Mathematical Modeling of Intracellular Calcium Signaling: A Study of $IP_3$ Receptor Models

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Doctor of Philosophy

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This dissertation titled Mathematical Modeling of Intracellular Calcium Signaling: A Study of $IP_3$ Receptor Models

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**ABSTRACT**

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Calcium signaling is one of the most important intracellular signaling mechanisms. A rich spatio-temporal repertoire of calcium signals is observed across cell types, with patterns exhibiting a hierarchical structure varying from single channel transients to $Ca^{2+}$ waves, sweeping the entire cell [36]. At the heart of the signaling mechanism is the inositol 1,4,5 trisphosphate ($IP_3$) receptor, a calcium channel regulated by a second messenger and calcium itself. Significant efforts have been made to characterize the properties of the $IP_3$ receptor and how it is regulated. Multi-scale computational modeling plays a significant role in testing hypotheses and providing feedback to experimental research.

The aim of this dissertation is to develop a simple mathematical model for an $IP_3$ receptor, which quantitatively describes the properties of elemental calcium release events, i.e $Ca^{2+}$ *puffs*. A simple, and computationally efficient and accurate model for the elementary $Ca^{2+}$ release events forms the building block for simulations on a larger organizational scale. A sequential binding model, for the $IP_3$ receptor, is proposed in this dissertation. The model is then compared to other models proposed in literature. On the organizational scale of a cluster of $IP_3$ receptors, predicted properties of the various models are compared. The role of buffers, in calcium cluster release is also investigated.

Approved: ________________________________

Peter Jung

Professor of Physics and Astronomy
To my parents
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The Figure shows the underlying fraction of open channels. Panel A) shows the underlying fraction of open channels for the 6-state model. During a puff 6-8 channels are open. Panels B) and C) show underlying fraction of open channels for the 6-state AICT model with higher values of $v_{channel}$. In panel B) $v_{channel}$ is 5000 $\text{s}^{-1}$. Duration of events is in the seconds regime with fluctuations (rapid opening and closing of 1-2 channels) lasting up to seconds. Panel C) shows results from simulation of 6-state AICT at $v_{channel}$ 20000 $\text{s}^{-1}$. Event duration is in the millisecond regime and duration of fluctuations is markedly reduced due to increased $Ca^{2+}$ values.

Fraction of uninhibited and inhibited subunits for high $v_{channel}$ values. Top Panel demonstrates the underlying fractions subunits for the 6-state AICT model for $v_{channel}=5000 \text{s}^{-1}$. Regimes in graph,15-20s and 31-34s, clearly show delayed recovery of the subunits post inhibition. The bottom panel shows the underlying subunit profile for $v_{channel}$ 20000 $\text{s}^{-1}$. On an average more subunits are inhibited due to an increase in $Ca^{2+}$ aided inhibition.
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<th>Acronym</th>
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<tr>
<td>IP₃</td>
<td>Inositol 1, 4, 5, triphosphate</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic Reticulum</td>
</tr>
<tr>
<td>CICR</td>
<td>Calcium-Induced Calcium Release</td>
</tr>
<tr>
<td>DYK</td>
<td>De-Young Keizer</td>
</tr>
<tr>
<td>PDE</td>
<td>Partial Differential Equation</td>
</tr>
<tr>
<td>PLT</td>
<td>Puff Lifetime</td>
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<tr>
<td>FDHM</td>
<td>Full Duration at Half Maximum</td>
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<tr>
<td>Pₒ</td>
<td>Steady State Open Probability</td>
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<td>AICT</td>
<td>Agonist Independent Conformational Transformation</td>
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<td>EGTA</td>
<td>Ethylene glycol tetraacetic acid</td>
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<tr>
<td>BAPTA</td>
<td>1,2-bis(o-aminophenoxy) ethane-N,N,N’,-N’-tetraacetic acid</td>
</tr>
<tr>
<td>TIRF</td>
<td>Total Internal Reflection Fluorescence Microscopy</td>
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1 INTRODUCTION

In living systems one of the most versatile and universal signaling mechanism is governed by intracellular calcium. Examples include contraction of the heart, information processing in the brain, synaptic plasticity and the release of digestive enzymes by the liver [28], [4]. To fulfill it’s vital role for cellular processes, calcium, acts as an intracellular messenger relaying information within cells. Upon fertilization of an egg for example, a calcium wave sweeps the cell and triggers embryonic development [34]. During the growth phase of an organism, cell differentiation [21] and proliferation [9] are controlled by calcium. Even as calcium is vital in the sustenance of life, increased calcium concentrations spell death. Calcium contributes to the process of programmed cell death, apoptosis, an important process during normal development of an organism [5]. The range of cellular activities controlled by calcium is wide and varied [5]. Muscle contraction, neurotransmitter release from neurons and astrocytes, gene expression and egg maturation are other important processes controlled by calcium.

To coordinate all these cellular activities calcium signals need to be flexible, yet precisely regulated [5], [4]. To be able to participate in this variety of cellular functions, calcium signals within cells exhibit diverse and complex spatio-temporal organization. Information is encoded in calcium signals through variations in frequency, amplitude, duration and spatial profile.

A cell has access to two sources of calcium: external and internal. Channels on the plasma membrane let extracellular calcium from the outside into the cell cytosol. Calcium can also be released into the cytosol through channels on the membranes of internal calcium rich sources like the endoplasmic reticulum(ER) or the sacroplasmic reticulum (SR) and the mitochondria [28], [22]. The surface membrane calcium channels are of three distinct types: voltage controlled channels which respond to changes in membrane potential, ligand controlled channels controlled by binding unbinding of external ligands
and the mechanically operated channels which respond to mechanical stimulation [28]. Calcium release from internal stores is mediated by the Inositol 1,4,5, trisphosphate (\(IP_3\)) receptors present on the membrane of the ER. The \(IP_3\) receptor is a ligand operated channel. Closing and opening of these channels controls calcium release into the cytosol.

The extracellular calcium concentration is on the order of 1 \(mM\) while cytosolic calcium concentrations are on the order of 0.1 \(\mu M\). Internal stores within the cell, like the ER, have calcium concentrations of the order of 500 \(\mu M\). Since, the cytosolic concentrations are low, a steep gradient exists from the outside to the inside of a cell. Similarly, a steep gradient exists across the ER membrane-cytosol interface. These steep gradients ensure a quick flow of calcium to the cytosol once a channel opens. Sustained high cytosolic calcium concentrations spell death for the cell. Therefore the cell expends energy to pump out excess cytosolic calcium, reloading the ER, in order to maintain low cytosolic calcium concentrations. Thus, a finely tuned mechanism operates to control the influx and removal of cytosolic calcium.

1.1 The Building blocks

1.1.1 The Inositol 1,4,5 trisphosphate receptor

The inositol-1, 4, 5 triphosphate receptors (\(IP_3R's\)) are intracellular calcium channels, regulated by both \(IP_3\) and \(Ca^{2+}\), governing release of calcium from the endoplasmic reticulum (ER) into the cytosol. Binding of an extracellular agonist, for example a hormone or a neurotransmitter, to the cell membrane evokes the production of \(IP_3\) through a chemical pathway. \(IP_3\) freely diffuses through the cytoplasm and binds to the \(IP_3\) receptors regulating their function. Clusters of \(IP_3R's\) are found on the ER membrane, with a typical strength of about 20-30 channels per cluster. The clusters are known to be randomly distributed on the ER membrane. The \(IP_3R's\) have also been
reported to mediate calcium release in other intracellular organelles like the nuclear envelope, golgi and secretory vesicles and plasma membrane of some cells [63].

Structurally the \( IP_3R \) is a large tetramer (~1MDa) [19]. It consists of four large subunits consisting of about 2700 residues each [63]. Each receptor subunit has a single \( IP_3 \) binding site and two binding sites for \( Ca^{2+} \): one stimulatory and another inhibitory. The exact location of the two \( Ca^{2+} \) binding sites is not known but what is known is that once \( IP_3 \) is bound it makes the subunit more susceptible to binding \( Ca^{2+} \) at the stimulatory/activation site [2], [37]. Across cell types three different \( IP_3R \) isomers are known to exist [66].

Despite the fact that the structure of \( IP_3R \) is not fully understood there are important functional aspects of the \( IP_3R \) which are known. Both \( IP_3 \) and \( Ca^{2+} \) regulate the receptor. \( IP_3 \) evoked \( Ca^{2+} \) release is positively co-operative, suggesting that all four subunits of the channel must bind to \( IP_3 \) before the channel can open [63]. \( IP_3 \) binding promotes \( Ca^{2+} \) binding to the activation site over \( Ca^{2+} \) binding to the inactivation site. Once \( Ca^{2+} \) is bound, the receptor channel opens and \( Ca^{2+} \) is released into the cytosol. Released \( Ca^{2+} \) binds to neighboring \( IP_3R \)'s, increasing their open probability. This phenomena is termed as calcium induced calcium release (CICR).

\( Ca^{2+} \) released by the opening of a single channel, termed a blip, has two consequences. First, more channels in the vicinity could be activated when \( Ca^{2+} \) binds to them through CICR. When a few channels (6-10) open from within a cluster, the resulting \( Ca^{2+} \) signal is termed a puff. Second, as elevated \( Ca^{2+} \) levels are also responsible for a channel to shut down, the channel may shut down with or without triggering a puff. Elevated \( Ca^{2+} \) levels, from a cluster that puffs, can recruit neighboring clusters to fire and thereby generate calcium waves within a cell. Figure 1.1 is a schematic diagram illustrating how the different \( IP_3 \) concentrations and calcium induced calcium release
Figure 1.1: A schematic illustrating basic tenets of calcium signaling. Panel A) shows at low $IP_3$ concentrations few channels have $IP_3$ bound and release events observed are generally single channel openings "blips". As $IP_3$ is increased more channels from within a cluster bind calcium and conduct leading to "puffs" (Panel B). Puffs signify a $Ca^{2+}$ release event evoked by the concerted opening of a few $IP_3$ receptors form with in the cluster. As $IP_3$ is further increased it enables more receptors across clusters to bind calcium and activate leading to global calcium waves in a cell (Panel C). Picture is extracted from [1].

work in tandem to create a repertoire of $Ca^{2+}$ signals. Recruitment of $IP_3$ channels is known to be a stochastic process. The random opening and closing of channels coupled with regulation by both $IP_3$ and $Ca^{2+}$ leads to a rich and interesting repertoire of $Ca^{2+}$ signals. Blips and puffs are referred to as elementary calcium release events. Duration of these events is in the millisecond regime. Whereas blips last for a mean time of few tens of milliseconds [54], puff lifetimes are up to a few hundred milliseconds [64], [57].
Global calcium waves, which are a result of synchronized calcium signaling across hundreds of clusters in a cell, last up to a few seconds [5], [34], [31].

1.1.2 The pump

As it is essential to maintain low cytosolic $Ca^{2+}$ concentrations, the cell recruits the sarcoendoplasmic reticulum calcium ATPases (SERCA or calcium pumps) present on the ER membrane, for the reuptake of $Ca^{2+}$ from the cytosol. The cell spends energy via ATP hydrolysis to replenish the internal store (ER) once $Ca^{2+}$ has been released into the cytosol. Three main SERCA isoforms are known to exist. All isoforms display qualitatively similar enzymatic properties and are activated by calcium in a cooperative manner [33].

1.1.3 Buffers

Cytosolic calcium concentrations are buffered to low levels by endogenous $Ca^{2+}$ binding proteins [53]. It has been estimated that about 1-5% of $Ca^{2+}$ is free [3], [72]. These proteins/buffers can be either mobile or immobile. Zhau and Neher [72] estimated that about 25% of all buffer in chromaffin cells is mobile. Upon release, calcium is immediately bound to the buffers due to fast binding time scales, creating a pool of mobile calcium (after binding to mobile buffers) and stationary calcium (after binding to immobile buffers). Dissociation of calcium from these complexes influences the functional behavior of the $IP_3$ receptor as $Ca^{2+}$ is an important regulatory agonist for the receptor. $Ca^{2+}$ binding and unbinding timescales therefore play a crucial role and add to the complexity of the system. Confocal line scan imaging has shown calcium release from a cluster of $IP_3$ channels to be diffusive in nature with the measured effective diffusion coefficient of $\sim 27 \mu m^2 s^{-1}$ [70].
1.2 Mathematical Modeling: An Overview of the Study

A wealth of experimental data is available detailing elementary $Ca^{2+}$ release events [57], [64], [54] and functional properties of the $IP_3$ receptors [6], [66], [27], [35]. Experiments are limited by protocol and therefore computational studies, across different spatial and temporal scales, are a significant and indispensable tool. Extensive computational work has been done [18], [65], [68], [14] and continuous to propel the symbiotic relationship between modeling and experimental studies.

In this thesis a systematic effort has been made to develop a simple binding model for the $IP_3$ receptor based on what is experimentally known of the receptor function. The model is used to quantify and reproduce statistics of experimentally observable $Ca^{2+}$ puff properties. The model is then further used to understand the behavior of a single cluster in a buffered environment, including the influence of different buffer kinetics and concentrations on single cluster behavior. I examine the extent to which a buffered system of a single cluster of $IP_3$ receptors can be simplified computationally without losing significant physiological properties of receptor function.

Traditional mathematical models of the $IP_3$ receptor predict that the receptor channel conducts after binding both $IP_3$ and $Ca^{2+}$. In recent theoretical work [49] it has been proposed that the conducting state of the $IP_3$ channel is independent of both $IP_3$ and $Ca^{2+}$. The proposed model was used to explain single channel open probabilities as recorded in nuclear patch clamp experiments [35]. I test the robustness of this model on a larger spatial scale by studying the impact this new introduced state has on the properties of $Ca^{2+}$ puffs detailed in chapter 4. A successful model should be able to predict and explain functional properties of the receptor across the different temporal and spatial scales involved in the process of calcium signaling.

Chapter 2 describes a simple binding model of the $IP_3$ receptor and the results were published as in reference [58] by Swaminathan et al. (2009). The chapter summarizes the
published work and presents additional analysis and discussions resulting from work done later. Further more the model presented in Chapter 2 was used to test the significance of a proposed new $IP_3$ subunit state. The results are presented in Chapter 4. Material covered in Chapter 4 has been submitted for peer review as a manuscript entitled "Role of Agonist-Independent Conformational Transformation (AICT) in $IP_3$ Cluster Behavior" to Cell Calcium [59]. In addition to the work presented in this thesis, the model was also used to study the underlying mechanisms of calcium spiking: comparing stochastic spiking in small periodic systems to small excitable systems [26]. This work has been accepted and will be published shortly [26].
2 A Simple Sequential Binding Model For Ca$^{2+}$ Puffs

2.1 Introduction

Each cell has internal calcium-rich sources like the mitochondria and the endoplasmic reticulum (ER). The $IP_3$ receptors are placed in small clusters of 20-30 channels each on the ER membrane. Elementary $Ca^{2+}$ release events from these clusters, be it blips or puffs, are the fundamental building blocks of intracellular $Ca^{2+}$ signaling. Local signals from a single cluster also have the potential to recruit neighbouring clusters leading to global $Ca^{2+}$ waves and oscillations. The main emphasis of this chapter is to present a simple markovian model of the $IP_3$ receptor kinetics to explain the observed statistics of $Ca^{2+}$ release events.

