

Supporting Information for

Differentiation of Short Single-Stranded DNA Homopolymers in Solid-State Nanopores

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Membrane Thinning and Characterization

To characterize our ultra-thin membranes, individual 85-nm-thick SiN_x windows are first patterned with small squares and plasma etched for a range of times. Thinning is performed by patterning with electron beam lithography followed by reactive ion etching on a Technics PEII-A plasma etcher.¹ TEM, focused ion beam (FIB),² He ion milling,³ or chemical etching can also be used to reduce the thickness of the SiN_x membrane.

Details of the calibration of this thinning procedure including electron beam lithography recipe, etch rate, AFM scans, ellipsometry measurements, and epi-fluorescence measurements were previously published.¹ The absolute etch depth is measured using a Veeco Enviroscope atomic force microscope (AFM). The AFM data is then correlated with the percent of the membrane thickness that was etched, as determined by high-

angular ADF-STEM mass contrast images, to give the absolute thickness of the membranes. The ADF-STEM mass contrast data also give information on the roughness (Figure S1). Thickness error is estimated to be 3 nm, with a contribution of 2 nm from surface roughness measured after etching, 10% (< 1 nm) from errors in the STEM measurements, 0.3 nm from variation in the thickness across a wafer from SiN_x deposition, and 1 nm from etching variability.¹

Nanopore Fabrication

Nanopores are drilled either in TEM mode or in scanning transmission electron microscopy (STEM) mode. Nanopores drilled in STEM mode were drilled in the thinnest area of the membrane, as determined by custom software. Thin membranes are drilled more easily than the standard 25- to 100-nm-thick membranes, and controlling the nanopore size to sub-2-nm-diameter is best achieved with the lowest current densities in the TEM. Figure S2 shows images of several nanopores fabricated in this manner.

Discussion of the Smallest Fabricated Nanopores ($d < 1.1$ nm)

We attempted to study translocation dynamics of single-stranded DNA in a nanopore with a calculated diameter of 0.8 nm, but no translocations were observed (see Figure S3). The smallest nanopore in which we observed translocations had a calculated diameter of 0.9 nm. Nevertheless, for the smallest pores we made, we did not observe translocations, while the larger pores readily allowed translocations of short ssDNA. This suggests that we have entered a range of diameters smaller than the cross-sectional size of ssDNA. In order to precisely determine the minimum pore diameter that allows ssDNA to pass through, we would need to develop a more precise calibration of the nanopore diameter in this size range and establish a minimum diameter that can be statistically validated from measurements on a large number of nanopores. In addition, the limits of nanopore thinning have not been fully explored.

Additional Experimental Data

Figure S4 shows noise power spectral densities for various nanopores measured with both the Chimera and Axopatch amplifiers. Figure S5 shows the histograms of even durations for translocations in Figure 4. Figure S6 examines current blockade as a function of nanopore diameter. Figure S7 shows another example of measured translocations.

