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ac separation of particles by biased Brownian motion in a two-dimensional sieve

Imre Derényi and R. Dean Astumian
Department of Surgery, MC 6035, University of Chicago, 5841 South Maryland Avenue, Chicago, Illinois 60637

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We propose a technique for separation of particles based on a nonlinear coupling between an applied force and the induced transverse drift in a two-dimensional system with broken reflection symmetry transverse to the force. Further, if the system has reflection symmetry parallel to the force, the drift velocity is independent of the sign of the force. Thus, an ac force causes a net drift only in the transverse direction, allowing for efficient separation even in a very small system. In an appropriately constructed device, particles with diffusion coefficients below and above a threshold move in opposite directions, allowing for continuous separation of significant amounts of molecules. [S1063-651X(98)14612-3]

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We propose a technique for separation of macromolecules in microfabricated sieves. The technique is based on the theory of ''Brownian ratchets'' [1–5], where nonequilibrium effects in systems possessing both broken time-reversal symmetry (usually imposed by a dissipative environment) and broken spatial reflection symmetry cause the Brownian motion of particles to be rectified. The possibility of separation by this phenomenon has recently been demonstrated experimentally with microelectrode devices [6] and optical traps [7], and an approach for continuous separation of particles has been discussed [8].

A recently introduced microlithographic technique [9] makes it possible to use ''Brownian ratchets'' for particle separation with quasi-two-dimensional sieves constructed such that the mobility tensor is nondiagonal [10,11]. The idea is to etch a two-dimensional array of micron sized obstacles on a silicon chip, which is then sealed and filled up with a medium containing the particles. If the arrangement of obstacles is asymmetric in both the x and y direction, a constant electrophoretic force in the $x(y)$ direction can cause a drift in the $y(x)$ direction that is sensitive to the particles' electrophoretic mobility and diffusion coefficient. Particles can be separated laterally (transverse to the force) with a good resolution compared to standard gel electrophoresis by injecting them in a localized entrance region at one end of the sieve and extracting them at the other end at locations that depend on their electrophoretic mobility and diffusion coefficient. For small applied force, the coupling between the force and the induced transverse drift is linear, with coupling coefficients that obey Onsager reciprocity relations.

The main advantages of this method of electrophoretic separation are that the shape of the obstacles can be chosen at will, the sieve can be reused after separation, and the particles can be sorted continuously, leading to separation that can be easily automated. However, there are two major concerns regarding this technique. The first is the throughput. Since the particles should start from the same point, rather than a band, they can be injected only at a very low rate. The second concern is the system size. To achieve a good resolution thousands of rows of obstacles must be built, and increasing the resolution, which is proportional to the square root of the system size, is very difficult.

Here we propose a new method that eliminates both concerns, and makes the separation very effective and competitive compared to other techniques. We design the system to have reflection symmetry in the y direction as shown in Fig. 1. In a system like this an applied force in the y direction induces a drift in the x direction that is independent of the sign of the force. Thus, applying a driving force in the y direction with constant magnitude but periodically alternating sign produces a constant drift in the x direction, but with a zero net velocity in the y direction. A force in the x direction, however, produces absolutely no drift in the y direction. This allows us to conclude that the linear coupling coefficients are identically zero. Unlike the approach of Duke and Austin [10] and Ertas [11], the mobility tensor for our system is strictly diagonal, and the coupling between the applied force and induced drift is clearly nonlinear.

For proper operation the frequency of the ac force should be small enough to let the particles pass several rows during a half-period, but large enough so that they advance only a small distance compared to the system size in the y direction. With this technique the system size parallel to the driving force is no longer a concern, and can be very small. The resolution increases continuously with (the square root of) time.

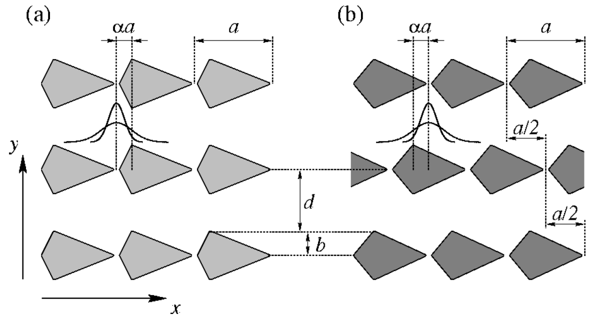

FIG. 1. Schematic illustrations of (a) system 1, and (b) system 2. Particles are driven downwards by an external force and drift to the right in both systems while going through the holes of the rows of obstacles. Since the probability density (indicated by the solid curves) of the smaller particles gets broader than that of the larger ones when they reach the next row, the smaller particles drift with larger velocity in system 1 but with smaller velocity in system 2.

