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Desalination by biomimetic aquaporin membranes: Review of status and prospects

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HIGHLIGHTS

► Aquaporin proteins may be used in biomimetic membranes for water treatment.
► We discuss the challenges in using aquaporin membranes for separation processes.
► We present various attempts to use aquaporins in membranes for desalination.
► We give an overview of our own recent developments in aquaporin-based membranes.
► We outline future prospects of aquaporin based biomimetic membranes.

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ABSTRACT

Based on their unique combination of offering high water permeability and high solute rejection aquaporin proteins have attracted considerable interest over the last years as functional building blocks of biomimetic membranes for water desalination and reuse. The purpose of this review is to provide an overview of the properties of aquaporins, their preparation and characterization. We discuss the challenges in exploiting the remarkable properties of aquaporin proteins for membrane separation processes and we present various attempts to construct aquaporin in membranes for desalination; including an overview of our own recent developments in aquaporin-based membranes. Finally we outline future prospects of aquaporin based biomimetic membrane for desalination and water reuse.

Contents

1. Introduction

Synthetic membranes have come a long way in the 50 years since the invention of the cellulose acetate reverse osmosis (RO) desalination membrane by Loeb and Sourirajan [1]. State-of-the-art synthetic membranes at optimal conditions [2] can now desalinate sea water with an energy demand about 15–20% of that used for the early (RO) membranes. However this is still 1.5 to 2.0 times the minimum energy dictated by thermodynamics [3]. Consequently, there is a continuing quest for membranes with improved performance to provide better separations at even lower energy demand.

In a thought-provoking paper in 2006 Bowen [4] discussed how we could learn from biological membranes in the development of

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membranes that benefit from biomimicry to achieve better selectivity and higher permeability. Shortly after that Kumar et al. published a paper that proposed the idea of incorporating aquaporin properties into desalination membranes [5]. Aquaporins are pore-forming proteins and ubiquitous in living cells. Under the right conditions they form 'water channels' able to exclude ionic species. In a series of simple characterization experiments Kumar showed the exceptional water permeability of aquaporins and extrapolated his observations to postulate desalination membranes with vastly improved performance. In a recent review [6] of membrane nanotechnologies, bio-inspired membranes, such as aquaporin-based, were judged to offer the best chance for revolutionary performance but were also seen as the furthest from commercialization. In fact there has been a surge of activity in the last half-decade attempting to develop practical biomimetic desalination membranes incorporating aquaporins, and it is timely to review the status of this new direction in desalination.

In this paper we first review the properties of aquaporins, their preparation and characterization. We then review the various attempts to exploit the remarkable properties of aquaporin in membranes for desalination; including an overview of our own recent developments in aquaporin-based membranes. Finally we discuss future prospects of this type of biomimetic membrane for desalination and water reuse.

2. Aquaporins: special properties, characterization and means of production

Several recent reviews have nicely summarized many fascinating aspects of aquaporin protein structure and function [7–11]. Here we will present only the basic features and discuss the permeability properties pertaining to biomimetic water transporting membranes. Aquaporins 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) [12] and subsequent expression of this protein in *Xenopus* oocytes [13] and liposomes [14] revealing rapid water diffusion along osmotic gradients, much has been discovered about this class of proteins for which the term *aquaporins* soon was coined [15].

