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Review

The promise of nanotechnology for separation devices – from a top-down approach to nature-inspired separation devices

An overview is given of the possible applications of nanotechnology to optimise existing separation methods and to enable new methods. Attention is paid to nanotechnological contributions in the fields of HPLC, CEC, sieves, Brownian ratchets and preconcentration units. A brief description is also given of some selection/separation mechanisms that occur in biological (cell) structures and possible future applications of these mechanisms in separation devices are investigated. Especially the active transport in discrete events occurring in cells is mentioned as a potentially powerful separating mechanism.

Keywords: Active transport/ Brownian ratchets / Nanofluidics / Review / Sieves
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1 Introduction: New forces or new methods?

In his impressive book on separation methods, Giddings [1] uses as the common denominator of separation methods that all of them apply a gradient in electrochemical potential to separate the analytes. This gradient and the resulting force can have various origins, for example van der Waals forces, gravitational forces, the action of an electrical field on a charged particle or the force resulting from the difference in analyte chemical standard potential between two phases. Analytes can only be separated if there is such a force. In micro- or nanomachined systems this situation is not different, meaning that also in these systems separation will only be possible if we can apply the forces mentioned and that no other forces are at our disposal. Of course the scale at which we apply them is different and it can thus be that in nanometer-size systems we will have to apply descriptions of the behaviour that are valid for single molecules, *e.g.*, based on molecular dynamics simulations, instead of the macroscopic descriptions that can be used for larger systems. However, even though no new forces are at our disposal in nanomachined devices it can still make sense to employ nanotechnology for a number of reasons. In this paper we will show that there are at least three reasons to do so. The first is to improve device behaviour by exactly determining its geometry down to the nanoscale. This can reduce band broadening in HPLC and CEC, or tailor the separating characteristics of entropic traps to certain sizes of DNA. The second reason is to apply clever design in order to extend the functionality of present devices as will be demonstrated for sieves. Especially the ability to make regularly structured devices can open up new alleys in this application area. The third reason is that truly new separation methods can become possible by micro- or nanomachining, such as Brownian ratcheting. Besides these arguments, nature is the true master in producing nanostructures, offering a dazzling array of functional proteins and compounds at the cellular level – in this sense 'Natura artis magistra' (nature teaches the arts) [2]. Many of these proteins perform separating actions, and the final paragraph of this paper will be devoted to some speculative thinking, as much as possible based on present research, on future separation devices performing biology-inspired nanotechnological separations.

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Abbreviation: COMOSS, collocated monolith support structures

2 Micro- and nanomachined columns for HPLC or CEC

Knox [3] showed in 1999 that at present the most important contribution to peak dispersion in HPLC stems from the imperfect packing of the stationary phase particles. He mentioned micromachining of the column packing as one of the possible approaches to solve this problem. More recent calculations by Gzil *et al.* [4] indeed showed that a perfectly ordered 2-D pillar array is expected to have a separation performance which is about ten times better than the best packed column. In 1998, He and Regnier [5] had already shown the first microfluidic structures with micromachined pillar arrays instead of conventional packing (Fig. 1). These so-called collocated monolith support structures (COMOSS) showed excellent separation efficiency in CEC separations. A disadvantage is that they rely on surface adsorption and hence have a much lower mass loadability than porous column materials. This could be solved by using a porous column material.

The micromachined column packings mentioned above have fluidic channels with a width w of 1–3 μm. Since the time required to obtain a certain resolution in HPLC in first-order approach scales with w^2, and the pressure needed with $1/w^3$, further downscaling of the channel width would make sense only for ultrarapid performance of easy separations, given the pressure limit inherent in HPLC [6]. For CEC separations it is not to be expected that separation efficiency or separation time will benefit from downscaling channel width below the micrometer range, since efficiency improvement here will be limited by the axial diffusion. However, mass loadability in CEC would benefit from the increased surface area, possibly obviating the need for porous pillar materials.

