Averaging and Monotonicity Analysis of $Ca^{2+}$/Calmodulin-Dependent Protein Kinase-Phosphatase System

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Ming Wu
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This thesis titled
Averaging and Monotonicity Analysis of $Ca^{2+}$/Calmodulin-Dependent Protein Kinase-Phosphatase System

by
MING WU

has been approved for
the School of Electrical Engineering and Computer Science
and the Russ College of Engineering and Technology by

Douglas A. Lawrence
Professor of Electrical Engineering and Computer Science

Dennis Irwin
Dean, Russ College of Engineering and Technology
**ABSTRACT**

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**Averaging and Monotonicity Analysis of Ca\(^{2+}\)/Calmodulin-Dependent Protein Kinase-Phosphatase System** (152 pp.)

Director of Thesis: Douglas A. Lawrence

Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII) is thought to be a key contributor to the induction of long-term potentiation (LTP). Researchers have developed a variety of mathematical models of CaMKII activation intended to produce simulation outputs that agrees with empirical observations. Our research focuses on one such model to which recent theoretical results for input-output monotone systems are applied. Several key findings in the literature are reproduced using simple algebraic computations as opposed to exhaustive, simulation-based analysis when the system input is constant.

However, the system input is often periodic in experimental settings, so another important part of our research is averaging analysis, which provides us a way to build up an average model that approximates the original system asymptotically as the perturbation tends to zero. Meanwhile, we intend to establish that the CaMKII activation system acts as a low-pass filter which filters out high frequency components in the input signal. Thus the CaMKII activation system with a periodic input can be approximated by an averaged system with a constant input. In this way, not only is the computational burden of the simulation greatly reduced, but also the system analysis can be simplified significantly.

Approved: ________________________________

Douglas A. Lawrence

Professor of Electrical Engineering and Computer Science
This thesis is dedicated to my father

whom I lost forever in the summer of 2006
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1 Introduction

Presently cell biology is one of the most heavily researched fields. How cells interact with the environment and other cells is a very complex process which involves many physical and chemical reactions. As more and more research results have revealed, the similarity between living cells and basic input/output control systems is astonishing. Thus, a new inter-disciplinary topic called systems biology has been developed to study the biological system from a system engineer’s perspective [3].

The objective of systems biology is to reveal and conceptualize basic dynamic processes underlying biological systems based on biological knowledge and experiments [4]. For instance, a cellular network can be modeled mathematically using methods coming from chemical kinetics and control theory. It also helps researchers to get system-level understanding of biological systems, which includes the structure, dynamics, control methods, and design methods of the system [5][6].

From the perspective of a system engineer, a living cell can be viewed as many interconnected networks which interact among proteins, RNA, DNA, and other smaller molecules. Due to the large number of parameters, variables and constraints involved in modeling cellular networks, numerical and computational methods are usually used in the modeling and simulating process. Moreover, viewed as an input-output system, the networks take environmental signals as inputs and produce appropriate responses to maintain the normal functions of the cell [7]. From control theory, an input-output system built out of interconnected simpler components is a natural analogy of a cellular network. As these kind of input-output systems have been well studied in control and system theories, engineers now have many proven effective methods to study the cellular dynamic systems, which makes system-level studies of the cellular system possible. The most direct and natural approach of studying cellular systems can be summarized as the following four steps [3]: modeling of the system, designing of large-scale simulation
models, simulating and analyzing the system, and reducing the dimension of the system models if possible.

The approach in most cases will produce a complicated large-scale model and require extensive numerical computations and simulations. For real-world biological dynamical systems, it is virtually impossible to perform experimental validation of the designed model and extremely hard to obtain accurate estimation of coefficients in vivo. In addition, due to the large scale of the model and the limitation of numerical algorithms, simulation outputs cannot be guaranteed to be accurate. All these difficulties have driven researchers to look for a more efficient analysis tool using which researchers can apply simple algebraic computations to study system properties. That will significantly reduce the time and computational burden in analyzing cellular dynamic systems.

1.1 Research Review

Systems biology is a broad field of study which includes many research trends that are actively investigated in bioscience. Here we will only briefly review one of them, i.e. the research efforts of applying monotone system theories to the study of biological dynamic systems.

Although there is a long history of applying monotone methods to dynamic systems since the 1920’s, the monotone systems theory was developed only about three decades ago by M.W. Hirsch. In his six-parts series of papers entitled ”Systems of differential equations which are competitive or cooperative”, he developed a theory which is now referred to as traditional monotone dynamic systems theory. Readers can get familiar with the theory via the textbook by Smith [8] and several papers by Hirsch and Smale [9][10][11]. However, the traditional monotone systems theory is defined only for systems with no external input functions, thus it is not a suitable tool to study biological dynamic systems because this kind of systems usually take environmental signals as inputs. The
situation changed once Sontag [12] extended the monotone system theory to ordinary input/output control systems in 2003. He and other researchers have been trying to apply the theory to the analysis of varieties of dynamical systems [3][13]. Among them, several interesting research topics are:

1. Biochemical interconnected networks. They are the primary interest because the newly extended monotone systems theory is very suitable for these problems which include chemical reaction systems and biological positive-feedback systems [14][15][16][17][18][19][20][21].

2. Methods and algorithms of decomposition of biological networks into monotone subsystems. Monotonicity is a surprisingly strong restriction and monotone systems are hard to find in reality, but researchers can decompose a complicated system into many simpler monotone subsystems and then interconnect them together to build up the whole dynamic system [22][23][24][25].

3. Multi-stability, bifurcations, and hysteresis of positive feedback systems [26][27][28][29][30][31][32][33][34][35].

4. Global convergence of monotone systems [36][37][38][39].

5. Application of monotone systems theory to small gain theorem [40][41][42][43][44], and small perturbation analysis method [45][46][47][48].

1.2 Research Motivation

We know that in all research fields, theories provide researchers fundamental understandings of system behaviors and help them obtain an useful estimation of system responses from different external inputs.

Considered as a typical input/output system, a cell receives environmental information as inputs that may be physical or chemical, and generates measurable outputs to other cells [49]. So each cell can be thought of as a complicated system composed of a
large number of subsystems. As mentioned before, for a really large-scale and complicated system, we can first break it down into several subsystems, each of which we can mathematically model from its behaviors or its internal structures. Then we can compose them in order to get the model for the whole system. This method of decomposition and reconnection has been one of the basic principles in systems and control theory.

In a way, long-term potentiation (LTP) can be defined as a permanent increase of signal transmission between two cells that results from stimulating them at the same time. LTP has been widely studied because it has been considered as one of the major mechanisms that underlies learning and memory. An important characteristic of LTP is that it consists of bistable elements. So, it is natural for researchers to look for some biophysical and biochemical systems that can operate as bistable switches to be candidates of the underlying mechanisms of LTP.

Several decades ago, scholars identified a biochemical system containing CaMKII that acts as a bistable switch over a range of concentrations of intracellular calcium ($[Ca^{2+}]$) and therefore may be the underlying mechanism of LTP’s bistability. The discovery has been widely accepted and validated by experiments.

In 2002, a CaMKII activation model of moderate complexity was developed by Zhabotinsky and it achieved satisfactory simulation outputs of bistability. Independently, Kubota also proposed a similar but more complicated model of CaMKII activation in 2004. Both models were able to reproduce system behaviors that matched empirical data. However, the theoretical analysis was not provided and to compute outputs of the models required extensive computations or tedious experiments. In this context, a research approach is motivated by the interest of studying the underlying theories of biological dynamic systems and of providing a new analysis tool for the systems that does not involve exhaustive computations.
Monotone systems theory has been the first candidate of our research because monotone systems are one of the most important dynamic systems in theoretical biology. The newly extended monotone systems theory that can deal with ordinary input-output systems thus becomes a very suitable choice for the analysis of enzymatic cascade networks and feedback loop problems.

With all considerations, the steps in our research are listed as the following:

1. Building up a model for the CaMKII activation system. We apply the model proposed by Zhabotinsky because of its moderate complexity and it is convenient for us to compare our results with that in his paper.

2. Applying the monotone systems theory to the model.

3. Analyzing the system using simple algebraic calculations rather than tedious numerical computation.

4. Validating the method. There are two ways to validate our research results: by extensive simulations and using experimental data. They will be discussed in detail in later chapters.

Another important part of our research is the averaging analysis, which is motivated by the fact that in biological experiments periodic exogenous inputs are widely applied by researchers. Given this situation, averaging analysis becomes very suitable to simplify the analysis process significantly because we can replace a periodic input with a constant input without losing much accuracy. Thus we can reduce a great deal of computational burden and provide a new method to study the system.

To summarize, our research will focus on the monotonicity analysis and averaging analysis of the CaMKII activation system using Zhabotinsky’s model. The main contributions of the research can be briefly summarized as the following:
(1) applying input-output monotone theories to analytically demonstrate some important properties of a complex biological system, which previously had to be derived numerically;

(2) performing averaging analysis of the system and providing a way to simplify the input-output analysis of complex biological systems.
2 Background

Systems biology is an interdisciplinary field of study which requires researchers to have some levels of knowledge of biology and system theories. In order to facilitate the presentation of the research efforts, some background knowledge will be briefly introduced in this chapter. That will not only help readers refresh their memories, but also get them familiar with the terms and symbols used in the following chapters.

2.1 Biology Background

In this section, some fundamental biology knowledge related to the CaMKII activation model will be introduced. This introductory knowledge can be found in many fundamental biology introductions. The following statements are from a textbook by T. Pollard [49] and several other papers [53][52].

It’s well known that all living organisms are made of cells which are also called the building blocks of life. They are the smallest units that can be regarded as living things. Among the cells, the one that is electrically excitable and processes and transmits signals is called a neuron, which exists in most animals’ nervous system. A neuron consists of a central part called soma, and long outgrowths which include multiple dendrites and an axon. In general, each neuron uses dendrites to carry signals into the soma, and an axon to transmit signals away from the soma towards another neuron.

In an animal’s nervous system, neurons can detect the presence of stimuli and send signals to the central nervous system. Then the central nervous system processes the signals (inputs) and sends responses (outputs) to other parts of the body for action. In the whole process, different signals are transmitted from the neurons to other neurons, which are produced and propagated by chemical ions that generate an electrical charge that moves along the network of neurons.
A neuron’s surface membrane is not uniform and can be divided into several specific areas, which are used to receive and send the signals by gated ion channels which are present within the neurons. Moreover, a neuron communicates with one another by synapses in a process known as synaptic transmission, which is a fundamental topic in neuroscience, and the reader can find detailed descriptions in all basic biology textbooks if interested.

Resulting from simultaneous activity of pre- and post-synaptic elements, long-term potentiation (LTP) is the long-lasting improvement in communication between two neurons. It brings about a facilitation of chemical transmission that lasts for hours in vitro, and can persist for periods of weeks or months in vivo. LTP has been one of the most studied topics in biology since its discovery in the 20th century because it involves a physical change in the structure of neurons, which has been proposed as the underlying mechanism of moving short-term memories into long-term storage, i.e. the process of learning and memory formation. Since neurons communicate via chemical synapses, and memories are believed to be stored within these synapses, LTP is widely considered to be one of the major cellular mechanisms that underlies learning and memory. Experimental data suggest that LTP relates to rewritable intermediate memory, which normally has a property of bistability. Accordingly, researchers proposed that protein phosphorylation models that are bistable might be the underlying mechanism of bistable synaptic memory since a protein phosphorylation model normally consists of a protein kinase that is capable of autophosphorylation and a phosphatase that dephosphorylates the kinase [53].

The dynamics system of protein phosphorylation and dephosphorylation are the focus of our studies, and the particular $Ca^{2+}/$Calmodulin-dependent protein kinase II (CaMKII) is chosen to be our study object because of the abundant available experimental data provided by many biologists.
**Enzyme Catalyzed Reaction**  In basic biochemistry, enzymes are defined as biomolecules that can catalyze chemical reactions and they are generally globular proteins. In enzymatic reactions, the molecules at the beginning of the process are called substrates, and the enzyme converts them into different products. Almost all processes and reactions in a cell require the presence of enzymes to occur at significant rates. The activity of enzymes is mainly determined by their 3D structure. However, many other factors such as temperature, chemical environment, pH value, and the concentration of substrate can also affect it. In addition, some other molecules can affect the enzymes activity too, for instance, inhibitors decrease enzyme activity while activators increase it.

The Michaelis-Menten type reaction, Eqn. 2.1, is often used to model the dephosphorylation reactions [55]. The enzyme (E) binds a substrate (S) and produces a product (P).

\[
E + S \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} ES \overset{k_2}{\underset{k_{-2}}{\rightleftharpoons}} E + P
\]

where \(k_1, k_{-1}, k_2\) and \(k_{-2}\) are the reaction rate constants. Usually, for most enzymes the second backward reaction rate is insignificant (let \(k_{-2} = 0\)).

We assume that the product does not bind to the enzyme and the concentration of the substrate-bound enzyme ([ES]) changes much more slowly than those of the product ([P]) and substrate ([S]). This allows us to set \(d[ES]/dt\) to zero

\[
\frac{d[ES]}{dt} = k_1[E][S] - (k_{-1} + k_2)[ES] \equiv 0
\]

\[
\frac{d[P]}{dt} = k_2[ES]
\]

Another assumption is that the total enzyme concentration ([E]_t) is constant, thus we can write the total concentration of enzyme [E]_t as

\[
[E]_t = [ES] + [E] \equiv const
\]
with some substituting and rearranging of Equation (2.2) and Equation (2.3), we have the well-known Michaelis-Menten equation

$$v = \frac{d[P]}{dt} = \frac{v_{\text{max}}[S]}{K_M + [S]}$$

(2.4)

where $v$ is the product reaction rate, the change of concentration of product in a given period of time. $V_{\text{max}}$ denotes the maximum reaction rate of the enzyme when all the enzyme active sites are bound to substrates, and the amount of substrate needed to achieve a given reaction rate is given by the Michaelis-Menten constant ($K_M$), which is the substrate concentration required for the reaction rate to reach half $V_{\text{max}}$.

Fig. 2.1 shows the saturation curve for an enzyme reaction, the curve relations are between substrate concentration $[S]$ and reaction rate $v$. Here $[S]$ denotes the concentration of substrate $S$, and this notation will be used in the sequel, for example, $[Ca^{2+}]$ denotes the concentration of calcium ion $Ca^{2+}$. Reaction rates depend on
solution conditions and substrate concentration, and saturation occurs as substrate concentration increases.

**Cooperative binding**  In biochemistry, the binding activity of a ligand (atom, ion, or molecule) to a large molecule which has multiple binding sites is often affected by the number of ligands already bound to the same molecule. The cooperativity can be positive, negative, or neither. Hill coefficient $n$ is used to quantify the cooperativity:

- $n > 1$ - Positively cooperative reaction: Once one ligand molecule is bound to the enzyme, its affinity for other ligand molecules increases.
- $n < 1$ - Negatively cooperative reaction: Once one ligand molecule is bound to the enzyme, its affinity for other ligand molecules decreases.
- $n = 1$ - Noncooperative reaction: The affinity of the enzyme for a ligand molecule is not dependent on whether or not other ligand molecules are already bound.

In practice, the Hill equation is used to determine the degree of cooperativity of the binding, and the equation can be written as:

$$\theta = \frac{[L]^n}{K_d + [L]^n} = \frac{[L]^n}{K_A^n + [L]^n} \quad (2.5)$$

where

- $\theta$ - fraction of ligand binding sites filled,
- $[L]$ - Ligand concentration,
- $K_d$ - Apparent dissociation constant,
- $K_A$ - Ligand concentration producing half occupation,
- $n$ - Hill coefficient.

**Protein Phosphorylation**  Phosphorylation is a process of appending a phosphate group ($PO_4$) to a protein or other organic molecule. It is the most common and important form of reversible protein posttranslational modification, and there are up to 30% of all proteins
being phosphorylated at any given time. Dephosphorylation, on the other hand, is a process of removing a phosphate group from a protein or molecule. Many enzymes and receptors are activated or deactivated by phosphorylation and dephosphorylation.

Two kinds of enzymes, kinases and phosphatases, are involved in the phosphorylation and dephosphorylation process. Protein kinases are the effectors of phosphorylation in proteins. In contrast, protein phosphatases are the primary effectors of dephosphorylation.

A protein kinase is an enzyme that modifies other proteins by phosphorylation which usually changes the target protein. A kinase can be turned on or off by binding activator proteins or inhibitor proteins. For instance, $Ca^{2+}/$calmodulin-dependent protein kinases (CaMKII) are protein kinases which are regulated by the $Ca^{2+}/$calmodulin complex. CaMKII is thought as having a ring structure and the following figure, Fig. 2.2 shows the proposed 3-D structure of CaMKII$^1$. From another perspective, a phosphatase is an

\[\text{Figure 2.2: CaMKII 3D structure [2]}\]

enzyme that helps remove a phosphate group from its substrate. Usually, a common phosphatase is alkaline phosphatase $^{[53]}$. Four kinds of protein phosphatases

$^{1}$ I am grateful for the kindness of Dr. M. Neal Waxham from Dept. of Neurobiology and Anatomy, The University of Texas Medical School at Houston. He generously let me use the figure in the thesis.
dephosphorylate CaMKII-P: PP1, PP2A, PP2C, and a specific CaMKII phosphatase. In the dephosphorylation, the activity of phosphatases is not the same. From the experiments of researchers, PP1 accounts for 20% and PP2A for 60% of the total phosphatase activity in cytosol; while in postsynaptic density (PSD) the number is 50% and 8% correspondingly [53]. Moreover, PP1 is the only protein phosphatase that dephosphorylates CaMKII in PSD. Activity of PP1 can also be controlled by $Ca^{2+}/CaM$ via inhibitor 1, calcineurin ($CaN$), and cAMP-dependent protein kinase ($PKA$). Meanwhile, PKA phosphorylates inhibitor 1 (I1) and $CaN$ dephosphorylates it. In addition, Phosphorylated inhibitor-1 (I1P) deactivates PP1.

**Bistable Protein Phosphorylation**  Now we have all the related biology knowledge to explain the bistability of protein phosphorylation with some simple algebraic calculations and figures.

In the following simplified analysis, we assume there exists a protein which can be phosphorylated by a kinase $S$ and dephosphorylated by a phosphatase $I$, and the concentrations of $S$ and $I$ are constant.

\[
P_0 + S \xrightleftharpoons[k_-]{k_+} P_1 + I
\]

We define two states of the protein: $P_0$ denotes unphosphorylated state and is inactive while $P_1$ denotes phosphorylated state and is active. We also define $k_+$ as the forward action rate coefficient and $k_-$ the reverse action rate coefficient.

\[
\frac{d[P_0]}{dt} = -k_+[S][P_0] + k_-[I][P_1]
\]
\[
\frac{d[P_1]}{dt} = k_+[S][P_0] - k_-[I][P_1]
\]

where $[S]$ is the concentration of kinase $S$ and $[I]$ is the concentration of phosphatase $I$.

Let $[P_t] = [P_0] + [P_1]$ be constant and define the phosphorylation action rate as

\[
R_+ = k_+[S][P_0] = k_+[S][P_t] - k_+[S][P_1]
\]
and the dephosphorylation rate is \( R_- = k_- [I] [P_1] \).

In the steady state, we have \( R_+ = R_- \), then

\[
\frac{[P_1]}{[P_t]} = \frac{[S]}{[S] + \frac{k_-[I]}{k_+}} \tag{2.6}
\]

Since all parameters in the equation are constants, we can get the stable state easily. Let \( k_-[I] = k_+ [S] \) then \( [P_1]/[P_t] = 50\% \), as shown in Fig. 2.3. In this situation, the dynamic system shows no bistability. In Fig. 2.3 and 2.4, the horizontal axis shows \( [P_1]/[P_t] \), left vertical axis denotes phosphorylation action rate, and the right vertical axis is the dephosphorylation action rate. However, if we put positive linear feedback into the system as in Fig. 2.4, then we have

\[
R_+ = k_+[S][P_0] + k_2[P_1][P_0] = (k_+[S] + k_2[P_1])([P_t] - [P_1]) \tag{2.7}
\]
where $k_2$ is the feedback reaction rate. The equation changes the forward rate curve from a line to a nonlinear curve, so we can have two stable states and one unstable state provided that parameters are in a limited range, as shown in Fig. 2.4. This type of bistable system has several key properties:

1. The system is bistable only for a limited range of kinetic parameters (Threshold);
2. The system converts continuous change in input $S$ into discontinuous change in output ($P_1$);
3. The path from one stable state (ON) to another state (OFF) is different from that from OFF to ON (Hysteresis);
4. The system stays in ON state after input $S$ is removed if a strong feedback is provided.

