Exploring Biological Polymers Through Hands-on Models Designed for 7th and 8th Grade Students

Authors: Dorottya B. Noble[1,2], Eric M. Johnson Chavarria[2], Anthony Schramm[2], Joshua Young[9], Douglas B. Noble[3], Nadia C. Abascal[2], Corey S. O'Hern[1,4,5,6,7], and Lynne Regan[1,2,7,8*]

Affiliations: [1]Integrated Graduate Program in Physical and Engineering Biology, [2]Department of Molecular Biophysics and Biochemistry, [3]Office of Environmental Health and Safety, [4]Department of Mechanical Engineering and Materials Science, [5]Department of Physics, [6]Department of Applied Physics, [7]Graduate Program in Computational Biology and Bioinformatics, [8]Department of Chemistry, Yale University, New Haven, CT 06520, USA; [9]Hopkins School, New Haven, CT 06515, USA

*corresponding author (lynne.regan@ed.ac.uk)
Current affiliation of corresponding author: Centre for Synthetic and Systems Biology, Institute for Quantitative Biology, Biochemistry and Biotechnology, School of Biological Sciences, University of Edinburgh

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Abstract: We designed an education module that enables middle school students (ages 12-14) to explore the physical properties of biological polymers by examining and experimenting with two 3D-printed models for actin molecules, a common biopolymer necessary for critical cellular processes. One model is held together by springs so that students can explore the flexibility of polymers and how the springs are strained as the polymer twists and bends. The other model consists of hand-sized actin monomers that allow students to explore the shape of an actin monomer and work together to build an actin filament. Students are taught how to assemble the biologically relevant nucleating fragment, comprised of three actin monomers, before growing the fiber by subsequent addition of one actin monomer at a time.

1) Introduction and learning objectives

Hands-on learning has been shown to be a powerful mode of classroom instruction.[1] With the advent of strategies like project-based learning and model-based instruction, teaching within STEM fields has benefitted from streamlined, clever applications of concepts that employ demonstrations and experimental set-ups that allow students to experience science first-hand.[2-3]

Here, we present an experimental module, using actin as a model system, which enables middle school students to explore the physical properties of biological polymers. Actin is an abundant protein that plays an important role in many cellular processes, including force generation, cell migration and division[4]. Furthermore, actin is a well-studied protein that is the focus of research projects at institutions worldwide, including in the Integrated Graduate Program in Physical and Engineering Biology[5] (IGPPEB) at Yale University. An important component of the IGPPEB is outreach and science communication to school-age children. In one of our outreach initiatives, IGPPEB graduate students developed instructional modules based on on-going research for a summer enrichment program, Pathfinder Hopkins School (formerly Breakthrough New Haven), for disadvantaged middle school students in New Haven, CT. The modules were designed so that they will be appealing to middle school students with the goal of fostering the intrinsic interest of this age group in the natural world.

Our approach was to first make prototypes, following discussions with teachers to ensure that the level of the modules and their implementation were appropriate for middle school students and the setting in which they would be offered. The IGPPEB students then tested the modules at one-day events for middle-school students and modified and improved them based on input from the students and instructors. The polished versions of the modules are now used in the science component of the summer enrichment program. In this article, we describe the module in detail and in particular how instructors should prepare for the modules and offer a lesson plan to teach the module.

This module introduces students to the basic concepts of protein self-assembly and polymer physics. As part of their natural function, proteins, such as actin, can assemble into long structures that are able to bend and twist. Students will explore the structure and properties of “actin filaments” – the self-assembled form of actin monomers. The module consists of two parts. In Part 1, the Polymer Spring Model, students will become familiar with ways of describing polymers and their physical characteristics and will develop an understanding of how bending forces deform polymer structure. In Part 2, the 3D Actin Model, students will be able to describe the structure of actin by examining a three-dimensional (3D) model of an actin monomer. They will determine the unique way in which actin monomers fit together to assemble into an actin filament.

2) Preparation prior to offering the module (see Table S1)

3D printing: Several components of the models used in Parts 1 and 2 must be 3D printed. They will take multiple days to print. For example, one actin monomer used in Part 2, printed on the MakerBot Replicator 2, takes 3 hours to print. 3D printing can be outsourced, for example, through shapeways.com.

