Major Intrinsic Proteins in Biomimetic Membranes

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Chapter

Major Intrinsic Proteins in Biomimetic Membranes

Claus Helix Nielsen*

Abstract

Biological membranes define the structural and functional boundaries in living cells and their organelles. The integrity of the cell depends on its ability to separate inside from outside and yet at the same time allow massive transport of matter in and out the cell. Nature has elegantly met this challenge by developing membranes in the form of lipid bilayers in which specialized transport proteins are incorporated. This raises the question: is it possible to mimic biological membranes and create a membrane based sensor and/or separation device?

In the development of a biomimetic sensor/separation technology, a unique class of membrane transport proteins is especially interesting—the major intrinsic proteins (MIPs). Generally, MIPs conduct water molecules and selected solutes in and out of the cell while preventing the passage of other solutes, a property critical for the conservation of the cells internal pH and salt concentration. Also known as water channels or aquaporins they are highly efficient membrane pore proteins some of which are capable of transporting water at very high rates up to 10^9 molecules per second. Some MIPs transport other small, uncharged solutes, such as glycerol and other permeants such as carbon dioxide, nitric oxide, ammonia, hydrogen peroxide and the metalloids antimonite, arsenite, silicic and boric acid depending on the effective restriction mechanism of the protein. The flux properties of MIPs thus lead to the question if MIPs can be used in separation devices or as sensor devices based on e.g., the selective permeation of metalloids.

In principle a MIP based membrane sensor/separation device requires the supporting biomimetic matrix to be virtually impermeable to anything but water or the solute in question. In practice, however, a biomimetic support matrix will generally have finite permeabilities to both electrolytes and non-electrolytes. The feasibility of a biomimetic MIP device thus depends on the relative transport contribution from both protein and biomimetic support matrix. Also the biomimetic matrix must be encapsulated in order to protect it and make it sufficiently stable in a final application. Here, I specifically discuss the feasibility of developing osmotic biomimetic MIP membranes, but the technical issues are of general concern in the design of biomimetic membranes capable of supporting selective transmembrane fluxes.

Introduction

Membranes for separation purposes require high permeability and high selectivity with sufficient mechanical stability to be functional. Reverse osmosis (RO) membranes fulfill these design criteria to a large extent by having relatively high water permeability while maintaining a good salt rejection. RO membranes have found use in drinking water production and waste water treatment for several decades. The driving force in RO operation is pressure, where a force exceeding

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the osmotic pressure difference across the membrane is applied and convective flux against the osmotic gradient is established. The operating mechanism in RO membranes is based on diffusion of water and small molecular weight organic molecules (non-electrolytes) through the membrane polymer by bonding transiently to the hydrophilic polymer meshwork. Electrolytes (ions) and larger non-electrolytes will not permeate easily due to charge and size exclusion, respectively. Classic RO is a fundamental separation process and in principle it is a realization of the semi-permeable membrane concept as introduced by Nernst a century ago.[1] Solvent (water) passes through the membrane leaving all other solutes behind.

In practice the ability of RO membranes to transport water and certain other molecules while rejecting others is not absolute. The passage of smaller molecules or less charged ions will be greater than the passage of larger molecules or highly charged ions. Thus Ca^{2+} will typically be rejected about three times better than Na^{+}. The passage of dissolved salts through an RO membrane may be as high as 5% as observed for nitrates depending on the particular membrane and on factors such as driving force (pressure), temperature and pH. Also, the closer an organic molecule structure resembles the structure of the RO polymer, the more readily it diffuses through the RO membrane.[2] However some types of RO membranes like sea water reverse osmosis (SWRO) membranes and some nanofiltration (NF) membranes are capable of some metal and metalloid removal.[3]

RO membranes have a biological counterpart—the set of membranes defining boundaries and organelles in living cells. Membrane transport (by e.g., ion channels and transporters) constitutes the basis for all living cells. Cellular membranes are highly specialized complexes of proteins and amphiphilic molecules (mostly lipids). The viability and function of all cells is critically dependent on very controlled fluxes of electrolytes, non-electrolytes and water across their membranes. While neutral gases and to some extent water can cross a protein-free lipid membrane, transmembrane flux of electrolytes is negligible due to the membranes hydrophobic interior. Electrolytes, most notably monovalent and divalent ions, are transported across membranes through highly specialized and ion-selective proteins. Channels facilitate the diffusion along concentration gradients whereas ATP-driven carriers and transporters catalyze uphill transport against concentration gradients. The cells basal need to adjust their osmotic balance (volume) as well as some cell's specific role in water reabsorption (e.g., renal concentration) requires a much larger transport of water than the pure lipid membrane supports. This transport is mediated by specialized water channel proteins—MIPs. In terms of large scale separation, ion channels and water channels are most interesting as their transport is decoupled from conformational changes in the protein—in contrast to the situation for carriers (e.g., the $Na^{+}K^{+}$ ATPase) where the catalytic event (the transfer of a molecule) is coupled to complex changes in protein structure. The result is a 10-1000 fold lower transport capacity in carriers compared to channel proteins. Although biomimetic membranes with transporters have interesting technological potential.[4] I will limit the scope here to MIP channel proteins in biomimetic membranes.