The canonical aquaporin sequence reveals two repeats each containing three transmembrane spanning α–helices (TM1-3), see Fig. 1. Each repeat contains a loop between TM2 and TM3 with an asparagine–proline–alanine (NPA) signature motif. The aquaporin protein folds as an hour-glass-shaped structure where the six TM segments surrounds a central pore structure defined by the two opposing NPA motifs, see Fig. 1a and b. (for a structural and chronological review see [7]). A conserved aromatic/arginine (ar/R) region defines a constrict-ion site or selectivity filter — the narrowest part of the channel lumen. Each six TM AQP unit functions as a pore and the predominant unit-assembly in biological membranes is a tetrameric arrangement [16], see Fig. 1c and d. Based on their permeability properties mammalian homologs can be classified into two groups: aquaporins and aquaglyceroporins. The *Escherichia coli* model system offers both variants [17]: the orthodox (i.e. 'water only') channel AqpZ [18,19] and the aquaglyceroporin GlpF also permeable to glycerol [20]. Although some can be classified as strictly water channels (e.g. AQP0, AQP4, and AqpZ), it is becoming increasingly clear that many aquaporins may have additional permeability properties [10]. In addition to the apparently complex permeability profile, several aquaporins display various forms of gating, e.g. as in [21] — analogous to the opening and closing of ion channels induced by external stimuli. Although many aspects of aquaporin gating and regulation of their permeability are still unknown, the function of some aquaporins has been demonstrated to depend on calmodulin [22,23], phosphorylation [24,25], and pH [22,26,27].

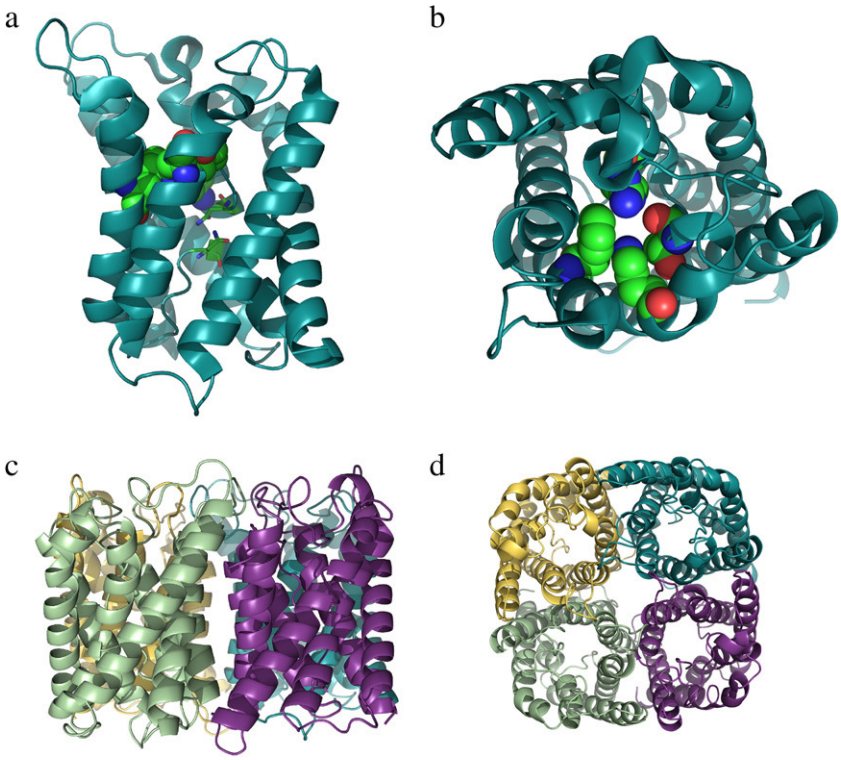

Fig. 1. Aquaporin protein structure. (a): Sideview of AqpZ monomer. Protein backbone (deep teal) with the two terminal asparagines from the NPA motifs shown in stick representation and the ar/R selectivity filter residues shown in spacefill representation. For stick and spacefill representations atoms are colored as carbon (green), oxygen (red) and nitrogen (blue). (b): Top view illustrating the selectivity filter (or constriction site) created by the four amino acids: F43, H174, R189 and T183. (c–d): Side and top view of the tetrameric AqpZ complex with the four monomers shown in deep teal, violet purple, pale green, and yellow. All renderings were generated using PyMol 1.5.0.2 using AqpZ PDB coordinates 2ABM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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2.1. Functional characterization

Water permeability and solute rejection of single aquaporins is not easily measured. Molecular dynamics simulations of aquaporins reveal diffusional water permeabilities corresponding to the transport of 10^8 to 10^9 water molecules/s [28]. In terms of the number of transported molecules this is about an order of magnitude higher than for typical ion channels where single channel pA currents on a ms time scale corresponds to the transmembrane displacement of ~10^7 ions [29]. While currents in the pA range are measurable by standard patch-clamp methods, the movement of 10^8 to 10^9 water molecules is not experimentally accessible by current methods. However the macroscopic transport mediated by an ensemble of aquaporins is measurable. Then by measuring osmotic transport arising from a large (known) number of aquaporins, single aquaporin permeabilities can be estimated. Two methods are currently used in this respect: *Xenopus* oocyte volume change and light scatter from proteoliposomes/proteopolymersomes.

