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Langevin, population density and moment-based modeling of local and global aspects of intercellular calcium signaling

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ABSTRACT

Markov chain models of the coupled gating of intracellular calcium ($Ca^{2+}$) channels are often used to study the stochastic dynamic of local $Ca^{2+}$ release events and whole cell $Ca^{2+}$ homeostasis. However, the runtime of the Markov chain description of $Ca^{2+}$ channel gating is exponential in the number of $Ca^{2+}$ channel states and may thus result in a combinatorial state space explosion when the number of channel states is large. This dissertation presents several novel stochastic modeling approaches that capture important aspects of $Ca^{2+}$ signaling while improving computational efficiency. This dissertation presents several novel stochastic modeling approaches that capture important aspects of calcium $Ca^{2+}$ signaling.

First, we present a $Ca^{2+}$ release site modeling approach based on a Langevin description of stochastic $Ca^{2+}$ release. This Langevin model facilitates our investigation of correlations between successive puff/spark amplitudes, durations and inter-spark intervals, and how such puff/spark statistics depend on the number of channels per release site and the kinetics of $Ca^{2+}$-mediated inactivation of open channels. Second, we show that when the $Ca^{2+}$ channel model is minimal, Langevin equations in a whole cell model involving a large number of release sites may be replaced by a single Fokker-Planck equation. This yields an extremely compact and efficient local/global whole cell model that reproduces and helps interpret recent experiments investigating $Ca^{2+}$ homeostasis in permeabilized ventricular myocytes. Last but not least, we present a population density and moment-based approach to modeling $L$-type $Ca^{2+}$ channels. Our approaches account for the effect of heterogeneity of local $Ca^{2+}$ signals on whole cell Ca currents. Moreover, they facilitate the study of domain Ca-mediated inactivation of $L$-type Ca channels.
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To my family and friends, without whom this would not have been possible.
Chapter 1

Introduction

Calcium (Ca$^{2+}$) ions are an ubiquitous intracellular signaling molecule that plays a key role in regulating cell functions. In the early development of eggs, for example, it has been observed that the fertilizing sperm triggers propagating Ca$^{2+}$ waves (Deguchi et al., 2000). The fertilization Ca$^{2+}$ signal restarts the cell cycle, via CaM/CaMKII pathways that interact with the cell cycle control machinery (Whitaker, 2006). During the development of cells, Ca$^{2+}$ stimulates the translocation of transcriptional factors from the cytoplasm into the nucleus (Berridge, 1993b). Increasing of cytosolic [Ca$^{2+}$] can activate hydrolytic enzymes and initiate cytoskeletal degradation that results in programmed cell death (apoptosis) (Nicotera and Orrenius, 1998).

Ca$^{2+}$ has been established as a key factor in regulating neurotransmitter release, excitability, plasticity and gene transcription in neurons (Mattson, 1988; Berridge, 1998). For instance, neuronal membrane depolarization and subsequent Ca$^{2+}$ influx into the cytoplasm can induce new gene transcription (West et al., 2001). Long-term potentiation (LTP) is caused by brief but large increases in postsynaptic [Ca$^{2+}$], while long-term depression (LTD) results from prolonged but smaller [Ca$^{2+}$] (Deguchi et al., 1999).

In cardiac cells, L-type Ca$^{2+}$ channels are activated upon membrane depolarization, and the Ca$^{2+}$ currents through these channels can trigger a larger amount of Ca$^{2+}$ release from the sarcoplasmic reticulum, a process known as Ca$^{2+}$-induced Ca$^{2+}$ release (CICR). CICR can
lead to the upstroke of Ca\(^{2+}\) transients and cause contraction when these Ca\(^{2+}\) ions binding to myofilaments. This physiological event is known as excitation-contraction (EC) coupling. EC coupling is important in regulating heart health. Abnormal EC coupling may trigger heart failure. For example, it has been observed that L-type Ca\(^{2+}\) currents are less effective at triggering sarcoplasmic reticulum (SR) Ca\(^{2+}\) release in hypertrophied and failing myocytes (Gómez et al., 1997, 2001). Moreover, there is typically a down regulation of SR Ca-ATPase and an up regulation of Na\(^{+}/Ca^{2+}\) exchanger function during heart failure (Pogwizd et al., 1999; Piacentino et al., 2003), both reducing SR Ca\(^{2+}\) content. Ca\(^{2+}\) alternans, on the other hand, can trigger T-wave alternans and further increase the risk of sudden cardiac death (Rovetti et al., 2010; Bers, 2008).

The time scale of Ca\(^{2+}\) signaling may vary over several orders of magnitude (Marchant and Parker, 2000). For example, Ca\(^{2+}\) is fundamental in electrical activation and ion channel gating that all occur on the millisecond time scale (Bers and Guo, 2005). On the other hand, Ca\(^{2+}\) also directly activates signaling via kinases and phosphatases that occur over a longer time scale (Bers and Guo, 2005).

1.1 Calcium homeostasis

Cytosolic [Ca\(^{2+}\)] is maintained between 10–100 nM, much lower than extracellular [Ca\(^{2+}\)] (1–2 mM) and intracellular Ca\(^{2+}\) stores (10–1000 μM) (Hille, 2001). Intracellular [Ca\(^{2+}\)] is regulated by multiple processes. In general, the endoplasmic reticulum (ER) or sarcoplasmic reticulum (SR) contributes to the dynamic of Ca\(^{2+}\) signaling by acting as either a source or a sink of Ca\(^{2+}\) release (Simpson et al., 1995). Ca\(^{2+}\) can enter into or leave the cytosol from the extracellular space or the ER/SR via Ca\(^{2+}\) channels, pumps or exchangers.

The pump and exchanger mechanisms maintain the resting level of cytosolic [Ca\(^{2+}\)] at approximately 100 nM (Bootman et al., 2001) and ensure that the internal stores are kept loaded. Four different pumping mechanisms are primarily responsible for regulating Ca\(^{2+}\) homeostasis, i.e., the plasma-membrane Ca\(^{2+}\)-ATPase (PMCA), the Na\(^{+}/Ca^{2+}\) exchanger.
(NCX), the sarcoplasmic edoplasmic reticulum Ca\textsuperscript{2+}-ATPase (SERCA) and mitochondrial uniporters. PMCA is a protein on the plasma membrane that couples the movement of Ca\textsuperscript{2+} ions into the extracellular space to the hydrolysis of ATP (Temple and Shilling, 2007). In this way, Ca\textsuperscript{2+} can be “pumped” against the extracellular/intracellular concentration gradient. In a similar manner, SERCA pumps Ca\textsuperscript{2+} into the ER/SR to maintain ER/SR stores (Periasamy and Kalyanasundaram, 2007). The Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (NCX) is a transporter that extrudes one Ca\textsuperscript{2+} ion in exchange for three Na\textsuperscript{+} ions, this process can generate an inward current and therefore prolongs action potential duration (Blaustein and Lederer, 1999). Mitochondrial uniporters located in the organelle’s inner membrane pass Ca\textsuperscript{2+} down the membrane’s electrochemical gradient without ATP hydrolysis or other transport proteins (Gunter and Gunter, 1994). Comparing the four types of transport proteins, the PMCA and SERCA pump have relatively lower rates but high affinities (Berridge, 1998). The NCX and mitochondrial uniporter have much greater transport rates and can thus limit Ca\textsuperscript{2+} transients over a wider range (Berridge, 1998). Mitochondrial uniporters also have high affinity and therefore enable high Ca\textsuperscript{2+} selectivity despite relatively low cytoplasmic [Ca\textsuperscript{2+}] (Kirichok et al., 2004).

In additional to Ca\textsuperscript{2+} channels and pumps, Ca\textsuperscript{2+} buffers are also important regulators of Ca\textsuperscript{2+} homeostasis. Ca\textsuperscript{2+} buffers are Ca\textsuperscript{2+}-binding proteins that chelate to free Ca\textsuperscript{2+} ions. Calbindin, for example, is a Ca\textsuperscript{2+} buffer that observed at high concentrations in neurons. Study has shown that the calbindin regulates Ca\textsuperscript{2+} influx through voltage-dependent Ca\textsuperscript{2+} channels in RIN pancreatic beta cells (Lee et al., 2006). Calbindin is also associated with faster age-related decline in hippocampus metabolism (Moreno et al., 2012). Calsequestrin, on the other hand, is a low-affinity Ca\textsuperscript{2+}-binding protein that is important in regulating Ca\textsuperscript{2+} content inside the SR lumen of cardiac and skeletal muscle (Fill and Copello, 2002; Györke et al., 2004). Calsequestrin can also regulate SR Ca\textsuperscript{2+} release channels independent of its function as a Ca\textsuperscript{2+} buffer in the SR lumen (Chopra et al., 2007). It has been shown that even modest reductions of cardiac calsequestrin can increase SR [Ca\textsuperscript{2+}] and therefore trigger ventricular arrhythmias in mice (Chopra et al., 2007).
1.2 Calcium channels

Ca\textsuperscript{2+} channels can be classified based on their activation and inactivation mechanisms. Voltage-gated channels (VOCCs), for example, are activated by the change of membrane potential and are employed largely by excitable cell types such as muscle and neuronal cells (Warrd et al., 1996). Voltage-gated Ca\textsuperscript{2+} channels have been classified into three subgroups based on their subunit composition and functions: the Ca\textsubscript{\textalpha}1 family includes L-type Ca\textsuperscript{2+} channels, the Ca\textsubscript{\textalpha}2 family includes N-, P/Q- and R-type Ca\textsuperscript{2+} channels, and the Ca\textsubscript{\textalpha}3 family includes T-type Ca\textsuperscript{2+} channels. The Ca\textsubscript{\textalpha}1 family is important in initiating contraction and secretion and in regulating gene expression (Calin-Jageman and Lee, 2008). The Ca\textsubscript{\textalpha}2 family is primarily responsible for initiation of synaptic transmission (Catterall and Few, 2008). The Ca\textsubscript{\textalpha}3 family plays a key role in repetitive firing of action potentials in cardiac myocytes and thalamic neurons (Catteral, 2011; Warrd et al., 1996). L-type Ca\textsuperscript{2+} currents have slow voltage-dependent inactivation and therefore are long lasting. N-type, P/Q-type and R-type Ca\textsuperscript{2+} currents need more negative potentials for complete removal of inactivation and strong depolarization for activation (Nowycky et al., 1985; Tsien et al., 1991). T-type currents inactivate at lower membrane potentials than L-type currents (Catteral, 2011).

Receptor-operated Ca\textsuperscript{2+} channels (ROCCs), on the other hand, are activated by binding of ligands. Different ROCCs can be activated by different agonists, e.g., ATP, serotonin and glutamate (Bootman et al., 2001). Two well known ROCCs are the N-methyl-D-aspartate receptor (NMDAR) and nicotinic acetylcholine receptor (nARhR). NMDARs are mainly expressed on postsynaptic dendrites and are activated by neurotransmitter glutamate (Zito and Scheuss, 2009). NMDARs are important in regulating long-term synaptic potentiation. It is well established that coincident activity in pre- and postsynaptic neurons resulting in Ca\textsuperscript{2+} influx through synaptic NMDARs is necessary for the triggering of both LTP and LTD (Luscher and Malenka, 2012). The nARhRs are activated by the binding of the neurotransmitter acetylcholine and can be divided into muscle receptors and neuronal receptors. Muscle receptors are mainly found at skeletal neuromuscular junction where they mediate
neuromuscular transmission; neuronal receptors, on the other hand, are mainly expressed in central nervous system where they are involved in fast synaptic transmission (Hogg et al., 2003).

Two important families of intercellular Ca\textsuperscript{2+} channels located on the ER membrane are inositol 1,4,5-trisphosphate receptors (IP\textsubscript{3}Rs) and ryanodine receptors (RyRs). Both of them play an important role in mobilizing stored Ca\textsuperscript{2+}. IP\textsubscript{3}Rs are activated by the binding of inositol 1,4,5-trisphosphate (InsP\textsubscript{3}). IP\textsubscript{3}Rs display a bell-shaped response to cytosolic Ca\textsuperscript{2+} which functions as a coagonist with InsP\textsubscript{3} (Finch et al., 1991). The InsP\textsubscript{3}-induced Ca\textsuperscript{2+} release displays all-or-none characteristics (Parker and Yao, 1991) and its sensitivity may change depending on the Ca\textsuperscript{2+} content of the ER (Missiaen et al., 1991). The RyRs are identified by their ability to bind the plant alkaloid ryanodine (McPherson and Campbell, 1993). Studies showed that low concentrations of ryanodine lock the RyRs into a long-lived open state (Wang et al., 1993), while higher ryanodine concentrations inhibit channel opening (Nagasaki and Fleischer, 1988). Both the IP\textsubscript{3}R and RyR have three major channel isoforms: the IP\textsubscript{3}R1, IP\textsubscript{3}R2, IP\textsubscript{3}R3; and the RyR1, RyR2 and RyR3, respectively. IP\textsubscript{3}R1s are mainly distributed in Purkinje neurons in the cerebellum and neurons in other regions, while IP\textsubscript{3}R2s are detected in glial cells and IP\textsubscript{3}R3s are mainly found in neurons with little detected in glial cells (Sharp et al., 1999). RyR1s are mainly found in skeletal muscle (Phillips et al., 1996), RyR2s are expressed in cardiac cells (Berridge, 1998), and RyR3s are mainly expressed in the neurons and glial cells (Murayama and Ogawa, 1996). IP\textsubscript{3}Rs and RyRs share considerable structural and functional homologies, suggesting a common evolutionary origin (Berridge, 1993b).

Fig. 1.1 illustrates the most important components of the intracellular Ca\textsuperscript{2+} signaling machinery including the Ca\textsuperscript{2+} channels, pumps and exchangers discussed above. Black arrows indicate the direction of Ca\textsuperscript{2+} flux.
1.3 Local and global calcium signaling

Intracellular Ca\textsuperscript{2+} signaling usually involves a complex interplay between global, cell-wide changes in [Ca\textsuperscript{2+}] and local, subcellular Ca\textsuperscript{2+} release events. Local signals are frequently caused by the release of Ca\textsuperscript{2+} from intracellular stores, primarily the ER/SR. Spatially localized Ca\textsuperscript{2+} release events are mediated by clusters of Ca\textsuperscript{2+} release channels located on the ER/SR. These clusters are called as Ca\textsuperscript{2+} release units (CaRUs) and they are usually composed of 50–200 intercellular Ca\textsuperscript{2+} channels depending on species, cell type, and receptor type (IP\textsubscript{3}Rs or RyRs) (Franzini-Armstrong et al., 1999). In healthy cardiac myocytes, CaRUs are relatively evenly spaced and close to T-tubule membranes. However, in diseased cells, structural alterations can occur. For example, RyR clusters can become smaller (Wu
et al., 2012) or move away from T-tubules (van Oort et al., 2011). The distance between neighboring release sites varies from 100 to 700 nm depending on the length of free T-tubule interactactions between the junctional T-tubule segments (Franzini-Armstrong et al., 1999).

Ca\(^{2+}\) release through CaRUs can occur via three different modes of Ca\(^{2+}\) mobilization, denoted by Berridge and colleagues (Berridge, 1997) as fundamental, elementary and global mode. The fundamental mode of Ca\(^{2+}\) mobilization involves Ca\(^{2+}\) elevations due to the activation of a single channel; a process known as Ca\(^{2+}\) blips or quarks depending on whether the events are mediated by IP\(_3\)Rs or RyRs (Niggli, 1999; Niggli and Shirokova, 2007). The elementary mode of Ca\(^{2+}\) mobilization involves Ca\(^{2+}\) elevations due to the activation of multiple IP\(_3\)Rs or RyRs in a single CaRU; these events are known as Ca\(^{2+}\) puff/sparks (Cheng et al., 1993a; Cannell et al., 1995a; Parker et al., 1996). The global mode of Ca\(^{2+}\) mobilization usually involves cell-wide Ca\(^{2+}\) elevations such as propagating Ca\(^{2+}\) waves or oscillations, that result from Ca\(^{2+}\) release via multiple CaRUs (Cheng et al., 1996).

1.4 Mathematical model of calcium signaling

1.4.1 Markov chain model of calcium channel gating

Stochastic Ca\(^{2+}\) signaling has been described by discrete state continuous time Markov chains. In this section, we will give a short review about the Markov chain description of stochastic gating of clusters of Ca\(^{2+}\) channels.

We will consider a CaRU composed of \(N\) two-state channels as an example. The single channel gating kinetics can be described by the following transition-state diagram

\[
\begin{align*}
\text{(closed) } C & \; \xrightleftharpoons[k^-]{k^+c^n} \; \text{(open) }, \\
\end{align*}
\]

where \(k^+c^n\) and \(k^-\) are transition rates with units of reciprocal time, \(k^+\) is an association rate
constant with units of \( \text{conc}^{-\eta} \text{time}^{-1} \), \( \eta \) is the cooperativity of \( \text{Ca}^{2+} \) binding, and \( c \) is the local \([\text{Ca}^{2+}]\). Under the assumption that a collection of \( N \) two-state RyRs are instantaneously coupled by a local \([\text{Ca}^{2+}]\) associated with the RyR cluster, the transition-state diagram for the \( \text{Ca}^{2+} \) release site as a collective entity is

\[
\begin{array}{cccc}
Nk^+c^n_0 & (N-1)k^+c^n_1 & 2k^+c^n_{N-2} & k^+c^n_{N-1} \\
0 & \rightarrow & \rightarrow & \rightarrow & \rightarrow & \rightarrow \\
0 & \equiv & 1 & \equiv & \equiv & \equiv & \equiv & \equiv & \equiv & N, & \equiv & N \\
k^- & 2k^- & (N-1)k^- & Nk^- & \\
\end{array}
\]

where the states \([0, 1, \ldots, N]\) indicate the number of open channels, \( N_0 \), and \( c_n \) is the local \([\text{Ca}^{2+}]\) experienced by channels in the release site when \( N_0 = n \). For simplicity, we can assume local \([\text{Ca}^{2+}]\) is a linear function of \( n \). That is, the \([\text{Ca}^{2+}]\) in the \( n \)th CaRU is given by \( c_n = c_{\infty} + nc_* \), where \( c_{\infty} \) is the bulk or background \([\text{Ca}^{2+}]\). The parameter \( c_* \) is referred to as the coupling strength, because it determines the increment in local \([\text{Ca}^{2+}]\) that occurs when an individual channel opens.

Eq. 1.2 specifies the infinitesimal generator matrix \( Q(t) = \{q_{ij}(t)\} (1 \leq i, j \leq N) \) for the Markov chain release site model. The relationship of these rate constants to the transition probabilities in a short increment of time \( \Delta t \) is \( \Pr(S_{t+\Delta t} = j | S_t = i) = q_{ij}(t)\Delta t \).

Assuming the Markov chain model has \( M \) states, and we may write \( \pi = [\pi_1, \pi_2, \ldots, \pi_M] \), a row vector collects the probability in each state \( \pi_i \). In this case the dynamic of the Markov chain probability distribution \( \pi \) is given by \( M \) ordinary differential equations (ODEs)

\[
\frac{d\pi}{dt} = \pi Q. \quad (1.3)
\]

At steady state, the stationary distribution \( \bar{\pi} \) satisfies

\[
\bar{\pi}Q = 0 \quad \text{and} \quad \bar{\pi}e = 1, \quad (1.4)
\]
where $e$ is a column vector with all element is 1.

### 1.4.2 Mathematical model of calcium homeostasis

We here present a simple two-compartment whole cell model to illustrate one method of simulating global $\text{Ca}^{2+}$ responses. More complicated models will be discussed in later chapters. Denote $c_{\text{cyt}}$ and $c_{\text{er}}$ as the $[\text{Ca}^{2+}]$ in the cytosol and ER, respectively. Assuming the whole cell $\text{Ca}^{2+}$ dynamics involve $\text{Ca}^{2+}$ release flux ($J_{\text{rel}}$) from the ER to the cytosol via IP$_3$Rs or RyRs, and the $\text{Ca}^{2+}$ flux ($J_{\text{pump}}$) from the cytosol to the ER via SERCA pump, the $\text{Ca}^{2+}$ concentration changes can then be expressed by the following ordinary differential equations:

\[
\frac{dc_{\text{cyt}}}{dt} = J_{\text{rel}} - J_{\text{pump}} \\
\frac{dc_{\text{er}}}{dt} = \frac{1}{\lambda_{\text{er}}}(-J_{\text{rel}} + J_{\text{pump}}),
\]

where $\lambda_{\text{er}}$ is the effective volume ratio between the ER and cytosol. The release flux $J_{\text{rel}}$ can be given by $J_{\text{rel}} = \pi_{\text{O}}(c_{\text{er}} - c_{\text{cyt}})$ where $\pi_{\text{O}}$ is the open probability for all the $\text{Ca}^{2+}$ channels on the ER. The restorative pump flux can by represented by $J_{\text{pump}} = v_{\text{pump}}c_{\text{cyt}}^2/(c_{\text{cyt}}^2 + k_{\text{pump}}^2)$. This two-compartment whole cell model is a “common pool” model. It may not represent the whole cell dynamics accurately since the $\text{Ca}^{2+}$ channels are in reality spatially localized and local $[\text{Ca}^{2+}]$ are heterogeneous due to local $\text{Ca}^{2+}$ release events. Previous studies of $\text{Ca}^{2+}$ handling in cardiac myocytes have shown that common pool models exhibit “all-or-none” $\text{Ca}^{2+}$ transients (Stern, 1992) while experimental results showing smoothly graded with changes in $\text{Ca}^{2+}$ influx (Fabiato, 1985).

To overcome this problem, stochastic models that account for the heterogeneous dyadic subspace and junctional SR $[\text{Ca}^{2+}]$ have been developed in recent years (Greenstein and Winslow, 2002a; Tanskanen et al., 2005; Hartman et al., 2010; Williams et al., 2011). In these models, $\text{Ca}^{2+}$ channel gating is typically described by a discrete-state Markov chain that accounts for large number of $\text{Ca}^{2+}$ release units. However, the runtime of Markov chain
simulations is exponential in the number of channel states and is therefore computationally expensive when the channel transition-state diagram is complicated.

This dissertation focuses on several novel modeling approaches that are more realistic than common pool models (Eqs. 1.5-1.6) but are nevertheless computationally efficient. In Chapter 2, we present a Langevin description of the collective gating of Ca$^{2+}$ channels with a Ca$^{2+}$ release site. We show that the Langevin model may be a good approximation to the corresponding Markov chain model when the number of Ca$^{2+}$ channels per CaRU is in the physiological range. Importantly, the computational efficiency of this approach does not depend on the number of RyRs per CaRU. In Chapter 3, we show that when the RyR model is minimal, Langevin equations in a whole cell model involving a large number of release sites may be replaced by a single Fokker-Planck equation. This yields an extremely compact and efficient local/global whole cell model that reproduces and helps interpret recent experiments investigating Ca$^{2+}$ homeostasis in permeabilized ventricular myocytes. In Chapter 4, we present a population density and moment-based approach to modeling domain Ca$^{2+}$-mediated inactivation of L-type Ca$^{2+}$ channels. The population density model involves a system of coupled advection-reaction equations for the time-evolution of the probability density of the domain [Ca$^{2+}$] associated with each channel and conditioned on channel state. The corresponding moment-based model is a system of ODEs that describe the time-evolution of the moments of the joint probability densities. Finally, Chapter 5 summarized the significant contributions of this dissertation and possible future directions.

