Improved reverse osmosis thin film composite biomimetic membranes by incorporation of polymersomes

Górecki, Radosaw Pawel; Reurink, Dennis Maik; Khan, Muntazim Munir; Sanahuja-Embuena, Victoria; Trzasku, Krzysztof; Hélix-Nielsen, Claus

Published in:
Journal of Membrane Science

Link to article, DOI:
[10.1016/j.memsci.2019.117392](https://doi.org/10.1016/j.memsci.2019.117392)

Publication date:
2020

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Górecki, R. P., Reurink, D. M., Khan, M. M., Sanahuja-Embuena, V., Trzasku, K., & Hélix-Nielsen, C. (2020). Improved reverse osmosis thin film composite biomimetic membranes by incorporation of polymersomes. *Journal of Membrane Science*, 593, [117392]. <https://doi.org/10.1016/j.memsci.2019.117392>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 *Improved reverse osmosis thin film composite biomimetic membranes by*
2 *incorporation of polymersomes*

3
4 Radosław Górecki^{* 1, 2}, Dennis Maik Reurink³, Muntazim Munir Khan², Victoria Sanahuja-
5 Embuena^{1, 2}, Krzysztof Trzaskus², Claus Hélix-Nielsen¹

6
7 ¹ Department of Environmental Engineering, Technical University of Denmark, Bygningstorvet 115, 2800 Kongens
8 Lyngby, Denmark

9 ² Aquaporin A/S, Nymøllevej 78, 2800 Kongens Lyngby, Denmark

10 ³ Membrane Science & Technology, University of Twente, MESA+ Institute for Nanotechnology, P.O. Box 217, 7500
11 AE Enschede, The Netherlands

12 * Corresponding author, e-mail: rgor@env.dtu.dk ; rgo@aquaporin.com

13
14 Authors e-mail addresses:

15 Dennis Maik Reurink: d.m.reurink@utwente.nl; Muntazim Munir Khan: mmk@aquaporin.com;

16 Victoria Sanahuja-Embuena: visc@env.dtu.dk; Krzysztof Trzaskus: ktr@aquaporin.com;

17 Claus Hélix-Nielsen: clhe@env.dtu.dk

18

Abstract

Biomimetic aquaporin-based membranes offer great promise as a disruptive water treatment technology, due to their potential of improving membrane permeability without compromising solute rejection. However, fabrication upscaling is challenging and therefore the technological potential of biomimetic membranes remains unused. We propose an easily upscalable process based on bulk hydration of diblock and triblock copolymer mixture for preparation of polymersomes which can reconstitute aquaporin proteins. Such polymersomes are incorporated into biomimetic membranes via polyamide active layer synthesis based on interfacial polymerization. By incorporation of blank polymersomes, it was possible to improve water permeability of the membrane by 30 %, and by incorporation of aquaporin reconstituting polymersomes by 50%, compared to the membranes without polymersomes. In both cases NaCl rejection was not affected. X-ray photoelectron spectroscopy measurements confirmed incorporation of copolymers prepared with aquaporins into the active polyamide layer without affecting the thickness of the membrane's active layer and surface zeta-potential.

Keywords: Aquaporin; Biomimetic Membrane; Block Copolymers; Polymersomes; Interfacial Polymerization.

1. Introduction

Ensuring access to water and sanitation for all is goal number 6 of the United Nation Sustainability Goals [1]. Not only the demand for water is increasing across all sectors [2], the problems with water quality are growing on a global scale, even in regions considered as water-rich. In both developing and industrialized nations more contaminants, including trace organics such as pharmaceuticals and pesticides to heavy metals, are entering the water due to human activities [3,4], which drives the development of more efficient water purification technologies [4] [5].

As a result of a high energy efficiency and manufacturing scalability combined with a small footprint [4,6], membrane technology becomes leading technology for high quality water purification [7]. For example, polyamide based reverse osmosis (RO) membranes are used worldwide for processes such as desalination [4], water reuse [8] and domestic tap water purification [9]. These membranes are mainly thin film composite (TFC) membranes with a thin (100-200 nm) polyamide (PA) active layer (AL) coated on a porous substrate which is supported by a non-woven fabric [10]. The broad application of TFC membranes has been accompanied by continuous performance improvements resulting in enhanced water permeability, high solute rejection, low energy demand and low fouling-ability [11].

Recently, biomimetic approaches have attracted considerable interest [12]. Here, the membrane AL is doped with transmembrane proteins facilitating water transport exemplified by the use of the bacterial aquaporin protein isoform Aquaporin Z (AqpZ) [12,13]. However, industrial scale production and incorporation of aquaporins into the AL is not a trivial task due to the need for elaborate protein purification and stabilization steps [14].

