Three Pool Model of Calcium Signaling

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Three Pool Model of Calcium Signaling

A thesis submitted in partial fulfillment of the requirement for the degree of Bachelors of Science in Mathematics from The College of William and Mary

by

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Accepted for __________________________________
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Three-pool model of calcium signaling

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Abstract

Mitochondria have been proposed to be an integral part of intracellular calcium signaling. We examine the two-pool model of intracellular calcium signaling by Goldbeter et al. 1990 [11] and propose a new model that uses the mitochondria. This creates a three-pool model for intracellular calcium signaling. We then proceed to discuss the differences between the two-pool and three-pool models by following the analysis of the two-pool model presented by Sneyd et al. 1993 [19]. The addition of the third pool does not change any of the dynamics of the two-pool model, but adds some functionality. The mitochondria acts like a buffer that has the ability to change the temporal dynamics, and we have a region of bistability that allows for dampened oscillations given the right stimulus and initial conditions.
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Chapter 1

Introduction

1.1 Background on Calcium Dynamics

Calcium is an essential ion for many of a cell’s different processes. Particularly, it is used as a secondary signal in response to stimuli in order to elicit a specific response from specific cells. This response can range from muscle contraction to synaptic transmission. The responses elicited from a cell can vary greatly from fast oscillations to relaxation. This response depends upon the type of stimulus and how it affects the cell.

External calcium concentrations are normally kept around 1mM, while intracellular concentrations are kept around .1µM. This difference in concentration allows for a fast influx of calcium when necessary. But while the cell is having a fast influx of calcium, we still have the mechanisms that pump out calcium active, so oscillations occur given the right stimulus. Oscillations will only occur if the stimulus is within a specific range (Dupont and Goldbeter 1989 [7]). If the stimulus is too weak or strong, we either do not enough influx of calcium to create oscillations, or we too much calcium influx and overload the mechanisms that create oscillations.

For this process of calcium oscillations to occur, we need mechanisms within the cell to regulate calcium carefully. The plasma membrane is responsible for allowing calcium
in from the extracellular space. The plasma membrane is a phospholipid bilayer that does not allow anything to cross except through selective protein channels. When these channels are activated, the channels selectively choose what ions or molecules to allow through. There are also organelles that act as calcium stores. The organelles we are interested in studying for our purposes are the endoplasmic reticulum (ER) and the mitochondria. The ER is the organelle responsible for protein translation, folding, and transport as well as many other specialized functions which includes storing calcium. The ER also has phospholipid bilayer that acts the same as the plasma membrane. Thus, there are also protein channels on the ER that must be activated for calcium transport. The mitochondria is responsible for most of the production of ATP within a cell, a molecule used for release of free energy within a cell. A cell can have anywhere from a few to thousands of mitochondria. The mitochondria also have a phospholipid bilayer that contains protein channels that must be activated. There are also buffers within the cytosol that attach to calcium at any given time and can either be mobile or immobile. The mobile buffers speed up the calcium signaling while the immobile buffers slow the calcium signaling (Goldbeter et al. 1990 [11]).

There are two ways a cell can influx intracellular calcium: calcium travels through a surface protein membrane channel or calcium is released from internal stores. The plasma membrane contains surface membrane channels that can be activated through a chemical stimulus, internal store release, and voltage dependence. These channels respond to their specific stimulus and influx calcium. The ER has two channels that influx calcium to the cytosol: the IP$_3$ channel and the calcium induced calcium release (CICR) channel. The IP$_3$ channel responds to the cytosolic release of the molecule Inositol 1,4,5-trisphosphate (IP$_3$) and releases calcium from the ER (Berridge and Galione 1988 [4]). The CICR channel responds to cytosolic calcium concentrations and opens in response. It is this channel that is mainly responsible for our oscillations. An increase in intracellular calcium occurs, these channels open in response, the intracellular calcium increases, and fast oscillations
occur. The mitochondria contains the sodium-calcium exchanger (NCX) that exchanges intracellular sodium for mitochondrial calcium.

There are two ways a cell can efflux intracellular calcium: calcium is pumped into the extracellular space or calcium is sequestered by internal stores. The plasma membrane contains the plasma membrane calcium ATP-ase (PMCA) which uses ATP hydrolysis to efflux calcium into the extracellular space. The ER also contains an ATP pump called the sarco-endoplasmic reticulum calcium ATP-ase (SERCA). This pump uses the same method of ATP hydrolysis to pump calcium into the ER store. The mitochondria contains the uniporter, which is a mechanism that responds to either membrane potential, stress, or ligand binding (Gunter and Pfeiffer 1990 [13]). Thus the uniporter responds to multiple stimuli and uptakes calcium into the mitochondria. An illustration of calcium channels is shown in Figure 1.1.

Figure 1.1: An illustration of calcium channels in a T-cell. From Quintana et al. 2005 [18].

