Electrical characterization of protein molecules by a solid-state nanopore

Daniel Fologea, Bradley Ledden, David S. McNabb,[a] and Jiali Li[b]
Department of Physics, University of Arkansas, Fayetteville, Arkansas 72701

(Received 3 April 2007; accepted 9 July 2007; published online 31 July 2007)

The authors measured ionic current blockages caused by protein translocation through voltage-biased silicon nitride nanopores in ionic solution. By calculating the mean amplitude, time duration, and the integral of current blockages, they estimated the relative charge and size of protein molecules at a single molecule level. The authors measured the change in protein charge of bovine serum albumin (BSA) protein induced by *p*H variation. They also confirmed that BSA molecules indeed traverse nanopores using an improved chemiluminescent analysis. They demonstrated that a larger protein fibrinogen could be distinguished from BSA by a solid-state nanopore measurement. © *2007 American Institute of Physics*. [DOI: 10.1063/1.2767206]

Measuring the charge and structural properties of individual protein molecules as well as the distribution of these properties in their native environment is currently a great challenge. Ionic current blockages measured in voltage biased nanofabricated pores have been used to sense nanoparticles and molecules of protein and DNA,[1–7] inspired by the pioneer work on measuring single polymers in protein channels.[8,9] There has been remarkable progress in the study of polymer translocation in protein pores and a recent study shows that polymer size can be resolved at high resolution.[10] However, the ionic current signature and the dynamics of a charged protein molecule moving in a solid-state nanopore need to be studied. In this letter, we report our observations of well-defined current blockage signals due to single protein molecules traversing silicon nitride nanopores. We measured the changes of bovine serum albumin (BSA) as a function of *p*H, and studied how the protein size and structure affect the blockage signal by comparing a larger fibrinogen protein with BSA. These studies are important milestones in the development of solid-state nanopore devices for fast protein characterization.

The main component of a nanopore sensing system [Fig. 1(a)] is a nanopore in a silicon nitride membrane that separates two chambers connected electrically by ionic solution inside the nanopore. When a voltage is applied across the membrane, a stable open pore current I_0 is observed. After the addition of negatively (or positively) charged protein molecules to the *cis* chamber, the molecules in the vicinity of the nanopore will be captured by the electric field, and forced to traverse the nanopore to the positively (or negatively) biased *trans* chamber. The interaction of protein molecules with the nanopore, either by reversible partitioning into or by translocation through, will result in transient current blockages, as shown in Fig. 1(b). Three parameters are calculated from a current blockage: the mean blockage current ΔI_b, the translocation duration t_d, and the integrated area of a blockage A_{ecd}.[6] A_{ecd} is the integral of $\Delta I_b(t)$ with respect to time in units of kiloelectron charge. The current blockages were recorded using an Axopatch 200B system (Molecular Devices) in voltage clamp mode at $V=120$ mV with its low pass filter set at 100 kHz for all measurements in this work. For each set of data, about 10 000 blockage events were recorded. The nanopores used in this work were about 10 nm in thickness and slightly larger in diameter than the protein molecules being studied. The details of silicon nitride nanopore fabrication and the single molecule detection apparatus were described in our previous work.[4,6] The concentration of protein molecules placed in the *cis* chamber was approximately 10 nM.

BSA (66 430 Da, Sigma) has an isoelectric point (PI) ranging from *p*H 5.1 to 5.5;[11] thus the protein has an overall negative charge ($-18e$) at *p*H 7. Applying 120 mV voltage to an ~16 nm diameter pore in a solution of 0.4*M* KCl at *p*H 7.0, $I_0 \sim 7.4$ nA was measured. After addition of BSA to the negatively biased *cis* chamber, downward blockage events occurred [Fig. 1(b)] indicating that the BSA molecules were negatively charged. When the *cis* chamber was positively biased, no blockages were observed at the beginning of the experiment. As the magnitude of the bias potential was decreased, smaller ΔI_b and longer t_d were observed. The cumulative results are presented in an event distribution plot [Fig. 1(d)]. Every dot in Fig. 1(d) represents one blockage event and every blockage is characterized by its ΔI_b and t_d. Figure 1(d) shows that there are two clusters of BSA blockage events: cluster 1 has most probable values of $\Delta I_b \sim 50$ pA and $t_d \sim 110$ μs, while cluster 2 has smaller $\Delta I_b \sim 20$ pA and shorter $t_d \sim 50$ μs. We attribute cluster 2 to the events that protein molecules partially entered the pore but failed to pass through it. Cluster 2 events will not be considered further in this study except to note that they can be easily distinguished from cluster 1 events, which were identified as free translocation of protein molecules through nanopores.

