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Tilted hexagonal post arrays: DNA electrophoresis in anisotropic media

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Abstract

Using Brownian dynamics simulations, we show that DNA electrophoresis in a hexagonal array of micron-sized posts changes qualitatively when the applied electric field vector is not coincident with the lattice vectors of the array. DNA electrophoresis in such “tilted” post arrays is superior to the standard “un-tilted” approach; while the time required to achieve a resolution of unity in a tilted post array is similar to an un-tilted array at a low electric field strengths, this time (i) decreases exponentially with electric field strength in a tilted array and (ii) increases exponentially with electric field strength in an un-tilted array. Although the DNA dynamics in a post array are complicated, the electrophoretic mobility results indicate that the “free path”, i.e., the average distance of ballistic trajectories of point sized particles launched from random positions in the unit cell until they intersect the next post, is a useful proxy for the detailed DNA trajectories. The analysis of the free path reveals a fundamental connection between anisotropy of the medium and DNA transport therein that goes beyond simply improving the separation device.

Keywords

Brownian dynamics; DNA electrophoresis; microfluidics; post array

1 Introduction

Microfabricated post arrays are one microfluidic method for separating long DNA [1,2]. The vast majority of experiments [3-13] focus on ordered hexagonal arrays, although there are some data for square arrays [14], quasi-hexagonal arrays [15-17] and disordered arrays [18]. In the case of hexagonal arrays like the one illustrated in Fig. 1, the separation medium is anisotropic with one lattice vector at $\theta = 0^\circ$ and another lattice vector at $\theta = 30^\circ$. The anisotropy of the medium contrasts sharply with gel electrophoresis [19], where the random fibers of the gel are normally modeled as an isotropic medium. DNA electrophoresis experiments in hexagonal post arrays [3-18] invariably use electric fields oriented along one of these lattice vectors. In the present contribution, we use Brownian dynamics simulations to show that the electrophoretic mobility and dispersivity undergo a qualitative shift when the electric field vector is no longer coincident with the lattice vectors of the post array. Our

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Abbreviations: none

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simulations indicate that these “tilted” post arrays should provide superior separations. Based on the understanding gained in periodic media, where we can perform accurate simulations that account for the electric field gradients [10], we also make a prediction about DNA electrophoresis in weakly ordered media [18], where the underlying lattice is partially “melted,” and random (isotropic) media.

There are a few examples of tilting the post array in the context of pulsed electric field electrophoresis in hexagonal post arrays [20,21], where the electric field vector alternates between a lattice vector, $\theta = 0^\circ$, and the angle $\theta = 135^\circ$, which is commonly used for pulsed field gel electrophoresis [19] and not coincident with the lattice vectors of a hexagonal array. (From symmetry, steady-state transport at an angle $\theta = 135^\circ$ is equivalent to an angle $\theta = 15^\circ$.) We recently investigated this system using Brownian dynamics simulations [22], with the aim of explaining the anomalous behavior of the DNA prism protocol [21] at high switching frequencies [23]. In the course of our work, we examined the zero-frequency DNA prism and noticed that the steady-state electrophoretic mobilities in a post array are strikingly different for $\theta = 0^\circ$ and $\theta = 15^\circ$ [22]. This observation led us to question the prevailing wisdom of applying the electric field along one of the lattice vectors of a hexagonal array.

2 Materials and Methods

2.1 Brownian Dynamics simulations

We used a standard Brownian dynamics simulation method for a bead-spring model of freely draining DNA electrophoresis in a post array that accounts for the non-uniform electric field. This model is known [13] to give very good agreement between simulation and experiments at the single-molecule level. The simulation algorithm has been described at length elsewhere [24,25], and the code we used here is identical to the one we used to study the DNA prism [22]. The cylindrical posts have diameter $1\ \mu\text{m}$ and they are distributed hexagonally (see Fig. 1) with a center-to-center distance $a = 3\ \mu\text{m}$. The separation channel height is $4.5\ \mu\text{m}$. We simulated λ DNA (48.5 kbp) with 37 beads and T4 DNA (169 kbp) with 126 beads. Most results will be reported in dimensionless form with the lengths scaled by the maximum extensible spring length ($0.58\ \mu\text{m}$) and times scaled with the bead diffusion time (approximately 20 ms). The dimensionless electric field is reported as a Péclet number, Pe , that represents the ratio of convective force (due to electric field) to thermal fluctuations on the length scale of a spring. For practical purposes, the value $Pe = 1$ corresponds to approximately 16 V/cm. These conversions from dimensionless notation to dimensional units were discussed previously [22,25].