Biological membranes can be seen as a lipid matrix with effective barrier properties in which proteins with very specific properties such as high selectivity and permeability for solutes (ions or water) are incorporated. This leads to the question whether biomimetic membranes can be used in separation/sensor applications. The high water permeability of many MIPs naturally raises the question if these proteins can be used in a water purification device for production of ultra pure water and seawater desalination. This has indeed been suggested[4,5] and attracted commercial interest.[6] Also MIP based up-concentration of contaminants e.g., metalloids as a step in early detection (i.e., at low feed water concentrations) may be feasible.

In this chapter I first briefly define the concept of biomimetic membranes and present some recent developments. Then I discuss issues involved in constructing a biomimetic osmotic type membrane in the form of MIPs embedded in lipid bilayers.

Biomimetic Membranes

The fundamental biomimetic approach is to extract guiding principles from nature in order to provide a basis for creating technological devices. Biomimetic membrane design takes cues from the self-assembly of lipids or other amphiphilic molecules into bilayer membranes and from the

rich repertoire of functions displayed by biomembrane embedded proteins.[4] The overwhelming complexity of a biological membrane with hundreds of lipid species[7] and extensive coupling between membrane components and cytoskeletal elements[8,9] have to some extent been an obstacle in understanding membrane function e.g., the reciprocal coupling in lipid-protein interactions, which means that the bilayer can regulate protein function and vice versa.[10,11] Nevertheless, by combining only a few biomembrane components several biosensors based on biomimetic membrane designs have successfully been developed (for a review see ref. 4).

Since the first reports on formation of bimolecular lipids extensive investigations of the physical properties of these films have provided a detailed picture of bilayer cohesive, elastic and structural properties.[12] At the same time techniques were developed for the reconstitution of proteins starting with incorporation of excitable protein material (voltage gated ion channels) over the first demonstration of single channel activity to the current plethora of reconstituted membrane proteins including receptors, ion channels and transporters.[13] With the introduction of microfluidic chip designs, formation of micron-sized membranes is now possible[14-18] and automation strategies enabling the creation of very large membrane arrays have recently been demonstrated.[19-21]

Recent developments in polymer research have resulted in the concept of polymer membranes formed from amphiphilic copolymers with barrier properties similar to lipid bilayers. Also, protein reconstitution in polymer membranes has been demonstrated.[5,22,23]

Sensor Applications

In most applications of biomimetic membrane biosensors, the membrane serves as a passive matrix for the embedded proteins where the protein typically a receptor can sense a signal and somehow transduce the information. Biosensors based on ion channels, where controlled flow of selected ions constitutes part of the mechanism, can sense low ionic concentrations as comparably few ions are needed to generate pA currents, which are easily detectable using standard voltage-clamp electrophysiological methods.[24] In either case, the biosensors do not require massive flux of matter across the membrane in order to function. Therefore, the membrane matrix can be supported on solid micro-patterned supports such as the smooth surfaces of cleaved mica, silicon or carbon nanotubes either directly or via an intermediate noncovalently bound or covalently tethered cushion for increased stability and portability.[25-32]

The application of supported membranes in the design of biosensors mounted on electro-optical devices is attracting considerable interest. One such example is surface plasmon resonance (SPR) a method that allows for real-time measurements of ligand binding to immobilized proteins[33] and thus opens for the possibility to detect ligand binding to membrane bound (both membrane spanning and membrane adsorbed) proteins. Immunosensing can be seen as a special case of ligand binding sensing. *Staphylococcus* enterotoxin B (SEB) has been detected in milk via a microfluidic system with supported bilayer membranes and biotinylated anti-SEB IgG.[34] A further strategy is to take advantage of the electrical properties of bilayers and use them as insulating surfaces. Any defect in this surface is easily detectable as a change in impedance and as the defect locations create strong nonspecific binding sites, the sensitivity of such a device is high.[35] Impedance analysis on supported lipid bilayers can also be used to dissect the action of channel forming peptides e.g., the bee venom melittin,[36] the potassium specific valinomycin[37] and channel forming proteins e.g., the bacterial outer membrane porin Omp F on the bilayer.[38] This approach has also been used in lipid films where protein-driven energy transduction was realized by incorporation of bacteriorhodopsin (BR) and cytochrome c oxidase (COX)[39] into supported polymer membranes. Recently, approaches based on interconnected inverted micelles formed in two-phase systems (e.g., electrolyte and lipid-alkene solution as the bulk phase) have resulted in very stable systems seemingly well suited for medium- to high-throughput screening of membrane proteins.[40,41] This approach has been extended to simply make the bilayer from an electrolyte drop falling though the lipid-alkane solution with the resulting inverted micelle landing on a lipid monolayer surface formed at the aperture, thereby separating the alkane lipid phase from a lower electrolyte solution and enabling easy buffer exchange and electrical measurements.[42]

