Chapter 9

Challenges and Opportunities for Biomimetic Membranes

Amira Abdelrasoul, Huu Doan and Ali Lohi

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.71721

Abstract

A brief overview of the fundamental and practical challenges as well as of the current status of biomimetic membrane technologies is presented.

Keywords: development, scale up, antifouling, nanopores, cost, challenge

1. Introduction

This chapter presents a brief overview of the fundamental and practical challenges as well as of the current status of biomimetic membrane technologies. An accompanying summary is also given in **Table 1**. The specific focus of this chapter is aquaporin-based membranes that have initiated a substantial increase in research activities and several recent and ongoing commercialization attempts [1, 2].

2. Biomimetic nanopores

The fabrication of biomimetic nanopores, including those created with solid-state materials such as silicon, is a relatively new research area. As a result, the scale-up of biomimetic nanopore to practical and implementable dimensions for separation applications could face several challenges. The making of nanoscale pores using current methods, such as i-beam and e-beam lithography, is currently a lab scale process which requires expensive infrastructure. Furthermore, one of the fundamental challenges still being explored is the functionalization of biomimetic nanopores using specialized biological molecules and chemistry, which

	Practical challenges	Fundamental challenges	Current status
Biomimetic nanopores (solid state)	Scale-up at reasonable cost	Selection of functionalization ligands to provide selectivity	Commercialization attempts for DNA sequencing; no separation applications yet
Carrier-mediated biomimetic membranes	Stability; process configuration; low transport rates would require large membrane areas	Overcome low transport rates for separation applications	Not commercialized Sensing electrode commercialization; no separation applications
Protein-mediated biomimetic membranes	Membrane protein production scale-up; large membrane scale-up; leakage prevention	Limited range of proteins and polymers	Commercialization attempts ongoing
Artificial channel membranes	Scale-up	Designing specificity into channels, packing channels in membranes, increasing permeability	A new research area for water channels, ion channels studied but not commercialized
Antifouling strategies	Cost and efficacy	Not substantial for most applications	Various stages of research and commercialization

Table 1. Challenges to development and scale-up of various biomimetic membranes.

may be difficult to implement on large scales. Questions regarding the ligands that can be used to functionalize pores are also difficult to address, in particular if discrimination such as that is seen in potassium channels, is desirable. On the other hand, their application to DNA sequencing has reached commercialization level with several technologies licensed to start-up organizations [3].

3. Carrier-mediated biomimetic membranes

Carrier-mediated biomimetic membranes include liquid membranes (LMs) and ionophore-based membranes. LMs have gained interest in researchers in the last several decades, and as a result, an excellent understanding of the transport process has been developed. However, commercialization efforts in separation processes have been hindered by the poor stability and other practical difficulties in the implementation of these membranes. The practical difficulties include unstable immobilization of LMs in supported liquid membranes (SLMs) and process inefficiencies in separation of the recovered materials from the emulsion phase in emulsion liquid membranes (ELMs). Nevertheless, some applications have been scaled up to the necessary pilot and larger scales in recent years. ELMs have been used for zinc, phenol, and cyanide removal from industrial waste streams [4]. Ionophore-based membranes are widely used in ion-selective electrodes. Ion-selective membranes are the gold standard for this type of application. However, their application in separation membranes has not yet progressed sufficiently due to the low transport rates of ions in polymeric matrices [5]. In order to provide fluidity to the polymer matrix, plasticizers can be used, but these still do not improve the transport to reasonable levels necessary for separation applications.

