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A DNA Prism: Physical Principles for Optimizing a Microfabricated DNA Separation Device

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Abstract

Recently, we reported a microfabricated "DNA prism" device that continuously sorts large DNA molecules (61 kilo-base pair to 209 kb) according to size in 15 seconds [1]. In this paper, we develop models to understand and optimize the device. The device's complicated characteristics are explained poorly by a simple model based on the assumption that DNA molecules are fully stretched. Assuming DNA molecules obey Hooke's law, a second model successfully explains the "biphasic" separation characteristics under low fields. Further, the model suggests the use of high fields combined with shorter pulse durations for more linear separation dependence on molecular weight, an advantage which is then confirmed by experiments.

Introduction

Because the separation of large DNA molecules according to size is essential in genomic projects, many methods have been developed for this goal. One approach, based on flow cytometry, measures the fluorescence intensity as stained individual DNA molecules pass through a focused laser beam [2]. The molecules can then be sorted into different reservoirs according to size [3]. A more commonly used approach, known as electrophoresis, exploits the fact that DNA molecules are negatively charged. The sizes of DNA molecules can be distinguished by measuring their migration speeds under electric fields. However, because DNA molecules move at the same speed in free solutions, this approach requires a sieving matrix, which conventionally consists of a cross-linked or entangled polymer. Although such DNA electrophoresis has long been adapted as the standard method for DNA separation, it is extremely time-consuming for molecules larger than 50 kb, with running times typically longer than 10 hours [4, 5, 6].

Recently, it has been demonstrated that microfabricated structures can be used as sieving matrices to increase the separation speed. In one design, entropic trap arrays consisting of many submicron-scale constrictions separated by wider regions, reduce the separation time to about 30 min [7]. Alternatively, we can replace the sieving matrix with an array of microposts in a microfabricated device, which can achieve separation in a few seconds in the 100 kb range [8, 9]. However, these are batch devices where loading, fractionation, and unloading occur sequentially.

The DNA prism

The DNA prism similarly consists of an array of micron-scale posts as the sieving matrix, and relies on integrated microfluidic channels to spatially tune uniform electric fields over the matrix (Fig. 1) [1, 9]. The microfluidic channels connect to electrolytic buffer reservoirs, where voltages are applied. DNA molecules are injected with a single channel connected to a DNA loading reservoir, and collected in different channels after separation.

Although the physical chip is the same as that for the conventional pulsed-field separation [9], a novel pulsing scheme enables continuous-flow fractionation instead of batch operations. This removes the limitation of the amount of sample the device can analyze. Further, a new microfluidic design has been used to generate and maintain uniform electric fields and precise sample injection [9]. Moreover, the speed and resolution, as well as the robustness

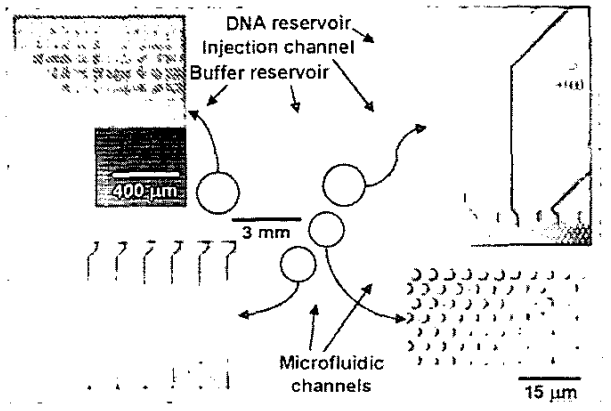

Fig. 1. Structure of the device illustrating the microfabricated sieving matrix integrated with the microfluidic channels [1, 9]. The post array is 3 mm x 9 mm, and the posts are 2 µm in diameter, 2 µm apart, and 2 µm tall. A single channel connecting to the DNA reservoir injects DNA through a 28 µm opening. The many microfluidic channels connecting to buffer reservoirs produce uniform electric fields over the sieving matrix by acting as electric current injectors.

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of the DNA prism has been demonstrated using bacterial artificial chromosomes (BAC) and P1-derived artificial chromosome (PAC) inserts isolated by a standard miniprep protocol [10]. 61 kb to 209 kb DNA molecules were separated in 15 sec, with ~13% resolution [1]. However, the device's separation characteristics are complicated and poorly understood. The goal of this paper is to understand and optimize these characteristics.

