NANO LETTERS

2009
Vol. 9, No. 12
4441-4445

Electrophoretic Force on a Protein-Coated DNA Molecule in a Solid-State Nanopore

Adam R. Hall, Stijn van Dorp, Serge G. Lemay, and Cees Dekker*

Kavli Institute of Nanoscience, Delft University of Technology, Lorentzweg 1, 2628 CJ Delft, The Netherlands

Received August 21, 2009; Revised Manuscript Received September 16, 2009

ABSTRACT

Using solid-state nanopores with optical tweezers, we perform force spectroscopy on DNA molecules that are coated with RecA proteins. We observe that the electrophoretic force is 2−4 times larger for RecA-DNA filaments than for uncoated DNA molecules and that this force increases at lower salt concentrations. The data demonstrate the efficacy of solid-state nanopores for locally probing the forces on DNA-bound proteins. Our results are described quantitatively by a model that treats the electrophoretic and hydrodynamic forces. The conductance steps that occur when RecA-DNA enters the nanopore change from conductance decreases at high salt to conductance increases at low salt, which allows the apparent charge of the RecA-DNA filament to be extracted. The combination of conductance measurements with local force spectroscopy increases the potential for future solid-state nanopore screening devices.

Since the fabrication of solid-state nanopores was first demonstrated,[1] they have come to represent an interesting new technique for single-molecule biophysics.[2] A nanopore consists of a single, nanometer-scale aperture in an insulating membrane that is used as a barrier between two reservoirs of ionic solution. The application of an electrical bias across the membrane results in an electric field that is highly localized at the region of the pore and can exert an electrophoretic force on nearby charged molecules, driving them through the opening. These translocations in turn result in a transient change in the measured transpore current. This technique has been used extensively in recent years to study a variety of biomolecular characteristics including contour length,[3] folding conformation,[4,5] strand configuration,[6] and intercalation state.[7] Such studies rely on the amplitude and residence time of translocation events to infer information about the geometry of the particular molecule in question. Important future directions will involve the application of this technique to measuring the sequence of DNA[8] and probing the variety of proteins that bind locally along the length of genomic DNA.

Recently, it has been demonstrated that the solid-state nanopore system can be made even more powerful through the incorporation of an optical tweezer.[9,10] In this approach, a microbead is conjugated with target molecules and then delivered near the nanopore with an optical tweezer. Application of transmembrane bias drives a molecule through the opening as in the case of free translocation, but the trapped bead prevents complete traversal of the molecule through the pore (Figure 1a). Not only does this allow for arbitrary control over the relative position of the molecule, but it also provides a method of directly measuring the force that acts on it. With different amounts of applied bias, the force applied to the molecule can be varied, thereby measurably moving the trapped bead from its equilibrium position in the optical tweezer. Measurements of the force can be related to the charge of the molecule in question, thus offering a second, parallel method of molecular differentiation which, for example, will be important for future nanopore-based molecular screening devices.

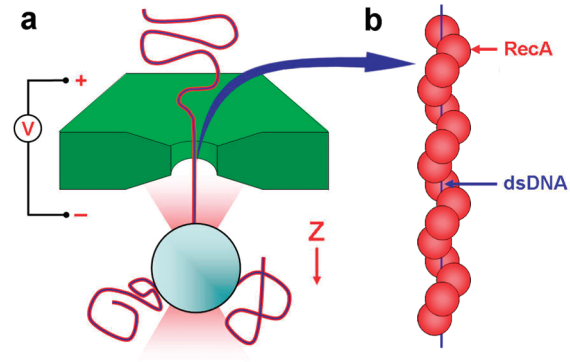

Figure 1. (a) Schematic of the experiment, showing a bead-conjugated molecule electrophoretically captured in a nanopore. (b) Representation of the RecA-dsDNA filament, where RecA proteins bind along the entire length of dsDNA.

* To whom correspondence should be addressed. E-mail: c.dekker@tudelft.nl.

10.1021/nl9027318 CCC: $40.75
Published on Web 09/25/2009

Here, we use the combination of solid-state nanopores with optical tweezers to study RecA protein filaments assembled along double-strand DNA (dsDNA). In vivo, RecA proteins play a central role in the homologous recombination and repair of prokaryote DNA.[11,12] It is able to bind to both duplex and single-stranded molecules in a cooperative, nonsequence-specific fashion along their entire contour length (Figure 1b). The RecA/dsDNA nucleoprotein filament has a local diameter of about 7 nm, as estimated from the crystal structure,[13] which is significantly larger than the 2.2 nm diameter of bare dsDNA. The charge density also differs from that of the uncoated dsDNA molecule.

