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Mathematical Modeling of Inositol Trisphosphate Receptor and Calcium Oscillations in Airway Smooth Muscle Cells

Pengxing Cao

Department of Mathematics
The University of Auckland

Abstract

Oscillations in cytoplasmic calcium concentration ($[\text{Ca}^{2+}]_i$) in airway smooth muscle cells (ASMC), primarily mediated by repetitive openings and closings of inositol trisphosphate receptor (IP$_3$R) channels situated in the sarcoplasmic reticulum membrane, have been found to be important in generating and maintaining airway contractile force. However, it has been unclear about the mechanisms accounting for such oscillations, especially how the IP$_3$R behaves in living cells to perform its function. In light of the extensive existence of calcium oscillations in many other cell types, although this thesis focuses on modeling calcium oscillations in ASMC due to their importance for the study of pathology of asthma, it also aims to solve some major questions in a wider context:

- What is the mechanism for the formation of the repetitive calcium releases? How is the mechanism connected to the dynamics of the IP$_3$R?
- How best (or simply) should the IP$_3$R be modeled for performing its function?
- Should we care about the channel stochasticity when making model predictions? That is, does the deterministic model (which is easier and faster to solve) have the same predictive power as the stochastic model?

In this thesis, by mathematical modeling, our primary achievements are:

- First, based on a very recent IP$_3$R model and available single-channel data, we developed a new IP$_3$R model by introducing time-dependent interstate transitions, and showed that the time-dependent feature is crucial for a quantitatively reproduction of behaviors and statistical properties of localized calcium events (called calcium puffs).
- Second, we showed that the existing 6-state IP$_3$R model could be reduced down to a simple 2-state model without losing its function. This result leads us around full circle of 20 years of detailed studies and modeling of the IP$_3$R, back to an early formulation where the calcium oscillations arise as a fast-slow dynamical system extensively seen in physiological processes.
- Third, we compared a stochastic ASMC calcium model and its associated deterministic form, and showed that both of the models successfully predicted the trends of frequency
change in response to a number of experimentally testable parameter perturbations. This allows a reliable use of deterministic models in making predictions.
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Nature of contribution by PhD candidate: designed and performed data analysis and model construction and simulations, wrote the paper.

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### CO-AUTHORS

<table>
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<tr>
<th>Name</th>
<th>Nature of Contribution</th>
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<tr>
<td>James Sneyd</td>
<td>general supervision of the work, oversight of revising and writing of the paper</td>
</tr>
<tr>
<td>Graham Donovan</td>
<td>discussion and certain aspects of the paper</td>
</tr>
<tr>
<td>Martin Falcke</td>
<td>discussion and certain aspects of the paper</td>
</tr>
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<tr>
<td>Graham Donovan</td>
<td>[Signature]</td>
<td>8 July 2014</td>
</tr>
<tr>
<td>Martin Falcke</td>
<td>[Signature]</td>
<td>14 July 2014</td>
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<td>General supervision of the work, oversight of revising and writing of the paper</td>
</tr>
<tr>
<td>Xiaohui Tan</td>
<td>Performed the experiments</td>
</tr>
<tr>
<td>Michael J. Sanderson</td>
<td>Performed the experiments, paper editing</td>
</tr>
<tr>
<td>Graham Donovan</td>
<td>Discussion and certain aspects of the paper</td>
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<td>Xiaohui Tan</td>
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<tr>
<td>Michael J. Sanderson</td>
<td></td>
<td>15/7/2014</td>
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Chapter 1

INTRODUCTION

Intracellular calcium ions (Ca$^{2+}$), as a cellular signal, play a significant role in regulating many physiological activities, such as synaptic transmission, muscle contraction, saliva secretion and cell fertilization and division [12, 13, 14, 15, 92]. Although the involvement of Ca$^{2+}$ is usually achieved by a transient increase in cytoplasmic Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) [12, 13, 14, 55], the patterns of Ca$^{2+}$ signals, interestingly, could be various for different cell types, like front-like waves during egg fertilization [66], spiral waves in Xenopus oocytes [95], periodic traveling waves upon airway smooth muscle cell (ASMC) contraction [10], and calcium transients in gonadotropin-releasing hormone (GnRH) neurons [97].

Despite of a huge variety of Ca$^{2+}$ patterns, it has been widely accepted that the patterns are primarily the results of coordinated Ca$^{2+}$ liberations from either an extracellular source or internal stores like the endoplasmic reticulum (ER) (or the sarcoplasmic reticulum (SR) in muscle cells). A general picture of calcium dynamics common for many cell types is shown in Fig. 1.1. First of all, large Ca$^{2+}$ concentration differences between the cytosol (on the order of 10$^{-7}$M, where M is mol/L) and the ER/SR or extracellular space (on the order of 10$^{-3}$M [113, 2]) form large concentration gradients along which Ca$^{2+}$ can rapidly flow into the cytosol upon openings of any calcium channels situated on the membranes of the ER/SR or the plasma membrane, leading to an increase in [Ca$^{2+}$]$_i$. In terms of the concentration gradient, there are two important pathways for Ca$^{2+}$ entry into the cytosol. One is Ca$^{2+}$ influx through the channel types including receptor-operated calcium channels (ROCC) requiring agonist binding to the plasma membrane receptors [141], store-operated calcium channels (SOCC) and/or reverse mode of the Na$^+$/Ca$^{2+}$ exchanger (NCX) in the case of (at least partial) ER/SR depletion [35, 78], voltage-gated calcium channels (VGCC) whose opening usually requires a moderate depolarization of cell membrane potential [31, 32], and a leak Ca$^{2+}$ inflow for maintaining the resting [Ca$^{2+}$]$_i$. The other pathway is ER/ SR Ca$^{2+}$ release through the inositol 1,4,5-trisphosphate receptor (IP$_3$R) and/or ryanodine receptor (RyR) which can act as calcium channels with ligands bound, such as inositol 1,4,5-trisphosphate (IP$_3$) or cyclic ADP-ribose (cADPR) [18, 96]. Besides these two, calcium liberations from the mitochondria and Ca$^{2+}$-bound buffers can also significantly
affect the properties of Ca^{2+} signals [89, 88, 38, 39].

Prolonged high \([\text{Ca}^{2+}]_i\) is toxic to cells, so they need pathways to either pump excessive Ca^{2+} back to the ER/SR or extrude it out of the cell. Two primary channels for this function are sarco(endo)plasmic reticulum calcium ATPase (SERCA) on the ER/SR membrane and plasma membrane calcium ATPase (PMCA) on the cell membrane [175, 22]. In addition, the mitochondrial uniporter, normal mode of NCX, and free Ca^{2+} buffers are all able to modulate and maintain the level of \([\text{Ca}^{2+}]_i\) [89, 38, 54, 188].

Figure 1.1: Schematic diagram showing the Ca^{2+} fluxes involved in the control of \([\text{Ca}^{2+}]_i\). Upon binding of agonist to a cell membrane receptor (R), G-protein is activated and induces the activation of phospholipase C (PLC), leading to the production of IP_3. IP_3 diffuses and binds to an IP_3R, triggering opening of the IP_3R to release Ca^{2+} from the endoplasmic reticulum. Such an initial Ca^{2+} release could then sensitize both IP_3R and Ryanodine receptor (RyR) to open for a larger Ca^{2+} release (J_{IPR} and J_{RyR}). Excessive Ca^{2+} are pumped either back to the ER by SERCA (J_{serca}) or out of the cell by PMCA (J_{pm}). \([\text{Ca}^{2+}]_i\) is also modulated by Ca^{2+} exchanges into and out of the cell. Mitochondria can also take up Ca^{2+} (J_{mito-in}) for the use of ATP production. Cytoplasmic Ca^{2+} is heavily buffered so that \([\text{Ca}^{2+}]_i\) is also significantly affected by binding (J_{on}) and dissociation (J_{off}) of Ca^{2+} and buffers.

Many types of cells have been shown to contain all the components listed here, but, depending on the situations, not all of them have to be involved, which is thus a reason for the versatile patterns of intracellular Ca^{2+} signaling. For example, VOCC is crucial for triggering elevations of \([\text{Ca}^{2+}]_i\) in cardiac pacemaker cells and GnRH neurons [106, 85, 34], but seems not to play a regulatory role during agonist-induced Ca^{2+} waves in ASMCs and pancreatic acinar cells (PAC) [120, 160].

The two antagonistic classes of calcium fluxes mentioned above could result in repetitive oscillatory changes of \([\text{Ca}^{2+}]_i\) (called Ca^{2+} oscillations), which have been found in many types of cells and are usually involved in regulating many downstream physiological processes,
like hormone production, saliva secretion, and airway contraction [92, 49]. One of the well-established processes for generating Ca\(^{2+}\) oscillations and waves is agonist/IP\(_3\)-induced Ca\(^{2+}\) release through IP\(_3\)R [60]. Binding of agonists (or neurotransmitters secreted from axon terminal) to receptors on the cell membrane activate guanine nucleotide binding proteins (G-proteins), leading to the activation of phospholipase C (PLC) which then catalyzes the conversion of phosphotidylinositol 4,5-bisphosphate (PIP\(_2\)) to diacylglycerol (DAG) and IP\(_3\) [11, 125]. The water-soluble IP\(_3\) diffuses through the cytosol and binds to IP\(_3\)R situated in the ER/SR membrane, leading to opening of IP\(_3\)R through which ER/SR Ca\(^{2+}\) release into the cytosol can occur [58, 130]. Ca\(^{2+}\) release is excitable, in the sense that a small increase in \([\text{Ca}^{2+}]_i\) has little effect, but once \([\text{Ca}^{2+}]_i\) increases over a threshold level (it is a threshold-like property but not a well-defined value), it opens the IP\(_3\)R, leading to the release of a much larger amount. This is due to two key properties of IP\(_3\)R that 1) its open probability is \([\text{Ca}^{2+}]_i\)-dependent in a bell-shaped manner that very low (\(< 10 \text{ nM}\)) or high \([\text{Ca}^{2+}]_i\) (\(> 100 \mu\text{M}\)) normally lead to a very low open probability whereas moderate \([\text{Ca}^{2+}]_i\) (the optimal value is different for different cell types and IP\(_3\) and ATP concentrations) can increase the open probability up to 70 – 85% [16, 105, 84, 179]; and 2) activation of IP\(_3\)R is significantly faster than its inactivation [83, 185, 107]. Therefore, an initial elevation of \([\text{Ca}^{2+}]_i\) can dramatically increase the open probability of unactivated IP\(_3\)R to greatly promote further release of Ca\(^{2+}\), which is a process called calcium-induced calcium release (CICR) [51]. Once \([\text{Ca}^{2+}]_i\) gets sufficiently high, IP\(_3\)R will be inactivated (or closed) again and experience a period of refractoriness, during which excessive calcium ions are removed from the cytosol mainly by SERCA and PMCA. Again, Ca\(^{2+}\) release for the next oscillation will occur by stochastic openings of IP\(_3\)Rs when the IP\(_3\)Rs recover from inactivation [109].

Such Ca\(^{2+}\) oscillations in many cells can propagate in the form of Ca\(^{2+}\) waves [10, 120, 124, 109]. Upon opening of first a few IP\(_3\)Rs, local Ca\(^{2+}\) liberated from the ER/SR will diffuse to neighboring IP\(_3\)Rs, sensitize those IP\(_3\)Rs to activate, trigger a wider release of Ca\(^{2+}\) by CICR and subsequent propagation of Ca\(^{2+}\) wave.

The site of wave initiation can be various for different cells, with on definite pattern. For instance, Ca\(^{2+}\) waves in ASMCs are usually initiated from either of the narrow ends of the fusiform cells (which behave like local oscillators to drive global waves), but there are many sites irregularly distributed in Xenopus oocytes to initiate a wave [109]. It is very likely to be the sites with a higher IP\(_3\)R density. The distribution of IP\(_3\)R has been found in some types of cell to be distributed on the ER/SR membrane in a clustered manner that approximately 10 – 30 IP\(_3\)Rs are located close to each other (within a diameter of 0.3 – 0.8 \(\mu\text{m}\)) to form a cluster [147, 177]. And the number of clusters in a cell varies; there are typically thousands in Xenopus oocytes, while there are only a few in SH-SY5Y cells [148, 147]. The clustered nature of IP\(_3\)R not only enhances CICR, but also acts as a repeating unit for assisting in wave propagation.

Ca\(^{2+}\) release through clustered IP\(_3\)Rs is hierarchically organized by the spatial arrangement and temporal recruitment of the IP\(_3\)R [20, 21, 174]. It usually starts from an initial Ca\(^{2+}\) release
through one IP$_3$R that randomly opens first (an event called a Ca$^{2+}$ blip, the smallest Ca$^{2+}$ release through IP$_3$R [119]). Such a release forms a Ca$^{2+}$ concentration profile that peaks over 100 $\mu$M at the IP$_3$R channel mouth but could drop down to a few $\mu$M at adjacent closed IP$_3$Rs about 100 – 200 nm away from the channel pore [168, 9, 133]. The huge pore $\left[\text{Ca}^{2+}\right]_i$ then rapidly shuts down the open channel, whereas the relatively low surrounding $\left[\text{Ca}^{2+}\right]_i$ dramatically activates other closed channels to open, leading to a larger Ca$^{2+}$ release (called Ca$^{2+}$ puffs, localized Ca$^{2+}$ releases through tightly clustered IP$_3$Rs [187, 163, 147]). By Ca$^{2+}$ diffusion, the Ca$^{2+}$ puff is then able to trigger openings of IP$_3$Rs in neighboring clusters (more puffs) to generate a global Ca$^{2+}$ release for oscillations and waves [26, 108]. However, how IP$_3$Rs are distributed in ASMCs is still unknown, as Ca$^{2+}$ blips and puffs that are usually used to evidence the IP$_3$R distribution have not been clearly found and identified in ASMCs [37].

The interest of researchers in ASMC is due to its crucial role in generating airway contraction force, a understanding of which is very important for understanding the pathology of asthma [3]. Our interest in Ca$^{2+}$ oscillations in ASMC is because such a Ca$^{2+}$ pattern plays an important role in generating and maintaining the contraction force in ASMC via activation of the actin-myosin crossbridges [71, 72, 73, 111, 180].

For a long time experimentalists have explored the mechanism of Ca$^{2+}$ oscillations in ASMC and their role in manipulating ASMC contraction. In the 1980s, cytoplasmic Ca$^{2+}$ had been known to be an important second messenger in regulating airway contraction. However, due to supramaximal concentrations of agonists commonly applied, scientists could only see a large Ca$^{2+}$ transient followed by a sustained elevation in $\left[\text{Ca}^{2+}\right]_i$ [115].

Later, progress was made by finding that, in the presence of relatively low concentrations of agonists, $\left[\text{Ca}^{2+}\right]_i$ experienced a large transient followed by oscillations with a smaller amplitude [50, 91, 142, 123]. Typically, those Ca$^{2+}$ oscillations originated from one end of the cell and propagated to the other end [124]. In the mean time, besides IP$_3$R, ryanodine receptors (RyR) were also found to be critical to acetylcholine (ACh, an agonist)-induced Ca$^{2+}$ oscillations [91].

However, in the early 2000s, Berger and Sanderson performed experiments using murine lung slices and found that IP$_3$R was essential for initiating Ca$^{2+}$ release from the SR and subsequent Ca$^{2+}$ oscillations and waves, and a sufficiently high frequency of oscillations was necessary for maintaining the contractile force [10]. Soon after that, it was found that the strength of contraction was closely related to the frequency of Ca$^{2+}$ oscillations [120, 5]. The findings were quite important so that scientists could artificially control the strength of airway contraction by controlling the frequency of Ca$^{2+}$ oscillations, which could provide a way of relieving the symptoms of asthma.

Following studies on Ca$^{2+}$ oscillations and airway contraction for different species (rats and mice) showed that Ca$^{2+}$ oscillation frequency and the mechanisms controlling airway contraction in different species were not exactly the same, indicating the necessity of a detailed study on human ASMC [6]. Furthermore, experimental data from mouse and human ASMCs showed that, despite frequency differences, agonist-induced Ca$^{2+}$ oscillations for both cell types were
not affected by the inhibition of RyR, and therefore most likely relied solely upon IP₃R [7, 127]. However, this finding contradicts those previously found in porcine tracheal smooth muscle [91]. The reason for this is still unclear, but we propose that it could be due to different SR Ca²⁺ loads (which could sensitively affect the open probability of RyR and thus the involvement of RyR in Ca²⁺ oscillations [181, 155]), different levels of expression of RyR, or even different properties of IP₃R and/or RyR.

Over the last two decades, IP₃R, because of its important role in Ca²⁺ signaling (not just ASMC, but many other cell types), has been paid more and more attention. Detailed studies of the structure and function of IP₃R have made a remarkable progress on our understanding about how the IP₃Rs are organized to generate Ca²⁺ oscillations and waves (see [60] for a thorough review). Although some researchers showed evidence that some ionic channels could also influence the Ca²⁺ signaling and in turn Ca²⁺ oscillations [78, 77, 79, 74, 121], the IP₃ receptor, as a primary Ca²⁺ channel regulating Ca²⁺ oscillations, was hardly challenged.

In this thesis, we try to solve several major questions in the studies of calcium oscillations. Firstly, what is the mechanism for the formation of the regenerative Ca²⁺ signals, especially the mechanism by which each Ca²⁺ upstroke induced by the positive feedback (i.e. CICR) is terminated. The debate over termination mechanisms of calcium oscillations has continued for over a decade, but has gradually moved to a debate over termination of individual Ca²⁺ puffs because of their role in generating repetitive waves [133, 134, 184]. In the literature, some mechanisms have been proposed:

• Excessive Ca²⁺ on the cytoplasmic side of the IP₃R could lead to inactivation (or inhibition) of the IP₃R (by either Ca²⁺ binding to the inactivation site [43] or some other complicated reactions) so that the IP₃R channel hardly reopens until [Ca²⁺]ᵢ returns back to a relatively low level. This mechanism makes use of the Ca²⁺-dependent properties of the IP₃R which is considered to be very important in terminating Ca²⁺ release, but it is still unable to explain the significant difference in termination time scale between Ca²⁺ puffs and oscillations [173], during which the IP₃Rs seem to experience the same [Ca²⁺]ᵢ.

• The high rate of Ca²⁺ release through open IP₃Rs could cause a temporary luminal Ca²⁺ depletion, which then leads to a decrease in [Ca²⁺]ᵢ due to both SERCA and lack of support for Ca²⁺ release. Although a recent study by Ullah et al. excluded this hypothesis based on the results of a computational model [178], it is still experimentally undetermined and thus might play a role.

• Stochastic attrition is another candidate. The generation of Ca²⁺ puffs and oscillations has been shown to exhibit a clear stochasticity [173, 145]. Therefore, Ca²⁺ release could be terminated when the IP₃Rs switch back to closed states by chance. However, it requires not only that the number of IP₃Rs underlying the process is very limited [42], but also a fine-tuned spatial arrangement of the IP₃Rs [116]. Also, many studies including ours
found it is unlikely the case for many cell types. In spite of this, it still plays a potential role but more probably not by itself.

- In addition to the above, Rüdiger et al., based on a computational model, found that prolong elevation in $[\text{Ca}^{2+}]_i$ (by possibly the residual Ca$^{2+}$ in the micro-domain of the IP$_3$R cluster) could cause dissociation of IP$_3$ from IP$_3$ binding site, leading to a relatively long closed time before the channel is able to reopen [133]. Although it provides a possible explanation to the terminations of the long-period oscillations, it relies on the choices of IP$_3$R model parameters and still needs experimental validation.

The difficulty of solving this question lies in not only lack of direct experimental data to solidly support any of the hypotheses, but also lack of an understanding of the real IP$_3$R kinetics in vivo. In this thesis, I will present our progress on solving this problem. Nevertheless, it is still far from giving a final answer, and the debate will continue.

The second question is how best IP$_3$R should be modeled. In other words, what fundamental properties of the IP$_3$R allow it to perform its function, and what are their quantitative properties? Models of the IP$_3$R have a long history, beginning with the heuristic models of [43, 47, 4]. With the recent appearance of single-channel data from IP$_3$R in vivo [105, 179], a new generation of Markov IP$_3$R models has recently appeared [177, 144]. These models show that IP$_3$Rs exist in different modes with different open probabilities. Within each mode there are multiple states, some open, some closed. Importantly, it was found [29] that time-dependent transitions between different modes are crucial for reproducing Ca$^{2+}$ puff data from [147]. However, it is not yet clear whether transitions between states within each mode are important, or whether all the important behaviors are captured simply by inter-mode transitions.

The third question is how important is the intrinsic IP$_3$R stochasticity for influencing Ca$^{2+}$ oscillations. Ca$^{2+}$ oscillations were being considered to be periodic, so that lots of deterministic models were developed to explain such phenomena. However, more and more studies have shown the oscillations (and puffs) are actually generated by stochastic openings of the IP$_3$Rs in a repetitive manner [109, 169, 170, 129, 145, 147], challenging the validity of the deterministic modeling approach. For ASMC, we have also found that oscillations and waves mediated by the IP$_3$R are generated by stochastic mechanisms (see chapter 5). Nevertheless, deterministic models of the IP$_3$R have been highly useful in advancing our understanding of intracellular Ca$^{2+}$ oscillations, and these models have made predictions which have been confirmed experimentally [24, 154, 181, 37]. Therefore, a detailed study of the role of IP$_3$R stochasticity in Ca$^{2+}$ oscillations (even if only in the specific context of ASMC) is the first step for modelers to establish a theory (or at least a guide) for the choice of appropriate models in the study of Ca$^{2+}$ dynamics, and will have an impact for future studies.

Focusing on the major questions, this thesis contains 6 chapters and are organized as follows,

- In Chapter 2, I will introduce mathematical background knowledge including IP$_3$R models and the general approach to modeling calcium dynamics, with emphasis on any mod-
els and approaches that will be used in our following study.