The research project in Chapter 2 is collaborated with Yan Hao, Seth H Weinberg. The research project in Chapter 3 is collaborated with Seth H Weinberg, Yan Hao and Eric A Sobie. The research project in Chapter 4 is collaborated with Kiah Hardcastle and Seth H Weinberg.
Chapter 2

Calcium-activation kinetics modulate successive puff/spark amplitude, duration and inter-event-interval correlations in a Langevin model of stochastic calcium release

2.1 Introduction

Intracellular Ca$^{2+}$ elevations known as Ca$^{2+}$ puffs and sparks (Cheng et al., 1993a; Yao et al., 1995) arise from the cooperative activity of inositol 1,4,5-trisphosphate receptors (IP$_3$Rs) and ryanodine receptors (RyRs) that are clustered in Ca$^{2+}$ release units (CaRUs) on the endoplasmic reticulum/sarcoplasmic reticulum (ER/SR) membrane (see Berridge (1993a); Bers (2002) for review). Single-channel Ca$^{2+}$ release events (Ca$^{2+}$ blips and quarks) are often observed as precursors to puffs, suggesting that these low-amplitude Ca$^{2+}$ release events trigger full-sized Ca$^{2+}$ puffs and sparks (Rose et al., 2006). This is consistent with
the observation that individual IP₃Rs and RyRs are activated by cytosolic Ca²⁺, that is, small increases in [Ca²⁺] near these channels promotes further release of intracellular Ca²⁺, a process known as Ca²⁺-induced Ca²⁺ release (Bezprozvanny et al., 1991; Finch et al., 1991; Parker and Yao, 1996; Parker et al., 1996).

Although the activation mechanism of Ca²⁺ puffs and sparks is agreed upon, the mechanism by which puffs and sparks terminate is understood to a lesser degree and may vary in different physiological contexts (see Stern and Cheng (2004) for review). The short duration of most stochastic Ca²⁺ release events (10-200 ms) suggests that puff/spark termination is facilitated by a robust negative feedback mechanism (Cheng et al., 1993a; Niggli and Shirokova, 2007). Because puff/sparks involve a finite number of channels, one possible termination mechanism is the simultaneous de-activation of all channels at a Ca²⁺ release site, a phenomenon referred to as stochastic attrition (DeRemigio and Smith, 2005; Groff and Smith, 2008a). Another possibility is that decreasing [Ca²⁺] in the SR/ER lumen reduces the driving force for Ca²⁺ release and/or the contribution of feed-through Ca²⁺-activation to channel closure (Huertas and Smith, 2007). The inhibitory role of cytosolic Ca²⁺-mediated inactivation of IP₃Rs and RyRs is also thought to contribute to puff/spark termination (Fill, 2002; Stern and Cheng, 2004; Fraiman et al., 2006). Termination of stochastic Ca²⁺ release could also be mediated by state-dependent allosteric interactions between adjacent intercellular Ca²⁺ channels (Wiltgen et al., 2014), the redox state of IP₃Rs and RyRs (Zima and Blatter, 2006; Hool and Corry, 2007), and luminal regulation mediated by calsequestrin or other ER/SR proteins (Györke et al., 2004).

Discrete-state continuous-time Markov chains (CTMCs) are often used to model the stochastic gating of plasma membrane and intercellular ion channels, including clusters of IP₃Rs and RyRs collectively gating within CaRUs (Groff et al., 2010). These theoretical studies help clarify the factors that contribute to the generation and termination of Ca²⁺ puffs and sparks. Simulations show that moderately fast Ca²⁺ inactivation leads to puffs and sparks whose termination is facilitated by the recruitment of inactivated channels during the puff/spark event, while slow Ca²⁺ inactivation facilitates puff/spark termination due to
stochastic attrition (DeRemigio and Smith, 2005; Groff and Smith, 2008b). Ca\(^{2+}\)-mediated coupling of IP\(_3\)Rs and RyRs also influences stochastic excitability of simulated CaRUs. The efficacy of this coupling is determined by the bulk ER/SR [Ca\(^{2+}\)], the dynamics of luminal depletion, and the number, density and spatial arrangement of channels within a CaRU (Nguyen et al., 2005; DeRemigio and Smith, 2008).

In this chapter, we present a Langevin formulation of the stochastic dynamics of Ca\(^{2+}\) release mediated by IP\(_3\)Rs and RyRs that are instantaneously coupled through a local ‘domain’ Ca\(^{2+}\) concentration (a function of the number of open channels). The Langevin approach assumes the number of Ca\(^{2+}\) channels in individual CaRUs is large enough that the fraction of channels in different states can be treated as a continuous variable. Importantly, the computational efficiency of the Langevin approach is linear in the number of channel states and independent of the number of Ca\(^{2+}\) channels per CaRU. This is quite distinct from compositionally defined Markov chain models, in which the number of CaRU states is exponential in the number of channel states and polynomial in the number of channels per CaRU. For this reason, the Langevin approach may be preferred for extensive parameter studies, provided the Langevin model of stochastic Ca\(^{2+}\) release is a sufficiently good approximation to the corresponding Markov chain.

This chapter is organized as follows. Section 2.2 presents a continuous-time Markov chain model (and the corresponding Langevin formulation) of a CaRU composed of \(N\) three-state channels, each of which exhibits fast Ca\(^{2+}\) activation and slower Ca\(^{2+}\) inactivation. Section 2.3.1 uses the Langevin CaRU model to illustrate how the mechanism of spark termination depends on the rate of Ca\(^{2+}\) inactivation. By comparing statistics of simulated puff/sparks (amplitude, duration and inter-event interval) generated by both models, Section 2.3.2 demonstrates that the Langevin description of the collective gating of Ca\(^{2+}\) channels is indeed a good approximation to the corresponding Markov chain model when the number of Ca\(^{2+}\) channels per release site is in the physiological range. Section 2.3.3 uses Langevin simulations of stochastic Ca\(^{2+}\) release to preform an investigation of the correlations between successive puff/spark amplitudes, durations and inter-spark intervals and the
dependence of these puff/spark statistics on the number of channels per release site and the kinetics of Ca\textsuperscript{2+}-mediated inactivation of open channels.

2.2 Model formulation

2.2.1 Markov chain model of a calcium release site

The stochastic gating of intracellular channels is often modeled by discrete-state continuous-time Markov chains. For example, the following state and transition diagram,

\[ \begin{align*}
    \text{C (closed)} & \quad \Rightarrow \quad \text{O (open)} & \quad \Rightarrow \quad \text{R (refractory)}, \\
    k_{a \text{a}}^+ c^n & \quad k_{b \text{b}}^+ c^n & \quad k_{a \text{a}}^- & \quad k_{b \text{b}}^-
\end{align*} \]

represents a minimal three-state channel that is both activated (C \( \rightarrow \) O) and inactivated (O \( \rightarrow \) R) by Ca\textsuperscript{2+} (Groff and Smith, 2008a). In this diagram, \( c \) is the local [Ca\textsuperscript{2+}]; \( \eta \) is the cooperativity of Ca\textsuperscript{2+} binding; \( k_{a \text{a}}^+ c^n, k_{a \text{a}}^- \) and \( k_{b \text{b}}^+ c^n, k_{b \text{b}}^- \) are transition rates with units of time\(^{-1}\); \( k_{a \text{a}}^+ \) and \( k_{b \text{b}}^+ \) are association rate constants with units of concentration\(^{-\eta}\) time\(^{-1}\); and the dissociation constants for Ca\textsuperscript{2+} binding are \( K_{a \text{a}}^\eta = k_{a \text{a}}^- / k_{a \text{a}}^+ \) and \( K_{b \text{b}}^\eta = k_{b \text{b}}^- / k_{b \text{b}}^+ \). For simplicity, the cooperativity of Ca\textsuperscript{2+} binding is the same for the activation and inactivation processes (\( \eta = 2 \)).

It is straightforward to construct a Ca\textsuperscript{2+} release unit (CaRU) model that includes an arbitrary number \( N \) of stochastically gating three-state channels. Because the channels are identical, such a model has \( (N + 2)(N + 1)/2 \) distinguishable states that may be enumerated as follows,

\[ (N, 0, 0), (N - 1, 1, 0), \ldots, (0, 1, N - 1), (0, 0, N), \]

where each state takes the form \( (N_C, N_O, N_R) \) and \( N_C, N_O \) and \( N_R \) are the number of channels in closed, open and refractory states, respectively. For example, let us assume that
when $N_o = n$, the local $[Ca^{2+}]$ experienced by channels in the CaRU is given by

$$c_n = c_\infty + nc_*, \quad (2.3)$$

where $c_\infty$ is the bulk $[Ca^{2+}]$. We will refer to the parameter $c_*$ as the coupling strength, because this parameter determines the increment in local $[Ca^{2+}]$ that occurs when an individual $Ca^{2+}$ channel opens. The transition rates for the compositionally defined Markov chain model with state space given by Eq. 2.2 are each the product of a transition rate of the single channel model (Eq. 2.1) and the number of channels that may make that transition. For example, in a release site composed of 20 channels, the transition rates out of the state $(15,3,2)$ would be $15k_a^+c_3^o = 15k_a^+(c_\infty + 3c_*)^o$, $3k_a^-, 3k_b^+c_3^o = 3k_b^+(c_\infty + 3c_*)^7$, and $2k_b^-$ respectively, with destination states $(14,4,2)$, $(16,2,2)$, $(15,2,3)$ and $(15,4,1)$.

### 2.2.2 The Langevin description of a calcium release site

A Langevin description of the CaRU is an alternative to the Markov chain model presented above (Gardiner, 1985; Dangerfield et al., 2012). The Langevin approach assumes that the number of channels in the CaRU is large enough so that the fraction of channels in each state can be treated as continuous randomly fluctuating variables that solve a stochastic differential equation (SDE) system. For example, the Langevin equation of a CaRU composed of $N$ three-state channels (Eq. 2.1) is given by (Keizer, 1987)

$$\frac{df}{dt} = fQ + \xi(t), \quad (2.4)$$

where $f$ is a row vector of the fraction of channels in each state, $f = (f_c, f_o, f_r)$, $Q$ is the infinitesimal generator matrix (Q-matrix) given by

$$Q = (q_{ij}) = \begin{pmatrix} \diamond & k_a^+c^o & 0 \\ k_- & \diamond & k_b^+c^o \\ 0 & k_b^- & \diamond \end{pmatrix}, \quad (2.5)$$
where the local \([\text{Ca}^{2+}]\) can be written as \(c = c_{\infty} + f_0 \bar{c}\) with \(\bar{c} = NC_*\), the off-diagonal elements are transition rates \((q_{ij} \geq 0)\), and the diagonal elements \((\delta)\) are such that each row sums to zero, \(q_{ii} = -\sum_{j \neq i} q_{ij} < 0\). In Eq. 2.4, \(\xi(t) = (\xi_c(t), \xi_\sigma(t), \xi_R(t))\) is a row vector of rapidly varying forcing functions with mean zero,

\[
\langle \xi(t) \rangle = 0, \tag{2.6}
\]

and two-time covariances,

\[
\langle \xi(t)\xi(t') \rangle = \Gamma(f)\delta(t - t'), \tag{2.7}
\]

where \(\Gamma(f) = (\gamma_{ij})\) and

\[
\gamma_{ij} = -\frac{(q_{ij}f_i + q_{ji}f_j)}{N} \quad (i \neq j) \tag{2.8}
\]
\[
\gamma_{ii} = -\sum_{j \neq i} \gamma_{ij} \tag{2.9}
\]

The Langevin model is simulated by integrating Eqs. 2.4–2.9 using a modification of the Euler-Maruyama method (Gillespie, 2000), appropriate for a stochastic ODE with dependent variables constrained to the unit interval, i.e., \(0 \leq f_i \leq 1\).

### 2.3 Results

The focus of this chapter is a theoretical analysis of spark statistics such as puff/spark duration, amplitude and inter-event interval. We are specifically interested in the relationship between successive puff/spark amplitudes, whether puff/sparks and inter-event intervals are positively or negatively correlated, and how such puff/spark statistics depend on the single channel kinetics (e.g., \(\text{Ca}^{2+}\) inactivation rate). The Langevin approach to modeling CaRU dynamics facilities the large number of Monte Carlo simulations required for this analysis. Below we will first show representative Langevin simulation and illustrate how sequences of spark amplitudes, duration, and inter-event intervals are obtained from Langevin release site...
Figure 2.1: The number of open channels ($N_O$, black line) and refractory channels ($N_R$, gray line) during simulated Ca\textsuperscript{2+} puffs/sparks obtained by numerically integrating the Langevin model (Eqs. 2.4–2.9 with integration time step $\Delta t = 0.1$ ms). Ca\textsuperscript{2+}-inactivation/de-inactivation rates are 10-fold slower in B ($k^+ = 0.0015 \mu M^{-n} ms^{-1}$, $k^- = 0.0005 ms^{-1}$) than A ($k^+ = 0.015 \mu M^{-n} ms^{-1}$, $k^- = 0.005 ms^{-1}$). Other parameters: $N = 20$, $k^+ = 1.5 \mu M^{-n} ms^{-1}$, $k^- = 0.5 ms^{-1}$, $c = 0.06 \mu M$, $c_\infty = 0.05 \mu M$, $n = 2$, $K_a = K_b = 0.58 \mu M$.

In prior work, Groff and Smith (2008a) found that Ca\textsuperscript{2+}-dependent inactivation may facilitate puff/spark termination in two distinct ways depending on Ca\textsuperscript{2+}-inactivation rates. Fig. 2.1A and B use the Langevin model (Eqs. 2.4–2.9) to illustrate these two different termination mechanisms. In Fig. 2.1A the number of inactivated channels ($N_R$, gray line) increases during each puff/spark event, and decreases during the inter-event intervals between

2.3.1 Representative Langevin simulations

In prior work, Groff and Smith (2008a) found that Ca\textsuperscript{2+}-dependent inactivation may facilitate puff/spark termination in two distinct ways depending on Ca\textsuperscript{2+}-inactivation rates.
puff/sparks. In this case, the Ca\(^{2+}\) inactivation rate is such that the accumulation of inactivated channels results in puff/spark termination. In Fig. 2.1B, the Ca\(^{2+}\) inactivation/de-inactivation rates are reduced by 10-fold compared with that of Fig. 2.1A. In this case the number of inactivated channels \((N_R, \text{gray line})\) is relatively constant; consequently, the CaRU composed of \(N\) three-state channels effectively reduces to a collection of \(N - N_R\) two-state channels. In Fig. 2.1B, the puff/spark termination is due to stochastic attrition (Stern, 1992; Stern and Cheng, 2004), that is, the coincident de-activation \((N_O \rightarrow N_C)\) of all channels in the CaRU that are not in the refractory state \(N_R\) (Groff and Smith, 2008a).

Fig. 2.2A shows a Langevin simulation of the fraction of open channels, \(f_O\), for a CaRU composed of 20 three-state Ca\(^{2+}\) channels. The duration of the \(i\)th Ca\(^{2+}\) release event \((D_i)\) is the time elapsed between the first channel opening (up arrows) and the last channel closing (down arrows) of each simulated spark. Because the fraction of open channels in the Langevin description is continuous (as opposed to discrete), the first/last channel opening is defined as \(f_O\) crossing the threshold \((1/N, \text{dashed line})\) in the upward/downward direction (vertical arrows). The amplitude of \(i\)th Ca\(^{2+}\) release event \((A_i)\) is defined as the integrated area under \(f_O(t)\) during the release event (gray). The inter-event interval \((I_i)\) is the length of time between the \((i-1)\)th and \(i\)th Ca\(^{2+}\) release events. Because in experimental studies many Ca\(^{2+}\) release events may be too small for detection, we specify an amplitude threshold, \(A_\theta\), and only the events with greater amplitude \((A_i \geq A_\theta)\) are used in the calculation of spark statistics. For example, using an amplitude threshold of \(A_\theta = 0.5\) ms, only three of four events are of sufficient magnitude to be included in the sequence of puff/spark duration, amplitudes and inter-event intervals chosen for further analysis (Fig. 2.2B). If \(A_\theta = 1\) ms, only two of the four events are included (Fig. 2.2C).
Figure 2.2: The puff/spark detectability threshold $A_\theta$ eliminates small Ca$^{2+}$ release events from the correlation analysis of the sequence of simulated spark amplitudes, durations and inter-event intervals. A: 20 three-state Ca$^{2+}$ channels simulated using the Langevin approach (Eqs. 2.4–2.9). The fraction of open channels, $f_O$, is shown as a function of time. The dashed line denotes $f_O = 1/N$, the threshold for identifying Ca$^{2+}$ release events. Up and down arrows indicate crossings that define the beginning and ending of Ca$^{2+}$ sparks. B: For an amplitude threshold $A_\theta = 0.5$ ms, the first Ca$^{2+}$ release event in A is discarded and three Ca$^{2+}$ spark events are considered detectable. C: For $A_\theta = 1$ ms, the first and last Ca$^{2+}$ release event in A are discarded, and two Ca$^{2+}$ spark events are detectable.
2.3.2 Validation of Langevin approach

Using CaRUs composed of \( N = 20 \) three-state channels and amplitude threshold of \( A_\theta \) = 1 ms (lower dashed line) that filters out small events, Fig. 2.3A shows a strong linear relationship between spark amplitudes and duration in both Markov chain (○) and Langevin (+) simulations. Using \( A_\theta = 1 \) or 2 ms, Fig. 2.3B–D compares the cumulative distribution functions of spark amplitude (B), duration (C) and inter-event interval (D) for CaRUs composed of 20 (thin) and 60 (thick lines) three-state channels. In these simulations, the aggregate coupling strength \( \bar{c} = Nc \) is fixed, as opposed to fixing the contribution to the local \([Ca^{2+}]\) made by a single open channel \( (c_\ast) \). The spark duration and inter-event interval distributions move to the right as the number of channels increases (Fig. 2.3C–D), that is, for larger \( N \), spark duration and inter-event intervals are typically longer. At the same
time, increasing $N$ leads to sparks that on average have smaller amplitudes (Fig. 2.3B). For high amplitude threshold $A_\theta$, the distributions move to the right because smaller events are filtered out. Most importantly, the agreement between Markov chain (black solid) and Langevin (gray dashed line) calculations of sparks statistics shown in Fig. 2.3B-D validates our use of Langevin approach for further analysis.

### 2.3.3 Analysis of spark statistics

Our analysis of puff/sparks statistics begins with Fig. 2.4A which shows the Pearson correlation coefficient between successive puff/spark amplitudes, $\rho_{A_n,A_{n+1}}$, when the standard parameters for Ca$^{2+}$-inactivation and de-inactivation are used (as in Fig. 2.1A, where sparks terminate through the accumulation of inactivated channels). The correlation between successive puff/spark amplitudes is small but negative regardless of the amplitude threshold ($A_\theta$), indicating event-to-event alternation of puff/spark amplitude (small, large,
small, large, etc.). The alternation in puff/spark amplitudes is most pronounced (i.e., the correlation is most negative) when $A_\theta$ is about 0.5 ms for $N = 20$ channels and 1 ms for 60 channels.

Fig. 2.4B shows how this tendency toward alternating puff/spark amplitude depends on the rate of Ca$^{2+}$-inactivation/de-inactivation (with dissociation constant $K_b$ fixed). As a summary, we plot the minimum value of the correlation coefficient observed over a range of amplitude thresholds ($0.05 \leq A_\theta \leq 5$ ms). Negative correlation between successive puff/spark amplitudes is observed for intermediate inactivation rates. This negative correlation occurs because large puff/sparks terminate with a relatively large fraction of inactivated channels (cf. Fig. 2.1A). Consequently, fewer channels are available to participate the next (small amplitude) Ca$^{2+}$ release event. Conversely, small puff/sparks terminate with fewer Ca$^{2+}$-inactivated channels, and the subsequent puff/spark amplitudes are thus likely to be larger. Negative amplitude-amplitude correlation is not observed when the inactivation rates are reduced or increased by 100-fold compared to the standard parameters. When the inactivation rate is slow enough that the number of refractory channels $N_R$ is essentially constant, the mechanism that may generate negative amplitude-amplitude correlation is no longer operative, because $A_n$ does not affect $N_R$ at puff/spark termination. Similarly, when the inactivation rates are very fast, $A_n$ can not influence $N_R$ at spark termination because $N_R$ is in quasistatic equilibrium with $N_\infty$ ($N_R = k_b^+ c^0 / k_b^-$). For both very slow and very fast inactivation rates, stochastic attrition is the mechanism of spark termination and spark amplitudes are less correlated. Interestingly, for a larger number of channels ($N = 60$), the most pronounced amplitude alternation is larger in magnitude (i.e., a stronger negative correlation) and occurs at slower inactivation rates than the $N = 20$ case.

Fig. 2.5 is similar in structure to Fig. 2.4, but focuses on the correlation between inter-event intervals and the subsequent puff/spark amplitudes, $\rho_{I_n,A_n}$, which are positively correlated regardless of amplitude threshold $A_\theta$ (Fig. 2.5A). Following a long inter-event interval, the channels that were inactivated at the end of the preceding puff/spark are more likely to be available for the subsequent Ca$^{2+}$ release event. Consequently, the spark amplitudes...
following long quiescent periods tends to be larger than those that follow brief quiescent periods. Fig. 2.5B shows that the interval-amplitude correlation becomes very small for sufficiently slow or fast inactivation rates, and a larger correlation peak is observed for larger $N$. Similarly, Fig. 2.6A shows a small positive correlation between puff/spark amplitudes and the subsequent inter-event intervals ($\rho_{A_n,I_{n+1}}$). Fig. 2.6B shows that this positive amplitude-interval correlation becomes negligible for sufficiently fast or slow inactivation rates (similar to the interval-amplitude correlation of Fig. 2.5B).

### 2.4 Discussion

This chapter presents a Ca$^{2+}$ release unit (CaRU) modeling approach based on a Langevin description of stochastic Ca$^{2+}$ release. This Langevin model facilitates our investigation of correlations between successive puff/spark amplitudes and inter-spark intervals, and how such puff/spark statistics depend on the number of channels per release site and the kinetics of Ca$^{2+}$-mediated inactivation of open channels. We find that when Ca$^{2+}$
inactivation/de-inactivation rates are intermediate—i.e., the termination of Ca\textsuperscript{2+} puff/sparks is caused by the recruitment of inactivated channels—the correlation between successive puff/spark amplitudes is negative, while the correlations between puff/spark amplitudes and the duration of the preceding or subsequent inter-spark interval are positive. These correlations are significantly reduced when inactivation/de-inactivation rates are extreme (slow or fast), that is, when puff/sparks terminate via stochastic attrition.

### 2.4.1 Comparison to experiment

Puff/spark amplitudes, durations and inter-event intervals have been extensively studied in recent years (Yao et al., 1995; Cheng et al., 1999; Rios et al., 2001; Shuai and Jung, 2002; Shen et al., 2004; Ullah and Jung, 2006). Positive correlations between spark amplitude and duration (Cheng et al., 1999) and rise time (Shkryl et al., 2012) have been observed. The rise time of spark fluorescence is interpreted as a proxy for the duration of Ca\textsuperscript{2+} release during the spark. Spark amplitude in our release site simulations is an increasing function of spark...
duration (i.e., positively correlated, with correlation coefficient of \( \rho = 0.95 \)).