In this letter, we present and compare measurements on these two types of molecules, allowing us to investigate nanopore sensitivity to molecular geometry and charge. We perform these experiments under salt conditions ranging from 100 mM to 1 M KCl. We find that the net forces acting on the nucleoprotein filament are 2−4 times larger than those acting on a bare dsDNA molecule and that forces increase for lower salt concentrations. We describe our results with a recently developed model and show that quantitative agreement is achieved for RecA-dsDNA without any fitting parameters. This is in contrast to measurements on bare dsDNA, which require a 50% charge reduction for agreement between model and data. Conductance steps due to insertion of molecules into a nanopore are also investigated. We show that the conductance change associated with RecA-coated dsDNA varies linearly with salt concentration. These conductance data provide an independent estimate of the charge of the nucleoprotein filament and a confirmation of the reduction in apparent charge for bare dsDNA. The results presented here provide a foundation for the detection of locally bound proteins along a single molecule, which would represent an important step toward the application of solid-state nanopores to single-molecule diagnostic technology.

Electrophoretic Force Measurement. We investigate electrophoretic forces by varying the voltage applied to a single molecule inside the nanopore and monitoring changes in the z-position of the attached microbead (Figure 1a). Microbead displacement is directly related to the applied force via the stiffness of the optical trap.[9] These measurements thus represent a direct determination of local force acting on a single molecule. A detailed description of the methods and materials are given in the Supporting Information.

Electrophoretic force measurements are performed on both protein-coated dsDNA and on bare dsDNA at KCl concentrations of 100 mM, 600 mM, and 1 M. Figure 2 shows the voltage dependence measured for each molecule under the three ionic conditions. The data consist of measurements on multiple beads, performed in different nanopores with diameters of 20−27 nm. In all cases, a linear increase in force is observed with increasing voltage with no detectable dependence on the distance z from the pore over the investigated range of 4 to 8 μm. The force on the RecA-dsDNA nucleoprotein filament is easily distinguishable from bare dsDNA under all conditions. Quantitatively, values of the force are found to be consistently higher for the

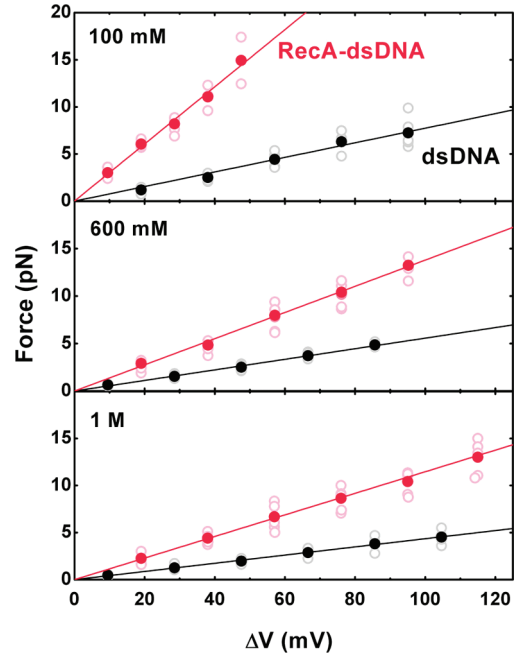

Figure 2. Nanopore force curves measured on bare dsDNA (black) and RecA-dsDNA (red) at KCl concentrations of 100 mM, 600 mM, and 1 M. Filled circles are averages of measurements on multiple individual molecules (open circles). Solid lines represent linear fits to the data. Nanopore diameters ranged from 20−27 nm.

nucleoprotein filaments by a factor of 2 to 4. A larger force is not surprising, as the RecA proteins that coat the negatively charged dsDNA are themselves negatively charged at pH 8.0.[14] Thus, the overall charge of the nucleoprotein filament is more negative than the bare dsDNA molecule, leading to a larger electrophoretic force.

The slopes of the force curves for bare dsDNA and RecA-coated dsDNA are plotted against salt concentration in Figure 3a,b, respectively. Error bars represent the spread in the individual force curves plus an estimated 10% error associated with the determination of the trap stiffness. The slightly larger spread in the case of the protein-coated molecule may be due to interactions with the interior of the nanopore. Qualitatively, a similar trend of increasing force per unit voltage for decreasing KCl concentration is observed for both types of molecule, although quantitatively, the effect is more pronounced for the RecA-dsDNA.

