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Direct observation of confinement-induced diffusophoresis

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Abstract

Nanofluidic devices have channel dimensions which come to within one order of magnitude of the Debye length of common aqueous solutions. Conventionally, external driving is used to create concentration polarization of ions and biomolecules in nanofluidic devices. Here we show that long-range ionic strength gradients intrinsic to all nanofluidic devices, even at equilibrium, also drive a drift of macromolecules. To demonstrate the effect, we confine long DNA to straight nanochannels of constant, rectangular cross-section ($100 \times 100 \text{ nm}^2$) which are connected to large microfluidic reservoirs. The motion of DNA is observed in absence of any driving. We find that at low ionic strengths, molecules in nanochannels migrate toward the nano-micro interface, while they are undergoing purely diffusive motion in high salt. Using numerical models, we demonstrate that the motion is consistent with the ionic strength gradient at the micro-nano interface even at equilibrium, and that the dominant cause of the drift diffusophoresis.

Nanofluidic devices with at least one nanoscale dimension are powerful tools in the analytical sciences [1,2]. The impact of the electric double layer (EDL) on ionic concentrations in nanochannels is central to understanding transport processes [3–6]. In contrast to microfluidic devices where all dimensions are at least about $1 \mu\text{m}$, the EDL can cover a significant portion of the nanochannel cross-section and its thickness cannot be ignored. Concentrations of anions and cations are substantially different in the EDL, and hence the effective ionic strength within a nanochannel is lower than that in adjacent microchannels containing the same buffer. In this publication, we focus on the transition region between micro- and nanofluidic regions. Diffusion limits the steepness of ion concentration gradients at the microchannel-nanochannel interface, and we will show that long-range gradients in ionic strength are present even at equilibrium.

Nanofluidic devices not only able to control the spatial distribution of macromolecules, but can also manipulate the configuration of macromolecules. A prominent example is the stretching of long ($>10 \text{ kbp}$) DNA molecules in nanofluidic channels with a channel width and height of a few DNA persistence lengths ($\sim 50 \text{ nm}$), and hundreds of micrometers long [7]. The technology has facilitated large-scale genome mapping [8] and the study of protein-DNA interaction [9,10]. The stretching is an equilibrium effect. DNA can be moved through the device using electrical [11], hydrodynamic [12], and thermophoretic [13] stimuli. Since

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Conflicts of interest

There are no conflicts to declare.

the deformation of DNA is linked to a free energy, DNA drift can also be induced by non-homogenous channel cross-sections [14].

Here we demonstrate that individual stretched DNA molecules undergo a directed motion along the nanochannel even if the channel cross-section is constant. The motion is toward the interface between nanochannel and an interfacing microchannel even if that interface is tens of microns away. By computing the ion distribution at the interface between nanochannels and the microfluidic loading channels, we will show that the drift of DNA is from regions of low to high ionic strength. We then compute a quasi-potential that includes both the energy associated with the DNA configuration as well as a contribution due to diffusophoresis [15–19], and demonstrate that the motion is dominated by diffusophoretic driving.

Figure 1 illustrates the layout of the micro- and nanofluidic device made out of fused silica. Nanochannels are 200 μm long with a cross-section of $100 \times 100 \text{ nm}^2$. The device is filled with Tris-Borate-EDTA (TBE) buffer at a nominal strength of $0.25 \times \text{TBE}$ or $2 \times \text{TBE}$. λ -DNA (48.5 kbp) is fluorescently stained using YOYO-1. Due to the anticipated variation in ionic strength along the axis of the nanochannels, we divide the dataset into groups according to the location of individual molecules from the micro-nano interface.

Figure 2 presents two kymographs for each combination of location and buffer (out of 20 molecules for each condition). Example video data is deposited as Supplementary Data. Figures 2a and 2b show that for both buffer conditions, DNA located in the central region of the nanochannel diffuses, but does not drift appreciably. For DNA locations within 45 μm of the micro-nano interface, devices with $0.25 \times \text{TBE}$ buffer showed apparent drift in addition to diffusion (Figure 2c,e,g). Drift appears faster closer to the micro-nano interface. Molecules in $2 \times \text{TBE}$ appeared to act purely diffusively, even when they were located within 15 μm of the micro-nano interface (Figure 2 d,f,h).

