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Short Term Mobility Program
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Scientific report

My stay in Pisa at the Scientific Visualisation laboratory of Monica Zoppe' lasted from the 23rd of February till the 7th of March 2009. During this period, we have been working on the visualisation of the cytokinesis event in a typical mammalian cell. In particular, the scene will depict a calmodulin molecule which upon calcium binding changes conformation. As a result, calmodulin induces the activation of myosin light chain kinase (MLCK). Finally, MLCK phosphorylates the regulatory light chain of myosin, leading to enhanced motor activity of the myosin filaments. This results in the contraction of the actin fibers of the cleavage furrow, eventually leading to the pinching off of the daughter cell. I have been working on the background of the scene, which is composed of the cleavage furrow that is being contracted by the activity of myosin filaments. One collegue from Monica's lab was charged with the contractile movement of the plasma membrane, whereas I have been working on the cytoskeleton as it occurs at the cleavage furrow.

In order to create a realistic view of the actin- and myosin filaments, we made extensive use of the protein database (PDB) crystal structures. Information about the positioning of the actin monomers with respect to each other in an actin filament was retrieved from the PDB file 1ALM. This model contains 5 actin monomers and was loaded in the molecular visualisation program Chimera (http://www.cgl.ucsf.edu/chimera/). Since the actin models only contain the alpha-carbon atoms, another, atomic resolution crystal structure of actin (PDB file 1ATN) was also loaded into Chimera. Five copies of this latter structure were aligned with the lower resolution filamentous actin structure, yielding a high resolution actin filament model. Since the cleavage furrow will be quite far away in the scene, there is no need to model it at atomic resolution. Therefore, all the models were redrawn using the Chimera built-in multiscale option, which produces surface models with resolutions that can be determined by the user. These final models were saved as wavefront objects and imported into Blender, which is the 3D animation program that was used to produce the scene. In Blender, one of the 5 aligned actin molecules was copied. Next, an array modifier was created, that multiplies the single actin model by a user defined number. By carefully adjusting the offset and rotation settings of the array, the actin filament could be exactly mimicked. The new array-based actin filament is very easily manipulated in Blender, and can be multiplied and elongated in a snap.

The myosin filaments were also created by making use of PDB structures. The PDB files 1I84 and 1SR6 were used to retrieve two extreme conformations of the myosin heavy chain head portion (which encompasses the motor domain). Furthermore, part of the coiled-coil tail structure from two intertwined myosin molecules was retrieved from PDB file 1NKN. These three structures were loaded into Chimera, together with the previously mentioned 1ALM file which contains the 5 aligned actin monomer. The latter file also contains a myosin head domain, which is bound to the actin filament. This gave us the opportunity to align the myosin head group to the actin filament. The other myosin structures were aligned to the 1ALM file and the surface models were created and exported as described previously for the actin molecules. The models also include the myosin essential light chain and regulatory light chain molecules, which are wrapped around the neck domain of the myosin heavy chain molecule. Again, the models were exported at a resolution that is lower than the atomic resolution. Once imported into Blender, the myosin motor domain movement had to be animated. For

this, an armature was created for one of the myosin surface models (the pre-power stroke model). An armature is comparable to a skeleton that directs the movement the softer body that surrounds it. Once the armature was connected to the myosin model, positioning of the bones lead to a smooth movement of the protein surface. The armature was subsequently posed in such a way that the connected protein surface model closely matched the other myosin surface model (the power stroke model). Care was taken to not only animate the large displacement of the head group, but also the more subtle displacements of smaller subdomains of the proteins. This was accomplished by adding armature bones into the bumps of the protein surface model. The resulting rigged model could now be moved from the pre-power stroke conformation to the powerstroke conformation by moving from one pose of the armature to the other. The final movement of the myosin molecule was composed of five partly overlapping steps:
1- power stroke (first 20 frames); the motion of the myosin head while it is attached to an actin filament; this motion causes the displacement of the actin filament.
2- release and lift (frame 20-40). The myosin head is released from the actin filament and the head is lifted away from the actin.
3- prepare for power stroke (frame 29-55). The myosin head is rotated around the neck to reposition it for the next power stroke.
4- drop (frame 41-66). The myosin head moves towards the actin filament.
5- connect (frame 66-67). The myosin again graps an actin filament; to visualize this, the motion of the bones stops for just one frame.

Of these five steps, only step 1 and 3 are based on crystal structure data; the others had to be animated manually, as there is no scientific data available on this movement.

The tail domains of two myosin heavy chains twist around each other to form a coiled coil. The 1NKN PDB file was used to model such a coiled coil. This model, however, only contains 89 amino acids per strand, whereas the tail domain of a myosin heavy chain molecule extends over hundreds of amino acids. Therefore, the model was imported into Blender and multiple copies of the small model were carefully aligned and connected manually until the correct tail length was reached. Myosin is active as bipolar filaments, which are composed of 10-20 pairs of myosin molecules (Cell Struct. Funct.(1990) 15, 343–354). To mimic this, a bundle of myosin tails was created by joining a number of tail models together. Twenty myosin head models were placed pairwise at the two ends of this filament model. The conformational movement of the headgroups was subsequently shifted with respect to each other to create a more realistic, shuffled animation. Finally, the acceleration of the movement by calcium waves that lead to MLCK activation had to be animated. For this, each armature object (which drive the movement of the myosin head groups) was given a so-called time-ipo. This allows for acceleration or deceleration of the movement of the head groups. As all the head groups were given the same time-ipo, the speed of movement can be globally changed easily.

We conclude that the project was successfully finished. All the basic cytoskeletal models and their movements have been finished and are ready to be incorporated into the final scene.