At present monolithic columns are widely investigated for use in HPLC. The external porosity of these columns makes them superior over conventional packed columns for separations that require plate numbers $N > 50\,000$ [7]. For more simple separations that require fewer plates however they are less efficient than the conventional columns packed with spherical particles [7]. The ultimate role of micro- and nanomachining could be to tailor external porosity and packing geometry to the separation problem at hand while at the same time maintaining mass loadability by using an internally porous column material [4].

3 Micro- and nanomachined sieving structures

In Section 2, it was shown that micromachining can be of benefit to increase separation efficiency. Here it will be shown that the possibility to make perfectly regular repetitive micro- or nanostructures can add functionality to an existing separation principle, namely sieving.

The term 'sieving' can be used to denote a whole range of size- and charge-based separation methods. When the size of the sieve pores is much larger than the electrical double layer thickness, sieving will occur based on molecular size. When the pore diameter approaches the double layer thickness, electrostatic effects will start playing a role and sieving will also be a function of molecular charge. Both effects form the basis of many technological and biological membrane separation systems. As we will show below micro- and nanomachining sieves can have two benefits: first it can improve sieving characteristics and second it can introduce new functionalities like size fractionation.

Micro- and nanomachining have been used to produce sieves for almost 20 years and an established sieve design is that of the nanomachined nanoporous membrane [8, 9]. Pore sizes can be sufficiently small to give double layer overlap and Desai *et al.* [10] for example demonstrated the continuous separation of glucose and

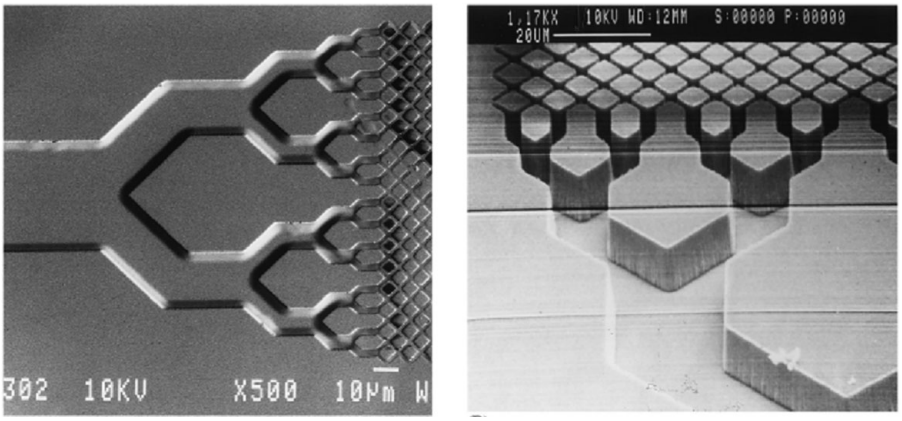

Figure 1. Micromachined COMOSS packings for on-chip HPLC and CEC. Reprinted from [5], with permission.

albumin using a sieve with nanopores of 24.5 nm. Regarding the small molecular dimensions of albumin (8 by 3.8 nm), electrostatic ion exclusion must play a role in the separation. Such membranes function not fundamentally different from conventional membranes that are used, *e.g.* for the fractionation of albumin and lysozyme (see, *e.g.* [11]) but the throughput can be much higher. In future it can furthermore be envisaged that a combination of micro- and nanomachining can create a series of nanosieves with holes of different diameter combined in a larger micromachined array to fractionate molecules from complex mixtures on size and charge.

Recently several devices have been demonstrated that contain sophisticated sieving structures for size-based separation of DNA-molecules and polystyrene beads. A device for continuous sieving that uses a regularly structured microarray is shown in Fig. 2 [12].