The signaling cascade is what we are interested in studying and expanding upon. The signaling cascade starts with an external stimulus stimulating the calcium response within the cell. The stimulus binds to an external site that then proceeds to release IP$_3$. The IP$_3$
release is mediated by a G-coupled protein to which the stimulus is bound. The increase in this IP$_3$ concentration then acts upon the ER’s IP$_3$ receptor that releases calcium into the cytosol (Berridge and Galione 1988 [4]). After the calcium is elicited from the ER, the CICR channels open up in response to the increase in intracellular calcium. This is seen as the major source of calcium spikes during the signaling cascade. While the channels are activated and increasing the intracellular calcium concentration, the PMCA, SERCA, and the uniporter are still active and work to effectively remove intracellular calcium. This influx and efflux occur simultaneously and lead to the oscillations of intracellular calcium. Lastly, there are buffers that act upon the calcium as it enters the cytosol. Most calcium that enters the cytosol is bound to buffers of which there are two types, mobile and immobile. Mobile buffers help to move the calcium throughout the cell, whereas immobile buffers will act to slow the calcium.

Many models have been proposed to simulate these responses within a cell. In general, there are two categories for these models: ones that use oscillations of IP$_3$ to create oscillations of calcium and ones that do not. It is possible that different mechanisms act in different cells, so both types of models could be correct. Goldbeter et al. 1990 [11] proposed an appealing model of the second variety. It uses the CICR method for generating calcium oscillations. This model is appealing because by using only the CICR method we can reduce the model to a system with two non-linear equations, rather than have a larger system that is more complicated.

Calcium induced calcium release has been proposed as the mechanism of cardiac cell and skeletal muscle fibre behavior (Endo et al. 1970 [8]; Fabiato and Fabiato 1975 [10]; Fabiato 1983 [9]). This mechanism has been used in other models (Cheer et. al. 1987 [5], Lane et al. 1987 [16], and Murray and Oster 1984 [17]), but Goldebeter’s implementation is the implementation that produces the most interesting results.
1.2 Summary of results

We will begin our discussion by examining the two-pool model of intracellular calcium signaling as proposed by Goldbeter et al. 1990 [11]. This involves the modeling of the ER and the plasma membrane to create signals in the intracellular space. Then we propose a new three-pool model based upon adding the mitochondria to the system. We examine the results of the analysis from Sneyd et al. 1993 [19] and examine the differences in our new model from the two-pool model. This leads to the discovery of the mitochondria acting as buffers that slow the dynamics of the system temporally. We also examine the bifurcations of various parameters to see which ones contribute to producing oscillations and find that the parameters which scale our functions within the equations are important. We also find a region of bistability produced by adding the third pool that leads to dampened oscillations adding some extra features to the model.
Chapter 2

Derivation of Mathematical Models

2.1 Two-Pool Model

In 1990, Goldbeter et al. [11] proposed a two-pool model to describe the calcium oscillations that occur during the signaling cascade. This model included the cytosol and the ER, modeling the pathways discussed above for these two pools. This model is the
basis for the one which we will develop to include the mitochondria. The model only
takes into account temporal behavior and does not show the spatial concerns of signaling.
The two-pool model was chosen for expansion, because it is one of the simplest models
that elicit the oscillations found in intracellular calcium signaling. It is simpler compared
to other models because of the use of a constant IP$_3$ term and also because it does not try to model the correct spatial aspects of the cell. The model makes the assumption
that channels only have two states, open or closed. Also, leak channels are used to model
release from the stores as a way of including channels that are not explicitly modeled.

The model is as follows:

\[
\begin{align*}
\frac{dCa_i}{d\tau} &= r - kCa_i - \frac{v_1Ca_i^n}{k_1^n + Ca_i^n} + \frac{v_2Ca_s^m}{k_2^m + Ca_s^m} \cdot \frac{Ca_i^p}{k_3^p + Ca_i^p} + k_fCa_s, \\
\frac{dCa_s}{d\tau} &= \frac{v_1Ca_i^n}{k_1^n + Ca_i^n} - \frac{v_2Ca_s^m}{k_2^m + Ca_s^m} \cdot \frac{Ca_i^p}{k_3^p + Ca_i^p} - k_fCa_s,
\end{align*}
\] (2.1)

This model contains all of the necessary pathways. It is a system of two non-linear
differential equations with the equations showing the dynamics of the two pools, the
cytosol and the ER (see Figure 2.1). Here $Ca_i(\tau)$ is the concentration of the intracellular
calcium ion in the cytoplasm, and $Ca_s(\tau)$ is the concentration of the calcium ion in the
calcium-sensitive pool; all parameters are positive constants, and $n, m, p > 1$. In the
model, the constant influx $r$ of calcium into the cytosol results from the IP$_3$
dependent release from the ER. The $kCa_i$ term illustrates the efflux of calcium out of the cell through
the PMCA pump. Next in the equation of $Ca_i$ are two Hill functions that model the
CICR channel. The first Hill function, $\frac{v_1Ca_i^n}{k_1^n + Ca_i^n}$, models the ER calcium binding, and
the second Hill function, $\frac{v_2Ca_s^m}{k_2^m + Ca_s^m} \cdot \frac{Ca_i^p}{k_3^p + Ca_i^p}$, models the intracellular calcium binding.
The Hill function will be explained in more details below. The last term, $k_fCa_s$, is a leak
term associated with the extrusion of calcium from the ER.

Looking closely, we notice the absence of terms for the buffers. Goldbeter et al. [11]
hypothesized that the buffers would only act as a constant term to either speed up or
slow down the oscillations associated with the model. So, they took the buffers to be 1
in this model since it would not produce any different results analytically if we take any other constant.

