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Spontaneous Stretching of DNA in a Two-Dimensional Nanoslit

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ABSTRACT

DNA molecules in silicon dioxide–glass fluidic nanoslits spontaneously extend at the lateral sidewalls of the slit. The nanoslit geometry, however, physically confines polymer molecules to two spatial dimensions; further reduction in configurational entropy resulting in axially stretched molecules arises spontaneously and appears to be electrostatically mediated. The observations not only shed light on electrostatic interactions of charged soft matter with like-charged confining walls but also offer a new method to stretch DNA in solution.

Stretching a DNA molecule in free solution unravels its contour length, normally stored in a random coil, and exposes it to observation by fluorescence microscopy. This has tremendous implications for studies on DNA−protein interactions at the single molecule level, sequence-related analytical operations such as restriction mapping, and more fundamental polymer dynamics studies on an individual DNA molecule.[1−3] Popular methods to stretch single DNA molecules include optical and magnetic tweezers or hydrodynamic flow past a tethered DNA molecule. Recently, entropic stretching of DNA confined in nanochannels—tubes of diameter smaller than the radius of gyration of the DNA molecule—was demonstrated.[4] Entropic stretching of DNA purely by physical confinement is achieved by introducing DNA molecules into fluidic channels of diameter smaller than the free solution radius of gyration of the molecule. The mass of the polymer molecule is distributed along the length of the channel resulting in an extended length that increases linearly with the polymer contour length as originally proposed by de Gennes.[5]

Here we report a regime of confined macromolecule behavior where a proportion of DNA molecules confined in solution to a two-dimensional fluidic slit—slits several micrometers in width, but less than 100 nm in depth—spontaneously assumes axially extended states at the lateral edges of the fluidic slit. The molecules thus localized at the edge distribute their mass preferentially along the major axis of the slit rather than sampling both dimensions of the fluidic plane in a two-dimensional random walk. This behavior is intriguing on account of the self-organization inherent in the process: molecules in solution spontaneously seek the lateral edges of the slit, shed their configurational entropy, and stretch out in one dimension.

Attractive interactions between charged macromolecules and oppositely charged surfaces are well documented in theory and experiment.[6−8] The wrapping of DNA around histones for compact storage within eukaryotic cells is a classic example from nature. Recently, Hochrein et al. reported the stretching of DNA adsorbed to groove regions of structured cationic lipid bilayers.[9] Here, the higher local curvature in groove regions acted as potential wells and served to localize and stretch the oppositely charged DNA molecules. Our experiments, however, involve negatively charged DNA molecules in solution bounded by negatively charged SiO_2 and glass walls, where the higher curvature at the lateral sidewall regions would be expected to repel nearby molecules more strongly than flat regions of the slit (Figure 1C). From classical electrostatic considerations this should then result in local potential maxima, rather than minima, that keep the molecules preferentially away from the walls. On the contrary, we observe what appears to be an attractive interaction between the slit edges and the negatively charged DNA, suggesting the presence of potential wells at the sidewalls that act as "traps", localizing and extending DNA molecules in solution. In addition, the absence of multivalent cations excludes the possibility of "charge inversion" of the slit walls[10] as a possible underlying mechanism for the phenomenon reported here. Can a surface and macroion of like charge attract?

In systems of like-charged colloidal particles, classical electrostatic considerations dictate that the particles repel each other in solution. However, several independent reports recently described anomalous long-ranged attractive components in the pair potential of like-charged confined

