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METHODS FOR SIMULATING ACTIN FILAMENT DYNAMICS IN MCELL

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Abstract.

Learning is mediated by activity-dependent changes in synaptic strength that rely on Calcium-dependent signalling in the postsynaptic neuron. In the hippocampus, synapses are located on dendritic spines, small mushroom-like structures that grow from the dendrite. Dendritic spines form micro-compartments that are - to some extent - chemically isolated from the rest of the dendrite due to the long and narrow spine neck limiting diffusion in and out. The shape and the physical structure of a dendritic spine are determined by the actin filaments that form the cellular cytoskeleton. Long-term potentiation (LTP) of the synapse leads to remodelling of the polymeric actin cytoskeleton, which increases the size of the dendritic spine. This process is regulated by interactions between actin and other postsynaptic proteins [1]. Here, addressing the lack of spatiotemporal models that combine structure with biochemical signalling, we present a set of methods that allows the modelling of the biochemistry and spatial dynamics of actin filament remodelling using the spatial stochastic simulator MCell [2, 3, 4]. These methods allow us to recapitulate the main events relevant for actin filament remodelling, including polymerisation, depolymerisation, branching and severing followed by filament displacement in space. They rely on the ability of MCell to model multi-state complex molecules [5] and on a system of virtual tags to label states of actin subunits. The "complex molecule" feature of MCell 3.3 was utilised by defining an immobile three-dimensional matrix with 8000 subunits, each belonging to one of the defined states. This provides a frame in which some of the subunits are occupied by actin monomers representing the cytoskeleton, while the rest are vacant. In response to biochemical reactions with freely diffusing modifier proteins, the state of the subunits can be changed allowing the rearrangement of the filaments. In addition, with the help of a tagging system for subunits, the monomers

forming a single filament can change their location inside the immobile matrix in corpore by occupying previously vacant slots. In the future, the model could be extended by incorporating more elements from signalling pathways and modelling filament bundles present in non-stimulated synapses. As such, the model can provide insights into how the actin cytoskeleton interacts with postsynaptic proteins that mediate LTP.

1 INTRODUCTION

Long-term changes in synaptic strength are thought to underlie many forms of learning and memory (reviewed in [6]). In the hippocampus, synapses terminate on specialised structures called dendritic spines. These serve as small diffusion-limited compartments that organise the postsynaptic molecular machinery in space (reviewed in [7]). Disruptions in the structure and function of dendritic spines have been linked to a wide range of diseases, from intellectual disabilities [8] and autism-spectrum disorders [9] to psychiatric conditions [10].

Within the dendritic spine, there is a complex interplay between biochemical signalling and structural change. Actin filaments provide structural stability and determine the size and physical shape of a spine. Other postsynaptic proteins interact with the actin cytoskeleton in an activity-dependent manner. Induction of long-term potentiation (LTP), for example, leads to actin cytoskeleton remodelling, which allows the dendritic spine head to change in size and shape [1, 11].

Actin is present both as free monomers termed G-actin (globular) and microfilaments called F-actin (filamentous). Filaments are formed from actin polymers that bundle together to give the cell its shape. Filaments are polar, with the end that faces the spine head ("barbed" end) growing more rapidly than the opposite end ("pointed" end) [12].

Both G-actin and F-actin monomers bind adenine nucleotides. G-actin monomers marked with ATP are incorporated into growing filaments. ATP is slowly hydrolysed into ADP which serves as the molecular tag of an ageing filament. Filament regions rich in F-actin-ADP monomers are subject to severing by cofilin. Severing into shorter filaments is vital for actin remodelling as it generates more ends for polymerisation and depolymerisation. Another way of generating barbed ends is branching. This is mediated by the Arp2/3 complex which nucleates new filaments from the sides of existing filaments, forming a branched network [12].

So far, most models of the dynamics of actin or other cytoskeletal proteins have taken one of two directions: Some have focused on the biochemical reaction network important for cytoskeletal dynamics, without explicitly considering the spatial arrangement of subunits (e.g. [13, 14]). Others have provided mechanical models of the cytoskeleton that predict the changes in cellular shape in response to stimulation. These models, however, focus on the intracellular force distributions and ignore many of the biochemical constituents of the cytoskeleton (reviewed in [15]). Therefore, there is currently a need for

spatiotemporal biochemical models of actin dynamics that are able to combine structural aspects with biochemical signalling.

