A novel Monte Carlo Simulation for Molecular Interactions and Diffusions in Postsynaptic Spine

Yoshihisa Kubota[1], Tara R. Gaertner[1], John A. Putkey[2] and M. Neal Waxham[1]

[1] Department of Neurobiology and Anatomy, and [2] Biochemistry and Molecular Biology, University of Texas Medical School, Houston, Texas 77030, USA

Abstract

We developed a new Monte Carlo simulator incorporating molecular interactions and diffusion of Ca^{2+} signaling molecules in dendritic spines. The simulator is based on two well-established Monte Carlo approaches, namely the Kopelman algorithm and the Kinetic Monte Carlo algorithm. This paper describes the basic features of the simulator and compares its performance for the general case of classical enzyme kinetics. We then explore anomalous diffusion of calmodulin (CaM) in the dendrite and quantify its interactions with Ca^{2+} and CaM-binding target proteins. The simulations suggest that the CaM-binding protein RC3 plays a significant role in determining the spatio-temporal dynamics of CaM-target interaction during Ca^{2+} oscillations.

***Keyword*:** Monte Carlo simulation; anomalous diffusion; range of action; calmodulin; dendritic spine

1. Introduction

Increased intracellular Ca^{2+} is required for the induction of synaptic plasticity, both long-term depression (LTD) and long-term potentiation (LTP). A long-standing question is: how is it that high levels of Ca^{2+} lead to LTP while intermediate levels lead to LTD? The distinction between LTP and LTD has long been hypothesized to do with how the distribution of Ca^{2+} in space and time within the spine compartment regulates downstream signaling machinery. However, the molecular mechanism that decodes the Ca^{2+}-signal in these two circumstances is not known, nor is there a plausible biological description for how two Ca^{2+}-dependent signaling systems are differentially engaged during LTP/LTD. A quantitative modeling approach is essential to examine this hypothesis.

The number of molecules involved in signaling pathways within the spine is small. Cytoplasm is a complex milieu and signaling molecules are not randomly distributed. These features demand that chemical reactions and diffusion of signaling molecules be computed individually. Monte Carlo approaches are currently the only computational strategy capable of efficiently incorporating these two key features of synaptic signaling. MCell (http://www.mcell.cnl.salk.edu) attempts to incorporate such features as highlighted in recent papers [1, 2]. Nevertheless, the current version of MCell cannot compute interactions of two freely diffusing molecules.

An additional critical point is that available data already indicate that diffusion of signaling molecules can deviate from Brownian motion. Instead, proteins in the cytoplasm can undergo anomalous diffusion or fractional Brownian motion [3, 4]. Furthermore, because of the complexities associated with diffusion of biological molecules or worse, macromolecular assemblies, reaction time distributions may be broader than a Poisson distribution [5]. These issues of single molecule dynamics and diffusions are, however, the reality of biologically realistic synaptic signaling mechanisms.

Here we propose to address these problems by creating a new Monte-Carlo simulation by fusing features of the Kopelman algorithm for molecular interactions and the Kinetic Monte-Carlo simulation of molecular diffusion (used in surface/material science). The first part of this paper describes the algorithm's basic architecture. Second, we compare simulated results against classical enzyme kinetics. Third, we explore the range of action of Ca^{2+}- calmodulin and its modulation by a unique calmodulin-binding protein, RC3 (neurogranin). The simulations suggest that RC3 plays a significant role in determining the spatio-temporal dynamics of CaM-target interactions during Ca^{2+} oscillations.

2. Simulation Details

A widely accepted exact stochastic simulation of molecular interactions is the Gillespie algorithm [6]. However, the Gillespie method is only valid for thermally equilibrated homogenous chemical systems.

In order to analyze molecular dynamics in a non-homogenous system, an alternative simulation method is needed.

