Stochastic binding of Ca^{2+} ions in the dyadic cleft continuous vs Random walk description of diffusion

Introduction

An important issue debated in the literature is whether diffusion in signaling micro domains can be modelled deterministically and continuously, or if stochastic and discrete Random walk (RW) methods should be employed(1-6). Signalling micro domains are used by the cell to convey information, and it is important to have accurate and reliable simulation methods when these processes are studied. Traditionally this has been done using Fick's second law of diffusion. This law predict the time evolution of the average number of molecules in a process deterministically, and reaction diffusion processes in macroscopic environments, where fluctuations from the predicted average number of particles in a solution are small, are successfully modelled by these laws.

During the last years, as smaller and smaller sub-cellular domains have been studied, the discreteness and stochasticity of the physiological processes have been in focus. This has raised issues with the deterministic models (4-8). In sub-cellular micro domains the number of involved molecules is small and the fluctuations from the predicted average number of molecules involved become dominant. Three dimensional (3D) RW simulators have been developed to incorporate the discreteness and stochasticity of the signaling in intracellular micro domains. Unfortunately simulations with these can be time consuming.

In this study we compare the use of a continuous model with the use of a RW model with respect to the Ca^{2+} ions that bind to single receptors in the cleft. These are important discrete events that can be modelled stochastically using both descriptions of diffusion. They are also direct related to the physiological functions of the cleft, such as the Ca^{2+} induced Ca^{2+} release, and the generation of a spark.

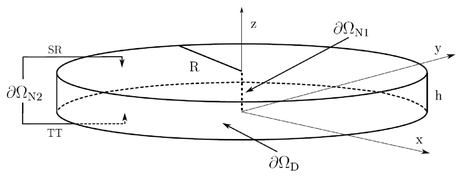

Figure 1: The figure present the disk geometry of the dyadic cleft we use in both the continuous model and in the RW model.

- $\partial\Omega_{N1}$: Ca^{2+} inflow
- $\partial\Omega_{N2}$: Reflecting boundaries
- $\partial\Omega_D$: Cytosole, $\left[Ca^{2+}\right] = 0$
- Buffers included as reactants in the domain
- Solved as a 1D problem

Methods

In both descriptions, i.e., continuous and discrete, we model the dyadic cleft as a flat disk, with h = 15 nm and R = 100 nm, See Fig. 1. The diffusion constant of Ca^{2+}, was set to $D_c = 10^5$ nm^2 ms^{-1}. Single LCC current amplitude was chosen to be $\bar{i}_{LCC} = 0.3$ pA, and was released in the center of the disk along the dashed line. Binding rate for the RyRs was set to 5 μM^{-1}s^{-1}, which corresponds to binding rates previously used in models for both RyR and LCC. The TT and SR membranes were modeled as reflective, no-flux, boundaries, $\partial\Omega_{N2}$ in Fig. 1. The cytosole was included in the model either as a zero concentration boundary, when a LCC Ca^{2+} source was used, or as a constant level corresponding to diastolic $\left[Ca^{2+}\right]$ of 0.1 μM

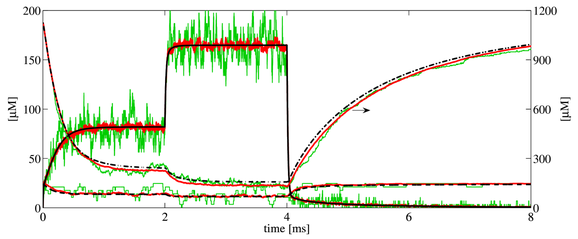

Figure 2: The figure presents simulation results from the continuous model, black lines and from the Random walk (RW) model, colored lines. The results represent the concentrations from the whole cleft.

Binding event comparison

To confirm that the solution from the continuous model coincided with the mean concentrations from the RW model, we did one run with the continuous model and 40 runs of the RW model. The result is presented in Fig. 2.

We tested how well the continuous model fit the equivalent binding events registered from the RW model. We used four tentative RyRs, positioned from the center of the cleft to the rim. Two different set of simulations were done correspondning to different physiological conditions, *i*) uniform $\left[Ca^{2+}\right]$ due to passive diffusion from cytosole, using very low diastolic $\left[Ca^{2+}\right]$ = 0.1 μM, and *ii*) transient $\left[Ca^{2+}\right]$, from three different LCCs. The statistical results from the simulations are presented in Fig. 3-4.

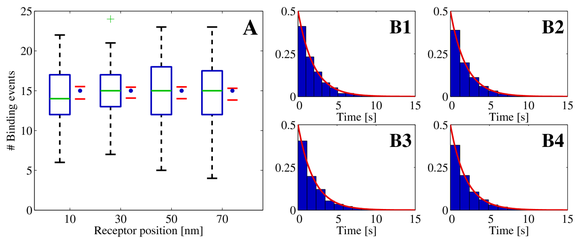

Figure 3: The figures present statistical data of binding events registered from Random walk simulations. The Ca^{2+} source was passive diffusion from cytosole during diastolic $\left[Ca^{2+}\right]$, i.e., 0.1 μM. No significant difference between the continuous model and the RW model were detected.

