Published in final edited form as:

Curr Opin Struct Biol. 2014 April ; 0: 92–97. doi:10.1016/j.sbi.2014.01.006.

Computational Modeling of Subcellular Transport and Signaling

Johan Hake^a, Peter M Kekenus-Huskey^b, and Andrew D. McCulloch^c

^aCenter for Biomedical Computing, Simula Research Laboratory, P.O. Box 134, 1325 Lysaker, Norway

^bHoward Hughes Medical Institute, University of California San Diego, CA, USA

^cDepartment of Bioengineering and Medicine, University of California San Diego, CA, USA

Abstract

Numerous signaling processes in the cell are controlled in microdomains that are defined by cellular structures ranging from nm to μm in size. Recent improvements in microscopy enable the resolution and reconstruction of these micro domains, while new computational methods provide the means to elucidate their functional roles. Collectively these tools allow for a biophysical understanding of the cellular environment and its pathological progression in disease. Here we review recent advancements in microscopy, and subcellular modeling based on reconstructed geometries, with a special focus on signaling microdomains that are important for the excitation contraction coupling in cardiac myocytes.

Keywords

Cell signaling; Microscopy; 3D reconstructions; Subcellular models; Molecular models; Excitation contraction coupling

Introduction

Recent advancements in electron microscopy (EM) and light microscopy have made it possible to resolve cellular micro-anatomic structures in great detail. 3D computational models leverage new structural data to examine their role in cell signaling, and their involvement in disease. While structural data are vital to computational modeling, the localization of key proteins as well as the kinetics of signaling reactions and second messenger fluxes are equally important. An example of a signaling microdomain in cardiac cells is the Ca^{2+} release unit (CRU) that drives excitation contraction coupling (ECC). At each CRU, electrical depolarization of the cell membrane during the cardiac action potential, drives a transient intracellular Ca^{2+} release event, known as a *spark* [1]. The coordinated and integrated release of Ca^{2+} from several thousand CRUs gives rise to the whole cell Ca^{2+}

© 2014 Elsevier Ltd. All rights reserved.

Correspondence to: Johan Hake.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

transient that triggers myofilament activation and contraction; computational models have been crucial to uncovering the quantitative determinants of this process [2].

Continued improvements in 3D microscopy and computational models are enabling a renaissance in the analysis of intracellular signaling events in near molecular detail [3]. Further integration of high-resolution bio-imaging and advanced modeling techniques will shepherd a fundamental understanding of cellular signaling enabled by the collective behavior of myriad molecular players. Here we review recent methods for subcellular modeling based on reconstructed geometries, with a special focus on signaling microdomains that are important for the ECC in cardiac myocytes.

High-Resolution Microscopy Techniques for Structural Imaging

Recent improvements in electron and light microscopy have increased the availability of structural data involved in sub cellular signaling. [4–6]. EM tomography is well-suited for identifying 3D nano-structures such as organelles and membrane systems [4]. The pixel resolution can approach $\simeq 1$ nm but the total sample size is limited to a few μm in the xy-plane and some 100 nm in the z-direction [4]. Different automated acquisition techniques can be applied to increase the volume, but at the cost of reduced resolution in one or more of the principal axes [7, 8]. To identify position of single channels, EM can be combined with immunolabeling techniques, but the staining percentage is low and labeling is limited to the cell surface [9]. A new labeling approach, mini Singlet Oxygen Generator, expresses genetically-encoded fluorescent labels in live cells and may expand the range of resolvable sub cellular structures [10].

Conventional confocal light microscopy (LM) can also be used to extract 3D features, but the resolution ($\simeq 100\text{nm}$) is not as good as in EM tomography [6]. With super resolution LM novel registration techniques can be used to bypass the diffraction limit of light, making it possible to identify features down to 20-nm resolution [5]. By correlating light and electron microscopy, co-labeling from LM can be used to complement the structural data from EM tomography and augment subcellular structural models with protein localization data [11].