A common approach for AqpZ membrane incorporation is based on reconstitution into amphiphilic liposomes or polymersomes preserving the ternary and quaternary structure of the AqpZ and thus their functionality [10,15]. Both liposomes and polymersomes have been applied in preparation for

61 RO, forward osmosis (FO) and nanofiltration (NF) biomimetic membranes [10]. Polymersomes
62 appear advantageous over liposomes for industrial applications due to their superior physical and
63 chemical stability [16] as well as the possibility of introducing active groups for covalent bonding to
64 the membrane AL [17]. However, the protein activity is generally reported to be lower in
65 polymersomes compared to liposomes, presumably due to the increased hydrophobic mismatch
66 between the hydrophobic polymer core layer thickness and transmembrane protein length [18]. In
67 order to achieve efficient functional reconstitution of AqpZ proteins into polymersomes, the polymer
68 matrix must resemble natural phospholipid membranes while preserving the advantages in stability.
69 For example, AqpZ can be successfully reconstituted in triblock copolymers containing
70 polydimethylsiloxane (PDMS) [19–22]. The conformational flexibility of the PDMS blocks allows
71 insertion of membrane proteins where the polymer chains can adapt to the fixed dimensions of the
72 proteins [18] and protein insertion can be further increased by addition of detergents [23]. Still,
73 reconstitution of AqpZ with triblock copolymers containing less flexible polymer chains is possible
74 and was reported for systems such as *block*-poly(isobutylene)-*block*-poly(ethyleneglycol)-*block*-
75 poly(isobutylene) (PIB-PEG-PIB) [24] or *block*-poly(ethyleneglycol)-*block*-poly(propyleneglycol)-
76 *block*-poly(ethyleneglycol) (PEG-PPG-PEG) [25].

77 The use of AqpZ liposomes (AQP-liposomes) has been reported for the manufacturing of TFC RO
78 membranes, TFC nanofiltration (NF) membranes, and FO membranes [26] [27] [28]. AQP-liposome
79 TFC RO membranes showed improvements in water permeability of up to 40 % when compared to
80 commercial membranes [26] and FO membranes with AQP-liposomes, achieved a water flux of 49.1
81 $\text{Lm}^{-2}\text{h}^{-1}$ coupled with specific reverse salt flux of 0.10 gL^{-1} (with 1 M NaCl as draw solution and
82 deionised (DI) water as feed) [28]. However, natural lipids are prone to oxidation which has inspired
83 the use of amphiphilic block copolymers, as they have a higher chemical and physical stability
84 [16,21,29]. Moreover, the use of amphiphilic block copolymers allows for the introduction of active

85 groups to the AQP-polymersomes that can participate in interfacial polymerization (IP) and thus be
86 covalently bonded to the PA layer [20,21,30,31].

87 AqpZ-embedded polymersomes (AQP-polymersomes) made by amphiphilic block copolymers have
88 been used in TFC membranes made via IP for FO [32]. However, for the applications in which
89 transport through the TFC membrane is driven by a hydraulic transmembrane pressure gradient, the
90 incorporation of AQP-polymersomes has not been reported yet. Moreover, the use of membranes
91 with AQP-polymersomes has been limited to NF membranes with limited selectivity towards
92 monovalent ions [30,31,33,34]. Another limitation for biomimetic membranes arises from challenges
93 in manufacturing process scalability. The preparation of AQP-polymersomes reported in the literature
94 has been limited to film rehydration techniques [19,21,22,30,31]. The major drawback of this
95 technique stems from the high cost due to several processing steps and product losses [35,36].

96 Here we propose a novel, fully scalable method to prepare and introduce AQP-polymersomes into
97 the AL of RO membranes. First, AqpZ proteins are functionally reconstituted into AQP-
98 polymersomes, using an industrially scalable process based on bulk hydration and self-assembly of
99 poly-2-methyl-2-oxazoline-*block*-poly(dimethylsiloxane) (PMOXA-PDMS) and poly(2-methyl-2-
100 oxazoline)-*block*-poly(dimethylsiloxane)-*block*-poly(2-methyl-2-oxazoline) (PMOXA-PDMS-
101 PMOXA) copolymers with the addition of amine terminated poly(dimethylsiloxane) (A-PDMS).
102 Secondly, AQP-polymersomes are successfully embedded into the membrane AL using the IP
103 reaction between m-phenylenediamine (MPD) and trimesoyl chloride (TMC). This results in
104 membranes with improved water permeability that maintain a high NaCl rejection.

