A Study of Protein Filaments Sliding through Solid-State Nanopores

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ABSTRACT

Solid-state nanopores have been used extensively over the past decade as powerful tools to study the structure and dynamics of single molecules of DNA. The use of nanopores has mostly been confined to the study of DNA due to the hope that they may eventually be used to cheaply sequence DNA. We have begun extending nanopores to the study of protein filaments, specifically filamentous actin (F-actin), one of three major components of the cytoskeleton. F-actin is a linear, negatively charged, helical polymer well suited for use in a nanopore setup. F-actin, however, behaves qualitatively different in solution than DNA as a result of their differences in persistence length. This enforces a different set of constraints on an F-actin molecule's interaction with a nanopore, allowing for new insights into the polymer dynamics of translocation through a nanometer-sized pore.

Keywords: single-molecule study, solid-state nanopores, actin, polymer physics, bio-sensing.

1 INTRODUCTION

Nanopores first emerged starting in 1996, when Kasianowicz *et al* [1] first observed a polynucleotide driven electrophoretically through an α-hemolysin protein channel that had self assembled in a lipid bilayer. Nanopores were quickly recognized as a powerful tool for probing the structure and electrophysical properties of single molecules of DNA, and this remains unchanged despite the transition from biological nanopores to solid-state nanopores [2-6]. Detailed work has demonstrated that nanopores are capable of assembling accurate information on a polynucleotide's length [7] and secondary structure [8,9], and even capable of shedding light on protein-DNA interactions [10-18]. These investigations have been vitally important for several reasons. First, solid-state nanopores serve as a biomimetic platform for studying the biologically relevant process of a polynucleotide translocating through a nanometer-sized pore, a process that occurs every time an RNA strand passes from the nucleus to the cytoplasm when a gene is expressed. Additionally, many nanopore based studies, both theoretical and experimental, use DNA as a model semi-flexible polymer to gain new insights into polymer physics [7, 22-34]. Finally, nanopores have attracted an incredible amount of interest because of their potential technological application to DNA sequencing [35-40]. The small diameter of a nanopore means that DNA must pass through the pore base by base. This basic aspect of nanopores led to the hope that they could be used to quickly and cheaply sequence entire genomes with minimal sample processing. This hope has yet to become a reality, but it continues to motivate an area of active research. Nanopores have mainly been applied to the study of polynucleotides because of this possibility of cheap sequencing, in addition to the fact that DNA is stable and commercially available.

In this proceeding article, we show that nanopores can also be used to study protein filaments in addition to DNA, and what new findings can be made from such an investigation. We will show preliminary data of the first ever, to our knowledge, translocations of filamentous actin through a solid-state nanopore. Finally, we discuss planned further studies to build on this work.

Specifically, in this preliminary survey we used solid-state nanopores to study filamentous actin instead of DNA. F-actin is a linear, charged biological polymer approximately 8 nm in width and variable in length from under a micron to over twenty microns [41]. Its charge and geometry make it well suited to study with solid-state nanopores.

F-actin provides essential biological functions in virtually all eukaryotic cells. It is most recognizable as the protein that works in conjunction with myosin to provide the force for muscle contraction. F-actin is also one of three protein filaments that provide the structural integrity of eukaryotic cells, the other two being microtubules and intermediate filaments [42]. Besides being arguably the most dominant among the three, it also provides the force that pushes out the cell membrane when a cell crawls. F-actin also has over a hundred accessory proteins, or actin-binding proteins, that regulate its function in the cell. The large variety of molecules that interact with F-actin provide countless potential subjects for nanopore based study once a nanopore based F-actin translocation assay is established.

In addition to its biological importance, F-actin represents an ideal stiff polymer, the study of which could yield deeper insights into polymer translocation through a pore. As mentioned previously, DNA has served as a model semi-flexible polymer in past theoretical and experimental work. F-actin, however, is much stiffer, with a persistence length of 17 μm as opposed to only 50 nm for dsDNA [43]. This several order of magnitude difference leads to qualitatively different behaviors in solution, as seen in the insets of Figure 1. Since dsDNA is relatively flexible, entropic forces keep the polymer from stretching out, causing it to form a tight ball in solution. F-actin, meanwhile, is much stiffer, behaving more like a rod in solution. These different behaviors present much different constraints on each molecule's interaction with a nanopore.