Recently, a theoretical model was developed for the electrophoretic force on molecules in solid-state nanopores.[15−18] Here, the nanopore is described as an annulus and the inserted molecule as a cylinder, both with uniform charge density. For a given salt concentration, this allows the counterion distribution between the cylinder and the annulus to be calculated numerically. This distribution can be used to solve the Stokes equation to yield an electroosmotic fluid flow profile and thus the level of viscous drag acting on the molecule at a given applied bias.[17] This viscous drag acts against the electrophoretic force. The force experimentally measured by our optical tweezer system is therefore a measure of the electrophoretic force acting on the bare charge of the molecule reduced by the counterforce of the electroosmotic

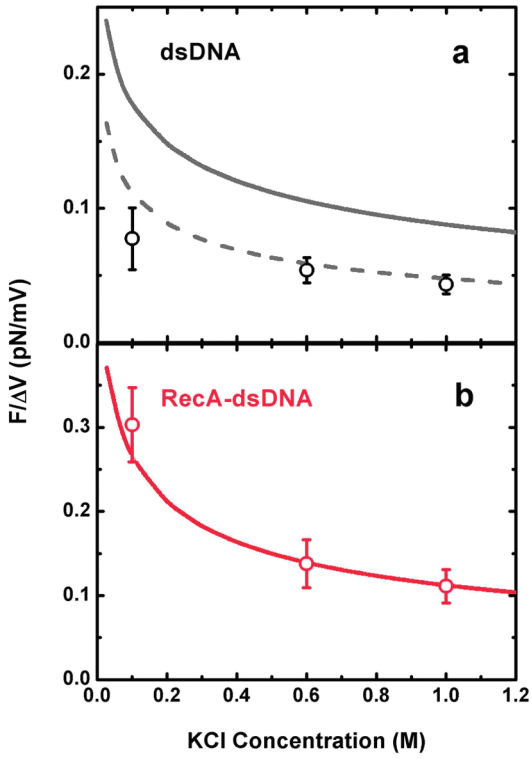

Figure 3. Electrophoretic forces as a function of salt concentration. (a) Measured forces per unit voltage for bare dsDNA (open circles) and modeling results. Calculations performed for the full dsDNA charge and for a 50% reduced charge are represented by the solid and dashed line, respectively (see text). (b) Measured forces per unit voltage for RecA-dsDNA (open circles). The solid line represents calculations performed for the expected full charge of the filament.

drag. Since the latter is dependent on the bulk ionic strength of the surrounding solution, the force measured in our experiments is expected to depend on KCl concentration.

So far, the above model has been tested only on measurements of dsDNA. We can now compare it quantitatively to our results on both RecA-coated and bare dsDNA molecules. All calculations are performed for an annulus with a diameter of 23.8 nm, which is the average diameter of all nanopores used in these experiments (see Supporting Information) as measured by TEM and the measured open-pore current. Annulus length is set at 20 nm, the thickness of the initial membrane. The bare dsDNA molecule is modeled as a cylinder with a diameter of 2.2 nm and a linear charge density of 0.98 nC/m, equivalent to 2 electron charges/bp. The model then yields the force per unit voltage shown as the solid line in Figure 3a. Our measured values show the same trend as the model but are lower by a factor of about 2, which agrees with earlier observations for bare dsDNA.[17] Indeed, the model was previously shown to agree with experimental measurements only after assuming a 50% reduction in the bare charge of the dsDNA. By adapting the same assumption to the current model (Figure 3a, dashed line), we find a good quantitative agreement to the measurements.[19] Note that no additional fitting parameters have been adjusted to obtain this result.

We now apply the same model to the RecA-dsDNA nucleoprotein filament, which we represent as a 7 nm diameter cylinder. The charge density applied to this model cylinder is crudely determined by adding the RecA protein charge (based on the charges of its amino acids at pH 8.0) to the charge of the underlying dsDNA. We take into account that the molecular length is increased by 50% over that of the bare dsDNA when coated with the protein[13] and we include the charge of the Mg^{2+} and ATPγS incorporated into the structure. Assuming a uniform charge distribution, this calculation yields a linear charge density of -1.83 nC/m. The results of the model calculation for the nucleoprotein filament of this size and charge are shown as the solid line in Figure 3b. Interestingly, no reduction in charge is necessary to obtain an excellent quantitative agreement in the case of the RecA-dsDNA complex, with no adjustable fitting parameters.