105

2. Experimental

2.1. Preparation of polymersomes

Polymersomes were prepared by the self-assembly method in an aqueous solution of phosphate buffered saline (PBS). PBS (pH 7.2, 136 mM NaCl, 2.6 mM KCl) was prepared by dissolving 8 g NaCl, 0.2 g KCl, 1.44 g Na₂HPO₄ and 0.24 g of KH₂PO₄ in 800 mL Milli Q purified H₂O, adjusting the pH to 7.2 with HCl and bringing the volume to 1 L with Milli Q H₂O[37]. All chemicals were analytical grade (Sigma Aldrich, Denmark). Polymersomes incorporating AqpZ (AQP-1) were prepared from a mixture of AqpZ protein and diblock PMOXA-PDMS and triblock PMOXA-PDMS-PMOXA amphiphilic copolymer, according to the procedure described in the patent of Spulber and Gerstandt [37] . Briefly, 112 mg of mixture of diblock PMOXA-PDMS and triblock amphiphilic copolymer PMOXA-PDMS-PMOXA (Aquaporin A/S, Denmark) and 100 µL of aminopropyl terminated polydimethylsiloxane (A-PDMS) (Aquaporin A/S, Denmark) were mixed with 100 µL of AqpZ-stock solution (5 mg/mL) (Aquaporin A/S, Denmark) and subsequently dissolved in 100 mL PBS with 0.05 % Lauryldimethylamine *N*-oxide (LDAO) (Carbosynth Ltd., United Kingdom) and 0.5 % solubilizer Kolliphor® HS 15 (KHS) (Sigma-Aldrich Denmark A/S, Denmark). The resulting solution was then stirred at 170 rpm for 10 hours at room temperature. Subsequently, the solution was firstly filtered through 0.5 µm polyethersulfone (PES) filter and secondly through 0.22 µm PES filter (Sarstedt, Germany). The polymersomes without AqpZ (AQP-0) were prepared in exactly the same procedure, without adding AqpZ-stock to the solution.

2.2. Characterization of polymersomes and functional reconstitution of Aquaporin Z proteins

Size distribution of polymersomes was characterized by Nanoparticle Tracking Analyzer (NTA) Nanosight 3.1 Build 3.1.46 (Malvern Panalytical Ltd, UK), equipped with a charge-coupled device

(CCD) camera. The camera level was set to 14, slider shutter to 1485, slider gain to 470, and the recording was done with 30 FPS and 3600 number of frames. The samples were diluted 400 times with PBS buffer, resulting in concentrations of 41 (for AQP-0) and 56 (for AQP-1) particles per frame. The measurements were done at 23°C. Zeta potential of the polymersomes solution has been measured with use of folded capillary cells DTS1070 on a Zeta Sizer Nano (Malvern Panalytical Ltd, UK). The functional reconstitution of AqpZ in polymersomes was analysed with the use of a Stopped-Flow Spectrometer SX20 device (Applied Biophysics Inc, USA), equipped with a 150 W xenon-arc lamp (Osram, Germany), using a monochromator set to a wavelength of 465 nm. For each SF measurement, 10 µL of polymersome solution and 10 µL of 0.5 M NaCl solution were rapidly mixed in a 20 µL optical cell at 25°C. When polymersomes are exposed to the NaCl solution, the osmotic pressure difference causes water efflux from the polymersomes, resulting in a decrease in volume, thereby causing a change in the refractive index of the solution [38]. The kinetics of these changes are measured by the light scattering detector. The higher the water efflux through the polymersome membrane observed – the more rapid volume change occurs. The kinetics of this process are obtained from the Stopped-Flow Light Scattering (SFLS) measurements by fitting the average of five readings to double exponential equation 1 [19,21,39,40] with use of OriginPro software (OriginLab Corporation, USA):

147

$$Y = \sum_{i=1}^N c_i e^{-k_i t} \quad (1)$$

149

where Y is light scattering intensity, N is the number of exponential terms ($N = 2$), t is time (s), c_i is relative amplitude and k_i are the exponential coefficients (s^{-1}) ($i = 1, 2$).

152 Faster exponential coefficient k_2 (s^{-1}) is attributed to the water efflux from the polymersomes [21].

153 The osmotic water permeability through polymersomes is calculated with equation 2 [21,39–41]:

$$P_f = \frac{k_2}{S_A/V_0} V_w \Delta_{osm} \quad (2)$$

where P_f is osmotic water permeability ($\mu\text{m}\cdot\text{s}^{-1}$), k_2 is the faster exponential coefficient (s^{-1}), S_A is vesicle surface area (μm^2), V_0 is initial vesicle volume (μm^3), V_w is molar volume of water ($0.018 \text{ L}\cdot\text{mol}^{-1}$), and Δ_{osm} is the osmolarity difference that drives the vesicle volume change ($\text{osmol}\cdot\text{L}^{-1}$).

2.3. Preparation of membrane TFC AL

MPD ($\geq 99\%$ for synthesis), ϵ -Caprolactam ($\geq 99\%$ for synthesis) and TMC ($\geq 99\%$ for synthesis) were obtained from Merck KGaA (Germany) and used as received. Isopar™ E was obtained from Brenntag Nordic A/S (Denmark) and filtered through a mechanical filter (approximately $500 \mu\text{m}$) prior to use. Anhydrous citric acid was obtained from Citrique Belge (Belgium) and used as received. A polysulfone (PSF) ultrafiltration membrane was obtained from Aquaporin A/S (Denmark) and used as the support membrane. The support membrane was stored in DI water overnight prior to AL fabrication.