Constant fraction elongation model

When electric pulses are applied to the sieving array, DNA molecules migrate at different directions according to their molecular weights (Fig. 2). The electric pulses are applied alternatively at two directions 120° apart, with stronger intensity along the first direction than the second. When the first electric pulse is applied, molecules of all sizes migrate similar distances because the DNA mobility is independent of the molecular weight (Fig. 2.a.ii and 2.b.ii). As the field direction is rotated by 120° and its intensity weakened, molecules backtrack through channels formed by the post array (Fig. 2.a.iii and 2.b.iii). The net motion of a molecule during a cycle is size-dependent. Mathematically, the net motion N in a cycle is the vector sum of the two distances,

$$N = (d_1 - L^*)\,\mathbf{e}_1 + (d_2 - L^*)\,\mathbf{e}_2 \qquad (1)$$

where L^* is the length of the molecule in the array, $\mathbf{e}_1$ and $\mathbf{e}_2$ are the unit vectors at the electric force directions, and d_1 and d_2 are the migration distances of point-like molecules during the first and second pulse respectively. When $L^* < d_2 < d_1$, N points to different directions according to the molecular length L^*. While small molecules ($L^* << d_2$) migrate at the average electric force direction ($d_1\ \mathbf{e}_1 + d_2\ \mathbf{e}_2$), large molecules move more towards the strong pulse direction ($\mathbf{e}_1$).

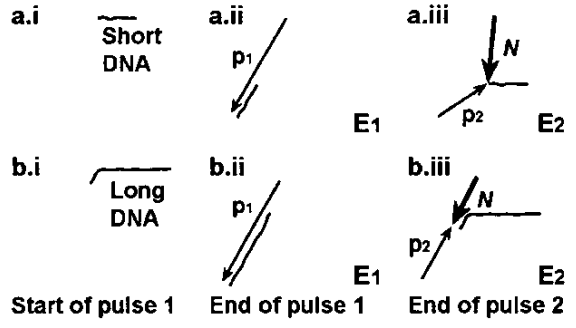

Fig. 2. Schematic contrasting motion of short (a.i, a.ii and a.iii) and long (b.i, b.ii and b.iii) DNA molecules over one cycle of electric pulses in the prism device, showing size-dependent direction of motion. (a.i, b.i) Starting position of molecule at beginning of pulse. (a.ii, b.ii) Position at end of first pulse with electric field E_1 and displacement during first pulse p_1 shown. (a.iii, b.iii) Position at end of second pulse with weaker electric field E_2 during second pulse. Molecules backtrack. Displacement during pulse is p_2 and net displacement in a cycle is N. Note p_2 differs for long and short molecules because long molecules do not backtrack off posts. Net motion over full cycle N therefore depends on size.

When $L^* > d_2$ (Fig. 2.b), molecules cannot reorient entirely, and move along $\mathbf{e}_1$.

If DNA molecules inside the sieving array are stretched to a constant fraction of their full lengths ($L^* = c\ L_0$, where $c \le 1$ and L_0 is the full length), the migration angle with respect to the average force direction would roughly be a linear function of molecular weight (Fig. 3.a). However, experiments showed "biphasic" separation characteristics under low fields (Fig. 3.a and 3.b) [1]: molecules smaller than a certain molecular weight threshold migrated towards one direction, and those larger than the threshold towards another direction. This "constant fraction elongation model" fits experimental data poorly.

Damped spring model

The discrepancy between the model and the experiment arises from the fact that molecules of different molecular weights are stretched to different extents. Although DNA molecules are linear strands whose full contour lengths L_0 scale with their molecular weights, they are randomly coiled by thermal agitation under no external forces. In fact, a DNA strand acts empirically like a spring—— the end-to-end distances L^* are linearly proportional to the force applied until L^* approaches the molecule's full length L_0 (Fig. 4.a) [11]. We gain understanding of the prism device by building a model that incorporates this restoring force, the viscous drag, and the electric force. When a molecule is moving in the post array under electric fields, it is going through cycles of elongation and relaxation (Fig. 4.b). The stretching phase occurs at the beginning of each pulse, when the molecule hooks on posts (Fig. 4.b.i). As soon as the molecule slides of the posts, it starts to relax (Fig. 4.b.ii).

We assume that DNA molecules obey Hooke's law. This assumption holds if the fields are low and the molecules are stretched to under ~50% of their full lengths (Fig. 4.a inset, $L^* < \frac{1}{2}\ L_0$) [11]. A segment of a DNA strand can be mathematically specified as its contour distance z from one

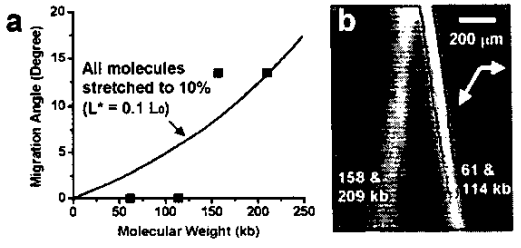

Fig. 3. "Biphasic" separation under low fields, poorly fitted by "constant fraction elongation model." (a) Migration angle dependence on molecular weight calculated by Equation (1) assuming all molecules stretched to 10 % of their full lengths (solid curve). The squares are the data points measured from (b). (b) Fluorescent micrograph of four sizes of DNA molecules separated into two bands in the array by alternating 250 msec pulses of 32 V/cm and 20 V/cm [1]. Arrows show directions of electric forces.

end of the molecule. The entire molecule's conformation state is described by the positions x of every segment z. The equation of motion for a molecule in one dimension is therefore

$$\rho E - \frac{\rho}{\mu}\frac{\partial x}{\partial t}\frac{\partial x}{\partial z} + k\frac{\partial^2 x}{\partial z^2} = 0 \qquad (2)$$

where ρ is the linear charge density of DNA, μ is the mobility in free solution, and k is the spring constant of a unit-length strand. The first term of the equation represents the electric force, the second term the viscous drag, and the third term the restoring force. The inertial term is ignored because of the extreme small Reynolds' number. Although the drag term is quadratic in x, rendering the equation unsolvable analytically, qualitative insights can be gained by assuming that $\frac{\partial x}{\partial z}$ is roughly a constant at all times. The linearized equation can be solved analytically by the technique of separation of variables.