2.2 Elementary $Ca^{2+}$ Release Events

The basic quantal event of $Ca^{2+}$ puffs has been studied extensively in experiments. Thomas et al. [64] studied $Ca^{2+}$ puffs in HeLa cells. A wide variation in puff amplitude, from 15 $nM$ to 600 $nM$, was reported in their experiments [64]. In experiments amplitudes are obtained by averaging over a length scale of 1 $\mu m$. The fluorescence magnitudes for puffs ($F/F_0$ of dye Oregon green 488 Bapta 1) as reported by Sun et al.[57] showed a skewed distribution with a peak at about 1.5$s$ and a tail extending up to 3.5$s$ [57]. Qualitatively, the spread of amplitudes observed by Sun et al.[57] in Xenopus oocytes cells is similar to the amplitude spread observed by Thomas et al.[64] in HeLa cells. Confocal line scans established the spatiotemporal distribution of $Ca^{2+}$ release events to be consistent with $Ca^{2+}$ diffusion from a point source [57]. Variability in the magnitude of $Ca^{2+}$ released during a release event correspond directly to the opening and closing of $IP_3$ channels from within a cluster [64], [57]. Experiments have clearly established that the release events are not stereotypical all or none events. Rather the underlying stochasticity accounts for the rich diversity observed.
Puff lifetimes provide an important experimental measure of the duration of the release events. The lifetime of an event is characteristically defined as the time course during which released Ca\(^{2+}\) measures more than half of the maximum Ca\(^{2+}\) concentration recorded: or the Puff Duration at Half Maximum (PDHM). In experiments with HeLa cells [64] the puff lifetime distribution showed a peak at about 150 ms followed by exponential decay with a very small fraction of puffs measuring greater than 600 ms. Similar puff lifetimes were reported by Sun et al. [57] in puff recordings from the Xenopus oocyte. In Xenopus oocytes [57] puff lifetime distribution peaks at about a 100 ms followed by an exponential decay cutting off at 600 ms. During a puff, the rise of cytosolic Ca\(^{2+}\) is fast and Ca\(^{2+}\) peaks within 20 ms [43]. The subsequent decline lasts a few hundred milliseconds. IP\(_3\) concentration impacts puff frequency [64]. With increasing IP\(_3\) more receptors activate increasing the frequency of puffs observed. Puffs at different sites, within a cell, show great variability as do puffs observed at the same cluster site [64], [57]. Stochastic opening and closing of channels underlies the variability exhibited in lifetimes and amplitudes of Ca\(^{2+}\) puffs [7].

The time interval distribution between consecutive puffs is also well characterized [36], [18]. In earlier experiments by Marchant et al. [36] the interpuff interval distribution was found to peak at 1.5s for focal Ca\(^{2+}\) puffs (puffs that initiate Ca\(^{2+}\) waves) and 3.5s for non-focal Ca\(^{2+}\) puffs (puffs that do not initiate a Ca\(^{2+}\) wave). In later studies with Xenopus Oocytes [18] the inter puff interval distribution was shown to peak at 1.5s. In experiments IP\(_3\) is released through flash photolysis. The actual IP\(_3\) concentration is unknown but flash strength is proportional to the amount of IP\(_3\) released.

Structural studies show the large IP\(_3\) receptor protein to be a tetramer [63]. Each subunit consists of approximately 2700 residues and a single IP\(_3\) binding site. Two calcium binding sites exist: one that activates the receptor and another that inhibits the receptor. The exact location of the two Ca\(^{2+}\) binding sites is not known but what is known
is that once $IP_3$ is bound it makes the subunit more susceptible to binding $Ca^{2+}$ at the stimulatory/activation site [37]. Three distinct isoforms of the $IP_3$ receptor are known to exist in mammalian cells [6], [67].

Available experimental data set a benchmark for mathematical models of the $IP_3$ receptor. Mathematical models should be able to reproduce statistical features of elementary release events observed in experiments. In 1992 De-Young and Keizer (DYK) proposed an eight state model for the $IP_3$ receptor subunit [12] based on the three distinct $IP_3$ and $Ca^{2+}$ binding sites. Stochastic simulation of the DYK model and its derivatives (like the Li-Rinzel (LR) model [32]) account for various puff properties like amplitudes and spreading but fail to account for puff lifetimes at realistic calcium concentrations. Puff duration distributions are in the millisecond regime [64], [57], [54]. As shown by Shuai et al. [46] and Ullah et al. [68] both the DYK and the LR model predict puff lifetimes in the seconds regime.

In this chapter I present a simple 6-state model of the $IP_3$ receptor subunit which generates puffs with amplitudes and puff lifetime durations consistent with experimental values. Gating of the $IP_3$ receptor as presented in the 6-state model is motivated by the DYK model but with two important constraints (based on experimental studies) imposed. The model is computationally inexpensive and the emphasis is on studying the behaviour of a single cluster of $IP_3$ receptors.

### 2.3 The 6-state $IP_3$ Receptor Model

The tetrameric $IP_3$ receptor is modeled as having four equal and independent subunits. Each subunit has three distinct binding sites. One for $IP_3$ activation, another for activating $Ca^{2+}$ and a third for binding inhibitory $Ca^{2+}$. Each of these sites are either occupied or unoccupied by their respective regulatory agonist ($IP_3$ or $Ca^{2+}$) [12], [28]. With three binding sites, the subunit can exist in a total of eight possible states. Based on
Figure 2.1: A schematic diagram illustrating the kinetics of a single \( IP_3 \) receptor subunit as described by the 6-state model. \( c \) denotes \( Ca^{2+} \) concentration and \( p \) denotes \( IP_3 \) concentration. Figure from reference [58].

The experimental studies [37] I impose first, the condition of sequential binding. Marchant et al. [37] observed in the absence of bound \( IP_3 \), \( Ca^{2+} \) does not bind to it’s activating site. \( Ca^{2+} \) binding to the activation site is sequential as it only binds once \( IP_3 \) is bound to the receptor. Figure 2.1 illustrates the kinetics of the 6-state \( IP_3 \) subunit model. Transition rates from one state to the other are governed by agonist (\( IP_3 \) and \( Ca^{2+} \)) concentrations. \( S_{ijk} \) represents the state of the subunit, with index \( i \) characterizing the \( IP_3 \) binding site (\( i = 0 \) represents \( IP_3 \) not bound / \( i = 1 \) represents \( IP_3 \) bound), index \( j \) the \( Ca^{2+} \) activating site and index \( k \) the \( Ca^{2+} \) inhibiting site [58]. Therefore the state \( S_{100} \) is representative of only \( IP_3 \) bound to the subunit. Enforcing sequential binding implies that states like \( S_{010} \)
and \( S_{011} \) do not exist (index \( i=0 \), \( IP_3 \) is not bound to the receptor). The rates governing transitions in between subunit states are listed in table 2.1.

### Table 2.1: Transition Kinetics: 6-state model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>6-state</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Receptor Binding constants (( a_i )'s)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( a_1 (\mu M s)^{-1} )</td>
<td>167.6</td>
<td>( IP_3 )</td>
</tr>
<tr>
<td>( a_2 (\mu M s)^{-1} )</td>
<td>3.81</td>
<td>( Ca^{2+} ) inhibition</td>
</tr>
<tr>
<td>( a_3 (\mu M s)^{-1} )</td>
<td>413.4</td>
<td>( IP_3 )</td>
</tr>
<tr>
<td>( a_4 (\mu M s)^{-1} )</td>
<td>0.3101</td>
<td>( Ca^{2+} ) inhibition</td>
</tr>
<tr>
<td>( a_5 (\mu M s)^{-1} )</td>
<td>53.9</td>
<td>( Ca^{2+} ) activation</td>
</tr>
<tr>
<td><strong>Receptor Dissociation constants (( b_i )'s)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( b_1 (s^{-1}) )</td>
<td>228.0</td>
<td>( IP_3 )</td>
</tr>
<tr>
<td>( b_2 (s^{-1}) )</td>
<td>0.409</td>
<td>( Ca^{2+} ) inhibition</td>
</tr>
<tr>
<td>( b_3 (s^{-1}) )</td>
<td>188.5</td>
<td>( IP_3 )</td>
</tr>
<tr>
<td>( b_4 (s^{-1}) )</td>
<td>0.096</td>
<td>( Ca^{2+} ) inhibition</td>
</tr>
<tr>
<td>( b_5 (s^{-1}) )</td>
<td>4.52</td>
<td>( Ca^{2+} ) activation</td>
</tr>
</tbody>
</table>

The \( IP_3 \) channel is activated when either three or all four subunits are activated [37], i.e. have both \( IP_3 \) and activating \( Ca^{2+} \) bound. Increase in cytosolic \( Ca^{2+} \) facilitated by the concerted opening of a few channels leads to channel inhibition. The 6-state model is a derivative of the standard DYK model. As life times predicted by the DYK model are in the seconds regime [46], [68] in contrast with the millisecond regime observed in experiments [64], [57], I incorporate a higher rate of inactivation in the 6-state model.
Increased inhibition shifts the puff lifetime distribution to the millisecond regime by reducing the mean open time of channels.

### 2.3.1 The Steady State Open Probability

![Normalized open probability response to increasing calcium for the IP$_3$ receptor model.](image)

Figure 2.2: Normalized steady state open probability response to increasing calcium for the 6-state IP$_3$ receptor model. IP$_3$ is fixed at 2 µM as in experiments[66]. Peak of the open probability curve lies at 120 nM [Ca$^{2+}$]. Following experimental results as reported in [66], open probability was normalized with respect to peak $P_0$ values and calcium is reported in units of pCa. pCa equals $-\log_{10}$[Ca$^{2+}$]. Figure from reference [58].

Single channel functional behaviour of a single IP$_3$ channel has been widely studied in the literature [6], [27], [67], [66]. Reconstitution of the IP$_3$ receptor into planar lipid bilayers is an important experimental technique used to study functional behaviour of an isolated channel. At clamped values of IP$_3$ and Ca$^{2+}$, the open probability of the receptor is recorded along with mean open and close times.

The single channel open probability of an IP$_3$ channel, $P_o$, can be calculated as follows. Taking into account that the channel opens when all or any three subunits conducts I have

$$P_o = q_o^4 + 4q_o^3(1 - q_o),$$

(2.1)
where, $q_a$ is the equilibrium probability for the subunit to be in the open state $S_{110}$.

Transitions between subunit states are governed by the law of mass action kinetics and detailed balance is imposed on the system. This allows for the equilibrium probability to be in the open state, $q_a$, to be expressed as the ratio of the unnormalized probability to be in the open state $S_{110}$, to the normalization factor $Z$ [49]. The unnormalized probability $w_{a(i,j,k)}$, to be in any state $S_{ijk}$ is defined, with respect to the state $S_{000}$, as the ratio of the forward to the backward rates along any path that connects the state $S_{i,j,k}$ with the state $S_{000}$ [49]. The normalization factor $Z$ is the sum total of all unnormalized probabilities.

\[
q_a = \frac{w_{a(110)}}{Z}
\]

\[
w_{a(110)} = \frac{pc}{d_1d_5}; \quad d_i = \frac{b_i}{a_i}; \quad i = 1, 2..5
\]

\[
Z = 1 + \frac{c}{d_4} + \frac{p}{d_1} \left(1 + \frac{c}{d_5}\right) \left(1 + \frac{c}{d_2}\right).
\] (2.2)

Figure 2.2 shows the normalized open probability $P_0$, obtained by the 6-state receptor model, as a function of increasing $Ca^{2+}$ with $IP_3$ clamped at 2 $\mu$M. In lipid bilayer experiments, reported open probabilities range from $<0.01$ [27] up to 0.4 [67], [66]. At an $IP_3$ concentration of 2 $\mu$M, $P_0$ in the 6-state model peaks at 0.042 at a $Ca^{2+}$ concentration of about 120 nM [58]. The peak $Ca^{2+}$ sensitivity as observed in the 6-state model correlates well with published experimental data [66]. At an $IP_3$ concentration of 2 $\mu$M, Tu et al.[66] report peak $Ca^{2+}$ sensitivity to be at 257 nM of $Ca^{2+}$ for the type 1 receptor isoform, 154 nM for the type 2 isoform and at 107 nM for the type 3 isoform. Based on these experimental results I conclude, for the parameters presented, the 6-state model works well within a relevant physiological range [58].
2.3.2 The Quasi Steady State Approximations

The following first order differential equations describe the subunit dynamics of the 6-state IP$_3$ receptor model. (Schematic in figure 2.1).

\[
\begin{align*}
\frac{dx_{000}}{dt} &= b_1 x_{100} + b_4 x_{001} - (a_1 p + a_4 c)x_{000} \\
\frac{dx_{100}}{dt} &= a_1 p x_{000} + b_2 x_{101} + b_3 x_{110} - (b_1 + a_5 c + a_2 c) \\
\frac{dx_{001}}{dt} &= b_3 x_{101} + a_4 c x_{100} - (b_4 + a_5 p)x_{001} \\
\frac{dx_{101}}{dt} &= b_5 x_{111} + a_2 c x_{100} + a_3 p x_{001} - (a_5 c + b_2 + b_3) \\
\frac{dx_{110}}{dt} &= b_2 x_{111} + a_5 c x_{100} - (a_2 c + b_5) x_{110} \\
\frac{dx_{111}}{dt} &= a_5 c x_{101} + a_2 c x_{110} - (b_5 + b_2) x_{111}
\end{align*}
\] (2.3)

For the 6-state model, IP$_3$ flux through a cluster of receptors is calculated as,

\[
J_{\text{cluster}} = v_{\text{channel}}(c_{ER} - c)[x_{110}^4 + 4x_{110}^3(1 - x_{110})]
\] (2.4)

where the subunit is activated in the $x_{110}$ state and a channel conducts when either three or all four subunits of the channel are activated.

In the above equations the fractions $x_{ijk}$ denote the probabilities of the subunit to be in states $S_{ijk}$. Observing the transition rates of the model (see table 2.1) it is clear that three distinct time scales exist. IP$_3$ binding is the fastest of the three binding processes followed by $Ca^{2+}$ activation. Inhibition by $Ca^{2+}$ binding is the slowest of the three processes. The differences in the agonist binding time scales can be used to simplify the system to reduced four or two state models. As IP$_3$ association and dissociation is faster than the other competing processes, transitions between states $x_{0jk}$ and $x_{1jk}$ are the first to reach an equilibrium. Therefore exploiting the faster time scale it is possible to define the four state system

\[
\sum_{j,k=0}^1 X_{jk} = x_{0jk} + x_{1jk}
\] (2.5)
together with the $IP_3$ equilibrium condition

$$a_i p x_{00k} = b_i x_{10k}; \quad i = 1, 3; \quad k = 0, 1. \quad (2.6)$$

Using equations 2.5 and 2.6, the reduced 4 state model is defined by the following set of equations.

$$\frac{dX_{00}}{dt} = \frac{(b_4 \kappa_3 + b_2) X_{01}}{1 + \kappa_3} + b_3 X_{10} - (a_4 \kappa_1 + a_2 c) \frac{X_{00}}{1 + \kappa_1}$$

$$\frac{dX_{10}}{dt} = b_2 X_{11} + a_5 c \frac{X_{00}}{1 + \kappa_1} - (a_2 c + b_5) X_{10}$$

$$\frac{dX_{01}}{dt} = \frac{(a_4 \kappa_1 + a_2) c X_{00}}{1 + \kappa_1} + b_5 X_{11} - (b_4 \kappa_3 + b_2 + a_5 c) \frac{X_{01}}{1 + \kappa_3}$$

$$\frac{dX_{11}}{dt} = a_5 \frac{X_{01}}{1 + \kappa_3} + a_2 c X_{10} - (b_5 + b_2) X_{11}, \quad (2.7)$$

where,

$$\kappa_j = \frac{b_j}{a_j p} \quad ........ \quad j = 1, 3 \quad , \quad (2.8)$$

and

$$J_{\text{cluster}} \equiv v_{\text{channel}}(c_{ER} - c)(X_{10}^4 + 4X_{11}^3) \quad (2.9)$$

Figure 2.3 shows the schematic diagram of the four state reduction of the full 6-state model. The second reaction to equilibrate is the $Ca^{2+}$ binding to the activation site, i.e. the second equilibration condition is

$$x_{10k} = \frac{d_5}{c} x_{11k}, \quad d_5 \equiv \frac{b_5}{a_5}, \quad k = 0, 1 \quad (2.10)$$

Defining $Y_j = X_{0j} + X_{1j} \ (j=0,1)$ and using the above equilibrium conditions the system can be further reduced to a two state system.

$$\frac{dY_0}{dt} = (b_4 \kappa_3 + b_2) \frac{X_{01}}{1 + \kappa_3} + b_2 X_{11} - (a_4 \kappa_1 + a_2 c) \frac{X_{00}}{1 + \kappa_1} - a_2 c X_{10} \quad (2.11)$$
Figure 2.3: Schematic diagram illustrating the kinetics of the four state reduction of the full 6-state $IP_3$ receptor model. Figure from reference [58].

