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An Artificial Nanopore for Molecular Sensing

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ABSTRACT

We have used micromolding techniques to embed a nanoscale pore in PDMS. This novel design allows rapid and reproducible fabrication of pores, is extremely flexible, and can be modified both structurally and chemically for a variety of single-molecule detection applications. We demonstrate the capabilities of the device to sense electronically single DNA molecules.

Because ion-channel proteins are so finely tuned to respond to a single molecule, they have served as a model for developing nanopore devices for biomolecular sensing.[1] Two strategies for engineered nanopores—transmembrane protein pores suspended in lipid bilayers[2−5] and molecular-scaled holes in silicon nitride[6]—have achieved success in detecting single biological molecules; however, further development of these strategies is impeded by several technological barriers, including difficulties in creating an effective pore or array of pores and stabilizing it over a period of time. Here we report on a fundamentally different artificial nanopore that can be fabricated with great ease and control using micromolding techniques, is capable of sensing single molecules of unlabeled λ-phage DNA, and provides opportunities for diverse single-molecule detection applications.

Figure 1 shows one of our devices: a pore of length 3 μm and diameter 200 nm connecting two 5-μm-deep reservoirs. Well-established lithographic techniques are used to create a negative master of the pore and reservoirs, which is subsequently cast into a poly(dimethylsiloxane) (PDMS) slab. The master is created in two steps: first, electron-beam lithography is used to pattern a 200-nm-wide, 200-nm-thick polystyrene line on a silicon substrate, creating the negative of the pore. Next, photolithography is used to pattern an SU-8 photoresist on the substrate to form the negatives of the reservoirs. Both resists (polystyrene and SU-8) are exceptionally durable once cross linked, allowing us to reuse each master indefinitely. Following standard micromolding techniques,[7] we pour PDMS (Sylgard 184) over the master and cure it at 80 °C for at least 24 h. The PDMS slab is then removed from the master and sealed to a glass substrate that has previously defined platinum electrodes. The device is now complete and can be wet with the solution to be studied.

Molecules in the solution are driven through the pore either electrophoretically or by applying pressure to one of the reservoirs. When in the pore, the molecules partially block the flow of current, leading to transient increases in the pore's electrical resistance. Molecular sensing is accomplished by performing a four-point measurement of the electrical current through the pore using the platinum electrodes. The current is low-pass filtered below 0.3 ms in rise time and is sampled at 1 kHz using a voltmeter.

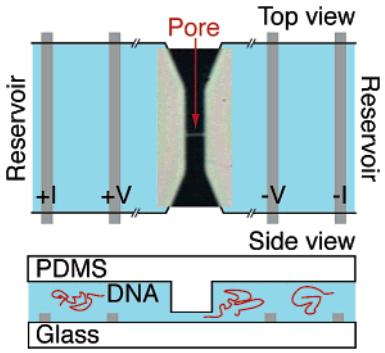

Figure 1. Schematic top and side views of our nanopore device, which consists of two 5-μm-deep reservoirs connected by a lateral pore of 3-μm length and 200-nm diameter; an optical image of an actual pore sealed to a glass coverslip is incorporated into the top view. Molecules in the reservoirs are electrophoretically drawn through the pore, partially blocking the flow of ions. The current through the pore is measured using a four-terminal technique, where the voltage and current controlling the platinum electrodes are as labeled.

To demonstrate the sensing capabilities of our nanopore, we have measured solutions of 2.5 μg/mL λ-phage DNA in a 0.1 M KCl, 2 mM Tris (pH 8.4) buffer. Typical traces of measured current are shown in Figure 2. The striking downward peaks, of height 10−30 pA and width 2−10 ms, correspond to individual molecules of DNA passing through the pore. In contrast, such peaks are absent when measuring only buffer. We further note that peaks are present only when using pores with diameters of 300 nm or less.

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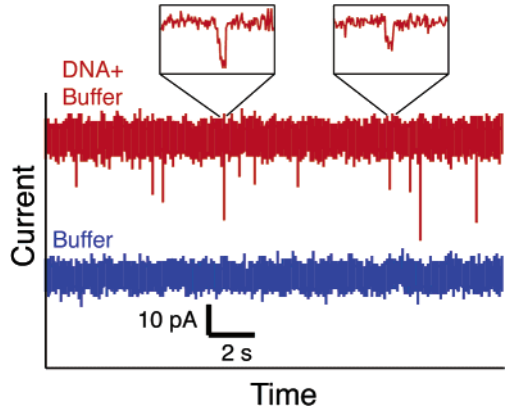

Figure 2. Typical traces of current vs time for solutions of buffer (lower trace) and buffer with λ-phage DNA molecules (upper trace) when 0.4 V is applied across the pore. The traces are offset for clarity; the total current in each case is ~15 nA. Each downward spike in the lower trace represents a DNA molecule passing through the pore. The spikes are typically 2−10 ms in duration and are well resolved, as shown in the insets. The variations in peak height most likely correspond to the different conformation of each molecule.