It employs a regular array of posts where each new line of posts is shifted for a fraction of the interpost spacing. Particles that just fit between the posts will 'go with the shift' and move diagonally through the device, while smaller particles will move with the average hydrodynamic flow which is straight down. Separations were demonstrated for polystyrene spheres with diameters from 600 to 1000 nm in 40 s in continuous flow (see Fig. 3A). An important difference between sieving-based separations and chromatographic methods like the related principles of hydrodynamic and size-exclusion chromatography [13] is that the sieving does not depend on diffusion into regions with different flow speed but on steric and hydrodynamic effects. This has the crucial advantage that the resolution improves with flow speed (see Fig. 3B and C). Two further important characteristics of this separation principle have to be pointed out. In the first place it is based on a perfectly regularly machined microstructure, and was therefore impossible to construct with classical technology which always produces random structures. Finally, flow field and separation force are perpendicular so that the device can be operated in continuous flow mode. It is important to emphasise this aspect, which makes this method akin to a method like free flow electrophoresis [14]. Interestingly, instead of solid obstacles, an array of optical traps can also be used for separations based on size and optical properties (see Fig. 4) [15, 16]. General theory on such regular array-based separation systems can be found in literature [17–19]. Since in the case of optical trap-based separations the minimal trap size is determined by the wavelength of the light used, such separations will be limited to particles of a few hundred nanometers diameter or larger.

A geometrically different sieving device was shown by Vankrunkelsven *et al.* [20]. Here human and bacterial cells are separated because they are stopped by micromachined steps of different height in the channel. Flow field and separation force in this case have the same direction (namely along the channel axis). Though the present device was operated with micron-sized steps, devices with submicron-sized steps would be possible.

Both the device of Huang and Vankrunkelsven employ micrometer-size obstacles and work mainly by steric exclusion forces. It is exciting to think of the possibilities when the devices are scaled down and electrostatic forces come into play. The sieving mechanism would then be quite akin to that occurring, *e.g.* in the basal membrane of the kidney, where the negatively charged albumin is prevented from passing from the blood to the primary urine by a combination of size filtering and co-ion exclusion by the negatively charged matrix [21]. The larg-

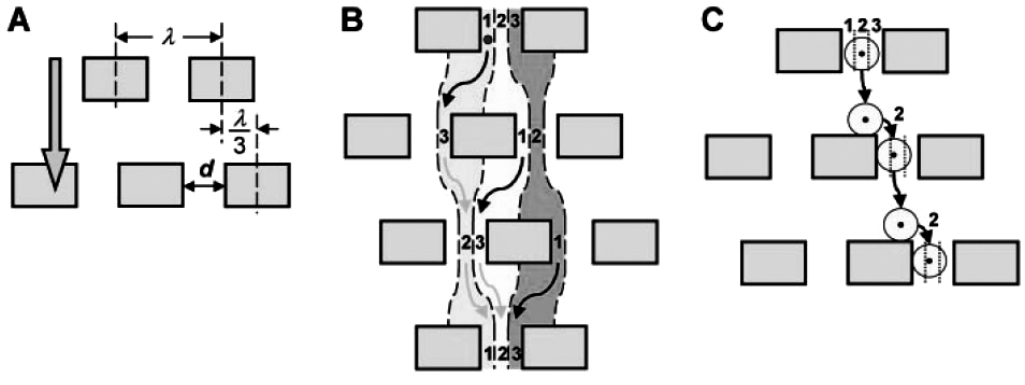

Figure 2. Continuous particle sieve. Principle of operation: (A) Design: a row of obstacles, gradually shifted in the flow direction; (B) small particles will move with the flow lines and would not be displaced horizontally; (C) particles with a size in the order of the gap will be continuously displaced. Reprinted from [12], with permission.