The PA AL layer was synthesized by an *in-situ* IP reaction between MPD monomer in the aqueous phase and TMC monomer in organic solvent. The PES support membrane was cut into $270\times 170 \text{ mm}$ pieces and clipped to stainless-steel frames on the edges. This membrane frame was immersed in a solution containing 3 wt% of MPD and 3 wt% of ϵ -Caprolactam. The solution also contained 3 wt% of AQP-1 or 3 wt% AQP-0 when polymersomes were loaded to the membrane. The immersion in aqueous solution was 30 seconds. The excess solution was removed from the surface of the membrane by air gun. Subsequently 50 mL of 0.09 wt% TMC organic solution (in Isopar™ E) was applied to the surface of the horizontally placed membrane frame. After 30 seconds, the excess of organic

177 solution was poured out and the surface of the membrane was dried with an air gun. The membrane
178 was then immersed for 4 minutes in the 20 wt% citric acid bath preheated to 70°C to remove the
179 unreacted amine monomers [42]. Afterwards, the membrane was immersed in the DI water at 70°C
180 for 2 minutes. Finally, the membranes were flushed with DI water, removed from the stainless-steel
181 frames and stored in the DI water overnight prior to testing.

182

183 **2.4.Membrane characterization**

184 **2.4.1. Scanning electron microscopy**

185 Cross-sections and surface micrographs of the polyamide layer were taken using a field emission
186 scanning electron microscope (JSM-7610F, JEOL, Japan). All samples were sputter coated (Quorum
187 Q150T ES, United Kingdom) with a 5 nm thick layer of chromium. Cross sections were prepared by
188 breaking the membrane samples in liquid nitrogen with a razor blade. All samples were vacuum dried
189 prior to sputter coating and images were taken at an accelerating voltage of 1.0 kV.

190

191 **2.4.2. X-ray photoelectron spectroscopy**

192 The X-ray photoelectron spectroscopy (XPS) measurements were done with use of Scanning XPS
193 microprobe Quanterra SXM (Physical Electronics, USA). The base pressure was $< 6.6 \times 10^{-8}$ Pa, the
194 power of monochromatic Al K α ($h\nu = 1486.6$ eV) was 25 W and the current was 2.6 mA. The used
195 beam size was 100 μm . A lower energy electron flood gun was used to supply the missing
196 photoelectrons and Auger electrons. Low energy argon ions were used to remove the surplus of
197 electrons from the electron flood gun. Reference elements were used to calibrate the energy scale.

198

2.4.3. Zeta (ζ) potential measurements

Zeta potential of the flat sheet membranes was measured using streaming potential measurements with an electrokinetic analyzer (SurPass, Anton Paar, Graz Austria). Two samples were affixed to 10 x 20 mm sample holders with double-sided tape using an adjustable gap cell. Inside the adjustable gap cell a rectangular silicone block ensures the the chamber is sealed. The two sample holders were placed opposite each other in the adjustable gap cell creating a rectangular slit between the two membrane samples. The slit, or gap height, was adjusted to 110 μm . A 5 mM KCl solution was allowed to flow through the adjustable gap cell under ambient conditions. Under a given pressure difference, the flow through the cell creates a streaming current. With the measured streaming current (A), dI , and the applied pressure difference (Pa) dp , the zeta potential (V) ζ , can be calculated according to equation 3:

$$\zeta = \frac{dI}{dp} \cdot \frac{\eta}{\varepsilon \cdot \varepsilon_0} \cdot \frac{L}{A} \quad (3)$$

where, η is the dynamic viscosity ($\text{Pa}\cdot\text{s}$) of the electrolyte, ε the dielectric constant (-) of the electrolyte, ε_0 the permittivity of vacuum ($\text{F}\cdot\text{m}^{-1}$), L is the length (m) of the streaming channel, and A is the cross-section area (m^2) of the streaming channel. Every membrane sample was measured a minimum of six times in the 5 mM KCl solution and for every measurement point three membrane samples were measured. From this data a standard deviation was derived.

2.5. Membrane performance evaluation

The performance of the prepared TFC membranes wasevaluated with a reverse osmosis testing setup containing six CF042P Sterlitech cross-flow cells (Sterlitech Corp., USA). Each sample of the

222 prepared membrane was cut into 3 coupons of 58 mm x 112 mm to fit the CF042P Sterlitech cells.
 223 The solution of 500 ppm NaCl (Akzo Nobel Salt A/S, Denmark) was used as a feed and has been
 224 circulated through the cells on the side facing the AL of the membrane. The testing pressure applied
 225 was 5 bar. The feed solution at 25°C was introduced into the filtration cell with a flow-rate
 226 of 60 Lh⁻¹, which corresponds to the cross-flow velocity of 20 cm/s. Before permeate sample
 227 collection, membrane samples were compressed for 30 minutes. Permeate samples were collected
 228 and weighted to obtain liquid permeability (L_p) according to equation 4:

$$230 \quad L_p = \frac{\Delta V}{\Delta t \cdot A \cdot (\Delta P - \Delta \pi)} \quad (4)$$

231
 232 where, L_p is the water permeability of the membrane (L·m⁻²·h⁻¹·bar⁻¹), ΔV is the volume of the
 233 collected permeate sample (L), Δt is the sampling time (s), A is the active area of the membrane (m²),
 234 ΔP is the applied pressure (bar), and $\Delta \pi$ is the osmotic pressure of the feed solution (bar).