This positive correlation is not always observed in experimental (Wang et al., 2002) and theoretical work (Shuai and Jung, 2002). Shen et al. (2004) suggests that spark amplitude is independent of rise time, but is strongly and positively related to the mean or maximal rising rate. Their results show that spark rising time is negatively related to the mean rising rate, suggesting that the regulation of \( \text{Ca}^{2+} \) termination is a negative feedback and the strength of which is proportional to the ongoing release flux or the number of activated RyRs (Shen et al., 2004). In contrast to prior experimental results (Shkryl et al., 2012), the amplitude-rising time relationship is negative in the simulation work presented by Stern et al. (2013), because a long rise time implies a slower release of approximately the same amount of junctional SR \( \text{Ca}^{2+} \).

In our simulations, successive puff/spark amplitudes are negatively correlated, but only weakly (the peak is less than 0.15). This is consistent with experimental measurements of the correlation between successive puff amplitude were not statistically significant (Callamaras and Parker, 2000).

Inter-event intervals are determined both by recovery from a refractory state established by the preceding puff/spark, and a stochastic triggering which leads to an exponential distribution at longer intervals (Yao et al., 1995; Parker and Wier, 1997). The histograms of inter-puff interval measured at individual puff site show an initial increase of the inter-puff interval distribution, which is compatible with recovery from a negative feedback occurring during the puff (Thurley et al., 2011). In ventricular myocytes, Sobie et al. (2005) found that the relative amplitude of the second spark tends to be small when the spark-to-spark delay is short and larger as this delay increases. Moreover, Fraiman et al. (2006) observed that in \emph{Xenopus} oocytes, puffs of large amplitudes tend to be followed by a long inter-puff time, and puffs that occur after a large inter-puff time are most likely large. One possible explanation of this positive interval-amplitude and amplitude-interval correlation is that high cytosolic \([\text{Ca}^{2+}]\) attained during a puff/spark inhibits channels within the CaRU, so that the amplitude and probability of occurrence of a subsequent puff recover with a long
time course (Fraiman et al., 2006). Another possible mechanism is local Ca\textsuperscript{2+} depletion of ER lumen leading to decreased channel open probability (Fraiman and Dawson, 2004). Parker and Wier (1997) studied the relationship between the preceding inter-spark interval and the amplitude peak of the spark at the end of the interval and found no correlation. Our simulation exhibit a positive correlation between the preceding inter-spark interval and the amplitude of the spark at the end of the interval, but the maximum correlation observed is always less than 0.3 (Fig. 2.5B). The correlation between the spark amplitude and the subsequent inter-event interval is even weaker and the peak is less than 0.2 (Fig. 2.6B).

2.4.2 Comparison to prior theoretical work

Several types of modeling approaches based on microscopic kinetics of channels have been developed to study puff/spark statistics. For example, Ullah and Jung (2006) simplified the Sneyd-Dufour model (Sneyd and Dufour, 2002) and utilized it to study the spread of Ca\textsuperscript{2+} in the cytosol. They computed the correlation between puff amplitude and lifetime which is measured as the full width at half-maximal amplitude (FWHM). The predicted correlation of 0.31 indicates that puffs with larger amplitudes are likely to have longer lifetimes.

Ullah et al. (2012) presented a model of IP\textsubscript{3}R derived directly from single channel patch clamp data. Their results suggest that puff terminations is due to self-inhibition rather than ER Ca\textsuperscript{2+} depletion (unlike cardiac muscle, where local SR depletion is important for spark termination (Zima et al., 2008)).

Stern et al. (2013) utilized a simplified, deterministic model of cardiac myocyte couplon dynamics to show that spark metastability depends on the kinetic relationship of RyR gating and junctional SR refilling rates. They found that spark amplitudes is negatively correlated to rise time, in spite of the fact that positive correlation between amplitudes and rise time was observed in chemically skinned cat atrial myocytes (Shkryl et al., 2012).

Some prior work utilizing the Langevin formulation to investigate puff statistics has focused on a reduced Hodgkin-Huxley-like IP\textsubscript{3} receptor model in which noise terms were added to the gating variable (Li and Rinzel, 1994; Shuai and Jung, 2002; Huang et al., 2011). By
comparing Langevin and Markov chain simulations, they determined that Langevin approach yields more puffs with larger amplitudes, which leads to a drop-off of distribution at smaller amplitude; we did not observe this discrepancy in our Langevin simulation. Jung and co-workers also investigated the correlation between puff amplitude and lifetime and found that the correlation values are typically smaller than 0.3 (Shuai and Jung, 2002).

2.4.3 Advantages and limitations of the Langevin approach

While the Markov chain and Langevin approaches lead to similar results, the runtimes for Langevin simulations is often shorter. One expects Markov chain simulation runtimes to be proportional to the number of CaRU states, a quantity that is exponential in the number of distinct channel states. To see this, consider a CaRU composed of \( N \) identical \( M \)-state channels, the number of distinguishable CaRU states is given by \( (N + M - 1)!/[N!(M - 1)!] \). For example, for 20, 60, and 100 identical three-state channels, there are 231, 1891, and 5151 distinguishable states, respectively. Conversely, Langevin simulation runtimes such as those presented in this study are independent of the number of channels (i.e., \( N \) is a model parameter) and proportional to the number of states \( M \). Table 2.1 illustrates this by comparing the simulation time of the Markov chain and the Langevin release site calculations shown here. The runtime of the Markov chain model increases significantly as the number of channels per release site \( N \) increases, while the simulation time of Langevin description is independent in \( N \).

The Langevin formulation presented here is applicable and efficient when the number of channels per release site is large enough so that the fraction of channels in each state can be treated as a continuous variable. When this condition is not met, the use of Markov chain simulation or the slightly less-restrictive \( \tau \)-leaping approach may be more appropriate (Gillespie, 2000).

This study has focus on correlations between spark statistics using relatively restrictive modeling assumptions, including a minimal three-state channel model and instantaneous coupling of channels. One important observation is that correlations between the ampli-
<table>
<thead>
<tr>
<th>Model</th>
<th>Simulation time (s)</th>
<th>Standard deviation (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Markov chain ($N = 20$)</td>
<td>11.78</td>
<td>0.56</td>
</tr>
<tr>
<td>Markov chain ($N = 60$)</td>
<td>148.86</td>
<td>2.37</td>
</tr>
<tr>
<td>Markov chain ($N = 100$)</td>
<td>416.80</td>
<td>4.26</td>
</tr>
<tr>
<td>Langevin ($N = 20, \Delta t = 0.1 \text{ ms}$)</td>
<td>4.38</td>
<td>0.32</td>
</tr>
<tr>
<td>Langevin ($N = 60, \Delta t = 0.1 \text{ ms}$)</td>
<td>4.28</td>
<td>0.35</td>
</tr>
<tr>
<td>Langevin ($N = 100, \Delta t = 0.1 \text{ ms}$)</td>
<td>4.30</td>
<td>0.36</td>
</tr>
<tr>
<td>Langevin ($N = 20, \Delta t = 0.01 \text{ ms}$)</td>
<td>43.98</td>
<td>3.14</td>
</tr>
<tr>
<td>Langevin ($N = 60, \Delta t = 0.01 \text{ ms}$)</td>
<td>43.26</td>
<td>3.89</td>
</tr>
<tr>
<td>Langevin ($N = 100, \Delta t = 0.01 \text{ ms}$)</td>
<td>44.47</td>
<td>3.52</td>
</tr>
</tbody>
</table>

Table 2.1: Simulation time of a CaRU composed of $N$ three-state channels, where $N$ is 20, 60 and 100 respectively. Time step $\Delta t$ is 0.1 or 0.01 ms. The reported simulation times are the average of 10 100 s trials. Parameters as in Fig. 2.1A.

Attitude, duration, and interval-event intervals of simulated Ca$^{2+}$ puffs and sparks are strongly influenced by spark termination mechanism (i.e., Ca$^{2+}$-dependent inactivation or stochastic attrition-like). Our formulation can be generalized to account for luminal depletion and/or regulation, both of which known to influence spark termination (Huertas and Smith, 2007), and finite system size effects (Weinberg and Smith, 2012).

2.5 Appendix

2.5.1 Derivation of the Langevin CaRU model

Assuming a time interval $\Delta t$ is small enough so that at most one event occurs in the interval $[t, t+\Delta t]$, a CaRU composed of $N$ three-state channels with generating matrix $Q$ in Eq. 2.5 will undergo 5 possible process, that is, $[N_C, N,O,N_R] \rightarrow [N_C - 1, N_O + 1, N_R]$ with probability $q_{CO} N_C \Delta t$; $[N_C + 1, N_O - 1, N_R]$ with probability $q_{OC} N_O \Delta t$; $[N_C, N_O, N_R] \rightarrow [N_C, N_O - 1, N_R + 1]$ with probability $q_{OR} N_R \Delta t$; and $[N_C, N_O, N_R] \rightarrow [N_C, N_O + 1, N_R - 1]$ with probability $q_{RO} N_O \Delta t$.

Let $N = [N_C, N_O, N_R]$, conditioning on the current state, the expected infinitesimal
change 
\( \Delta N = [\Delta N_C, \Delta N_O, \Delta N_R] \) & Probability \\
\( \Delta_1 = [-1, 1, 0] \) & \( p_1 = q_{CO} N_C \Delta t \) \\
\( \Delta_2 = [1, -1, 0] \) & \( p_2 = q_{OC} N_O \Delta t \) \\
\( \Delta_3 = [0, -1, 1] \) & \( p_3 = q_{OR} N_O \Delta t \) \\
\( \Delta_4 = [0, 1, -1] \) & \( p_4 = q_{RO} N_R \Delta t \) \\
\( \Delta_5 = [0, 0, 0] \) & \( p_5 = 1 - \sum_{i=1}^{4} p_i \) \\

**Table 2.2: Infinitesimal change of three-state channel**

mean of \( \Delta N \) is thus

\[
\lim_{\Delta t \to 0} \frac{1}{\Delta t} E[\Delta N|N(t)] = \lim_{\Delta t \to 0} \frac{1}{\Delta t} \sum_{k=1}^{5} p_k \Delta k = NQ.
\]

The deterministic part of the right hand side of Eq. 3.5 is derived as the corresponding infinitesimal expected increment in \( f = N/N \). Similarly, the infinitesimal variance of \( \Delta N \)

\[
\lim_{\Delta t \to 0} \frac{1}{\Delta t} E[\Delta N^T N|N(t)] = \lim_{\Delta t \to 0} \frac{1}{\Delta t} \sum_{k=1}^{5} p_k \Delta k^T \Delta k
\]

\[
\lim_{\Delta t \to 0} \frac{1}{\Delta t} E[\Delta N^T N|N(t)] = \lim_{\Delta t \to 0} \frac{1}{\Delta t} \sum_{k=1}^{5} p_k \Delta k^T \Delta k
\]

\[
\begin{pmatrix}
q_{CO} N_C + q_{OC} N_O & -q_{CO} N_C - q_{OC} N_O & 0 \\
-q_{CO} N_C - q_{OC} N_O & q_{CO} N_C + q_{OC} N_O + q_{OR} N_O + q_{RO} N_R & -q_{OR} N_O - q_{RO} N_R \\
0 & -q_{OR} N_O - q_{RO} N_R & q_{OR} N_O + q_{RO} N_R
\end{pmatrix}
\]

The function \( \Gamma(f) \) that occurs in Eq. 2.7 is derived from this quantity using \( E[\Delta f^2|f(t)] = E[\Delta N^2|N(t)]/N^2 \).

### 2.5.2 Langevin equation boundary conditions

Because solutions of the Langevin CaRU model \( (f_i) \) represent the fraction of channels in state \( i \), physical values are in the range \( 0 \leq f_i \leq 1 \) and, formally, the stochastic processes that solve the Langevin CaRU models (Eqs. 3.5 and 3.39) have this property. However, numerical integration via the Euler-Maruyama method (Gillespie, 2007) involves a finite time step; consequently, there is a small probability of crossing \( f_i = 0 \) or \( 1 \), thereby exiting
the physical range.

In the context of stochastic ODE modeling of ion channel dynamics, several modifications of the Euler-Maruyama scheme are commonly used to address this numerical issue. These include rejection and projection methods as well as more sophisticated approaches such as equilibrium noise approximations (Goldwyn et al., 2011) and reflected stochastic differential equations (reviewed in Dangerfield et al. (2012)). Unfortunately, these methods yield solutions that may disagree with the corresponding Markov chains when \( N = 20-200 \) (Franzini-Armstrong et al., 1999). In the context of Langevin CaRU models, a superior approach is to define auxiliary variables (observables) restricted to the physical range, i.e., \( \hat{f}_i = \max(0, \min(1, f_i)) \), for evaluation of state-dependent rates, without projecting the stochastic trajectory \( f_i \) to the boundary.

## 2.5.3 Numerical scheme of the Langevin model

We here give a short introduction of the Euler-Maruyama Method (Gillespie, 2000) that numerically integrates the Langevin model (Eqs. 2.4-2.9). The Euler-Maruyama method is a generalization of the Euler method for ordinary differential equations to stochastic differential equations. In general, we assume a CaRU has \( N \times M \)-state channels, where the states are \( S_1, S_2, \ldots, S_M \). Let \( f = [f_1, f_2, \ldots, f_M]^T \), where \( f_i \) denotes the fraction of channels in the \( i \)th state and \( i = 1, 2, \ldots, M \). If we consider each state transition as an elementary (one-way) reaction and assume there are \( l \) reactions in total, we can compactly write all reactions as:

\[
Y_1 S \rightarrow Y_2 S, \tag{2.11}
\]

where \( S = [S_1, S_2, \ldots, S_M]^T \) is a vector that collects all the channel states. \( Y_1 \) and \( Y_2 \) are two \( l \times M \) matrices of stoichiometric coefficients. The reaction system is characterized by two sets of quantities. The first are the update vectors \( v_1, v_2, \ldots, v_l \) for each of the \( l \) reactions. Let update matrix \( V = [v_1, v_2, \ldots, v_l] \) and \( V \) can be derived from \( V = Y_2^T - Y_1^T \). The second set of quantities are the propensity functions \( \alpha(f) = [\alpha_1(f), \alpha_2(f), \ldots, \alpha_l(f)] \), where \( \alpha_k(f) \) is
the propensity function of the \( k \)th reaction. For example, for the three-state model (Eq. 2.1) used in this chapter, we can decompose the model into four one-way reactions, i.e., \( \mathcal{C} \rightarrow \mathcal{O} \), \( \mathcal{O} \rightarrow \mathcal{C} \), \( \mathcal{O} \rightarrow \mathcal{R} \), and \( \mathcal{R} \rightarrow \mathcal{O} \). Furthermore, we have \( \mathbf{v}_1 = [-1, 1, 0]^T \), \( \mathbf{v}_2 = [1, -1, 0]^T \), \( \mathbf{v}_3 = [0, -1, 1]^T \) and \( \mathbf{v}_4 = [0, 1, -1]^T \). The propensity functions are given by \( \alpha_1 = k_{o}^+ c^0 f_C \), \( \alpha_2 = k_{o}^- f_C \), \( \alpha_3 = k_{o}^+ c^0 f_C \) and \( \alpha_4 = k_{o}^- f_C \).

The Euler-Maruyama algorithm is therefore given by (Gillespie, 2000)

\[
\mathbf{f}(t + \Delta t) = \mathbf{f}(t) + \Delta t \sum_{k=1}^{l} \mathbf{v}_k \alpha_k(f) + \sqrt{\frac{\Delta t}{N}} \sum_{k=1}^{l} \mathbf{v}_k \sqrt{\alpha_k(f)} Z_k, \quad (2.12)
\]

where \( Z_k \) (\( k = 1, 2, ..., l \)) are independent random variables with normal distribution \( Z_k \sim N(0, 1) \) and \( \Delta t \) is the time step. Note that the first two terms on the right side of Eq. 2.12 are the same as the Euler algorithm to integrate ODE, and the third term represents random fluctuations due to the small number of channels. As \( N \rightarrow \infty \), this term would approach zero, and the integration is identical to a standard forward Euler method.
Chapter 3

Calcium homeostasis in a local/global whole cell model of permeabilized ventricular myocytes

3.1 Introduction

Intracellular calcium (Ca\(^{2+}\)) signaling involves a complex interplay between global (cell-wide) changes in \([\text{Ca}^{2+}]\) and local (subcellular) \(\text{Ca}^{2+}\) release events. Local signals are caused by plasma membrane \(\text{Ca}^{2+}\) influx and release of \(\text{Ca}^{2+}\) from intracellular stores, primarily the endoplasmic/sarcoplasmic reticulum (ER/SR). Spatially localized \(\text{Ca}^{2+}\) release events mediated by clusters of intracellular \(\text{Ca}^{2+}\) channels, IP\(_3\) receptors (IP\(_3\)Rs) or ryanodine receptors (RyRs) on the ER/SR membrane, are referred to as “\(\text{Ca}^{2+}\) sparks” or “puffs” (see Berridge (2006) for review).

While plasma membrane ion channels in a small cell experience essentially the same time-course of membrane voltage, intracellular \(\text{Ca}^{2+}\) channels experience radically different local \([\text{Ca}^{2+}]\), even during global \(\text{Ca}^{2+}\) responses, and clusters of IP\(_3\)Rs and RyRs are in fact only locally coupled via the buffered diffusion of intracellular \(\text{Ca}^{2+}\). That is, when
one or several of the channels in a Ca$^{2+}$ release unit (CaRU) are open, the [Ca$^{2+}$] experienced by spatially localized channels is dramatically different from the [Ca$^{2+}$] in the bulk myoplasm. For this reason, conventional whole cell modeling of Ca$^{2+}$ dynamics based on Hodgkin-Huxley-like gating variables for the dynamics of intracellular channels is not always appropriate.

Mechanistic models of ER/SR Ca$^{2+}$ release often represent the stochastic gating of Ca$^{2+}$ channels using Monte Carlo methods. When these approaches are applied to cardiac myocytes, voltage-gated L-type Ca$^{2+}$ channel(s) interact with a cluster of RyRs through changes in [Ca$^{2+}$] in small “dyadic subspaces” between the sarcolemmal and SR membranes. These models also sometimes consider depletion of junctional SR [Ca$^{2+}$] that may influence Ca$^{2+}$ spark termination and refractoriness (Sobie et al., 2002; Sobie and Lederer, 2012; Terentyev et al., 2002). Realistic global (cell-wide) SR Ca$^{2+}$ release can be reproduced by Monte Carlo simulation of the stochastic triggering of sparks from hundreds to thousands of CaRUs (Rice et al., 1999; Sobie et al., 2002; Greenstein and Winslow, 2002b; Greenstein et al., 2006). However, such simulations of local control of excitation-contraction coupling are computationally demanding, especially when each CaRU is composed of interacting Markov chain models of individual RyRs (e.g., see Hinch (2004)).

Population density approaches are an alternative to Monte Carlo simulations that produce realistic and computationally efficient models by using a master equation to represent heterogeneous local Ca$^{2+}$ signals in dyadic subspaces and junctional SR domains (Williams et al., 2007). This approach involves the numerical solution of advection-reaction equations for the time-dependent bivariate probability density of subspace and junctional SR [Ca$^{2+}$] conditioned on CaRU state, coupled to ordinary differential equations (ODEs) for the bulk myoplasmic and network SR [Ca$^{2+}$]. This methodology was validated in prior work (Williams et al., 2007) and an associated moment-based approach to simulating the probability distribution of junctional SR [Ca$^{2+}$] was benchmarked to be several orders of magnitude faster than conventional Monte Carlo simulation of the dynamics of local Ca$^{2+}$ associated with a physiological number of CaRUs (Williams et al., 2008).

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One disadvantage of the population density approaches to modeling local control is that their runtimes (computational efficiency) are proportional to the number of CaRU states. When realistically modeled as the collective gating of identical and indistinguishable RyRs, the number of CaRU states is exponential in the number of channel states. Population density and moment-based methods for multiscale (i.e., local/global) whole cell modeling are limited by this state-space explosion.

Here we present an alternative local/global whole cell modeling approach based on a Langevin formulation of the stochastic Ca\(^{2+}\) release via CaRUs. We assume that the number of RyRs per CaRU is large enough that the fraction of channels in each state can be treated as a continuous variable. We show that the Langevin description of the collective gating of RyRs is a good approximation to the corresponding discrete-state continuous-time Markov chain model when the number of RyRs per release site is in the physiological range. By coupling the numerical solution of such Langevin equations to balance equations for the bulk myoplasmic and network SR [Ca\(^{2+}\)], a local/global whole cell model is produced whose runtimes scale with the number of states in the Markov chain model for an individual RyR, as opposed to the far greater number of states in a compositionally defined CaRU. When the RyR model is minimal, these Langevin equations may be replaced by a single Fokker-Planck equation for a randomly sampled CaRU, yielding an extremely compact and efficient local/global whole cell model. We illustrate the usefulness and computational efficiency of the Fokker-Planck equation-based local/global whole cell model by performing parameter studies motivated by recent experiments (Zima et al., 2010; Bovo et al., 2011).

In intact ventricular myocytes of the healthy heart, the balance of diastolic SR Ca\(^{2+}\) leak and uptake maintains the appropriate SR Ca\(^{2+}\) load. While the SR Ca\(^{2+}\) leak is mediated primarily by RyRs, the contributions of spark- and non-spark-mediated SR Ca\(^{2+}\) release depends on the concentration of both myoplasmic and SR [Ca\(^{2+}\)] (Loughrey et al., 2002; Shannon et al., 2002; Zima et al., 2010; Dibb and Eisner, 2010). When SR [Ca\(^{2+}\)] is low, SR Ca\(^{2+}\) leak occurs primarily through spark-independent pathways. Conversely, when SR [Ca\(^{2+}\)] is high, spontaneous Ca\(^{2+}\) sparks make a large contribution to SR leak. In
pathophysiological conditions that include SR Ca\(^{2+}\) overload, increased SR Ca\(^{2+}\) leak may generate spontaneous sparks that triggers Ca\(^{2+}\) induce Ca\(^{2+}\) release (CICR) from neighboring CaRUs, thereby initiating arrhythmogenic spontaneous Ca\(^{2+}\) waves (Díaz et al., 1997).

Using permeabilized ventricular myocytes, a reduced experimental preparation that allows precise control of myoplasmic [Ca\(^{2+}\)], Bovo et al. (2011) observed that increasing myoplasmic [Ca\(^{2+}\)] results in an exponential increase in spark-mediated release and a linear increase in non-spark-mediated release. These results are reproduced by the Fokker-Planck equation-based local/global whole cell model that is the focus of this chapter. In addition, the model predicts potentially significant characteristics of Ca\(^{2+}\) homeostasis in permeabilized cells. For example, in the local/global whole cell model, two distinct steady-states may exist for a given network SR [Ca\(^{2+}\)]. One steady-state corresponds to low myoplasmic [Ca\(^{2+}\)] and small SR Ca\(^{2+}\) release flux that is dominated by stochastic leak, while the other corresponds to high myoplasmic [Ca\(^{2+}\)] and large release flux mediated by Ca\(^{2+}\) sparks. Interestingly, for any clamped myoplasmic [Ca\(^{2+}\)] that is large enough to trigger spark-mediated release, the local/global model predicts that the resulting spontaneous stochastic Ca\(^{2+}\) release tends to decrease the network SR Ca\(^{2+}\) load just enough to maintain robust Ca\(^{2+}\) sparks.

