(N) \) such that \( W^T \cdot x = c \) and \( c = W^T \cdot x(0) \). The rank \( s \) of the stoichiometric matrix \( N \) can be found by reducing it to row echelon form. In the row echelon form each
leading entry in a column is to the right of the leading entry in the previous row and rows with all zeros are below rows with non-zero elements. This is done by row operations, i.e. arithmetic computations replacing each row until row echelon form is satisfied. Row echelon form of $N$ from (1.16) for the example is

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

(1.23)

Since the rank of $N$ is $s = \text{rank}(N) = 2$, number of non-zero rows, and the number of species is $n = 4$ it is inferred that the matrix $W \in \mathbb{R}^{4 \times 2}$. Further, the transpose of $W$ is $W^T \in \mathbb{R}^{2 \times 4}$ which is substituted into (1.22) to confirm the entries satisfy

$$
W^T \cdot N = 0
$$

(1.24)

$$
[w_{ij}]^T \cdot 
\begin{bmatrix}
-1 & 1 & 1 \\
1 & -1 & -1 \\
-1 & 1 & 0 \\
0 & 0 & 1
\end{bmatrix}
= 0
$$

(1.25)

Next, $W$ is determined by establishing a system of linear combinations of differential equations that equal to zero.

$$
\dot{x}_1(t) + \dot{x}_2(t) = 0
$$

(1.26)

$$
\dot{x}_2(t) + \dot{x}_3(t) + \dot{x}_4(t) = 0
$$

(1.27)
This suggests that $W$ satisfying (1.22) leads to the following matrix

$$W = \begin{bmatrix} 1 & 1 & 0 & 0 \\ 0 & 1 & 1 & 1 \end{bmatrix}$$ (1.28)

Finally, integration, i.e. anti-differentiation, must be used to obtain the reaction network’s conservation relations. Equations (1.26) and (1.27) are easily integrated from 0 to $t$ to find our conservation relations for $c_1$, $c_2$ respectively.

$$\int_0^t \dot{x}_1(t) + \dot{x}_2(t)dt = \int_0^t 0dt$$ (1.29)

$$x_1(t) - x_1(0) + x_2(t) - x_2(0) = 0$$ (1.30)

$$x_1(t) + x_2(t) = x_1(0) + x_2(0)$$ (1.31)

$$x_1(t) + x_2(t) = c_1$$ (1.32)

Similarly, we obtain the second conservation relation

$$\int_0^t \dot{x}_2 + \dot{x}_3 + \dot{x}_4 dt = \int_0^t 0 dt$$ (1.33)

$$x_2(t) - x_2(0) + x_3(t) - x_3(0) + x_4(t) - x_4(0) = 0$$ (1.34)

$$x_2(t) + x_3(t) + x_4(t) = x_2(0) + x_3(0) + x_4(0)$$ (1.35)

$$x_2(t) + x_3(t) + x_4(t) = c_2$$ (1.36)

The linear equations (1.32) and (1.36) are the conservation relations for the reaction network consisting of (1.1) and (1.2).
1.3 General Model

Overall, the work and concepts introduced here can be applied to any generic mass action kinetic network in biology or chemistry.

A series of steps are required to create a thorough model including

1. Given a chemical reaction network representing a biological process made up of \( m \) reactions and \( n \) chemical species \( A_1, A_2, \ldots, A_n \), it can be succinctly represented by the formula:

\[
\sum_{i=1}^{n} \alpha_{ij} A_i \xrightarrow{k_j} \sum_{i=1}^{n} \beta_{ij} A_i, \quad j = 1, 2, \ldots, m. \tag{1.37}
\]

Both \( \alpha_{ij} \geq 0, \beta_{ij} \geq 0 \) are the stoichiometric coefficients that are small integers and \( k_j > 0 \) the rate constant of a reaction.

2. Next, denote each concentrate of species, \([A_i]\), as \( x_i(t) \), a function of time, \( t \), for each \( i = 1, \ldots, n \), to be used as a variable function to model the rate of change in an ordinary differential equation system.

3. Then express a rate law for each reaction with the assumption that they are governed by the law of mass action, i.e. that the reaction rate is proportional to the product of each species concentration raised to the power of their molecularity. Therefore, the rate law of each elementary reaction is simply a product of the concentration, \( x_i \), raised to \( \alpha_{ij} \), multiplied by the rate constant of reaction \( j, k_j \), for each \( i = 1, \ldots, n \) and \( j = 1, \ldots, m \). The rate law of a given network is detailed by the formula:

\[
r_j(k, x) = k_j \prod_i x_i^{\alpha_{ij}}, \quad j = 1, 2, \ldots, m. \tag{1.38}
\]
Vector of rate functions, an $m \times 1$ matrix, are referred to
\[ r(k, x) = (r_1(k, x), r_2(k, x), ..., r_m(k, x)) \]. The stoichiometric coefficients $\alpha_{ij}$ are used to
determine the kinetic order matrix $Y = [\alpha_{ij}]$ where $Y \in \mathbb{R}^{n \times m}$.

4. Construct a stoichiometric matrix containing $n$ rows and $m$ columns corresponding to
the number of species, $x_i$, and to the number of reactions, $r_j$, for each $i = 1, ..., n$ and
$j = 1, ..., m$. The net production of species $x_i$ in reaction $r_j$, labelled $N_{ij}$, is called the
stoichiometry. The $N_{ij}$ form an $n \times m$ stoichiometric matrix, which we will refer to as
$N$, and each entry can be determined by the difference of the stoichiometry coefficients:

\[ N_{ij} = \beta_{ij} - \alpha_{ij} \]  \hspace{1cm} (1.39)

The stoichiometric coefficients, $\alpha_{ij} \geq 0$ and $\beta_{ij} \geq 0$, are integer coefficients of the
chemical reactions in a given network.