Here, we present a method for achieving this using the particle-based spatial stochastic simulator MCell [2, 3, 4]. MCell 3.3 provides a syntax for multi-subunit complexes that allows users to specify both macromolecular complexes and molecules that can adopt a large number of biological states (reviewed in [5]). We use this syntax and a system of subunits and tags to model actin cytoskeleton remodelling, including actin polymerisation and depolymerisation, branching of actin filaments, and filament severing and shifting. Our model includes a relatively small number of molecular species and biochemical interactions, but can be extended to include more components of the postsynaptic proteome in the future.

2 METHODS

We used MCell 3.3 [2, 3, 4]. MCell enables users to track the trajectory and state of every single molecule in a reaction compartment, making it possible to model molecules that can exist in many different states [5]. MCell also allows the use of immobile complex molecules which can consist of any desired number of subunits with defined states and orientations in space relative to each other. Here, we used this feature to create a 3D matrix of "subunits" as a frame for the cytoskeleton, and to develop a system with rule sets which would represent actin remodelling, as explained below.

MCell simulation files were written in GNU Emacs text editor and the definition of the 3D matrix cube with 8000 ($20 \times 20 \times 20$) subunits was written into a separate file using a C script. MCell simulations were run on standard laptop and desktop computers and took a few hours to complete for 10^7 simulations (1 s of model time). Results were visualised using the CellBlender add-on for Blender 2.78.

The biochemical components of the model are simplified and include the free monomeric molecules Arp2/3, cofilin, and G-actin (bound to either ADP or ATP). In addition, filamentous (F) actin exists as part of a macromolecular complex that interacts with the monomeric molecular components as described below. At the beginning of the simulation, 39 molecules of ADP-bound G-actin, and four molecules each of Arp2/3 and cofilin were released into the simulation volume. In addition, the simulation volume was seeded with a short, branched F-actin filament consisting of 15 subunits. We use a simplified representation that disregards the double-helical nature of actin filaments for the purpose of easier spatial representation.

Where possible, we took reaction parameters from previous models [14, 16]. However, since the focus of this work was to establish a general method to model filament dynamics in MCell, we did not work on refining estimates for unknown parameters or on determining parameter robustness.

The model files are available on github under the following link: `https://github.com/MelanieIStefan/actin_model`.

3 RESULTS

3.1 Model of actin filament structure using virtual subunits

As the MCell complex molecule feature has so far not been used for filament remodelling, the main focus was to develop a set of rules to model actin filament dynamics in MCell.

Whilst MCell allows users to specify multi-subunit complexes, the size and number of subunits of those complex needs to be specified from the start. We thus pre-defined a "virtual complex" of potential actin subunits. This was done by specifying a $20 \times 20 \times 20$ matrix, which represented possible sites for actin filament growth. This spanned the entire reaction volume. At the start of the simulation, only 15 of those sites were occupied with actual F-actin subunits (bound to either ATP or ADP). All other sites were occupied by "virtual subunits" (named "empty" in our simulation) and were hidden in CellBlender visualisations. Since subunits occupy no actual space in MCell, those virtual subunits do not impact the diffusional properties of other molecules. They can, however, undergo reactions to change, for instance, from an "empty" (virtual) subunit into an F-actin subunit, thus representing extension of the actin filament into a previously empty unit of space.

The outermost subunits of the "virtual complex" received a special designation as "border", in order to prevent errors that might arise from trying to extend filaments outside the original bounds of the $20 \times 20 \times 20$ matrix.

3.2 Actin polymerisation and depolymerisation

The actin filament matrix has directionality and the subunits were defined using x, y and z coordinates. To simplify modelling, the filaments were represented as linear strands of monomers growing upwards at a 45° in four directions: +x (right) and -x (left) on the xy-plane, and +z (forward) and -z (backward) on the yz-plane.

In our model, actin polymerisation and depolymerisation are taking place independently with defined kinetic parameters. Polymerisation occurs when a freely diffusing G-actin-ATP molecule collides with an "empty" subunit that is on the tip of a filament. As a result, a previously "empty" subunit will become occupied by an F-actin-ATP molecule. In order to avoid filament collisions, a polymerisation reaction can only occur when no other molecules occupy subunits in the immediate vicinity of the growing end. In contrast, depolymerisation occurs spontaneously from pointed ends that are occupied by an F-actin-ADP subunit. In this reaction, a previously occupied subunit becomes "empty" and one molecule of freely diffusing G-actin-ADP will be released. To simplify the model, G-actin-ADP molecules will spontaneously be phosphorylated with a defined kinetic parameter in order to be reused for filament growth. This scheme is illustrated in Figure 1 (left panel).