In the *Xenopus* oocyte expression, frog oocytes (~1 mm diameter) are cytoplasmically injected with mRNA that has been transcribed in vitro from a cDNA clone [30]. In the case of aquaporins the resulting expression renders the oocyte membrane significantly more permeable to water compared to control oocytes [13]. Upon an osmotic challenge the oocyte will change size (diameter) and by employing small osmotic gradients for short periods of time (e.g. 2.5 mosM for 5 s) the transport parameters (water permeability and solute rejection) can be determined from the initial rate of oocyte volume changes in both swelling and shrinkage experiments [31].

Water permeabilities of proteoliposomes/proteopolymersomes can also be measured by detecting the light scattering of the preparations in a stopped-flow apparatus (see reference [32] and Fig. 2a). Thus if a suspension of aquaporin containing vesicles with initial diameters around 200 nm is rapidly mixed with the same volume of a hyperosmolar solution with membrane impermeant solutes (e.g. sorbitol, sucrose or mannitol) for proteoliposomes, the resulting transmembrane osmotic gradient will generate water efflux, and the consequent reduction in vesicle volume can be measured as an increase in the intensity of scattered light. The rate constant k of the normalized light intensity increase indicates the rate constant of water efflux, which is proportional to the water permeability coefficient. The light intensity increases exponentially as a function of k with time (Fig. 2b). The response from protein free controls is fitted to a single exponential whereas a double-exponential function is used for proteoliposomes/proteopolymersomes (vesicles) reflecting the dual pathways for water transport (membrane mediated and protein mediated). The k values can then be used to calculate osmotic permeability P_f :

$$P_f = \frac{k}{\frac{S}{V_0} \cdot V_w \cdot \Delta osm} \quad (1)$$

where S/V_0 is the surface area to initial volume ratio of the vesicle, V_w is the partial molar volume of water (18 cm^3/mol), and Δosm is the difference in osmolarity between the intravesicular and extravesicular aqueous solutions. Based on stopped-flow measurement, the water permeability of AqpZ is estimated to be in the range of $2–10\times10^{-14}$ cm^3/s [33–35], which is in reasonable agreement with reported molecular dynamics simulation results ($3–30\times10^{-14}$ cm^3/s).

2.2. Production

Until now, most recombinant aquaporins have been expressed only in lab-scale quantities for screening, functional, regulatory or structural studies [36,37]. One of the main obstacles in protein production is that membrane protein overexpression in vivo is hampered by their complex structure, hydrophobic transmembrane regions,