- In Chapter 3, we introduce a new modeling idea to incorporate the non-stationary single-channel data from [105] into the Siekmann model which is built based upon stationary single-channel data, in an attempt to construct a new IP$_3$R model that satisfies both single channel data. The work presented in Chapter 3 and Chapter 4 has been published in Biophysical Journal (Cao P, Donovan G, Falcke M, and Sneyd J. 2013. Also see [29] for full publication details).

- In Chapter 4, we construct a stochastic puff model based on our new IP$_3$R model. We show that the puff model is able to reproduce experimental puff statistics, and the reproduction closely relies on the new properties that we introduce in the newly constructed IP$_3$R model. To the best of our knowledge, our IP$_3$R model is the first model satisfying both single-channel data and Ca$^{2+}$ puff data, and is thus a relatively reliable model to use in modeling Ca$^{2+}$ oscillations in ASMCs.

- In Chapter 5, based on the new IP$_3$R model, we construct an ASMC calcium model in both stochastic and deterministic versions. We first show that calcium oscillations in ASMC, as well as the statistics of calcium puffs in other cell types, can be quantitatively reproduced by a two-state model of the IP$_3$R, and thus the behavior of the IP$_3$R is essentially determined by its modal structure. The structure within each mode is irrelevant for function. We then show that the termination of each oscillation is primarily modulated by a rapid calcium-inhibition in a deterministic way, whereas the initiation of each oscillation is significantly affected by stochastic openings of IP$_3$Rs. Also, we find that the stochasticity of IP$_3$R is not essential for a qualitative prediction of change of oscillation frequency, showing that the deterministic models have almost the same predictive power as stochastic models. This work allows a reliable use of deterministic models in model predictions and time-consuming multi-scale simulations to improve computational efficiency. The work presented in Chapter 5 has been accepted for publication in PLoS Computational Biology (Cao P, Tan X, Donovan G, Sanderson MJ, and Sneyd J. 2014. Also see [30] for full publication details).

- In Chapter 6, I will briefly summarize our primary results and discuss some remaining problems in the field.
Chapter 2

MATHEMATICAL BACKGROUND

In this chapter, I will introduce some background about IP$_3$R models and calcium dynamics modeling which are the basis of my studies and are also helpful for general readers to know the modeling approaches that we use quite often in the field. In detail, the chapter contains three main sections. The first section introduces models of the IP$_3$R but with emphasis on the details of three IP$_3$R models: the most widely used De Young-Keizer model and its simplified version named the Li-Rinzel model, and the single-channel data-driven Siekmann model. The second section introduces the general ways of modeling calcium dynamics, including both deterministic and stochastic methods. The last section introduces the numerical method applied to solve the stochastic calcium models.

2.1 Models of IP$_3$R

Along with the development of experimental studies of IP$_3$R physiology, mathematical/physical models of IP$_3$R play an important role in advancing our understanding of kinetics of the IP$_3$R and its physiological importance. One of the questions modelers are interested in is how the IP$_3$R behaves in regulating $[\text{Ca}^{2+}]_i$. Therefore, most of the IP$_3$R models constructed in different ways are primarily for the purpose of answering this question. Hence, we emphasize that none of the models (even any new models) are able to fully reflect the complex reactions and conformations of the molecular structure, but instead they provide new insights into the role of IP$_3$R in controlling Ca$^{2+}$ signaling.

The construction of an IP$_3$R model relies on an understanding of the physiology of the IP$_3$R. Most of the early models proposed in the 1990s were based on two primary findings; one is the bell-shaped Ca$^{2+}$-dependency of the stationary open probability of the IP$_3$R [82, 118, 16]; the other is that the IP$_3$R experiences a rapid activation followed by a relatively slow inactivation in response to a step increase in Ca$^{2+}$ concentration [83, 185]. The first model taking the two properties into account was the De Young–Keizer model, which assumed an IP$_3$R contains three subunits, each of which consists of three binding sites, one for IP$_3$ and two for activation and
inactivation by Ca$^{2+}$ respectively [43]. Whether the three binding sites are bound or not by an IP$_3$ or a Ca$^{2+}$ determines in total eight states. The model clearly proposed detailed kinetics of the IP$_3$R (even though incorrectly due to the existence of modal structure [84]) and successfully reproduced Ca$^{2+}$ oscillations, thus it became the first choice for modelers to use in simulations, even nowadays.

The De Young–Keizer model was then simplified by Li and Rinzel [99], who found that the property of fast activation and slow inactivation could be captured by a rather simple model in a conductance-based form of the famous Hodgkin–Huxley model [80] or more appropriately the Fitzhugh-Nagumo model [59]. This reduction significantly advanced the understanding of the dynamics of the De Young–Keizer model. The formalism was also used by Atri et al. [4] in their IP$_3$R model where an inactivation variable evolving on a relatively slow time scale was heuristically introduced to model slow inactivation.

Besides the above, sequential binding models, including the Othmer–Tang model [117] and the Bezprozvanny model [17], were proposed during the same period. Those models assumed binding of a Ca$^{2+}$ to the activation site of the IP$_3$R required an initial binding of an IP$_3$, different from the De Young–Keizer model which assumed that binding of IP$_3$ and Ca$^{2+}$ were independent.

These early models assumed, based on the law of mass action, that the transition rate was a linear function of either Ca$^{2+}$ concentration or IP$_3$ concentration. This assumption would lead to the same order of magnitude of changes in both [Ca$^{2+}$], and the rate of Ca$^{2+}$-activation, which conflicted with experimental data [46, 164]. Thus, to overcome this problem, in the early 2000s, Sneyd and Dufour proposed a model with saturating binding rates [149, 150]. By using the model, they also showed that steady-state data (like the open probability) is insufficient to characterize the functional properties of the IP$_3$R.

In addition to the models discussed above, a number of other models appeared in the early 2000s. For example, models by Kaftan et al. [90] or Moraru et al. [114] assumed a large number of states for the purpose of capturing as many properties revealed by data as they could. However, their complexities limited their applications in modeling calcium dynamics. Models by Dawson et al. [41] or Mak et al. [104] included interconvertible conformational states (thus called allosteric models). More details about early models are given in detail by Sneyd and Falcke in a review [153].

Before the middle 2000s, single-channel measurements of the IP$_3$R gating properties were primarily done with either planar lipid bilayers or patch-clamp of isolated nuclei [60]. However, the two methods could give divergent results, which in turn misled the constructions of the IP$_3$R models. Since both experimental approaches were not performed in the original environment in living cells (i.e. in vivo), lots of questions remained unclear, especially how the IP$_3$R behaves in real cells, which could be the key for knowing the real mechanism of Ca$^{2+}$ oscillations and waves.

The field of IP$_3$R modeling was dramatically changed by the appearance of new data of
2.1. MODELS OF IP$_3$R

single-channel openings and closings from IP$_3$R \textit{in vivo} [60, 105, 179]. For the first time, modelers were able to construct models of a single IP$_3$R that could reproduce the correct statistical single-channel behavior \textit{in vivo}. In the late 2000s, Gin et al. proposed two different 4-state Markov models (both of which consist of three closed states and one open state) and all the transition rates of each model were obtained by using Markov chain Monte Carlo (MCMC) fitting the model to the open-time and closed-time distributions extracted from stationary single-channel data (measured with clamped [Ca$^{2+}$]$_i$) [69, 70]. However, it turned out that the open-time and closed-time distributions and stationary open probabilities were still coarse descriptions of single-channel behavior, incapable of capturing some features like mode switching [84].

Therefore, later models by Ullah et al. [177] or Siekmann et al. [144] took into account the modal feature by fitting their own models to both stationary and dynamic single-channel data (dynamic data refers to the data measured in response to a step change in either Ca$^{2+}$ or IP$_3$ concentrations [105]). The Ullah–Mak–Pearson model consists of 12 states which are divided into three modes, low activity mode, intermediate activity and high activity mode, whereas the Siekmann–Wagner–Yule–Crampin–Sneyd model (we simply call it the Siekmann model in the thesis) has only two modes, Park mode included 3 closed states and one open state, and Drive mode included one open state and one closed state. Moreover, besides the difference in model structure, the two models used different fitting approaches to obtain transition rates.

The story will not stop here, and more modelers including us will keep moving it forward (which is part of the aim of the thesis). In the following, I will introduce in detail several IP$_3$R models, which are helpful for general readers to have a better understanding of our following studies.

2.1.1 The De Young–Keizer model

In 1992, De Young and Keizer proposed a model to capture the fast Ca$^{2+}$-activation and slow inactivation of IP$_3$R [43]. They assumed that an IP$_3$R consists of three equivalent and independent subunits involved in controlling the channel conductance. Each of the subunits has one IP$_3$ binding site and two Ca$^{2+}$ binding sites, and thus have in total 8 states. By introducing the notation $S_{ijk}$ to represent the states, where $i$ (indicating IP$_3$ binding site), $j$ (indicating Ca$^{2+}$ activation site), $k$ (indicating Ca$^{2+}$ inactivation site) are either 0 (empty site) or 1 (bound by a ligand), the model of each subunit is schematically presented by a Markov model shown in Fig. 2.1. Note that the original diagram in [43] is a cubic shape, but we present it using a unfolded form from [153] (the states of the two sides are the same) for a better view.

Transition rates are either constants or linear functions of [Ca$^{2+}$]$_i$ ($c$) or [IP$_3$] (IP$_3$ concentration, $p$) based on mass action kinetics. An subunit in $S_{000}$ could be activated by IP$_3$ ($S_{100}$) and then rapidly activated to open by Ca$^{2+}$ ($S_{110}$ which is the only state for the opening of the subunit), or it could be directly inhibited by high Ca$^{2+}$ ($S_{001}$). After opening of the subunit
in $S_{110}$, it could either lose a Ca$^{2+}$ ion ($S_{100}$) or be inactivated by one more Ca$^{2+}$ binding to its inactivation site ($S_{111}$). The model achieved the properties of fast Ca$^{2+}$-activation and slow inactivation by assuming a larger value of $k_5$ ($= 20 \mu M^{-1} \cdot s^{-1}$), two orders of magnitude than that of $k_2$ ($= 0.2 \mu M^{-1} \cdot s^{-1}$) (All the parameter values can be seen in the original article [43], so are not presented here).

Figure 2.1: A schematic diagram of the kinetics of the De Young-Keizer IP$_3$R model. Adopted from [153]. The notation $S_{ijk}$ represents the states, where i (indicating IP$_3$ binding site), j (indicating Ca$^{2+}$ activation site), k (indicating Ca$^{2+}$ inactivation site) are either 0 (empty site) or 1 (bound by a ligand). Transition rates are either constants or linear functions of $[Ca^{2+}]_i$ (c) or [IP$_3$] (p) based on mass action kinetics. See [43] for all the parameter values.

Another assumption is that the IP$_3$R channel opens when all the three subunits are in $S_{110}$. Therefore, if denoting $x_{110}^\infty$ to be the stationary fraction of each subunit in $S_{110}$, the stationary open probability of the channel ($P_o^\infty$) is given by

$$P_o^\infty = (x_{110}^\infty)^3 = \frac{pcK_2}{(pc + pK_2 + K_1K_2 + cK_3)(c + K_5)},$$

where $K_i = k_{-i}/k_i$, $i = 1, 2, 3, 4, 5$. The $P_o^\infty$ curve is bell-shaped in terms of c, and thus could fit to the stationary open probability data to obtain some of the parameter values (as De Young and Keizer did). As we can see, the clever way of modeling IP$_3$R based on experimental quantitative measurements excellently achieved the goals of that time, which is why, unsurprisingly, it has been the most influential IP$_3$R model to date.

2.1.2 The Li-Rinzel model

In 1994, Li and Rinzel showed that the function of the De Young-Keizer IP$_3$R model in generating Ca$^{2+}$ oscillations could be simplified down to one gating variable that is for inactivating the IP$_3$R on a relatively slow time scale [99]. The model reduction mainly relies on time scale separation so that relatively fast evolved variables could be assumed to instantaneously follow
2.1. MODELS OF IP$_{3}$R

their equilibria (which is called the quasi-steady-state approximation, a common mathematical method used to reduce complex dynamical systems with multiple time scales). Hence, strictly speaking, it is not a new IP$_{3}$R model, but a simplified version of the De Young-Keizer model. However, due to its concise form and predictive power in modeling Ca$^{2+}$ oscillations, it can be seen as a special IP$_{3}$R model, even though use of it needs extra cautions in situations where the original quasi-steady-state approximations fail.

Letting $h = x_{000} + x_{100} + x_{010} + x_{110}$ (where $x_{ijk}$ is the fraction of each subunit in $S_{ijk}$) be the proportion of IP$_{3}$Rs that have not been inactivated by Ca$^{2+}$, the original De Young-Keizer model can be reduced to one ODE (see [99] for details of the model derivations),

$$\frac{dh}{dt} = h_{\infty} - \frac{h}{\tau_h}, \quad (2.2)$$

where $p$ is [IP$_3$] and $c$ is [Ca$^{2+}$], and

$$\tau_h = 1/(k_2(Q + c)),$$
$$h_{\infty} = Q/(Q + c),$$
$$Q = K_2(p + K_1)/(p + K_3).$$

The open probability of the Li-Rinzel model is given by

$$P_o = \left(\frac{p}{p + K_1}\right)^3 \left(\frac{c}{c + K_5}\right)^3 h^3, \quad (2.3)$$

$K_i$ and $k_i$ are the same as those in the De Young-Keizer model. Note that this open probability is a dynamic variable instead of the one in Eq. 2.1. However, by letting $h = h_{\infty}$ (its stationary function), it is easy to derive the stationary open probability ($P_o^\infty$) of the Li-Rinzel model which is exactly the same as Eq. 2.1.

2.1.3 The Siekmann model

The Siekmann IP$_{3}$R model is a six-state Markov model (see Fig. 2.2) comprised of two modes, park and drive, each of which has multiple closed and open states [144]. This model can be applied to two different isoforms of the IP$_{3}$R, IP$_{3}$R-1 and IP$_{3}$R-2, by MCMC fitting the model to the corresponding in vivo single-channel data [143].

In drive mode, there are three closed states and one open state. The main states dominating the activity of the IP$_{3}$R in drive mode are $C_2$ and $O_6$, whereas $C_1$ and $C_3$ are rarely visited. Due to the fast transition pair of $q_{26}$ and $q_{62}$, the stationary open probability of the drive mode is around 70%. Conversely, in park mode which consists of only one closed state ($C_4$) and one open state ($O_5$), the stationary open probability of the IP$_{3}$R is almost zero, as $q_{54}$ is approximately 300 times greater than $q_{45}$. 
The two modes are connected by $q_{24}$ and $q_{42}$ which are dependent on $[\text{Ca}^{2+}]_i$, [IP$_3$] and [ATP] [179]. Except $q_{24}$ and $q_{42}$, all the other transitions are constant and are given in either [144] or Table 3.1. The transition rates are obtained by MCMC fitting the model to the in vivo stationary single-channel data from [179].

One way in which this model could be considered to be superior to previous models is the approach of model construction. Previous models, without exception, are first proposed to be some certain structures and are then fitted to experimental data. Thus, those models were only the “best” fits for the specific model structures. In contrast, Siekmann et al. derived the structures of the drive mode and the park mode directly from the single-channel data, with no structural assumptions in advance. The number of states and topology of the model was derived by achieving a minimum likelihood. Although the Siekmann model is still the “best” approximated model in the sense of MCMC fitting, it is, to the best of our knowledge, the most reliable stationary model to date.

### 2.2 Modeling calcium dynamics and IP$_3$-induced oscillations

The most common way of modeling the dynamics of $[\text{Ca}^{2+}]_i$ (denoted by $c$) is based on the fact that the transient change of $[\text{Ca}^{2+}]_i$ is equal to the transient balance of all the major Ca$^{2+}$ fluxes
regulating $[\text{Ca}^{2+}]_i$. Mathematically, it is described, in terms of Fig. 1.1, by a system of ODEs,

$$\frac{dc}{dt} = J_{\text{IPR}} + J_{\text{RyR}} + J_{\text{leak}} - J_{\text{serca}} + J_{\text{in}} - J_{\text{pm}} + J_{\text{mito-in}} - J_{\text{mito-out}} + J_{\text{on}} - J_{\text{off}}, \quad (2.4)$$

where a leak flux $J_{\text{leak}}$ mentioned in Chapter 1 is there to balance the $[\text{Ca}^{2+}]_i$ at a physiological level in the resting state. This equation is usually the governing equation in various calcium models, however, for modeling different phenomena in different cell types, it could contain quite different fluxes or take different forms. For example, Ca$^{2+}$ oscillations are usually propagating waves, so that the diffusion of Ca$^{2+}$ needs to be taken into account. This is achieved by a reaction-diffusion equation (subject to some boundary conditions),

$$\frac{\partial c}{\partial t} = \nabla \cdot (D_c \nabla c) + J_{\text{IPR}} + J_{\text{RyR}} + J_{\text{leak}} - J_{\text{serca}} + J_{\text{in}} - J_{\text{pm}} + J_{\text{mito-in}} - J_{\text{mito-out}} + J_{\text{on}} - J_{\text{off}}, \quad (2.5)$$

where $D_c$ is the diffusion coefficient of Ca$^{2+}$. If assuming $D_c$ is constant everywhere (a very common assumption in many computational models), the diffusion term becomes a Laplacian form $D_c \Delta c$. Note that if calcium buffers are implicitly included in Eq. 2.5 by rapid buffer approximation [92], $D_c$ represents the effective diffusivity, smaller than the diffusivity of free Ca$^{2+}$ and all the fluxes are effective fluxes that substantially contribute the change of $[\text{Ca}^{2+}]_i$.

The governing equation is usually coupled with another ODE describing the change of ER Ca$^{2+}$ concentration ($c_e$),

$$\frac{dc_e}{dt} = \gamma (J_{\text{serca}} - J_{\text{IPR}} - J_{\text{RyR}} - J_{\text{leak}}), \quad (2.6)$$

where $\gamma$ is a parameter representing the cytosol-to-ER volume ratio. The choice of $\gamma$ seems to be arbitrary due to lack of knowledge, but about 5 – 10 is usually used in most of the models. ER Ca$^{2+}$ is also heavily buffered, even though it is not usually explicitly shown in the model by using an approximation approach mentioned later in subsection "Calcium buffers". Moreover, similar to Eq. 2.5, Ca$^{2+}$ diffusion in the ER could also be included, depending on different purposes of the studies.

Mitochondrial Ca$^{2+}$ uptake by calcium uniporters and Mitochondrial Ca$^{2+}$ release can modulate $[\text{Ca}^{2+}]_i$, but the detailed dynamics of those calcium channels are very complicated [52, 64, 94, 86, 65, 128, 126]. Mathematical models were also developed for the understanding of mitochondrial Ca$^{2+}$ handling and the channel dynamics [52, 110, 56, 40, 122, 183]. Due to lack of knowledge of mitochondrial regulation for Ca$^{2+}$ puffs in some cells like SH-SY5Y cells and HEK293 cells and Ca$^{2+}$ oscillations in ASMC, it will not be included in Ca$^{2+}$ models. Nevertheless, for any studies relating to the mitochondria, it should be included in the model.

Removing mitochondrial flux terms, the model reads,

$$\frac{dc}{dt} = J_{\text{IPR}} + J_{\text{RyR}} + J_{\text{leak}} - J_{\text{serca}} + J_{\text{in}} - J_{\text{pm}} + J_{\text{on}} - J_{\text{off}}, \quad (2.7)$$
\[
d\frac{dc_e}{dt} = \gamma (J_{\text{serca}} - J_{\text{IPR}} - J_{\text{RyR}} - J_{\text{leak}}).
\] (2.8)

Transmembrane fluxes \(J_{\text{in}}\) and \(J_{\text{pm}}\) are found to modulate \(\text{Ca}^{2+}\) oscillations on a slower time than that of the fluxes across the ER membrane [19, 135]. By introducing a slow variable \(c_t = c + c_e/\gamma\), Eq. 2.8 can be replaced by

\[
d\frac{dc_t}{dt} = J_{\text{in}} - J_{\text{pm}}.
\] (2.9)

Hence, \(c_t\) (named total intracellular \(\text{Ca}^{2+}\) concentration or total intracellular \(\text{Ca}^{2+}\) load) evolves far slower than \(c\). Due to the separation of the time scale, in the studies of some fast processes, like \(\text{Ca}^{2+}\) puffs and oscillations, \(d c_t / dt \approx 0\), so that \(c_t\) could be treated as a parameter instead of a variable for the analysis of bifurcations [151].

We also need detailed formulations of individual fluxes (sub-models), which provide another dimension of the model variability. All the sub-models of \(\text{Ca}^{2+}\) fluxes form a tool set named the \(\text{Ca}^{2+}\) signaling toolkit from which we can select useful tools to build special calcium models to investigate specific phenomena [15]. In the following, I will briefly introduce how the individual fluxes are usually modeled.

**Calcium flux through the IP\(_3\)R**

\(\text{Ca}^{2+}\) flux through the IP\(_3\)R is usually assumed to be a linear function of the concentration difference between \(c_e\) and \(c\), i.e. \(J_{\text{IPR}} = P_{\text{ipr}} P_o (c_e - c)\), where \(P_{\text{ipr}}\) is the maximum conductance and \(P_o\) is the transient open probability of the IP\(_3\)R (see Section 2.1, Models of IP\(_3\)R). Therefore, the variability of the flux mainly lies in the complexity of the IP\(_3\)R model. As we have seen, different IP\(_3\)R models could have very different structures and thus completely different expressions for the open probability.