When the *p*H of the chamber solution was lowered to acidic conditions (*p*H<5), current blockages disappeared if the *trans* chamber remained positively biased. However, when the *trans* chamber was switched to negatively biased, current blockages appeared again, as shown in Fig. 1(c), indicating that the net charge of BSA protein had changed to positive at *p*H<5. This measurement is consistent with the fact that BSA is positively charged when the *p*H is lower than its PI (*p*H<5).[12] We studied the translocation of BSA through the same nanopore at three different acidic *p*H values (4.5, 4.1, and 2.4). The BSA molecules proved to be

[a]Department of Biological Science, University of Arkansas, Fayetteville, Arkansas 72701.
[b]Author to whom correspondence should be addressed; electronic mail: jialili@uark.edu

Nanopore sculpting with noble gas ions

Qun Cai,[a] Brad Ledden, and Eric Krueger
Department of Physics, University of Arkansas, Fayetteville, Arkansas 72701

Jene A. Golovchenko
Department of Physics, Harvard University, Cambridge, Massachusetts 02138

Jiali Li[b]
Department of Physics, University of Arkansas, Fayetteville, Arkansas 72701

(Received 9 January 2006; accepted 18 May 2006; published online 27 July 2006)

We demonstrate that 3 keV ion beams, formed from the common noble gases, He, Ne, Ar, Kr, and Xe, can controllably "sculpt" nanometer scale pores in silicon nitride films. Single nanometer control of structural dimensions in nanopores can be achieved with all ion species despite a very wide range of sputtering yields and surface energy depositions. Heavy ions shrink pores more efficiently and make thinner pores than lighter ions. The dynamics of nanopore closing is reported for each ion species and the results are fitted to an adatom diffusion model with excellent success. We also present an experimental method for profiling the thickness of the local membrane around the nanopore based on low temperature sputtering and data is presented that provides quantitative measurements of the thickness and its dependence on ion beam species. © *2006 American Institute of Physics*. [DOI: 10.1063/1.2216880]

INTRODUCTION

Recently we reported that a low energy Ar^+ ion beam could "sculpt" nanometer scale pores in silicon nitride and silicon dioxide films.[1,2] The Ar^+ ion beam incident on these solid membranes was shown to induce lateral mass transport on the surface of a membrane containing a prefabricated ~100 nm pore extending through its thickness. The transport was discovered by observing the continuous closing of this pore down to nanometer diameters during Ar^+ ion beam exposure. The utility of the method for creating single digit solid state nanopore single molecule detectors has been demonstrated.[3–5] Other methods of nanopore formation are also being actively explored.[6] The physics responsible for the ion beam sculpting process appears to be consistent with an adatom diffusion model.[1,7,8] However, another approach based on the notion of stimulated viscous flow has been advanced to account for the potentially related phenomena of ion beam induced ripple formation,[9–11] and it must be admitted that the explanation for the ion sculpting phenomena may yet to be fully elucidated.

Low energy noble gas ion beams have been applied as an effective tool to modify surface structures and properties on various materials at the nanoscale.[7–9,12–15] Here we present ion beam sculpting results for a variety of noble gas ions and ion beam fluxes to drastically change some of the potentially relevant parameters of the process. We also introduce a method for thickness profiling the material near a nanopore and show that these profiles can be very sensitive to the nanopore forming ion beam species used.

