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Computational modeling of subcellular transport and signaling

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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 on the basis of reconstructed geometries, with a special focus on signaling microdomains that are important for the excitation contraction coupling in cardiac myocytes.

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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+} 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 on the basis of 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 ≃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 subcellular structures [10].

Conventional confocal light microscopy (LM) can also be used to extract 3D features, but the resolution (≃100 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 subcellular simulations of Ca^{2+} signaling in cardiomyocytes [18••,19–22]. `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` (http://www.blender.org). Such visualization tools allow annotation of functionally important regions in the surface mesh, for example, 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 [26,2]. These 3D metrics were estimated by extrapolating geometric data from serial 2D images. By assuming a regular, for example, 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 μm thick, and contained a single intact CRU with detailed structures of neighboring Sarcoplasmic reticulumn (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 [32,31].

A single t-tubule hosts several CRUs and facilitates the spread of excitation throughout the myocyte. Using reconstructed geometries of t-tubules on the basis of 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,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