ND*nano* Undergraduate Research Fellowship (NURF)
2015 Project Summary

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Picometer-diameter Pore-based Protein Sequencing

4. Briefly describe any new skills you acquired during your summer research:

My summer research exposed me to a variety of software used to acquire and analyze data. I learned how to observe the ionic current across a synthetic membrane using Clampex and to further analyze the signals I acquired using MATLAB codes I helped develop. I learned image processing techniques to gather information from AFM images and TEM micrographs. On the practical side, I learned how to make microfluidic channels out of PDMS using a mold and to prepare denaturant solutions for proteins, as well as the proper preparation of the microfluidic/pore apparatus for data acquisition. I learned methods for culturing bacteria and how to take optical density measurements using a UV spectrophotometer. I was also able to practice my oral presentation skills through presenting in group meetings and at the NURF research presentations at the end of the summer.

5. Briefly share a practical application/end use of your research:

Nanopores make great single molecule sensors and have demonstrated their ability to observe DNA sequences and protein domains, however as of yet they are unable to observe the sequence of amino acids in a protein. My research helped advance pore-based protein sequencing toward realization which will ultimately allow for another effective means of protein sequencing to and eventually the production of devices for commercial sequencing.

Summary:

My research concerns the sequencing of protein molecules using a sub-nanometer-diameter pore sputtered in an ultra-thin (10 nm thick) silicon nitride membrane. The geometry of these pores only allows a single molecule to occupy the pore at a time. To detect the protein sequence, we measure the current through the pore when the membrane is placed between two electrolytic solutions and an electrical field is imposed across the membrane. The electric force on the protein molecules impel them through the pore. When a protein occludes the pore volume, discrete blockades in the fundamental ion-flow occur can be observed. Further, fluctuations in the electrolytic current through the pore are observed during translocation events, which correspond to the number of amino acids (AAs) in the protein. The amplitude of these current fluctuations correlates highly with the volume of the AA residues along the protein molecule. However, the finite vertical dimension of the pore means that 3-5 AAs occupy the pore waist at a time, such that at each point on the chain, the observed current blockade results from multiple AAs. Additionally, this sequencing method is prone to skips and repeats in which some AAs are read not at all (because the molecule moves too quickly for our instruments to observe) or more than once (because the molecule lingers in the pore). Noise further confounds the electrical measurements taken and necessitates careful handling of the data.

Our work focused on the observation of post-translational modifications (PTMs) at a single site along a protein molecule using electrical measurements from a sub-nanometer pore.

For this experiment, we used three versions of a short (21 AA) peptide derived from the Histone H3 protein. One peptide had no PTMs, the second had undergone acetylation at the 9th AA residue (K9) and the last was tri-methylated at the same position (K9). The proteins were denatured using sodium dodecyl sulfate (SDS), β-mercaptoethanol (bME), and heat in the presence of NaCl electrolyte. One protein solution at a time was added to the electrolyte reservoir and a bias was imposed across the membrane, driving the protein molecules through the pore. Electrophoresis enabled nearly linear translocation velocities, as the SDS imparted a uniform negative charge along the protein. The low current (50-500 pA) associated with ionic flow through the pore at 0.5-1 V was recorded at 10 KHz when the pore was immersed in proteinaceous solution (50-150 pM), and the resulting blockades of the open pore current were categorized. Different protein solutions were run on multiple pores ranging in diameter from 0.5-0.7 nm, and by the end of the summer I had helped successfully gathered data from all three H3 variants on a single pore. My data analysis involved using MATLAB to fit peaks to the current measurements during the translocation events, thereby suppressing noise and allowing me to explore the nature of the fluctuations further. Since the amplitude of the peaks has already been shown to correlate with AA volume, I analyzed the time between two consecutive fluctuations in an event. If the protein moves through the pore at a constant velocity, the fluctuations are expected to occur at regular intervals. However, I observed some variation in the peak inter-arrival times of events that were not present in a similar sample of empty noise, indicating both that the current fluctuations observed are above the noise and that some force interaction between the pore and the protein during translocation. Significant effort was put into investigating the relationship between the peak delays and the occluded volume due to the AAs in the pore, but results were inconclusive.

In addition to this work with sub-nanometer-diameter pores and proteins, I was able to gain experience in cell culture for another experiment going on in my lab involving liquid cell imaging of viable bacteria in a TEM. Over a period of several days I measured the growth of bacteria cultured in varying concentrations of media using a UV spectrophotometer to create growth curves for the bacteria. Although I did not image the bacteria myself, I was able to observe the preparation of liquid cells for imaging in the TEM and I wrote MATLAB codes to overlay imaging conditions on TEM micrographs of bacteria and phages.

Figure 1: The experimental apparatus. A microfluidic channel connects the two volumes of electrolyte solution, right and center. The silicon nitride chip containing the drilled pore is affixed to the PDMS microfluidic through a process of oxygen plasma sealing and sequential heat treatment. The pore is located in the center of the black chip in the protein reservoir, center.

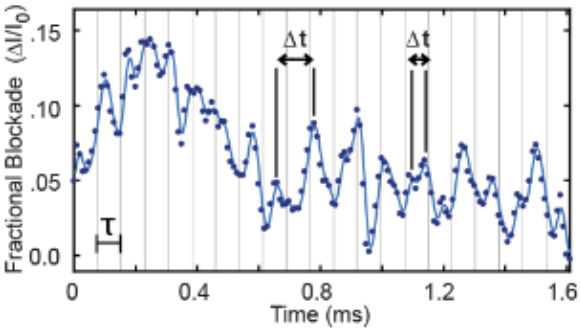

Figure 2: An H3 event fitted for peaks. Gray lines display the expected time between consecutive peaks for a linear translocation velocity (τ). The variation of the time between actual peaks (Δt) and τ is shown.

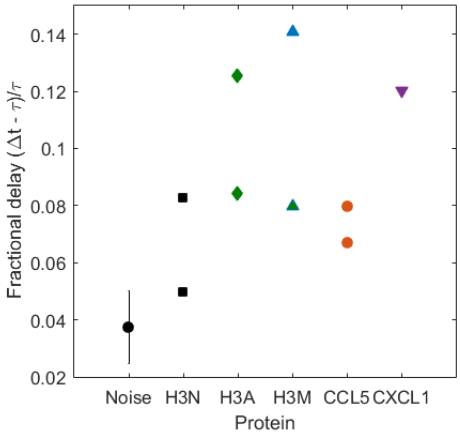

Figure 3: Mean fractional delays for datasets of several kinds of proteins. Proteins exhibit on average more deviation from the expected peak delay time than noise, indicating 'jitter' in the peak positions.

Output

- Oral Presentation "Single Molecule Protein Sequencing with a Picopore." 22 July 2015.
- Authorship on paper submission to Nature Biotechnology