5. Subsequently, the ordinary differential equation system of a given chemical reaction
network is represented by the compact formula:

\[ \dot{x}(t) = N \cdot r(k, x) \]  \hspace{1cm} (1.40)

6. Conservation of mass in a given reaction network is determined by $W^T$ from the equa-
tion:

\[ W^T \cdot N = 0 \]  \hspace{1cm} (1.41)

Here, $W^T x = c$, such that $c = W^T x(0)$, constitutes the conservation relations for
species of the elementary reactions in the network (1.37) where the matrix
$W \in \mathbb{R}^n \times \mathbb{R}^{n-s}$ with $s = \text{rank}(N)$. 
CHAPTER 2
PHOSPHORYLATION NETWORKS

For cells to continuously function properly, the protein activity has to be modified constantly. One mechanism by which protein activity is highly regulated within a cell is known as phosphorylation [6]. Phosphorylation is possible when adenosine triphosphate (ATP) breaks down into adenosine diphosphate (ADP) as a result of cellular energy making a phosphate group available [6]. The process of phosphorylation occurs when a phosphate group is added to an amino acid side chain, which is structurally made up of a terminal hydroxyl group [6]. Specifically, serine, threonine, and tyrosine are the three common amino acid residues able to accept a phosphate group where binding triggers the process of phosphorylation [7]. Phosphorylation is essential for a change in the protein’s shape leading to an increase, decrease, or support in protein activity within that cell [6]. Protein phosphorylation is also reversible and occurs when the phosphate group is removed from the active site of the protein. This type of reaction is referred to as dephosphorylation and has the opposite effect on protein activity of the original reaction [6]. Depending on the protein and its function, phosphorylation or dephosphorylation can turn on or off the protein’s activity [6].

Phosphorylation and dephosphorylation of protein substrates require the assistance of activated enzymes [8]. Specifically, an enzyme known as a protein kinase adds the phosphate group in phosphorylation and the enzyme phosphatase removes the phosphate group in dephosphorylation [8]. The figure below illustrates the activity of a protein kinase and phosphatase in phosphorylation/dephosphorylation reactions.
Figure 2.1: Single Phosphorylation/Dephosphorylation Reaction

(a) exposed polar end of the amino acid

(b) phosphate group transferred by protein kinase

(c) bound phosphate group (P)

(d) removed by phosphatase

(e) serine, threonine, tyrosine are amino acid side chains

While some phosphorylation/dephosphorylation reaction involve only a single phosphate group to be added or removed, protein substrates in intracellular signaling may require two or more phosphatase groups to be added or removed [9]. This type of reaction, the addition or subtraction of two phosphate groups, is known as dual phosphorylation/dephosphorylation.
respectively [9]. When a protein substrate requires more than two phosphatase groups to be added or removed the process is known as *multisite phosphorylation/dephosphorylation* [10]. This paper will continue with its focus on the process of dual phosphorylation/dephosphorylation.

In their paper, Ferrel, Jr. and Bhatt present two processes for dual phosphorylation/dephosphorylation [9]. In the first mechanism, one protein kinase or phosphatase is utilized. In the dual phosphorylation reaction, a protein kinase picks up two available phosphatase groups, collides with a protein substrate and then transfers each phosphate group one at a time. Specifically, a *bind and slide* or *processive mechanism* is adapted here [9]. Once the first phosphate group is transferred, it is believed that while still bound, the protein kinase slides to the next active site and transfers the second phosphate group. The same occurs in dephosphorylation with one active phosphatase [9].

Unlike the bind and slide mechanism, the second type of dual phosphorylation/dephosphorylation discussed in Ferrel Jr. and Bhatt, requires two protein kinases/phosphatases to be active [9]. This is referred to as a *distributive mechanism*. In this type of process, an activated protein kinase binds and adds the first phosphate group before detaching. A second activated protein kinase follows by binding and transferring the required second phosphate group. Once both phosphate groups are bound to the protein substrate, protein activity is either turned on or shut off. In dual dephosphorylation, the mechanism is distributive when two phosphatases are needed to remove the two bound phosphate groups [9]. The action of the enzymes is the same as described here.

Since protein substrates along a signaling pathway are activated/deactivated by dual phosphorylation/dephosphorylation, it is important to understand which mechanism is acting for the response. In their paper, Conradi and Mincheva contend that dual phosphorylation of protein substrates in signaling pathways is a distributive one rather than a processive one [11]. Conradi and Mincheva further state that a distributive mechanism leads to bistability, one in which two stable steady states exists meets the conditions necessary for ensuring
the right response, on or off of protein activity, occurs [11]. Conradi and Mincheva also show in their paper that the presence of at least two positive steady states also known as multistationarity signals that dual phosphorylation of protein substrates in cellular pathways is distributive and not a processive one [11].
CHAPTER 3
ANALYZING A DUAL
PHOSPHORYLATION/DEPHOSPHORYLATION MODEL

In Chapter 1, a series of steps for constructing a general biological reaction network model was established. These same steps are applied here to build a model representative of a dual phosphorylation/dephosphorylation reaction network system. The model adopts key concepts and work from Mincheva and Conradi’s research in [11]. The model also utilizes convex parameters and degree theory to obtain the conditions of multistationarity as a key component in dual phosphorylation/dephosphorylation reaction network.