The term $Y_0$ stands for the fraction of open subunits in a two state reduction of the full six-state model. Likewise $Y_1$ represents fraction of closed subunits and the sum total of open and closed fractions should equal one.

Using the $IP_3$ equilibration condition 2.6 we have,

$$\frac{dY_0}{dt} = (b_4\kappa_3 + b_2)x_{101} + b_2x_{111} - (a_4\kappa_1 + a_2)c x_{100} - a_2cx_{110} \quad (2.12)$$

Using the $Ca^{2+}$ equilibration condition 2.10 the above equation can be written as,

$$\frac{dY_0}{dt} = [(b_4\kappa_3 + b_2)\frac{d_5}{c} + b_2]x_{111} - [(a_4\kappa_1 + a_2)d_5 + a_2c]x_{110}. \quad (2.13)$$

The two conditions of equilibration are now used to express $x_{111}$ in terms of $Y_1$ and $x_{110}$ in terms of $Y_0$. The following set of equations completely describe the reduced two state
version of the 6-state model, i.e.

\[
\frac{dY_0}{dt} = \frac{[(b_4\kappa_3 + b_2)\frac{d_5}{c} + b_2]}{[1 + \kappa_3\frac{d_5}{c} + 1]} Y_1 - \frac{[(a_4\kappa_1 + a_2)\frac{d_5}{c} + a_2]}{[1 + \kappa_1\frac{d_5}{c} + 1]} cY_0.
\]

\[Y_1 = 1 - Y_0\]

\[m = [(1 + \kappa_1)\frac{d_5}{c} + 1]^{-1}\]

\[J_{\text{cluster}} = v_{\text{channel}}(c_{ER} - c)[m^4 Y_0^4 + 4m^3 Y_0^3(1 - mY_0)].\]

(2.14)

Figure 2.4 shows the bifurcation diagram obtained by the full 6-state model and its quasi steady four state and two state reductions. The minimum and maximum of the \(Ca^{2+}\) concentrations during the oscillations are plotted as a function of \(IP_3\) concentration. At low \(IP_3\) values \(< 0.2 \mu M\) \(Ca^{2+}\) settles into a steady state and no oscillations are observed. At \(IP_3 = 0.24 \mu M\), the steady state loses stability via a subcritical Hopf bifurcation [58]. At this point, an unstable periodic orbit collapses onto the equilibrium, and both disappear [58]. \([Ca^{2+}]\) is subsequently attracted toward a large-amplitude stable periodic orbit. \(Ca^{2+}\) oscillations persist for an \(IP_3\) concentration less than 0.48 \(\mu M\) for both the six and four state models. At this point another sub critical Hopf bifurcation is observed and \(Ca^{2+}\) approaches steady state values with further increasing \(IP_3\) concentrations [58]. For the two state reduction of the full model, oscillations are observed up to 0.64 \(\mu M\) of \(IP_3\), \(Ca^{2+}\) settling into a steady state value beyond 0.64 \(\mu M\) of \(IP_3\), in sharp contrast with the oscillations obtained from the other two models. The two state model predicts unsatisfactory results and therefore is not considered further in the text [58].

### 2.4 Stochastic Simulation of the 6-State Model

Spatio-temporal \(Ca^{2+}\) dynamics is described by the following reaction-diffusion equation.

\[
\frac{dc}{dt} = D \nabla^2 c + \delta(\vec{x} - \vec{x}_0)J_{\text{cluster}} + J_{\text{leak}} - J_{\text{pump}},
\]

(2.15)
Figure 2.4: The bifurcation diagram for \([Ca^{2+}]\) concentrations as a function of the \([IP_3]\) concentrations for the six-state model (in red), the four-state reduction, (in black) and the two-state reduction (in blue). Parameter values are \(v_{\text{channel}}=5.88\ s^{-1}\), \(c_{ER}=11\ \mu M\), \(v_{\text{leak}}=0.02035\ s^{-1}\), \(v_{\text{pump}}=0.9\ \mu M\) and \(k_{\text{pump}}=0.1\ \mu M\). Peak amplitude for \(Ca^{2+}\) oscillations is similar in both the 6-state and the four state reduction at \(\sim 0.8\ \mu M\). For the two state reduction (blue), the oscillation amplitude is markedly higher at about \(1.15\ \mu M\). The range of \(IP_3\) concentration over which oscillations are observed is markedly different for the two state reduction. Solid lines represent stable states. Dotted lines represent unstable state. Figure from reference [58]

where \(c\) defines cytosolic \(Ca^{2+}\) concentration.

In equation 2.15 the term \(J_{\text{cluster}}\) describes the release of \(Ca^{2+}\) into the cytosol via a small cluster of \(IP_3\) receptors placed on the ER membrane at \(\vec{x}_0\). The total \(Ca^{2+}\) flux, \(J_{\text{cluster}}\), through the single cluster is directly proportional to the \(Ca^{2+}\) gradient that exists across the membrane and the number of channels that open from within a cluster. For the numerical solution of eqn 2.15, a discrete representation of Dirac’s delta function is used. For a single cluster of \(N\) channels, the \(Ca^{2+}\) flux through a single cluster is defined as,
\[ J_{\text{cluster}} = N(\pi r_c^2) v_{\text{channel}} (c_{\text{ER}} - c) \frac{N_o}{N} \]
\[ J_{\text{leak}} = v_{\text{leak}} (c_{\text{ER}} - c) \]
\[ J_{\text{pump}} = \frac{v_{\text{pump}} c^2}{k_{\text{pump}}^2 + c^2} \]  \hspace{1cm} (2.16)

where, \( r_c = 6 \text{ nm} \) is the radius of a single channel, \( v_{\text{channel}} \) is the flux conducted by a single channel and \( N_o \) represents the total number of open channels at a given instant of time. The number of open channels is obtained by stochastically simulating the kinetics of the \( IP_3 \) receptor. Subunit kinetics are based on the 6-state model (see schematic in fig 2.1). The term \( J_{\text{leak}} \) accounts for unspecified calcium leak from the ER into the cytosol. To restore low basal \( Ca^{2+} \) concentration, upon release of \( Ca^{2+} \) into the cytosol, the cell recruits sarcoplasmic reticulum calcium ATPases (SERCA or calcium pumps) present on the ER membrane. A uniform distribution of pumps is assumed and they are modeled with a hill coefficient of two as shown in eqn 2.16 above. For a cluster of 20 channels, a channel radius of 6nm gives us a cluster area of \( \sim 50 \text{nm} \times 50 \text{nm} \).

I do not model the cluster as a distributed source, i.e channels within the cluster are not treated to be spatially distinct. This approach in computationally very expensive. Instead, I model the cluster as a point source where all channels are exposed to a homogenous \( Ca^{2+} \) concentration. In a detailed modeling study, Swillens et al. [60] show that within 15 \( \mu s \), after \( Ca^{2+} \) release, concentration at channel mouth is indistinguishable from concentration about 100 nm away from the channel. Similar observations have also been made in other modeling studies [56], [65]. In a later work, Swillens et al.[61] show the equivalence of results obtained by resolving individual channels on a finer spatial domain to a cluster on a larger spatial domain exposed to a homogenous \( Ca^{2+} \) concentration. Time scale of calcium release events are on the order of tens of
milliseconds, implying $Ca^{2+}$ concentrations on the length scale of a cluster can be treated as spatially homogenous [60], [68].

Cellular environments are heavily buffered. Once $Ca^{2+}$ is released into the cytosol it diffuses away. In the cytosol, diffusion coefficient of free $Ca^{2+}$ has been measured to be $\sim 250 \ \mu m^2 s^{-1}$ [3]. The presence of buffers slows down the effective diffusion of free $Ca^{2+}$ [69], [53]. Radial profiles of $Ca^{2+}$ dependent fluorescence at different times throughout a puff show gaussian distributions, whose width increases as a square root function of time, consistent with diffusion from a point source [38]. The rate of spread as observed in experiments corresponds to an apparent diffusion coefficient of $27 \ \mu m^2 s^{-1}$ [70]. The first term in equation 2.15 accounts for the diffusive spread of cytosolic $Ca^{2+}$. Effective coefficient of diffusion is taken to be $25 \ \mu m^2 s^{-1}$ to be consistent with experimental measurements.

The partial differential equation 2.15 is solved on a square area of $5 \mu m \times 5 \mu m$ using a four point discretization of the Laplacian and a fully explicit forward difference solver [58]. No flux boundary conditions are applied. The delta function in equation 2.15 is implemented in a discreet manner, by (i) limiting the cluster flux to a bin area $\delta x^2$, where $\delta x$ is the spatial grid size and (ii) dividing the total cluster flux by the bin area $\delta x^2$ such that the integral over the cluster is independent of grid size [58]. The square area was discretized into a bin size of $50nm \times 50nm$, close to the actual size of a cluster. The time step used in the simulations was $10 \ \mu sec$. To solve a PDE on a 2-dimensional grid the stability criterion states that $(D\delta t/\delta x^2) \leq 0.25$ [8], [40]. All simulations presented in the thesis strictly adhere to the above criterion.

For the stochastic simulations each single $IP_3$ channel was modeled with four equal and independent subunits where the kinetics of each subunit are determined by the 6-state model (see figure 2.1). A single channel is considered to be open when either any three or all four subunits have $IP_3$ and activating $Ca^{2+}$ bound (i.e are in the state $S_{110}$). Stochastic
transitions in between subunit state are governed by the stochastic association and dissociation of the agonist ($IP_3$ and $Ca^{2+}$). For sufficiently small time step, transition probabilities have a linear time dependence [58]. Specifically, if the subunit is in the state $S_i$ with access to state $S_j$ with a forward transition rate $r_{ij}$ then the probability to transition from $i \rightarrow j$ is $r_{ij}\delta t$ where $\delta t$ is small. The probability to remain in state $S_i$ is $1 - \sum_j r_{ij}\delta t$ for sufficiently small $\delta t$ [58].

Table 2.2: Parameters for stochastic simulation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$v_{channel}$</td>
<td>5689.0 $s^{-1}$</td>
<td>Maximal single channel flux.</td>
</tr>
<tr>
<td>$v_{leak}$</td>
<td>0.02035 $s^{-1}$</td>
<td>$Ca^{2+}$ leak flux constant</td>
</tr>
<tr>
<td>$v_{pump}$</td>
<td>45.5$\mu m s^{-1}$</td>
<td>Maximum $Ca^{2+}$ uptake (SERCA)</td>
</tr>
<tr>
<td>$k_{pump}$</td>
<td>0.1 $\mu M$</td>
<td>Activation constant for $Ca^{2+}$ pump</td>
</tr>
<tr>
<td>$D$</td>
<td>25.0 $\mu m^2 s^{-1}$</td>
<td>Effective $Ca^{2+}$ diffusion</td>
</tr>
<tr>
<td>$IP_3$</td>
<td>5 $\mu M$</td>
<td>$IP_3$ concentration.</td>
</tr>
<tr>
<td>$c_{ER}$</td>
<td>11$\mu M$</td>
<td>Lumenal $Ca^{2+}$ concentration.</td>
</tr>
</tbody>
</table>

2.5 Results

A single cluster of 20 $IP_3$ channels was placed at the center of a membrane patch of dimensions 5$\mu m \times 5\mu m$ and stochastically simulated with an effective $Ca^{2+}$ diffusion coefficient of 25 $\mu m^2 s^{-1}$. The membrane patch was discretized into a fine spatial grid with grid size $\delta \chi = 50$ nm. $IP_3$ concentration was a constant at 5 $\mu M$. All model parameters used in the simulation are presented in tabel 2.2 [58].
Figure 2.5: \( Ca^{2+} \) puffs (upper panel) as predicted by the 6-state model. For consistency with experiments \( Ca^{2+} \) is averaged over an area of 1 \( \mu m^2 \). The lower panel shows the underlying fraction of open channels accompanying each release event. Simulated at parameters as reported in Tables 2.1 and 2.2.

Figure 2.5 presents a representative trace of \( Ca^{2+} \) release events as generated by the 6-state model. To be consistent with experiments, as experiments record fluorescence from a length scale \( \sim 1 \mu m \) [57], \( Ca^{2+} \) values are averaged over a 1 \( \mu m^2 \) area. The time course of a puff is in the regime of a few hundred of milliseconds as shown by in Figure 2.6. The lower panel of Figure 2.5 shows the underlying fractions of open channels that correspond to the \( Ca^{2+} \) trace of the upper panel. Figure 2.6 presents an isolated puff and the underlying fraction of open channel. A few channels open leading to a rise in cytosolic \( Ca^{2+} \). \( Ca^{2+} \) release has the potential to recruit more neighboring channels through CICR. Upon further release, elevated \( Ca^{2+} \) levels lead to inhibition of the cluster and therefore termination of the puff. In the representative puff in Figure 2.6 up to 10 channels (50%) of the channels are open. Experimental studies point to graded recruitment of \( IP_3 \) receptors.
Figure 2.6: Temporal evolution of a single release event as predicted by the 6-state model, together with underlying channel activity. $Ca^{2+}$ is averaged over 1 $\mu m^2$. At peak calcium value up to 10 channels from within the 20 channel cluster are open. Time course of the $Ca^{2+}$ release event lasts a few hundred milliseconds.

during a puff [64]. During a puff 2 -8 channels are recruited ([57], [54]), a feature reproducible by the 6-state model. Stochastic opening and closing of channels leads to variability in time course, amplitude and spatial profile of a $Ca^{2+}$ puff. In Figure 2.5 isolated single channel events blips are also visible as shown at about 16s and 32s during the time course presented. The steady state basal level cytosolic calcium is at 20 nM.

I simulated a thousand puffs and studied the statistical distributions of their amplitudes and lifetimes, properties measured extensively in experiments. The puff amplitudes are defined as follows. A puff starts at a time $t_a$ when greater than or equal to two channels open. The puff terminates at a time $t_b$ when averaged $Ca^{2+}$ concentration falls off to under 10 nM above basal level. The puff amplitude is defined as the maximum of the averaged $Ca^{2+}$ concentration recorded during the time interval $[t_a,t_b]$. Puff lifetimes are defined as the time duration during which the averaged $Ca^{2+}$ concentrations are equal
to or greater than half of the maximum puff amplitude observed in \([t_0,t_f]\). Experiments on HeLa cells by Thomas et al. [64] report puff lifetimes to peak at about 100 ms. A similar peak puff lifetime of 100 ms was reported by Sun et. al. [57] for measurements made in Xenopus oocytes. Haak et al.[23] report mean puff lifetimes of 60ms and recent experiments with neuroblastoma cells by Smith et. al. [54] show a puff lifetime distribution peaking at 50ms. Figure 2.7 shows the puff amplitude and puff lifetime durations obtained for the simulated puffs. For the parameter set reported, the puff lifetime distribution peaks at ∼ 100 ms with a mean of 157 ms in excellent agreement with experimental results (fig 2.7, left panel).