Equation (2) shows that the degree to which molecules are stretched depends on the molecular weight. For example, a tethered molecule will be stretched to $\frac{\rho E}{2k} L_0^2$, where L_0 is the full contour length of the molecule. The end-to-end distance L* scales with the square of molecular weight L_0 because the electric force is proportional to the molecular weight, while the spring constant are inversely proportional to it. Moreover, by solving Equation (2), we find that relaxation time constants (for different modes) of a molecule scale with L_0^2. Small molecules relax much faster than larger ones. This result is similar to experimental measurements found in the literature [12], which reports an exponent of 1.6. From this simple consideration, we see that the end-to-end distance L* at the change of electric fields is

$$L^* = \alpha L_0^2 e^{-\frac{\beta}{L_0^2}t} \qquad (3)$$

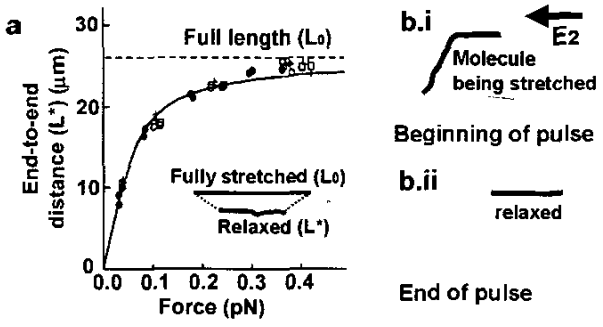

Fig. 4. (a) End-to-end distance of a 97 kb DNA molecule vs. stretching force [11]. (b.i) A DNA strand in the array is being stretched at the beginning of each pulse, because each segment aligned with the field experiences stronger force than those at 60° with respect to the field. (b.ii) The DNA molecules starts to coil once it reorients completely.

where t is the pulse duration, and α and β are fitting parameters.

Equation (3) qualitatively explains the "biphasic" separation character under low fields (Fig. 5). According to the model, the biphasic behavior arises from the poor stretching and the rapid relaxation of small molecules. Further, molecules shorter than the center-to-center post spacing will not deviate from the average force direction ($d_1 e_1 + d_2 e_2$), whereas those longer than the migration distance during the weak pulse ($L^* > d_2$) will co-migrate towards the same direction (Fig. 5.a, b). The maximum migration angle in the model is the strong field direction (e_1). The measured angle is always smaller than the model predicted. Although the mechanism for this behavior is poorly understood, we speculate that it is due to the herniation of large molecules.

High field behavior

To achieve approximately linear separation, molecules should be highly stretched. Therefore high fields should be used to more fully stretched all molecules. Fig. 6 shows improved linearized performance achieved by increasing fields from 32 V/cm (Fig. 3) to 240 V/cm (Fig. 6) [1].

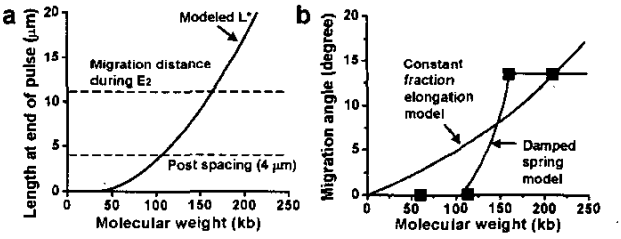

Fig. 5. (a) End-to-end distance of molecule calculated from Equation (3), with α = 0.004 μm^{-1}, and βt = 320 μm^2. (b) Migration angle (solid curve) calculated from the length modeled in (a) fits data (solid squares) for 250 msec pulses of 32 V/cm and 20 V/cm [1].

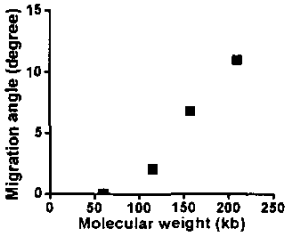

Fig. 6. Improved device characteristics under high fields and shorter pulses. The high-field pulses used are 40 msec of 240 V/cm and 150 V/cm [1].

Conclusions

A model has been developed to qualitatively understand the behavior of the DNA prism device that continuously fractionate DNA in an array of microposts. The critical parameters is the degree of stretching of DNA, which depends on the electric field strengths and pulsing durations. While low fields result in biphasic separation behavior, high fields create more linear separation characteristics due to increased stretching. Future work includes quantitative understanding of the frequency dependence of stretching, and the effect of post size on DNA separation.

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