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Multi-scale continuum modeling of biological processes: from molecular electro-diffusion to sub-cellular signaling transduction

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Abstract. This paper presents a brief review of multi-scale modeling at the molecular to cellular scale, with new results for heart muscle cells. A finite element-based simulation package (SMOL) was used to investigate the signaling transduction at molecular and sub-cellular scales (http://mccammon.ucsd.edu/smol/, http://FETK.org) by numerical solution of the time-dependent Smoluchowski equations and a reaction-diffusion system. At the molecular scale, SMOL has yielded experimentally validated estimates of the diffusion-limited association rates for the binding of acetylcholine to mouse acetylcholinesterase using crystallographic structural data. The predicted rate constants exhibit increasingly delayed steady-state times, with increasing ionic strength, and demonstrate the role of an enzyme’s electrostatic potential in influencing ligand binding. At the sub-cellular scale, an extension of SMOL solves a nonlinear, reaction-diffusion system describing Ca²⁺ ligand buffering and diffusion in experimentally derived rodent ventricular myocyte geometries. Results reveal the important role of mobile and stationary Ca²⁺ buffers, including Ca²⁺ indicator dye. We found that alterations in Ca²⁺-binding and dissociation rates of troponin C (TnC) and total TnC concentration modulate sub-cellular Ca²⁺ signals. The model predicts that reduced off-rate in the whole troponin complex (TnC, TnI, TnT) versus reconstructed thin filaments (Tn, Tm, actin) alters cytosolic Ca²⁺ dynamics under control conditions or in disease-linked TnC mutations. The ultimate goal of these studies is to develop scalable methods and theories for the integration of molecular-scale information into simulations of cellular-scale systems.
1. Introduction

Diffusion of enzymes and ions plays an important role in a variety of biological processes and has been studied extensively using various biophysical, biochemical and computational approaches. Computational models of diffusion have been widely used in both discrete [1] and continuum methods [2]. Discrete methods describe the stochastic trajectories of individual particles and include methods such as Monte Carlo [3], Brownian dynamics [4] and Langevin dynamics [5]. Continuum approaches model particle diffusion by the evolution of continuous probability distributions and include the Smoluchowski and Fokker–Planck formalisms. Relative to discrete methods, continuum approaches provide an inexpensive alternative for the modeling of individual Brownian particles.

In our previous work, we applied adaptive finite-element methods to solve the time-dependent Smoluchowski equation on a single enzyme molecule (mouse acetylcholinesterase (mAChE)) or clusters of mAChEs [6–8]. We observed both steady-state and time-dependent diffusive particle distributions on the diffusion domain and calculated the rate association coefficient for acetylcholine (ACh) diffusive particles. Including the electrostatic field determined by solution of the Poisson–Boltzmann equation allowed us to examine the role of electrostatics in guiding diffusive particles to the active center. This study resulted in the SMOL software package, which facilitates the finite-element solution of steady-state and time-dependent Smoluchowski equations for molecular species.

Recently, the SMOL package was generalized to model signaling transduction processes on the sub-cellular level and, specifically, the role of calcium (Ca$^{2+}$) diffusion and binding to mobile and stationary buffers during the cardiac excitation–contraction cycle [9–11]. Previous approaches employed a systems biology approach, in which components of the myocyte are represented by simple geometries [12–14]. In recent years, advancements in the electron microscopy community have enabled the three-dimensional (3D) structure determination of cells and sub-cellular organelles across a wide range of spatial scales [15]. Simulations utilizing experimentally derived structures offer a degree of realism that cannot be easily captured with reduced representations.

The extended simulation tool SMOL represents progress toward unifying molecular-level and cellular-level dynamics via the solution of partial differential equations on 3D tetrahedral meshes representative of realistic systems. In this paper, we summarize the capabilities of SMOL in modeling molecular-level and cellular-level events, provide new data for myocyte cellular modeling and discuss potential further applications and extensions of the SMOL multi-scale modeling tool.
2. Methods and software

2.1. Time-dependent Smoluchowski equation

The starting point for solving the time-dependent SMOL equation is based on the steady-state Smoluchowski equation solver described by Song et al [16, 17]. The original time-dependent Smoluchowski equation has the form of a continuity equation,

$$\frac{du(\vec{R}; t)}{dt} = -\vec{\nabla} \cdot \vec{j}(\vec{R}; t),$$

where the particle flux $\vec{j}(\vec{R}; t)$ is defined as

$$\vec{j}(\vec{R}; t) = D(\vec{R}) \left[ \vec{\nabla} u(\vec{R}; t) + \beta \vec{\nabla} W(\vec{R}) u(\vec{R}; t) \right] = D(\vec{R}) \exp[-\beta W(\vec{R})] \vec{\nabla} \exp[\beta W(\vec{R})] u(\vec{R}; t).$$

Here $u(\vec{R}; t)$ is the distribution function of the ensemble of Brownian particles, $D(\vec{R})$ is the diffusion coefficient, $\beta = 1/k_B T$ is the inverse Boltzmann energy, $k_B$ is the Boltzmann constant, $T$ is the temperature and $W(\vec{R})$ is the potential of mean force (PMF) for a diffusing particle due to solvent-mediated interactions with the target molecule. For simplicity, $D(\vec{R})$ is assumed to be constant. The two terms contributing to the flux have clear physical meaning. The first is due to free diffusion processes, as quantified by Fick’s first law. The second contribution is due to the drift velocity, $\vec{\nabla} W(r)/\gamma$, induced by the systematic forces, $\vec{\nabla} W(r)$, and friction quantified by the friction constant $\gamma$. The relation between the diffusion coefficient $D(\vec{R})$ and the friction constant $\gamma$ is given by the Stokes–Einstein equation $D\beta \gamma = 1$.