### 3.2 Model formulation

#### 3.2.1 Markov chain description of a calcium release site

The most straightforward starting point for the presentation of the Langevin description of a Ca\(^{2+}\) release site (CaRU) is the following two-state Markov chain model of a stochastically gating RyR,

\[
\begin{align*}
\text{(closed)} \ C & \quad \overset{k^+}{\Rightarrow} \quad \text{(open)} \ O
\end{align*}
\]

where \(c\) is the local [Ca\(^{2+}\)], \(k^+\) and \(k^-\) are transition rates with units of reciprocal time, \(k^+\) is an association rate constant with units of concentration\(^{-\eta}\) time\(^{-1}\), and \(\eta\) is the coop-
erativity of Ca\textsuperscript{2+} binding. Under the assumption that a collection of \( N \) two-state RyRs are instantaneously coupled by a local [Ca\textsuperscript{2+}] associated with the RyR cluster, the transition diagram for the CaRU as a collective entity is (DeRemigio and Smith, 2005)

\[
\begin{array}{cccc}
Nk^+ c_0^n & (N-1)k^+ c_1^n & 2k^+ c_{N-2}^n & k^+ c_{N-1}^n \\
0 & 1 & \ldots & N-1 \\
k^- & 2k^- & (N-1)k^- & Nk^-
\end{array}
\]

where the states \{0, 1, ..., \( N \}\} correspond to the number of open channels \( \( N_\text{O} \) \), and \( c_n \) is the local [Ca\textsuperscript{2+}] experienced by RyRs when \( N_\text{O} = n \).

Fig. 3.1A shows a Markov chain simulation of a CaRU composed of \( N = 20 \) two-state channels. For simplicity we here assume that the local [Ca\textsuperscript{2+}] is a linear function of \( N_\text{O} \), that is,

\[
c_n = c_\infty + nc_*,
\]

where \( c_\infty \) is the bulk or background [Ca\textsuperscript{2+}] and \( c_* \) determines the increment in local [Ca\textsuperscript{2+}] following an individual RyR opening. The corresponding relationship between \( N_\text{O} \) and local [Ca\textsuperscript{2+}] is more realistic in the local/global whole cell model (Eqs. 3.24 and 3.25).

### 3.2.2 Langevin calcium release site model

We will write \( f_\text{O}(t) \) as the time-varying fraction of open RyRs, that is,

\[
f_\text{O}(t) = \frac{N_\text{O}(t)}{N}.
\]

The Langevin equation that corresponds to a CaRU composed of \( N \) two-state channels (see above) is a stochastic ordinary differential equation (SDE) of the form

\[
\frac{df_\text{O}}{dt} = k^+(c_\infty + \bar{c}f_\text{O})^n (1 - f_\text{O}) - k^- f_\text{O} + \xi(t),
\]

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where $\bar{c} = Nc_*$ and $\xi(t)$ is a rapidly varying forcing term (Gaussian white noise) with zero mean

$$\langle \xi(t) \rangle = 0. \quad (3.6)$$

The magnitude of the noise term, $\xi(t)$, is characterized by the two-time covariance (Keizer, 1987; Gillespie, 2007),

$$\langle \xi(t)\xi(t') \rangle = \gamma(f_0)\delta(t - t'), \quad (3.7)$$
where \( \delta \) is the Dirac delta function and \( \gamma(\phi) \) is the infinitesimal variance of \( \phi \) and is given by

\[
\gamma(\phi) = \frac{k^+(c_\infty + \bar{\phi})\eta (1 - \phi) + k^ - \phi}{N}.
\]

Using parameters that lead to \( \text{Ca}^{2+} \) sparks, Fig. 3.1B shows that the Langevin simulation of a 20-channel CaRU is qualitatively similar to the corresponding Markov chain simulation (Fig. 3.1A).

### 3.2.3 Equivalence of Markov chain and Langevin formulations

The Langevin CaRU model is expected to well-approximate the Markov chain model when the number of RyRs per CaRU \( (N) \) is sufficiently large. To determine whether this convergence occurs for a physiological number of RyRs \( (10-200 \text{ per } \text{CaRU in skeletal and cardiac myocytes } (\text{Franzini-Armstrong et al., 1999}), \text{we compare the stationary distributions for } N_\phi \text{ (Fig. 3.1C). The white histogram of Fig. 3.1C shows that the bimodal Markov chain stationary distribution has a local maxima near } N_\phi = 0 \text{ and } N_\phi = 15. \text{ This distribution reflects the fact that the } N \text{ RyRs are usually closed, but occasionally open in concert as is characteristic for } \text{Ca}^{2+} \text{ sparks. Comparison to the corresponding distribution of the Langevin model (black histogram) shows that the SDE formulation is a good approximation to the Markov chain, even when the number of RyRs per CaRU is on the low end of the physiological range.}

In the Langevin CaRU formulation, the state space for \( \phi \) is continuous \( (0 \leq \phi \leq 1) \). The Fokker-Planck equation solved by the probability density function for the fraction of open channels, \( \rho(f,t) \), is given by \((\text{Gardiner, 1985})\)

\[
\frac{\partial \rho}{\partial t} = -\frac{\partial}{\partial f} [\alpha \rho] + \frac{1}{2} \frac{\partial^2}{\partial f^2} [\gamma \rho]
\]

where \( \rho(f,t) df = \text{Pr}\{f \leq \phi(t) < f + df\} \). Note that in these expressions, \( \phi \) is the random variable, and \( f \) is the independent variable of the probability density. The drift and diffusion
terms in Eq. 3.9 are given by

\begin{align*}
\alpha(f) &= \nu^+ - \nu^-, \quad (3.10) \\
\gamma(f) &= (\nu^+ + \nu^-)/N, \quad (3.11)
\end{align*}

where \( \nu^\pm(f) \) are the rates of the elementary processes leading to an increase and decrease in the fraction of open channels, that is,

\begin{align*}
\nu^+(f) &= k^+ (c_\infty + \bar{c} f)^\eta (1 - f), \quad (3.12) \\
\nu^-(f) &= k^- f, \quad (3.13)
\end{align*}

and \( \bar{c} = c_s N \) as above (Eq. 3.5).

Setting the left-hand side of Eq. 3.9 equal to zero \((\partial \rho/\partial t = 0)\), denoting the stationary density by \( \rho_{ss}(f) \), and applying boundary conditions \( \rho_{ss}(f) \to 0 \) as \( f \to \pm \infty \), it can be shown that (Gardiner, 1985)

\[ \rho_{ss}(f) = \frac{\theta}{\gamma} \exp\{2U\} \quad (3.14) \]

where \( \theta \) is a normalization constant such that \( \int \rho_{ss}(f) df = 1 \) and

\[ U(f) = \int_a^f \frac{\alpha(f')}{\gamma(f')} df' \quad (3.15) \]

is an accumulation function with a lower limit of integration satisfying \( \alpha(f)/\gamma(f) = 0 \) for \( f \leq a \). In fact, \( U \) may be any antiderivative satisfying \( U' = \alpha/\gamma \), because the normalization of \( \rho_{ss} \) determines the constant of integration.

Fig. 3.1D shows the stationary density \( \rho_{ss}(f) \) for the 20-channel Fokker-Planck CaRU model described above. The + symbols in Fig. 3.1C are binned values of \( \rho_{ss}(f) \) that may be compared to (and agree with) the stationary distributions of the Markov chain (white histogram) and Langevin (black histogram) descriptions. Appendix B provides more comparisons of Markov chain, Langevin and Fokker-Planck CaRU simulations.
3.2.4 Full local/global whole cell model

Having validated the Langevin CaRU model in the previous sections, we are prepared to construct the local/global whole cell model of Ca\(^{2+}\) homeostasis in permeabilized ventricular myocytes that is the focus of this chapter. Fig. 3.2 shows the relationship between the bulk Ca\(^{2+}\) concentrations of the myoplasm \((c_{\text{myo}})\) and the network SR \((c_{\text{nsr}})\) and the local Ca\(^{2+}\) concentrations associated with each CaRU. With respect to global aspects of Ca\(^{2+}\) signaling, the material balance equations of the whole cell model are

\[
\frac{dc_{\text{myo}}}{dt} = J_{\text{myo}}^T - J_{\text{pump}} + J_{\text{pm}} \tag{3.16}
\]

\[
\frac{dc_{\text{nsr}}}{dt} = \frac{1}{\lambda_{\text{nsr}}}( -J_{\text{nsr}}^T + J_{\text{pump}} ) \tag{3.17}
\]

where \(\lambda_{\text{nsr}}\) is an effective volume ratio that accounts for both physical volume and Ca\(^{2+}\) buffering capacity of the myoplasm and network SR. A plasma membrane flux may take the form \(J_{\text{pm}} = k_{\text{pm}}(c_{\text{ext}} - c_{\text{myo}})\). The SERCA type Ca\(^{2+}\) ATPase flux is (Williams et al., 2007)

\[
J_{\text{pump}} = v_{\text{pump}} \frac{(c_{\text{myo}}/K_f)^{\eta_f} - (c_{\text{nsr}}/K_r)^{\eta_r}}{1 + (c_{\text{myo}}/K_f)^{\eta_f} + (c_{\text{nsr}}/K_r)^{\eta_r}}. \tag{3.18}
\]

The aggregate fluxes \(J_{\text{myo}}^T = \sum_{m=1}^M J_{\text{myo}}^m\) and \(J_{\text{nsr}}^T = \sum_{m=1}^M J_{\text{nsr}}^m\) in Eqs. 3.16–3.17 account for the stochastic dynamics of Ca\(^{2+}\) release, where \(J_{\text{myo}}^m = v_{\text{myo}}(c_{\text{myo}}^m - c_{\text{myo}})\) with \(v_{\text{myo}} = v_{\text{myo}}^T/M\) is the flux from the \(m\)th dyadic subspace into the bulk myoplasm and \(J_{\text{nsr}}^m = v_{\text{nsr}}(c_{\text{nsr}} - c_{\text{nsr}}^m)\) with \(v_{\text{nsr}} = v_{\text{nsr}}^T/M\) is the flux from the network SR to the \(m\)th junctional SR \((m = 1, 2, \ldots, M)\). See Table 3.1 for parameters.
Figure 3.2: Diagram of compartments and Ca$^{2+}$ fluxes in the local/global whole cell model. The model includes Ca$^{2+}$ concentrations in two bulk compartments: network SR (c$_{sr}$) and myoplasm (c$_{myo}$). M CaRUs are coupled to these bulk compartments; each includes a dyadic subspace (c$_{ds}$) and junctional SR (c$_{j sr}$). The bulk myoplasmic [Ca$^{2+}$] is a model parameter, as this quantity is clamped in permeabilized ventricular myocytes. Fluxes include: passive exchange between network and junctional SR (J$_{n sr}$) and between dyadic subspace and myoplasm (J$_{m y o}$); release fluxes between junctional SR and dyadic subspace (J$_{re l}$); SR uptake from myoplasm to network SR via SERCA (J$_{pump}$); and (for intact cells) plasma membrane fluxes (J$_{p m}$).
Table 3.1: Parameters for the local/global whole cell model of calcium homeostasis in permeabilized ventricular myocytes.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(c_{\text{myo}})</td>
<td>myoplasmic ([\text{Ca}^{2+}])</td>
<td>(\mu\text{M})</td>
<td>varied</td>
</tr>
<tr>
<td>(\Lambda_{\text{nsr}})</td>
<td>effective volume ratio of network SR and myoplasm</td>
<td>-</td>
<td>1.46</td>
</tr>
<tr>
<td>(v_{\text{myo}}^T)</td>
<td>rate of myoplasmic domain collapse</td>
<td>(s^{-1})</td>
<td>31.25</td>
</tr>
<tr>
<td>(v_{\text{nsr}}^T)</td>
<td>rate of SR domain recovery</td>
<td>(s^{-1})</td>
<td>0.45</td>
</tr>
<tr>
<td>(v_{\text{rel}}^T)</td>
<td>maximum release rate via RyRs</td>
<td>(s^{-1})</td>
<td>1.56</td>
</tr>
<tr>
<td>(v_{\text{pump}})</td>
<td>maximum pump rate via SERCA</td>
<td>(\mu\text{M} \cdot s^{-1})</td>
<td>161.25</td>
</tr>
<tr>
<td>(K_{fs}, K_{rs})</td>
<td>forward and reverse half-saturation constant</td>
<td>(\mu\text{M})</td>
<td>0.17, 1702</td>
</tr>
<tr>
<td>(\eta_{fs}, \eta_{rs})</td>
<td>forward and reverse cooperativity constant</td>
<td>-</td>
<td>0.75</td>
</tr>
<tr>
<td>(N)</td>
<td>number of RyRs per CaRU</td>
<td>-</td>
<td>varied</td>
</tr>
<tr>
<td>(k^+)</td>
<td>association rate constant for ([\text{Ca}^{2+}]) binding to RyRs</td>
<td>(\mu\text{M}^{-\eta} \cdot s^{-1})</td>
<td>0.4</td>
</tr>
<tr>
<td>(k^-)</td>
<td>disassociation rate constant for ([\text{Ca}^{2+}]) unbinding</td>
<td>(s^{-1})</td>
<td>50</td>
</tr>
<tr>
<td>(\eta)</td>
<td>cooperativity of ([\text{Ca}^{2+}]) binding to RyRs</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>(M)</td>
<td>number of CaRUs in Langevin simulations</td>
<td>-</td>
<td>200</td>
</tr>
</tbody>
</table>

Myoplasmic \([\text{Ca}^{2+}]\) \((c_{\text{myo}})\) is under experimental control in permeabilized myocytes and thus it is a parameter of the whole cell model. The effective volume ratio that accounts for \([\text{Ca}^{2+}]\) buffering in Eq. 3.17 is given by \(\lambda_{\text{nsr}} = (V_{\text{nsr}}/\beta_{\text{nsr}})/(V_{\text{myo}}/\beta_{\text{myo}})\), where \(V_{\text{nsr}}\) and \(V_{\text{myo}}\) are the volume of network SR and myoplasm respectively, and \(\beta_{\text{nsr}}\) and \(\beta_{\text{myo}}\) are buffering factors. The rate constants \(v_{\text{myo}}^T, v_{\text{nsr}}^T\) and \(v_{\text{rel}}^T\) scale the fluxes between domains and bulk, given by integrals over the density function \(\rho(f, t)\): \(J_{\text{myo}}^T(t) = \int v_{\text{myo}}^T(c_{\text{myo}}) \rho(f, t) df\), \(\tilde{J}_{\text{rel}}^T(t) = \int v_{\text{rel}}^T \rho(f, t) df\), and \(\tilde{J}_{\text{nsr}}^T(t) = \int v_{\text{nsr}}^T (c_{\text{nsr}} - c_{\text{jsr}}) \rho(f, t) df\) where \(c_{\text{myo}}(f)\) and \(c_{\text{jsr}}(f)\) are given by Eqs. 3.32 and 3.33. The flux via SERCA is governed by \(v_{\text{pump}}, K_{fs}, K_{rs}, \eta_{fs}\) and \(\eta_{rs}\) (Eq. 3.18). \([\text{Ca}^{2+}]\) activation/de-activation of the two-state RyR channel model is governed by \(k^+, k^-\) and \(\eta\) (Eq. 3.1). The number of CaRUs \((M)\) in the Fokker-Planck formulation is large but unspecified.
Each CaRU in the whole cell model is a collection of $N$ RyRs with open fraction $f^m_O$ and associated dyadic subspace ($c_{ds}^m$) and junctional SR ($c_{jsr}^m$) Ca$^{2+}$ concentrations:

$$\frac{dc_{ds}^m}{dt} = \frac{1}{\lambda_{ds}}(J_{rel}^m - J_{myo}^m)$$  \hspace{0.5cm} (3.19)
$$\frac{df_O^m}{dt} = k^+(c_{ds}^m)^\eta (1 - f_O^m) - k^- f_O^m + \xi^m(t)$$ \hspace{0.5cm} (3.20)
$$\frac{dc_{jsr}^m}{dt} = \frac{1}{\lambda_{jsr}}(-J_{rel}^m + J_{nsr}^m).$$  \hspace{0.5cm} (3.21)

In these equations, $\lambda_{ds}$ and $\lambda_{jsr}$ are effective volume ratios, that is, $\lambda_{ds} = (V_{ds}/\beta_{ds})/(V_{myo}/\beta_{myo})$ where $V_{ds} = V_{ds}^T/M$ and $V_{ds}^T$ is the aggregate volume of the diadic subspaces (similarly for $\lambda_{jsr}$). $J_{rel}^m$ is the release flux through the $m$th RyR cluster given by $J_{rel}^m = v_{rel}f^m_O(c_{jsr}^m - c_{ds}^m)$ for $m = 1, 2, \ldots, M$ and $v_{rel} = v_{rel}^T/M$. The random functions of time $\xi^m(t)$ are independent Gaussian white noise terms with zero mean, $\langle \xi^m(t) \rangle = 0$ for all $m$, and the two-time covariances are

$$\langle \xi^m(t)\xi^{m'}(t') \rangle = \begin{cases} 0 & \text{for } m \neq m' \\ \gamma(f_O^m)\delta(t-t') & \text{for } m = m' \end{cases}$$  \hspace{0.5cm} (3.22)

where

$$\gamma(f_O^m) = \frac{k^+(c_{ds}^m)^\eta (1 - f_O^m) + k^- f_O^m}{N}.$$  \hspace{0.5cm} (3.23)

Note that the dyadic subspaces only influence each other through the bulk concentrations $c_{myo}$ and $c_{nsr}$. Below we refer to Eqs. 3.16–3.23 as the “full local/global whole cell model.”

### 3.2.5 Reduced local/global whole cell model

The Langevin description of each CaRU (Eqs. 3.19–3.21) in the full local/global model may be simplified by assuming that the dyadic subspace and junctional SR rapidly equilibrate with the bulk myoplasmic and network SR [Ca$^{2+}$], that is, $J_{rel}^m = J_{myo}^m$ and $J_{rel}^m = J_{nsr}^m$. These balanced fluxes relate the $2M$ domain Ca$^{2+}$ concentrations, $c_{ds}^m$ and $c_{jsr}^m$, to the bulk concentrations, $c_{myo}$ and $c_{nsr}$, and the fraction of open channels $f_O^m$ in the $m$th CaRU as
follows Hartman et al. (2010),

\[
\begin{align*}
\tilde{c}_{ds}^m &= (1 - \chi_{myo}^m)c_{myo} + \chi_{myo}^mc_{nsr} \\
\tilde{c}_{jsr}^m &= \chi_{nsr}^mc_{myo} + (1 - \chi_{nsr}^mc_{nsr},
\end{align*}
\]

where \(\chi_{myo}^m = \tilde{v}_{nsr}^m/(v_{myo} + \tilde{v}_{myo}^m)\) and \(\chi_{nsr}^m = \tilde{v}_{myo}^m/(v_{nsr} + \tilde{v}_{myo}^m)\), \(\tilde{v}_{myo}^m = \tilde{v}_{rel}^m/(\tilde{v}_{rel}^m + v_{myo})\), and \(\tilde{v}_{rel}^m = v_{rel}f_{\mathcal{O}}^m\). Eqs. 3.24–3.25 eliminate 2\(M\) of the 3\(M\) ODEs representing the population of \(M\) CaRUs, with the remaining ODEs,

\[
\frac{df_{\mathcal{O}}^m}{dt} = k^+ (\tilde{c}_{ds}^m)^{\eta} (1 - f_{\mathcal{O}}^m) - k^- f_{\mathcal{O}}^m + \xi^m(t),
\]

dependent upon the rapidly equilibrated dyadic subspace concentration \(\tilde{c}_{ds}^m\) that is an algebraic function of \(f_{\mathcal{O}}^m\), \(c_{myo}^m\) and \(c_{nsr}\). Realizations of this “reduced local/global whole cell model” are obtained by numerically integrating Eqs. 3.16–3.17 and Eq. 3.26.

### 3.2.6 Fokker-Planck local/global whole cell model

The full and reduced local/global whole cell models presented above include heterogeneous local \(\text{Ca}^{2+}\) signaling and stochastic \(\text{Ca}^{2+}\) release. Unfortunately, a physiologically realistic ventricular myocyte simulation would involve \(M \approx 20,000\) CaRUs (Chen-Izu et al., 2006). Rather than perform Monte Carlo simulations with a lesser, unphysiological value for \(M\) that is computationally feasible, we recognize that a Fokker-Planck equation similar to Eq. 3.9 is the master equation for a CaRU and its associated domains. Because the \(M\) CaRUs in the whole cell model are identical and independent except for fluxes to and from the bulk myoplasm and network SR, we replace the \(M\) SDEs representing these CaRUs (Eq. 3.26) with this Fokker-Planck equation (a good approximation for large \(M\) that is exact as \(M \to \infty\)). In this way, we obtain the “Fokker-Planck local/global whole cell model.”

In the study of \(\text{Ca}^{2+}\) homeostasis in permeabilized ventricular myocytes presented below, the governing equations are Eq. 3.9 and Eqs. 3.16–3.17, with the fluxes \(J_{myo}^T\) and \(J_{nsr}^T\)
redefined as functions of the probability distribution function for \( f_0 \) in a randomly sampled CaRU. In permeabilized myocytes, the bulk myoplasmic [Ca\(^{2+}\)] is clamped \((k_{pm} \text{ is large})\) and \( c_{myo} \approx c_{ext} \) is no longer a variable but a parameter. Consequently, Eq. 3.16 is superfluous and the governing equations for the Fokker-Planck equation description of the local/global model of permeabilized ventricular myocytes are therefore given by

\[
\frac{dc_{nsr}}{dt} = \frac{1}{\Lambda_{nsr}} (-J_{nsr}^T + J_{pump}) \quad (3.27)
\]

\[
\frac{\partial \rho}{\partial t} = -\frac{\partial}{\partial f} [\alpha \rho] + \frac{1}{2} \frac{\partial^2}{\partial f^2} [\gamma \rho] \quad (3.28)
\]

where

\[
J_{nsr}^T (t) = \nu_{nsr}^T \int (c_{nsr} - \bar{c}_{jsr}) \rho(f,t) df. \quad (3.29)
\]

In Eq. 3.28, \( \alpha(f) \) and \( \gamma(f) \) are given by Eqs. 3.10–3.11, with

\[
\nu^+ = k^+ \bar{c}_{ds} (1 - f), \quad (3.30)
\]

\[
\nu^- = k^- f. \quad (3.31)
\]

The equilibrated domain concentrations are given by

\[
\bar{c}_{ds} = [(1 - \chi_{myo}) c_{myo} + \chi_{myo} c_{nsr}] \quad (3.32)
\]

\[
\bar{c}_{jsr} = [\chi_{nsr} c_{myo} + (1 - \chi_{nsr}) c_{nsr}] \quad (3.33)
\]

where \( \chi_{myo} \) and \( \chi_{nsr} \) are the following functions of \( f \),

\[
\chi_{myo} = \frac{\nu_{nsr}^T / (\nu_{myo}^T + \nu_{nsr}^T)}{\bar{c}_{nsr} / \bar{c}_{jsr}} \quad (3.34)
\]

\[
\chi_{nsr} = \frac{\nu_{nsr}^T / (\nu_{nsr}^T + \nu_{myo}^T)}{\bar{c}_{nsr} / \bar{c}_{jsr}} \quad (3.35)
\]
where

\[ \tilde{v}_{rel} = \frac{\tilde{v}_{rel}^T \nu_{nsr}^T}{\tilde{v}_{rel}^T + \nu_{nsr}^T} \quad (3.36) \]

\[ \tilde{v}_{myo} = \frac{\tilde{v}_{rel}^T \nu_{myo}^T}{\tilde{v}_{rel}^T + \nu_{myo}^T} \quad (3.37) \]

and \( \tilde{v}_{rel}^f = v_{rel}^f \) (cf. Eqs. 3.24–3.25). In the local/global whole cell model calculations presented below, the Fokker-Planck equation was numerically integrated using a total variation diminishing scheme (Williams et al., 2007).

