VOLUME 5, NUMBER 7, JULY 2005

NANO LETTERS

Fast DNA Translocation through a Solid-State Nanopore

Arnold J. Storm,[†] Cornelis Storm,[‡,§] Jianghua Chen,[†] Henny Zandbergen,[†] Jean-François Joanny,[‡] and Cees Dekker*[,†]

Kavli Institute of Nanoscience, Delft University of Technology, Lorentzweg 1, 2628 CJ Delft, The Netherlands, and Physicochimie Curie (CNRS-UMR168), Institut Curie Section de Recherche, 26 Rue d'Ulm, 75248 Paris Cedex 05, France

Received November 26, 2004; Revised Manuscript Received January 25, 2005

ABSTRACT

We report experiments and modeling of translocation of double-strand DNA through a siliconoxide nanopore. Long DNA molecules with different lengths ranging from 6500 to 97000 base pairs have been electrophoretically driven through a 10 nm pore. We observe a power-law scaling of the translocation time with the length, with an exponent of 1.27. This nonlinear scaling is strikingly different from the well-studied linear behavior observed in similar experiments performed on protein pores. We present a theoretical model where hydrodynamic drag on the section of the polymer outside the pore is the dominant force counteracting the electrical driving force. We show that this applies to our experiments, and we derive a power-law scaling with an exponent of 1.22, in good agreement with the data.

Translocation of biopolymers such as polypeptides, DNA, and RNA is an important process in biology. For example, viral infection by phages, DNA transduction between bacteria, RNA translation, and protein secretion all involve the migration of biopolymers through pores of 1−10 nm size. Translocation of DNA and RNA molecules can be studied in vitro, as first demonstrated by Kasianowicz et al.[1] using an α-hemolysin pore in a lipid bilayer membrane. By measuring the ionic current through a voltage-biased nanopore, one can detect individual single-strand molecules that are pulled through the pore by the electric field. Such single-molecule studies have yielded a wealth of information on the dynamics and structure of oligonucleotides.[2−6] Recently, various groups have started to use solid-state nanopores for DNA translocation experiments.[7−13] Such pores offer a range of obvious advantages such as tunable pore size and stability over a wide range of voltages, temperatures, and buffers of varying salinity and pH.

Here, we report a set of experiments on double-strand DNA molecules with various lengths that translocate through siliconoxide nanopores. Surprisingly, we find a nonlinear relationship between the most probable translocation time τ and the polymer length L_0, in contrast to the linear behavior observed for all experiments on α-hemolysin.[1,2] We observe a clear power-law relation $\tau \sim L_0^{1.27}$ for DNA fragments from 6557 to 97000 base pairs (bp). We propose a scaling model based on the assumption that the dominant contributions to the force balance are the hydrodynamic drag and the driving. The model accurately reproduces the observed power-law scaling.

* Corresponding author, email address dekker@mb.tn.tudelft.nl.
[†] Delft University of Technology.
[‡] Institut Curie Section de Recherche.
[§] Present address: Instituut-Lorentz for Theoretical Physics, LION, Universiteit Leiden, P.O. Box 9506, NL-2300 RA Leiden, The Netherlands.

10.1021/nl048030d CCC: $30.25
Published on Web 02/10/2005

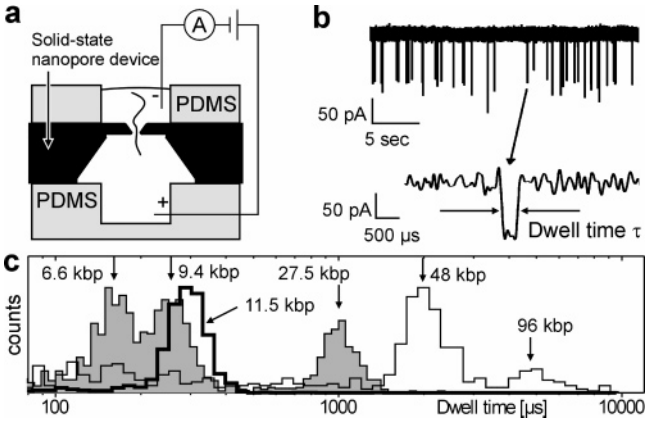

Figure 1. (a) Cross-sectional view of the experimental setup (not to scale). A negatively charged DNA molecule is driven by an electric field through a 10 nm aperture. Both reservoirs are filled with an aqueous buffer solution (1 M KCl, 10 mM Tris-HCl pH 8.0, 1 mM EDTA). (b) Measured ionic current versus time, after the addition of 11.5 kbp DNA to the top reservoir. An individual event is shown with increased time resolution. (c) Histograms of measured dwell times for unfolded translocation events. The bold line corresponds to the 11.5 kbp DNA experiment ($N = 1146$), the thin line to the 48.5 kbp DNA experiment ($N = 569$) and the gray-filled histogram to the mixture experiment ($N = 2117$).