http://dx.doi.org/10.5772/intechopen.71721

4. Membrane protein-mediated biomimetic membranes

Protein-based biomimetic membranes, and in particular aquaporin-based membranes, have gained significant interest in recent years leading to multiple attempts at their commercialization. However, there are several fundamental and practical challenges that need to be addressed before large-scale membranes suitable for industrial applications can be developed. Applications of aquaporin biomimetic membranes face many critical challenges, primarily because of the limited scope of research studies conducted in this area. In particular, block copolymers (BCPs) that have been used for inserting membrane proteins have been limited a single polymer type with polydimethyl siloxane (PDMS) hydrophobic block [6]. Recent reports have shown that the mammalian eye lens aquaporin (AQP0) was successfully incorporated into poly(butadiene)-block-poly(ethylene oxide) block copolymer (PB-PEOBCP) membranes [7]. While these polymers have been shown to insert membrane proteins, it is not well understood what dictates membrane protein polymer interactions and compatibilities. Perhaps other polymers with superior characteristics have not been explored because a rational basis for polymers election does not yet exist. More experimental and theoretical explorations are required to develop this rapidly growing field. A related question is how to quantify the insertion efficiency of membrane proteins in BCPs in order to determine the best and most compatible polymer for a particular membrane protein. No effective method currently exists for quantifying the amount of protein inserted per unit membrane area with sufficient accuracy. Biochemistry-based methods such as stern blotting [8, 9], antibody-gold labeling [10], and freeze fracture [11] are difficult to implement and do not provide relevant quantitative information. A new method is needed to accurately determine insertion efficiency and compatibility of membrane proteins in various polymers and to provide a rational basis for BCP selection. A successful biomimetic membrane would require a high level of protein packing in the membrane. In most studies, full function of aquaporins in BCP membranes has only been demonstrated at packing densities that are relatively low and when the concentration of membrane proteins in native membrane systems, such as eye lenses, retina, and bacterial photosynthetic membranes, is considered. A typical packing density showing the expected function was demonstrated for AqpZ reconstituted into poly-(2-methyloxazoline)-block-poly-(dimethyl siloxane)-block-poly-(2-methyloxazoline) (PMOXA-PDMS-PMOXA) triblock copolymer membranes at a molar polymer-to-protein ratio (PoPR) adjusted for triblock architecture of 50–100, beyond which permeability has been shown to decrease [12]. In a recent study, AQP0 function was shown to persist for PoPR of 15 in a PB-PEO polymer [7]. This study also indicated that, while the constitution methodology is critical, polymer block length and chemistry may also be the important factors that determine how much protein could be functionally reconstituted into BCP membranes. The possibility of obtaining a high density of functional membrane proteins in BCP membranes has significant implications for applications of such systems. High level of protein packing density has been shown in lipid bilayers of protein-based membrane protein, using 2D crystallization [13–15] and several native membranes described earlier [13, 16, 17]. A more comprehensive understanding and characterization of membrane protein-BCP compatibility will also assist in making highly packed aquaporin-based membranes, similar to lipid-based membrane protein 2D crystals. There is also a need to explore aquaporin membranes beyond the traditionally used *Escherichia coli*

AqpZ, which may feature higher permeability and better insertion efficiency in BCPs. Another key challenge is the use of systems for expressing large amounts of membrane proteins. As has been reported, AqpZ is well expressed in *E. coli* and yields up to 200 mg L^{-1} of culture in a fusion form [18]. Results like these can be promising for scale-up of this particular aquaporin. In general, yield values are much lower (typically 1 mg L^{-1} of culture) for membrane proteins. The major membrane protein expression systems that have been developed and used widely in laboratory research setting are *E. coli*, yeast, and mammalian cells (Chinese hamster ovary cells in particular). However, membrane protein production is limited by both the cells' ability to survive membrane protein overproduction and the lack of coordination between membrane protein production and cell membrane production to accommodate membrane proteins. Alternative approaches to produce membrane proteins in general and aquaporins in particular should be further explored. The main practical challenge is in the scale-up of defect free membranes. So far, the sizes that are being realized are in the scale of square millimeter, even though rapid strides are being made in this research direction [19–30]. The methods used for membrane fabrication-vesicle position, monolayer formation, and pore suspended bilayers are all challenging to replicate at larger scales. Furthermore, most substrates used to support or immobilize active AqpZ are highly specialized and range from gold-coated track-etched membranes [23, 29] to polymer-based membranes [22, 24–28, 30]. The more scalable approach may be achieved through the use of polymer membranes, if technical concerns with regards to sealing around deposited vesicles and bilayers are solved. The economic aspects of making such membranes can likewise be problematic. Membrane protein purification is expensive, primarily due to the necessary process of disrupting cell membranes, use of specialty nonionic detergents ultracentrifugation, and chromatography. A thorough analysis of membrane protein scale-up has not been conducted before and should be a critical step forward if this class of membrane progresses to larger production scales and commercialization. Another challenge may be the unknown landscape of regulations regarding the use of membrane proteins, particularly in water treatment and industrial applications. The possibility of release of these materials is real and must be regulated. This issue is similar to the one faced by membranes that incorporate nanomaterials and are under specific regulations.