### 3.3 Results

#### 3.3.1 Calcium homeostasis in the local/global whole cell model

We use the Fokker-Planck version of the reduced local/global model (Eqs. 3.27–3.33) to investigate \( \text{Ca}^{2+} \) homeostasis in permeabilized ventricular myocytes, in particular, the influence of \( c_{myo} \) on SR \( \text{Ca}^{2+} \) load and release. The relationship between \( c_{myo} \) and \( \text{Ca}^{2+} \) homeostasis is complex, as \( c_{myo} \) can promote elevated \( c_{nsr} \) through increased SERCA uptake. On the other hand, a sufficiently elevated \( c_{nsr} \) also promotes \( \text{Ca}^{2+} \) sparks that may deplete the network SR (i.e., decrease \( c_{nsr} \)).

Using an intermediate value for the myoplasmic \( [\text{Ca}^{2+}] \) \( (c_{myo} = 0.18 \, \mu\text{M}) \) in the permeabilized ventricular myocyte model, Fig. 3.3A shows the bimodal steady-state probability density function for the fraction of open channels, \( \rho_{ss}(f) \), calculated via the Fokker-Planck version of the whole cell model (solid line). This bimodal density reflects the dynamics of CaRUs composed of RyRs that are usually closed, but occasionally open in a concerted fashion. For comparison, Fig. 3.3A also shows a (nearly identical) estimate of the steady-state density function obtained from a whole cell model with the corresponding Langevin description of \( M = 200 \) release sites (dashed curve). Fig. 3.3B compares the stationary distribution for a whole cell model that uses a Markov chain description of release sites (white bars) and the corresponding distribution calculated via the Fokker-Planck version of
Figure 3.3: Spark- and non-spark-mediated release from the population of CaRUs represented in the local/global whole cell model. A: The steady-state density function $\rho_{ss}(f)$ of the Fokker-Planck-based whole cell model (solid line) and the corresponding density in the Langevin model (dashed line, $M = 200$ release sites). B: The binned (discretized) steady-state density of the Fokker-Planck model (black histogram) and distribution of the number of open channels in the Markov chain-based whole cell model (white histogram, average of $M = 200$ release sites). C-D: The RyR release rate function (C) given by $v_{rel}(\bar{c}_{jSR} - \bar{c}_{ds})$ and the steady-state release flux density (D) given by $v_{rel}(\bar{c}_{jSR} - \bar{c}_{ds}) \rho_{ss}$, plotted as functions of the fraction of open channels ($f_o$). Parameters: $c_{myo} = 0.18$ $\mu$M, $N = 40$ RyRs per CaRU. In this figure and those follow, see Table 3.1 for other parameters.

Using the Fokker-Planck-based whole cell model, Fig. 3.3C shows the monotone increasing relationship between the fraction of open channels and stochastic Ca$^{2+}$ release rate, given by $v_{rel}(\bar{c}_{jSR} - \bar{c}_{ds})$ where $\bar{c}_{jSR}$ and $\bar{c}_{ds}$ are functions of $f$ (Eqs. 3.32–3.33). Fig. 3.3D shows the steady-state release flux density, given by $v_{rel}(\bar{c}_{jSR} - \bar{c}_{ds}) \rho_{ss}$, that is, the product of the curves in Fig. 3.3A and C. Note that the steady-state release flux density is also a bimodal
Fig. 3.4 shows steady-state values for total release flux $J_{rel}^T$, network SR $[Ca^{2+}]$ ($c_{nsr}$), and the spark Score as a function of $c_{myo}$, obtained from simulation of the local/global model using the Langevin ($+$ symbols) and Fokker-Planck (solid lines) descriptions of the CaRU population. The spark Score is the index of dispersion of the fraction of open channels ($f_O$),

$$Score = \frac{\text{Var}[f_O]}{\text{E}[f_O]},$$  \hspace{1cm} (3.38)$$

where $E[f_O] = \int f \rho_{ss} df$, $\text{Var}[f_O] = \int (f - E[f_O])^2 \rho_{ss} df$, and $\rho_{ss}(f)$ is the steady-state probability density of open channels. The spark Score takes values between 0 and 1, and a Score greater than approximately 0.25 indicates the presence of robust Ca$^{2+}$ sparks (Groff and Smith, 2008a). Over a wide range of $c_{myo}$ values, there is agreement between $J_{rel}^T$, $c_{nsr}$ and the spark Score calculated using the Langevin and Fokker-Planck approaches, validating
Figure 3.5: Mean spark amplitude (A), duration (B) and inter-event interval (C) as a function of myoplasmic \([\text{Ca}^{2+}]\) \((c_{\text{myo}})\), calculated via the Langevin version of the local/global whole cell model (average over 1000 s simulations).

the use of the Fokker-Planck version of the model and our implementation of both methods. Note that \(J_{\text{rel}}^T\) is a monotone increasing function of \(c_{\text{myo}}\) (Fig. 3.4A), while \(c_{\text{nsr}}\) is biphasic, increasing for \(c_{\text{myo}} < 0.2 \mu\text{M}\) and decreasing for \(c_{\text{myo}} > 0.2 \mu\text{M}\) (Fig. 3.4B). The spark \(\text{Score}\) shows similar biphasic dependence on \(c_{\text{myo}}\) (Fig. 3.4C).

The biphasic dependence of \(c_{\text{nsr}}\) and the spark \(\text{Score}\) on \(c_{\text{myo}}\) can be understood by considering the representative stochastic trajectories for the fraction of open channels in a randomly sampled CaRU in the Langevin model (Fig. 3.4A) or, alternatively, the steady-state population density function \(\rho_{ss}(f)\) in the Fokker-Planck model (Fig. 3.4C). For a low myoplasmic \([\text{Ca}^{2+}]\) \((c_{\text{myo}} = 0.1 \mu\text{M})\), \(\rho_{ss}(f)\) is located near \(f = 0\), consistent with few channel openings (insets, Fig. 3.4A and C). As \(c_{\text{myo}}\) increases to an intermediate value of 0.2 \(\mu\text{M}\), increased SERCA uptake elevates \(c_{\text{nsr}}\), and \(\rho_{ss}(f)\) is distinctly bimodal, consistent with robust sparks and the observed increase in \(J_{\text{rel}}^T\) and \(\text{Score}\). However, a further increase in myoplasmic \([\text{Ca}^{2+}]\) \((c_{\text{myo}} = 0.6 \mu\text{M})\) promotes tonic activation of CaRUs (as opposed to sparks, for a decreasing \(\text{Score}\)). The resulting increase in release flux \((J_{\text{rel}}^T)\) depletes the network SR \([\text{Ca}^{2+}]\) (lower values of \(c_{\text{nsr}}\)) and eliminates robust sparks.

Fig. 3.5 shows the mean steady-state spark amplitude (A), spark duration (B) and inter-event intervals (C) as a function of \(c_{\text{myo}}\), calculated via the Langevin version of the local/global whole cell model. The duration of the \(i\)th \(\text{Ca}^{2+}\) release event is the time elapsed between the first channel opening and last channel closing of each simulated spark, here
Figure 3.6: Balance between SR Ca\(^{2+}\) release and uptake at steady-state. The SR Ca\(^{2+}\) release flux \(J_{rel}^T\) (black dashed lines) and SERCA uptake flux \(J_{pump}\) (solid gray lines) are shown as a function of a fixed (clamped) network SR [Ca\(^{2+}\)] (as though \(c_{nsr}\) were a parameter) for different values of myoplasmic [Ca\(^{2+}\)] (\(c_{myo}\)). Steady-state release fluxes (solid black lines) are shown as a function of clamped \(c_{nsr}\), for increasing values of \(c_{myo}\) from 0.06 \(\mu\)M to 1.2 \(\mu\)M (arrows). Each intersection of these curves (three open circles) indicates a steady-state release flux \((J_{rel}^T)\) and corresponding unclamped SR Ca\(^{2+}\) load \((c_{nsr})\) solving Eqs. 3.27–3.37 for a particular \(c_{myo}\).

Defined as \(f_o\) crossing the threshold \(1/N\) in the upward/downward direction. The amplitude of \(i\)th Ca\(^{2+}\) release event is the integrated area under \(f_o(t)\) during the event. The \(i\)th inter-event interval is the length of time between the \((i - 1)\)th and \(i\)th Ca\(^{2+}\) release events. Note that spark amplitude and spark duration are biphasic functions of \(c_{myo}\), peaking at \(c_{myo} \approx 0.25 \mu\)M, similar to the steady-state \(c_{nsr}\) and spark Score (Fig. 3.4B and C). Increasing \(c_{myo}\) up to 0.2 \(\mu\)M elevates \(c_{nsr}\) (increasing SERCA pump update), and this increases the amplitude and duration of Ca\(^{2+}\) sparks. However, high \(c_{myo}\) promotes spark-mediated release that ultimately depletes network SR Ca\(^{2+}\).

In Fig. 3.6, \(J_{rel}^T\) (black dashed lines) and \(J_{pump}\) (solid gray lines) are shown as a function of \(c_{nsr}\) for three values of \(c_{myo}\). \(J_{rel}^T\) is a monotone increasing function of \(c_{nsr}\); the increasing slope at high \(c_{nsr}\) levels is due to spark-mediated Ca\(^{2+}\) release. \(J_{pump}\) decreases approximately
Figure 3.7: SR Ca\(^{2+}\) depletion following inhibition of SERCA uptake in permeabilized ventricular myocytes. Network SR Ca\(^{2+}\) concentration, \(c_{\text{nsr}}\), is shown as a function of time, for different values of myoplasmic Ca\(^{2+}\) concentration, \(c_{\text{myo}}\). At \(t = 30\) s, SERCA inhibition by thapsigargin (TG) is simulated by setting uptake rate constant \(v_{\text{pump}} = 0\).

linearly with \(c_{\text{nsr}}\), and both \(J_{\text{rel}}^T\) and \(J_{\text{pump}}\) increase for increasing \(c_{\text{myo}}\). The intersection of the \(J_{\text{rel}}^T\) and \(J_{\text{pump}}\) curves (open circles) indicate the steady-state total release flux and SR Ca\(^{2+}\) load (\(c_{\text{nsr}}\)) for a given value of \(c_{\text{myo}}\) (solid black line, arrow indicates increasing \(c_{\text{myo}}\)). For a given \(c_{\text{nsr}}\), two distinct steady-states are possible—one with low \(c_{\text{myo}}\) and \(J_{\text{rel}}^T\) (primarily non-spark-mediated release) and another with high \(c_{\text{myo}}\) and \(J_{\text{rel}}^T\) (primarily spark-mediated release). The next section further explores the dependence of spark- and non-spark-mediated release on \(c_{\text{myo}}\).

### 3.3.2 Spark- and non-spark-mediated SR calcium release

In a recent experimental study, Bovo et al. (2011) demonstrated that myoplasmic Ca\(^{2+}\) levels augment both spark-mediated SR Ca\(^{2+}\) release and non-spark-mediated SR Ca\(^{2+}\) release in ventricular myocytes (Bovo et al., 2011). While controlling myoplasmic [Ca\(^{2+}\)] (\(c_{\text{myo}}\))
by permeabilization of the cell plasma membrane, the time-course of network SR $[\text{Ca}^{2+}]$ ($c_{\text{nsr}}$) depletion was measured following application of the SERCA inhibitor, thapsigargin (cf. reference Bovo et al. (2011), Fig. 1A). Assuming negligible SERCA activity (i.e., $J_{\text{pump}} = 0$), the rate of change of $c_{\text{nsr}}$ was used as a measure of the SR $\text{Ca}^{2+}$ release flux (see Eq. 3.27), and further analysis was performed to distinguish spark- and non-spark-mediated release as functions of $c_{\text{myo}}$ and $c_{\text{nsr}}$. Fig. 3.7 uses a similar protocol (setting $v_{\text{pump}} = 0$) to elucidate the influence of $c_{\text{myo}}$ on spark- and non-spark-mediated release. Consistent with Bovo et al. and Fig. 3.4B, Fig. 3.7 shows that steady-state $c_{\text{nsr}}$ increases as $c_{\text{myo}}$ increases from 0.12 to 0.18 $\mu$M (compare initial values, solid, dashed and thick solid lines). Consistent with experiment, increasing $c_{\text{myo}}$ in this range of concentrations also leads to increased $\text{Ca}^{2+}$ release rate, as evidenced by faster SR depletion upon simulated block of SERCA with thapsigargin (TG in Fig. 3.7).

Fig. 3.8 shows the total release flux, $J_{\text{rel}}^T$, and the spark- and non-spark-mediated release ($J_{\text{rel}}^S$ and $J_{\text{rel}}^{NS}$ as defined in Fig. 3.3C) as a function of $c_{\text{nsr}}$ during the SR depletion simulation of Fig. 3.7, for different values of $c_{\text{myo}}$ (cf. reference Bovo et al. (2011), Fig. 3). While $J_{\text{rel}}^T$ increases as a function of both $c_{\text{nsr}}$ and $c_{\text{myo}}$ (Fig. 3.8A), the contributions of the spark- and non-spark-mediated release ($J_{\text{rel}}^S$ and $J_{\text{rel}}^{NS}$) are highly dependent on $c_{\text{nsr}}$. At low network SR $[\text{Ca}^{2+}]$ ($c_{\text{nsr}}$), the spark-mediated release flux ($J_{\text{rel}}^S$) is negligible, but it increases exponentially as $c_{\text{nsr}}$ increases (Fig. 3.8B). The non-spark-mediate release ($J_{\text{rel}}^{NS}$) is small for low $c_{\text{nsr}}$ levels and increases as a linear function of $c_{\text{nsr}}$ (Fig. 3.8C). When the SR load is clamped at $c_{\text{nsr}} = 950$ $\mu$M, both $J_{\text{rel}}^S$ and $J_{\text{rel}}^{NS}$ increase as $c_{\text{myo}}$ increases (Fig. 3.8D). However, the spark-mediated release flux ($J_{\text{rel}}^S$), increases to a greater extent than the non-spark-mediated release ($J_{\text{rel}}^{NS}$). Steady-state calculations of $J_{\text{rel}}^T$, $J_{\text{rel}}^S$, and $J_{\text{rel}}^{NS}$ closely agree with time-varying simulations (Fig. 3.8, + symbols). In summary, when the SR is depleted, most SR $\text{Ca}^{2+}$ release occurs via non-spark-mediate release; conversely, when the SR is replete, most SR $\text{Ca}^{2+}$ release occurs via $\text{Ca}^{2+}$ sparks, more so as $c_{\text{myo}}$ increases.

The number of RyRs per CaRU, $N$, can vary over a wide physiological range Franzini-Armstrong et al. (1999). Fig. 3.9 shows the steady-state values for $J_{\text{rel}}^T$, $J_{\text{rel}}^S$, and $J_{\text{rel}}^{NS}$.
for different values of $N$. As $N$ increases (scaling $v_{rel}$ appropriately such that $J_{rel}^{T}$ when all $N$ channel are open is unchanged), $J_{rel}^{T}$ becomes a steeper function of $c_{nsr}$ (Fig. 3.9A).

Interestingly, when the network SR [Ca$^{2+}$] is higher ($c_{nsr} = 1000$ M), $J_{rel}^{T}$ is larger for large $N$, but when $c_{nsr}$ is slightly lower ($c_{nsr} = 950$ M), $J_{rel}^{T}$ is smaller for large $N$ (Fig. 3.9A, arrows). Spark-mediated release ($J_{rel}^{S}$) varies with $N$ in a manner similar to $J_{rel}^{T}$ (Fig. 3.9B), while non-spark-mediated release ($J_{rel}^{NS}$) generally decreases as $N$ increases (Fig. 3.9C).

Fig. 3.10 shows how steady-state probability density function, $\rho_{ss}(f)$, and the release flux density, $v_{rel}^{T}(\tilde{c}_{jsr} - \tilde{c}_{ds})\rho_{ss}$, depend on the number of RyRs per release site ($N$) when the total release rate $v_{rel}^{T}$ is fixed (i.e., $MN$ is a constant). For network SR [Ca$^{2+}$] of $c_{nsr} = 950$ M (Fig. 3.10A), a larger number of channels per CaRU ($N$) decreases the “diffusion” term (channel gating fluctuations) in Eq. 3.11 and both spark- and non-spark-mediated SR Ca$^{2+}$

Figure 3.8: Spark- and non-spark-mediated release during SR Ca$^{2+}$ depletion. Total ($J_{rel}^{T}$, A), spark-mediated ($J_{rel}^{S}$, B), and non-spark-mediated ($J_{rel}^{NS}$, C) release flux are shown as functions of network SR [Ca$^{2+}$], $c_{nsr}$, during SR depletion simulations (see Fig. 3.7), for different values of myoplasmic [Ca$^{2+}$], $c_{myo}$. Steady-state calculations of $J_{rel}^{T}$, $J_{rel}^{S}$, and $J_{rel}^{NS}$ ("+") symbols) are shown for $c_{myo} = 0.18$ M. (D) $J_{rel}^{S}$ (filled circles) and $J_{rel}^{NS}$ (open circles) as functions of $c_{myo}$ for $c_{nsr} = 950$ M. Solid and dash lines indicate steady-state $J_{rel}^{S}$ and $J_{rel}^{NS}$, respectively.
release. However, for a slightly larger value of \( c_{n_{sr}} = 1020 \, \mu M \), larger \( N \) decreases non-spark-mediated release \( J^{NS}_{rel} \) while promoting robust sparks and increasing spark-mediated release \( J^{S}_{rel} \).

Finally, Fig. 3.11 shows the steady-state spark Score for “clamped” \( c_{myo} \) and \( c_{nsr} \) and illustrates the interplay of bulk concentrations and Ca\(^{2+}\) sparks. For a given value of \( c_{myo} \), the Score is a bell-shaped function of \( c_{nsr} \), that is, there is a specific range of SR Ca\(^{2+}\) load that supports robust sparks. As observed in prior work (Groff and Smith, 2008a), the range for robust sparks decreases as \( N \) is increased (Fig. 3.11B and C). Most importantly, the solid black lines indicate the steady-state (unclamped) network SR [Ca\(^{2+}\)] \( (c_{nsr}) \) as a function of \( c_{myo} \) (cf. Fig. 3.4B). When \( c_{myo} \) is sufficiently elevated that further increase leads to decreased \( c_{nsr} \), the SR Ca\(^{2+}\) load equilibrates to a value that maximizes the Score, that is, the steady-state \( c_{nsr} \) decreases (with increasing \( c_{myo} \)) just enough to maintain robust sparks. This intriguing and potentially significant result is also observed when the total release flux \( v_{rel}^{T} \) is proportional to \( N \) (not shown).
Figure 3.10: Steady-state density of the fraction of open channels and release flux density for $c_{nsr} = 950$ (A and B) and $c_{nsr} = 1020 \mu M$ (C and D). Parameters: $N = 40$ (thick line), 80 (dash line) and 120 (thin line); $c_{myo} = 0.18 \mu M$, other parameters as in Table 3.1.

3.4 Discussion

3.4.1 Summary of main findings

In this chapter, we present a novel local/global whole cell model of Ca$^{2+}$ homeostasis based on a Langevin description of stochastic Ca$^{2+}$ release that includes both spark-mediated and non-spark-mediated release dynamics. The Fokker-Planck equation associated with the Langevin formulation of stochastic Ca$^{2+}$ release is coupled to balance equations for the bulk myoplasmic and network SR [Ca$^{2+}$]. Using this approximate representation of the collective dynamics of a large number of identical CaRUs, this whole cell modeling approach avoids Monte Carlo simulation of a large population of CaRUs and facilitates our study of Ca$^{2+}$ homeostasis in permeabilized ventricular myocytes.

In permeabilized myocytes, the interplay between bulk myoplasmic [Ca$^{2+}$] ($c_{myo}$), and
Figure 3.11: Score as function of \( c_{\text{myo}} \) and \( c_{\text{nSR}} \) when \( N \) is 20 (A), 60 (B) and 100 (C), respectively. The solid line indicates the steady-state value for \( c_{\text{nSR}} \) as a function of \( c_{\text{myo}} \) (cf. Fig. 3.4).

network SR \( [\text{Ca}^{2+}] \) (\( c_{\text{nSR}} \)) on SR \( \text{Ca}^{2+} \) release is complex, in spite of the fact that myoplasmic \( [\text{Ca}^{2+}] \) is under experimental control (i.e., \( c_{\text{myo}} \) is not a dynamic variable but a model parameter). Elevated \( c_{\text{myo}} \) promotes \( \text{Ca}^{2+} \) uptake into the network SR via the SERCA pump, and this may elevate \( c_{\text{nSR}} \). On the other hand, high \( c_{\text{myo}} \) and high \( c_{\text{nSR}} \) both promote increased SR \( \text{Ca}^{2+} \) release and depletion of SR \( \text{Ca}^{2+} \).

We use the Langevin and Fokker-Planck local/global whole cell model of a permeabilized ventricular myocyte to characterize the depletion of network SR \( [\text{Ca}^{2+}] \) (\( c_{\text{nSR}} \)) that occurs via both spark-mediated release and non-spark-mediated release, as well as dependency of SR \( \text{Ca}^{2+} \) load on myoplasmic \( [\text{Ca}^{2+}] \) (\( c_{\text{myo}} \)). In agreement with recent experimental work (Bovo et al., 2011), we find that spark-mediated release increases exponentially as \( c_{\text{myo}} \) increases, while non-spark-mediated release increases linearly (Fig. 3.8).

The interplay between \( c_{\text{myo}} \), \( c_{\text{nSR}} \), and spark- and non-spark-mediated release in the local/global whole cell model generates several phenomena of \( \text{Ca}^{2+} \) homeostasis in permeabilized cells that are worth highlighting. For example, the model predicts the presence of two distinct stable steady-states that lead to the same SR \( \text{Ca}^{2+} \) load—one with low myoplasmic \( [\text{Ca}^{2+}] \) and predominantly non-spark-mediated SR \( \text{Ca}^{2+} \) release and another with high myoplasmic \( [\text{Ca}^{2+}] \) and release that is primarily spark-mediated (Fig. 3.6). Significantly, in
our permeabilized ventricular myocyte model, for any clamped myoplasmic $[\text{Ca}^{2+}]$ ($c_{\text{myo}}$) that is large enough to trigger spark-mediated release, the resulting spontaneous stochastic Ca$^{2+}$ release tends to decrease the network SR Ca$^{2+}$ load just enough to maintain robust Ca$^{2+}$ sparks (Fig. 3.11). To our knowledge this potentially significant characteristic of Ca$^{2+}$ homeostasis in permeabilized cells has not previously been identified.

3.4.2 Physiological significance

Significant effort in recent years has been devoted to understanding the mechanisms influencing RyR regulation and SR Ca$^{2+}$ release. Abnormal regulation of RyRs can lead to aberrant SR Ca$^{2+}$ release that directly contributes to excitation-contraction coupling dysfunction (George et al., 2007; George, 2008). Previous studies have shown RyR-mediated Ca$^{2+}$ release was enhanced in myocytes from failing rabbit hearts (Zima et al., 2010), which increases the likelihood of Ca$^{2+}$-dependent arrhythmias (George, 2008). Recent experiments suggested that hidden RyR release contributes to the total release flux and influences Ca$^{2+}$ homeostasis (Zima et al., 2010; Bovo et al., 2011; Brochet et al., 2011).

