DNA Electrophoresis on a Flat Surface

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We report a new approach for performing DNA electrophoresis. Using experimental studies and molecular dynamics simulations, we show that a perfectly flat silicon wafer, without any surface features, can be used to fractionate DNA in free solution. We determine that the ability of a flat surface to separate DNA molecules results from the local friction between the surface and the adsorbed DNA segments. We control this friction by coating the Si surface with silane monolayer films and show that it is possible to systematically change the size range of DNA that can be separated.

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Methods to fractionate mixtures of biomolecular components typically fall into two classes: Chromatographic methods, which utilize surface interactions between the biomolecules and a particulate phase [1], and electrophoretic methods which rely on topological restrictions, such as the entanglements in a polymer solution, the junction points of a gel, or obstacles on a surface, to separate the components [2–6]. The strong adsorption of DNA chains to the particulate phase in a chromatographic column coupled with the large surface area presented by the complex packing of the column severely limits the use of chromatographic principles for fractionating DNA. Consequently, electrophoretic methods have become the primary means of separating and sequencing DNA. Yet, despite the advances made in the design of electrophoretic separation media, separating large DNA fragments still presents a problem since the molecules get trapped by the topological restrictions of the media [2,4]. In this Letter, we propose a new approach to performing electrophoresis of DNA. We show that it is possible to exploit the conformational entropy of an adsorbed DNA molecule such that a perfectly flat surface can separate DNA fragments, when an electric field is applied in the plane of the surface. We demonstrate that the critical factor that controls the fractionation of DNA on a flat surface is the local friction between the adsorbed DNA segments and the surface. Since this friction is controlled by the surface energy of the substrate, we show that it is possible to design a surface to separate DNA fragments over a size range much larger than conventional methods.

When a DNA molecule is adsorbed on a flat surface, the balance between the loss of entropy due to the localization of the DNA at the surface and the energetic gain on adsorption of the molecule results in the classic picture of DNA segments being present as either loops (that extend into the solution) or trains (that are contiguous segments adsorbed on the surface) [7]. For a fixed surface attraction, shorter chains will maximize their entropy and have a larger number of loops, while longer chains will exploit the energetic gains on adsorption and have larger trains. Consequently, if an electric field were applied in the plane of the surface, the response of the molecule to the field should be a function of its conformation on the surface, and therefore there should be, in theory, a length dependent mobility.

To test this hypothesis, electrophoresis on a broad series of double stranded DNA fragments with sizes ranging from $N = 1$ kbase pairs (kbp) to 164 kbp was performed on a flat Si wafer. Si strips (2 cm long by 0.4 cm wide) were cleaned of organic contaminents using a modified Shiraki technique [8]. They were then used as is, or covered with an additional evaporated layer of unadecyltrichlorosilane (UTS) or octadecyltricholorosilane (OTS). The DNA was dissolved in tris-borate-EDTA buffer solution (TBE) at concentrations ranging from 50 to 500 μg/ml and a 100–200 nL drop was loaded on the Si substrate at the negative electrode. The solution was allowed to remain on the surface for at least 10 min for adsorption to occur. Atomic force microscopy (AFM) revealed that the DNA chains were concentrated by the drying liquid into an initial diameter ≤ 50 μm independent of the size of the initial droplet or the diameter of the pipette. The Si strips were placed at the bottom of a 0.5 cm deep groove of an electrophoretic chamber. The groove was then filled with buffer solution (TBE) at a concentration of 10^{-2} M. Ethidium bromide dye at a concentration of 7 μg/ml was added to the solution. The mobility of the DNA chains, in a dc field of 4.5 V/cm, was then measured by laser induced fluorescence with a confocal microscope.

In Fig. 1 we plot the background substracted fluorescence intensity spectra as a function of time t for a 1 kbp DNA ladder, with the detector located at a distance of 1 cm from the droplet. From the figure we can see that this method can produce well-resolved peaks corresponding to different base pairs [9]. In order to compare with standard electrophoresis, we calculated the mobility of λ-DNA, to be $\mu = 3.31 \times 10^{-5}$ cm^2/V s, which is similar to the mobility of λ-DNA in a 2% agarose gel at the same field strength [3]. The resolution (RSL) was determined as the peak width at half maximum (W) of a single peak divided by the slope of the migration function, $x(N)$ using the

