Active transport: a new chemical separation method?

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Introduction

The biological world offers continuous inspiration for investigators in the field of lab-on-a-chip and especially in nanofluidics where the old adage 'Natura Artis Magistra' (nature teaches the art) certainly seems to hold true.[1] An active area of exploration is for example the use of motor proteins, that perform functions on chip fuelled by ATP.[2] In this focus paper we want to draw the attention of the lab-on-a-chip community to a biochemical phenomenon that also involves the consumption of ATP but that to our knowledge has not yet been applied in analytical chemistry or lab-on-a-chip, namely active transport. We will use this expression here in its common *specialized sense*, namely the active transport that takes place across the lipid bilayer membranes of biological organisms.[3] Both primary and secondary active transport will be considered, that are performed by special families of proteins called, respectively, pumps and transporters. Living organisms operate these transporters and pumps amongst others to separate and concentrate ions and nutrients at locations where they benefit their survival. In this paper we propose to apply the same proteins for separation and concentration in chemical analysis. It will be argued that the properties of these molecules carry quite some promise for analysis, if we are able to contain them in a suitable environment. First we will introduce the proteins and their general characteristics, and show some applications that have already been demonstrated especially in conversion of photonic to chemical energy.[4] Subsequently we will discuss some possible applications for chemical analysis. The considerable technological challenges and hurdles will in this context be considered.

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Primary active transport

Primary active transport occurs in biological organisms by ATP-powered pump ATPases, that transport substances across bilayer membranes. They exist in eukaryotic cells as well as in bacteria, and both in the plasma membrane that separates the cell from the outside world and in the membranes of organelles like mitochondria, vesicles and chloroplasts. In eukaryotic cells the plasma membrane-spanning Na^+K^+ ATPase is responsible for the difference in K^+ and Na^+ concentration between cellular and interstitial fluid. The protein pumps three molecules of Na^+ out of the cell and two molecules of K^+ into the cell at the cost of hydrolyzing one molecule of ATP. As a result the K^+ and Na^+ cytoplasm-over-blood concentration ratios respectively are 140 mM/5 mM and 10 mM/145 mM. Due to the presence of a transmembrane K^+ channel, a small amount of K^+ again diffuses out of the cell under the influence of this concentration difference, giving rise to the intracellular potential of -70 mV *vs.* the extracellular fluid. In view of the very thin lipid bilayer membrane the final situation is characterized by very large ionic concentration and electrical potential gradients. Many ATPases pump protons, *e.g.* across bacterial plasma membranes and across intracellular vesicles in eukaryotes. As a result the interior of the vesicle for example is acidified and obtains a positive potential of about 50 mV with respect to the cytosol (Fig. 1a). Other examples are the H^+ ATPase in the mitochondrial inner membrane, and the H^+ K^+ ATPase in the parietal cells of the gastric gland. All the ATPases mentioned thus far pump inorganic ions, but the family of ABC (ATP binding cassette) transporter proteins transports organic molecules over the plasma membrane by direct consumption of ATP. In this family both excretion and take-up proteins are found, and a wide variety of substrates is transported (*e.g.* amino acids, sugars, ions, peptides, hydrophobic organic substances, vitamins).[5] Primary active transport can also occur with other sources of energy than the hydrolysis of ATP, *e.g.* by electron transport (oxidation) especially in the mitochondrial membrane, or by photon absorption, *e.g.* by bacteriorhodopsin (Fig. 1b and c).

Secondary active transport

The cell exploits the steep electrochemical gradients generated by primary active transport amongst others for so-called *secondary active transport* in which substances are excreted or taken up by special membrane-spanning transporter proteins. Membrane transporters utilize the energy of a H^+, Na^+, Cl^- or K^+ ion moving down its electrochemical potential gradient to move *e.g.* an amino acid molecule against its concentration gradient, and/or they allow charged particles to move in the direction of the coulomb force that is exerted by the electrical field. These transporter proteins can be either symporters, in which the inorganic ions move in the same direction as the transported species, or antiporters in which the direction is opposite. Specific transporters exist for many substrates, *e.g.* amino acids, neurotransmitters, sugars, peptides, ions, *etc.* Fig. 1a–c show examples of secondary active transport of glutamate and lactose, and serves to demonstrate the versatility of the concept of active transport, which can use photonic, electronic (redox) or chemical (ATP hydrolysis) energy to create an electrochemical ion gradient, subsequently using it for specific transport.