An IP\(_3\)R-based calcium model is obtained by coupling the calcium model (Eqs. 2.7 and 2.8) and an IP\(_3\)R model. In general, there are two different ways of coupling based on different assumptions. The first is assuming infinitely many IP\(_3\)Rs everywhere and \([\text{Ca}^{2+}]_i\) in the oscillatory region is homogeneous (well-mixed) so that the region can be seen as a point where \(P_o\) is always equal to the proportion of the open IP\(_3\)Rs. This allows us to convert the Markov IP\(_3\)R model to a set of ODEs, which, together with the two calcium ODEs, become a large system of ODEs that can be solved simultaneously (see Section 5.3 for an example). This type of model is called a deterministic model, as the solutions are completely determined by the choice of the parameters and initial conditions, and the simulated \(\text{Ca}^{2+}\) oscillations are exactly periodic. This model has an advantage of fast simulation (as many efficient numerical methods have been developed for the ODEs), but its underlying assumptions are wrong and the simulated periodic oscillations are seriously challenged by experimental data which show the oscillations are fundamentally stochastic [109, 145].
The second way is based on a more realistic assumption that there are only a limited number of independent IP$_3$Rs underlying the oscillations, which is called a stochastic model. A stochastic model can take many different forms, and the intuitive one could be a fully numerical realization of the stochastic openings and closings of individual IP$_3$Rs distributed randomly in 3-dimensional space. In this case, the calcium model becomes PDEs with discrete Ca$^{2+}$ release sites at individual IP$_3$R channel pores. The current state of IP$_3$R is only affected by its last state due to the Markov property, and Ca$^{2+}$ release at each IP$_3$R channel is determined only by the current state of the IP$_3$R. Interactions among the IP$_3$Rs by Ca$^{2+}$ is accomplished by Ca$^{2+}$ diffusion from open IP$_3$Rs to closed ones. The states of the IP$_3$Rs can be calculated using the Gillespie method [67] or the hybrid Gillespie method [131], and the calcium PDEs can be solved using finite difference or finite element methods. To get sufficient accuracy, a fine spatial and temporal discretization is required, which, however, is still computationally intractable.

To overcome the computational issues, many attempts have been made to simplify the full stochastic model. Upon opening of an IP$_3$R, the Ca$^{2+}$ profile around the channel could be assumed to be quickly established due to a fast local Ca$^{2+}$ diffusion. This allows us to simplify the complicated time/spatial-dependent solutions (solved by finite differences or finite elements) to a superposition of several local quasi-stationary concentration profiles (determined by the current states of the IP$_3$Rs, and thus speed up the simulations [9, 157, 146]. In addition, the feature of the tightly clustered IP$_3$Rs implies that [Ca$^{2+}$]$_i$ in each cluster is approximately homogeneous so that each cluster could be modeled by either a ball of varying radius depending on the number of open channels or a point-source calcium model. This allows us to either completely remove Ca$^{2+}$ diffusion for the studies of Ca$^{2+}$ puffs or dramatically decrease the resolution of the spatial mesh required for simulating Ca$^{2+}$ waves [138, 53, 168, 162, 146].

Another simplification I would like to emphasize is the use of two [Ca$^{2+}$]$_i$ to model the heterogeneity of the intra-cluster Ca$^{2+}$ concentration [116, 132, 133]. One is an instantaneous high [Ca$^{2+}$]$_i$ at any open IP$_3$R channel mouth for determining the next state of each open IP$_3$R, and the other is an average low [Ca$^{2+}$]$_i$ at any closed IP$_3$R mouth for determining the next states of all the closed IP$_3$Rs. The instantaneous high pore [Ca$^{2+}$]$_i$ can be modeled by a simple large value (say > 100 µM), whereas the average low [Ca$^{2+}$]$_i$ needs to be updated by solving Eq. 2.7. This simplification is based on a concept of scale separation. Temporally, the Ca$^{2+}$ release through an open channel to reach a steady level of [Ca$^{2+}$]$_i$ at the channel mouth is far faster (usually within 1 µs) than the average elevation of [Ca$^{2+}$]$_i$ over the domain of the cluster (taking on average about 1 ms). Spatially, the concentration profile around an open channel exhibits a very steep gradient from > 100 µM at the channel mouth down to < 1 µM at 100 – 200 nm away from the mouth. This simplification allows us to use a rather simple point-source model without loss of a detailed description of the intra-cluster heterogeneity.
Calcium flux through the RyR

Similar to the IP$_3$R, RyR flux normally takes the form that $J_{\text{RyR}} = P_{\text{ryr}} O_{\text{ryr}} (c_e - c)$, where $P_{\text{ipr}}$ is the maximum conductance and $O_{\text{ryr}}$ is the transient open probability of the RyR. The key component is $O_{\text{ryr}}$ whose calculation needs an RyR model. In the literature, there have been a considerable number of RyR models constructed for different purposes [62, 63, 93, 87, 159, 156, 76, 137, 8, 181], and most of the models are similar to the early IP$_3$R models in terms of modeling approaches because of their similarities in triggering CICR. However, the use of those models needs more caution, as almost all the RyR models were derived for dyadic clefts in cardiac myocytes where RyR experience a specific local environment, and might be thus poor candidates for RyR expressed on general parts of the ER. Details of the RyR models will not be provided here, but will be given whenever necessary.

Calcium leak flux through ER membrane

Although the real mechanism of the Ca$^{2+}$ leak is hard to investigate and thus is still not fully clear in many cells, it seems that the leak is a relatively slow process and is not mediated by the IP$_3$R or RyR [81, 27]. Therefore, the leak including the leak currents through the IP$_3$R, RyR and many other pathways is usually treated as a single flux term for balancing the flux generated by the SERCA pumps at resting state. The models usually take the simplest forms adequate for the purposes of studies. For example, the most intuitive model is a linear function of the concentration difference between $c_e$ and $c$ (i.e. $J_{\text{leak}} = P_{\text{leak}} (c_e - c)$ where $P_{\text{leak}}$ is the total conductance); Also, the flux could be assumed to be constant or voltage-dependent, or even mediated by the reverse mode of the SERCA [136].

SERCA pump

The simplest model of the SERCA pump giving a good approximation to the experimental data is the Hill function,

$$J_{\text{serca}} = \frac{V_s c^n}{K_s^n + c^n}, \quad (2.10)$$

where $K_s$ is the concentration value at which $J_{\text{serca}}$ attains a half of its maximum capacity $V_s$, and the Hill coefficient $n$ is around 2 [100, 33]. Due to a relatively large variability among different species, the choice of the values of $V_s$ and $K_s$ is still a bit arbitrary, but $K_s$ is usually assumed to be relatively small ($< 0.5 \mu M$). Although this simple model is adequate for most studies of calcium oscillations, this model has some drawbacks that it is a unidirectional flux and is independent of the ER Ca$^{2+}$ concentration, which are oversimplifications of the experimental data [57, 112, 136]. Thus, for some specific studies that touch the weak points of the Hill function model, one may use a more detailed and complicated SERCA model, such as the one proposed by MacLennan et al. (although it has 10 parameters to determine) [101] and the one
2.2. MODELING CALCIUM DYNAMICS AND IP$_3$-INDUCED OSCILLATIONS

by Tran et al. which took more factors like metabolic-sensitivity, Ca$^{2+}$- and H$^+$-sensitivity and thermodynamic constraints into consideration [176]. Besides the above, there are some relatively simple SERCA models available to use [186, 28].

**Calcium influx**

Ca$^{2+}$ entry into the cells occurs in many different ways via different types of channels, some of which are voltage-dependent and some are ligand- or even ER Ca$^{2+}$ content-dependent. Thus, their models are also quite different.

- Voltage-gated calcium channels are expressed in almost all the types of cells, and they are particularly important for excitable cells, like neurons, to induce Ca$^{2+}$ entry by a depolarization of the membrane potential. Most of the models of those channels are based on the classical Hodgkin–Huxley formalism [182, 161].

- Receptor-operated calcium channels (ROCC) require binding of ligand (e.g. agonist) to open. Although the relation of the ROCC flux (denoted by $J_{rocc}$) and the concentration of the agonist ($a$) is not known and may vary for different cell types, it has been known that $J_{rocc}$ is an increasing function of $a$. Given that IP$_3$ concentration ($p$) is also an increasing function of $a$, $J_{rocc}$ could then be simply assumed to be an increasing function of $p$. The choice of the increasing function is still arbitrary, and a simple way is to assume a linear function, i.e. $J_{rocc} = a_1 + a_2 p$.

- Store-operated calcium channels (SOCC), as it is named, are a type of channel sensitive to the ER Ca$^{2+}$ load. Since they open when ER is highly depleted, the SOCC flux ($J_{socc}$) is a decreasing function of $c_e$. An example can be seen in [37].

In addition, a small basal influx is assumed for balancing the PMCA flux at resting state. Hence, we can see that $J_{in}$ is usually a combination of the fluxes through several major calcium channels, which is thus modeled by a sum of different inward fluxes with different mathematical models. An example is given in Chapter 5, where the influx consists of receptor-operated Ca$^{2+}$ entry, store-operated Ca$^{2+}$ entry, and a leak flux.

**PMCA pump**

Although the PMCA shows some molecular differences from the SERCA, like the presence of one instead of two Ca$^{2+}$ binding sites, and has smaller Ca$^{2+}$ binding affinity than that of SERCA, they have very similar kinetics and functions [98, 23]. Therefore, PMCA usually takes the same model form as that of SERCA, and the simplest one is

$$J_{pm} = \frac{V_p c^n}{K_p^n + c^n},$$  \hspace{1cm} (2.11)
where $K_p$ is the concentration value at which $J_{pm}$ attains a half of its maximum capacity $V_p$, and the Hill coefficient $n$ is about 2. Still, the choice of $V_p$ and $K_p$ could be relatively arbitrary within their physiological ranges.

Here I would like to comment that, for modeling Ca$^{2+}$ puffs and oscillations, the exact forms of the SERCA or PMCA models are not crucial, if the models are able to capture the feature of the continuously increasing Ca$^{2+}$-dependency in Eqs. 2.10 and 2.11. Thus, linear functions of $c$ have been used in many simulations and have shown a great power in fire-diffuse-fire modeling of Ca$^{2+}$ waves and developing analytical solutions for model simplifications [36, 9, 167, 172].

**Calcium buffers**

Ca$^{2+}$ buffering is usually simply modeled as the chemical reaction

$$B + Ca^{2+} \xrightleftharpoons{} k_{on} \xleftleftharpoons{} k_{off} CaB,$$

where B represents the unbounded buffer and CaB is the bounded buffer. $k_{on}$ and $k_{off}$ are the rates of Ca$^{2+}$-binding and Ca$^{2+}$-dissociation, indicating how fast the time scale of the buffer dynamics is. Buffers with relatively large (small) $k_{on}$ are called fast (slow) buffers. The ratio of $k_{off}$ to $k_{on}$ is the dissociation constant $K_d$ ($= k_{off}/k_{on}$), a measure of the affinity between the buffering protein and Ca$^{2+}$. In living cells, there are many type of buffers with different $k_{on}$ and $K_d$ modulating Ca$^{2+}$–signaling in different manners [54, 188]. But here I will present an example with only one buffer as an introduction, as more buffer types can be easily added in the model in the same way.

Letting $b$ and $B_t$ be the concentrations of CaB and the total buffer (i. e. B + CaB) respectively, based on the mass action kinetics, the inclusion of a calcium buffer in calcium models is usually achieved by the coupling of the calcium equation and the buffer equation,

$$\frac{dc}{dt} = J_{PR} + J_{RYR} + J_{leak} - J_{serca} + J_{in} - J_{pm} - k_{on}(B_t - b)c + k_{off}b, \quad (2.12)$$

$$\frac{db}{dt} = k_{on}(B_t - b)c - k_{off}b. \quad (2.13)$$

Without buffer diffusion, it refers to a stationary buffer. However, a considerable number of buffers are mobile, the models of which require a diffusion term in Eq. 2.13 and thus,

$$\frac{\partial b}{\partial t} = \nabla \cdot (D_b \nabla b) + k_{on}(B_t - b)c - k_{off}b, \quad (2.14)$$

where $D_b$ is the diffusivity of the buffer.

Buffers can be simplified under some assumptions, so that the calcium model does not need to include buffer terms explicitly [92, 68]. One common assumption is that the buffer is immobile and has sufficiently fast kinetics (called rapid buffer approximation), i.e. binding rate
and dissociation rate of the buffer are sufficiently larger than the rates of other Ca\(^{2+}\) fluxes. Taking \(b\) to be in quasi-steady state, we have

\[
    k_{\text{on}}(B_t - b)c - k_{\text{off}}b = 0,
\]

which gives \(b = B_t c / (c + K_d)\). Adding Eq. 2.12 and Eq. 2.13 and letting \(J_{\text{IPR}} + J_{\text{RYR}} + J_{\text{leak}} - J_{\text{serca}} + J_{\text{in}} - J_{\text{pm}}\) be \(F(c)\), we have

\[
    \frac{d(c + b)}{dt} = F(c).
\]

Replacing \(b\) by its quasi-steady level, \(B_t c / (c + K_d)\), we can obtain a new ODE describing the evolution of \(c\) with the buffer implicitly treated in the model,

\[
    \frac{dc}{dt} = \frac{F(c)}{\theta},
\]

where \(\theta = 1 + B_t K_d / (c + K_d)^2\). We can note that all the original Ca\(^{2+}\) fluxes have been rescaled by a factor of \(1/\theta\). If we re- denote the scaled flux by \(f(c)\), i.e. \(f(c) = F(c)/\theta\), then we have a simpler form of the model \(dc/dt = f(c)\) which is the common form extensively used in the study of Ca\(^{2+}\) dynamics.

Another buffer approximation is assuming excess buffer is present (so called excess buffer approximation) so that \(B_t - b \approx B_t\). In this case, Eq. 2.14 becomes

\[
    \frac{\partial b}{\partial t} = \nabla \cdot (D_b \nabla b) + k_{\text{on}}B_t c - k_{\text{off}}b,
\]

and the buffer term in Eq. 2.12 is accordingly changed.
Chapter 3

AN IMPROVED IP$_3$R MODEL BASED ON THE SIEKMANN MODEL

Due to the importance of the IP$_3$R in regulating Ca$^{2+}$ puffs and oscillations, a better understanding of such Ca$^{2+}$ signals relies on an accurate model of the IP$_3$R, a model that can generate the correct statistical properties of the openings and closings of a single IP$_3$R.

The most recent IP$_3$R model is due to Siekmann et al. [144]. The advantage of the Siekmann model is that its topological structure and transition rates are determined by Markov chain Monte Carlo (MCMC) fitting [143] directly to the stationary single-channel current traces instead of fitting only to statistical distributions. However, the Siekmann model fails to describe some earlier findings of transient behaviors shown in [105].

Although, in theory, the openings and closings of the IP$_3$R (in a stationary state) will contain enough information to characterize completely the rate constants in the Markov model, and thus determine the non-stationary behavior also, in practice this is not the case here. Our stationary data, although sufficient to characterize the stationary behavior of the IP$_3$R, does not contain enough information also to determine the transient behavior of the receptor measured in [105].

To overcome this problem, we develop a new IP$_3$R model with time-dependent state-transitions by incorporating the recent non-stationary single-channel data from [105] into the Siekmann model. Both of these data sets are measured from intact cells, and thus represent a significant improvement on the data used to construct previous models of the IP$_3$R.

The chapter is part of the co-authored work published in Biophysical Journal (see [29] for publication details). Details about authors’ contribution are given in a signed co-authorship form provided right before the main text of this thesis.

3.1 More details about the Siekmann model

I have introduced the Siekmann model in Chapter 2, but left some details which are inappropriate a simple introduction but are important for the new model construction. Therefore, they
will be introduced here.

Recall that in the Siekmann model (Fig. 2.2), the transitions $q_{24}$ and $q_{42}$ connecting the park and drive modes determine the open probability of the IP$_3$R, and are dependent on $[Ca^{2+}]_i$, [IP$_3$] and [ATP] [179]. For given $[Ca^{2+}]_i$, [ATP] and [IP$_3$], the posterior distributions of $q_{24}$ and $q_{42}$ can be computed using the MCMC fitting, and then the mean values of the distributions are obtained. By varying $[Ca^{2+}]_i$, different means of the posterior distributions of $q_{24}$ and $q_{42}$ are all computed and plotted as a function of $[Ca^{2+}]_i$ (see symbols in Fig. 3.1). Smooth $Ca^{2+}$-dependencies can then be obtained by the functions fitting to the symbols.

The experiments tried two different [IP$_3$]s, and thus two sets of $Ca^{2+}$-dependencies were obtained by MCMC fitting (see Fig. 3.1). We can see that a higher [IP$_3$] leads to a higher rate of $q_{42}$ responsible for opening the IP$_3$R channel. The IP$_3$ dependency can then be interpolated by functions.

Moreover, the stationary data were measured only at 0.1 mM and 5 mM [ATP]s [179, 144]. We use 0.1 mM [ATP], because it is closer to the [ATP] of 0.5 mM used for obtaining the dynamic data of [105] which we will use to develop our IP$_3$R model. Investigating the effect of varying [ATP] needs more data and is beyond our current scope.

![Figure 3.1: Stationary transition rates, $q_{24}$ and $q_{42}$, as functions of Ca$^{2+}$ concentration (c). Circles and squares represent the means of $q_{24}$ and $q_{42}$ distributions computed by MCMC simulation. The corresponding fitting curves are produced using Eqs. 3.4 – 3.9. Note that the data point for 100 µM in (a) is not considered during fitting. First, our current model cannot well include this data point without violating good fits for other data points. Second, data from [105] showed that the channel would never open for $c = 300µM$ where $q_{42}$ should be very small. In conjunction with small value of $q_{42}$ at $c = 10μM$, one would expect a similar small value of $q_{42}$ at $c = 100µM$. Third, for $c = 100µM$ where inhibitory effect is relatively large, limited duration of single-channel measurement could not contain enough information about the channel kinetics, which could also lead to inaccurate parameter estimation.](image-url)
3.2 Incorporation of non-stationary data

Mak et al. [105] measured dynamic properties of the response of the IP$_3$R to step changes in [Ca$^{2+}$]$_i$ or [IP$_3$]. Fig. 3.2 shows examples of the experimental data. In response to a step change of Ca$^{2+}$ concentration that either from under 0.01 µM to 2 µM or from 2 µM to under 0.01 µM (indicated by colored bars) at 10 µM IP$_3$ (green bar), the channel experienced a latency (grey bar) before switching its mode (inactivated or highly activated). By gathering sufficiently many latency samples, the latency distributions were then used to reveal single-channel dynamics.

We modify the Siekmann model to incorporate these dynamic data. We accomplish this by assuming that $q_{24}$ and $q_{42}$ are controlled by gating variables which evolve on different timescales, which can be determined from the data of [105], without changing their steady-state properties which are determined by the data of [144].

Incorporation of these dynamic properties does not change the stationary behavior of the IP$_3$R model. Hence, the fact that $q_{24}$ and $q_{42}$ are saturating functions of [Ca$^{2+}$]$_i$, which was an important feature of the Siekmann model, is preserved in our new model. It turns out that these dynamic data are in fact crucial for the proper behavior of puffs and oscillations in the models, which will be seen in Chapters 4 and 5.

It is important to note that this modification of the Siekmann model is not based on rigorous MCMC fits to the data of [105]. Instead, we choose the gating variables so as to give approximately correct distributions for the response to step changes of [Ca$^{2+}$]$_i$. It is left for future work to do rigorous MCMC fits to both the steady-state and dynamic data simultaneously.

The transition rates $q_{24}$ and $q_{42}$ are given by

$$ q_{24} = a_{24} + V_{24}(1 - m_{24}h_{24}), $$

$$ q_{42} = a_{42} + V_{42}m_{42}h_{42}, $$

where $m_{24}$, $h_{24}$, $m_{42}$ and $h_{42}$ are the gating variables that determine the values of $q_{24}$ and $q_{42}$. $a_{24}$, $a_{42}$, $V_{24}$ and $V_{42}$ are either functions of [IP$_3$] or constants, and are given later.

We assume they obey the following differential equations,

$$ \frac{dG}{dt} = \lambda_G(G_{\infty} - G), \quad (G = m_{24}, h_{24}, m_{42}, h_{42}), $$

where $G_{\infty}$ is the equilibrium and $\lambda_G$ is the rate at which the equilibrium is approached. The $G_{\infty}$ are functions of [Ca$^{2+}$]$_i$ and are given by

$$ m_{24\infty} = \frac{c_{m24}^{n24}}{c_{m24}^{n24} + k_{24}^{m24}}, $$

$$ h_{24\infty} = \frac{k_{-24}^{n-24}}{c_{-24}^{n-24} + k_{-24}^{n-24}}, $$
Figure 3.2: Latency of mode-switch of a single IP₃R channel in response to a step change in Ca²⁺ concentration at fixed 10 µM IP₃ concentration and its distributions. Green bars indicate IP₃ concentration. Grey bars in (a) and (b) indicate latency duration. Other color bars indicate changes of Ca²⁺ concentration (in µM). Note that the "< 10" should be "< 0.01" in (d) which is an error in the original article [105]). Blue curves are the theoretical fits obtained by using different linear Markov chains with the tabulated number of functionally indistinguishable states (see [105] for details). Black curves are the sums of related fits. For (c)–(f), y axis is number of observations and x axis is latency (in second). This figure is adapted from [105].
3.2. INCORPORATION OF NON-STATIONARY DATA

\[ m_{42\infty} = \frac{c^{n_{42}}}{c^{n_{42}} + k_{42}^{n_{42}}} \]  
\[ h_{42\infty} = \frac{k_{n_{-42}}^{n_{-42}}}{c^{n_{-42}} + k_{-42}^{n_{-42}}} \]  

(3.6)

(3.7)

Hence, the stationary expressions of \( q_{42} \) and \( q_{24} \) are given by

\[ q_{24\infty} = a_{24} + V_{24}(1 - m_{24\infty}h_{24\infty}) \]  
\[ q_{42\infty} = a_{42} + V_{42}m_{42\infty}h_{42\infty} \]  

(3.8)

(3.9)

where the expressions of \( a_s, V_s, n_s \) and \( k_s \) are chosen by interpolating data at two \([IP_3]\)s using Hill functions, which guarantees that Eqs. 3.8 and 3.9 give good fits to the experimental values of \( q_{24} \) and \( q_{42} \) shown in Fig. 3.1.

\[ V_{24} = 60 + 437/(p^3 + 1.73), \quad a_{24} = 1 + 7.5/(p^2 + 0.25), \]
\[ V_{42} = 100, \quad a_{42} = 1.8p^2/(p^2 + 0.34), \]
\[ n_{24} = 6.3 + 1.72p^2/(p^2 + 1.44), k_{24} = 0.48 + 0.1/(p^2 + 1.44), \]
\[ n_{-24} = 8.2p^2/(p^2 + 2.25), k_{-24} = 79.75 + 25/(p^2 + 1.44), \]
\[ n_{42} = 5.9 + 7.6/(p^2 + 1.44), k_{42} = 0.4 + 0.26p^4/(p^4 + 168), \]
\[ n_{-42} = 3.2 + 4.88p^2/(p^2 + 1.69), k_{-42} = 0.17 + 70p^3/(p^3 + 274.6). \]

Other constant transitions are given in Table 3.1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value/Units</th>
<th>Parameter</th>
<th>Value/Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>( q_{12} )</td>
<td>1240 s(^{-1} )</td>
<td>( q_{21} )</td>
<td>88 s(^{-1} )</td>
</tr>
<tr>
<td>( q_{23} )</td>
<td>3 s(^{-1} )</td>
<td>( q_{32} )</td>
<td>69 s(^{-1} )</td>
</tr>
<tr>
<td>( q_{26} )</td>
<td>10500 s(^{-1} )</td>
<td>( q_{62} )</td>
<td>4010 s(^{-1} )</td>
</tr>
<tr>
<td>( q_{45} )</td>
<td>11 s(^{-1} )</td>
<td>( q_{54} )</td>
<td>3330 s(^{-1} )</td>
</tr>
</tbody>
</table>

Table 3.1: All the transition rates in the Siekmann model except \( q_{24} \) and \( q_{42} \) [144].