3D computational geometries from microscopy data

Computational models that integrate sub cellular structural data often rely on meshes that denote surface topology or cellular volumes. In general, the mesh construction procedure begins with *segmentation* of individual image frames into regions of interest. Segmentation is either done manually, or by using automated or semi automated procedures [12], or even *crowd-sourcing* [13]. Automated procedures are attractive as they facilitates mass processing of large microscopy data sets. However these methods are highly dependent on contrast and resolution, and may ultimately require some level of manual segmentation [8].

The segmented 3D model is suitable for visualization and for measuring quantities such as distances, areas and volumes, however the quality of the mesh is generally too poor for numerical simulations. This motivates the need for mesh refinement tools that render segmented 3D models into high quality meshes [14–17]. The surface mesh improvement library, GAMer, has enabled sub cellular simulations of Ca^{2+} signaling in cardiomyocytes

[18–21] [22]. GAMer is a light weight C-library providing smoothing and coarsening algorithms suitable for improving the quality of surface meshes. The library is wrapped in Python, making it possible to be used as a plug-in in other mesh-manipulation visualization tools such as Blender (www.blender.org). Such visualization tools allow annotation of functionally-important regions in the surface mesh, e.g the localization of subcellular fluxes important for the computational models. MCell, a particle-based simulation tool, which also use Blender as a GUI, provides a number of surface mesh improvement methods of its own. In a recent study where the precise volume and structure of the extracellular volume were important, a method for correcting overlapping and incorrect segmented contours was developed [23].

Instead of generating a computational mesh, necessary for FEM or particle-based simulations, segmented images can be used directly in software packages such as VirtualCell [24]. Here subcellular regions reconstructed from imaging data can be integrated via a GUI with complex reaction schemes coupled to compartmental models. An advantage of the compartmentalized models supported by VirtualCell is the ease of building complex, but well constrained models that are informed by experimental data [25].

An Example from Cardiac Myocyte Excitation-Contraction Coupling

With early 2D EM images it was possible to estimate 3D structural properties such as the size and distribution of CRUs, which guided the construction of numerous of different subcellular models of intracellular Ca^{2+} cycling [2, 26]. These 3D metrics were estimated by extrapolating geometric data from serial 2D images. By assuming a regular, e.g. circular, geometry the extrapolation often overestimated the size of CRUs and the distances between them [27]. Similarly, basic assumptions of RyR packing density within each CRU led to overestimates of the number of RyR per dyad [26]. With the use of 3D EM tomography, more refined structural information of the CRU became available, revising our understanding of the CRU size and distribution [27]. The more detailed 3D measurements suggested that CRU are smaller and much more densely distributed than previously assumed, which was further confirmed by super resolution LM [28]. These new geometric observations were integrated into computational models leading to a refinement of our understanding of a single release event [29, 30]. However, these studies used a reconstructed 3D geometry and by reducing the CRU to a single compartment connected to a lumped cytosolic compartment, important structural details were lost from the analyses.

Using publicly available EM tomograms from ventricular cardiomyocytes Hake *et al* [18] reconstructed a 3D model of a single CRU unit (see Figure 1A). The EM tomogram was a few μm in size in the xy-plane but only $0.43 \mu\text{m}$ thick, and contained a single intact CRU with detailed structures of neighboring Sarcoplasmic reticulum (SR), mitochondria and transverse tubules (t-tubule) (see Figure 1B). By explicitly modeling a Ca^{2+} sensitive-dye inside the SR, we established a correlation between local Ca^{2+} movement inside the SR with functional measurements [31]. The model predicted near total junctional Ca^{2+} depletion after the spark while keeping a regional Ca^{2+} reserve, reconciling previous model predictions with experimental measurements [31, 32].

A single t-tubule hosts several CRUs and facilitates the spread of excitation throughout the myocyte. Using reconstructed geometries of t-tubules based on LM data from rat [21] and rabbit [19], effects of geometrical variations on single t-tubules were investigated. In smaller mammals like mice and rats, t-tubules are branched and excitation follows a more complex pattern, which contrasts with the more linear t-tubules in higher mammals such as rabbits (see Figure 2A and B). By embedding a model of reconstructed t-tubules geometry into a coarser model of the whole cell Yu *et al* [20] constructed a multilevel model of the early stage of the ECC. Here, the coarser model provided boundary conditions to simulations on the reconstructed geometry.