The first step in developing an alternative is to re-examine Gillespie's original derivation of the algorithm. To derive the probability of chemical reactions within a given time period, Gillespie [6] calculated the collision probability of two reacting molecules and then computed the conditional probability of chemical reactions given the collision. To account for homogeneity in the Gillespie algorithm, the probability of collision of two reacting molecules must be explicitly calculated. One means of accomplishing this calculation is to simulate the motion of molecules by random walks. In the simulation, a collision occurs when two molecules come within a certain distance defined by the radius of the reacting molecules.

Computing the probability of chemical reactions conditional to a collision is a difficult task even with quantum molecular dynamics simulations [6]. Therefore, a conditional probability is empirically assigned as being proportional to the macroscopic reaction rate for each chemical interaction. This is a well-established method employed by previous models of chemical reactions in disordered media (Kopelman Algorithm) [7-9] and this approach was experimentally validated in one of these works [9].

The basic architecture of the Kopelman algorithm is as follows: Suppose we have a pair of association and dissociation reactions.

$$A + B \xrightarrow{k_1} AB \qquad (1)$$

$$AB \xrightarrow{k_{-1}} A + B \qquad (2)$$

where A and B may represent CaM and RC3 (or other CaM-binding targets) in our simulation. The k_1 and k_{n1} are corresponding on- and off-rates.

We simulate reaction (1) and (2) using a Monte Carlo algorithm on 3D cubic lattices with reflective boundary conditions. The lattices of our typical simulation contain 1×10^6 cubic volumes of 5 nm each side. The unit volume size and minimal time increment during the simulation are

constrained by the size of CaM and each CaM target protein (e.g. CaMKII) as well as by the largest diffusion coefficient in the system. Each molecular species (A, B and C) is mobile on the lattice through diffusion, which is modeled by independent (generalized) random walks of individual molecules. The probabilities *f* and *g* are set to be proportional to the corresponding rate coefficients k_1 and k_{n1} respectively (equations 1 and 2). The A and B molecules are placed on the lattice by randomly choosing the coordinates for each of them at the beginning of the simulation. At each Monte Carlo sequence, an occupied lattice site is chosen at random (excluding obstacles). The rules for movement and reaction of the molecules are:

1. If the molecule occupying the chosen site is an A, a destination site is chosen at random among its nearest neighbors. If this destination site is unoccupied, the molecule moves to it directly. If a B molecule occupies the destination site, a random number is chosen between 0 and 1. If this number is lower than the reaction probability *f*, the destination site is turned into a C molecule and the initial A site becomes unoccupied. In all other cases, the A molecule remains at its initial position.
2. If the molecule occupying the chosen site is a B, the process is identical to the case for: movement if unoccupied, reaction with a probability *f* if occupied by A, or immobility in all other cases.
3. If the molecule occupying the chosen site is a C, a random number is chosen between 0 and 1. If this number is lower than the reaction probability *g*, and provided that at least one of its nearest neighbors is unoccupied, the C molecule dissociates into an A and a B. The new A molecule is placed on the initial C site, whereas the new B molecule randomly moves to one of the unoccupied sites. If the random number is greater than *g*, the C molecule is allowed to move to a randomly chosen unoccupied nearest-neighbor site.
4. After each sequence, the time is incremented by the current number of molecules on the lattice (excluding obstacles) and another sequence begins.

The extension of this scheme to more complicated enzyme kinetics is straightforward. The elementary reaction schemes are either in the form of equation 1 and 2 or are monomolecular reactions.

To speed up the simulations, Lin et al. [7] developed a new strategy whereby the coordinates of the position/activation status of every molecule and the occupancy status of each lattice site are stored in two arrays. Though it may appear redundant to store same information in two arrays; Lin et al. [7] showed that it is much faster than storing information in a single array: we do not need to scan the whole lattice array for unreacted particles.