Previous work on colloids[8,9] has shown that, for particles of diameter much smaller than that of the pore, the ratio of peak height to baseline current is approximately equal to the volume ratio of particle to pore: $\delta I/I \approx V_{\text{particle}}/V_{\text{pore}}$. We can estimate the volume of a single λ DNA molecule by approximating it as a cylinder with a 2-nm radius (which includes a 1-nm ionic, or Debye, layer) and a height equal to the contour length of the molecule (~16 μm). Given the known pore volume and a total current of $I = 15$ nA, we can expect a decrease in current of $\delta I \approx 30$ pA when a DNA molecule fully inhabits the pore. This estimate agrees well with the upper range of measured peak heights. Further corroboration for this model comes from the fact that no peaks are observed when using larger pores (pores >300 nm in diameter). When a molecule inhabits a pore with a diameter >300 nm, the expected response in current is less than 40% of that for a 200-nm-diameter pore. Therefore, at 15 nA of total current, the maximum peak heights for a λ DNA molecule will be less than 12 pA, a value not well resolvable above the noise. Our results suggest that the measured variation in δI is most likely due to differences in molecular conformation: maximum peak heights arise when an entire molecule inhabits the pore, whereas smaller peak heights occur when only a portion of a molecule resides within the pore. Future experiments will focus on controlling the conformation of each molecule to relate the measured peak height to the length of each DNA molecule. Thus, our nanopore device may provide a simple and quick method for the coarse sizing of large DNA molecules.

The results described here represent the first step toward a host of single-molecule sensing applications. By relying on common microfabrication techniques, we can easily create arrays of pores for the simultaneous measurement of many different molecules.[10] Decreasing the pore size will allow us to detect and size smaller molecules such as proteins or viruses. The minimum achievable pore diameter for the PDMS used here (Sylgard 184) is ~150 nm, but recent work has shown that other PDMS formulations can maintain features as small as 80 nm.[11] Finally, we can add chemical specificity in two ways: First, by covalently attaching molecules of interest to the pore wall, we expect to see changes in the transit times of molecules in solution that interact with the immobilized molecules. Second, we can measure changes in the diameter of chemically functionalized colloids upon binding of molecules in the solution, as we have already done using micrometer-scale colloids and pores.[12] The ease and reproducibility of micromolding and the simplicity of our device greatly enhance the capabilities of artificial nanopores for molecular sensing.

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References

(1) Bayley, H.; Cremer, P. S. *Nature (London)* **2001**, *413*, 226−230.
(2) Bezrukov, S. M.; Kasianowicz, J. J. *Phys. Rev. Lett.* **1993**, *70*, 2352−2355.
(3) Bezrukov, S. M.; Vodyanoy, I.; Parsegian, V. A. *Nature (London)* **1994**, *370*, 279−281.
(4) Braha, O.; Walker, B.; Cheley, S.; Kasianowicz, J. J.; Song, L.; Gouaux, J. E.; Bayley, H. *Chem. Biol.* **1997**, *4*, 497−505.
(5) Kasianowicz, J. J.; Brandin, E.; Branton, D.; Deamer, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 13770−13773.
(6) Li, J.; Stein, D.; McMullan, C.; Branton, D.; Aziz, M. J.; Golovchenko, J. A. *Nature (London)* **2001**, *412*, 166−169.
(7) Xia, Y. N.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 551−575.
(8) Deblois, R. W.; Bean, C. P. *Rev. Sci. Instrum.* **1970**, *41*, 909−913.
(9) Saleh, O. A.; Sohn, L. L. *Rev. Sci. Instrum.* **2001**, *72*, 4449−4451.
(10) Saleh, O. A.; Sohn, L. L.; Dunkleberger, L. N. I. To be submitted for publication.
(11) Schmid, H.; Michel, B. *Macromolecules* **2000**, *33*, 3042−3049.
(12) Saleh, O. A.; Sohn, L. L. Submitted for publication.

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