Polymerization of Filaments

Julian Charles Shillcock 18.10.1960
1982: B.Sc (Hons), Physics (Kings College London)
1985: M.Sc, Nuclear Physics (Simon Fraser University, Canada)
Thesis: Hanbury-Brown Twiss Effect in Heavy-Ion Collisions
1986-1990: Research Scientist (British Aerospace, Space Systems Division, U.K.)
1995: PhD, Biophysics (Simon Fraser University, Canada)
Thesis: Elastic Properties of Fluid and Polymerised Membranes under Stress
1995-1997: Postdoc (Max Planck Institute of Colloids and Interfaces, Potsdam)
1998-1999: Senior Scientist (Molecular Simulations Inc., Cambridge, U.K.)
1999-2003: Group Leader (Max Planck Institute of Colloids and Interfaces, Potsdam)
2004-2007: External Research Associate (Max Planck Institute of Colloids and Interfaces, Potsdam)

The cytoskeleton of a cell is a major structural component that gives rigidity and support to the plasma membrane and participates in numerous cellular processes. It is composed of rodlike filaments of varying degrees of rigidity that self-assemble, and disassemble, in response to cellular signals. Actin filaments form one part of the cytoskeleton, and are composed of many hundreds of actin monomers that bind together into linear and branched filaments. Each monomer is a globular protein approximately 5 nm in diameter that contains a bound ATP molecule whose hydrolysis, and subsequent phosphorylation, provides the energy required to drive filament growth. In motile cells, actin filaments continually form and disassemble in a process that requires the consumption of ATP. This process is referred to as treadmilling, and is the basis for cell crawling. Although experiments have revealed many fascinating aspects of actin treadmilling in generating cellular motion, the molecular details of the process are still unclear. Molecular Dynamics simulations of small sections of filaments have shown the importance of electrostatic interactions in guiding the monomers onto the ends of the filament, and the kinetics of monomer addition and loss at the two ends of a short filament **[1]**. However, these highly-detailed simulations are limited to short lengths of filament because of their computational cost.

In order to visualize F-actin growth and treadmilling in filaments containing hundreds or thousands of monomers, we are using Brownian Dynamics simulations without an explicit solvent. Each actin monomer moves under the influence of forces between monomers, but has a bulk diffusion coefficient that is a parameter of the simulation. The absence of solvent particles allows simulations of filament growth over times approaching several milliseconds. Actin monomers are represented as polar rigid bodies that diffuse freely around the simulation box and, if they encounter the ends of a filament, can bind to it. The terminal monomers can also unbind from a filament at a constant rate (**Fig. 1**).

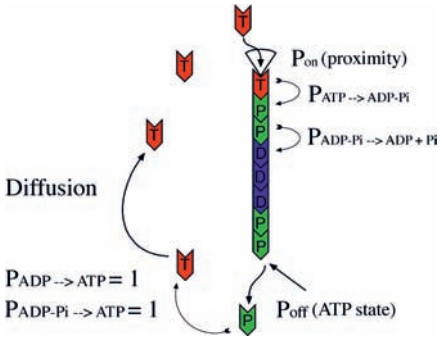

Fig. 1: Diagram showing how the attachment and detachment of actin monomers from a filament is modelled in the simulations. Monomers diffusing in the bulk possess a bound ATP molecule (red monomers). Once a monomer binds to a filament, its ATP molecule has a certain probability of being hydrolysed to ADP with a bound Pi (green monomers). Later, the bound Pi can dissociate leaving the monomer with a bound ADP. The probability for the two terminal monomers of a filament to detach may depend on the monomer's internal state. The ATP molecules are not explicitly modelled in the simulations, but each actin monomer has an internal flag that represents its ATP state. Monomers may be restricted to a single state by setting the probability of ATP hydrolysis to zero, or may be given two states if the probability of the transition from ADP with bound Pi to ADP is set to zero. In the most general case, the internal flag has three states with three transition probabilities. All monomers that detach from a filament are instantaneously converted to ATP monomers as the phosphorylation of the freely-diffusing actin monomers is expected to occur more rapidly than the attachment of monomers to a growing filament in the experiments of interest.

The two ends of F-actin filaments are referred to as the barbed and pointed ends, and are not equivalent. The rates of monomer attachment and detachment are typically different for the two ends, attachment being faster at the barbed end while detachment occurs faster at the pointed end. Monomers have an internal flag that represents the state of a bound ATP molecule: it takes the values ATP, ADP with bound inorganic phosphate, ADP-Pi, and ADP with the phosphate released. The unbinding rates at the filament's ends depend on the terminal monomer's internal state.