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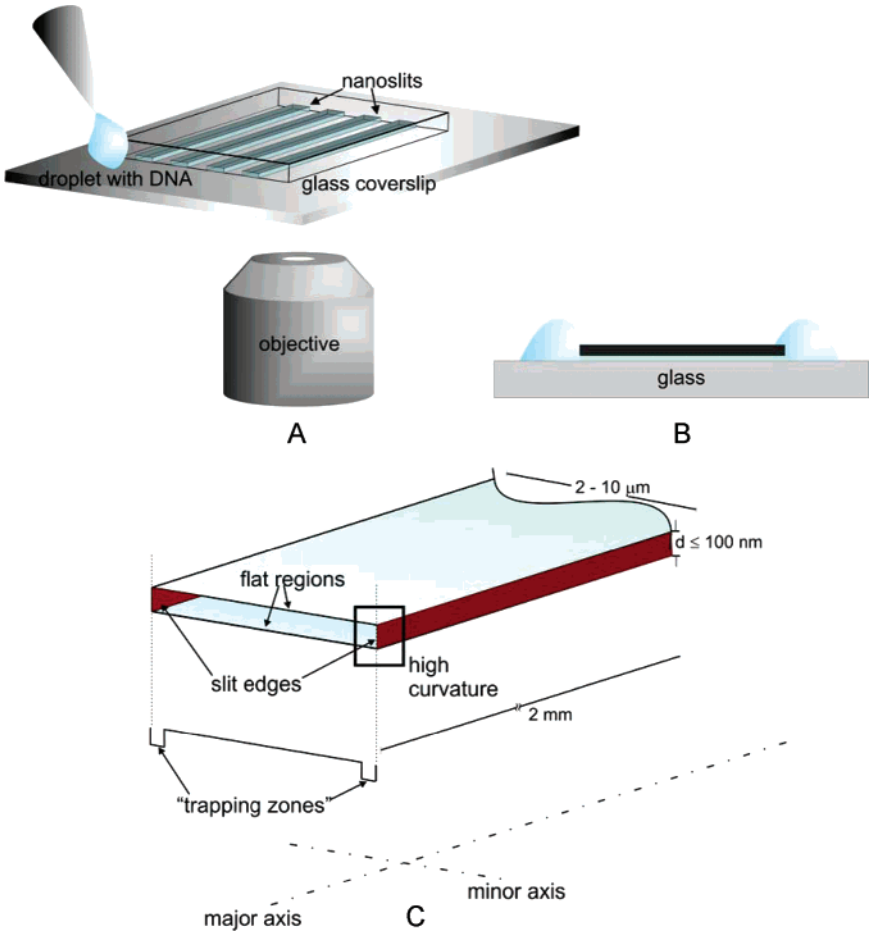

Figure 1. (A) Schematic representation of device operation. (B) Longitudinal section of the device through a slit. (C) Detail of an individual slit highlighting the "edge" or "lateral sidewall" regions (dark brown) of higher local curvature than the adjacent flat regions (blue). Also sketched in is a conceptualized profile of the potential across the slit width, emphasizing regions of lower potential or "traps" near the slit edges that localize and stretch DNA molecules.

colloids.[11,12] Although subsequent scrutiny attributed these observations to possible biases in the interaction potential measurements arising from uncorrected experimental data,[13] the phenomenon of like-charge attraction remains both experimentally and theoretically largely unresolved. The spontaneous localization and extension of DNA molecules at the edges of fluidic nanoslits under conditions of weak electrostatic screening reported here could support the idea of anomalous attractive interactions between charged macroions and like-charged confining walls. The format of the experiment permits direct optical observation of the interaction of negatively charged polyelectrolyte molecules with high curvature (concave) regions of negatively charged confining surfaces using real-time fluorescence imaging. The direct, rather than inferred, evidence of the presence of what appears to be "traps" or "potential wells" at nanoslit edges, which localize and stretch DNA molecules in solution, could offer fundamentally different insight into the nature of electrostatic interactions between confined macroions and like-charged bounding sidewalls.

Devices were fabricated by patterning silicon substrates bearing approximately 1000 nm silicon dioxide using laser lithography. Silicon dioxide was etched by two different methods: reactive ion etching in CHF_3 plasma using a photoresist mask and wet etching in HF using a 50 nm Cr mask. Wafers were diced so as to expose the extremities of the etched channel regions to the lateral edges of the substrate. Fluidic slits were produced by anodic bonding of patterned substrates to cover glass suitable for microscopy. Devices consisted of several parallel fluidic slits of width 2, 5, and 10 μm.

λ-DNA (Fermentas Life Sciences) was labeled with YOYO-1 (Invitrogen) at a ratio of 1:10 (dye:basepair). A drop of solution containing labeled DNA (10 ng/μL) in TrisHCl at the relevant concentration was placed at the entry to the nanoslits. Solution drawn into the nanoslits by capillary effect was allowed to flow for at least 1 min. The pressure gradient over the length of the slit was eliminated by placing a drop of buffer of the same concentration at the inlets and outlets of the slits (parts A and B of Figure 1). When the pressure was equalized at both ends of the slits, DNA molecules confined in the nanoslits showed an abrupt transition from flowing to diffusive behavior. Dynamics of DNA molecules in the nanoslits was studied using fluorescence microscopy on a Zeiss Axiovert inverted fluorescence microscope using a 60× N.A. 1.2 water immersion objective (Carl Zeiss AG) and a Cascade II EMCCD camera (Visitron Systems GmbH) for imaging. A schematic representation of

