Reduction of calcium release site models via moment fitting of phase-type distributions

M Drew LaMar\textsuperscript{1}, Peter Kemper\textsuperscript{2} and Gregory D Smith\textsuperscript{1}

\textsuperscript{1} Department of Applied Science, The College of William and Mary, Williamsburg, VA 23187, USA
\textsuperscript{2} Department of Computer Science, The College of William and Mary, Williamsburg, VA 23187, USA
E-mail: mdlama@wm.edu

Received 19 October 2010
Accepted for publication 11 March 2011
Published 6 April 2011
Online at stacks.iop.org/PhysBio/8/026015

Abstract
Models of calcium (Ca\textsuperscript{2+}) release sites derived from continuous-time Markov chain (CTMC) models of intracellular Ca\textsuperscript{2+} channels exhibit collective gating reminiscent of the experimentally observed phenomenon of Ca\textsuperscript{2+} puffs and sparks. In order to overcome the state-space explosion that occurs in compositionally defined Ca\textsuperscript{2+} release site models, we have implemented an automated procedure for model reduction that replaces aggregated states of the full release site model with much simpler CTMCs that have similar within-group phase-type sojourn times and inter-group transitions. Error analysis based on comparison of full and reduced models validates the method when applied to release site models composed of 20 three-state channels that are both activated and inactivated by Ca\textsuperscript{2+}. Although inspired by existing techniques for fitting moments of phase-type distributions, the automated reduction method for compositional Ca\textsuperscript{2+} release site models is unique in several respects and novel in this biophysical context.

1. Introduction
Cell signal transduction is often mediated by molecular assemblies composed of multiple interacting transmembrane receptors \cite{1, 2}. For example, in coliform bacteria, the high sensitivity of lattices of plasma membrane proteins to chemoattractants is due to allosteric protein–protein interactions leading to conformational spread through the receptor array \cite{3}. In rat basophilic leukemia (RBL) cells, cross-linking of immunoglobulin E receptors (F\textsubscript{cR1}) with multivalent antigen leads to tyrosine kinase-dependent activation of phospholipase C\textsubscript{\gamma}, production of inositol 1,4,5-trisphosphate (IP\textsubscript{3}), release of Ca\textsuperscript{2+} from intracellular stores, clustering of IP\textsubscript{3} receptor Ca\textsuperscript{2+} channels (IP\textsubscript{3}Rs), sustained Ca\textsuperscript{2+} influx, and secretion of histamine and other mediators of inflammation \cite{4, 5}. Systems biologists seek principles that govern the emergent behavior of signaling complexes and a robust conceptual framework for biological modeling of these molecular assemblies.

Signaling complexes are not restricted to the plasma membrane (PM), but are also found on mitochondrial and nuclear membranes and on the endoplasmic (ER) or sarcoplasmic (SR) reticulum \cite{6–8}. Clusters of 5–50 IP\textsubscript{3}Rs on both the cortical ER \cite{9, 10} and outer nuclear membrane \cite{11} of immature \textit{Xenopus laevis} oocytes exhibit coordinated gating that gives rise to spatially localized Ca\textsuperscript{2+} elevations known as ‘Ca\textsuperscript{2+} puffs.’ During cardiac excitation–contraction coupling, PM depolarization leads to Ca\textsuperscript{2+} influx via L-type Ca\textsuperscript{2+} channels that triggers ‘Ca\textsuperscript{2+} sparks,’ i.e. the release of SR Ca\textsuperscript{2+} from two-dimensional arrays of ryanodine receptors (RyRs).

While there is consensus that Ca\textsuperscript{2+} regulation of IP\textsubscript{3}Rs and RyRs, multiple cytoplasmic and luminal accessory proteins, and allosteric protein–protein interactions generate the concerted activity of Ca\textsuperscript{2+} release sites \cite{12–14}, the biophysical theory relating single channel kinetics of intracellular Ca\textsuperscript{2+} channels to the emergent dynamics of Ca\textsuperscript{2+} puffs and sparks is in development \cite{15–22}. Ca\textsuperscript{2+} signaling complexes can be idealized as stochastic automata networks (SANs) that involve many functional transitions whose rates depend on the state of other channels in the release site \cite{16}. This promising theoretical perspective combines Markov
chain modeling of the stochastic gating of intracellular Ca2+ channels with an idealized representation of the coupling of channels via allosteric interactions and/or localized elevations in intracellular Ca2+. However, the compositional nature of Ca2+ signaling complexes and the resulting combinatorial state-space explosion leads to significant challenges for the analysis and reduction of SAN models [23, 24]. This paper focuses on the automated reduction of compositionally defined Ca2+ signaling complexes, an important step toward mechanistic modeling of Ca2+ dynamics that traverses the molecular and cellular levels of biological organization.

1.1. Markov chain models of single channel gating

The stochastic gating of voltage- and ligand-gated ion channels—experimentally observed using single channel recording techniques—is often modeled using discrete-state continuous-time Markov chains (CTMCs) [25–27]. The topology of Markov chain models of single channel gating can be summarized by a diagram that indicates the possible states a channel may occupy and allowed transitions between these states. For example, the state–transition diagram for a minimal model of a Ca2+-regulated intracellular Ca2+ channel,

$$\begin{align*}
C &\to O \to R,
\end{align*}$$

includes one open state O and two closed states: a short-lived closed state C and a long-lived closed (i.e. refractory) state R. The C → O and O → R transitions correspond to Ca2+-mediated activation and inactivation of the channel, respectively. In this state–transition diagram, $k_{co}([\text{Ca}^{2+}])^2$, $k_{oc}$, $k_{or}$, and $k_{ro}$ are transition rates with units of $1$/time of a transition from state i to state j, and $[\text{Ca}^{2+}]$ is the local concentration of Ca2+ experienced by the channel. For simplicity, this model does not explicitly account for the homotetrameric structure of individual IP3Rs and RyRs and it is assumed that the cooperativity of Ca2+ binding is the same for the activation and inactivation processes.