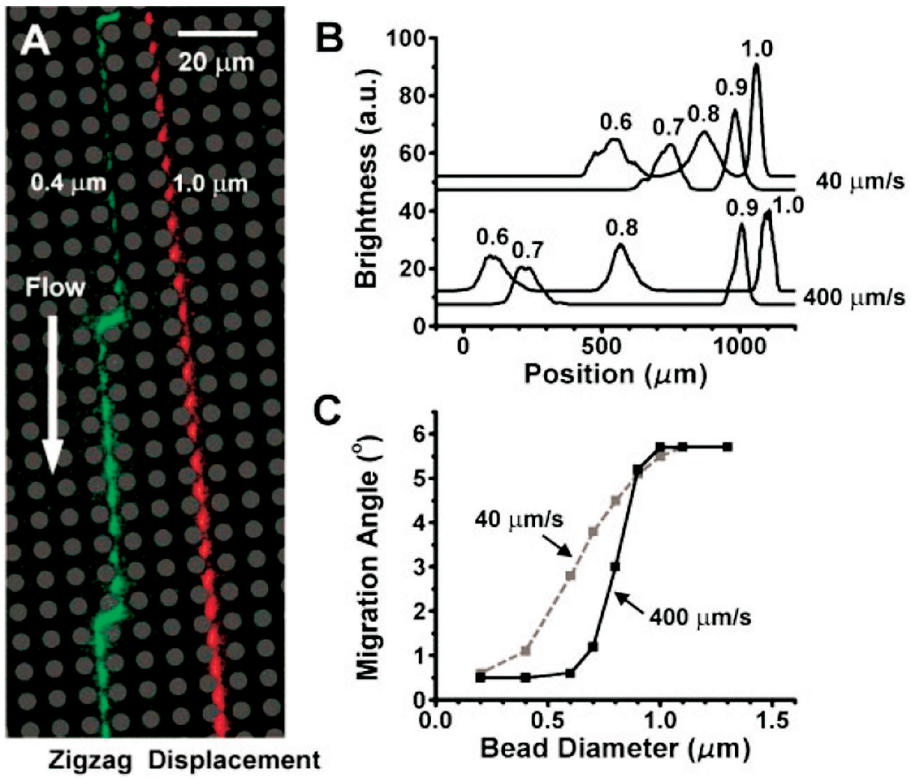

Figure 3. Continuous particle sieve employing an obstacle array as shown in Fig. 2. (A) Polystyrene beads (400 nm) move straight down, while 1 μm particles deviate; (B) separation of four different sizes of beads; (C) crucially separation improves with flow speed since separation depends on viscous sorting and not on diffusion. Reprinted from [12], with permission.

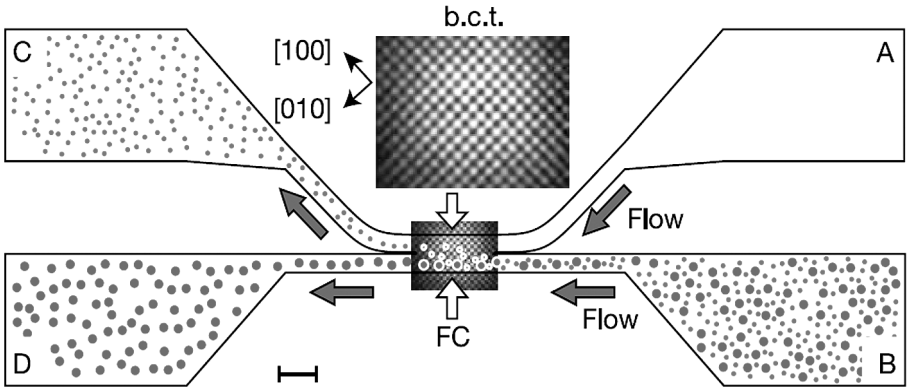

Figure 4. Continuous flow particle fractionation using a 3-D array of optical traps (in a body-centred tetragonal lattice = b.c.t.) in a fractionation chamber (FC). Continuous laminar flow is maintained from reservoir A to C and from reservoir B to D. Trap array selectively pushes one species from the mixture in B into the upper flow field, separating it from the other species. Reprinted from [16], with permission.

est obstacles to creating such devices probably are technological. The minimum feature size of standard photolithographically produced devices is 1–2 μm. E-beam lithography and nanoimprint lithography could be used to produce devices with smaller dimensions (down to 50 nm) [22]. Repetitive structures can also be produced using interferometric lithography [23].

4 Ratchets

For separating by ratcheting a regular micro- or nanostructure is needed and hence this principle only became possible due to the availability of microtechnology. Here we will introduce this method and then discuss whether downscaling to the nanoscale would be beneficial.