3.1 Mathematical Model of a Dual
Phosphorylation/Dephosphorylation Network

A dual phosphorylation/dephosphorylation reaction network model is illustrated in (3.1), (3.2), (3.3), and (3.4) as in [11]. The model involves four sets of reactions, comprising of twelve separate reactions. Each set includes a reversible and irreversible reaction. Phosphorylation reactions consist of (3.1) and (3.2) and dephosphorylation reactions consist of (3.3) and (3.4). The aforementioned reactions are

\[
A + E_1 \xrightleftharpoons[k_2]{k_1} AE_1 \xrightarrow{k_3} A_p + E_1 \quad (3.1)
\]

\[
A_p + E_1 \xrightleftharpoons[k_4]{k_5} A_pE_1 \xrightarrow{k_6} A_{pp} + E_1 \quad (3.2)
\]
\[ A_{pp} + E_2 \xrightleftharpoons[k_8]{k_7} A_{pp}E_2 \xrightarrow{k_9} A_p + E_2 \]  
(3.3)

\[ A_p + E_2 \xrightleftharpoons[k_{11}]{k_{10}} A_pE_2 \xrightarrow{k_{12}} A + E_2 \]  
(3.4)

Information given in the reactions demonstrate what occurs in the network model. Specifically, an activated protein kinase \([E_1]\) in (3.1) collides and binds to substrate \(A\) in the first reaction and leads to the complex \(AE_1\). Once phosphorylation occurs, kinase \(E_1\) and \(A_p\), the partially phosphorylated substrate, are released. In reaction (3.2), the \(A_p\) product substrate from (3.1) is phosphorylated by a second activated kinase, \(E_1\). The final products released in the reaction are \(E_1\) and \(A_{pp}\) showing the completion of the dual phosphorylation event. In contrast, reactions (3.3) and (3.4) remove the phosphate groups with activated enzymes called phosphatases designated as \(E_2\). The final product released include \(E_2\) and substrate \(A\) in reaction (3.4).

The table below summarizes the main steps in the reaction network.

| Reactants | Enzyme \((E_1)\) + Substrate \((A)\) | Enzyme \((E_2)\) + Substrate \((A_{pp})\) | Reaction Type | Reaction Type | Reaction Type |
|-----------|-----------------------------------|-------------------------------------|---------------|---------------|
| \(E_1\) + \(A\) | \(E_1\) + \(A_p\) | Reversible | Adds phosphate group | Monophosphorylated | Irreversible |
| \(E_2\) + \(A_{pp}\) | \(E_2\) + \(A_p\) | Reversible | Removes phosphate group | Monodephosphorylated | Irreversible |

Next, the ODE model of the dual phosphorylation reaction (3.1)-(3.4) is constructed. First, each species concentration will be denoted as

\[ x_1 = [A] \quad x_2 = [E_1] \quad x_3 = [AE_1] \]

\[ x_4 = [A_p] \quad x_5 = [A_pE_1] \quad x_6 = [A_{pp}] \]

\[ x_7 = [E_2] \quad x_8 = [A_{pp}E_2] \quad x_9 = [A_pE_2] \]

Then, relying on mass action kinetics, the reaction rates are described as
Then the vector of reaction rates is given as \( r(k, x) = (r_1, r_2, ..., r_{12})^T \).

Recall the stoichiometric matrix in [11] for the dual phosphorylation/dephosphorylation reaction is constructed as follows

\[
N = \begin{bmatrix}
-1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
-1 & 1 & 1 & -1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 \\
1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & -1 & 1 & 0 & 0 & 1 & -1 & 1 & 0 \\
0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 1 & -1 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1
\end{bmatrix}. \tag{3.5}
\]
Using the reaction rate vector $r(k, x)$ and the stoichiometric matrix $N$ the ordinary differential equation system, $\dot{x} = N \cdot r(k, x)$, is determined to be

$$\dot{x} = \begin{bmatrix} 
\dot{x}_1 \\
\dot{x}_2 \\
\dot{x}_3 \\
\dot{x}_4 \\
\dot{x}_5 \\
\dot{x}_6 \\
\dot{x}_7 \\
\dot{x}_8 \\
\dot{x}_9 
\end{bmatrix} = \begin{bmatrix} 
-x_1(k_1x_2) + k_2x_3 + k_{12}x_9 \\
-x_2(k_1x_1 + k_4x_4) + k_2x_3 + k_3x_3 + (k_5 + k_6)x_5 \\
-x_3(k_2 + k_3) + k_1x_1x_2 \\
-x_4(k_4x_2 + k_{10}x_7) + k_3x_3 + k_5x_5 + k_9x_8 + k_{11}x_9 \\
-x_5(k_5 + k_6) + k_4x_2x_4 \\
-x_6(k_7x_7) + k_6x_5 + k_8x_8 \\
-x_7(k_{10}x_4 + k_7x_6) + k_8x_8 + k_9x_8 + (k_11 + k_{12})x_9 \\
-x_8(k_8 + k_9) + k_7x_6x_7 \\
-x_9(k_{11} + k_{12}) + k_{10}x_4x_7 
\end{bmatrix} \tag{3.6}$$