While models for intracellular Ca2+ channels such as the IP3R and the RyR can be significantly more complicated [28–30], they often can be written in the form

$$Q([\text{Ca}^{2+}]) = K_+ + ([\text{Ca}^{2+}])^2K_-, \quad (2)$$

where $K_+$ is a square matrix of bimolecular (Ca2+-dependent) transition rates, and $K_-$ collects the unimolecular, Ca2+-independent transition rates [16]. For example, the $K_-$ and $K_+$ matrices corresponding to the three-state model in (1) are

$K_+ = \begin{pmatrix} -k_{co} & k_{co} & 0 \\ 0 & -k_{oc} & k_{or} \\ 0 & 0 & 0 \end{pmatrix}$

and

$K_- = \begin{pmatrix} 0 & 0 & 0 \\ k_{oc} & -k_{oc} & 0 \\ 0 & k_{ro} & -k_{ro} \end{pmatrix}$.

where the rows and columns of each matrix correspond to the states C, O, and R (in that order). Because the cooperativity of Ca2+ binding is $\eta = 2$ in (1), the $Q$-matrix for the three-state channel in (2) is

$$Q(c) = \begin{pmatrix} -k_{co}([\text{Ca}^{2+}])^2 & k_{co}([\text{Ca}^{2+}])^2 & 0 \\ k_{oc} & -k_{oc} - k_{or}([\text{Ca}^{2+}])^2 & k_{or}([\text{Ca}^{2+}])^2 \\ 0 & k_{ro} & -k_{ro} \end{pmatrix}. \quad (3)$$

In the general case, the matrix $Q = (q_{ij})$ is referred to as the infinitesimal generator matrix (or $Q$-matrix) for the CTMC $X(t)$ that takes integer values between 1 and $M$ (the number of states). The off-diagonal elements of $Q$ give the probability per unit time of a transition from state $i$ to state $j$,

$$q_{ij} = \lim_{\delta t \to 0} P\{X(t + \delta t) = j|X(t) = i\}/\delta t \quad (i \neq j), \quad (4)$$

while the absolute value of the diagonal elements correspond to the probability per unit time of a transition out of each state,

$$|q_{ii}| = \lim_{\delta t \to 0} P\{X(t + \delta t) \neq i|X(t) = i\}/\delta t. \quad (5)$$

If we write the probability distribution over channel states as a row vector, e.g., $\pi(t) = (\pi_0, \pi_O, \pi_R)$, then the time evolution of this distribution can be found by solving the ordinary differential equation system

$$\frac{d\pi}{dt} = \pi Q, \quad (6)$$

where $\pi(0)$ is an initial distribution satisfying $\sum \pi_i = 1$, that is, $\pi e = 1$ where e is a commensurate column vector of ones. Equations (4) and (5) imply that the diagonal entries of $Q$ are the opposite of the sum of the off-diagonal entries in the same row, that is, $q_{ii} = -\sum_{j \neq i} q_{ij}$ (cf (3)). This condition ensures conservation of probability, as can be seen by multiplying (6) on the right by e to give $d(\pi e)/dt = \pi Q e = 0$. For further review of Markov chains from a mathematical perspective see [31].

Markov chain models of single channel gating have a finite number of states and are irreducible, that is, it is possible to move between any two states via one or more transitions. The limiting probability distribution of such Markov chains (found by integrating (6) for a long time) does not depend on the initial condition $\pi(0)$ and is equal to the unique stationary distribution $\bar{\pi}$ satisfying

$$\bar{\pi} Q = 0 \quad \text{subject to} \quad \bar{\pi} e = 1. \quad (7)$$

For review of numerical methods for calculating the stationary distribution of Markov chains see [32].

For the three-state model in (1), an analytical expression for the stationary distribution is easily found. In particular, the open probability for this single channel model is

$$\pi_{CO} = \frac{(\text{Ca}^{2+})^2 K_{inact}}{K_{act} K_{inact} + (\text{Ca}^{2+})^2 K_{inact} + (\text{Ca}^{2+})^4},$$

where $K_{act} = \sqrt{k_{oc}/k_{co}}$ and $K_{inact} = \sqrt{k_{ro}/k_{or}}$ are dissociation constants for Ca2+ activation and inactivation, respectively. The standard parameter values of $K_{act} = 0.58 \mu M$ and $K_{inact} = 0.76 \mu M$ result in a single channel open probability that is a bell-shaped function of $[\text{Ca}^{2+}]$, consistent with experimental observations of intracellular Ca2+ channels such as the IP3R and the RyR.
1.2. Compositional Ca\textsuperscript{2+} release site models

Clustered IP\textsubscript{3}Rs and RyRs participate in forming a dynamic Ca\textsuperscript{2+} microdomain that influences the local [Ca\textsuperscript{2+}]\textsubscript{cyt} experienced by each channel [33, 34]. Assuming that the dwell times of channel states are long compared to the time scale of local [Ca\textsuperscript{2+}] changes, the microdomain [Ca\textsuperscript{2+}] profile can be calculated from the position and source amplitude of open channels using well-known equations for the buffered diffusion of Ca\textsuperscript{2+} [35, 36]. The coupling of Ca\textsuperscript{2+}-regulated channels that are distinguishable due to their spatial location can lead to release site models with large state spaces. For example, a release site composed of 20 instantaneously coupled three-state channels has \(3^{20} \approx 3.5 \times 10^9\) states. Due to this combinatorial state-space explosion, Ca\textsuperscript{2+} release site models are often constructed under the assumption of mean-field coupling [16, 37]. In the simplest case, each channel is assumed to experience a domain [Ca\textsuperscript{2+}] that is an algebraic function of the number of open channels at the release site. For example,

\[
[\text{Ca}\textsuperscript{2+}] (t) = \begin{cases} 
 c_\infty + c_o C_o (t), & \text{when channel closed,} \\
 c_\infty + c_o (C_o (t) - 1) + c_d, & \text{when channel open,}
\end{cases}
\]

where \(c_\infty\) is the background cytosolic [Ca\textsuperscript{2+}], \(c_o\) is the coupling strength and \(c_d\) is the substantially elevated [Ca\textsuperscript{2+}] that the channel experiences when open. Prior work has investigated the validity of various mean-field formulations for allosteric channel interactions and those mediated through elevated local [Ca\textsuperscript{2+}] [17, 38]. For the purposes of this paper it is sufficient to restrict attention to channels coupled via (8).