2.1.1 Hill Functions

A Hill function is defined as

$$\theta(l) = \frac{v_0 l^n}{k_a^n + l^n}.$$  (2.2)

The graph of the Hill function is a sigmoidal shaped curve that returns a fraction of $v_0$ (see Figure 2.2). With this function we are able to manipulate the kinetics of the curve fairly easily since all of the constants are easily accessible. Each constant has its own importance in determining the kinetics of the curve. The $v_0$ term is the maximal value of the function. We can see from the formula that if we take $l \to \infty$, then $\theta(l) \to v_0$. This will allow us to change the maximum transport rate of calcium provided by each channel. The parameter $n$ is called the Hill coefficient. If we take $n \geq 1$, we have a monotonic curve with a non-negative slope at every point in $[0, \infty)$. If we take $n < 1$, we have a monotonic curve with a non-positive slope at every point in $[0, \infty)$. The parameter $k_a$ is called the dissociation constant and is a measure of how easily the ligand dissociates from the channel. We can see that it will also give us our half occupation point on the curve, because when we take $l = k_a$ we receive:

$$\theta(l) = \frac{v_0 k_a^n}{k_a^n + k_a^n} = \frac{v_0}{2}.$$  (2.3)

Hill functions are used to model activating channels because calcium channels have the property of cooperative binding. This means that when a ligand binds, it is most likely that more ligand will bind, further activating the channel. This results in a sigmoidal activation curve and thus Hill functions are effective in modeling this activation of the channels. Hill functions provide a way to model these sigmoidal activation curves and also allow for the kinetics to be changed fairly easily.
Figure 2.2: Three Hill functions with $k_a = 15$ and $v_0 = 1$. The Hill coefficients are $n = 1$, $n = 2$, and $n = 4$.

## 2.2 Three-Pool Model

From the Goldbeter two-pool model, we can make various improvements. The one we are focusing on is to include the mitochondria in the model. This involves adding a third term with its own dynamics and integrating it with the two-pool model. This results in a new three equation system:

\[
\begin{align*}
\frac{dCa_i}{d\tau} &= r - kCa_i - \frac{v_1Ca_i^n}{k_1^n + Ca_i^n} + \frac{v_2Ca_s^m}{k_2^m + Ca_s^m} \frac{Ca_i^p}{k_3^p + Ca_i^p} + k_fCa_s - \frac{v_3Ca_i^q}{k_4^q + Ca_i^q} + k_mCm, \\
\frac{dCa_s}{d\tau} &= \frac{v_1Ca_i^n}{k_1^n + Ca_i^n} - \frac{v_2Ca_s^m}{k_2^m + Ca_s^m} \frac{Ca_i^p}{k_3^p + Ca_i^p} - k_fCa_s, \\
\frac{dCa_m}{d\tau} &= \frac{v_3Ca_i^q}{k_4^q + Ca_i^q} - k_mCm,
\end{align*}
\]

(2.4)
This is the three-pool model based upon the Goldbeter two-pool model. This model contains all of the functions of the Goldbeter model, but it has an added function for the mitochondria sequestering calcium (see Figure 2.3). With other notations as in (2.1), the new variable $Ca_m(\tau)$ is the concentration of the calcium ion in the mitochondria. This is modeled through a Hill function, $\frac{v_3Ca^q}{k_3^q + Ca^q}$, taking up cytosolic calcium and having a leak term, $k_mC_m$, on the mitochondria. We assume the membrane potential is fixed and that it is passively uptaking calcium. It essentially acts like the SERCA pump, but uptakes calcium into the mitochondria. This is not the most complete model of a mitochondria, but it is one way to implement it in the two-pool model and see how it affects the model.

The system (2.4) is a generalization of (2.1) in the sense that if $v_3 = 0$ and initially $Ca_m(0) = 0$, then (2.4) then reduces to (2.1). Thus, the system (2.4) has all the dynamical behavior of (2.1).
Chapter 3

Analysis of the Model

3.1 Analysis of the Two-Pool Model

The two-pool model (2.1) was analyzed thoroughly by Sneyd et al. [19]. They found that there is a unique equilibrium solution for any given set of parameters within the physical constraints of a cell. Oscillations occur for certain parameters, and they found that oscillatory solutions emerge through Hopf bifurcations. The analysis in this section is essentially from [19]. We reproduce the analysis for the sake of completeness, and also for comparison to our extended model. When we perform numerical simulations, we use MATLAB for phase portraits and time series, and MatCont for bifurcations. MatCont [6, 12] is a MATLAB toolbox that is very effective at drawing numerical bifurcation diagrams.