Next, the mass conservation relations based on the understanding that $W \in R^9 \times R^3$ is determined. To satisfy $W^T \cdot N = 0$, it is necessary to calculate or determine $W^T$. Solving for the matrix $W^T$ produces

$$W^T = \begin{bmatrix} 
0 & 1 & 1 & 0 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 \\
1 & 0 & 1 & 1 & 1 & 1 & 0 & 1 & 1 
\end{bmatrix} \tag{3.7}$$

Since $W^T$ satisfies $W^T \cdot N \equiv 0$ then $W^T \cdot x = c$ where $c$ is a constant vector and the $c_i > 0$ from $i = 1, 2, 3$. Thus, having worked out $W^T \cdot N = 0$ for $W^T$ this means that
the conservation relations for the total concentrations of kinase ($c_1$), phosphatase ($c_2$), and protein substrate ($c_3$) calculated are

$$x_2 + x_3 + x_5 = c_1$$

$$x_7 + x_8 + x_9 = c_2$$

$$x_1 + x_3 + x_4 + x_5 + x_6 + x_8 + x_9 = c_3$$

(3.8)

### 3.2 Multistationarity

A reaction network system is said to exhibit *multistationarity* when two or more positive stable steady states are present in the model. To analyze the model for multistationarity, two parametric components become important in the analysis and those are species concentration and rate constants. Earlier in Chapter 2, steady states were defined biologically. Mathematically, a steady state occurs when the rate of change, $\dot{x} = 0$, is identified to be zero for $t \geq 0$. Methods used in this chapter incorporate guidelines provided in Conradi and Mincheva in [11] for determining when an ODE model exhibits multiple steady states. The next sections in this chapter considers mathematical tools and methods found in [11] that are suitable for investigating the possibility of the existence of multistationarity in the reaction network model of dual phosphorylation/dephosphorylation reaction network system. Specifically, Clarke’s Stoichiometric Network Analysis, Banaji and Pantea’s network modifications for easier computation, determinant of the Jacobian with convex parameters, and degree theory are adopted to determine multistationarity in the reaction network model.
3.2.1 Convex Parameters

Using a systematic approach as in Clarke, the opportunity exists to investigate the relationships between reactants and products in the reactions that make up the network in a model [5]. Clarke’s Stoichiometric Network Analysis (SNA) is a valuable mathematical tool that allows for modifications within the chemical reaction network system [5]. Often, the SNA is applied to analyze the stability part of the model network system. The application takes into account conservation constraints, inflow/outflow equality, in determining stability/instability [5]. Further, convex parameterization is a topic of mathematics that will be used in the SNA. Using the SNA, the results show that the proposed reaction network for some values of convex parameters system exhibits multistationarity. In fact, the model indicates the presence of several steady states for some set of the convex parameters. This section will focus on Stoichiometric Network Analysis which can also be used to investigate the number of steady states in a chemical reaction network [5].

Mathematically, a non-negative steady state is defined as $x^* \in \mathbb{R}_{\geq 0}^n$ where

$$\mathbb{R}_{\geq 0}^n = \{x \in \mathbb{R}^n : x_i \geq 0 \ \forall i = 1, \ldots, n\}.$$ 

The steady state $x^*$ must satisfy $\dot{x} = N \cdot r(k, x^*) = 0$ such that

$$N \cdot r(k, x^*) = 0, \ r \geq 0 \ (3.9)$$

where each component of $r$ is non-negative because $r = r(k, x^*)$ and $x^* \in \mathbb{R}_{\geq 0}^n$. Similarly, we define a positive steady $x^*$ such that $\dot{x} = N \cdot r(k, x^*) = 0$ and all $x_i^* > 0$, $i = 1, 2, \ldots, n$.

In this section ahead, a convex polyhedral cone is constructed. The convex polyhedral cone represents the set of non-negative rate functions $r \geq 0$ in the chemical reaction network.

First, a convex set $E$ is defined where $E \subset \mathbb{R}^n$. The set $E$ is convex only if it contains a line segment for all $x, y \in E$ then $z = \alpha x + (1 - \alpha)y \in E$ where $\alpha \in [0, 1]$. An extreme point $z$ of the convex set $E$ exists if $z = x = y$ for $\alpha \in [0, 1]$. 

A convex cone can be constructed using the set $E$ if $x \in E$ then $\lambda x \in E$ for $\lambda \geq 0$ [19]. Therefore, if $\{x \in E : \lambda \cdot x \in E, \lambda \geq 0\}$ is a polyhedral set, the convex cone is polyhedral.

A convex polyhedral cone is a set $E \subset R^n$ such that $E = \{x \in R^n : A \cdot x \leq 0, A \in R^{n \times n}\}$. Therefore, the proper form of the solution set is $E = \{r \geq 0 : N \cdot r = 0\}$ for (3.9) which constructs the convex polyhedral cone.

The solutions for (3.9) form a convex polyhedral cone $E = \{r : N \cdot r(k, x^*) = 0\}$, which is referred to as a flux cone by Clarke [5]. The flux cone has a finite number of edges/points $r$ where each of these constitutes an extreme vector/point of the non-empty convex set $E$ since it is polyhedral [5]. Therefore, $E$ is the set of convex combinations of extreme vectors/points that constructs the flux cone.