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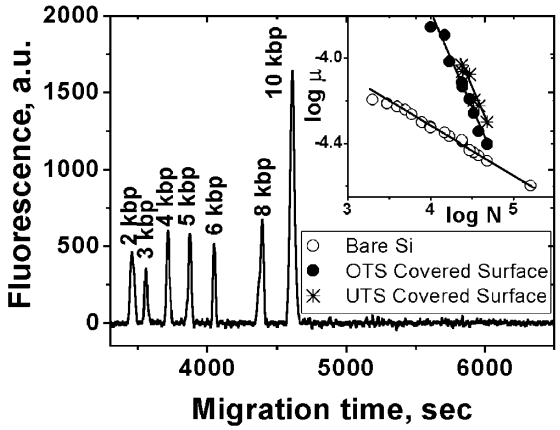

FIG. 1. The fluorescence intensity spectra as a function of time for a 1 kbp ladder on a bare Si surface. In the inset is a log-log plot of the mobility μ ($cm^2/V\,s$) as a function of the number of base pairs N (kbp). The straight lines are linear fits. The three surfaces used here are a bare Si wafer, a Si wafer covered by a monolayer of OTS, and a Si wafer covered by a monolayer of UTS. In all cases the electric field used was $E = 4.5$ V/cm.

equation RSL $= W/(\Delta x/\Delta N)$ [10,11]. From our data we obtained RSL $= 324$ base pairs in the range of 10–48 kbp which is an order of magnitude better than that previously reported for dc field separation (RSL > 1 kbp) [12] and compares favorably with that obtained in pulsed field electrophoresis separation at much higher fields (100 V/cm) [13]. The log of the mobility on different substrates is plotted versus log N in the inset of Fig. 1. From the figure we can see that the slope on the most interactive surface, native oxide covered silicon scales as $N^{-0.22}$. Except for the first data point at $N = 2000$, the slope appears fairly constant up to $N = 164$ kbp (T2 DNA), or in a range spanning almost 2 orders of magnitude.

The relatively strong interactions between the polar silicon oxide surface and DNA can be partially screened by coating the surface with an organic self-assembled monolayer. Screening reduces both the surface friction coefficient and the electro-osmotic flow (EOF). The UTS and OTS layers were chosen since they have chemically identical repeat units and differ only in the total carbon chain length. Earlier studies have shown that OTS and UTS self assemble into layers that are 1.5 and 2.5 nm thick, respectively [14]. From the figure we can see that the scaling with chain length is now $N^{-0.87}$ which is comparable to that reported for slab gel or capillary electrophoresis [11]. It is interesting to note that the slope is identical on the two monolayers despite the fact that the thicker OTS layer should provide a stronger screen. The decrease in interaction strength though is manifested by a decrease in the range of chains that can be fractionated on OTS (Fig. 1). Figure 2a is a scan at the edge of the Si substrate close to the positive electrode which shows the short chains that have migrated together and reached the positive electrode. On this surface, electrophoresis yields only a large single peak for chains with $N < 20$ kbp with a mobility

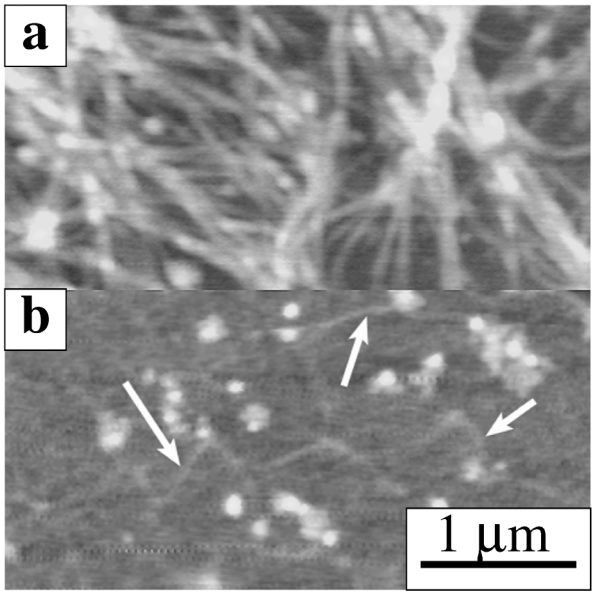

FIG. 2. AFM scans of DNA adsorbed on an OTS covered Si layer after an electric field ($E = 4.5$ V/cm) is applied. The arrows indicate the location of the chains. (a) The shorter chains are all moving together, while (b) the longer chains move separately.

corresponding to that of free draining DNA. Figure 2b was obtained near the negative electrode and shows well-separated longer chains which are elongated in the field directions. These chains are moving far more slowly across the surface and correspond to the peaks observed in the fluorescence spectra for $N > 20$ kbp.