In this chapter, we are particularly interested in how myoplasmic $[\text{Ca}^{2+}]$ ($c_{\text{myo}}$) influences SR Ca$^{2+}$ release via regulation of stochastic Ca$^{2+}$ release mediated by CaRUs composed of clusters of RyRs. Our model shows that RyRs may produce both visible (spark-mediated) and invisible (non-spark-mediated) stochastic Ca$^{2+}$ release. High $c_{\text{myo}}$ increases both spark- and non-spark-mediated release by increasing the open probability of Ca$^{2+}$-activated RyRs. However, $c_{\text{myo}}$ affects these pathways in two distinct and characteristic ways. Non-spark-mediated Ca$^{2+}$ release increases linearly as a function of $c_{\text{myo}}$, while spark-mediated release increases exponentially with $c_{\text{myo}}$.

We investigated how the number of RyRs in each individual CaRU influences network SR Ca$^{2+}$ depletion and stochastic Ca$^{2+}$ release. When $\nu_{\text{req}}^T$ is fixed (single channel conductance inversely proportional to $N$), we found that a larger number of RyRs per CaRU results in a steeper release flux (primarily spark-mediated release) as a function of network SR [Ca$^{2+}$], when the SR is replete. However, when network SR [Ca$^{2+}$] is depleted, and the release flux

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is primarily non-spark-mediated, increasing the number of RyRs per CaRU decreases the total release flux, due to reduced triggering of Ca\textsuperscript{2+} sparks (Figs. 3.9 and 3.10). When $v_{rel}^T$ is proportional to $N$ (fixed single channel conductance), SR Ca\textsuperscript{2+} decreases with increasing $N$, due to higher release rates (not shown).

3.4.3 Comparison to other whole cell models

A number of mathematical and computational whole cell models have been developed to understand Ca\textsuperscript{2+} homeostasis and the cardiac Ca\textsuperscript{2+} cycle. For example, computational models of excitation-contraction (EC) coupling in ventricular myocytes have been developed in which SR Ca\textsuperscript{2+} release depends directly on the average myoplasmic [Ca\textsuperscript{2+}] (Jafri et al., 1998b; Tang and Othmer, 1994). These "common pool" models (Stern, 1992) exhibit all-or-none triggered SR Ca\textsuperscript{2+} release, contrary to experiments showing that release is smoothly graded with changes in Ca\textsuperscript{2+} influx (Wier et al., 1994; Cannell et al., 1995b). This discrepancy is a consequence of the "local control" mechanism of Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release (CICR). In ventricular myocytes, the cellular SR Ca\textsuperscript{2+} release flux is not a function of the spatially-averaged intracellular [Ca\textsuperscript{2+}], but instead depends on thousands of different local Ca\textsuperscript{2+} concentrations fluctuating in response to stochastic openings and closings of RyRs located on the SR membrane. The picture is further complicated by dynamic changes in localized SR [Ca\textsuperscript{2+}] that are also spatially heterogeneous and thought to influence the gating of RyRs (Sobie and Lederer, 2012).

To overcome this problem, stochastic models that account for the heterogeneous dyadic subspace and junctional SR [Ca\textsuperscript{2+}] have been developed (Greenstein and Winslow, 2002b; Hartman et al., 2010; Williams et al., 2011). Similar to the Langevin model that is the focus of this chapter, these local control models include a large number of CaRUs. In such models, RyR stochastic gating is typically described by a discrete-state Markov chain. This approach has recently been used to examine issues such as allostERIC coupling between RyRs (Williams et al., 2011) and refractoriness of Ca\textsuperscript{2+} release after termination (Ramay et al., 2011).
While Markov chain and Langevin models of CaRUs may lead to similar results (Fig. 3.1), the runtime for Markov chain simulations is proportional to the number of CaRU states, a quantity that is exponential in the number of distinct RyR states. To see this, consider a CaRU composed \( N \) identical \( K \)-state channels (and thus \( K^N \) states). It is well-known that the number of distinguishable CaRU states is given by \( (N + K - 1)!/[N!(K - 1)!] = [(N + K - 1) \cdots (N + 1)]/(K - 1)! \), a quantity that includes a term proportional to \( N^{K-1} \) (the numerator has \( K - 1 \) terms) and is thus exponential in \( K \). On the other hand, the runtime for Langevin simulations is independent of the number of RyRs (\( N \) is a model parameter that scales the channel noise) and proportional to the number of RyR states \( K \) (the required number of SDEs). Similarly, the runtime of the Langevin local/global model does not scale with \( N \), and the model may be extended to include RyRs with more than two states (see below). Because the Langevin version of the local/global model that has been our focus involves only a single SDE (two-state RyR model), the probability density function for CaRU state is univariate. For this reason, the Fokker-Planck local/global whole cell model is extremely computationally efficient. Because a \( K \)-state RyR model leads to a Fokker-Planck equation with \( K - 1 \) independent variables (conservation of probability), the Langevin version of the local/global model is likely to be more straightforward than the Fokker-Planck version when \( K \geq 3 \) (see Eq. 3.41 below).

It is instructive to compare the local/global model presented here with our prior work. In Hartman et al. (2010), we presented a similar minimal model of a permeabilized myocyte, in which bulk myoplasmic and network SR Ca\(^{2+} \) levels were coupled to a Markov chain CaRU model with \( N \) Ca\(^{2+} \)-activated RyRs per release site. The master equation in this case was a linear system of \( N + 1 \) ODEs. The Langevin and Fokker-Planck local/global models presented here are also distinct from prior work of Williams et al. (2007) and Williams et al. (2008). In these studies, Ca\(^{2+} \) release dynamics were described by a set of coupled multivariate probability density functions (advection-reaction equations) for the dyadic subspace and junctional SR [Ca\(^{2+} \)], \( c_{ds} \) and \( c_{jsr} \), conditioned on CaRU state. This population density method and the associated moment-based reductions (Williams et al., 2008) are limited by
a state-space explosion that is exponential in $K$, while the computational efficiency of the Langevin local/global model is linear in $K$.

### 3.4.4 Limitations and extensions of the model

In the Langevin model, we assume that the number of channels in each CaRUs is large enough that the fraction of RyRs in different states can be treated as a continuous variable. When the number of RyRs per CaRU is small, the error associated with the Langevin approximation to the Markov chain CaRU model may not be acceptable (Gillespie, 2000). In the local/global whole cell model presented here, the Langevin formulation was validated using a physiologically realistic numbers of RyRs per CaRU (Fig. 3.1). The number of RyRs per CaRU required for the Langevin formulation to be highly accurate likely depends on the details of the RyR model used, but is easily determined in any specific case.

In the derivation of the reduced local/global model, we assume that the dynamics of dyadic subspace $[\text{Ca}^{2+}]$ and junction SR $[\text{Ca}^{2+}]$ are fast compared to the gating of RyRs. However, slow translocation of junctional SR $[\text{Ca}^{2+}]$ can be incorporated into the Langevin local/global whole cell model through the addition of an additional SDE (Huertas et al., 2010). This extension might be important if the chosen RyR model includes luminal regulation, that is, transitions whose rate is a function of junctional SR $[\text{Ca}^{2+}]$. Accounting for slow junctional SR dynamics would increase the dimensionality of the probability density function (Eq. 3.28) used in the corresponding Fokker-Planck whole cell model.

Upgrading the Langevin formulation of the local/global whole cell model to accommodate more complex RyR models is straightforward. A $K$-state RyR model leads to a linear system of $K$ SDEs,

$$\frac{df}{dt} = fQ + \xi(t)$$

(3.39)

where $f = (f_1, f_2, ..., f_K)$ and $\xi = (\xi_1, \xi_2, ..., \xi_K)$ are row vectors, $Q = (q_{ij})$ is the RyR model's transition matrix (the Markov chain's infinitesimal generator), the random term is
mean zero ($\langle \xi(t) \rangle = 0$) with two-time covariance matrix,

$$\langle \xi^T(t)\xi(t') \rangle = \Gamma(f) \delta(t - t'), \quad (3.40)$$

where $\Gamma = (\gamma_{ij})$, $\gamma_{ij} = -(q_{ij} f_i + q_{ji} f_j)/N$ for $i \neq j$ and $\gamma_{ii} = -\sum_{j \neq i} \gamma_{ij}$ (the $\gamma_{ii}$ are positive) (Keizer, 1987). The corresponding Fokker-Planck equation for the $K$-state RyR is

$$\frac{\partial}{\partial t} \rho(f, t) = -\sum_{i=1}^{K} \frac{\partial}{\partial f_i} [(fQ)_i \rho(f, t)] + \frac{1}{2} \sum_{i=1}^{K} \sum_{j=1}^{K} \frac{\partial^2}{\partial f_i \partial f_j} [\gamma_{ij} \rho(f, t)], \quad (3.41)$$

where $(fQ)_i$ is the $i$th element of the row vector $fQ$.

### 3.5 Appendix

#### 3.5.1 Comparison of Markov chain and Langevin CaRU models

Fig. 3.12A compares the spark $Score$ calculated via the Langevin (+ symbols) and the Markov chain (lines) description of a CaRU composed of two-state channels. The $Score$
Figure 3.13: Comparison of the Langevin (+ symbols) and the Markov chain (lines) description of an individual CaRU composed of $N = 20$ (solid) or 60 (dashed lines) three-state channels that include fast Ca$^{2+}$ activation and slow Ca$^{2+}$ inactivation (Eq. 2.1). The dissociation constant of inactivating Ca$^{2+}$ is fixed ($K_b = 5.8$); however, the thick lines indicate inactivation rates slowed ten-fold compared to thin lines. Parameters: $c_{\infty} = 0.05 \, \mu\text{M}$, $\eta = 2$, $k_+^\gamma = 1.5 \, \mu\text{M}^{-n} \text{ms}^{-1}$, $k_-^\gamma = 0.5 \, \text{ms}^{-1}$; thin lines: $k_+^\gamma = 0.015 \, \mu\text{M}^{-n} \text{ms}^{-1}$, $k_-^\gamma = 0.005 \, \text{ms}^{-1}$; thick lines: $k_+^\gamma = 0.0015 \, \mu\text{M}^{-n} \text{ms}^{-1}$, $k_-^\gamma = 0.0005 \, \text{ms}^{-1}$. Error bars as in Fig. 3.12.

Figure 3.12B shows that the Score calculated via the stationary distribution of the Markov chain and the Fokker-Planck equation are in agreement.

Fig. 3.13 plots Score versus coupling strength ($c_*$) for this Langevin model of a CaRU composed of $N$ three-state channels with Ca$^{2+}$ inactivation (see Eq. 2.1 for the definition of three state model). This may be compared to the result for a CaRU composed of $N$ two-state channels with no inactivation (Fig. 3.12). Consistent with a previous computational study Groff and Smith (2008a), Fig. 3.13 shows that Ca$^{2+}$-dependent inactivation facilitates spark termination (i.e., CaRUs spark for a wider range of coupling strengths). Most importantly, the Langevin (+ symbols) and Markov chain (lines) simulations agree.
3.5.2 Calcium release flux and CaRU size

Most of the parameter studies presented in this chapter assume that the total number of RyRs per cell is fixed. When the number of channels per CaRU ($N$) is varied, the number of CaRUs per cell ($M$) is changed so that $MN$ is a constant (i.e., the total release flux rate $v_{rel}^T$ is fixed). Alternatively, $M$ may be fixed; in this case, $v_{rel}^T$ is proportional to CaRU size ($N$). Fig. 3.14 shows the total release flux ($J_{rel}^T$), spark-mediated release ($J_{rel}^S$), and non-spark-mediated release ($J_{rel}^{NS}$) when the number of channels per CaRU ($N$) is varied under this assumption (fixed single channel conductance). In this case, regardless of $c_{nsr}$, the total release flux and spark-mediated release are higher for larger $N$. Conversely, when $v_{rel}^T$ is fixed (Fig. 3.9), the clamped network SR [$Ca^{2+}$] $c_{nsr}$ determines whether CaRU size $N$ increases or decreases the total release flux $J_{rel}^T$. Fig. 3.15 shows release flux density increases with CaRU size when $v_{rel}^T$ is proportional to $N$ (cf. Fig. 3.10).
Figure 3.15: Probability density function for the fraction of open channels $\rho_{os}(f)$ (A) and the release flux density (B) for $c_{\text{tar}} = 950 \mu M$ calculated under the assumption that $v_{\text{rel}}^T$ is proportional to $N$. Parameters as in Fig. 3.14.
Chapter 4

A population density and moment-based approach to modeling domain calcium-mediated inactivation of L-type calcium channels

4.1 Introduction

Voltage-gated Ca\textsuperscript{2+} channels fall into three main groups: Ca\textsubscript{\textalpha}1 (L-type, L for "long lasting"), Ca\textsubscript{\textalpha}2 (P-, N-, and R-type), and Ca\textsubscript{\textalpha}3 (T-type, T for "transient") (Lipscombe et al., 2002). Among them, plasma membrane L-type Ca\textsuperscript{2+} channels (LCCs) are widely expressed in many tissues and are known to play an important role in Ca\textsuperscript{2+}-dependent responses of electrically excitable cells. In cardiac myocytes, for example, Ca\textsuperscript{2+} influx via L-type Ca\textsuperscript{2+} channels into the dyadic subspace triggers sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} release and muscle cell contraction (Bers, 2002; Cheng et al., 1993b; Cannell et al., 1995b). L-type Ca\textsuperscript{2+} channels also play a key role in coupling synaptic excitation to activation of transcriptional events that contribute to neuronal plasticity (Murphy et al., 1991). The activation of LCCs
is voltage-dependent while the inactivation occurs via both voltage- and Ca$^{2+}$-dependent mechanisms; consequently, the formation of Ca$^{2+}$ microdomains following LCC influx can greatly influence the stochastic gating of LCCs and the physiology of excitable cells (Haack and Rosenberg, 1994; Budde et al., 2002).

There are four subtypes of LCCs that are denoted Ca$\alpha$1.1–1.4. Ca$\alpha$1.1 is primarily found in skeletal muscle and Ca$\alpha$1.4 is mainly found in retinal cells (Lipscombe et al., 2004; Baumann et al., 2004). Ca$\alpha$1.2 and 1.3 are highly expressed in cardiac myocytes and cells of the central nervous system (Ertel et al., 2000; Simon et al., 2003). In neuroendocrine cells, Ca$\alpha$1.2 and 1.3 are both involved in action potential generation, bursting activity and hormone secretion (Lipscombe et al., 2004; Marcantoni et al., 2007). Ca$\alpha$1.3 is biophysically and pharmacologically distinct from Ca$\alpha$1.2. For example, Ca$\alpha$1.3 activates at a more hyperpolarized voltage, has faster activation, and slower and less complete voltage-dependent inactivation than Ca$\alpha$1.2 (Koschak et al., 2001; Vandael et al., 2010). In the heart, Ca$\alpha$1.2-mediated Ca$^{2+}$ currents play an important role in cystolic events such as EC coupling (the triggered release of SR Ca$^{2+}$) (Huang et al., 2014) and the plateau depolarization (phase 2) of the action potential (Christel and Lee, 2012). Ca$\alpha$1.3, on the other hand, is highly expressed in cardiac pacemaker cells and is the major regulator of RyR-dependent local Ca$^{2+}$ release during the diastolic phase (Torrente et al., 2011). Inactivation of Ca$\alpha$1.2 channels is both voltage- and Ca$^{2+}$-dependent (Budde et al., 2002); however, certain Ca$\alpha$1.4 L-type channels do not exhibit Ca$^{2+}$-dependent inactivation (Lipscombe et al., 2004). L-type Ca$^{2+}$ channels that undergo Ca$^{2+}$-dependent inactivation do not in fact result in long lasting currents, in spite of the traditional nomenclature (Lipscombe et al., 2004).

Models of Ca$^{2+}$-inactivation often assume a high density of Ca$^{2+}$ channels and the slow accumulation of intracellular Ca$^{2+}$ in a cortical shell near the plasma membrane (Li et al., 1995). In the context of low density Ca$^{2+}$ channels, it may be assumed that spatially localized high [Ca$^{2+}$] regions (Ca$^{2+}$ domains) form near any individual channel when that particular channel is open (Fig. 4.1, left panel). In both shell and domain models, it is usually assumed that stochastic gating of L-type channels and the dynamics of the associated domains are
Figure 4.1: Comparison of equilibrium and dynamic domain models for Ca\textsuperscript{2+}-mediated inactivation of L-type Ca\textsuperscript{2+} channels. In equilibrium domain models, low density channels are not only locally controlled, but also inactivated by a domain \([\text{Ca}^{2+}]\) that is slaved to the channel state (high concentration when open and low concentration when closed). In the dynamic domain model presented here, low density channels experience heterogeneous domain \([\text{Ca}^{2+}]\) that depend on channel state in time-dependent manner. The right panel shows the fluxes associated with a minimal formulation of single domain. Extracellular, cytosolic, and \([\text{Ca}^{2+}]\) in the \(n\)\textsuperscript{th} domain are denoted by \(c_{\text{ext}}\), \(c_{\text{cyt}}\), and \(c^n\), respectively. The domain influx rate \(j_{\text{influx}}^{n}\) is nonzero when the Ca\textsuperscript{2+} channel in the \(n\)\textsuperscript{th} domain is open. The diffusion-mediated flux of the \(n\)\textsuperscript{th} domain Ca\textsuperscript{2+} to the cytosol is denoted by \(j_{\text{cyt}}^{n}\).

Independent except through global coupling via the bulk \([\text{Ca}^{2+}]\) and plasma membrane voltage (Zweifach and Lewis, 1995). For example, the domain model proposed and investigated by Sherman et al. (1990) took this form. Sherman et al. further assumed that Ca\textsuperscript{2+} domains form instantaneously when a channel activates, and collapse instantaneously when a channel deactivates or inactivates. This equilibrium formulation of domain Ca\textsuperscript{2+}-mediated inactivation of L-type Ca\textsuperscript{2+} channels is viable and often utilized as an alternative to shell models. Nevertheless, when the dynamics of Ca\textsuperscript{2+} channel activation and inactivation are not slow compared to domain formation and collapse, the assumption of rapidly equilibrating domain \([\text{Ca}^{2+}]\) might be inadequate.

In recent years, computational models of cardiac myocytes have been developed to account for local control of Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release and heterogeneous dyadic subspace and junctional SR \([\text{Ca}^{2+}]\) (Greenstein and Winslow, 2002b; Tanskanen et al., 2005; Hartman...
et al., 2010; Williams et al., 2011). In these models, a large number of Ca$^{2+}$ release units (CaRUs) are simulated, each of which is represented by a discrete-state continuous time Markov chain and a compartmental representation of the dyadic subspace and junctional SR. Unfortunately, when the description of CaRU gating includes many channel states, the runtime using a Markov chain approach can be excessive.

To avoid the computationally demanding task of performing Monte Carlo simulations of a large number of CaRUs, Williams et al. (2007) presented an approach to modeling local control and EC coupling in cardiac myocytes that uses probability densities to represent heterogeneous time-dependent local Ca$^{2+}$ signals in a large number of dyadic subspaces and junctional SR domains. This approach involves numerical solution of advection-reaction equations for time-dependent bivariate probability densities of subspace and junctional SR [Ca$^{2+}$] conditioned on CaRU state, densities that are coupled to ordinary differential equations (ODEs) for the bulk myoplasmic and network SR [Ca$^{2+}$]. Subsequently, a moment-based approach to simulating the dynamics of local Ca$^{2+}$ signals was found to be several orders of magnitude faster than conventional Monte Carlo simulation (Williams et al., 2008).

In this chapter, we apply a population density and moment-based modeling formalism that extends the framework for domain Ca$^{2+}$-mediated inactivation of LCCs to represent the time-dependent dynamics of domain formation and collapse (Fig. 4.1, middle panel). Using this modeling approach, we investigate the dependence of the inactivation function on the exponential time constant of domain collapse.

The remainder of this chapter is organized as follows. First, we formulate a population density approach to modeling domain Ca$^{2+}$-medicated inactivation of LCCs. Next, we derive the associated ODEs for the moments of these densities, and truncate and close the moment equations to produce reduced models that faithfully reproduce population density results. Using both the population density and moment-based models, we investigate the voltage-dependence of Ca$^{2+}$-inactivation that may occur through local Ca$^{2+}$ signaling in heterogeneous domains, and how Ca$^{2+}$-inactivation of L-type channels may be influenced by non-equilibrium dynamics of domain formation and collapse.
Model formulation

The compartments and fluxes included in the model formulation are shown in Fig. 4.1 (right panel), which includes the $[\text{Ca}^{2+}]$ in the extracellular space, the cytosol, and individual domains denoted by $c_{\text{ext}}$, $c_{\text{cyt}}$, and $c^n$, respectively. The modeling work presented in this paper assumes that the $c_{\text{ext}}$ and $c_{\text{cyt}}$ are clamped. However, it is straightforward to extend the model to account for the dynamic of $c_{\text{cyt}}$ (see Discussion). Fluxes between compartments include the influx from the extracellular space to the $n^{th}$ individual domain ($j^n_{\text{influx}}$), and the flux from each domain to the cytoplasm ($j^n_{\text{cyt}}$).

Consistent with Fig. 4.1, the time-dependent dynamics of the $[\text{Ca}^{2+}]$ in the $n^{th}$ domain is governed by the following ODE,

$$\frac{dc^n}{dt} = \frac{1}{\lambda_d} (\xi^n j^n_{\text{influx}} - j^n_{\text{cyt}}),$$

where $\xi^i = 0$ or 1 depending on whether the associated LCC is closed or open. In Eq. 4.1, $\lambda_d = (\Omega_d/\beta_d)/(\Omega_{\text{cyt}}/\beta_{\text{cyt}})$ is the effective volume ratio between the domain and cytoplasm that accounts for both physical volume and (constant fraction) buffering capacity. The flux from the domain to the cytoplasm is given by $j^n_{\text{cyt}} = v_{\text{cyt}}(c^n - c_{\text{cyt}})$ where $v_{\text{cyt}}$ is the rate of $\text{Ca}^{2+}$ domain collapse. The voltage- and $\text{Ca}^{2+}$-dependent influx, $j^n_{\text{influx}}$, is given by Goldman-Hodgkin-Katz current equation Hille (2001). That is, if the $n^{th}$ LCC is open, $j^n_{\text{influx}} = -A_m v^n_{\text{influx}}/(zF)$ where $A_m = C_m \beta_{\text{cyt}}/\Omega_{\text{cyt}}$ is a whole-cell capacitance scaling factor, $C_m$ is the capacitive membrane area, $z = 2$ is the valence of $\text{Ca}^{2+}$ and $F$ is Faraday’s constant. The $\text{Ca}^{2+}$ current, $i^n_{\text{influx}}$, is given by $i^n_{\text{influx}} = z^2 F P V (c^n - c_{\text{ext}} e^{-zV/V_\theta})/[V_\theta (1-e^{-zV/V_\theta})]$ where $P$ is the permeability, $V$ is the membrane voltage, $V_\theta = RT/F$, $R$ is the gas constant and $T$ is the absolute temperature.
Figure 4.2: Gating scheme of the L-type channel. The 12-state L-type \( \text{Ca}^{2+} \) channel includes \( \text{Ca}^{2+} \)-unbound and \( \text{Ca}^{2+} \)-bound states (denoted mode normal and mode \( \text{Ca} \), respectively). In both modes there are five closed states (\( C_0, \ldots, C_4 \) and \( C_{\text{Ca}0}, \ldots, C_{\text{Ca}4} \)) and one open state (\( O \) and \( O_{\text{Ca}} \)). Transitions from mode normal to mode \( \text{Ca} \) depend on the rate constants \( \gamma \) (proportional to domain \( [\text{Ca}^{2+}] \)) and \( \omega \). Voltage-dependent transitions are determined by rate constants \( \alpha(V) \) and \( \beta(V) \) (mode normal) and \( \alpha'(V) \) and \( \beta'(V) \) (mode \( \text{Ca} \)). Parameters follow Greenstein and Winslow (2002b), \( \alpha = \alpha_0 \exp(\alpha_1(V - V_0)) \), \( \beta = \beta_0 \exp(\beta_1(V - V_0)) \), \( \alpha' = \alpha a \), \( \beta' = \beta/b \), \( \gamma = \gamma_0c^n \), \( g_+ = 0.85 \text{ ms}^{-1} \), \( g_- = 2 \text{ ms}^{-1} \), \( g'_+ = 0.005 \text{ ms}^{-1} \), \( g'_- = 7 \text{ ms}^{-1} \), \( a_0 = 2.0 \), \( a_1 = 0.0012 \), \( \beta_0 = 0.0882 \), \( \beta_1 = -0.05 \), \( a = 2 \), \( b = 1.9356 \), \( \gamma_0 = 0.44 \text{ mM}^{-1} \text{ ms}^{-1} \), \( \omega = 0.01258 \text{ ms}^{-1} \) and \( V_0 = 35 \text{ mV} \).