Part 1. *Polymer spring models and components:* Build two different types of actin polymer models for each group of students. The first model, called the actin-actin polymer, consists of oval shaped 3D printed pieces with four pegs (each oval represents one actin monomer) (Figure 1A and 1B; supplementary file: “BareActin.thing”). The other model, the actin-phalloidin polymer, consists of

oval (actin) (supplementary file: “ActinPhalloidin_Actin.thing”) and spherical (phalloidin) (supplementary file: “ActinPhalloidin_Phalloidin.thing”) shaped 3D printed pieces, with six pegs on each oval shaped piece and two pegs on each spherical piece (Figure 1A, 1C, and 1D). The pegs are used to connect the 3D printed pieces in the model via springs that are ~1cm in length. The springs come in 20-inch-long sections. Cut them to the appropriate length (~1 cm) using a diagonal cutting plier. Each group of students requires approximately 20 oval actin pieces each with four pegs, 20 oval actin pieces each with six pegs, and 20 spherical phalloidin pieces each with two pegs. Monomers are printed with a raft (base) and supports (which support the overhangs in the monomer, such as the pegs). After manually breaking off the base and supports from each monomer, the pegs may need to be filed down if excess support structure remains.

Assembling the actin-actin polymer spring model: Connect the pegs on the oval pieces (with 4 pegs each, Figure 1B), as shown in Figure 1A, using the pre-cut springs.

Assembling the actin-phalloidin polymer spring model (Figure 2): The pegs on the actin monomer are connected either to other oval shaped actin pieces or to the spherical shaped phalloidin pieces, using the pre-cut springs. The pegs that are further apart (1.8 cm) should be connected to the phalloidin pieces. The pegs that are closer together (1.3 cm) should be connected to other actin pieces. Connect the actin and phalloidin pieces, as shown in Figure 2.

Note that each actin monomer has a “top” and “bottom” resulting from the 3D printing process. The bottoms will be rough where the supports were attached. When assembling the polymer, one strand should be composed of all “tops” and others all “bottoms”. If the polymer is not assembled in this manner, it will not attach properly without significant strain on the springs connecting the monomers. To make sure that the springs attach to the pegs correctly, the instructor should make sure that the pegs have the correct shape and are smooth. We used the MakerBot Replicator 3D printer, with a resolution of 0.2 mm per layer. Leave the spring on the pegs once assembled, since repeated assembly and disassembly can deform the springs and loosen the connections.

Part 2. To prepare the 3D actin model, in which students will assemble actin monomers into an actin fiber, the first step is to 3D print actin monomers found on the Thingiverse repository, thing:45393 (makerware.thingiverse.com). The 3D printable file can be downloaded and printed on a 3D printer (“actin_notelevated.stl”). Importantly, prior to 3D printing, the dimensions of the model should be rescaled to approximately 9.5 cm by 9 cm by 4.5 cm. When printing, do not rotate the downloaded model. The orientation provided in the file will result in the best strength and surface quality. At least 3 actin monomers should be given to each group of students. Once the actin monomers have been 3D printed, they need to be modified. To complete the lateral connection between two actin monomers (see ball 2, joint 2 in Figure 3), using the locations already provided by the peg and hole connections of the model, cut/shave off the peg using the Dremel tool (see Section 5 for Safety Tips) and if needed, file down the already existing, original holes so that they are smooth. Snap one joint magnet in the original hole of the actin monomer so that the countersink faces out (see joint 2 in Figure 3). Glue this joint magnet in place by applying gorilla super glue to the edge of the hole. Wait 10 minutes for the glue to set. Next, place one ball magnet on the joint to ensure proper alignment of the magnets for creating an attractive force. Place gorilla super glue in the recess that remains after cutting or shaving off the peg and stick the proper side of the ball magnet into the recess (see ball 2 in Figure 3). To complete the longitudinal connection, place the second joint magnet in the natural recess area of the pointed side and secure with gorilla glue (see joint 1 in Figure 3). Then, place the ball magnet on the joint magnet to ensure proper magnetic alignment and find the corresponding connection on the opposite end of the other monomer (see ball 1 in Figure 3). You may need to shave down this area and add gorilla glue. Finally, bring the longitudinal area into contact with the ball magnet

that is resting on the joint magnet. Repeat these steps to glue all magnets in place. Ultimately, each actin monomer will have two joint and two ball magnets.

