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Towards Rapid DNA Sequencing: Detecting Single-Stranded DNA with a Solid-State Nanopore

Hao Yan* and Bingqian Xu*

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The rapid developments in nanotechnology have led to exciting progress in the investigation of biological events at the single-molecule level. When studying systems in which molecular individuality matters,^[1] single-molecule experiments offer several important advantages over ensemble measurements. First, single-molecule measurements provide important information that is “averaged out” in ensemble results. Second, by conducting many sequential measurements, they allow one to determine the distribution of molecular properties and investigate the inhomogeneous systems. Finally, they permit observation of rarely populated transients that are difficult or impossible to capture using bulk measurements. Traditional single-molecule detection techniques can be roughly grouped into two categories: applied optical spectroscopies, which provide optical and spectroscopic information,^[2–4] and scanning probe microscopies, which yield force and electrical current data.^[5]

High-speed and reliable genome sequencing is one of the grand challenges in the 21st century. Recently, a number of groups have started to use nanopores for rapid detection of single DNA molecules and their sequences. Two types of nanopores have been used for this purpose: nanopores embedded in an insulating membrane, for example, biological nanopores such as α -hemolysin protein nanopores in lipid membranes;^[6–8] or solid-state nanopores in Si_3N_4 ^[9,10] and SiO_2 .^[11,12] While most of the research has focused on double-stranded DNA (dsDNA) translocations, rapid DNA sequencing with nanopores requires single-stranded DNA

(ssDNA) molecules to pass through the nanopores and be detected with single-base resolution. Solid-state nanopores possess advantageous features for robust DNA detection and sequencing. They are chemically, thermally, and mechanically stable, which makes it possible to denature DNA under conditions of high pH and/or increased temperature. It is also possible to incorporate solid-state nanopores with local electrical and optical probes to form an integrated circuit.

In a recent paper,^[13] Li and co-workers demonstrated an exciting experimental investigation for detecting ssDNA using voltage-biased solid-state Si_3N_4 nanopores. A single nanopore was fabricated using focused ion-beam (FIB) milling followed by feedback-controlled ion-beam sculpting in an insulating Si_3N_4 membrane.^[9] The membrane was then immersed in an ionic solution, which was then divided into two isolated reservoirs with a Ag/AgCl electrode inserted in each of the two reservoirs. By applying a bias voltage over the two electrodes, an electric field was established in the area between the electrodes, ideally, in the nanopore and near it. When DNA molecules were introduced to the reservoir with the negative electrode they diffused toward the nanopore and were captured by the local electric field near it. The electric field within the nanopore then forced the DNA molecules to pass through the pore and translocate into the positive electrode reservoir. The ionic current through the nanopore during the translocation event was monitored to study the history of the molecule’s interaction with the nanopore (Figure 1 A).

In the design by Li and co-workers,^[13] they studied both single dsDNA and ssDNA molecule translocations by performing the experiments under different pH values at room temperature. Figure 1 B and C are two-dimensional (translocation time and mean ionic current blockage) histograms of the translocation event density at pH 7 (dsDNA, Figure 1 B) and pH 13 (ssDNA, Figure 1 C) environments. The DNA molecules used in the experiments are the same length (3-kilobase long) and the bias voltage applied was fixed at 120 mV. At pH 7, as Figure 1 B shows, a well-defined peak reveals a translocation time of about 170 μs and a current

[*] Prof. H. Yan
 Department of Chemistry and Biochemistry and
 The Biodesign Institute
 Arizona State University, Tempe, AZ 85287 (USA)
 Fax: (+1) 480-965-2747
 E-mail: hao.yan@asu.edu
 Dr. B. Xu
 Department of Biological & Agricultural Engineering
 University of Georgia, Athens, GA 30602 (USA)
 Fax: (+1) 706-542-8806
 E-mail: bxu@engr.uga.edu