Clarke refers to the vector $r = r(k, x^*)$ as a flux vector in his theory of SNA [5]. Any flux vector, $r$, is a non-negative linear combination of the extreme vectors $\{E_1, E_2, ..., E_l\}$ [5]. This linear combination is defined as

$$r = \sum_{i=1}^{l} r_i E_i$$

(3.10)

where $l$ is the number of elementary reactions minus both the number of reverse reactions and rank of the stoichiometric matrix, $E$ is a matrix with columns $E_1, ..., E_l$ the extreme vectors, and $r(k, x^*)$ is the vector of rate functions $r_i \geq 0$. The vector of rate functions $(r_1, r_2, ..., r_l)$ evaluated at steady states is considered part of the convex parameters. There is another group of convex parameters in [5] and they are the reciprocal of each positive steady state coordinate, $x_k^* > 0$, denoted by $h_k = \frac{1}{x_k^*}$ for each $k = 1, 2, 3, ..., n$. Therefore, we have a set of convex parameters taking the form $(h, r) = (h_1, ..., h_n, r_1, ..., r_l)$ such that $h \in R^n_{>0}$ and $r \in R_l^{\geq 0}$.
The convex parameterization is applicable in the evaluation of the Jacobian matrix $J(k, x^*)$. The Jacobian matrix, $J(k, x^*)$, using convex parameters is

$$J(k, x^*) = N \cdot \text{diag}(r(k, x^*)) \cdot Y^T \cdot \text{diag}\left(\frac{1}{x^*}\right)$$

(3.11)

The notation $\text{diag}(\ldots)$ introduced here in the Jacobian is a shorthand for a diagonal matrix. Furthermore, it can be expressed functionally as

$$\text{diag} : \mathbb{R}^n \rightarrow \mathbb{R}^{n \times n}$$

(3.12)

illustrated by the following

$$\text{diag}(\alpha, \ldots, \omega) := \begin{bmatrix} \alpha \\ \vdots \\ \omega \end{bmatrix}$$

(3.13)

### 3.2.2 Modifying a Network and Computing the Jacobian

The concept of inheriting multiple positive steady state equilibria researched by Banaji and Pantea is useful in simplifying the search for multistationarity [17]. The authors state that the reverse reactions of a chemical reaction network can be removed or added as dependent reactions [17]. This suggests that if it is shown that there exists multistationarity for a network that contains only forward reactions, multistationarity in the full network exists [17]. This means that based on this research, multistationarity is an inherited property, thus
the dual phosphorylation network can be modified to make computations simpler [17]. The modified reaction network is written below:

\[
A + E_1 \xrightarrow{k_1} AE_1 \xrightarrow{k_3} A_p + E_1 \quad (3.14)
\]

\[
A_p + E_1 \xrightarrow{k_5} A_pE_1 \xrightarrow{k_6} A_{pp} + E_1 \quad (3.15)
\]

\[
A_{pp} + E_2 \xrightarrow{k_8} A_{pp}E_2 \xrightarrow{k_9} A_p + E_2 \quad (3.16)
\]

\[
A_p + E_2 \xrightarrow{k_{11}} A_pE_2 \xrightarrow{k_{12}} A + E_2 \quad (3.17)
\]

Therefore, the reverse reaction rates will be zero, \( r_2 = r_5 = r_8 = r_{11} = 0 \), because \( k_2 = k_5 = k_8 = k_{11} = 0 \) which changes the constant rate vector to the following:

\[
r(k, x) = [k_1x_2, 0, k_3x_3, k_4x_2x_4, 0, k_6x_5, k_7x_6x_7, 0, k_9x_8, k_{10}x_4x_7, 0, k_{12}x_9]^T \quad (3.18)
\]

Subsequently, the ordinary differential equation system also changes. The following is the new system representing the forward reactions of the dual phosphorylation model:

\[
\begin{bmatrix}
\dot{x}_1 \\
\dot{x}_2 \\
\dot{x}_3 \\
\dot{x}_4 \\
\dot{x}_5 \\
\dot{x}_6 \\
\dot{x}_7 \\
\dot{x}_8 \\
\dot{x}_9
\end{bmatrix} =
\begin{bmatrix}
x_1(k_1x_2) + k_{12}x_9 \\
-x_2(k_1x_1 + k_4x_4) + k_6x_5 \\
-k_3x_3 + k_1x_2 \\
-x_4(k_4x_2 + k_{10}x_7) + k_3x_3 + k_9x_8 + k_{11}x_9 \\
-k_6x_5 + k_4x_2x_4 \\
-x_6(k_7x_7 + k_6x_5) \\
-x_7(k_{10}x_4 + k_7x_6) + k_{12}x_9 \\
-k_9x_8 + k_7x_6x_7 \\
-k_{12}x_9 + k_{10}x_4x_7
\end{bmatrix} \quad (3.19)
\]
The stoichiometric matrix also changes accordingly

\[
N = \begin{bmatrix}
-1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
-1 & 1 & 1 & -1 & 0 & 1 & 0 & 0 & 0 & 0 \\
1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & -1 & 0 & 0 & 0 & 1 & -1 & 0 \\
0 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 1 & -1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & -1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
\end{bmatrix}
\]  
(3.20)

The kinetic order matrix was introduced in (1.2.2) and we use it to compute the Jacobian (3.11). The kinetic order matrix for the dual phosphorylation/dephosphorylation reaction network is

\[
Y = \begin{bmatrix}
1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
\end{bmatrix}
\]  
(3.21)
Computing the Jacobian with convex parameters is possible since the reaction rate vector from (3.18), the stoichiometric matrix from (3.20), and the kinetic order matrix from (3.21) have been determined.