Experimental Results. Figure 1a shows the experimental layout for our translocation studies. At the heart of the setup is a solid-state nanopore device that has been fabricated by shrinking a 20−50 nm pore in siliconoxide in a transmission electron microscope to a final diameter of 10 nm.[9] Based on tilting experiments inside the TEM, we estimate that the typical depth of our nanopores is on the order of 20 nm, much shorter than the detected molecules. The nanopore is situated within an insulating Si/SiO_2 membrane that separates two macroscopic reservoirs filled with a high-salt aqueous buffer solution. When a voltage bias is applied across the membrane in the presence of DNA molecules in the negative compartment, the DNA is electrophoretically drawn through the pore due to its negative charge.

The detection of single DNA molecules is straightforward: A DNA polymer traversing the pore lowers the amount of conducting solution present inside the pore and thereby reduces the ionic conductivity between the reservoirs. Passing molecules are thus detected as short dips in the ionic current that is induced by the externally applied voltage (see Figure 1b). Analogous to Li et al.,[8] we find that the dsDNA molecules, which have a diameter of about 2 nm, can pass the 10 nm wide pore either in a linear or in a folded fashion. Using an event sorting algorithm discussed elsewhere,[14] we exclude folded translocations based on their deeper current blockage and we restrict our analysis here to the simple linear (unfolded) translocation events. Figure 1b shows an example of such a linear translocation event for 11.5 kbp linear DNA. The width of the dip is a measure for the duration of the translocation. We performed three experiments, all at room temperature and at 120 mV bias: one on linear 11.5 kbp DNA, one on linear 48.5 kbp λ-DNA (here we detected both 16.5 μm long individual molecules as well as 33 μm long dimers that are serially connected through their complementary sticky ends), and one on a mixture containing 27491, 9416, 6557, 2322, and 2027 bp fragments in equal concentrations (full details given in ref 14). For each experiment, the durations of individual linear translocation events were collected in a histogram as shown in Figure 1c. Each DNA length gives rise to a separate peak in the histogram. The peak position in these histograms sets the most probable dwell time[15] for the DNA molecule of a certain length.

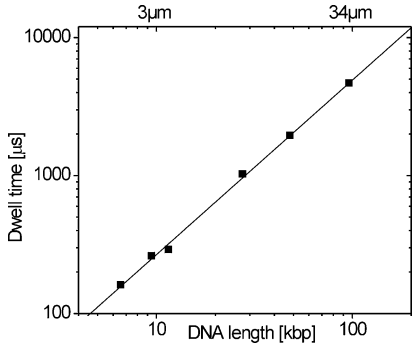

Figure 2. Dwell time versus DNA length. The line shows the result of a power-law fit to the data, with a best-fit exponent of $\alpha = 1.27 \pm 0.03$.

Figure 2 shows the dwell time versus polymer length for all DNA fragments. We observe a clear power-law scaling of the dwell-time τ with the contour length of the DNA, viz., $\tau \sim L_0^{\alpha}$.[16] From a least-squares fit to the data, we obtain a value of 1.27 ± 0.03 for the exponent α. The error bar corresponds to one standard deviation, as determined in the fitting procedure. This error estimate may be considered a lower bound since it does not include any systematic errors. The root-mean-square deviation between the data and the fitted line is only about 5%, which is of the order of 1 bin size in the histogram depicted in Figure 1c. It is gratifying to observe that the data from our three independent experiments fit the same power law $\tau(L_0)$ dependence well. Most importantly, the exponent that we experimentally deduce clearly deviates from 1. The behavior that we observe thus qualitatively differs from the well-studied linear behavior that is observed in all translocation experiments reported so far for α-hemolysin nanopores. We now turn to a theoretical discussion of our experimental observation of this intriguing nonlinearity.