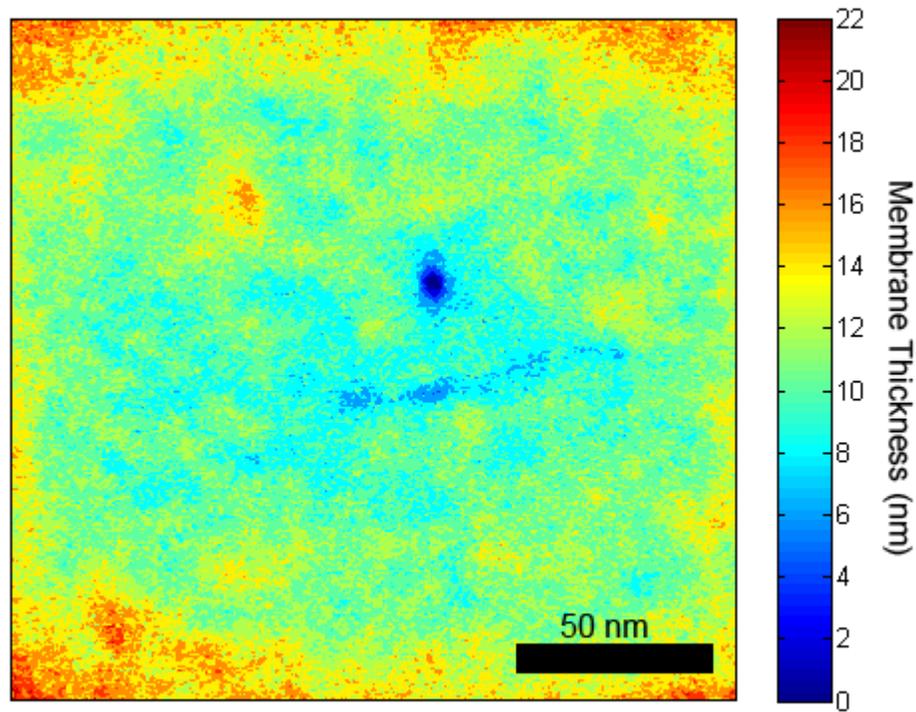


FIGURE S1 Scaled mass contrast contour plot of the thinned area of a membrane with a nanopore (blue spot). The rms surface roughness, h_{rms} , is found to be 2 nm. Nanopores drilled in STEM mode were drilled in the thinnest area of the membrane, as determined by custom software. The raw data from the mass contrast image is given in intensity. To translate to nm, the background intensity (found from the intensity through the nanopore) is subtracted off, and the intensity values outside the etched region are scaled to our original membrane thickness of 85 nm.