5. Artificial channel-based membranes

The research of artificial channel-based biomimetic membranes is relatively new, and so far most of the work has focused on synthesis and characterization. Transport measurements are still rudimentary in this field [31], and more studies are necessary to be able to compare their efficiency to membrane protein channels. Artificial water channels attract significant interest since they might prove to be the key materials for water purification. The challenges of carbon nanotubes (CNTs) for desalination applications, where it could have the most impact, include insufficient salt rejection levels and the inability to be used in manufacturing large-scale aligned CNT membranes [32]. Organic nanochannels-based water channels, in particular, are just in the early stages of being explored [33–37]. The only semiempirical principle available is the mimicking of natural selective filters. However, the current structures are still far from the perfect design models. Current data indicate that they suffer

from low permeability (43 orders of magnitude lower than the aquaporins) and possibly imperfect rejection of solutes in some cases where channel diameters are large [36]. As mentioned by LeDuc in 2011, extensive hydrogen bonding helps encapsulate water within the channel, but also reduces the mobility of water molecules. This is probably the reason why the channels showed very low water permeability values (44 orders of magnitude lower than the lipid background permeability). This also leads to another challenge of finding a way to measure the permeability of low-permeable channels. A systematic platform for water permeability measurement needs to be established [31]. The next generation of water channels is expected to improve the design of the pore structure in order to increase water permeability while maintaining or improving solute rejection values. The geometry of the channels is also one possible area of improvement, as this will assist in packing these channels with very high density in lipid or polymer matrix for membrane fabrication. None of these ion or water channels have been tested in a practical membrane form since they are currently being studied in lipid vesicles. However, they hold great promise for separation applications due to their higher stability, properties potentially matching natural channels, scalability of their production, and ability of immobilization in a membrane-like support in a scalable manner.

6. Biomimetic antifouling strategies

Bioinspired antifouling strategies proposed for existing membranes are also generating greater interest in this research field. Many of the approaches proposed, and specifically surface modification, have the potential of being technically feasible. A cost-benefit analysis and their practical implementation may be important to consider prior to advancing them to the application level, in particular, because some of these approaches may actually decrease the initial permeability of membranes.

Author details

Amira Abdelrasoul[1]*, Huu Doan[2] and Ali Lohi[2]

*Address all correspondence to: amira.abdelrasoul@usask.ca

1 Department of Chemical and Biological Engineering, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

2 Department of Chemical Engineering, Ryerson University, Toronto, Ontario, Canada

References

[1] Shannon MA, Bohn PW, Elimelech M, Georgiadis JG, Marinas BJ, Mayes AM. Science and technology for water purification in the coming decades. Nature. 2008;**452**:301-310

[2] Kasemset S, Lee A, Miller DJ, Freeman BD, Sharma MM. Effect of polydopamine deposition conditions on fouling resistance, physical properties, and permeation properties of reverse osmosis membranes in oil/water separation. Journal of Membrane Science. 2013;**425-426**:208-216

[3] McCloskey BD, Park HB, Ju H, Rowe BW, Miller DJ, Chun BJ, Kin K, Freeman BD. Influence of polydopamine deposition conditions on pure water flux and foulant adhesion resistance of reverse osmosis, ultrafiltration, and microfiltration membranes. Polymer. 2010;**51**: 3472-3485