3) Lesson Plan

Part 1. Polymer Spring Model. (~20-30 minutes) In Part 1, students are introduced to polymers and their physical properties, exploring two different versions of an actin polymer spring model.

Background on polymers in biology: The instructor should introduce students to polymers, and specifically to the actin polymer. Polymers are chain-like structures made of smaller, repeating units. The instructor should ask the students whether they know any examples of polymers. Students may be familiar with "slime" or may know that plastic used to make water bottles, and fabric, such as wool or silk, are made of polymers. Next, the instructor should introduce the actin filament, which is an example of a biological polymer. It is made of the protein actin and is needed by cells to function properly. The actin polymer forms a network that provides firmness and organization to cells. It also allows cells to generate forces needed to move, grow and divide. Polymers are often flexible and can be bent or twisted. Depending on the monomers from which they are constructed, polymers have different bending and twisting rigidities, which determine how much they bend and twist in response to applied forces and torques.

Examining the actin-actin polymer spring model: Each group of students should be given an actin-actin polymer spring model to examine. The instructor should explain that in this model, each oval shaped piece represents a single actin protein, or actin monomer. The instructor can point out that the prefix "mono" means "one," whereas "poly" means "many," thus the actin monomer refers to a single actin protein, but the actin polymer refers to the actin filament consisting of many individual actin proteins or monomers. The instructor should ask students to examine their model and describe their observations. These can be shared aloud or written down. Important things to notice are: 1) the model is flexible, 2) the model has two identical strands of actin monomers, 3) there is a twist within the polymer, 4) springs hold the actin monomers together, and 5) there are two different types of springs (see Figure 4). If students need guidance to help discover these aspects of the model, questions can be posed, such as "How are the individual actin monomers connected in the model?" "Can you identify two different types of springs that connect the actin monomers differently?" The instructor should point out that the monomers form connections with each other, both within a single strand – which we call longitudinal links – and between the two parallel strands – which we call lateral links[6] (Figure 4). Therefore, although the monomers may look the same and have identical properties as individual units, different monomers may experience different forces within a polymer, which students will investigate.

Bending and twisting the actin-actin polymer spring model: The instructor should ask students to gently bend the actin-actin polymer spring model and describe what they observe. There are several things to notice. First, when the polymer is bent, the springs become stretched around the bent portion. This type of deformation, which affects the area around the bend the most, is called strain. We have found that students who are keen observers may also notice that the longitudinal springs are more strained than the lateral springs (Figure 5). This suggests that the longitudinal links are more important in determining how much force is required to bend the polymer[7]. The instructor can help students see this detail by asking them to describe how much the two different types of springs stretch when bending the fiber. Next, the instructor should ask the students to gently twist the actin-actin polymer spring model and make observations about what happens to the filament. They should notice that when the filament is twisted with the natural twist of the model, both longitudinal and lateral springs are strained (Figure 6), indicating that both

the longitudinal and lateral links are important in determining how easily the filament can twist. However, if the polymer is twisted against its natural twist, the lateral springs become more bent than the longitudinal ones (Figure 6), meaning that the polymer experiences more force within the lateral springs. This is in contrast to bending, where the longitudinal springs are strained more. Both types of springs contribute to twisting stiffness, which quantifies how hard it is to twist the polymer.

Just as manipulating the actin-actin polymer spring model required energy, because students had to physically bend or twist the model using their muscles, inside the cell, energy is also needed to change the shape of the actin filament. If a large force is applied to bend or twist the actin-actin polymer spring model, it is possible to overstretch the springs, have them pop off, or even break the model. This effect can also occur in real actin filaments inside our bodies, which can also break under large forces that strain individual links in actin filaments. Ongoing research is being carried out to understand how real actin filaments break[7-11].

Actin-phalloidin polymer spring model: The instructor should pass out the actin-phalloidin polymer spring model to students and ask them to describe how this model is different from the actin-actin polymer spring model. Students should look at it and bend and twist it. The main differences are: 1) there are extra spherical pieces connected to the outer surface of the oval shaped actin monomers, and 2) the actin-phalloidin polymer spring model is slightly harder to bend, is much harder to twist, and is significantly stiffer overall. The instructor should explain that molecules that bind the actin filament together can change its physical properties, for example, making it more or less stiff. Such changes can affect its function. In the actin-phalloidin model, phalloidin, a peptide toxin produced in the death cap mushroom *Amanita phalloides*, is bound to the actin filament[12]. Phalloidin is toxic to humans because it prevents filament breakage, which is a normal, "healthy" process in our cells.

