DNA Electrodiffusion in a 2D Array of Posts

W. D. Volkmuth, T. Duke, M. C. Wu, and R. H. Austin
Department of Physics, Princeton University, Princeton, New Jersey 08544

Attila Szabo
National Institutes of Health, Bethesda, Maryland 20892
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We consider some of the fundamental statistical mechanics of the electrodiffusion of a long polyelectrolyte, DNA, in a microlithographically constructed 2D rectangular array of cylindrical posts. The DNA polymer is shown to be free draining when not hooked on a post, and the mean time to unhook is explicitly calculated and compared to our measurements.

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Perhaps the most important application of electrodiffusion of polyelectrolytes in confining environments is the length-dependent fractionation of DNA via gel electrophoresis. This paper will demonstrate that electrophoresis of DNA within microlithographically constructed synthetic lattices can be understood qualitatively and described quantitatively owing to the precisely characterized environment.

A polymer can be characterized by its persistence length $\mathcal{P}$, contour length L, and diameter d. In the case of DNA under normal physiological buffer conditions, d is 2.4 nm and $\mathcal{P} = 0.060$ μm [1]. We are concerned here with "long" polymers, where $L/\mathcal{P} = N \gg 1$. The velocity of the polymer center of mass $v_{\text{c.m.}}$ is on the order of μm/sec in water (viscosity $\eta = 1 \times 10^{-3}$ Pa sec at 20°C). Since the Reynolds number $\mathcal{R} = \rho v_{\text{c.m.}} L/\eta$ (ρ is the mass density of the solvent) is exceedingly small, $\sim 10^{-10}$ [2], the viscous drag forces are much larger than any inertial terms and the velocity of the center of mass is determined by $\lambda E L = \zeta v_{\text{c.m.}}$. Here ζ is the friction coefficient of the polymer, λ is the effective charge/length of the polymer, and E is the applied electric field. A rod of length L at very low $\mathcal{R}$ has $\zeta \sim \eta L \ln(\frac{L}{d})$ due to hydrodynamic coupling between rod segments [3]. However, in the case of electrophoresis of polyelectrolytes such as DNA there is a compensating flow of ions on the surface which screens the hydrodynamic coupling on scales longer than the Debye length [4], making ζ for the free-draining Gaussian coil to a good approximation $\sim 3\pi\eta L$. The electrophoretic mobility μ ($\mu = \frac{v_{\text{c.m.}}}{E}$) of the polymer free in solution is then *independent* of L and no length-dependent electrophoretic fractionation occurs.

To circumvent this problem, gels have traditionally been used to separate DNA, for a polyelectrolyte moving in a restricting environment *can* have a length-dependent electrophoretic mobility $\mu(L)$. Three separate mechanisms have been identified by which gels fractionate polymers depending on the ratio $\mathcal{S} = R_g/a$, the ratio of the radius of gyration R_g of the polymer to the characteristic pore size a of the gel. In three dimensions and when $N = L/\mathcal{P} \gg 1$, $R_g = \mathcal{P}(\frac{N}{3})^{1/2}$.

When $\mathcal{S} < 1$, polymers are fractionated by a sieving process. The mobility is usually described by the Ogston model [5,6]. When $\mathcal{S} > 1$, fractionation takes place by the process of reptation if the electric field is weak [7–9]. A weak field is one in which the dimensionless electric field strength $E^* \ll 1$ [10]. If $a \gg \mathcal{P}$ then $E^* = \frac{\lambda E a^3}{\mathcal{P} kT}$ where kT is the thermal energy. The electrophoretic mobility μ of the polymer in the reptative regime is $\sim \frac{\lambda L}{3\zeta}[\frac{1}{N} + E^*]$ [11,12]. Note that since ζ scales with L, μ becomes *independent* of the length L of the polymer for $N > 1/E^*$. The lack of ready fractionation of long DNA molecules [13] is disastrous for gel electrophoresis from an applied view and has spawned numerous attempts to circumvent the problem [14,15].

As an alternative to trying to extend the regime of reptative separation, one might consider increasing the pore spacing in order to extend the sieving regime. Unfortunately, the pore size in agarose gels cannot be made larger than about 0.3 μm without loss of gel structural integrity. Lithographic arrays, however, offer the possibility of constructing restrictive environments with much bigger effective pore sizes. We have used optical microlithography to construct rectangular arrays of cylindrical posts of height h 0.15 μm and radius r 0.5 μm hermetically sealed to a clear Pyrex coverslip [16,17]. The cylinders were separated by a distance of 2 μm center to center so the pore opening a and separation b was 1.0 μm, far larger than can be achieved in agarose gels. The DNA molecules were imaged using the intercalating fluorescent dye ethidium bromide.