[4] Kislik VS. Liquid Membranes: Principles and Applications in Chemical Separations and Wastewater Treatment. UK: Elsevier Science; 2009

[5] Armstrong RD, Todd M. Ionic mobilities in PVC membranes. Electrochimica Acta. 1987;**32**:155-157

[6] Kumar M, Payne M, Poust S. Polymer-based biomimetic membranes for desalination. In: Hélix-Nielsen C, editor. Zilles J, Biomimetic Membranes for Sensor and Separation Applications. Netherlands: Springer; 2012. p. 43-62

[7] Kumar M, Habel JEO, Shen YX, Meier WP, Walz T. High-density reconstitution of functional water channels into vesicular and planar block copolymer membranes. Journal of the American Chemical Society. 2012;**134**:18631-18637

[8] Gershoni JM, Palade GE. Protein blotting: Principles and applications. Analytical Biochemistry. 1983;**131**:1-15

[9] Nakamura K, Tanaka T, Kuwahara A, Takeo K. Micro assay for proteins on nitrocellulose filter using protein dye-staining procedure. Analytical Biochemistry. 1985;**148**:311-319

[10] Stoenescu R, Graff A, Meier W. Asymmetric ABC-triblock copolymer membranes induce a directed insertion of membrane proteins. Macromolecular Bioscience. 2004;**4**:930-935

[11] Severs NJ. Freeze-fracture electron microscopy. Nature Protocols. 2007;**2**:547-576

[12] Kumar M, Grzelakowski M, Zilles J, Clark M, Meier W. Highly permeable polymeric membranes based on the incorporation of the functional water channel protein Aquaporin Z. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**:20719-20724

[13] Jap BK, Zulauf M, Scheybani T, Hefti A, Baumeister W, Aebi U, Engel A. 2D crystallization: From art to science. Ultramicroscopy. 1992;**46**:45-84

[14] Hasler L, Heymann JB, Engel A, Kistler J, Walz T. 2D crystallization of membrane proteins: Rationales and examples. Journal of Structural Biology. 1998;**121**:162-171

[15] Abeyrathne PD, Chami M, Pantelic RS, Goldie KN, Stahlberg H. Preparation of 2D crystals of membrane proteins for high-resolution electron crystallography data collection. In: Grant JJ, editor. Methods in Enzymology. Vol. 1. Academic Press; 2010. p. 25-43. DOI: 10.1016/S0076-6879(10)81001-8

[16] Raunser S, Walz T. Electron crystallography as a technique to study the structure on membrane proteins in a lipidic environment. Annual Review of Biophysics. 2009;**38**:89-105

[17] Renault L, Chou HT, Chiu PL, Hill R, Zeng X, Gipson B, Zhang Z, Cheng A, Unger V, Stahlberg H. Milestones in electron crystallography. Journal of Computer-Aided Molecular Design. 2006;**20**:519-527

[18] Lian J, Ding S, Cai J, Zhang D, Xu Z, Wang X. Improving aquaporin Z expression in *Escherichia coli* by fusion partners and subsequent condition optimization. Applied Microbiology and Biotechnology. 2009;**82**:463-470

[19] Kaufman Y, Berman A, Freger V. Supported lipid bilayer membranes for water purification by reverse osmosis. Langmuir. 2010;**26**:7388-7395

[20] Wang H, Chung TS, Tong YW, Meier W, Chen Z, Hong M, Jeyaseelan K, Armugam A. Preparation and characterization of pore-suspending biomimetic membranes embedded with Aquaporin Z on carboxylated polyethyleneglycol polymer cushion. Soft Matter. 2011;**7**:7274-7280

[21] Duong PHH, Chung TS, Jeyaseelan K, Armugam A, Chen Z, Yang J, Hong M. Planar biomimetic aquaporin-incorporated triblock copolymer membranes on porous alumina supports for nano filtration. Journal of Membrane Science. 2012;**409-410**:34-43