The above simple explanation will only give readers a basic idea of bistable protein phosphorylation activation systems, the real problem is more complicated and will be discussed later in the thesis.

### 2.2 Monotone Systems Theory

In this section we will focus on monotone systems, which is regarded as one of the most important classes of dynamic systems in systems biology. Monotone systems are referred as systems whose trajectories preserve a partial ordering on states, and they are usually defined on subsets of ordered Banach spaces. As we mentioned before, Sontag [12] extended the traditional monotone systems theory to dynamic systems with inputs and outputs, which made the monotone systems theory more useful in the study of system biology. In the following, we will briefly introduce some basic knowledge of monotone
Figure 2.4: Bistability of Protein Phosphorylation
systems. For readers who want to know more details of monotone system theories and applications, please refer to other related documents [8][9][10][11].

2.2.1 Mathematical Preliminaries

**Banach spaces** Banach spaces are defined as complete normed vector spaces. This means that a Banach space $B$ is a vector space $V$ over a field $F$ (such as the real field $\mathbb{R}$ or complex field $\mathbb{C}$) with a norm $\| \cdot \|$ such that every Cauchy sequence (with respect to the metric $d(x, y) = \|x - y\|$) in $V$ has a limit in $V$.

**Ordered Set** An ordered set $(T, \succeq)$ is a set $T$ with an order operation “$\succeq$” on it such that the following hold for all $x, y, z \in T$:

1. $x \succeq x$. (reflexivity)
2. If $x \succeq y$ and $y \succeq x$, then $x = y$. (antisymmetry)
3. If $x \succeq y$ and $y \succeq z$, then $x \succeq z$. (transitivity)
4. Either $x \succeq y$ or $y \succeq x$. (comparability)

The order operation above is usually called "total order". In addition, a partially ordered set or a poset is a set taken together with a "partial order" on it, which also has reflexivity, antisymmetry, and transitivity.

The difference between "total order" and "partial order" is that partial order implies that not every pair of elements of a partially ordered set need be related, i.e. for some pairs, sometimes neither element precedes the other in the poset while in total orders every pair is related.

**Positive Cone** Positive Cone $K$ is a nonempty closed subset of a Banach space $B$ and has the following properties:

1. it is a cone, i.e., $\alpha K \subseteq K$ for any positive scalar $\alpha \in F$
2. it is a convex cone, i.e., $K + K \subset K$, given that $K$ is a cone

3. it is a pointed cone, i.e., $K \cap -K = \{0\}$

4. it is positive, i.e., $x \succeq 0$ if $x \in K$

**Ordered Banach Space**  
Ordered Banach Space $(K, B, \succeq)$ is the combination of Banach space $B$, positive cone $K$ and order operation $\succeq$. The order operation is defined as $x_1 \succeq x_2$ iff $x_1 - x_2 \in K$; another strict ordering "$>$" is roughly defined as: $x_1 > x_2$ means that $x_1 \geq x_2$ and $x_1 \neq x_2$. An example of ordered Banach space is $B = \mathbb{R}^n$, $K = \mathbb{R}^n_{\geq 0}$, and for two vectors $x_1 \succeq x_2$ means that each coordinate of $x_1$ is greater than or equal to the corresponding coordinate of $x_2$.

$\mathbb{R}^n_{\geq 0}$ denotes the first orthant of the Euclidean vector space $\mathbb{R}^n$, i.e. the subset of $\mathbb{R}^n$ consisting of n-tuples with all nonnegative components.

**Tangent Cone**  
Tangent Cone is a set of vectors that is tangent to a set at a specified point (in that set). Let $S$ be a subset of a Euclidean space $\mathbb{R}^n$, and $\xi \in S$, then the tangent cone to $S$ at point $\xi$ is denoted by $T_\xi S$, which is a set consists of all limits of type

$$
\lim_{i \to \infty} (1/t_i)(\xi_i - \xi) \text{ such that } \xi_i \to \xi \text{ and } t_i \to 0, \text{ where } \xi_i \in S.
$$

Note that a vector $v \in T_\xi S$ if and only if there is a sequence $v_i \in V$, with $v_i \to v$ and $t_i \to 0$ such that $\xi + t_i v_i \in S$ for all $i$. So, $T_\xi S = \mathbb{R}^n$ when $\xi$ is in the interior of $S$, which means only the boundary points are of interest.

**Approximability Property**  
Let $V = \text{int}X$, the interior of $X$, then for all $\xi_1, \xi_2 \in X$ such that $\xi_1 \succeq \xi_2$, there exist sequences $\xi_1^i, \xi_2^i \subseteq V$ such that $\xi_1^i \succeq \xi_2^i$ for all $i$ and $\xi_1^i \to \xi_1$ and $\xi_2^i \to \xi_2$ as $i \to \infty$. 
2.2.2 Monotone System Properties

Definition 1 [12] A controlled dynamical system given by [12]

\[
\begin{align*}
\dot{x} &= f(x, u) \\
y &= h(x)
\end{align*}
\]  

(2.8)

is monotone if for all \( t \geq 0 \):

\[
u_1(t) \geq u_2(t), x_1(0) \geq x_2(0) \Rightarrow x_1(t) \geq x_2(t)
\]  

(2.9)

and the system is input-output monotone if in addition

\[
y_1(t) \geq y_2(t)
\]  

(2.10)

where map \( f : X \times U \rightarrow X \), and map \( h : X \rightarrow Y \).

\( X \) is state space in some open subset of \( \mathbb{R}^n \) and \( U \) is an ordered input value space.

While \( Y \), the set of output values, is also a subset of some ordered Banach space. Thus we can define ordering in \( X, U \) and \( Y \) respectively.

We assume \( f(x, u) \) is continuous in \( (x, u) \) and locally Lipschitz continuous in \( x \) locally uniformly in \( u \), which can be written as

\[
|f(\xi, u) - f(\zeta, u)| \leq k|\xi - \zeta|
\]  

(2.11)

for all \( \xi, \zeta \in C_1 \subseteq X \) and all \( u \in C_2 \subseteq U \).

Assume that the solution of system \( x(t) = \phi(t, x_0, u) \) with initial condition \( x(0) = x_0 \) is defined for all inputs \( u(.) \) and all times \( t \geq 0 \). This means the set \( X \) is forward invariant and forward complete.

If in system (2.8), we define \( K = \mathbb{R}_{\geq 0}^n \) and \( K_u = \mathbb{R}_{\geq 0}^m \), then the system is called cooperative systems.
Theorem 1 [12] System (2.8) is monotone if and only if for all \( \xi_1, \xi_2 \in V \)
\[
\xi_1 \geq \xi_2, u_1 \geq u_2 \Rightarrow f(\xi_1, u_1) - f(\xi_2, u_2) \in T_{\xi_1-\xi_2}K
\]  
(2.12)

or equivalently
\[
\xi_1 \geq \xi_2 \in \partial K, u_1 \geq u_2 \Rightarrow f(\xi_1, u_1) - f(\xi_2, u_2) \in T_{\xi_1-\xi_2}K
\]  
(2.13)

where "\( \xi_1 - \xi_2 \in \partial K \)" means \( \xi_1 \geq \xi_2 \) and \( \xi_1^i = \xi_2^i \) for at least one \( i \in 1, \ldots, n \), where \( i \)
denotes the \( i^{th} \) element of the vector. If we define \( \xi_1^i = \xi_2^i \) for \( i \in I \) and \( \xi_1^i \geq \xi_2^i \) for
\( i \in \{1, \ldots, n\} - I \), the tangent cone \( T_{\xi_1-\xi_2}K \) consists of all vectors \( v = (v_1, \ldots, v_n) \in R^n \) such
that \( v_i \geq 0 \) for \( i \in I \) and \( v_i \) is arbitrary in \( R \) otherwise. Thus equation(2.13) can be changed into
\[
\xi_1 \geq \xi_2, \xi_1^i = \xi_2^i, u_1 \geq u_2 \Rightarrow f^i(\xi_1, u_1) - f^i(\xi_2, u_2) \geq 0
\]  
(2.14)

Proposition [12] Considering system (2.8), suppose \( U = R^m \) and \( U \) satisfies the
approximability property, and both \( V \) and \( W = \text{int}U \) are order-convex. Assume that \( f \)
is continuously differentiable, then system (2.8) is cooperative if and only if
\[
\frac{\partial f^i}{\partial x^j}(x, u) \geq 0, \forall x \in V, \forall u \in W, \forall i \neq j
\]
\[
\frac{\partial f^i}{\partial u^j}(x, u) \geq 0, \forall x \in V, \forall u \in W
\]  
(2.15)

for all \( i \in 1, \ldots, n \) and \( j \in 1, \ldots, m \).

We can extend the proposition a little further by changing positive cone \( K \) to the
more general orthants. We write orthants as \( K^{(e)} \), the set of all \( x \in R^n \) so that \((-1)^{e_i}x_i \geq 0 \)
where \( e = (e_1, \ldots, e_n) \in (0, 1)^n \). For instance, orthant \( R_{\leq 0} \times R_{\geq 0} \) can be written as \( K^{(e)} \),
where \( e = (1, 0) \), while for \( K = R_{\geq 0} \), \( e = (0) \). Then we have

Corollary [12] Under the assumptions in Proposition, and for the orders induced from
\( K^{(e)} \) and \( K^{(e)} \). Then system (2.8) is monotone if and only if \( \forall i \neq j \)
\[
(-1)^{e_i + e_j} \frac{\partial f^i}{\partial x^j}(x, u) \geq 0, \forall x \in V, \forall u \in W
\]
\[
(-1)^{e_i + e_j} \frac{\partial f^i}{\partial u^j}(x, u) \geq 0, \forall x \in V, \forall u \in W
\]  
(2.16)
for all $i \in 1, \ldots, n$ and $j \in 1, \ldots, m$.

The detailed proofs of Theorem 1, Proposition and Corollary can be found in [12].

In this subsection, we briefly introduced monotone systems and provided a method to identify whether a dynamic system is a monotone system. Please note that monotonicity is a considerably strong restriction and it is difficult to find a monotone system in reality.

### 2.2.3 Input-State and Input-Output Characteristics

**Definition 2** [12] System (2.8) admits a non-degenerate input-state(I/S) characteristics $K_x(\cdot)$ such that for each constant input $u$, there is an unique globally asymptotically stable equilibrium state $K_x(u)$ for which the Jacobian matrix denoted $f_x(K_x(u), u)$ is nonsingular.

We also define input-output(I/O) characteristics by $K_y(u) = h(K_x(u))$.

**Note 1:** If the system (2.8) is monotone and has the I/S characteristics $K_x$, the $K_x$ must be nondecreasing with respect to the orders in equation, i.e.,

$$u \succeq v \Rightarrow K_x(u) \succeq K_x(v) \quad (2.17)$$

**Note 2:** $K_x$ is continuous.

**Definition 3** [12] The I/O characteristics $K_y(\cdot)$ has **non-degenerate fixed points** if for all $u \in U$ with $K_y(u) = u$ we have $K_y'(u)$ exists and $K_y'(u) \neq 1$.

The Input-State and Input-Output characteristics are useful in analyzing monotone systems. In many conditions, they can provide a visual assistance for researchers. For example, Fig. 2.6 shows the I/O characteristics of a monotone system with positive unity feedback. Fig. 2.5 is the block diagram of a positive unity feedback system, where $v$, and $w$ are exogenous input and output which can be used to connect to other systems. Fig. 2.6 demonstrated that the described system has three equilibria; two of them are stable and another is unstable.
The following theorem can provide a global tool for positive unity feedback of monotone systems. The fixed points of the input-output characteristics will play an important role in the analysis.

![Block Diagram of Positive Unity Feedback](image-url)

**Figure 2.5: Block Diagram of Positive Unity Feedback**

**Theorem 2 [28]** For a strongly monotone SISO system in the form of Eqn. 2.8 with a non-degenerate I/S and I/O characteristics, consider the unity positive feedback configuration $u = y$. Then the equilibria are in a 1−1 correspondence with fixed points of the I/O characteristic. Moreover, if $K_y$ has non-degenerate fixed points, the closed-loop system is strongly monotone, and all trajectories are bounded, then for almost all initial conditions, solutions converge to the set of equilibria corresponding to inputs for which $K_y'(u) < 1$.

A typical application of Theorem 2 is when a monotone system with a well-defined I/O characteristic of sigmoidal shape is closed under unitary feedback. If the sigmoidal function is sufficiently steep, this configuration is known to yield three equilibria, two of
them are stable and another is unstable, as shown in Fig. 2.6, where the straight line is the unity feedback. The system is regarded as a multi-stable system.

2.3 Averaging analysis

Averaging is an important tool to analyze time-varying systems. It is the procedure of replacing a vector field by its average value over time with the goal of obtaining an averaged system that approximates the original system asymptotically as the perturbation tends to zero. It should be noted that classical averaging results apply only to systems without inputs. In the thesis we will concentrate on averaging of systems with inputs, especially periodic inputs.

In general, consider an ordinary differential equation with a time-varying input

\[
\dot{x} = f(t, x, u) \\
x(0) = x_0
\]  

(2.18)
where \( x, x_0 \in D \subset \mathbb{R}^n \), \( D \) is an open set with compact closure on which \( f \) is defined. In addition, system input \( u \) is periodic in \( t \) with period \( T \).

\[
 u(t + T) = u(t)
\]

Although the vector field \( f \) is assumed to be differentiable with respect to all variables, this can be relaxed under specific situations.

Since map \( f \) depends explicitly on time \( t \), Eqn. (2.18) is called the non-autonomous differential equation. In most cases, this type of equation is very difficult to analyze, so researchers are interested in finding an averaged autonomous system, the solutions of which approximate the original system asymptotically.

The natural idea of averaging is to average the effect of the periodic input on the system vector field in order to obtain the approximation

\[
 \tilde{f}(x) = \frac{1}{T} \int_0^T f(s, x, u(s))ds.
\]

(2.19)

If the response of a system is much slower than the input excitation, then the response will be mainly determined by the average value of the excitation (i.e. DC component of the input signal) rather than high-frequency components. It can be explained in linear system theory as the bandwidth of system: if the bandwidth of the system is much smaller than the input signal’s bandwidth, the system will act as a low-pass filter that eliminates the high-frequency components of the input signals.
3 CaMKII Activation Model and Monotonicity Analysis

3.1 CaMKII Activation Model

There are several approaches to building a model of a CaMKII activation system, all of them agree with the experimental data and can reproduce the multi-stability phenomenon of the system. In the following, one of the models will be examined in detail to illustrate the method of performing monotonicity and averaging analysis. Here we will study the CaMKII activation system using the model proposed by A. M. Zhabotinsky [1]. The model uses 10 subunits with each subunit occurring in either a phosphorylated or unphosphorylated state. Subunit phosphorylation is governed by two competing processes: autophosphorylation and dephosphoryation by a protein phosphatase. During the process of autophosphorylation, one subunit will act as catalyst and another unit acts as a substrate. It is understandable that some simplifications and assumptions will have to be applied to build the model. As described in [1], we summarize the assumptions as the following:

1. states of other subunits do not affect the binding rate of a \((\text{Ca}^{2+})_4\text{CaM}\) complex to a subunit;

2. cooperative binding of \((\text{Ca}^{2+})_4\text{CaM}\) to subunits can be modeled by the empirical Hill equation;

3. binding of a phosphatase to a phosphorylated subunit is independent of binding of \((\text{Ca}^{2+})_4\text{CaM}\);

4. the rate of dephosphorylation is independent of binding of \((\text{Ca}^{2+})_4\text{CaM}\);

5. binding opportunities of \((\text{Ca}^{2+})_4\text{CaM}\) to the unphosphorylated subunit and phosphorylated subunits are equal.
3.1.1 Autophosphorylation

Autophosphorylation must first be initiated by having two neighboring subunits each bind the calcium-calmodulin complex \((\text{Ca}^{2+})_4\text{CaM}\). This process is described by the reactions

\[
4\text{Ca}^{2+} + \text{CaM} \rightleftharpoons C
\]

\[
P_0 + C \rightleftharpoons P_0C
\]

\[
P_0C + C \rightleftharpoons P_0C_2
\]

\[
P_0C_2 \xrightarrow{k_1} P_1C_2
\]

(3.1)

in which \(C, P_0, P_1\) denote the calcium-calmodulin complex, unphosphorylated CaMKII, and 1-fold phosphorylated CaMKII, respectively.

Cooperative binding of \((\text{Ca}^{2+})_4\text{CaM}\) to CaMKII subunits is modeled by the empirical Hill equation with positive Hill number \(n = 4\) and Hill constant \(K_{H1}\). This yields the fraction of CaMKII subunits bound to \((\text{Ca}^{2+})_4\text{CaM}\).

\[
\theta = \frac{([\text{Ca}^{2+}]/K_{H1})^4}{1 + ([\text{Ca}^{2+}]/K_{H1})^4}
\]

(3.2)

The velocity of the initiation step is given by

\[
V_0 = \frac{10k_1([\text{Ca}^{2+}]/K_{H1})^8}{(1 + ([\text{Ca}^{2+}]/K_{H1})^4)^2p_0}
\]

(3.3)

in which \(k_1\) is the rate constant of the last reaction in Eqn. 3.1 and \(p_0\) is the concentration of \(P_0\).

Since two neighboring subunits are required to initiate autophosphorylation, the second autophosphorylation step is

\[
P_1 + C \rightleftharpoons P_1C
\]

\[
P_1C \xrightarrow{k_1} P_2
\]

(3.4)
with the rate
\[ V_1 = \frac{10k_1([Ca^{2+}]/K_{H1})^8}{(1 + ([Ca^{2+}]/K_{H1})^4)^2p_1} \] (3.5)
in which \( k_1 \) is the rate constant of the last reaction in Eqn. 3.4, which is the same as Eqn. 3.1; and \( p_1 \) is the concentration of \( P_1 \).

Once initiated and otherwise unimpeded, autophosphorylation is assumed to propagate in one direction. Further, under the assumption that the ability of a phosphorylated subunit to act as a catalyst in the phosphorylation of its neighbor is \((Ca2+)_4CaM\)-independent, propagation of autophosphorylation is

\[ P_i + C \rightleftharpoons P_iC \]
\[ P_iC \rightarrow P_{i+1} \] (3.6)

for \( i = 2, \ldots, 9 \) in which \( P_i \) represents \( i \)-fold phosphorylated CaMKII. However, dephosphorylation occurs at random subunit locations which results in a random distribution of phosphorylated and unphosphorylated subunits in the CaMKII enzyme. To address this under the assumption that all distinguishable configurations having the same number of phosphorylated subunits occur with equal probability, an effective number of phosphorylating pairs for \( i \) phosphorylated subunits is determined from

\[ w_i = w_{10-i} = \frac{\sum_{j=1}^{i} j m_j}{\sum_{j=1}^{i} m_j} \quad i = 1, \ldots, 9 \] (3.7)

where \( m_j \) is the number of distinguishable configurations with \( j \) autophosphorylating pairs. This yields the values \( w_1 = w_9 = 1.0, w_2 = w_8 = 1.8, w_3 = w_7 = 2.3, w_4 = w_6 = 2.7, \) and \( w_5 = 2.8 \) [1]. These weights scale the rate of the second reaction in Eqn. 3.6 according to

\[ V_i = w_i \frac{k_1([Ca^{2+}]/K_{H1})^4}{1 + ([Ca^{2+}]/K_{H1})^4} p_i \quad i = 1, \ldots, 10 \] (3.8)

where \( p_i \) is the concentration of \( P_i \).
3.1.2 Dephosphorylation

Of the four protein phosphatases that dephosphorylate phosphorylated CaMKII, PP1 is the only one known to dephosphorylate CaMKII in postsynaptic densities (PSDs) and is the one incorporated into this model. Dephosphorylation proceeds according to the reactions

\[
P_i + PP1 \rightleftharpoons P_i \cdot PP1
\]

\[
P_i \cdot PP1 \rightarrow P_{i-1} + PP1
\]

(3.9)

for \(i = 1, \ldots, 10\). Upon invoking the standard Michaelis-Menten approximation for this class of reactions, the dephosphorylation rate for \(i\)-fold phosphorylated CaMKII is

\[
V_{-i} = \frac{k_2 e_p}{K_M + \sum_{k=1}^{N} kP_k} i p_i
\]

(3.10)

in which \(e_p\) is concentration of free PP1, \(k_2\) is the associated catalytic rate constant, and \(K_M\) is the associated Michaelis constant.