Part 2. 3D Actin Model. (~20-30 min) In Part 2, students become familiar with the 3D structure of an actin monomer and will discover how to build an actin filament using actin monomers.

Examining the structure of an actin monomer: The instructor should give each student a 3D printed actin monomer and explain that it is a model of the actin monomers inside our cells. To help grasp the tiny size of an actin monomer, the instructor can ask the students to guess how many actin monomers would need to be lined up, with their longest sides touching, to be as wide as a human hair. The answer is approximately 15,000 (assuming that an average human hair is 100 micrometers and the longest side of an actin monomer is 67 Angstroms[13]). The instructor should ask students to explore their 3D printed actin monomer and describe it in their own words. They should notice that there are many small bumps and grooves on the surface and some larger indentations. These arise from the shapes and arrangement of amino acids, which make up proteins. The instructor can tell students that each actin monomer is made up of 375 amino acids, giving rise to its complex surface.

Assembling three actin monomers: The instructor should ask student groups to find as many different orientations as possible, in which to assemble the three actin monomers. Students should wiggle the structures around and determine the orientation that forms the most stable structure. Such a structure should wiggle minimally with minimal space between the monomers (Figure 7). The assembly of such a short, stable fragment, formed in a process called nucleation, is the first step in building the actin fiber. Once the nucleating fragment is formed, actin monomers are added one at a time in a specific orientation. The instructor should ask students to examine the structure of the nucleating fragment and describe its ends. The students should see that the two ends are not identical, rather one end sticks out more than the other and has a large bump

on the bottom actin monomer (see red monomer in Figure 7). The observation that the two ends are not identical is important because inside the cell, it is the presence of distinct ends that gives rise to directionality, allowing for the addition of monomers to the same end, thus growing the actin filament in a specific direction. This type of directional growth allows cells to exert forces and move in specific directions.

Assembling the actin filament: The instructor should encourage students to build a long actin filament by adding all available 3D printed actin monomers to the nucleating fragment. Students should work together, adding one actin monomer at a time until all available actin monomers have been used (Figure 8).

4) Safety Considerations

Be careful not to come in contact with the hot glue. When working with the Dremel tool, wear appropriate personal protective equipment, including protective eyewear and a mask. Avoid breathing in any dust that may result from cutting, drilling or filing. Do not wear gloves when working with the Dremel tool as they can get caught in the spinning part of the tool. Magnets, particularly neodymium magnets (sold by K&J Magnetics, for example) should be handled with care. Anyone with a pacemaker or other implanted device should not be in close proximity to these magnets. Also, do not put the magnets in your mouth or swallow them!

Figures

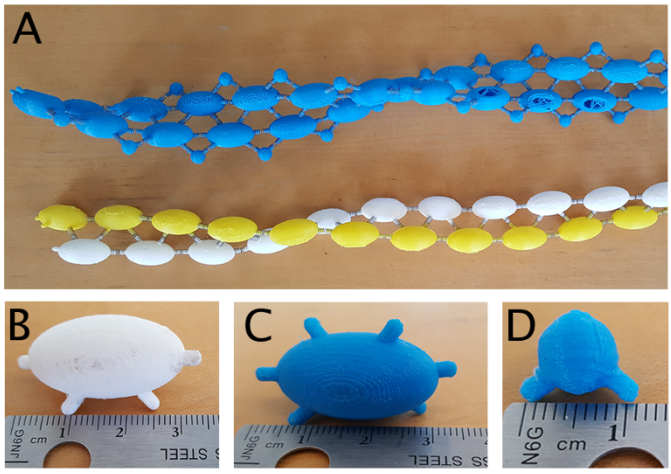

Figure 1. Polymer spring models and 3D printed components. A) The actin-actin polymer (bottom, here the actin monomers are white or yellow ovals) and actin-phalloidin polymer (top, here the actin monomers are blue ovals and the phalloidin are small blue spheres). 3D printed components are connected by approximately 1 cm long springs. B) Oval shaped 3D printed piece with four pegs, representing one actin monomer in the actin-actin polymer. C) Oval shaped 3D printed piece with six pegs, representing one actin monomer in the actin-phalloidin polymer. D) Spherical piece with two pegs, representing one phalloidin molecule in the actin-phalloidin polymer.