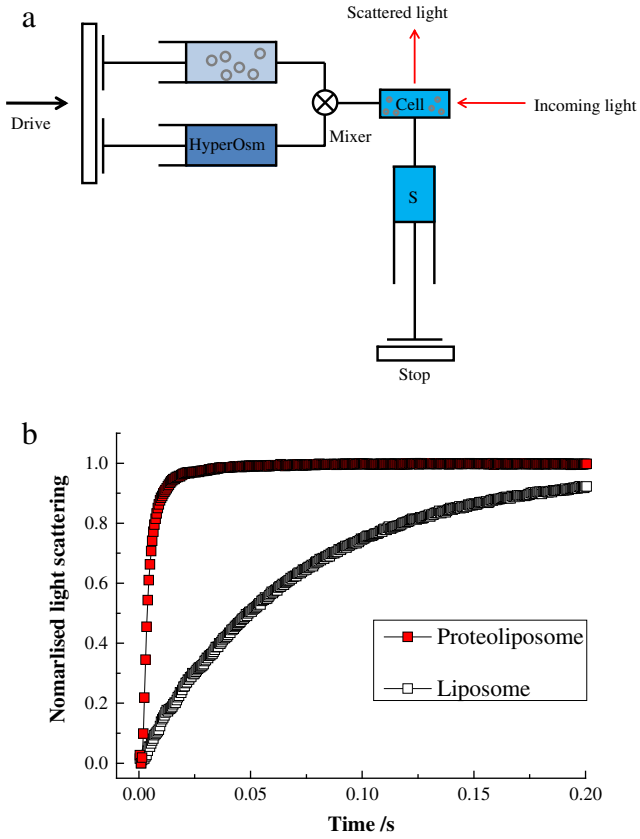

Fig. 2. Stopped-flow characterization. (a): Schematics of stopped-flow measurement; (b): Typical stopped-flow results for lipid vesicles with (i.e., proteoliposomes) and without aquaporin incorporated (i.e., liposomes).

host toxicity, and the time consuming and low efficiency refolding steps required. Recent developments of high-expression systems may however provide insights into how large-scale AQP production may be realized. These include *E coli*, *Saccharomyces cerevisiae*, *Pichia pastoris*, and baculovirus/insect cell based systems, for a recent review see [38].

E. coli expression methods providing milligram quantities of protein have been successfully employed to solve the X-ray structure

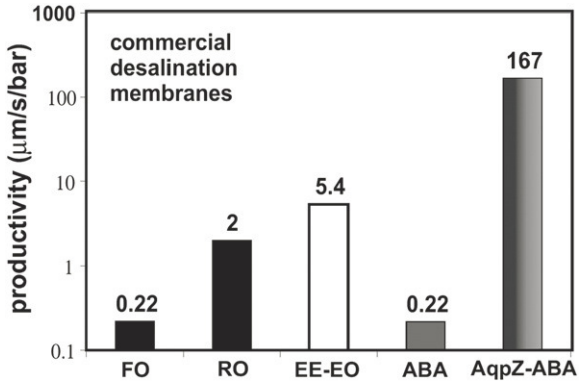

Fig. 3. Comparison water permeability of polymer vesicles with AqpZ (AqpZ-ABA) or without AqpZ (ABA) to those of polymeric membranes. FO is a commercial forward osmosis membrane; RO is a commercial reverse osmosis membrane, and EE-EO is a polyethylethylenepolyethylene oxide diblock polymer. Permission for reprint of figure will be obtained after the paper is accepted for publication.
Reproduced from Ref. [5].

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of the AqpZ and GlpF channels, AqpZ [19] and GlpF [20], as well as of the archaeal aquaporin AqpM [39]. High expression (200 mg/L) of the orthodox aquaporin AqpZ was recently achieved by the *E. coli* system using maltose binding protein (MBP) as fusion partner protein and subsequent condition optimization [5,40]. Also the *S. cerevisiae* system can be used to produce high amounts of functional aquaporins [36,41–45]. The methylotrophic yeast *P. pastoris* has been successfully employed to produce a large number of distinct aquaporins. These include all thirteen human aquaporins [46] as well as a range of active plant aquaporins [47–53]. Finally large-scale expression of many functional recombinant aquaporins has been achieved using the baculovirus/insect cell system [54–61].

Recent evidence suggests the possibility of high-level membrane protein expression using cell-free (CF) production. The key idea is to synthesize membrane proteins in the presence of natural or synthetic lipids and/or detergents that help solubilize the membrane protein. CF production of aquaporins has been demonstrated at analytical levels [37,62–64], and recently high expression of correctly folded AqpZ and a plant aquaporin have been obtained with *E. coli* CF protocols using different fusion vectors [65,66]. Milligram quantities of highly efficient AqpZ have been produced in synthetic liposomes by a CF approach [67]. The demonstration of cost effective cell-free protein synthesis in a 100-liter reaction by Sutro Biopharma Inc. [68] shows the potential of CF systems to become a powerful recombinant protein industrial scale production platform.