The diagonal of the modified constant rate vector is the following expression

$$\text{diag}(r(k, x^*)) = \text{diag}(r_1, 0, r_3, r_4, 0, r_6, r_7, 0, r_9, r_{10}, 0, r_{12})$$  \hspace{1cm} (3.22)$$

Then, the diagonalization of the vector for the set of parameters \(h_k = \frac{1}{x_k}\) for all \(k = 1, 2, \ldots, 9\) leads to

$$\text{diag}(h_1, \ldots, h_9) = \text{diag}\left(\frac{1}{x_1^*}, \ldots, \frac{1}{x_9^*}\right)$$  \hspace{1cm} (3.23)$$

Finally, the Jacobian is computed in Mathematica by the proper implementation of (3.11) and aforementioned matrices. The Jacobian, \(J(k, x^*) = N \cdot \text{diag}(r(k, x^*)) \cdot Y^T \cdot \text{diag}(h_1, \ldots, h_9)\), output is the following

$$J(k, x^*) = \begin{bmatrix}
-h_1 r_1 & -h_2 r_1 & 0 & 0 & 0 & 0 & 0 & 0 & h_9 r_1 \\
-h_1 r_1 & h_2 (-r_1 - r_4) & h_3 r_1 & -h_4 r_1 & h_5 r_4 & 0 & 0 & 0 & 0 \\
h_1 r_1 & h_2 r_1 & -h_3 r_4 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & -h_2 r_4 & h_3 r_1 & h_4 (-r_1 - r_4) & 0 & 0 & -h_7 r_1 & h_8 r_4 & 0 \\
0 & h_2 r_4 & 0 & h_4 r_4 & -h_6 r_4 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & h_5 r_4 & -h_6 r_4 & -h_7 r_4 & 0 & 0 \\
0 & 0 & 0 & -h_4 r_1 & 0 & -h_6 r_4 & h_7 (-r_1 - r_4) & h_8 r_4 & h_9 r_1 \\
0 & 0 & 0 & 0 & 0 & h_6 r_4 & h_7 r_4 & -h_8 r_4 & 0 \\
0 & 0 & 0 & h_4 r_1 & 0 & 0 & h_7 r_1 & 0 & -h_9 r_1
\end{bmatrix}$$  \hspace{1cm} (3.24)$$

The Mathematica syntax used for these calculations is outlined in the Appendix.
### 3.2.3 Degree Theory

One of the applications worth considering for determining whether multistationarity maybe present in a dual phosphorylation/dephosphorylation reaction networks is degree theory. Degree theory is considered most applicable for examining solution sets of differential and algebraic equations. Specifically, the topological definition of degree theory will be applied in this section. Furthermore, the Jacobian's determinant, which defines the degree, is appropriate for examining whatever multistationarity exists in a reaction network. The work here builds on applicable work accomplished by Conradi and Mincheva in [11]. In their work, Conradi and Mincheva take advantage of positive parameterization and degree theory to determine multistationarity, in dual phosphorylation. Further, their paper demonstrates that steady states in dual phosphorylation/dephosphorylation can be determined by utilizing positive parameterization and employing degree theory. In [11], the presence of several steady states or multistationarity in a reaction network system such as for dual phosphorylation depends on the reaction rate constants and total enzyme and substrate concentrations within the network. In this research, multistationarity will be shown using the degree described in this section.

Constructing the degree integrates the Jacobian with convex parameters as defined in Section (3.2.1). The degree requires an open and bounded set $D$ a subset of $\mathbb{R}^n$, $D \subset \mathbb{R}^n$. The closure of $D$ is a compact set represented by the union between set $D$ and its boundary represented by $\partial D$, i.e. $\overline{D} = D \cup \partial D$. Now, let there be a smooth function $f : \overline{D} \mapsto \mathbb{R}^n$ such that $f \in C^1(\overline{D})$. As previously discussed, the Jacobian matrix in the modeled network is represented by $J(x) = \left[ \frac{\partial f_i}{\partial x_j} \right]$ and the determinant of this matrix is denoted as $\text{det}(J(x))$. Then let $\text{det}(-J(x))$ be used to avoid the multiplication by $(-1)^n$ [11]. The regular point $x$ for a function $f(x)$ only occurs if $\text{det}(-J(x)) \neq 0$ for $x \in D$ [11]. A point $y \in \mathbb{R}^n$ is a regular
value when all of \( x \in D \) for \( f(x) = y \) are regular [11]. Now, if \( y \notin f(\partial D) \) and \( y \) is a regular value, the degree of \( f \) at \( y \) relative to \( D \) is defined as

\[
\deg(f) = \deg(f, D, y) = \sum_{\{x^* : f(x^*) = y\}} \text{sign}(\det(-J(x)))
\]  

(3.25)

where

\[
\text{sign}(x) = \begin{cases} 
-1 & \text{if } x < 0 \\
0 & \text{if } x > 0 \\
1 & \text{if } x > 0 
\end{cases}
\]

The \( \deg(f) \) in (3.25) is the sum over all solutions \( x^* \in D \) of \( f(x^*) = y \), the number of steady states \( f(x^*) = 0 \) if \( y = 0 \), such that the \( \det(-J(x^*)) \neq 0 \) [11]. Otherwise, if \( f(x^*) = y \) has no solutions \( x^* \in D \) we then set \( \deg(f) = 0 \).

Before we introduce the next lemma, we need the definition of a forward invariant set: \( S \) is forward invariant for \( \dot{x} = f(x) \) if whenever \( x(0) \in S \), \( x(t) \in S \), \( \forall t \geq 0 \).