3.1.1 Non-dimensionalization

Through the use of nondimensionalization, the model (2.1) can be rewritten as:

\[
\begin{align*}
\frac{du}{dt} &= \mu - u - \frac{\gamma}{\varepsilon} f(u, v), \\
\frac{dv}{dt} &= \frac{1}{\varepsilon} f(u, v),
\end{align*}
\]  
(3.1)
where

\[ f(u, v) = \frac{\beta u^n}{1 + u^n} - \frac{v^m}{1 + v^m} \cdot \frac{u^p}{\alpha^p + u^p} - \delta v, \tag{3.2} \]

and the new dimensionless variables and parameters are defined through the old ones in the following way:

\[
\begin{align*}
    u &= \frac{C a_i}{k_1}, \quad v = \frac{C a_s}{k_2}, \quad t = \tau k, \\
    \alpha &= \frac{k_3}{k_1}, \quad \beta = \frac{v_1}{v_2}, \quad \gamma = \frac{k_2}{k_1}, \\
    \delta &= \frac{k f k_2}{v_2}, \quad \mu = \frac{r}{k k_1}, \quad \varepsilon = \frac{k k_2}{v_1}. 
\end{align*} \tag{3.3} \]

### 3.1.2 Equilibrium

This nondimensionalized system (3.1) makes solving for the equilibrium solutions slightly easier. We can see from the equations when we solve for the steady state \((u^*, v^*)\), we obtain \(u^* = \mu\) and \(0 = f(\mu, v^*)\). From our choice of \(f(u, v)\) we have only one solution for \(v^*\) that must satisfy the equation

\[
\frac{\beta \mu^n}{1 + \mu^n} - \frac{v^m}{1 + v^m} \cdot \frac{\mu^p}{\alpha^p + \mu^p} - \delta v = 0. \tag{3.4} \]

From this, we can see that the equilibrium solution depends on \(\mu\). Thus, we construct a bifurcation diagram for this parameter to see how the oscillations arise (Figure 3.1). We see that we need a large enough \(\mu\) value in order to elicit oscillations. This \(\mu\) value corresponds to our stimulus as defined by \(r\) in (2.1), thus we need a stimulus within a specific range which follows from experimental observations.

We will use the following values of parameters for numerical simulation or bifurcation diagrams unless we specify other parameter values:
3.1.3 Bifurcations

Figure 3.1: Bifurcation diagram of steady states and limit cycle constructed using MatCont. Parameter $\gamma = 2$. Here the horizontal axis is $\mu$, and the vertical axis is $u$. The line $u = \mu$ is the steady state; Hopf bifurcation points are $\mu_1 = 0.3109$, $\mu_2 = 0.6652$; and each vertical line between two Hopf bifurcation points represents the $u$-amplitude of the limit cycle.

Because of the oscillations, we receive a periodic orbit in our phase portrait for $u$ and $v$. According to Sneyd et al. [19], the Hopf bifurcations at both Hopf bifurcation points are supercritical, which means we have a stable periodic orbit that arises when the equilibrium

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$\varepsilon$</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>$\delta$</th>
<th>$m$</th>
<th>$n$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>0.04</td>
<td>0.9</td>
<td>0.13</td>
<td>0.004</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 3.1: Parameter used for simulations for (3.1).
changes the stability [20]. This means we either have a limit cycle (Figure 3.2) or a stable node, but never both at the same time. This is verified numerically by looking at the first Lyapunov coefficient which Matcont [6] calculates when it detects a Hopf bifurcation. If the first Lyapunov coefficient is negative at the Hopf bifurcation point, then we have a supercritical Hopf point [15]. This becomes important later when we compare across both models.

Further analysis presents us with another bifurcation parameter $\gamma$. Decreasing $\gamma$ (Figure 3.3) decreases the range of the existence of limit cycles for $\mu$, and when $\gamma$ is smaller than a threshold value ($\gamma < \gamma_0$), then there is no Hopf bifurcation occurring any more and the equilibrium is always stable. The critical threshold value $\mu_0$ satisfies $1.015 < \gamma_0 < 1.02$ for the parameter values in Table 3.1. The parameter $\gamma$ scales the ER function and thus scales the CICR kinetics. The CICR channel is what is responsible for our oscillations, thus when we decrease the values low enough by decreasing $\gamma$, we lose our oscillations.

Figure 3.2: Phase portraits of limit cycle. Here the horizontal axis is $u$, and the vertical axis is $v$. Parameters are $\gamma = 2$, (left) $\mu = 0.4$, (right) $\mu = 0.64$. 

Figure 3.3: Phase portraits of limit cycle. Here the horizontal axis is $u$, and the vertical axis is $v$. Parameters are $\gamma = 2$, (left) $\mu = 0.4$, (right) $\mu = 0.64$. 

Further analysis presents us with another bifurcation parameter $\gamma$. Decreasing $\gamma$ (Figure 3.3) decreases the range of the existence of limit cycles for $\mu$, and when $\gamma$ is smaller than a threshold value ($\gamma < \gamma_0$), then there is no Hopf bifurcation occurring any more and the equilibrium is always stable. The critical threshold value $\mu_0$ satisfies $1.015 < \gamma_0 < 1.02$ for the parameter values in Table 3.1. The parameter $\gamma$ scales the ER function and thus scales the CICR kinetics. The CICR channel is what is responsible for our oscillations, thus when we decrease the values low enough by decreasing $\gamma$, we lose our oscillations.
Figure 3.3: Evolution of bifurcation diagrams when $\gamma$ changes. Here the horizontal axis is $\mu$, and the vertical axis is $u$. Parameters are (left) $\gamma = 1.5$ and (right) $\gamma = 1.08$.

3.1.4 Excitability

Also evident in our model is the existence of excitability. When we have $\mu < \mu_1$, we have excitable behavior arise where large enough superthreshold perturbations produce large transient excursions away from equilibrium before returning to the equilibrium.

Figure 3.4: Time series of $u$ with $\gamma = 2$, $\mu = .316$, and $(u_0, v_0) = (2.5, .75)$. 