Spatially detailed subcellular ECC models using qualitative structural representation have been a useful complement to simulations with reconstructed geometries. Hatano *et al* [33] for instance combined a reaction-diffusion model of ECC and energy production in the mitochondria, with a mechanical model for contraction. Similarly, a CRU geometry from EM tomography was used in a recent model of a Ca^{2+} spark [34] to suggest a novel mechanism for spark termination, where the geometry of the CRU played a crucial role [35]. Using RyR distribution data from LM, Soeller *et al* [36] demonstrated that RyR puncta are distributed in a staggered fashion along the z-disks, which together with diffusion simulations could explain the anisotropic diffusion measured experimentally.

Combining discrete atomistic models with continuous models

The cell cytosol is crowded with proteins and other species that influence substrate diffusion through electrostatic, van der Waals and hydrodynamic interactions [37]. As a first step towards accounting for these influences in a model of ECC, Tanskanen *et al* [38] used a particle-based Monte Carlo method with nanometer resolution of the CRU. A simple box geometry was used to represent the geometry, but cryo-EM reconstructions of the channel proteins were included as well as the electrostatic potential (ESP), owing to the charged sarcolemmal lipid bilayer. It was shown that the protein structures and the ESP both impacted the effectiveness of the ECC. Similarly it was shown that densely-packed myofibril lattice with high resolution details of the filament proteins are important for accurate predictions of subcellular diffusion [39, 40]. By applying homogenization methods to a generated 2D geometry of the myofilaments experimentally-observed anisotropic diffusion coefficient could be explained [39]. Using a reconstructed 3D geometry of the thin filament from cryo-EM (see Figure 3), the estimated diffusion coefficient was recently refined [40]. Progress toward accounting for the ESP surrounding a protein was reported in [41], where electrostatic interactions were found to accelerate Ca^{2+} association rates to Troponin C and SR Ca^{2+} -ATPase (SERCA) by orders of magnitude. The rate was, however, reduced upon including the proteins in its native environment, thin filament and lipid-bilayer, respectively.

Proteins are not only passive bystanders in subcellular modeling they also serve specific functional roles through conformational changes. Silva *et al* [42] were among the first to incorporate the structural changes in the potassium pump that were involved in the gated K^+ current. By Monte Carlo sampling of the movement of the voltage gate helix, they quantified the free energy barriers for transitions between the proteins functional states. The

barriers were then used to determine the rates in a Markov model of the ion channel. An alternative to this approach was recently published by the same lab [43] in which new governing equations were presented, leading to better predictions of channel openings. Extensive studies linking molecular dynamics (MD) simulations of the KcsA K⁺ channel to its selectivity for K⁺ relative to other endogenous cations were reviewed by Roux *et al* [44] and provide compelling evidence that ionic conduction can be predicted from first principles via MD. Several approaches have incorporated finite-volume, diffusing particles in the simulation of proteins through Brownian dynamics (BD) methods. Recent examples include BD predictions of diffusion-limited Ca²⁺ binding to TnC [45, 46] and SERCA [47], as well as current-voltage relationships for K⁺ diffusion through the inwardly-rectifying potassium channel [48]. Given the computational expense of explicit particle simulations, efforts to combine continuum and discrete simulations could potentially offer the best of both worlds, see for example Bauler *et al* [49].

Conclusion

The convergence of new microscopy methodologies and multi-scale modeling techniques is offering the opportunity to help elucidate the functional roles of cellular microdomains in numerous important signaling processes. The ability to directly image near atomistic resolution protein structural data has the potential to unwind the molecular basis of biological function and disease. Equally important are the modeling tools that can bridge a breadth of imaging techniques examining disparate time and length scales. In recent years, exciting progress has been made on both fronts, as well as techniques to couple the tools.

Acknowledgments

This work was supported by NIH grants (ADM) from the National Institute for General Medical Sciences and the National Heart Lung and Blood Institute and by AHA award 13POST14510036 (PKH). The work was also partially supported by the Evita programme of the Research Council of Norway, Center of Excellence grant from the Research Council of Norway to the Center for Biomedical Computing at Simula Research Laboratory (JH).