Nevertheless, the Kopelman algorithm still results in a large number of simulation steps during which no reaction or movement of molecules take place, especially if some of the reaction rates are very small. This results in a significant waste of computational time. Furthermore, in the original Kopelman algorithm, each molecule moves with the same diffusion coefficient and this crude approximation will not serve our purpose. To solve these problems, we took an approach similar to the n-fold way (continuous-time Monte Carlo algorithm) [10] from which the Kinetic Monte Carlo simulation [11, 12] was derived. In brief, we rearrange and modify the Kopelman algorithm into the following simulation steps:

a. Select a jump path at random, weighting by individual reaction rules

b. Make the jump

c. Update and recalculate the next possible jump probability

d. Increment time (by the number of molecules)

e. Repeat Step (a) through (d).

This is very similar to the Kinetic Monte Carlo simulation (e.g., atomic scale surface diffusion and island growth) [11, 12] except that jump rate/probabilities are explicitly calculated in the latter. If we compute jump probability according to the measured diffusion coefficient, molecular diffusion that drives chemical interactions can be accurately simulated. We therefore, created a novel interface of the Kopelman algorithm and the Kinetic Monte Carlo simulation.

3. Results & Summary

3-1 Initial test of the hybrid MC simulator using classical enzyme kinetics

We verify our algorithm in several steps. First, the simulation must reproduce classical enzyme kinetics in a well-stirred homogeneous system. We generated a Kopelman-type Monte Carlo simulation of a Michaelis-Menten enzyme reaction ($E + S \leftrightarrow C \rightarrow E + P$) and compared it with the law of mass action. Since we are interested in a small number of molecules, the Gillespie-type stochastic model is used to simulate product (P) formation. Fig. 1 compares means of 1000 simulation runs of each method. The Gillespie simulation and the Kopelman algorithm are in a good agreement.

3-2 Anomalous Diffusion of Calmodulin (CaM) Molecules

Second, the capability of the simulator to reproduce molecular diffusion with or without obstacles was assessed. Fig. 2 uses CaM as an example (diffusion coefficients in the dendrite = 0.2×10^{-7} cm^2s^{-1}) to simulate a random walk. A single molecule is deposited in the lattice at a random site at t = 0. The mean squared displacement $\langle R^2 \rangle$ of the molecule is plotted as a function of time. In the simulation, we generated obstacles as randomly distributed immobile lattice sites with prescribed densities. The observed power-law time dependence can be expressed by $\langle R^2 \rangle \sim t^{\alpha}$, where α is the slope of the curve. Without obstacles, the simulation accurately reproduces classical Brownian motion (α =1). With increasing obstacle densities, the slope of the curve becomes lower, and diffusion becomes increasingly anomalous (α < 1). These results are in agreement with previously published simulations of anomalous diffusion [13].

3-3 Range of Action of CaM

We are interested in simulations that contain many molecules (e.g., Ca^{2+}, CaM and CaMKII) that have different diffusion coefficients and that undergo chemical reactions at the same time. The molecular diffusion and chemical reactions need to be explicitly coupled. A solution to this problem was

accomplished by calculating collision rates for each pair of molecules in a well-stirred system as our calculation of reaction rate is based on the Gillespie's original formulation of collision conditioned reaction probability [6].

As an example of this type of analysis, we calculated Ca^{2+}-CaM's range of action (defined below) for various Ca^{2+} concentrations in the presence and absence of the CaM-binding protein RC3. RC3 (neurogranin) is enriched in dendritic spines but it has no identified enzymatic activity, although recent knockout experiments suggested a role for RC3 in synaptic plasticity [14]. Detailed kinetic analysis revealed that the rate of Ca^{2+} dissociation from C-terminal lobe of CaM was increased by 30-fold in the presence of RC3 (k_{off} rates: from 8.5 s^{-1} to 270 s^{-1}) [15]. We therefore hypothesized that the range of action, the mean distance CaM can travel before Ca^{2+} dissociates from it, should be regulated by RC3. This provides an interesting problem to apply our computational strategy to gain potential insight into CaM's function in the spine.