Once produced the (detergent stabilized) protein must be reconstituted into its host biomimetic membrane and this poses challenges for industrial upscaling: in terms of detergent stabilized intermediates where stability and detergent cost are main concerns [69,70]; in terms of optimizing the interaction between membrane and protein (c.f. [71,72]) and in terms of yield — i.e. how much functional protein can be incorporated in the final product (c.f. [73]).

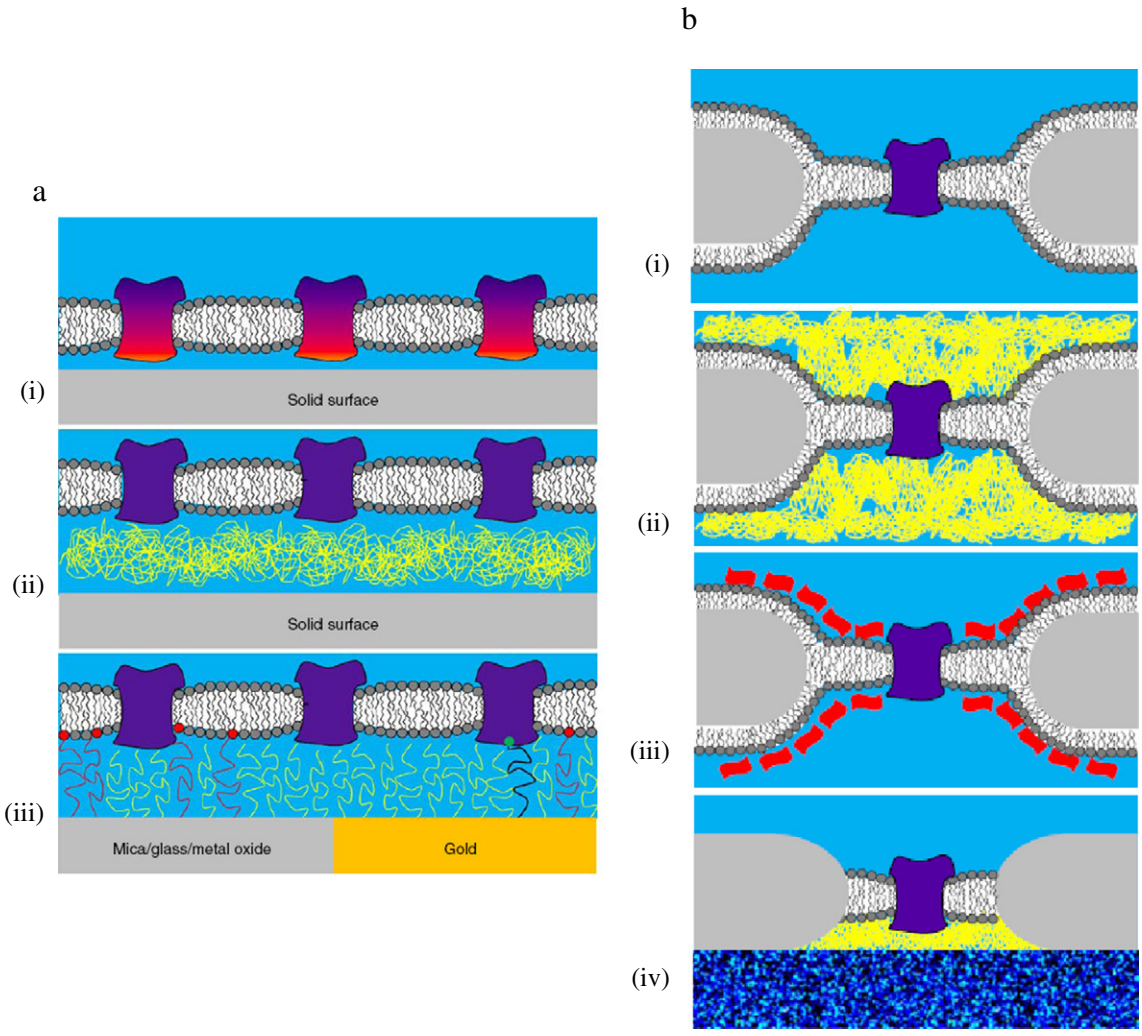

Fig. 4. Summary of existing designs of biomimetic membranes (Reproduced from Ref. [75] with permission). (a): Cross-sectional examples of solid-supported biomimetic membranes. (i) Direct deposit on a hydrophilic surface (light gray). This method may bring part of the integral membrane proteins (red protein shaded areas) embedded in the matrix formed from the self-assembly of lipids (dark gray molecules) too close to the surface, potentially inactivating (or even denaturing) the protein. (ii) Cushion-supported biomimetic membrane. Here a polymer forms a cushion between the support material. (iii) Layers grafted covalently on to the support using spacers with silane groups reacting with hydroxyl surfaces (light gray) or spacers with thiol groups bonding on gold surfaces (orange). Various hydrophilic spacers (e.g. poly(ethylene glycol) (PEG)) may be used as cushion material. This cushion can be non-covalently interacting with the biomimetic membrane (yellow spacers) or covalently attached to lipids (red lipid headgroups) or proteins (green bonds) in the biomimetic membrane directly or through intermediates e.g. biotin–avidin complexes. (b): Cross-sectional examples of porous supported biomimetic membranes with an embedded protein (blue). (i) Free-standing membrane formed across a (micro or nano) porous support. The membrane (solvent-free or solvent-containing) is formed in an aperture (light gray). (ii) Hydrogel-encapsulated biomimetic membrane. A hydrogel polymer meshwork (yellow) encapsulates the biomimetic membrane. (iii) A surface (S) layer-encapsulated membrane. The monomolecular layer of protein or glycoproteins (red) self-assembles into a two dimensional lattice creating identical pores 2–8 nm in diameter. (iv) A cushioned membrane on a porous support. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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3. Status of Aquaporin membrane development