In order to optimize this method for DNA separation and explore its potential for fractionation we must determine the conditions to optimize both throughput and resolution. We used a molecular dynamics (MD) simulation to isolate the critical factors that determine the dynamics of DNA electrophoresis on a surface. In the simulation, we model the DNA by a linear polymer chain with N segments. Monomers of mass m and an effective charge q separated by a distance r interact through a truncated Lennard-Jones (L-J) potential of the form $V(r) = 4\epsilon[(\sigma/r)^{12} - (\sigma/r)^6]$ for $r < r^c = 2.2\sigma$. Here ϵ and σ are the characteristic energy and length scales, and the potential is zero for $r > r^c$. Adjacent monomers along the chain are coupled by an additional FENE potential which prevents chain breaking and yields realistic dynamics for polymers [15].

The surface consists of atoms forming two (111) planes of an fcc lattice, with the atoms of the surface connected to the lattice sites by stiff springs [16]. An L-J potential with modified parameters ϵ_s, $\sigma_s = \sigma$, and $r_s^c = 2.2\sigma_s$ was used to model the interactions between the monomers and the surface atoms. The value of the surface-polymer coupling ϵ_s was varied from 2.0 to 3.0 ϵ. Increasing the value of the surface-polymer coupling ϵ_s increases the attraction between the polymer and the surface. The temperature T was kept constant by coupling the polymer to

a heat bath [15]. Periodic boundary conditions were applied within the plane of the walls to eliminate edge effects. The equations of motion were integrated using a fifth-order predictor-corrector algorithm with a time step of $\Delta t = 0.005\tau$ where $\tau = (m\sigma^2/\epsilon)^{1/2}$. In all the runs we fixed the temperature $T = 4.0\epsilon/k_B$ and used a scaled electric field $E_s = q\sigma E/k_B T$. We note that for computational simplicity we do not include the details of the solvent or the counterions in this simulation. Thus, these simulations will not provide a quantitative result, but rather provide a guide to future experiments by elucidating the mechanisms that control the separation of DNA on a flat surface.

To understand the processes by which a flat surface causes fractionation of DNA we first consider the following limiting cases of surface interactions (while keeping the length of DNA fixed). In the first limit, when the attraction between the DNA segments and the surface is weak ($\epsilon_s = 2.0$), the conformation of DNA consists of loops that extend into the solution. When a constant electric field is applied in the plane of the surface, sections of the DNA can be easily detached from the surface, and can then subsequently reattach downstream (Fig. 3a). For this weak interaction, the mobility of DNA is controlled primarily by the unadsorbed segments. Consequently, we can expect the surface only to have a minimal affect on the mobility of the DNA and this mobility should approach the free draining limit of the chain in solution (where there is no length dependence). Indeed, the simulation shows no fractionation is observed in this limit (Fig. 4). At the opposite limit, when the surface is very strongly attractive to the DNA molecule ($\epsilon_s = 2.5$), almost all the segments are adsorbed on the surface. As the interaction between the DNA segments and the surface is very large, even under an applied electric field, only a few segments at a time can become detached from the surface (Fig. 3b). As a result, the motion of the DNA is effectively limited to the plane of the surface. Since the surface is flat, there are no restrictions to this in-plane motion, and so the dynamics of DNA undergoing electrophoresis on a strongly attractive surface can be mapped onto a DNA molecule undergoing electrophoresis in a two-dimensional medium with a reduced mobility. In this limit we also expect to see free draining DNA and no fractionation should be possible (Fig. 4). We note that we expect the mobility of the DNA in the limit of strong attraction to be most sensitive to any topological obstacles present, as the motion of DNA in this limit is truly two dimensional [4]. These two limits result in a possible window of resolution for a flat surface. When the attraction between the DNA and the surface is in the intermediate regime, chains will have a distribution of loops and trains. The train segments, as they are close to the surface feel the presence of the surface in the form of a local friction coefficient that resists motion. Thus, as chains of increasing length have a larger number of train segments [7], the mobility of longer chains should be reduced relative to the shorter chains and we should see separation. We tested this in the simulation by using an intermediate surface attraction ($\epsilon_s = 2.25$) and we see clear separation between DNA molecules of different length (Fig. 4 and inset). If, however, the local friction coefficient is the critical factor in the separation, increasing the field strength should result in all chains having the same mobility, as this resistance to motion will be overwhelmed. Indeed, when we doubled the field strength (keeping $\epsilon_s = 2.25$) we found that fractionation in this length range was no longer possible.