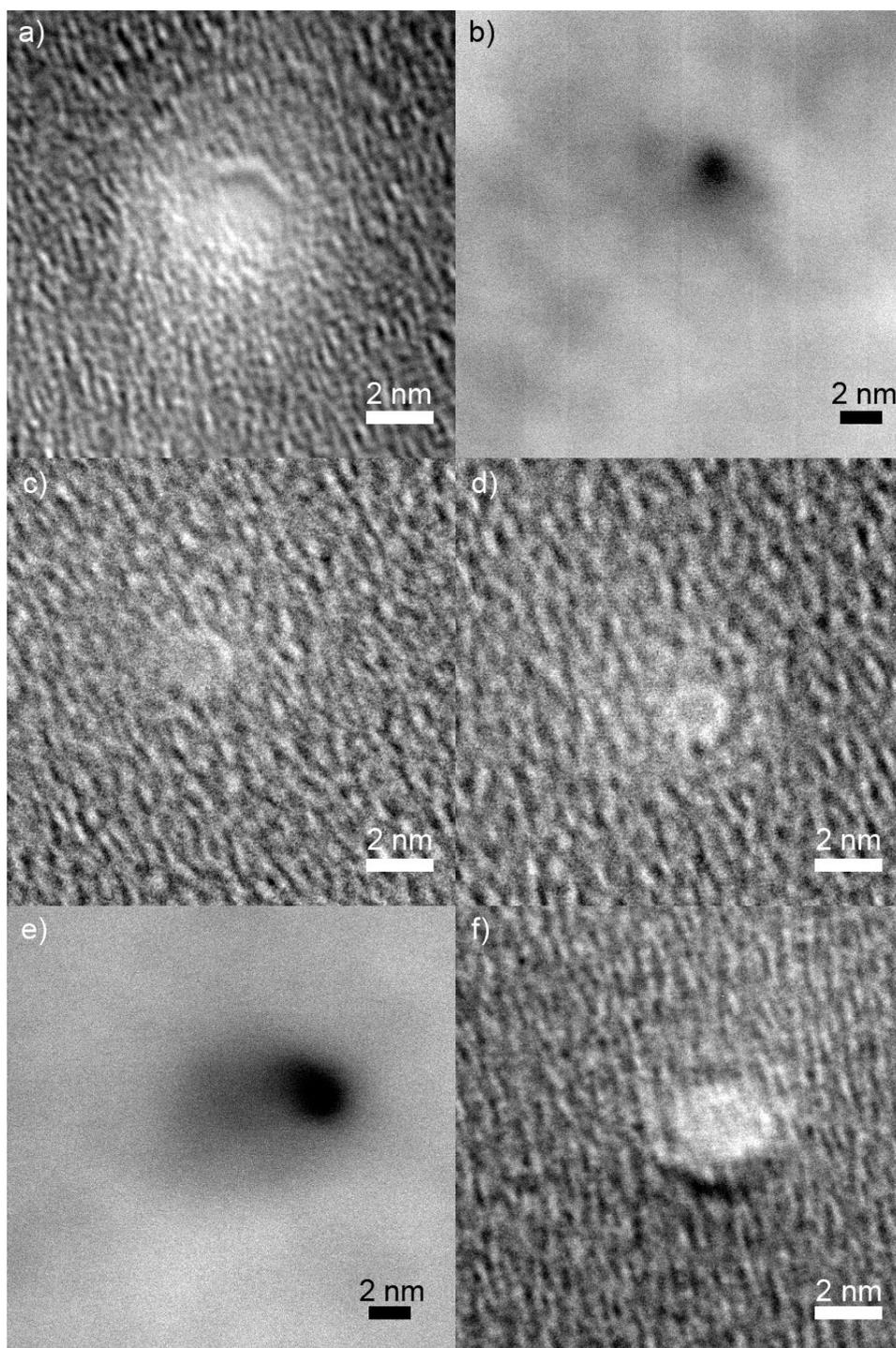


FIGURE S2 TEM images of sub-2-nm-diameter pores in various thicknesses of SiN_x . Most pores fabricated were not imaged to avoid altering the pore size, and many may be smaller than those shown here, based on their appearance while drilling and conductance measurements. Pore diameters from these images are calculated from the average diameter of a fit to an ellipse. (a) 0.9-nm-diameter pore in 30-nm-thick SiN_x (b) STEM image of a 1.8-nm-diameter pore in 5-nm-thick SiN_x drilled in a thinner region of the thinned square (c) 1.6-nm-diameter pore in 20-nm-thick SiN_x (d) 1.3-nm-diameter pore in 20-nm-thick SiN_x (e) STEM image of a 1.3-nm-diameter pore in 8-nm-thick SiN_x drilled in a thinner region of the thinned square (f) TEM image of a 2-nm-diameter pore in 10-nm-thick SiN_x .

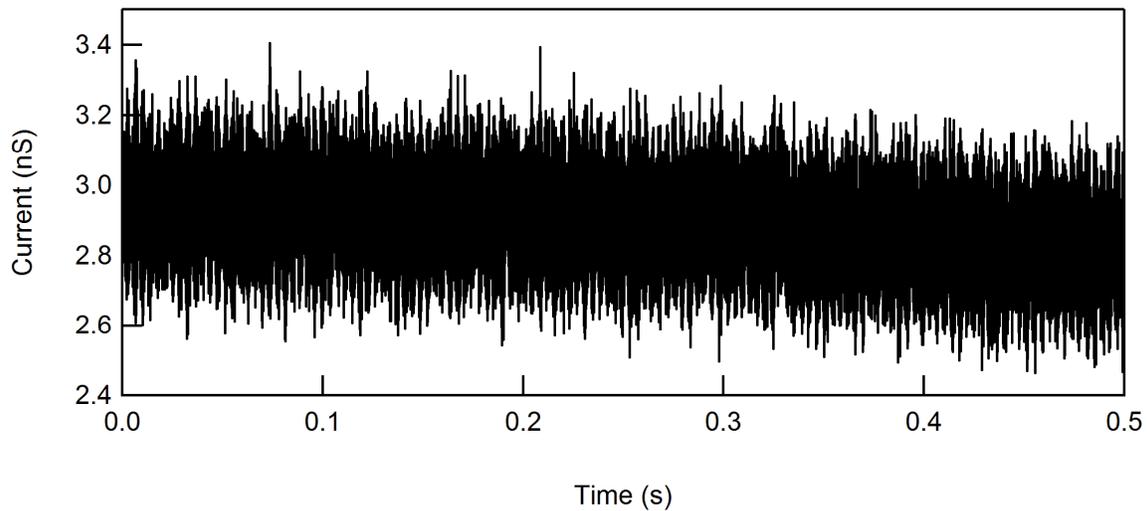


FIGURE S3 Ionic current vs time trace for a nanopore apparently too small to allow translocations. From conductance calculations^{1,4}, we estimate the nanopore diameter to be 0.8 nm. This nanopore was not imaged by TEM to avoid altering its size. The nanopore has 600 mV applied voltage across it, and approximately 2 μ M of an 180-mer single-stranded DNA composed of 75 thymine nucleotides on either side of a 30 cytosine nucleotide block in the correct chamber to drive translocations. For over 18 minutes, although the current gradually varied by about 10% (as seen in trace), no translocations were observed, whereas the larger nanopores readily allowed DNA translocations. Note that the conductance of such small diameter nanopores in thin membranes is rather high compared to the typical SiN_x nanopores of the same diameter in much thicker membranes used in most literature.