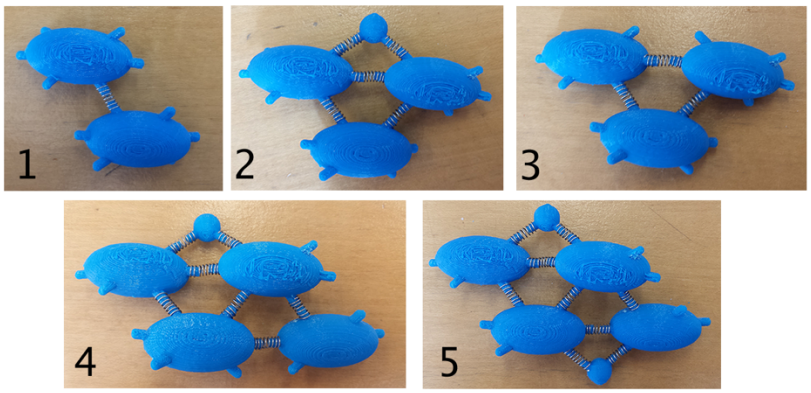

Figure 2. Step-by-step assembly of the actin-phalloidin polymer spring model, performed sequentially in steps 1-5.

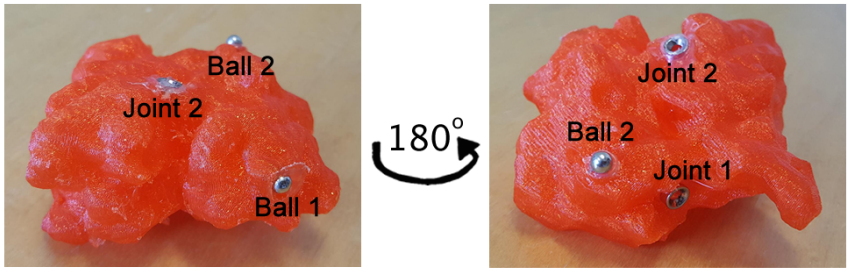

Figure 3. Image of the actin-actin polymer spring model. Two strands of the polymer are shown in yellow and white. Each oval shaped piece represents an actin monomer. The inset shows longitudinal (green box) and lateral (purple box) springs connecting monomers within and across strands, respectively.

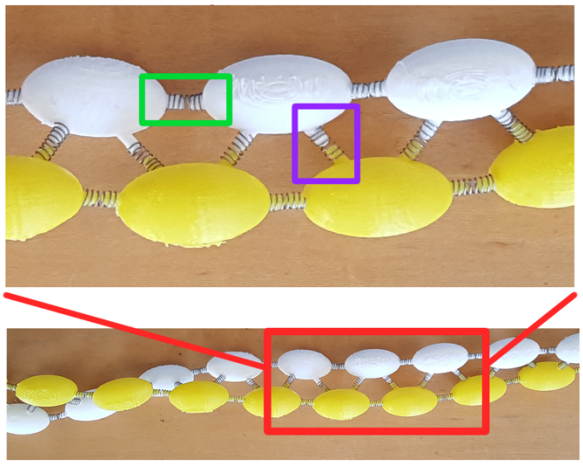

Figure 4. An actin monomer from the 3D actin model, showing the two ball and two joint magnet positions. The image on the right depicts the actin monomer after rotating 180 degrees to the right relative to the monomer pictured on the left.