PP1 activity is indirectly influenced by calcium via an inhibitor I1 as follows. The inhibitor I1 is phosphorylated by the c-AMP protein kinase PKA to produce I1P which deactivates PP1. Calcineurin (CaN) is activated by cooperative binding with the calcium-calmodulin complex denoted C3 (with empirical Hill constant \(\approx 3\)). This, in turn, dephosphorylates I1P. This process is represented by the reactions:

\[
I1 + PKA \rightleftharpoons I1 \cdot PKA \rightarrow I1P
\]

\[
3Ca^{2+} + CaM \rightleftharpoons C_3
\]

\[
CaN + C_3 \rightleftharpoons CaN \cdot C_3
\]

\[
I1P + CaN \cdot C_3 \rightleftharpoons I1P \cdot CaN \cdot C_3 \rightarrow I1
\]

\[
P_i + PP1 \rightleftharpoons P_i \cdot PP1 \rightarrow PP1 + P_{i-1}
\]

\[
PP1 + I1P \xrightarrow{k_3}{k_4} PP1 \cdot I1P
\]

\[
P_i \cdot PP1 + I1P \rightleftharpoons P_i \cdot PP1 \cdot I1P
\]

(3.11)
As asserted in [1], when the concentration of free I1 is constant and much less than the Michaelis constant of PKA and the concentration of free I1P is much less than Michaelis constant of CaN then the phosphatase-inhibitor kinetics can be represented by

\[
\dot{e}_p = -k_3 I e_p + k_4 (e_{p0} - e_p)
\]

\[
I = -k_3 I e_p + k_4 (e_{p0} - e_p) + v_{PKA} I_0 - v_{CaN} I \frac{([Ca^{2+}]/K_{H1})^3}{1 + ([Ca^{2+}]/K_{H1})^3}
\]

(3.12)

in which \(e_p\) is the concentration of free PP1, \(e_{p0}\) is the total concentration of PP1, I is the concentration of free I1. Remaining parameters are listed in Table 3.1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Symbol</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Concentration of CaMKII</td>
<td>(e_k)</td>
<td>20</td>
<td>(\mu M)</td>
</tr>
<tr>
<td>Total concentration of protein phosphatase</td>
<td>(e_{p0})</td>
<td>0.05</td>
<td>(\mu M)</td>
</tr>
<tr>
<td>Concentration of free inhibitor 1</td>
<td>(I_0)</td>
<td>0.1</td>
<td>(\mu M)</td>
</tr>
<tr>
<td>Activity of calcineurin divided by its Michaelis constant</td>
<td>(v_{CaN})</td>
<td>1.0</td>
<td>(s^{-1})</td>
</tr>
<tr>
<td>Activity of PKA divided by its Michaelis constant</td>
<td>(v_{PKA})</td>
<td>1.0</td>
<td>(s^{-1})</td>
</tr>
<tr>
<td>The Michaelis constant of protein phosphatase</td>
<td>(K_M)</td>
<td>0.4</td>
<td>(\mu M)</td>
</tr>
<tr>
<td>The (Ca^{2+}) activation Hill constant of CaMKII</td>
<td>(K_{H1})</td>
<td>4.0</td>
<td>(\mu M)</td>
</tr>
<tr>
<td>The (Ca^{2+}) activation Hill constant of calcineurin</td>
<td>(K_{H2})</td>
<td>0.7</td>
<td>(\mu M)</td>
</tr>
<tr>
<td>The catalytic constant of autophosphorylation</td>
<td>(k_1)</td>
<td>0.5</td>
<td>(s^{-1})</td>
</tr>
<tr>
<td>The catalytic constant of protein phosphatase</td>
<td>(k_2)</td>
<td>2.0</td>
<td>(s^{-1})</td>
</tr>
<tr>
<td>The association rate constant of the (PP1 \cdot I1P) complex</td>
<td>(k_3)</td>
<td>1.0</td>
<td>(\mu M^{-1}s^{-1})</td>
</tr>
<tr>
<td>The dissociation rate constant of the (PP1 \cdot I1P) complex</td>
<td>(k_4)</td>
<td>0.001</td>
<td>(s^{-1})</td>
</tr>
</tbody>
</table>
3.1.3 State Equations

The autophosphorylation and dephosphorylation processes described in the preceding subsections are collectively represented by the block diagram in Fig. 3.1. In this subsection, we cast the autophosphorylation and dephosphorylation processes described in the preceding subsections, specifically the rate equations associated with the reaction rates Eqn. 3.3, 3.5, 3.8, and 3.10 in the form of a nonlinear state equation amenable to monotonicity and bistability analysis.

Figure 3.1: Model Block Diagram

For CaMKII activation which initializes autophosphorylation, we introduce the calcium-dependent exogenous input

\[ w_a = \frac{([Ca^{2+}]/K_{H1})^4}{1 + ([Ca^{2+}]/K_{H1})^4} \]

in term of which we define the function

\[ a_0(w_a) = 10k_1 w_a^2 \]

along with constants \( \alpha_i = w_i k_1, i = 1, \ldots, 9. \)

To include the effects of dephosphorylation in the state equation formulation, we set \( w_d = e_p \) for notational consistency (which is generated by the calcium-dependent...
phosphatase-inhibitor kinetics (3.12)) and also define the function

$$\delta(p) = \frac{k_2}{K_M + \sum_{k=1}^{10} k p_k}$$

(3.14)

in which \( p = (p_1, p_2, \ldots, p_{10}) \).

Under the assumption that the total CaMKII concentration is a constant denoted \( p_{tot} \) (= \( e_k \) in Table 3.1), this constraint can be used to eliminate \( p_0 \) from the analysis via

\[ p_0 = p_{tot} - (p_1 + \cdots + p_{10}) \]

and the interaction between autophosphorylation and dephosphorylation is governed by a system of coupled first-order ordinary differential equations in the concentration variables \( p = (p_1, p_2, \ldots, p_{10}) \) which evolve on

\[ \{ p = (p_1, \ldots, p_{10}) \mid p_i \geq 0 \text{ and } p_1 + \cdots + p_{10} \leq p_{tot} \} \]

While \( p_1, p_2, \ldots, p_{10} \) seem to be a natural choice for the state variables, it will prove to be more convenient to adopt \( x_i := p_i + \cdots + p_{10}, \ i = 1, \ldots, 10 \) which yields

\[
\begin{align*}
\dot{x}_1 &= -\alpha_0(w_a)x_1 - \delta(x)w_d(x_1 - x_2) + \alpha_0(w_a)p_{tot} \\
\dot{x}_2 &= \alpha_1 w_a(x_1 - x_2) - 2\delta(x)w_d(x_2 - x_3) \\
\dot{x}_3 &= \alpha_2 w_a(x_2 - x_3) - 3\delta(x)w_d(x_3 - x_4) \\
\dot{x}_4 &= \alpha_3 w_a(x_3 - x_4) - 4\delta(x)w_d(x_4 - x_5) \\
\dot{x}_5 &= \alpha_4 w_a(x_4 - x_5) - 5\delta(x)w_d(x_5 - x_6) \\
\dot{x}_6 &= \alpha_5 w_a(x_5 - x_6) - 6\delta(x)w_d(x_6 - x_7) \\
\dot{x}_7 &= \alpha_6 w_a(x_6 - x_7) - 7\delta(x)w_d(x_7 - x_8) \\
\dot{x}_8 &= \alpha_7 w_a(x_7 - x_8) - 8\delta(x)w_d(x_8 - x_9) \\
\dot{x}_9 &= \alpha_8 w_a(x_8 - x_9) - 9\delta(x)w_d(x_9 - x_{10}) \\
\dot{x}_{10} &= \alpha_9 w_a(x_9 - x_{10}) - 10\delta(x)w_d x_{10}
\end{align*}
\]

(3.15)

in which, with a slight abuse of notation, we now write

$$\delta(x) = \frac{k_2}{K_M + \sum_{k=1}^{10} x_k}$$
By definition of the state variables $x_i$, $i = 1, \ldots, 10$ it follows that $\delta(x) = \delta(p)$. Upon defining, with $e_1, \ldots, e_{10}$ denoting the standard basis vectors on $\mathbb{R}^{10}$,

$$A_d(w_d) = w_d \left( \sum_{i=1}^{9} i e_i (e_i - e_{i+1})^T + 10 e_{10} e_{10}^T \right)$$

and finally

$$B_a(w_a) = \alpha_0(w_a) e_1$$

Eqn. 3.15 can be written compactly as

$$\dot{x} = (A_a(w_a) - \delta(x)A_d(w_d)) x + B_a(w_a)p_{tot}$$

(3.16)

which evolves on the convex, compact subset of $\mathbb{R}^{10}$

$$X = \{ x = (x_1, \ldots, x_{10}) \mid 0 \leq x_{10} \leq \cdots \leq x_1 \leq p_{tot} \}$$

In the analysis to follow, it will be useful to view Eqn. 3.16 as resulting from the open-loop system

$$\dot{x} = (A_d(w_d) - uA_d(w_d)) x + B_d(w_d)p_{tot}$$

$$y = \delta(x)$$

(3.17)

together with unity feedback $u = y$.

### 3.2 Analysis

In this section, we will apply the monotone systems theory established in [56][12] to the dynamic model developed in the preceding section to confirm the bistability properties presented in [1] without resorting to require exhaustive, simulation-based steady-state analysis.

#### 3.2.1 Monotonicity Analysis

Input-Output monotonicity of a dynamic system represented by a nonlinear state equation is defined in terms of closed convex cones in the state, input and output spaces that define a partial ordering on the respective space.
With the transformed state variables \( x_i := p_i + \ldots + p_{10}, i = 1, \ldots, 10 \), we can work with the nonnegative orthant \( K = R_{\geq 0}^{10} \) in the state space. Henceforth, the notation \( x^1 \geq x^2 \) means that \( x^1 - x^2 \in K \) so that each component of \( x^1 \) is greater than or equal to the corresponding component of \( x^2 \). For the inputs \((u, w_a, w_d)\), we also define input cone by \( K_{in} = K^{(1,0,1)} = \{(u, w_a, w_d) \in R^3 | u \leq 0, w_a \geq 0, w_d \leq 0 \} \). Thus \( (u^1, w_a^1, w_d^1) \geq (u^2, w_a^2, w_d^2) \) corresponds to \( (u^1, w_a^1, w_d^1) - (u^2, w_a^2, w_d^2) \in K_{in} \) from which \( u^1 \leq u^2, w_a^1 \geq w_a^2, \) and \( w_d^1 \leq w_d^2 \).

At last, for scalar output \( y \) we define output cone by \( K_{out} = \{ y \in R | y \leq 0 \} \). Thus we can write it directly as \( y^1 \leq y^2 \).

With the definitions, and the monotonicity theory introduced in section 2.2, we can conclude that the system (3.17) is monotone if the following implication holds for all \( t \geq 0 \):

\[
x^1(0) \geq x^2(0)
\]

and

\[
(u^1(t), w_a^1(t), w_d^1(t)) \geq (u^2(t), w_a^2(t), w_d^2(t))
\]

imply that for all \( t \geq 0 \) the respective solutions satisfy

\[
x^1(t) \geq x^2(t)
\]

Meanwhile, the output is monotone if \( x^1 \geq x^2 \) implies \( y^1 \leq y^2 \).

The conclusion can be verified by checking whether the following identities hold for all \( i, j \in \{1, 2, \ldots, 10\} \) for all \( x \) in interior of \( \chi \) and for all \( u > 0, w_a > 0, w_d > 0 \) ([12], Corollary III.3).

\[
\frac{\partial f_i}{\partial x_j}(x, u, w_a, w_d) \geq 0, i \neq j
\]

\[
\frac{\partial f_i}{\partial u}(x, u, w_a, w_d) \leq 0
\]

\[
\frac{\partial f_i}{\partial w_a}(x, u, w_a, w_d) \geq 0
\]

\[
\frac{\partial f_i}{\partial w_d}(x, u, w_a, w_d) \leq 0
\]
For the first set of relationships

\[ \frac{\partial f_i}{\partial x_{i+1}}(x, u, w_a, w_d) = iuw_d \geq 0, \forall i = 1, \ldots, 9 \]

\[ \frac{\partial f_i}{\partial x_{i-1}}(x, u, w_a, w_d) = w_{i-1}k_1w_a \geq 0, \forall i = 2, \ldots, 10 \]

with all other partial derivatives zero for \( i \neq j \). For the second set of relationships,

\[ \frac{\partial f_i}{\partial u}(x, u, w_a, w_d) = -iw_d(x_i - x_{i+1}) \leq 0, \forall i = 1, \ldots, 9 \]

\[ \frac{\partial f_{10}}{\partial u}(x, u, w_a, w_d) = -10w_dx_{10} \leq 0 \]

Next,

\[ \frac{\partial f_i}{\partial w_a}(x, u, w_a, w_d) = 20k_1w_a(p_{tot} - x_1) \geq 0 \]

\[ \frac{\partial f_i}{\partial w_d}(x, u, w_a, w_d) = w_ik_1(x_{i-1} - x_i) \geq 0, \forall i = 2, \ldots, 10 \]

and finally

\[ \frac{\partial f_i}{\partial w_d}(x, u, w_a, w_d) = -iu(x_i - x_{i+1}) \leq 0, \forall i = 1, \ldots, 9 \]

\[ \frac{\partial f_{10}}{\partial w_d}(x, u, w_a, w_d) = -10ux_{10} \leq 0 \]

where these inequalities follow from the definition of the set \( \chi \). Thus we conclude that the system (3.17) is monotone. Also, we observe the output map \( \delta(x) \) is monotone since \( x^1 \geq x^2 \) gives \( \delta(x^1) \leq \delta(x^2) \).

It is of further interest to investigate monotonicity of the overall system consisting of the cascade interconnection of the combined CaMKII and CaN activation subsystems (with input \( v = [Ca^{2+}] \) and output \( (w_a, w_d) \)) and the autophosphorylation-dephosphorylation dynamics (3.15). The former is represented by
the nonlinear state equation derived from (3.12) and (3.13)

$$\dot{e}_p = -k_3 I e_p + k_4 (e_{p0} - e_p)$$

$$\dot{I} = -k_3 I e_p + k_4 (e_{p0} - e_p) + v_{PKA} I_0 - v_{CaN} I \frac{(v/K_{H2})^3}{1 + (v/K_{H2})^3}$$

$$w_a = \frac{(v/K_{H1})^4}{1 + (v/K_{H1})^4}$$

$$w_d = e_p$$

(3.18)

Another application of the infinitesimal monotonicity characterization (3.19) reveals that (3.18) is monotone with respect to the orthant $K_1$

$$K_1 = K^{(1,0)} = \{(\xi_1, \xi_2) \in \mathbb{R}^2 | \xi_1 \leq 0, \xi_2 \geq 0\}$$

for the state $(e_p, I)$ and the negative ordering on $\mathbb{R}$ for the scalar input $v$. Further, the CaMKII/CaN activation subsystems are I/O monotone with respect to the orthant

$$K_{out} = K^{(1,1)} = \{(\xi_1, \xi_2) \in \mathbb{R}^2 | \xi_1 \leq 0, \xi_2 \leq 0\}$$

for the output $(w_a, w_d)$.

$$\frac{\partial f_1}{\partial x_2} (x, v) = -k_3 x_1 \leq 0$$

$$\frac{\partial f_2}{\partial x_1} (x, v) = -k_3 x_2 - k_4 \leq 0$$

$$\frac{\partial f_1}{\partial v} (x, v) = 0 \geq 0$$

$$\frac{\partial f_2}{\partial v} (x, v) = -v_{CaN} x_2 \left[ \frac{3v^2/(K_{H2})^3}{1 + (v/K_{H2})^3} - \frac{3v^5/(K_{H2})^6}{(1 + (v/K_{H2})^3)^2} \right] \leq 0$$

(3.19)

The fact that $K_{out}$ and the $(w_a, w_d)$ portion of $K_{in}$ are incompatible seemingly precludes the possibility that the cascade interconnection of system (3.18) and (3.16) is input-output monotone from the calcium input (with respect to the negative ordering on $\mathbb{R}$) to the composite state with positivity cone $K \times K_1$. Nevertheless, several interesting conclusions can be reached for the unity feedback system (3.17) with $(w_a, w_d)$ treated as external stimuli [28]. For instance, strong monotonicity of the closed-loop system (3.18) for constant $(w_a, w_d)$ can be concluded essentially because the discussion surrounding the
second example in [28] applies to (3.16). Moreover, it follows that \( int(\chi) \) is positively invariant and so trajectories of (3.16) are bounded.

### 3.2.2 I/S and I/O Characteristics

In the case of constant calcium concentration, \( w_a \) is given by equation (3.13) and \( w_d \) is given by the phosphatase equilibrium derived from Eqn. (3.20)

\[
w_d = \frac{k_4 v_{CaN} e_{p0} \gamma}{k_3 v_{PKA} I_0 + k_4 v_{CaN} \gamma} = \frac{e_{p0} \gamma}{(k_3 v_{PKA} I_0)/(k_4 v_{CaN}) + \gamma}
\]

where

\[
\gamma = \frac{([Ca^{2+}]/K_{H2})^3}{1 + ([Ca^{2+}]/K_{H2})^3}
\]

For system (3.17), the open-loop equilibria for constant \( u \) and \( w := (w_a, w_d) \) are characterized by

\[
(A_a(w_a) - uA_d(w_d))x + B_a(w_a)p_{tot} = 0
\]

from which we derive the input-state (I/S) and input-output (I/O) characteristics, each parameterized by constant \( w \), as follows

\[
x = -(A_a(w_a) - uA_d(w_d))^{-1}B_a(w_a)p_{tot} = k_x(u, w)
\]

\[
y = (\delta \circ k_x)(u, w) = k_y(u, w)
\]

It can be confirmed that for any nonnegative, constant values for \((u, w)\), the equilibrium state \( x = k_x(u, w) \) is globally exponentially stable because the CaMKII activation system reduce to the parameterized linear time-invariant system.

\[
\frac{d}{dt}[x - k_x(u, w)] = (A_a(w_a) - uA_d(w_d))[x - k_x(u, w)]
\]

and matrix \( A_a(w_a) - uA_d(w_d) \) is a constant matrix since \((u, w)\) is constant. It also can be verified that the constant matrix has strictly negative real-part eigenvalues for each constant \((u, w)\), i.e.

\[
\text{Re}[\lambda_i] < 0
\]
where $\lambda_i$ is an eigenvalue of matrix $A_a(w_a) - uA_d(w_d)$.

Notice that when dephosphorylation is not included (i.e. $u = 0$), equilibrium concentrations of phosphorylated subunits are given by

$$k_s(0, w) = p_{tot}[1  1  1  1  1  1  1  1  1  1]^T$$

which leads to the equilibrium concentration of $i$-fold phosphorylated holoenzyme molecules given by $p_i = 0$, $i = 1, \cdots, 9$ and $p_{10} = p_{tot}$. This implies that all enzyme molecules will become fully phosphorylated eventually in the absence of dephosphorylation provided $[Ca^{2+}]$ is high enough.

---

**Figure 3.2:** Input-output (I/O) characteristic with fixed points
3.2.3 Bistability and Hysteresis

Equilibria of the dynamic system 3.17 correspond to fixed points of the relation

\[ u = k_y(u, w), \]

as shown in Fig. (3.2). To investigate further, we let \( C = [1, 1, 1, \cdots 1] \) and

\[
H(u, w) = K_M - C(A_a(w_a) - uA_d(w_d))^{-1}B_a(w_a)p_{tot}
\]

in terms of which, exploiting the form of the nonlinearity \( \delta(x) \),

\[
\delta(x) = \frac{k_2}{K_M + \sum_{k=1}^{10} x_k}
\]

fixed points of \( u = k_y(u, w) \) satisfy

\[
H(u, w)u = k_2
\]

\( H(u, w) \) is a proper rational function in \( u \) of degree 10 for all constant calcium-dependent \( w = (w_a, w_d) \). Letting \( n(u, w) \) and \( d(u, w) \) denote the degree-10 numerator and denominator polynomials in \( u \), respectively, fixed points of \( u = k_y(u, w) \) correspond to real roots of the degree-11 polynomial

\[
n(u, w)u - k_2d(u, w) = 0 \quad (3.24)
\]

The I/S characteristic \( k_y(u, w) \) is non-degenerate by virtue of the global stability property mentioned above. The I/O characteristic \( k_y(u, w) \) is non-degenerate provided that \( \left( \frac{dk_y}{du}(u, w) \right) \neq 1 \) at each fixed point, which can be easily noticed in the Fig. 3.2.