Transport characteristics

In order to understand its potential usefulness, it is important to know the maximum concentration ratio, transport rate and specificity that can be obtained by primary and secondary active transport. The energetics of primary and secondary active transport are understood on the basis of the pioneering work on mitochondrial metabolism (the so-called chemiosmotic theory) by Mitchell,[9] and have been presented in a clear way *e.g.* by Rottenberg.[10] Using a proton pump as an example for primary active transport, we can write the reaction/transport equation[10]

$$\mathrm{ATP} + n\mathrm{H^+_{out}} \leftrightarrows \mathrm{ADP} + \mathrm{P_i} + n\mathrm{H^+_{in}}$$

where n protons are pumped across the membrane per hydrolyzed ATP molecule. The change of free energy in this process is obtained by adding the term for the chemical reaction to the one for the proton electrochemical potential on both sides of the membrane. In equilibrium the free energy change is zero and we obtain[10]

$$n(q\Delta\psi - 2.3kT\Delta\mathrm{pH}) = \Delta g^0_\mathrm{p} + kT\ln\frac{[\mathrm{ATP}]}{[\mathrm{ADP}][\mathrm{P_i}]}$$

where Δg^0_p is the reaction standard free energy, $\Delta\mathrm{pH} = \mathrm{pH_{in}} - \mathrm{pH_{out}}$, and the potential difference $\Delta\psi = \psi_\mathrm{in} - \psi_\mathrm{out}$. With typical physiological concentrations of ATP and ADP, the right-hand side of the equation amounts to approximately 21 kT, and furthermore often approximately 3 protons are pumped per ATP ($n = 3$).[10] It is conceptually helpful to define a so-called proton-motive force Δp over the membrane (the proton electrochemical potential in units of volts).[9] In our case Δp amounts to

$$\Delta p = \Delta\psi - \frac{2.3kT}{q}\Delta\mathrm{pH} = \frac{7kT}{q} = 173\,\mathrm{mV} \quad (1)$$

For example, without a pH difference the potential difference can be up to 173 mV and if a potential difference is absent, the pH can differ by more than three units between inside and outside.

When sufficient substrate and source of energy is available (leading to saturated transporters and a maximal rate), the transport rate per protein molecule across the membrane for primary active transport typically is 10^2–10^4 $\mathrm{s^{-1}}$.[3]

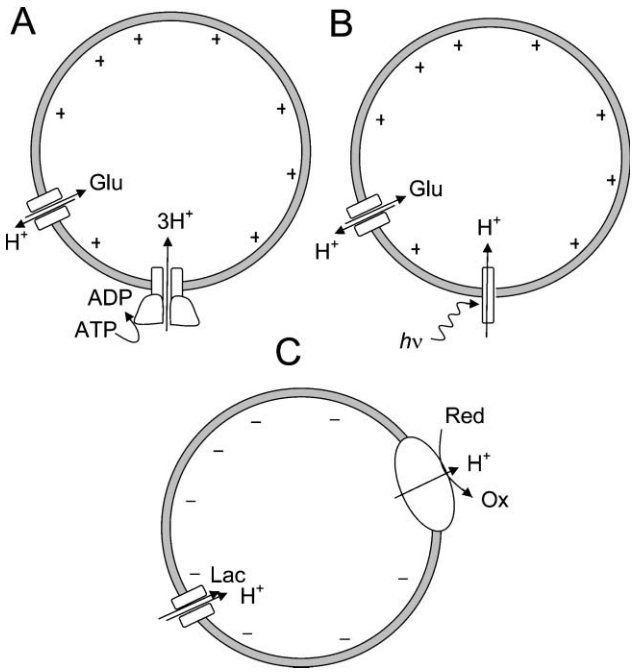

Fig. 1 Some combinations of primary and secondary active transport that have been demonstrated using proteoliposomes (phospholipids vesicles with reconstituted membrane proteins). (A) Primary proton transport by a vesicular H^+ ATPase coupled to the secondary active transport of glutamate by the glutamate antiporter.[6] (B) Primary proton transport by bacteriorhodopsin coupled to the secondary active transport of the glutamate antiporter.[7] (C) Primary active transport by cytochrome oxidase coupled to secondary active transport of lactose by the lactose symporter.[8]