EXPERIMENTAL METHODS AND RESULTS

Samples with a single large (~100 nm diameter) hole were prefabricated in 30×30 μm^2 free standing silicon nitride membranes supported on 380 μm (100) silicon substrates. The 275 nm thick, low stress (~200 MPa tensile) silicon nitride membranes were grown by low pressure chemical vapor deposition at the Cornell Nanofabrication Center. Photolithography and anisotropic wet KOH etching

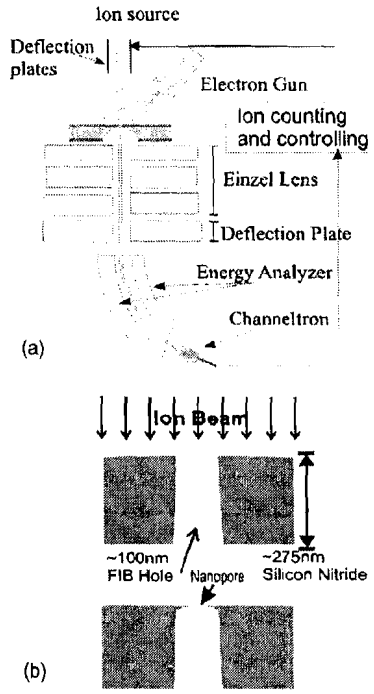

FIG. 1. (Color online) (a) Illustration of ion beam sculpting apparatus. (b) Shrinking a larger hole to a smaller one.

[a]Present address: Surface Physics Laboratory, Fudan University, Shanghai, China.

[b]Author to whom correspondence should be addressed; electronic mail: jialili@uark.edu

NANO LETTERS

2005
Vol. 5, No. 10
1905−1909

Detecting Single Stranded DNA with a Solid State Nanopore

Daniel Fologea

Department of Physics, University of Arkansas, Fayetteville, Arkansas 72701

Marc Gershow

Department of Physics, Harvard University, Cambridge, Massachusetts 02138

Bradley Ledden

Department of Physics, University of Arkansas, Fayetteville, Arkansas 72701

David S. McNabb

Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas 72701

Jene A. Golovchenko

Department of Physics, Harvard University, Cambridge, Massachusetts 02138 and Division of Engineering and Applied Sciences, Harvard University, Cambridge, Massachusetts 02138

Jiali Li*

Department of Physics, University of Arkansas, Fayetteville, Arkansas 72701

Received June 24, 2005; Revised Manuscript Received August 15, 2005

ABSTRACT

Voltage biased solid-state nanopores are used to detect and characterize individual single stranded DNA molecules of fixed micrometer length by operating a nanopore detector at pH values greater than ~11.6. The distribution of observed molecular event durations and blockade currents shows that a significant fraction of the events obey a rule of constant event charge deficit (ecd) indicating that they correspond to molecules translocating through the nanopore in a distribution of folded and unfolded configurations. A surprisingly large component is unfolded. The result is an important milestone in developing solid-state nanopores for single molecule sequencing applications.

Single molecule methods based on nanopore detectors provide a new approach to rapid characterization and perhaps even sequencing of long biomolecules such as DNA.[1] Nanopores capable of single biomolecule detection fabricated from solid-state materials such as silicon nitride[2−5] or silicon dioxide[6,7] offer several potential advantages over biological pores, like alpha hemolysin, for which many elegant and beautiful single molecule results have already been reported.[1,8−16] These advantages include chemical, mechanical, and thermal robustness and the potential ability to articulate the solid-state nanopores with local electron tunneling and optical molecular probes. In order to one day apply these methods to rapid DNA sequencing, it is important to explore and understand the conditions under which long single stranded DNA (ss-DNA) molecules can be passed through and detected by solid-state nanopores.

Denaturation, or the transformation of double stranded DNA (ds-DNA) to ss-DNA, is routinely accomplished using alkaline pH and/or increased temperature.[17] These conditions are difficult if not impossible environments in which to operate biological nanopores. Here we show that a highly alkaline environment is compatible with the operation of a voltage biased silicon nitride nanopore, which enables the observation of freely translocating, long ss-DNA molecules through the nanopore. We find well defined ionic current blockades and translocation times for ss-DNA molecules that differ significantly from those of the same length ds-DNA molecules. The room-temperature denaturation of ds-DNA

* Corresponding author. E-mail: jialili@uark.edu.