There are two possible configurations for arranging identical obstacles in a two-dimensional lattice (supposing that each unit cell contains one obstacle) compatible with the desired symmetry. In the first (system 1), shown in Fig. 1(a), neighboring rows of obstacles are shifted only in the y direction relative to each other. In the second [system 2 in Fig. 1(b)] neighboring rows are shifted also in the x direction by half a period. The deltoid shape of the obstacles also reflects the symmetry of the system. All parameters of the array are defined in Fig. 1, except the size of the holes between neighboring obstacles in the rows, which should be smaller than αa, but larger than the particles. (Note that with holes that are smaller than the effective radius of polymers such as DNA one can design a ‘‘steric ratchet’’ [12] that can also be used for separation.)

To analyze the separation we determine the average trajectory of a particle with diffusion coefficient D and electrophoretic mobility μ. Suppose that the electric field has magnitude E and points downward in Fig. 1. Then, after a particle passes a hole (see the left hole of the top row in Figs. 1(a) and 1(b) it migrates down with an average velocity $v=\mu E$, and concomittantly undergoes lateral diffusion. Neglecting reflection from the surrounding obstacles that the particle leaves and diffusion in the y direction (which are good approximations for not too large D) the probability distribution of the particle in the x direction after advancing a distance $y=vt$ is

$$\varrho(x|y)=\sqrt{\frac{\mu E}{4\pi D y}}\exp\left(-\frac{\mu E x^2}{4Dy}\right). \tag{1}$$

When the particle reaches the line of the top corners of the obstacles in the next row, it is trapped by the two surrounding obstacles and continues through the hole between them. If D is not too large (which should hold for proper separation), the particle is captured either by the trap below its starting position (with probability P^{left}) or by the next trap on the right (with probability P^{right}), but the probability of being trapped elsewhere is negligibly small. Thus, we can write that $P^{\mathrm{left}}+P^{\mathrm{right}}=1$. All particles drift to the right, but because the probability distribution of the larger particles (with smaller D) spreads out more slowly (as indicated by the Gaussian curves in Fig. 1) their drift velocity is smaller in system 1, but larger in system 2.

Introducing the parameters

$$v_{\mathrm{max}}=\frac{a}{2}\,\frac{\mu E}{d+b} \quad \text{and} \quad A=\frac{\alpha a}{2\sqrt{\dfrac{dD}{\mu E}}} \tag{2}$$

the probability P^{right} in system 1 and the probability P^{left} in system 2 can be expressed as

$$P_1^{\mathrm{right}}=P_2^{\mathrm{left}}=\tfrac{1}{2}\operatorname{erfc}(A), \tag{3}$$

and the drift velocity in systems 1 and 2 are

$$v_1=a\frac{\mu E}{d+b}P_1^{\mathrm{right}}=\operatorname{erfc}(A)v_{\mathrm{max}}, \tag{4}$$

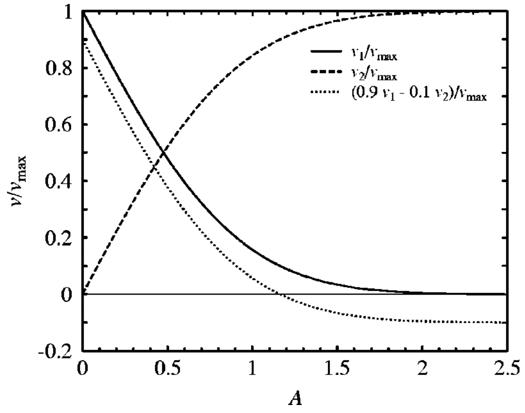

FIG. 2. The lateral velocity as a function of the dimensionless parameter A in system 1 (solid line), in system 2 (dashed line), and in a system composed of 90% system 1 and 10% oppositely directed system 2 (dotted line).

$$v_2=a\frac{\mu E}{d+b}\left(\tfrac{1}{2}-P_2^{\mathrm{left}}\right)=[1-\operatorname{erfc}(A)]v_{\mathrm{max}}. \tag{5}$$

These velocities are plotted in Fig. 2 as a function of the dimensionless parameter A.