In general, a release site composed of \(N\) identical and indistinguishable \(M\)-state channels has

\[\beta (N, M) = \binom{M + N - 1}{N}\]

states. More concretely, a release site composed of \(N\) identical mean-field coupled three-state channels (see (1)) has \(\beta (N, 3) = (N + 2)(N + 1)/2\) distinct states that may be enumerated anti-lexicographically as

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

where each configuration takes the form \((N_c, N_o, N_R)\) with \(0 \leq N_c \leq N, 0 \leq N_O \leq N, 0 \leq N_R \leq N\), and \(N_c + N_o + N_R = N\). When these \(N\) channels are coupled via (8), the nonnegative elements of an expanded generator matrix for this release site are

\[
Q[[N_c, N_o, N_R], (N_c - 1, N_o + 1, N_R)] = N_c k_{oc} (c_\infty + N_o c_o)^2
\]

\[
Q[[N_c, N_o, N_R], (N_c + 1, N_o - 1, N_R)] = N_o k_{oc}
\]

\[
Q[[N_c, N_o, N_R], (N_c, N_o - 1, N_R + 1)] = N_R k_{ro}
\]

\[
Q[[N_c, N_o, N_R], (N_c, N_o + 1, N_R - 1)] = N_R k_{ro},
\]

where it is understood that the origin and destination configurations of the release site must both be valid (i.e. must both occur in (9)). In the case of \(N = 20\) three-state channels, there are \(\beta (20, 3) = 231\) distinguishable release site states. Figure 1 shows two representative release site simulations with 20 three-state channels exhibiting stochastic excitability that is reminiscent of Ca\textsuperscript{2+} puffs and sparks.

IP\textsubscript{3}R and RyR models can be significantly more complicated than the three-state model in (1); when composed of multiple subunits they may involve tens or even hundreds of states [28–30]. Because physiologically realistic values for \(N\) are in the range 20–200, and \(\beta (N, M)\) is on the order of \(N^M - 1\), even these ‘minimal’ release site models that assume mean-field coupling may have extremely large state spaces.

1.3. Automated reduction of Ca\textsuperscript{2+} release site models

This paper presents a novel approach to the automated reduction of Ca\textsuperscript{2+} release site models that are composed of stochastically gating channels as in section 1.2. The complexity of a release site model is not a significant concern when Monte Carlo simulation techniques are used to simulate the stochastic dynamics of individual Ca\textsuperscript{2+} release events. However, multiscale whole cell simulations that include both local and global Ca\textsuperscript{2+} signals require release site models that are as compact as possible while retaining the physiological realism of interacting channels and collective gating [40–44]. Indeed, for computational modeling to contribute to mechanistic understanding of the cellular effect of pharmacological manipulations of single channel gating, a Ca\textsuperscript{2+} release site model must be compositional. In addition, direct calculation of release site properties (e.g., mean puff/spark duration) is most efficiently performed using matrix analytic expressions that scale with the number of release site states. Consequently, development of methods for the automated reduction of compositional Ca\textsuperscript{2+} release site models (e.g., application of lumpability results for structured Markov chains [45–47]) is an important research objective.

In prior work we have investigated the automated reduction of Ca\textsuperscript{2+} release site models that begins with categorization of the single channel model rate constants as either fast or slow [24]. Groups of states connected by fast transitions are aggregated and transition rates between reduced states are chosen consistent with the conditional probability distribution among states within each group. For small test problems, these conditional probability distributions can be calculated from the full model without approximation. For the larger problems, the conditional probability distributions can be approximated without the construction of the full model by assuming rapid mixing of states connected by fast transitions; alternatively, memory-efficient iterative aggregation/disaggregation may be employed [48, 49].

Fast–slow reductions, however, may not preserve the group structure corresponding to the number of open channels (as seen in [24], for example), which is an important quantity for comparison with experimental observables [50] as well as insertion into multiscale models of whole cell response (via (8)). There exist other techniques for model simplification.
for example invariant manifold reduction [51, 52]. In these references, single channel models as well as collections of independent channels or subunits have solutions consisting of parameterized distributions, where the parameters satisfy a much smaller system of differential equations than the original master equation. While exploration in this direction with our compositionally defined release sites is of interest, it is currently not useful in our whole-cell context since our channels are coupled in a non-trivial manner.

In contrast to fast–slow reductions, this paper introduces a technique for automated Ca\textsuperscript{2+} release site reduction that does not require a separation of time scales. While inspired by approximate constructions such as Markov arrival processes [53], the method is unique in several respects and novel in this biophysical context. As presented in section 2, reduction is achieved by replacing aggregated states of the full release site model with much simpler CTMCs that have similar phase-type sojourn times between inter-group transitions. Section 3 validates the method when applied to release site models composed of two mean-field coupled three-state models leads to full generality using release sites composed of N arbitrary yet identical channels. We will, however, refer to the three-state model in (1) as needed for clarification and validation.

The method begins by partitioning the model state space into \( N + 1 \) groups \( \{P_0, P_1, \ldots, P_N\} \) where \( P_i \) includes all release site states with \( i \) open channels \( (N_O = i, \text{ see figure 2}) \). This partitioning strategy is consistent with our primary interest in the stochastic dynamics of the number of open channels and the local \([\text{Ca}\textsuperscript{2+}]\) (the experimental observable). The \( Q \)-matrix is permuted into the following matrix:

\[
\begin{align*}
Q_{\text{permuted}} &= \begin{pmatrix}
0 & \cdots & 0 \\
\vdots & \ddots & \vdots \\
0 & \cdots & 0
\end{pmatrix}
\end{align*}
\]
where the off-diagonal blocks $Q_{i,j}$ ($i \neq j$) are nonnegative matrices whose positive entries correspond to transitions from aggregate states $P_l$ to $P_j$. Note that $Q$ has row sum zero by (4) and (5) and thus the row sums of the diagonal blocks $Q_{i,i}$ can be negative. To illustrate, with $N = 2$ three-state channels (see figure 2), a permuted $Q$-matrix has the form

$$Q = \begin{pmatrix} Q_{0,0} & Q_{0,1} & Q_{1,0} & Q_{1,1} \\ Q_{0,1} & Q_{0,2} & Q_{1,1} & Q_{1,2} \\ \vdots & \vdots & \ddots & \vdots \\ Q_{N-1,N-2} & Q_{N-1,N-1} & Q_{N-1,N} & Q_{N,N} \end{pmatrix},$$

where a filled circle denotes a non-zero transition rate, and diagonal entries (denoted by an asterisk) are defined such that the row sums are zero. For this example, there are no transitions between states in the $Q_{i,i}$ blocks. In general, however, there will be transitions in the diagonal blocks for single channel models with transitions between multiple closed or open states (e.g. the three-state model $C_1 \equiv C_2 \equiv O_3$ with two closed states $C_1$, $C_2$ and one open state $O_3$).

