Probing the Microscopic Origins of Strain Stiffening in Biopolymer Gels

Victoria Reeves
Chemistry, Benedict College

NNIN REU Site: Center for Nanoscale Systems, Harvard University, Cambridge, MA
NNIN REU Principal Investigator(s): Professor David A. Weitz, School of Engineering and Applied Sciences/ Department of Physics, Harvard University
NNIN REU Mentor(s): Louise Jawerth, School of Engineering and Applied Sciences/ Department of Physics, Harvard University
Contact: vreeves16@yahoo.com, weitz@seas.harvard.edu, ljawerth@fas.harvard.edu

Abstract:

Using microscopic imaging of sparsely cross-linked actin networks, we looked to better understand what is responsible for strain stiffening in biopolymer gels. Actin networks were labeled with fluorescent dye to see how the individual filaments reacted to shearing. By polymerizing these filaments, *in vitro*, within a larger sea of unlabelled actin, we were able to observe the deviations of thermal fluctuations in these filaments.

With further research, the non-linear rheology of gels formed by these viscoelastic biopolymers could allow for further mechanisms by which cells can be made to respond to, and model, the mechanical characteristics of these networks.

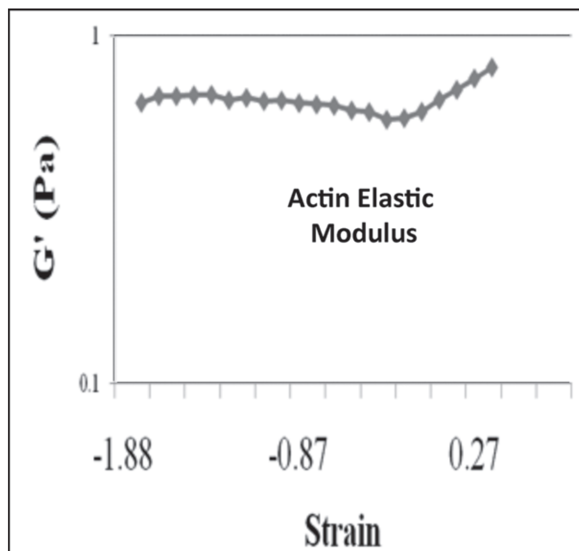

Figure 1: Nonlinear correlation between the strain applied to the network and stress until breakage.

Introduction:

Cytoskeletal microfilaments, located just below the cell membrane, are responsible for resisting tension and maintaining cellular shape. Actin, a subunit of microfilaments, contributes in major aspects of cellular functions, including muscle contraction and motility. The mechanical elements and elasticity of these networks are, more specifically, critical in determining how forces act on and effect living cells.

In vivo, the mechanical characteristics of the cytoskeletal network are not only controlled by the concentration of actin, but by the cross-linkers that bind the actin filaments into networks. Networks of biopolymers, like those of f-actin, have viscoelastic properties that are very different from those of other materials. When strain is applied to f-actin, the network is stressed more or less linearly, and then begins to stiffen, causing a nonlinear correlation between the strain applied to the network and stress until breakage, as shown in Figure 1. Models have been developed which explain this phenomenon from the dynamics of individual filaments. Rheological methods have been used to analyze this phenomenon, but no one, until now, has looked at individual actin filaments bound into a network by specific cross-linkers, in order to determine how the stress-strain relation actually effects the motility of the filament, network, and, in essence, the entire cell. *In vitro* methods were used to image the purely thermal fluctuations of actin, caused by Brownian motion.

Materials/Methods:

We crosslinked the actin filaments with heavy meromyosin, the larger of two fragments, acquired from the full-length protein, myosin II, which comes from rabbit skeletal muscle. This *in vitro* actomyosin network was prepared from 93 μM actin, obtained using purified monomeric actin from rabbit skeletal muscle, according to Spudich and Watt, and gel-filtered to remove capping proteins. All actin samples in this experiment were diluted with a combination of F-buffer and G-buffer. 93 μM actin was prepared with Alexa 488 Phalloidin and buffer to yield 0.28 μM F-actin, which was then added to a Rhodamine 532 fluorescent network prepared with buffer, heavy meromyosin (HMM/Actin ratio 1/100), and 93 μM F-actin. Oxygen scavengers were prepared using 2.25 M glucose, 7.15 M 2-mercaptoethanol, 20 mg/ml glucose oxidase, and 3.5 mg/ml catalase. (Diluted x10) Upon