![Graphs](image)

**Figure 2.7:** Simulated puff lifetime distribution (left panel) and puff amplitude distribution (right panel) for the 6-state model. \(c_{ER}=11 \mu M, IP_3 = 5\mu M\) and \(v_{channel} = 5689s^{-1}\). Puffs are \(Ca^{2+}\) release events when two or more channels open to conduct. Single channel events are not considered. Figure from reference [58]

In experiments, puff amplitudes have been found to range from 20 nM to 700 nM [64]. Figure 2.7(b) shows the puff amplitude distribution obtained. Simulated puffs range from 16nM to 300 nM with a mean of 144.19 nM [58]. The puff amplitude distribution also compares qualitatively with results of Sun et. al. [57] (Fig 8A in [57]). In my results a small peak is observed at ∼ 30 nM. This peak is a result of 2 channel events. It is possible that these small peak events are not resolved in experiments due to optical
limitations or the peak may be a signature of channels in the 6-state model being poorly synchronized. In a computational study, Swillens et al.[61] have also shown a peak of low amplitude events (< 50 nM). For the set of parameters reported up to 44% of the events were single channel events. Due to increased inhibition in the model, single channel events in particular after a large amplitude event, fail to recruit more channels as cluster inhibition sets in for the duration of seconds. Larger amplitudes are followed by longer periods of inhibition. A previous study by Fraiman et. al.[18] reports similar correlations between larger events and long periods of cluster inhibition.

IP$_3$ binding is prerequisite in activating the channels in the cluster. Therefore puff properties vary with IP$_3$ concentrations [58]. At low IP$_3$ concentrations i.e, < 0.5 µM no puffs were observed. As IP$_3$ is increased to 0.5 µM I observed the rate of puffs to be 1 in 750 seconds (no single channel events considered) with a mean amplitude of 50 nM and a lifetime of 171 ms. Increasing IP$_3$ concentration further increases the frequency and amplitude of the elementary release events. At 1 µM IP$_3$, puff rate is at about 1 per ~ 83 seconds. The mean amplitude was 108 nM with a mean puff lifetime of 193.62 ms. For 2 µM IP$_3$, I obtain a puff rate of about 1 in 22 seconds, with a mean lifetime of 158 ms and a mean amplitude of 132.9 nM Increase in IP$_3$ leads to a decay in the fraction of single channel events. At 2 µM IP$_3$, 50% of the events recorded are single channel events, a percentage that decreases to 44% for 5 µM IP$_3$. Remarkably, properties like puff durations and amplitude saturate once a certain level of IP$_3$ is reached. For a range of IP$_3$ between 3 µM and 5 µM no change in mean puff lifetimes and amplitudes is recorded consistent with observations from some experimental studies [43].

2.5.1 The Reduced Models

Figure 2.8 illustrates the puff lifetimes and amplitude distributions obtained with the four state reduction of the full 6-state model. As shown in a previous section the reduction
Figure 2.8: Puff lifetime (left panel) and puff amplitude (right panel) distributions for the 4 state reduction of the full 6-state model. All parameters are as reported in tables 2.1 and 2.2.

works in predicting a valid $IP_3$ concentration regime for $Ca^{2+}$ oscillations (see fig. 2.4). For a complete comparison it is vital to compare results from the stochastic implementation of the four state model with the full model. Overall the results are in reasonable agreement with results obtained by the stochastic implementation of the full model. The 6-state model predicts a mean puff lifetime of 157 ms and a mean puff amplitudes of 144 nM. The four state reduced model predicts a mean puff lifetime of 135 ms and a mean puff amplitude of 134 nM. An important difference between the distributions of fig 2.7 and fig 2.8 is a higher percentage of small duration small amplitude events. This feature explains the difference observed in the mean puff lifetimes and amplitudes. $IP_3$ binding and unbinding is a fast equilibrating process, assumed instantaneous on the time scale of $Ca^{2+}$ binding to the activating site. Therefore $IP_3$ dissociates faster than $Ca^{2+}$ binds to activate thereby leading to a higher proportion of small duration/amplitude events failing to elicit a larger puff. For both models, puff lifetimes peak at 100 ms while puff amplitudes peak at $\sim 180-190$ nM. The results agree well with experiments and the full model.
2.5.2 Local $Ca^{2+}$ Concentrations and Maximum Cluster Flux.

Detailed computational studies for a single open channel predict extremely high $Ca^{2+}$ concentrations at channel mouth upon release [65], [60]. At the mouth of the channel predicted concentrations are about 100 $\mu$M, decreasing to fractions of 1 $\mu$M at a distance of about a micron from the channel mouth [44], [56]. Steep gradients exist across channel mouth. In our 6-state model at $c_{ER} = 11 \mu M$ and a $v_{channel}$ value of 5689 s$^{-1}$, puffs generated have statistically similar properties as observed in experiments. The cluster is modeled as a point source with individual channels being indistinguishable. Cluster site (to be distinguished from a single channel) concentrations for the results reported in the earlier section are in the range of a few micro mols.

In view of the analysis of the decay of the $Ca^{2+}$ profile around the length scale of a cluster [44] a few tens of microMolars ($\sim$10-20 $\mu$M) is a reasonable estimate for cluster site $Ca^{2+}$ concentrations. In a recent modeling study it was shown that for a distributed source, averaged $Ca^{2+}$ concentrations on the spatial scale of a cluster, are of the order of a few microMolars, in the presence of high $Ca^{2+}$ concentrations ($\sim$ 100 $\mu$M) at channel mouth [45].

Figure 2.9 shows the puff lifetime distributions obtained when the 6-state model is simulated with a realistic value of lumenal calcium ($c_{ER} \sim 500 \mu M$) with $v_{channel} = 2500$ s$^{-1}$. Cluster site $Ca^{2+}$ concentrations are in the 7-15 $\mu$M regime. But at these high values of $v_{channel}$, for $c_{ER} \sim 500 \mu M$, the mean puff lifetime is 31 ms with a mean puff amplitude of 1.25 $\mu M$. The lifetime distribution peaks at 20-30 ms (see fig 2.9) whereas the amplitude distribution peaks in the range of 1.5-1.6 $\mu M$. These values are inconsistent with experimental measures of puff lifetimes and amplitudes. The right panel in fig 2.9 shows the puff lifetime distribution for all release events with $\geq 2$ channels conducted. When all two channel events are also neglected the mean puff lifetime drops to $\sim 27$ ms.
Figure 2.9: Puff lifetime distribution as obtained by simulating the 6-state model at $c_{ER} \sim 500 \, \mu M$ and $v_{\text{channel}} = 2500 \, s^{-1}$. The distribution in the left panel records all events with the exception of single channel events. The panel on the right is a distribution of all release events with greater than 2 channels open. All parameters are as reported in tables 2.1 and 2.2. Mean puff lifetimes are in the 30 ms range.

and mean puff amplitude is $\sim 1.56 \, \mu M$. In conclusion, when simulated with a realistic value of $c_{er}$, high $v_{\text{channel}}$ values (of the order of a few thousand) lead to puff lifetime and amplitude distributions inconsistent with experimental measures. For a realistic value of lumenal calcium, $c_{ER} \sim 500 \, \mu M$, a smaller value of $v_{\text{channel}}$ is better suited to match simulated results with experimental measures of puff frequency, lifetime and amplitude distributions. Figure 2.10 shows puff lifetime distributions obtained by simulations of the six and four state models with $c_{er} \sim 500 \, \mu M$ and a low $v_{\text{channel}}$ value of 300 $s^{-1}$. Cluster site $Ca^{2+}$ concentrations are in the 2-3 $\mu M$ range. Puff lifetimes peak at 100 ms with puff amplitudes ranging from 20 $nM$-580 $nM$ (measured from basal level $Ca^{2+}$). A similar observation was also made in a previous study [68].
2.6 Discussion

Elementary calcium release events like blips and puffs are signatures of local $Ca^{2+}$ signaling and act as the building blocks of intracellular $Ca^{2+}$ signaling [5], [7], [4]. The basic characteristics of these release events convey important information about the gating and spatial extend of the $IP_3$ receptors.

In this chapter I have presented results from a simple, sequential 6-state binding model for $Ca^{2+}$ puffs. The model is computationally inexpensive and succeeds in generating statistical measures of puff properties as observed in experimental studies. The 6-state model is a derivative of the traditional De-Young Kiezer model. The model is derived after imposing two conditions (i) sequential binding: $Ca^{2+}$ binds to the activating site once $IP_3$ binds to the receptor and (ii) increased inhibition. At parameter values of $v_{\text{channel}}$ and $c_{ER}$ for which the 6-state model agrees well with experimental observations,
the DYK model predicts puff lifetimes and amplitudes which are about an order of magnitude large. The simple sequential binding model is computationally inexpensive and can be used to predict whole cell signaling behaviour.

In simulations of the 6-state model the \( \text{Ca}^{2+} \) concentration across the spatial extend of the cluster is taken to be homogenous. Results of the simulations are consistent with different grid sizes (100 \( nm \), 50 \( nm \) and 25 \( nm \)) with no significant statistical differences observed in the distributions. To facilitate computational simplicity individual buffers were not modeled, instead an effective diffusion coefficient for \( \text{Ca}^{2+} \) is used \([69], [70]\) in conjunction with the point source approximation. Our results show, for the set of parameters presented in this chapter, the model generates puff lifetimes and amplitudes in fair agreement with measurements from experiments. In a later chapter (chapter 3) I study the system in presence of mobile and immobile buffers and compare results obtained with results presented in this chapter.

To be able to simulate \( \text{Ca}^{2+} \) signaling on a larger spatial scale, i.e modeling of multi-cluster dynamics, based on accurate elemental event properties, 2D simulations of simple rate models like the 6-state and it’s four state reduction are a viable option. Though in the use of the point cluster and an effective diffusion coefficient, details around single channel nano-domains are lost, accurate predictions are obtained over single cluster micro-domains. The approach is practical and computationally viable for simulation of global \( \text{Ca}^{2+} \) signals and therefore important.
3 THE ROLE OF BUFFERS IN INTRACELLULAR CALCIUM SIGNALING.

3.1 Introduction

The intricate calcium signaling processes, within a cell, take place in strongly buffered environments. Buffers are large proteins which bind to calcium within cells. Buffer concentrations within a cell are expected to be hundreds of μM’s [72]. Up to 99% of calcium in cells (cytosolic or in stores) may be buffered [3]. Zhou and Neher [72] have estimated that only about 1-5% of calcium ions in the cytoplasm are free. Two distinct kinds of buffers are present within cells: stationary and mobile buffers. Buffers are also characterized by their binding rates as slow or fast. Upon release, if calcium binds to the stationary buffer, a pool of non transportable calcium is created. This is in contrast to the transportable pool of calcium that binds to mobile buffers. It has been estimated that about 25% of all buffer in the cytoplasm is mobile [72]. The presence of buffers alters free calcium concentrations in the vicinity of the channel or a cluster, influencing the dynamics of the IP₃ receptor. Buffer concentrations, binding kinetics and respective diffusive properties play a significant role in the spatial and temporal aspects of calcium release events.

Diffusion coefficient of free Ca²⁺ in the cytosol is in the range of 225-300 μm²s⁻¹ [3]. Fast buffering time scales allow for the simplification of the full model (see equations 3.1) to a single calcium transport equation with an effective diffusion coefficient for calcium as proved by Wagner and Keizer [69]. Experiments measure effective Ca²⁺ diffusion coefficient to be 27 μm²s⁻¹ [70]. Results presented in chapters 2 and 3, incorporate rapid buffering approximation in simulating a single transport equation of free calcium, with an effective diffusion coefficient of 25-30 μm²s⁻¹.
The emphasis of this chapter is to examine the 6 state model in the presence of both stationary and mobile buffers. My main aim is to compare the reduced system (as presented in chapters 2 and 4) to a system in the presence of buffers. The term ”reduced system” refers to employing a single transport equation for $Ca^{2+}$ without modeling buffers in the medium explicitly. Such a direct comparison will help us examine the limits within which use of an effective diffusion coefficient (the rapid buffering approximation) holds for elemental calcium release events: $Ca^{2+}$ puffs. Previously published modeling studies in literature [46], [68], [58] use the point source model in conjunction with an effective diffusion coefficient, but the role of buffer concentrations, binding rates and diffusion coefficients has not been explored in detail. I set out to explore the range of buffer parameters for which a single calcium transport equation with a small effective diffusion coefficient can be used.

3.2 The System in the Presence of Buffers

In the simplest form the following equation describes the buffering of $Ca^{2+}$.

$$Ca^{2+} + b_i \rightleftharpoons Cab_i$$

where, $Cab_i$ represents calcium bound to buffer $b_i$. Subscript $i$ represents the various mobile and immobile buffers considered. Incorporating mass action kinetics and diffusion,
in the presence of buffers, the various transport equations defining the system are given by,

\[
\frac{dc}{dt} = D_c \nabla^2 c + \delta(\vec{x} - \vec{x}_0)J_{\text{cluster}} - J_{\text{pump}} + J_{\text{leak}} + \sum_i (-k_{i}^{\text{on}}(B_i - [cb_i])c + k_{i}^{\text{off}}[cb_i])
\]

\[
\frac{d[cb_s]}{dt} = k_{s}^{\text{on}} c(B_s - [cb_s]) - k_{s}^{\text{off}}[cb_s]
\]

\[
\frac{d[cb_m]}{dt} = D_m \nabla^2 [cb_m] + k_{m}^{\text{on}} c(B_m - [cb_m]) - k_{m}^{\text{off}}[cb_m]
\]

\[
\frac{d[cb_d]}{dt} = D_d \nabla^2 [cb_d] + k_{d}^{\text{on}} c(B_d - [cb_d]) - k_{d}^{\text{off}}[cb_d]
\]

\[
J_{\text{cluster}} = N_0(\pi r_c^2)v_{\text{channel}}(c_{ER} - c)
\]

\[
J_{\text{pump}} = \frac{c^2}{v_{\text{pump}}k_{\text{pump}}^2 + c^2}
\]

\[
J_{\text{leak}} = v_{\text{leak}}(c_{ER} - c).
\]  

(3.1)

In the equations above, the total amount of buffer, \(B_i\), is conserved implying \(b_i = B_i - [cb_i]\), where subscript \(i\) refers to the type of buffer. \(Ca^{2+}\) concentration is denoted by \(c\). As previously mentioned different types of buffers are present in the cellular medium. Troponin C and Sacrolemmal phospholipids are examples of stationary buffers while EGTA, BAPTA, Calmodulin and various fluorescence dyes used in experiments are examples of mobile buffers. It is the \(Ca^{2+}\)-dye complex that is imaged during experiments, with the buffer on and off rates used to determine the underlying free \(Ca^{2+}\) concentration [64]. In the equations [ eqn3.1] above, subscript \(m\) refers to the mobile buffer, subscript \(d\) is for the dye and subscript \(s\) denotes the stationary buffer.

In the literature several modeling studies have been presented which study the impact of buffers on the functional aspects of a single \(IP_3\) channel [50], [53]. The role buffers play in inter cluster coupling and calcium oscillations has also been studied [71], [13]. In this chapter the focus is to study the role of buffers and their influence on cluster release of \(Ca^{2+}\). I study in detail, the impact the presence of buffers has on the statistical properties of puffs.
3.3 Methods

A single cluster of 20 IP₃R's is simulated at the center of a membrane patch of 5µm × 5µm. The patch is discretized with a grid size of 50 nm. The cluster is placed on the center of the grid and all channels are exposed to a homogenous calcium concentration. Receptor kinetics are modeled based on the 6 state model. Schematic of receptor dynamics has been presented in chapter 2, figure 2.1. Binding and disassociation rates for the 6 state model are as given in table 4.1. I simulate the system as described by equations 3.1 with a time step of 2.5 µs. All buffers are assumed to be homogeneously spread, in equilibrium, across the membrane patch, i.e

\[ [Cab_i] = \frac{k^\text{on}_i [Ca] B_i}{k^\text{on}_i [Ca] + k^\text{off}_i} \]  

(3.2)

where, \( i \) denotes the species of buffer, either stationary, EGTA, BAPTA or Fluo-4 dye. All parameters used in the simulations are presented in table 3.1 unless specifically mentioned. Values correspond to buffer kinetics parameters used widely in other computational studies [44], [56]. It is assumed that diffusion coefficients of the buffer and the respective \( Ca^{2+} \)-buffer complex are equal.