Note that for the case of 10 \( \mu M \) IP\(_3\), the MCMC method fails to work out convergent distributions of \( q \)'s when \([Ca^{2+}]_i \) is between 1 – 50 \( \mu M \), as the receptor is almost always in the drive mode so that limited length of the single-channel data fail to show any mode switches. Therefore, for this range, we assume a saturated large \( q_{42} \) value and a saturated small \( q_{24} \).

Note that the choice of gating variables for \( q_{24} \) and \( q_{42} \) is purely a heuristic idea. Bell-shaped/bowl-shaped curves have seen in many experimental studies, and are a result of a combination of activation and inhibition processes. By introducing time-dependent gating variables to
control the two processes, we found it could not only incorporate non-stationary single-channel data (non-exponential latency distributions) into the stationary IP3R model, but also reproduce the puff data that were not used for channel model construction (see Chapter 4).

The rates $\lambda_{m24}$, $\lambda_{h24}$ and $\lambda_{m42}$ in Eq. 3.3 are evaluated based on the latency distributions of mode-switches of an IP3R from [105] (i.e. Fig. 3.2c–f). Considering the data and the IP3R model together, we see that different changes of $[Ca^{2+}]_i$ actually reveal different values of the $\lambda$s. For example, a step increase of $[Ca^{2+}]_i$ from 10 nM to 2 $\mu$M leads to a mode switch of an IP3R from the park mode (or some undetected inactivation mode, as no stationary single channel data at 10 nM $[Ca^{2+}]_i$ is provided) to the drive mode. During the process, activation latency is solely influenced by the activation of $m_{42}$ (as $k_{-42} \gg 2 \mu$M so $h_{42}$ is nearly constant in this range of $[Ca^{2+}]_i$), which therefore tells us that the value of $\lambda_{m42}$ could be roughly estimated if we can generate a simulated latency distribution in good agreement with the experimental data. Fig. 3.3a shows the activation latency histogram using $\lambda_{m42} = 100$ s$^{-1}$. It exhibits a good agreement on the range and the location of the peak with the experimental histogram in [105] except showing a smaller dispersion.

Similarly, $\lambda_{m24}$ and $\lambda_{h24}$ are roughly estimated to be 100 s$^{-1}$ and 40 s$^{-1}$ respectively (by comparing Fig. 3.2e–f with Fig. 3.3b–c). We note that experimental latency histogram for $\lambda_{h24}$ (i.e. Fig. 3.2e) clearly contains more than one peak which the current two-mode IP3R model cannot achieve. To make sure the IP3R model captures the most dominant feature, we only consider the largest peak and use it to estimate $\lambda_{h24}$. Possible reasons for explaining the multimodal distribution could be mode changes caused by randomness of IP3 binding or unbinding or the existence of some undetected inactivation modes at such a high $[Ca^{2+}]_i$ of 300 $\mu$M (which is beyond the range used to get the stationary single channel data [179]).

The estimation of $\lambda_{h42}$ is unsuccessful, as no value can give an acceptable histogram comparable to the experimental data (see Fig. 3.3d and Fig. 3.2f). This could be due to the simplicity of the model wherein only two modes are considered. On the other hand, it is also possible that some unusual modes mislead the estimation of $\lambda_{h42}$, as the experiments in [105] use a wider range of $[Ca^{2+}]_i$ than that in [179] and [144] which constrain it to a physiological range. Since only two modes are unambiguously found by the MCMC methods (for the physiological range of $[Ca^{2+}]_i$), we will not add any more states or modes to the Siekmann model. Instead, we shall estimate $\lambda_{h42}$ from puff data in [147], where it is found that most puffs exhibit fast increases from baseline to the peak with sharp peaks instead of plateaus. This reveals that IP3Rs are inhibited by high $[Ca^{2+}]_i$ very quickly and are hard to reopen immediately, telling us that $\lambda_{h42}$ is relatively small for low $[Ca^{2+}]_i$ level but relatively large for high $[Ca^{2+}]_i$. Therefore, we model $\lambda_{h42}$ by

$$\lambda_{h42} = a_{h42} + \frac{V_{h42}c^7}{c^7 + 20^7}.$$  \hspace{1cm} (3.10)

This is merely a heuristic way of modeling a step-wise rate that is low at low $[Ca^{2+}]_i$ and high at high $[Ca^{2+}]_i$. Due to a very fast $Ca^{2+}$ release rate at open channel mouth, the channels seem
3.2. INCORPORATION OF NON-STATIONARY DATA

Figure 3.3: Simulated latency histograms for different step changes of $[\text{Ca}^{2+}]_i$ labelled in the titles of the subfigures. We set $p = 10 \mu\text{M}$. For (d), $\lambda_{h_{42}} = 1 \text{s}^{-1}$.

to only experience two levels of $[\text{Ca}^{2+}]_i$, and the values of $\lambda_{h_{42}}$ for intermediate $[\text{Ca}^{2+}]_i$ thus play a little role. Because we have no information about the exact concentration at which this transition occurs, we arbitrarily assume it to occur at 20 $\mu\text{M}$. In addition, we choose $V_{h_{42}} = 100 \text{s}^{-1}$ to represent fast inhibition by $\text{Ca}^{2+}$. Due to lack of information about $d_{h_{42}}$, we use it as a parameter. Later studies of $\text{Ca}^{2+}$ puffs (Chapter 4) will show that the parameter dominates the recovery rate of an IP$_3$R inhibited by $\text{Ca}^{2+}$, and in turn has a significant effect on interpuff intervals.

In this chapter, I present the details of the construction of the new IP$_3$R model. A validation of the IP$_3$R model needs applications in modeling $\text{Ca}^{2+}$ puffs and oscillations and to see if experimental data could be quantitatively reproduced, which will be shown in the following chapters.
Chapter 4

MODELING CALCIUM PUDDS

Ca\(^{2+}\) signaling is organized in a hierarchical manner [174]. At the lowest level, stochastic release of Ca\(^{2+}\) through a single IP\(_3\)R results in a small localized increase in cytoplasmic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)), called a Ca\(^{2+}\) “blip”. Due to the tightly clustered arrangement of IP\(_3\)R, a Ca\(^{2+}\) blip can stimulate the release of additional Ca\(^{2+}\) through neighboring IP\(_3\)Rs, to cause release from a cluster of IP\(_3\)Rs, giving a larger, but still localized, increase in [Ca\(^{2+}\)]\(_i\), called a Ca\(^{2+}\) “puff”. At the highest level of organization, if enough puffs are generated, they can form a propagating wave of increased [Ca\(^{2+}\)]\(_i\) across an entire cell.

Recent studies have shown that Ca\(^{2+}\) puffs reflect the quantal Ca\(^{2+}\) releases by stochastic openings of IP\(_3\)Rs [147]. This is very important to reveal how the IP\(_3\)Rs behave in vivo. Although there are a number of studies of Ca\(^{2+}\) puffs already in the literature [168, 116, 139, 140, 157, 75, 132, 178, 133], they are, without exception, all based on older IP\(_3\)R models, and thus fail to capture important aspects of the stationary behavior of single IP\(_3\)R. Hence, in light of recent data and models, it is necessary to reexamine the question of Ca\(^{2+}\) puff formation, and how the hierarchy of Ca\(^{2+}\) signaling is constructed. Moreover, a correct reproduction of puff statistics is essential for confirming the validity of an IP\(_3\)R model, which is another purpose of building a puff model based on the improved IP\(_3\)R model.

In this chapter, we particularly wish to address some outstanding questions in the field. There are a number of such questions, but the ones we address here are:

- What is the mechanism of puff termination? Do the IP\(_3\)Rs close due to inhibition by Ca\(^{2+}\), by stochastic attrition, or by some other inherent process?

- What determines the distribution of interpuff intervals? Thurley et al. [173] performed a detailed analysis of interpuff intervals (IPI), i.e., the waiting time between successive puffs, and found that some puff sites exhibit exponential IPI distributions but most sites have non-exponential distributions with a maximum at times larger than 0 s. This shows the stochastic occurrence of puffs is usually but not necessarily influenced by an inhibitory effect from the previous puffs, implying that the time constant of the inhibitory effect could be different for different puff sites. What kind of mechanism can generate both
exponential and non-exponential IPI distributions? Is it possible that the mechanism is intrinsic to the IP$_3$R instead of local depletion of the ER/SR which has been shown to be unlikely by [178]?

I will answer the questions by using a stochastic puff model based on the IP$_3$R model proposed in chapter 3.

The chapter is also part of the co-authored work published in Biophysical Journal (see [29] for publication details). Details about authors’ contribution are given in a signed co-authorship form provided right before the main text of this thesis.

4.1 The puff model

In Chapter 2, we have mentioned a method of using two different $[\text{Ca}^{2+}]_i$ to model Ca$^{2+}$ release; a high constant $[\text{Ca}^{2+}]_i$ at each open channel mouth and a low average $[\text{Ca}^{2+}]_i$ for all the closed channels. This method ignores Ca$^{2+}$ diffusion and the spatial distribution of the IP$_3$Rs, and therefore dramatically increases the computational efficiency. Here we apply the same idea to build our puff model. A detailed justification of this assumption is given in Chapter 2 and [116, 132, 133].

Let $c$ be the average low $[\text{Ca}^{2+}]_i$ and let $c_m$ be the $[\text{Ca}^{2+}]_i$ at the IP$_3$R mouth. Before formulating the model, some assumptions need to be made.

- Ca$^{2+}$ fluxes through the plasma membrane do not influence Ca$^{2+}$ puffs.

- The rate of Ca$^{2+}$ release through single IP$_3$R is a constant. In other words, there is sufficiently high $[\text{Ca}^{2+}]_i$ in the ER/SR to keep a nearly constant flux. Although local depletion of the ER/SR is possible for some types of cells, it should not be the case for puffs observed in [44, 147], as $[\text{Ca}^{2+}]_i$ can be kept at an elevated level when the channel is sustainedly open and the stepwise increments of those puffs do not get progressively smaller.

- The endogenous fast buffers are immobile, unsaturated and in quasi-steady state. Relaxation of these assumptions needs more detailed studies on buffer effect. Our preliminary results show that buffers do not qualitatively alter the properties of IPI, which are consistent with the results from Skupin et al. [146] that buffers have little effect on the signal-to-noise ratio.

- The limited effect of endogenous slow buffers on puff dynamics is ignored. We also ignore the effect of EGTA, a slow buffer which is used in the experiments to isolate different puff sites by decreasing the effective Ca$^{2+}$ diffusivity [147].
With these assumptions, the differential equations governing the dynamics of $c$ are

$$\frac{dc}{dt} = J_{\text{increase}}N_o + J_{\text{leak}} - J_{\text{decrease}} - k_{\text{off}}b_{\text{fluo4}}, \quad (4.1)$$

$$\frac{db_{\text{fluo4}}}{dt} = k_{\text{on}}(B_{\text{fluo4}} - b_{\text{fluo4}})c - k_{\text{off}}b_{\text{fluo4}}. \quad (4.2)$$

$J_{\text{increase}}$ is the Ca$^{2+}$ flux contributing to the increase of $c$. $J_{\text{decrease}}$ represents the flux (mainly via diffusion and SERCAs) removing Ca$^{2+}$ from the puff site and is modeled by $V_d c/(c + K_d)$, where $V_d$ and $K_d$ are constants. In addition, a linear model of $J_{\text{decrease}}$ with an appropriate conductance can also be used. $J_{\text{leak}}$ is Ca$^{2+}$ leakage from the ER/SR, and is necessary for establishing a stable resting $[\text{Ca}^{2+}]_i$. A Ca$^{2+}$ dye (fluo-4) is added to the model, as all the experimental statistical analyses are done by using the fluorescence ratio instead of $[\text{Ca}^{2+}]_i$. $B_{\text{fluo4}}$ and $b_{\text{fluo4}}$ represent the total fluo-4 concentration and Ca$^{2+}$-bound fluo-4 concentration respectively.

$N_o$ denotes the number of open IP$_3$Rs and satisfies $0 \leq N_o \leq N_{\text{IPR}}$ (total number of functional IP$_3$Rs). It is computed by direct counting of the IP$_3$R states, and then is used to calculate $c$ by integrating Eq. 4.1. To determine the state of each IP$_3$R, we use $c_m$ which is modeled by

$$c_m = c + c_h \delta, \quad (4.3)$$

where $\delta$ indicates whether the IP$_3$R is open ($\delta = 1$) or closed ($\delta = 0$). $c_h$ is the constant high $[\text{Ca}^{2+}]_i$ at an open receptor mouth. $\delta$ is a stochastic variable that depends on $c_m$ via the stochastic solution of the IP$_3$R model. All the parameter values are given in Table 4.1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value/Units</th>
<th>Parameter</th>
<th>Value/Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c_0$</td>
<td>0.1 $\mu$M</td>
<td>$V_d$</td>
<td>4000 $\mu$M $\cdot$ s$^{-1}$</td>
</tr>
<tr>
<td>$J_{\text{increase}}$</td>
<td>200 $\mu$M $\cdot$ s$^{-1}$</td>
<td>$K_d$</td>
<td>12 $\mu$M</td>
</tr>
<tr>
<td>$J_{\text{leak}}$</td>
<td>33 $\mu$M $\cdot$ s$^{-1}$</td>
<td>$k_{\text{on}}$</td>
<td>150 $\mu$M$^{-1}$ $\cdot$ s$^{-1}$</td>
</tr>
<tr>
<td>$B_{\text{fluo4}}$</td>
<td>20 $\mu$M</td>
<td>$k_{\text{off}}$</td>
<td>300 s$^{-1}$</td>
</tr>
<tr>
<td>$c_h$</td>
<td>120 $\mu$M</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1: Parameter values for the puff model. Parameters relating to fluo-4 are obtained from [139]. Resting $[\text{Ca}^{2+}]_i$, $c_0$, is set to be a typical value of 0.1 $\mu$M. The value of $c_h$ is obtained from [132]. The remainder of the parameters are determined by requiring physiologically realistic simulations.

We solve Eqs. 4.1–4.2 in a deterministic way but apply a stochastic solver to the IP$_3$R dynamics. Eqs. 4.1–4.2 are solved by the fourth-order Runge-Kutta method (RK4). To solve
the stochastic IP$_3$R model, a hybrid Gillespie method (see Appendix A) with adaptive timing is used, in order that we may take into account the IP$_3$R-dependencies of transitions $q_{24}$ and $q_{42}$ [131]. We choose a maximum time stepsize of $10^{-4}$ s to guarantee accuracy. In addition, the 4 differential equations in Eq. 3.3 in the last chapter are solved by RK4 as well. All the numerical results are obtained using the software MATLAB (The MathWorks, Natick, MA).

4.2 Results

We study a number of aspects of calcium puffs. In particular, we focus on investigating how the puff statistics are influenced by the following three parameters:

- $[IP_3](p)$,
- recovery rate of an IP$_3$R from Ca$^{2+}$-inhibition ($a_{h24}$),
- the number of IP$_3$Rs at a puff site ($N_{IPR}$),

as they are found experimentally to be important to the puff dynamics.

4.2.1 Statistics of calcium blips

In our puff model, by letting $N_{IPR} = 1$, we can simulate blips and their statistics. Similar to the IPI, the interblip interval (IBI) is the waiting time between successive blips. Fig. 4.1a shows the IBI distribution is almost an exponential, demonstrating that the average recovery time of an IP$_3$R from self-inhibition is far shorter than the average IBI.

The blip duration distribution is also found experimentally to be an exponential [147], which is qualitatively reproduced by the model (see Fig. 4.1b). But the simulated average blip duration, about 63 ms, is significantly longer than 17 ms found in the experiments [147]. This could be because the residual $[Ca^{2+}]_i$ caused by a relatively slow drop of $[Ca^{2+}]_i$ after the closing of the channel induces stochastic reopening of the channel. To match the data, we need to either increase the rate of Ca$^{2+}$ removal or decrease the open time of the IP$_3$R, which requires new single-channel data in support. The quantitative difference could also cause a relatively longer puff duration, but this quantitative difference will not affect the fundamental properties of the puff model which will be shown to be able to qualitatively and even quantitatively reproduce many aspects of the Ca$^{2+}$ puffs.

The fitting curve is given by the following exponential distribution,

$$P_{BD} = \gamma e^{-\gamma t}, \quad (4.4)$$

which is derived from the below probability density function of puff duration proposed by Thurley et al. [173],

$$P_{PD} = N\gamma e^{-\gamma t}(1 - e^{-\gamma t})^{N-1}, \quad (4.5)$$
4.2. RESULTS

Figure 4.1: Interblip interval and blip duration distributions obtained by model simulation. We set $p = 0.2 \mu M$ and $a_{h_42} = 1 \text{ s}^{-1}$ (which is the benchmark value for $a_{h_42}$, as the parameters of IPI distribution obtained using this value lie roughly in the middle of experimental ranges observed in SH-SY5Y cells) for both cases. (a) The IBI distribution is fit by $\lambda e^{-\lambda t}$ with a $\lambda$ of $0.23 \text{ s}^{-1}$. (b) The blip duration distribution is fit by $\gamma e^{-\gamma t}$ with a $\gamma$ of $0.015 \text{ ms}^{-1}$.

Figure 4.2: Blip amplitude distribution obtained by model simulation. Samples are gathered from two simulations using two IP$_3$ concentrations, 0.1 $\mu M$ or 0.2 $\mu M$. Blip amplitude is defined to be $(F - F_0)/F_0$, the relative difference between $b_{fluor}$ and its resting level (the reference value). Error bar shows the mean of about 1.6 and standard deviation of about 0.9. We set $a_{h_42} = 1 \text{ s}^{-1}$.

where $N$ is the number of IP$_3$Rs that are open at the peak of the puff and $\gamma$ is the average closing rate of a single IP$_3$R. When $N = 1$, Eq. 4.5 becomes Eq. 4.4 containing only one parameter $\gamma$.

Fig. 4.2 shows the blip amplitude distribution with mean of about 1.6 and standard deviation of about 0.9. Based on the mean of blip amplitude, we choose 3 as the sampling threshold to
determine which are acceptable puffs in the simulated traces for statistical analysis.

### 4.2.2 Calcium puffs

Fig. 4.3 shows an example of simulated puff traces. $F/F_0$ represents the ratio of $b_{\text{fluor}}$ to its resting value. We can see a large puff usually needs openings of sufficiently many receptors. To investigate how puffs are initiated and terminated, we rescale the puff amplitude and plot it, together with $m_{42}$ and $h_{42}$, in Fig. 4.4 ($m_{42}$ and $h_{42}$ are calculated by averaging over all the IP$_3$Rs. Although averaging a gating variable over all the receptors does not reflect the behavior of individual receptors, it is a good indicator to show the potential ability of all the receptors to induce or inhibit Ca$^{2+}$ release.). In the lower panel of Fig. 4.4 we can see a “trigger” event at the beginning of the puff, which leads to a fast upstroke of the activation variable $m_{42}$, which then further increases the open probability of IP$_3$Rs. This trigger event has been found experimentally [129]. Termination of Ca$^{2+}$ release is achieved when $h_{42}$ gets close to zero. During the falling phase, open IP$_3$Rs close randomly and independently with an average open time determined by $m_{24}$ and $h_{24}$, as they are hard to open again when $h_{42}$ is very small. The slow recovery of $h_{42}$, which is due to a small value of $a_{h_{42}}$ used, influences the average IPI and the next puff amplitude. This will be investigated later.

![Figure 4.3: An example of simulation results of calcium puff traces. The top panel shows an example of the $[\text{Ca}^{2+}]_i$ trace (variable $c$ in Eq. 4.1). $F/F_0$ represents the ratio of $b_{\text{fluor}}$ to its resting value. We set $N_{\text{IPR}} = 10$ [147, 178], $p = 0.2 \mu\text{M}$, and $a_{h_{42}} = 1 \text{s}^{-1}$.](image)

We can see in Fig. 4.3 there are many small blips and other noise in the puff trace. To eliminate this noise, we choose puffs with amplitude larger than 3, i.e., $(F - F_0)/F_0 > 3$. The choice of this value is based on the simulated blip amplitude distribution and the mean blip amplitude of 1.6.
4.2. RESULTS

Figure 4.4: A close-up of some puffs in Fig. 4.3. The lower panel is an enlargement of the rectangular portion of the upper panel. The ratio $F/F_0$ (solid curve) is rescaled into the interval $[0,1]$ for ease of comparison with $m_{42}$ and $h_{42}$.