For \([Ca^{2+}]\) ranging from approximately 0.09\( \mu M \) to 0.7 \( \mu M \) and other parameter values in Table 3.1, the polynomial equation (3.24) has three real roots for each \([Ca^{2+}]\) value. This is shown in Fig. 3.2 for \([Ca^{2+}] = 0.2\mu M \) in which the I/O characteristic is plotted on a log-log scale. The three fixed points correspond to intersections of the I/O characteristic with the line \( y = u \). The I/O characteristic essentially shifts from left to right with increasing \([Ca^{2+}]\) value with a corresponding effect on the fixed points. Since the logarithmic scaling on both axes preserves the slope of the tangent to the characteristic at
each point, Fig. 3.2 indicates that the I/O characteristic is non-degenerate whenever the $[Ca^{2+}]$ values yields three fixed points.

The total concentration of phosphorylated subunits is an important parameter and is in general determined from the $i$-fold phosphorylated CaMKII concentration via

$$p_1 + 2p_2 + 3p_3 + \cdots + 10p_{10} = x_1 + x_2 + x_3 + \cdots + x_{10}$$

The equilibrium concentration of phosphorylated subunits as a function of $u$ and $w$ is therefore determined from the I/S characteristic according to

$$Ck_x(u, w)$$

The value of $w$ determined by the constant calcium concentration and the $u$-values determined by the fixed points of the I/O characteristic yield closed-loop equilibrium concentration of phosphorylated subunits. These values are plotted versus constant calcium concentration in Fig. 3.3. This graph is identical to Fig. 2 in [1], and was generated by polynomial root finding as opposed to exhaustive simulation-based asymptotic (steady-state) analysis. It is also possible, as an application of the main result which described in [[56], Theorem 3] to validate the stability claims made in [1]. For constant calcium concentration values in the range from $0.09 \mu M$ to $0.7 \mu M$ yielding a non-degenerate I/O characteristic with three fixed points, these fixed points are in a one-to-one correspondence with closed-loop equilibria (as determined from the I/S characteristic). Then, having already asserted that the closed-loop system is strongly monotone with bounded trajectories, almost all initial conditions yield trajectories that converge to the two stable equilibria corresponding to the two fixed points at which $(\partial k_y/\partial u)(u, w) < 1$ which is easily determined graphically as indicated in Fig. 3.2.

Critical calcium concentration values of $\approx 0.09 \mu M$ and $0.7 \mu M$ yield the two I/O characteristic plotted in Fig. 3.4 that each display a degenerate fixed point along with a
Figure 3.3: Bistability shown in a range of $[Ca^{2+}]$
non-degenerate fixed points with \( (\partial k_y/\partial u)(u, w) \neq 1 \). These critical \([Ca^{2+}]\) values correspond to equilibrium bifurcations. For \([Ca^{2+}] < 0.09\mu M\), there is a single stable equilibrium corresponding to a low level of CaMKII phosphorylation (inactive state). For \([Ca^{2+}] > 0.7\mu M\), there is a single stable equilibrium corresponding to a high level of CaMKII phosphorylation (active state). These critical \([Ca^{2+}]\) values can be interpreted as switching thresholds between inactive and active states, with the intermediate range functioning as hysteresis band as depicted in Fig. 3.5. It is noted in [1] that the resting \([Ca^{2+}]\) nominally lies within the hysteresis band. Thus if the intracellular calcium concentration exceeds the upper threshold long enough, the state trajectory will be attracted to an equilibrium corresponding to the active state. Even as \([Ca^{2+}]\) retreats to the resting value, a high level of activation will persist. LTP has been attributed to this type of phenomena. It is also observed in [1] the cellular mutations leading to off-nominal parameter values can have the effect of shifting or narrowing the hysteresis band in such a way that a high level of activation is no longer maintained by the resting \([Ca^{2+}]\) level.
Figure 3.4: Threshold values of $[Ca^{2+}]$

Figure 3.5: Hysteresis curve
4 Averaging Analysis

The model and its analysis in Chapter 3 provided us a simple method to study the steady states without applying simulation-based numerical methods when the calcium input signals are constant. However, periodic inputs such as pulse trains rather than constant inputs have been frequently applied in empirical biological experiments. In that case, numerical methods are normally applied to analyze the nonlinear system. The idea of averaging analysis is to replace the periodic input by an averaged constant input, thus we can use the results from the preceding chapter to compute the system response with respect to the periodic inputs.

The chapter is organized as follows. In the first section, we discuss the averaging and linearizing of the CaN activation system and also examine the bandwidth of the linearized system. In the second section, we conduct monotonicity analysis of the averaged system. While in the third section two simulation examples are used to validate our averaging analysis.

4.1 Averaging and Linearizing

4.1.1 Pulse train signal

Before we start to discuss the averaging problem, we need to introduce some knowledge of pulse train signals. A typical pulse train signal, shown in Fig. 4.1, can be defined by four parameters: resting value $[Ca^{2+}]_{\text{resting}}$, amplitude $[Ca^{2+}]_{\text{amp}}$, frequency $f$ (Hz) and duty cycle $D = \tau/T$, where $\tau$ is the pulse-width. Also we have the period $T = 1/f$ (s). It is easy to calculate the average value of calcium concentration

$$\overline{[Ca^{2+}]} = [Ca^{2+}]_{\text{resting}} + D \times [Ca^{2+}]_{\text{amp}}$$

However for higher powers of $[Ca^{2+}]$, we need to note that

$$[Ca^{2+}]^n \neq \overline{[Ca^{2+}]^n}$$
and

\[
[Ca^{2+}]^n = [Ca^{2+}]_{resting}^n + D \times [Ca^{2+}]_{amp}^n
\]

where \( n > 1 \) and \( n \) is a integer.

### 4.1.2 CaN Activation subsystem

For the CaMKII activation system, we can interpret it as a cascade interconnection of two subsystems: autophosphorylation-dephosphorylation subsystem (ADS) and CaN activation subsystem (CAS). The whole system has only one scalar input: calcium concentration \([Ca^{2+}]\). When the \([Ca^{2+}]\) is a constant signal as discussed in Chapter 3, the exogenous input \( w_d \) is considered to be a constant and can be obtained by finding the stable equilibrium of the CAS, as shown in Eqn. 3.20. However, for a nonconstant input \([Ca^{2+}]\), we cannot use the result directly.
We write the CaN activation subsystem

\[\begin{align*}
\dot{e}_p &= -k_3 I e_p + k_4 (e_{p0} - e_p) \\
\dot{I} &= -k_3 I e_p + k_4 (e_{p0} - e_p) + v_{PKA} I_0 - v_{CaN} I_u d \\
u_d &= \frac{(v/K_{H2})^3}{1 + (v/K_{H2})^3} \\
w_d &= e_p
\end{align*}\]

(4.1)

where state is \(x = (e_p, I)\), input is \(v\), and output is \(w_d\).

To analyze the CAS explicitly is not easy, so we seek some help from the averaging method. When the calcium input is a pulse train, \(u_d\) is a pulse train too. It is natural to replace the periodic \(u_d\) with its averaged value \(\bar{u}_d\), then we can apply the result in Chapter 3 to get

\[w_d = e_{p0} \bar{u}_d \left( \frac{k_3 v_{CaN} k_4 \bar{u}_d e_{p0}}{(k_4 v_{CaN} k_4 \bar{u}_d) + \bar{u}_d} \right)\]

(4.2)

where \(\bar{u}_d \in (0, 1]\).

For a certain value of \(\bar{u}_d\), there is a stable equilibrium \(x_0\)

\[x_0 = \left( \frac{v_{CaN} k_4 \bar{u}_d e_{p0}}{(v_{PKA} I_0 k_3 + v_{CaN} k_4 \bar{u}_d) \bar{u}_d v_{CaN}} \right)\]

and we can linearize CAS at the equilibrium \(x_0\) to get

\[\frac{d(x - x_0)}{dt} = A(x - x_0)\]

where

\[A = \begin{bmatrix}
\frac{\partial f_1}{\partial x_1} & \frac{\partial f_1}{\partial x_2} \\
\frac{\partial f_2}{\partial x_1} & \frac{\partial f_2}{\partial x_2}
\end{bmatrix}_{x = x_0}\]

\[= \begin{bmatrix}
\frac{-v_{PKA} I_0}{v_{CaN}} & \frac{-v_{PKA} k_3 \bar{u}_d e_{p0}}{v_{CaN} k_4 \bar{u}_d} \\
\frac{-v_{PKA} I_0}{v_{CaN}} & \frac{-v_{PKA} k_3 \bar{u}_d e_{p0}}{v_{CaN} k_4 \bar{u}_d}
\end{bmatrix}\]

is the jacobian matrix \(J|_{x_0}\).
For this linear system, we can use the parameters value in the Table 3.1 to examine the eigenvalues of the matrix $A$. It can be proved that the matrix has two negative real eigenvalues $\lambda_1$ and $\lambda_2$ which have the property $\lambda_2 < \lambda_1 < 0$, which also means that the linear system is exponentially stable.

The system response of the linear system can be written as

$$ x(t) = Me^{\lambda_1 t}M^{-1}x_0 $$

where $M = [v_1, v_2]$, $v_1$ and $v_2$ are real eigenvectors associated with $\lambda_1$ and $\lambda_2$, and

$$ J_r = \begin{bmatrix} \lambda_1 & 0 \\ 0 & \lambda_2 \end{bmatrix} $$

We know $e^{\lambda_1 t}$ and $e^{\lambda_2 t}$ tend to zero as $t \to \infty$ and $e^{\lambda_2 t}$ tends to zero faster than $e^{\lambda_1 t}$. That is why $\lambda_2$ is called the fast eigenvalue and $\lambda_1$ the slow eigenvalue. The fast eigenvalue $\lambda_2$ describes the short-term behavior and the slow eigenvalue shows the transient to the global steady state. Thus the slow eigenvalue dominates the system response and determines the bandwidth of the linear system.

Fig. 4.2 and Fig. 4.3 show how the eigenvalues change as $u_d$ changes. From the figures we can conclude that the fastest slow eigenvalue is $\lambda_1 = -0.3097$ when $u_d = 0.316$, which corresponds to $[Ca^{2+}] = 0.5412 \mu M$. We know that the bandwidth of the linear system is related to the slow eigenvalue and

$$ \omega_B = 2\pi f_B \approx -\lambda_1 $$

then we can get the bandwidth in hertz $f_B = 0.05Hz$.

The following figures, Fig. 4.4 to Fig. 4.7, show the time history of $w_d$ as the frequency of the pulse train changed from $1f_B$, $3f_B$, $7f_B$ to $20f_B$, where $f_B$ is the bandwidth of CAS. In the simulations, we set three parameters of the pulse trains constant: $[Ca^{2+}]_{resting} = 0.1 \mu M$, $[Ca^{2+}]_{amp} = 1.0 \mu M$ and $D = 44.12\%$, so the average
value of calcium is also fixed $[Ca^{2+}] = 0.5412\mu M$. From the simulation results, we can conclude that $w_d$ approaches $\overline{w}_d$ eventually, and as the frequency of a input gets well above the CAS bandwidth the difference between $w_d$ and $\overline{w}_d$ becomes insignificant.

![Figure 4.2: Eigenvalues of the Jacobian matrix for pulse train frequency $u_d$ changes. Shown in the complex plane.](image)

4.1.3 Combined CaMKII/CaN activation subsystem

The CaN activation subsystem discussed in Chapter 3 has a output $(w_a, w_d)$, where $w_a$ and $w_d$ are exogenous inputs of ADS. Here we will extend the CAS to a combined CaMKII/CaN subsystem to include two more parameters, $\overline{w}_a$ and $w_a^2$, which are also exogenous inputs of approximated ADS.
For a pulse train $[Ca^{2+}]$ signal, we know that $w_a(t)$ is a pulse train. So we can get the averaged value of $w_a(t)$

$$\bar{w}_a = (1 - D) \frac{([Ca^{2+}]_{resting}/Kh1)^4}{1 + ([Ca^{2+}]_{resting}/Kh1)^4} + D \frac{([([Ca^{2+}]_{resting} + [Ca^{2+}]_{amp})/Kh1)^4}{1 + ([([Ca^{2+}]_{resting} + [Ca^{2+}]_{amp})/Kh1)^4}

Similarly for $w_a^2(t)$

$$\bar{w}_a^2 = (1 - D) \frac{([Ca^{2+}]_{resting}/Kh1)^8}{(1 + ([Ca^{2+}]_{resting}/Kh1)^4)^2} + D \frac{([([Ca^{2+}]_{resting} + [Ca^{2+}]_{amp})/Kh1)^8}{(1 + ([([Ca^{2+}]_{resting} + [Ca^{2+}]_{amp})/Kh1)^4)^2}

\bar{w}_a, \bar{w}_a^2, \text{and } \bar{w}_d \text{ are the outputs of the combined subsystem and the inputs of the approximation ADS.}

In the combined subsystem, the state is still $x = (e_p, I)$, input is also $v$, and output becomes $(\bar{w}_a, \bar{w}_a^2, \bar{w}_d)$.

Using the infinitesimal monotonicity characterization equations (4.3), we can prove that the combined subsystem is also monotone with respect to the orthant.
Figure 4.4: $\overline{w_d}$ and $w_d$ for pulse train frequency $f = 0.05$Hz

Figure 4.5: $\overline{w_d}$ and $w_d$ for pulse train frequency $f = 0.15$Hz
Figure 4.6: $\bar{w}_d$ and $w_d$ for pulse train frequency $f = 0.35Hz$

Figure 4.7: $\bar{w}_d$ and $w_d$ for pulse train frequency $f = 2Hz$
$K_1 = K^{(1,0)} = \{(\xi_1, \xi_2) \in \mathbb{R}^2 | \xi_1 \leq 0, \xi_2 \geq 0\}$ for the state $(e_p, I)$ and the negative ordering on $\mathbb{R}$ for the scalar input $v$.

$$\frac{\partial f_1}{\partial x_2}(x, v) = -k_3 x_1 \leq 0$$

$$\frac{\partial f_2}{\partial x_1}(x, v) = -k_3 x_2 - k_4 \leq 0$$

$$\frac{\partial f_1}{\partial v}(x, v) = 0 \geq 0$$

$$\frac{\partial f_2}{\partial v}(x, v) = -v_{CaN} x_2 \frac{3v^2/(K_{H2})^3}{1 + (v/K_{H2})^3} - \frac{3v^5/(K_{H2})^6}{(1 + (v/K_{H2})^3)^2} \leq 0 \quad (4.3)$$

Further, we observe that the CaMKII/CaN activation subsystems are I/O monotone with respect to the orthant $K_{out} = K^{(1,1,1)} = \{(\xi_1, \xi_2, \xi_3) \in \mathbb{R}^3 | \xi_1 \leq 0, \xi_2 \leq 0, \xi_3 \leq 0\}$ for the output $(\overline{w_a}, \overline{w_d})$ as the averaged values have the same monotonicity as the original values.

### 4.1.4 Approximated Autophosphorylation-Dephosphorylation Subsystem

Building up the approximated ADS is not difficult because the inputs are also constant. So we can still use the unity feedback system

$$\dot{x} = (A_a(\overline{w_a}, \overline{w_d}^2) - uA_d(\overline{w_d}))x + B_a(\overline{w_d})p_t$$

$$y = \delta(x) \quad (4.4)$$

One can notice that in the original ADS the input is $(u, w_a, w_d)$, while in the approximated ADS the input becomes $(u, \overline{w_a}, \overline{w_a^2}, \overline{w_d})$ because $\overline{w_a^2} \neq \overline{w_a^2}$. The differences between original and approximated ADS are minor, we just need to make several slight changes to build the new model.

#### 4.2 Averaged System

##### 4.2.1 Monotonicity Analysis

For the approximated ADS, we also use nonnegative orthant $K = R_{\geq 0}^{10}$ in the state space. For the inputs $(u, u_1, u_2, u_3) = (u, \overline{w_a}, \overline{w_a^2}, \overline{w_d})$, we also define input cone by
\[ K_{in} = K^{(1,0,0,1)} = \{(u, u_1, u_2, u_3) \in \mathbb{R}^4 | u \leq 0, u_1 \geq 0, u_2 \geq 0, u_3 \leq 0\}. \]

Thus

\[
(u_1^1, u_1^2, u_2^1) \geq (u_2^2, u_2^3, u_3^2) \mbox{ corresponds to } (u_1^1, u_1^2, u_2^1) - (u_2^2, u_2^3, u_3^2) \in K_{in}
\]

from which \( u_1^1 \leq u_2^2, u_1^2 \geq u_2^3 \) and \( u_1^3 \leq u_3^2 \).

At last, for scalar output \( y \) we define output cone by

\[ K_{out} = \{y \in \mathbb{R} | y \leq 0\}. \]

Thus we can write it directly as \( y^1 \leq y^2 \).

Here we rewrite the nonlinear averaged system as

\[
\begin{align*}
\dot{x} &= f(x, u) = (A_a(u_1, u_2) - u A_d(u_3)) x + B_a(u_2) p_t \\
y &= \delta(x)
\end{align*}
\]

Writing \( \alpha_0(u_2) = 10k_1 u_2 \), we have

\[
\begin{align*}
A_a(u_1, u_2) &= -\alpha_0(u_2)e_1 e_1^T + u_1 A_1 \\
B_a(u_2) &= \alpha_0(u_2)e_1
\end{align*}
\]

where

\[
A_1 = \begin{bmatrix}
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\alpha_1 & -\alpha_1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & \alpha_2 & -\alpha_2 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & \alpha_3 & -\alpha_3 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & \alpha_4 & -\alpha_4 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & \alpha_5 & -\alpha_5 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & \alpha_6 & -\alpha_6 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & \alpha_7 & -\alpha_7 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & \alpha_8 & -\alpha_8 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \alpha_9 & -\alpha_9
\end{bmatrix} \quad (4.5)
\]}
in which \( \alpha_i = w_i k_1, i = 1, \ldots, 9 \). Also

\[
A_d(u_3) = u_3 A_2 = u_3 \times \begin{bmatrix}
1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 2 & -2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 3 & -3 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 4 & -4 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 5 & -5 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 6 & -6 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 7 & -7 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 8 & -8 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 9 & -9 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 10
\end{bmatrix}
\] (4.6)

Now to prove monotonicity, we need to check whether the following inequalities hold for all \( i, j \in \{1, 2, \ldots, 10\} \) for all \( x \) in interior of \( X \) and for all \( u > 0, u_1 > 0, u_2 > 0, u_3 > 0 \)

\[
\frac{\partial f_i}{\partial x_j}(x, u, u_1, u_2, u_3) \geq 0, \forall i \neq j
\]

\[
\frac{\partial f_i}{\partial u}(x, u, u_1, u_2, u_3) \leq 0
\]

\[
\frac{\partial f_i}{\partial u_1}(x, u, u_1, u_2, u_3) \geq 0
\]

\[
\frac{\partial f_i}{\partial u_2}(x, u, u_1, u_2, u_3) \geq 0
\]

\[
\frac{\partial f_i}{\partial u_3}(x, u, u_1, u_2, u_3) \leq 0
\]

where \( X \) is the set

\[
X = \{x \in \mathbb{R}^{10} | 0 \leq x_1 \leq x_2 \leq \ldots \leq x_9 \leq x_{10} \leq p_1\}
\]

For the first set of inequalities

\[
\frac{\partial f_i}{\partial x_{i+1}}(x, u, u_1, u_2, u_3) = i u u_3 \geq 0, \forall i = 1, \ldots, 9
\]

\[
\frac{\partial f_i}{\partial x_{i-1}}(x, u, u_1, u_2, u_3) = w_{i-1} k_1 u_1 \geq 0, \forall i = 2, \ldots, 10
\]
with all other partial derivatives zero for $i \neq j$. For the second set of inequalities,

\[ \frac{\partial f_i}{\partial u}(x, u, u_1, u_2, u_3) = -iu_3(x_i - x_{i+1}) \leq 0, \forall i = 1, \ldots, 9 \]

\[ \frac{\partial f_{10}}{\partial u}(x, u, u_1, u_2, u_3) = -10u_3x_{10} \leq 0 \]

For the third,

\[ \frac{\partial f_1}{\partial u_1}(x, u, u_1, u_2, u_3) = 0 \geq 0 \]

\[ \frac{\partial f_i}{\partial u_1}(x, u, u_1, u_2, u_3) = \alpha_{i-1}(x_{i-1} - x_i) \geq 0, \forall i = 2, \ldots, 10 \]

Next,

\[ \frac{\partial f_1}{\partial u_2}(x, u, u_1, u_2, u_3) = 10k_1(p_{\text{tot}} - x_1) \geq 0 \]

\[ \frac{\partial f_i}{\partial u_2}(x, u, u_1, u_2, u_3) = 0 \geq 0, \forall i = 2, \ldots, 10 \]

and finally

\[ \frac{\partial f_i}{\partial u_3}(x, u, u_1, u_2, u_3) = -iu(x_i - x_{i+1}) \leq 0, \forall i = 1, \ldots, 9 \]

\[ \frac{\partial f_{10}}{\partial u_3}(x, u, u_1, u_2, u_3) = -10ux_{10} \leq 0 \]

where these inequalities follow from the definition of the set $X$. Thus we conclude that the system (4.5) is monotone. Also, we observe the output map $\delta(x)$ is monotone since $x^1 \geq x^2$ gives $\delta(x^1) \geq \delta(x^2)$.