The paper by Kumar et al. [5] suggested that membranes with very high permeability and salt rejection may be constructed based on aquaporin protein function. Based on the measured water permeability of AqpZ containing proteoliposomes, these authors postulated that AqpZ based biomimetic membranes can potentially achieve a membrane permeability as high as 167 $\mu m \cdot s^{-1} \cdot bar^{-1}$ (i.e., 601 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$, see Fig. 3), which is about two orders of magnitude more permeable compared to existing commercially available seawater RO membranes [74]. However a major issue still remained: since the membrane is constructed from nanoscale elements (the aquaporins) how is the biomimetic membrane scaled-up and stabilized to m^2 dimensions suitable for industrial applications [75]?

Several design strategies have recently been proposed, see Fig. 4. These include membranes established across multiple micron scale apertures either as free-standing lipid or polymer membranes [73,76–80], or as membranes stabilized by polymeric support materials [81,82]. Other approaches rely on nanoporous support material onto which membranes are deposited. These include charged lipid vesicle depositions onto commercially available nanofiltration membranes where the recipient surface was either cross-linked polyamide or sulfonated polysulfone both negatively charged at pH 7 [83]; rupture of aquaporin containing polymersomes on methacrylate functionalized cellulose acetate membranes [84]; detergent-stabilized His-tagged aquaporin added to monolayers with nickel-chelating lipids [85]; and proteopolymersome deposition onto polycarbonate track-etched substrates coated with gold and functionalized with photo-active acrylate groups [86]. It is also found in our recent work [87] that the use of applied pressure and spin coating enhances vesicular coating/fusion onto the substrate and that surface charge and hydrophilicity play a critical role in determining the quality of the supported lipid layer.

Table 1 summarizes the existing approaches of preparing aquaporin based biomimetic membranes. In general, most of the above-mentioned membranes have relatively low NaCl rejection (or rejection information not available), which does not allow them to be used for desalination applications. In addition, most of these membranes are not sufficiently stable for industrial applications. In many cases, only small membrane areas have been prepared, and most of the techniques require the use of highly specialized nanofabrication techniques and are difficult and (prohibitively) expensive to scale up both for RO and forward osmosis (FO) membrane fabrication.