For secondary active transport we can write for the example of a symporter of neutral molecules S[10]

$$\mathrm{S_{out}} + n\mathrm{H^+_{out}} \leftrightarrows \mathrm{S_{in}} + n\mathrm{H^+_{in}}$$

At equilibrium we can equate the sum of electrochemical potentials of both species on either side of the membrane and obtain

$$\frac{2.3kT}{q}\log\left(\frac{S_\mathrm{in}}{S_\mathrm{out}}\right) = n\left(\Delta\psi - \frac{2.3kT}{q}\Delta\mathrm{pH}\right) = n\Delta p \quad (2)$$

Depending on the value of n, a neutral molecule can be enriched by several orders of magnitude on the inside of the membrane. An enrichment of neurotransmitter concentration by five orders of magnitude has been reported in neuronal vesicles.[11] In saturation the transport rate of secondary active transport typically is 10^0–10^2 $\mathrm{s^{-1}}$.[3] When the substrate concentration drops below the Michaelis constant K_M of the transporter, the transport rate will drop as well and will become proportional to the substrate concentration.[3] Some typical values for K_M are 0.1–1 μM for neurotransmitter transporters, 1–1000 μM for amino acid transporters and 1 mM for the Na^+ glucose symporter. K_M differs strongly between transporters in different organs and species. Also the substrate specificity widely differs and as a rule of thumb is comparable to that of enzymes.

Comparison with classical separation methods

It is helpful to compare active transport in this specialized biochemical sense with active transport in a *general sense*, which is any transport of chemical species under the influence of an applied directional force. Active transport then stands opposed to diffusional transport which occurs as a result of random collisions and imparts no directionality. Active transport in this general sense is used in chemical analysis to obtain separation, *e.g.* in electrophoresis when a coulomb force is applied or in chromatography when a pressure gradient is applied in

combination with a differential affinity for a stationary phase. Diffusional transport that takes place during these processes is randomizing and counteracts separation. Two disadvantageous properties generally characterize our present analytical systems that could be overcome should biochemical active transport be used for separation. Firstly, the pressure gradient or the electrical field is mostly applied to many more molecules than the ones that are separated. This makes the process energetically highly inefficient due to power dissipation in processes that do not cause separation, for example in the current carried by the background electrolyte. The generated heat furthermore causes a temperature rise, which increases the randomizing diffusion. Secondly, the separation typically takes place within one continuous compartment (column or channel), so that diffusion continuously works against the achieved separation. The downscaling applied in microTAS or lab-on-a-chip devices brings improvement on both points and benefits therefore both analysis quality and time. The scale at which biological systems apply separating forces however again is orders of magnitude smaller. Thus considering applied separation force and obtained concentration gradients the biochemical active transport results in separations superior to anything achieved in lab-on-a-chip.[12] This superiority derives from the fact that a very large force is locally applied to transport just a single target molecule, and that the transported molecule is compartmentalized behind the bilayer membrane, preventing diffusion. For example, the proton-motive force that results from primary active transport is exerted over a membrane with a thickness of only 4 nm. Its equivalent electrical field is 4.4×10^7 V m^{-1}, which is about two orders of magnitude higher than the maximal field generally applied in lab-on-a-chip applications.

Table 1 and Fig. 2 compare primary and secondary active transport with the separation processes of liquid/liquid extraction and antibody capture. By active transport the separated species is stored inside a liquid volume and therefore the separated amount is comparable to extraction but much larger than can be obtained by specific adsorption, which occurs at a surface. In active transport the specificity is larger than in extraction but smaller than in the case of antibody binding. Comparisons of separation speed cannot be made because they will depend too much on the geometry used. It must furthermore be realized that a class of low molecular weight species is targeted by active transport that normally lies outside the range of antibody binding.