The basic picture of the motion that emerged from the analysis of the DNA molecules diffusing in an applied electric field E of 1.0 V/cm was that the polymers episodically "hooked" on the posts forming metastable U-shaped structures, and that between hooks the polymer moved as an extended, entropically collapsing string between the posts until hooking again.

The first issue that must be addressed is the applicability of the free-draining approximation in two dimensions for very long DNA molecules. Figure 1 shows a plot of the measured center of mass velocities $v_{\text{c.m.}}$ for

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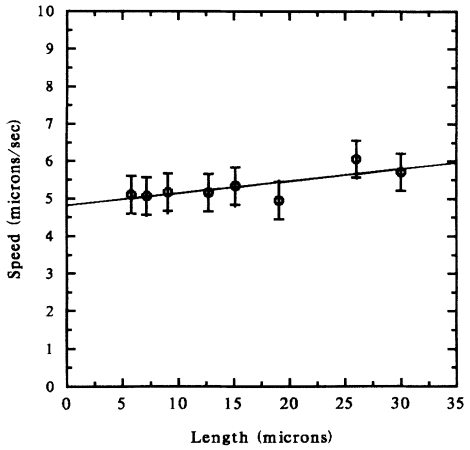

FIG. 1. Center of mass velocity $v_{\text{c.m.}}$ of DNA fragments as a function of DNA length during movement between hooks. The solid line is a linear fit.

several different length polymers freely moving between hooks in an applied electric field $E = 1.0$ V/cm. A fit of the observed velocities by a linear relation of the form $v_{\text{c.m.}}(L) = gL + f$ yields a very small value for g of 0.03 $\pm$ 0.02 sec^{-1}, indicating virtually no velocity dispersion as a function of length L in the arrays when the polymer is not hooked on a post. If we assume that $\zeta = 3\pi\eta L$ for a randomly oriented polymer then from our measured average value of $v_{\text{c.m.}} = 5.2$ μm/sec we find that $\lambda = 4.6\times10^{-10}$ C/m, or 0.3 e^-/Å. Note that the uncompensated value for DNA due to 2 electrons per base pair is 0.6 e^-/Å. Counterion condensation not only removes the $\ln L$ term in the free-diffusion expression for ζ but also reduces λ from the uncompensated value. Manning's theory predicts a substantially lower value for λ then we use [18]; this may be due to our neglect of forces due to electroendosmosis or hydrodynamic Burgers-Oseen terms [19] involving interactions of the DNA with the posts [20]. However, since simple modeling of the force equations using a reduced λ gives excellent fits to the data as shown below, we cannot determine at this time the true origin of the anomalously high λ.

Substitution of λ into our expression for E^* then gives that E^* in these experiments is much bigger than 1, approximately 2.0×10^2. The consequence of this large E^* is that the polymers never achieve anything resembling a Gaussian coil as they move through the array and are consequently not sieved according to their equilibrium radii of gyration as a simple sieving theory would predict. One reason for this is that the Rouse relaxation time τ_1 of large polymers [3] is longer than the time that it takes these molecules to traverse a pore, so that any deformation of the coil caused by contact with an obstacle will persist until the polymer impinges on a subsequent obstacle. Second, when R_g is not considerably smaller than the obstacle size, the flexible molecule is able to pass simultaneously on both sides of an obstacle. In this case the polymer may get very deformed—how much so depends on L and E^* [21].

We see many instances of almost complete extension of hooked polymers to the full contour length L, even at $E = 1$ V/cm. The applied forces due to the external electric field E acting on the polymer generate a tension dependent on the distance s along the contour length, $\mathcal{T}(s)$. When this tension exceeds the internally generated entropic tension $\mathcal{T}_{\text{ent}}$ of a random coil, the coil will straighten [22]. Since a hung molecule has zero external tension at the loose ends $[\mathcal{T}(0) = \mathcal{T}(L) = 0]$ the polymer is disordered for some distance κ, after which the increasing externally applied tension dominates. This characteristic distance $\kappa = \frac{kT}{\lambda E P}$.

κ is the length of disorder seen at the two free ends of the molecule which appear anomalously bright. From the observed value of κ of approximately 1 μm in an E field of strength 1 V/cm we find that $\lambda \sim 0.2$ e^-/Å, in good agreement with the value deduced from the mobility of the polymer diffusing when not hooked on a post. Let $N^* = \frac{L}{\kappa}$. If $N^* \gg 1$ the polymer will extend to virtually the full contour length when hooked, with an easily observed center of mass and well defined "arms" extended on either side of the post. The conclusion is that Ogston sieving for DNA polymers longer than a few microns in environments of pore size 1 μm and above will not occur for E fields greater than about 0.01 V/cm, impractically low for reasonable separation times.