We simulated applied electric fields with angles between $\theta = 0^\circ$ and $\theta = 90^\circ$ in increments of 10° , which allows us to confirm our claims about the symmetry of the array. For λ DNA, we simulated Péclet numbers from 0.5 to 4.0 in increments of 0.5. For T4 DNA, there are numerical instabilities at high electric fields that lead to unphysically long hooking collisions [26], similar to what has been observed for similar simulations using a bead-rod model [27]. As a result, we limited our simulations of T4 DNA to Péclet numbers from 0.5 to 2.0 in increments of 0.5. To test the robustness of our conclusions, we also simulated λ

DNA at $Pe = 2.0$ in a square post array (with the same post size and post density) for the same values of θ as well as the square array lattice vector at $\theta = 45^\circ$.

To compute the average velocity v of the DNA, we used a finish-line approach by computing the average time required to reach the dimensionless distance $L = 2000$. The electrophoretic mobility μ is then computed by dividing the velocity by the electric field. To compute the effective diffusion (dispersion) coefficient, D , we used a snapshot approach where we measure the variance of the positions of the DNA, $\sigma^2(t) = \langle s^2 \rangle - \langle s \rangle^2$, where s is the position of the DNA along the direction of the electric field and the time data are obtained at intervals of 0.4 to 2.0 dimensionless time units, depending on the Péclet number. The diffusion coefficient is then obtained from the linear regression of $\sigma^2 = 2Dt$. To render the diffusion coefficient dimensionless, we divide it by the free-solution Rouse diffusion coefficient [28].

The mobility and diffusivity data for λ DNA and T4 DNA can be combined to compute the separation resolution [29],

$$R_s = \frac{(v_\lambda - v_{T4}) t}{2(\sigma_\lambda + \sigma_{T4})} \quad (1)$$

Equation (1) can be inverted to determine the required time, t_R , to achieve a resolution R . If we also define an average velocity $\bar{v} = (v_\lambda + v_{T4})/2$, the array length required to reach this resolution is approximately $L_R = \bar{v}t_R$.

For a given combination of array geometry, angle, Péclet number and molecular weight, we relaxed an ensemble of 96 non-interacting molecules inside the array [22] and then simulated them under the electric field until their center of mass reached a dimensionless length $L = 2000$ in the direction (opposite) the applied electric field. We found that this sample size was large enough so that the standard error of the mean for the number of collisions, the collision time [30], and the electrophoretic mobility were smaller than the symbol sizes in the plots. To compute the standard error of the mean for the effective diffusion coefficient, we divided the ensemble of 96 molecules into 4 groups. The diffusivity of each group $i = (1, 2, 3, 4)$ was computed from the slope of $\sigma_i^2(t)$ obtained by ensemble average for those molecules, and the standard error of the mean for the entire simulation was obtained from these four measurements of D . The standard error of the mean for the diffusivity is sometimes larger than the symbol sizes in the plots.

2.2 Computing the free path in an array

It will prove illuminating to connect the results for the DNA trajectories, which are expensive to compute, to the ballistic motion of point-sized particles in the array. To this end, we define the free path inside an array as the average distance for a ballistic trajectory of a point-size particle, launched from within the unit cell in the direction of the applied electric field, from its initial position until the point where the trajectory intersects the next post. For example, supplemental Fig. A1 shows 400 randomly chosen initial conditions within a unit cell of a hexagonal post array. From these points, we draw straight lines along the given direction until they intersect with a post. The average length of these lines is the

“free path” of the corresponding post array and direction θ , which we then render dimensionless with the post spacing a . If the angle θ is a lattice vector, some of the straight lines will be infinitely long. To mimic the DNA diffusion, we replace any infinite length trajectory with the arbitrary cutoff value $10a$ to avoid having diverging values of the free path. The particular choice of the cutoff value proves to be irrelevant in the subsequent analysis.

In addition to computing free paths in ordered arrays, we also computed free paths in arrays that correspond to “melting” the original lattice. To create these disordered arrays, we follow an approach analogous to our previous experimental work [18] and let each post move n random steps with step size $a/40$. Therefore, n controls the degree of disorder. Since the disordered array has no unit cell, we select an arbitrary origin and use the region within a circle with radius a centered on the origin to launch trajectories. For each value of n , we measured the free path for 50 realizations of the disordered array to generate sufficient statistics.