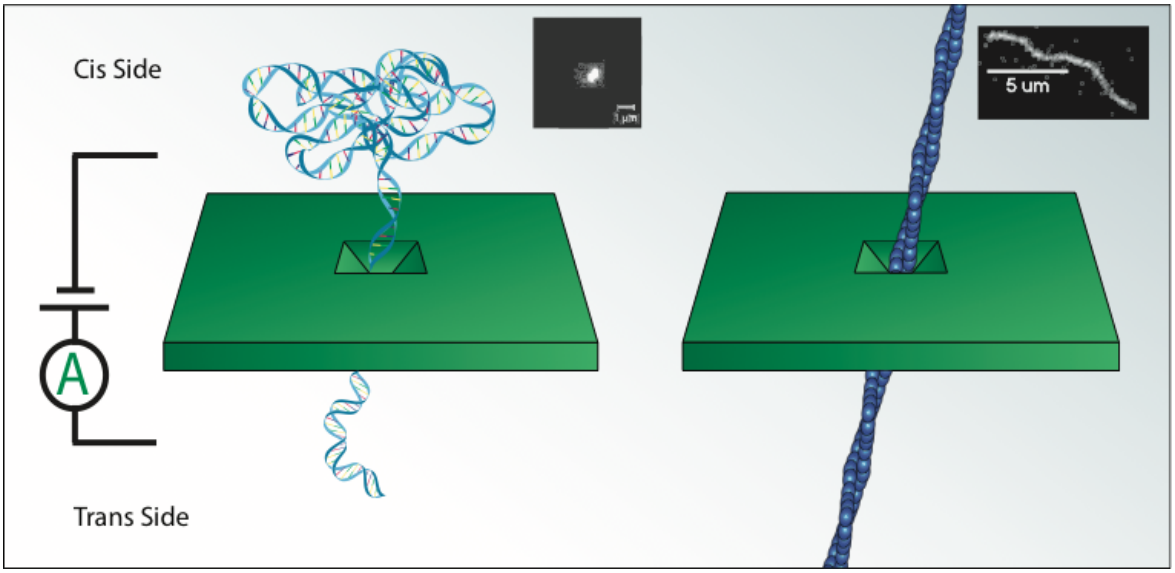

Figure 1: A schematic showing the qualitative differences in translocation due to differences in persistence length between DNA (left) and F-actin (right). The insets are fluorescence optical microscopy images of these two molecules.

Specifically, because of the entropic effects of the coil above the pore, the translocation time of DNA scales as L^{α}, where L is the contour length and α has been experimentally found to be 1.27 [44] and theoretically predicted to be some function of the Flory exponent [45]. Since these entropic effects are absent in F-actin's translocation, its translocation time should scale simply as L^2. This prediction can be obtained from simple scaling arguments. The electric force that pulls the filament through is proportional to the charges on the segment inside the pore, since the electric field is concentrated there. For a given nanopore with a given voltage applied over it, the force exerted on a particular filament sliding through the pore is of a constant value. Meanwhile, the viscous drag force opposing the electric field scales roughly as $1/L$, causing the velocity to scale likewise. The translocation time Δt should therefore scale as $\Delta t \propto L/v \propto L^2$. Therefore, once fully implemented, nanopore studies of F-actin should provide direct test on these expected properties based on polymer physics. The experimental results using F-actin as a different test polymer from DNA may provide new insights on the process.