From the simulation results, we see that for separation to occur between different lengths, the chains have to adopt conformations on the surface that have both loops and trains. Even for a weak surface interaction, as the length of

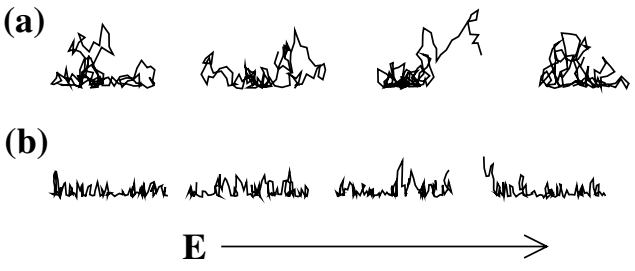

FIG. 3. MD snapshots of DNA undergoing electrophoresis on a flat surface. (a) The surface interaction is $\epsilon_s = 2.0$. (b) The surface interaction is $\epsilon_s = 2.5$. In both (a) and (b) the atoms of the surface are not shown for clarity. Projections of the DNA molecules in the xz plane (normal to the surface) are shown. In both (a) and (b), the electric field ($E_s = 0.02$) is applied in the direction indicated by the arrow.

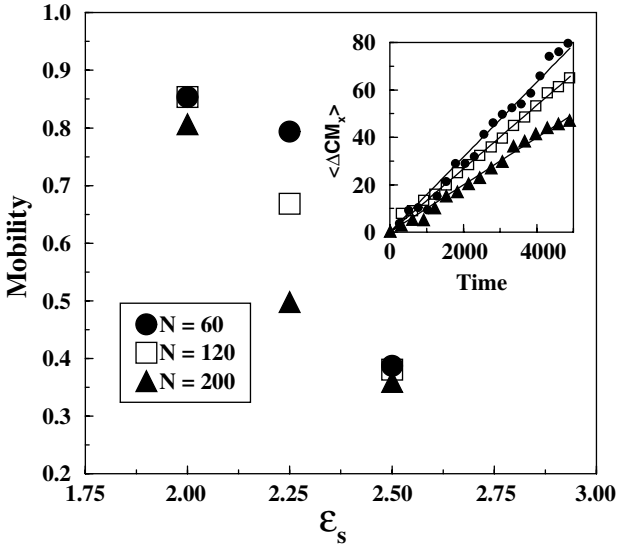

FIG. 4. Plot of the mobility of DNA on a flat surface from the MD simulation as a function of surface attraction ϵ_s averaged over 10 random initial chain conformations. The inset shows the motion of the center of mass of the chain, $\langle \Delta CM \rangle_x$ in the direction of the field, for the intermediate regime. In all these runs, the field strength used was $E_s = 0.02$, and the field was applied along the positive x axis. The size of the error bars is on the order of the size of the symbols used.

the adsorbed chain is increased, the probability of forming train segments will also increase [7]. This implies that any flat surface (below the strong adsorption limit) can separate chains in a particular size range. Therefore, as one changes the surface-DNA attraction, there should be no change in the scaling exponent, but the mobility and the size range of the DNA that can be separated will be affected. This is borne out by the results shown in Fig. 1 where changing from a bare Si surface to a coated surface changes the size range of DNA that can be resolved. We note, however, that while the OTS and the UTS covered surface show a similar slope (with only a vertical shift indicating that chains move faster on the UTS surface) the slope of the Si surface is much lower. This, we believe, is a result of EOF [17] that is strongest on a bare Si surface, but dissapears when the surface is coated with the silane monolayers. Studies have shown that the EOF is small near the surface region and then increases to its bulk value towards the center of the capillary [18]. Since the DNA adsorbed on Si consists of loops that are a function of the chain length, the adsorbed DNA will encounter an EOF environment that varies with the distance from the surface. Consequently, the EOF can affect the scaling behavior of the mobility of the DNA. On the other hand, on the coated surfaces, since EOF is absent, the scaling is the same on both surfaces.

In summary, we have demonstrated that it is possible to separate DNA fragments on a flat surface using friction rather than topological constraints. Since the geometry of the flat surface is ideal for chemical patterning techniques [19], this method offers the advantage of providing a cheap, reusable medium for electrophoresis that can be easily integrated in a microfluidic system [20].

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