The diffusion-determined reaction rate constant during the simulation time can be obtained from the flux $\vec{j}(\vec{R}; t)$ by integration over the active site boundary ($\Gamma$), i.e.

$$k_{on}(t) = u_{bulk}^{-1} \int_{\Gamma} n(\vec{R}) \cdot \vec{j}(\vec{R}; t) \, dS,$$

where $u_{bulk}$ is the bulk concentration at the outer boundary and $n(\vec{R})$ is the surface normal [6]. The variation of reaction rates with ionic strength is often interpreted via the Debye–Huckel limiting law [18, 19].

For molecular simulations, the long-range contributions to the PMF are electrostatic in nature and thus may be estimated by solving the Poisson–Boltzmann equation. Typical approaches consider the positions of atoms from high-resolution x-ray crystal structures available in the Protein Data Bank (http://www.rcsb.org) and their corresponding partial charges, as well as the dielectric constant of the surrounding solvent. For subcellular systems, we assume that the electrostatic potential arising from the cell membrane is screened beyond the characteristic Debye length, which is less than 1.0 nm at physiological ionic strengths. As such, the $W(\vec{R})$ term defined in (2) may be neglected and one arrives at Fick’s law of diffusion [6].

2.2. Reaction-diffusion equations

In this section, we derive a model that describes the diffusive transport of Ca$^{2+}$ in the presence of stationary and mobile Ca$^{2+}$ buffers. Assuming mass action kinetics and Fickian diffusion [20], we can write the transport equations,

$$\frac{\partial [\text{Ca}^{2+}]}{\partial t} = D_{\text{Ca}} \nabla^2 [\text{Ca}^{2+}] - \sum_{m=1}^{m_{\text{max}}} R_{B_m} - \sum_{s=1}^{s_{\text{max}}} R_{B_s} + J_{\text{Ca}_{\text{max}}},$$

$$\frac{\partial [\text{Ca}B_m]}{\partial t} = D_{\text{Ca}B_m} \nabla^2 [\text{Ca}B_m] + R_{B_m},$$
\[ \frac{\partial [\text{CaB}_s]}{\partial t} = R_{B_s}, \]

\[ R_{B_m} = k_{\text{on}}^\text{m} ([B_m] - [\text{CaB}_m]) [\text{Ca}^{2+}], \]

\[ R_{B_i} = k_{\text{on}}^\text{i} ([B_i] - [\text{CaB}_i]) [\text{Ca}^{2+}], \]

where \([\text{Ca}^{2+}]\) is the intracellular \(\text{Ca}^{2+}\) concentration, \(k_{\text{on}}\) and \(k_{\text{off}}\) are the association and dissociation buffer rate constants, \([B]\) is the concentration of stationary buffer, \([B_m]\) is the concentration of mobile buffer, \(D_{\text{Ca}}\) and \(D_{\text{CaB}}\) are the diffusion constants for free \(\text{Ca}^{2+}\) and \(\text{Ca}^{2+}\) bound to mobile buffer, and \(I_{\text{Ca,in}}\) is the total \(\text{Ca}^{2+}\) flux via the cell membrane.

2.3. Numerical algorithms, software and meshing tools

In 3D finite-element methods, the geometry is discretized into polyhedrons, over which the local solution to the partial differential equations may be estimated. This process is usually referred to as mesh generation [21, 22]. Although different types of meshes may be generated depending on the numerical solvers to be employed, we restrict ourselves to triangular (surface) and tetrahedral (volumetric) mesh generation as commonly used in biomedical simulation (http://fetk.org/codes/gamer/index.html). Of particular interest are 3Dscalar volumes obtained by imaging techniques such as the 3D electron tomography (cellular level) and x-ray crystal structures (molecular level).

The time-dependent Smoluchowski equation and nonlinear reaction-diffusion system were solved by a finite-difference method in time and a finite-element method in space using our SMOL software tool. The SMOL program utilizes libraries from the finite-element tool kit (FEtK; http://FETK.org), which previously has been used in several molecular level studies [6–8, 23–25]. To extend SMOL to sub-cellular simulations, the reaction terms due to buffering were decoupled from the diffusion and flux boundary conditions defined in (4)–(8). Simulation results were visualized using OpenDX and GMV mesh viewers, whereas post-processing and data analyses were implemented by customized Python, MATLAB 2008b (MathWorks, Natick, MA) scripts and Xmgrace software [26].

3. Results and discussion

3.1. Continuum diffusion on the molecular level

This section demonstrates SMOL’s proficiency in the numerical modeling of diffusion-limited binding kinetics of a single enzyme molecule (in this particular case, mAChE) under various ionic strength conditions [6, 19]. Acetylcholinesterase is an enzyme that hydrolyzes ACh during neuron signaling. Under physiological conditions, the experimentally suggested association rate constant \((k_{\text{on}})\) is \(\sim 10^{12} \text{M}^{-1} \text{min}^{-1}\), which is close to the diffusion limit. Therefore, it is not easy to accurately measure \(k_{\text{on}}\). By numerically solving the Poisson–Boltzmann and time-dependent Smoluchowski equations, we calculated \(k_{\text{on}}\) under the influence of a molecular electrostatic field. The tetrahedral meshes in this numerical experiment were obtained from the inflated van der Waals-based accessibility data for the mAChE monomer and tetramers using the level-set boundary interior exterior-mesher (figure 1) [6].