Separation Applications

The use of biomimetic membranes in separation applications requires a different approach. Even though a cushioned membrane (see above) on a solid support would have space for some transport on the cushion side, the demand for massive flux across the biomimetic membrane for separation precludes the use of nonporous support material. The extreme alternative—a free standing lipid bilayer[12] with incorporated MIPs (or ion channels) formed across an aperture—is not sufficiently stable to be used in a technological separation device. For planar membranes, this implies that the support material must be sufficiently porous in order to allow substantial vectorial flow. At the same time, the support material needs to be sufficiently dense to support the membrane in the presence of transmembrane pressure gradients. An early attempt to stabilize biomimetic membranes on porous supports included the use of polycarbonate and nitrocellulose filters in various protein reconstitution experiments (for a review see ref. 13). Although protein function could be demonstrated, the stabilization was in fact realized as an impregnation of the support with a lipid-hydrocarbon solvent resulting in lipid structures with complex water transport properties and consequently limited control over non-electrolyte passage. However, recent advances in membrane encapsulation methods and support material development open the possibility of implementing efficient biomimetic separation processes in the form of long-term stabilized membranes with embedded proteins on an industrial scale. Interesting developments in membrane encapsulation have been demonstrated using bacterial S-layer proteins[43-45] or hydrogels[46-48] and porous polymers[49] may be used as porous support material for stabilization of biomimetic membranes.

Many issues are still not resolved. Simple model proteins notably β-barrel proteins or small peptides are inherently more stable than α-helical bundle proteins. Insertion and stabilization of the latter class of proteins which includes all MIPs and most of the ligand- and voltage-gated ion channels still poses many problems which must be resolved before large scale protein containing membranes can be produced and stored on a commercial basis. Thus a biomimetic device will have to meet design criteria based on both protein-based requirements as well as the requirement for well-defined and stable barrier properties. In addition the protein-matrix complex must be encapsulated in such a way that the encapsulation process does not compromise protein structure and function.

MIP Biomimetic Membranes and Osmotic Processes

MIP Basic Properties

Chapters in this book as well as several recent reviews have nicely summarized many fascinating aspects of MIP structure and function.[50-54] In this section I will present only the basic properties and discuss the permeability properties pertaining to biomimetic water/metalloid transporting membranes. MIPs constitute a family of 24-30 kDa pore forming integral membrane proteins. Since the purification of a red blood cell membrane protein: channel-forming Integral membrane Protein of 28 kDa (CHIP28)[55] and subsequent expression of this protein in Xenopus oocytes[56] and liposomes[57] revealing rapid water diffusion along osmotic gradients much has been revealed about this class of proteins for which the term *aquaporins* soon was coined.[58] Thirteen mammalian homologs (denoted AQP0-AQP12) are now known[59] and in total more than 450 members of the MIP family have been identified to date.[60]

The primary sequence of AQP1 cDNA reveals the canonical MIP structure in the form of two tandem repeats each containing three transmembrane spanning α-helices (TM1-3). Each tandem repeat contains a loop between TM2 and TM3 with an asparagine-proline-alanine (NPA) signature motif. Biochemical analysis and later crystal structures revealed an hour-glass structure with pseudo two-fold symmetry where the six TM segments surround a central pore structure defined by the two opposing NPA motifs (for a structural and chronological review see 50). Each six TM AQP unit functions as a pore and the predominant unit-assembly in biological membranes is a tetrameric arrangement.[61] Higher order arrays (aggregates) have been described for AQP4.[62,63] For AQP0 coaxial octamers formed by two juxtaposed tetramers have been proposed.[64]

Based on their permeability properties, mammalian homologs can be classified into two groups: aquaporins and aquaglyceroporins. The *Escherichia* coli model system offers both variants:[65] the orthodox (i.e., 'water only') channel AqpZ[66] and the aquaglyceroporin GlpF.[67] Although some MIPs can be classified as strict water channels (e.g., AQP0, APP4 and AqpZ), it is becoming increasingly clear that many MIPs may have additional permeability properties.[53] For example the nonglycerol transporting AQP1 may transport cations[68] as well as carbon dioxide,[69] nitric oxide[70] and ammonia[71] and AQP6 may transport chloride at low pH.[72] It should, however, be stressed that cation/anion transport by MIPs is an exception. The water and glycerol transporting AQP7, AQP9 and GlpF may also transport metalloids e.g., hydroxylated forms of arsenic and antimony;[73] and GlpF transports urea and glycine in addition to glycerol.[74,75] While the implications of these observations are still under debate,[53,76-78] there is clear evidence that the physiological role of some MIPs stems from both their metalloid and water permeability properties.[78]