Fig. 4.5 shows the details about the IP$_3$R states (1 for open state; 0 for closed state) and modes (red for drive mode; black for park mode) during puffs. We can see the occurrence of each puff requires synchronized mode-switches from park mode to drive mode (indicated by red). Hence, the drive mode that exhibits a high open probability and a relative long dwell time behaves like an “open” state for triggering a substantial Ca$^{2+}$ release and thus subsequent CICRs to initiate puffs, but short openings in $O_5$ in park mode can hardly achieve this.

4.2.3 Dependence of IPI distribution on $a_{h_{42}}$

Experimental IPI distributions exhibit two different shapes, exponential and non-exponential [173]. The former indicates the channels recover back to their resting state very soon after each puff, whereas the latter implies a slow recovery process back to resting their state is involved
Figure 4.5: Details about the states and modes of the 10 IP₃Rs during simulated puffs from a part of Fig. 4.3 (0 – 20 s). For each IP₃R, 1 and 0 indicate open and closed respectively. If an IP₃R enters the drive mode, it is colored in red; otherwise, in black. The last panel summarizes the number of the IP₃Rs in either O₅ or O₆ (Nₒ), the same as the last panel of Fig. 4.3 but with colors to indicate the mode-switches.
Figure 4.6: Various IPI distributions are well fit by Eq. 4.6 or 4.7 in the main text with appropriate values of $\lambda$ and/or $\xi$ shown in each of the subfigures. 5 values of $a_{h_{42}}$, 0.1 s$^{-1}$ (a), 0.5 s$^{-1}$ (b), 1 s$^{-1}$ (c), 2 s$^{-1}$ (d) and 5 s$^{-1}$ (e), are chosen. We set $N_{IPR} = 10$ and $p = 0.1 \mu M$. A fitting method proposed by Thurley et al. [173] is used here, that $\lambda$ is first equal to the sample mean and $\xi$ is then obtained by least squares fitting (for Fig. 4.6e where an exponential distribution is more appropriate, only $\lambda$ is used). Note that the residuals seem not to be normally distributed, which might be due to the inability of the proposed Eq. 4.6 to fully reflect the complexity of the system.
CHAPTER 4. MODELING CALCIUM PUFFS

after each puff. A formula which gives excellent fits to experimental IPI distributions has been proposed by Thurley et al. [173] as follows,

\[ P_{IPI} = \lambda (1 - e^{-\xi t}) e^{[-\lambda t + \lambda (1 - e^{-\xi t})/\xi]}, \]  

(4.6)

where \( \lambda \) is the puff rate, a measure of the typical IPI (similar to average puff frequency), and \( \xi \) is the recovery rate. If \( \xi \gg \lambda \), Eq. 4.6 can be reduced to a simple exponential distribution,

\[ P_{IPI} = \lambda e^{-\lambda t}. \]  

(4.7)

We then use Eqs. 4.6 and 4.7 to fit the simulated IPI distributions (shown in Fig. 4.6). Each of the fit curves is obtained by choosing appropriate values of \( \lambda \) and/or \( \xi \) in Eq. 4.6 or 4.7 so that the corresponding IPI histogram can be fit nicely. A summary of the fits is shown in a different figure for a better comparison (see Fig. 4.7).

![Figure 4.7: Various simulated IPI distributions for different \( a_{h_{L2}} \). We set \( N_{PR} = 10, \rho = 0.1 \) \( \mu \)M. The values of \( a_{h_{L2}} \) are indicated in the legend. For a given \( a_{h_{L2}} \), we choose appropriate values of \( \lambda \) and \( \xi \) to fit to the corresponding simulated IPI distributions using Eq. 4.6 or 4.7. Then the values of \( \lambda \) and \( \xi \) are used to plot these curves in the figure.](image)

According to Fig. 4.6, the simulated values for \( \lambda \) vary from 0.1 s\(^{-1}\) to 0.5 s\(^{-1}\) and values for \( \xi \) vary from 0.5 s\(^{-1}\) to 2.2 s\(^{-1}\). These results are quantitatively consistent with the experimental ranges of \( \lambda \) and \( \xi \) found in SH-SY5Y cells, that from 0.18 s\(^{-1}\) to 0.5 s\(^{-1}\) for \( \lambda \) and from 0.4 s\(^{-1}\) to 4 s\(^{-1}\) for \( \xi \). Moreover, it is also found that in HEK 293 cells values for \( \lambda \) and \( \xi \) are in the ranges from 0.5 s\(^{-1}\) to 3 s\(^{-1}\) and from 1 s\(^{-1}\) to 90 s\(^{-1}\) respectively [173, 174].
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Refactoriness of simulated puffs comes from the slow recovery of $h_{42}$ from inhibition by $\text{Ca}^{2+}$. This implies that varying $a_{h42}$ could change the duration of refractoriness, and in turn change the shape of IPI distribution. This is confirmed by Fig. 4.7 which shows that increasing $a_{h42}$ from 0.1 $\text{s}^{-1}$ to 5 $\text{s}^{-1}$ leads to a change of the simulated IPI distribution from non-exponential to exponential.

4.2.4 Coefficient of variation is independent of $[\text{IP}_3]$

![Graph showing the relationship between standard deviation and mean of IPIs.](image)

Figure 4.8: Relation of standard deviation and mean of IPI is approximately linear and seems to be independent of $[\text{IP}_3]$. 6 $[\text{IP}_3]$s, 0.05, 0.1, 0.15, 0.2, 0.3 and 0.5 ($\mu$M), are used to generate the 6 points (from right to left) respectively. The points are expressed as mean ± standard error (SE) and are fit by a solid line with slope of 0.79 compared to the dashed line of CV = 1. The slope is significantly different from 1 ($p<0.005$). We set $N_{\text{IPR}} = 10$ and $a_{h42} = 1 \text{s}^{-1}$.

For a puff site, the IPI standard deviation has been shown to be approximately linearly related to the IPI mean [173]. A consistent result is given by the model (Fig. 4.8), where we plot the IPI standard deviation against the IPI mean. The coefficient of variation (CV) is defined to be the ratio of the standard deviation to the mean of IPIs. When CV = 1 the IPI is a homogeneous Poisson process (the dashed line), while when CV = 0 the IPI is a constant, giving an entirely periodic series of puffs. Other values of the CV between 0 and 1 indicate an inhomogeneous Poisson process with relative refractoriness. In our model, the CV is 0.79 which is in reasonable agreement with experimental values (that vary from 0.42 to 0.94 for different types of cells [173]). By increasing $a_{h42}$ to a large value (like 100), all the CVs will be almost 1 (on the dashed line in Fig. 4.8). Therefore, the model result not only confirms the
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non-exponential IPI distribution in Fig. 4.7, but also implies a relatively slow recovery of IP$_3$R from Ca$^{2+}$-inhibition could be a key mechanism of modulating IPI. We also find that changing the value of $a_{h42}$ can vary the CV between 0.65 and 0.95. The relation is not very clear, as $a_{h42}$ can also change some other statistics, like average IPI and puff amplitude, which could in turn influence the CV.

4.2.5 Dependence of puff amplitude on $a_{h42}$

To understand how $a_{h42}$ influences puff amplitude, we plot average puff amplitude and maximum puff amplitude for different values of $a_{h42}$ in Fig. 4.9. We find that, as $a_{h42}$ increases, both the average and maximum initially increase but then get saturated at about $a_{h42} = 0.5$ s$^{-1}$. Moreover, this trend seems to be independent on IP$_3$ concentration. The results show that very small recovery rate from Ca$^{2+}$-inhibition could eventually decrease observed puff amplitude, whereas not too small recovery rate would not significantly change puff amplitude. Therefore, If $a_{h42}$ can be known experimentally, we could then know whether the number of IP$_3$Rs is severely underestimated by observed puff amplitude and to what extent it is underestimated. Accordingly, if the number of IP$_3$Rs is known, we could also roughly estimate the range of $a_{h42}$.

4.2.6 Dependence of IPI on the number of IP$_3$Rs at a puff site

It has been found experimentally and by model simulations that the IPI mean is a hyperbolic function of $N_{IPR}$, the number of IP$_3$Rs at a puff site [44]. This implies a linear relationship between the IPI mean and $1/N_{IPR}$. This relationship is reproduced by our model (Fig. 4.10). Moreover, we find that varying [IP$_3$] changes the slope of the linear fit, but has little effect on the linearity of the relationship. We explain this as follows. If $P_o$ is the probability per unit time for opening of a single channel at baseline, then $N_{IPR}P_o$ is the probability per unit time for opening of one out of $N_{IPR}$ channels. Hence, the average IPI is proportional to $1/N_{IPR}$. Since a saturated low calcium sensitivity near the baseline of 0.1 $\mu$M is assumed in the IP$_3$R model (see Fig. 3.1), $P_o$ is nearly a constant for [Ca$^{2+}$]$_i$ close to baseline, which implies the average IPI is simply proportional to $1/N_{IPR}$.

For small numbers of IP$_3$R, the model (Fig. 4.10) has longer IPIs than does the data (Fig. 4E in [44]). There could be two reasons for this. One is that the applied [IP$_3$] is different. The other is that the cluster size estimated in the experiments is severely underestimated due to a very slow recovery rate (see Fig. 4.9). For either of these reasons, the discrepancy can be easily fixed by changing model parameters. But due to lack of information about either of them, a quantitative modification is beneficial for model improvement.
Figure 4.9: Dependence of puff amplitude on $a_{h42}$. Average puff amplitude (filled circle with error bar) and maximum puff amplitude (empty circle) are plotted for various values of $a_{h42}$. The average puff amplitude is expressed as mean ± standard deviation (SD). Puff amplitudes have been normalized to the mean blip amplitude of 1.6. We perform two simulations with two different IP$_3$ concentrations, 0.05 µM (a) and 0.1 µM (b), respectively. We set $N_{IPR} = 10$.

4.2.7 The relationship between IPI and latency

Puff latency is defined to be the waiting time from addition of IP$_3$ to the occurrence of the first puff [44]. The biggest difference between IPI and latency is that the former could contain some inhibition effect from the preceding puff whereas the latter definitely does not contain such an
Figure 4.10: Linear relationship between mean of IPIs and $1/N_{IPR}$, the reciprocal of the number of IP$_3$R at a puff site. $N_{IPR}$ is chosen to be 3 – 15, 20 and 25. For each value of $N_{IPR}$, means of IPIs for two different [IP$_3$]s, 0.1 µM and 0.2 µM, are computed and expressed as mean ± SE. For each [IP$_3$], a least-square linear fitting is performed and plotted using a solid line. We set $a_{h42} = 1$ s$^{-1}$.

Similarly to Fig. 4D in [44], we plot the IPI mean and latency for different $N_{IPR}$ (Fig. 4.11), and find that they are linearly related with a slope of about 1.2, and a positive IPI-axis-intercept of about 0.35 s (which are pretty close to the slope of 1.1 and intercept of 0.25 s found experimentally). The intercept is significantly different from 0 ($p<0.001$) and gives the average effective time of the inhibitory effect of the preceding puffs on the next IPI. Note that since the slope of the linear fit is close to 1, the intercept nicely reflects the difference between IPI and latency for 6-20 receptors. However, it cannot be seen as a difference for very large receptor numbers, because as the receptor number increases the linear relation will be no longer valid and the difference will tend to 0. The case of 25 receptors has indicated this trend. The quantitative agreement between model results and experimental data shows that the preceding puff has a clear inhibitory effect on the occurrence of the next puff. The dependence of puff amplitude on the preceding IPI will be investigated in the following section, where we will find that the inhibitory effect is time dependent, which is also found experimentally [61].

### 4.2.8 Dependence of puff amplitude on IPI

Model results have shown the preceding puff has an inhibitory effect on the occurrence of the next puff (see 4.11) and this effect comes from the slow recovery of $h_{42}$ (see Fig. 4.4). This
means a long IPI is easier to lead to a larger puff, as most IP\textsubscript{3}Rs have been restored to resting states. A more straightforward relation of puff amplitude and IPI can be seen in Fig. 4.12 wherein puff amplitude is directly plotted in terms of the preceding IPI. We can see in the scatter plot that as the preceding IPI increases, puff amplitude also follows a gradually increasing trend but seems to get saturated when IPI is longer than 2 s. The saturation can be seen clearly in Fig. 4.13a wherein we group those points in Fig. 4.12 in bins of IPI and then calculate average puff amplitude in each bin. This result is consistent with the experimental data in [61].

We also find that where the saturation occurs depends on $a_{h42}$. In Fig. 4.13, we test two cases of $a_{h42}$, 1 s\textsuperscript{−1} (a) and 0.1 s\textsuperscript{−1} (b). Noting that the saturation occurs at about 1 − 2 s for $a_{h42} = 1$ s\textsuperscript{−1} but at about 8 − 12 s for $a_{h42} = 0.1$ s\textsuperscript{−1}, we predict that the saturation should occur at about $1/a_{h42}$. Importantly, this provides a way of estimating the slow recovery rate $a_{h42}$ experimentally, as Fig. 4.13 can be easily obtained using experimental data.

In addition, we find the following IPI seems to be independent of the preceding puff amplitude by looking at the scatter plot similar to Fig. 4.12. The result is also found experimentally [61, 173]. This could be explained by Fig. 4.4 where we can see the inactivation variable $h_{42}$ will quickly drop down to be very close to 0 during a puff regardless of the puff amplitude. Therefore, the inhibitory effect from preceding puffs with various amplitudes on the following
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Figure 4.12: Scatter plot shows the relation of puff amplitude and the preceding IPIs. Puff amplitude has been normalized to the mean blip amplitude of 1.6. We set $N_{IPR} = 10$, $p = 0.1 \mu M$ and $a_{h_{42}} = 1 \text{s}^{-1}$. IPIs is nearly identical.

4.2.9 Dependence of puff amplitude on number of IP$_3$Rs at a puff site

Fig. 4.14 shows the dependence of average puff amplitude and the largest puff amplitude (both normalized to mean blip amplitude), on the number of IP$_3$Rs at a puff site ($N_{IPR}$). Both amplitudes increase as $N_{IPR}$ increases. The average amplitude is linearly related to $N_{IPR}$ for $N_{IPR} < 12$. However, when $N_{IPR} \geq 12$, it behaves like the square root of $N_{IPR}$. Since our model assumes that the rate of Ca$^{2+}$ release through a single channel is a constant, we rule out ER/SR depletion as a reason for the nonlinearity. To check whether it is due to the nonlinear relation between Ca$^{2+}$ and buffer, we plot the puff [Ca$^{2+}$]$_i$ amplitude instead of the Ca$^{2+}$-bound buffer (shown in Fig. 4.15) and find that average puff [Ca$^{2+}$]$_i$ amplitude is linearly dependent on $N_{IPR}$. This result shows the nonlinear relation of puff amplitude and $N_{IPR}$ in Fig. 4.14 is caused by using the Ca$^{2+}$ buffer to indicate puff amplitude. Although no severe buffer saturation is observed, the nonlinearity of the buffering indicator is inevitably affecting the observed results, as the height of [Ca$^{2+}$]$_i$ for large puffs can easily become higher than the dissociation constant of fluo-4 of 2 $\mu M$.

Another important relationship obtained by combining Fig. 4.14 and Fig. 4.15 is that the average puff amplitude is nonlinearly related to the average maximum Ca$^{2+}$ current in a similar
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Figure 4.13: Average puff amplitude is plotted in terms of grouped preceding IPIs. Simulations are performed for two different values of \(a_{h_{2}}\), 1 s\(^{-1}\) (a) and 0.1 s\(^{-1}\) (b). Puff amplitude has been normalized to the mean blip amplitude of 1.6. In each bin, there are about 10 – 30 samples. Results are expressed to be mean ± SE. We set \(N_{IPR} = 10\) and \(p = 0.1 \mu\text{M}\).

way, as maximum Ca\(^{2+}\) current can be roughly assumed to be proportional to puff \([\text{Ca}^{2+}]_i\) amplitude.
4.2.10 Dependence of puff duration on the number of IP$_3$Rs at a puff site

Experimental puffs usually exhibit a rapid increase to the peak but a relatively slow return to baseline [147]. This property is also seen in the model (Fig. 4.16). As $N_{IPR}$ increases, the average rise time gets saturated to about 130 ms whereas the average decay time keeps increasing linearly. This gives rise to an asymmetric puff shape for larger $N_{IPR}$. However, for smaller $N_{IPR}$, the two times are close to each other, which indicates that small puffs are relatively symmetric. The linear dependence of average decay time on the number of receptors is primarily a consequence of the fact that the IP$_3$Rs close randomly and independently [147]. The saturation of rise time could be caused by two reasons. One is the puff amplitude saturation observed in Fig. 4.14. The other is that sufficient high average $[\text{Ca}^{2+}]_i$ during Ca$^{2+}$ release through a few initially activated channels prevents other closed channels from opening. The former seems not to be very convincing, as the amplitude saturation occurs when $N_{IPR} = 12$ whereas the rise time saturation takes place when $N_{IPR}$ is only about 8. Hence, the latter is
4.3 Discussion

Ca$^{2+}$ puffs are local transient Ca$^{2+}$ release events from internal Ca$^{2+}$ stores such as the ER or the SR through a cluster of open IP$_3$Rs. For a better understanding of the mechanisms underlying this physiological phenomenon, we first construct a new mathematical model of the IP$_3$R, based mostly on the Siekmann IP$_3$R model [143], but also incorporating the time-dependent data of Mak et al. [105]. By construction, we know that our new model fits the stationary data equally as well as the original Siekmann model. It merely has additional time-dependencies to allow for the correct transient behavior. We then show how our model qualitatively and quantitatively reproduces experimentally observed puff statistics. The most important feature of our model is that the IP$_3$R can recover from inhibition by Ca$^{2+}$ only on a relatively slow time scale. This time scale cannot be identified from the stationary data of [143], but depends on the non-stationary data of [105].

This is an important point, worth emphasizing. We know (theoretically) that a sufficiently
Figure 4.16: Puff rise time and decay time are differently dependent on the number of IP$_3$R. Results are plotted as mean ± SE. Points of decay time is fit linearly (the solid line). We set $p = 0.1 \, \mu$M and $a_{hi} = 1 \, s^{-1}$.

A long experimental trace will contain information about the behavior of the IP$_3$R on both long and short time scales, and thus will be sufficient to determine both steady-state and transient behavior. However, the reality is more complex. In practice, such long traces are not obtainable, and, even if they were, fitting to the data would require unrealistically large amounts of computer time, due to the rarity of the slow transitions. Thus, fitting the stationary data alone will determine only some of the important receptor properties.

Interestingly, it turns out that the slow processes that are vital for controlling the inhibition of the IP$_3$R, and thus puffs, do not occur often enough so that they are not well captured by limited size of stationary single-channel data and thus are not well characterized by MCMC fitting.

There are thus two options. The first option would be to incorporate non-stationary data (i.e., the responses to steps of $[\text{IP}_3]$ and/or $[\text{Ca}^{2+}]_i$) into the full fitting process, and thereby construct an extended Markov model, with more than the present six states. One would then continue to include additional states until the MCMC fits to the data indicated that additional states were unnecessary, or that the additional rate constants could not be unambiguously determined. Ullah et al. [177] took an approach similar to this (although their method of fitting to data is different from that of Siekmann et al. [143]), constructing a Markov model with 12 states. However, the model of Ullah et al. [177] cannot successfully reproduce calcium puffs. This failure is primarily due to lack of an effective transition from the inactivated state to the resting
4.3. DISCUSSION

state, without which transition the receptors lose the excitability that is crucial for generating repetitive puffs and waves.

The second option, which we took, is to construct a hybrid model, partaking both of the nature of Markov models (such as the De Young-Keizer model) and of heuristic models (such as the Atri et al. model). This has the advantage that we need not introduce any additional states into the Markov model, but has the disadvantage that the time-dependent transitions we introduce have no biophysical basis. However, we have lost less than one might think. It is highly unlikely that an actual IP$_3$R exists in exactly six states (or even 60 states), with well-defined transitions between them. In general, it is thus more accurate to interpret Markov models as useful descriptions, rather than as exact biophysical reality, in which case a hybrid Markov/heuristic model is just as useful.

Finally, we note that although the new model still does not reproduce every aspect of the non-stationary data (i.e., it does not reproduce multimodal waiting time distributions), it still captures the most important features of the dynamic data by reproducing the most dominant modes of those distributions.

The hybrid nature of our model raises some interesting questions. Which part of our model is primarily responsible for the puff dynamics? We know that the Markov model, with time-independent rate constants, does not provide an adequate description of puff dynamics. This is why we introduced the heuristic time dependencies in the first place. However, can the Markov model skeleton be replaced by a simpler model (most likely with the incorrect stationary behavior), as long as the heuristic time dependencies are retained?

We can answer only some of these questions. For example, one implication of Fig. 4.7 is that the original Siekmann IP$_3$R model (that fits only to stationary single-channel data) cannot be used to reproduce the non-exponential IPI distributions. In Eq. 3.3, if $\lambda_G$ is sufficiently larger than the average change velocity of $c$, $G$ can be reasonably assumed to follow its equilibrium at any time. Thus, $m_{42}$, $m_{24}$ and $h_{24}$ evolve nearly as their equilibria whereas $h_{42}$ evolves on a much slower time scale indicated by a small value of $a_{h_{42}}$. As $a_{h_{42}}$ increases, the evolution of $h_{42}$ become closer to its equilibrium. However a consequence of this change is the feature of the non-exponential IPI distribution gradually disappears and become closer to an exponential distribution. This implies that the inhomogeneity of the occurrence of puffs is primarily caused by the slow recovery of $h_{42}$. Therefore, assuming the IP$_3$R can instantaneously follow the steady state, cannot reproduce results that fully explain the experimental data, which confirms the necessity of introducing a relatively slow recovery rate $a_{h_{42}}$.