Recently, a new approach for fabricating aquaporin based biomimetic membranes has been developed in our laboratory. This involves embedding aquaporin-containing proteoliposomes or proteopolymersomes in a crosslinked polyamide matrix [88]. A microporous substrate was first soaked in an aqueous solution of m-phenylene-diamine (MPD) that also contains a given amount of aquaporin containing vesicles, see Fig. 5. Soaked substrates were then exposed to a tri-mesoyl chloride (TMC) solution to form an interfacially polymerized polyamide rejection layer, where the vesicles were dispersed in the thin rejection layer. In this design, the aquaporin-containing vesicles provide preferential water paths through the polyamide layer and thus significantly enhance the membrane water permeability. Meanwhile, the crosslinked polyamide provides a scaffold to support the aquaporin-containing vesicles and protect them in the environment, and this is expected to significantly enhance the membrane's stability. The membrane prepared showed a permeability of ~4 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$, see Table 1, ~40% higher than a commercial brackish water reverse osmosis membrane BW30 coupon tested under identical conditions while maintaining similar or better NaCl rejection. Membranes with this design have been tested for periods of weeks to months with stable flux and rejection performance. The water enhancement effect of aquaporins was also demonstrated by comparing to membranes loaded with vesicles containing inactive R189A AqpZ mutants [88]. Due to the simple fabrication procedure, this technique can be easily scaled up to produce large membrane areas.

4. Prospects

The application of biomimetic membranes, based on aquaporin, is yet to be realized commercially. However recent promising results suggest that the time-frame to practical application may be relatively short. Initially the most likely membrane preparation strategy is to incorporate vesicles into thin-film composites. This approach is relatively low-cost and amenable to scale-up. Optimization of vesicle formulation and thin-film incorporation could produce permeabilities >100% higher than current commercial membranes. The immediate applications include FO (at atmospheric pressure), and pressure

Table 1
Examples of biomimetic membrane designed for water reuse and desalination. Performance data are presented as water permeability (WP) [$L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$], NaCl rejection ($R_{NaCl}$) [%], membrane area ($A$) [$cm^2$], and maximal external pressure applied (P_{Max}) [bar] when operated in RO. CA: cellulose acetate, PC: polycarbonate.

Approach	WP ($L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$)	R_{NaCl} (%)	Area (cm^2)	P_{Max} (bar)	Upscaling issues	Remarks	Reference
Charged lipid mixture vesicles depositions onto NF membranes	0.83	n.d.	3.5	10	Difficult to produce large defect-free membranes	No aquaporin incorporated.	[83]
Vesicle fusion facilitated by hydraulic pressure on hydrophilic NF membranes coated with positively charged lipids	3.6 ± 0.2	35 ± 8	12.6	1	Difficult to produce large defect-free membranes	Low R_{NaCl}. Only suitable for NF.	[87]
Membranes across multiple micron scale apertures either as free-standing lipid or polymer membranes	n.d.	n.d.	4[a]	n.d.	Nanofabrication required. Low robustness	WP/R_{NaCl} not tested. Not suitable for RO.	[73,76–81]
Membranes across multiple micron scale apertures and stabilized by hydrogel encapsulation	12–40	n.d.	3.5[a]	2	Nanofabrication required. High robustness	Characterized with gramicidin channels. No aquaporin incorporated.	[82]
Aquaporin containing polymersomes on methacrylate functionalized CA membranes	34.2 ± 6.9	32.9 ± 9.1	0.07	5	Medium robustness	Small area. High WP but low R_{NaCl} . Only suitable for NF.	[84]
Detergent-stabilized His-tagged aquaporin added to monolayers with nickel-chelating lipids	n.d.	n.d	n.d.	n.d.	Complex fabrication. Low robustness	WP/R_{NaCl} not tested. May not be suitable for desalination.	[85]
Proteopolymersome deposition onto gold-functionalized PC track-etched substrates	n.d.[b]	n.d.[b]	0.096	n.d.	Complex fabrication. Low robustness	Small area. Relatively high WP in FO. No RO data.	[86]
Interfacial polymerization method with embedded proteoliposomes	4 ± 0.4	96.3 ± 1.2	>200	14	Simple fabrication. High robustness	Combined high WP and R_{NaCl}. Suitable for RO.	[88]