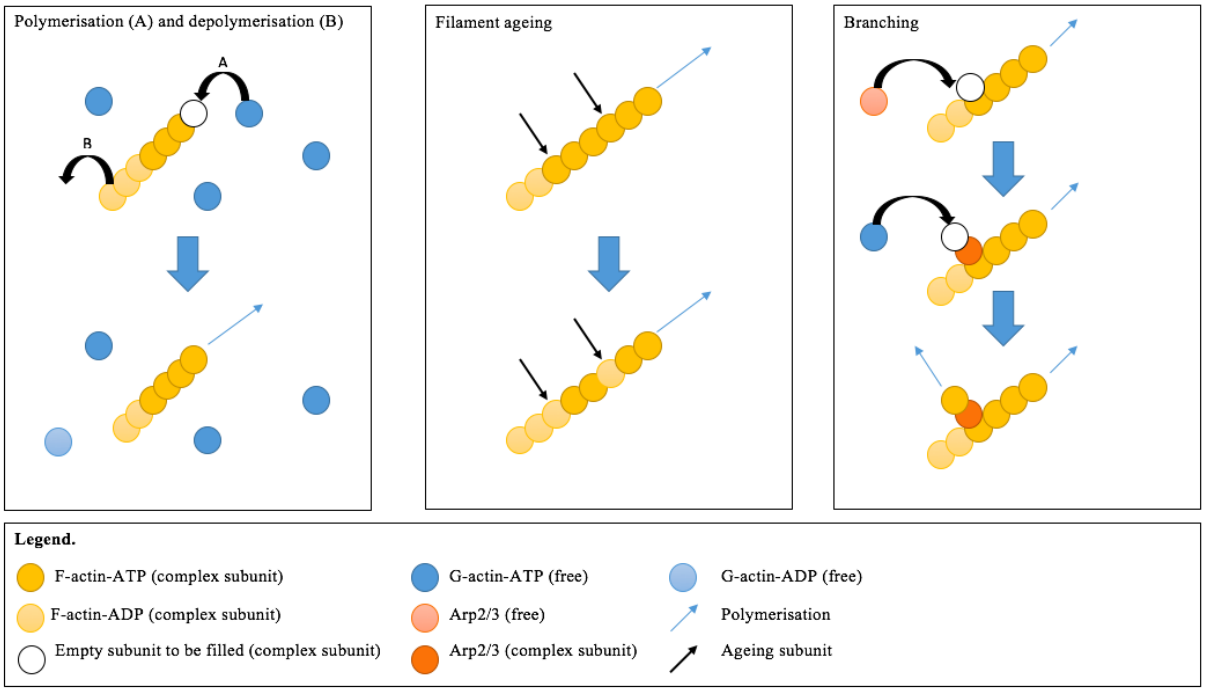

Figure 1: Method for modelling actin polymerisation and depolymerisation (left panel), ageing of actin filaments (middle panel) and branching of actin filaments (right panel).

3.3 Filament ageing

Filament ageing (middle panel of figure 1) involves the hydrolysis of ATP into ADP. In our model, this takes place with three different kinetic parameters depending on the subunit composition of the region. If a monomer is surrounded by F-actin-ADP monomers on both sides, then hydrolysis is taking place with the fastest parameter. If it is surrounded by F-actin-ATP and F-actin-ADP, then the kinetic parameter governing that reaction is half of the previous one. Lastly, if the monomer is surrounded by F-actin-ATP on both sides, then the speed is half of the intermediate speed. This ensures that hydrolysis is more likely to happen around subunits that are already hydrolysed, i.e. the parts of the filament that are older and closer to the pointed end. This is necessary since in memoryless stochastic models the reaction probability does not increase with time and hydrolysis would otherwise take place with similar kinetics throughout the filament.

3.4 Filament branching

Branching of actin filaments takes place when a freely diffusing Arp2/3 molecule collides with an "empty" subunit that is on the side of a filament that faces the spine head. Arp2/3 nucleation is followed by G-actin-ATP monomer binding to either of the three upward directions that are not parallel with the mother filament. After nucleation, polymerisation of the daughter filament continues in a similar manner to the mother filament (see figure 1, right panel). One filament can be branched from different locations and in different directions, resulting in the formation of a 3D network.