235 Conductivity of the obtained samples was measured by Ultrameter II 6PFCE (Myron L[®] Company,
 236 USA) and was applied for calculation of NaCl concentration in feed and permeate samples, which
 237 was later used to calculate NaCl rejection according to equation 5:

$$239 \quad R (\%) = \left(1 - \frac{C_p}{C_f} \right) \times 100 \% \quad (5)$$

240
 241 where, R is the membrane salt rejection, C_p is the concentration of NaCl in the permeate, and C_f is
 242 the concentration of the NaCl in the feed solution.

243

3. Results and discussion

3.1. Functional reconstitution of Aquaporin Z into polymersomes

The characteristics of polymersomes are summarized in Table 1.

Table 1. Size distribution, ζ -potential, pH and osmotic water permeability of PMOXA-PDMS-PMOXA polymersomes. D10, D50 and D90 are particle size distribution intercepts of 10 %, 50 % and 90 %, $\pm$ values indicate standard error.

Type of polymersomes	Mean size (nm)	D10 (nm)	D50 (nm)	D90 (nm)	Conc. of polymersomes (particles $\cdot$ mL $^{-1}$)	ζ -potential (mV)	pH	Osmotic water permeability P_f (μ m $\cdot$ s $^{-1}$)
AQP-1	154 $\pm$ 1	99 $\pm$ 1	126 $\pm$ 2	234 $\pm$ 3	3.26 $\times$ 10 11 $\pm$ 7.08 $\times$ 10 9	+ 0.9	8.03	143 $\pm$ 5
AQP-0	153 $\pm$ 3	102 $\pm$ 1	134 $\pm$ 1	208 $\pm$ 8	4.44 $\times$ 10 11 $\pm$ 1.24 $\times$ 10 10	+ 0.4	8.10	109 $\pm$ 3

The measurements obtained by NTA show that AQP-1 and AQP-0 have very comparable mean sizes and size distribution, namely 154 $\pm$ 1 nm for AQP-1 and 153 $\pm$ 3 nm for AQP-0. In both solutions concentration of polymersomes was comparable as well, 3.26 $\times$ 10 11 $\pm$ 7.08 $\times$ 10 9 nm for AQP-1 and 4.44 $\times$ 10 11 $\pm$ 1.24 $\times$ 10 10 nm for AQP-0, respectively. This indicates that the presence of AqpZ in the solution does not affect the self-assembly process, and that the formation of polymersomes does not influence the average size nor the concentration of polymersomes. The ζ -potential of both AQP-1 and AQP-0 was similar, +0.9 mV and +0.4 mV respectively, confirming no difference in surface charge between AQP-1 and AQP-0 polymersomes. Measured ζ -potential suggest that the electrostatic repulsion between polymersomes are negligible. It is suspected that the stability of the solution is maintained by steric stabilization induced by the presence of copolymers and surfactants [43]. The faster exponential coefficient obtained from the SFLS experiment (see Figure 1) was larger for AQP-1 ($k_2 = 54 \pm 2$ s $^{-1}$) than for AQP-0 ($k_2 = 41 \pm 1$ s $^{-1}$). As a result, osmotic water permeability calculated from the faster exponential coefficient (k_2) was about 32 % larger for AQP-1 polymersomes than for AQP-0 polymersomes – 143 $\pm$ 5 μ m $\cdot$ s $^{-1}$ and 109 $\pm$ 3 μ m $\cdot$ s $^{-1}$, respectively (Table 1).

Figure 1. Stopped-flow light scattering readings for AQP-0 and AQP-1 polymersomes – measured against 0.5 M NaCl at 25 °C. The data points presented here are average values from 5 separate measurements for each sample, while dashed lines represent the fits made with double exponential equation 1.

The increased permeability of AQP-1 compared to AQP-0 indicates a successful functional reconstitution of AqpZ into amphiphilic membrane of polymersomes, without influencing other characteristics of polymersomes such as hydrodynamic diameter and zeta potential. In this case, the observed difference in water permeability between AQP-0 and AQP-1 is significantly lower than usually reported in the literature for PMOXA-PDMS-PMOXA polymersomes containing aquaporins. In multiple articles, authors have reported greater increases in permeabilities of the polymersomes made of PMOXA-PDMS-PMOXA amphiphilic block copolymers. In the early work on AqpZ

reconstituting polymersomes, Kumar et al. reported permeabilities of $0.8 \mu\text{ms}^{-1}$ for blank polymersomes that increased to $74 \mu\text{ms}^{-1}$, $1500 \mu\text{ms}^{-1}$ and $2500 \mu\text{ms}^{-1}$ with increased AqpZ loading in the PMOXA₁₅-PDMS₁₁₀-PMOXA₁₅ measured at 5.5°C [19]. In a paper from 2012, Zhong et al. reported an increased permeability from $0 \mu\text{ms}^{-1}$ for PMOXA₁₀-PDMS₇₀-PMOXA₁₀ blank to $1673 \mu\text{ms}^{-1}$, $2049 \mu\text{ms}^{-1}$ and $2350 \mu\text{ms}^{-1}$, however, the temperatures at which the measurements were done are not specified [31]. In another article by Duong and co-workers, no flux is reported for the control disulphide functionalized PMOXA₁₀-PDMS₇₅-PMOXA₂₀ polymersomes, whereas the flux increase to 2595 and $4680 \mu\text{ms}^{-1}$ when AqpZ are incorporated (measured at 25°C) [30]. Grzelakowski et al., using amine functionalized PMOXA-PDMS-PMOXA reports fluxes of $184 \mu\text{ms}^{-1}$ for control and $4788 \mu\text{ms}^{-1}$ for AqpZ-loaded polymersomes, however, no block lengths are specified, and neither were the temperatures at which the permeabilities were measured [21].