Why is there a deviation between model and data for dsDNA but not for RecA-coated dsDNA? A first possible reason is that fixed charges on the nanopore surface will produce an additional electroosmotic fluid flow, thereby increasing the opposing fluid drag force.[17] However, this effect would produce a systematic deviation in the measurements on both types of molecules, inconsistent with what we observe. A second possibility is a reduction in the counterion mobility at the surface of the bare molecule.[17] This is thought to be due to the very high surface charge density of dsDNA (-0.14 C/m^2), resulting in a nonlinear charge accumulation at the molecule.[20] In such an arrangement, counterions will interact strongly with the dsDNA leading to a reduced mobility.[21] While the RecA-dsDNA nucleoprotein filament has a higher line charge density, it also has a significantly larger diameter, resulting in a lower surface charge density (-0.08 C/m^2) as compared to that of bare dsDNA. This suggests a weaker coupling and thereby a mobility closer to that of bulk. Our results are thus consistent with the hypothesis that a reduced ion mobility near the molecule surface is responsible for the observed variation between experimental results and calculation on bare dsDNA.

Conductance Blockades. In performing the force measurements described above, target molecules are electrophoretically captured in the nanopore and the resulting ionic conductance steps are recorded. Such measurements yield important independent information about the molecule. Here, we report and analyze these data. Typical captures observed at KCl concentrations of 100 mM, 600 mM, and 1 M are shown in Figure 4a. As with the direct force measurements, the RecA-dsDNA filaments yield larger signals and can be distinguished well from the bare molecules above the noise level under all investigated conditions. Some increased noise is observed during the RecA-dsDNA measurements, especially at high ionic strength, probably due to interactions between the pore and free proteins in the solvent.

The conductance change ΔG measured for both types of molecules is plotted against KCl concentration in Figure 4b. At each salt concentration, at least five capture events are used to determine the average value. We note that the conductance blockade values for RecA-dsDNA at 1 M salt (-6.5 ± 0.9 nS) agree quantitatively with the distribution

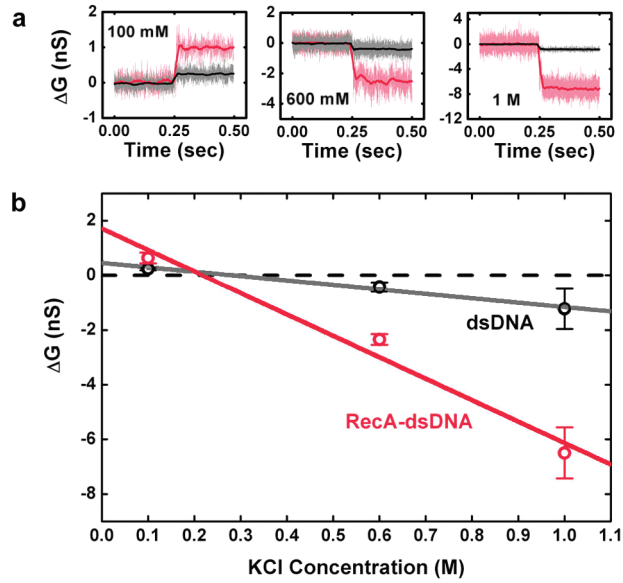

Figure 4. (a) Typical conductance changes measured for bare dsDNA (black) and RecA-dsDNA (red), measured at KCl concentrations of 100 mM, 600 mM, and 1 M. Measurements were performed on nanopores of 20−27 nm in diameter. (b) Conductance change for both studied molecules as a function of KCl concentration. The lines are fits to eq 1.

found previously (−7.5 ± 1.8 nS).[22] For both types of molecules, a qualitative transition from conductance decreases at high salt to conductance increases at low salt is apparent. The data indicate a crossover ($\Delta G = 0$) at 274 ± 56 mM for bare dsDNA and at 218 ± 81 mM for RecA-coated dsDNA.