3.4 Results

Figure 3.1 presents, averaged \( Ca^{2+} \) profiles and a trace of the fraction of open channels for a single cluster, simulated with an effective \( Ca^{2+} \) diffusion coefficient of 25 µm² s⁻¹, using a single transport equation for \( Ca^{2+} \). The puff lifetime and amplitude distributions are presented in Figure 3.2. As reported in chapter 2, the puff lifetime distribution peaks at 100 ms in agreement with experimental data. All single channel events were neglected. Puff lifetimes range from 22.5 ms to 807.5 ms. The mean of puff lifetime increases to 148.74 ms from 135.42 ms once all two channel events are also eliminated. The mean puff amplitude increases to 676.9 nM from 573.6 nM after the
Table 3.1: Numerical parameters used to solve the full reaction diffusion system (eqn 3.1). All buffer parameters taken from references [44] and [56].

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
<th>Description</th>
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<td>$v_{\text{cluster}}$</td>
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<td>maximum single channel flux</td>
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<td>$v_{\text{leak}}$</td>
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<td>$[Ca^{2+}]$ leak flux constant</td>
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<td>$v_{\text{pump}}$</td>
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</table>
exclusion of two channel events. Of all events recorded up to 50% are single channel events and 8.73% are two channel events.

Both, the stationary and dye buffers are fast buffers with a $K_d$ value of 2 $\mu M$. $K_d$ is defined as the ratio of the dissociation rate to the association rate, i.e $k_{off}^{i} / k_{on}^{i}$. EGTA is a slow mobile buffer; $K_d = 0.15 \mu M$ with $k_{on}^{i} = 5 \mu M^{-1} s^{-1}$. Dissociation of $Ca^{2+}$ once buffered by EGTA is a slow process. BAPTA has a $K_d$ value of 0.2 $\mu M$ though it is a fast binding buffer with a $k_{on}^{i}$ value of 500 $\mu M^{-1} s^{-1}$, about a hundred times more than the binding rate of EGTA. Figure 3.3 top panel shows $Ca^{2+}$ profiles as generated by the full model in the presence of a stationary buffer, a dye (Flou-4 dextran) and EGTA. Calcium is averaged over an area of 1 $\mu m^2$. The bottom panel of figure 3.3 shows the underlying fraction of open channels. A visual inspection, between figures 3.1 and 3.3, tells us that the puff amplitudes and underlying open fractions are in good agreement with profiles generated by the reduced system.

![Figure 3.1](image.png)

Figure 3.1: Averaged $Ca^{2+}$ profiles as generated by the reduced model (top panel). The corresponding underlying fraction of open channel (bottom panel). The effective diffusion coefficient for $Ca^{2+}$ is 25 $\mu m^2 s^{-1}$. 
Figure 3.2: Puff lifetime and amplitude distributions obtained for the simplified system. Effective diffusion coefficient, $D = 25 \mu m^2 s^{-1}$. $v_{channel}$ is $200 s^{-1}$. For distributions in the top panel all single channel release events are neglected ($gt1$). For the distributions in the bottom panel, all events with greater than two channels open, were considered. There is a significant drop in events below 100 $nM$ and 50 $ms$ once all two channel events are removed.

About a thousand puffs were generated sequentially and amplitudes and lifetimes are measured as described in chapter 2. Distributions obtained from the buffered system are shown in figure 3.4.

Remarkably, in the presence of buffers, puff lifetime distributions peak at 100 $ms$ in excellent agreement with the reduced model. Compare top left and bottom left panels of figure 3.2 with figure 3.4. Puff amplitudes were measured from 100 $nM$ up to 1.4 $\mu M$. When all single channel events are neglected the mean puff lifetime was 125.76 $ms$ with
Figure 3.3: Averaged Ca$^{2+}$ profiles as generated by the full model (top panel). Basal Ca$^{2+}$ level is at 25 nM. Underlying fractions of open channels (bottom panel). The system is simulated with stationary and mobile buffers. Parameters used are as reported in table 3.1

lifetimes ranging from 10 ms to 584.74 ms. The mean puff amplitude was 703.73 nM. When all two channel events are also neglected, the mean puff lifetime increased to 130.72 ms and the mean puff amplitude increased to 755.31 nM. Of all recorded events, 33% were single channel events and 6% of the events were two channel events. Two channel events make up a significant fraction of the 25-50 ms duration events with amplitudes in the 100-300 nM range.

In the results presented in figure 3.2, $v_{\text{channel}} = 200 \text{ s}^{-1}$. Addition of different buffers leads to a higher value of $v_{\text{channel}}$. At low $v_{\text{channel}}$ values, the presence of buffers quenches the free Ca$^{2+}$ at the cluster site. The buffered system is simulated with a $v_{\text{channel}}$ value of 4625 s$^{-1}$. Increasing $v_{\text{channel}}$ increases the maximum channel flux and restores release events in the system. Simulating the reduced system with increased $v_{\text{channel}}$ values leads to puffs in the 30 ms and 1.5-2.0 $\mu$M regime, characteristics inconsistent with experimental results. The parameter $v_{\text{channel}}$ is not experimentally measurable. Detailed theoretical
Figure 3.4: Puff lifetime and amplitude distributions as obtained by the full system in the presence of a stationary buffer, Fluo-4 dye and EGTA. All single channel events are neglected for the distributions marked by \(gt1\) (top left and top right). For the distributions marked by \(gt2\) events recorded have more than two channel open (bottom left and bottom right). Puff lifetimes peak at a 100 ms. Puff amplitudes spread from 100 nM to 1.4 \(\mu\)M.

studies though have estimated high values of \(v_{channel}\) running up to a few hundred thousand [50], [65]. In the results presented above it is clear that a reduced system simulated with a lower value of \(v_{channel}\) and a buffered system with a higher value of \(v_{channel}\), both give \(Ca^{2+}\) puffs lifetimes and amplitudes in the experimentally measurable range. Puff lifetime distributions, for both the reduced and buffered systems, are in excellent agreement. In the buffered system fractions of single and two channel events observed are significantly less. Short amplitude and short duration events are a marker of poor channel synchronization within the cluster [61]. The peak corresponding to two channel events is markedly
reduced in the buffered system. Compare amplitude distributions from figure 3.2 to the amplitude distributions in figure 3.4.

### 3.4.1 Response to EGTA and BAPTA.

Binding kinetics of buffers have a significant impact on $Ca^{2+}$ release events from a cluster. EGTA and BAPTA are both mobile buffers with different binding kinetics. EGTA has slow binding kinetics and BAPTA is a fast binding buffer. $K_d$ values for both are well under 1$\mu$M (see table 3.1). The order of magnitude difference in the binding kinetics of BAPTA and EGTA has an impact on puff profiles. In this section I investigate the response of a single cluster of $IP_3$ receptors to the presence of EGTA and BAPTA.

Figures 3.5 and 3.6 show the time trace of different complex species present in a the buffered system. Concentrations of the different buffers used are as given in table 3.1.

Figure 3.5 presents traces when the system is simulated in the presence of 100 $\mu$M EGTA. Puff statistics obtained from this system are presented in figure 3.4 above. Averaged $Ca^{2+}$ profiles in fig 3.5(F) show puffs in the $ms$-$nM$ regime. Upon release, the uptake of $Ca^{2+}$ by EGTA ($k_{on}=5 \mu M^{-1}s^{-1}$) is fast in comparison to the dissociation of $Ca^{2+}$ from the $Ca^{2+}$-$EGTA$ complex. fig 3.5(A) presents the time trace of the $Ca^{2+}$-$EGTA$ complex. Cluster site release values are of the order of 8-10 $\mu$M as shown in fig 3.5(B). Association and disassociation of $Ca^{2+}$ to the dye and stationary buffer is a fast process, as shown by the $Ca^{2+}$-buffer profiles of fig 3.5(D) and fig 3.5(E).

Figure 3.6 presents traces of an equivalent system with 100 $\mu$M BAPTA in place of EGTA. fig 3.6(A) represents the time trace of $Ca^{2+}$-BAPTA complex. The uptake of $Ca^{2+}$ is fast and so is the dissociation. First upon release, $Ca^{2+}$ rapidly binds to BAPTA and the stationary buffer depleting the cluster macro domain of released $Ca^{2+}$. $k_{on}$ for BAPTA is
Figure 3.5: Profiles from the full system in the presence of 100 µM slow mobile buffer EGTA. (A) Time trace of Ca²⁺-EGTA complex at cluster site. (B) Trace of Ca²⁺ at cluster site upon release. (C) The underlying fraction of open channels. (D) Time trace of calcium bound to the stationary buffer upon release denoted by Ca²⁺ − stat. (E) Time trace of Ca²⁺ − dye complex at cluster site. (F) Ca²⁺ puffs observed in the system. Free Ca²⁺ is averaged over an area of 1 µm².

two orders of magnitude greater to the on rate for EGTA, a difference one can see in Ca²⁺ uptake, as fig 3.5(A) and fig 3.6(A) are compared with each other.
Figure 3.6: Profiles from the full system in the presence of 100 μM fast mobile buffer BAPTA. (A) Time trace of \(Ca^{2+}\)-BAPTA complex at cluster site. (B) Trace of \(Ca^{2+}\) at cluster site upon release. (C) The underlying fraction of open channels. (D) Time trace of calcium bound to the stationary buffer upon release denoted by \(Ca^{2+} - \text{stat}\). (E) Time trace of \(Ca^{2+} - \text{dye}\) complex at cluster site. (F) \(Ca^{2+}\) puffs observed in the system. Free \(Ca^{2+}\) is averaged over an area of 1 μm².

For BAPTA, the averaged free \(Ca^{2+}\) signal is strongly suppressed (see fig 3.6(F)) as opposed to EGTA (see fig 3.5(F)). This difference in forward binding rates is therefore
crucial for the free $Ca^{2+}$ response of the system. The buffers are homogeneously spread on
the membrane patch therefore across spatial scales the fast uptake of $Ca^{2+}$ results in a
quenched $Ca^{2+}$ signal. In both systems, be it the presence of BAPTA or EGTA, both the
stationary and dye buffers have fast dissociation rates. A fast dissociation rate implies that
free $Ca^{2+}$ is released into the cluster macro domain within a few milliseconds leading to
inhibition of the cluster. Underlying open channel fractions have a similar time course for
both the systems. Compare figures 3.5(C) and 3.6(C).

In conclusion, high concentrations of a fast mobile buffer like BAPTA quench the
averaged $Ca^{2+}$ signal from a cluster of IP$_3$ receptors though release site values are well
over 1 $\mu M$. Based on the results presented here one can conclude, in a multi-cluster
system, at high concentrations the presence of BAPTA inhibits cluster cluster interaction.

3.4.2 The Rapid Buffering Approximation.

Based on the different time scales involved in the buffered system, Wagner and
Keizer presented a rapid buffering approximation [69]. A buffered system has different
diffusion and buffering time scales. Wagner and Keizer assume, fast buffering time scales,
rapid equilibrium at all points in space before appreciable diffusion occurs and a constant
total $Ca^{2+}$ concentration, to reduce the full system to a single transport equation for $Ca^{2+}$
[69]. The reduction is valid in the presence of fast buffers and high concentrations of low
affinity mobile buffers ($K_d \geq 5 \mu M$). For the sake of completeness I am comparing my
results to the theoretical study of Wagner and Keizer [69] and Smith et al.[53].

Wagner and Keizer [69] show the effective $Ca^{2+}$ diffusion coefficient to be

$$D_{eff} = \beta(D_e + \sum_j \gamma_j D_{cb_j})$$  \hspace{1cm} (3.3)
where for the above system of equations 3.1,

\[
\beta = \left(1 + \frac{K_d^s B_s^T}{(K_d^s + c)^2}\right) + \sum_j \frac{K_d^j B_j^T}{(K_d^j + c)^2}^{-1}
\]

\[
\gamma_j = \frac{K_d^j B_j^T}{(K_d^j + c)^2}
\]

Subscript \(j\) stands for either EGTA or BAPTA or the dye Fluo-4. \(D_{cbj}\) is the diffusion coefficient of the respective mobile buffer.

For the parameters presented in table 3.1, \(D_{eff}\) for \(Ca^{2+}\) as calculated by equation 3.3 is about 180 \(\mu m^2 s^{-1}\), far greater than the measured experimental value of 27 \(\mu m^2 s^{-1}\) [70]. The lower the affinity of the mobile buffer, the better the effective diffusion coefficient becomes, i.e for \(K_d^m \geq 5 \mu M\) [69]. Both BAPTA and EGTA are high affinity buffers.

Dissociation from the \(Ca^{2+}\) buffer complex is a slow process with \(K_d\) values for both well under 1 \(\mu M\). Moreover at high concentrations of mobile buffers the approximation begins to break down [69],[72].

To obtain an effective diffusion coefficient of 25-30 \(\mu m^2 s^{-1}\), using the fast buffer approximation, the total concentration of the mobile buffer, be it EGTA or BAPTA, has to be low. For the parameter values presented in table 3.2, with the approximation, \(D_{eff}\) for the system is 25 \(\mu m^2 s^{-1}\). Total buffer concentrations were selected to get puff lifetime and amplitude distributions similar to the reduced system (see figure 3.2).

Figure 3.8 shows the puff lifetime and amplitude distributions for low values of EGTA. The corresponding \(Ca^{2+}\) profiles are presented in Fig 3.7(D). The puff lifetime distribution peaks at \(\sim 140 ms\) with a mean puff lifetime of 233.67 \(ms\). Puff lifetimes range from 40.6 \(ms\) to a maximum of 1.71 \(s\). The mean puff amplitude is 412.42 \(nM\). Amplitudes spread from a minimum of 640.\(nM\) to a maximum of 717.53 \(nM\). The puffs
Table 3.2: Set of buffer parameters for which $D_{eff}$ is 25 $\mu m^2 s^{-1}$. $D_{eff}$ (eqn 3.3) calculated with the stationary buffer, the dye and either EGTA or BAPTA. Concentrations of stationary and dye buffers are chosen such as puff characteristics are similar to the reduced system. $v_{channel}$ is 5550 $s^{-1}$.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D$</td>
<td>200.0 $\mu m^2 s^{-1}$</td>
<td>diffusion coefficient for free cytosolic $[Ca^{2+}]$</td>
</tr>
<tr>
<td>$D_d$</td>
<td>15 $\mu m^2 s^{-1}$</td>
<td>diffusion coefficient for $Fluo$ – 4 dye [44], [56]</td>
</tr>
<tr>
<td>$D_{EGTA}$</td>
<td>113.0 $\mu m^2 s^{-1}$</td>
<td>diffusion coefficient for $EGTA$ [53]</td>
</tr>
<tr>
<td>$D_{BAPTA}$</td>
<td>95 $\mu m^2 s^{-1}$</td>
<td>diffusion coefficient for $BAPTA$ [53]</td>
</tr>
<tr>
<td>$B_s$</td>
<td>140 $\mu M$</td>
<td>total concentration stationary buffer</td>
</tr>
<tr>
<td>$k^{on}_s$</td>
<td>50 ($\mu M s)^{-1}$</td>
<td>on rate stationary buffer [44]</td>
</tr>
<tr>
<td>$k^{off}_s$</td>
<td>100 $s^{-1}$</td>
<td>off rate stationary buffer</td>
</tr>
<tr>
<td>$B_d$</td>
<td>140 $\mu M$</td>
<td>total concentration $Fluo$ – 4 dye</td>
</tr>
<tr>
<td>$k^{on}_d$</td>
<td>150.0 ($\mu M s)^{-1}$</td>
<td>on rate $Fluo$ – 4 dye [44],[56]</td>
</tr>
<tr>
<td>$k^{off}_d$</td>
<td>300 $s^{-1}$</td>
<td>off rate $Fluo$ – 4 dye</td>
</tr>
<tr>
<td>$B_{EGTA}$</td>
<td>10.0 $\mu M$</td>
<td>total concentration mobile buffer $EGTA$</td>
</tr>
<tr>
<td>$k^{on}_{EGTA}$</td>
<td>5.0 ($\mu M s)^{-1}$</td>
<td>on rate $EGTA$</td>
</tr>
<tr>
<td>$k^{off}_{EGTA}$</td>
<td>1.6 $s^{-1}$</td>
<td>off rate $EGTA$</td>
</tr>
<tr>
<td>$K_{d(EGTA)}$</td>
<td>0.32 $\mu M$</td>
<td>affinity for $EGTA$ [53]</td>
</tr>
<tr>
<td>$B_{BAPTA}$</td>
<td>6.0 $\mu M$</td>
<td>total concentration mobile buffer $BAPTA$</td>
</tr>
<tr>
<td>$k^{on}_{BAPTA}$</td>
<td>600 ($\mu M s)^{-1}$</td>
<td>on rate $BAPTA$</td>
</tr>
<tr>
<td>$k^{off}_{BAPTA}$</td>
<td>100 $s^{-1}$</td>
<td>off rate $BAPTA$</td>
</tr>
<tr>
<td>$K_{d(BAPTA)}$</td>
<td>0.167 $\mu M$</td>
<td>affinity for $BAPTA$ [53]</td>
</tr>
</tbody>
</table>
Figure 3.7: Time traces of a full buffered system when effective diffusion coefficient is 25 \( \mu m^2 s^{-1} \). The system is simulated with 180 \( \mu M \) dye, 140 \( \mu M \) stationary buffer and 10 \( \mu M \) EGTA or 6 \( \mu M \) BAPTA. (A) Time trace of the \( Ca^{2+} \) Bapta complex. (B) time trace of the \( Ca^{2+} \) Egta complex. (C) \( Ca^{2+} \) release events in the presence of 6 \( \mu M \) BAPTA. (D) \( Ca^{2+} \) release events in the presence of 10 \( \mu M \) EGTA.

recorded are slightly longer than the once presented earlier but not out of experimental range.
Figure 3.8: Puff lifetime (A) and amplitude (B) distributions for low concentrations of EGTA. $D_{eff}$ equals 25 $\mu m^2 s^{-1}$. All parameters as listed in table 3.2.