However, we do not yet know the simplest possible version of our model that can generate correct IPI distributions or other puff statistics. Preliminary results indicate that the modal nature of the IP$_3$R plays little role in the dynamics of puffs and can thus reasonably be ignored in studies of periodic Ca$^{2+}$ waves. In this case, the simplest Markov scheme is just a two-state open/closed model, with time-dependent transitions. A complete study of this question will be given in chapter 5.
A major assumption in our model is that $\lambda_{h42}$ is heuristically modeled by Eq. 3.10 wherein an important parameter, $a_{h42}$, is introduced to indicate the recovery rate of a single IP$_3$R from inhibition by Ca$^{2+}$. This assumption is based on the appearance and statistics of observed puffs, rather than on non-stationary single channel data in [105], because a relatively long (about 2.4 s) recovery time given by the data in [105] cannot be achieved otherwise by our model. The failure is mainly due to the saturation of $q_{42}$ curves for high $[\text{Ca}^{2+}]_i$, as seen in Fig. 3.1. To resolve the problem, we need to consider two aspects. One is whether the stationary single-channel data supports a smaller value of $q_{42}$ for 300 $\mu$M $[\text{Ca}^{2+}]_i$. The other is whether $[\text{Ca}^{2+}]_i$ at the channel mouth during Ca$^{2+}$ release can reach 300 $\mu$M. The former needs more stationary data, whereas the latter is still not clearly known. If $[\text{Ca}^{2+}]_i$ can reach 300 $\mu$M, then we need to modify the existing model, especially the values of $q_{42}$ for high $[\text{Ca}^{2+}]_i$. But if $[\text{Ca}^{2+}]_i$ can only reach about 100 $\mu$M, the dynamic data for 300 $\mu$M is not sufficient to reveal the actual recovery rate. Because of this uncertainty, we use Eq. 3.10 as an alternative way of modeling the slow recovery process.

Our new model is, to our knowledge, the first to reproduce, simultaneously, the correct statistics of IP$_3$R opening and closing, as well as the correct puff statistics. Although most of the model results could qualitatively be reproduced by some older models, none of these older models demonstrate the correct IP$_3$R statistical behavior. In addition, our model demonstrates that slow recovery of an IP$_3$R from Ca$^{2+}$-inhibition is a crucial feature for Ca$^{2+}$ puffs, and shows also how the IPI distribution is affected by the recovery rate (Fig. 4.7). We find that various IPI distributions (either exponential or non-exponential, both of which are seen experimentally [173]) obtained by varying the recovery rate, $a_{h42}$, reveal that different puff sites could exhibit different average recovery rates. In addition, we also find that puff amplitude initially increases but then becomes saturated as $a_{h42}$ increases (Fig. 4.9). This suggests that the number of IP$_3$R at a puff site could be severely underestimated if the average recovery process is sufficiently slow.

We investigate the relation of puff amplitude and IPI (see Fig. 4.9), based on which we find a saturation in Fig. 4.9 occurs at about $1/a_{h42}$. This could be a way of estimating $a_{h42}$ from experimental data. In addition, we find the following IPI seems to be independent on the preceding puff amplitude. This could be explained by Fig. 4.4 where we can see the inactivation variable $h_{42}$ will quickly drop down to be very close to 0 during a puff regardless of the puff amplitude. Therefore, the inhibitory effect from preceding puffs with various amplitudes on the following IPIs is nearly identical.

Puff amplitude has been reported to be nonlinearly related to the maximum Ca$^{2+}$ current such that, as maximum Ca$^{2+}$ current increases, puff amplitude initially increases linearly but then becomes proportional to the square root of maximum Ca$^{2+}$ current [168, 25, 158]. Our model gives the same result. Thul et al. [168] suggested the nonlinearity is due to local Ca$^{2+}$ depletion in the ER/SR. However, this conclusion was challenged by Solovey et al. [158] who showed that this nonlinearity could be generated by a mean field model, or by using a stochastic...
model with a constant single channel Ca\(^{2+}\) flux. They also concluded that the nonlinear relation was due to the dynamics of the Ca\(^{2+}\)-bound dye. By comparing the nonlinear relation between average puff amplitude and the number of IP\(_3\)Rs (Fig. 4.14), and the linear relation between average puff [Ca\(^{2+}\)]\(_i\) amplitude and the number of IP\(_3\)Rs (Fig. 4.15), our model supports the conclusion of [158] by showing that nonlinearity arises from the nonlinear relationship between [Ca\(^{2+}\)]\(_i\) and Ca\(^{2+}\)-bound buffer concentration, even when Ca\(^{2+}\)-bound buffer is not saturated.

Ca\(^{2+}\) oscillations and waves usually exhibit a relatively long decay time, and periods ranging from a few seconds to a few minutes. The mechanisms underlying long-period waves remain unclear; this is perhaps the most important unsolved problem in the theoretical study of Ca\(^{2+}\) waves. One can obtain stochastically generated long-period waves in models which do not have the correct puff statistics, but there is as yet no model which has the correct IP\(_3\)R statistics, the correct puff statistics, and can generate long-period waves. Although our preliminary computations in simulating ASMC oscillations (see chapter 5) indicate that our new model can generate short-period oscillations (around a few seconds) but not oscillations of longer period, the variability of the period will still be a major question in the field.
Chapter 5

MODELING CALCIUM OSCILLATIONS IN ASMC

As I have introduced in Chapter 1, the oscillations in cytoplasmic calcium concentration mediated by IP$_3$R play an important role in cellular function in ASMC. Hence, a thorough knowledge of the behavior of the IP$_3$R is a necessary prerequisite for an understanding of intracellular Ca$^{2+}$ oscillations and waves. Mathematical and computational models of the IP$_3$R play a vital role in studies of Ca$^{2+}$ dynamics. However, over the past decade, two major questions about IP$_3$R models have arisen.

Firstly, how best should the IP$_3$R be modeled? Models of the IP$_3$R have a long history, beginning with the heuristic models of [43, 47, 4]. With the recent appearance of single-channel data from IP$_3$R in vivo [105, 179], a new generation of Markov IP$_3$R models has recently appeared [178, 144]. These models show that IP$_3$Rs exist in different modes with different open probabilities. Within each mode there are multiple states, some open, some closed. Importantly, it was found in the last chapter that time-dependent transitions between different modes are crucial for reproducing Ca$^{2+}$ puff data from [147]. However, it is not yet clear whether transitions between states within each mode are important, or whether all the important behaviors are captured simply by inter-mode transitions.

Secondly, why do deterministic models of the IP$_3$R perform so well as predictive models? Deterministic models of the IP$_3$R have proven to be useful predictive models in a range of cell types. For example, IP$_3$R-based models have been developed to study Ca$^{2+}$ oscillations in ASMC [24, 154, 181, 37], and these models have made predictions which have been confirmed experimentally. This shows the usefulness of such models in advancing our understanding of how intracellular Ca$^{2+}$ oscillations and waves are initiated and controlled in ASMC. However, these models are deterministic models which assume infinitely many IP$_3$Rs per unit cell volume, an assumption that contradicts experimental findings in many cell types showing that Ca$^{2+}$ puffs and spikes occur stochastically, and that intracellular Ca$^{2+}$ waves and oscillations arise as an emergent property of fundamental stochastic events [147, 109, 145].
Here, we answer these two fundamental modeling questions using data and models from ASMC. Firstly, we show that a simple model of the IP$_3$R, involving only two states with time-dependent transitions, suffices to generate correct dynamics of Ca$^{2+}$ puffs and oscillations. Secondly, we show that, although Ca$^{2+}$ oscillations in ASMC are generated by a stochastic mechanism, a deterministic model can make the same qualitative predictions as the analogous stochastic model, indicating that deterministic models, that require much less computational time and complexity, can be used to make reliable predictions. Although we work in the specific context of ASMC, our results are applicable to other cell types that exhibit similar Ca$^{2+}$ oscillations and waves.

The chapter is co-authored work published in PLOS Computational Biology (see [30] for publication details). Details about authors’ contribution are given in a signed co-authorship form provided right before the main text of this thesis.

5.1 Materials and methods

5.1.1 The calcium model

Inhomogeneity of cytoplasmic Ca$^{2+}$ concentration not only exists around individual channel pores of the IP$_3$Rs, where a nearly instantaneous high Ca$^{2+}$ concentration at the pore (denoted by $c_p$) leads to a very sharp concentration profile, but is also seen inside an IP$_3$R cluster where the average cluster Ca$^{2+}$ concentration ($c_b$) is apparently higher than that of the surrounding cytoplasm ($c$) [45]. This indicates that during Ca$^{2+}$ oscillations each IP$_3$R is controlled by either the pore Ca$^{2+}$ concentration (when it is open) or the cluster Ca$^{2+}$ concentration (when it is closed). Neither of these local concentrations influence cell membrane fluxes or the majority of SERCAs, which we assume to be distributed outside the cluster.

The scale separation between the pore Ca$^{2+}$ concentration and the cluster Ca$^{2+}$ concentration allows to treat $c_p$ as a parameter, providing a simpler way of modeling local Ca$^{2+}$ events (like Ca$^{2+}$ puffs) that has been used in several previous studies[132, 133]. However, evolution of the cluster concentration and wide-field cytoplasm Ca$^{2+}$ concentration are not always separable, so an additional differential equation for the cluster Ca$^{2+}$ is necessary.

A schematic diagram of the model is shown in Fig. 5.1. The corresponding ODEs are

$$\frac{dc}{dt} = J_{\text{diff}} + J_{\text{leak}} - J_{\text{serca}} + J_{\text{in}} - J_{\text{pm}},$$  \hspace{1cm} (5.1)

$$\frac{dc_b}{dt} = \gamma_1 (J_{\text{IPR}} - J_{\text{diff}}),$$  \hspace{1cm} (5.2)

$$\frac{dc_t}{dt} = J_{\text{in}} - J_{\text{pm}},$$  \hspace{1cm} (5.3)

where $c_t = c + c_b/\gamma_1 + c_s/\gamma_2$ representing total intracellular Ca$^{2+}$ concentration, and thus SR
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Figure 5.1: Schematic diagram of the Ca\(^{2+}\) model. \(c\) represents cytoplasmic Ca\(^{2+}\) concentration, excluding a small local Ca\(^{2+}\) (whose concentration is denoted by \(c_b\)) close to the Ca\(^{2+}\) release site (i.e., an IP\(_3\)R cluster). Upon coordinated openings of the IP\(_3\)Rs, SR Ca\(^{2+}\) (\(c_s\)) is first released into the local domain \(J_{IPR}\) to cause a rapid increase in \(c_b\). High local Ca\(^{2+}\) then diffuses to the rest of the cytoplasm \(J_{diff}\), and is eventually pumped back to the SR \(J_{serca}\).

Ca\(^{2+}\) concentration, \(c_s\) is given by
\[
c_s = \gamma_2 (c_t - c - c_b / \gamma_1)
\]
where \(\gamma_1\) and \(\gamma_2\) are the volume ratios given in Table 5.1. \(J_{IPR}\) is the flux through the IP\(_3\)R, \(J_{leak}\) is a background Ca\(^{2+}\) leak out of the SR, and \(J_{serca}\) is the uptake of Ca\(^{2+}\) into the SR by SERCA pumps. \(J_{pm}\) is the flux through plasma pump, and \(J_{in}\) represents a sum of main Ca\(^{2+}\) influxes including \(J_{rocc}\) (receptor-operated Ca\(^{2+}\) channel), \(J_{socc}\) (store-operated Ca\(^{2+}\) channel) and \(J_{leakin}\) (Ca\(^{2+}\) leak into the cell). \(J_{diff}\) coarsely models the diffusion flux from cluster microdomain to the cytoplasm. Details of the fluxes are

- Different formulations of \(J_{IPR}\) give different types of models:
  
  a) For the stochastic model, \(J_{IPR} = (k_{IPR}/N_t)N_o(c_s - c)\) where \(k_{IPR}\) is the maximum conductance of a cluster of \(N_t\) IP\(_3\)Rs (here \(N_t = 20\)). \(N_o\) is the number of open IP\(_3\)Rs determined by the states of IP\(_3\)Rs.
  
  b) For the deterministic model we set \(J_{IPR} = k_{IPR}P_o(c_s - c)\) where \(P_o\) is the IP\(_3\)R open probability, a continuous analogue of \(N_o/N_t\).

To calculate \(N_o\) and \(P_o\), we use the IP\(_3\)R model of [144, 29], with minor modifications described later.

- \(J_{diff} = k_{diff}(c_b - c)\).
- \(J_{serca} = V_s^n c_n / (K_s^{ns} + c_n)\) where \(K_s\) and \(n_s\) are obtained from [33].
- \(J_{leak} = k_{leak}(c_s - c)\).
- \(J_{in}\) includes a basal leak \(J_{leakin}\), receptor-operated calcium channel (ROCC, \(J_{rocc}\)), store-operated calcium channel (SOCC, \(J_{socc}\)). By using the IP\(_3\) concentration \(p\) as a surrogate
indicator of MCh concentration, we assume that $J_{rocc} = V_{rocc} p$. SOCC is modeled by $J_{socc} = V_{socc} \frac{K_{socc}^4}{(K_{socc}^4 + c_{socc}^4)}$ [37].

- $J_{pm} = V_{pm} c_{np} / (K_{np}^p + c_{np})$.

Calcium concentration at open channel pore ($c_p$) does not explicitly appear in the equations but is used in the IP$_3$R model introduced later. $c_p$ is assumed to be proportional to SR Ca$^{2+}$ concentration ($c_s$) and is therefore simply modeled by $c_p = c_{p0} (c_s / 100)$ where $c_{p0}$ is the value corresponding to $c_s = 100 \mu$M. Alternatively, $c_p$ can also be assumed to be a large constant (say greater than 100 \mu M) without fundamentally altering the model dynamics. The choice of $c_{p0}$ is not critical as long as it is sufficiently large to play a role in inactivating the open channels. All the parameter values are given in Table 5.1.

### 5.1.2 The data-driven IP$_3$R model

The IP$_3$R model used in our ASMC calcium model is an improved version of the Siekmann IP$_3$R model which is a 6-state Markov model derived by fitting to the stationary single channel data using Markov chain Monte Carlo (MCMC) [179, 144, 29]. Fig. 2.2 has shown the structure of the IP$_3$R model which is comprised of two modes; the drive mode, containing three closed states $C_1$, $C_2$, $C_3$ and one open state $O_6$, and the park mode, containing one closed state $C_4$ and one open state $O_5$. The transition rates in each mode are constants (shown in Table 5.2), but $q_{42}$ and $q_{24}$ which connect the two modes are Ca$^{2+}$-/IP$_3$-dependent and are formulated as

$$q_{24} = a_{24} + V_{24}(1 - m_{24} h_{24}), \quad (5.4)$$

$$q_{42} = a_{42} + V_{42} m_{42} h_{42}, \quad (5.5)$$

where $m_{24}$, $h_{24}$, $m_{42}$ and $h_{42}$ are Ca$^{2+}$-/IP$_3$-modulated gating variables. $a_{24}$, $a_{42}$, $V_{24}$ and $V_{42}$ are either functions of $p$ or constants and are given later. We assume the gating variables obey the following differential equation,

$$\frac{dG}{dt} = \lambda_G (G_\infty - G), \quad (G = m_{24}, h_{24}, m_{42}, h_{42}), \quad (5.6)$$

where $G_\infty$ is the equilibrium and $\lambda_G$ is the rate at which the equilibrium is approached. Those equilibria are functions of Ca$^{2+}$ concentration at the cytoplasmic side of the IP$_3$R ([$Ca^{2+}$], denoted by $\hat{c}$ in the equations, equal to either $c_p$ or $c_b$ depending on the state of the channel). They are assumed to be

$$m_{24\infty} = \frac{\hat{c}^3}{\hat{c}^3 + k_{24}^3}, \quad (5.7)$$

$$h_{24\infty} = \frac{k_{24}^2}{\hat{c}^2 + k_{24}^2}, \quad (5.8)$$
### 5.1. MATERIALS AND METHODS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value/Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{IPR}$</td>
<td>IP$_3$R flux coefficient</td>
<td>0.05 s$^{-1}$</td>
</tr>
<tr>
<td>$k_{diff}$</td>
<td>Ca$^{2+}$ diffusional flux coefficient</td>
<td>10 s$^{-1}$</td>
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<tr>
<td>$k_{leak}$</td>
<td>SR leak flux coefficient</td>
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<tr>
<td>$V_s$</td>
<td>maximum capacity of SERCA</td>
<td>10 µM·s$^{-1}$</td>
</tr>
<tr>
<td>$K_s$</td>
<td>SERCA half-maximal activating [Ca$^{2+}$]$_i$</td>
<td>0.26 µM</td>
</tr>
<tr>
<td>$n_s$</td>
<td>Hill coefficient for SERCA</td>
<td>1.75</td>
</tr>
<tr>
<td>$J_{leakin}$</td>
<td>plasma membrane leak influx</td>
<td>0.03115 µM·s$^{-1}$</td>
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<tr>
<td>$V_{rocc}$</td>
<td>ROCC flux coefficient</td>
<td>0.2 s$^{-1}$</td>
</tr>
<tr>
<td>$V_{socc}$</td>
<td>maximum capacity of SOCC</td>
<td>1.6 µM·s$^{-1}$</td>
</tr>
<tr>
<td>$K_{socc}$</td>
<td>SOCC dissociation constant</td>
<td>100 µM</td>
</tr>
<tr>
<td>$V_p$</td>
<td>maximum capacity of plasma pump</td>
<td>0.8 µM·s$^{-1}$</td>
</tr>
<tr>
<td>$K_p$</td>
<td>plasma pump half-maximal activating [Ca$^{2+}$]$_i$</td>
<td>0.5 µM</td>
</tr>
<tr>
<td>$n_p$</td>
<td>Hill coefficient for plasma pump</td>
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</tr>
<tr>
<td>$\gamma_1$</td>
<td>the cytoplasmic-to-microdomain volume ratio</td>
<td>100</td>
</tr>
<tr>
<td>$\gamma_2$</td>
<td>the cytoplasmic-to-SR volume ratio</td>
<td>10</td>
</tr>
<tr>
<td>$c_{p(0)}$</td>
<td>an instantaneous high [Ca$^{2+}$]$_i$ at open channel pore when $c_s = 100$ µM</td>
<td>120 µM</td>
</tr>
<tr>
<td>$N_t$</td>
<td>total number of IP$_3$R channels</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 5.1: Parameter values of the stochastic calcium model

\[
\begin{align*}
  m_{42\infty} &= \frac{\hat{c}_3}{\hat{c}_3 + k_{42}^3}, \quad (5.9) \\
  h_{42\infty} &= \frac{k_{-42}^3}{\hat{c}_3 + k_{-42}^3}. \quad (5.10)
\end{align*}
\]
Hence, we have stationary expressions of $q_{24}$ and $q_{42}$,

$$q_{24\infty} = a_{24} + V_{24}(1 - m_{24\infty}h_{24\infty}), \quad (5.11)$$

$$q_{42\infty} = a_{42} + V_{42}m_{42\infty}h_{42\infty}. \quad (5.12)$$

The expressions of $a_s, V_s, n_s$ and $k_s$ are chosen as follows so that Eq. 5.11 and Eq. 5.12 capture the correct trends of experimental values of $q_{24}$ and $q_{42}$ (see Fig. 5.2) and generate relatively smooth open probability curves (see Fig. 5.3),

$$V_{24} = 62 + 880/(p^2 + 4) \quad a_{24} = 1 + 5/(p^2 + 0.25)$$

$$V_{42} = 110p^2/(p^2 + 0.01) \quad a_{42} = 1.8p^2/(p^2 + 0.34)$$

$$k_{24} = 0.35 \quad k_{42} = 0.49 + 0.543p^3/(p^3 + 64)$$

$$k_{-24} = 80 \quad k_{-42} = 0.41 + 25p^3/(p^3 + 274.6)$$

Note that the above formulas are different from the relatively complicated formulas used in [29]. The rates, $\lambda_{m_{24}}, \lambda_{h_{24}}$ and $\lambda_{m_{42}}$, are constants estimated by using dynamic single channel data [105] and given in Table 5.2, whereas $\lambda_{h_{42}}$ is not clearly revealed by experimental data. However we have shown that it should be relatively large for high $\hat{c}$ but relatively small for low $\hat{c}$ for reproducing experimental puff data [29]. By introducing two Ca$^{2+}$ concentrations, $c_b$ and $c_p$, $\lambda_{h_{42}}$ and the state of the IP$_3$R channel become highly correlated, so that we can assume $\lambda_{h_{42}}$ is a relatively large value $H$ if the channel is open and is a relatively small value $L$ if the channel is closed. Hence, $\lambda_{h_{42}}$ is modeled by the logic function

$$\lambda_{h_{42}} = \begin{cases} 
H, & \text{if the channel is open;} \\
L, & \text{if the channel is closed.}
\end{cases}$$

Values of $L$ and $H$ are chosen so that simulated Ca$^{2+}$ oscillations in ASMC are comparable to experimental observations.

### 5.1.3 The IP$_3$R model reduction

Here we reduce the 6-state model to a 2-state open/closed model. The reduction takes the following steps:

- The sum of the probabilities of $C_1$, $C_3$ and $O_5$ is less than 0.03 for any $\hat{c}$, so they are either rarely visited by the IP$_3$Rs or have a very short dwell time. This implies they have
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Figure 5.2: Stationary data and fits of $q_{24}$ and $q_{42}$. Stationary transition rates of $q_{24}$ and $q_{42}$, $q_{24\infty}$ and $q_{42\infty}$, as functions of Ca$^{2+}$ concentration were estimated and fitted for two [IP$_3$]s, 1 µM (A) and 10 µM (B). Circles and squares represent the means of $q_{24}$ and $q_{42}$ distributions computed by MCMC simulation [144]. Note that MCMC failed to determine the values of $q_{24}$ and $q_{42}$ at [Ca$^{2+}$] = 1, 10 µM for 10 µM IP$_3$, as the IP$_3$R was almost in the drive mode for these cases. The corresponding fitting curves (solid for $q_{42}$; dashed for $q_{24}$) are produced using Eqs. 5.7 – 5.12. The data point of $q_{42}$ at [Ca$^{2+}$] = 100 µM and [IP$_3$] = 1 µM is excluded in fitting process because of the same reason mentioned in Fig. 3.1.