The steady-state \(k_{\text{on}}\) value \((t > 10 \mu\text{s})\) for a charged ligand in a 0.0 M ionic strength solution was estimated to be \(9.56 \times 10^{11} \text{M}^{-1} \text{min}^{-1}\), which is consistent with the experimentally suggested value of \(9.8 \pm 0.6 \times 10^{11} \text{M}^{-1} \text{min}^{-1}\) [18] and the predicted value from the time-independent solution of the Smoluchowski equation [16]. Our model studies also indicate that the mAChE association rate was rapidly attenuated by increasing the ionic strength (ranging in this numerical experiment from 0.0 to 0.67 M), which supports the idea of electrostatically guided molecular diffusion [6]. We concluded that accurate estimation of the electrostatic potential and of the time to reach steady state are important factors in modeling the activity of enzymes and buffers in cellular volumes.

To further test the SMOL solver, recently we also applied it to study the drug molecular diffusion in wild-type neuraminidase and its mutants [27]. We observed quantitatively consistent trends compared with
Figure 1. Left to right: mAChE monomer; the molecular surface and outer sphere; magnification of the active site gorge; the tetrahedral mesh of the internal volume between the molecular surface and the outer sphere.

experimental measurements, although the predictions were an order of magnitude higher than experiment. This indicates that the finite size of the drug molecule, as well the conformational changes of the active site of the target protein, may be significant. These attributes are neglected in the current SMOL tool. To overcome this limitation, we are developing a new multi-scale algorithm to couple the SMOL continuum formulation with particle-based Brownian dynamics. The simulation of explicit particles naturally accommodates finite-size and conformational effects that may influence association rate constants. These studies also lay the foundation for integrating molecular-scale information into cellular-scale systems such as Ca$^{2+}$ signaling transduction in cardiac myocytes [9–11].

3.2. Continuum diffusion on sub-cellular level

In cardiac muscle cells, calcium (Ca$^{2+}$) is best known for its role in contraction activation [28]. A remarkable number of quantitative data on cardiac cell structure, ion-transporting protein function and distribution and intracellular Ca$^{2+}$ dynamics have become available [15, 28–30]. Alterations in myocyte ultra-structure and in protein function and distribution are now recognized to be the primary mechanisms of cardiac dysfunction in a diverse range of common pathologies including cardiac arrhythmias and hypertrophy [28].

3.2.1. Cardiomyocyte ionic model with realistic t-tubule geometry. In this paper, we used our published computational model with realistic transverse-axial t-tubule geometry and experimentally suggested ion-transporting protein distributions, to analyze several important spatial and temporal features of Ca$^{2+}$ signaling, buffering and diffusion in rat ventricular myocytes [11]. We considered a small compartment containing a single t-tubule and its surrounding half-sarcomeres (figure 2, middle panel). The t-tubule diameter varied from 0.19 to 0.469 µm and the t-tubule depth was 5.645 µm. The surrounding half-sarcomeres were modeled as a rectangular-shaped box of 2 µm × 2 µm in the plane of external sarcolemma and 5.96 µm in depth. Because the original t-tubule model did not include the realistic cell surface, one of the box faces (top red surface in figure 2) was assumed to be the external cell membrane [11]. To generate the high-fidelity and -quality tetrahedral meshes for a 3D t-tubular system, we used GAMer [22].

The overall scheme of the ionic model is shown in figure 2 (right panel). In this study, we examined Ca$^{2+}$ diffusion in rat cells that were treated with ryanodine and thapsigargin to eliminate the release and uptake of Ca$^{2+}$ by the sarcoplasmic reticulum (e.g. Ca$^{2+}$ fluxes via the ryanodine receptors and the SR Ca$^{2+}$ pumps blocked) [11]. At rest, Ca$^{2+}$ influx via Ca$^{2+}$ leak was adjusted to match Ca$^{2+}$ efflux via NCX; thus no net movement across the cell membrane would occur. The depolarization of the sarcolemma activates L-type Ca$^{2+}$ channels. The subsequent influx of Ca$^{2+}$ increases the intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$). Free Ca$^{2+}$ diffuse and react throughout the cytoplasm. The equations describing Ca$^{2+}$ fluxes via the t-tubule and surface membrane ($J_{Ca_{tub}} = J_{Ca} + J_{NCX} + J_{M-leak}$, where $J_{Ca}$ is LCC Ca$^{2+}$ influx, $J_{NCX}$ is NCX Ca$^{2+}$ flux and $J_{M-leak}$ is membrane Ca$^{2+}$ leak) were the same as those in Cheng et al (see also appendix). The generalized SMOL package was used to solve the nonlinear reaction-diffusion system defined in (4)–(8) [11]. Unless specified
also shows that strongly non-uniform Ca$^{2+}$ were computed from Cheng et al. The buffer Ca$^{2+}$$\approx$M SCH (76 ms) by 2.68-fold, SCH (100 ms) by 4.46-fold and SCH (200 ms) by 28.65-fold.