In general two types of ratchets are distinguished: ones that rectify externally applied fields and ones that use Brownian motion or diffusion. Both types are depicted in Fig. 5 [27]. The first type (Fig. 5A) is much like the ratchet from our everyday experience, namely a rectifying structure where movement is easier in one direction than the other because a larger force is necessary for transport in one direction than in the other. This type of ratchet can use an oscillating time-averaged zero force as its input (*e.g.* an E-field or a pressure gradient) which it rectifies

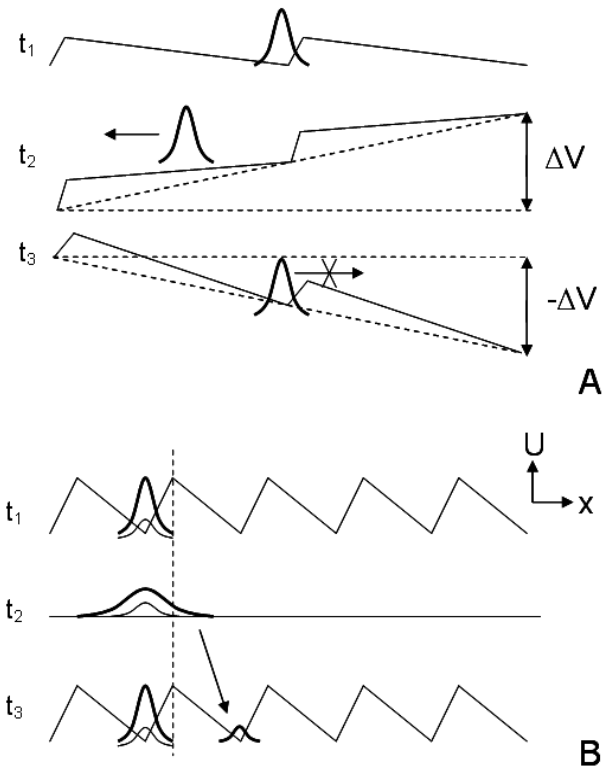

Figure 5. Two types of 1-D ratchet devices. (A) Rectifying ratchet, employing an asymmetric potential profile U rectifying an oscillating net-zero force $F(t) = -\mathrm{d}V/\mathrm{d}x$, where $-\mathrm{d}V/\mathrm{d}x$ is an applied potential gradient; (B) Brownian ratchet, employing a time-varying asymmetric potential profile to rectify Brownian motion (Gaussian distribution curves of a slow and fast diffusing species are drawn); t_1, t_2 and t_3 denote consecutive moments in time.

[24]. The other ratchet type (Fig. 5B) is more subtle and employs a periodic change of the height of the ratchet teeth combined with a period for diffusion. This type uses Brownian motion as its input (Brownian ratchet) and switches from the state where the energy barriers (ratchet teeth) are lower than the thermal energy kT, to a state where they are higher. Because of the spatial asymmetry of the teeth, a net transport of particles takes place. In 1993 Magnasco *et al.* [24, 25] showed that if a certain period of Brownian motion was combined with a periodically applied potential with broken symmetry, unidirectional particle movement was possible. This treatment concerned the description of cellular motor proteins, which are thought to use the thermally 'noisy' cellular environment to their advantage by rectifying the thermal (diffusional) energy through molecular shape changes consuming ATP. In 1992, Ajdari and Prost [25] had already suggested a new separation principle that operated along these lines, namely a dielectrophoretic separation with fluctuating barriers and without a macroscopically applied field, which in 1994 was experimentally demonstrated by Rousselet *et al.* [26]. Other treatments on this subject have followed [27, 28]. The Brownian ratchet separation principle is fundamentally different from the ones described by Giddings and mentioned at the introduction of this paper (occurring under the influence of a gradient in chemical potential) in the respect that the two different time phases only cause separation if applied in succession. Parrondo and de Cisneros [29] have given an interesting analysis on this phenomenon, showing how two losing games can be combined to a winning strategy (Parrondo's games).