Figure 5.3: Open probability curves for various [IP$_3$]s. $P_o$ is equal to the sum of probabilities of the IP$_3$R in $O_5$ and $O_6$. Three representative curves correspond to 0.1 µM, 1 µM and 10 µM [IP$_3$] (from bottom to top) respectively. Data (average open probability) are from [179].
very little contribution to the Ca\(^{2+}\) dynamics. Therefore, we completely remove the three states from the full model.

- Transition rates of \(q_{26}\) and \(q_{62}\) are about 2 orders larger than that of \(q_{24}\) and \(q_{42}\), which allows us to omit the fast transitions by taking a quasi-steady state approximation. This change will affect two aspects. First, we have \(O_6 = C_2 q_{26}/q_{62}\) which allows us to combine \(C_2\) and \(O_6\) to be a new state \(D\), which satisfies \(D = O_6(q_{62} + q_{26})/q_{26}\). Although this means \(D\) is a partially open state with an open probability of \(q_{26}/(q_{62} + q_{26})\), it can be used as an fully open state in the stochastic simulations by multiplying the maximum IP\(_3\)R flux conductance \(k_{IP}\) by a factor of \(q_{26}/(q_{62} + q_{26})\). Secondly, \(q_{24}\) needs to be rescaled by \(q_{62}/(q_{62} + q_{26})\), i.e., the effective closing rate is \(q_{24}q_{62}/(q_{62} + q_{26})\).

- Due to the combination of \(C_2\) and \(O_6\), \(\lambda_h\) is accordingly modified to

\[
\lambda_{h_{42}} = \begin{cases} 
H, & \text{if the channel is in } D \text{ (the drive mode)} \\
L, & \text{if the channel is in } C_4
\end{cases}
\]

Hence, the reduced two-state model contains one “open” state \(D\) and one closed state \(C_4\) with the opening transition rate of \(q_{42}\) and the closing transition rate of \(q_{24}q_{62}/(q_{62} + q_{26})\).
5.1.4 Deterministic formulation of the stochastic model

Based on the stochastic calcium model and the reduced 2-state IP$_3$R model, we construct a deterministic model. We need to modify three things that are used in the stochastic model but inapplicable to fast simulations of the deterministic model. The first is the discrete number of open channels; the second is state-dependent use of $c_b$ and $c_p$ in calculating $q_{42}$ and $q_{24}$; the last is the logic expression of $\lambda_{h42}$. Details of the modifications are as follows,

- The fraction of open channels ($N_o/N_t$) is replaced by open probability $P_o$ which is $(q_{26}/(q_{26} + q_{62}) = 70\%$ of the probability of state $D$.

- In the stochastic simulations, $q_{24}$ which only controls the IP$_3$R closing is primarily governed by $c_p$, whereas $q_{42}$ which controls IP$_3$R opening is mainly governed by $c_b$. Therefore, in the deterministic model, we separate the functions of $c_p$ and $c_b$ by assuming $m_{24\infty}$ and $h_{24\infty}$ are functions of $c_p$ only whereas $m_{42\infty}$ and $h_{42\infty}$ are functions of $c_b$ only. That is, $m_{24\infty} = m_{24\infty}(c_p)$, $h_{24\infty} = h_{24\infty}(c_p)$, $m_{42\infty} = m_{42\infty}(c_b)$ and $h_{42\infty} = h_{42\infty}(c_b)$. Here $c_p = c_p0(c_s/100)$ as defined before.

- To describe an average rate that infinitely many receptors are rapidly inhibited by high Ca$^{2+}$ concentration but slowly restored from Ca$^{2+}$-inhibition. $\lambda_{h42}$ is proposed to be

$$\lambda_{h42} = (1 - D)L + DH.$$ 

Based on the above changes, the full deterministic model containing 8 ODEs is presented as follows,

$$\frac{dc}{dt} = J_{\text{diff}} + J_{\text{leak}} - J_{\text{serca}} + J_{\text{in}} - J_{\text{pm}}, \quad (5.13)$$

$$\frac{dc_b}{dt} = \gamma_1(J_{\text{IPR}} - J_{\text{diff}}), \quad (5.14)$$

$$\frac{dc_t}{dt} = J_{\text{in}} - J_{\text{pm}}, \quad (5.15)$$

$$\frac{dD}{dt} = q_{42}(1 - D) - \left(\frac{q_{24}q_{62}}{q_{62} + q_{26}}\right)D, \quad (5.16)$$

$$\frac{dm_{24}}{dt} = \lambda_{m_{24}}\left(\frac{c_p^3}{c_p^3 + h_{24}^3} - m_{24}\right), \quad (5.17)$$

$$\frac{dh_{24}}{dt} = \lambda_{h_{24}}\left(\frac{k_{24}^2}{c_p^2 + h_{24}^2} - h_{24}\right), \quad (5.18)$$

$$\frac{dm_{42}}{dt} = \lambda_{m_{42}}\left(\frac{c_b^3}{c_b^3 + h_{42}^3} - m_{42}\right), \quad (5.19)$$

$$\frac{dh_{42}}{dt} = \lambda_{h_{42}}\left(\frac{k_{42}^3}{c_p^3 + h_{42}^3} - h_{42}\right), \quad (5.20)$$
where \(q_{24}\) and \(q_{42}\) are functions of the gating variables given by Eqs. 5.4 and 5.5. All the fluxes are the same as those of the stochastic model except \(J_{\text{IPR}} = k_{\text{IPR}}(Dq_{26}/(q_{62} + q_{26}))(c_x - c_b)\). All the parameter values of the deterministic model are the same as those of the stochastic model and are therefore given in Tables 5.1 and 5.2.

### 5.1.5 Reduction of the full deterministic model

The full deterministic model contains 8 variables which make the model difficult to implement and analyze. Thus, we reduce the full model to a minimal model that still captures the crucial features of the full model. First of all, \(\lambda_{m_{24}}, \lambda_{m_{42}}\) and \(\lambda_{h_{24}}\) are sufficiently large so that we can assume they instantaneously follow their equilibrium functions. Therefore, by taking quasi-steady state approximation to \(m_{24}, h_{24}\) and \(m_{42}\), we remove the three time-dependent variables from the full model.

By now, the full model has been reduced to a 5D model,

\[
\frac{dc}{dt} = J_{\text{diff}} + J_{\text{leak}} - J_{\text{serca}} + J_{\text{in}} - J_{\text{pm}},
\]

\[
\frac{dc_b}{dt} = \gamma_1(J_{\text{IPR}} - J_{\text{diff}}),
\]

\[
\frac{dc_t}{dt} = J_{\text{in}} - J_{\text{pm}},
\]

\[
\frac{dD}{dt} = q_{42}(1 - D) - \left(\frac{q_{24}q_{62}}{q_{62} + q_{26}}\right)D,
\]

\[
\frac{dh_{42}}{dt} = \lambda_{h_{42}}\left(\frac{k_{-42}^3}{c_b^3 + k_{-42}^3} - h_{42}\right).
\]

Second, the rate of change of \(D\) approaching its equilibrium, \(\lambda_D = (q_{42}q_{62} + q_{42}q_{26} + q_{24}q_{62})/(q_{62} + q_{26})\) (calculated from Eq. 5.24), is at least one order larger than those of \(c, c_t\) and \(h_{42}\), indicating that taking the quasi-steady state approximation to Eq. 5.24 will not significantly affect the evolutions of \(c, c_t\) and \(h_{42}\). That is,

\[
D = \frac{q_{42}(q_{62} + q_{26})}{q_{42}q_{62} + q_{42}q_{26} + q_{24}q_{62}}.
\]

We emphasize here that the theory of the quasi-steady state approximation has not yet been well established, particularly about the rigorous conditions under which such a reduction is valid. Thus, our criterion of judging the validity of the reduction is checking whether the solutions of the reduced model are capable of qualitatively reproducing that of its original model. For this model, we find the reduction works. Hence, the full model is eventually reduced to a 4D model summarized as follows,

\[
\frac{dc}{dt} = J_{\text{diff}} + J_{\text{leak}} - J_{\text{serca}} + J_{\text{in}} - J_{\text{pm}},
\]
5.1. MATERIALS AND METHODS

\[ \frac{dc_b}{dt} = \gamma_1(J_{IPR} - J_{diff}), \quad (5.28) \]

\[ \frac{dc_t}{dt} = J_{in} - J_{pm}, \quad (5.29) \]

\[ \frac{dh_{42}}{dt} = \lambda_{h_{42}} \left( \frac{k^3_{42}}{c^2_b + k^3_{42}} - h_{42} \right), \quad (5.30) \]

where \( D \) is given by Eq. 5.26.

5.1.6 Inclusion of calcium buffers

To check the effect of calcium buffers on oscillation frequency, we introduce a stationary buffer (no buffer diffusion), as mobile buffers are too complicated to be included in the current deterministic model. Since we have two different cytoplasmic \( \text{Ca}^{2+} \) concentrations, \( c \) and \( c_b \), two pools of buffer with the same kinetics should be considered. Hence, the inclusion of a stationary calcium buffer is modeled by the following system,

\[ \frac{dc}{dt} = J_{diff} + J_{leak} - J_{serca} + J_{in} - J_{pm} - k_+(B_t - b_1)c + k_- b_1, \quad (5.31) \]

\[ \frac{dc_b}{dt} = \gamma_1(J_{IPR} - J_{diff}) - k_+(B_t - b_2)c_b + k_- b_2, \quad (5.32) \]

\[ \frac{dc_t}{dt} = J_{in} - J_{pm} - k_+(B_t - b_1)c + k_- b_1 - \frac{1}{\gamma_1}(k_+(B_t - b_2)c_b - k_- b_2), \quad (5.33) \]

\[ \frac{db_1}{dt} = k_+(B_t - b_1)c - k_- b_1, \quad (5.34) \]

\[ \frac{db_2}{dt} = k_+(B_t - b_2)c_b - k_- b_2, \quad (5.35) \]

where \( b \) (\( b_1 \) and \( b_2 \)) and \( B_t \) represent the concentrations of \( \text{Ca}^{2+} \)-bound buffer and total buffer respectively. \( k_+ \) and \( k_- \) are the rates of \( \text{Ca}^{2+} \)-binding and \( \text{Ca}^{2+} \)-dissociation, indicating how fast the time scale of the buffer dynamics is. Fast buffer refers to the buffer with relatively large \( k_+ \). In the simulations, we use a fast buffer with \( k_+ = 100 \mu \text{M}^{-1} \cdot \text{s}^{-1} \) and \( k_- = 100 \text{ s}^{-1} \) and vary \( B_t \) to test if the stochastic model and the deterministic model have a qualitatively similar \( B_t \)-dependency.

5.1.7 Numerical methods and tools for deterministic and stochastic simulations

For the stochastic model, Eqs. 5.1–5.3 and ODEs of the four gating variables in the IP_3R model are solved by the fourth-order Runge-Kutta method (RK4) and the stochastic states of IP_3Rs determined by the IP_3R model are solved by using a hybrid Gillespie method (see Appendix A) with adaptive timing [131]. The maximum time step size is set to be either \( 10^{-4} \) s (for
the 6-state IP$_3$R model) or 10$^{-3}$ s (for the reduced 2-state IP$_3$R model). All the computations are done with MATLAB (The MathWorks, Natick, MA). For the deterministic model, we use ode15s, an ODE solver in MATLAB. Accuracy is controlled by setting an absolute tolerance of 10$^{-8}$ applied to all the variables.

5.1.8 Statistical analysis

Data analysis is performed on the Ca$^{2+}$ traces with relatively stable baselines and less noise. A moving average of every 3 data points is used to improve the data by smoothing out short-term fluctuations (Fig. 5.4A is an improved result, shown later). Due to large variations in baseline, amplitude, and level of noise in data, we used two thresholds to get samples: a low threshold, 20% of the amplitude of the largest spike above the baseline, to initially filter baseline noise out; and a relatively high threshold, 50% of the amplitude of the largest spike above the baseline, to further remove small spikes that cannot initiate waves. For simulated stochastic traces of variable $c$, we first convert it to fluorescence ratio ($F/F_0$) by using $F/F_0 = c(c_0 + K_d)/(c_0c + c_0K_d)$ where the dissociation constant of Oregon Green $K_d = 0.17$ µM and resting $[Ca^{2+}]_i = c_0 = 0.1$ µM. We then used the same sampling procedure mentioned above to obtain samples. After samples are chosen, ISIs and spike durations are calculated based on the low threshold. The frequency in the stochastic model is obtained by counting the number of spikes over a long period of time (200-400s) and converting the total number to the number of spikes per minute. All the samplings and linear least-squares fittings are implemented using MATLAB.

5.2 Results

5.2.1 A two-state model of the IP$_3$R is sufficient to reproduce function

We have shown in Chapter 4 that the statistics of Ca$^{2+}$ puffs in SH-SY5Y cells can be reproduced by a Markov model of the IP$_3$R based on the steady-state data of [179] and the time-dependent data of [105]. We showed that, to reproduce the experimentally observed non-exponential ISI distribution and CV of ISI smaller than 1, the time-dependent intermodal transitions are crucial. Lack of time dependencies in the Siekmann model leads to exponential ISI distributions and CV=1, which is not the case for calcium spikes in ASMC. Fig. 5.4A shows an example of Ca$^{2+}$ oscillations generated by 50 nM methacholine (MCh, an agonist that can induce the production of IP$_3$ by binding to a G protein-coupled receptor in the cell membrane) in ASMC. By gathering data from 14 cells in 5 mouse lung slices, we found that the standard deviation of the ISI is approximately a linear function of the ISI mean, with a slope clearly between 0 and 1 (i.e. CV<1), indicating that the spikes are generated by an inhomogeneous Poisson process (a slope of 1 would denote a pure Poisson process) (see Fig. 5.4B). This shows the necessity of inclusion of time-dependent transitions for mode-switching.
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Figure 5.4: Ca\(^{2+}\) oscillations in ASMC in lung slices are generated by a stochastic mechanism. A: experimental Ca\(^{2+}\) spiking in ASMC in lung slices, stimulated with 50 nM MCh. Relative fluorescence intensity was expressed as a ratio of the fluorescence intensity (F/F\(_0\)) at a particular time (F) normalized to the initial fluorescence intensity (F\(_0\)). In the upper panel we filter out baseline noise by using a low threshold of 1.42 (relative fluorescence intensity) and then choose samples with amplitude larger than 1.75. The ISI calculated from the upper panel is shown in the lower panel. B: relationship between the standard deviation and the mean of experimental ISIs. Data obtained from 14 ASMC in 5 mouse lung slices. The relationship is approximately linear with a slope of 0.66, which implies that an inhomogeneous Poisson process governs the generation of oscillations. The dashed line indicates where the coefficient of variation (CV) is 1 (as it is for a pure Poisson process). The slope is significantly different from 1 (p<0.001). Variation in ISI is mainly caused by both use of different doses of MCh and different sensitivities of different cells to MCh. Error bars indicate the standard errors of the means (SEM) of ISI.

Using a quasi-steady-state approximation, and ignoring states with very low dwell times, it is possible to construct a simplified two-state version of the full six-state model (see Materials and Methods). In the simplified model the intramodal structure is ignored, and only the intermodal transitions have an effect on IP\(_3\)R behavior. In Fig. 5.5 we compared the simplified IP\(_3\)R model to the full six-state model. Both models have the same distribution of interspike interval, spike amplitude and spike duration. Moreover, by looking at a more detailed comparison between the two model results (Figs. 5.6A, C and E) and experimental data (Figs. 5.6B, D and F), we found the 2-state model not only can reproduce the behavior of the 6-state model, but can also qualitatively reproduce experimental data. The average experimental ISI shows a clear decreasing trend as MCh concentration increases (although a saturation occurs in the data for high MCh), a trend that is mirrored by the model results as the IP\(_3\) concentration increases. Unfortunately, since the exact relationship between MCh concentration and IP\(_3\) concentration is uncertain, a quantitative comparison is not possible. In both model and experimental results, the average peak and duration of the oscillations are nearly independent of agonist concentration. The quantitative difference in spike duration between the model results and the data in Figs. 5.6E and F are most likely due to choice of calcium buffering parameters. For example, adding 3 or 5 µM fast Ca\(^{2+}\) buffer (see Materials and Methods) increases the average spike duration...
to 0.54 s or 0.7 s respectively, which are close to the levels shown in the data.

Figure 5.5: A 2-state open/closed model quantitatively reproduces the 6-state IP$_3$R model. **A**: histograms of interspike interval (ISI) distribution for both the 6-state and the simplified models. The ISI is defined to be the waiting time between successive spikes. Each histogram contain an equal number of samples (180). **B**: comparison of average ISI, average peak value of [Ca$^{2+}$]$_i$ (c in the model) and average spike duration. All distributions were computed at a constant [IP$_3$] = 0.15 μM.

Thus, the intramodal structure of the six-state model is essentially unimportant, as the model behavior (in terms of the statistics of puffs and oscillations) is governed almost entirely by the time dependence of the intermode transitions, particularly the time dependence of the rapid inhibition of the IP$_3$R by high [Ca$^{2+}$]$_i$, and the slow recovery from inhibition by Ca$^{2+}$. The multiple states within each mode are necessary to obtain an acceptable quantitative fit to single-channel data, but are nevertheless of limited importance for function. Hence, even when simulating microscopic events such as Ca$^{2+}$ puffs it is sufficient to use a simpler, faster, two-state model, rather than a more complex six-state model. In the following, we will use the 2-state IP$_3$R model to generate all the simulation results.

### 5.2.2 Prediction of stochastic Ca$^{2+}$ behavior by a deterministic model

Although the data (Fig. 5.4) show that Ca$^{2+}$ oscillations in ASMC are generated by a stochastic process, not a deterministic one, we wish to know to what extent a deterministic model can be used to make qualitative (and experimentally testable) predictions. Our simplified 2-state Markov model of the IP$_3$R can be converted to a deterministic model (see Materials and Methods). The result is a system of ordinary differential equations (ODEs) with four variables, which takes into account the increased [Ca$^{2+}$]$_i$ at an open IP$_3$R pore, as well as the increased [Ca$^{2+}$]$_i$ within a cluster of IP$_3$R; the four variables are the [Ca$^{2+}$]$_i$ outside the IP$_3$R cluster (c), the [Ca$^{2+}$]$_i$ within the IP$_3$R cluster (c$_b$), the total intracellular Ca$^{2+}$ concentration (c$_t$) and an IP$_3$R gating variable (h$_{42}$). We refer to the reduced 4D model as the deterministic model for all the results and analyses.
Figure 5.6: More detailed comparisons between the 2-state and the 6-state IP$_3$R models, and a comparison to experimental data. As a function of IP$_3$ concentration ($p$), the two models give the same ISI (A), peak $[Ca^{2+}]_i$ (C) and spike duration (E). These results agree qualitatively with experimental data, as shown in panels B, D and F respectively. Quantitative comparisons are generally not possible as the relationship between IP$_3$ concentration and agonist concentration is not known. Error bars represent mean ± SEM. Data for each MCh concentration are obtained from at least three different cells from at least two different lung slices.

Note that there is no physical or geometric constraint enforcing a high local $[Ca^{2+}]_i$; in this case the spatial heterogeneity arises solely from the low diffusion coefficient of Ca$^{2+}$. Our
use of $c_b$ is merely a highly simplified way of introducing spatial heterogeneity of the Ca$^{2+}$ concentration. Since the IP$_3$R can only “see” $c_b$ (as well as the Ca$^{2+}$ concentration right at the mouth of an open channel, which we denote by $c_p$), but cannot be influenced directly by $c$ (the experimentally observed Ca$^{2+}$ signal), our approach allows for the functional differentiation of the rapid local oscillatory Ca$^{2+}$ in the cluster, from the slower Ca$^{2+}$ signal in the cytoplasm, without the need for computationally intensive simulations of a partial differential equation model. Quantitative accuracy is thus sacrificed for computational convenience.

Figure 5.7: Stochastic and deterministic simulations exhibit similar dynamic properties. A: simulated stochastic (upper panel) or deterministic (lower panel) Ca$^{2+}$ oscillations at 0.1 µM IP$_3$. B: a typical stochastic solution projected on the $c_b$−$h_{42}$ plane. The average $h_{42}$ represents the average value of $h_{42}$ over the 20 IP$_3$Rs. Statistics (mean ± SD) of the initiation point (blue square), the peak (red square) and termination point (green square) are shown in the inset. 116 samples are obtained by applying a low threshold of 0.15 µM and a high threshold of 0.8 µM to $c_b$. C: a typical periodic solution of the deterministic model (black curve), plotted in the $c, c_b, h_{42}$ phase space. The arrow indicates the direction of movement. $c_t$ is the slowest variable so that its variation during an oscillation is very small. This allows to treat $c_t$ as a constant ($c_t = 53.12$ µM in this case) and study the dynamics of the model in the $c, c_b, h_{42}$ phase space. The color surface is the surface where $dc_b/dt = 0$ (called the critical manifold). The white N-shaped curve is the intersection of the critical manifold and the surface $dc/dt = 0$. D: projection of the periodic solution to the $c_b, h_{42}$ plane. The red N-shaped curve is the projection to the $c_b, h_{42}$ plane of the white curve shown in C. The evolution of the deterministic solution exhibits three different time scales separated by green circles (labelled by a, b and c) and indicated by arrows (triple arrow: fastest; double arrow: intermediate; single arrow: slowest).
Calcium oscillations in the stochastic and deterministic models are shown in Fig. 5.7A. According to our previous results in Chapter 4, the average value of $h_{42}$ over the cluster of IP$_3$R primarily regulates the termination and regeneration of individual spikes. This can be seen in the stochastic model by projecting the solution on the $c_b, h_{42}$ phase plane (Fig. 5.7B). Upon an initial Ca$^{2+}$ release from one or more IP$_3$Rs, a large spike is generated by Ca$^{2+}$-induced Ca$^{2+}$ release (via the IP$_3$R) during which time a decreasing $h_{42}$ gradually decreases the average open probability of the clustered IP$_3$Rs. The spike is terminated when $h_{42}$ is too small to allow further Ca$^{2+}$ release. This phenomenon is qualitatively reproduced by the deterministic model (Fig. 5.7D). In both the stochastic and deterministic models the decrease in average IP$_3$R open probability of a cluster of IP$_3$R caused by Ca$^{2+}$ inhibition is the main reason for the termination of each spike.