2 EXPERIMENTAL METHOD

True to its name, a nanopore is simply a nanometer scale pore in an impermeable membrane. The basic premise of the experiment is shown in Figure 1. The nanopore-containing chip is used as a membrane between two chambers containing 1 M KCl Tris buffered pH 8.0 solution. A sample of actin monomers is polymerized with 50 mM $MgCl_2$ and 150 mM KCl, stabilized against depolymerization with the phallotoxin phalloidin, and then diluted to approximately 0.01 mg/ml concentration with the same 1 M KCl Tris buffered solution used above. The F-actin sample is then injected on only one side of the pore. When a voltage of 100 mV is applied across this membrane, an ionic current of a few nanoamps begins flowing through the pore. This voltage also draws the highly negatively charged F-actin through the pore, allowing it to overcome entropic and steric barriers against threading through the pore. Once an actin filament is inside the pore, it blocks a certain amount of cross sectional area. The decrease in area available leads to a smaller available area for other ions to flow through, causing an increase in electrical resistance and a drop in ionic current. This drop in current is measured with a sensitive patch clamp instrument, and should be around the same magnitude for every actin filament that translocates, since they all have the same diameter.

Solid-state nanopores can be made using standard microfabrication techniques [4, 46-48]. A stack of 20 nm of silicon nitride, 200 nm of LPCVD silicon oxide, and another 600 nm of silicon nitride is deposited on a silicon substrate. A micron scale "window" of this thin silicon nitride layer is opened up, and then an approximately 20 nm sized hole is drilled into this thin layer with a Transmission Electron Microscope (TEM) to create a nanopore. A cross section and TEM image of a typical nanopore is seen in

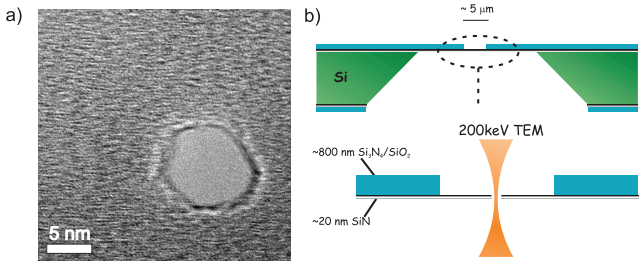

Figure 2: a) A TEM image of a typical nanopore. b) A schematic of the cross section and TEM drilling of the nanopore.

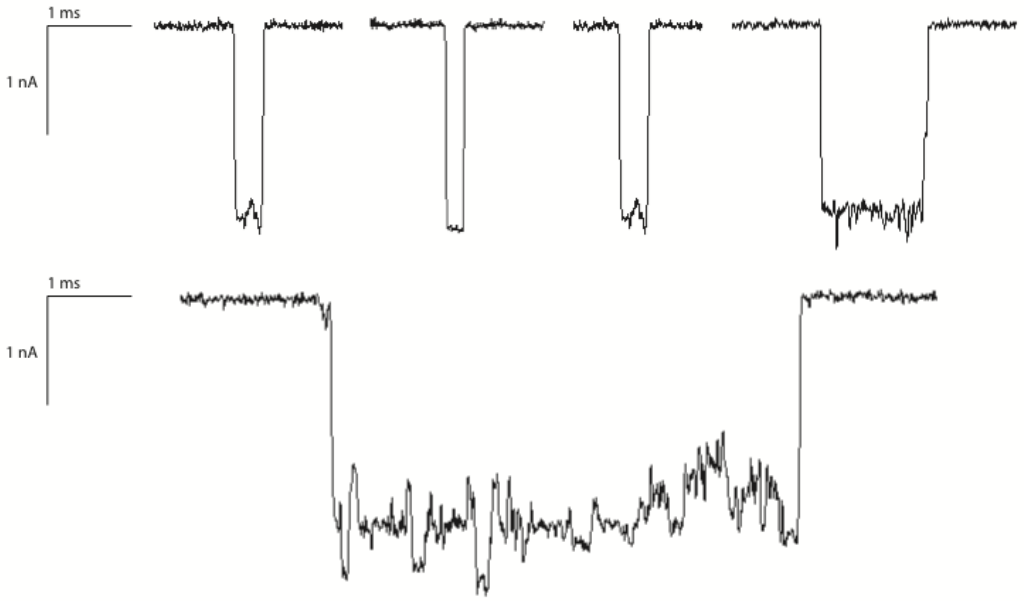

Figure 3: Representative preliminary data for the ionic current blockages caused by the translocation of actin filaments through a solid-state nanopore. The pore diameter was 20 nm.