Examples of rectifying ratches (Fig. 5A) have appeared in literature. A silicon wafer with asymmetric pores enabled vectorial transport of micronsize colloidal particles when an oscillating net-zero pressure difference or electrical field was applied [30, 31]. More recently the latter system was scaled down and it was shown that potassium ions can be transported against their concentration gradient through a conical surface-charged nanopore (tapered from 2 to 500 nm) when subjected to an oscillating (time-averaged zero) electrical field [32]. Simulations furthermore predict that an asymmetric nanopore will rectify an oscillating pressure difference to create net liquid flow [33]. It is to be expected that exciting devices can be produced when more nanoscale ratchet devices are produced, since nature also employs the concept for transport and pumping, rectifying the abundant kT noise at the molecular level [24].

All analytical Brownian ratchets (Fig. 5B) manufactured up till now had micrometer-scale structures and their separation performance, *e.g.* for long DNA was worse than in classical methods. Forces employed in Brownian ratchets for DNA analysis have been dielectrophoretic and electrostatic [34, 35]. Recently optical trapping has also been used in a ratchet [36]. Large improvements are however to be expected on downscaling. In the following calculations we will distinguish between 1-D Brownian ratchets (*e.g.*, periodic electrode structures along a microfluidic channel axis) and 2-D Brownian ratchets (where a 1-D ratchet structure is repeated in a spatially shifted fashion along a perpendicular axis, enabling continuous flow operation) [37, 38]. The lateral displacement x (m) along the separation axis of a Brownian ratchet is [35]

$$x(n) = nd\alpha$$

where n is the number of cycles, d (m) is the unit length of the periodic structure and α the probability that a species particle moves one unit length. As well as the lateral dis-

placement, the variance σ_x^2 (m) also increases with each cycle, by [35]

$$\sigma_x^2(n) = nd^2\alpha(1 - \alpha)$$

The speed of separation depends on the number of cycles that can be performed *per* unit time. In a 1-D horizontal array this corresponds to the operating frequency f (/s) of, *e.g.* an electrode array and in a 2-D array on the vertical flow velocity u (m/s). If f or u are too high, particles will have insufficient time for diffusion, and if they are too low time is wasted and dispersion increases. An optimal frequency or velocity can be derived from simulations and can be shown to depend on the species diffusion coefficient D (m^2/s) and the length of the steep ratchet slope d_1 for 1-D arrays (Eq. 1a), or on the ratio of vertical flow velocity u and vertical ratchet spacing d_2 which determines the frequency of meeting the array for 2-D arrays (Eq. 1b).

$$f_{opt} \propto \frac{D}{d_1^2} \tag{1a}$$

$$\left(\frac{u}{d_2}\right)_{opt} \propto \frac{D}{d_1^2} \tag{1b}$$

It can clearly be seen that downscaling the device will increase the speed with the square of the typical length $d_1 \approx d$. Apart from the diffusion time needed as discussed above, some time is also needed in which the asymmetric potential gradient is applied. This time also scaled favorably with d^2 because the particle velocity v (assuming electrophoretic transport) equals $\mu_{ep}V/d$ and the transport time needed equals d/v. Here μ_{ep} ($m^2/(V \times s)$) is the electrophoretic mobility and V (V) the electrical potential difference applied between two neighbouring electrodes.

Limits to the development of Brownian ratchets will certainly be posed by available technology. At present many cleanrooms can photolithographically produce electrodes of 1 μm width, but for narrower electrodes less widely available methods must be employed like nanoimprinting or ion-beam lithography.

5 Nanomachined devices for entropy-controlled separation

As pointed out by Giddings [39], size exclusion is also influenced by changes in entropy. Molecules will less easily enter a pore when they have to surrender more conformational (for flexible molecules) or orientational (*e.g.*, for elongated molecules) freedom causing a loss in entropy.

DNA is a flexible molecule, and entropy changes play a large role in DNA separations. Baumgärtner and Muthukumar [40] developed the theory of entropic trapping of DNA molecules in the cavities inside porous gels. DNA-separating devices containing such entropic traps were later machined by Han and Craighead [41], and an entropic recoil device was also constructed by the same group [42]. In both devices cleanroom machining is used which is preferable over the use of gels because the size of the traps and the column spacing in the recoil device can be precisely tailored [43]. Both separation methods employ the specific properties of DNA, which has a long flexible chain with many distributed charges and these methods will therefore only be possible for DNA or comparable (bio)polymers. Since the dimensions of the cavity traps and connecting nanochannels of the entropic trap device must be tailored to the size of the DNA molecules, typical dimensions are in the order of 50–100 nm which are relatively easy to produce in a standard cleanroom.