According to Figs. 5.7B and D, regeneration of each spike requires a return of $h_{42}$ back to a relatively high value (i.e., recovery of the IP$_3$R from inhibition by Ca$^{2+}$). The deterministic model sets a clear threshold for the regeneration, as can be seen in Fig. 5.7C, where an upstroke in $c_b$ occurs when the trajectory creeps beyond the sharp “knee” of the white curve. When the trajectory reaches the knees of the white curve it is forced to jump across to the other stable branch of the critical manifold, resulting in a fast increase in $c_b$ followed by a relatively fast increase in $c$ (seen by combining Figs. 5.7C and D).

In contrast, the stochastic model enlarges the contributions of individual IP$_3$Rs so that the generation of each spike is also effectively driven by random Ca$^{2+}$ release through the IP$_3$Rs, which can be seen in the inset of Fig. 5.7B where the site of spike initiation (blue bar) exhibits significantly greater variation than that of spike termination (green bar). In spite of this, the essential similarities in phase plane behavior result in both deterministic and stochastic models making the same qualitative predictions in response to perturbations, such as changes in IP$_3$ concentration ([IP$_3$]), Ca$^{2+}$ influx or efflux. In the following, we illustrate this by investigating a number of experimentally testable predictions. Due to the extensive importance of frequency encoding in many Ca$^{2+}$-dependent processes, we focus particularly on the change of oscillation frequency in response to parameter perturbations. As a side issue we also investigate how the oscillation baseline depends on physiologically important parameters.

### 5.2.3 Dependence of oscillation frequency on IP$_3$ concentration

In many cell types a moderate increase in [IP$_3$] increases the Ca$^{2+}$ oscillation frequency (see Fig. 2A in [154], Fig. 4E in [120] and Fig. 6B in [7]), a result that is reproduced by both model types (Fig. 5.8A). As [IP$_3$] increases, the stochastic model increases the probability of the initial Ca$^{2+}$ release through the first open IP$_3$R and of the following Ca$^{2+}$ release, thus shortening the average ISI. Although the oscillatory region of the deterministic model is strictly confined by bifurcations which do not apply to the stochastic model, the deterministic model can successfully replicate an increasing frequency by lowering the “knee” of the red curve in
Figure 5.8: Comparison of parameter-dependent frequency changes in the stochastic and deterministic models. All curves are computed at 0.12 µM IP$_3$ except in panel A, which uses a variety of [IP$_3$]. Other parameters are set at their default values given in Table 1. A: as [IP$_3$] increases, Ca$^{2+}$ oscillations in both models increase in frequency. B: as Ca$^{2+}$ influx increases (modeled by an increase in receptor-operated calcium channel flux coefficient $V_{rocc}$), so does the oscillation frequency in both models. C: as Ca$^{2+}$ efflux increases (modeled by an increase in plasma pump expression $V_{p}$), oscillation frequency decreases. D: as SERCA pump expression, $V_{s}$, increases, so does oscillation frequency. E: as total buffer concentration, $B_t$, increases, oscillation frequency decreases.
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Fig. 5.7D and shortening the time spent from the termination point c to the initiation point a (thus shortening the ISI). The difference between the two models also gives rise to a difference in oscillation frequency (see Fig. 5.8). Hence, although the deterministic model cannot be used to predict the exact values of [IP$_3$] at which the oscillations begin and end, as stochastic effects predominate in these regions [171], it can be used to predict the correct qualitative trend in oscillation frequency.

5.2.4 Dependence of oscillation frequency on Ca$^{2+}$ influx and efflux

In many cell types, including ASMC, transmembrane fluxes modulate the total intracellular Ca$^{2+}$ load ($c_t$) on a slow time scale [120, 19], and thereby modulate the oscillation frequency [151]. Experimental data can be seen in Fig. 8 in [120] and Fig. 2 in [19]. Figs. 5.8B and C show that both stochastic and deterministic models predict the same qualitative changes in oscillation frequency in response to changes in membrane fluxes (through membrane ATPase pumps and/or Ca$^{2+}$ influx channels such as receptor-operated channels or store-operated channels).

5.2.5 Dependence of oscillation frequency on SERCA expression

The level of sarco/endoplasmic reticulum calcium ATPase (SERCA) expression (or capacity) is important for airway remodeling in asthma [102] and ASMC Ca$^{2+}$ oscillations [135]. We thus investigated the predictions of the two models in response to changes in SERCA expression ($V_s$). As $V_s$ decreases, the deterministic model exhibits a decreasing frequency, in agreement with experimental data (see Figs. 3 and 4 in [135]). The same trend is seen in the stochastic model with only 20 IP$_3$Rs (see Fig. 5.8D).

5.2.6 Dependence of oscillation frequency on Ca$^{2+}$ buffer concentration

Calcium buffers have been shown to be able to change the ISI and spike duration, which in turn change the oscillation frequency [145, 188]. We compared the effects on the two models of varying total buffer concentration ($B_t$) by adding one buffer with relatively fast kinetics to the models (see Materials and Methods for details). In both models the frequency decreases as $B_t$ increases (see Fig. 5.8E), which is consistent with experimental data (Fig. 2B in [19]). This is not surprising, because increasing $B_t$ can decrease the effective rates of SR Ca$^{2+}$ release and reuptake.

5.2.7 Dependence of oscillation baseline on Ca$^{2+}$ influx and SERCA expression

Sustained elevations of baseline during agonist-induced Ca$^{2+}$ oscillations or transients have been observed experimentally, and are believed to be a result of an increase in Ca$^{2+}$ influx
caused by opening of membrane Ca\(^{2+}\) channels [37, 120]. Furthermore, there is evidence showing that decreased SERCA expression could also increase the baseline (Fig. 4 in [135]). Those phenomena are successfully reproduced by both models (see Fig. 5.9).

5.3 Discussion

In this chapter we address two current major questions in the field of Ca\(^{2+}\) modeling. Firstly, we show that Ca\(^{2+}\) puffs and stochastic oscillations can be reproduced quantitatively by an extremely simple model, consisting only of two states (one open, one closed), with time-dependent transitions between them. This model is obtained by removing the intramodal structure of a more complex model that was determined by fitting a Markov model to single-channel data [144]. We thus show that the internal structure of each mode is irrelevant for function and mode switching is the key mechanism for the control of calcium release. The necessity for time-dependent mode switching is shown not only by the dynamic single-channel data of [105]), but also by the puff data of [147] and our ASMC data.

Secondly, we investigate the role of stochasticity of IP\(_3\)R in modeling Ca\(^{2+}\) oscillations in ASMC by comparing a stochastic IP\(_3\)R-based Ca\(^{2+}\) model and its associated deterministic version, for parameters such that both of the models exhibit Ca\(^{2+}\) spikes but the stochastic model cannot necessarily be replaced by a mean-field model. We find that a four-variable deterministic model has the same predictive power as the stochastic model, in that it correctly reproduces the process of spike termination and predicts the same qualitative changes in oscillation frequency and baseline in response to a variety of perturbations that are commonly used experimentally. The mechanism for termination of individual spikes is fundamentally a deterministic process controlled by a rapid inhibition induced by the high local \([\text{Ca}^{2+}]_i\) in the IP\(_3\)R cluster, whereas...
spike initiation is significantly affected by stochastic opening of IP$_3$Rs. Hence, repetitive Ca$^{2+}$ cycling is primarily induced by the time-dependent gating variables governing transitions of the IP$_3$R from one mode to another.

Our simplified two-state model of the IP$_3$R is identical in structure (although not in parameter values) to the well-known model of [99]. It is somewhat ironic that after 20 years of detailed studies of the IP$_3$R, and the construction of a plethora of models of varying complexity, the single-channel data have led us around full circle, back to these original formulations. The IP$_3$R model modulated by $[\text{Ca}^{2+}]$, shows a fast activation followed by a slower recovery from inhibition, a combination often seen in physiological processes [92]. Encoding of this fundamental combination results directly from the two-mode structure of the IP$_3$R. Although similar single-channel data have been used to construct three-mode models [177, 84], neither of these models has yet been used in detailed studies of Ca$^{2+}$ puffs and waves, and it remains unclear whether or not they have a similar underlying structure.

In contrast to previous deterministic ODE models, our four-variable Ca$^{2+}$ model includes a more accurate IP$_3$R model, as well as local control of clustered IP$_3$Rs by two distinct Ca$^{2+}$ microdomains; one at the mouth of an open IP$_3$R, the other inside a cluster of IP$_3$Rs. Neglect of either of these microdomains leads to models that either exhibit unphysiological cytoplasmic Ca$^{2+}$ concentrations or fail to reproduce reasonable oscillations. This underlines the importance of taking Ca$^{2+}$ microdomains into consideration when constructing any model. Our microdomain model is highly simplified, with the microdomain being treated simply as a well-mixed compartment. More detailed modeling of spatially-dependent microdomains is possible, and not difficult in principle, but requires far greater computational resources. It is undeniable that a more detailed model, incorporating the full spatial complexity – and possibly stochastic aspects as well – would make, overall, a better predictive tool. However, our goal is to find the simplest models that can be used as predictive tools.

An important similar study is that of Shuai and Jung [138]. They compared the use of Markov and Langevin approaches to the computation of puff amplitude distributions, compared their results with the deterministic limit, and showed that IP$_3$R stochasticity does not qualitatively change the type of puff amplitude distribution except for when there are fewer than 10 IP$_3$Rs. Here, we significantly extend the scope of their study by exploring the effects of IP$_3$R stochasticity on the dynamics of Ca$^{2+}$ spikes, and we do this in the context of an IP$_3$R model that has been fitted to single-channel data. Although this is true in a general sense for the Li-Rinzel model, which is based on the DeYoung-Keizer model, which did take into account the opening time distributions of IP$_3$R in lipid bilayers, neither model can reproduce the more recent data obtained from on-nuclei patch clamping. When these recent data are taken into account one obtains a model with the same structure, but quite different parameters and behavior.

We find that, in spite of a relatively large variation in spike amplitude which is partially caused by a large variation in ISI (Fig. 5.7B), the mechanism governing individual spike terminations is the same for both a few or infinitely many IP$_3$Rs, which explains why the one-peak
type of amplitude distribution is independent of the choice of IP$_3$R number (see Fig. 6A in [138]).

Another important relevant study was done by Dupont et al. [48], who compared the regularity of stochastic oscillations in hepatocytes for different numbers of IP$_3$R clusters. They found that the impact of IP$_3$R stochasticity on global Ca$^{2+}$ oscillations (in terms of CV) increases as the total cluster number decreases. Our study here extends these results, and demonstrates how well stochastic oscillations can be qualitatively described by a deterministic system, even when there is only a small number of IP$_3$R (which appears to be the case for ASMC, in which the wave initiation site is only 2 – 4 µm in diameter). Indeed, as we have shown, for the purposes of predictive modeling a simple deterministic model does as well as more complex stochastic simulations.

Ryanodine receptors (RyR) are another important component modulating ASMC Ca$^{2+}$ oscillations [120, 91, 166] but are not included in our model. This is because the role of RyR is not fully understood and may be species-dependent; for example, in mouse or human ASMC, RyR play very little role in IP$_3$-induced continuing Ca$^{2+}$ oscillations [7, 127], but this appears not to be true for pigs [91]. Our study focuses on the calcium oscillations in mouse and human (as we did in our experiments) where inclusion of a deterministic model of RyR should have little effect. An understanding of the role of RyR stochasticity and how the IP$_3$R and the RyR interact needs a reliable RyR Markov model, exclusive to ASMC, which is not currently available. Multiple Markov models of the RyR have been developed for use in cardiac cells [156], but these are based on single-channel data from lipid bilayers, and are adapted for the specific context of cardiac cells. Their applicability to ASMC remains unclear.

Although we have not shown that the deterministic model for ASMC has the same predictive power as the stochastic model in all possible cases (which would hardly be possible in the absence of an analytical proof) the underlying similarity in phase plane structure indicates that such similarity is plausible at least. Certainly, we have not found any counterexample to this claim. However, whether or not this claim is true for all cell types is unclear. Some cell types exhibit both local Ca$^{2+}$ puffs and global Ca$^{2+}$ spikes (usually propagating throughout the cells in the form of traveling waves), showing that initiation of such Ca$^{2+}$ spikes requires a synchronization of Ca$^{2+}$ release from more than one cluster of IP$_3$Rs [109]. This type of spiking relies on the hierarchical organization of Ca$^{2+}$ signal pathways, in particular the stochastic recruitment of both individual IP$_3$Rs and puffs at different levels [174], and therefore cannot be simply reproduced by deterministic models containing only a few ODEs. However, Ca$^{2+}$ oscillations in ASMC, as observed in lung slices, may not be of this type, as IP$_3$R-dependent puffs have not been seen in these ASMC. It thus appears that, in ASMC in lung slices, every Ca$^{2+}$ “puff” initiates a wave, resulting in periodic waves with ISI that are governed by the dynamics of individual puffs.
Chapter 6

SUMMARY AND FUTURE WORK

Cytoplasmic Ca\(^{2+}\) oscillations mediated by repetitive openings and closings of IP\(_3\)R channels situated in the endo/sarcoplasmic reticulum membrane are found in many cell types. However, the mechanisms accounting for such oscillations remain unclear, especially how the IP\(_3\)R behaves in living cells to perform its function. In light of the extensive existence of calcium oscillations in many other cell types, although this thesis focuses on modeling calcium oscillations in ASMC due to their importance for the study of pathology of asthma, it also aims to solve some major questions in a wider context:

- First of all, what is the mechanism for the formation of the repetitive Ca\(^{2+}\) releases? How is the mechanism connected to the IP\(_3\)R dynamics? The key to answering these questions is to understand how each Ca\(^{2+}\) release is terminated. In Chapter 1, we mentioned several proposed mechanisms in the literature for oscillation termination, but lack of understanding of IP\(_3\)R kinetics in vivo remains an obstacle for the full solution of this problem.

- Second, how best (or simply) should the IP\(_3\)R be modeled for performing its function? Models of the IP\(_3\)R have a long history, beginning with the heuristic models of [43, 47, 4]. With the recent appearance of single-channel data from IP\(_3\)R in vivo [105, 179], a new generation of Markov IP\(_3\)R models has recently appeared [178, 144]. In light of the behaviors of these recent models, it is important to know which one is more important in generating oscillations, intra-mode transitions within each mode or ligand-gated inter-mode transitions.

- How much does the channel stochasticity matter for the construction of a useful predictive model? In other words, does the deterministic model (which is easier and faster to solve) have the same predictive power as the stochastic model? Many examples have shown deterministic models of the IP\(_3\)R are useful in advancing our understanding of Ca\(^{2+}\) oscillations and making predictions for a range of cell types. However, the deterministic assumption contradicts experimental findings in many cell types showing that Ca\(^{2+}\) events (puffs and oscillations) arise as an emergent property of fundamental stochastic
events. Thus, it is important to reexamine the reliability of the deterministic models by comparison with stochastic models.

Here, we answer the above questions using data and models from either in vivo single-channel measurement or ASMC. Although we mostly work in the specific context of ASMC, our results are applicable to other cell types that exhibit similar Ca\textsuperscript{2+} oscillations and waves. Our primary findings are

- First, based on a very recent IP\textsubscript{3}R model (the Siekmann model, see Chapter 2) that fits to stationary single-channel data and available nonstationary single-channel data, we developed a new IP\textsubscript{3}R model by introducing time-dependent inter-mode transitions. We applied the new IP\textsubscript{3}R model to modeling calcium puffs (localized calcium events) and showed that the time-dependent feature is crucial for a quantitatively reproduction of behaviors and statistical properties of calcium puffs. The fits both to single-channel data as well as to puff data provide some reassurance that our new IP\textsubscript{3}R model can be reliably used to study the link between IP\textsubscript{3}R dynamics and oscillatory Ca\textsuperscript{2+} signals.

- Second, we showed that the existing 6-state IP\textsubscript{3}R model could be reduced down to a simple 2-state model without losing its function. The multiple states within each mode are necessary to obtain an acceptable quantitative fit to single-channel data, but are nevertheless of limited importance for function. This result leads us around full circle, back to an early formulation where the calcium oscillations arise as a fast-slow dynamical system extensively seen in physiological processes.

- Third, we compared a stochastic ASMC calcium model and its associated deterministic model, and showed that 1) the two models have qualitatively the same dynamical changes in the phase plane; and 2) both of the models successfully predicted the trends of frequency change in response to a number of experimentally testable parameter perturbations. This allows reliable use of deterministic models in making predictions. Although we cannot develop a general theory for this conclusion which would hardly be possible in the absence of an analytical proof, this could be one of the steps towards the ultimate goal and will have an impact.

Although progress has been made, many questions, including the ones mentioned above are far from being answered. One of the most important questions is how the Ca\textsuperscript{2+} spike exhibiting long duration, like those in Xenopus oocytes, pancreatic acinar cells and HEK293 cells, emerges from relatively rapid IP\textsubscript{3}R kinetics revealed by single-channel data and puff data. One plausible explanation is the long spikes result directly from coordinated occurrence of multiple puffs, but its mechanism is still being challenged by emerging data (especially single-channel data and puff data). Therefore, given the data-driven IP\textsubscript{3}R model, an investigation on how the time scale of spikes is dependent on the time scales of the puffs, and also the number and cooperativity
of the puffs is absolutely necessary as the next task, though the numerical realization of the scenario requires a lot of time- and memory-consuming computations.

Another urgent question is how ryanodine receptors (RyR) influence Ca$^{2+}$ dynamics. As mentioned in Chapters 1 and 5, the role of RyR depends on cell types and species. This could be due to difference in RyR kinetics or difference in level of RyR expression. However, a reliable RyR Markov model constructed based on *in vivo* single-channel data has not been available. Hence, constructing such a RyR model (similar to building the IP$_3$R model), checking its reliability, and investigating the role of RyR and interaction between the IP$_3$R and the RyR in Ca$^{2+}$ oscillations, will be an important next step.

Last but not least, as mentioned in Chapter 5, a general theory about the usability of deterministic calcium models in making reliable predictions is still far from being established. A stochastic model is more realistic, whereas a deterministic model is more efficient to solve and analyze analytically. Although people have their preferences in choosing models to achieve their specific purposes, it is always helpful if there is a theory letting them know which model, either the deterministic and stochastic, is a better choice (or whether there is any major difference).
Appendix A

Hybrid Gillespie method for model simulation

When simulating IP$_3$R-mediated stochastic calcium release (which is the focus of this thesis), the most important step is to determine the state of each IP$_3$R which will give the fraction of open IP$_3$Rs and thus the flux through IP$_3$R (i.e. $J_{IPR}$). Each channel will stay in one state for a random time and then jump to a different state in a probabilistic way. To simulate such a transition, an efficient method is Gillespie’s algorithm [67]. This method takes into account the exponential distribution of dwell times of a state, making use of which the transition time escaping from the state can be determined by simply picking a random number from the uniform distribution on $[0,1]$.

The limitation of the classical Gillespie method lies in its assumption of constant interstate transition rates that well define the exponential dwell time distributions used to determine the transition time. However, for the case that the transition rates are ligand-dependent and change dramatically in response to a rapid change in ligand concentration (which is the case where some transition rates in IP$_3$R models are very sensitive to the change of $[Ca^{2+}]_i$), an improved method [1, 131] (called the hybrid Gillespie method) has to be used. In the following, I will use the Siekmann model (Fig. 2.2) as an example to show how the hybrid method works.

First of all, according to Fig. 2.2, we define the transition matrix of the model to be

$$T = (T_{ij}) = \begin{pmatrix}
0 & q_{12} & 0 & 0 & 0 & 0 \\
q_{21} & 0 & q_{23} & q_{24}(c) & 0 & q_{26} \\
0 & q_{32} & 0 & 0 & 0 & 0 \\
0 & q_{42}(c) & 0 & 0 & q_{45} & 0 \\
0 & 0 & 0 & q_{54} & 0 & 0 \\
0 & q_{62} & 0 & 0 & 0 & 0 \\
\end{pmatrix}, \quad (A.1)$$

where all the elements are constants except $q_{24}$ and $q_{42}$ which are functions of cytoplasmic Ca$^{2+}$ concentration ($c$). Let $S_i$ be the probability of staying in a state $i$ ($i = 1, 2, ..., 6$ corresponding to...
the six state in the model), then evolution of $S_i$ is described by an ordinary differential equation

$$\frac{dS_i}{dt} = - \sum_j T_{ij} S_i. \quad (A.2)$$

Therefore we have the solution of Eq. 2.16 subject to the initial condition $S_i(0) = 1$,

$$S_i = e^{-\int_0^t \sum_j T_{ij} ds}. \quad (A.3)$$

Here we use the integral form because $\sum_j T_{ij}$ could be a function of $c$ which could be a function of time. Note that we set the integral interval starting from 0 because of the memorylessness of the Markov process. Two random numbers $r_1$ and $r_2$ are first drawn from a uniform distribution between 0 and 1 to determine the transition time and which state it will switch to. By introducing a tracking variable $g_i = \int_0^t \sum_j T_{ij} ds$, as time increases, $g_i$ keeps being accumulated until $S_i \leq r_1$, (or $g_i \geq \ln(1/r_1)$) (which indicates the condition for state transition). By recording the time spent in $S_i$ (denoted by $\tau$), the new state $S_k$ is obtained by choosing a $k$ such that $\sum_{j < k} T_{ij}(c(\tau)) < r_2 \sum_j T_{ij}(c(\tau)) \leq \sum_{j < k+1} T_{ij}(c(\tau))$, where $T_{ij}(c(\tau))$ represents the transition rate evaluated at the transition time $\tau$ (when the value of $c$ is $c(\tau)$).
BIBLIOGRAPHY