Here, a relatively low increase in permeability of the polymersomes after reconstitution of the AqpZ and high permeability of the blank polymersomes implies that an amphiphilic membrane of prepared polymersomes is already highly permeable to water. It differs from the previously reported approaches, in which polymersomes show no transport or very limited transport of water through amphiphilic membranes. We speculate that the reason behind increased permeability of blank polymersomes is the effect of the surfactants on the amphiphilic copolymer wall of the vesicles. The surfactant molecules are known to incorporate into the outer leaflet of the polymeric bilayer causing its partial solubilisation [44]. This will cause an enhanced permeability and as the concentration of the surfactant increase, may also lead to disintegration of polymersomes [45]. However, this is not the case in this study, as the size measurement does not imply the presence of a significant population of structures below 100 nm (Table 1).

3.2. Membrane characterization

Biomimetic TFC RO membranes were prepared according to the protocol described in section 2.2. The TFC layer was doped with AQP-0 and AQP-1 polymersomes in order to investigate the effect of aquaporin proteins on the performance and properties of the RO membranes. Membranes without polymersomes were also prepared as a control of the experiment.

3.2.1. Scanning electron microscopy

SEM micrographs of surface and cross-section of the control membranes, membranes with AQP-0, AQP-1 are presented in Figure 2.

Figure 2. SEM micrographs of prepared polyamide membranes - Control, AQP-1 and AQP-0. A - top views of the surfaces of polyamide active layer; B - top views of the surface of polyamide active layer; C - cross section of the membranes.

310 The addition of polymersomes did not affect the thickness of the polyamide layer. For all membranes,
311 the thickness of the polyamide was comparable - ranging from 200 to 350 nm. However, from the
312 cross-section micrographs it can be seen that the morphology of the control membrane is different
313 than for the AQP-0 and AQP-1 membranes. When polymersomes are introduced, there is a visible
314 increase in the number of hollow spaces within the bulk polyamide layer – both for AQP-0 and AQP-
315 1. The surface morphology of the control membrane was also different when compared to AQP-1 and
316 AQP-0, whereas AQP-1 and AQP-0 are comparable. As polymersomes are expected to take part in
317 the interfacial polymerization reaction through amino-terminated chains of A-PDMS, it is likely that
318 the resulting polyamide layer has a different appearance compared to the membrane without
319 polymersomes. Since AQP-0 and AQP-1 have the same characteristics and the only difference
320 between them is the reconstitution of AqpZ proteins for AQP-1, the same type of TFC layer
321 morphology is observed. The single polymersomes are not visible, as they become an integral part of
322 the polymer matrix when they are covered by it. Due to the size of the AqpZ protein tetramer, which
323 only spans a few nm [46], the morphology difference between AQP-0 and AQP-1 cannot be observed
324 on the SEM micrographs.

325

3.2.2. X-ray photoelectron spectroscopy

The XPS analysis of the selective layer was made to determine the elemental composition of the formed polyamide film. The concentrations of C, N, O, S and Si in the selective layer, obtained with XPS core spectra, are listed in Table 2.

Table 2. X-ray photoelectron spectroscopy spectra results. Concentrations of C, N, O, S and Si of the support membrane, control membrane, and membranes loaded with AQP-0 and AQP-1.

<i>Sample name</i>	<i>C concentration</i> (%)	<i>N concentration</i> (%)	<i>O concentration</i> (%)	<i>S concentration</i> (%)	<i>Si concentration</i> (%)
Support membrane	77.6 ± 0.6	2.1 ± 0.2	18.1 ± 0.5	2.2 ± 0.1	-
Control membrane	74.3 ± 0.7	11.4 ± 0.3	14.0 ± 0.5	-	0.3 ± 0.1
AQP-0 membrane	74.6 ± 0.7	10.1 ± 0.3	15.2 ± 0.6	-	0.1 ± 0.1
AQP-1 membrane	73.7 ± 0.7	10.1 ± 0.3	16.2 ± 0.8	-	0.1 ± 0.1

In comparison to the support, the concentration of C and O dropped for the control membrane and AQP-0 and AQP-1 membranes, while the concentration of N increased due to the polyamide layer formed on top of the membrane. Regarding the spectra for the polyamide membranes, there is no longer a peak from S, indicating a full coverage of the support with a polyamide thin film for all membrane samples. Concentrations of N decreased by 1.5 %, while concentration of O increased by 1-2 % for both AQP-0 and AQP-1 membranes in comparison to the control. This indicates that the elemental composition of polyamide formed when polymersomes are introduced differs from the polyamide without polymersomes. We speculate that the elevated concentration of oxygen indicates incorporation of PMOXA-PDMS-PMOXA and amino-PDMS via reaction with TMC. Counterintuitively, the content of Si is the highest for the control membrane and drops for AQP-0 and AQP-1, where Si-containing polymers have been introduced. However, the content of Si is at the

344 lower limit of detection, with a high standard deviation, which may be a result of sample cross
345 contamination, as all of the samples were stored together overnight prior testing.