Our reduction technique replaces the blocks $Q_{i,j}$ with significantly reduced blocks $Q_{i,j}$ such that the dwell time distribution in each group $P_l$ and the probability of moving to $P_{l-1}$ versus $P_{l+1}$ upon exiting $P_l$ are well approximated, thereby capturing the statistics of the time evolution of the number of open channels in the release site ($N_0(t)$, see figure 1). To understand how this is accomplished, it is important that the reader be familiar with phase-type distributions.

### 2.1. Phase-type distributions

It is well-known that the cumulative probability of sojourn time ($\tau$) in the transient states of an absorbing Markov chain with absorbing state $U$

$$Q'' = \begin{pmatrix} 0 & 0 \\ u & T \end{pmatrix}$$

is given by the \textit{Erlang} or \textit{phase-type distribution} [54],

$$F_t(x) = \mathbb{P}[\tau \leq x] = 1 - \exp(tT)e,$$

where $\alpha = (\alpha_i)$ is a row vector indicating the initial probability distribution, $\sum \alpha_i = 1$, $u = (u_i)$ is a column vector of exit rates ($u_i \geq 0$), and $T = (t_{ij})$ are the rate constants for transient states ($t_{ij} \geq 0$ for $i \neq j$ and $t_{ii} = -u_i - \sum_{j=1}^{m} t_{ij} < 0$). In (12), $\exp(tT)$ is a matrix exponential and $e$ is a commensurate column vector of ones. The corresponding probability density function for $\tau$,

$$f(x)dx = \mathbb{P}[\tau \leq x + dx],$$

is given by

$$f(x) = \begin{cases} \alpha \exp(tT)u & \text{if } x > 0 \\ 0 & \text{if } x \leq 0. \end{cases}$$

We will write $\tau \sim PH(\alpha, T)$ to indicate that the random variable $\tau$ is phase-type distributed with parameters $\alpha$ and $T$. The probability density $f(x)$ integrates to unity as required, because $u = -Te$ and $\int_0^\infty \exp(tT)dx = -T^{-1}$ and thus

$$\int_0^\infty f(x)dx = -\alpha T^{-1}u = 1.$$ Furthermore, the expected sojourn time until absorption is

$$\mathbb{E}[\tau] = \int_0^\infty xf(x)dx = -\alpha T^{-1}e,$$

where we have used $\int_0^\infty x \exp(tT)dx = -T^{-2}$ and $u = -Te$.

In the case of a Markov chain with two distinct absorbing states $U^\pm$, the exit rate vector $u$ can be written as $u = u^- + u^+$ where $u^- > 0$ and $u^+ > 0$. The probability density for the dwell time $\tau$ jointly distributed with the absorbing states ($U^-$ and $U^+$),

$$f^\pm(x)dx = \mathbb{P}[U^\pm \text{ and } x < \tau \leq x + dx],$$

is given by

$$f^\pm(x) = \begin{cases} \alpha \exp(xT)u^\pm & \text{if } x > 0 \\ 0 & \text{if } x \leq 0, \end{cases} \text{ where } u^- + u^+ = -Te. \quad (13)$$

The probabilities of absorption into states $U^\pm$ are given by the positive scalars

$$\mathbb{P}[U^\pm] = \int_0^\infty f^\pm(x)dx = -\alpha T^{-1}u^\pm > 0,$$

where $\alpha e = 1$ and $-Te = u^- + u^+$ guarantees that $\mathbb{P}[U^-] + \mathbb{P}[U^+] = 1$. The expected sojourn times in transient states given absorption into states $U^\pm$ are

$$\mathbb{E}[\tau | U^\pm] = \frac{\int_0^\infty xf^\pm(x)dx}{\mathbb{P}[U^\pm]} = \frac{-\alpha T^{-2}u^\pm}{-\alpha T^{-1}u^+},$$

where both the numerator and denominator are positive scalars. We will write $(\tau, U^\pm) \sim PH(\alpha, T, u^\pm)$ to indicate a random dwell time, $\tau \in [0, \infty)$, with absorbing states $U^\pm$, jointly distributed according to (13).
2.2. The homogenized Ca\textsuperscript{2+} release site model

Returning to the generator matrix for the full release site model in (10), we note that the diagonal blocks \( Q_{i,i} \) can each be interpreted as a transient Markov chain with two exits by identifying

\[
T_i = Q_{i,i},
\]

\[
u_i^- = Q_{i,i-1} e_{i-1},
\]

\[
u_i^+ = Q_{i,i+1} e_{i+1},
\]

where the zero row sum of \( Q \) ensures that \( Q_{i,i-1} e_{i-1} + Q_{i,i} e_i + Q_{i,i+1} e_{i+1} = 0 \) and thus \( u_i^- + u_i^+ = -T_i e_i \) (see (13)). Next, we construct pairs of initial probability distributions, \( \alpha_i^- \) and \( \alpha_i^+ \), associated with entrances into \( P_i \) from \( P_{i-1} \) and \( P_{i+1} \), respectively, given by

\[
\alpha_i^- = \frac{\pi_i^- Q_{i,i-1}}{\pi_{i-1} Q_{i-1,i} e_i} \quad \text{and} \quad \alpha_i^+ = \frac{\pi_i^+ Q_{i,i+1}}{\pi_{i+1} Q_{i+1,i} e_i},
\]

(14)

where \( \pi_i \) is the portion of the release site model stationary distribution \( \pi \) (see (7)) associated with aggregated states \( P_i \). In this construction, the elements of \( \alpha_i^- \) are the probabilities that each substate begins the sojourn in \( P_i \), given that the sojourn was initiated by a \( P_{i-1} \rightarrow P_i \) transition (i.e. the number of open channels in the release site increases from \( N_O = i - 1 \rightarrow i \)). Similarly, the elements of \( \alpha_i^+ \) are the probabilities that each substate begins the sojourn in \( P_i \), given that the sojourn was initiated by a \( P_{i+1} \rightarrow P_i \) transition (\( N_O = i + 1 \rightarrow i \)).