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3.2 Analysis of Three-Pool Model

We now analyze the three-pool model in order to compare the changes that may have arisen in modifying the model. We will use some techniques in Sneyd et al. [19] to compare the models. Also when we select parameters, we use the same parameters used in Sneyd’s analysis (see Table 3.1) and we use approximate values for the new terms from experimental observations presented in a review by Gunter and Pfeiffer 1990 [13].

3.2.1 Non-dimensionalization

Through the use of non-dimensionalization, we can examine how the parameters affect the equations. Here we take

\[ u = \frac{Ca_i}{k_1}, \quad v = \frac{Ca_s}{k_2}, \quad w = \frac{Ca_m}{k_4}, \quad t = \tau k. \]  

(3.5)

After applying dimensional analysis techniques we arrive at the following equations:

\[
\begin{cases}
\frac{du}{dt} = \mu - u - \frac{\gamma_1}{\varepsilon_1} f(u, v) - \frac{\gamma_2}{\varepsilon_2} g(u, w), \\
\frac{dv}{dt} = \frac{1}{\varepsilon_1} f(u, v), \\
\frac{dw}{dt} = \frac{1}{\varepsilon_2} g(u, w),
\end{cases}
\]

(3.6)

where

\[ f(u, v) = \frac{\beta u^n}{1 + u^n} - \frac{v^m}{1 + v^n} - \frac{u^p}{\alpha_1^p + u^p} - \delta_1 v, \]

\[ g(u, w) = \frac{w^q}{\alpha_2^q + w^q} - \delta_2 w. \]

(3.7)

Here \( \varepsilon_1, \varepsilon_2, \gamma_1, \gamma_2, \mu, \beta, \delta_1, \delta_2, \alpha_1, \alpha_2 \) are positive parameters, and \( m, n, p, q > 1 \). From the original equations we have \( m, n, p, q \) remain the same, and the other parameters are:

\[
\varepsilon_1 = \frac{kk_2}{v_2}, \quad \varepsilon_2 = \frac{kk_4}{v_3}, \quad \gamma_1 = \frac{k_2}{k_1}, \quad \gamma_2 = \frac{k_4}{k_1}, \quad \mu = \frac{r}{kk_1},
\]

\[
\beta = \frac{v_1}{v_2}, \quad \delta_1 = \frac{k_1k_2}{v_2}, \quad \delta_2 = \frac{k_mk_4}{v_3}, \quad \alpha_1 = \frac{k_3}{k_1}, \quad \alpha_2 = \frac{k_4}{k_1}.
\]

(3.8)
3.2.2 Equilibrium and Stability

The nondimensionalized equation (3.6) has a unique equilibrium point \((\mu, v^*(\mu), w^*(\mu))\), where \((v^*(\mu), w^*(\mu))\) satisfies

\[
\frac{\beta \mu^n}{1 + \mu^n} - \frac{v^m}{1 + v^m} \cdot \frac{\mu^p}{\alpha_1^p + \mu^p} - \delta_1 v = 0, \quad w = \frac{\mu^q}{\delta_2(\alpha_2^q + \mu^q)}.
\] (3.9)

The Jacobian matrix \(J\) has the form:

\[
J = \begin{pmatrix}
-1 - \frac{\gamma_1}{\varepsilon_1} f_u - \frac{\gamma_2}{\varepsilon_2} g_u & -\frac{\gamma_1}{\varepsilon_1} f_v & -\frac{\gamma_2}{\varepsilon_2} g_w \\
\frac{1}{\varepsilon_1} f_u & \frac{1}{\varepsilon_1} f_v & 0 \\
\frac{1}{\varepsilon_2} g_u & 0 & \frac{1}{\varepsilon_2} g_w
\end{pmatrix}.
\] (3.10)

The terms \(f_u, f_v, g_u, \) and \(g_w\) are the partial derivatives of the functions \(f(u, v)\) or \(g(u, w)\) with respect to the variable in the subscript. These partial derivatives work out to be:

\[
\begin{align*}
f_u &= \frac{nu^{n-1}}{(1 + u^n)^2} - \frac{v^m}{1 + v^m} \cdot \frac{\alpha_1^p u^{p-1}}{(\alpha_1^q + u^q)^2}, \\
f_v &= -u^p \cdot \frac{mv_{n-1}}{(1 + v^m)^2} - \delta_1, \\
g_u &= \frac{\alpha_2^q u^{q-1}}{(\alpha_2^q + u^q)^2}, \\
g_w &= -\delta_2.
\end{align*}
\] (3.11)

For \(\mu = 0\) we obtain a stable equilibrium. This can be shown through the Jacobian matrix. When we take \(\mu = 0\), we obtain \(f_u = g_u = 0, f_v = -\delta_1, \) and \(g_w = -\delta_2.\) This