**Lemma 3.2.1.** [11] Let the set \( D \) be bounded, open, convex and forward invariant and the boundary \( \partial D \) of \( D \) does not contain any steady states. Moreover, if \( x \in \partial D \) then \( f(x) \neq 0 \), it follows \( \deg(f, D, 0) = 1 \).

The conservation relations (3.8) for the dual phosphorylation network form the following set

\[
\omega_c = \{ x \in R^9_{\geq 0} : W^T x = W^T x_0 \}
\]  

(3.26)

Whereas the set \( \omega_c \) is convex, compact and forward invariant whilst the boundary, \( \partial \omega_c \), does not contain any steady state solutions of the equation \( \dot{x}(t) = N \cdot r(k, x) = 0 \) as shown in [11]. Using the properties of the set \( \omega_c \) as defined by Lemma (3.2.1) we obtain the following corollary.
Corollary 3.2.1.1. [11] Let \( \dot{x} = f(x) \) defined in Section (3.6). Then, \( \text{deg}(f, D, 0) = 1 \) where \( D \) is the set \( \omega_c \). If \( \det(-J(x)) \) changes sign from positive to negative while \( x \in D \), then \( D \) contains more than one regular steady state and if all steady states are regular, then the number of steady states will be odd.

3.2.3.1 Projection of the Jacobian onto the \((\xi, \eta)\) Parameter Space

We will project the Jacobian onto the parameter space \((\xi, \eta)\). This is transformation through a change of variables. This transformation is represented by a set of matrices, \((S, Z)\), of full rank such that the columns of \( S \) are an orthonormal basis of \( \text{im}(N) \) and the columns of \( Z \) are an orthonormal basis of \( \text{im}(N)^\perp \). This leads to \( x = x(\xi, \eta) = S \cdot \xi + Z \cdot \eta \).

Let’s consider the function \( g_\eta(k, \xi) = S^T N r(k, x(\xi, \eta)) \) with the dynamical system to be

\[
\dot{\xi} = g_\eta(k, \xi)
\]  

(3.27)

such that \( \xi(0) = S^T x_0, \eta = \eta_0 = Z^T x_0 \). It follows that the solutions of \( \xi(k, \xi_0) \) corresponding to solutions of \( x(\xi, \eta) \) remain in \( \Omega_\eta = \{\xi \in \mathbb{R}^r : S \xi \geq -Z \eta\} \).

The Jacobian \( J(k, x) \) and \( G_\eta(k, \xi) \) denote the Jacobian matrices of (1.40). It follows from \( \text{im}(Z) \) the orthogonal complement of \( \text{im}(S) \in \mathbb{R}^n \) where \( n \) represents the order of \( N \). Therefore, \( \mathbb{R}^n = \text{im}(S) \oplus \text{im}(S)^\perp = \text{im}(S) \oplus \text{im}(Z) \). By definition of \( g_\eta(k, \xi) \) the Jacobian matrix is given by \( G_\eta = S^T J(k, x(\xi, \eta)) S \). According to Conradi and Mincheva in [11] \( \det(-G_\eta(k, \xi)) = \det(-S^T J(k, \xi) S) = a_r(k, x) \) is the last non-zero coefficient of (3.29). To conclude, the coefficient factored from the characteristic polynomial is used in place of the \( \det(-J(x)) \) in the degree theory arguments.
The characteristic polynomial of the Jacobian matrix $J(k, x^*)$ was computed using Mathematica as

$$p(\lambda) = det(\lambda I_n - J(k, x^*))$$

(3.28)

where $I_n$ is the $n \times n$ identity matrix. The last non-zero coefficient (3.28) will be used to identify the existence and number of steady states in the modeled network. Furthermore,

$$p(\lambda) = \lambda^{n-r}(\lambda^r + a_1\lambda^{r-1} + \ldots + a_{r-1}\lambda + a_r) = \lambda^{n-r}q(\lambda)$$

(3.29)

is a simplified characteristic polynomial where $a_i = a_i(k, x), i = 1, \ldots, r$ such that $n$ is the number of rows of $N$ and $r = rank(N)$. The number of species is $n = 9$ and the rank of $N$ is $r = rank(N) = 6$ which leads to

$$p(\lambda) = \lambda^{9-6}q(\lambda) = \lambda^3q(\lambda).$$

Moreover, simplification of $p(\lambda)$ by successively factoring $\lambda^3, r_1^3, r_4^3, h_2,$ and $h_7$ into Mathematica yields the following negative coefficient for the leading term in $a_6$

$$-(h_3h_8 + h_1(h_4 + h_6))(h_3h_8 - h_5h_9).$$

(3.30)

Thus, we can conclude that $h_3h_8 - h_5h_9 < 0$, $a_6(h, r_1, r_4) < 0$ for large values of $h_2, h_7$.

### 3.2.3.2 Application of Degree Theory to the Concept of Multistationarity

In this section, Theorem 1 and Corollary 1 from Conradi and Mincheva in [11] are modified and applied to the factored coefficient from the characteristic polynomial. The following theorem summarizes the conditions necessary for a chemical reaction network to have mult-
tistationarity. The proof of the theorem follows from the previous lemmas and corollaries in
this section.

**Theorem 3.2.2.** If for some positive parameter values $a_6(k, x)$ satisfy $\text{sign}(a_6(k, x)) < 0$
then that means there exist values of total concentrations $c$ such that the system exhibits
multiple positive steady states. In other words, there exists $c \in \mathbb{R}_{>0}$ and $x^*_k \in \omega_c$ such that
$N \cdot r(k, x^*_k) = 0$ for $k = 1 > 0$.