Figure 3. State–transition diagram for the homogenized renewal process characterizing the full Ca\textsuperscript{2+} release site model in (15). The state \( PH(\alpha_i^-, T_i, u_i^+) \) indicates \( N_O = i \) open channels and dwell time that is \( PH(\alpha_i^-, T_i, u_i^+) \) distributed. The two directed edges leaving this state labeled \( u_i^- \) and \( u_i^+ \) correspond to a decrease or increase in \( N_O \), respectively.

As a result of this procedure, the homogenized full model \( \hat{Q} \) is reduced by replacing the diagonal blocks \( (T_i) \) with the generator matrices for transient Markov chains that have similar phase-type sojourn times but fewer states. The general technique is to use the moments of the phase-type distributions that appear in the homogenized model, \( PH(\alpha, T, u^\pm) \), to fit phase-type distributions of smaller size, \( PH(\hat{\alpha}, \hat{T}, \hat{u}^\pm) \), where the carets on \( \alpha, \hat{T} \), and \( \hat{u} \) indicate that these are parameters of the reduced model.

First we compute the target moments of the phase-type distributions in the homogenized full model in (15). For example, for each interior diagonal block \( T_i, 1 \leq i \leq N - 1 \), we numerically calculate the moments for the pair of single-exit phase-type distributions, \( PH(\alpha_i^-, T_i) \) and \( PH(\alpha_i^+, T_i) \), where \( \alpha_i^- \) and \( \alpha_i^+ \) are the initial probability distributions when entering this block from below or above, respectively, and the exit rate vector is \( u_i = -T_i e_i \). This is a straightforward numerical calculation, because the moments of a phase-type distribution \( PH(\alpha, T) \) are given by

\[
m_k = k! \alpha (-T)^{-k} e = k! \alpha (-T)^{-k} e.
\]

where \( \alpha e = 1 \) and \( u = -Te \).

2.4. Moment fitting constrains reduced model parameters

Moment fitting per se requires specification of the class of phase-type distributions to be used in the reduced model.
For example, if we construct the reduced model so that the probability distribution of the sojourn time in each block is given by a two-state hyper-exponential distribution, $PH(\hat{\alpha}, \hat{T})$, then using the constraints that $\hat{\alpha}e = 1$ and $\hat{u} = -\hat{T}e$ we have
\[
\hat{\alpha} = (\hat{\alpha}^1 1 - \hat{\alpha}), \quad \hat{T} = \begin{pmatrix} -\hat{u}_1 & 0 \\ 0 & -\hat{u}_2 \end{pmatrix}, \quad \hat{u} = \begin{pmatrix} \hat{u}_1 \\ \hat{u}_2 \end{pmatrix},
\]
with three scalar parameters to be determined: $\hat{u}_1 > 0$, $\hat{u}_2 > 0$, and $0 < \hat{\alpha} < 1$. Using (16) and elementary properties of diagonal matrices, the moments of a two-state hyper-exponential distribution are
\[
\hat{m}_k = k!(\hat{\alpha} 1 - \hat{\alpha}) \begin{pmatrix} \hat{u}_1 & 0 \\ 0 & \hat{u}_2 \end{pmatrix}^{-k} \begin{pmatrix} 1 \\ 1 \end{pmatrix},
\]
(17)

Setting the first and second moments ($k = 1, 2$) of this expression equal to the first and second moments of the phase-type distributions of the full model, simultaneously for both $PH(\alpha^1, T)$ and $PH(\alpha^2, T)$, leads to the following four equations:
\[
m_1^\pm = \frac{\hat{\alpha}^\pm}{\hat{u}_1} + \frac{1 - \hat{\alpha}^\pm}{\hat{u}_2},
\]
\[
m_2^\pm = 2 \left( \frac{\hat{\alpha}^\pm}{\hat{u}_1} + \frac{1 - \hat{\alpha}^\pm}{\hat{u}_2} \right),
\]
with four unknowns: $\hat{\alpha}^-$, $\hat{\alpha}^+$, $\hat{u}_1$, and $\hat{u}_2$. These equations can be rewritten as
\[
m_1^1 \hat{u}_1 \hat{u}_2 = \hat{\alpha}^+ (\hat{u}_2 - \hat{u}_1) + \hat{u}_1,
\]
(19)
\[
m_2^1 \hat{u}_1 \hat{u}_2 = 2\hat{\alpha}^+ (\hat{u}_2 - \hat{u}_1) + 2\hat{u}_1^2.
\]
(20)

Substituting $\hat{\alpha}^+ (\hat{u}_2 - \hat{u}_1) = m_1^1 \hat{u}_1 \hat{u}_2 - \hat{u}_1$ from (19) into (20) gives
\[
m_2^1 \hat{u}_1 \hat{u}_2 = 2m_1^1 (\hat{u}_1 + \hat{u}_2) - 2.
\]
Writing the unknowns as $x = \hat{u}_1 + \hat{u}_2$ and $y = \hat{u}_1 \hat{u}_2$, we find that choosing the reduced model parameters requires finding $x$ and $y$ that minimize
\[
\begin{pmatrix} 2m_1^1 \\ 2m_2^1 \\ -m_1^2 \\ -m_2^2 \end{pmatrix} \begin{pmatrix} x \\ y \end{pmatrix} - \begin{pmatrix} 2 \\ 2 \end{pmatrix},
\]
subject to inequality constraints $0 < \hat{\alpha}^\pm < 1$, $x, y > 0$ and $x^2 > 4y$ where
\[
\hat{\alpha}^\pm = \frac{\hat{u}_1 (m_1^1 \hat{u}_2 - 1)}{\hat{u}_2 - \hat{u}_1} \quad \text{and} \quad \hat{u}_{1/2} = \frac{x \pm \sqrt{x^2 - 4y}}{2}.
\]