### 4.2.2 Averaged Values of Exogenous Inputs

For a pulse train $[Ca^{2+}]$ signal, if we set a fixed resting value we can sweep a certain range of averaged calcium concentration value by changing amplitude and duty cycle. One way is to fix the amplitude and sweep the duty cycle; another is to fix the duty cycle and change the amplitude. However, for the same $[Ca^{2+}]$, the averaged values of exogenous inputs can be different, which means if we sweep $[Ca^{2+}]$ in different ways we
could get different I/O and I/S characteristics of the system. Fig. 4.8 shows how $\overline{w_a}$ varies as $[Ca^{2+}]$ changes in a certain range while Fig. 4.9 shows $\overline{w_a^2}$ vs. $[Ca^{2+}]$ and Fig. 4.10 shows $\overline{w_d}$ vs. $[Ca^{2+}]$. To plot the figures, we set $[Ca^{2+}]_{resting} = 0.0\mu M$ and in fixed amplitude case: $[Ca^{2+}]_{amp} = 0.9\mu M$; Sweep D from 0% to 100%; while in fixed duty cycle case: $D = 50\%$; Sweep $[Ca^{2+}]_{amp}$ from 0.0$\mu M$ to 1.8$\mu M$.

![Graph showing $\overline{w_a}$ vs. $[Ca^{2+}]$.](image)

Figure 4.8: $\overline{w_a}$ vs. $[Ca^{2+}]$

As we can see, fixing the amplitude and sweeping the duty cycle generate the averaged exogenous outputs that are closer to those in the corresponding constant input case. In the following analysis, we will fix the resting value to 0.09$\mu M$, the amplitude of the pulse train to 0.62$\mu M$ and sweep the duty cycle from 0% to 100% to cover the bistable range of calcium concentration $[Ca^{2+}]$ discussed in Chapter 3, which is 0.09 – 0.70$\mu M$. The other parameters used in the computations are listed in Table 3.1.
Figure 4.9: $w_a^2$ vs. $[Ca^{2+}]$  

Figure 4.10: $w_d$ vs. $[Ca^{2+}]$
4.2.3 I/S and I/O Characteristics

Similar to the discussion in Chapter 3, for system (4.5), the open-loop equilibria for constant $u$ and $w := (\overline{w_d}, \overline{w^2_d}, \overline{w_d}) = (u_1, u_2, u_3)$ are characterized by

$$(A_a(u_1, u_2) - uA_d(u_3))x + B_a(u_2)p_{\text{tot}} = 0$$

(4.7)

from which

$$x = -(A_a(u_1, u_2) - uA_d(u_3))^{-1}B_a(u_2)p_{\text{tot}} = k_x(u, w)$$

$$y = (\delta \circ k_x)(u, w) = k_y(u, w)$$

(4.8)

It can be confirmed that for any nonnegative, constant values for $(u, w)$, the equilibrium state $x = k_x(u, w)$ is globally exponentially stable because the CaMKII activation system reduce to the parameterized linear time-invariant system.

$$\frac{d}{dt}[x - k_x(u, w)] = (A_a(u_1, u_2) - uA_d(u_3))[x - k_x(u, w)]$$

and matrix $A_a(u_1, u_2) - uA_d(u_3)$ is a constant Hurwitz matrix for all constant $(u, u_1, u_2, u_3)$.

We refer to the map $k_x(\cdot, \cdot)$ as the input-state (I/S) characteristics and $k_y(\cdot, \cdot)$ as the input-output (I/O) characteristics from $u$ to $y$, in this case parameterized by averaged calcium concentration. We can also verify that that all enzyme molecules will become fully phosphorylated eventually in the absence of dephosphorylation.

Closed-loop equilibria correspond to fixed points of the relationship $u = k_y(u, w)$, and they can be shown by Fig. 4.11. Comparing Fig. 4.11 with Fig. 4.12, which is the corresponding constant input case, we can notice that the I/O characteristics curve in the periodic input case shifts to the right significantly. That can be explained by Fig. 4.8 to Fig. 4.10: the values of averaged exogenous inputs in the periodic-input case are greater than those in the corresponding constant-input case.
Figure 4.11: Input-output (I/O) characteristics with fixed points. Pulse train parameters: $[Ca^{2+}]_{resting} = 0.09 \mu M$, $[Ca^{2+}]_{amp} = 0.61 \mu M$, duty cycle $D = 1\%$ and frequency $f = 0.5 Hz$

Figure 4.12: Input-output (I/O) characteristics with fixed points. Parameters: $[Ca^{2+}] = 0.0961 \mu M$
4.2.4 Bistability and Hysteresis

Equilibria of the dynamic system correspond to fixed points of the relation $u = k_y(u, w)$. Additionally, we conclude that the I/O characteristic $k_y(u, w)$ is non-degenerate provided that $(\partial k_y/\partial u)(u, w) \neq 1$ at each fixed point, which can be easily noticed in Fig. 4.11. Recall that for pulse-train $[Ca^{2+}]$ signals, the resting value is $0.09\mu M$, the amplitude is to $0.62\mu M$ and the duty cycle ranges from 0% to 100%. Other parameters are given in Table 3.1.

In the figure, the three fixed points correspond to intersections of the I/O characteristic with the line $y = u$. The I/O characteristic essentially shifts from left to right with increasing $[Ca^{2+}]$ value with a corresponding effect on the fixed points.

The value of $w$ determined by the averaged calcium concentration and the $u$-values determined by the fixed points of the I/O characteristic yield closed-loop equilibrium concentration of phosphorylated subunits values. These values are plotted versus averaged calcium concentration in Fig. 4.13. In addition, Fig. 4.14 is the same figure but shows some details that are too small to see in Fig. 4.13.

The threshold values of averaged calcium concentration are plotted in Fig. 4.15. These critical $[Ca^{2+}]$ values can be interpreted as switching threshold between inactive and active states, with the intermediate range functioning as a hysteresis band as depicted in Fig. 4.16.

4.3 Simulation of the averaged system

In this section, some simulation results are used to validate our averaging analysis. The parameters used in the simulations are the same as that in the preceding chapter and are given in Table 3.1.
Figure 4.13: Bistability shown in a range of duty cycle

Figure 4.14: Bistability shown in a range of duty cycle
Figure 4.15: Threshold values

Figure 4.16: Hysteresis curve
4.3.1 High-Frequency Pulse Train Signal

We consider a relatively high-frequency calcium pulse train signal with the parameters listed in Table 4.1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Symbol</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting value</td>
<td>([Ca^{2+}]_{resting})</td>
<td>0.1</td>
<td>(\mu M)</td>
</tr>
<tr>
<td>Amplitude</td>
<td>([Ca^{2+}]_{amp})</td>
<td>1.9</td>
<td>(\mu M)</td>
</tr>
<tr>
<td>Frequency</td>
<td>(f)</td>
<td>1</td>
<td>Hz</td>
</tr>
<tr>
<td>Duty Cycle</td>
<td>(D)</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Average value</td>
<td>(\bar{w}_a)</td>
<td>0.0265</td>
<td></td>
</tr>
<tr>
<td>Average value</td>
<td>(\bar{w}_d)</td>
<td>0.0016</td>
<td></td>
</tr>
<tr>
<td>Average value</td>
<td>(\bar{w}_d)</td>
<td>(4.29 \times 10^{-4})</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4.17 and Fig. 4.18 show the time history of state variables from simulations of the original model and the averaged model respectively. Fig. 4.19 and Fig. 4.20 show the time history of output from simulations of the original model and the averaged model respectively. From the figures, we observe that the results from the averaged system are almost identical to those from the original system. The influences of the periodic signals are insignificant as the frequency of the input signal is relatively high. In this case, it is about 20 times the system bandwidth.

4.3.2 Low-Frequency Pulse Train Signals

Here we consider a pulse train signal with a relatively low frequency. The pulse train parameters values in Table 4.2.
Figure 4.17: Time history of state variables from simulation of the original model

Figure 4.18: Time history of state variables from simulation of the averaged model
Figure 4.19: Time history of output from simulation of the original model

Figure 4.20: Time history of output from simulation of the averaged model
Table 4.2: Low-frequency pulse train signal parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Symbol</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting value</td>
<td>$[Ca^{2+}]_{\text{resting}}$</td>
<td>0.1</td>
<td>$\mu M$</td>
</tr>
<tr>
<td>Amplitude</td>
<td>$[Ca^{2+}]_{\text{amp}}$</td>
<td>1.9</td>
<td>$\mu M$</td>
</tr>
<tr>
<td>Frequency</td>
<td>$f$</td>
<td>0.01</td>
<td>Hz</td>
</tr>
<tr>
<td>Duty Cycle</td>
<td>$D$</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Average value</td>
<td>$\bar{w}_a$</td>
<td>0.0265</td>
<td></td>
</tr>
<tr>
<td>Average value</td>
<td>$\bar{w}_a^2$</td>
<td>0.0016</td>
<td></td>
</tr>
<tr>
<td>Average value</td>
<td>$\bar{w}_d$</td>
<td>$4.29 \times 10^{-4}$</td>
<td></td>
</tr>
</tbody>
</table>

The averaged values are the same as that in the high-frequency case, so the state and output responses of the averaged system remain unchanged (Fig. 4.18 and Fig. 4.20).

Fig. 4.21 shows the time history of state variables from simulations of the original model while Fig. 4.22 shows the time history of output. We can observe that as the pulse train frequency gets relatively low, 20% of the bandwidth in this case, the differences between the outputs of the two systems is significant. Thus the averaging analysis becomes inapplicable. That is because the low-frequency perturbations in the input signal cannot be filtered out and have a significant effect on the system response.

From the analysis and simulation results of the average model, we can conclude that averaging analysis is a very suitable tool to simplify the system analysis when the frequency of a periodic input is significantly higher than the bandwidth of the dynamic system.
Figure 4.21: Time history of state variables from simulation of the original model

Figure 4.22: Time history of output from simulation of the original model
5 SIMULATION

In the above chapters, we have built up a model for the CaMKII activation system and conducted an averaging analysis of the model. In this chapter, extensive simulations will be applied to analyze system properties and validate the averaged model obtained from the analysis. As always, MATLAB/SIMULINK tools have been applied to simulate both the original model and the corresponding averaged model. In the averaging simulations, periodic signals have been introduced as inputs to both the original and averaged model to compute the respective system responses. In this way, we can easily put the outputs together and examine the differences between them. Furthermore, studying how inputs of the system affect the differences will aid us to get a better understanding of the underlying mechanism.

The chapter is organized as follows. In Section 5.1, bistability, threshold and hysteresis are shown by simulations and the effects of variable parameters on the size and position of the bistability domain are discussed. In Section 5.2, an averaging analysis of the system will be conducted. Some typical periodical signals such as sine waves, pulse train signals and tetanic excitations will be applied in the simulation to validate the averaged model.

5.1 System Properties

5.1.1 Bistability

We recall the closed-loop system

\[ \dot{x} = [A_d(w_a) - \delta(x)A_d(w_d)]x + B_d(w_a)p_{tot} \]

From the discussion before, the system has three equilibria when \([Ca^{2+}] \in (0.09, 0.7) \mu M\) with a set of variable parameters: \(e_k = 20, I_0 = 0.1, K_M = 0.4, e_{p0} = 0.05, K_{h2} = 0.7\), all in \(\mu M\). Two of the equilibria are globally exponentially stable and the other one is unstable.
When $[Ca^{2+}] \in (0, 0.09) \mu M$ the system has only one equilibrium that is close to the zero point, while for $[Ca^{2+}] \in (0.7, \infty) \mu M$ the system has a only equilibrium that is near the fully phosphorylated point, as can be shown in Fig. (5.1).

![Figure 5.1: Bistability shown in a certain range of $[Ca^{2+}]$. Parameters (in $\mu M$): $e_k = 20$, $I_0 = 0.1$, $K_M = 0.4$, $e_{\rho_0} = 0.05$, $K_{h2} = 0.7$](image)

Fig. (5.2) and Fig. (5.3) show that for a constant input $[Ca^{2+}] = 0.5 \mu M$ the system will reach different stable equilibria from different initial conditions, which is called bistability. For convenience, from now on in the thesis, we name the stable state near the zero point bottom steady state (BSS) and the one close to the full phosphorylation point top steady state (TSS).

In the captions of the figures, $\mathbf{0}_{10}$ denotes a 10-dimensional vector which has all components equal to zero while $\mathbf{1}_{10}$ is a 10-dimensional vector which has all components equal to 1.
Figure 5.2: System dynamics with initial state $x_0 = 0_{10}$ and a constant input $[Ca^{2+}] = 0.5 \mu M$

Figure 5.3: System dynamics with initial state $x_0 = 20 \times 1_{10}$ and a constant input $[Ca^{2+}] = 0.5 \mu M$
Fig. (5.4) and (5.5) show that when the signal input $[Ca^{2+}]$ is greater than the upper threshold value $0.7 \mu M$, the trajectories converge to TSS from different initial values. In a similar way, when the signal input $[Ca^{2+}]$ is less than the lower threshold value $0.09 \mu M$, all trajectories will reach BSS.

![Graph showing system dynamics](image)

**Figure 5.4:** system dynamics with initial state $x_0 = 0_{10}$ and a constant input $[Ca^{2+}] = 2\mu M$

### 5.1.2 Region of Bistability

In the many parameters involved in the model, some parameters are variable. The figures in this section will show how boundaries of the bistability domain depend on variable parameters of the system, which include total concentration of CaMKII denoted $e_k$, concentration of PP1 $e_{p0}$, Michaelis constant of protein phosphatase $K_M$, the $Ca^{2+}$ activation Hill constant of CaN $K_{h2}$, and concentration of free inhibitor 1 $I_0$. The last parameter, $I_0$, is a special parameter. If its value is set to zero, inhibitor 1 will be
eliminated in the model. So all the PP1 becomes unbound, thus the model gets simplified with a Ca$^{2+}$-independent protein phosphatase. The truncated model will be used in several simulations that are used to show hysteresis.

Fig. (5.6), Fig. (5.7), and Fig. (5.8) are the reproductions of Fig. 3 in Zhabotinsky’s paper [1]. The bistability domain in the plane of [CaMKII] ($e_k$) versus [Ca$^{2+}$] at three values of $K_M$ is shown in Fig. (5.6). It is obvious that the domain considerably gets smaller when $e_k$ decreases under 5 $\mu$M. In addition, when parameter $K_M$ increases, the right boundary of domain goes to the left significantly. On the other hand, the left boundary hardly moves at low values of $e_k$. Fig. (5.7) reinforces the statement that the region of bistability shrinks when $K_M$ increases. Even further, the bistability domain does not exist when $K_M > 12 \mu M$ and $e_k = 1 \mu M$. Fig. (5.8) demonstrates positions of the
bistability domain in the plane concentration of PP1 ($e_{p0}$) versus $[Ca^{2+}]$ at three different values of $e_k$. The domain boundaries move to the right when $e_{p0}$ increases or $e_k$ decreases.

As we can conclude from these three figures, in simulations we should carefully choose appropriated parameters to ensure that a bistability range includes the desired resting value of the intracellular $[Ca^{2+}]$ and is wide enough to prevent autophosphorylation by random fluctuations of $[Ca^{2+}]$. Fig. (5.9) is another view of how $e_{p0}$ and $e_k$ affect bistability domain of the system. It is shown in the plane $e_k$ versus $[Ca^{2+}]$ with three values of $e_{p0}$. One can conclude that the domain boundaries shift to the right considerably when $e_{p0}$ increases, and the domain shrinks significantly when $e_k$ drops below $10\mu M$.

Fig. (5.10) shows the effect of $e_{p0}$ to the bistability domain in the plane $K_M$ versus $[Ca^{2+}]$ at three values of $e_{p0}$. The domain boundaries shift to the right and the domain gets wider when $e_{p0}$ increases. Meanwhile, it is obvious that as $K_M$ increases, the bistability domain diminishes significantly.

Fig. (5.11) is another view of Fig. (5.10). The right boundary of domain shifts to right while the left boundary hardly moves when $K_M$ increases. Also, one can observe that $e_{p0}$ should be high enough and $K_M$ should be small enough to ensure an applicable bistable range.

Fig. (5.12) shows positions of the bistability domain in the plane $e_k$ versus $[Ca^{2+}]$ at three values of $K_{h2}$. The domain boundaries shift to right significantly when $K_{h2}$ decreases. It also shows that $e_k$ should be greater than $10\mu M$ to ensure an applicably wide domain.

Fig. (5.13) shows positions of the bistability domain in the plane $K_M$ versus $[Ca^{2+}]$ at three values of $K_{h2}$. The domain boundaries shift to the left significantly when $K_{h2}$
Figure 5.6: Effect of Michaelis constant of protein phosphatase ($K_M$) on size and position of the bistable region on ($e_k$, $[Ca^{2+}]$)-plane. The lines on the left (dashed) denote the down-switching threshold values and the lines on the right (solid) the up-switching threshold values. Parameters (in $\mu M$): $K_{h2} = 1.4$, $I_0 = 0.1$, $e_{p0} = 0.3$. $K_M$ takes value of 0.4, 2 and 10
Figure 5.7: Effect of concentration of CaMKII ($e_k$) on size and position of the bistable region on ($K_M, [Ca^{2+}]$)-plane. The lines on the left (dashed) denote the down-switching threshold values and the lines (solid) on the right the up-switching threshold values. Parameters (in $\mu M$): $K_{h2} = 1.4, I_0 = 0.1, e_{p0} = 0.3$. $e_k$ takes value of 20, 5 and 1.
Figure 5.8: Effect of concentration of CaMKII ($e_k$) on size and position of the bistable region on $(e_{p0}, [Ca^{2+}])$-plane. The lines on the left (dashed) denote the down-switching threshold values and the lines (solid) on the right the up-switching threshold values. Parameters (in $\mu M$): $K_{h2} = 1.4$, $I_0 = 0.1$, $K_M = 0.4$. $e_k$ takes value of 20, 5 and 1.
Figure 5.9: Effect of concentration of PP1 ($e_{p0}$) on size and position of the bistable region on ($e_k$, $[Ca^{2+}]$)-plane. The lines on the left (dashed) denote the down-switching threshold values and the lines on the right (solid) the up-switching threshold values. Parameters (in $\mu$M): $K_{h2} = 1.4$, $I_0 = 0.1$, $K_M = 0.4$. $e_{p0}$ takes value of 0.05, 0.4 and 1.2
Figure 5.10: Effect of concentration of PP1 ($e_{p0}$) on size and position of the bistable region on ($K_M$, $[Ca^{2+}]$)-plane. The lines on the left (dashed) denote the down-switching threshold values and the lines on the right (solid) the up-switching threshold values. Parameters (in $\mu M$): $K_{h2} = 1.4$, $I_0 = 0.1$, $e_k = 20$. $e_{p0}$ takes value of 0.05, 0.4 and 1.2.
Figure 5.11: Effect of Michaelis constant of protein phosphatase ($K_M$) on size and position of the bistable region on ($e_{p0}, [Ca^{2+}]$)-plane. The lines on the left (dashed) denote the down-switching threshold values and the lines on the right (solid) the up-switching threshold values. Parameters (in µM): $K_{h2} = 1.4, I_0 = 0.1, e_k = 20$. $K_M$ takes value of 0.4, 2 and 10.
Figure 5.12: Effect of the \(Ca^{2+}\) activation Hill constant of CaN (\(K_{h2}\)) on size and position of the bistable region on (\(e_p, [Ca^{2+}]\))-plane. The lines on the left (dashed) denote the down-switching threshold values and the lines on the right (solid) the up-switching threshold values. Parameters (in \(\mu M\)): \(e_p0 = 0.3\), \(I_0 = 0.1\), \(K_M = 0.4\). \(K_{h2}\) takes value of 0.3, 0.7 and 1.4.
increases and the domain shrinks considerably as $K_M$ increases. The figure again shows that $K_M$ has to be significantly lower than $1\mu M$ to provide a proper bistability range.