[a] Including membrane scaffold.
[b] RO tests were not performed. Based on FO tests, a WP of 16.4 $L \cdot m^{-2} \cdot h^{-1}$ and a salt flux of 6.6 $g \cdot m^{-2} \cdot h^{-1}$ were obtained for membrane prepared with a protein to polymer molar ratio of 1:100 with 0.3 M sucrose as draw and 200 ppm NaCl as feed.

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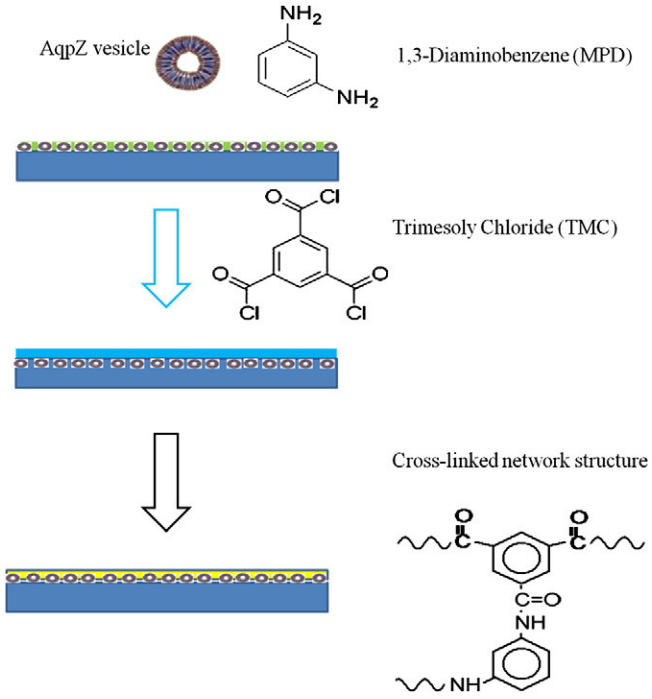

Fig. 5. Preparation of aquaporin based biomimetic membranes by interfacial polymerization. The polymerization results in a thin matrix (yellow) with aquaporin-containing forming the active layer supported by a microporous substrate, see [88] for details. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

retarded osmosis (PRO), brackish water desalination and used-water reclamation (where modest pressures, ≤10 bar, are required). In FO and PRO applications the low salt/water permeability ratio promised by aquaporin would be very attractive. Seawater desalination, requiring pressures >50 bar, should also be achievable with vesicles in thin-film composites that provide a supporting environment. The vesicle-containing films will have an overall permeability that combines that of the vesicles alone and the film material (cross-linked polyamide). To achieve a performance closer to the intrinsic vesicle permeability will probably require elaborate soft-matter and microfabrication techniques, and these are a longer-term prospect.

5. Conclusions

Based on their unique combination of offering high water permeability and high solute rejection aquaporin proteins have attracted considerable interest over the last years as functional building blocks of biomimetic membranes for water desalination and reuse. Several design approaches have been pursued in facing the challenge of making the biomimetic membranes as stable, robust, scalable, and cost-effective as their polymeric counterparts in the form of existing technologies such as RO membranes. One type of approach aims at making ultrathin <10 nm supported films with incorporated aquaporins. While attractive in terms of flux potential this approach rests on the ability to form large defect free membranes – a technical challenge yet to be met – even at the square micron scale. Other designs use aquaporins stabilized in vesicular structures as a structural and functional element. Recent progress with this type of design, involving interfacial polymerization, has led to large (>400 cm^2) robust membranes (with lifetime of months) with water permeabilities >4 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ and salt rejection values >96%. Production of these membranes can easily be established on an industrial scale thus paving the way for biomimetic aquaporin membranes out of the laboratory and into full scale applications.

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