Given that we are forced to deal with highly stretched polymers, we can ask if the time to "unhook" can give rise to a length-dependent mobility. Let x represent the difference in the positions of the ends of a polymer hung asymmetrically over a post. We will show that the present experiments within error bars are adequately fit without post friction, unlike other work that has attempted to understand the motion of hooked polymers [23].

The net force acting along the polymer due to the applied electric field is λEx, and the polymer moves on an inverted harmonic potential surface of the form $U(x) = -\frac{1}{2}\lambda Ex^2$; thus $\lambda Ex = 3\pi\eta L\frac{dx}{dt}$. This yields $x(t) = x_0\exp(\frac{t}{\tau})$ where $\tau = \frac{3\pi\eta L}{\lambda E}$ and x_0 is the initial length difference of the polymer when it becomes fully extended after uncurling on the post. Figure 2 shows data and a least-squares fit of a single exponential to the motion of a 100 kilobase long DNA molecule moving off a post after full extended hooking. Figure 3 shows the time constants τ determined from fitting of the data vs various lengths of the polymer L. The predicted linearity of τ with length L is good as long as the length L is much greater than κ. Since $\tau = L/v_{\text{c.m.}}$, from our measured value of $v_{\text{c.m.}} = 5.2$ μm/sec it follows that a polymer of length $L = 30$ μm should have a time constant τ of 6 sec, in good agreement with our measured value of 7$\pm$1 sec.

The above analysis ignores thermal fluctuations. Consequently, we incorrectly predict that if $x_0 = 0$ the system is infinitely metastable. Since the DNA irreversibly falls off a post when $x = \pm L$, its average lifetime on the post is

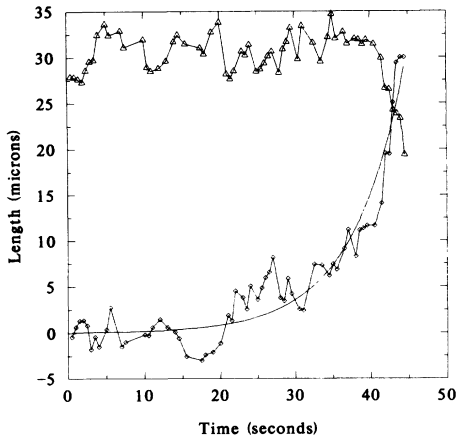

FIG. 2. $x(t)$ vs time for a 100 kilobase (30 μm) long DNA molecule, and an exponential fit to the plot (solid line). The upper curve is the observed contour length of the molecule L.

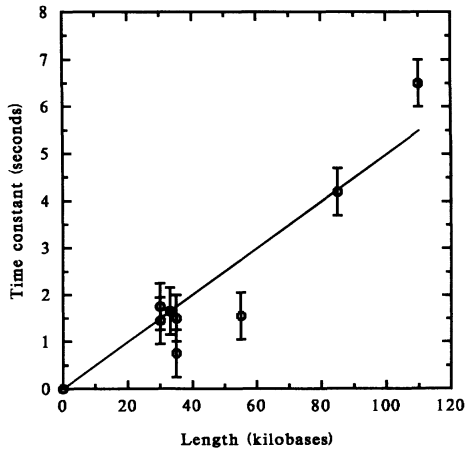

FIG. 3. τ vs the length. The solid line gives a linear fit.

the *mean first passage time* $\langle t(x_0)\rangle$ of a particle initially at x_0 to reach $x = \pm L$. In the high friction limit, the correct description of the Brownian motion of the molecule is provided by the Smoluchowski equation [24]. Using the theory of first passage times [25,26] it is possible to show in the case of an inverted harmonic potential that

$$\langle t(x_0)\rangle = 2\tau \int_0^\infty \frac{e^{-2y^2}}{y} \sin[\gamma y(L - x_0)] \sin[\gamma y(L + x_0)] dy \tag{1}$$

where $\gamma = (\lambda E/kT)^{1/2}$.

For our standard condition of $E = 1$ V/cm and the value for λ extracted from the measured value of $v_{\text{c.m.}}$ of unhooked polymers we find that $\gamma = 3.5\times10^6$ m^{-1}. Thus $\gamma L \gg 1$ for our DNA molecules, and in this case $\langle t(x_0)\rangle$ approaches $\tau \ln(L/x_0)$ for $\gamma x_0 \gg 1$ and $\tau \ln(\gamma L)$ for $x_0 \ll L$ with no singularity at $x_0 = 0$. In fact, for $x_0 = 0$, $L = 30$ μm, and $\tau = 7$ sec we predict that $\langle t(x_0)\rangle \sim 25$ sec, in good agreement with our measured value of 30 $\pm$ 10 sec. Thus, the unhooking times of the polymers is understood even for the $x_0/L = 0$ case.