References

1. Cheng H, Lederer WJ. Calcium sparks. *Physiol Rev.* 2008; 88(4):1491–1545. [PubMed: 18923188]
2. Williams GSB, Smith GD, Sobie EA, Jafri MS. Models of cardiac excitation-contraction coupling in ventricular myocytes. *Math Biosci.* 2010; 226(1):1–15. [PubMed: 20346962]
3. Michailova, AP.; Edwards, AG.; Hake, J.; Hoshijima, M.; McCulloch, AD. Calcium Signaling in Cardiomyocyte Models With Realistic Geometries. Elsevier; 2013. *Cardiac Electrophysiology: From Cell to Bedside*, chap. 33; p. 33.1–33.10.
4. Das T, Hoshijima M. Adding a new dimension to cardiac nano-architecture using electron microscopy: Coupling membrane excitation to calcium signaling. *J Mol Cell Cardiol.* 2013; 58:5–12. [PubMed: 23201225]
5. Soeller C, Baddeley D. Super-resolution imaging of EC coupling protein distribution in the heart. *J Mol Cell Cardiol.* 2013; 58:32–40. [PubMed: 23159441]
6. Savio-Galimberti E, Frank J, Inoue M, Goldhaber JJ, Cannell MB, Bridge JHB, Sachse FB. Novel features of the rabbit transverse tubular system revealed by quantitative analysis of three-dimensional reconstructions from confocal images. *Biophys J.* 2008; 95(4):2053–2062. [PubMed: 18487298]
7. Briggman KL, Bock DD. Volume electron microscopy for neuronal circuit reconstruction. *Curr Opin Neurobiol.* 2012; 22(1):154–161. [PubMed: 22119321]