Figure 2. Generally, the pores would need to be much smaller for DNA, but F-actin's greater diameter allows us to use larger pores. The larger the pore is, the larger the baseline ionic current is and the more noise is in the signal relative to the constant amount of current blocked by a single translocating filament. Since the blockage signal should be proportional to the cross sectional area of the polymer, however, an F-actin translocation should yield a signal 16 times greater than dsDNA. This allows for the use of larger pores than those necessary for DNA translocation, pores that are generally on the order of a few nm and much harder to produce routinely.

3 PRELIMINARY RESULTS

Current traces of what we believe to be F-actin translocating through a solid-state nanopore are seen in Figure 3. The magnitude of the blockades are in line with expected values. As mentioned above, since F-actin is 8 nm in diameter and dsDNA is 2 nm in diameter, we expect the current blockage to be 16 times greater for F-actin. Working off of a current blockade of about 120 pA at 100 mV for dsDNA [44], we would expect a current blockage of approximately 1.9 nA for F-actin, in line with what we observe. Thus we are confident that these signals correspond to the translocation of F-actin through a nanopore. The blockage durations, however, are a bit more complicated. F-actin has an electrophoretic mobility an order of magnitude lower than that of DNA, so a F-actin strand of comparable length should translocate much slower than a DNA molecule. Typically, DNA nanopore experiments are done with λ-DNA, a strand approximately 16 μm in contour length that translocates completely in about 2 ms with a voltage bias of 100 mV. In DNA experiments, however, one can make a sample that is monodisperse in length: every molecule is known to be 16 μm long, for instance. This cannot be done with F-actin. Every sample made has a distribution of lengths, ranging from under a micron to over 20 microns, with an average length of about 5 microns (when stabilzed with phalloidin). It is therefore impossible to correlate the duration of a translocation event to the contour length of F-actin with our current setup. Keeping this in mind, however, and estimating that smaller filaments would be more likely to overcome the steric constraints to thread through the pore, the durations of events seen in Figure 3 are deemed reasonable.

4 SUMMARY AND FUTURE STEPS

We have reported here, to our knowledge, the first ever report of F-actin translocations through a solid-state nanopore. We believe that this work will lay the groundwork for extending the use of nanopores beyond the study of DNA alone and into the study of protein filaments. Of course, this is simply a preliminary work. Much work remains to be done to take full advantage of this new experimental system. First, we plan on incorporating epifluorescence imaging with the nanopore setup. This will allow imaging filaments both before and after they translocate through the pore, allowing us to accurately determine the dependence of translocation time on contour length.

We also believe that solid-state nanopores will provide a unique platform for studying the interactions of actin-binding proteins and F-actin. As mentioned earlier, actin-

binding proteins are a large class of accessory proteins that help regulate and direct the function of F-actin in the cell [49-51]. Nanopores should provide an interesting and novel way of studying their interaction, much as they have been used already to probe the interactions of DNA with various DNA accessory proteins. A specific molecule of interest would be myosin, an important protein because of its roles in the cell and its properties as a molecular motor. Nanopores would provide a fresh way of performing single molecule studies on the interactions between conventional myosin and F-actin. Normally, single molecule studies are done with unconventional myosins because of the low duty ratio of conventional myosin [52]. The confinement provided by the nanopore might allow us to overcome this complication and provide new single molecule data on the interaction between conventional myosin and F-actin.