Fig. 3 shows the range of action of CaM as a function of Ca^{2+}-concentraion in a well-stirred system calculated by our new Monte Carlo algorithm. In the absence of RC3, the greater the number of Ca^{2+} ions, the longer the life time of Ca^{2+} bound calmodulin. In the presence of RC3, the simulation shows that the range of action is significantly decreased by RC3 in a Ca^{2+} concentration dependent manner. The result confirms our predication concerning RC3's role in the regulation of Ca^{2+}/CaM's range of action. It indicates that, in the dendritic spine, Ca^{2+} ions most likely stimulate CaM within or in close proximity to the site of Ca^{2+} entry and that RC3 acts as a filter so that Ca^{2+} simply passes through the cytoplasm of a RC3 rich region without fully binding to, or rapidly dissociating from, CaM.

Thus far, we have tested our algorithm for basic functionality for molecular diffusion and interaction. Future work will more systematically examine molecular diffusion coupled to chemical reactions, in the presence of various forms of obstacles, together with theoretical and experimental validation of the simulation. Once confirmed, the simulator will provide a useful computational tool to analyze molecular diffusion and dynamics in the dendritic spine of other intracellular compartments.

4. Conclusion

We have created a novel Monte-Carlo algorithm that simulates molecular interactions and diffusion in a non-homogeneous media. The simulation algorithm was shown to reproduce classical enzyme kinetics and molecular diffusion. The preliminary results suggest that this new Monte Carlo simulation will provide a powerful tool to investigate spatio-temporal dynamics of Ca^{2+}-CaM-target biochemical network in the dendritic spine.

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Figure Legends

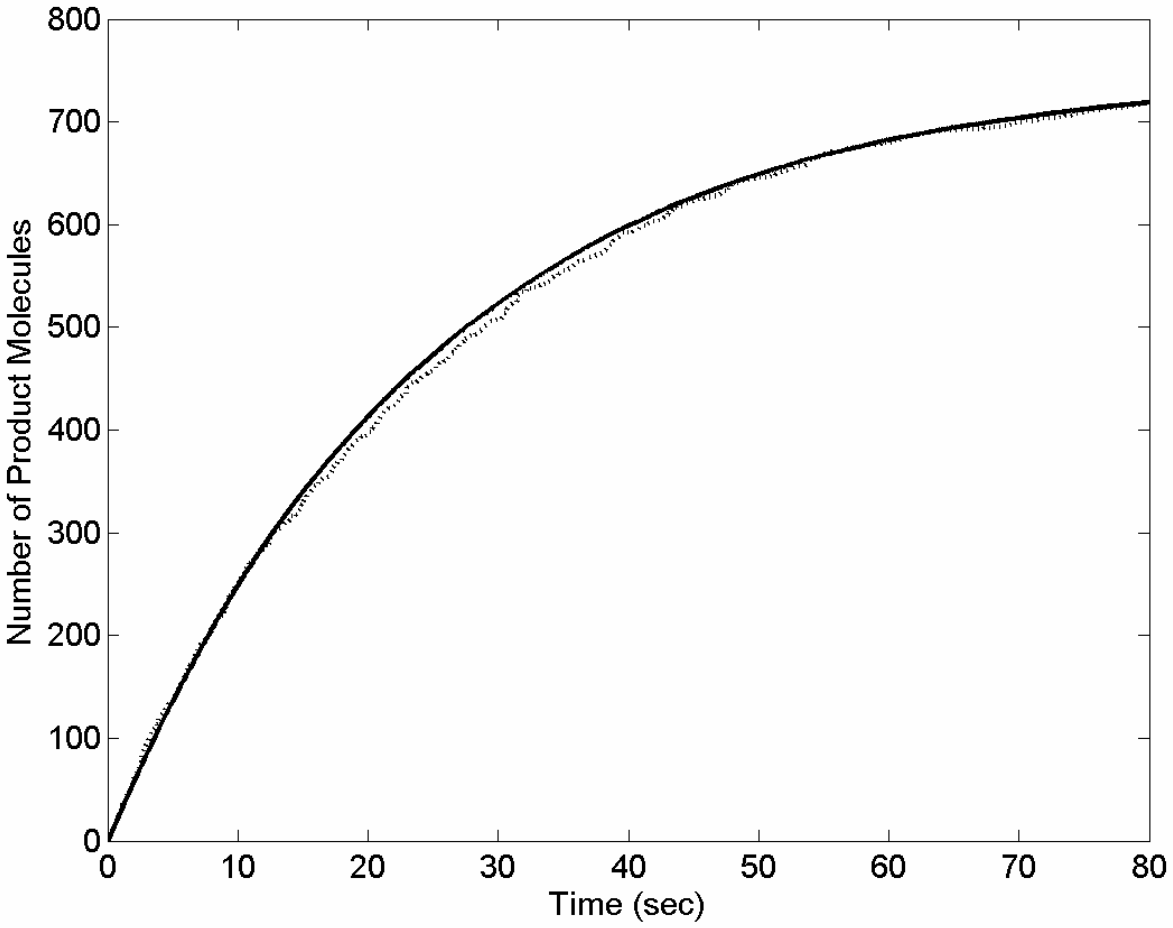
Fig. 1 The Kopelman-type simulation of Michaelis-Menten enzyme kinetics. 750 molecules of enzyme (E) and substrate (S) (equivalent to 10 μM each) are placed randomly in a 0.125 μm^3 lattice cube (0.5 μm side, $1x10^6$ lattice volumes) without obstacles. The reaction probability of enzyme-substrate complex (C) formation, dissociation, and product (P) formation, per simulation step, are 1.0, 0.02 and 0.04, respectively. The simulation was run 1000 times and the average time evolution is plotted (dotted line). The Gillespie-type model of the same reaction was generated and an average of