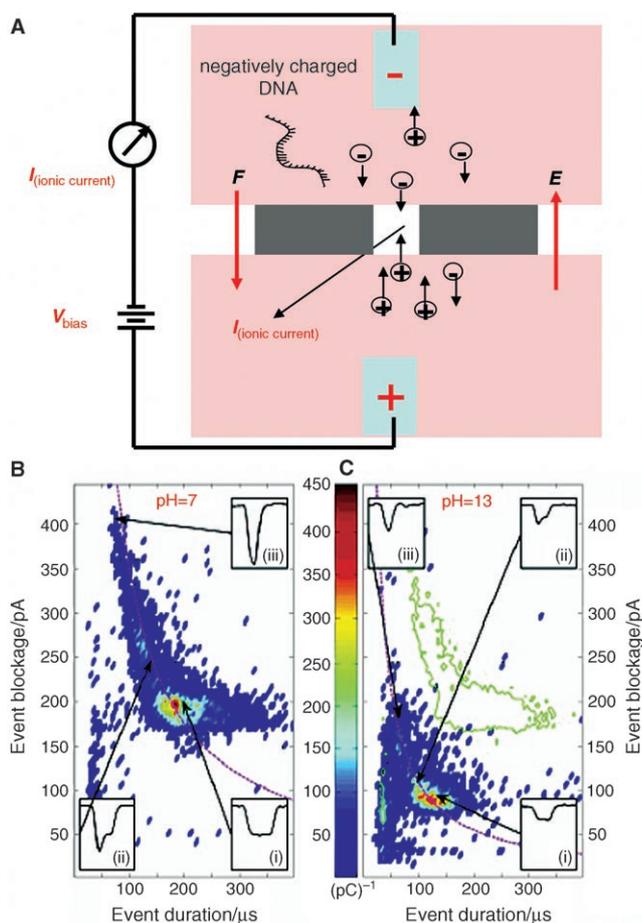


Figure 1. A) Schematic diagram of the experimental setup of a nanopore used to detect single DNA molecules. A DNA molecule was driven through a solid-state nanopore by an electric field between the electrodes on the opposite sides of the pore. B) Translocation event density plot versus translocation time and average blockage current for 3 kb dsDNA in a pH 7 electrolyte solution (taken from Ref. [13]). C) A similar plot for ssDNA in a pH 13 electrolyte solution. The dashed lines express the constant event charge deficit. Inserted curves are representative time traces for individual events: i) unfolded, ii) partially folded, and iii) completely doubled-over molecules (taken from Ref. [13]).

blockage of about 200 pA. At pH 13, the values of translocation time and current blockage at the peak (Figure 1C) change to about 120 μs and 100 pA, respectively. The large current blockage difference is due to the change from dsDNA to ssDNA because the pH value change of the solution produces only slight changes (<2%) in the ionic current.^[13] The denaturation of DNA by increasing the pH value of the solution was also confirmed by optical absorption measurements (see Figure 3 in Ref. [13]).

Another interesting finding was that the molecular translocations for both dsDNA and ssDNA through the nanopore reveal different molecular configurations. When the DNA is folded it passes through the nanopore more quickly and with larger current blockage. This result was explained by the fact that the molecular cross-sectional area

of folded DNA is larger, and thus the overall length is shorter than those of unfolded DNA.^[14] These translocation differences roughly obeyed a constant event charge deficit^[13] and were expressed by the events that fall close to the region of the dashed lines in the figures. This phenomenon proved that the DNA molecules pass through the nanopore freely. The study also showed that the DNA molecules stuck to the nanopore walls resulting in a longer translocation time with the same current blockage (as shown in Figure 1B and C in the areas above and to the right of the dashed lines). Therefore, the DNA molecules can pass through the nanopore folded or unfolded, freely or slowed by interactions with the nanopore walls. However, the majority are unfolded events (>50%), which is very important for single-molecule sequencing applications.^[13] Another study indicates the retarded translocation event distribution for dsDNA is greatly enhanced by using very small nanopores with diameters of 3 nm, close to the dsDNA diameter.^[14] Li and co-workers,^[13] however, investigated DNA translocation by increasing the nanopore diameter up to 10 nm and found that the unfolded molecule translocation events prevail. This suggests that long molecules are often unfolded by the nanopore capture process itself and it is not necessary to squeeze the nanopore in order for the DNA molecules to remain unfolded when they translocate through the pore.

The study by Li and co-workers clearly demonstrated the effects of pH and pore size on DNA translocation through nanopores.^[13] With pH values between 7 and 13 at room temperature, DNA translocation through nanopores was able to distinguish between dsDNA and ssDNA. DNA molecules were also found to translocate through the pore both folded and unfolded, with most of them unfolded. Most importantly, ssDNA does not fold up on itself prior to passing through the hole. Therefore, solid-state nanopores are a promising method for high-speed DNA sequencing applications. Recently, Ashkenasy et al.^[15] successfully demonstrated that using α -hemolysin protein nanopores, a single adenine nucleotide at a specific location on a single stand of polycytosine can be detected and distinguished by monitoring its characteristic ionic current blockage of the nanopore. In this case, the DNA molecule was prepared as a long ssDNA segment and had a hairpin structure at one end. Potentially, solid-state nanopores are robust and could be incorporated with the mature silicon technology and offer better versatility. We expect to see more interesting developments in solid-state nanopore research for single-molecule characterization and sequencing. For instance, using solid-state nanopore arrays to separate different molecules simultaneously would be an attractive application. For ssDNA sequencing purposes, it is necessary to demonstrate single-base sensitivity and resolution while translocating through a nanopore by incorporating local nanoscale electrodes within or near the nanopore. Although it was demonstrated that DNA translocation speeds can be tuned by controlling the solution temperature, salt concentration, viscosity, and the bias voltage,^[16] the challenge exists to develop methods to turn on/off the DNA translocation by means of "gates".

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