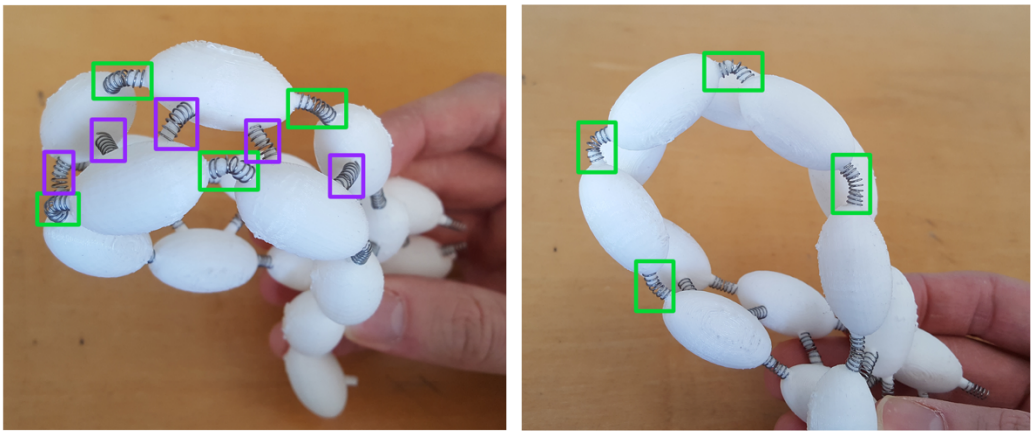

Figure 5. Bending the actin-actin polymer spring model. Longitudinal (green boxes) springs stretch more than lateral (purple boxes) springs.

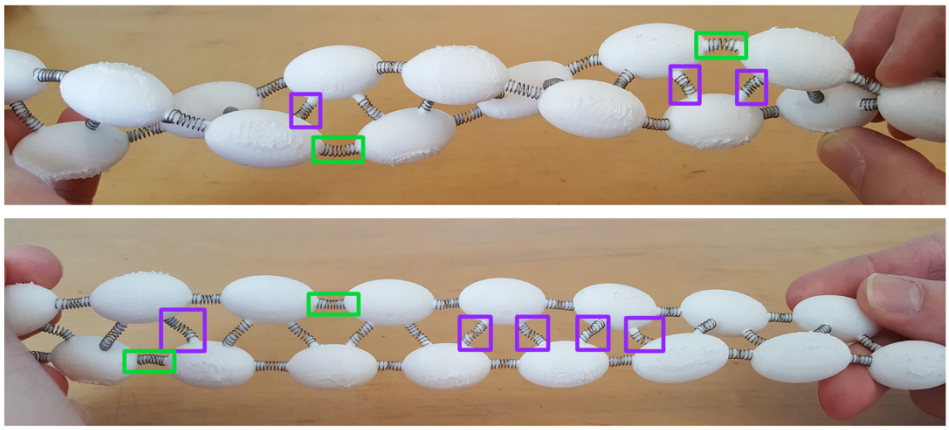

Figure 6. Twisting the actin-actin polymer spring model. The most strained longitudinal (green boxes) and lateral (purple boxes) springs are highlighted when the polymer is twisted in the direction of its natural twist (top) or against it (bottom).

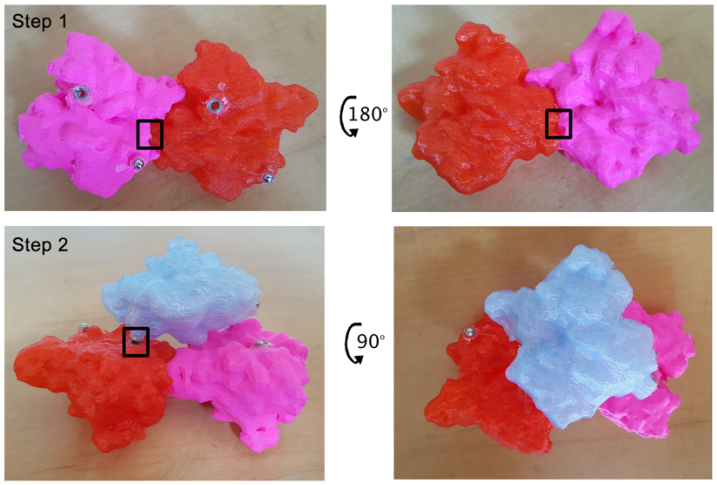

Figure 7. Assembling the nucleating fragment composed of three actin monomers. In step 1 (top images), place two 3D printed actin monomers together such that ball 1 and joint 1 magnets (see Figure 4) along the interface snap together (black box). In step 2 (bottom images), add a third actin monomer such that it is offset and ball 2 of the blue monomer snaps together with joint 2 of the orange monomer (black box and see Figure 4). Each actin monomer is a different color in the images for ease of visualization.

Figure 8. The actin fiber assembled by sequentially adding one actin monomer to the nucleating fragment (see Figure 7). In total, 12 actin monomers were used.

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