Microfabricated Sieve for the Continuous Sorting of Macromolecules

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In a two-dimensional periodic but asymmetric environment, a Brownian particle that is driven in one direction by a potential gradient will also drift in the orthogonal direction at a rate that depends on its diffusion coefficient. On this basis, we propose a new method for separating biological macromolecules according to size. A fine stream of molecules is electrophoresed through a microfabricated sieve, etched from a silicon chip by lithography. The sieve consists of a periodic array of oblong obstacles, which deflect the molecules so that each species follows a different trajectory, oblique to the flow. Advantages promised by the technique include improved efficiency, continuous sorting and ready automation. [S0031-9007(98)05341-1]

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Efficient methods to separate and analyze the different components of a mixture of biological macromolecules are of paramount importance, both for research and in biomedical applications. To meet this need, a concerted effort is being made to develop miniaturized bioanalytical devices [1], using the technology of microfabrication. These devices aim to supersede the present methods, which are time consuming and difficult to automate. The separation of DNA molecules, for example, is currently accomplished by gel electrophoresis [2]; molecules of different size migrate at different speeds through the gel, and can be distinguished from one another after a certain time has elapsed. Using a gel to sieve the DNA (a necessity, since the electrophoretic mobility μ_0 in free solution is independent of size) complicates the procedure: A new matrix must be made each time, and recovery of the DNA is awkward, since the gel must either be sliced or blotted [2].

In this Letter, we propose a fresh approach to separating biological macromolecules, based on the technology of microfabricated arrays introduced by Volkmuth and Austin [3]. Lithography is used to etch a pattern of obstacles on a silicon chip, which is then sealed to make a quasi-two-dimensional "sieve" through which a solution of molecules can be electrophoresed. One obvious benefit compared to gels is the regularity of the sieve. The major advantage, however, is that the pattern of obstacles can be chosen at will. This allows an *alternative method* of electrophoretic separation. By disposing the obstacles so that the molecules are deflected away from the field direction, a separation can be effected in the direction *transverse* to the field. Quite generally, this can be done by choosing a periodic array of obstacles, each of which is *asymmetric* with respect to reflection in the field direction. The combination of the spatial asymmetry and the broken time-reversal symmetry (imposed by the flow) causes the Brownian motion of the molecules to be rectified. Since the effect depends on the thermal motion, molecules with different diffusion coefficients are deflected by different amounts, and, consequently, a mixture of molecules is sorted according to size.

On one hand, this result reflects the fact that the mobility tensor in an anisotropic medium is nondiagonal. Alternatively, the force-free transverse flux of driven Brownian particles in a two-dimensional, periodic, asymmetric environment can be regarded as an especially simple instance of a "thermal ratchet" [4,5]. Ratchets that work by constantly switching on and off a one-dimensional, asymmetric potential [5] have recently been demonstrated to be effective as devices for separating colloidal particles [6,7]. "Steric ratchets," which employ a fluctuating driving force in an asymmetric, confining channel, have also been proposed as a way of separating particles according to size [8]. In these devices, as in traditional electrophoresis methods, molecules of different types migrate in a single direction at varying speeds. The device that we propose, in which different species move in *different* directions, has two advantages: Sorted molecules are carried to specific locations in the device, where they can be collected for analysis or subsequent manipulation; and the separation need not be carefully timed, since the device can be operated in continuous mode.

In the following, we discuss a particular realization of this idea, designed with both performance and ease of manufacture in mind. The silicon chip is etched to make a periodic pattern of rectangular obstacles, shown in Fig. 1. The scale is set by the grid size a, which can be chosen as small as 1 μm using standard optical lithography, or smaller still using electron-beam lithography. When sealed and filled with buffer solution, the chip forms a miniature chamber into which a mixture of molecules can be fed. The array of oblong barriers constitutes an obstacle course which the molecules must negotiate as they move through the chamber. An electric field is applied at 45° to the principal axes of the array, as indicated. In this direction, there is a clear "line of sight" through narrow gaps between adjacent obstacles. Molecules

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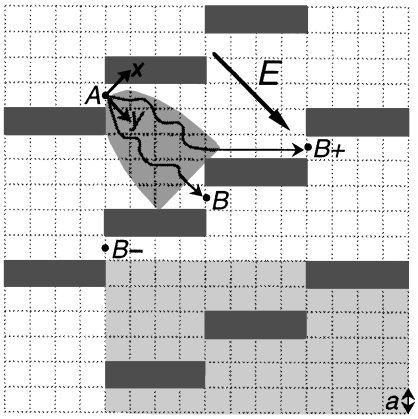