REFERENCES

[1] J. Kasianowicz, et al., P Natl Acad Sci USA, 1996. 93: 13770.
[2] J. Li, *et al.*,. Nature, 2001. **412**: 166.
[3] Z. Siwy and A. Fulinski,. Phys Rev Lett, 2002. **89**:
[4] A. J. Storm, *et al.*, Nat Mater, 2003. **2**: 537.
[5] S. S. Wu, S. R. Park, and X. S. Ling, Nano Lett, 2006. **6**: 2571.
[6] C. Ho, *et al.*, P Natl Acad Sci USA, 2005. **102**: 10445.
[10] S. Benner, *et al.*, Nat Nanotechnol, 2007. **2**: 718.
[11] F. Olasagasti, *et al.*, Nat Nanotechnol, 2010. **5**: 798.
[12] R. M. M. Smeets, *et al.*, Nano Lett, 2009. **9**: 3089.
[13] A. R. Hall, *et al.*, Nano Lett, 2009. **9**: 4441.
[14] S. W. Kowalczyk, A. R. Hall, and C. Dekker, Nano Lett, 2010. **10**: 324.
[15] S. Howorka, *et al.*, J Am Chem Soc, 2000. **122**: 2411.
[16] L. Movileanu, *et al.*, Nat Biotechnol, 2000. **18**: 1091.
[17] L. Movileanu, Soft Matter, 2008. **4**: 925.
[18] L. Movileanu, J Biomol Struct Dyn, 2009. **26**: 804.
[19] W. A. Vercoutere, *et al.*, Nucleic Acids Research, 2003. **31**: 1311.
[20] N. Ashkenasy, *et al.*, Angewandte Chemie-International Edition, 2005. **44**: 1401.
[21] I. M. Derrington, *et al.*,. P Natl Acad Sci USA, 2010. **107**: 16060.
[22] W. Sung and P. J. Park, Physical Review Letters, 1996. **77**: 783.
[23] C. Y. Kong and M. Muthukumar, Electrophoresis, 2002. **23**: 2697.
[24] M. Muthukumar, J Chem Phys, 1999. **111**: 10371.
[25] M. Muthukumar, Physical Review Letters, 2001. **86**: 3188.
[26] J. B. Heng, *et al.*, Biophysical Journal, 2006. **90**: 1098.
[27] A. Meller and M. W. Bates, Biophysical Journal, 2002. **82**: 188A.
[28] M. Bates, M. Burns, and A. Meller, Biophysical Journal, 2003. **84**: 303A.
[29] M. Wanunu, *et al.*, Nat Nanotechnol, 2010. **5**: 160.
[30] I. Huopaniemi, *et al.*, Phys Rev E, 2007. **75**:
[31] A. Bhattacharya, *et al.*, Eur Phys J E, 2009. **29**: 423.
[32] J. Comer, *et al.*, Biophysical Journal, 2009. **96**: 593.
[33] S. van Dorp, *et al.*, Nat Phys, 2009. **5**: 347.
[34] L. Movileanu and H. Bayley, P Natl Acad Sci USA, 2001. **98**: 10137.
[35] D. Branton, *et al.*, Nat Biotechnol, 2008. **26**: 1146.
[36] M. Wanunu, *et al.*,. Nat Nanotechnol, 2010. **5**: 807.
[37] V. S. K. Balagurusamy, P. Weinger, and X. S. Ling, Nanotechnology, 2010. **21**:
[38] Q. Zhao, *et al.*,. Nano Lett, 2007. **7**: 1680.
[39] G. Sigalov, *et al.*, Nano Lett, 2008. **8**: 56.
[40] H. Yan and B. Xu, Small, 2006. **2**: 310.
[41] J. X. Tang and P. A. Janmey, Journal of Biological Chemistry, 1996. **271**: 8556.
[42] B. Alberts, *et al.*, *Molecular biology of the cell*. 3 ed. 1994, New York: Garland.
[43] H. Isambert, *et al.*, J Biol Chem, 1995. **270**: 11437.
[44] Dekker C. Nature Nanotechnology. 2007. **2**(4):209-215.
[45] Huopaniemi I, Luo K, Ala-Nissila T, Ying S-C. The Journal of chemical physics. 2006. **125**(12):124901.
[46] A. J. Storm, *et al.*, J Appl Phys, 2005. **98**:
[47] M. van den Hout, *et al.*, Nanotechnology, 2010. **21**:
[48] Z. J. Jiang, *et al.*, J Phys-Condens Mat, 2010. **22**:
[49] M. Balland, A. Richert, and F. Gallet, Eur Biophys J, 2005. **34**: 255.
[50] D. Humphrey, *et al.*, Nature, 2002. **416**: 413.
[51] T. Yanagida, *et al.*, Nature, 1984. **307**: 58.
[52] A. Yildiz, *et al.*, Science, 2003. **300**: 2061.