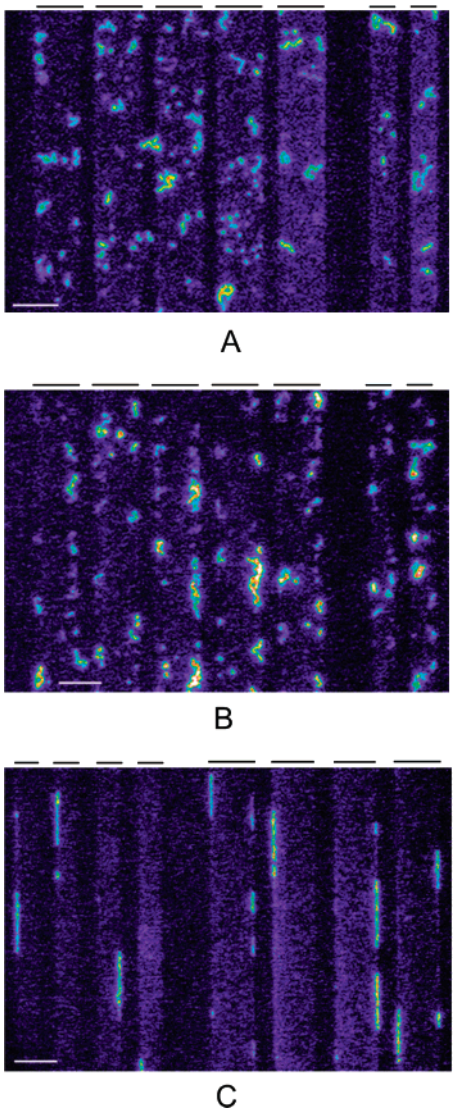

Figure 2. Representative fluorescence micrographs of DNA molecules in 0.35 mM TrisHCl in slits of different depths, (A) 350 nm, (B) 200 nm, and (C) 100 nm, reflecting the transition of molecules from random coils occupying the area of the slit, to one-dimensional extended states at the slit sidewalls with decreasing slit-depth. Scale bars represent 10 μm. Horizontal lines above the images denote the locations of the fluidic slits.