Moreover, the Ca$^{2+}$$\approx$M conditions \[\text{including calmodulin, myosin, TnC high-affinity sites and ADP}\] are controversial or unknown. In agreement with experiment \[the association rate \(k_{\text{on}}\), the model predicts that spatially uniform Ca$^{2+}$ \[\text{on}\] and rapid changes in the Ca$^{2+}$ occupancy of these sites can occur during the Ca$^{2+}$ transient \[fig.2\]. We assume otherwise in the figure legends or in the text, all initial conditions and values of the parameters that are not included in this paper correspond to those used in Cheng et al \[11\].

In agreement with experiment \[31\], the model predicts that spatially uniform Ca$^{2+}$ transients can be achieved with 100 \(\mu\)M Fluo-3 when total Ca$^{2+}$ flux \(J_{\text{Ca,flu}}\) was heterogeneously distributed along the sarcolemma (fig. 3). Figure 3 also shows that strongly non-uniform Ca$^{2+}$ signals are predicted in the absence of a fluorescent indicator. The surface plots in fig. 3 were computed from Cheng et al line-scan images (figures 4(F) and 6(F) in Cheng et al \[11\]). To delineate further the suggested spatial differences in \([\text{Ca}^{2+}]_i\) (see figures 3(A) and (B)), we introduced a quantity called ‘spatial Ca$^{2+}$ heterogeneity’ (SCH). The SCH is defined to be the difference of the maximal and minimal \([\text{Ca}^{2+}]_i\) values, normalized by the maximal value at a given reference point along the scanning line in a given moment \(t_j\) of interest. High SCH value suggests non-uniform \([\text{Ca}^{2+}]_i\); distribution and an SCH of zero indicates spatially uniform \([\text{Ca}^{2+}]_i\); distribution. The histogram in fig. 3(C) shows that in the absence of dye, SCH (10 ms) increased by 1.64-fold, SCH (70 ms) by 2.63-fold, SCH (76 ms) by 2.68-fold, SCH (100 ms) by 4.46-fold and SCH (200 ms) by 28.65-fold.

In this particular model, the effects of exogenous and endogenous Ca$^{2+}$ buffers (Fluo-3, ATP and troponin C) were considered. The buffer Ca$^{2+}$ dissociation constants \(K_{C\text{Fluo}}^{\text{CaFluo}} = 0.739 \, \mu\text{M}, \ K_{C\text{ATP}}^{\text{CaATP}} = 200 \, \mu\text{M} \) and \(K_{C\text{Tn}}^{\text{CaTn}} = 1 \, \mu\text{M}\) and on-rate constants \(k_{\text{onFluo}} = 0.23 \, \mu\text{M}^{-1} \text{ms}^{-1}, k_{\text{onATP}} = 0.225 \, \mu\text{M}^{-1} \text{ms}^{-1} \) and \(k_{\text{onTn}} = 0.04 \, \mu\text{M}^{-1} \text{ms}^{-1}\) used here are average values measured in different cardiac species under physiological conditions \[9–11\]. Theoretical estimates of \(k_{\text{onTn}}, k_{\text{onATP}}\) or \(k_{\text{onFluo}}\) rates in the literature, however, are lacking. Moreover, the Ca$^{2+}$-binding buffer constants for several other important buffers regulating cardiac cell cycle (including calmodulin, myosin, TnC high-affinity sites and ADP) are controversial or unknown \[12, 28\]. Thus, in the event that the association rate \(k_{\text{on}}\) is unknown, we assume a typical near-diffusion-limited on-rate value of 0.125 \(\mu\text{M}^{-1} \text{ms}^{-1}\) \[12\]. Hence, the computational estimation of these kinetic parameters presents an exciting opportunity to leverage our advanced molecular-level tools.

Two classes of Ca$^{2+}$-binding sites have been identified on cardiac TnC subunit (fig. 2, left panel): one low-affinity Ca$^{2+}$-specific site on the amino end of TnC (N-domain) and two high-affinity Ca$^{2+}$/Mg$^{2+}$ binding sites on the carboxy end of TnC (C-domain) \[28, 32\]. The high-affinity Ca$^{2+}$/Mg$^{2+}$ sites are saturated at resting \([\text{Ca}^{2+}]_i \approx 100 \, \text{nM}\). Therefore, in our model only the low-affinity Ca$^{2+}$-specific site was included since large and rapid changes in the Ca$^{2+}$ occupancy of these sites can occur during the Ca$^{2+}$ transient \[32\]. We assume

\begin{table}[h]
\centering
\begin{tabular}{lll}
  \hline
  \textbf{Troponin C} & \textbf{T-tubule} & \textbf{Ventricular myocyte} \\
  \hline
  0.1 nm - 10 nm & 100 nm - 10 \mu m & 10 \mu m - 100 \mu m \\
  \hline
\end{tabular}
\end{table}
Figure 3. Ca\textsuperscript{2+} signals in the presence (A) and absence of dye (B). Estimated SCH with 100 \(\mu\)M Fluo-3 and zero Fluo-3 (C). In (A) and (B), the line scan was positioned at 200 nm from the surface of the \(t\)-tubule. Featured spots along the scanning line: 17, 3.09 and 5.45 \(\mu\)m. Moments \(t_j\) of interest: 10 ms—L-type Ca\textsuperscript{2+} current peak \((t_{I_{Ca-peak}})\); 70 ms—duration of L-type Ca\textsuperscript{2+} current \((t_{I_{Ca-max}})\); 76 ms—[Ca\textsuperscript{2+}]\textsubscript{i} peak \((t_{[Ca^{2+}]_i-peak})\); 100 and 200 ms—relaxation times for [Ca\textsuperscript{2+}]\textsubscript{i} \((t_{100\,ms}, t_{200\,ms})\).

also that TnC is immobile because it is attached to the thin filament [28]. Little is known, however, about how alterations in TnC on- and off-rates in the whole Tn complex (TnC, TnI, TnT) modulate the spatial and temporal features of the Ca\textsuperscript{2+} signaling, buffering and diffusion in rats. Therefore, our goal here was to examine these effects from a modeling perspective. In this study, we used published experimental measurements for \(k_{CaTn}^{\text{on}}\) and \(k_{CaTn}^{\text{off}}\) because currently at the molecular scale we do not yet have theoretical estimates as in the case of mAChE monomer on-rate [6].