Theoretical Modeling. The translocation process consists of two separate stages. First, in the capture stage, a DNA molecule initially in solution in the negative reservoir has to come close enough to the pore to experience the electrostatic force and get pulled in. The reservoirs are good ionic conductors, and the driving force is only felt in the direct vicinity of the pore. Capture is thus a stochastic process, since the pore has to be reached by diffusion. In this work, we focus on the second stage, where the DNA passes the pore until it has reached the other side. We assume that one end of the DNA has entered the pore and calculate the time required for complete translocation.

Slow vs Fast Translocations. We now address the dependence of this duration on the length of the polymer. To this end, consider a linear polymer consisting of N monomers, each of which has a Kuhn length b. This polymer is partially threaded through a narrow pore. Time $t = 0$ sets

the moment of initial capture. We will let $L(t)$ denote the contour length of the untranslocated part of the polymer, so that $L(0) = Nb \equiv L_0$. The dwell time τ is therefore determined by $L(\tau) = 0$. A second time scale in the problem is the characteristic relaxation time scale of the translocating polymer. This Zimm time,[17] given approximately by $t_Z \approx 0.4\eta R_g^3/k_BT$, can be considered an upper bound on the time it takes the polymer to relax to an entropically and sterically favored configuration. In this expression, η is the solvent viscosity and R_g is the radius of gyration of the polymer. This is the radius of the typical blob-like configuration that a long polymer will assume in a good solvent, and it scales with the polymer length as $R_g \sim L_0^{\nu}$, which defines the (Flory) swelling exponent ν. In three dimensions, a value of 0.588 is theoretically found for self-avoiding polymers in good solvent.[18] Smith et al.[19] have measured the diffusion constant D for stained DNA molecules with lengths ranging from 4.3 kbp to 300 kbp. They report a scaling with length L_0 as $D \sim L_0^{\nu}$ with $\nu = 0.611 \pm 0.016$, and conclude that Flory scaling is appropriate for DNA molecules longer than about 4 kbp. For translocations of DNA through α-hemolysin at room temperature, the measured velocity is about 0.8 μs per base or slower.[20] A 100-base, single-stranded DNA fragment therefore takes around 80 μs to fully translocate. When we compare this to the Zimm time for the same polymer fragment, about 0.2 μs, we see that relaxation is much quicker than the translocation. We will call such events, for which $\tau \gg t_Z$, *slow* translocations. Lubensky and Nelson[21] have argued that for single-stranded DNA and RNA through α-hemolysin, the criterion for slow translocation is indeed satisfied for polymer lengths up to hundreds of nucleotides. They show that the Zimm time for a polynucleotide of roughly 300 bases is comparable to the translocation time per nucleotide.

The criterion for slow translocation is evidently not met in our experiments on solid-state nanopores. A full λ-phage genome (48.5 kbp, or 16.5 μm of double-stranded DNA) is found to take only around 2 ms to traverse a 10 nm SiO_2 pore. The Zimm time for this molecule, in comparison, is about 700 ms, clearly much longer than the translocation time. Even the translocation of the shortest molecules studied in our experiments (6557 bp) can be considered fast, with a translocation time of about 162 μs and a Zimm time about 20 ms. We therefore refer to this second regime, where $\tau \ll t_Z$, as *fast* translocations. We should point out that an important reason for the fastness of our system is the fact that we use double-stranded DNA, which has a much larger persistence length, and consequently a longer relaxation time, than single-stranded DNA.

Model. Let us estimate the magnitudes of the possibly relevant forces, following Lubensky and Nelson.[21] First, consider the driving force. As stated, a potential difference across the pore exerts a highly localized force on the negatively charged DNA molecule. We assume the potential drop to occur entirely inside the pore, and therefore only the part of the polymer inside experiences the driving force. This force can then be estimated to be maximally equal to $F_{driving} = 2eV/a$, where e is the elementary charge, V is the potential difference, and $a = 0.34$ nm is the spacing between nucleotides. A bias voltage of 120 mV, as is used in experiments, then yields a force value of around 110 pN. This value is an upper bound of the actual force, as screening effects will greatly reduce the effective charge on the DNA, and thereby the driving force. Simulations of Manning condensation on double-stranded DNA yield charge reduction values between 53% and 85%,[22,23] leading to a force estimate of 20−50 pN. It is thus clear that, barring complete screening, our DNA translocations are strongly driven, and it is justified to ignore diffusive contributions.