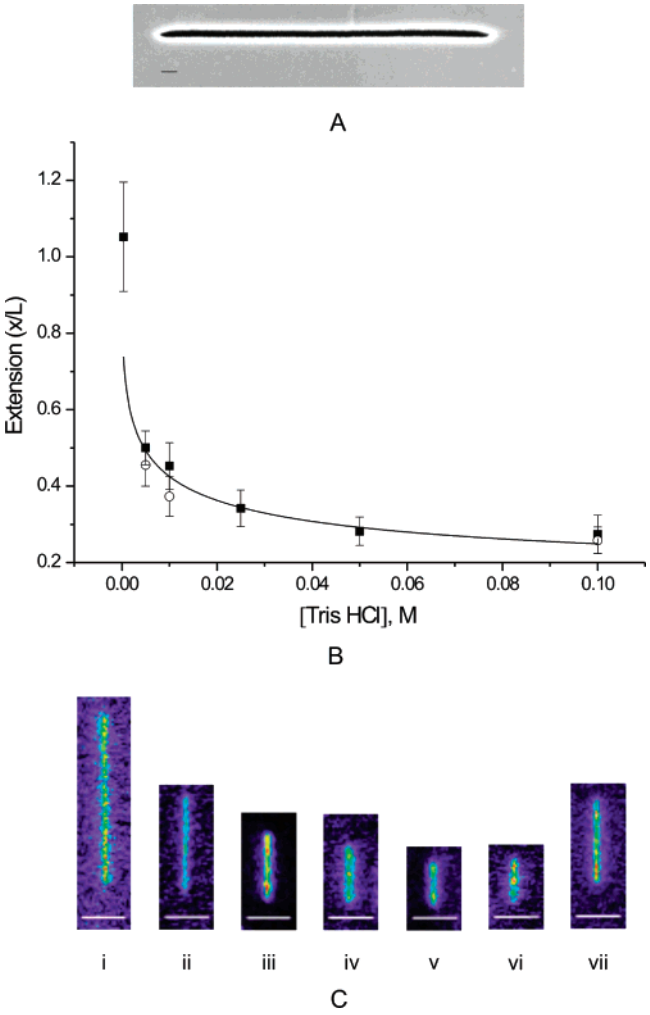

Figure 3. (A) Scanning electron micrograph of a 2 μm wide and 50 nm deep slit. Scale bar represents 100 nm. (B) Average extended lengths of DNA molecules as a function of ionic strength of the medium. Data are displayed for the following conditions: 100 $\pm$ 10 nm deep slits (black squares), 50 $\pm$ 5 nm deep slits (open circles). Each data point represents averages over 15−40 molecules. The line represents a fit of the data to eq 1. (C) Fluorescence micrographs of representative individual extended molecules in 100 nm deep slits for ionic strengths ranging from 0.35 to 100 mM TrisHCl (i−vi) and in a representative biochemical buffer (Lambda exonuclease reaction buffer, 67 mM glycine-KOH, 2.5 mM $MgCl_2$, 50 μg/mL BSA) (vii). Scale bars represent 5 μm.

the experimental setup is shown in parts A and B of Figure 1. Groups of molecules were imaged for about 5 s at 10−20 frames/s. The lower limit on the solution ionic strength in our experiments was established by the 10 mM TrisHCl content of our commercially obtained λ-DNA stock solution.

λ-DNA molecules, fluorescently labeled with YOYO-1, were introduced into slit-shaped channels of depth ranging from 50 to 350 nm. In slits of depth 200 nm and higher, molecules occupied the footprint of the slit in coiled configurations, as intuitively expected (panels A and B of Figure 2). In slits of depth 100 nm and less, a proportion of molecules was observed to adopt axially extended configurations along the edges of the slit and exhibited thermally induced fluctuations in contour length along the major axis of the slit (Figure 2C and Supporting Information video 1). The extended length of individual molecules, which we define as the linear projected length along the slit edge over which the polymer mass was distributed, was found to depend strongly on ionic strength of the medium. The data show that the magnitude of molecular extension does not depend strongly on slit depth below a depth of 100 nm. Our measurements of the average observed extended length of DNA molecules were influenced by random breakage of DNA molecules mainly due to photocleavage[14] and to some extent physical breakage during flow. Smaller fragments were eliminated by visual inspection, and only the largest extended molecules under a given set of conditions were used to obtain estimates of the average end-to-end length shown in Figure 3. The measurements involved experiments performed in several different devices, and the spread of the distribution of molecular extension at any given salt con-

centration inherently incorporates small variations associated with device-to-device variability.

Unstained λ-DNA has a contour length of 16.5 μm. Previous work on the stretching of DNA stained with intercalating dyes suggests that the dye increases the contour length and persistence length up to a saturating value of 30%.[15] This corresponds to a total extended length of 18.7 μm at the staining ratio used in these experiments. Average molecular extension in slits of depth 100 nm ranged from complete stretching in 0.35 mM TrisHCl to around 8 μm in 10 mM TrisHCl and 5 μm at 100 mM TrisHCl (Figure 3B). We also performed proof-of-concept experiments using DNA covalently labeled with AlexaFluor 488 that ruled out the possibility that staining DNA with YOYO-1 or the presence of small amounts of positively charged free YOYO-1 in solution could produce these effects.

Recently scaling arguments were used to arrive at the following approximate expression for the relative elongation of a polymer in a two-dimensional nanoslit[16]

$$\frac{x}{L} = 1 - 0.085\left[\left(\frac{A}{P}\right)^{2/3} + \left(\frac{B}{P}\right)^{2/3}\right] \qquad (1)$$

where x is this observed extension at a given salt concentration, L is the total contour length (18.7 μm in this case), A and B represent the width and depth of the confining slit, respectively, and P represents the persistence length. Recognizing that persistence length of DNA, P, is itself a function of salt concentration allows us to obtain a relation between molecular extension, x/L, and salt concentration. Using the expression $P = 37.0 + (2.94/C^{1/2})$ nm, relating persistence length of DNA to salt concentration, C,[17] and fixing the value of the depth of confinement, $B = 100$ nm, we found the data fits eq 1 in the range 5−100 mM with a value of $A = 900 \pm 67.5$ nm for the width of the confinement (Figure 3B). The width of the confinement A, here, does not represent a physical confinement in the lateral dimension, rather it could be taken as a measure of the range of the lateral attractive potential confining the DNA molecule to the sidewall. The fact that a constant value of A in (1) does not fit the extension data over the entire range of salt concentrations investigated strongly suggests that the width of the confinement is itself a function of salt concentration. The observed maximal extension at the lowest salt concentration investigated is, for example, consistent with assuming a value of, say, $A \approx 100$ nm in (1). We might add that the exclusion of the extension data corresponding to the lowest salt concentration, 0.35 mM, did not significantly alter the fit or the value of A reported here. Recently, the extended length of DNA molecules confined in nanochannels was reported to exhibit a stronger scaling with channel diameter than that expected from polymer scaling arguments.[18] The extension of confined DNA molecules at high curvature regions of negatively charged bounding nanoslit sidewalls, even under conditions of strong electrostatic screening, demonstrated in this work, could suggest the involvement of similar curvature-related phenomena in the observed stronger scaling of molecular extension with diameter of confinement.