At this point we ask whether the apparent drift can be described either as a directed translocation with a constant speed, or rather a classical diffusion with modified diffusion coefficient. To do so, we calculate mean square displacement $MSD = \langle \Delta x_{cm}^2 \rangle$ for each condition (Figure 2 i–p). In the Supplementary Data we show that the resulting graphs can be described by a subdiffusive, a diffusive and a superdiffusive component corresponding to measurement error, Brownian motion, and drift, respectively [20]. To compare the motion for different conditions, we fitted $MSD = c + 2D t + (v t)^2$. The parameters for diffusion coefficient and drift velocity are presented in Table 1.

Figure 2 shows the composite fits, as well as contributions of the individual components. We find that the magnitude of the drift component only becomes dominant for $0.25 \times \text{TBE}$, and only locations within 30 μm of the micro-nano interface. This finding mirrors the initial visual inspection of Figure 2. For $2 \times \text{TBE}$, no drift velocity was determined during the fixed-exponent fitting. We note that the diffusivities in $2 \times \text{TBE}$ were lower than we would have anticipated based on the diffusivities in $0.25 \times \text{TBE}$ and the shorter extension along the nanochannel. We believe that the discrepancy may be due to the reduced screening length at

high salt, combined with inhomogeneities in the channel surface and the resulting variation of ζ -potential.

In order to link the local ionic strength to the motion of DNA, we compute the ionic strength profile using finite-element methods. The governing equations of the electric double layer and ionic strength in the channels have been presented before [6,21]. Final results were obtained by solving the Poisson-Boltzmann and the mass transfer equations in the steady state form. In particular, we used an approach of non-overlapping EDL layers, ionic-strength dependent ζ -potentials, and open boundary conditions in places where the simulation domain was not bounded by a channel wall. Only 5 μm of channel nanochannel length and a similar distance into the microchannel were simulated due to computational constraints. Similarly, the ionic system was reduced to TRIS and borate ions to enable convergence of the solution in the challenging geometry. Details are given in the Supplementary Materials.

In Figure 3 we present the ionic strengths at the micro-nanointerface for both buffer concentrations, and confirm the initial assumption the ionic strength in nanochannels is lower than in microchannels. An ionic strength gradient is formed near the interface of microchannels and nanochannels which extends both into the nanochannel and the microchannel. The differences in ionic strength between microchannel and nanochannel, as well as the strength of the gradient values are a strong function of the bulk ionic strength. While nanoconfinement has a small influence for $2\times\text{TBE}$, the ionic strength at $0.25\times\text{TBE}$ is 10% lower in the nanochannel than in the microchannel. Note that the leading contributor to this result is the strong buffer dependence of the ζ -potential, as it is the main source of EDL strength and determines the surface charge density through the Poisson-Boltzmann equation.

Even though the largest gradients of the ionic strength are found at the micro-nano interface, the gradients extend far into the nanochannels. We find that ionic strength in the nanochannel between 0.5 μm and 5.0 μm from the micro-nano interface can be described as $5.43\text{ mM}(1 - x \cdot 2.6 \times 10^{-4}\mu\text{m}^{-1})$ for $0.25\times\text{TBE}$, while $47.33\text{ mM}(1 - x \cdot 4.0 \times 10^{-5}\mu\text{m}^{-1}(1 - x \cdot 0.05\mu\text{m}^{-1}))$ is a good description for $2\times\text{TBE}$.

The DNA drift in a gradient of ionic strength may be caused by three distinct mechanisms: it can be due to diffusophoresis, due to electrostatic forces, or due to the free energy of confinement that is dependent on the ionic strength. Diffusophoresis drives DNA to regions of high ionic strength, in agreement with our observation [22]. No electrostatic forces are acting in accordance with the electroneutrality of our ion model. Free energy of confinement is anticipated to translocate DNA because rising ionic strength shortens DNA stretched in nanochannels [23].

We test the plausibility of both the diffusophoresis and free-energy of confinement hypotheses by using the linear and parabolic approximations of the ionic strength reported above. In the Supplementary Materials we derive a quasi-free-energy density expression which includes entropic, excluded-volume [24–28], and diffusophoretic contributions [15] of the form $E = -L T \delta \log \kappa$. Here L is a contour length along the polymer, is a constant coefficient characteristic of diffusophoresis, and is the Debye-Hückel parameter. We then note that drift is slow when compared to length fluctuations, and thus propose that the

instantaneous configuration is given by the minimum of the quasi-free-energy if an appropriate Lagrange multiplier λ is used to satisfy the boundary condition that the molecule is not tethered at either end. For a center-of-mass trajectory that can be described by $\langle \Delta x_{cm}^2 \rangle = 2Dt + (vt)^2$ we find that the Lagrange multiplier is observable as $\lambda \approx v/DL$, where L is the total contour.