3.5 Filament severing and shifting

Another crucial event in cytoskeletal remodelling is the dislocation of parts of a filament. In this model, the concept of shifting involves vacating of the original set of positions in the matrix and instantly occupying neighbouring empty slots further away while maintaining the original structure. This required the development of a rule set for monomer tagging that allowed for full filament shift. We first developed a set of rules governing severing events. Severing divides filaments into two providing more barbed ends for polymerisation as well as more pointed ends for depolymerisation. Filament severing is mediated by cofilin and is preferably taking place in the older parts of the filament. Here, it is modelled as a reaction between freely diffusing cofilin and F-actin-ADP, whereupon F-actin-ADP instantly receives a tag (tag 1), which marks it for severing (figure 2). The tag is instantly transmitted to all neighbouring F-actin subunits towards the pointed end, regardless of their nucleotide phosphorylation status, until the tagging chain reaches Arp 2/3. An Arp2/3 molecule that is in the immediate vicinity of a subunit with tag 1 is instantly tagged as well. The untagged subunits from another branch next to the tagged Arp2/3 molecule will then be instantly tagged with tag (2) and now, tagging can take place in both directions and will also include all the other branches attached to tagged monomers. This ensures that only the parts below the site of severing will shift, leaving

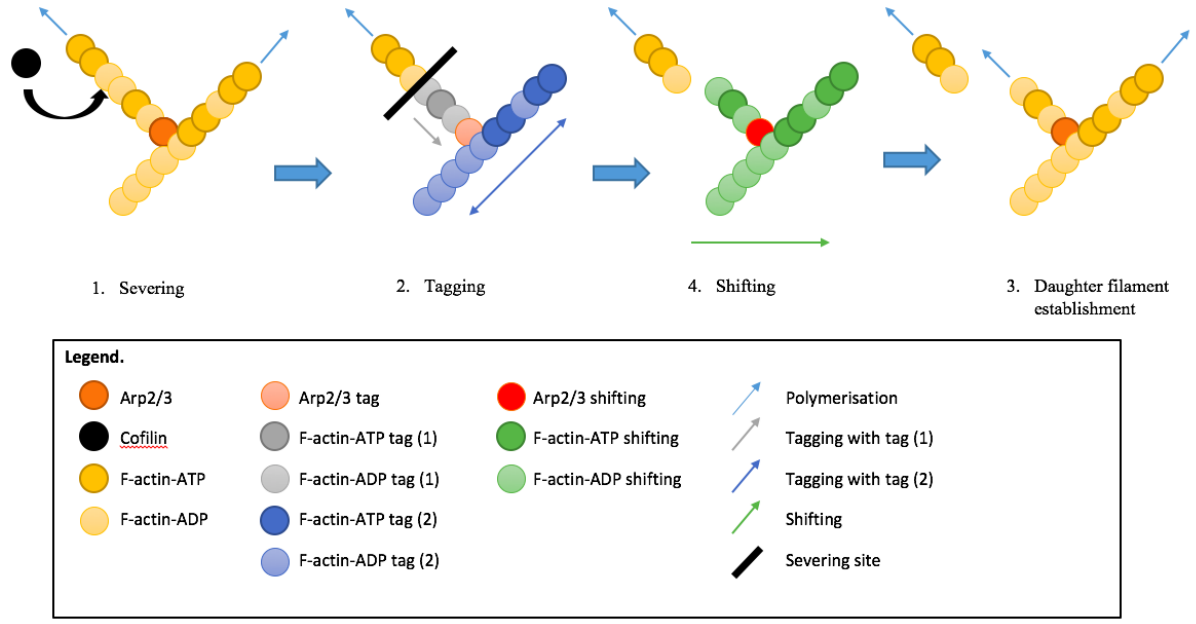

Figure 2: Method for modelling actin filament severing and shifting.

the upper part of the filament in its place.

The kinetic parameter for the tagging reaction is a large arbitrary number, chosen to be faster than any other reaction in the simulation. This is to prevent all the other possible changes that could take place within the filament during the same short time window. Once a molecule receives a tag, it cannot be subject to any other reaction events besides shifting. However, in order to avoid errors, the rule set includes rules that ensure that no other molecule is occupying the subunits through which shifting will take place. In current models, molecules are only shifting in the +x direction. Therefore, each subunit will only shift if the next slot in +x direction is unoccupied.