8. Pinali C, Bennett H, Davenport JB, Trafford AW, Kitmitto A. 3-D Reconstruction of the Cardiac Sarcoplasmic Reticulum Reveals a Continuous Network Linking T-Tubules: This Organization is Perturbed in Heart Failure. *Circulation Research*. 2013 published online ahead of print. . • This paper uses automated segmentation methods to reconstruct the whole sarcoplasmic reticulum (SR) from a healthy and failing cardiomyocytes from serial block face electron microscopy data, showing that SR is disrupted in failing myocytes.
9. Ladinsky MS, Howell KE. Electron tomography of immunolabeled cryosections. *Methods Cell Biol*. 2007; 79:543–558. [PubMed: 17327173]
10. Shu X, Lev-Ram V, Deerinck TJ, Qi Y, Ramko EB, Davidson MW, Jin Y, Ellisman MH, Tsien RY. A genetically encoded tag for correlated light and electron microscopy of intact cells, tissues, and organisms. *PLoS Biol*. 2011; 9(4):e1001041. [PubMed: 21483721]
11. Wong J, Baddeley D, Bushong EA, Yu Z, Ellisman MH, Hoshijima M, Soeller C. Nanoscale Distribution of Ryanodine Receptors and Caveolin-3 in Mouse Ventricular Myocytes: Dilatation of T-Tubules near Junctions. *Biophys J*. 2013; 104(11):L22–L24. [PubMed: 23746531] . • This paper combines electron tomography with super-resolution light microscopy to quantify the relationship between ryanodine receptors and caveolin-3, which are proteins involved in the excitation contraction coupling.
12. Fernandez JJ. Computational methods for electron tomography. *Micron*. 2012; 43(10):1010–1030. [PubMed: 22658288]
13. Giuly RJ, Kim KY, Ellisman MH. DP2: Distributed 3D image segmentation using micro-labor workforce. *Bioinformatics*. 2013; 29(10):1359–1360. [PubMed: 23574738]
14. Gao Z, Yu Z, Holst M. Feature-preserving surface mesh smoothing via suboptimal Delaunay triangulation. *Graph Models*. 2013; 75(1):23–38. [PubMed: 23580890]
15. Kinney, JP. Ph. D. thesis. San Diego: University of California; 2009. Investigation of neurotransmitter diffusion in three-dimensional reconstructions of hippocampal neuropil.
16. Yu Z, Holst MJ, Cheng Y, McCammon J. Feature-preserving adaptive mesh generation for molecular shape modeling and simulation. *Journal of Molecular Graphics and Modelling*. 2008; 26(8):1370–1380. [PubMed: 18337134]
17. Liao T, Zhang Y, Kekenus-Huskey PM, Cheng Y, Michailova A, McCulloch AD, Holst M, McCammon JA. Multi-core CPU or GPU-accelerated Multiscale Modeling for Biomolecular Complexes. *Molecular Based Mathematical Biology*. 2013; (1):164–179.
18. Hake J, Edwards AG, Yu Z, Kekenus-Huskey PM, Michailova AP, McCammon JA, Holst MJ, Hoshijima M, McCulloch AD. Modelling cardiac calcium sparks in a three-dimensional reconstruction of a calcium release unit. *J Physiol*. 2012; 590(Pt 18):4403–4422. [PubMed: 22495592] . •• This study developed an unprecedentedly detailed computational model of a cardiac Ca^{2+} spark based on a reconstructed geometry of a Ca^{2+} release unit from electron tomography data. The model was used to reconcile previous model predictions with experimental measurements.
19. Kekenus-Huskey PM, Cheng Y, Hake JE, Sachse FB, Bridge JH, Holst MJ, McCammon JA, McCulloch AD, Michailova AP. Modeling effects of L-type Ca^{2+} current and Na^{+} - Ca^{2+} exchanger on Ca^{2+} trigger flux in rabbit myocytes with realistic T-tubule geometries. *Front Physiol*. 2012; 3:351. [PubMed: 23060801]
20. Yu Z, Yao G, Hoshijima M, Michailova A, Holst M. Multiscale modeling of calcium dynamics in ventricular myocytes with realistic transverse tubules. *IEEE Trans Biomed Eng*. 2011; 58(10): 2947–2951. [PubMed: 21632291]
21. Cheng Y, Yu Z, Hoshijima M, Holst MJ, McCulloch AD, McCammon JA, Michailova AP. Numerical analysis of Ca^{2+} signaling in rat ventricular myocytes with realistic transverse-axial tubular geometry and inhibited sarcoplasmic reticulum. *PLoS Comput Biol*. 2010; 6(10):e1000972. [PubMed: 21060856]
22. Yu Z, Holst MJ, Hayashi T, Bajaj CL, Ellisman MH, McCammon JA, Hoshijima M. Three-dimensional geometric modeling of membrane-bound organelles in ventricular myocytes: Bridging the gap between microscopic imaging and mathematical simulation. *Journal of Structural Biology*. 2008; 164(3):304–313. [PubMed: 18835449]
23. Kinney JP, Spacek J, Bartol TM, Bajaj CL, Harris KM, Sejnowski TJ. Extracellular sheets and tunnels modulate glutamate diffusion in hippocampal neuropil. *J Comp Neurol*. 2013; 521(2):448–