Twelve-state LCC model

The LCC model used in this paper was introduced by Jafri et al. (1998a) and reparameterized by Greenstein and Winslow (2002b). In this model, the gating of the LCC is represented by a continuous-time, discrete-state Markov chain with twelve states, ten of which are non-conducting (closed) and two of which are conducting (open). As illustrated in Fig. 4.2, the upper and lower rows of states are \( \text{Ca}^{2+} \)-unbound (mode normal) and \( \text{Ca}^{2+} \)-bound (mode \( \text{Ca} \)), respectively. When in mode \( \text{Ca} \), transitions to the open state \( O_{\text{Ca}} \) are extremely rare, because \( g'_+ \ll g_+ \). Transitions from mode normal to mode \( \text{Ca} \) depend on the rate constant \( \gamma = \gamma_0c^n \), which is a linear function of the domain \( [\text{Ca}^{2+}] \), that is, high \( [\text{Ca}^{2+}] \) induces more transitions to mode \( \text{Ca} \) (more \( \text{Ca}^{2+} \)-dependent inactivation). In both mode normal and mode \( \text{Ca} \), there are five closed states (\( C_0, \ldots, C_4 \) and \( C_{\text{Ca}0}, \ldots, C_{\text{Ca}4} \)) and one open state (\( O \) and \( O_{\text{Ca}} \)). Voltage-dependent transitions are determined by rate constants \( \alpha(V) \) and \( \beta(V) \), which are increasing and decreasing functions of membrane voltage, respectively (see Fig. 4.2, caption).

The transition rates between the 12 states of the LCC model can be written as a 12 x 12 matrix.
infinitesimal generator matrix ($Q$ matrix) that takes the form

$$Q(V, c) = K_\phi(V) + c K_c,$$  \hspace{1cm} (4.2)

where $K_\phi(V)$ includes the Ca$^{2+}$-independent transitions (both voltage-dependent and voltage-independent with units of time$^{-1}$), and $K_c$ collects the association rate constants for the transitions mediated by domain Ca$^{2+}$.

**Population density formulation**

We here present a population density approach to modeling the domain Ca$^{2+}$-mediated inactivation of L-type Ca$^{2+}$ channels that is an alternative to Monte Carlo simulation of the Markov chain model. Assuming a large number ($N$) of domains, we define a continuous univariate probability density function for the domain [Ca$^{2+}$] of a randomly sampled channel,

$$\rho^i(c, t) \, dc = \Pr\{c < \tilde{c}(t) < c + dc \text{ and } \tilde{S}(t) = i\},$$  \hspace{1cm} (4.3)

where the index $i \in \{C_0, C_1, ..., O_{Ca}\}$ runs over the twelve states of the LCC, and the tildes on $\tilde{c}(t)$ and $\tilde{S}(t)$ indicate random quantities. The time-evolution of these joint probability densities is governed by the following system of advection-reaction equations (Bertram and Sherman, 1998; Mazzag et al., 2005; Williams et al., 2007; Huertas and Smith, 2007),

$$\frac{\partial \rho^i}{\partial t} = - \frac{\partial}{\partial c} \left[ f^i \rho^i \right] + [\rho Q]^i,$$  \hspace{1cm} (4.4)

where $i$ is an index over channel states, $Q$ is the generator matrix given by Eq. 4.2, the row-vector $\rho = (\rho^{C_1}, \rho^{C_1}, ..., \rho^{O_{Ca}})$ collects the time-dependent joint probability densities for domain Ca$^{2+}$, and $[\rho Q]^i$ is the $i^{th}$ element of the vector-matrix product $\rho Q$. In Eq. 4.4, the reaction terms $[\rho Q]^i$ account for the probability flux associated with channel state changes. The advection terms of the form $-\partial(f^i \rho^i)/\partial c$ represent the divergence of the probability flux $\phi^i(c, t) = f^i(c) \rho^i(c, t)$ where the advection rate $f^i(c)$ account for the state-dependent
deterministic dynamics of domain $\text{Ca}^{2+}$,

$$f^i = \frac{1}{\lambda_d^T} (\xi^i J_{\text{influx}} - J_{\text{cyt}}), \quad (4.5)$$

where $\lambda_d^T = N\lambda_d$. The flux term $J_{\text{cyt}}$ is total flux from $\text{Ca}^{2+}$ domains to cytosol and is given by $v_{\text{cyt}}^T(c - c_{\text{cyt}})$ where $v_{\text{cyt}}^T = N\nu_{\text{cyt}}$ is the total rate of $\text{Ca}^{2+}$ domain collapse. Let $\tau = \lambda_d^T/v_{\text{cyt}}^T$, $\tau$ can be interpreted as the exponential time constant of domain collapse. A large $\tau$ indicates slow domains and a small $\tau$ indicates fast domains. The total influx term $J_{\text{influx}}$ is linear in domain $[\text{Ca}^{2+}]$ and can be written as $J_{\text{influx}} = J_0 - J_1 c$ where $J_0 = zA_mP^T V_{\text{ext}} e^{-zV/V_e} / [V_0 (1 - e^{-zV/V_e})]$ and $J_1 = zA_mP^T V e^{-zV/V_e} / [V_0 (1 - e^{-zV/V_e})]$ where $P^T = N\nu$ is the total permeability. Consequently, the whole cell $\text{Ca}^{2+}$ current is given by

$$I_{\text{influx}} = \frac{zF}{A_m} \int (-J_0 + J_1 c) (\rho^O + \rho^{O_{\text{Ca}}}) \, dc. \quad (4.6)$$

The time evolution of the joint densities $\rho^i(c,t)$, i.e., the dependent variables of the population density model are found by integrating Eqs. 4.4-4.5 using a total variation diminishing scheme that has been described previously Williams et al. (2007); Huertas and Smith (2006). The most important observable of the model is the probability that a randomly sampled LCC is in a given state,

$$\text{Pr}(S(t) = i) = \int \rho^i(c,t) \, dc, \quad (4.7)$$

where $i \in \{C_0, C_1, ..., O_{\text{Ca}}\}$. Another important observable is the expected $[\text{Ca}^{2+}]$ in a randomly sampled domain,

$$E[c](t) = \sum_i \int c \rho^i(c,t) \, dc. \quad (4.8)$$

The expected $[\text{Ca}^{2+}]$ conditioned on a randomly sampled channel being in state $i$ is

$$E^i[c](t) = E[c|S(t) = i](t) = \frac{\int c \rho^i(c,t) \, dc}{\int \rho^i(c,t) \, dc}. \quad (4.9)$$
Moment-based LCC model

The probability density approach described above is generally fast compared to Monte Carlo simulation, in part because the joint densities are univariate. However, this computational advantage diminishes when an LCC model is complex, because one joint density is required for each state. In this section, we develop a moment-based modeling approach that is computationally more efficient than the population density approach.

We begin by writing the $q^{th}$ moment of the $i^{th}$ joint density as

$$
\mu_q^i(t) = \int c^q \rho^i(c,t) dc.
$$

This expression implies that the zeroth moments $\mu_0^i$ are the time-dependent probabilities that a randomly sampled channels is in state $i$ (Eq. 4.7). The first moments, $\mu_1^i(t) = \int c \rho^i dc$ are related to the expected value of domain $[Ca^{2+}]$ conditioned on channel state though $E^i[c] = \mu_1^i / \mu_0^i$ (cf. Eq. 4.9). The conditional variance in a randomly sampled domain is a function of the first three moments: $\text{Var}^i[c] = \mu_2^i / \mu_0^i - (\mu_1^i / \mu_0^i)^2$.

The derivation of the moment-based LCC model begins by differentiating Eq. 4.10 with respect to time,

$$
\frac{d \mu_q^i}{dt} = \int c^q \frac{\partial \rho^i}{\partial t} dc.
$$

The ODEs of the moment-based model are found by replacing the factor $\partial \rho^i / \partial t$ in the integrand of Eq. 4.11 by the advection-reaction equation of the population density model (Eq. 4.4), which yields

$$
\frac{d \mu_q^i}{dt} = \int c^q \left[ -\frac{\partial (f^i \rho^i)}{\partial c} + [\rho Q]^i \right] dc
$$

$$
= -\int c^q d(f^i \rho^i) + \int c^q [\rho (K_\phi + cK_c)]^i dc.
$$
Integrating by part gives

\[
\frac{d\mu^i}{dt} = q \int f^i e^{\alpha - 1} \rho^j dc + [\mu_q K_{\phi}]^i + [\mu_{q+1} K_c]^i, \tag{4.12}
\]

where we have eliminated boundary terms using the fact that \(\phi^i(c, t) = f^i(c)\rho^i(c, t) = 0\) on the boundary (conservation of probability). We evaluate the first integral of Eq. 4.12 by substituting for \(f^i\) (Eq. 4.5) and simplifying,

\[
q \int f^i e^{\alpha - 1} \rho^j dc = q \left( \frac{\xi^i (\xi^0 - \xi^1 c)}{\lambda_d^T} - \frac{c - c_{\text{cyt}}}{\tau} \right) e^{\alpha - 1} \rho^j dc \\
= q \left( \frac{\xi^i \xi^0}{\lambda_d^T} + \frac{c_{\text{cyt}}}{\tau} \right) \mu^i_{q-1} - q \left( \frac{\xi^i \xi^1}{\lambda_d^T} + \frac{1}{\tau} \right) \mu^i_{q+1}. \tag{4.13}
\]

Finally, substituting Eq. 4.13 into Eq. 4.12 results in the following equation for \(\mu^i_q\),

\[
\frac{d\mu^i_q}{dt} = q \left( \frac{\xi^i \xi^0}{\lambda_d^T} + \frac{c_{\text{cyt}}}{\tau} \right) \mu^i_{q-1} - q \left( \frac{\xi^i \xi^1}{\lambda_d^T} + \frac{1}{\tau} \right) \mu^i_q + [\mu_q K_{\phi}]^i + [\mu_{q+1} K_c]^i. \tag{4.14}
\]

where \(\xi^i = 0\) for \(i \in \{C_0, ..., C_4, C_{c_0}, ..., C_{c_04}\}\) and \(\xi^i = 1\) for \(i \in \{O, O_{c_0}\}\), \(\mu_q = (\mu^0_{c_0}, ..., \mu^0_{c_04})\), \(\mu_{q+1} = (\mu^1_{c_0}, ..., \mu^1_{c_04})\), and \([\mu_q K_{\phi}(V)]^i\) and \([\mu_{q+1} K_c]^i\) are the \(i^{th}\) element of the vector-matrix product of \(\mu_q K_{\phi}(V)\) and \(\mu_{q+1} K_c\), respectively. Note that Eq. 4.14 is an open system of ODEs that takes the form,

\[
\frac{d\mu^i_0}{dt} = f^i_0(\mu_0, \mu_1), \tag{4.15}
\]
\[
\frac{d\mu^i_q}{dt} = f^i_q(\mu_{q-1}, \mu_q, \mu_{q+1}), \quad q = 1, 2, 3, .... \tag{4.16}
\]

In particular, note that the equations for the \(q^{th}\) moments depend on the \((q+1)^{th}\) moments.

**Truncation and closure of moment ODEs**

Eqs. 4.15–4.16 can be closed by assuming the \((q+1)^{th}\) central moment is zero, so that \(\mu^i_{q+1}\) can be expressed as an algebraic function of lower moments. For example, if we assume
that the conditional variance, given by \( \mu_i^2/\mu_0^4 - (\mu_1^i/\mu_0^4)^2 \), is zero for each state \( i \), then the second moments are \( \mu_i^2 = (\mu_1^i)^2/\mu_0^4 \). In this case, Eqs. 4.15-4.16 can be truncated and closed as follows:

\[
\begin{align*}
\frac{d\mu_0^i}{dt} &= f_0^i(\mu_0, \mu_1), \\
\frac{d\mu_1^i}{dt} &= f_1^i(\mu_0, \mu_1, \mu_2(\mu_0, \mu_1)).
\end{align*}
\]

(4.17)

(4.18)

Closing the moment equations in this manner results in two ODEs per channel state—one for the zeroth moment \( \mu_0^i \), and the other one for the first moment \( \mu_1^i \) (24 ODEs in total):

\[
\begin{align*}
\frac{d\mu_0^i}{dt} &= [\mu_0K_c]^i + [\mu_1K_c]^i, \\
\frac{d\mu_1^i}{dt} &= \left( \frac{\xi_{j_0}^i}{\chi_d^i} + \frac{c_{\text{ref}}}{\tau} \right) \mu_0^i - \left( \frac{\xi_{j_1}^i}{\chi_d^i} + \frac{1}{\tau} \right) \mu_1^i + [\mu_1K_c]^i + [\mu_2K_c]^i,
\end{align*}
\]

(4.19)

(4.20)

where \( \mu_2 \) is a row vector with elements \( \mu_2^i = (\mu_1^i)^2/\mu_0^4 \).

Alternatively, we could assume the 3rd central moments are zero. In that case, the truncated and closed moment equations take the form,

\[
\begin{align*}
\frac{d\mu_0^i}{dt} &= f_0^i(\mu_0, \mu_1), \\
\frac{d\mu_1^i}{dt} &= f_1^i(\mu_0, \mu_1, \mu_2), \\
\frac{d\mu_2^i}{dt} &= f_2^i(\mu_1, \mu_2, \mu_3(\mu_0, \mu_1, \mu_2)),
\end{align*}
\]

(4.21)

(4.22)

(4.23)

where

\[
\mu_3^i = \frac{3\mu_0^4\mu_1^i - 2(\mu_1^i)^3}{\mu_0^i},
\]

(4.24)
Table 4.1: Parameters for the population density and moment-based model. See Fig. 4.2 for the parameters of the 12-state L-type Ca^{2+} channel.

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Definition</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F$</td>
<td>Faraday’s constant</td>
<td>coul mol$^{-1}$</td>
<td>96,480</td>
</tr>
<tr>
<td>$R$</td>
<td>gas constant</td>
<td>mJ mol$^{-1}$ K$^{-1}$</td>
<td>8314</td>
</tr>
<tr>
<td>$T$</td>
<td>absolute temperature</td>
<td>K</td>
<td>310</td>
</tr>
<tr>
<td>$V_0$</td>
<td>$RT/F$</td>
<td>mV</td>
<td>26.72</td>
</tr>
<tr>
<td>$P^T$</td>
<td>total permeability / specific capacitance</td>
<td>cm$^3$ s$^{-1}$ µF$^{-1}$</td>
<td>10$^{-4}$</td>
</tr>
<tr>
<td>$C_m$</td>
<td>capacitance</td>
<td>µF</td>
<td>1.534 x 10$^{-4}$</td>
</tr>
<tr>
<td>$A_m$</td>
<td>capacitive to volume ratio</td>
<td>mF L$^{-1}$</td>
<td>356.7</td>
</tr>
<tr>
<td>$\lambda_d^T$</td>
<td>effective volume ratio of domain and cytosol</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td>$c_{ext}$</td>
<td>extracellular Ca$^{2+}$ concentration</td>
<td>mM</td>
<td>2</td>
</tr>
<tr>
<td>$c_{cyt}$</td>
<td>bulk Ca$^{2+}$ concentration</td>
<td>µM</td>
<td>0.1</td>
</tr>
<tr>
<td>$c_{ss}$</td>
<td>maximum Ca$^{2+}$ concentration (Eq. 4.30)</td>
<td>µM</td>
<td>35</td>
</tr>
</tbody>
</table>

This assumption results in a moment-based model that includes 36 ODEs:

\[
\frac{d\mu_0^i}{dt} = [\mu_0 K_{\phi}]^i + [\mu_1 K_c]^i
\]

\[
\frac{d\mu_1^i}{dt} = \left(\frac{\xi_j j_0}{\lambda_d^T} + \frac{c_{cyt}}{\tau}\right) \mu_0^i - \left(\frac{\xi_j j_1}{\lambda_d^T} + \frac{1}{\tau}\right) \mu_1^i + [\mu_1 K_{\phi}]^i + [\mu_2 K_c]^i
\]

\[
\frac{d\mu_2^i}{dt} = 2 \left(\frac{\xi_j j_0}{\lambda_d^T} + \frac{c_{cyt}}{\tau}\right) \mu_1^i - 2 \left(\frac{\xi_j j_1}{\lambda_d^T} + \frac{1}{\tau}\right) \mu_2^i + [\mu_2 K_{\phi}]^i + [\mu_3 K_c]^i
\]

where $\mu_3$ is row vector with elements $\mu_3^i = 3\mu_2^i \mu_1^i / \mu_0^i - 2(\mu_1^i)^3 / (\mu_0^i)^2$.

Below, Eqs. 4.25–4.27 are referred to as the “third-order moment truncation approach” while in the sequel, Eqs. 4.19–4.20 are called the “second-order moment truncation approach.”

## Results

### Representative population density simulation results

To illustrate the population density approach to modeling domain Ca$^{2+}$-mediated inactivation, we first show simulations of a two-pulse voltage clamp protocol, analogous to those used in the experimental quantification of Ca$^{2+}$-inactivation of LCCs Sherman et al. (1990); Plant (1988). As shown in the top panel of Fig. 4.3A, the simulated command voltage began
Figure 4.3: Representative simulation results. (A) The response of the whole cell current (middle panel) and expected [Ca\textsuperscript{2+}] (bottom panel) to the two-pulse voltage clamp protocol (top panel). (B) The peak current (top panel) and the inactivation function (Eq. 4.28, bottom panel) to a range of prepulse potentials (-40 \leq V_p \leq 80 \text{ mV}). Parameters: \( V_h = -40 \text{ mV}, V_t = 0 \text{ mV}, V_p = -40 \text{ to } 80 \text{ mV}, \tau = 10 \text{ ms} \) and as in Fig. 4.2 and Table 4.1.

at the holding potential of \( V_h = -40 \text{ mV} \), and the joint densities of the model equations were equilibrated with this voltage. The command voltage was then stepped up to various prepulse potentials, \( V_p \), and held at \( V_p \) for a prescribed length of time, \( t_p \). The voltage was then stepped back down to the holding potential, \( V_h \), for duration \( t_h \), and then up to the test potential given by \( V_t \). Channel inactivation was measured by estimating the inactivation function, \( h_\infty(V_p) \), defined as the normalized peak current during the test voltage pulse as a function of the prepulse potential (Sherman et al., 1990),

\[
h_\infty(V_p) = \frac{\text{peak}[I(V_p)]}{\text{peak}[I(V_p = V_h)]}. \tag{4.28}
\]

The inactivation function \( h_\infty(V_p) \) gives the fraction of channels that are not inactivated and takes a value between 0 and 1. When \( h_\infty = 1 \), none of the channels are inactivated; when \( h_\infty = 0 \), all of the channels are inactivated.
The middle and bottom panel of Fig. 4.3A show the whole cell \( \text{Ca}^{2+} \) current \( (I_{\text{influx}}) \) and the mean domain \([\text{Ca}^{2+}]\) \( (E(c)) \) during the simulated two-pulse protocol. The largest inward currents during the test phase occurred when the prepulse voltage \( V_p \) was very low or very high (Fig. 4.3B top panel). This is consistent with the observation that during the prepulse phase little current was expressed at extreme voltages, preventing an accumulation of domain \( \text{Ca}^{2+} \) that could potentially inactivate LCCs.

The lower panel of Fig. 4.3B shows the inactivation curve \( h_\infty(V_p) \) calculated via Eq. 4.28. Similar to the peak current, the inactivation function is biphasic with minimal \( \text{Ca}^{2+} \) inactivation \( (h \approx 1) \) when the repulse potential is very low or high, and maximum \( \text{Ca}^{2+} \) inactivation \( (h \approx 0.6) \) for intermediate repulse potentials.

Fig. 4.4A shows the model response to the two-pulse voltage clamp protocol using a range of domain time constants \( (\tau) \). Slower domain time constants (large \( \tau \), purple line) lead to decreased inward whole cell currents during the prepulse phase (compare green and red lines). This is consistent with the observation that a slow domain time constant leads to higher expected domain \([\text{Ca}^{2+}]\) and more \( \text{Ca}^{2+} \) inactivation.

Fig. 4.4B shows the sum of the joint density functions of open states (i.e., \( \rho^O + \rho^O_{\text{Ca}} \)) for three different domain time constants at three different times during the two-pulse protocol (arrows labeled a, b, c in panel A). Note that these densities have been normalized for clarity, so the integrated areas no longer correspond to channel open probability, which is shown as text. Consistent with Fig. 4.4A, the open probability at time \( t = 80 \text{ ms} \) (b) is higher than at times \( t = -50 \) and 750 ms (a and c, respectively) regardless of the domain time constant. When \( \tau \) is small (fast domain), the density functions (red and green shaded regions) are narrow and delta-function-like (small variance). When \( \tau \) is large (slow domain), the densities have greater variance (purple shaded regions).

**Comparison of population density and moment closure approaches**

Fig. 4.5 compares the moment-based model that uses second-order and third-order truncation methods to the corresponding population density and Markov chain model. The
Figure 4.4: (A) Command voltage traces, Ca\(^{2+}\) current and expectation [Ca\(^{2+}\)] when domain time constant \(\tau\) is varied. (B) Snapshot of the sum of the joint densities for open states, \(p^O + p^{OCa}\), at three different times (a, b, c) and three domain time constants. Parameters: \(\tau = 1\) ms (red), 10 ms (green) and 100 ms (purple), times a, b and c are shown as arrows at -50, 80 and 750 ms, in (A), \(V_h = -40\) mV, \(V_p = 20\) mV, \(V_i = 0\) mV and as in Fig. 4.2 and Table 4.1.

agreement between the Markov chain (o) and the population density simulation (+ and x) validates our model. When \(\tau\) is fast or intermediate (e.g., \(\tau = 10\) ms), the assumption of zero variance (green) leads to nearly the same result as the population density model (+ symbols). However, when \(\tau\) is slow (e.g., \(\tau = 1\) s), the result computed from the second-order moment truncation approach (khaki) deviates slightly different from the population density model (x symbols). As might be expected, this small error is eliminated using the third-order moment truncation approach (purple). Moment-based calculations in the remainder of the chapter will utilize the third-order truncation method, which accurately approximate the population density model for domain time constants in the physiological range (\(\tau = 0.1\) ms to 1 s).