Figure 3.9 presents the puff lifetimes and amplitude distributions for low values of BAPTA. The mean puff lifetime is 193.38 ms with a maximum of 1.38 s. The mean puff amplitude is 297.8 nM. Amplitudes spread from a minimum of 56.54 nM to a maximum of 604.13 nM. There are two outstanding features of the above result. First, at low concentrations of both the slow and fast buffers $Ca^{2+}$ puffs are retained. This is in contrast to puffs not being observed when BAPTA concentrations are high $\sim$ 100 $\mu M$. Second, when the fast buffer is used, the two channel events are distinctly observed in the lifetime and amplitude distributions (see fig 3.9) unlike distributions recorded in the presence of the slow buffer EGTA (see fig 3.8 and fig 3.4).

Fast buffer depletes the $Ca^{2+}$ macro domain faster than a slow buffer. Presence of the two channel events can be accounted by this fact. $Ca^{2+}$ is a vital agonist required for the activation of the channels. Neighbouring channels within the cluster are recruited using CICR. The fast buffer acts on a time scale faster than the slow buffer in binding released calcium, leading to the possibility that enough activating $Ca^{2+}$ is not bound to the receptors. If fewer channels in the cluster have been activated by $Ca^{2+}$, a higher
proportion of single and two channel events will be observed, as is the case in the presence of BAPTA. In the presence of EGTA, since depletion of \( Ca^{2+} \) from the macro domain is a slow process, one does not observe a distinct two channel events peak. In other words, the channels are better synchronised by the slow buffer.

![Graphs](image)

Figure 3.9: Puff lifetime (A) and amplitude (B) distributions for low concentrations of BAPTA. \( D_{eff} \) equals 25 \( \mu m^2 s^{-1} \). All parameters as listed in table 3.2

Low fast buffer concentrations also yield puffs. The reason for this lies is the buffering capacity of the buffer which is not only a function of the kinetic rates for the buffer but also the total concentration of the buffer present in the medium. To test this, a trial simulation was run. For a period of 5 \text{ ms}, 10 channels conduct from within the cluster. The subsequent response of the system is recorded for two different concentrations, 10 \( \mu M \) and 100 \( \mu M \), of both EGTA and BAPTA. All other buffer concentrations are as reported in table 3.2. Spatial profiles for the \( Ca^{2+} \)-buffer complex were recorded. Steeper gradients are established in the case of the fast buffer. For the slow buffer the gradient is not so steep as shown in figure 3.10. The profiles presented in figure 3.10 are normalised to show the effect of buffer concentrations on the spatial gradient of the \( Ca^{2+} \)-buffer complex upon the release of \( Ca^{2+} \). At release site the peak value of the
$Ca^{2+}$-EGTA complex was $\sim 1.5 \mu M$ for 10$\mu M$ EGTA and about 12 $\mu M$ for 100 $\mu M$ EGTA. When the two profiles were normalized, the resulting spatial gradient is similar. Buffers were spread homogeneously in space, in equilibrium (see eqn.3.2). The basal equilibrium levels are a function of the total buffer concentration, hence different in the two cases. When normalised, in the case of EGTA, it is clear that the slow dynamics ensure a very small fraction of $Ca^{2+}$ is buffered for both concentration (fig 3.10, bottom panel). As spatial gradients are not significantly different, for the slow buffer, therefore high concentrations do not quench averaged $Ca^{2+}$ signals.

For the fast buffer, steeper spatial gradients exist. Across the spatial extent of the membrane, increase in the fast buffer concentration leads to more $Ca^{2+}$ being bound to the buffer. This is clear in fig 3.10 (top panel), wherein the blue line refers to the $Ca^{2+}$ buffer complex for 100 $\mu M$ of BAPTA. In terms of absolute concentrations a higher amount (about 5 times) of $Ca^{2+}$ was buffered in the presence of high concentrations of the fast buffer, in stark contrast to the slow buffer case. High concentrations of fast buffer have a high buffering capacity and therefore underlying $Ca^{2+}$ signals are strongly suppressed.

### 3.4.3 On the Significance of $K_d$ values.

BAPTA and EGTA have similar $K_d$ values. The rates and concentrations effect puff profiles as discussed in the section above. In this section I examine the effect of $K_d$ values on the profile of a puff. A trial code was run in the presence of only a dye buffer present with two different values of $K_d$. For a $K_d$ value of 2 $\mu M$, $k_{on} = 150.0(\mu M s)^{-1}$ and $k_{off} = 300.0s^{-1}$. The rates were reversed to obtain a $K_d$ value of 0.5 $\mu M$. All parameter are as presented in table 3.1.

A single puff is isolated from the simulation to study the impact of $K_d$ values on puff profile. In figure 3.11 (top panel) the averaged $Ca^{2+}$ profile is presented for the two $K_d$
Figure 3.10: Normalized spatial profiles of $Ca^{2+}$ Bapta complex (top panel) and $Ca^{2+}$ – Egta complex (bottom panel). The total concentration together with the binding rates determines buffering capacity of the buffer. High concentrations of the fast buffer have a depleting affect on the $Ca^{2+}$ macro domain of a cluster (left panel).

values. A higher $K_d$ value implies that less $Ca^{2+}$ is picked up by the buffer hence a greater amplitude is measured for the underlying signal. A lower $K_d$ value is representative of a
Figure 3.11: Figure showing the impact of different $K_d$ values on a representative puff. The top panel shows averaged calcium values for two different $K_d$ values. A low affinity buffer leads to a higher puff amplitude in contrast to a higher affinity buffer. When normalized the equivalence of puff life times in both the cases is clear, though decay profiles are influenced by the respective $K_d$ values (lower panel).
high affinity buffer. This leads to a reduced amplitude of the averaged $Ca^{2+}$ signal. It is interesting to note that the lifetime, full duration at half maximum (FDHM), for both the puffs in figure 3.11 (top panel) is same at 60 $ms$. Figure 3.11 (lower panel) shows the normalised profile for the puffs presented in the top panel. Once normalised, it is clear that the FDHM is the same for both puffs, though the decay characteristics are not. In the presence of a high affinity buffer, the decay is marginally faster with a lower $Ca^{2+}$ value. A higher $K_d$ value ensures a faster dissociation and therefore more $Ca^{2+}$ is present during the decay phase of the puff. It can be concluded that statistically significant puff properties like puff lifetimes are not affected by a change in $K_d$ values.
3.5 Discussion.

Internal cellular environments are heavily buffered. Buffers therefore play an important role in intracellular calcium signaling. It was shown in chapter 2 that statistically accurate distributions of $Ca^{2+}$ puffs can be generated by solving a single transport equation for $Ca^{2+}$ with an effective diffusion coefficient as proposed in [69]. The aim of this chapter was to explore the robustness of the reduced approach used in chapters 2 and 4.

Following different experimental and modeling studies ([44], [56],[10]) a full system with a dye, a stationary and a mobile buffer present was simulated. In the presence of all three, at varying buffer concentrations, puffs in the $ms – nM$ regime were reproduced. Buffer binding kinetics play an important role. For both low and high concentrations of the slow buffer EGTA, puffs with accurate statistics are observed. At low concentrations of the fast buffer, puffs are observed. A high fast buffer concentration suppresses the underlying $Ca^{2+}$ signal. In a multi-cluster environment therefore large quantities of fast buffer would decouple the clusters as $Ca^{2+}$, an important agonist in recruiting neighboring clusters, would be suppressed.

Puff amplitudes in particular are determined by total buffer concentrations, buffer affinity and $v_{\text{channel}}$ values. A distinct and clear variation is observed in the spread of puff amplitudes, see fig 3.11(top panel). A higher concentration of buffer binds more $Ca^{2+}$ modulating the underlying free $Ca^{2+}$ signal amplitude. A low affinity buffer also leads to higher amplitudes as opposed to a high affinity buffer. A higher $Ca^{2+}$ flux, i.e. $v_{\text{channel}}$ is also a control parameter for puff amplitudes and remains an experimental unknown. Puff lifetime statistics are more robust. Across the parameter range of buffer concentrations, I was able to reproduce puffs in the 100 – 200$ms$ range. Affinity of a buffer does not have an impact on puff lifetime as $K_d$ values impact decay and spatial spread but not the full duration at half maximum.
Summarising, in the present chapter I have shown that the use of a simple single transport equation to study the properties of elementary $Ca^{2+}$ release events and the underlying $IP_3$ cluster behaviour is accurate and efficient. A buffered system was simulated and results compared with the reduced system. First, the simple model drastically reduces computation time without compromising on essential details of puff behaviour. Second, equipped with an efficient model, with basic puff properties explained, we can employ it to study large multi-cluster behaviour and study the basics of $Ca^{2+}$ signalling (eg. $Ca^{2+}$ waves) on a larger spatial scale.
4 THE AICT MODELS

4.1 Introduction

The $IP_3$ receptor plays a crucial role in intracellular calcium signalling. In light of its significance it is vital to experimentally characterize the properties of a single channel. To characterize the working of a channel in physiological environments, single channel open probabilities and open-times has been studied in great detail. The two most widely used experimental protocols for the functional analysis of a single $IP_3$ channel are i) reconstitution of the channel in planar lipid bilayers [6], [66], [67], [27] and ii) single channel recordings in native cellular environments through nuclear patch clamp recordings [35], [42]. Results obtained by the two different protocols differ significantly. In the experiments, $IP_3$ is typically clamped and response of the receptor to varying $Ca^{2+}$ concentrations is recorded. In lipid bilayer experiments, Bezprozvanny et al. [6] record a maximum open probability of 15% for a single $IP_3$ channel. Tu et al. [67] record single channel open probabilities in the 30% - 40% regime for the type-1 and type-2 $IP_3$ isoforms and as low as 4% for the type-3 $IP_3$ isoform (recordings from mammalian $IP_3$ receptors). Kaftan et al. [27] record open probabilities in the range of 1-4% for a single receptor (recordings from mammalian receptors). In nuclear patch clamp recordings Mak et al. [35] record single channel open probabilities to peak at around 80% for type-1 $IP_3$ receptor (receptors from xenopus oocyte). In both the lipid bilayer and nuclear patch clamp experiments, the mean open-time of an $IP_3$ channel has been reported in the 7-10 $ms$ regime [66], [42], [35].

The results obtained from the lipid bilayer experiments are in marked contrast with results obtained from nuclear patch clamp recordings. In lipid bilayer experiments, the open probability of a receptor has a bell shaped response to gradually increasing calcium concentrations. The bell shaped open probability curve indicates biphasic regulation of
the receptor by calcium. At lower concentrations of calcium the channel is activated whereas higher calcium concentrations inhibit the channel. In nuclear patch clamp experiments, bell shaped dependence of open probability on calcium is a feature observed with lower $IP_3$ concentrations ($IP_3$ few tens of nM). At higher $IP_3$ concentrations (in the $\mu M$ regime) the bell shaped dependence flattens out at the peak leading to a broader curve [35]. Flattening of the open probability curve is indicative of a low affinity $Ca^{2+}$ inhibitory site, a feature not observed in lipid bilayer studies.

Mathematical modeling is an important tool used in the study of calcium signaling. Based on the experiments of Bezprozvanny et al. [6], the very first mathematical model of the $IP_3$ receptor was developed by De-Young and Keizer [12]. The DYK model and its variants have been used extensively in the literature to investigate the role of $IP_3$ receptors in calcium release [32], [14], [55]. In the standard DYK model a single $IP_3$ receptor subunit consists of three binding sites. The $IP_3$ binding site, the activating $Ca^{2+}$ binding site and the inhibitory $Ca^{2+}$ binding site. Based on the three binding sites, a single receptor subunit can exist in eight states. The subunit conducts when both $IP_3$ and activating $Ca^{2+}$ are bound to it. A single channel consists of four such equal and independent subunits and conducts when either three or all four subunits have $IP_3$ and activating $Ca^{2+}$ bound. The low open probability response of the standard DYK model [12] is in excellent agreement with measurements from Bezprozvanny’s lipid bilayer experiment [6], but not with those observed in nuclear patch clamp experiments.

Recently, Shuai et al. [49], presented a variant of the standard DYK model to account in particular for the high single channel open probabilities of nuclear patch clamp recordings from Xenopus oocytes [35]. In the new kinetic model an extra subunit ”active state” was introduced. It was proposed that after binding both $IP_3$ and activating $Ca^{2+}$, the subunit undergoes a reversible agonist independent conformational transformation (AICT) to an ”active state” and conducts therein. Transition rates to and from the active
state do not depend on the concentrations of $IP_3$ and $Ca^{2+}$ and were chosen to fit nuclear patch clamp data obtained from Mak et al. [35]. Henceforth in the text the ”active state” shall be referred to as the $AICT$.

Our primary aim, in this chapter, is to study whether $IP_3$ receptor models with an ”$AICT$” can reproduce $Ca^{2+}$ signaling patterns on the organizational scale of a cluster. We do so by focusing on the role of the $AICT$ in collective $Ca^{2+}$ release from a cluster of $IP_3$ receptors. First we model the response of a single cluster of 20 $IP_3$ channels using both, the DYK and the 6-state kinetic scheme for the receptor subunit. In the next step we introduce the agonist-independent conformational transformation to the receptor subunit and study the role of the $AICT$ in $IP_3$ cluster behavior.

4.2 The Two Models

Figure 4.1: Schematic illustrating the receptor kinetics of the De-Young Keizer (left) and the 6-state model (right). The original receptor kinetics as presented in the literature [12], [58] do not have the AICT present.

The kinetic schemes of the DYK and the 6-state $IP_3$ receptor models are described in detail in chapter 2. Figure 4.1 shows the schematic of the DYK and the 6-State model with
the AICT introduced. After binding both $IP_3$ and $Ca^{2+}$ the subunit, $x_{110}$, undergoes a conformational change to the AICT. The transition rates for both the models are given in table 4.1.