There are two basic modes of separation. In the first, particles are initially injected into the sieve at a band along the left side of the chip, from where they will migrate to the right due to the alternating driving force. Since the particles with different diffusion coefficients migrate with different velocities, they form different bands that move apart with time. However, because of diffusion, the bands become broader, but the width increases with only the square root of time, so the resolution improves with time. A good measure of the efficiency of the separation is the resolution parameter R, which we define through the following equation:

$$|x(D,N)-x(D(1+R),N)|=4\,\sigma(D,N), \tag{6}$$

where $x(D,N)$ denotes the average distance (in the x direction) that a particle with diffusion coefficient D advances after going through $N=\mu Et/(d+b)$ rows, and $\sigma(D,N)$ is the standard deviation. The meaning of this equation is that two kinds of particles with a relative diffusion coefficient difference larger than R are considered to be separated from each other, because the distance between the middle of their bands is larger than twice the width (or 4 times the standard deviation) of the bands. Thus smaller R means better resolution. For small R (or large N) it can be expressed as

$$R=\frac{8}{\sqrt{N}}\,\frac{\sqrt{P_i^{\kappa}(1-P_i^{\kappa})}}{\left|\dfrac{\partial P_i^{\kappa}}{\partial A}\right|A}, \tag{7}$$

where $i=1,2$ and the index κ denotes either left or right. Thus, the resolution parameter

$$R=4\sqrt{\frac{\pi}{N}}\frac{\exp(A^2)}{A}\sqrt{\operatorname{erfc}(A)\operatorname{erfc}(-A)} \tag{8}$$

is identical for both systems 1 and 2. Since R is proportional to $N^{-1/2}$ it decreases with the square root of time. A plot of

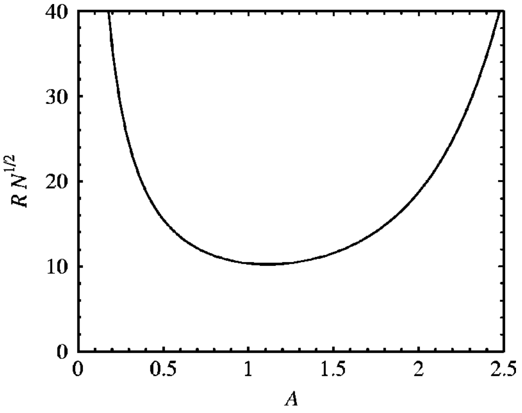

FIG. 3. The resolution parameter R (multiplied by $\sqrt{N}$) as a function of A for both systems, and thus for all mixtures of these two systems.

$RN^{1/2}$ is shown in Fig. 3 as a function of the dimensionless parameter A. Since this function has a broad minimum at $A \approx 1.1$, the parameters of the system should be chosen in such a way that the values for A for the particles fall around this value. Note that in the determination of the resolution we presumed identical electrophoretic mobilities (which holds, e.g., for DNA molecules), however, different electrophoretic mobilities usually make the resolution even better.

The particles can be extracted from the sieve in two ways. They can be collected at the right side of the chip, because different particles reach it at different times; or, in system 1, by simply applying a big constant electric field in the y direction, different particles can be collected at different locations along the bottom (or top) side of the chip.

To reduce the system size in the x direction we can do the following trick. Let us embed some rows from system 2 into system 1 in the opposite direction, as illustrated in Fig. 4. Since the relationship between the velocities in systems 1 and 2 is $v_2(D) = v_{\max} - v_1(D)$, in a combined sieve, where the ratio of the number of rows from system 2 is γ (and that from system 1 is $1-\gamma$) the drift velocity is $v(D) = (1-\gamma)v_1(D) - \gamma v_2(D) = v_1(D) - \gamma v_{\max}$. This allows us to shift the $v_1(D)$ curve downwards (as shown in Fig. 2 for $\gamma = 0.1$) without changing the resolution, but setting a threshold diffusion coefficient D_{th} [defined by $v(D_{\mathrm{th}}) = 0$] below which all particles drift to the left and above which particles drift to the right. In this way, by decreasing the drift velocity for all the particles simultaneously, one can make a longer run and achieve better resolution in a system with the same size. Note that for a fixed system one can also tune the threshold by changing the magnitude of the electric field or by applying a constant field in the x direction.

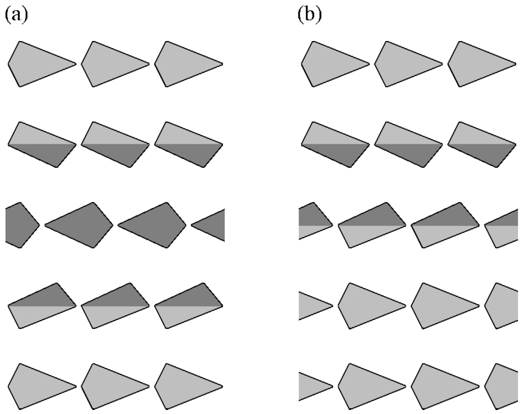

FIG. 4. Illustration of how (oppositely directed) rows of obstacles from system 2 can be embedded in system 1.