In addition to the apparently complex permeability profile, several MIPs display various forms of gating—analogous to the opening and closing of ion channels induced by external stimuli; for ion channels typically in the form of transmembrane potential and/or chemical signals. Although many aspects of aquaporin gating and regulation of their permeability are still unknown, the function of some MIPs has been demonstrated to depend on calmodulin[79,80] phosphorylation[81,82] and pH.[79,83,84]

The application of MIPs and other helical bundle integral membrane proteins (e.g., ion channels) in a biomimetic separation membrane depends on controlling protein stability. Membrane proteins are inherently unstable and may require modifications to ensure effective insertion of stable constitutively active proteins.[85] In addition, the interaction (hydrophobic coupling) between the protein and the biomimetic membrane can affect protein stability and conformational equilibrium.[11,86] In the design of a biomimetic water filtration membrane which is based on MIPs, the issues of protein oligomerization, aggregation, selectivity, regulation and stability must be resolved in order to ensure efficient protein-mediated water filtration.

Considerations Regarding Permeability

A fundamental question to be addressed in the development of biomimetic membrane devices is how much transport is mediated by the proteinaceous pathways inserted into the membrane matrix and how much transport it mediated by the membrane matrix itself? A general answer to this question depends on the solute, the transporting protein and the structure of the support material. Although the phenomenon seems quite simple: solutes and water move in response to transmembrane gradients (electrochemical, hydrostatic and osmotic gradients), the intricate mechanistic details of protein and membrane selectivity have remained a major obstacle for understanding biological membrane transport for more than a century.

The simplicity of the MIP structure as outlined above is only apparent. The structure is the basis of the remarkable ability to transport water molecules and simultaneously reject charged species including protons. Molecular dynamics (MD) simulation based on the experimental structure of AQP1 strongly suggests single-file transport of water molecules through a narrow <3Å pore in which steric and electrostatic factors prevent electrolyte passage.[87] MD simulations have provided values for single channel water osmotic permeabilities p_f. Experimental values for p_f have also been obtained for several MIPs (see Table 1). Theoretical (MD) p_f values may not be accurate as they to some extend depend on the choice of MD force fields. Experimentally obtained single channel values are also characterized by some uncertainties due to the difficulty in quantifying the number of channels in a given preparation. Nevertheless the values listed in Table 1 obtained for various MIPs provide a quantitative basis for designing biomimetic MIP membranes for water separation/purification purposes.

Over the last decade it has become increasingly clear that some MIPs e.g., several plant Nodulin-26-like Intrinsic Proteins (NIPs) are specifically involved in the regulated uptake and distribution of selected metalloids, e.g.,[88-91] (reviewed in refs. 78,92,93). From a separation perspective it is interesting that some NIPs from the thale cress (*Arabidopsis thaliana*) transport boric acid $B(OH)_3$ while possessing a low water permeability (AtNIP5; 1)[89] or no water permeability at all (AtNIP6;1).[90] Also some rice (*Oryza sativa*) NIPs have metalloid transport properties:

Table 1. Aquaporin single channel (subunit) osmotic water permeability constants

	p_f $10^{-14}cm^3/s$	Method	Reference
AQP0	0.25	Xenopus Oocytes	135
	0.2	MD[c]	136
AQP1[a]	4.6	Proteoliposomes	137
	5.43	Proteoliposomes	138
	6	Xenopus Oocytes	135
	11.7	Proteoliposomes	57
	10.3	MD[c]	136
AQP2	3.3	Xenopus Oocytes	135
AQP3	2.1	Xenopus Oocytes	135
AQP4	24	Xenopus Oocytes	135
	7.4	MD[c]	136
AQP5	5	Xenopus Oocytes	135
AqpZ	2	Planar bilayers	139
	>10	Proteoliposomes	140
	15.9	MD[c]	136
GlpF	0.7	Planar bilayers	141
	16	MD[c]	136

[a]Also known as Major Intrinsic Protein (MIP); [b]Also known as CHannel forming Integral Protein of 28 kDa (CHIP28); [c]Based on a channel length of 16Å.