6 Nanomachined preconcentrators

When electrical double-layer overlap occurs in nanofluidic systems, be it nanochannels or nanoporous membranes, the system locally is semipermeable meaning ions of only one sign are present in its channels or pores. If an electrical current is sent through such a system, ions will be concentrated on one side and depleted at the other side of the nanopores. This effect was recently demonstrated for nanochannels [44]. Microfluidic devices with integrated nanochannels and silica membranes for analyte preconcentration based on semipermeability have been demonstrated, in the former case showing a concentration by a factor of 10^6 [45, 46]. Of interest is also the method shown by Leinweber *et al*. (accepted by *Anal. Chem.*), employing an array of electrically floating metal electrodes in a micrometer-high CE channel to concentrate ionic substances in a continuous-flow fashion by nonlinear electrokinetics (Leinweber *et al.*, accepted).

7 Separations inspired by biological systems

Biological systems show a number of amazing properties from the point of view of a separation scientist. In the first place biological systems come into existence by a process of dynamic (energy-dissipating) self-assembly, implying separation and compartmentalization of many substances over several tens of micrometers [47]. Also, they contain a number of nanosystems devoted to locomotion, like actin moving along myosin and kinesin moving along microtubules. Active transport occurs in many

places in the cell, for example across its membrane by the Na^+ K^+ ATPase protein which pumps three sodium atoms out of the cell and two potassium atoms in while consuming one ATP molecule. Thus, over the cell membrane which has a thickness of about 4 nm the sodium concentration differs with a factor of about 10, and the potassium concentration with a factor of about 40. These concentration differences are created and maintained between ions in free solution, not chemically bound to any molecule. To give another example, the body is able to specifically and actively take up many neurotransmitters at the nerve endings bordering the synaptic cleft, and to concentrate these substances in vesicles located in the cell body [48]. Two active transport mechanisms by which the cells perform these separations are schematically depicted in Fig. 6.

If we take a closer look at Na^+ K^+ ATPase as an example of active transport, a number of important properties become manifest that make it highly attractive for separation purposes. It is selective since it pumps three Na^+ out of the cell and two K^+ in, exerts a very large force pumping against a high chemical potential gradient, and is very efficient (operating practically isothermally its efficiency is thought to approach 100%). Furthermore important for the overall efficiency of the system is that the separating force is only exerted when an instance of separation takes place, so that no energy is wasted in other places. This situation stands in strong contrast with, *e.g.*, CE where a macroscopic force field is applied and the efficiency is low due to Ohmic heating. The separating force (or electrochemical potential gradient) that is exerted by the Na^+ K^+ ATPase equals the energy stored in one ATP molecule ($14\,kT = 5.76 \times 10^{-20}$ J) divided by the membrane thickness d_m of 4 nm, and can be compared to the force exerted by an equivalent electrical field E_{equiv} (V/m) of 8.8×10^7 V/m on an ion of unit charge q (1.60×10^{-19} C)

$$\frac{14\,kT}{d_m} = qE_{equiv}$$

$$E_{equiv} = \frac{kT}{q} \cdot \frac{14}{d_m} = 8.8 \times 10^7\,\mathrm{V/m}$$

A field of this magnitude cannot be applied in macroscopic systems, where fields are practically limited to the 3×10^5 V/m reached in chip-based CE systems because of the low efficiency causing Ohmic heating. It does locally exist in cells, however, since in fact the activity of the Na^+ K^+ ATPase creates the electrical field of 70 mV/4 nm (or 1.75×10^7 V/m) that exists over the cell membrane. It is the high efficiency of the Na^+ K^+ ATPase that enables this high field, because it limits the heat production.