We can now estimate the effective mobility of the hooked DNA molecules. Let $\langle v_{\text{c.m.}}(x_0)\rangle$ be the *net* center of mass velocity of the stretched polymer as it unhooks, measured from the time $t(x = x_0)$ where the hook is formed to $t(x = L)$, that is,

$$\langle v_{\text{c.m.}}(x_0)\rangle = \frac{\Delta x_{\text{c.m.}}}{\langle t(x_0)\rangle}, \tag{2}$$

where $\Delta x_{\text{c.m.}}$ is the distance the center of mass moves, easily seen to be equal to $\frac{L}{4}[1 - (\frac{x_0}{L})^2]$. We then have

$$\langle v_{\text{c.m.}}(x_0)\rangle \sim \frac{\lambda E[1 - (\frac{x_0}{L})^2]}{12\pi\eta \ln(\frac{L}{x_0})} \tag{3}$$

for $\gamma x_0 \gg 1$ and

$$\langle v_{\text{c.m.}}(x_0)\rangle \sim \frac{\lambda E}{12\pi\eta \ln(\gamma L)} \tag{4}$$

for $\gamma x_0 \ll 1$.

Inspection of $\langle v_{\text{c.m.}}(x_0)\rangle$ in the above equations reveals that for $\gamma x_0 \gg 1$, $\langle v_{\text{c.m.}}(x_0)\rangle$ is only dependent on the ratio $\frac{x_0}{L}$ and thus any length-dependent mobility can only occur indirectly through some L dependence of the probability distribution of achieving a particular value of x_0/L. However, in the special case where $\gamma x_0 \ll 1$ we expect the mobility to depend directly on the inverse log of the polymer contour length.

Figure 4 shows a three-dimensional plot of the predicted $\langle v_{\text{c.m.}}(x_0)\rangle$ vs x_0/L and L. The surface is calculated from Eq. (5) with the value measured for γ, and the data are observed values of $\langle v_{\text{c.m.}}\rangle$ from measurements with $E = 1.0$ V/cm. The statistical errors in the measurements result in an estimated error for $\langle v_{\text{c.m.}}\rangle$ of $\pm$ 0.2 μm/sec. Unfortunately hooking with equal arm

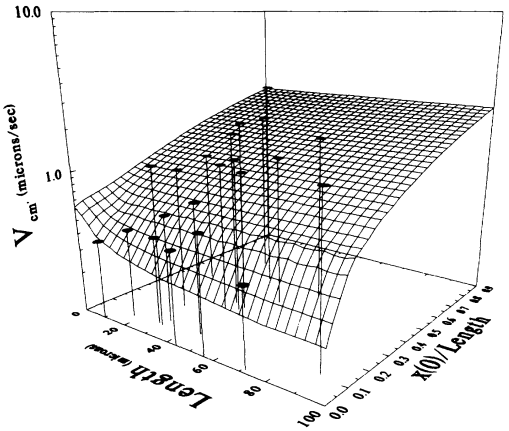

FIG. 4. The surface predicted by Eqs. (1) and (2). Experimental measurements are denoted by the stick points.

length ($\frac{x_0}{L} \sim 0$) is statistically a rare event and thus there are few points in the region where a $\ln(\gamma L)$ dependence of the center of mass velocity is expected; nonetheless, the theory and data are seen to be in good agreement. The reduced $(\chi^2)^{1/2}$ between the data and the theoretical surface using no free fitting parameters is 1.7.

Although we have shown good agreement between theory and experiment for part of the problem, calculation of the *net* mobility of the polymer moving through the lattice is a much more complex problem involving both the mean *hooking* times and the unhooking times, convoluted in with the probability that a polymer will hook with initial arm inequality x_0. Moreover, the hooking dynamics can be more complicated for larger molecules since multiple hooks can occur and the single hooking model described here becomes less appropriate. This investigation indicates that within the constraints of constant electric fields and micron sized obstacles a simple post array will probably *not* give rise to *strongly* dispersive electrophoretic mobilities of long polymers and indicates that a geometry other than a simple array of posts must be considered in order to extend the range of length-dependent fractionation in synthetic lattices. Fortunately, as we have shown here, the main features of the polymer dynamics *can* be understood quantitatively, and since arbitrary control of the geometry down to micron length scales is easy with microlithography, the prospects are excellent that a geometry can be identified and optimized for length fractionation.

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