10.1021/nl051199m CCC: $30.25
Published on Web 08/31/2005

NANO LETTERS

2005
Vol. 5, No. 9
1734−1737

Slowing DNA Translocation in a Solid-State Nanopore

Daniel Fologea,[†] James Uplinger,[†] Brian Thomas,[†] David S. McNabb,[‡] and Jiali Li*,[†]

Department of Physics and Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas 72701

Received June 7, 2005; Revised Manuscript Received July 25, 2005

ABSTRACT

Reducing a DNA molecule's translocation speed in a solid-state nanopore is a key step toward rapid single molecule identification. Here we demonstrate that DNA translocation speeds can be reduced by an order of magnitude over previous results. By controlling the electrolyte temperature, salt concentration, viscosity, and the electrical bias voltage across the nanopore, we obtain a 3 base/μs translocation speed for 3 kbp double-stranded DNA in a 4–8 nm diameter silicon nitride pore. Our results also indicate that the ionic conductivity inside such a nanopore is smaller than it is in bulk.

A nanopore-based sensor can detect single DNA molecules, and nanopore sensing represents a potential future technology for rapid DNA sequencing. Since Kasianowicz et al.[1] demonstrated that individual DNA molecules could be electrophoretically driven through a single ~2 nm diameter α-hemolysin protein nanopore, several studies have clarified and extended the utility of this nanopore.[2−6] Recently, solid-state[7−14] nanopores have also been used to detect DNA molecules. The DNA nanopore translocation process has also been investigated theoretically.[15−19] Several serious technical problems remain to be solved if the goal of rapid molecule characterization and sequencing is to be achieved in solid-state nanopores. One is that the measured DNA translocation speed of ~30 bases/μs requires an electronic sensing system at extremely high bandwidth, and the concomitant electronic noise poses serious limitations in electrically discriminating between bases. Below we demonstrate how the bandwidth requirements can be reduced by an order of magnitude by slowing down the molecule translocation speed.

The detection of a DNA molecule is performed by placing a nanopore chip between two separated chambers, electrically connected only by an ionic solution inside the nanopore. When a voltage is applied, a negatively charged DNA molecule in the vicinity of the nanopore will be captured by the electric field, and forced to pass through the nanopore from the negative (cis) side to the positive (trans) side. A molecule inside the nanopore causes a detectable ionic current blockade. Both the translocation time (dwell time, t_d) and the amplitude of the blockade (current drop, ΔI_b) are dependent on the solution conditions (ionic concentration, viscosity, and temperature), properties of the nanopore, bias voltage, and the passing molecule. DNA translocation is a very complex process, but it can be envisioned as resulting from a balance between the electric driving force and viscous drag. The nanopore electrical behavior in ionic solution seems to be an ohmic one, with the electrical current blockages proportional to the applied voltage[14]

$$\Delta I_b = \sigma V A_{DNA}/H \tag{1}$$

where σ is the solution conductivity, V the applied voltage across the nanopore, H the effective thickness of the nanopore, and A_{DNA} the hydrodynamic cross section of the translocating molecule. Using a simple equation of force balance between the electric force in the nanopore and the viscous drag over the whole molecule, one finds for the translocation time[7]

$$t_d = K\frac{\eta L_{DNA}}{\lambda V} \tag{2}$$

where η is the viscosity of the solution, λ and L_{DNA} are the linear charge density and length of the DNA molecule, respectively, and K is a constant of proportionality accounting for complex issues beyond the capabilities of the simple model. Equations 1 and 2 are coupled by the fact that σ will depend on η ($\sigma \sim 1/\eta$).[20] In addition, σ, η, and λ will depend on the temperature and the concentration of ions in the nanopore.

In this work we explore the various accessible experimental factors in eqs 1 and 2 for slowing DNA molecule

* Corresponding author. E-mail: jialili@uark.edu.
† Department of Physics.
‡ Department of Biology.