1000 simulations (solid line) is overlaid on the Kopelman simulation. The two curves are not statistically different ($p <= 0.05$).

Fig. 2 Simulated diffusion of a single molecule. A cubic lattice (0.5 μM side) with reflective boundaries was seeded with different densities of randomly distributed obstacles. A single molecule was randomly placed in the lattice and the mean squared displacement $<R^2>$ is plotted as a function of time (double logarithmic plot). The data represent averages of 200 simulations for each condition. The simulations were run with 3 different densities of obstacles (none, 50 % of 3D percolation threshold (θ_c), i.e., 0.15 or 15 % of total lattice sites occupied; and 200 % of percolation threshold, i.e., 0.62 or 62 % of total lattice sites occupied) as indicated. For comparison, the thin solid line is a slope of mean square displacement following classical Brownian motion.

Fig. 3 Range of action of Ca^{2+}-saturated CaM The mean distance traveled by Ca^{2+}-saturated CaM is plotted as a function of the steady-state level of Ca^{2+} concentration in a cubic lattice of $0.125\ \mu m^3$ at different concentrations of RC3. Solid line; no RC3. Dashed line; 750 molecules of RC3, equivalent to 10 μM. Dotted line;1500 molecules of RC3, equivalent to 20 μM, dotted line.

Fig. 1


Number of Product Molecules
Time (sec)
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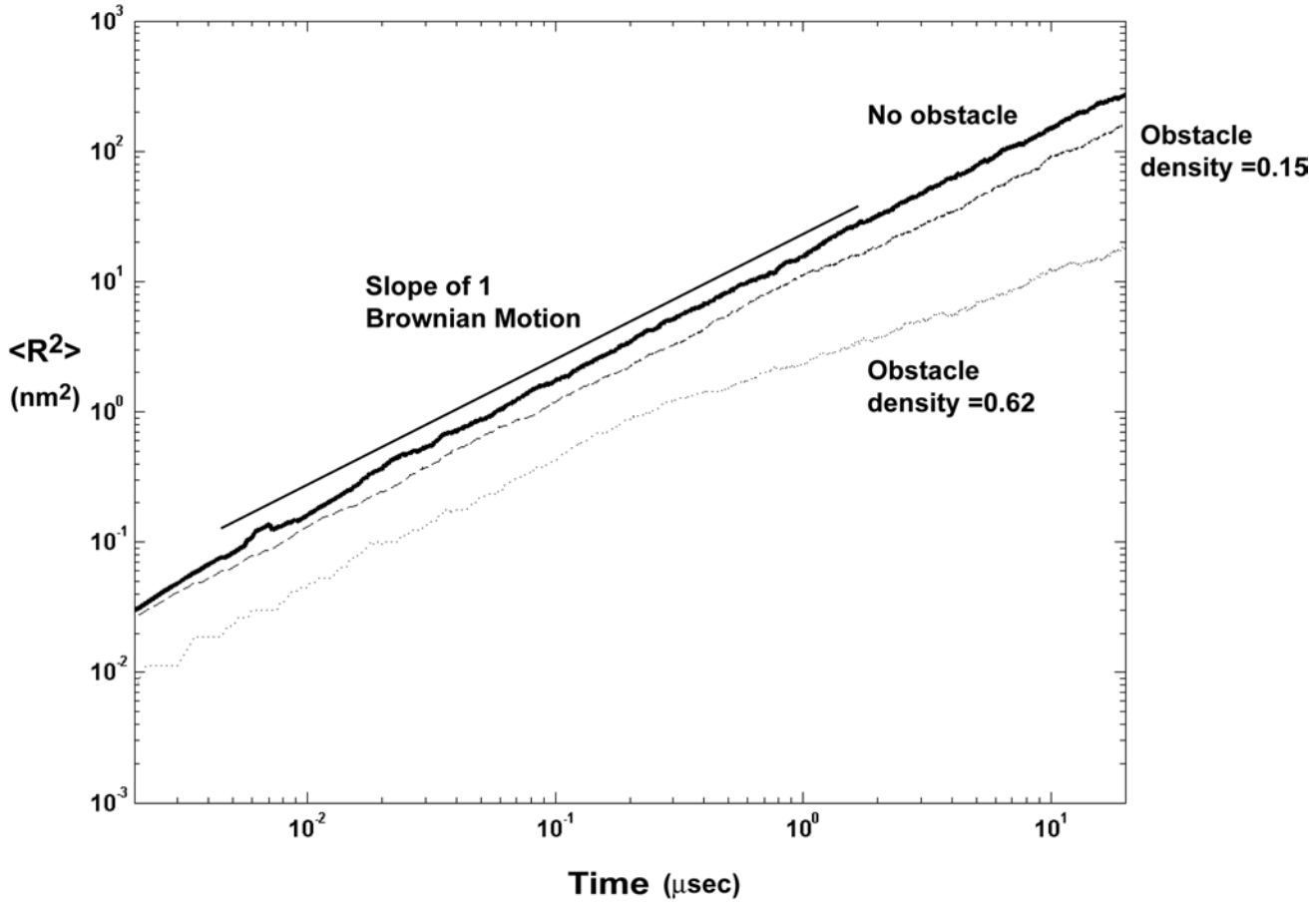
200
300
400
500
600
700
800
0
10
20
30
40
50
60
70
80