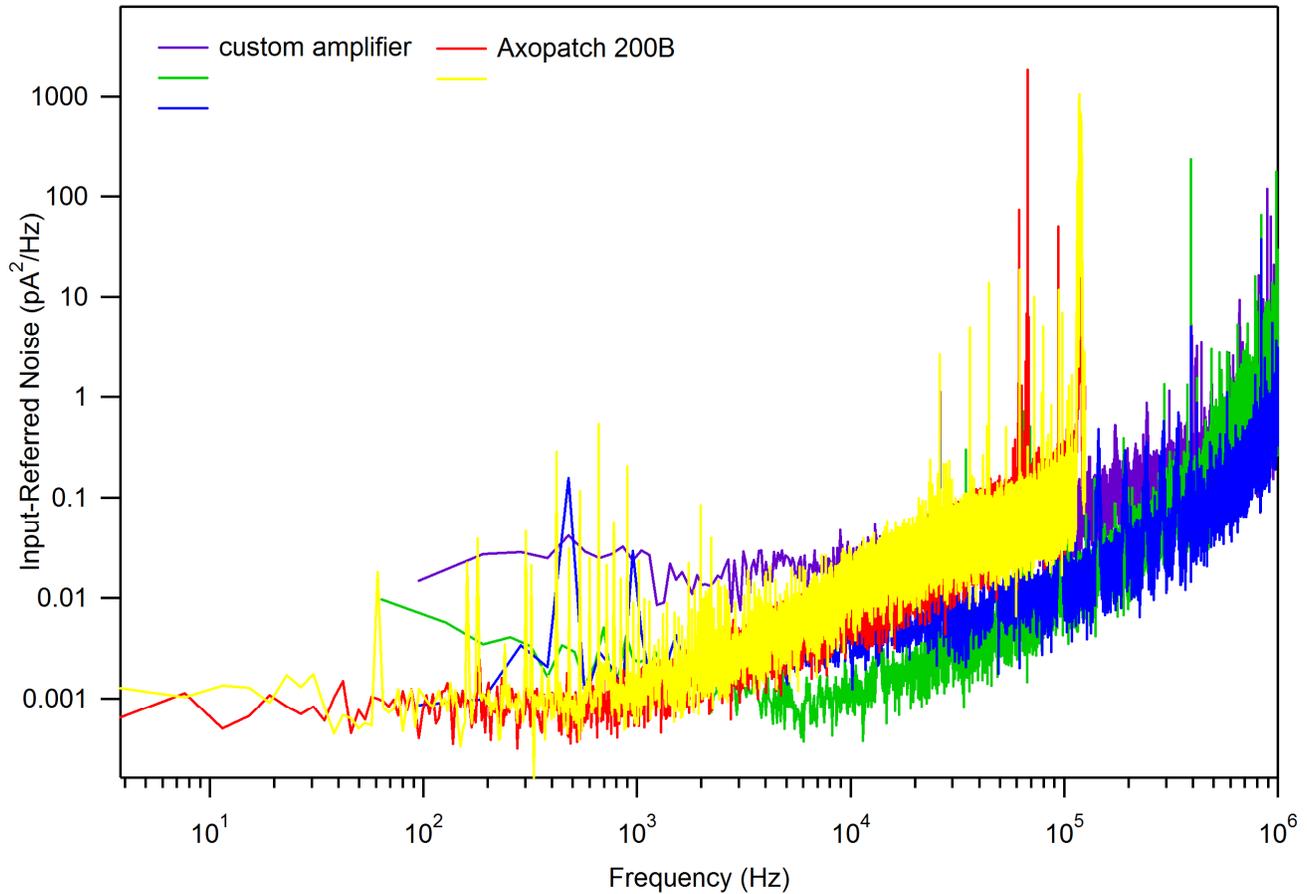


FIGURE S4 Input-referred noise power spectral density of various nanopores tested with either an Axopatch 200B or a Chimera VC100 amplifier. The observed noise was largely a function of electronic and ionic capacitances, and as a result it did not depend strongly on the dimensions of the nanopore. Parasitics varied slightly with each experiment, but data from the Chimera amplifier typically exhibited moderately lower noise density than the Axopatch.