FIG. 1. Design of the sieve. The unit cell of the periodic array, which contains three rectangular obstacles, is indicated by the shaded area at the lower right. Molecules are propelled by an electrophoretic force, which acts along the diagonal, and are channeled through the narrow gaps between obstacles. A molecule which passes through gap A subsequently executes a biased random walk and visits, with high probability, points located in the parabolic shaded region. It is most likely that the molecule arrives at B, but some paths pass around an obstacle and lead the molecule to $B+$. The probability of reaching $B+$ is higher for smaller, more swiftly diffusing species.

migrating electrophoretically naturally tend to follow this pathway along the diagonal, passing through successive gaps (e.g., from A to B in Fig. 1). However, as they drift, they also diffuse. The probability distribution of a molecule, initially localized in gap A, spreads in the direction transverse to the field as the molecule advances. Consequently, there is a probability that the molecule passes around the upper left-hand corner of the obstacle located immediately above B, in which case the field will carry it to gap $B+$. There is also a possibility—more remote, since it requires diffusion through a greater distance in a shorter time—of the molecule diffusing around the top left corner of the obstacle just below B, in which case it will proceed to gap $B-$. Owing to the different probabilities of passage through the three gaps B, $B+$, and $B-$, the molecule's line of motion will, on average, be deflected upwards, away from the field direction. Most importantly, since the deflection probabilities depend on the diffusion coefficient, the mean trajectory depends on the molecular size. Smaller, more rapidly diffusing species are deflected through a larger angle.

Figure 2 shows how a device could be constructed to continuously sort a mixture of molecules into its individual components. A square array of obstacles is surrounded by point electrodes configured to produce a uniform field acting along its diagonal [9]. The mixture of species is injected into the top corner of the chamber via a narrow conduit. Molecules with different diffusion coefficients follow distinct straight line trajectories across the array and the sorted components are collected at different locations along the opposite side. The entire device, comprising sieve, electrodes, supply conduit, and collection channels could readily be integrated into a single silicon chip.

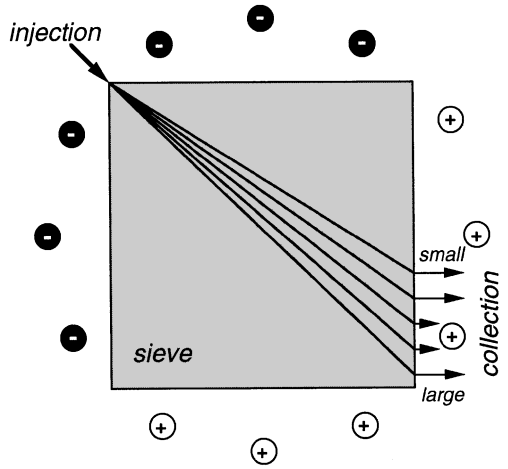

FIG. 2. Operation of the sorting device. A mixture of molecules is injected into the microchamber at the top corner. The fractionated components of the mixture are collected at intervals along the opposite edge.

An analysis of the molecular sorting provided by the sieve of Fig. 1 requires the determination of the average trajectory of a molecule with diffusion coefficient D and electrophoretic drift velocity $v = \mu_0 E$. We proceed by calculating the respective probabilities p_+ and p_- of the molecule traveling from A to $B+$ and A to $B-$. Consider the coordinate system (x, y) with origin at gap A, shown in Fig. 1. A molecule initially at the origin diffuses laterally as it drifts in the field direction, so that, after time t, at which it has advanced a distance $y = vt$ from the gap, the probability distribution of its transverse coordinate x is

$$P(x, y) = \left(\frac{v}{4\pi D y}\right)^{1/2} \exp\left(-\frac{x^2 v}{4Dy}\right).$$

Here, we have neglected the effect of reflections from the surrounding barriers, which is a valid approximation for small D. Let $(x_{\rm obs}, y_{\rm obs})$ be the coordinate of the top left corner of an obstacle. Then, the probability that the molecule passes around the obstacle is

$$p = \int_{|x_{\rm obs}|}^{\infty} P(x|y_{\rm obs})\, dx\,.$$

Substituting for the coordinates of the relevant obstacles, we obtain

$$p_\pm = \frac{1}{2}\,\mathrm{erfc}\left(c_\pm \sqrt{\frac{va}{D}}\right), \qquad (1)$$

where $c_+ = 0.35$ and $c_- = 1.26$. Note that the deflection probabilities depend only on the dimensionless parameter D/va.