OsNIP2;1 not only transports $B(OH)_3$ but also silicic acid $Si(OH)_4$, urea and $As(OH)_3$ (see below) and OsNIP3;1 is a boric acid channel required for efficient growth under limited boron conditions.[94] Antimonite $Sb(OH)_3$ is transported by AtNIP7;1[95] and the *E.coli* GlpF.[88]

Of particular interest is the transport of arsenic via MIPs. The toxic and carcinogenic arsenic[96,97] occurs in natural aqueous environments in +5 and +3 oxidation states. The most common inorganic arsenic compounds found in water are arsenite As(III) and arsenate As(V).[98] Arsenic may be methylated[99] as monomethylarsonic acid MMA(V), monomethylarsonous acid MMA(III), dimethylarsinic scid DMA(V), dimethylarsinous acid DMA(III) and trimethylarsine oxide TMAO. As(III) exists in reduced waters (low oxygen tension) and species may include H_3AsO_3 ($As(OH)_3$) (pK = 9.23), $H_2AsO_3^-$ (pK = 12.13) and $HAsO_3^{2-}$ (pK = 13.4).[100] At neutral pH As(III) is present as $As(OH)_3$.

$As(OH)_3$ is transported by the mammalian aquaglyceroporins AQP7 and AQP9,[73,101] the aquaglyceroporin homologue AqpS from *Sinorhizobium meliloti*,[102] OsNIP1;1[103] and OsNIP2;1.[104] Also GlpF has been suggested as the basis for $As(OH)_3$ uptake in *E. coli*.[105] The closely related antimonite $Sb(OH)_3$ is also transported by AqpS,[102] AQP9[73] and GlpF.[105] The overlap between $As(OH)_3$, $Sb(OH)_3$ and glycerol transport capability points to some shared properties important for permeation. This is also illustrated by the fact that the methylated form MMA(III) is in fact transported more efficiently than As(III) by rat AQP9.[106]

When compared, $As(OH)_3$, $Sb(OH)_3$ and glycerol share significant physico-chemical properties.[107] $As(OH)_3$ and $Sb(OH)_3$ both adopt a trigonal pyramidal structure with the metalloid atom at the apex[107,108] and glycerol may adopt a 'retracted' configuration in which the two carbons

Table 2. Permeability coefficients for protein-free phosphatidyl choline (PC) membranes

	Permeability Coefficeint cm/s	Remarks	Reference
Water	$4\ 10^{-3}$		115
	$2\ 10^{-3}$	PC:Chol 1:1	115
	$7.5\ 10^{-4}$	PC:Chol 1:8	115
	$8\ 10^{-5}$	ABA BPM[a]	5
Urea	$4\ 10^{-6}$		142
Glycerol	$5.4\ 10^{-6}$		142
Tetraphenyl-phosphonium (TPP^-)	10^{-7}		143
Na^+	10^{-14}		144
Cl^-	10^{-11}		144
H^+/OH^-	10^{-4}-10^{-8}		145
	10^{-9}	PLFE vesicles[b]	118

[a]$PMOXA_{15}$-$PDMS_{110}$-$PMOXA_{15}$ triblock copolymer; [b]PLFE Polar lipid fraction E from *Sulfolobus acidocalarius* membranes.

share the apex position and the three oxygen atoms defining a triangular surface plane with an area comparable to the corresponding area in $As(OH)_3$ and $Sb(OH)_3$ (~3.2-3.7 $Å^2$). The molecular volumes of $As(OH)_3$, $Sb(OH)_3$ and 'retracted' glycerol are similar (98-118 $Å^3$) which is consistent with all three molecules meeting the same steric constraints during the passage through an AQP. However it should be pointed out that the dipole moment of 'retracted' glycerol (4.07 Debye) is larger than for the metalloids with 2.32 and 2.15 Debye for $As(OH)_3$ and $Sb(OH)_3$, respectively, whereas the hydration energies are higher for the hydroxylated metalloids (~117 kJ mol^{-1}) than for 'retracted' glycerol (~84 kJ mol^{-1}).[107] This illustrates the complex interactions involving water and protein moieties (e.g., carbonyls) between the permeating molecules and the channel. From a biomimetic point this also illustrates the motivation to learn from biological structures and construct de novo channel structures with finely tuned permeation properties based on coordination between the permeating molecules and the proteins.[109,110]

Water, non-electrolyte and electrolyte permeabilities in protein-free lipid bilayers have been investigated intensely over the last 40 years. The permeability for water has been determined both by tracer diffusion measurements yielding the diffusional membrane permeability P_d and by osmotic flow measurements giving the osmotic membrane permeability P_f. Early experimental work reported that $P_f > P_d$ (e.g.,[111]) which could suggest a pore based (single-file) transport mechanism,[112,113] but this is an artifact due to unstirred layers (see also below). There is no evidence for aqueous single-file transport in protein-free bilayers (for a comprehensive review see ref. 114). P_f is a function of bilayer composition.[115] Thus, increasing the cholesterol content decreases the water permeability (see Table 2). Permeabilities for non-electrolytes have always been determined in diffusion measurements[116] and are therefore potentially erroneous due to unstirred layers. Generally, however, permeabilities measured for non-electrolytes are lower than those for water. In general, the barrier for hydrophilic solutes represented by the membrane interior while the barrier for hydrophobic solutes will be the membrane interfacial regions.[116] The high electrical resistance of lipid bilayers is reflected in its very low permeability coefficients for electrolytes i.e., small inorganic anions and cations (see Table 2). The electrolyte permeability can be affected by

amphiphilic anesthetics where physiologically relevant concentrations may increase potassium permeabilities 2-5 fold.[117] Also the membrane structure is important, both ether linked lipids and natural hydrocarbon solvents in the membranes can affect proton permeability.[4,118]