3 Results and Discussion

3.1 Transport parameters and separation resolution

The most natural starting point for examining the role of the electric field angle is the electrophoretic velocity of the DNA. Figure 2 presents the velocity data obtained for λ DNA at four values of the Péclet number. Based on the symmetry of the hexagonal array, we would expect the velocity to have a period of 60° and a mirror symmetry about 30° . Supplemental Fig. A2 demonstrates the periodicity and mirror symmetry in an alternate form. Supplemental Fig. A3 confirms that the symmetry of the lattice is reflected also in the electrophoretic mobility for a square array. We thus assume that the periodicity is valid for all angles in Fig. 2, and restrict our subsequent discussion of hexagonal arrays to values of θ between 0° and 30° .

The standard experimental values correspond to $\theta = 0^\circ$ and $\theta = 30^\circ$, which are the local maxima in the electrophoretic velocity as a function of angle. In contrast, cases where the electric field vector is not coincident with the lattice vectors of the array feature lower mobilities that are relatively insensitive to the electric field. In what follows, we selected $\theta = 20^\circ$ for a more detailed study.

The electrophoretic mobility trend for λ DNA is also apparent in the data for T4 DNA, plotted in Fig. 3. When the electric field is applied along the lattice vector at $\theta = 0^\circ$, both the λ DNA and T4 DNA mobilities increase with Péclet number due to channeling [10,11,27,31,32]. At the highest Péclet numbers, the two mobilities are converging and the separation is lost. In contrast, the mobilities for $\theta = 20^\circ$ decrease with increasing Péclet numbers. Previous simulation work on collisions for $\theta = 0^\circ$ suggest that the electrophoretic mobility is a non-monotonic function of electric field [27,33,34]; there is an initial decrease in the electrophoretic mobility with electric field as the collisions become more effective, but then an eventual increase in electrophoretic mobility as the DNA tends to move long distances between collisions (i.e., channeling). For the $\theta = 0^\circ$ case, scaling theories and simulation data [27,33] suggest the minimum for our model of λ DNA should be around Pe^*

$= 0.6$, so the data in Fig. 4 correspond to the onset of channeling. For the $\theta = 20^\circ$ case, it appears that the obstacles in the tilted array break up the channels. As a result, when the Péclet number is large, the mobilities for $\theta = 20^\circ$ reach molecular-weight dependent plateau values instead of increasing towards unity. Indeed, it also appears that the mobility minimum is shifted to a larger value of Pe^* in the tilted array. The $\theta = 20^\circ$ case is thus qualitatively different than the canonical $\theta = 0^\circ$ case.

The deviation of the electrophoretic mobility from its free solution value arises from the collisions with the post. In Fig. 4, we plot the number of collisions during the simulation and the average holdup time [30] as a function of Péclet number for three different angles. The holdup time is relatively insensitive to the angle, indicating single post collision statistics [35-37]. There is a slight decrease in the average holdup time for $\theta = 30^\circ$, which we attribute to the greater likelihood of having a higher impact parameter [38] in the sparser array. In other words, due to the wider channel, the DNA have more opportunities to make glancing collisions for $\theta = 30^\circ$. Therefore, the average holdup time is slightly lower.

The number of collisions depends very strongly on the angle of the electric field. For $\theta = 0^\circ$ and $\theta = 30^\circ$, the frequency of collisions decreases with Péclet number, reflecting the rapid transport of the relatively small λ DNA through the channels of the array [10,11,27,31,32]. The effect is most dramatic for $\theta = 30^\circ$, which has wider channels than $\theta = 0^\circ$, making this angle less efficient for separations than the typical choice of $\theta = 0^\circ$. In contrast, for $\theta = 20^\circ$, the number of collisions becomes effectively independent of the Péclet number once the transport becomes convection-dominated. Moreover, the number of collisions at $\theta = 20^\circ$ is substantially higher than the standard cases of $\theta = 0^\circ$ and $\theta = 30^\circ$. Thus, the local maxima in electrophoretic mobility at $\theta = 0^\circ$ and $\theta = 30^\circ$ are due to the collision frequency, not the duration.

Compared to the electrophoretic mobility, Fig. 5 shows that the effective diffusivity is relatively insensitive to the angle. In all cases, the effective diffusion coefficient scales linearly with the Péclet number, as predicted by theory [39]. Since we already know from Fig. 4 that the holdup times are similar for different angles but the collision frequencies differ, the similar effective diffusivities at both angles in Fig. 5 suggest that the holdup time distribution is the dominant contribution [33].