In general, the runtime of the population density model is fast when \(\tau\) is large and slow when \(\tau\) is small. However, even when \(\tau = 1\) s and \(V = 0\) mV, the population density model takes 79 s on average to simulate a 2 s trail (10 trails in total), while the mean simulation
Steady-state Ca\textsuperscript{2+}-inactivation and the domain time constant

When an LCC is open, the time-dependence of domain [Ca\textsuperscript{2+}] can be rewritten as

\[
\frac{dc}{dt} = \frac{1}{\lambda_d} \left( j_0 - j_{1c} \right) - \frac{c - c_{\text{cyt}}}{\tau},
\]

(4.29)
Figure 4.6: Comparison of steady-state probabilities of L-type channels states when the domain time constant τ is varied. The fraction of channel in closed states of mode normal (P_{\text{normal}}^C, A) and mode Ca (P_{\text{Ca}}^C, B), and the fraction of channels in open state of mode normal (P_{\text{normal}}^O, C) and mode Ca (P_{\text{Ca}}^O, D), as a function of V_m. The khaki, blue and purple lines are the simulation results of the moment-based model when τ = 0.1 ms, 10 ms and 1 s, respectively. The corresponding population density simulation results are given by open circles. Parameters as in Fig. 4.2 and Table 4.1.

where j_0 and j_1 are defined above. From Eq. 4.29, it is straightforward to derive the steady state domain [Ca^{2+}] for an open LCC,

\[ c_{ss} = \frac{j_0/\lambda_d^T + c_{cys}/\tau}{j_1/\lambda_d^T + 1/\tau}. \]  

(4.30)

The concentration \( c_{ss} \) is the maximum [Ca^{2+}] that can be achieved in a domain, its value depends on membrane voltage, the domain time constant τ and the total permeability \( P^T \), where \( V \) and \( P^T \) occur as parameters in \( j_0 \) and \( j_1 \). In this section, we investigated in how the domain time constant influences steady-state Ca^{2+}-inactivation under the assumption of fixed total permeability. In the following section, we considered the related question of the domain time constant’s impact on steady-state Ca^{2+} inactivation when LCC permeability is adjusted so that the steady-state domain [Ca^{2+}] (\( c_{ss} \)) is fixed.
Figure 4.7: Steady-state Ca\(^{2+}\)-inactivation and domain time constant \(\tau\) with fixed \(P^T\). Ca\(^{2+}\)-inactivation function \(h_\infty\) (A), Ca\(^{2+}\) influx current \(I_{influx}\) (B), expected \([Ca^{2+}]\) (E(c)) (C) and the variance of \([Ca^{2+}]\) in different domains \((V_\text{ar}(c))\) (D) calculated via the moment-based model as a function of \(V\). The corresponding population density simulation results are given by open circles. Parameters: \(\tau = 0.1\) ms (khaki), 10 ms (blue) and 1 s (purple) and others as Fig. 4.2 and Table 4.1.

Fig. 4.6 shows how the domain time constant \((\tau)\) influences the voltage-dependence of the steady-state Ca\(^{2+}\)-dependent inactivation of LCCs in the population density and moment-based models. For each domain time constant and voltage, the steady-state fraction of LCCs in four lumped states are shown, namely, mode normal open \((P^O_\text{normal})\), mode Ca open \((P^O_{Ca})\), mode normal closed \((P^C_\text{normal})\), including contributions from states \(C_0, ..., C_4\), and mode Ca closed \((P^C_{Ca}, \text{states } C_{Ca0}, ..., C_{Ca4})\). For all domain time constants studied, increasing the voltage leads to increased steady-state open probabilities \((P^O_\text{normal} + P^O_{Ca})\).

Slowing the domain time constant increases the probability that a randomly sampled channel is in mode Ca \((P^C_{Ca} + P^O_{Ca})\) regardless of voltage, consistent with our prior observation that slower domain time constants result in higher domain \([Ca^{2+}]\) (Fig. 4.7C) and decreased open probability \((P^O_\text{normal} + P^O_{Ca})\).

Fig. 4.7A shows the inactivation function \((h_\infty)\) at steady state when \(\tau\) is varied from
Figure 4.8: Steady-state of Ca\textsuperscript{2+}-inactivation and domain time constant $\tau$ with $c_{ss}$ and voltage fixed. $h_\infty$, $I_{influx}$, $E^O(c)$ and $Var(c)$ calculated via the moment-based model as a function of the maximum domain [Ca\textsuperscript{2+}], $c_{ss}$. The corresponding population density simulations are given by open circles. Parameters: $\tau = 10$ ms (blue), 100 ms (red) and 1 s (purple line), $V = -10$ mV, and others as Fig. 4.2 and Table 4.1.

0.1 ms to 1 s. As the domain time constant $\tau$ increases, the inactivation function shifts downwards, corresponding to increased Ca\textsuperscript{2+} channel inactivation. This results from residual Ca\textsuperscript{2+} lingering in the domain, increasing the expected [Ca\textsuperscript{2+}] (Fig. 4.7C). Although the expected domain [Ca\textsuperscript{2+}] increases with $\tau$, the total Ca\textsuperscript{2+} current decreases (Fig. 4.7B) due to decreased open probability. Fig. 4.7D shows that the domain Ca\textsuperscript{2+} concentrations are more heterogeneous (higher variance) with slow domain collapse time regardless of voltage. This is consistent with Fig. 4.4 where small $\tau$ results in narrow distribution and low variance and large $\tau$ yields broader distribution and higher variance.

Ca\textsuperscript{2+}-inactivation when maximum [Ca\textsuperscript{2+}] is fixed

In the parameter studies of Fig. 4.6 and 4.7, the permeability $P^T$ was held constant as the domain time constant $\tau$ was varied. Structuring the parameter study in this manner allows $\tau$ to influence the domain dynamics by changing the rate of domain formation and collapse
as well as the steady-state domain \([\text{Ca}^{2+}]\), given by \(c_{ss} = \left( j_0 / \lambda_d^T + c_{cyt}/\tau \right) / \left( j_1 / \lambda_d^T + 1/\tau \right)\).

Fig. 4.8 presents an alternative parameter study that controls for the effect of the domain time constant on the steady state domain \([\text{Ca}^{2+}]\), thereby highlighting the manner in which the rate of domain formation and collapse influences \(\text{Ca}^{2+}\)-mediated inactivation of LCCs.

Fig. 4.8 shows that for a given voltage and domain time constant \(\tau\), increasing the permeability of the channel (and thus \(c_{ss}\), the maximum domain \([\text{Ca}^{2+}]\) that can be achieved) leads to an increase in \(\text{Ca}^{2+}\)-mediated inactivation (decreased \(h_\infty\)). On the other hand, when the permeability is adjusted so that the maximum domain \([\text{Ca}^{2+}]\) is fixed decreasing \(\tau\) (faster domain) increases both the expected domain \([\text{Ca}^{2+}]\) at open state (Fig. 4.8C) and \(\text{Ca}^{2+}\)-dependent inactivation (Fig. 4.8A). When \(c_{ss}\) is fixed, a slower domain leads to smaller variance, i.e., \(\text{Ca}^{2+}\) channels in different domains are likely to experience similar \([\text{Ca}^{2+}]\) (Fig. 4.8D).

4.2 Discussion

4.2.1 Summary of results

In this chapter, we have shown how a population density approach (Eq. 4.4) to modeling \(\text{Ca}^{2+}\)-mediated inactivation of L-type \(\text{Ca}^{2+}\) channels is an extension of (and improvement upon) biophysical theory that assumes that domain \([\text{Ca}^{2+}]\) is proportional to single channel current (recall Fig. 4.1). The population density approach is similar to traditional domain models of \(\text{Ca}^{2+}\)-mediated inactivation (Sherman et al., 1990) in that both assume a large number of low-density \(\text{Ca}^{2+}\) channels and a minimally represent action of the heterogeneity of domain \([\text{Ca}^{2+}]\)—a potentially important feature of \(\text{Ca}^{2+}\)-mediated inactivation that is not captured by common pool models.

However, the population density approach is distinct from traditional multiscale models of \(\text{Ca}^{2+}\)-inactivation in its representation of the time-dependent formation and collapse of \(\text{Ca}^{2+}\) domains associated with L-type channels. Similar to previous work focused on local control of excitation-contraction coupling in cardiac myocytes (Williams et al., 2007),
the population density approach to modeling Ca\textsuperscript{2+} inactivation of L-type channels is often preferable to Monte Carlo simulation of the stochastic dynamics of channels and domains. This is due to the fact that the computational efficiency of a population density model scales with the number of states in the Markov chain model of the L-type channel, as opposed to the (far greater) number of channels present in the plasma membrane of the cell. Traditional equilibrium domain models also have this advantage, but do not account for the dynamics of domain formation and collapse that may in some cases influence the kinetics of Ca\textsuperscript{2+} inactivation (Mazzag et al., 2005; Bertram and Sherman, 1998).

The population density formalism allows the derivation of moment-based models of domain Ca\textsuperscript{2+} inactivation that are extremely computationally efficient. We have derived two different moment-based models that are distinguished by the number of ODEs per channel state retained after truncation of the open system of moment equations as well as the assumptions made to close the moment equations. Both the second-order (Eqs. 4.19–4.20, zero variance) and third-order (Eqs. 4.25–4.27, zero third central moment) moment-based models performed well when validated by comparison to corresponding population density simulations, but the third-order moment-based model was extremely accurate and valid for a wider range of domain time constants (Fig. 4.5). The second-order moment-based model is most accurate when the domain time constant is relatively small (fast domain, $\tau < 100$ ms), because in that case the joint distributions for domain [Ca\textsuperscript{2+}] conditioned on channel state are very focused (low variance, recall Fig. 4.5).

Using both the population density and moment-based models, we investigated the dependence of the steady-state inactivation of the 12-state L-type Ca\textsuperscript{2+} channel model (Greenstein and Winslow, 2002b) on the exponential time constant ($\tau$) for domain formation and collapse. When the study was performed using a fixed permeability for the L-type channel, faster domains (smaller $\tau$) leads to less inactivation for a wide range of clamped voltages. When the channel permeability is chosen to be a function of $\tau$ that results in a fixed maximum domain [Ca\textsuperscript{2+}], a smaller domain time constant leads to increased Ca\textsuperscript{2+}-mediated inactivation, presumably because the kinetics of domain formation subsequent to channel
opening are more rapid.

4.2.2 Limitations and possible extensions

Although the computational efficiency of the probability density and moment-based calculations is notable, the runtimes of both models are proportional to the number of states in a given L-type channel model. Consequently, both methods may have little computational advantage if the LCC model of interest is extremely complex. In addition, the efficiency of the probability density approach is dependent upon the number of meshpoints used in solving the advection-reaction equations. For simplicity, we have illustrated the population density and moment-based models under the assumption that plasma membrane fluxes do not change the bulk cytosolic [Ca$^{2+}$] (that is, $c_{cyt}$ is clamped). However, it is straightforward to relax this assumption and thereby allow a dynamic bulk intercellular [Ca$^{2+}$]. For example, assuming the rate of ATP-dependent plasma membrane Ca$^{2+}$ efflux is given by $J_{out} = k_{out} c_{cyt}$, the ODE for bulk cytosolic Ca$^{2+}$ is

$$\frac{dc_{cyt}}{dt} = J_{cyt}^* - J_{out},$$

(4.31)

where $J_{cyt}^*$ is the total flux from domains to cytosol,

$$J_{cyt}^*(t) = \sum_i \int J_{cyt} \rho^i(c, t) \, dc$$

$$= \tau^{-1} \sum_i \int [c - c_{cyt}(t)] \rho^i(c, t) \, dc$$

$$= \tau^{-1} \left[ \sum_i \mu^i_1 - c_{cyt}(t) \right].$$

In spite of the fact that we have chosen to illustrate the population density and moment-based models through simulated voltage clamp recordings, the modeling formalism is easily modified to simulate current clamp recordings.
Chapter 5

Epilogue

5.1 Summary of results

Discrete-state continuous-time Markov chain models of Ca\(^{2+}\) release sites reminiscent the physiological realism of Ca\(^{2+}\) channels. However, large number of Ca\(^{2+}\) channel states can impede the simulations. This dissertation proposes several novel modeling approach to describe stochastic Ca\(^{2+}\) release. These modeling approaches facilitate our investigation on certain important aspects of Ca\(^{2+}\) signaling that are otherwise computationally expensive for the corresponding Markov chain model.

Chapter 2 presents a Ca\(^{2+}\) release site modeling approach based on a Langevin description of stochastic Ca\(^{2+}\) release. By comparing the cumulative probability distributions of spark amplitude, duration and inter-event interval, we show that the Langevin description of individual CaRU agree with the corresponding Markov chain description in a wide range of channels. Importantly, this Langevin modeling approach facilitates our investigation of correlations between successive puff/spark amplitudes and inter-spark intervals, and how such puff/spark statistics depend on the number of channels per release site and the kinetics of Ca\(^{2+}\)-mediated inactivation of open channels.

The results in Chapter 2 demonstrate that when Ca\(^{2+}\) inactivation/de-inactivation rates are intermediate, i.e., the Ca\(^{2+}\) puff/spark is terminated by the recruitment of inactivated
channels, the correlation between successive puff/spark amplitudes is negative, while the correlations between puff/spark amplitudes and the duration of the preceding or subsequent inter-spark interval are positive. This is because sparks of large amplitude are terminated with a relatively high fraction of channels in the refractory state, therefore there are less channels available to participate for the next release event. A long inter-event interval provides a longer time for inactivated channels to be de-inactivated, thus the subsequent Ca$^{2+}$ release event is more likely to have large spark amplitude. Similarly, if the current spark amplitude is large, the subsequent inter-event interval is more likely to be longer due to Ca$^{2+}$-mediated inactivation.

These correlations are significantly reduced when inactivation/de-inactivation rates are extreme (slow or fast), that is, when puff/sparks terminate via stochastic attrition. When the inactivation/de-inactivation rates are extremely slow, the number of channels in the refractory state is essentially a constant and consequently, the CaRU composed of $N$ three-state channels effectively reduces to a collection of $N - N_R$ two-state channels. In this case, the mechanism that may generate the positive/negative correlations is no longer operative, because the Ca$^{2+}$ spark amplitude does not affect the number of channels in the refractory state at puff/spark termination. On the other hand, when inactivation/de-inactivation rates are extremely fast, the spark amplitude does not influence $N_R$ at spark termination because $N_R$ is in quasistatic equilibrium with $N_O$.

Chapter 3 presents a novel local/global whole cell model of Ca$^{2+}$ homeostasis based on a Langevin description of stochastic Ca$^{2+}$ release that includes both spark-mediated and non-spark-mediated release dynamics. The Fokker-Planck equation associated with the Langevin formulation of stochastic Ca$^{2+}$ release is coupled to balance equations for the bulk myoplasmic and network SR [Ca$^{2+}$]. Using this approximate representation of the collective dynamics of a large number of identical CaRUs, this whole cell modeling approach avoids Monte Carlo simulation of a large population of CaRUs and facilitates our study of Ca$^{2+}$ homeostasis in permeabilized ventricular myocytes.

We utilize the Langevin and Fokker-Planck local/global whole cell model to study
network SR [Ca\textsuperscript{2+}] depletion that occurs via both spark-mediated release and non-spark-mediated release, as well as the dependency of SR Ca\textsuperscript{2+} load on myoplasmic [Ca\textsuperscript{2+}]. In agreement with recent experimental work Bovo et al. (2011), we find that spark-mediated release increases exponentially as myoplasmic [Ca\textsuperscript{2+}] increases, while non-spark-mediated release increases linearly. Moreover, the model predicts the presence of two distinct stable steady-states that lead to the same SR Ca\textsuperscript{2+} load—one with low myoplasmic [Ca\textsuperscript{2+}] and predominantly non-spark-mediated SR Ca\textsuperscript{2+} release and another with high myoplasmic [Ca\textsuperscript{2+}] and release that is primarily spark-mediated. Interestingly, in our permeabilized ventricular myocyte model, for any clamped myoplasmic [Ca\textsuperscript{2+}] that is large enough to trigger spark-mediated release, the resulting spontaneous stochastic Ca\textsuperscript{2+} release tends to decrease the network SR Ca\textsuperscript{2+} load just enough to maintain robust Ca\textsuperscript{2+} sparks.

Chapter 4 first presents a population density approach to modeling Ca\textsuperscript{2+}-mediated inactivation of L-type Ca\textsuperscript{2+} channels that is an extension of (and improvement upon) biophysical theory that assumes that domain [Ca\textsuperscript{2+}] is proportional to single channel current. This modeling approach assume a large number of low-density Ca\textsuperscript{2+} channels and a minimally represent action of the heterogeneity of domain [Ca\textsuperscript{2+}]. Importantly, the population density formalism allows the derivation of moment-based models of domain Ca\textsuperscript{2+} inactivation that are extremely computationally efficient. We show two different moment-based models that are distinguished by the number of ODEs per channel state retained after truncation of the open system of moment equations as well as the assumptions made to close the moment equations. Both the second-order and third-order moment-based models performed well when validated by comparison to corresponding population density simulations, but the third-order moment-based model was extremely accurate and valid for a wider range of domain time constants.

Using both the population density and moment-based models, we investigated the dependence of the steady-state inactivation of the 12-state L-type Ca\textsuperscript{2+} channel model on the exponential time constant for domain formation and collapse. When the study was performed using a fixed permeability for the L-type channel, faster domains leads to less
inactivation for a wide range of clamped voltages. This is because slow domain results in more Ca\textsuperscript{2+} accumulation which inactivates the L-type Ca\textsuperscript{2+} channel. On the other hand, when the channel permeability is chosen to be a function of domain time constant that results in a fixed maximum domain [Ca\textsuperscript{2+}], a smaller domain time constant leads to increased Ca\textsuperscript{2+}-mediated inactivation. This is because the domain time constant is negative correlated to the membrane permeability, fast domain results in a high membrane permeability which promotes Ca\textsuperscript{2+} influx and inactivates the L-type Ca\textsuperscript{2+} channel.

5.2 Direction of future work

5.2.1 Incorporating the Langevin/Fokker-Planck model into spatial whole cell models

The Langevin and Fokker-Planck descriptions of the local/global whole cell model presented in Chapter 3 are not explicitly spatial. That is, a large population of CaRUs are assumed to influence one another indirectly via the spatially-averaged bulk myoplasmic and network SR [Ca\textsuperscript{2+}] (the CaRUs are mean-field coupled). This form of the local/global model is not well-suited to investigate macrosparks and other explicitly spatial phenomena that might occur in permeabilized ventricular myocytes when myoplasmic [Ca\textsuperscript{2+}] is very high. By partitioning (discretizing) the bulk myoplasm and SR into regions that interact via buffered Ca\textsuperscript{2+} diffusion, the formalism would allow for propagation of intercellular Ca\textsuperscript{2+} waves, subcellular alternans, and so on. Such extensions of the Langevin local/global model approach would be straightforward and robust. Extending the Fokker-Planck local/global model in this way require a discretization sufficiently coarse that number of CaRUs per sub-compartment remains large.
5.2.2 Calcium release sites with varied number of channels

Because recent studies have shown that the number of RyRs per CaRU is variable (Baddeley et al., 2009), the local/global whole cell model presented in Chapter 3 can be modified to account for CaRUs of different size by simultaneously solving multiple Fokker-Planck equations, each with a different value for N. Assuming \( M = \sum_i M_i \) CaRUs, with CaRU's of type \( i \) composed of \( N_i \) RyRs, the population densities \( \rho_i \) solve

\[
\frac{\partial \rho_i}{\partial t} = -\frac{\partial}{\partial f} [\alpha_i \rho_i] + \frac{1}{2} \frac{\partial^2}{\partial f^2} [\gamma_i \rho_i],
\]

where \( \alpha_i = \nu_i^+ - \nu^- \), \( \gamma_i = (\nu_i^+ + \nu^-)/N \), \( \nu_i^+ = k^+(\bar{c}_{ds}^i)^\eta (1 - f) \) and \( \nu^- = k^- f \). The stochastic Ca\(^{2+}\) release flux (Eq. 3.27) becomes

\[
J_{nsr}^T(t) = \frac{1}{M} \sum_i M_i \int v_{nsr}^T \left( c_{nsr} - \bar{c}_{jsr}^i \right) \rho_i(f, t) df
\]

\[
= v_{nsr}^T \left( c_{nsr} - \frac{1}{M} \sum_i M_i \int \bar{c}_{jsr}^i \rho_i(f, t) df \right)
\]

where \( \int \rho_i df = 1 \) and thus \( M^{-1} \sum_i M_i \rho_i df = 1 \). In these equations, \( \bar{c}_{ds}^i(f) \) and \( \bar{c}_{jsr}^i(f) \) are given by indexed versions of Eqs. 3.32 and 3.33 where \( \bar{v}_{rel}^i = v_{rel}^0 N_i f \) and \( v_{rel}^0 \) is analogous to the RyR unitary conductance. Writing \( v_{myo}^i \) and \( v_{nsr}^i \) as the domain time constants for a representative of the \( i \)th class of CaRU, \( \chi_{myo}^i \) and \( \chi_{nsr}^i \) are given by Eqs. 3.34–3.37 upon replacement of \( i \) for \( T \). The Fokker-Planck equations are coupled, because \( \alpha_i \) is a function of \( c_{nsr} \) through \( \bar{c}_{ds}^i \), and \( dc_{nsr}/dt \) depends on the \( \rho_i \) through \( J_{nsr}^T \) (Eq. 3.32).

5.2.3 Modeling calcium-dependent potassium channels

The population density approach presented in Chapter 4 is well-suited to investigate whole-cell potassium currents that arise through voltage- and Ca\(^{2+}\)-dependent stochastic gating of SK and BK channels, both of which play important physiological roles in the heart, brain and muscle cells and are often spatially co-localized with L-type Ca\(^{2+}\) channels.
(Vandael et al., 2010; Vergara et al., 1988; Qi et al., 2014; Hammond et al., 2006; Pribnow et al., 1999). Previous work by Stanley et al. (2011) has shown that the stochastic gating of Ca$^{2+}$ channels increases the activation of SK channels. Cox recently presented a Ca$\nu$2.1/BK$\text{Ca}$ model and that suggested that Ca$^{2+}$ channels will open during a typical cortical neuron action potential, while the associated BK$\text{Ca}$ channel opens in only 30% of trials (Cox, 2014). Furthermore, this percentage is sensitive to the action potential duration, the distance between the two channels in the signaling complex, and the concentration of intercellular Ca$^{2+}$ buffers (Cox, 2014). Extensions of the population density and moment-based model that account for the dynamic of Ca$^{2+}$ buffering and the geometric relationship between channels is an important avenue for future research.

The examples given above provide some promising directions and confirm that the theoretical models of stochastic Ca$^{2+}$ release presented in this manuscript are essential. They help clarify the connection between channels gating to local Ca$^{2+}$ release and Ca$^{2+}$ homeostasis. They also establish a foundation on which whole cell model Ca$^{2+}$ release can be built.


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