Next, we use the zeroth moment ($k = 0$) to split the exit rate vector $\hat{u}$ into two components, $\hat{u}^-$ and $\hat{u}^+$. The joint moments for phase-type distributions with two exit rate vectors, $PH(\alpha^\pm, T, u^\pm)$, are
\[
m_k^\pm = k!\alpha^\pm (-T)^{-(k+1)} u^\pm,
\]
where $\alpha^\pm e = 1$, $u^+ + u^- = -Te$. Thus, the first target moments from the homogenized full model are
\[
m_0^\pm = \alpha^\pm (-T)^{-1} u^\pm.
\]

The corresponding moments of the two-state hyper-exponential distributions of the reduced model can be written as
\[
\begin{pmatrix} \hat{m}_0^- \\ \hat{m}_0^+ \end{pmatrix} = \begin{pmatrix} \hat{\alpha}^- \\ \hat{\alpha}^+ \end{pmatrix} \begin{pmatrix} 1 - \hat{\alpha}^- \\ 1 - \hat{\alpha}^+ \end{pmatrix}
\times \begin{pmatrix} \hat{u}_1 \\ 0 \\ \hat{u}_2 \end{pmatrix} - 1 \begin{pmatrix} \hat{u}_1^1 \\ \hat{u}_2^1 \\ \hat{u}_1^2 \end{pmatrix} \begin{pmatrix} \hat{u}_1^+ \\ \hat{u}_2^+ \end{pmatrix},
\]
(23)

subject to the constraints $0 < \hat{\alpha}_i^\pm < 1$ and $0 < \hat{u}_i^- < \hat{u}_i^+$. The minimizations necessary for moment fitting in (21) and (24) can often be attained by solving the corresponding linear algebraic systems. If this solution does not satisfy the inequality constraints, minimization can be performed using an appropriate optimization routine (e.g., linear least-squares in (24)). The moment fitting scheme employed for the interior blocks $T_i$ for $1 \leq i \leq N - 1$ are modified when $i = 0$ or $N$ (see the appendix) as there is only one way to enter and leave the blocks in these special cases.

2.5. Diagrammatic representation of the dynamics of the reduced model

Figure 4 shows a diagrammatic representation of the hyper-exponential distributions in the reduced model. The transient matrix $\tilde{T}$ corresponding to $N = i$ open channels has two nonzero elements, $\tilde{u}_1$ and $\tilde{u}_2$ (the index $i$ is dropped for clarity),
with dwell times given by \( \tilde{\tau}_1 = 1/\tilde{u}_1 \) and \( \tilde{\tau}_2 = 1/\tilde{u}_2 \). If the \( \tilde{i} \)th block is entered from below \( (i - 1) \), the substate with mean dwell time \( \tilde{\tau}_1 \) and \( \tilde{\tau}_2 \) is entered with probability \( \tilde{\alpha}^- \) and \( 1 - \tilde{\alpha}^- \), respectively. If the \( \tilde{i} \)th block is entered from above \( (i + 1) \), these substates are entered with probabilities \( \tilde{\alpha}^+ \) and \( 1 - \tilde{\alpha}^+ \).

The exit rate vectors \( \tilde{\mu}_i^\pm \) associated with the \( \tilde{i} \)th block determine if the subsequent block has \( N_O = i - 1 \) or \( i + 1 \) open channels. Upon entry into the \( \tilde{u}_l \) substate of the \( \tilde{i} \)th block, the probabilities of a decreasing or increasing number of open channels are

\[
\tilde{p}_l^- = \frac{\tilde{\mu}_l^-}{\tilde{\mu}_l^- + \tilde{\mu}_l^+} \quad \text{and} \quad \tilde{p}_l^+ = 1 - \tilde{p}_l^- = \frac{\tilde{\mu}_l^+}{\tilde{\mu}_l^- + \tilde{\mu}_l^+}, \tag{25}
\]

where again we drop the index \( i \) for clarity. Similarly, entry of the \( \tilde{u}_l \) substate results in a decreasing or increasing number of open channels with probabilities \( \tilde{p}_2 = \tilde{u}_2 / (\tilde{u}_2 + \tilde{u}_3) \) and \( \tilde{p}_2^+ = 1 - \tilde{p}_2^- = \tilde{u}_2^+ / (\tilde{u}_2^+ + \tilde{u}_3^+). \)

### 3. Validation of the Ca\(^{2+}\) release site reduction method

The validation of the Ca\(^{2+}\) release site reduction method described in the previous section was performed by comparing the dynamics of full models composed of \( N = 20 \) mean-field coupled three-state channels in (1) leading to release sites with 231 total states. The reduced model consists of \( N + 1 = 21 \) diagonal blocks \((T_i)\), 20 of which are two-state hyper-exponential distributions \((N_O = 0, \ldots, 19)\). Because the \( N_O = 20 \) partition has only one state in the full model, no reduction is required for the \( T_{20} \) block. The reduced model has \( 2 \times 20 + 1 = 41 \) states, which is an 82% reduction in size compared to the full model \((41/231 \approx 0.18)\).

Figure 5 shows the number of open channels \( N_O(t) \) over time for model Ca\(^{2+}\) release sites composed of 20 three-state channels. Simulations of the 231-state full model and the 41-state reduced model are presented in the left and middle panels, respectively. The right panels show good agreement between the steady state distributions of \( N_O \) in the full and reduced models (black and white bars). The parameters used in figures 5(a) and (b) correspond to the two possible roles of Ca\(^{2+}\) inactivation in the dynamics of Ca\(^{2+}\) spark termination (cf figure 1) [17]. The reduced model reproduces the distribution of \( N_O \) in the full model when (a) spark termination occurs through the process of stochastic attrition on a relatively constant background of refractory channels, and also (b) when spark termination occurs via recruitment of refractory channels during each event.

Figure 6 shows a scatter plot of Ca\(^{2+}\) event amplitude and duration for simulations of both the full and reduced models. Sparks initiate with an \( N_O = 0 \rightarrow 1 \) transition at time \( t_{init} \) and terminate upon the first subsequent \( N_O = 1 \rightarrow 0 \) transition at time \( t_{term} \). Spark duration = \( t_{term} - t_{init} \) and spark amplitude is the integral of the number of open channels between these times. The marginal distributions of spark duration and amplitude show general agreement between the full and reduced models (black and white bars). Comparison of figures 6(a) and (b) shows that the reduced model captures the dynamics of Ca\(^{2+}\) sparks more accurately when spark termination is via stochastic attrition (a) and slightly less accurately when spark termination is due to the accumulation of refractory channels (b).