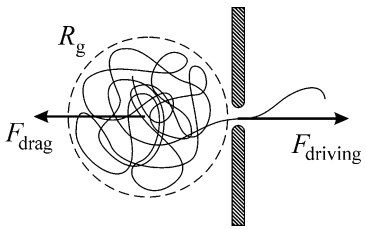

Figure 3. The balance between the two dominating forces determines the dynamics of translocation of a DNA molecule through a nanopore: A driving force that locally pulls the DNA through the pore and a viscous drag force that acts on the entire DNA blob. At time t, the DNA on the left side of the pore has a radius of gyration of R_g, indicated by the dashed line.

In the absence of specific DNA−pore interactions, the viscous drag per unit length in the pore can be estimated as $2\pi\eta r v_{lin}/(R - r)$, where R is the pore radius, r is the polymer's cross-sectional radius, η the solvent viscosity, and v_{lin} is the linear velocity of the polymer inside the pore. Substituting typical values ($\eta = 1 \times 10^{-3}$ Pa·s, $r = 1$ nm, $v_{lin} = 10$ mm/s, $R = 5$ nm, and a pore depth d_{pore} of 20 nm), we estimate this drag force to be around 0.3 pN, decidedly smaller than the driving force. We feel this constitutes an essential difference between solid-state pores and protein pores: In sufficiently shallow solid-state pores, the effect of friction inside the pore is negligible.

Finally, we estimate the hydrodynamic drag on the untranslocated part of the polymer outside the pore. To this end, we approximate the untranslocated part as a sphere of radius R_g (see Figure 3). As the polymer threads through the pore, the center of mass of this sphere moves toward the pore at a velocity dR_g/dt. Assuming that the solvent inside the coil moves with the polymer, the coil experiences a Stokes drag force of $6\pi\eta R v_{blob} = 6\,\pi\eta R_g\, dR_g/dt$, which for typical parameters yields a drag force of about 24 pN. This assumption is justified by experiments by Smith et al.,[19] who found clear evidence for Zimm dynamics for DNA longer than 4.3 kbp. Clearly, in this case the hydrodynamic friction on the part of the polymer outside the pore is the dominant force counteracting the driving force.

We therefore choose to model fast translocation dynamics as determined only by the cumulative effect of driving at the pore and hydrodynamic friction outside. Figure 3 depicts the simplified system we consider. The part of the polymer inside the pore experiences a driving force to the right, while the length of polymer before the pore is coiled up. The pore is sufficiently small to allow only linear (i.e., unfolded) passage of a single molecule at a time. As the polymer is

pulled through the pore, the blob before the entrance shrinks in size, and its center of mass moves toward the pore with a velocity $v_{blob} = dR_g/dt$. Motivated by our consideration of the relative magnitudes of the counteracting forces, we propose that the principal effect of hydrodynamics is to resist motion with a hydrodynamic drag on the DNA coil. Such a drag force can, quite generally, be expressed as $\xi_{eff}\, v_{blob}$, where ξ_{eff} is an effective friction coefficient proportional to the relevant length scale in the direction of motion, and v_{blob} is the velocity. The relevant length scale is R_g, and the relevant hydrodynamic velocity cannot be anything other than R_g/τ, as the polymer's radius must decrease from R_g to 0 during the translocation time τ. Thus, we write $F_{drag} = \xi_{eff}\, v_{blob} \sim R_g \cdot R_g/\tau$. Force balance must be met at all times, and since there are only two major forces the driving force should balance the hydrodynamic friction: $F_{drag} = -F_{driving}$. As the driving force is constant during the whole process, the same should hold for F_{drag}. This leads us to conclude that $R_g \cdot R_g/\tau$ = constant, or equivalently

$$\tau \sim R_g^{\,2} \tag{1}$$

Note that this argument is presented as a scaling theory only. We can, however, compute a rough estimate for the prefactor, assuming the hydrodynamic friction term is of the same order of the Stokes drag on a sphere of radius R_g (ignoring for now the effect of the wall). Equating again the driving and the drag, we find that for a full length of λ-genome, the translocation time is $0.38/\varphi$ ms. Here we have introduced the screening factor $0 < \varphi < 1$, where $\varphi = 1$ corresponds to no screening of the charge on the DNA at all. From this we see that for realistic values of φ ($0.15 < \varphi < 0.47$, see above) we do indeed find a translocation time on the order of 1−2 ms. As we have argued in the preceding, translocation is generally too fast for R_g to follow its equilibrium length dependence adiabatically. As a consequence, part of the polymer coil is frozen in its initial configuration. This frozen part will obviously not contribute to the blob's motion. At any given time t, however, those chain strands with relaxation times smaller than t have had time to fully relax, and the blob motion induced by these relaxing strands will follow the Flory scaling. This, however, does not invalidate our argument; in fact, the scaling that we obtain does not require any assumptions on the relaxational dynamics of the translocating polymer. Rather, it is a necessary consequence of having a constant driving force balanced by a hydrodynamic drag.