Figure 5.13: Effect of the $Ca^{2+}$ activation Hill constant of CaN ($K_{h2}$) on size and position of the bistable region on ($K_M$, [Ca$^{2+}$])-plane. The lines on the left (dashed) denote the down-switching threshold values and the lines on the right (solid) the up-switching threshold values. Parameters (in $\mu M$): $e_{p0} = 0.3, I_0 = 0.1, e_k = 20$. $K_{h2}$ takes value of 0.3, 0.7 and 1.4.

Fig. (5.14) shows positions of the bistability domain in the plane ($e_{p0}$, [Ca$^{2+}$]) at three values of $K_{h2}$. One can see that activity of PP1 has to decrease with decreasing $K_{h2}$ in order to obtain a proper $Ca^{2+}$ range of bistability; if $K_{h2}$ equals 1.4 $\mu M$, $e_{p0}$ must be about 0.3 $\mu M$, if $K_{h2}$ equals 0.7 $\mu M$, $e_{p0}$ must be about 0.05 $\mu M$, and if $K_{h2}$ equals 0.3 $\mu M$, $e_{p0}$ must be below 0.01 $\mu M$. 
Figure 5.14: Effect of the $Ca^{2+}$ activation Hill constant of CaN ($K_{h2}$) on size and position of the bistable region on ($e_{p0}, [Ca^{2+}]$)-plane. The lines on the left (dashed) denote the down-switching threshold values and the lines on the right (solid) the up-switching threshold values. Parameters (in $\mu M$): $K_M = 0.4, I_0 = 0.1, e_k = 20$. $K_{h2}$ takes value of 0.3, 0.7 and 1.4.
Fig. (5.15) shows positions of the bistability domain in the plane $K_{h2}$ versus $[Ca^{2+}]$ at three values of $e_k$. The domain boundaries shift to the left significantly when $e_k$ increases. One can claim that $e_k$ must be significantly higher than $5\mu M$ to obtain a proper bistability range. Fig. (5.16) shows positions of the bistability domain in the plane $K_{h2}$ versus $[Ca^{2+}]$. The right boundary shifts to left significantly while the right sides stay as $K_M$ increases, while the left boundary only moves slightly. The figure again demonstrates that $K_M$ must be significantly lower than $2\mu M$ and $K_{h2}$ should exceed $1\mu M$ to ensure the system has a applicable bistability range. Fig. (5.17) shows positions of the

Figure 5.15: Effect of concentration of CaMKII ($e_k$) on size and position of the bistable region on $(K_{h2},[Ca^{2+}])$-plane. The lines on the left (dashed) denote the down-switching threshold values and the lines on the right (solid) the up-switching threshold values. Parameters (in $\mu M$): $e_{p0} = 0.3, I_0 = 0.1, K_M = 0.4, e_k$ takes value of 20, 5 and 1 $[Ca^{2+}]$ at three values of $K_M$. The right boundary shifts to left significantly while the right sides stay as $K_M$ increases, while the left boundary only moves slightly. The figure again demonstrates that $K_M$ must be significantly lower than $2\mu M$ and $K_{h2}$ should exceed $1\mu M$ to ensure the system has a applicable bistability range. Fig. (5.17) shows positions of the
Figure 5.16: Effect of Michaelis constant of protein phosphatase ($K_M$) on size and position of the bistable region on ($K_{h2}, [Ca^{2+}]$)-plane. The lines on the left (dashed) denote the down-switching threshold values and the lines on the right (solid) the up-switching threshold values. Parameters (in μM): $e_{p0} = 0.3, I_0 = 0.1, e_k = 20$. $K_M$ takes value of 0.4, 2 and 10.

Bistability domain in the plane $K_{h2}$ versus $[Ca^{2+}]$ at three values of $e_{p0}$. Both domain boundaries shift to right significantly and as $e_{p0}$ increases. The figure demonstrates that $K_{h2}$ should increase as $e_{p0}$ increases. Fig. (5.18) shows the bistability domain in the plane: ($e_{p0}, K_{h2}$) at the resting $Ca^{2+}$ concentration equal to 0.1 μM and $e_k$ at three different values. It demonstrates that the bistability domain is quite wide at $e_k = 20μM$ and diminishes significantly when $e_k = 1.0μM$. Fig. (5.19) shows the bistability domain in the plane: ($e_{p0}, K_{h2}$) at the resting $Ca^{2+}$ concentration equal to 0.1 μM and $K_M$ at three different values. It shows that the bistability domain shrinks as $K_M$ increases.
Figure 5.17: Effect of concentration of PP1 ($e_{p0}$) on size and position of the bistable region on $(K_{h2}, [Ca^{2+}])$-plane. The lines on the left (dashed) denote the down-switching threshold values and the lines on the right (solid) the up-switching threshold values. Parameters (in $\mu M$): $K_M = 0.4, I_0 = 0.1, e_k = 20$. $e_{p0}$ takes value of 0.05, 0.4 and 1.2.
Figure 5.18: Effect of concentration of CaMKII ($e_k$) on size and position of the bistable region on $(e_{p0}, K_{h2})$-plane. Parameters (in $\mu M$): $K_M = 0.4$, $I_0 = 0.1$. $e_k$ takes value of 20, 10 and 1

5.1.3 Hysteresis

Fig. (5.20) shows the steady-state curve of the system for a set of variable parameters in which $I_0 = 0.0$. Other values of parameters are listed in the figure captions. The system moves along a loop of hysteresis when $[Ca^{2+}]$ gradually changes. When $[Ca^{2+}]$ increases, the system moves along the path ABCDE; when $[Ca^{2+}]$ decreases, the path becomes EDFBA.

As we can see, the bistability range of the system is $(\sim 1.6, 2.1) \mu M$, in which we designed several simulations. In each simulations, a constant input $[Ca^{2+}]$ has been
Figure 5.19: Effect of Michaelis constant of protein phosphatase ($K_M$) on size and position of the bistable region on ($e_{p0}, K_{h2}$)-plane. Parameters (in $\mu M$): $e_k = 20, I_0 = 0.1$. $K_M$ takes value of 0.4, 2 and 10
introduced to the model first. When a steady state has been reached and kept for 100 seconds, the input $[Ca^{2+}]$ was changed to a new constant value to record the system responses. Fig. (5.21) demonstrates the results of the simulations. When $[Ca^{2+}]$ jumps from 1.3 $\mu M$ to 1.8 $\mu M$, the concentration of phosphorylated subunits increases only slightly, denoted by line (A), because the system remains on BSS; when $[Ca^{2+}]$ jumps from 1.3 $\mu M$ to 2.2 $\mu M$, the system moves to the TSS, as line (B). When $[Ca^{2+}]$ is switched from 2.3 $\mu M$ to 1.8 $\mu M$, the system moves to a new steady state of TSS, as line (C); when $[Ca^{2+}]$ jumps from 2.3 $\mu M$ to 1.5 $\mu M$, the system transits to BSS, as line (D).

The two figures are actually reproductions of Fig. 9 in Zhabotinsky’s paper [1]. The simulation figures are almost identical to the original figures. In Fig. (5.20) the curve (FC) in the hysteretic loop (BCDF) denotes the change of unstable equilibrium with respect to $[Ca^{2+}]$.

The property of hysteresis offers the system the ability to stay at the current steady state even when the excitation returns to the resting value after the system get activated. That can be shown in Fig. (5.22) and (5.23). Fig. (5.22) shows that system stays in TSS if the value of resting $[Ca^{2+}]$ is in the bistability region while Fig. (5.23) and Fig. (5.24) show that the system will not stay in TSS when the resting $[Ca^{2+}]$ is not in the bistability range anymore.

Please note that the above figures are obtained when $I_0$ is set to zero, which means the protein phosphatase is $[Ca^{2+}]$-independent. In the following case, we let $I_0 = 0.1$ and set other parameters’ value as $K_M = 0.4, e_k = 20, e_{p0} = 1.2, K_{h2} = 0.7$, all in $\mu M$, then we have a significantly different bistability range which can be shown by Fig. (5.25). The parameters are chosen to obtain higher threshold values so the convergence time of simulation becomes shorter.
Figure 5.20: The steady-state curve with the hysteretic loop. Arrows show direction of movement when $[Ca^{2+}]$ slowly increases or decreases. Parameters (in $\mu M$): $K_M = 0.4, e_k = 20, e_{p0} = 0.3, I_0 = 0.0, K_{h2} = 0.7$
Figure 5.21: States Transients over shifts of \([Ca^{2+}]\): A, the systems remains on BSS when \([Ca^{2+}]\) jumps from 1.3 \(\mu M\) to 1.8 \(\mu M\); B, when \([Ca^{2+}]\) jumps from 1.3 \(\mu M\) to 2.2 \(\mu M\), the system transits to the TSS; C, when \([Ca^{2+}]\) is switched from 2.3 \(\mu M\) to 1.8 \(\mu M\), the system keeps on TSS; D, \([Ca^{2+}]\) jumps from 2.3 \(\mu M\) to 1.5 \(\mu M\), and the system state transits to BSS. Parameters (in \(\mu M\)): \(K_M = 0.4\), \(e_k = 20\), \(e_{p0} = 0.3\), \(I_0 = 0.0\), \(K_{h2} = 0.7\).
Figure 5.22: System dynamics when excitation is removed after system reaches TSS (resting $[Ca^{2+}]$ is 1.9 $\mu M$, that is in the bistability range). Parameters (in $\mu M$): $K_M = 0.4, I_0 = 0.0, e_k = 20, e_{\rho 0} = 0.3, K_{h2} = 0.7$.

Figure 5.23: System dynamics when excitation is removed after system reaches TSS (resting $[Ca^{2+}]$ is 1.0 $\mu M$, that is under the bistability range). Parameters (in $\mu M$): $K_M = 0.4, I_0 = 0.0, e_k = 20, e_{\rho 0} = 0.3, K_{h2} = 0.7$. 
Figure 5.24: System dynamics when excitation is removed after system reaches TSS (resting \([Ca^{2+}]\) is 0.1 \(\mu M\), that is below the bistability range). Parameters (in \(\mu M\)): \(K_M = 0.4, I_0 = 0.0, e_k = 20, e_{p0} = 0.3, K_{h2} = 0.7\)

Figure 5.25: Threshold values of system. Parameters (in \(\mu M\)): \(K_M = 0.4, I_0 = 0.1, e_k = 20, e_{p0} = 1.2, K_{h2} = 0.7\)
Fig. (5.26) shows the system dynamics when the resting value of $[Ca^{2+}]$ is in the bistability range while Fig. (5.27) shows the dynamics when the resting $[Ca^{2+}]$ value is lower than the lower threshold value.

In addition, Fig. (5.28) shows the system state transition when the resting $[Ca^{2+}]$ is zero. One can observe that the transition of states takes a very long time to complete. For instance, in Fig. (5.27), more than 30 hours are needed to finish the state change. For a special case that when the calcium input is completely removed and no calcium concentration is present, the system keeps in TSS. This is because the protein phosphatase is calcium dependent thus phosphorylation and dephosphorylation all cease in this situation. That may not happen in reality but worth pointing out in the system analysis.

![Graph showing system dynamics](image)

**Figure 5.26:** System dynamics when excitation is removed after system reaches TSS (resting $[Ca^{2+}]$ is 0.6 $\mu M$, that is in the bistability range). Parameters (in $\mu M$): $K_M = 0.4$, $I_0 = 0.1$, $e_k = 20$, $e_{p0} = 1.2$, $K_{h2} = 0.7$
Figure 5.27: System dynamics when excitation is removed after system reaches TSS (resting \([Ca^{2+}]\) is 0.5 \(\mu M\), that is not in the bistability range). Parameters (in \(\mu M\)): \(K_M = 0.4, I_0 = 0.1, e_k = 20, e_{p0} = 1.2, K_{h2} = 0.7\)

Figure 5.28: System dynamics when excitation is removed after system reaches TSS (resting \([Ca^{2+}]\) is 0.0 \(\mu M\)). Parameters (in \(\mu M\)): \(K_M = 0.4, I_0 = 0.1, e_k = 20, e_{p0} = 1.2, K_{h2} = 0.7\)
5.2 Averaging Analysis Simulation

In this section, several typical periodic calcium inputs will be introduced to the simulation to examine the system dynamics. After that, the simulation outputs from the averaged model will be used to compare with those from the original model.

5.2.1 Pulse Train Input

We start with a pulse train. In the following, we set two parameters constant by letting $[Ca^{2+}]_{resting} = 0.1\mu M$, and duty cycle $D = 0.25$. In simulation, I let $[Ca^{2+}]_{amp} = 0.8\mu M$, then we have $\bar{w}_a = 2.35 \times 10^{-4}, \bar{w}_u = 2.19 \times 10^{-7}$ and $\bar{w}_d = 6.33 \times 10^{-5}$. One should note that the averaged values are frequency independent. The amplitude value is set to ensure that system stays in the bistable region of the full model. Putting the results together, we have Fig. (5.29) and Fig. (5.30). We can conclude that the output from the averaged

![Graph](image)

Figure 5.29: System dynamics of original and averaged model when $x_0 = 0$. Parameters: $f = 1Hz$, $[Ca^{2+}]_{amp} = 0.8\mu M$
model matches the output of the original model very well as the frequency $f = 1Hz$.

Besides, the averaged model still has the property of bistability.

The four figures (Fig. 5.30 - 5.33) try to show the effect of input signal’s frequency on the system outputs. The figures show that the differences between the outputs of original model and that from the averaged model increase as the frequency decreases as the system filters out less of the high frequency signal components.

### 5.2.2 Sine Wave Input

If we put a DC offset to a regular sine wave, we can build up a sine wave $[Ca^{2+}]$ signal. It can be shown by Fig. (5.34) and defined by three parameters: resting value
Figure 5.31: System dynamics of original and averaged model, all systems reach BSS. Parameters: $f = 1Hz, [Ca^{2+}]_{amp} = 0.8\mu M$

Figure 5.32: System dynamics of original and averaged model, all systems reach TSS. Parameters: $f = 0.001Hz, [Ca^{2+}]_{amp} = 0.8\mu M$
Figure 5.33: System dynamics of original and averaged model, all systems reach BSS.

Parameters: \( f = 0.001\,\text{HZ}, [\text{Ca}^{2+}]_{\text{amp}} = 0.8\mu M \)

\([\text{Ca}^{2+}]_{\text{resting}}\), amplitude \( A \) and frequency \( f \).

\[ [\text{Ca}^{2+}] = [\text{Ca}^{2+}]_{\text{resting}} + A\sin(2\pi ft) \]

It is easy to calculate the average values of calcium concentration

\[ \overline{[\text{Ca}^{2+}]} = [\text{Ca}^{2+}]_{\text{resting}} \]

on the other hand, finding an explicit way of calculating \( \overline{w_a}, \overline{w_a^2} \) and \( \overline{w_d} \) is very complicated. So we instead use a numerical method to compute them. Taking \( \overline{w_a} \) as an example,

\[ \overline{w_a} = \frac{\int_0^T w_a(t)dt}{T} \]

where \( T \) is the period. For \([\text{Ca}^{2+}]_{\text{resting}} = 1.8\mu M, A = 0.2\mu M \) and \( f = 1\,\text{Hz} \), we have \( \overline{w_a} = 0.0406, \overline{w_a^2} = 0.0018 \) and \( \overline{w_d} = 4.6692 \times 10^{-4} \). At last, in the simulations the variable parameters are set as: \( K_M = 0.4, e_k = 20, e_{p0} = 1.2, I_0 = 0.1, K_{h2} = 0.7 \), all in \( \mu M \).
Figure 5.34: Sine wave $[Ca^{2+}]$ Signal. Parameters: $f = 1$Hz, $A = 0.2\mu M$, $[Ca^{2+}]_{resting} = 0.4\mu M$

Fig. (5.35) and Fig.(5.36) show the time history of concentration of phosphorylated subunits when we change the frequency of the inputs.

In these simulations, the averaged input calcium concentration is set to be greater than the upper threshold value thus the system will reach TSS eventually. From the figures, one can observe that the differences between outputs increase when the input frequency goes lower.

In addition, if we set the averaged value of $[Ca^{2+}]$ to be in the bistability range, we can observe that the averaged model still shows bistability. That can be shown by Fig. (5.37) - Fig. (5.40), the averaged model reaches different equilibria from different initial states.
Figure 5.35: System dynamics of original and averaged model, all systems reach TSS. Parameters: $x_0 = 0_{10}$, $f = 1 HZ$, $[Ca^{2+}]_{rest} = 1.8 \mu M$, $A = 0.2 \mu M$

Figure 5.36: System dynamics of original and averaged model, all systems reach TSS. Parameters: $x_0 = 0_{10}$, $f = 0.01 HZ$, $[Ca^{2+}]_{rest} = 1.8 \mu M$, $A = 0.2 \mu M$
Figure 5.37: System dynamics of original and averaged model, all systems reach BSS. Parameters: $x_0 = 0_{10}, f = 1HZ, [Ca^{2+}]_{rest} = 1.2\mu M, [Ca^{2+}]_{amp} = 0.2\mu M$

Figure 5.38: System dynamics of original and averaged model, all systems reach TSS. Parameters: $x_0 = 20 \times 1_{10}, f = 1HZ, [Ca^{2+}]_{rest} = 1.2\mu M, [Ca^{2+}]_{amp} = 0.2\mu M$
Figure 5.39: System dynamics of original and averaged model, all systems reach BSS. Parameters: $x_0 = 0_{10}$, $f = 0.01Hz$, $[Ca^{2+}]_{rest} = 1.2 \mu M$, $[Ca^{2+}]_{amp} = 0.2 \mu M$

Figure 5.40: System dynamics of original and averaged model, all systems reach TSS. Parameters: $x_0 = 20 \times 1_{10}$, $f = 0.01Hz$, $[Ca^{2+}]_{rest} = 1.2 \mu M$, $[Ca^{2+}]_{amp} = 0.2 \mu M$
5.2.3 Mixed Sine Wave Input

In the preceding section, we only apply one sine wave to a resting value; in this section, we first mix several sine waves with different frequency to obtain a periodic signal and then add it to a constant input (resting value) to achieve a mixed sine wave $[\text{Ca}^{2+}]$ input, which is shown by Fig. (5.41). The equation for the signal can be written as

$$[\text{Ca}^{2+}] = [\text{Ca}^{2+}]_{\text{resting}} + \sum_{i=1}^{n} A_i \sin(2\pi f_i t)$$

and can be defined by a group of parameters: resting value $[\text{Ca}^{2+}]_{\text{resting}}$, amplitudes $A_i$ and frequencies $f_i$ for each sine wave component. Shown in the figure and applied to the simulation, three sine waves are mixed in the signal: base sine wave with $f_1 = 1\text{Hz}, A_1 = 0.2\mu M$, third harmonic component $f_2 = 3f_1, A_2 = 0.1\mu M$ and high-frequency component $f_3 = 30f_1, A_3 = 0.1\mu M$. The integration method discussed in the preceding section is applied to calculate the averaged parameters. For the parameters value in the caption of Fig. 5.41, we have $\bar{w}_a = 0.0408, \bar{w}_a^2 = 0.0019$ and $\bar{w}_d = 4.6709 \times 10^{-4}$. The variable parameters’ values are set as in the preceding section (in $\mu M$): $(K_M = 0.4, I_0 = 0.1, e_k = 20, e_{\rho 0} = 1.2, I_0 = 0.1, K_{R2} = 0.7)$. In addition, the parameters of input signal are set as $(f_1 = 1\text{Hz}, A_1 = 0.2\mu M, f_2 = 3\text{Hz}, A_2 = 0.1\mu M, A_3 = 30\text{Hz}, 4A_3 = 0.1\mu M, [\text{Ca}^{2+}]_{\text{resting}} = 1.8\mu M)$.

Applying the signal to the simulations and plotting the outputs from the original and averaged models, we can get Fig. (5.42) and Fig.(5.43). In Fig. (5.42), the base sine wave frequency is $1\text{Hz}$ while in Fig.(5.43) the frequency is $0.01\text{Hz}$.