The data in Figs. 3 and 5 suggest that the tilted post arrays should be superior for DNA separations. To illustrate this point in a concrete manner, Fig. 6 plots the time required to reach unit resolution in Eq. (1) (with the corresponding array length) as a function of electric field from a hypothetical delta function injection. The key experimental parameter is the time required for the separation; the available array length is typically an upper bound set by the fabrication procedure. Since real separations include additional sources of band width, especially due to the injection, we should view the estimates in Fig. 6 as lower bounds for the required time. For the standard case of $\theta = 0^\circ$, the time and array size required to achieve unit resolution increases almost exponentially with the electric field, which explains why successful separations using this angle [4,5,12] require weak electric fields in order to achieve unit resolution in centimeter-long (or smaller) arrays. In contrast, the time required to achieve unit resolution in the tilted post array (with $\theta = 20^\circ$) decreases almost

exponentially with electric field. While the corresponding array length increases with electric field, it remains well within the bounds for microfabrication. Indeed, Fig. 6 suggests that tilted arrays could provide fast separations (at high electric fields) or compact separation devices (at low electric fields) when compared to existing post array technologies.

3.2 Free Path and the Role of Order

The results thus far indicate that the dominant contribution to the electrophoretic mobility is the distance between collisions; the holdup times in Fig. 4 and the effective diffusion coefficients in Fig. 5 indicate that the DNA interacts with isolated posts rather than by multipost entanglements [25,40]. As a result, the separation is governed by the collision rate. We thus suspected that the mobility data could be understood with a simple transport model that captures how the pore width, projected onto the direction of motion, changes with the electric field direction at a fixed pore size. The free path model, described in Section 2.2, is one possible approach.

As we can see in Fig. 7, the free path is a remarkably accurate proxy for the trend in the electrophoretic mobility for a sufficiently large Péclet number, where the transport is dominated by convection. While the free path simulations do not provide the conversion factor between the free path and the electrophoretic mobility, they accurately capture the trends in the mobility as a function of angle. We were quite surprised by the ability of such a simple concept to capture the mobility trend, so we performed a set of companion Brownian dynamics simulations and free path simulations for a square lattice of posts. Supplemental Fig. A3 shows that the free path is also a robust metric for a square post array, accurately capturing the mobility maxima along the lattice vectors at $\theta = 0^\circ$ and $\theta = 45^\circ$ of the square lattice.

The free path concept also allows us to speculate about the role of order on DNA electrophoresis in post arrays. The key challenge in simulating DNA electrophoresis in post arrays is accounting for the non-uniform electric field; it is known [10,37,41] that models that use a uniform electric field [27,31,32] can lead to different conclusions about the DNA deformation and the dynamics in a post array. In an ordered array, we can take advantage of the symmetry to reduce the computational cost for the simulation [25]. In the absence of symmetry and with the large unit cells required to model periodic pseudo-random media, it is prohibitively expensive to compute the electric field in the Brownian dynamics simulation. If we assume that the free path is a reasonable proxy for the mobility in disordered arrays, then we can use these inexpensive simulations to compare perfectly ordered arrays (where we know from Fig. 7 and supplemental Fig. A3 that the assumption is valid) to examine post arrays without lattice symmetries.

Figure 8 plots the free path for the hexagonal array ($n = 0$), a “melted” version of the hexagonal array ($n = 40$), and an array that is effectively disordered ($n = 160$). The free path for the disordered array is essentially independent of the angle of the electric field, as we would expect for an isotropic medium. The melted array has modest peaks in the free path at the locations of the original lattice vectors, while the original hexagonal array has strong peaks at the locations of the lattice vectors. (Without the arbitrary cutoff used to mimic DNA diffusion, the free path in the ordered array diverges at the lattice vectors.) For the

standard angle $\theta = 0^\circ$, the free path in the hexagonal array is larger than the free path for the disordered array. Based on our discussion thus far, we might expect that the disordered array gives better separations than an ordered array with $\theta = 0^\circ$ due to more frequent collisions, in agreement with simulations in disordered arrays [27,31,32]. However, experiments [18] suggest that this conclusion is specious. First, disordered arrays feature more frequent multipost collisions, and thus a higher effective diffusivity [18,25], than an ordered array due to the fluctuations in the pore size. Second, the latter experiments [18] also concluded that ordered and disordered arrays exhibit similar mobilities to within the experimental error. It is not obvious whether the latter behavior results from the lower post density ($a = 5 \mu\text{m}$) in the experiments or the complicated electric field in a disordered array that is neglected in the free path analysis and extant simulations in disordered arrays [27,31,32].