Our experimental conditions are such that the DNA is in the swollen coil regime, and the equilibrium relation between R_g and L_0 is best described by $R_g \sim L_0^{\nu}$, where ν is the Flory exponent. Thus, using eq 1, our model predicts a power-law relation between the dwell time τ and the contour length L_0: $\tau = L_0^{2\nu}$. If we adopt the experimentally obtained value for ν of 0.61,[19] we find $\alpha = 1.22$, in good agreement with our experiments where we find $\alpha = 1.27 \pm 0.03$.

Scaling Regimes for Translocation. Our results suggest a straightforward way of predicting the outcome of a wide range of translocation experiments. First, one determines the dominant contribution to the friction. In most cases, it suffices to compare the hydrodynamic friction inside the pore $F_{pore} = \xi_{eff}\, v_{lin}$ (where now $\xi_{eff} = 2\pi\eta d_{pore} r/(R - r)$ in the absence of specific interactions) to the Stokes drag on the coil $6\pi\eta R_g\, dR_g/dt$. If the pore friction dominates, force balance with respect to the constant driving force implies that the translocation time scales linearly with the polymer's length $\tau \sim L_0$. A possible reason for a large pore friction could be the presence of specific interactions, but because of the geometric factor in the effective friction constant ξ_{eff}, the shape of the pore can also lead to pore-friction-dominated translocation. Such linear dependence of τ on the length for single-stranded DNA ranging from 12 to 400 bases has been reported experimentally by Kasianowicz[1] and Meller.[2] For the α-hemolysin pore they used, it is indeed speculated that significant specific interactions with the passing DNA occur.

When hydrodynamic drag dominates, we have shown that $\tau \sim R_g^2$, without any assumptions on the polymer statistics. Depending on the length of the polymer, different regimes are thus obtained: when the polymer is short compared to its persistence length $R_g \sim L_0$, and we find that $\tau \sim L_0^2$. For polymers of intermediate length, the radius of gyration follows the scaling for a Gaussian chain, $R_g \sim L_0^{1/2}$, and consequently the translocation time is predicted once again to scale linearly with length (note, however, that this is a qualitatively different regime than the pore-friction-dominated regime identified before). For long polymers (such as those considered in the preceding sections) we have shown that $\tau \sim L_0^{2\nu}$.

So far we have presented scaling arguments assuming Zimm (non-free draining) dynamics. Kantor and Kardar have identified and numerically confirmed yet another regime[24] where $\alpha = \nu + 1$. This behavior can be understood assuming Rouse dynamics (stationary solvent). In this case, the effective hydrodynamic friction coefficient is proportional to L rather than R_g, as now the entire untranslocated length of the polymer has to be dragged through the solvent. Consequently, $F_{drag} \sim L v_{blob}$, and one recovers $\tau \sim L_0 R_g = L_0^{\nu+1}$, in agreement with their findings. We speculate that this regime might be observable for semidilute solutions close to the critical concentration.

Concluding Remarks. We have obtained a simple model description that appears to describe our data very well. There are, however, several effects that we neglect but which could have an additional influence on the process that we consider. For instance, we expect an electro-osmotic flow to be generated inside the pore. This effect is caused by an electrophoretic force on the ions screening the charge on the surface of our pore. As siliconoxide is known to be negatively charged in water, there is a surplus of positive ions near the surface. These positive ions generate a flow of water inside the pore, slowing down the DNA that moves in the other direction. While we have not explored the consequences of this and other possibilities, the observed agreement between theory and experiment suggests that, at least for the fast polymer translocations considered here, hydrodynamic drag does indeed dominate the dynamics.

Identification and understanding of the dominant effects in polymer translocation through nanopores is relevant not

only for biological processes but also for potential analytical techniques based on nanopores. Rapid oligonucleotide discrimination on the single-molecule level has been demonstrated with α-hemolysin,[20] and more recently solid-state nanopores were used to study folding effects in double-stranded DNA molecules.[8,14] Future applications of this technique may include DNA size determination, haplotyping, and sequencing.