In the simulations, the resting value of $[\text{Ca}^{2+}]$ is already greater than the threshold value, thus the system will reach TSS eventually. Also, one can conclude that the differences between outputs increase when the input frequency goes down. Furthermore, we can also observe the bistability from the simulation results of the averaged model. That
can be shown by Fig. (5.44) to Fig. (5.47), which also show how the differences change with respect to the frequencies.

Figure 5.41: Mixed Sine wave \([Ca^{2+}]\) Signal. Parameters: \(f_1 = 1\text{HZ}, A_1 = 0.2\mu\text{M}, f_2 = 3\text{Hz}, A_2 = 0.1\mu\text{M}, f_3 = 30\text{Hz}, A_3 = 0.1\mu\text{M}, [Ca^{2+}]_{\text{resting}} = 1.8\mu\text{M}\)

5.2.4 Tetanus Input

A tetanus signal can be shown by Fig. (5.48) and Fig. (5.49). The two figures show the leading part and ending part of a 60 second long tetanus respectively.

The input signal can be treated as a combination of two signals: a pulse train with zero resting value (defined by amplitude \(A_p\), duration time \(\tau\) and period \(T\)) and a repeating sequence (defined by frequency \(f_s\) and amplitude \(A_s\)). As before, the same
Figure 5.42: System dynamics of original and averaged model, all systems reach TSS. Parameters: $x_0 = 0_{10}$, $f_1 = 1 Hz$, $A_1 = 0.2\mu M$, $f_2 = 3 Hz$, $A_2 = 0.1\mu M$, $f_3 = 30 Hz$, $A_3 = 0.1\mu M$, $[Ca^{2+}]_{rest} = 1.8\mu M$

Figure 5.43: System dynamics of original and averaged model, all systems reach TSS. Parameters: $x_0 = 0_{10}$, $f_1 = 0.01 Hz$, $A_1 = 0.2\mu M$, $f_2 = 0.03 Hz$, $A_2 = 0.1\mu M$, $f_3 = 0.3 Hz$, $A_3 = 0.1\mu M$, $[Ca^{2+}]_{rest} = 1.8\mu M$
Figure 5.44: System dynamics of original and averaged model, all systems reach BSS. Parameters: $x_0 = 0, f_1 = 1\text{Hz}, A_1 = 0.2\mu M, f_2 = 3\text{Hz}, A_2 = 0.1\mu M, f_3 = 30\text{Hz}, A_3 = 0.1\mu M, [Ca^{2+}]_{\text{rest}} = 1.2\mu M$

Figure 5.45: System dynamics of original and averaged model, all systems reach TSS. Parameters: $x_0 = 10 \times 1, f_1 = 1\text{Hz}, A_1 = 0.2\mu M, f_2 = 3\text{Hz}, A_2 = 0.1\mu M, f_3 = 30\text{Hz}, A_3 = 0.1\mu M, [Ca^{2+}]_{\text{rest}} = 1.2\mu M$
Figure 5.46: System dynamics of original and averaged model, all systems reach BSS. Parameters: \( x_0 = 0.10, f_1 = 0.01\text{Hz}, A_1 = 0.2\mu M, f_2 = 0.03\text{Hz}, A_2 = 0.1\mu M, f_3 = 0.3\text{Hz}, A_3 = 0.1\mu M, [Ca^{2+}]_{\text{rest}} = 1.2\mu M \)

integration method is applied to calculate the averaged parameters: \( \bar{w_a} = 0.0019, \) \( \bar{w_a}^2 = 1.8477 \times 10^{-4} \) and \( \bar{w_d} = 3.7851 \times 10^{-6} \). The parameters are set as before (in \( \mu M \)): \( K_M = 0.4, I_0 = 0.1, e_k = 20, e_{p0} = 0.3, I_0 = 0.1, \) and \( K_{h2} = 1.4 \).

Similarly, we can get Fig. (5.50) and Fig. (5.51). Fig. (5.50) shows the system dynamics in one period while Fig. (5.51) shows the dynamics in seven periods during which the systems reached TSS.

In the simulations, the averaged value of \([Ca^{2+}]\) is about half of \(K_{h1}\), and the system takes 60 seconds to reach the level of total phosphorylated subunits concentration of about 60\(\mu M\). We can also observe that the concentration of phosphorylated subunits gets "frozen" after the end of excitation. This phenomena has already been mentioned in Section 6.1, which is that the system will keep its state when input \([Ca^{2+}]\) drops to a very low resting value.
Figure 5.47: System dynamics of original and averaged model, all systems reach TSS. Parameters: $x_0 = 10 \times 1_{10}, f_1 = 1Hz, A_1 = 0.2\mu M, f_2 = 3Hz, A_2 = 0.1\mu M, f_3 = 30Hz, A_3 = 0.1\mu M, [Ca^{2+}]_{rest} = 1.2\mu M$

In addition, Fig. (5.53) shows the output from a 100Hz 1 second tetanus input. Combined with Fig. (5.52), we can observe that what is frequency’s impact on the differences between the original and the averaged model. Fig. (5.52) to Fig. (5.55) also show that bistability still holds in the averaged model.
Figure 5.48: Tetanus \([Ca^{2+}]\) Signal (leading parts): Sequence Frequency 10Hz, Sequence amplitude \(1\mu M\), duration 60s, pulse period 70s, pulse amplitude \(2\mu M\)

Figure 5.49: Tetanus \([Ca^{2+}]\) Signal (ending parts): Sequence Frequency 10Hz, Sequence amplitude \(1\mu M\), duration 60s, pulse period 70s, pulse amplitude \(2\mu M\)
Figure 5.50: System dynamics in one signal period. ($f_s = 10Hz$, $\tau = 60s$ tetanus)

Figure 5.51: System dynamics in seven signal periods (TSS reached). ($f_s = 10Hz$, $\tau = 60s$ tetanus)
Figure 5.52: System dynamics of original and averaged model, all systems reach TSS. Parameters: \( f_s = 10Hz, A_s = 1\mu M, \tau = 60s, T = 70s, A_p = 2\mu M \)

Figure 5.53: System dynamics of original and averaged model, all systems reach TSS. Parameters: \( f_s = 100Hz, A_s = 1\mu M, \tau = 1s, T = 2s, A_p = 20\mu M \)
Figure 5.54: System dynamics of original and averaged model, all systems reach BSS. Parameters: $f_s = 10Hz, A_s = 0.2\mu M, \tau = 60s, T = 70s, A_p = 1\mu M$.

Figure 5.55: System dynamics of original and averaged model, all systems reach BSS. Parameters: $f_s = 100Hz, A_s = 0.2\mu M, \tau = 1s, T = 2s, A_p = 1\mu M$.
6 Conclusion

In the thesis, we first reviewed several typical and important ways of applying monotone system theory to nonlinear dynamic systems. Then we analyzed the processes of CaMKII phosphorylation and dephosphorylation separately and built up a model by putting them together. After that, we applied the monotone systems theory to the model and proved that the protein activation dynamic system is a monotone system. We were therefore able to use the established tools to study the system and obtain some properties analytically using simple algebraic calculations instead of exhaustive numerical simulations.

6.1 Significance of research

The research is focused on the monotonicity analysis and averaging analysis applied to Zhabotinsky’s model of the CaMKII activation system. The main contributions of the research can be briefly summarized as the following:

(1) applying input-output monotone theories to analytically demonstrate some important properties of a complex biological system while previously they have to be derived numerically;

(2) performing averaging analysis of the system and providing a way to simplify the input-output analysis of complex biological systems.

As an example, a simulation shown by Fig. (5.27) in Chapter 5 which may take many hours of computation to obtain the equilibrium points if we solve it numerically. On the other hand, if we solve it by simple algebraic calculations, it only takes one thousandth of a second.

Consequently, the averaging analysis provides us the possibility to replace a periodic input by a constant input, which enables us to continuously apply analytical methods to analyze the model even if the inputs are not constant anymore.
6.2 Call for Further Research

As mentioned before, the model we discussed in the thesis has a moderate complexity. Because we assume that CaMKII has ten subunits and each subunit of CaMKII has only two states: unphosphorylated and phosphorylated, the number of states in state equations becomes only ten if we define the state variables as the number of phosphorylated subunits. Meanwhile we use a statistical method to simulate the random subunits positions in which dephosphorylation occurs.

On the other hand, Kubota [54] in 2002 independently proposed another CaMKII activation model. The model is more straightforward in the modeling process and uses fewer simplifications. For instance, no statistical method is used to simulate the randomness of dephosphorylation. In his model, a subunit has five states which increases the complexity of the model dramatically. In that case, if we still assume that CaMKII has ten subunits the total states will be $5^{10}$. That is too complicated to compute efficiently. Kubota did some studies and concluded that changing the quantity of subunits will only affect the system quantitatively and not the fundamental system properties. So in his model, he assumed that CaMKII has only 4 subunits. That will generate up to 625 state variables; with the help of rotational symmetry there will still be over one hundred state variables. With this model, some works have been done and the results suggested that the system also exhibits monotonicity and bistability.

The same method discussed in the thesis should be applicable to Kubota’s model too. If we will be able to successfully apply the method to this complicated CaMKII activation model, we can conclude that they are effective and valuable in this type of system analysis.
REFERENCES


Appendix: Matlab Codes

A.1 Codes in Chapter 3

To plot Fig. 3.2 and Fig. 3.3

```
% Figure 1: I/O characteristic plot
% Figure 2: Bistability
% clc
clear
close all
zhabotinsky_init

u = logspace(-3,1,1000);
Ca_1 = 0.2;
[y1, p1] = IOcharacteristic(u, Ca_1, param);
z1 = (1:N) * p1;

[z_e, p_e, u_e] = equilibria(Ca_1, param);

lwidth = 2.0;
fsize = 14;

u1 = logspace(-3,1,1000);
Ca_1 = 0.2;
[y1, p1] = IOcharacteristic(u, Ca_1, param);
z1 = (1:N) * p1;

[z_e, p_e, u_e] = equilibria(Ca_1, param);

lwidth = 2.0;
fsize = 14;

%subplot(2,2,1)
figure(1)
loglog(u, y1, u, u, u_e, u_e, 'ro',
       'LineWidth', lwidth)
legend(['
        [Ca^2+] = ', num2str(Ca_1), ' \muM'], 2)
ylabel('y', 'FontSize', fsize)
xlabel('u', 'FontSize', fsize)
axis([min(u) max(u) min(u) max(u)])
v1 = axis;
for k = 1:3
    line([u_e(k) u_e(k)], [v1(3) u_e(k)],
         'Linewidth', 2,
         'LineStyle', '--', 'Color', 'r')
    line([v1(1) u_e(k)], [u_e(k) u_e(k)],
         'Linewidth', 2,
         'LineStyle', '--', 'Color', 'r')
end
```
Ca = 0.093:0.005:0.703;
Z = [];

for k = 1:length(Ca)
    zk = equilibria(Ca(k), param);
    leng = length(zk);
    if(leng<3)
        zk=[zk zk zk];
    end
    Z = [Z; zk];
end

%subplot(2,2,4)
figure(2)
plot(Ca, Z(:,1), 'k', Ca, Z(:,2), Ca, Z(:,3),
     Ca_1*ones(1,3), z_e, 'ro',
     'LineWidth', lwidth)
axis([0 1 -5 200]);
ylabel('Concentration of phosphorylated subunits (\muM)',
     'FontSize', fsize)
xlabel('[Ca^{2+}] (\muM)', 'FontSize', fsize)
v3 = axis;
for k = 1:3
    line([Ca_1 Ca_1], [v2(3) zk(k)], 'Linewidth', 2,
         'LineStyle', '--', 'Color', 'r')
    line([v3(1) Ca_1], [zk(k) zk(k)], 'Linewidth', 2,
         'LineStyle', '--', 'Color', 'r')
end

To plot Fig. 3.4
%
% I/O characteristic plots showing multi-stability
% threshold values
% clc
clear
close all
zhobotinsky_init

u = logspace(-3,1,1000);
Ca1 = 0.093179;
[y1, p1] = IOcharacteristic(u, Ca1, param);
\[ z_1 = (1:N) \times p_1; \]
\[ \text{Ca}_2 = 0.702687; \]
\[ [y_2, p_2] = \text{IOcharacteristic}(u, \text{Ca}_2, \text{param}); \]
\[ z_2 = (1:N) \times p_2; \]
\[ \text{LineWidth} = 2.0; \]
\[ \text{fsize} = 14; \]

```matlab
figure(1)
loglog(u, y1, u, y2, 'r', u, u,
    'LineWidth', linewidth)
legend('Ca \approx 0.09 \mu M', 'Ca \approx 0.70 \mu M', 2)
ylabel('y', 'FontSize', fsize')
xlabel('u', 'FontSize', fsize')
```

To plot Fig. 3.5

% % I/O hesteresis plot %
clear
close all
zhabotinsky_init

\[ \text{Ca} = 0.01:0.01:0.9; \]
\[ \text{Zmin} = \text{zeros(size(Ca))}; \]
\[ \text{Zmax} = \text{zeros(size(Ca))}; \]

```matlab
for k = 1:length(Ca)
    zk = equilibria(Ca(k), param);
    zk(zk<0)=[];
    \[ \text{Zmin}(k) = \text{min}(zk); \]
    \[ \text{Zmax}(k) = \text{max}(zk); \]
end
```

\[ \text{LineWidth} = 2.0; \]
\[ \text{fsize} = 14; \]

```matlab
plot(Ca, Zmin, Ca, Zmax, '--',
    'LineWidth', linewidth)
axis([0 1 -1 200]);
```
The subroutines:

% Initialize Zhabotinsky (2000) model
% clc
% clear

% Parameter values from Table 1 on p. 2214 of Zhabotinsky (2000)
% Note: Table 1 provides a range of values for parameters marked with asterisks below.

ek = 20; % 1. total CaMKII concentration (uM) (*)
ep0 = 0.05; % 2. total protein phosphatase concentration (uM) (*)
I0 = 0.1; % 3. free inhibitor I1 concentration (uM) (*)
vCaN= 1.0; % 4. CaN activity divided by its Michaelis constant (1/s)
vPKA= 1.0; % 5. PKA activity divided by its Michaelis constant (1/s)
Km = 0.4; % 6. protein phosphatase Michaelis constant (uM) (*)
Kh1 = 4.0; % 7. Hill constant for Ca2+ activation of CaMKII (uM)
Kh2 = 0.7; % 8. Hill constant for Ca2+ activation of CaN (uM) (*)
K1 = 0.5; % 9. catalytic constant of autophosphorylation (1/s)
K2 = 2.0; % 10. catalytic constant of protein phosphatase (1/s)
K3 = 1.0; % 11. association rate constant of PP1-I1P complex (1/uM/s)
K4 = 1.0e-3; % 12. dissociation rate constant of PP1-I1P complex (1/s)
N = 10; % 13. number of subunits per CaMKII holoenzyme molecule
param = [ek ep0 I0 vCaN vPKA Km Kh1 Kh2 K1 K2 K3 K4 N];

% Initialize Simulink Simulation
Ca_rest = 0.1;
Ca_amp = 5;
gam = (Ca_rest / Kh2)^3 /
     (1 + (Ca_rest / Kh2)^3);
I_equ = vPKA / vCaN / gam * I0;
ep_equ = K4*ep0 / (K3*I_equ + K4);

[z, p, u] = equilibria(Ca_rest, param);
p_init = p(:, 1);
x_init = [ep_equ; I_equ];
toff = 1800;
h = 1.5;
tstop = toff + h*3600;

Ca = Ca_rest;
pt = ek;
w_a = (Ca / Kh1)^4 / (1 + (Ca / Kh1)^4);
v1 = 10 * K1 * w_a^2;
v2 = K1 * w_a;
w = [1.0 1.8 2.3 2.7 2.8 2.7 2.3 1.8 1.0];
S = [zeros(1,N); eye(N-1) zeros(N-1,1)];
Aa = v2 * ( -diag([0 w]) + S*diag([w 0]) ) -
    v1 * eye(N,1)*ones(1,N);
Ba = v1 * eye(N,1);

A01 = dAa*x0 + dBa*pt;
A11 = dAa;
A02 = ddAa*x0 + ddBa*pt;

2. IOCharacteristic.m
function [y, P] = IOcharacteristic(u, Ca, param);
%
% Index Parameter Description
%
% 1. ek (pt) - total CaMKII concentration (uM) (*)
% 2. p0 - total protein phosphatase concentration (uM) (*)
% 3. I0 - free inhibitor I1 concentration (uM) (*)
% 4. vCaN - CaN activity divided by its Michaelis constant (1/s)
% 5. vPKA - PKA activity divided by its Michaelis constant (1/s)
% 6. Km - protein phosphatase Michaelis constant (uM) (*)
% 7. Kh1 - Hill constant for Ca2+ activation of CaMKII (uM)
% 8. Kh2 - Hill constant for Ca2+ activation of CaN (uM) (*)
% 9. K1 - catalytic constant of autophosphorylation (1/s)
% 10. K2 - catalytic constant of protein phosphatase (1/s)
% 11. K3 - association rate constant of PP1-I1P complex (1/uM/s)
% 12. K4 - dissociation rate constant of PP1-I1P complex (1/s)
% 13. N - number of subunits per CaMKII holoenzyme molecule
%
pt = param(1);
ep0 = param(2);
I0 = param(3);
vCaN = param(4);
vPKA = param(5);
Km = param(6);
Kh1 = param(7);
Kh2 = param(8);
K1 = param(9);
K2 = param(10);
K3 = param(11);
K4 = param(12);
N = param(13);
% Autophosphorylation Dynamics
\[
w_a = \frac{(Ca / Kh1)^4}{1 + (Ca / Kh1)^4}
\]
\[
v1 = 10 \cdot K1 \cdot w_a^2
\]
\[
v2 = K1 \cdot w_a
\]
\[
w = \begin{bmatrix} 1.0 & 1.8 & 2.3 & 2.7 & 2.8 & 2.7 & 2.3 & 1.8 & 1.0 \end{bmatrix}
\]
\[
S = \begin{bmatrix} zeros(1,N); & eye(N-1); & zeros(N-1,1) \end{bmatrix}
\]
\[
Aa = -v2 \cdot \left( \text{diag}([w 0]) - S \cdot \text{diag}([w 0]) \right) - v1 \cdot \text{eye}(N,1) \cdot \text{ones}(1,N)
\]
\[
Ba = v1 \cdot \text{eye}(N,1)
\]
% Phosphase/Inhibitor concentrations at equilibrium
\[
C0 = \frac{(Ca / Kh2)^3}{1 + (Ca / Kh2)^3}
\]
\[
I_equ = \frac{vPKA}{vCaN} \cdot \frac{C0}{I0}
\]
\[
ep_equ = \frac{K4 \cdot ep0}{K3 \cdot I_equ + K4}
\]
% Dephosphorylation Dynamics
\[
w_d = ep_equ
\]
\[
D = \text{eye}(N) - \begin{bmatrix} zeros(N-1,1) & eye(N-1); & zeros(1,N) \end{bmatrix}
\]
\[
L = \text{diag}(1:N)
\]
\[
Ad = w_d \cdot D \cdot L
\]
% Equilibria
\[
m = \text{length}(u);
\]
\[
P = \text{zeros}(N, m);
\]
\[
y = \text{zeros}(\text{size}(u));
\]
for k = 1:m
\[
P(:,k) = - (Aa - u(k) \cdot Ad) \backslash Ba \cdot pt;
\]
\[
y(k) = K2 / (Km + (1:N) \cdot P(:,k))
\]
end

3. equilibria.m

function [z, p, u] = equilibria(Ca, param);
% Syntax
% [z, p, u] = equilibria(Ca, param)
%
% Index Parameter  Description
%
% 1.ek (pt) -  total CaMKII concentration (uM) (*)
% 2.p0 -  total protein phosphatase concentration (uM) (*)
% 3.I0 -  free inhibitor I1 concentration (uM) (*)
% 4.vCaN -  CaN activity divided by its Michaelis constant (1/s)
% 5.vPKA -  PKA activity divided by its Michaelis constant (1/s)
% 6.Km -  protein phosphatase Michaelis constant (uM) (*)
% 7.Kh1 -  Hill constant for Ca2+ activation of CaMKII (uM)
% 8.Kh2 -  Hill constant for Ca2+ activation of CaN (uM) (*)
% 9.K1 -  catalytic constant of autophosphorylation (1/s)
% 10.K2 -  catalytic constant of protein phosphatase (1/s)
% 11.K3 -  association rate constant of PP1-I1P complex (1/uM/s)
% 12.K4 -  dissociation rate constant of PP1-I1P complex (1/s)
% 13.N -  number of subunits per CaMKII holoenzyme molecule