3.2.1.1 Effects of changes in Ca\textsuperscript{2+} on-rate for TnC in the whole Tn complex on local Ca\textsuperscript{2+} signals. A recent study has clarified the role of a specific, disease-associated mutation of the TnC regulatory domain in altered Ca\textsuperscript{2+} binding, signal transmission and myocyte contractile dysfunction in rats [33]. Lim and collaborators, by combining targeted expression/integration of recombinant mutant TnC in cardiomyocytes and in vitro functional analysis, found a decrease in myofilament Ca\textsuperscript{2+} sensitivity and Ca\textsuperscript{2+} binding affinity. It is difficult, however, to derive from the Lim \textit{et al} studies an understanding of how changes in the Ca\textsuperscript{2+} on-rate for TnC \((k_{CaTn}^{\text{on}})\) modulate spatial Ca\textsuperscript{2+} distribution in rats. Our studies imply that in the absence of dye with 70 \(\mu\)M
TnC (see figure 4(A)), inflated \( k_{\text{CaTn}} \) values tend to decrease SCH within the initial 10 ms of simulation, during which the calcium concentration ([Ca\(^{2+}\))] is low. SCH, however, increases at the total [Ca\(^{2+}\)], beyond 10 ms.

Measurements have demonstrated also that the total TnC concentration ([TnC]\(_{\text{tot}}\)) can range from 30 to 150 \( \mu \)M [28, 32]. With this in mind, we computed SCH decreasing or increasing [TnC]\(_{\text{tot}}\). Figures 4(A)–(C) show that at \( t_{\text{Ca-peak}} \) (10 ms) 35 \( \mu \)M [TnC]\(_{\text{tot}}\) enhanced SCH, whereas 140 \( \mu \)M [TnC]\(_{\text{tot}}\) tends to decrease SCH as predicted with 70 \( \mu \)M [TnC]\(_{\text{tot}}\). Interestingly, with \( k_{\text{CaTn}} \) ranging from 0.02 to 0.06 \( \mu \)M\(^{-1}\) ms\(^{-1}\): (i) beyond 10 ms [Ca\(^{2+}\)] was more uniformly distributed with 35 \( \mu \)M [TnC]\(_{\text{tot}}\) than was predicted for 70 \( \mu \)M [TnC]\(_{\text{tot}}\); (ii) 140 \( \mu \)M [TnC]\(_{\text{tot}}\) also decreased SCH (70 \( \mu \)M [TnC]\(_{\text{tot}}\) at \( t_{\text{Ca-max}} \), \( t_{\text{Ca-peak}} \) and \( t_{100\text{ms}} \), whereas at 200 ms SCH remained almost unchanged. Additional model findings are that with 35 \( \mu \)M [TnC]\(_{\text{tot}}\) SCH increased at the total [Ca\(^{2+}\)] beyond 10 ms (figure 4(B)), while the changes in \( k_{\text{CaTn}} \) slightly affected the predicted SCH (70, 76, 100 and 200 ms) when control [TnC]\(_{\text{tot}}\) increased 2-fold (figure 4(C)). Taken together, our numerical work and analyses suggest that the disease-linked changes in \( k_{\text{CaTn}} \) (respectively in \( K_{\text{CaTn}} = k_{\text{off}} / k_{\text{on}} \)) and the alterations in [TnC]\(_{\text{tot}}\) level at low free [Ca\(^{2+}\)] (SR activity disabled) alter the effective diffusion coefficient for free Ca\(^{2+}\) (\( D_{\text{eff}} \)) [8, 9]. The changes in \( D_{\text{eff}} \) will affect local flow rates of membrane Ca\(^{2+}\) transporters and local Ca\(^{2+}\) trigger fluxes (\( d[Ca^{2+}]_i/dt \)) controlling SR Ca\(^{2+}\) release and ultimately the normal myocyte function [33].