A more accurate calculation should take account of the restrictions to movement imposed by the nearby obstacles. A numerical solution, obtained by Monte Carlo simulation, is shown in Fig. 3. Here, as in the above analysis, we have neglected diffusion in the y direction and assumed that the molecules drift along the field at an even velocity. Longitudinal diffusion admits the possibility of a molecule moving *backwards* out of a gap and around an obstacle if

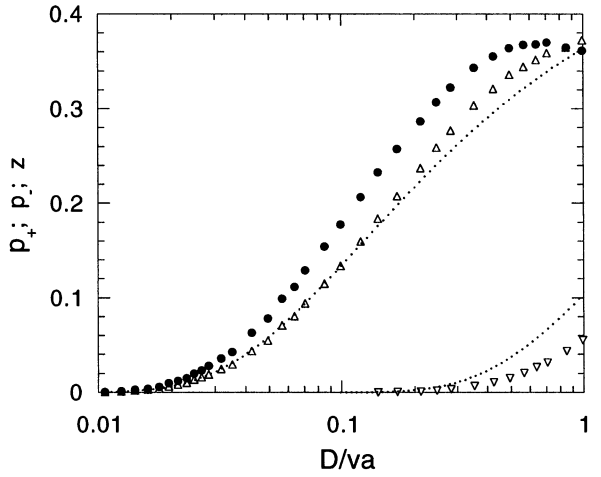

FIG. 3. Deflection probabilities p_+ (triangles) and p_- (inverse triangles), and predicted exit position z (circles), as functions of the dimensionless variable D/va. The dotted lines are Eq. (1).

the diffusion coefficient is high enough. We have verified that the probability of such an event is insignificant (less than p_-) if $D/va < 1$, in which case it suffices to consider forward motion alone, the stochastic passage through successive gaps can be modeled as a Markov process, and the trajectory can be calculated straightforwardly from the deflection probabilities p_+ and p_-. Let the total number of gaps along the diagonal be N. Then, on traversing the obstacle course, a molecule will shift away from the diagonal, on average, by $n = N(p_+ - p_-)$ gaps. Given this, it is a simple geometrical calculation to determine the angle of the trajectory and the exit location. The fractional distance z along the side of the chamber (measured from the bottom corner) at which the molecule leaves the sieve is $z = 3n/2(N + n)$. The exit location is plotted as a function of D/va in Fig. 3. Notice that z depends strongly on D over a wide range of values: The device can be used to sort a mixture of molecules, which have diffusion coefficients that differ by more than an order of magnitude. An important feature is that, for a given obstacle size a, the range of separation can be shifted by varying the drift velocity v.

The utility of the device depends upon its capacity to resolve two molecular species with closely similar diffusion coefficients. Thus, it is important to consider the variations in trajectory due to the stochastic motion. The variance of n is $\sigma^2(n) = N[p_+(1 - p_+) + p_-(1 - p_-) + p_+p_-]$. Identical molecules in the injected sample will spread apart as they travel through the sieve and emerge in a narrow band. Two different types of molecules will be sorted only if the separation of their respective bands exceeds the bandwidth. Using the Rayleigh criterion, the fractional amount by which the diffusion coefficient of two species must differ in order for them to be resolved can be expressed as

$$\mathrm{Res}(D) = \sigma(n) \Big/ \frac{dn}{d(\ln D)} \propto N^{-1/2}.$$

The resolution improves with the square root of the number of obstacles, and varies with diffusion coefficient as shown in Fig. 4. Good resolution is obtained in the range

$$0.02 < D/va < 0.3. \qquad (2)$$

Note that the resolution is better than 3% over most of this range if $N = 10^4$, a typical number for a device measuring a few centimeters. An unusual and useful feature is that, since the variance declines as the deflection probability decreases, the resolution is actually *higher* for larger molecules (smaller D).

Let us now evaluate how well this device could perform the task of separating DNA molecules with lengths varying from 100 to 20 000 base pairs—the range for which agarose gel electrophoresis is routinely used [2]. In the buffer solution, these molecules adopt random coil conformations, with size $R = (M/M_0)^{1/2}b$, where M is the number of base pairs, $b = 100$ nm is the Kuhn length [10], and $M_0 = 300$ is the number of base pairs per Kuhn length. According to the Zimm model [11,12], their drag coefficient is proportional to the coil size, so that their diffusion coefficient is $D = k_BT/5.1\eta R$, where $\eta = 10^{-3}$ kg s^{-2} is the viscosity of the buffer and k_BT is the thermal energy. So, DNA molecules with $M = 100$–$20\,000$ have sizes $R = 0.06$–1.0 μm and diffusion coefficients $D = 14$–1.0 μm^2 s^{-1}. A device manufactured with maximal optical-lithographic resolution, $a = 1$ μm, is an appropriate choice, since the gaps between obstacles would be wide enough to permit all of the molecules in the mixture to pass through without significant deformation of their conformation. Condition (2) indicates that this sieve would sort DNA molecules in the above range if the electrophoretic flow velocity were set to the value $v = 50$ μm s^{-1} (which, since $\mu_0 = 2.5 \times 10^{-8}$ m^2 s^{-1} V^{-1} [13], corresponds to a field strength $E = 20$ V/cm) [14]. A chamber measuring 4×4 cm would have $N = 10^4$ obstacles along the