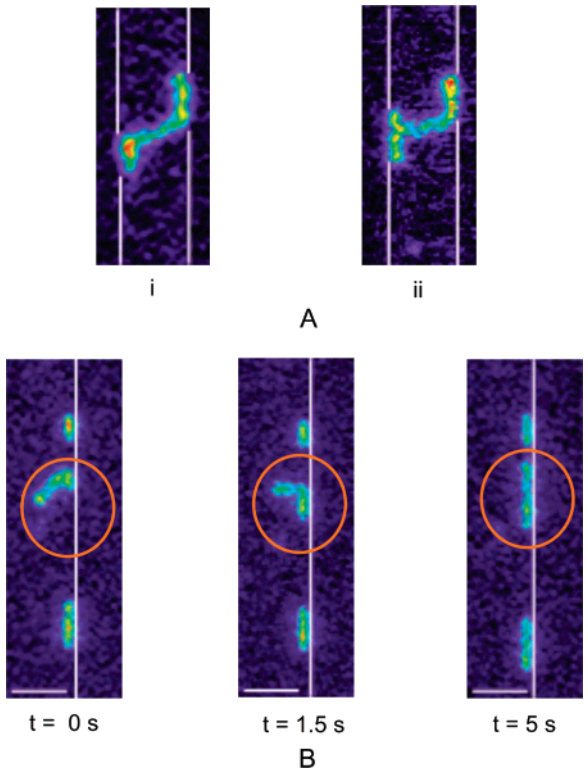

Figure 4. (A) A single DNA molecule in 0.35 mM TrisHCl straddling the breadth of a 100 nm deep and 5 μm wide slit. Portions of each end of the molecule are trapped and extended in the potential well associated with the two bounding lateral sidewalls and diffuse along the sidewall. $\Delta t = 1.5$ s between images (i) and (ii). (B) Time course of the trapping of a single DNA molecule (circled in red) in 1 mM KCl in a 100 nm deep slit. The images depict a molecule beginning to interact with a nearby sidewall and proceeding to be "fully trapped" at the sidewall on a time scale of about 5 s. Vertical lines superimposed on the images indicate locations of the lateral sidewalls. Scale bars represent 5 μm.

At the lowest salt concentration, individual extended molecules frequently adopt configurations that span the width of the channel, with segments at each of the molecule trapped and extended at each edge of a given slit (Figure 4A and Supporting Information video 2). The lengths of the individual arms of a molecule associated with each sidewall fluctuated rapidly. The dynamics usually continued for a few seconds until a photocleavage event occurred that separated the molecule into independent entities. This behavior was found to occur in channels 2 and 5 μm in width but not in channels 10 μm in width. The dependence of the occurrence of such "crossover" states on the slit width directly reflects the likelihood that the two ends of a given DNA molecule simultaneously interact with both bounding edges of a given fluidic slit. The apparent similarity to the behavior of DNA molecules adsorbed on cationic lipid bilayers[9] suggests the involvement of attractive interactions between the like-charged molecule and slit edge regions which compensate the loss in configurational entropy associated with stretching the DNA molecule. The overall reduction in system free energy arising from these attractive interactions results in one-dimensional extended configurations of DNA molecules at the slit edge.