The interaction of arsenic compounds with lipid membranes has been investigated and it appears that thermodynamic parameters for lipids with ethanolamine headgroups change upon exposure to arsenic compounds while choline headgroup lipids are unaffected.[119,120] The effect seems to be related to both direct interactions between polar moieties of the arsenic compounds and the lipids and indirect modification of the interface via changes in water bonding structure in the lipid headgroup region.[121] The water-octanol partitioning, log *P* which is a measure for molecular hydrophobicity[122] and thus partitioning into the lipid bilayer, reveals that methylation is an important factor for bilayer perturbation induced by arsenic compounds. Both As(III) ($\log P < -3$) and As(V) ($\log P < -7$) are highly hydrophilic compared to DMA(V) ($\log P = 0.36$).[120] Thus in biomimetic designs involving separation/sensing arsenic compounds the log *P* (and thus potential bilayer permeation) must be considered in addition to the MIP permeation profiles per se.

Osmotic Processes

The large ratios between MIP-mediated water permeabilities and bilayer ionic permeabilities suggest that it should indeed be possible to construct an osmosis-based biomimetic membrane. MIP-mediated osmoregulation is vital to all living organisms (for recent reviews see refs. 123,124). In pure terms 'osmosis' describes the selective movement of water from regions with high water chemical potential (i.e., a dilute salt solution) to regions with low water chemical potential (i.e., concentrated salt solution). In water treatment/purification this process is typically reversed and realized using RO membranes as mentioned above. RO uses hydraulic pressure to oppose and exceed the osmotic pressure of an aqueous feed solution. Thus in RO the driving force is the applied pressure, whereas in osmosis the osmotic pressure is the driving force. A third osmotic process is called forward osmosis (FO) sometimes also referred to direct osmosis.[125] FO is based on using a draw solution on the permeate side of the membrane as the driving force. The force arises as the draw solution has a higher osmotic pressure than the feed solution. The main advantage of FO is that the hydraulic pressure is low (or even zero). This implies that the supported biomimetic MIP membrane will not be subjected to as high pressures as would be required in typical RO applications and the feed solution is treated 'gently'. In addition the fouling propensity (i.e., the clogging of the membrane) is reduced. Finally, pressure retarded osmosis (PRO)[126] is emerging as a promising process in power generation. PRO uses the osmotic pressure difference between seawater and fresh water to pressurize the saline stream thereby converting the osmotic pressure difference into a hydrostatic pressure capable of driving a turbine.[127]

All of the above mentioned osmotic processes can be described using the relation between volume (water) flux $\overline{J}$ [$cm^3 s^{-1}$] across a membrane separating aqueous phases 1 and 2 due to osmotic and pressure driving forces:

$$\overline{J}_v = P_f A_M V_w / RT[(P_1 - P_2) + \sum_i \sigma_i(\Pi_{2i} - \Pi_{1i})], \quad (1)$$

where P_f is the membrane permeability [cm s^{-1}], A_M membrane area [cm^2], V_w the partial molar volume of water [$cm^3 mol^{-1}$], P the pressure [Pa], RT the product of the molar gas constant R [JK^{-1} mol^{-1}] and temperature T [K], σ_i the reflection coefficient for the i'th solute and $\Pi = \phi RTC$ is the van't Hoff osmotic pressure [Pa] where ϕ is the molar osmotic coefficient and C the concentration.

Let $J_v = \overline{J}_v / A_M$ [cm s^{-1}] be the volume flux per unit area. In the case of a pressure difference only (i.e., no concentration difference)

$$J_v = P_f V_w (P_1 - P_2)/RT = L_p (P_1 - P_2), \quad (2)$$

where $L_p = P_f V_w / RT$ [$cm^3 N^{-1} s^{-1}$] is the hydraulic permeability of the membrane. Thus we may rewrite

$$J_v = L_p[(P_1 - P_2) + RT \sum_i \phi_i \sigma_i (C_{2i} - C_{1i})]. \quad (3)$$

In the case of a concentration difference only (i.e., no pressure difference)

$$J_v = L_p \cdot RT \cdot \sum_i \phi_i \sigma_i (C_{2i} - C_{1i}) = P_f \sum_i \phi_i \sigma_i (C_{2i} - C_{1i}), \quad (4)$$

where $P_f^* = P_f \phi_i \sigma_i$ is the osmotic permeability of the membrane for the *i*'th solute. In the case of an ideal semi-permeable membrane ($\sigma_i = 1$) separating two ideal solutions ($\phi_i = 1$), the osmotic permeability is equal to the membrane permeability and P_f is sometimes in the literature also referred to as the osmotic permeability of the membrane.