Fig. 2


No obstacle
Obstacle density =0.15
Slope of 1 Brownian Motion
Obstacle density =0.62
<R2>
(nm2)
Time (μsec)
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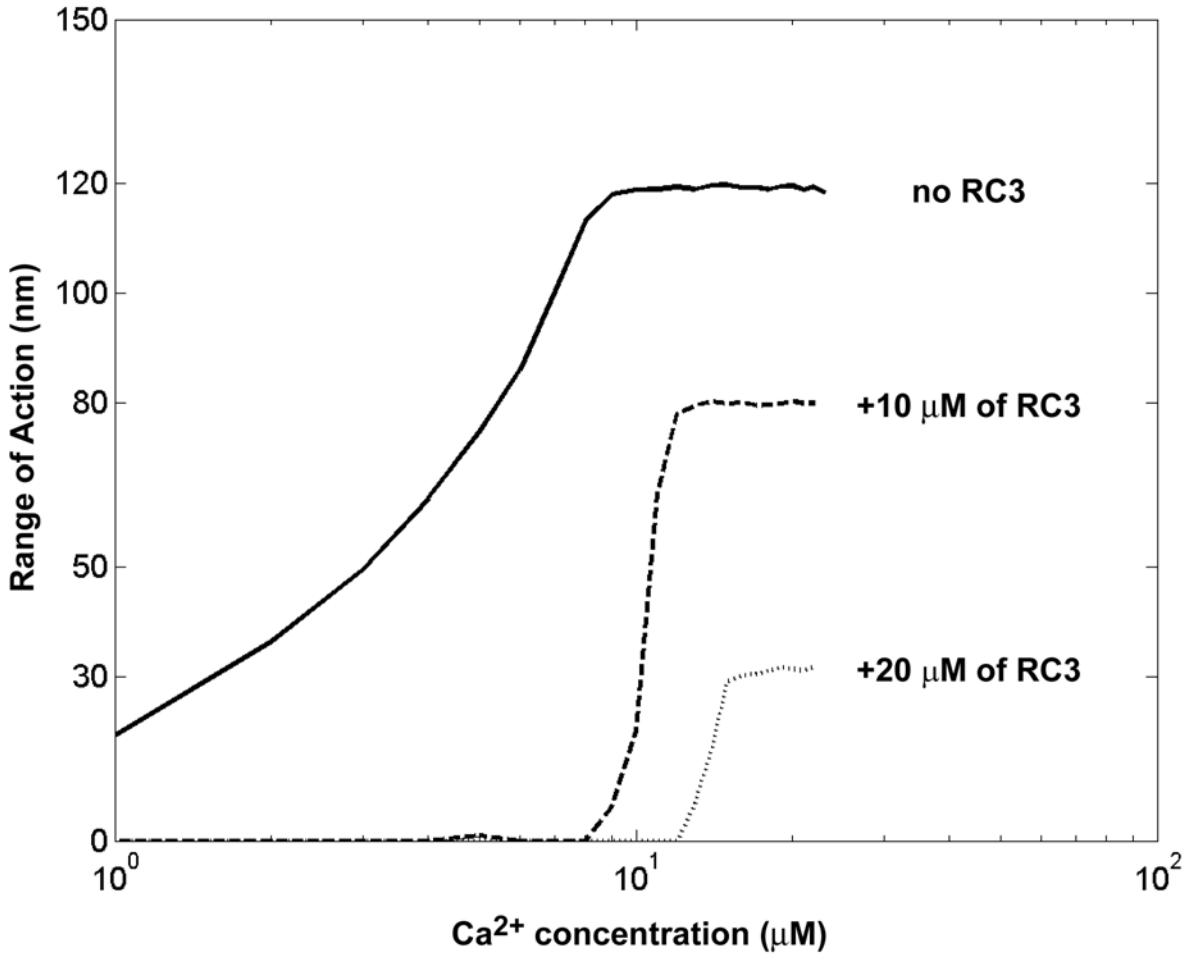
10^2
10^1
10^0
10^-1
10^-2
10^-3
10^-2
10^-1
10^0
10^1

Fig. 3


150
120
100
80
50
30
0
Range of Action (nm)
no RC3
+10 μM of RC3
+20 μM of RC3
10^0
10^1
10^2
Ca2+ concentration (μM)