Importantly, Fig. 8 indicates that the tilted array has a smaller mean path than the disordered system, implying that tilted ordered arrays should be superior to both (i) ordered arrays at $\theta = 0^\circ$ and (ii) disordered arrays with the same post density. We have already established conclusion (i) in Fig. 6. Figure 8 suggests that there will be the same (or possibly even more) collisions in a tilted array than a random array, leading to a bigger mobility difference as a function of molecular weight in the tilted array. Moreover, we know from Figs. 3-5 that a tilted array features single post collisions. As a result, we do not expect tilted arrays to exhibit the increased band broadening [18] observed in random arrays caused by locally dense regions of posts and the ensuing multi-post collisions. Conclusion (ii) is supported further by simulation work [31] showing that short-range correlations can increase the collision probability and aid the separation; such correlations appear naturally in the tilted, ordered array.

4 Conclusions

In the present contribution, we have shown that DNA electrophoresis in ordered post arrays, which are anisotropic media, qualitatively changes in so-called “tilted” post arrays, where the electric field vector is not coincident with the lattice vectors of the array. This behavior contrasts sharply with electrophoresis in an isotropic medium such as a gel. Our simulation data suggest that tilted post arrays are superior to existing devices for separating long DNA and operate best at high electric fields. We also anticipate that our predictions can be tested easily in the lab; one can either use a microchannel containing the tilted post array or use a large area array with a tunable electric field direction [21]. Tilted arrays are especially attractive from a fabrication standpoint because they reduce the free path (and thus increase the number of collisions) without requiring an expensive increase in the post density.

Overall, our analysis suggests that the fundamental property is not the order (or lack thereof) of the array, but rather the free path and the type of post collisions (single post versus multipost) occurring in the array. The type of post collision depends primarily on the local post density relative to the contour length of the DNA; if the posts are sufficiently close together, the DNA can become entangled with multiple posts. The free path is intimately related to the global arrangement of these posts. For isotropic media, the free path is, by definition, independent of the direction of the electric field. For anisotropic media, the free path depends strongly on the direction of the electric field, with the local maxima occurring

when the electric field vector is coincident with the lattice vectors of the array. For the other angles used for tilted arrays, the free path may be smaller than an isotropic medium with the same post density.

The free path concept may also aid in identifying improved post array geometries for separating long DNA. While we have focused here on hexagonal arrays, it is straightforward to compute the free path for other two-dimensional crystal symmetry classes. While the resulting calculations would need to be confirmed by Brownian dynamics simulations of the DNA and, if the prediction is robust, subsequent experiments, the free path provides an attractive route to screen new device designs and determine optimal orientations with respect to the electric field.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Schematic view of a hexagonal post array. The dashed line denotes the direction (opposite) of the applied electric field, which is defined by the angle θ between the dashed line and x -axis. The angles corresponding to the lattice vectors are indicated.

Figure 2.

Polar plot of the dimensionless velocity (left) and the normalized mobility (right) of λ DNA for $Pe = 1, 2, 3$ and 4 . The polar angle corresponds to the angle θ of the vector opposite the electric field and the radial distance corresponds to the value of the velocity or mobility. The data points (stars) are simulation data obtained for $\theta = 0^\circ, 10^\circ, \dots$ and 90° . The lines connecting these data points are only to guide the eye.

Figure 3. Electrophoretic mobility, made dimensionless with the free solution mobility, versus Péclet number for λ DNA (circles) and T4 DNA (squares) at $\theta = 0^\circ$ (open markers) and 20° (filled markers).

Figure 4.

Average number of collisions for traveling a fixed distance $L = 2000$ (left panel) and the elapsed time of each holdup (right panel) as a function of Péclet number for λ DNA at $\theta = 0^\circ$ (stars), 20° (circles), and 30° (triangles).

Figure 5. Effective diffusion coefficient in the array, made dimensionless with the free solution diffusion coefficient, versus Péclet number for λ DNA (circles) and T4 DNA (squares) at $\theta = 0^\circ$ (open markers) and 20° (filled markers).

Figure 6. Time needed to reach a unit resolution from a delta function injection for $\theta = 0^\circ$ (open markers) and 20° (filled markers). The numbers above each data point indicate the corresponding length of the array in millimeters. The conversion to dimensional quantities uses the conversion factors in Section 2.1.

Figure 7.

Normalized mobility for λ DNA at $Pe = 1$ (top) and the free path (bottom) versus θ in a hexagonal post array. For the mobility plot, the standard error of the mean is small compared to the symbol size. For the free path, the results from 500 sampling points (circles) and 1200 sampling points (stars) are plotted.

Figure 8.

Free path as a function of θ for 3 different post arrays with increasing disorder: $n = 0$ (dashed line, ordered), $n = 40$ (circles, partially melted), and $n = 160$ (stars, disordered).