The conductance change ΔG is a well-defined value experimentally, but is difficult to model without simplifications. In the past, the linear relationship between ΔG and ionic strength has been expressed as[23]

$$\Delta G = \frac{1}{L_{\text{pore}}}\left(-\frac{\pi}{4}d^2(\mu_K + \mu_{Cl})n_{KCl}e + \mu^*_K q^*_l\right) \qquad (1)$$

where L_{pore} is the length of the pore, d is molecular diameter, μ_K and μ_{Cl} are electrophoretic mobilities of potassium and chlorine ions, respectively, n_{KCl} is the number density of ions in solution, μ^*_K is the effective mobility of the counterions near the molecule and q^*_l is the net charge per unit length of excess ions around the molecule. For the analysis presented here, we assume that μ^*_K equals the bulk mobility μ_K. The value q^*_l is a complicated quantity that includes contributions from both attracted counterions and displaced co-ions.[16] The expression for ΔG does not treat this complexity explicitly (and it also neglects other difficulties, such as mobile surface charges on the membrane outside of the nanopore and the contributions of the access regions to the measured conductance), and thus q^*_l does not strictly correspond to a physical charge. However, to a first approximation, this apparent charge is equal to the charge of the molecule itself for highly charged systems like the ones considered here. The above expression is therefore a useful parametrization for comparing experimental results across molecules in the same system.

Equation 1 is first fit to the data for bare dsDNA molecules with the diameter d constrained to the known value of 2.2 nm. The results yield an apparent charge of −0.20 ± 0.04 nC/m or 0.42 ± 0.09 electron charges/bp. This agrees favorably with previous translocation measurements[23] which yielded a value of 0.58 ± 0.02 electrons/bp. An apparent charge reduction is thus observed as compared to the expected line charge of 2 electron charges/bp. Equation 1 is also fit to the data for RecA-dsDNA, where the diameter d is constrained to 7 nm. Now, an effective charge of −1.65 ± 0.61 nC/m is obtained. Within error, this value matches the expected value of −1.83 nC/m, obtained from the simple charge summation described above. It is gratifying to find that these data independently show a charge reduction for bare dsDNA but not for RecA-dsDNA, similar to what we found in the force measurements.

Previous measurements have used the conductance change upon molecular capture to differentiate RecA-dsDNA from bare dsDNA at high ionic concentrations.[22] The present data support this finding and add to it the dependence on salt concentration, which allows the apparent charge of the molecules to be extracted. Molecular discrimination is possible sufficiently far from the concentration range in which $\Delta G \cong 0$ and far from where the two dependencies cross each other. Valid concentrations for RecA-coated dsDNA and bare dsDNA thus lie roughly outside of the range 200−400 mM KCl. This range may be relevant only to static capturing of molecules, however, as the conductance change associated with RecA-dsDNA has been shown to differ somewhat between the cases of optical tweezer measurements and free translocation.[22] The quantitative differences between the two are the subject of current study.

Conclusion. We have successfully demonstrated electrophoretic force measurements of individual molecules of protein-coated DNA using a solid-state nanopore/optical tweezer system. We show that the measured force on RecA-dsDNA is 2−4 times larger than on bare dsDNA, and is thus distinguished easily under all investigated ionic strengths from 100 mM to 1 M KCl. The electrophoretic force is shown to increase with lower salt concentration and the observed behavior can be described well by a model that takes into account electroosmotic fluid drag forces. We have also measured conductance changes caused by single-molecule capture events of the RecA-DNA complex. Individual nucleoprotein filaments were captured statically at multiple ionic strengths and we have employed a theoretical treatment to extract an apparent charge from the conductance steps. Quantitative agreement was found between the expected charge and the value extracted from measurements. RecA-dsDNA molecules were found to be discernible from bare dsDNA under a range of conditions, due to their different dimensions and different charge characteristics.

The ability to differentiate molecules based on their size in solid state nanopores has been demonstrated previously and is largely the focus of current efforts to utilize nanopores in sequencing technology.[24] The results presented here add the ability to distinguish molecules based on charge density in the same system. Simultaneous measurements of both size

and charge will result in the ability to differentiate between proteins with similar dimensions. We expect these capabilities to be valuable in future proteomic screening, in which a variety of DNA-bound proteins could be discriminated in situ at local positions along a single molecule.

Acknowledgment. We thank M. Y. Wu and V. Svechnikov for contributions to nanopore fabrication and M. van den Hout, S. W. Kowalczyk, G. M. Skinner, U. F. Keyser, and N. H. Dekker for valuable discussions. We also acknowledge D. Krapf, J. van der Does, and J. Beekman for contributions to instrument construction. This work was supported by the EC project READNA.