464. [PubMed: 22740128] . •• This paper presents a massive 3D model of a hippocampal Neuropil based on data from serial section transmission electron microscopy and shows that specialized membrane structures such as tunnels and sheets are important for neuronal signaling.
24. Cowan, AE.; Moraru, II.; Schaff, JC.; Slepchenko, BM.; Loew, LM. Chapter 8 - Spatial Modeling of Cell Signaling Networks. In: Asthagiri, AR.; Arkin, AP., editors. Computational Methods in Cell Biology, vol. 110 of Methods in Cell Biology. Academic Press; 2012. p. 195-221.
 25. Ditlev J, Mayer B, Loew L. There is More Than One Way to Model an Elephant. Experiment-Driven Modeling of the Actin Cytoskeleton. Biophysical Journal. 2013; 104(3):520–532. [PubMed: 23442903]
 26. Franzini-Armstrong C, Protasi F, Ramesh V. Shape, size, and distribution of Ca^{2+} release units and couplons in skeletal and cardiac muscles. Biophys J. 1999; 77(3):1528–1539. [PubMed: 10465763]
 27. Hayashi T, Martone ME, Yu Z, Thor A, Doi M, Holst MJ, Ellisman MH, Hoshijima M. Three-dimensional electron microscopy reveals new details of membrane systems for Ca^{2+} signaling in the heart. J Cell Sci. 2009; 122(Pt 7):1005–1013. [PubMed: 19295127]
 28. Baddeley D, Jayasinghe ID, Lam L, Rossberger S, Cannell MB, Soeller C. Optical single-channel resolution imaging of the ryanodine receptor distribution in rat cardiac myocytes. Proc Natl Acad Sci U S A. 2009; 106(52):22275–22280. [PubMed: 20018773]
 29. Ramay HR, Liu OZ, Sobie EA. Recovery of cardiac calcium release is controlled by sarcoplasmic reticulum refilling and ryanodine receptor sensitivity. Cardiovasc Res. 2011; 91(4):598–605. [PubMed: 21613275]
 30. Louch WE, Hake J, Mrk HK, Hougen K, Skrbic B, Ursu D, Tnnessen T, Sjaastad I, Sejersted OM. Slow Ca^{2+} sparks de-synchronize Ca^{2+} release in failing cardiomyocytes: Evidence for altered configuration of Ca^{2+} release units? J Mol Cell Cardiol. 2013; 58:41–52. [PubMed: 23376034]
 31. Zima AV, Picht E, Bers DM, Blatter LA. Termination of cardiac Ca^{2+} sparks: role of intra-SR [Ca^{2+}], release flux, and intra-SR Ca^{2+} diffusion. Circ Res. 2008; 103(8):e105–e115. [PubMed: 18787194]
 32. Sobie EA, Dilly KW, dos Santos Cruz J, Lederer WJ, Jafri MS. Termination of cardiac Ca^{2+} sparks: an investigative mathematical model of calcium-induced calcium release. Biophys J. 2002; 83(1):59–78. [PubMed: 12080100]
 33. Hatano A, Okada Ji, Washio T, Hisada T, Sugiura S. A three-dimensional simulation model of cardiomyocyte integrating excitation-contraction coupling and metabolism. Biophys J. 2011; 101(11):2601–2610. [PubMed: 22261047]
 34. Cannell M, Kong C, Imtiaz M, Laver D. Control of Sarcoplasmic Reticulum Ca^{2+} Release by Stochastic RyR Gating within a 3D Model of the Cardiac Dyad and Importance of Induction Decay for CICR Termination. Biophysical Journal. 2013; 104(10):2149–2159. [PubMed: 23708355]
 35. Laver DR, Kong CHT, Imtiaz MS, Cannell MB. Termination of calcium-induced calcium release by induction decay: an emergent property of stochastic channel gating and molecular scale architecture. J Mol Cell Cardiol. 2013; 54:98–100. [PubMed: 23123322] . • This paper uses a detailed 3D model of a Ca^{2+} release unit to present a novel Ca^{2+} spark termination theory termed *induction decay*.
 36. Soeller C, Jayasinghe ID, Li P, Holden AV, Cannell MB. Three-dimensional high-resolution imaging of cardiac proteins to construct models of intracellular Ca^{2+} signalling in rat ventricular myocytes. Experimental Physiology. 2009; 94(5):496–508. [PubMed: 19139064]
 37. McGuffee SR, Elcock AH. Diffusion, crowding & protein stability in a dynamic molecular model of the bacterial cytoplasm. PLoS Comput Biol. 2010; 6(3):e1000694. [PubMed: 20221255]
 38. Tanskanen AJ, Greenstein JL, Chen A, Sun SX, Winslow RL. Protein geometry and placement in the cardiac dyad influence macroscopic properties of calcium-induced calcium release. Biophys J. 2007; 92(10):3379–3396. [PubMed: 17325016]
 39. Shorten PR, Sneyd J. A mathematical analysis of obstructed diffusion within skeletal muscle. Biophys J. 2009; 96(12):4764–4778. [PubMed: 19527637]