A significant difference in the behavior of DNA molecules trapped and extended in solution at the edges of SiO_2 nanoslits as compared to those adsorbed to cationic lipid bilayers however lies in the faster timescales involved in trapping and extension of DNA in fluidic slits. This originates from the fact that the diffusion coefficient for DNA diffusing in solution in a 100 nm deep slit is at least an order of magnitude larger than that for DNA adsorbed on cationic lipid bilayers.[19,20] The longest time scale involved in the trapping and extension of a molecule at the sidewall is determined by the diffusive transport of a molecule located at the center of the slit to a location close enough to the slit edge such that at least a portion of its contour length begins to interact with the edge. Assuming a diffusion distance of 2 μm, the measured diffusion coefficient of λ-DNA diffusing in a 100 nm slit places this time scale at about 1 min. In our experiments however, we observe that trapping and extension of DNA molecules at the edge of a nanoslit is practically instantaneous, dominated by molecules closest to edge. Nevertheless, we were occasionally able to observe the dynamics associated with the trapping and extension of a molecule diffusing a few micrometers away from a slit edge. Figure 4B shows a representative case of a molecule beginning to interact with a nearby lateral sidewall and sliding into the "trap". The molecule is fully trapped on a time scale of about 5 s, while its two smaller neighbors above and below diffuse along the sidewall (Supporting Information video 3).

Furthermore, we analyzed the diffusion behavior of individual trapped extended molecules at the slit edges in 100 nm deep slits. Initial analyses on trapped DNA molecules exhibiting an extended length of approximately 10 μm in 0.35 mM TrisHCl reveal a diffusion coefficient for one-dimensional diffusion of the order of 10^{-9} cm^2/s which is comparable to the calculated value for free diffusion of an ellipsoid of length 10 μm and width 2 nm diffusing along its long axis, given by $D = kT/\xi L$, where ξ is the frictional drag per unit extended length given by $(2\pi\eta)/(\ln(L/w))$. Here, η is the viscosity of water, L the extended length of the molecule, and w the width of the DNA molecule, assumed to be 2 nm to place an upper limit on the value of the calculated diffusion coefficient. The fact that the overall friction for one-dimensional diffusion of trapped and extended molecules seems dominated by the hydrodynamic contribution suggests that molecules are confined to the slit edges by forces acting normal to the edges but that molecular motion along the sidewall remains unconstrained, permitting the extended molecules to diffuse freely along the axis of extension. The corresponding drag per unit extended length, ξ, was around 1 fN s/μm^2, at least an order of magnitude less than that reported for the motion of extended DNA molecules in nanochannels.[21]

Our observations on the spontaneous extension of DNA molecules confined in solution to two dimensions in nanoslits present intriguing evidence of anomalous interactions, electrostatic in origin, between like charged macroions and confining sidewalls. In slits of depth 100 nm and less, attractive interactions, whose origins remain unclear, arise between the molecules and high curvature edge regions of slit. This attractive potential results in virtual quasi-one-dimensional nanochannel zones at the slit edges, within whose confines the molecules shed configurational entropy and extend out along the edge. Slit depth and Debye length are two important system dimensions that influence the behavior of trapped polyelectrolyte molecules. The fact that the propensity of DNA molecules for extended states at the walls is attenuated with increasing slit depth at a given ionic strength (Debye length) suggests that the coupling or interaction of counterion clouds may be important in producing this effect. Increasing slit depth also reflects the transition from two-dimensional confinement to three-dimensionality relative to the polymer molecule, which suggests that the intrinsic configurational entropy of the polymer chain could also play a role in the process. Development of a theoretical framework adequately describing such interactions not only would greatly enhance our understanding of the forces governing the interactions of charged colloidal objects and macromolecules in confinements but could also shed light on how nature orchestrates complex processes involving many body interactions among charged biological macromolecules packaged in small spaces.

From a technological standpoint, silicon dioxide−glass nanoslits present a simple system to stretch DNA in solution. Capillary flow transports dozens of DNA molecules simultaneously for stretching into nanoslits enabling high throughput single molecule DNA−protein interaction studies. The ability to stretch DNA in planar fluidic nanoslits could facilitate the direct observation of association and dissociation of proteins on the backbone of DNA and the formation of induced tertiary structures such as loops that may not be possible using existing methods. In addition, the fact that the information to stretch DNA in solution—a combination of confinement and electrostatics, in this case—is built into the structure of the device offers an alternative to macroscopic experimental setups involving externally applied forces and fields to stretch DNA: a nanostructured coverslip that exploits the fortuitous interplay of physical phenomena that dominate at small length scales.

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Supporting Information Available: Movies displaying trapping and extension of DNA at nanoslit sidewalls. This material is available free of charge via the Internet at http://pubs.acs.org.

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