% pt = param(1);
ep0 = param(2);
I0 = param(3);
vCaN = param(4);
vPKA = param(5);
Km = param(6);
Kh1 = param(7);
Kh2 = param(8);
K1 = param(9);
K2 = param(10);
K3 = param(11);
K4 = param(12);
N = param(13);
% Autophosphorylation Dynamics

\[
\begin{align*}
    w_a &= \frac{(Ca / Kh1)^4}{1 + (Ca / Kh1)^4}; \\
    v1 &= 10*K1 * w_a^2; \\
    v2 &= K1 * w_a; \\
    w &= [1.0 1.8 2.3 2.7 2.8 2.7 2.3 1.8 1.0]; \\
    S &= \text{zeros}(1,N); \text{eye}(N-1) \text{ zeros}(N-1,1) ; \\
    Aa &= -v2 * ( \text{diag}([w \ 0]) - S*\text{diag}([w \ 0]) ) - v1 * \text{eye}(N,1)*\text{ones}(1,N); \\
    Ba &= v1 * \text{eye}(N,1); \\
\end{align*}
\]

% Phosphase/Inhibitor concentrations at equilibrium

\[
\begin{align*}
    C0 &= \frac{(Ca / Kh2)^3}{1 + (Ca / Kh2)^3}; \\
    I\_equ &= \frac{vPKA}{vCaN / C0} * I0; \\
    ep\_equ &= \frac{K4*ep0}{K3*I\_equ + K4};
\end{align*}
\]

% Dephosphorylation Dynamics

\[
\begin{align*}
    w_d &= ep\_equ; \\
    D &= \text{eye}(N) - [\text{zeros}(N-1,1) \text{ eye}(N-1); \text{zeros}(1,N)]; \\
    L &= \text{diag}(1:N); \\
    Ad &= w_d*D*L;
\end{align*}
\]

% Find equilibria

\[
\begin{align*}
    A &= \text{Ad} \text{ \backslash} \text{ Aa}; \\
    B &= \text{Ad} \text{ \backslash} \text{ Ba} * \text{ pt}; \\
    C &= 1:N; \\
    D &= \text{Km}; \\
    \text{sys} &= \text{tf} (\text{ss}(A, B, C, D) ); \\
    \text{poly}\_u &= \text{[sys}\text{.num}\{1\} \ 0] - K2*[0 \text{ sys}\text{.den}\{1\}]; \\
    \text{u}\_\text{roots} &= \text{roots}(\text{poly}\_u); \\
    \text{idx} &= \text{setdiff}(1:\text{length}(\text{u}\_\text{roots}), \\
                     \text{find}(\text{u}\_\text{roots} - \text{real}(\text{u}\_\text{roots}))); \\
    \text{u} &= \text{u}\_\text{roots}(\text{idx})'; \\
    \text{z} &= []; \\
    \text{p} &= [];
\end{align*}
\]

if \( \text{isempty(u)} \)
    for \( k = 1:\text{length(u)} \)
\[ pk = - (Aa - u(k)*Ad) \backslash Ba \ast pt; \]
\[ p = [p \; pk]; \]
\[ z = [z \; (1:N)*pk]; \]
end
end

A.2 Codes in Chapter 4

To plot Fig. 4.2 and 4.3

clear all;
syms ep0 k3 k4 ep I0 ud vpka vcan
f1 = -k3*I*ep + k4*(ep0-ep);
f2 = -k3*I*ep + k4*(ep0-ep) +
    vpka*I0 - vcan*I*ud;
equ = solve(f1,f2,ep,I);

f = [f1;f2];
x = [ep, I];
R = jacobian(f,x);
R = subs(R,ep,equ.ep);
R = subs(R,I,equ.I);
bb=eig(R);

ep0 = 0.05; k3=1; k4=0.001;
vpka=1.0; vcan=1.0;
I0 = 0.1;
sd = 0.1:0.05:1;
for i=1:1:length(sd)
    ud=sd(i);
    bb1(i) = subs(bb(1));
    bb2(i) = subs(bb(2));
end

figure(1); hold on; grid on;
plot(real(bb1), imag(bb1),'ro')
hold on
plot(real(bb2), imag(bb2), 'b+');
axis([[-1.1 0.1 -0.5 0.5]]);
legend('Slow Eigenvalue', 'Fast Eigenvalue');

figure(2); hold on; grid on;
plot(sd, bb1,'ro')
hold on
plot(sd, bb2,'b+');
axis([0.1 1.1 -1 0.2]);
legend('Slow Eigenvalue','Fast Eigenvalue');

To plot Fig. 4.8, 4.9, and ??

clc
clear
close all
zhabotinsky_init;
resting = 0.00;
amp = 0.90;
per = 10;
pul = 0:5:100;

for i=1:1:length(pul)
    % calculate awa awa2 awd of Pulse train
    aca(i) = (100-pul(i))*resting/100 + pul(i)*(resting+amp)/100;
    temp = (resting/Kh1)^4/(1+(resting/Kh1)^4);
    temp2 = ((resting+amp)/Kh1)^4/(1+((resting+amp)/Kh1)^4);
    awa(i) = (100-pul(i))*temp/100 + pul(i)*temp2/100;
    temp = (resting/Kh1)^8/(1+(resting/Kh1)^8 + 2*(resting/Kh1)^4);
    temp2 = ((resting+amp)/Kh1)^8/(1+((resting+amp)/Kh1)^8 + 2*((resting+amp)/Kh1)^4);
    awa2(i) = (100-pul(i))*temp/100 + pul(i)*temp2/100;
    temp = (resting/Kh2)^3/(1+(resting/Kh2)^3);
    temp2 = ((resting+amp)/Kh2)^3/(1+((resting+amp)/Kh2)^3);
    ud(i) = (100-pul(i))*temp/100 + pul(i)*temp2/100;
    awd(i) = ep0*ud(i)/((K3/K4)*(vPKA/vCaN)*I0+ud(i));
end
figure(1); hold on; grid on;
plot(aca, awa, 'r', 'LineWidth', 2);
figure(2); hold on; grid on;
plot(aca, awa2, 'r', 'LineWidth', 2);
figure(3); hold on; grid on;
plot(aca, awd, 'r', 'LineWidth', 2);

Ca = resting:0.01:resting+amp;
for i = 1:length(Ca)
    wa(i) = (Ca(i)/Kh1)^4/(1+(Ca(i)/Kh1)^4);
    wa2(i) = wa(i)^2;
    ud(i) = (Ca(i)/Kh2)^3/(1+(Ca(i)/Kh2)^3);
    wd(i) = ep0*ud(i)/((K3/K4)*(vPKA/vCaN)*I0+ud(i));
end
figure(1); hold on;
plot(Ca, wa, 'r--', 'LineWidth', 2);
figure(2); hold on;
plot(Ca, wa2, 'r--', 'LineWidth', 2);
figure(3); hold on;
plot(Ca, wd, 'r--', 'LineWidth', 2);

pul = 50;
amp = 0:0.05:1.8;
for i = 1:length(amp)
    % calculate awa awa2 awd of Pulse train
    aca(i) = (100-pul)*resting/100 + pul*(resting+amp(i))/100;
    temp = (resting/Kh1)^4/(1+(resting/Kh1)^4);
    temp2 = ((resting+amp(i))/Kh1)^4/(1+((resting+amp(i))/Kh1)^4);
    awa(i) = (100-pul)*temp/100 + pul*temp2/100;
    temp = (resting/Kh1)^8/(1+(resting/Kh1)^8+2*(resting/Kh1)^4);
    temp2 = ((resting+amp(i))/Kh1)^8/(1+((resting+amp(i))/Kh1)^8+2*((resting+amp(i))/Kh1)^4);
    awa2(i) = (100-pul)*temp/100 + pul*temp2/100;
    temp = (resting/Kh2)^3/(1+(resting/Kh2)^3);
    temp2 = ((resting+amp(i))/Kh2)^3/
\begin{verbatim}
(1+((resting+amp(i))/Kh2)^3);
ud(i) = (100-pul)*temp/100 + pul*temp2/100;
awd(i) = ep0*ud(i)/((K3/K4)*(vPKA/vCaN)*I0+ud(i));
end

figure(1); hold on;
plot(aca, awa,'b:','LineWidth', 2);
xlabel('Average [Ca^{2+}](\mu M)');
ylabel('w_a');
legend('Fix amplitude, sweep duty cycle',
   'Constant',
   'Fix duty cycle, sweep amplitude',
   'Location','NorthWest');

figure(2); hold on;grid on;
plot(aca, awa2,'b:','LineWidth', 2);
xlabel('Average [Ca^{2+}](\mu M)');
ylabel('w_a^2');
legend('Fix amplitude, sweep duty cycle',
   'Constant',
   'Fix duty cycle, sweep amplitude',
   'Location','NorthWest');

figure(3); hold on;grid on;
plot(aca, awd,'b:','LineWidth', 2);
xlabel('Average [Ca^{2+}](\mu M)');
ylabel('w_d');
legend('Fix amplitude, sweep duty cycle',
   'Constant',
   'Fix duty cycle, sweep amplitude',
   'Location','NorthWest');

To plot Fig. 4.11 and Fig. 4.13

% I/O characteristic plots
clc
clear
close all
zhabotinsky_init;
\end{verbatim}
resting = 0.09;
amp = 0.62;
per = 2;
pul = 1;
cal_average;
u = logspace(-3,1,1000);
[y1, p1] = IOcharacteristic(u, aca, awa, awa2, ud, awd, param);
[z_e, p_e, u_e] = equilibria(aca, awa, awa2, ud, awd, param);
lwidth = 2.0;
fsise = 14;
figure(1)
%loglog(u, y1, u, u, 'LineWidth', lwidth)
loglog(u, y1, u, u, u_e, u_e, 'ro',
    'LineWidth', lwidth)
legend(['Averaged [Ca$^{2+}$] = ',
    num2str(aca), '
    \muM'], 2)
ylabel('y', 'FontSize', fsize')
xlabel('u', 'FontSize', fsize')
axis([min(u) max(u) min(u) max(u)])
v1 = axis;
for k = 1:3
    line([u_e(k) u_e(k)], [v1(3) u_e(k)],
    'Linewidth', 2,
    'LineStyle', '--', 'Color', 'r')
end

Pul = 0.0:0.001:5;
Z = [];

for k = 1:1:length(Pul)
pul=Pul(k);
cal_average;
zk = equilibria(aca, awa, awa2, ud, awd, param);
leng = length(zk);
if(leng<3)
    zk=[zk zk zk];
end
Z = [Z; zk];
end
axis([-5 100 -5 205]);
v3=axis;

figure(3); hold on;
plot(Pul, Z(:,1), 'k', Pul, Z(:,2), Pul, Z(:,3), 1*ones(1,3), z_e, 'ro', 'LineWidth', lwidth)
for k = 1:3
    line([1 1], [0 z_e(k)], 'Linewidth', 2, 'LineStyle', '--', 'Color', 'r')
    line([v3(1) 1], [z_e(k) z_e(k)], 'Linewidth', 2, 'LineStyle', '--', 'Color', 'r')
end
Pul = 5:1:100;
Z = [];

for k = 1:length(Pul)
pul=Pul(k);
cal_average;
zk = equilibria(aca, awa, awa2, ud, awd, param);
leng = length(zk);
if(leng<3)
    zk=[zk zk zk];
end
Z = [Z; zk];
end

figure(3); hold on;
plot(Pul, Z(:,1), 'k', Pul, Z(:,2), Pul, Z(:,3), 'LineWidth', lwidth)
axis([-5 100 -5 205]);
ylabel('Concentration of phosphorylated subunits (\mu M)', 'FontSize', fsize)
xlabel('Averaged [Ca^{2+}] (\mu M)', 'FontSize', fsize)

Subroutines:
1. Zhabotinsky-init

\begin{verbatim}
% Initialize Zhabotinsky (2000) model
%
clc
clear
%
Parameter values from Table 1
% on p. 2214 of Zhabotinsky (2000)
% Note: Table 1 provides a range
% of values for parameters marked with
% asterisks below.
%
ek = 20; % 1. total CaMKII concentration (uM) (*)
ep0 = 0.05; % 2. total protein phosphatase concentration (uM) (*)
I0 = 0.1; % 3. free inhibitor I1 concentration (uM) (*)
vCaN= 1.0; % 4. CaN activity divided by its Michaelis constant (1/s)
vPKA= 1.0; % 5. PKA activity divided by its Michaelis constant (1/s)
Km = 0.4; % 6. protein phosphatase Michaelis constant (uM) (*)
Kh1 = 4.0; % 7. Hill constant for Ca2+ activation of CaMKII (uM)
Kh2 = 0.7; % 8. Hill constant for Ca2+ activation of CaN (uM) (*)
K1 = 0.5; % 9. catalytic constant of autophosphorylation (1/s)
K2 = 2.0; % 10. catalytic constant of protein phosphatase (1/s)
K3 = 1.0; % 11. association rate constant of PP1-I1P complex (1/uM/s)
K4 = 1.0e-3; % 12. dissociation rate constant of PP1-I1P complex (1/s)
N = 10; % 13. number of subunits per CaMKII holoenzyme molecule
param = [ek ep0 I0 vCaN vPKA Km Kh1 Kh2 K1 K2 K3 K4 N];

2. cal-average
\end{verbatim}
% calculate awa awa2 awd of a Pulse train
%
%Average calcium
aca = (100-pul)*resting/100 + pul*(resting+amp)/100;

%average wa
temp = (resting/Kh1)^4/(1+(resting/Kh1)^4);
temp2 = ((resting+amp)/Kh1)^4/
    (1+((resting+amp)/Kh1)^4);
awa = (100-pul)*temp/100 + pul*temp2/100;

%average wd^2
temp = (resting/Kh1)^8/(1+(resting/Kh1)^8+
    2*(resting/Kh1)^4);
temp2 = ((resting+amp)/Kh1)^8/
    (1+((resting+amp)/Kh1)^8 +
    2*((resting+amp)/Kh1)^4);
awa2 = (100-pul)*temp/100 + pul*temp2/100;

%average wd
temp = (resting/Kh2)^3/(1+(resting/Kh2)^3);
temp2 = ((resting+amp)/Kh2)^3/
    (1+((resting+amp)/Kh2)^3);
ud = (100-pul)*temp/100 + pul*temp2/100;
awd = ep0*ud/((K3/K4)*(vPKA/vCaN)*I0+ud);

3. equilibria

function [z, p, u] = equilibria(Ca, awa,
    awa2, ud, awd, param);

% Syntax
%
% [z, p, u] = equilibria(Ca, param)
%
% Index Parameter Description
%
% 1. ek (pt) - total CaMKII concentration (uM) (*)
% 2. p0 - total protein phosphatase concentration (uM) (*)
% 3. I0 - free inhibitor I1 concentration (uM) (*)
% 4. vCaN - CaN activity divided by its Michaelis
% constant (1/s)
% 5. vPKA - PKA activity divided by its Michaelis constant (1/s)
% 6. Km - protein phosphatase Michaelis constant (uM) (*)
% 7. Kh1 - Hill constant for Ca2+ activation of CaMKII (uM)
% 8. Kh2 - Hill constant for Ca2+ activation of CaN (uM) (*)
% 9. K1 - catalytic constant of autophosphorylation (1/s)
% 10. K2 - catalytic constant of protein phosphatase (1/s)
% 11. K3 - association rate constant of PP1-I1P complex (1/uM/s)
% 12. K4 - dissociation rate constant of PP1-I1P complex (1/s)
% 13. N - number of subunits per CaMKII holoenzyme molecule

pt = param(1);
ep0 = param(2);
I0 = param(3);
vCaN = param(4);
vPKA = param(5);
Km = param(6);
Kh1 = param(7);
Kh2 = param(8);
K1 = param(9);
K2 = param(10);
K3 = param(11);
K4 = param(12);
N = param(13);

% Autophosphorylation Dynamics

w_a = awa;
v1 = 10*K1 * awa2;
v2 = K1 * awa;
w = [1.0 1.8 2.3 2.7 2.8 2.7 2.3 1.8 1.0];
S = [zeros(1,N); eye(N-1) zeros(N-1,1)];
Aa = -v2 * (diag([w 0]) - S*diag([w 0])) -
    v1 * eye(N,1)*ones(1,N);
Ba = v1 * eye(N,1);

x0 = [pt 0 0 0 0 0 0 0 0]';
% Phosphase/Inhibitor concentrations at equilibrium
% C0 = ud;
I_equ = vPKA / vCaN / C0 * I0;
ep_equ = K4*ep0 / (K3*I_equ + K4);
%
% Dephosphorylation Dynamics
%
w_d = ep_equ;
D = eye(N) -
    [zeros(N-1,1) eye(N-1); zeros(1,N)];
L = diag(1:N);
Ad = w_d*D*L;
%
% Find equilibria
%
A = Ad \ Aa;
B = Ad \ Ba * pt;
C = 1:N;
D = Km;
sys = tf( ss(A, B, C, D) );
poly_u = [sys.num{1} 0] - K2*[0 sys.den{1}];
u Roots = roots(poly_u);
idx = setdiff(1:length(u Roots),
    find(u Roots - real(u Roots)));

if isempty(u)
    for k = 1:length(u)
        pk = - (Aa - u(k)*Ad) \ Ba * pt;
        p = [p pk];
        z = [z (1:N)*pk];
    end
end

4. IOcharacteristic

function [y, P] = IOcharacteristic(u, aca,
    awa, awa2, ud, awd, param);
<table>
<thead>
<tr>
<th>Index Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.ek (pt)</td>
<td>total CaMKII concentration (uM) (*)</td>
</tr>
<tr>
<td>2.p0</td>
<td>total protein phosphatase concentration (uM) (*)</td>
</tr>
<tr>
<td>3.I0</td>
<td>free inhibitor I1 concentration (uM) (*)</td>
</tr>
<tr>
<td>4.vCaN</td>
<td>CaN activity divided by its Michaelis constant (1/s)</td>
</tr>
<tr>
<td>5.vPKA</td>
<td>PKA activity divided by its Michaelis constant (1/s)</td>
</tr>
<tr>
<td>6.Km</td>
<td>protein phosphatase Michaelis constant (uM) (*)</td>
</tr>
<tr>
<td>7.Kh1</td>
<td>Hill constant for Ca2+ activation of CaMKII (uM)</td>
</tr>
<tr>
<td>8.Kh2</td>
<td>Hill constant for Ca2+ activation of CaN (uM) (*)</td>
</tr>
<tr>
<td>9.K1</td>
<td>catalytic constant of autophosphorylation (1/s)</td>
</tr>
<tr>
<td>10.K2</td>
<td>catalytic constant of protein phosphatase (1/s)</td>
</tr>
<tr>
<td>11.K3</td>
<td>association rate constant of PP1-I1P complex (1/uM/s)</td>
</tr>
<tr>
<td>12.K4</td>
<td>dissociation rate constant of PP1-I1P complex (1/s)</td>
</tr>
<tr>
<td>13.N</td>
<td>number of subunits per CaMKII holoenzyme molecule</td>
</tr>
</tbody>
</table>

pt = param(1);
ep0 = param(2);
I0 = param(3);
vCaN = param(4);
vPKA = param(5);
Km = param(6);
Kh1 = param(7);
Kh2 = param(8);
K1 = param(9);
K2 = param(10);
K3 = param(11);
K4 = param(12);
N = param(13);

% Autophosphorylation Dynamics
%  
w_a = awa;
v1 = 10 * K1 * awa2;
v2 = K1 * awa;
w = [1.0 1.8 2.3 2.7 2.8 2.7 2.3 1.8 1.0];
S = [zeros(1,N); eye(N-1) zeros(N-1,1)];
Aa = -v2 * ( diag([w 0]) - S*diag([w 0]) )
    - v1 * eye(N,1)*ones(1,N);
Ba = v1 * eye(N,1);
%
% Phosphase/Inhibitor concentrations at equilibrium
%
C0 = ud;
I_equ = vPKA / vCaN / C0 * I0;
ep_equ = K4*ep0 / (K3*I_equ + K4);
%
% Dephosphorylation Dynamics
%
w_d = ep_equ;
D = eye(N) -
    [zeros(N-1,1) eye(N-1); zeros(1,N)];
L = diag(1:N);
Ad = w_d*D*L;
%
% Equilibria
%
m = length(u);
P = zeros(N, m);
y = zeros(size(u));
for k = 1:m
    P(:,k) = - (Aa - u(k)*Ad) \ Ba * pt;
    y(k) = K2 / ( Km + (1:N)*P(:,k) );
end