In order to accurately quantify the error that results from model reduction, we define the error measure \( E_{max}(t) = \)
max_{ij} |E_{ij}(t)| \) where \( E(t) \) compares the dynamics of \( N_0(t) \) in the full and reduced models as follows:

\[
E(t) = U \exp(tQ) V - \hat{U} \exp(t\hat{Q}) \hat{V}, \tag{26}
\]

where \( Q \) and \( \hat{Q} \) are the generator matrices for the full and reduced models that have \( \beta = 231 \) and \( \hat{\beta} = 41 \) states, respectively. In this expression, \( V \) is a \( \beta \times N + 1 \) collector matrix,

\[
V = \begin{pmatrix}
e_0 & 0 & \cdots & 0 \\
0 & e_1 & \cdots & 0 \\
\vdots & \vdots & \ddots & \vdots \\
0 & 0 & \cdots & e_N
\end{pmatrix},
\]

where \( N = 20 \) is the number of channels in the release site model, and each \( e_i \) is a column vector of ones commensurate with \( Q_{i,i} \) (see (10)). The \( N + 1 \times \beta \) distributor matrix \( U \) is given by

\[
U = \begin{pmatrix}
\bar{\pi}_0 & 0 & \cdots & 0 \\
0 & \bar{\pi}_1 & \cdots & 0 \\
\vdots & \vdots & \ddots & \vdots \\
0 & 0 & \cdots & \bar{\pi}_N
\end{pmatrix},
\]

where the row vector \( \bar{\pi} = (\bar{\pi}_0 \bar{\pi}_1 \cdots \bar{\pi}_N) \) is the stationary probability distribution of the full model (see (7)). The \( \hat{\beta} \times N + 1 \) collector \( (\hat{V}) \) and \( N + 1 \times \hat{\beta} \) distributor \( (\hat{U}) \) matrices for the reduced model are similarly defined.

The solid lines of figures 7(a) and (b) show the error between the full and reduced models \( (E_{\text{max}}(t)) \) calculated using (26) for the two different release site parameter sets discussed above. In both cases, the error is initially \( (t = 0) \) and ultimately \( (t \to \infty) \) very small \((< 10^{-7})\), but grows modestly to 0.0059 \((t = 74 \text{ ms})\) in the case of stochastic attrition (a) and 0.041 \((t = 29 \text{ ms})\) when recruitment of refractory channels terminates sparks (b). The broken lines in figure 7 show the error between the homogenized full model and the reduced model calculated using (26) with the replacement of \( \hat{Q} \) for \( Q \). The small size of this error \((< 10^{-3} \text{ for all } t)\) indicates that the reduction error is primarily attributable to the homogenization of the full model in (15) as opposed to the moment fitting procedure per se (section 2.4).

4. Discussion

We have described a novel reduction technique applicable to Markov chain models of Ca\(^{2+}\) release sites composed of instantaneously mean-field coupled intracellular Ca\(^{2+}\) channels. Using 20 three-state channels that are both activated and inactivated by Ca\(^{2+}\), the full and reduced models have nearly identical stationary distributions for the number of open channels (figure 5). The dynamics of \( N_0(t) \) and the distribution of spark durations and amplitudes (figure 6) are...
also quite similar in considering the 82% reduction in model size obtained.

The Ca\(^{2+}\) release site model reduction procedure introduced in this paper includes three major steps: choosing a partitioning scheme that determines how full model states are aggregated (figure 2 and (10)), calculation of target moments using the homogenized full model (sections 2.2 and 2.3), and moment fitting to constrain the parameters of the phase-type distributions in the reduced model (section 2.4).

4.1. Partitioning schemes

Aggregating full model states according to the number of open channels leads to a block tridiagonal matrix with skip-free transitions between blocks. Consequently, the phase-type distributions for the interior blocks 1 \(\leq i \leq N - 1\) have two starting distributions (\(\alpha^+\)) and two exit vectors (\(\alpha^-\)) in both the homogenized full model and the reduced model.

While it is important to choose a partition structure that does not combine full model states that correspond to different \(N_O\), it is not required to combine full model states that correspond to a given \(N_O\) into a single aggregate class. If a large block contributes substantially to the reduction error, the partition can be refined to subdivide this block (e.g., into components with lesser or greater numbers of refractory channels). Such partitioning may be useful when the refractory channels play a complex dynamic role in spike termination as in figure 1(b). Of course, when multiple diagonal blocks correspond to the same \(N_O\), the homogenized full model is no longer tridiagonal (cf (15)).

In the general case, a partitioning scheme will result in \(n_a\) ways to enter and \(n_a\) ways to leave a given block. Associated with this block are \(n_a\) phase-type distributions, each involving a transient matrix \(T\), \(n_a\) exit vectors, and one of the \(n_a\) initial distributions.

4.2. Moment fitting

The moment fitting procedures that are appropriate to constrain parameters of the reduced model depend on the chosen partition structure, the block size used in the reduced model, and the class of phase-type distribution used in the reduced model. While moment-fitting techniques exist to fit arbitrary phase-type distributions [55, 56], we use two-state hyper-exponential distributions in the reduced model because this results in a tractable moment fitting problem (section 2.4). If the block size used in the reduced model were larger than 2, a direct extension of the approach used here would require further analysis. A less direct extension that bypasses this block (e.g., into components with lesser or greater numbers of refractory channels). Such partitioning may be useful when the refractory channels play a complex dynamic role in spike termination as in figure 1(b). Of course, when multiple diagonal blocks correspond to the same \(N_O\), the homogenized full model is no longer tridiagonal (cf (15)).

In the general case, a partitioning scheme will result in \(n_a\) ways to enter and \(n_a\) ways to leave a given block. Associated with this block are \(n_a\) phase-type distributions, each involving a transient matrix \(T\), \(n_a\) exit vectors, and one of the \(n_a\) initial distributions.