For $0.25\times$ TBE we observe a drift motion with $\lambda_{O,0.25\times} = 0.11 \mu m^{-2}$ in the bin adjacent to the micro-nano interface and determine that $\delta=420 \mu m^{-1}$ is consistent with the observed drift. We can determine the relative importance of diffusophoresis and drift based on confinement free energy by inverting the problem to ask what $\lambda_{\delta=0,0.25\times}$ is expected for $\delta=0$. We find that $\lambda_{O,0.25\times} = 325 \lambda_{\delta=0,0.25\times}$, and conclude that the drift for $0.25\times$ TBE is almost entirely due to diffusophoresis. Since the coefficient δ is independent of the buffer strength, we can test whether the value of δ determined at $0.25\times$ TBE is consistent with the observation of no drift in $2\times$ TBE. We find an expected $\lambda_{E,2\times} = 1.3 \times 10^{10} m^{-2}$. Within our observation time, we thus expect that diffusion is dominant. However, in the very long-time limit (longer than practical here), drift should also be observable.

Conclusions

In summary, we have directly demonstrated diffusophoresis of single DNA due to ionic strength gradients that are induced by nanoconfinement. The drift occurs predominantly adjacent to the transition region between nanochannel and microchannel, and is characterized by a gradual transition region. We anticipate this effect to be universal to all solvated molecules or objects in a nanofluidic devices that employ a microfluidic infrastructure. However, its detection here is enabled by the favorable magnitudes of the diffusion and diffusophoretic coefficients. Furthermore, the use of a slowly diffusing test object enabled the direct observation of the trajectory of single molecules.

It is interesting to consider the impact of diffusophoresis in DNA analysis using manufactured nanopores [29]. First-generation sequencing devices typically are nanofluidic devices that typically have dimensions that are smaller than the Debye length scale, and thus the majority of the DNA molecule would be unconfined and unaffected by the mechanism described here. However, future devices may contain multiple, integrated nanofluidic regions such as described by some groups [30].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Fig. 1.

Integrated micro-nanofluidic device. (a) Schematic of the device. Shading indicates the anticipated ionic strength distribution. (b) Phase-enhanced brightfield micrograph of liquid-filled nanofluidic device. (c) Electron micrograph of micro-nano interface before the device was sealed using fused silica coverslip. Imaging performed with discharge layer, and imaging from the left in panel (b).

Fig. 2.

(a)-(h) Two representative kymographs for each combination of location and buffer out of at least twenty molecules that we recorded for each condition. The micro-nano interface is located to the left. (i)-(p) MSD(Δt) graphs including the fits. Black markers are data, blue curves are the full fit, green curves follow $(v \Delta t)^2$, yellow lines follow $2D \Delta t$, and red lines are constant. (a), (b), (i), (j) are for central location, (c), (d), (k), (l) are 30 to 45 μm from the micro-nano interface, (e), (f), (m), (n) are 15 to 30 μm from the interface, and (g), (h), (o), (p) are within 15 μm of the interface. (a), (c), (e), (g), (i), (k), (m), (o) are for 0.25 $\times$ TBE, and (b), (d), (f), (h), (j), (l), (n), (p) are for 2 $\times$ TBE.

Fig. 3.

Finite-element simulation of ionic strength distributions at the micro-nano interface.

Microchannels lie to the left, and nanochannels to the right. (a) In the symmetry plane of a single nanochannel at $0.25 \times \text{TBE}$. (b) Average across nanochannel cross-section as function of position along the nanochannel axis at $0.25 \times \text{TBE}$. (c) In symmetry plane at $2 \times \text{TBE}$. (d) Average over cross-section at $2 \times \text{TBE}$.

Table 1.

Drift velocity v and diffusion coefficient D derived from curve fitting shown in Figure 2 i–p. Errors as measured by the 95% confidence interval were less than 1 significant digit of the table.

	v ($\mu\text{m/s}$)		D ($\mu\text{m}^2/\text{s}$)	
	$0.25 \times \text{TBE}$	$2 \times \text{TBE}$	$0.25 \times \text{TBE}$	$2 \times \text{TBE}$
Center	0.02	0.00	0.08	0.06
30–45 μm	0.09	0.00	0.08	0.07
15–30 μm	0.11	0.00	0.07	0.05
0–15 μm	0.13	0.00	0.08	0.13