Table 4.1: Kinetics of the DeYoung-Keizer and 6-state models.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DYK</th>
<th>6-state</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_1(\mu Ms)^{-1}$</td>
<td>400</td>
<td>81.16</td>
<td>$IP_3$</td>
</tr>
<tr>
<td>$a_2(\mu Ms)^{-1}$</td>
<td>0.2</td>
<td>1.845</td>
<td>$Ca^{2+}$ inhibition</td>
</tr>
<tr>
<td>$a_3(\mu Ms)^{-1}$</td>
<td>400</td>
<td>200</td>
<td>$IP_3$</td>
</tr>
<tr>
<td>$a_4(\mu Ms)^{-1}$</td>
<td>0.2</td>
<td>0.15</td>
<td>$Ca^{2+}$ inhibition</td>
</tr>
<tr>
<td>$a_5(\mu Ms)^{-1}$</td>
<td>20</td>
<td>53.9</td>
<td>$Ca^{2+}$ activation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DYK</th>
<th>6-state</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b_1(s^{-1})$</td>
<td>52</td>
<td>228</td>
<td>$IP_3$</td>
</tr>
<tr>
<td>$b_2(s^{-1})$</td>
<td>0.2098</td>
<td>0.409</td>
<td>$Ca^{2+}$ inhibition</td>
</tr>
<tr>
<td>$b_3(s^{-1})$</td>
<td>377.4</td>
<td>188.5</td>
<td>$IP_3$</td>
</tr>
<tr>
<td>$b_4(s^{-1})$</td>
<td>0.0289</td>
<td>0.096</td>
<td>$Ca^{2+}$ inhibition</td>
</tr>
<tr>
<td>$b_5(s^{-1})$</td>
<td>1.647</td>
<td>4.52</td>
<td>$Ca^{2+}$ activation</td>
</tr>
</tbody>
</table>

4.2.1 The Open Probabilities

Introduction of the AICT changes the steady state open probability ($P_o$) of the receptor. The steady-state open probability of a single channel can be calculated as

$$P_o = q_a^4 + 4q_a^3(1 - q_a).$$  \hspace{1cm} (4.1)
where $q_a$ is the equilibrium probability for the subunit to be in the open state, $x_{110}$ in both models (DYK and 6-state). Equilibrium probability for a state denoted by $(ijk)$ is:

$$q_{a(ijk)} = \frac{w_{a(ijk)}}{Z}$$

where, $w_{a(ijk)}$ is the unnormalized probability to be in the state $(ijk)$ and $Z$ is the normalization factor [49]. The unnormalized probability of state $(ijk)$, $w_{a(ijk)}$ relative to the state $(000)$ is the ratio of forward to backward rates along any path connecting $(000)$ to $(ijk)$. Therefore for the state $x_{(110)}$

$$w_{a_{110}} = \frac{pc}{d_1d_5}; \quad d_i = \frac{b_i}{d_i}, \quad \text{for } i = 1, 2 \ldots 5. \quad (4.2)$$

The normalization factor $Z$ is defined as the sum of all unnormalized probabilities. For the two models,

$$Z_{dyk} = (1 + \frac{c}{d_4})(1 + \frac{c}{d_5}) + \frac{p}{d_1}(1 + \frac{c}{d_2})(1 + \frac{c}{d_5})$$

$$Z_{6st} = 1 + \frac{c}{d_4} + \frac{p}{d_1}(1 + \frac{c}{d_5})(1 + \frac{c}{d_2}). \quad (4.3)$$

In presence of the AICT the expressions for unnormalized probability $w_a$ and normalization factor $Z$ are given by,

$$w_a = \frac{pc}{d_1d_5d_6}; \quad Z_{dyk, ICT} = (1 + \frac{c}{d_4})(1 + \frac{c}{d_5}) + \frac{p}{d_1}(1 + \frac{c}{d_2})(1 + \frac{c}{d_5}) + w_a$$

$$Z_{6st, ICT} = 1 + \frac{c}{d_4} + \frac{p}{d_1}(1 + \frac{c}{d_5})(1 + \frac{c}{d_2}) + w_a. \quad (4.4)$$

Under standard experimental recording conditions, i.e. $2 \mu M IP_3$ and $0.5 mM ATP$, in lipid bilayer experiments Tu et al. [67] measure the open probability of a single receptor to be in the 0.3-0.4 range for type-1 and type-2 receptors. They measure an open probability of about 0.04 for the type-3 receptor. The mean open-time of the channel is between 7 and 10 ms [67]. At $2 \mu M IP_3$, the open probability response of the channel
Figure 4.2: The open probability of the $IP_3$ receptor has a bell shaped response to $Ca^{2+}$. The left panel shows the open probability response of the DYK model at $2\mu M IP_3$. $P_o$ peaks at 0.36 (left panel). Addition of the AICT to the DYK model, with $a_0 = 540 ~s^{-1}$ and $b_0 = 80 ~s^{-1}$, increases peak $P_o$ values to 0.79 as shown in the right panel. Ratio of $a_0$ to $b_0$ is 6.75. $pCa = -\log_{10}(c)$.

Figure 4.3: The open probability $P_o$ response for the 6-state model (left panel) peaks at 0.075 (see inset). For the AICT variant, of the 6-state model, $P_o$ increases to 0.58 (right panel). $a_0 = 540 ~s^{-1}$ and $b_0 = 80 ~s^{-1}$, a ratio ($a_0/b_0$) of 6.75.

predicted by the DYK model, is bell shaped and peaks at 0.36. Peak sensitivity is achieved at a pCa value of $\sim 6.6$ and the mean open-time of a channel equals 6.19 ms. The DYK model is in good agreement with the results of lipid bilayer experiments as shown in fig
4.2 (left panel). The 6-state model predicts lower open probabilities closer to values predicted for the type-3 receptor (see fig 4.3).

Motivated by Shuai et al. [49], we introduced the AICT in both the DYK and the 6-state models and take the forward transition rate \( a_0 \) to be 540 s\(^{-1}\) and the backward transition rate \( b_0 \) to be 80 s\(^{-1}\). Introduction of the AICT, to the DYK model, with fast transitioning rates, increases the open probability of the receptor from 0.36 to 0.79 (fig 4.2). For the 6-state model, the open probability changes from 0.075 to 0.58 at 2 \( \mu M \) IP\(_3\) (fig 4.3). For a bell shaped response to increasing \( Ca^{2+}\), it is sufficient to introduce the AICT for a high maximum \( P_o \).

From the expressions of open probability above it is clear that in models with the AICT, single channel open probabilities \( P_o \), increase with the ratio of \( a_0 \) to \( b_0 \). In the next section I study the impact of the AICT on cluster release. A single cluster of 20 IP\(_3\)R\(\prime\)s is simulated with the DYK and 6-state channel kinetics and the new AICT introduced versions of the models.

### 4.2.2 Modeling of a Single Cluster

Spatio-temporal dynamics of \( Ca^{2+}\) released into the cytosol, from a cluster of channels, can be modelled by the following set of equations as also described in chapter 2, i.e.

\[
\frac{dc}{dt} = D \nabla^2 c + \delta(\vec{x} - \vec{x}_0) J_{\text{cluster}} + J_{\text{leak}} - J_{\text{pump}}
\]

\[
J_{\text{cluster}} = N_0 (\pi r_c^2) v_{\text{channel}} (c_{\text{ER}} - c)
\]

\[
J_{\text{pump}} = v_{\text{pump}} \frac{c^2}{k_{\text{pump}}^2 + c^2}
\]

\[
J_{\text{leak}} = v_{\text{leak}} (c_{\text{ER}} - c).
\]  

(4.5)
A single cluster of 20 $IP_3R$'s is placed at the center of a $5\mu m \times 5\mu m$ membrane patch with an effective $Ca^{2+}$ diffusion coefficient of $25 \mu m^2 s^{-1}$. The reaction diffusion equation was discretized with a four point scheme for the Laplacian with a grid size of $\delta x = 100 nm$ and a time step of $25 \mu s$ using a fully explicit solver. The results presented in this chapter were also tested at a smaller grid size of $\delta x = 50 nm$. All simulations are at a constant $IP_3$ concentration of $2 \mu M$. Parameter values are reported in table 4.2.

### Table 4.2: Model Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$v_{channel}$</td>
<td>1000$s^{-1}$</td>
<td>maximum $Ca^{2+}$ flux from a channel</td>
</tr>
<tr>
<td>$v_{leak}$</td>
<td>0.0613$s^{-1}$</td>
<td>$Ca^{2+}$ leak flux constant</td>
</tr>
<tr>
<td>$v_{pump}$</td>
<td>35$\mu M s^{-1}$</td>
<td>Maximum $Ca^{2+}$ uptake</td>
</tr>
<tr>
<td>$k_3$</td>
<td>0.02$\mu M$</td>
<td>Activation constant for $Ca^{2+}$ pump</td>
</tr>
<tr>
<td>$c_{ER}$</td>
<td>500$\mu M$</td>
<td>Lumenal $Ca^{2+}$ concentration</td>
</tr>
<tr>
<td>$D$</td>
<td>25$\mu m^2 s^{-1}$</td>
<td>Effective $Ca^{2+}$ diffusion coefficient</td>
</tr>
<tr>
<td>$p$</td>
<td>2$\mu M$</td>
<td>$IP_3$ concentration</td>
</tr>
</tbody>
</table>

### 4.3 Results

#### 4.3.1 $Ca^{2+}$ Puffs: The Standard Models

The standard DYK and 6-state model (with no AICT) have been used to model receptor kinetics. Table 4.2 lists the parameter values for the simulations. Binding affinities for both the models are given in table 4.1.

Figure 4.4 shows the averaged cytosolic $Ca^{2+}$ profiles obtained from the DYK (top panel) and the 6-state models (bottom panel). The plots show the profile of $Ca^{2+}$ released
into the cytosol, through a cluster of $IP_3$ receptors, on the ER membrane. As in experiments fluorescence is typically collected from a 1 $\mu m^3$ volume, in our simulation we average calcium over a 1 $\mu m^2$ area for consistency. When two or more channels from within the cluster open, a $Ca^{2+}$ puff is observed. An increased cytosolic $Ca^{2+}$ concentration inhibits the cluster leading to puff termination. Cytosolic $Ca^{2+}$ levels return to basal level and it takes a few seconds before the cluster conducts and fires again to give rise to another puff. A visual comparison of the $Ca^{2+}$ profiles, in the top and bottom panels, tells us that the DYK model predicts longer puff lifetimes as opposed to the 6-state model. The individual binding kinetics of the two models are responsible for the differences in puff profiles (see table 4.2).

Calcium profiles that are generated can be directly linked to channel and subunit behavior within a cluster as channel activity is directly linked to subunit activity. In Figure 4.5 we show the fraction of inhibited and uninhibited subunits. The number of inhibited subunits, is the number of all subunits with $Ca^{2+}$ bound to the inhibitory site, while all other subunits are characterized as uninhibited. Right before a few channels open to fire a puff, a large fraction of subunits are uninhibited. Following the release of $Ca^{2+}$, immediately the number of inhibited subunits increases while the number of uninhibited subunits decreases and puff termination ensues. Puff termination is followed by a recovery phase lasting up to a few seconds. Recovery time of uninhibited subunits is determined by model construct (DYK or 6-state). The resulting synchronized, rhythmic dynamics of subunits results in temporally limited and distinct $Ca^{2+}$ release events.

4.3.2 $Ca^{2+}$ puffs: The AICT models

A single cluster of 20 $IP_3$ receptors is simulated on a membrane patch of $5\mu m \times 5\mu m$. The channel subunits are modeled with the AICT introduced variant of DYK and the
Figure 4.4: Averaged $Ca^{2+}$ profiles obtained from the DYK (top panel) and the 6-state model (bottom panel).

6-state models, respectively (see figure 4.1). The channel conducts when either 3 or all 4 subunits have undergone an agonist independent conformation transition to the active/AICT state. Transition to and from the AICT depend on the forward transition rate $a_0 = 540 \text{ s}^{-1}$ and the backward transition rate $b_0 = 80 \text{ s}^{-1}$. Values of $a_0$ and $b_0$ were motivated by the work of Shuai et al. [49]. As mentioned in an earlier section, $a_0$ and $b_0$ were selected to reproduce the high open probabilities observed in nuclear patch clamp.
Figure 4.5: Subunit profiles corresponding to $Ca^{2+}$ release events. DYK (top panel) and 6-state (bottom panel). Uninhibited fraction increases sharply right before the cluster fires. $Ca^{2+}$ release is immediately followed by an increase in the fraction of inhibited subunits leading to puff termination. Termination is followed by a period of recovery before the cycle repeats itself.

The left panel in figure 4.6 shows the averaged $Ca^{2+}$ profile as generated by the DYK model after the introduction of the AICT. The right panel of figure 4.6 shows $Ca^{2+}$ profile for the 6-state model with the AICT introduced. It is significant that with the introduction
of the AICT no temporally restricted $Ca^{2+}$ release events are observed. As both $Ca^{2+}$ traces suggest, the cluster never inhibits and a fluctuating $Ca^{2+}$ profile is recorded.

The observed $Ca^{2+}$ profile is a direct signature of the underlying opening and closing of channels. Figure 4.7 presents the underlying inhibited (in black) and uninhibited (in red) subunit fractions for the cluster. With the AICT state introduced, there is a clear and distinct change in the fraction of inhibited subunits. In the standard models (Figure 4.4) a puff is preceded by a rise in fraction of uninhibited subunits. $Ca^{2+}$ release is immediately followed by an increase in the inhibited subunit fraction and a corresponding dip in the uninhibited fraction. A recovery phase follows puff termination. This characteristic behavior is not maintained in the presence of the AICT. In presence of the AICT, on an average, more subunits are uninhibited than inhibited (compare figures 4.5 and 4.7). Less inhibition leads to more channels being open at all times.

Introducing the AICT to the subunit structure, shifts the frequency with which all subunit states are accessed. It is this shift in the dwell times that leads to the fluctuating
calcium profiles. In the DYK model, without AICT, dwell time in the open state, i.e. $x_{110}$, of the subunit is 18.57 ms, at standard conditions. With the introduction of the AICT, at $a_0 = 540 \text{ s}^{-1}$, the dwell time for the subunit in the state $x_{110}$ is drastically reduced to 1.68 ms. The forward rate $a_0$ dominates all the other ($a_2c, b_5, b_1$) competing rates out of the $x_{110}$ state. A direct consequence is a decrease in the probability to access the inhibited state (governed by the rate $a_2c$) leading to a lack of inhibition and therefore the loss of inherent synchrony and distinct $Ca^{2+}$ release events.

I conclude that introduction of an additional state with transition rates chosen to fit experimental nuclear patch clamp open probability data fails to generate puffs consistent with experimental measurements.

4.3.3 Variation of Transition Rates $a_0$ and $b_0$

In this section I examine how different choices of rates $a_0$ and $b_0$ effect cluster calcium release. It is intuitive that extremely large values of $a_0$ and $b_0$ will not work. If $a_0$ is very large (in the range of 1000 s$^{-1}$) in comparison with $b_0$, the fast transition rate will ensure that the subunit is always in the AICT state. In reverse, if $b_0$ is much larger (in the
range of 1000 s\(^{-1}\)) than \(a_0\), the fast backward transition rate ensures the subunit is almost never active. A fast forward or backward transition rate effectively decouples the AICT from the rest of the eight or six subunit states (See figure 4.1).