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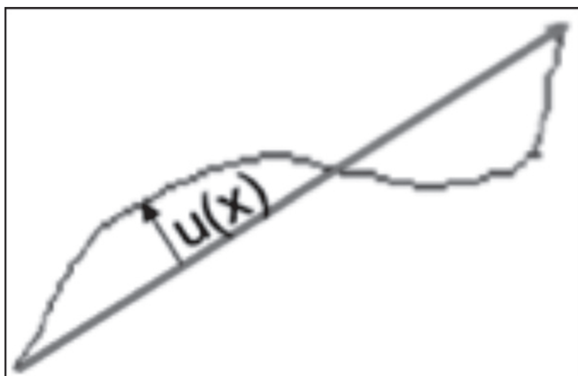

Figure 2: The straight line is the end-to-end distance of an individual filament while the function u(x) represents the deviation of this.

placing the network on the glass slide, 1.0 μl of OS was added to help control bleaching of the filaments. Filament fluctuations were analyzed using the model shown in Fig. 2.

Results/Discussion/Conclusions:

Using the parameters described, the total average deviation of a single actin filament from its original position was found to be approximately 1 μm. This was calculated using the imaging program *imageJ*. The maximum projection of an image is done by taking the maximum intensity throughout an entire stack (Z axis) and making that the pixel value. If there is something moving, relative to time, within a given stack, its maximum projection indicates the magnitude of its motion, and roughly the size of its fluctuations. The deviation of these fluctuations, from specific end-to-end coordinates, more simply, represents their amplitude. More calculations must be conducted to better quantify the size of these fluctuations.

From this experiment we have concluded that the imaging of individual actin filament is very possible, though precise sample preparation is required, based on the concentration of the gels, and imaging equipment being utilized. Photo-bleaching inhibitors were of great necessity with the imaging of our particular actin gels. With the incorporation of catalase-based inhibitors, the available imaging time producing quantifiable images and videos improved from a mere 30 seconds to over five minutes for a single gels sample. We have also concluded rough measurements of filamentous

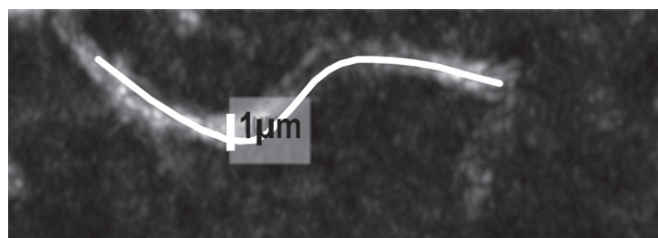

Figure 3: Max Projection Image Image of the greatest intensity values throughout the X, Y and Z positions of a single fluctuating filament in time.

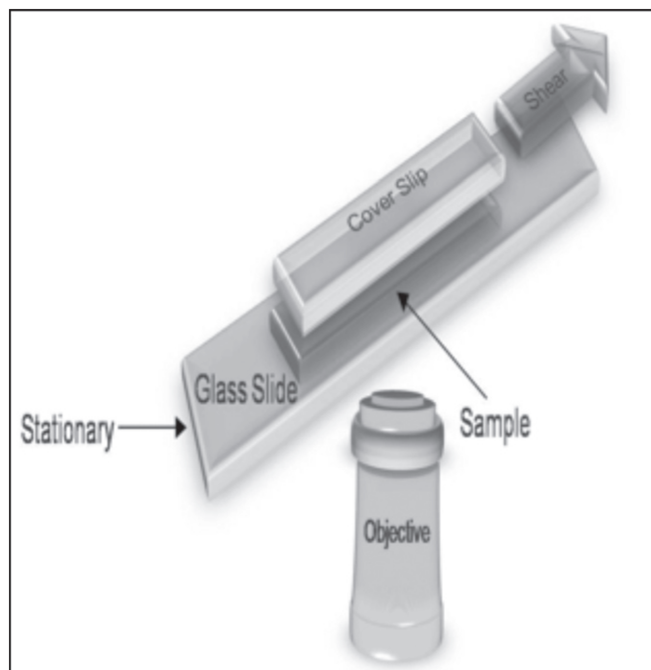

Figure 4: Our hypothesis is that the maximum projection of these filament fluctuations will decrease as a result of shearing.

fluctuation amplitudes can be conducted. Finally, we found that in a single Z-stack, the average fluctuation deviation from start to finish is 1 μm.

Future Work:

For future work, we would like to attach a shear cell, similar to the one in Figure 4, to a confocal microscope (Leica SP5, Germany) in order to apply stress to the network, and analyze the changes that occur in comparison to our un-sheared network. Confocal images would then be taken at different shear positions, until the network reaches critical strain and breaks. The shear cell would require a 40 μl sample at the same concentrations described above.

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