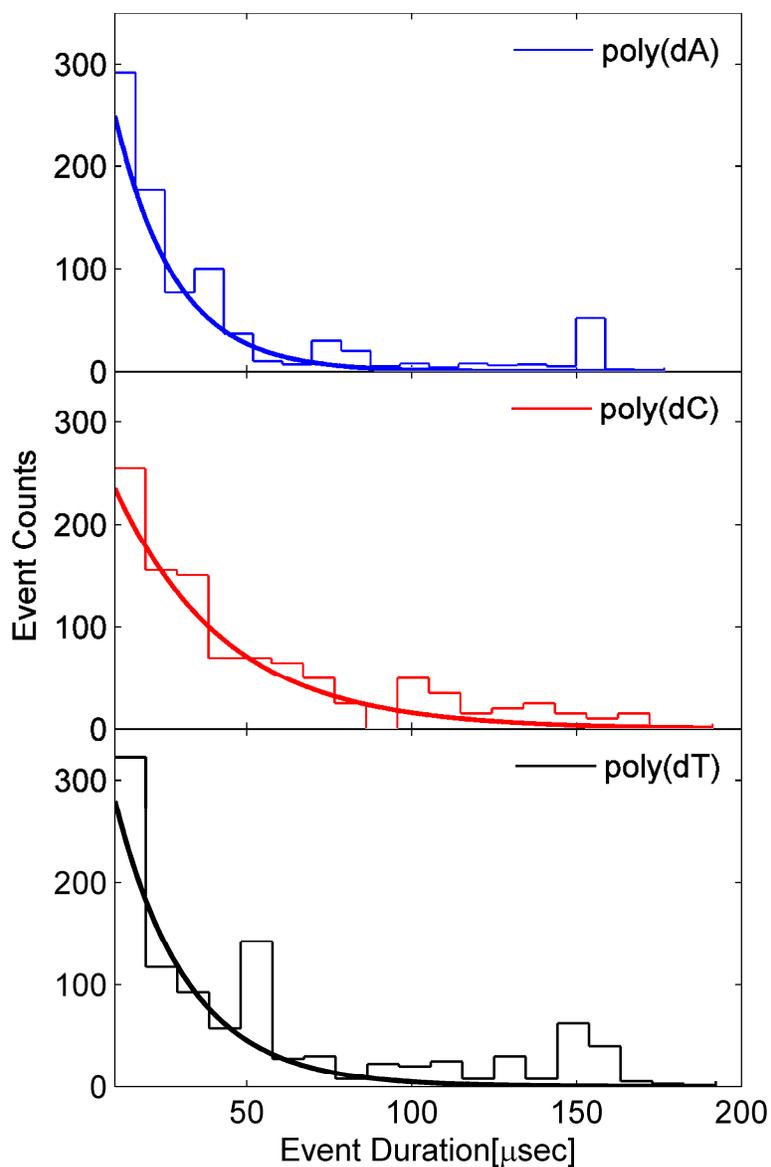


FIGURE S5 Histogram of event duration for each of the homopolymers. The histograms were fitted using a single exponential decay function ($e^{-t/\tau}$). The time scales, τ , for the different homopolymers were 18 μsec ($\text{poly}(\text{dA})_{30}$), 33 μsec ($\text{poly}(\text{dC})_{30}$), and 22 μsec ($\text{poly}(\text{dT})_{30}$), close to values previously obtained¹ for similar conditions.