Equation (1) can be simplified to describe water transport across an ideal membrane as

$$J_w = L_p(\sigma\Delta\Pi - \Delta P) \quad (5)$$

From this expression we can now directly relate the various osmotic processes to relations bet-ween ΔP and ΔΠ. For FO ΔP = 0, for RO ΔP > ΔΠ and for PRO ΔΠ > ΔP (see Fig. 1A,B).

In principle Eqns 1-5 constitute the very basis for osmotic driven water flow through a selectively water permeable membrane.

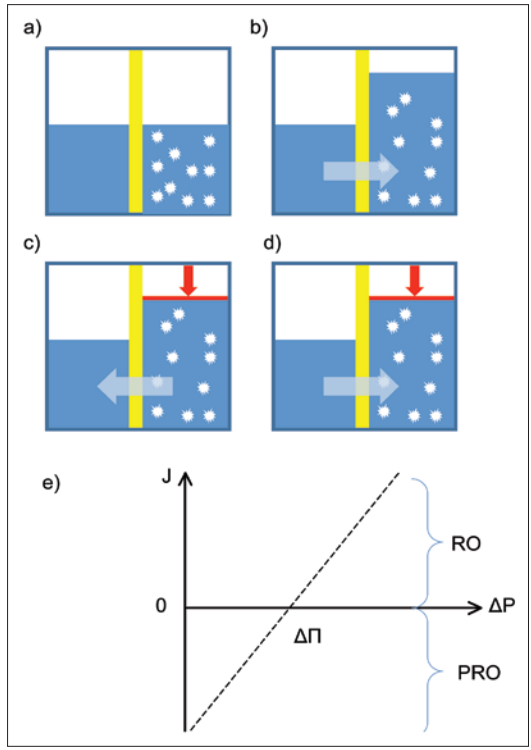

Figure 1. Osmotic membrane processes (after ref. 125). Solvent flow (A) initial condition with water impermeable membrane**.** (B-D): Flow through an ideal semipermeable (biomimetic aquaporin) membrane. B) Forward osmosis (FO), water moves into the concentrated solution. C) Reverse osmosis (RO), water is convectively moving into the diluted solution driven by pressure. D) Pressure retarded osmosis. (PRO): water moves as in (A), but whereas the movement is retarded by external pressure, it still moves into the concentrated solution (from high to low water potential). E) Direction of flow in FO, RO and PRO. For FO there is no hydraulic pressure across the membrane. For RO the applied pressure must exceed the osmotic pressure and convectively drive the water from low to high water potential. For PRO the hydraulic pressure retards the FO process but the hydraulic pressure applied is still less than the osmotic pressure.

Combining Eq (5) with the equation for solute flux J_s:

$$J_s = \omega RT(C_2 - C_1) + J_w(1-\sigma)\bar{C}, \quad (6)$$

where ω is the solute permeability coefficient and $\bar{C}$ is the average concentration of the two solutions on either side of the membrane, we arrive at the Kedem-Katchalsky equations for coupled water and solute movement (eqns 5-6).[128,129] The first term in (6) represents the concentration driven solute movement and the second term describes the solvent drag of the respective solute. The latter as σ describes both the efficacy of the solute to induce the osmotic flow water movement and the drag of the solvent induced by volume movement.

Polarization Effects

The Kedem-Katchalsky framework describes transmembrane transport of homogeneous solutions. Experimentally this can be realized by vigorous mechanical stirring of solutions. Lack of stirring causes formation of concentration boundary layers or unstirred layers as coined by Walther Nernst in 1904[130] on both sides of the membrane, directly adjacent to the membrane surface. The thickness of these layers depends on concentration, density, viscosity and temperature, on diffusive permeability coefficients of the membrane and Rayleigh numbers. This implies that the extent of unstirred layers will have direct influence on the volume fluxes passing across the membrane.

For membranes separating two aqueous solutions there will always be a region of incomplete mixing[131] and for most biological membranes there is only very little stirring at membrane solution interfaces. In the original (oversimplified) approximation by Nernst the unstirred layer was considered as a region with diffusion only and thus no convection. Recently, a more accurate hydrodynamic model for simultaneous diffusion and convection has been described.[132,133] A test of this model has been realized using ion-selective electrodes where the polarization was directly measured in the presence of an osmotic gradient consistent with the hydrodynamic model.[134] For biomimetic MIP membranes the polarization arises as a consequence of water flow through the MIPs. The ions are rejected and build up a concentration gradient in the unstirred layer on the hypotonic side relative to bulk ion concentrations.