Kunkun Guo, a post-doctoral fellow, has been exploring various quantitative measures of a filament's properties and growth behaviour. The stiffness of a single filament is measured from its shape fluctuations in an external potential, and the attachment and loss of actin monomers to a filament is studied as a simple model of treadmilling. Our preliminary results on filament growth are in agreement with previous theoretical models **[2]** in which multiple states of bound ATP/ADP in the actin monomers are required in order to reproduce the observed properties of actin filaments, including the fluctuations in length of a filament as a function of bulk monomer concentration. It currently appears that a filament composed of actin monomers with only one internal ATP state grows tran-

siently but then disintegrates. Monomers that have two internal states appear to show transient periods of treadmilling. A snapshot of a growing filament that consists of monomers with three internal states is shown in **Fig. 2**.

Fig. 2: Snapshot of a growing filament composed of monomers with 3 internal states. The bulk of interior of the filament is made up of monomers with bound ADP (shown in blue) whereas the two ends are composed of monomers with bound ATP (red) or ADP-Pi (green). The sizes of the caps are different at the two ends because the probability of the terminal monomer detaching depends on the state of the monmer, and the precise values are chosen to be different for the two ends.

The bulk of the filament consists of ADP monomers (shown in blue), while the two ends consist of short caps of ADP-Pi (green monomers) and ATP monomers (red). The lengths of the caps, and their proportions of red to green monomers, are different because the detachment probabilities of the monomers depend on the monomer internal state and are chosen to be different at each end to reflect the polar character of actin monomers in the experiments. We are exploring the model's parameter space to see if treadmilling can be observed as a steady-state phenomenon, and to measure quantitative properties of the process **[3]**. **Fig. 3** shows preliminary results for the fluctuating length of a filament composed of monomers with only a single internal state.

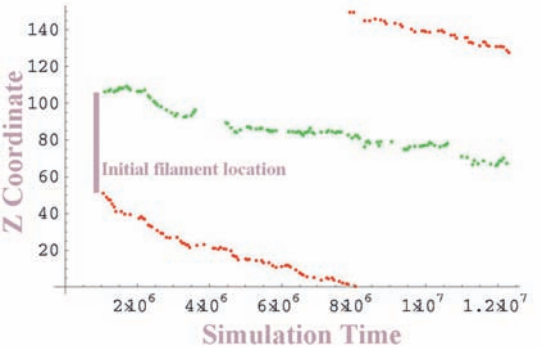

Fig. 3: Plot of the z coordinates of the newly-attached terminal monomers of a growing filament as a function of time. When a monomer attaches to the filament its instantaneous z coordinate is recorded. The two ends of the filament are shown in different colours with the pointed end in green and the barbed end in red, but these do not correspond to the orientation of the barbed and pointed ends shown in Fig. 1. We allow the filament to grow to a certain length before we start measuring its properties. An increasing gap between the two curves indicates that the filament is increasing in length, whereas a decreasing gap shows that it is shrinking. The discontinuity in the red curve at approximately 8,000,000 timesteps is due to the filament extending across the periodic boundary at the z ends of the simulation box.

The filament appears to increase in length continuously throughout the simulation period (the red and green curves move apart). This indicates that this particular filament is not treadmilling. **Fig. 4** shows the distribution of the time intervals between monomer-binding events for the two ends of the same filament as **Fig. 3**. The distribution is approximately exponential, although the relatively small number of data points (65) does not allow a definitive conclusion. This work is continuing.

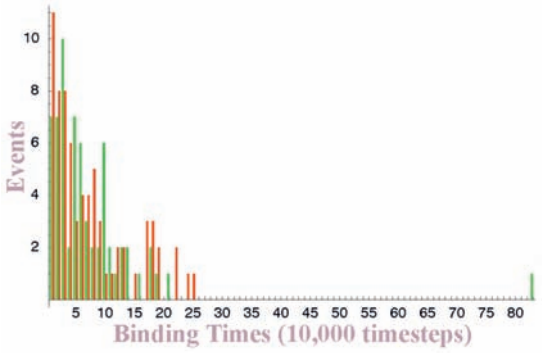

Fig. 4: Histogram of the distribution of time intervals between successive monomers attaching to the two ends of a growing filament (green curve is the filament's pointed end, the red curve is its barbed end). The width of the bins is 10,000 timesteps, and the probability of attachment is seen to be approximately exponentially distributed.

J. C. Shillcock, K. Guo, R. Lipowsky.
Julian.Shillcock@mpikg.mpg.de

References:

[1] Sept, D. and McCammon, J. A., Thermodynamics and kinetics of actin filament nucleation. Biophys. J. **81**, 667-674 (2001).
[2] Vavylonis, D., Yang, Q. and O'Shaughnessy, B. Actin polymerization kinetics, cap structure and fluctuations. PNAS. **102**, 8543-548 (2005).
[3] Guo, K., Shillcock, J. C. and Lipowsky, R. Self-assembly, growth and treadmilling of actin filaments in coarse-grained simulations. JACS. (to be published 2007).