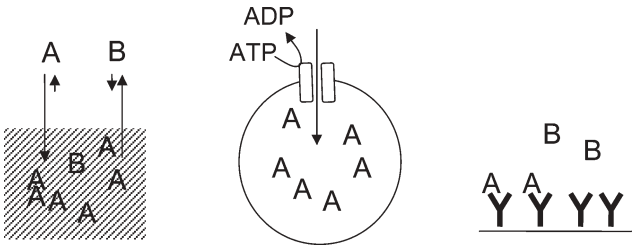

Fig. 2 Schematic comparison of active transport (middle), liquid/liquid extraction (left) and antibody capture (right).

Some possible applications in lab-on-a-chip

How can active transport be employed in lab-on-a-chip devices? Here we will mention just a few possibilities. Every reader from his or her personal expertise undoubtedly will be able to add numerous other ideas. As mentioned above, lipid bilayer systems with reconstituted membrane proteins could be used for specific analyte concentration and separation. Analytical systems would need to be built around these bilayer systems to enable sampling and detection. In the first place different geometries could be used for the lipid bilayers, for example liposomes, surface-tethered membranes or parallel membranes. Liposome suspensions would have the advantage of rapid mass transport because of the short diffusion distance. A back-of-the-envelope calculation can be made for the speed with which a 1% suspension of 100 nm diameter liposomes depletes a 1 μM solution of a neurotransmitter. If each liposome contains one transporter (K_M = 0.1 μM; saturated transport rate 10 s^{-1}) and one molecule of bacteriorhodopsin, we would have 10^{16} transporters L^{-1} and therefore 10^{17} molecules s^{-1} transported, which would deplete the surrounding liquid in 6 seconds! The 'safe storage' of the analyte in a diffusion-free area furthermore presents a large advantage for analysis. A suspension of proteoliposomes could be manipulated by electroosmotic flow or AC electroosmotic flow.[13,14] Detection of the enriched liposomes could be performed by mass spectrometry, where bursts of the carried species would be observed. If the analyte is electroactive detection could also be performed by antibody capture of the liposome using a specific hapten-tag, followed by liposome lysis and electrochemical detection.[15] Possibly intraliposomal dyes could be employed: dyes exist that are sensitive to the local pH,[16] electrical potential[8] and redox potential.[17]

Considering geometries with flat membranes, flat membranes tethered to a surface would enable conductance measurements.[18] It might also be possible to create a parallel membrane array oriented in the direction of an applied flow field. Analytes would then selectively be transported at right angles to the flow field, creating a continuous flow separation device.

The first steps towards the local generation of ATP in a lab-on-a-chip device have already been made by incorporating proteoliposomes containing bacteriorhodopsin and F0F1 ATPase in a solid state sol–gel matrix.[19] The liposomes generated ATP when

Table 1 Some properties of three different separation methods compared

	L/L extraction	Active transport	Antibody capture
Molecular weight	All	Low (<200)	Medium to high
Specificity	—	±	+
Amount	+	+	—

illuminated. This work builds on the pioneering work of Racker and Stoeckenius.[20] Such ATP could be used to locally power other pumps. On-chip transport of the liposomes by ATP-driven molecular motors could also be envisaged.[2,21,22]

Hurdles and outlook

Many hurdles will have to be overcome to make active transport a feasible option for lab-on-a-chip. Membrane proteins are inherently instable in aqueous solution because they contain a significant hydrophobic domain. Thus only a few score have been crystallized and structurally resolved in contrast to 10^4 soluble proteins. The preparation of proteoliposomes is an art that takes some time to master.[4,23] Transmembrane protein orientation can differ and is a factor to consider since it can reduce the net transport rate to zero.[4] The lifetime of the proteoliposomes and the protein stability are furthermore important factors. Protein stability can be increased by selecting proteins from extremophils. Detection at present is generally performed by biochemists employing radioactive tracers, which will not be an option for analytical chemical applications. Several alternative detection options have already been mentioned above.

Many question marks remain where it considers the feasibility of using sensitive proteins from the biochemical domain in lab-on-a-chip systems where robustness is of prime importance. Close multi-disciplinary cooperation will certainly be needed to create working devices. Perhaps bioanalytical research will be the first area of application, for example in the measurement of neurotransmitter release. Whatever its final usefulness however, active transport is certainly too interesting an option not to explore.

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