Acknowledgment. We thank the groups of D. Branton and J. A. Golovchenko at Harvard University for their hospitality and advice on nanopore experiments. We also thank Nynke Dekker, Sean Ling, John van Noort, and Peter Veenhuizen for their support and discussions. This research was financially supported by the Dutch Foundation for Fundamental Research on Matter (FOM). C.S. acknowledges funding from the European PHYNECS research network. J.H.C. is grateful to The Netherlands Institute for Metals Research (NIMR) for financial support under Project MC4.98047.

References

(1) Kasianowicz, J. J.; Brandin, E.; Branton, D.; Deamer, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 13770.
(2) Meller, A.; Nivon, L.; Branton, D. *Phys. Rev. Lett.* **2001**, *86*, 3435.
(3) Deamer, D. W.; Branton, D. *Acc. Chem. Res.* **2002**, *35*, 817.
(4) Nakane, J. J.; Akeson, M.; Marziali, A. *J. Phys.-Condens. Matter* **2003**, *15*, R1365.
(5) Sauer-Budge, A. F.; Nyamwanda, J. A.; Lubensky, D. K.; Branton, D. *Phys. Rev. Lett.* **2003**, *90*, 238101.
(6) Mathe, J.; Visram, H.; Viasnoff, V.; Rabin, Y.; Meller, A. *Biophys. J.* **2004**, *87*, 3205.
(7) Li, J. L.; Stein, D.; McMullan, C.; Branton, D.; Aziz, M. J.; Golovchenko, J. A. *Nature* **2001**, *412*, 166.
(8) Li, J. L.; Gershow, M.; Stein, D.; Brandin, E.; Golovchenko, J. A. *Nature Mater.* **2003**, *2*, 611.
(9) Storm, A. J.; Chen, J. H.; Ling, X. S.; Zandbergen, H. W.; Dekker, C. *Nature Mater.* **2003**, *2*, 537.
(10) Polk, B. J.; Bernard, M.; Kasianowicz, J. J.; Misakian, M.; Gaitan, M. *J. Electrochem. Soc.* **2004**, *151*, C559.
(11) Chang, H.; Kosari, F.; Andreadakis, G.; Alam, M. A.; Vasmatzis, G.; Bashir, R. *Nano Lett.* **2004**, *4*, 1551.
(12) Heng, J. B.; Ho, C.; Kim, T.; Timp, R.; Aksimentiev, A.; Grinkova, Y. V.; Sligar, S.; Schulten, K.; Timp, G. *Biophys. J.* **2004**, *87*, 2905.
(13) Chen, P.; Gu, J.; Brandin, E.; Kim, Y. R.; Wang, Q.; Branton, D. *Nano Lett.* **2004**, *4*, 2293.
(14) Storm, A. J.; Chen, J. H.; Zandbergen, H. W.; Dekker, C., **2004**.
(15) Note that, due to the asymmetric shape of the peaks in the histogram, the *most probable* translocation time does not necessarily coincide with the *average* translocation time. We have chosen to work with the most probable time because the overlap between the various peaks, in particular at shorter DNA lengths, complicates an accurate determination of the average. Fitting the mean translocation times yields an exponent of 1.30 ± 0.03.
(16) Quantitatively, our results are in good agreement with those published in ref 8, which studies translocation of 3000 bp and 10000 bp DNA fragments through a 10 nm silicon-nitride nanopore, for which translocation times of 100 μs and 400 μs are reported, respectively.
(17) Doi, M.; Edwards, S. *The Theory of Polymer Dynamics*; Clarendon: Oxford, 1986.
(18) le Guillou, J. C.; Zinn-Justin, J. *Phys. Rev. Lett.* **1977**, *39*, 95.
(19) Smith, D. E.; Perkins, T. P.; Chu, S. *Macromolecules* **1996**, *29*, 1372.
(20) Meller, A.; Nivon, L.; Brandin, E.; Golovchenko, J. A.; Branton, D. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 1079.
(21) Lubensky, D. K.; Nelson, D. R. *Biophys. J.* **1999**, *97*, 1079.
(22) Padmanabhan, S.; Richey, B.; Anderson, C. F.; Record, J. M. T. *Biochemistry* **1988**, *27*, 4367.
(23) Ravishanker, G.; Auffinger, P.; Langley, D. R.; Jayaram, B.; Young, M. A.; Beveridge, D. L. *Rev. Comput. Chem.* **1997**, *11*, 317.
(24) Kantor, Y.; Kardar, M. *Phys. Rev. E* **2004**, *69*, 021806.

NL048030D