**Figure 4.** (A–C) Estimated spatial Ca\(^{2+}\) heterogeneity index with respect to ±25% and ±50% changes in control Ca\(^{2+}\) on-rate for TnC (\( k_{\text{CaTn}} = 0.04 \mu \text{M}^{-1} \text{ms}^{-1} \), assuming the basic \( k_{\text{CaTn}} \) value of 0.04 ms\(^{-1}\) is unchanged) in the absence of dye. In panels (A)–(C), Ca\(^{2+}\) flux was heterogeneously distributed, line scan positioned at 200 nm away from the \( t \)-tubule membrane and the featured spots along the scan line were chosen to be the same as in figure 3.
3.2.1.2 Effects of changes in Ca\(^{2+}\) off-rate for TnC in whole Tn complex on local Ca\(^{2+}\) signals. Kreutziger and colleagues introduced site-directed mutations in the N-terminus of rat TnC (L48Q TnC, I61Q TnC) that altered the control Ca\(^{2+}\) off-rate value [34]. Using stopped-flow spectroscopy, they measured Ca\(^{2+}\) dissociation rates from the whole troponin complex containing recombinant WT TnI and WT TnT and any one among WT TnC, L48Q TnC and I61Q TnC (in the paper, Ca\(^{2+}\) off-rates are denoted as \(k_{\text{CaTn} \text{off}}\), \(k_{\text{CaTn} \text{off(L48Q)}}\) or \(k_{\text{CaTn} \text{off(I61Q)}}\), respectively). These experiments demonstrated that the mutations in TnC altered the basic \(k_{\text{CaTn} \text{off}}\) value, reducing it by 75% for L48Q TnC and increasing it by 3.2-fold for I61Q TnC. It is difficult, however, to derive from these studies an understanding of how these site-directed mutations in TnC (e.g. altered \(k_{\text{CaTn} \text{off}}\), \(K_{\text{CaTn}}\) and \(D_{\text{Ca}}^\text{eff}\)) modulate the cytosolic Ca\(^{2+}\) dynamics that ultimately will affect the normal cell function. Here, we used our 3D reaction-diffusion model to investigate this. Figure 5(A) shows that with 70 \(\mu M\) [TnC]\(_\text{tot}\): (i) both the alterations in \(k_{\text{CaTn} \text{off}}\) enhanced SCH at \(t_{\text{I_{Ca}-peak}}\) (10 ms); (ii) at \(t_{\text{I_{Ca}-max}}\) (70 ms), \(t_{[\text{Ca}^{2+}]_\text{r-peak}}\) (76 ms) and \(t_{200\text{ms}}\)
Figure 6. Estimated spatial Ca\(^{2+}\) heterogeneity index for all TnC variants in whole Tn complex (A) and reconstructed thin filaments (B) in the absence of dye. Panel (A): \(k_{\text{on}}^{\text{CaTn}} = 0.0297\ \text{ms}^{-1}\), \(k_{\text{off}}^{\text{CaTn}}\) = 0.0073 ms\(^{-1}\) and \(k_{\text{off}(I61Q)}^{\text{CaTn}} = 0.067\ \text{ms}^{-1}\). Panel (B): \(k_{\text{off}(L48Q)}^{\text{CaTn(TF)}} = 0.028\ \text{ms}^{-1}\) and \(k_{\text{off}(I61Q)}^{\text{CaTn(TF)}} = 0.2377\ \text{ms}^{-1}\). In this set of simulations: \(k_{\text{on}}^{\text{CaTn}}\) value was 0.04 \(\mu\text{M}^{-1}\\text{ms}^{-1}\); Ca\(^{2+}\) flux was heterogeneously distributed; the line scan positioned at 200 nm from the surface of the \(\tau\)-tubule; reference points along the scanned line were chosen to be the same as in figure 3.

Inflated \(k_{\text{off}(I61Q)}^{\text{CaTn}}\) decreased SCH, while no visible differences in SCH were found when the control \(k_{\text{off}}^{\text{CaTn}}\) was reduced by 75% (L48Q Tn mutation); (iii) at 100 ms both the alterations in \(k_{\text{off}}^{\text{CaTn}}\) decreased SCH.

The model also predicts that at \(I_{\text{Ca-peak}}\) the decrease in control TnC buffer capacity to 35 \(\mu\text{M}\) increased SCH, whereas 140 \(\mu\text{M}\) [TnC]\(_{\text{tot}}\) decreased SCH for all TnC variants (figures 5(A)–(C)). In addition, figure 5(B) demonstrates that with 35 \(\mu\text{M}\) [TnC]\(_{\text{tot}}\), SCH decreased at the total [Ca\(^{2+}\)]\(_{\text{tot}}\) beyond 10 ms. The predicted changes in SCH beyond 10 ms with 140 \(\mu\text{M}\) [TnC]\(_{\text{tot}}\) are shown in figure 5(C). This set of simulations demonstrates that: (i) at 70 and 76 ms, inflated \(k_{\text{off}(I61Q)}^{\text{CaTn}}\) increased SCH, while [Ca\(^{2+}\)]\(_{\text{tot}}\) was more uniformly distributed when \(k_{\text{off}}^{\text{CaTn}}\) was decreased (L48Q Tn mutation); (ii) at 100 ms no visible differences in SCH were found when \(k_{\text{off}}^{\text{CaTn}}\) was increased 2.3-fold (I61Q Tn mutation), while a 75% drop in the basic \(k_{\text{off}}^{\text{CaTn}}\) (L48Q Tn mutation) decreased SCH notably; (iii) at 200 ms both the alterations in \(k_{\text{off}}^{\text{CaTn}}\) tended to decrease the SCH. In summary, our results demonstrate that the mutation-linked changes in \(k_{\text{off}}^{\text{CaTn}}\) (in \(k_{\text{D}}^{\text{CaTn}}\), respectively) and the variations in [TnC]\(_{\text{tot}}\) levels alter control \(D_{\text{Ca}}^{\text{CaTn}}\) thereby altering the local sarcoglemmal fluxes, SR Ca\(^{2+}\) trigger fluxes and local [Ca\(^{2+}\)]\(_{i}\), inside the cell with SR disabled. The model also demonstrates that the alterations in \(k_{\text{off}}^{\text{CaTn}}\) may affect quite differently local Ca\(^{2+}\) distributions ([Ca\(^{2+}\)]\(_{i}\) and d[Ca\(^{2+}\)]\(_{i}\)/dt) with respect to those when control \(k_{\text{on}}^{\text{CaTn}}\) is varied (compare figures 4 and 5).