4.3. Validation to experimental observations

While the three-state model (1) was chosen for illustrative simplicity rather than biophysical realism, it is informative to see if the full (and more importantly reduced) model can reproduce some recent experimental observations. For example, in [50] measurements using total internal reflection fluorescence microscopy were able to capture the quantal structure of calcium release from clusters of IP\(_3\)R channels in mammalian cells. Subsequent analysis showed that for closing events that showed no channel openings during the falling phase, the histogram of puff durations (more specifically time from peak amplitude to closure, scaled by the mean blip duration) was well approximated by a distribution corresponding to the closing time for a representative number of stochastic and independent channels, each closing with time constant given by the mean blip duration. The same analysis applied to simulations of the full three-state model (1) showed similar results (not shown). More importantly, the results were nearly identical for the reduced model as well.

It was posited in [50] that this close approximation may be due to the strong Ca\(^{2+}\)-dependent inhibition seen in type I IP\(_3\)R channels. Surprisingly, this agreement of the closure of stochastic and independent channels with simulated events was seen for both parameter sets corresponding to closure via the gradual recruitment of inactivated channels and via stochastic attrition.

4.4. Ca\(^{2+}\) release site reduction and multiscale modeling

The reduction strategy presented and validated here is an important step toward the goal of including realistic compositional release site models in multiscale whole cell simulations (see section 1). With this objective in mind, an avenue for future research is the extension of this reduction technique to Ca\(^{2+}\) release site models that take the form

\[
Q([Ca^{2+}]) = K_+ + [Ca^{2+}]K_-, 
\]

where \([Ca^{2+}](t)\) is an external ‘environmental’ variable, as opposed to an intrinsic variable that is an instantaneous function of release site state (cf (8)). This could be accomplished by performing model reduction for a number of physiologically realistic Ca\(^{2+}\) concentrations and interpolating the rate constants so that, e.g., \(Q([Ca^{2+}]) = \epsilon Q(c_i) + (1 - \epsilon) Q(c_j)\) where \(c_i \leq [Ca^{2+}] \leq c_j\) and \(\epsilon = (c_j - [Ca^{2+}])/(c_j - c_i)\). A more interesting approach would be to assume that the free parameters of the phase-type distributions of the reduced model \((\tilde{\alpha}^+, \tilde{\alpha}_1^+, \tilde{\alpha}_2^+)\) can be written as simple functions of \([Ca^{2+}]\) (e.g., polynomials of specified degree). The challenge of this approach would be to generalize (18) and develop moment fitting techniques that constrain the coefficients of these polynomial functions of \([Ca^{2+}]\).
Acknowledgments

This research was supported in part by the National Science Foundation under grant no 0443843 to GDS and a Howard Hughes Medical Institute grant through the Undergraduate Biological Sciences Education Program to The College of William and Mary. This work was performed in part using computational facilities provided with the assistance of the National Science Foundation, the Virginia Port Authority, Sun Microsystems, and Virginia’s Commonwealth Technology Research Fund.

Appendix. Moment-fitting for first and last blocks

The scheme employed to fit the moments of the two-state hyper-exponential distributions in the reduced model to the moments calculated from the interior blocks of the full model $T_i$, for $1 \leq i \leq N - 1$ are modified when $i = 0$ or $N$. The reduction of the block row corresponding to $N_0 = N$ is not required, because the full model has only one state in this group (all channels in state $O$) and $T_N = -u_N$ and $\alpha_N = 1$ are scalars. The reduction of the block row corresponding to $N_0 = 0$ requires a different scheme than the interior blocks because the transient model $T_0$ is associated with one starting distribution ($\alpha_0$) and one exit vector ($u_0$). Dropping the superscript and subscript for clarity and using (17) and (18) we have

$$m_1 = \frac{\hat{\alpha}}{\hat{u}_1} + 1 - \frac{\hat{\alpha}}{\hat{u}_2},$$

$$m_2 = 2 \left( \frac{\hat{\alpha}}{\hat{u}_1^2} + \frac{1 - \hat{\alpha}}{\hat{u}_2^2} \right),$$

$$m_3 = 6 \left( \frac{\hat{\alpha}}{\hat{u}_1^3} + \frac{1 - \hat{\alpha}}{\hat{u}_2^3} \right),$$

where we have equated the first three reduced model moments to the target moments of the full model ($\hat{m}_i = m_i$). These expressions are partially inverted to obtain

$$m_1 \hat{u}_1 \hat{u}_2 = \hat{\alpha} \hat{u}_1 + (1 - \hat{\alpha}) \hat{u}_2,$$

$$m_2 \hat{u}_1^2 \hat{u}_2^2 = 2 \hat{\alpha} \hat{u}_1^2 + 2 (1 - \hat{\alpha}) \hat{u}_2^2,$$

$$m_3 \hat{u}_1^3 \hat{u}_2^3 = 6 \hat{\alpha} \hat{u}_1^3 + 6 (1 - \hat{\alpha}) \hat{u}_2^3.$$

After some algebra, we write $x = \hat{u}_1 + \hat{u}_2$ and $y = \hat{u}_1 \hat{u}_2$ and simultaneously solve

$$0 = 2m_1 x - m_2 y - 2,$$

$$0 = m_3 y^2 - 6 m_1 (x^2 - y) + 6x.$$

We let $x$ be the larger of the two solutions to the following quadratic equation:

$$(2m_3 m_1^2 - 3m_1 m_2) x^2 + (6m_1 m_2 + 3m_2^2 - 4m_1 m_3) x + 2m_3 - 6m_1 m_2 = 0,$$

and $y$ is given by

$$y = \frac{2 (m_1 x - 1)}{m_3}.$$

Finally, the parameters of the hyper-exponential distribution are given by

$$\alpha = \frac{\hat{u}_1 (m_1 \hat{u}_2 - 1)}{\hat{u}_2 - \hat{u}_1} \quad \text{and} \quad \hat{u}_{1/2} = \frac{x \pm \sqrt{x^2 - 4y}}{2}.$$

References


[18] Shuai J W and Jung P 2002 Stochastic properties of Ca2+ release of inositol 1,4,5-trisphosphate receptor clusters Biophys. J. 83 87–97


[23] DeRemigio H, Kemper P, LaMar M D and Smith G D 2008 Markov chain models of coupled intracellular calcium