40. Keken-Huskey P, Liao T, Hake J, Gillette A, Zhang J, Michailova A, McCulloch A, McCammon A. Molecular and sub cellular-scale modeling of nucleotide diffusion in the cardiac myofilament lattice. 2013 accepted in Biophysical Journal.
41. Keken-Huskey PM, Gillette A, Hake J, McCammon JA. Finite Element Estimation of Protein-Ligand Association Rates with Post-Encounter Effects: Applications to Calcium binding in Troponin C and SERCA. *Comput Sci Discov*. 2012; 5:014015. [PubMed: 23293662]
42. Silva JR, Pan H, Wu D, Nekouzadeh A, Decker KF, Cui J, Baker NA, Sept D, Rudy Y. A multiscale model linking ion-channel molecular dynamics and electrostatics to the cardiac action potential. *Proc Natl Acad Sci U S A*. 2009; 106(27):11102–11106. [PubMed: 19549851]
43. Nekouzadeh A, Rudy Y. Continuum molecular simulation of large conformational changes during ion-channel gating. *PLoS One*. 2011; 6(5):e20186. [PubMed: 21625456] . •• Created multi-physics model for cardiac potassium channel gating, based on atomic resolution structural data, electrostatic energies and a kinematic theory that better models slow conformational changes, as an alternative to Langevin and Smoluchowski approaches. The method approach predicted the open probability over a range of applied voltages, which was in close agreement with experimentally-measured currents.
44. Roux B, Bernche S, Egwolf B, Lev B, Noskov SY, Rowley CN, Yu H. Ion selectivity in channels and transporters. *J Gen Physiol*. 2011; 137(5):415–426. [PubMed: 21518830]
45. Lindert S, Keken-Huskey PM, Huber G, Pierce L, McCammon JA. Dynamics and calcium association to the N-terminal regulatory domain of human cardiac troponin C: a multiscale computational study. *J Phys Chem B*. 2012; 116(29):8449–8459. [PubMed: 22329450]
46. Keken-Huskey PM, Lindert S, McCammon JA. Molecular basis of calcium-sensitizing and desensitizing mutations of the human cardiac troponin C regulatory domain: a multi-scale simulation study. *PLoS Comput Biol*. 2012; 8(11):e1002777. [PubMed: 23209387]
47. Keken-Huskey PM, Metzger VT, Grant BJ, Andrew McCammon J. Calcium binding and allosteric signaling mechanisms for the sarcoplasmic reticulum Ca^{2+} ATPase. *Protein Sci*. 2012; 21(10):1429–1443. [PubMed: 22821874]
48. Hilder TA, Chung SH. Conductance properties of the inwardly rectifying channel, Kir3.2: molecular and Brownian dynamics study. *Biochim Biophys Acta*. 2013; 1828(2):471–478. [PubMed: 23022491]
49. Bauler P, Huber GA, McCammon JA. Hybrid finite element and Brownian dynamics method for diffusion-controlled reactions. *J Chem Phys*. 2012; 136(16):164107. [PubMed: 22559470] . • Devised a technique for coupling explicit-particle Brownian dynamics simulations with continuum diffusion models. The explicit and implicit particle domains are solved concurrently, and transport between domains occurs in a buffer region, in which particles are dually represented by continuous distributions and points.

Highlights

- Electron and light microscopy yield 3D reconstructions of signaling microdomains
- Imaging techniques provide further high-resolution structural data suitable for 3D modeling
- Advancements in computational geometry enable generation of 3D meshes suitable for numerical solutions
- Computational models with molecular details provide integral insight into cellular signaling

Figure 1. Modeling Ca^{2+} sparks in a 3D reconstruction of a Ca^{2+} release unit (CRU)

A. 3D EM tomography data was used to segment a single Ca^{2+} release unit. Features like transverse tubule (TT), Mitochondria (Mit), and sarcoplasmic reticulum (SR) were identified. **B.** The annotated computational mesh included a complex network of SR, two distinct Mit, and two distinct TTs. **C.** Together with junctional SR on one of the TTs formed the CRU. Ca^{2+} was released from SR at the jSR boundary (red). **D.** Finite element simulations of a Ca^{2+} spark revealed huge spatial gradients within the CRU and its immediate vicinity. Adapted from [18].

Figure 2. The geometry of transverse tubule (t-tubules) is species dependent

A. The computational geometry of the t-tubules from a rat is branched and **B.** linear in rabbit ventricular myocyte. From [21] and [19] respectively.

Figure 3. Homogenization of myofilament lattice improved the estimate of the diffusion coefficient in cytosol

An reconstruction with atomistic-resolution of the thin filaments in a myofibril. From [40].