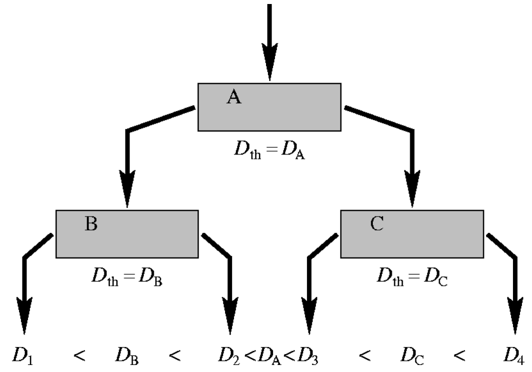

FIG. 5. Illustration of how large amounts of particles can be sorted continuously by devices (A, B, and C) with different thresholds ($D_{\mathrm{B}} < D_{\mathrm{A}} < D_{\mathrm{C}}$) arranged in a tree topology.

To describe the second mode of separation we first consider the case when only two different kinds of particles should be separated. By using a chip that contains a combined sieve with a D_{th} that falls between the diffusion coefficients of the two kinds of particles, the system can be continuously fed with a mixture of particles along a band near the middle of the system and one kind continuously extracted along the left side of the chip and the other kind extracted along the right side. For optimal separation the parameters of the system should be chosen such that the magnitude of the lateral velocity of each kind of particles, is the same (but of course their directions are opposite), and that the resolution parameter for D_{th} is minimal. This latter automatically holds for $\gamma \approx 0.1$ (compare Figs. 2 and 3). Since the two kinds of particles drift in opposite directions the system size in the x direction can also be very small.

For the separation of more than two kinds of particles several sieves with different thresholds can be used simultaneously, arranged, e.g., in a tree topology as depicted in Fig. 5. The first sieve (A) would separate one-half of the particles from the other half, then the next two sieves (B and C) would continue the separation on these two outputs, and so on.

A major application of this new technique may be the separation of biological macromolecules, such as DNA or proteins. DNA molecules have an electrophoretic mobility $\mu \approx 2.2$ (μm/s)/(V/cm), which is independent of size [13]. This makes their separation in free solution impossible. According to the Zimm model [14,15] the diffusion coefficient of large DNA molecules is $D = D_0/\sqrt{n}$, where $D_0 \approx 8$ $\mu\mathrm{m}^2$/s and n is the number of Kuhn segments. The length of a Kuhn segment is ~ 0.1 μm and contains about 300 base pairs. Thus optimal resolution for DNA fragments having 100–20 000 base pairs in a sieve with parameters $a = d = 10$ μm and $\alpha = 0.2$ can be achieved by applying an $E \approx 10$ (V/cm) electric field. A stronger electric field is required to separate smaller DNA fragments or protein molecules. Since the height of the obstacles cannot be much larger than 10 μm [9], the heat conductivity of the sieve is very good and the field can be increased up to a few kV/cm, as in capillary electrophoresis [16,17]. Moreover, a larger field results in larger drift velocity, allowing the molecules to go through more rows, which makes the resolution better.

Though the resolution of our technique is estimated to be an order of magnitude lower than that with capillary electrophoresis [16,17], the throughput with the second mode of separation (because the sieves can be fed continuously along a band) is much larger, which makes this technique very useful for preparative purposes. E.g., to separate two different DNA fragments with about 1000 base pairs and with 10% difference in their diffusion coefficient in a 10 μm high and 1 cm long (in the y direction) sieve, the system should be fed with the solution at a rate ~ 0.1 μl/h. The separation of smaller molecules can be achieved with an even larger throughput. In addition, the separation can be easily automated because it can run continuously.

The magnitude of the transverse drift velocity induced by an external electric field in our system is essentially the same as that in the systems of Duke and Austin [10] and Ertas [11]. We thus conclude that although the mobility tensor in their systems is indeed nondiagonal, the coupling in the experimentally relevant range of driving forces is strongly nonlinear, and the mobility tensor is totally irrelevant.

Because of the reflection symmetry in the y direction a driving force in the x direction cannot generate drift in the y direction in our system, which seems to contradict the Onsager reciprocity law. The resolution of this paradox is that the first nonvanishing term of the lateral velocity (when the force is applied in the y direction) is at least quadratic, and therefore the Onsager coefficients are zero. Thus the symmetry of the systems (in the y direction) makes the response to a driving force asymmetric. To the best of our knowledge, our model is the first to explore the possibility of clearly non-Onsager coupling in a physical system.

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