346

347 3.2.3. ζ -potential measurements

348 The ζ -potential measurements of polyamide membranes and support are presented in Table 3.

349 *Table 3. ζ -potential of the support, control, AQP-0 and AQP-1 membranes measured at pH 5.5.*

Sample name	ζ -potential (mV)	Standard Deviation
Support Membrane	- 20.13	± 1.63
Control Membrane	- 28.93	± 0.34
AQP-0 membrane	- 36.05	± 1.97
AQP-1 membrane	- 34.50	± 0.77

350

351 As expected, measured ζ -potential for all of the polyamide coated membranes was about 9 - 16 mV
352 more negative than ζ -potential for the support membrane. Lower ζ -potential of polyamide coated
353 membranes indicated a more negatively charged surface of the membrane and in fact again confirms
354 the successful coating of the polyamide layer on top of the membrane support. The polyamide coated
355 membranes had similar ζ -potential, but for the AQP-0 and AQP-1 doped membranes, the ζ -potential
356 was 5.57 – 7.12 mV more negative than for the control membrane. Taking into account the accuracy
357 of the measurement method and standard deviations, we assume that this slight change has a minor
358 impact on the separation properties between control membrane and membranes doped with
359 polymersomes: AQP-0 and AQP-1.

360

361

362

3.3. Membrane performance

Water permeability and NaCl rejection of the control membranes, membranes doped with AQP-0 and membranes doped with AQP-1 polymersomes are presented in Figure 3. For each type of RO membrane, 9 coupons were tested.

Figure 3. Water permeability and NaCl rejection of membrane samples without polymersomes, with AQP-0 polymersomes and AQP-1 polymersomes. The membranes were tested at 5 bars with 500 ppm NaCl as feed solution, at 25 °C and feed flow rate of 60 Lh⁻¹.

The control membranes, which are composed of a polyamide layer without polymersomes, resulted in an average water permeability of 4.20 ± 0.25 Lm²h⁻¹bar⁻¹ and a NaCl rejection of 92.7 ± 3.9 %. The membranes with AQP-0 polymersomes exhibited increased water permeability in comparison to the control membrane: 5.59 ± 0.42 Lm²h⁻¹bar⁻¹, while maintaining comparable rejection of NaCl:

376 92.2 ± 4.1 %. The highest average water permeability was observed for the samples prepared with
 377 AQP-1, which was 6.38 ± 0.55 Lm⁻²h⁻¹bar⁻¹ and NaCl rejection was also maintained at 93.5 ± 3.4 %.
 378 Statistical analysis with One-Way ANOVA and the Tukey Test was made for each pair of membrane
 379 permeability datasets and confirmed that each set of prepared membranes is significantly different
 380 from the others (for all comparisons of the pairs, the p-value was always < 0.05, with an overall
 381 ANOVA p-value of 4.34 · 10⁻¹⁰ and an F-value of 60.33 with F-critical of 3.43) (Figure 4).

382
 383 *Figure 4. Tukey test means comparison plot for permeability of different membrane sets. If an interval does not*
 384 *contain 0, the corresponding means of pairs are significantly different.*

385 Compared to the membranes without polymersomes (control), addition of AQP-0 polymersomes
 386 increased water permeability by 33 %, while the NaCl rejection was maintained at the same level.
 387 Membranes prepared with AQP-1 polymersomes resulted in a further increase in performance,
 388 compared to the control, with an improved water permeability of 51 % and comparable NaCl
 389 rejection. The permeability increase with AQP-1 compared to AQP-0 was 14 %. The increased water
 390 permeability after addition of AQP-0, clearly indicates that the polymersomes contribute to
 391 facilitation of water transport through the TFC active layer, due to high water permeability through
 392 their triblock copolymer membrane wall, confirmed by the SFLS. Similarly, Qi et al. used PMOXA-
 393 PDMS-PMOXA polymersomes to improve water permeability [39]. Additionally, the improved

water permeability can be explained by the incorporation of the hollow nanostructures in the polyamide layer, corresponding to the case recently reported by Ma et al.; in their work, the nanobubbles formed during IP reactions were responsible for the facilitation of water permeability [47]. Here, the AQP-0 polymersomes may play a similar role. Furthermore, NH₂ groups from amino-modified PDMS chains within polymersomes, may compete with MPD in interfacial polymerization reactions. The resulting membrane will be less densely cross-linked, as long PDMS chains will be introduced in the polyamide – the results obtained from XPS clearly indicate that the structure of the formed polyamide has changed (Table 2).