10.1021/nl051063o CCC: $30.25
Published on Web 08/09/2005

REVIEW OF SCIENTIFIC INSTRUMENTS VOLUME 75, NUMBER 4 APRIL 2004

Feedback-controlled ion beam sculpting apparatus

Derek M. Stein
Harvard University, Division of Engineering and Applied Sciences, 17 Oxford Street, Cambridge, Massachusetts 02143 and Department of Nanoscience, Delft University of Technology, Lorentzweg 1, 2628 CJ, Delft, The Netherlands

Ciaran J. McMullan
Harvard University, Department of Physics and Division of Engineering and Applied Sciences, 17 Oxford Street, Cambridge, Massachusetts 02138

Jiali Li
Harvard University, Department of Physics and Division of Engineering and Applied Sciences, 17 Oxford Street, Cambridge, Massachusetts 02138 and Department of Physics, University of Arkansas, Fayetteville, Arkansas 72701

Jene A. Golovchenko
Harvard University, Department of Physics and Division of Engineering and Applied Sciences, 17 Oxford Street, Cambridge, Massachusetts 02138

(Received 22 October 2003; accepted 4 December 2003; published 11 March 2004)

We report the design of an "ion sculpting" instrument that enables the controlled fabrication of nanometer-sized structures in solid-state materials. The instrument employs a beam of kilo-electron-volt argon ions that impinge on a solid-state membrane containing prefabricated structures such as holes, slits, or cavities whose properties are to be modified. By controlling both the ion beam parameters and sample temperature, the instrument can be adjusted to either deliver or remove material from these articulations, for example opening or closing holes of various shapes. The instrument is unique in its use of feedback control for the crafting of structures that define a hole through which a component of the incident ion beam is permitted to pass and be monitored. Electrostatic ion optics refocus ions transmitted unimpeded through the hole, onto a detector capable of registering single ions. The transmission rate is a direct, real-time measure of the transmitting area that is used as a feedback signal to trigger the termination of the ion irradiation process precisely when a desired dimension is obtained. The ions thus serve the dual role of modifying and measuring the size of the nanoscale structures. The sensitivity of the ion beam sculpting apparatus to atomic-scale material rearrangement at the perimeter of a hole also enables the study of ion beam induced material transport at solid-state surfaces. The utility of the instrument as a fabrication tool has been demonstrated by the fabrication of nanopores used for recent single-molecule biophysics studies. © *2004 American Institute of Physics.* [DOI: 10.1063/1.1666986]

I. INTRODUCTION

We required isolated single-nanometer-scale holes, or "nanopores," in thin insulating solid-state membranes for use at the heart of a device capable of detecting and manipulating individual DNA molecules. This need presented a significant technological challenge since the minimal feature size accessible by standard fabrication techniques is typically limited to tens of nanometers, as in the case of electron beam lithography. The fabrication of ~nanometer scale holes in synthetic polymer films has been reported using a high-energy ion track etch technique,[1] however the ultra-high aspect ratio structures do not offer the necessary spatial resolution nor the compatibility with semiconductor processing techniques that we sought. The only established technique for fabricating suitable holes ~10 nm in diameter was developed by Ralls *et al.*,[2] who used electron beam lithography to expose a small spot on a free-standing membrane of silicon nitride. A reactive ion etch process that etched through the membrane was timed to stop shortly after the formation of a hole. This method is limited, however, by the fact that it is "open-loop" because there is no way of directly knowing when it would be optimal to stop the process—a limitation that is common to all standard open-loop fabrication techniques.

A nanofabrication technique that we call "ion beam sculpting" was developed to circumvent the limitations of an open-loop etch. The ion beam sculpting apparatus (Fig. 1) incorporates feedback into the fabrication process to gain dimensional control over synthesized holes at the single nanometer length scale: In the instrument, a freestanding membrane surface containing an initial ~100 nm hole or bowl-shaped cavity is exposed to a normal beam of low energy ions. From simple geometrical considerations, the area of the hole is equal to the argon transmission rate divided by the incident flux. The rate of argon transmission through the hole is therefore a direct measure of its size, and is detected to provide the feedback signal necessary to trigger the extinction of the ion beam when the desired hole size is obtained. By controlling important experimental parameters such as