**Proof.** Using (3.30) we know there exists a steady state $x^*$ such that $a_6(k, x^*) < 0$. By
definition of degree (3.25), Lemma (3.2.1.1), and Corollary (3.2.1.1) it follows that

$$1 = \sum_{\{x^*: f(x^*)=0\}} \text{sign}(a_6(k, x^*)) = 1 - 1 + 1 - 1 + 1 \ldots$$

which contains an odd number of positive, negative 1’s. Since the degree is the sum over
steady states, there are multiple positive steady states $x^*$, $x^*_i = \frac{1}{h_i}$ for $i = 1, 2, \ldots, n$.
Furthermore, let the convex parameters $(h, r)$ be such that $a_6(k, x^*)$ is the last non-zero
coefficient of Jacobian’s characteristic polynomial. Pick any such $x^*$ such that $W^T \cdot x^* = c$.
The hyperplane $W^T x = c$ where $W^T x^* = c$ contains multiple positive steady states. This
means that $x^*_k$ for each $k > 1$ satisfy $N \cdot r(k, x^*_k) = 0$. Otherwise, there does not exist a
steady state $x^*$ such that $a_6(k, x^*) < 0$ while

$$1 = \sum_{\{x^*: f(x^*)=0\}} \text{sign}(a_6(k, x^*))$$

will not contain an odd number of positive, negative 1’s and by Corollary (3.2.1.1) there is
no multistationarity. In this case, there exists only one positive steady state. \qed

The dual phosphorylation/dephosphorylation network exhibits multistationarity for each
total concentration protein kinase, phosphatase, and substrate by Theorem (3.2.2). There-
fore, the dual phosphorylation/dephosphorylation system exhibits multistationarity and the concepts learned here can be applied to other models.

### 3.3 Conclusion

A reaction network model of dual phosphorylation/dephosphorylation is presented. Key components of ordinary differential equations, mass action kinetics, the stoichiometric matrix, and mass conservation are utilized to construct the mathematical model. The model and concepts not only examines enzymatic activity, but also explores the possibility that multistationarity explains the behavior of mass action kinetics in a dual phosphorylation/dephosphorylation reaction system. With this in mind, the model first employs Clarke’s theory of SNA to analyze convex parameters which are important in determining or evaluating the Jacobian matrix [5]. Next, a research concept by Banaji and Pantea in [17] is utilized in which all reactions were modified to be forward in the network model. Finally, building on previous work completed by Conradi and Mincheva in [11], the degree is constructed. Computations were completed and the Jacobian was calculated in a common mathematical software named Mathematica. Mathematica is used to compute large matrices and simplify the large characteristic polynomials to obtain the last non-zero coefficient. The resulting sign of the degree provided proof that multistationarity is present in dual phosphorylation/dephosphorylation.
REFERENCES


APPENDIX

MATHEMATICA CODE
The Mathematica code outlined below is numbered by cell input. It is important to note that http://reference.wolfram.com/language/ref is a great reference site regarding the built-in functions used for this thesis. Also, $x^*$ is used in place of $\lambda$ for the characteristic polynomial whilst using Mathematica.

1. \(Nmat = \{\{-1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1\}, \{-1, 0, 1, -1, 0, 1, 0, 0, 0, 0, 0, 0\}, \{1, 0, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0\}, \{0, 0, 1, -1, 0, 0, 0, 1, -1, 0, 0, 0\}, \{0, 0, 0, 0, 0, 0, -1, 0, 1, -1, 0, 1\}\}

\(Nmat \) // MatrixForm

\(Ymat = \{\{1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0\}, \{1, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0\}, \{0, 0, 1, 0, 0, 0, 0, 0, 0, 1, 0, 0\}, \{0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0\}, \{0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0\}\}

\(Ymat \) // MatrixForm

\(Hmat = \{h_1, h_2, h_3, h_4, h_5, h_6, h_7, h_8, h_9\}\)

\(Rmat = \{r_1, r_2, r_3, r_4, r_5, r_6, r_7, r_8, r_9, r_{10}, r_{11}, r_{12}\}\)

\(Rmat \) // MatrixForm

2. \(Nmat.Rmat \) // MatrixForm

\(NSolve[Nmat.Rmat == 0]\)

\(JacMat = \text{DiagonalMatrix}\\{r_1, 0, r_1, r_4, 0, r_4, 0, r_4, 0, r_1, 0, r_1\\}\)

\(JacMat \) // MatrixForm

3. \(\text{MatrixRank}[JacMat]\)

\(\text{MatrixRank}[Nmat]\)
4. Det[-JacMat]

\[ \text{JacMatPolynomial} = \text{CharacteristicPolynomial}[\text{JacMat}, x^*] \]
\[ \text{CPolyReductionXThree} = \text{Coefficient}[\text{JacMatPolynomial}, x^*, 3] \]
\[ \text{CPolyReductionROneThree} = \text{Coefficient}[\text{CPolyReductionX}, r_1, 3] \]
\[ \text{CPolyReductionRFourThree} = \text{Coefficient}[\text{CPolyReductionROneThree}, r_4, 3] \]
\[ \text{CPolyReductionHTwo} = \text{Coefficient}[\text{CPolyReductionRFourThree}, h_2] \]
\[ \text{CPolyReductionHSeven} = \text{Coefficient}[\text{CPolyReductionHTwo}, h_7] \]
\[ \text{FullSimplify}[\%\text{Line}\#] \]