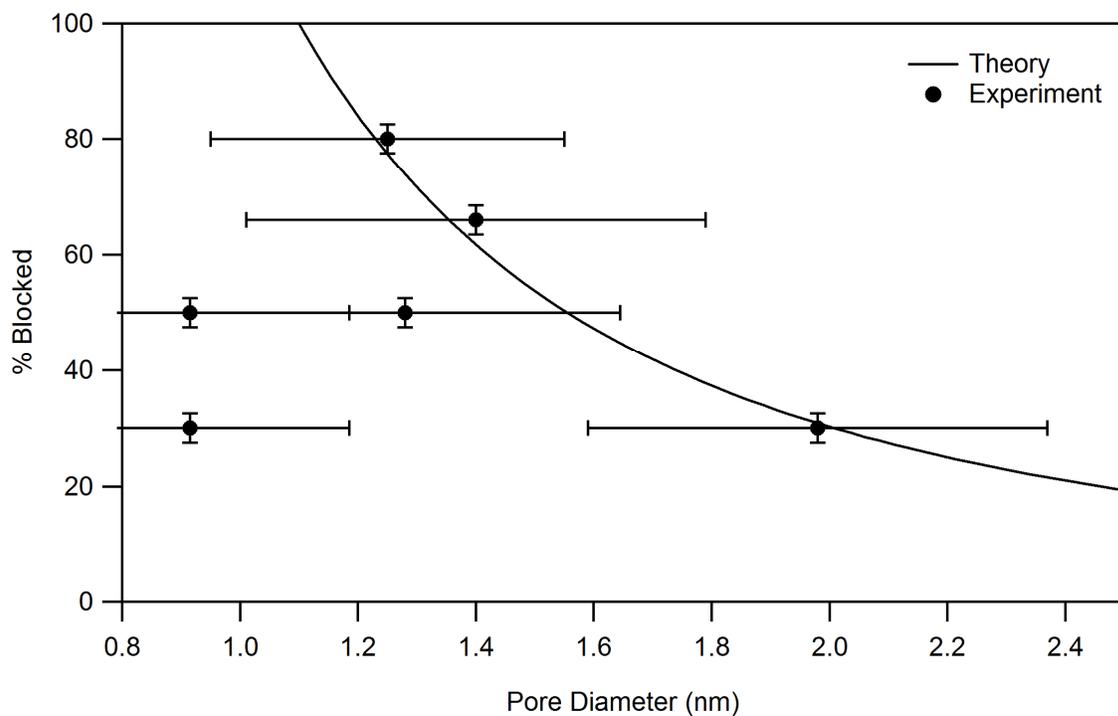


FIGURE S6 Percent of the open pore current blocked by homopolymers in six experiments. All nanopores are between 5 and 10 nm thick, and the nanopore diameter is determined from ionic conductance measurements.^{1,4} The homopolymers used vary by experiment. Theory line, $(1.1)^2/d^2 \times 100\%$, where d is the pore diameter, is a calculation of the percent blocked as determined from the ratio of cross-sectional areas of the single stranded DNA and the nanopore.

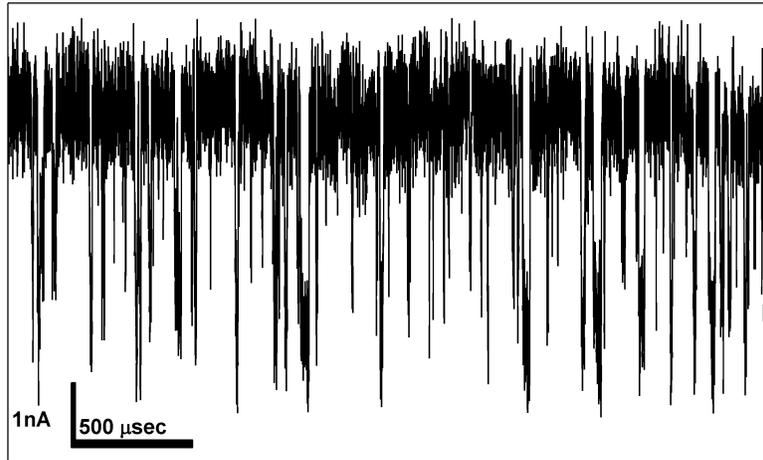


FIGURE S7 Current trace of a 180-mer ssDNA molecule (the same molecule as in Figure S3) sampled at a bandwidth of 1 MHz under a bias voltage of 1000 mV. The open pore current for this nanopore is about 5.5 nA and ssDNA was added at a concentration of 3 μ M. The nanopore diameter is 1.3 nm and the membrane thickness is 7 nm.

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