Supporting Information Available: A detailed description of experimental materials and methods. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Li, J.; Stein, D.; McMullan, C.; Branton, D.; Aziz, M. J.; Golovchenko, J. A. *Nature* **2001**, *412* (6843), 166–169.
(2) Dekker, C. *Nat. Nanotechnol.* **2007**, *2* (4), 209–215.
(3) Storm, A. J.; Storm, C.; Chen, J. H.; Zandbergen, H.; Joanny, J. F.; Dekker, C. *Nano Lett.* **2005**, *5* (7), 1193–1197.
(4) Storm, A. J.; Chen, J. H.; Zandbergen, H. W.; Dekker, C. *Phys. Rev. E* **2005**, *71* (5), 051903.
(5) Fologea, D.; Brandin, E.; Uplinger, J.; Branton, D.; Li, J. *Electrophoresis* **2007**, *28*, 3186–3192.
(6) Skinner, G. M.; van den Hout, M.; Broekmans, O.; Dekker, C.; Dekker, N. H. *Nano Lett.* **2009**, *9* (8), 2953–2960.
(7) Wanunu, M.; Sutin, J.; Meller, A. *Nano Lett.* [Online early access.] DOI: 10.1021/nl901691v. Published Online: July 8, 2009.
(8) Clarke, J.; Wu, H. C.; Jayasinghe, L.; Patel, A.; Reid, S.; Bayley, H. *Nat. Nanotechnol.* **2009**, *4* (4), 265–270.
(9) Keyser, U. F.; Koeleman, B. N.; Van Dorp, S.; Krapf, D.; Smeets, R. M. M.; Lemay, S. G.; Dekker, N. H.; Dekker, C. *Nat. Phys.* **2006**, *2* (7), 473–477.
(10) Keyser, U. F.; van der Does, J.; Dekker, C.; Dekker, N. H. *Rev. Sci. Instrum.* **2006**, *77* (10), 105105.
(11) Bell, C. E. *Mol. Microbiol.* **2005**, *58* (2), 358–366.
(12) Cox, M. M. *Nat. Rev. Mol. Cell Biol.* **2007**, *8* (2), 127–138.
(13) Chen, Z. C.; Yang, H. J.; Pavletich, N. P. *Nature* **2008**, *453* (7194), 489–496.
(14) Simonson, T.; Kubista, M.; Sjoback, R.; Ryberg, H.; Takahashi, M. *J. Mol. Recognit.* **1994**, *7*, 199–206.
(15) Viovy, J. L. *Rev. Mod. Phys.* **2000**, *72* (3), 813–872.
(16) Ghosal, S. *Phys. Rev. E* **2007**, *76* (6), 061916.
(17) van Dorp, S.; Keyser, U. F.; Dekker, N. H.; Dekker, C.; Lemay, S. G. *Nat. Phys.* **2009**, *5* (5), 347–351.
(18) Keyser, U. F.; van Dorp, S.; Lemay, S. G. *Chem. Soc. Rev.*, in press.
(19) A best fit to our experimental data corresponds to a charge reduction of 58 ± 6%, very close to the 50% found by van Dorp et al in ref 17.
(20) Manning, G. S. *Q. Rev. Biophys.* **1978**, *11* (2), 179–246.
(21) Boroudjerdi, H.; Kim, Y. W.; Naji, A.; Netz, R. R.; Schlagberger, X.; Serr, A. *Phys. Rep.* **2005**, *416* (3−4), 129–199.
(22) Smeets, R. M. M.; Kowalczyk, S. W.; Hall, A. R.; Dekker, N. H.; Dekker, C. *Nano Lett.* **2009**, *9* (9), 3089–3095.
(23) Smeets, R. M. M.; Keyser, U. F.; Krapf, D.; Wu, M. Y.; Dekker, N. H.; Dekker, C. *Nano Lett.* **2006**, *6* (1), 89–95.
(24) Branton, D.; Deamer, D. W.; Marziali, A.; Bayley, H.; Benner, S. A.; Butler, T.; Di Ventra, M.; Garaj, S.; Hibbs, A.; Huang, X. H.; Jovanovich, S. B.; Krstic, P. S.; Lindsay, S.; Ling, X. S. S.; Mastrangelo, C. H.; Meller, A.; Oliver, J. S.; Pershin, Y. V.; Ramsey, J. M.; Riehn, R.; Soni, G. V.; Tabard-Cossa, V.; Wanunu, M.; Wiggin, M.; Schloss, J. A. *Nat. Biotechnol.* **2008**, *26* (10), 1146–1153.

NL9027318