<table>
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<tr>
<th>Parameter</th>
<th>(\varepsilon_1)</th>
<th>(\varepsilon_2)</th>
<th>(\alpha_1)</th>
<th>(\alpha_2)</th>
<th>(\beta)</th>
<th>(\delta_1)</th>
<th>(\delta_2)</th>
<th>(\gamma_1)</th>
<th>(\gamma_2)</th>
<th>(m)</th>
<th>(n)</th>
<th>(p)</th>
<th>(q)</th>
</tr>
</thead>
<tbody>
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<td>.9</td>
<td>10</td>
<td>.13</td>
<td>.004</td>
<td>2</td>
<td>2</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 3.2: Parameter used for simulations of (3.6).
simplifies our Jacobian matrix to:

\[
J = \begin{pmatrix}
-1 & \frac{\gamma_1}{\varepsilon_1} \delta_1 & -\frac{\gamma_2}{\varepsilon_2} \\
0 & -\frac{1}{\varepsilon_1} \delta_1 & 0 \\
0 & 0 & -\frac{1}{\varepsilon_2} \delta_2
\end{pmatrix}
\]  

This gives us an upper triangular matrix, and the eigenvalues of this matrix can be taken directly from the diagonals of the matrix since when we take the determinant |\(J - \lambda I\)|, we obtain \((-1-\lambda)(-\frac{1}{\varepsilon_1} \delta_1 - \lambda)(-\frac{1}{\varepsilon_2} \delta_2 - \lambda)\). Thus the roots to our characteristic polynomial are 

\(-1, -\frac{1}{\varepsilon_1} \delta_1, \quad \text{and} \quad -\frac{1}{\varepsilon_2} \gamma_2\)

which are all negative real numbers so we have a stable equilibrium when \(\mu = 0\) or \(\mu\) is near 0 from perturbation.

We also have a stable equilibrium as \(\mu \to \infty\). Similarly to when \(\mu = 0\), we receive an upper triangular matrix with negative terms along the diagonal. This occurs because when \(\mu\) approaches \(\infty\), we obtain \(f_u = g_u = 0\), \(g_w = -\delta_2\), and \(f_v = -\frac{m(v^*)^{m-1}}{(1 + (v^*)^m)^2} - \delta_1\) where \(v^*\) is positive, thus \(f_v\) is negative. Our Jacobian matrix then becomes:

\[
J = \begin{pmatrix}
-1 & -\frac{\gamma_1}{\varepsilon_1} \delta_1 & -\frac{\gamma_2}{\varepsilon_2} \\
0 & \frac{1}{\varepsilon_1} f_v & 0 \\
0 & 0 & -\frac{1}{\varepsilon_2} \delta_2
\end{pmatrix}
\]  

The eigenvalues are \(-1, -\frac{1}{\varepsilon_1} f_v, \text{and} -\frac{1}{\varepsilon_2} \delta_2\), which are real and negative therefore we have a stable equilibrium as \(\mu \to \infty\).

### 3.2.3 Hopf Bifurcation and Periodic Orbits

A Hopf bifurcation occurs at \(\mu = \mu^*\) if the characteristic polynomial, \(P(\mu, \lambda)\), has a pair of complex eigenvalues \(\lambda = \zeta(\mu) \pm \eta(\mu)i\) for \(\mu\) near \(\mu = \mu^*\), such that \(\zeta(\mu^*) = 0\), \(\eta(\mu^*) \neq 0\), and \(\zeta'(\mu^*) \neq 0\). This shows that a pair of complex eigenvalues move across the imaginary axis, and as a result a small amplitude periodic orbit emerges near the equilibrium point.
We can find $P(\mu, \lambda)$ by taking our Jacobian matrix (3.10) and taking the determinant $|J - \lambda I|$ and solve for $\lambda$. Rather than solving the third degree polynomial, we can use the Routh-Hurwitz criteria to determine whether the equilibrium’s stable or not. This criteria states that an equilibrium point is stable in the three equation case if the characteristic polynomial is given by $P(\lambda) = \lambda^3 + a_1 \lambda^2 + a_2 \lambda + a_3$ and the inequalities $a_1 > 0$, $a_3 > 0$, and $a_1 a_2 - a_3 > 0$ hold ([1] Section 4.5, page 150–151). The interesting case from this criteria is when $a_1 a_2 - a_3 = 0$, as a Hopf bifurcation occurs when this happens.

After solving the determinant for $a_1$, $a_2$ and $a_3$, we receive the expressions for each term:

\[
\begin{align*}
  a_1 &= 1 + \frac{\gamma_1}{\varepsilon_1} f_u + \frac{\gamma_2}{\varepsilon_2} g_u - \frac{1}{\varepsilon_1} f_v - \frac{1}{\varepsilon_2} g_w, \\
  a_2 &= -\frac{1}{\varepsilon_1} f_v - \frac{1}{\varepsilon_2} g_w + \frac{1}{\varepsilon_1 \varepsilon_2} f_v g_w - \frac{\gamma_1}{\varepsilon_1 \varepsilon_2} f_u g_w - \frac{\gamma_2}{\varepsilon_1 \varepsilon_2} g_u f_v, \\
  a_3 &= \frac{1}{\varepsilon_1 \varepsilon_2} f_v g_w.
\end{align*}
\]