In the AICT models, the open probability \(P_o\) is directly proportional to the ratio of \(a_0\) to \(b_0\) (see equation 4.4). The DYK model, at 2\(\mu\)M IP\(_3\) predicts channel open probability to be 0.36 and mean open-time to be 6.19 ms. For the DYK AICT model, rates \(a_0\) and \(b_0\), can be constrained to a ratio of \(\sim 7\), to obtain a maximum \(P_o\) as measured in nuclear patch clamp experiments (see fig 4.2). The mean open time of a channel, in presence of the AICT, is \(1/(3b_0)\). Constraining the open-time to 6.19 ms, gives \(b_0= 53.85\) s\(^{-1}\). At a ratio of \(a_0=7b_0\), to maintain the high \(P_o\), \(a_0=377.0\) s\(^{-1}\). At these values of \(a_0\) and \(b_0\), a fluctuating calcium profile is recorded and the cluster never inhibits. Details of cluster response are the same as described in the preceding section. Reducing the rates further, increases the dwell time in the subunit state \(x_{110}\), increasing the probabilities to access neighboring states \(x_{010}, x_{111}\) and \(x_{100}\). Rates \(a_2c, b_5, b_1\) and \(a_0\) determine which subunit state will be accessed from \(x_{110}\). For synchrony to be restored \(a_0\) has to be of the order of \(a_2c\) and \(b_5\). At various values of \(b_0\), 40 s\(^{-1}\), 25 s\(^{-1}\), 10 s\(^{-1}\), 5 s\(^{-1}\) and 2.5 s\(^{-1}\), with the ratio of \(a_0\) to \(b_0\) maintained at 7, the cluster does not inhibit. For a \(b_0\) value of 2.5 s\(^{-1}\), the mean open-time increases to 133.3 ms, inconsistent with experimental measurements [67], [35], [42]. A further decrease in \(b_0\) would lead to uncharacteristically long open times for the channel.

Figure 4.8 shows, at a ratio of 2, i.e \(a_0 = 2b_0\), the AICT model predicts \(P_o\) to be 0.34, comparable to the open probability of 0.36 predicted by the DYK model. Therefore, for a \(b_0\) value of 53.85 s\(^{-1}\), at a ratio of 2, \(a_0\) is 107.7 s\(^{-1}\). Once again a fluctuating calcium profile is recorded with the cluster never inhibiting. At various values of \(a_0\), ranging from 80 s\(^{-1}\) to 10 s\(^{-1}\) for a ratio of 2, the cluster does not inhibit. The cluster inhibits, at the reduced transition rate \(a_0 = 5\) s\(^{-1}\) and \(b_0 = 2.5\) s\(^{-1}\), albeit for a few milliseconds. As discussed above, mean open time of a channel is now too high at 133.3 ms.
Figure 4.8: Steady state open probability for the DYK model (blue line) and the DYK AICT model (dotted line). At a ratio of $a_0/b_0$ of 2, peak value of $P_o$ for the DYK AICT model is 0.34, close to the DYK model’s $P_o$ of 0.36.

Figure 4.9: Puffs as generated by the DYK AICT model with rescaled parameters (left panel). $a_0 = 5.0 \text{ s}^{-1}$ and $b_0 = 2.5 \text{ s}^{-1}$. Inhibition was increased by a factor of five and detailed balance imposed. The right panel shows the underlying fraction of uninhibited and inhibited subunits. $P_o$ for a channel is 0.2.

In experiments the inter-puff time interval have also been characterized. Studies [18], [36] have shown the inter-puff interval distribution to peak at 1.5 s. Based on experimental evidence when a cluster inhibits it typically fires after a second or two. In the AICT model, at a lower $P_o$ and at much lower values of $a_0$ and $b_0$, the cluster inhibits, but does
so for only a few milliseconds. To restore inter-puff intervals to the order of a second, inhibition was increased by a factor of five and detailed balance imposed. Resulting puff profiles are shown in Figure 4.9 (left panel). Figure 4.9 (right panel) shows the underlying fractions of uninhibited and inhibited subunit. With an increase in inhibition, $P_o$ decreases from 0.34 to 0.2 as expected.

### 4.3.4 Variation of Maximum Single Channel Flux: $v_{channel}$

Detailed theoretical studies propose that when a channel conducts, $Ca^{2+}$ concentration at channel mouth is of the order of 100-120 $\mu M$ [60], [56], [44]. $Ca^{2+}$ release is followed by a sharp instantaneous decay, from about 100$\mu M$ to under 10 $\mu M$ over the spatial spread of about 100 nm away from the channel [56], [44]. In the present modeling study I do not distinguish one channel from the other within a cluster, therefore single channel calcium profiles are not resolved. The point source model paradigm helps to resolve calcium release through a cluster of IP$_3$ receptors over a micro domain (i.e. cluster site). Upon release, cluster-site peak $Ca^{2+}$ concentrations are in the range of 10-20 $\mu M$. Calcium averages over the spatial extend of puff like events are expected to be much lower. For the set of parameter values reported, cluster site $Ca^{2+}$ release is in the range of 8-12 $\mu M$. Averaged calcium concentrations over a 1 $\mu m^2$ area are well under 5$\mu M$ (See Fig 4.4).

Simulation results presented in this chapter were done at $v_{channel} = 1000$ s$^{-1}$. The parameter $v_{channel}$ quantifies maximum single channel flux. Increasing $v_{channel}$ leads to increased $Ca^{2+}$ release into the cytosol thereby aiding $Ca^{2+}$ dependent inhibition. Increased $v_{channel}$ values should therefore lead to restoration of $Ca^{2+}$ puffs, albeit high transitioning rates, in the AICT models. To verify this hypothesis, cluster behavior at increasing $v_{channel}$ values was simulated.
In the 6-state AICT model, increasing $v_{channel}$ values to 5000 $s^{-1}$ puffs are established but have longer lifetimes. The mean puff lifetime is 400 $ms$ with a maximum duration of 3.5$s$ and the mean inter puff interval is 500 $ms$. This behavior does not match experimentally observable puff properties. Increasing $v_{channel}$ further up to 20000 $s^{-1}$ restores events in the millisecond regime and the average inter puff intervals to 1-1.5$s$. For the DYK AICT model, puffs are restored at $v_{channel}= 25000$ $s^{-1}$, with a mean lifetime of 1$s$ and the average inter puff interval of 1.25$s$. But at these high values of $v_{channel}$ micro domain (cluster site) $Ca^{2+}$ concentrations are too high, in the range of a 100 $\mu M$, strikingly inconsistent with theoretical predictions.

Figure 4.10 shows the fraction of open channels for various values of $v_{channel}$. Panel A) shows the underlying open channels for the standard 6-state model. Puffs are in the millisecond regime with post inhibition recovery phase lasting for a few seconds. Panel B) shows the underlying fraction of open channels for the 6-state AICT model for $v_{channel} = 5000$ $s^{-1}$. Two observations can be made from the channel profile. First, event durations are in the seconds regime. Second and more interesting, after a release event inhibition does not immediately set in, instead single or two channel fluctuations are observed. These fluctuations, last for seconds, delaying the onset of the recovery phase and can be accounted for by fast transitions to and from the AICT state. Panel C) shows the underlying open fractions for $v_{channel} = 20000$ $s^{-1}$. Event durations are in the milliseconds regime. Burst like activity of the cluster is observed though is significantly reduced (compare panels B and C). Cluster site release values are now in the 100 $\mu M$ regime much larger than theoretical micro domain estimates of 10-20 $\mu M$.

Figure 4.11 shows the corresponding uninhibited and inhibited fraction of subunits for the 6-state AICT model at $v_{channel}= 5000$ $s^{-1}$ (panel A) and at $v_{channel}= 20000$ $s^{-1}$ (panel B). In panel A) one can clearly see delayed recovery post inhibition, in particular from
Figure 4.10: The Figure shows the underlying fraction of open channels. Panel A) shows the underlying fraction of open channels for the 6-state model. During a puff 6-8 channels are open. Panels B) and C) show underlying fraction of open channels for the 6-state AICT model with higher values of $v_{\text{channel}}$. In panel B) $v_{\text{channel}}$ is 5000 s$^{-1}$. Duration of events is in the seconds regime with fluctuations (rapid opening and closing of 1-2 channels) lasting up to seconds. Panel C) shows results from simulation of 6-state AICT at $v_{\text{channel}}$ 20000 s$^{-1}$. Event duration is in the millisecond regime and duration of fluctuations is markedly reduced due to increased $Ca^{2+}$ values.

15-20s and from 30-35s. With increasing $v_{\text{channel}}$, this behavior subsides due to an increase in $Ca^{2+}$ aided inhibition. Fast single channel transitions are still observed though with a lower frequency and smaller duration. The time scales of the collective subunit behavior at higher $v_{\text{channel}}$ values do not match the time scales of the standard 6-state model (compare panel fig 4.10 A with fig 4.11 B).
Figure 4.11: Fraction of uninhibited and inhibited subunits for high $v_{channel}$ values. Top Panel demonstrates the underlying fractions subunits for the 6-state AICT model for $v_{channel}= 5000 \text{ s}^{-1}$. Regimes in graph, 15-20s and 31-34s, clearly show delayed recovery of the subunits post inhibition. The bottom panel shows the underlying subunit profile for $v_{channel} 20000 \text{ s}^{-1}$. On an average more subunits are inhibited due to an increase in $Ca^{2+}$ aided inhibition.

4.4 Discussion

The $IP_3$ receptor plays a central role in calcium signaling. The rich dynamic variety observed in amplitude, durations and spatial spread of $Ca^{2+}$ signals, derives from interactions of the receptors within a cluster and on larger spatial and temporal scale, through inter cluster interactions. It is vital to use theoretical modeling studies to investigate kinetic mechanisms that regulate receptor behavior. Recently Shuai et al. [49] proposed the existence of an “active” state to account for data obtained for the single
channel in nuclear patch clamp experiments [35]. The focus of this chapter has been to study the role of the recently proposed agonist-independent conformation transformation for the $IP_3$ receptor subunit, in $IP_3$ cluster behavior. The driving ambition has been, to test the validity of a model that reproduced single channel behavior at predicting cluster behavior on a larger spatial scale.

$Ca^{2+}$ release events from single clusters are well characterized in literature [57], [64], [54]. Well known features of $Ca^{2+}$ puffs/ calcium cluster release were used to study and constrain the behavior of the AICT models in an $IP_3$ receptor cluster.

The presence of the fast agonist-independent conformational transformation explains high open probabilities ($P_o$) observed in nuclear patch clamp experiments, albeit breaking the inherent synchrony that exists between uninhibited and inhibited subunit states of the receptors within a cluster. The time scales associated with high $P_o$ as observed in nuclear patch clamp experiments, when associated with $IP_3$ cluster function, bias the subunits against inhibition. No distinct $Ca^{2+}$ release event is observed. Reducing the time scales to restore synchrony in the cluster leads to a lower single channel $P_o$, typically in ranges observed in patch clamp recordings from lipid bilayer studies [6], [67].

Increasing $v_{channel}$ values in conjunction with fast transition rates restores $Ca^{2+}$ puff like events in principle due to $Ca^{2+}$ aided inhibition. At high $v_{channel}$ values two significant features emerge. First, micro domain (cluster release site) $Ca^{2+}$ values are in the 100 $\mu M$ range, significantly larger than predictions from detailed theoretical studies [44], [45], [56], [60]. Second, an increased intrinsic propensity for single channel fluctuations, following a puff, lasting up to hundreds of milliseconds, is observed. The observed burst like behavior is a direct consequence of the presence of the AICT and is absent in vivo.

Shuai et al. [51] have proposed a series of $IP_3$ receptor models with fast transitioning rates to explain high $P_o$ from nuclear patch clamp experiments. I conclude, based on the study presented, that such models suffer from the same shortcomings as detailed in this
chapter. Biasing the system against inhibition may account for high open probabilities, but
does not reproduce features of collective cluster calcium release. In conclusion, single
channel models with parameters fitted to explain single channel experimental recordings
may not necessarily explain receptor function at a higher organizational scale of a cluster
or a cell.
5 Conclusion and Future Work

The $Ca^{2+}$ ion is of vital significance in cellular signaling processes. Regulation of the inositol 1,4,5 trisphosphate ($IP_3$) receptor by $IP_3$ and $Ca^{2+}$ is at the heart of the rich spatiotemporal complexity observed in $Ca^{2+}$ signaling patterns.

A simple markovian model was proposed to explain the basic kinetics of the $IP_3$ receptor. The model succeeds in reproducing statistically observed puff properties as measured in experiments. The 6-state model is an improvement over other markovian models for the $IP_3$ receptor in that it retains all major features of elementary calcium release events while being computationally inexpensive.

The modeling scheme employed to study the six state model was based on two vital assumptions. First, individual molecular buffer species were not explicitly modeled. Following the work of Wagner and Keizer [69] and Yao et al. [70], I model release from a single cluster to be diffusive in nature, with a single transport equation describing the release and spread of $Ca^{2+}$. Second, all channels from within the cluster experience a homogenous distribution of calcium. As explained in chapter 2, this assumption works well on the temporal and spatial scale dealt with in calcium release from a cluster of $IP_3$ receptors. In chapter 3, a full system with various molecular buffer species was modeled. I have shown the equivalence of puff properties as obtained in the presence of buffers to the simplified approach of chapter 2. On the temporal scale of a puff $\sim 100 ms$, presence or absence of buffers does not have a significant effect on the full width at half maximum of the release event. Binding affinities have a significant impact on the decay profile of $Ca^{2+}$ as detailed in chapter 3. Buffer concentrations and affinities impact puff amplitudes, with high concentrations of fast binding buffer strongly quenching the underlying free $Ca^{2+}$ signal. The study is an ongoing work but preliminary results support the validity of a simplified reduced system in studying properties of cluster release.
In chapter 4, the recently proposed existence of an "active" (AICT) state was tested. In the literature, the model was proposed [49] to explain single channel properties in particular, the open times and the high open probability of the receptor, observed in a specific set of experiments [35]. I studied the impact of the proposed "active" state on the behavior of calcium release from a cluster of $IP_3$ receptors. For targeted cluster release of $\sim 10 \, \mu M$, the AICT with fast transitioning rates fails to elicit puffs from a single cluster, in marked contrast with what is known of a single cluster behavior. As calcium induced inhibition is increased, puffs are observed but cluster site release values are in violation of theoretical estimates. Though introduced to explain single channel behavior, the model fails on the organizational scale of a cluster.

Better imaging techniques, like the TIRF [54], are making it possible to image calcium release from individual channels within a cluster. Important questions still remain, in particular related to the construct of a cluster of $IP_3R$’s. Immediate future work will focus on studying, based on available data, the underlying construct of a single cluster. Does the spacing between channels effect the overall profile of a puff, if yes then how? Do the differences on these nano domains, in the vicinity of a single channel, have an impact on microdomain $Ca^{2+}$ signaling characteristics? In the next stage of this work I will model on finer spatial scales typically on the order of a few nm’s ($\sim 5 \, nm$), a factor of 10 less than the work presented in this thesis.

$Ca^{2+}$ plays a significant role in the maturation of T-cells [17]. In Future, I want to use my work on $Ca^{2+}$ signaling, to study the role of $Ca^{2+}$ in different immune signaling pathways.
REFERENCES


[27] Kaftan EJ, BE Ehrlich and J Watras. **Inositol 1,4,5- trisphosphate and calcium interact to increase the dynamic range of InsP3 receptor-dependent calcium signaling.** *Journal of general physiology*, 110, 529-538, 1997.


[38] Parker I, J Choi, and Y Yao. Elementary events of InsP$_3$-induced Ca$^{2+}$ liberation in Xenopus oocytes: hot spots, puffs and blips. Cell Calcium, 20(2), 105–21, 1996.


[59] Swaminathan D and P Jung. Role of agonist independent conformational transformation (AICT) in $IP_3$ cluster behavior. Submitted


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- **Swaminathan D**, G Ullah & P Jung, Chaos, 19, 037109 September 2009
  *A Simple Sequential Binding Model for Calcium Puffs.*

- Contributed Poster ”The role of AICT in IP₃ cluster behavior” February 2010
  Annual Biophysical Society Meeting.
• Jung P, D Swaminathan & A Ullah. *Chemical Physics* In Press; 2010
  
  *Calcium Spikes: Chance or Necessity?*

• Swaminathan D & P Jung. Submitted; 2010
  
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