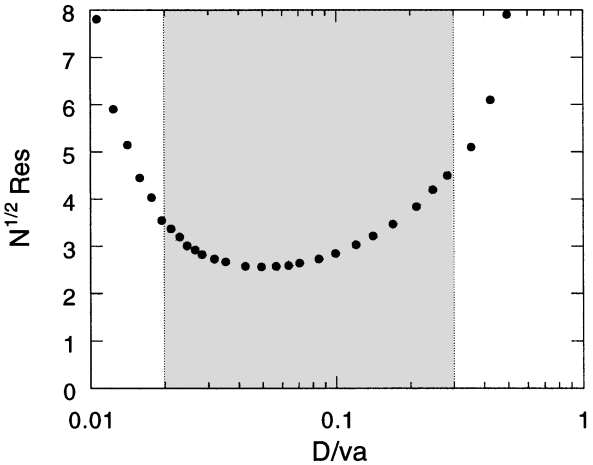

FIG. 4. Fractional resolution (Res) as a function of the dimensionless variable D/va and the number of obstacles N. The range of effective sorting is indicated by the shaded area.

diagonal, providing a resolution of ~3% in the diffusion coefficient D, which corresponds to ~6% in molecular length M. The passage of a molecule through the chamber, from injection to collection, would take about 20 min. These figures compare favorably with those for agarose gel electrophoresis [2], roughly matching the resolution and largely exceeding the rapidity of separation.

An obstacle course of the same dimensions could equally well be used to separate protein molecules. Since these diffuse quickly, a fast flow would be required, and since the electrophoretic mobility of proteins is variable, they would be sorted according to the value of μ_0/D [15]. Using electron-beam lithography, a reduction in grid size to $a = 0.1$ μm would be feasible. By permitting the use of higher velocities and increasing the number of obstacles in the array, this would simultaneously reduce the transit time and improve resolution.

A device of this design is less well suited for separating very large DNA fragments. Long molecules can easily be deformed when they collide with an obstacle, stretching to almost their full contour length and, subsequently, relaxing only slowly back to coiled conformations [16]. Such events would upset the stochastic process by which separation occurs and seriously limit the effectiveness of the technique. Sorting of globular, colloidal particles with sizes up to 10 μm would be feasible, however, by suitably enlarging the scale of the obstacles. Thus, microfabricated sieves could potentially be used for cell sorting, although the performance would be less effective than for small molecules; the slow diffusion of large particles demands the use of a low flow velocity, which would decrease the rate at which sorting could be achieved.

The principal limitation of this type of sieve is that the overall throughput of molecules is low, since the mixture must be injected in a very localized region and the solution should be dilute enough that steric intermolecular interactions are rare. To maximize the cross-sectional area of the injection conduit, the chip should be etched as deeply as possible. Current technology permits the obstacles to be made as tall as 10 μm when $a = 1$ μm. A deep etch has the additional advantage of minimizing hydrodynamic interactions between the molecules and the surfaces of the chamber, which slow down diffusion and thereby diminish the drift speeds that can be used [17]. The problem with throughput is alleviated, somewhat, by the fact that the device can be operated continuously. More molecules can be sorted simply by running the device for a longer time. A number of chips could also be stacked and run in parallel. Even so, the typical amount of molecules collected would be about one femtomole. Consequently, the device is probably more appropriate as an analytical tool than as a preparative one. It might, though, prove useful for recovering DNA from scarce samples for subsequent amplification by the polymerase chain reaction, a task for which agarose gel electrophoresis is wholly unsuited.

Alternative obstacle geometries could be considered, but the rectilinear design proposed in this Letter is appealing since it facilitates accurate manufacture. Prototype devices have been constructed and are currently being tested in our laboratory. The sorting that such sieves offer, in which molecules are transported to specific locations within the chamber, makes them especially suitable for integrating molecular separation with subsequent analytical steps. Hence, we believe that they will be useful in the development of "lab-on-a-chip" devices, which, by automating many laborious experimental procedures, promise new levels of efficiency and convenience to researchers in the biological sciences.

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