In Kreutziger et al’s experiment, \(k_{\text{off}}^{\text{CaTn}}\) was determined for each Tn \(k_{\text{off}}^{\text{CaTn}} = 0.0297\ \text{ms}^{-1}\); \(k_{\text{off}(L48Q)}^{\text{CaTn}} = 0.0073\ \text{ms}^{-1}\); \(k_{\text{off}(I61Q)}^{\text{CaTn}} = 0.067\ \text{ms}^{-1}\) by fitting fluorescence data (at 15 °C) with exponential curves [34]. We then calculated SCH replacing the model \(k_{\text{off}}^{\text{CaTn}}\) values (0.01 ms\(^{-1}\) L48Q Tn, 0.04 ms\(^{-1}\) WT Tn and 0.092 ms\(^{-1}\) I61Q Tn) with the experimentally measured values. However, only minor effects in predicted SCH were found with 70 \(\mu\text{M}\) [TnC]\(_{\text{tot}}\) (compare figures 5(A) and 6(A)).

### 3.2.1.3 Effects of changes in Ca\(^{2+}\) off-rate for TnC in reconstructed thin filaments on local Ca\(^{2+}\) signals

Recently, Kreutziger et al [34] measured the off-rates in reconstructed thin filaments with WT or mutant whole TnC, WT Tm and WT actin (in the paper these Ca\(^{2+}\) off-rates are denoted as \(k_{\text{off}(L48Q)}^{\text{CaTn(TF)}}\), \(k_{\text{off}(I61Q)}^{\text{CaTn(TF)}}\) respectively). They found 2.2–3.4-fold increases in off-rates for all TnC variants versus the isolated whole Tn complex. The relative effect of the mutations in TnC remained almost the same as for whole Tn, such that \(k_{\text{off}(L48Q)}^{\text{CaTn(TF)}}\) was reduced by 63% for L48Q (versus 75% in isolated Tn complex) and increased by 3.2-fold for I61Q Tn in the thin filaments. It remains unclear, however, whether the changes in off-rates in reconstructed...
thin filaments would have a different effect on Ca\(^{2+}\) dynamics relative to those predicted with the isolated whole Tn complex. Our numerical results demonstrate that with 70 \(\mu\)M [TnC]\(_{\text{tot}}\) visible differences in the calculated SCH values compared to those in isolated whole Tn complex were found. At \(I_{\text{Ca}}\) peak (10 ms) a \(\sim\)2.5-fold increase in WT variant had little or no effect on SCH, a \(\sim\)3.85-fold increase in L48Q variant decreased SCH, while a \(\sim\)3.55-fold increase in I61Q variant increased SCH (figures 6(A) versus 6(B)).

Figures 5 and 6 also show that at the moment of \(I_{\text{Ca}}\) closing (70 ms) and at [Ca\(^{2+}\)]\(_i\) peak (76 ms), SCH decreased for WT and I61Q variants versus the whole Tn complex. The model also predicts that: (i) at \(t_{100\,\text{ms}}\) \(\text{SCH}(k_{\text{off}}^{\text{CaTn(TF)}}) \sim\text{SCH}(k_{\text{off}}^{\text{CaTn(TF)}})\), \(\text{SCH}(k_{\text{off}}^{\text{CaTn(L48Q)}}) < \text{SCH}(k_{\text{off}}^{\text{CaTn(L48Q)}})\) and \(\text{SCH}(k_{\text{off}}^{\text{CaTn(I61Q)}}) > \text{SCH}(k_{\text{off}}^{\text{CaTn(I61Q)}});\) (ii) at \(t_{200\,\text{ms}}\) \(\text{SCH}(k_{\text{off}}^{\text{CaTn(TF)}}) > \text{SCH}(k_{\text{off}}^{\text{CaTn(TF)}})\), \(\text{SCH}(k_{\text{off}}^{\text{CaTn(L48Q)}}) \sim \text{SCH}(k_{\text{off}}^{\text{CaTn(L48Q)}})\) and \(\text{SCH}(k_{\text{off}}^{\text{CaTn(I61Q)}}) > \text{SCH}(k_{\text{off}}^{\text{CaTn(I61Q)}})\). These findings suggest that the use of whole Tn off-rates versus reconstructed thin filament off-rates into cardiac cellular and sub-cellular models may alter the predicted control and disease-linked Ca\(^{2+}\) transporter rates, Ca\(^{2+}\) trigger fluxes for SR release and local Ca\(^{2+}\) signals.

In summary, our studies lay the foundations for a more comprehensive understanding of signaling transduction at molecular and sub-cellular scales. Our studies also illustrate the importance of improved experimental measurements of Ca\(^{2+}\) buffering kinetics and of complementary theoretical estimates of Ca\(^{2+}\) buffer reaction rates. Together, this will lead to a better understanding of Ca\(^{2+}\) signaling in the heart under normal or pathological conditions.