The kinetic parameter for shifting is also very fast but slightly slower than the tagging speed to ensure that no movement occurs before the whole filament is tagged. Briefly after the shifting intermediate is generated and labelled with a shifting tag, the original subunit disappears by being tagged as "empty".

The next step shifts the subunit by one more slot in the +x direction and converts it back to its original molecular state (e.g. F-actin-ATP). The shifting intermediate is again converted back to vacant subunits. This ensures that the original filaments and the part that shifted away from it are two subunits away from each other to allow polymerisation from the newly generated barbed end.

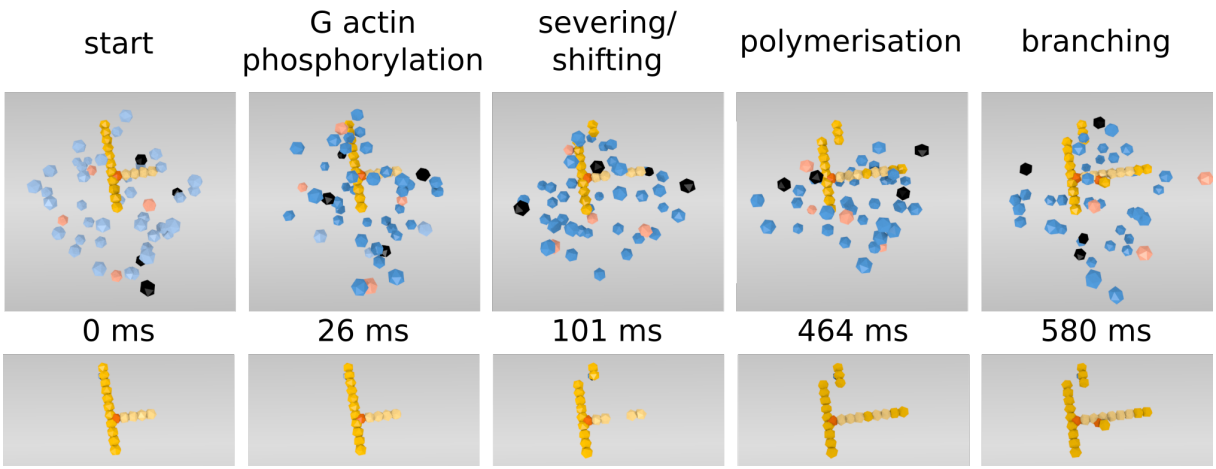

Figure 3: Stills from an MCell simulation showing G-actin phosphorylation, filament severing/shifting, polymerisation and branching.

3.6 Simulation results

In order to test the implementation of our rule set in MCell, we ran 10 simulations using different random seeds. Simulations were run with a time interval of 10^{-7} s, for a total of 1 s each. Within this time frame, we were typically able to observe one or several instances of filament severing and shifting, branching, and elongation. Filament ageing and depolymerisation have much slower reaction rates, and are therefore not observed in this time frame. As an example, still images from one of the simulations (using random seed 4) are shown in figure 3.

4 DISCUSSION

This work presents a proof of concept that the "complex molecule" syntax in MCell 3.3 [2, 3, 4] can be used to model actin cytoskeleton dynamics and, more generally, assembly, disassembly and movement of any type of biopolymer. Thus, the particle-based simulator MCell can act as a bridge between structure-based models of biopolymer assembly and models of cellular biochemical pathways.

Our present model is an implementation of the methods we have developed to model different aspects of actin dynamics in MCell and serves as a prototype. The representation of actin filament dynamics could be extended, e.g. by allowing shifting of severed polymers over wider ranges. There is also work to be done in determining and refining the parameters governing all reactions in order to create a model that closely matches results from previous experiments.

Finally, the model can be combined with models of biochemical pathways within the dendritic spine. We have previously worked on Calcium-dependent signalling pathways in dendritic spines [17, 18, 19]. For instance, Calcium signalling leads to activation of CaMKII, a key protein for initiating synaptic long-term potentiation [6]. CaMKII inter-

acts with the actin cytoskeleton and thereby regulates its ability to remodel and to regulate spine structure. Combining our model of actin dynamics with a model of CaMKII activation could give a unique insight into how biochemical regulation and structural changes interact to regulate the synaptic machinery that controls learning and memory.

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