The practical realization of the biomimetic membrane requires porous support materials on one or both sides of the biomimetic support matrix. This implies that the solvent (water) filled porous support will effectively act as an unstirred (and hence polarized) layer thereby affecting transmembrane fluxes (see Fig. 2).

This concentration polarization occurs on both sides of the membrane so for example in RO an increased osmotic pressure occurs on the feed side that must be overcome with hydraulic pressure. Alternatively in FO a dilution occurs on the draw side reducing the osmotic driving force. Both cases are examples of external concentration polarization. When the membrane is asymmetrical (see Fig. 2B,C) also internal concentration polarization can occur. These issues must be addressed in any future development of biomimetic MIP membranes, but in principle these issues are not unique to biomimetic membranes. In this respect the biomimetic membrane can be seen as the equivalent to the active layer in conventional RO membranes and the porous support as the equivalent to the support layers in RO membranes (see Fig. 3).

Conclusion

The many outstanding challenges in creating stable, selectively permeable and functional biomimetic membranes for sensor/separation purposes may seem daunting and even discouraging. On the positive side, however, are the recent scientific and technological advances discussed there. First, our increased understanding of the unique permeability properties for MIPs, notably a very high water permeability for some of the orthodox aquaporins and the complex permeation profiles for some MIPs e.g., selectivity for metalloids. Second, the recent progress in membrane stabilization methods opens for the possibility to create membranes with long-term stability. By merging insights from both MIP research and biomimetic membrane design, many potential applications can be envisaged, not only in water purification, but also in sensor devices built on the concept of up-concentrating selected permeants.

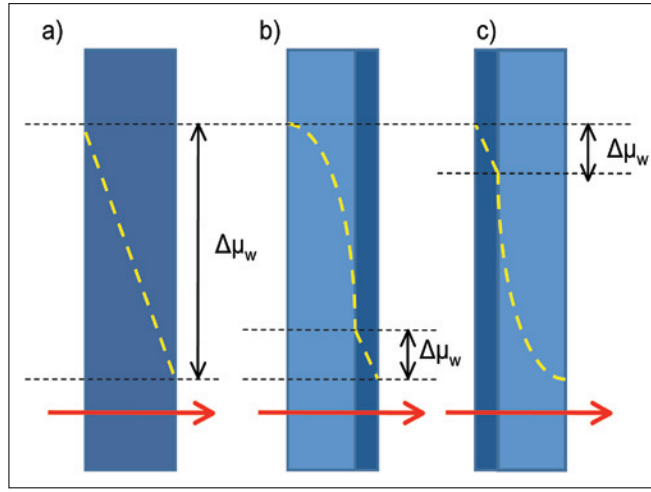

Figure 2. Polarization effects for symmetric and asymmetric membranes. A) In a symmetrical membrane with no unstirred layers osmosis is driven by the full water potential $\Delta\mu_w$. In asymmetrical membranes (e.g., with a biomimetic membrane (dark blue) as the active layer on a porous support (light blue), the effective water potential is changed due to polarization concentration profiles in the unstirred water layer wetting the porous support. Thus in (B) the salt concentration increases up to the active layer, whereas in (C) the salt concentration decreases up to the active layer. A color version of this image is available at www.landesbioscience.com/curie.

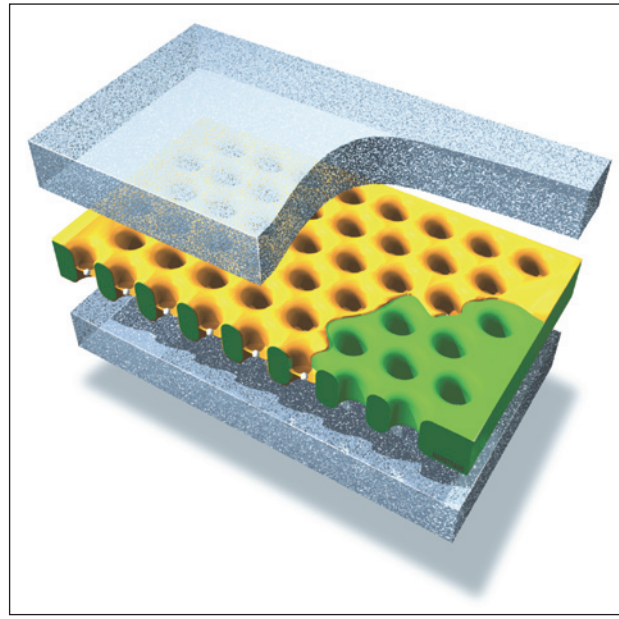

Figure 3. Principal sketch of a biomimetic aquaporin membrane. The aquaporins (white) are embedded in a biomimetic matrix (yellow) formed across a partition (green). Encapsulation material (grey) must be able to support the membrane without compromising transmembrane flow of water. A color version of this image is available at www.landesbioscience.com/curie.

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