Molecular-Scale Simulation of Calcium Ions within Cardiac Tissue
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Introduction:

Within the heart, cells undergo a process called **calcium-induced calcium release (CICR)**, which causes the cardiac muscle to contract and, as a result, allows the heart to beat. In particular, this process is mediated by a protein called a **ryanodine receptor (RyR)**, which resides on the membrane between the cell's myoplasm and an intracellular compartment called the junctional sarcoplasmic reticulum (JSR), where a high concentration of Ca^{2+} ions are stored. When the RyR is opened, free calcium ions flow out of the JSR and into a subdomain of the myoplasm adjacent to the RyR, called the dyad. On average, one dyad contains approximately 20 separate ryanodine receptors. Figure 1A shows an abstract illustration of the geometry of the dyad; 1B shows one suggested layout of RyRs on the JSR membrane.
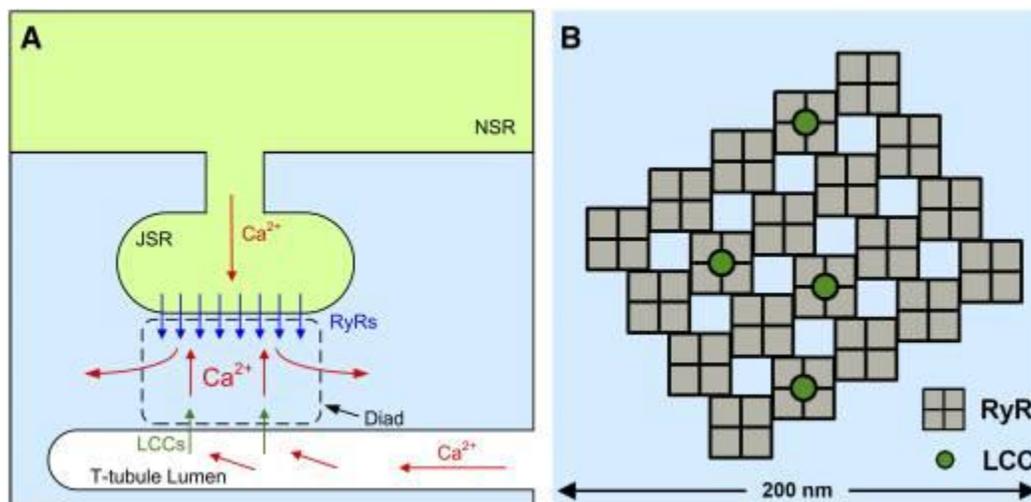

Figure 1: from Tanskanen, et al (1)

The goal of this project was to develop a simulation for this process and investigate how different arrangements of ryanodine receptors impact the patterns of CICR, specifically the intensity and frequency of **calcium sparks**, where the influx of calcium through one open RyR catalyzes the opening of other RyRs in the dyad.

Simulation:

The simulation was developed using a program called Smoldyn (2) which allows users to simulate chemical reactions on a microscopic scale. Each simulation featured a single independent dyad (200x200x15 nm) and adjacent JSR (200x200x40 nm) using a simplified geometrical model of two connected rectangular boxes. 20 RyRs were arranged on the center membrane in the configuration shown above. Each receptor was also modelled as a rectangular box with cross-sectional dimensions 27x27 nm and extending 12 nm into the dyad. In the centre of the front face of each RyR box was a volume-less molecule used to represent the receptor in Smoldyn reactions. Calcium ions are also modelled as volume-less point sources undergoing Brownian motion. Initially, both the JSR and the dyad contained 100 calcium ions; the concentration within the JSR was held constant throughout. The simulations were run for 2 seconds each, with time step 0.0001 ms.

The ryanodine receptors were modelled as having two states, open and closed. A RyR transitions from the closed state to the open state at rate 0.65 ms^{-1} and back to closed at rate 0.06 ms^{-1}. Each RyR is modelled to possess 4 activating binding sites to which calcium ions must be bound in order for it to transition to the open state. In this model, binding is considered separately from state transition, so all 4 binding sites must be bound before this transition may occur. The location of these activating binding sites is uncertain, so instead a radius was chosen from the center of the front face such that all calcium ions within that radius would bind to the receptor with a constant rate per binding site. Hence, a calcium ion would bind with higher probability to a receptor with 4 vacant binding sites than to a receptor with only 2 vacant binding sites. The reaction network required to facilitate these interactions was developed using a program called BioNetGen (3). A Perl script, originally written by a Smoldyn contributor, was edited to correctly convert the reactions generated by BioNetGen into a form recognized by Smoldyn. It has been suggested that RyRs may also be inactivated via inactivating binding sites, but the mechanisms by which this occurs and the role of inactivation in CICR are not yet understood. We therefore chose to ignore receptor inactivation to maintain the simplicity and limited scope of this model.

The opening and closing rates of the ryanodine receptors and the diffusion constant of the free calcium ions were taken directly from the Tanskanen article; all other parameters were approximated and then varied over different simulations.

Results:

After trials with various parameter values and ultimately settling on the binding/unbinding rates and transmission rate in the table below, the simulation was successful in modelling the domino-effect

of RyR opening, where the opening of one RyR triggered the opening of other RyRs. Using our current approach, however, it has not been possible to produce a realistic and complete calcium spark; in particular, the time elapsed before the release event is triggered is far longer than expected, on average around 1 s. Although we were unable to achieve the full results we had desired, this project allowed us to discover the surprisingly substantial importance of the basic geometry of the RyR in CICR; in our attempt to generalize the binding domain of the ryanodine receptor, we lost a key aspect of the process. In future efforts, it has become clear that consideration of the full, complex geometry of these receptors will be imperative; likewise, it will be necessary to utilize a software tool that precisely models the microscopic physics within the cell rather than one that focusses solely on biochemical reactions.

Parameter	Value
Active site binding rate	3×10^4 ms^{-1}
Active site unbinding rate	0.001 ms^{-1}
Transmission rate	50000 ms^{-1}
Opening rate	0.65 ms^{-1}
Closing rate	0.06 ms^{-1}

References:

1. Tanskanen, Antti J., et al. "Protein geometry and placement in the cardiac dyad influence macroscopic properties of calcium-induced calcium release" *Biophys. J.* 92:3379-3396, 2007.
2. Andrews, Steven S., et al. "Detailed simulations of cell biology with Smoldyn 2.1" *PLoS Comp. Biol.* 6:e1000705, 2010.
3. J. R. Faeder, M. L. Blinov, and W. S. Hlavacek. "Rule-based modeling of biochemical systems with BioNetGen," *Methods Mol. Biol.* 500:113–67, 2009.