Looking at these expressions, we can see the importance of certain parameters, particularly $\gamma_1$ and $\gamma_2$. Since we need $a_1 a_2 - a_3 \leq 0$, we need the terms $a_1 a_2$ to be negative and $a_3$ to be positive. $a_3$ is always positive as long as $\delta_1$, $\delta_2$, and $m$ are positive. Since $a_1$ and $a_2$ contain various terms we need more rigorous results. To simplify the equations, we can eliminate the $a_3$ term by taking $(a_1 - 1)a_2 + a_2 - a_3$, where the $a_3 = \frac{1}{\varepsilon_1 \varepsilon_2} f_v g_w$ term is part of the $a_2$ term. This leads to the expression:

\[
a_1 a_2 - a_3 = (a_1 - 1)a_2 + a_2 - a_3
= \left(\frac{\gamma_1}{\varepsilon_1} f_u + \frac{\gamma_2}{\varepsilon_2} g_u - \frac{1}{\varepsilon_1} f_v - \frac{1}{\varepsilon_2} g_w\right)\left(-\frac{1}{\varepsilon_1} f_v - \frac{1}{\varepsilon_2} g_w + \frac{1}{\varepsilon_1 \varepsilon_2} f_v g_w\right)
- \frac{\gamma_1}{\varepsilon_1 \varepsilon_2} f_u g_w - \frac{\gamma_2}{\varepsilon_1 \varepsilon_2} g_u f_v.
\]

Examining the values we have for our parameters, the functions $f_v$ and $g_w$ are negative and $g_u$ is positive. The value of $f_u$ cannot be as easily determined as it involves two Hill functions and it could be positive or negative depending upon parameters. Thus, the parameters that have the most obvious importance in this expression are $\mu$, $\varepsilon_1$, $\varepsilon_2$, $\gamma_1$, and $\gamma_2$. The parameter $\mu$ is involved in every term and needs to be within a range so that
the $f_u$ term is negative and produces a negative value for our expression. The parameters \( \varepsilon_1, \varepsilon_2, \gamma_1, \) and \( \gamma_2 \) all act to scale the terms in the expression (3.15). Thus we need \( \varepsilon_1 \) and \( \varepsilon_2 \) to be sufficiently small, and \( \gamma_1 \) and \( \gamma_2 \) to be sufficiently large. Looking back to the non-dimensionalized model, the \( \varepsilon_1, \varepsilon_2, \gamma_1, \) and \( \gamma_2 \) scale the kinetics of the internal stores, so they need to be of the right scale in order for the kinetics of the internal stores to be significant.

Nevertheless through numerical bifurcation analysis using MatCont, Hopf bifurcations occurs for the parameters given in Table 3.2, see the bifurcation diagram in Figure 3.5 and the phase portraits of limit cycle in Figure 3.6.

![Bifurcation diagram](image)

Figure 3.5: Bifurcation diagram of the three-pool system with parameters in Table 3.2. Here the horizontal axis is $\mu$, the vertical axis is $v$, and each vertical line between two Hopf bifurcation points represents the $v$-amplitude of the limit cycle.
Figure 3.6: Phase portrait of the three-pool system: (left) 3-dimensional portrait with \(u\), \(v\), and \(w\) coordinates; (right) 2-dimensional portrait with \(v\) and \(w\) coordinates. Here we take \((u_0, v_0, w_0) = (.25, .85, .1)\) with \(\mu = .36\) and the rest of the parameters are specified in Table 3.2.

### 3.2.4 Bistability Dynamics and Multiple Periodic Orbits

While the bifurcation diagram Figure 3.5 is similar to the one in Figure 3.1, a careful examination shows that the Hopf bifurcation at the left Hopf bifurcation point is subcritical when we use the parameter values in Table 3.2. The Hopf bifurcation points are \(\mu_1 = 0.3167\), and \(\mu_2 = 0.61\). The bifurcation at \(\mu = \mu_2\) is supercritical with negative first Lyapunov coefficient, but the bifurcation at \(\mu = \mu_1\) is subcritical with a positive first Lyapunov coefficient. The curve of periodic orbits emerging from \(\mu = \mu_1\) goes to the left initially, then at \(\mu_* = 0.31538\) it turns back and continues without more turning points until it reaches the other Hopf bifurcation point \(\mu = \mu_2\). So for \(\mu \in (\mu_*, \mu_1)\), there exist two periodic orbits, with one stable and one unstable, while the equilibrium is also stable. Hence there are bistable dynamics for \(\mu \in (\mu_*, \mu_1)\). Since the range of the bistability is very narrow, it is hard to see it from Figure 3.5. A much clearer picture of bistability can be viewed from Figure 3.7 with a different value of \(\alpha_2\), and we use the period of the periodic orbits as the vertical axis.

In Figure 3.8, two solutions with same parameter values (Table 3.2 and \(\mu = .316\) but
Figure 3.7: Bifurcation diagram showing bistability. Parameters are the same as Figure 3.5 except $\alpha_2 = 5$. Here the horizontal axis is $\mu$, the vertical axis is the period of the periodic orbits. The value of the turning point is $\mu_* = .33552$. The first Lyapunov coefficient at the first Hopf point $\mu_1 = .33997$ is 6.41047, a positive term indicating the subcritical nature of the bifurcation [15].

Different initial values are shown. The bistability is clear from their different asymptotically behavior.