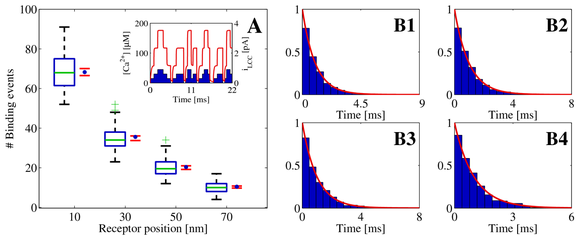

Figure 4: The figures present statistical data of binding events registered from Random walk simulations and the Ca^{2+} source is zero to three open LCCs, situated in the center of the cleft, see inset of A. No significant difference between the continuous model and the RW model were detected.

Single rate comparison

Our goodness-of-fit tests revealed that there are no significant differences between the registration of stochastic binding events from the two models. This is a result that holds on the level of single runs and at the level of single IEI. To get a better understanding of how this could be true we examined the binding rate, registered by a single RyR positioned 10 nm from the center during a run with one constantly open LCC. The result is presented in Fig. 5. A

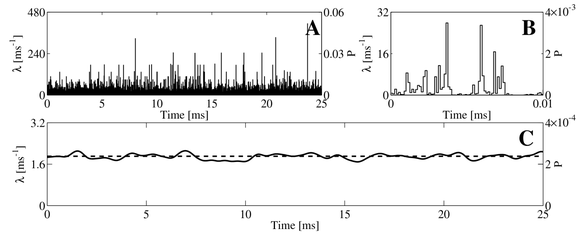

Figure 5: The A and B figures present the lumped binding rates for each time step, registered from one RyR during a single Random walk simulation. In the C figure a filtered version of the binding rate is presented together with the one predicted from the continuous model, dashed line.

Single rate comparison cont.

The mean averaged rate from 100 runs was $[1.904 \pm 0.019]$ ms^{-1}, and this value was not significantly different from the rate the continuous model, $\lambda_c = 1.91$ ms^{-1}. On a smaller time scale, we expected that the averaged rate would fluctuate more. For example the mean rate for the interval shown in Fig. 5 B, i.e., $t = [0,\ 0.01]$, is 2.70 ms^{-1}. We filtered the registered rate with a Gaussian filter, which act as a weighted mean over a certain time window, with a width corresponding to the mean intervall between the binding events. The filtered rate is a continuous function of time and does not vary by far as much as the unfiltered rate in Fig. 5 A.

Parameter dependency

To check the dependency of some of the parameters we have used, we made five runs where we altered the diffusion constant, D_c, together with the maximal input current from one open LCC, $\bar{i}_{LCC}$. We scaled the parameters with a factor of $[0.1, 0.5, 1, 2, 5]$ and made 100 runs, where one LCC was constantly open. The spatial resolution for the registration of binding events was set to $\sigma = 5$ nm for every run. Fig. 6 A. shows a box-plot of the number of binding events registered in the RW simulations versus the predicted number from the continuous model. We recognize a significant difference for scale = 0.1.

To further investigate the dependency of parameters we also altered the spatial resolution, σ. We used $[5, 2, 1, 0.5]$ for the σ and here we also did 100 runs for each different value. The result is presented in a similar box plot in Fig. 6 B. From the figure we see that the number of registered binding events steadily falls. This illustrates that the binding event registration is not only dependent on physical parameters, but also on the spatial resolution of the RW method.

When a one single ion contributes enough to the binding rate a difference between the two model emerges. When we simulate the RW model without registereing any binding events, i.e., not removing them from the solution, but registering the binding rate from the ions, we see that the binding rate do not differ from the continuous model, see Fig 6 C.

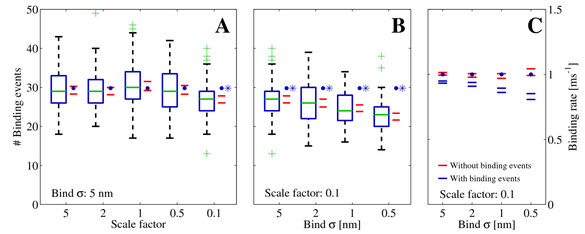

Figure 6: The A and B figures present the number of registered binding events from 100 runs each, where we altered different parameters. The data were collected from the receptor 30 nm from the center and are represented by the box plots together with a 95 % confidence interval for the true means, i.e., the red horizontal lines. In C we did 100 runs where we collected the mean binding rates the receptor at 30 nm from the cleft were exposed to. The blue horizontal lines represents the binding rates collected from runs where we did register binding events, i.e., as in B, and the red horizontal lines represent binding rates collected from runs where we did not register binding events.

Parameter dependency cont.

The binding rate registered from the Ca^{2+} ions can be expressed by a dimensionless on rate,

$$k^{+*} = k^{+} / (4\pi D \sigma N a)\,. \qquad (1)$$

We recognized a difference between the two models when this value exceeded 0.02. This is probably a conservative measure, as we in our simulations do not close a receptors for registration after an ion is bound. This makes the effect of removing an ion from the vicinity of an unbound receptor larger than it would have been if the receptor was in a bound state.

Conclusion

For a certain range of parameters we show that one can use a continuous model of diffusion to register discrete binding events in the dyadic cleft.

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