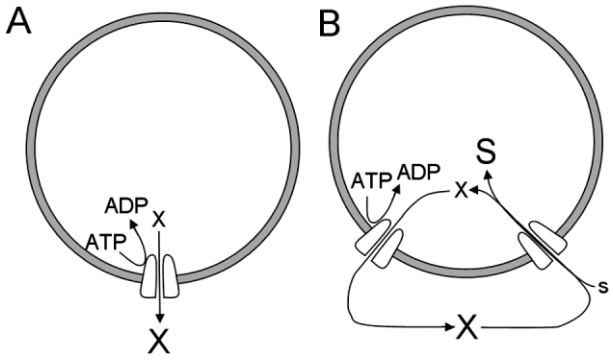

Figure 6. Two active transport mechanisms employed for molecular transport by cells. (A) In primary active transport, species *x* is transported against its concentration gradient by expenditure of one ATP of energy; (B) in secondary active transport the energy stored in the chemical potential difference of X across the membrane is used to transport species S against its concentration gradient.

Future separating devices can be envisaged that apply protein structures analogous to the Na^+ K^+ ATPase in a highly parallel and/or serial fashion for ionic separation. The function could be switched on and off *via* the fluidic pathway creating very efficient devices. Such nanomachined structures would present the ultimate limit of molecular separation. Other functionalities could be added like channels that employ the ionic concentration gradients created for selective transport of other molecules, like the cells do, *e.g.*, for neurotransmitter reuptake.

For this projection to become reality some formidable hurdles have to be overcome. Proteins generally quickly denature when in contact with the inorganic materials that are often used as the wall material in micro- or nanofluidic devices. Special coatings therefore need to be applied or noninorganic wall materials chosen. Here coatings with PEG are one possibility [49]. Lipid bilayers can be employed to contain the membrane proteins but also these are not very stable. At present many investigations concern their stability increase, which can be done by the choice of lipids, by cross-linking, by reducing the membrane size and employing many membranes in parallel or by the use of block-copolymers [50–52]. It is encouraging that a biosensor employing the transmembrane protein gramicidin in a lipid bilayer membrane tethered to a gold electrode has already been demonstrated [53]. Also, functional bacterial porins have been incorporated in the walls of vesicles that were stabilized by polymerization [54].

Another type of biology-inspired separation mechanism could be based on the Brownian ratchet principle. At present this principle is thought to be employed on the

molecular level in cells for transport by actin/myosin [25, 27]. In micromachined devices it has been employed in a not very successful way for DNA separation but downscaling into the nanometer scale would be of much benefit since as mentioned above, separation speed scales with the square of the typical feature dimension. Top-down nanomachining methods however presently limit the minimal feature size to about 50 nm. If bottom-up methods could be employed instead or in combination, molecular separating machines could possibly be constructed that resemble the transport proteins. Such separators could extract their energy, *e.g.*, from ATP, causing highly efficient operation as was mentioned above for Na^+ K^+ ATPase.

8 Separation in discrete events

On the cellular level discrete separation events take place, *e.g.*, by Na^+ K^+ ATPase. The single molecular events needed to achieve this can be regarded as embodying the ultimate level of separational control and efficiency. If future devices can be designed with a nanoarchitecture in which such control is possible, and if furthermore the separate discrete events can be spatially or temporally coordinated to obtain parallel and serial functioning, the ultimate separation device can be constructed. Interestingly, present top-down micromachined devices like the sieve of Huang already separate in discrete steps [12]. Also Brownian ratchets separate in discrete steps n (Eqs. 1, 2), with the actin movement along myosin in discrete steps of 5 nm as the example of the thermal ratchet scaled down to molecular size [55].

9 Conclusions

Nanotechnology can have a large impact on separation science. The possibility to freely machine separating structures down to the nanometer-scale for classical separation methods like HPLC or CEC can lead to an optimised design tailored to every type of separation. Sieving devices and Brownian ratchets are areas where the possibility to machine regular nanostructures can have a large impact. Benefits of nanotechnology for preconcentrators have already been demonstrated. Finally, nature offers examples of highly efficient separating structures that beg to be included in man-made (nanotechnological) separating devices. In the opinion of the writers especially the concept of active transport occurring in discrete events merits attention by separation scientists.

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