### 3.2.5 Other Bifurcations

We can also produce bifurcations for the other parameters to see which ones also affect the dynamics of our model. Using MatCont, we can produce these diagrams numerically and determine which parameters affect the dynamics of our system. When we do this, the parameters that are determined to affect the stability of the system are $\alpha_1$, $\beta$, $\varepsilon_1$, and $\gamma_1$. Looking at what these parameters mean biologically, they all relate either the dissociation constants or velocity terms of the various pathways. $\alpha_1$ is the ratio of the dissociation constants of the CICR and the SERCA pump, $\beta$ is the ratio of the velocities of the CICR and the SERCA pump, $\varepsilon_1$ is the ratio of the dissociation constants on the CICR and the PMCA pump to the velocity of the CICR, and $\gamma_1$ is similar to $\alpha_1$. Looking at each bifurcation we need $\alpha_1$ and $\beta$ to be within a specific range, $\gamma_1$ to be sufficiently
large, and $\varepsilon_1$ to be sufficiently small. These bifurcations tell us what constraints need to be placed on our parameters to elicit oscillations.

We also have parameters that do not produce Hopf bifurcations, but do affect the dynamics of our system. The parameter $\varepsilon_2$ scales the mitochondria dynamics in the intracellular equation. When we change $\varepsilon_2$, we lose our bistability, but we do not lose our Hopf bifurcation points. The first Lyapunov coefficients at our Hopf point becomes negative when we vary $\varepsilon_2$ in either direction (see Figure 3.11). The $\varepsilon_2$ parameter needs to be within a certain range that will scale the mitochondrial equations to the right kinetics.
in order for us to have bistability. This means that our bistability is dependent upon the mitochondrial dynamics. The same bifurcation occurs for $\delta_2$ as it acts in the same way that $\varepsilon_2$ does.

### 3.2.6 Excitability

The three-pool system also exhibits excitability. This means that a large enough superthreshold perturbation will elicit a large transient excursion before going to equilibrium. This behavior is most evident in the region before bistability where our $\mu$ parameter is less than the $\mu$ at the turning point of the limit cycles.
Figure 3.11: Bifurcation diagrams for $\mu$ showing the absence of bistability when $\varepsilon_2$ is not within range given by [13] ($\varepsilon_2 = .2$). The top graph has $\varepsilon_2 = .01$ with a first Lyapunov coefficient of $-6.5921$ and the bottom graph has $\varepsilon_2 = 1$ with a first Lyapunov coefficient of $-6.4459$.

Figure 3.12: Times series of $u$. Here $(u_0, v_0, w_0) = (.3, 1.4, .01)$ and $\mu = .316$ which is in our region of bistability.
Chapter 4

Conclusion

Comparing both models we can see that the addition of the third pool does provide significant changes to our model. With our three-pool model we preserved the important points of the two-pool model along with adding the third pool. The importance of the $\mu$ parameter remains. The addition of the third pool does not change the fact that we need a large enough stimulus to elicit calcium oscillations. We also do not lose our Hopf bifurcation on either $\gamma$ or $\mu$. This keeps the oscillations related to the parameters that were used in the two-pool model. We also have the excitability preserved, which shows that the model still produces predictions in line with experimental observation.

The main differences that arise between the models are related to bistability. We receive a different form of equilibrium that gives us different behavior. For the most part in our bistable region, we have a very small window to receive a stable spiral and initial conditions will result in a periodic orbit. But we do have this stable spiral arise that produces damped oscillations in the concentrations of each pool. This is significant because if we have a stimulus that is in our bistable region, we could have an oscillation that eventually deactivates on its own and would not need to have another signal to deactivate the oscillations. This could be useful for a process like apoptosis where we would need a stimulus to elicit the signals and subsequently we would not need another
stimulus to deactivate it, since the target of the stimulus would be to terminate the cell.

Other differences that arise are the mitochondria scaling the intracellular spikes. Looking at Figure 3.4 and Figure 3.12, we can see the period between spikes is the same, but the spikes reach a lower height in the three-pool model. This is evident mathematically because we have another uptake term that subtracts from the intracellular calcium where our oscillations occur. We have the spikes waiting for two pools to respond to the intracellular levels. We still have fast spikes, but they are smaller. This is similar to the effect of having buffers in the cytosol. As stated before, the presence of buffers would scale the model’s kinetics very much in the way that the mitochondria’s presence does. Thus, we have the mitochondria acting similarly to buffers in the model. Also, the parameters can be adjusted to produce larger spikes, so this could also be an issue of parameter selection as well.

To further improve the model we can add many different aspects to our model. The biggest improvement could be to implement a spatial version of our model. This would be especially interesting with our three-pool model since we would have two pools competing for intracellular calcium as it enters the cytosol. This could provide many interesting results; in particular, it could provide a way to change equilibrium without using another stimulus. Another improvement to this model would be to use oscillating IP$_3$ concentrations. This is a mechanism that has been proposed in various other models, but was not added to this model. Lastly, more descriptive equations for the pathways of the mitochondria could be used. In our model we have one pathway solely dependent on intracellular calcium, but in descriptions of the mitochondria, there are multiple pathways. The problem with adding these other pathways are the complications that arise from the addition of the pathways. The pathway that would be most beneficial would be the sodium-calcium exchanger. This poses a problem because we would need to keep track of sodium levels as well as calcium levels in the cell that would lead to a model that would double in equations. It would also be beneficial to more effectively model
the uniporter in the mitochondria. This would create the need to model the membrane potential and other ions that act with the uniporter and produces the same concern as with the sodium-calcium exchanger with the added complication of membrane potential.

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Bibliography