4. Conclusions

In this paper, we applied the SMOL package to molecular-level reaction kinetics of ACh and cellular-level Ca\(^{2+}\) dynamics. Our data demonstrate that a continuum-based description of ion diffusion, the Smoluchowski equation, captures molecular-level details impacting reaction rates. This suggests that alternative descriptions of electrodynamics, such as the Poisson–Nernst–Planck equation, could further improve our estimates. These data also demonstrate progress toward sub-cellular modeling of the heart, in which buffering dynamics are handled at a molecular level. Future applications of SMOL will feature spatially coupled 3D structural and functional models of ventricular cardiac myocytes with (i) realistic sub-cellular anatomical structures, such as Ca\(^{2+}\)-signaling micro-domains and cell organelles; (ii) sarcoplasmic reticulum fluxes via ryanodine receptors (RyR) and the SERCA pump; and (iii) spatial and temporal scales spanning from single-channel ion fluxes to tension development in actin and myosin filaments. These developments would facilitate modeling excitation–contraction coupling influenced by mutations, phosphorylation and alterations in protein expression. Other molecular-level approaches that could further benefit excitation–contraction facilitation include modeling excitation–contraction coupling influenced by mutations, phosphorylation and alterations in protein expression. Our studies also illustrate the importance of improved experimental measurements of Ca\(^{2+}\) buffering kinetics and of complementary theoretical estimates of Ca\(^{2+}\) buffer reaction rates. Together, this will lead to a better understanding of Ca\(^{2+}\) signaling in the heart under normal or pathological conditions.

Acknowledgments

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Appendix

Equations describing total Ca\(^{2+}\) flux \(J_{\text{Caflux}}\), L-type Ca\(^{2+}\) current \(J_{\text{Ca}}\), Na\(^{+}\)/Ca\(^{2+}\) exchanger flux \(J_{\text{NCX}}\) and Ca\(^{2+}\) leak current \(J_{\text{M-leak}}\) throughout the t-tubule and external membrane:

\[
J_{\text{Caflux}} = J_{\text{Ca}} + J_{\text{NCX}} + J_{\text{M-leak}},
\]

\[
I_{\text{Ca}}(t) = I_{\text{Ca}} f(t)
\]
Table 1. Membrane calcium fluxes parameters

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I_{Ca}$</td>
<td>Constant</td>
<td>1</td>
<td>[9]</td>
</tr>
<tr>
<td>$t_a$</td>
<td>Constant</td>
<td>4 ms</td>
<td>[40]</td>
</tr>
<tr>
<td>$t_b$</td>
<td>Constant</td>
<td>70 ms</td>
<td>[40]</td>
</tr>
<tr>
<td>$I_{Na/Ca}$</td>
<td>Extracellular Na$^+$ concentration</td>
<td>140 mM</td>
<td>[28]</td>
</tr>
<tr>
<td>$[Na^+]_i$</td>
<td>Resting Na$^+$ concentration</td>
<td>10 mM</td>
<td>[28]</td>
</tr>
<tr>
<td>$g_{NCX}$</td>
<td>Pump rate of NCX</td>
<td>38.5 µM ms$^{-1}$</td>
<td>[41]</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Voltage dependence of NCX control</td>
<td>0.35</td>
<td>[41]</td>
</tr>
<tr>
<td>$k_{m,Na}$</td>
<td>Na$^+$ half saturation of NCX</td>
<td>87.5 mM</td>
<td>[41]</td>
</tr>
<tr>
<td>$k_{m,Ca}$</td>
<td>Ca$^{2+}$ half saturation of NCX</td>
<td>1380 µM</td>
<td>[41]</td>
</tr>
<tr>
<td>$k_{sat}$</td>
<td>Low potential saturation factor of NCX</td>
<td>0.1</td>
<td>[41]</td>
</tr>
</tbody>
</table>

Membrane Ca$^{2+}$ leak

$g_{M-leak}$ | Conductance | 3.4e – 6 µM mV$^{-1}$ ms$^{-1}$ | Estimated |

\[
I(t) = \begin{cases} 
1.14380t, & 0 < t < t_a, \\
1.73189 + 5.11444 e^{-t_a/T}, & t_a \leq t < t_b, \\
0, & t \geq t_b,
\end{cases}
\]  
(A.3)

\[
I_{NCX} = g_{NCX} \frac{e^{\eta V_F / RT} [Na^+]_i^3 [Ca^{2+}]_e - e^{(\eta - 1) V_F / RT} [Na^+]_i^3 [Ca^{2+}]_i}{(k_{m,Na}^3 + [Na^+]_e)(k_{m,Na} + [Ca^{2+}]_e)(1 + k_{sat} e^{(\eta - 1) V_F / RT})},
\]  
(A.4)

\[
I_{M-leak} = g_{M-leak} ([Ca^{2+}]_e - [Ca^{2+}]_i).
\]  
(A.5)

Flux parameter values were estimated or taken from the literature (see table 1). In this study, the Ca$^{2+}$ leak is not actually a particular ‘leak protein’. The Ca$^{2+}$ leak was included and adjusted so that at rest Ca$^{2+}$ influx via Ca$^{2+}$ leak matches Ca$^{2+}$ efflux via NCX and thus no net movement across the cell membrane occurs.

In the model, each current density ($I_i$) was converted to Ca$^{2+}$ flux ($J_i$) by using the experimentally suggested surface to volume ratio ($C_m / V_{cell} \sim 8.8$ pF/pL) in adult rat ventricular myocytes [39]:

\[
J_i = \left( \frac{1}{2 F V_{cell}} \right) I_i.
\]  
(A.6)

The voltage-clamp protocol (holding potential –50 mV; electric pulse of 10 mV for 70 ms) and whole-cell L-type Ca$^{2+}$ current were derived from the Zahradnikova et al data with the blocked SR activity [40].

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