[22] Li XS, Wang R, Tang CYT, Vararattanavech A, Zhao Y, Torres J, Fane T. Preparation of supported lipid membranes for aquaporin Z incorporation. Colloids and Surfaces B: Biointerfaces. 2012;**94**:333-340

[23] Wang HL, Chung TS, Tong YW, Jeyaseelan K, Armugam A, Chen ZC, Hong MH, Meier W. Highly permeable and selective pore-spanning biomimetic membrane embedded with aquaporin Z. Small. 2012;**8**:1185-1190

[24] Zhao Y, Qiu C, Li X, Vararattanavech A, Shen W, Torres J, Hélix-Nielsen C, Wang R, Hu X, Fane AG, Tang CY. Synthesis of robust and high- performance aquaporin-based biomimetic membranes by interfacial polymerization-membrane preparation and RO performance characterization. Journal of Membrane Science. 2012;**423-424**:422-428

[25] Zhong PS, Chung TS, Jeyaseelan K, Armugam A. Aquaporin-embedded biomimetic membranes for nano filtration. Journal of Membrane Science. 2012;**407-408**:27-33

[26] Sun G, Chung TS, Jeyaseelan K, Armugam A. A layer-by-layer self-assembly approach to developing an aquaporin-embedded mixed matrix membrane. RSC Advances. 2013;**3**: 473-481

[27] Sun G, Chung TS, Chen N, Lu X, Zhao Q. Highly permeable aquaporin-embedded biomimetic membranes featuring a magnetic-aided approach. RSC Advances. 2013;**3**:9178-9184

[28] Sun G, Chung TS, Jeyaseelan K, Armugam A. Stabilization and immobilization of aquaporin reconstituted lipid vesicles for water purification. Colloids and Surfaces B: Biointerfaces. 2013;**102**:466-471

[29] Wang HL, Chung TS, Tong YW, Jeyaseelan K, Armugam A, Duong HHP, Fu F, Seah H, Yang J, Hong M. Mechanically robust and highly permeable Aquaporin Z biomimetic membranes. Journal of Membrane Science. 2013;**434**:130-136

[30] Xie W, He F, Wang B, Chung TS, Jeyaseelan K, Armugam A, Tong YW. An aquaporin-based vesicle-embedded polymeric membrane for low energy water filtration. Journal of Materials Chemistry A1. 2013;**26**:7592-7600

[31] Sakai N, Matile S. Synthetic ion channels. Langmuir. 2013;**29**:9031-9040

[32] Pendergast MM, Hoek EMV. A review of water treatment membrane nanotechnologies. Energy Environmental Sciences. 2011;**4**:1946-1971

[33] Fei Z, Zhao D, Geldbach TJ, Scopelliti R, Dyson PJ, Antonijevic S, Bodenhausen G. A synthetic zwitterionic water channel: characterization in the solid state by X-ray crystallography and NMR spectroscopy. Angewandte Chemie, International Edition. 2005;**44**:5720-5725

[34] Kaucher MS, Peterca M, Dulcey AE, Kim AJ, Vinogradov SA, Hammer DA, Heiney PA, Percec V. Selective transport of water mediated by porous dendritic dipeptides. Journal of the American Chemical Society. 2007;**129**:11698-11699

[35] LeDuc Y, Michau M, Gilles A, Gence V, Legrand YM, vanderLee A, Tingry S, Barboiu M. Imidazole-quartet water and proton dipolar channels. Angewandte Chemie, International Edition. 2011;**50**:11366-11372

[36] XB H, Chen Z, Tang G, Hou JL, Li ZT. Single-molecular artificial transmembrane water channels. Journal of the American Chemical Society. 2012;**134**:8384-8387

[37] Zhou X, Liu G, Yamato K, Shen Y, Cheng R, Wei X, Bai W, Gao Y, Li H, Liu Y, Liu F, Czajkowsky DM, Wang J, Dabney MJ, Cai Z, Hu J, Bright FV, He L, Zeng XC, Shao Z, Gong B. Self-assembling sub nanometer pores with unusual mass-transport properties. Nature Communications. 2012;**3**:949