Finally, the use of AQP-1 can increase the water permeability even further than AQP-0. This indicates that AqpZ activity is maintained when AQP-1 are incorporated into the active layer matrix of the membrane. The additional increase in water permeability suggests that the water transport is facilitated through the AqpZ channels, which have been functionally reconstituted to the ABA copolymer membrane of the polymersomes. We speculate that the addition of the amino-modified PDMS during reconstitution of the AQP-0 and AQP-1 polymersomes and the incorporation of those polymer chains into the vesicles allowed for covalent bonding of the polymersomes to the polyamide matrix. As a result, rejection of the NaCl was maintained at the same level for all the tested membranes, even though water permeability increased significantly.

411

412 **4. Conclusions**

Highly permeable NH₂-functionalized triblock PMOXA-PDMS-PMOXA copolymer polymersomes prepared by a bulk hydration method can functionally reconstitute AqpZ proteins. In this study, it has been shown that such polymersomes, which can reconstitute AqpZ water channel proteins can be successfully used for improving the permeability of reverse osmosis membranes without affecting the thickness nor the surface charge of the resulting polyamide layer. Changed elemental composition

418 of the polyamide layer proves that the triblock copolymer is being incorporated into the polyamide.
419 Additionally, incorporation of the blank polymersomes improves water permeability, but an even
420 greater increase in permeability can be achieved with the introduction of Aquaporin Z proteins. The
421 approach for biomimetic membrane fabrication used in this study is based on industrially well-known
422 interfacial polymerization synthesis of polyamide and is therefore relatively easy to upscale to full-
423 size membrane production. Furthermore, since the bulk hydration self-assembly method for
424 preparation of polymersomes does not require multiple steps (such as film rehydration), upscaling of
425 the polymersome production process is also possible. The proposed approach offers great promise
426 for water treatment technology due to the combination of improved membrane performance coupled
427 with process scalability for large-scale industrial biomimetic membrane manufacturing.

428

429 **Acknowledgements**

430 The authors would like to thank Dr. Jörg Vogel for support and feedback during the research work
431 and writing this article, Scott Treven Myers for scientific feedback and proof reading and
432 Katrine Veis for language related feedback.

433

434 This work is co-founded by Aquaporin A/S and the Innovation Fund Denmark under the project
435 *Development of Next Generation of Aquaporin InsideTM membranes* and EU Horizon 2020 project
436 *Aquaporin-InsideTM Membranes for Brackish water Reverse Osmosis Application (AMBROSIA)*,
437 *grant agreement ID 783848*.

438

439

440

441

References

- [1] B.F. Milan, *Clean water and sanitation for all: interactions with other sustainable development goals*, Sustain. Water Resour. Manag. 3 (2017) 479–489. doi:10.1007/s40899-017-0117-4.
- [2] United Nations Environment: UN-WATER, *The United Nations World Water Development Report 2018: Nature-Based Solutions for Water*, UNESCO, Paris, France, 2018. www.unesco.org/open-access/.
- [3] H.T. Madsen, N. Bajraktari, C. Hélix-Nielsen, B. Van der Bruggen, E.G. Søgaaard, *Use of biomimetic forward osmosis membrane for trace organics removal*, J. Memb. Sci. 476 (2015) 469–474. doi:10.1016/j.memsci.2014.11.055.
- [4] M. Shannon, P.W. Bohn, M. Elimelech, J.G. Georgiadis, B.J. Mariñas, A.M. Mayes, *Science and technology for water purification in the coming decades*, Nature. 452 (2008) 301–310. doi:10.1038/nature06599.
- [5] T. Hey, N. Bajraktari, J. Vogel, C. Hélix Nielsen, J. la Cour Jansen, K. Jönsson, *The effects of physicochemical wastewater treatment operations on forward osmosis*, Environ. Technol. 0 (2016) 1–13. doi:10.1080/09593330.2016.1246616.
- [6] H.B. Park, J. Kamcev, L.M. Robeson, M. Elimelech, B.D. Freeman, *Maximizing the right stuff: The trade-off between membrane permeability and selectivity*, Science. 356 (2017). doi:10.1126/science.aab0530.
- [7] M.M. Pendergast, E.M.V. Hoek, *A review of water treatment membrane nanotechnologies*, Energy Environ. Sci. 4 (2011) 1946–1971. doi:10.1039/c0ee00541j.
- [8] H.L. Wang, T.S. Chung, Y.W. Tong, K. Jeyaseelan, A. Armugam, H.H.P. Duong, F. Fu, H. Seah, J. Yang, M. Hong, *Mechanically robust and highly permeable Aquaporin Z biomimetic membranes*, J. Memb. Sci. 434 (2013) 130–136.

- doi:10.1016/j.memsci.2013.01.031.
- [9] H. Elfil, A. Hamed, A. Hannachi, *Technical evaluation of a small-scale reverse osmosis desalination unit for domestic water*, Desalination. 203 (2007) 319–326.
doi:10.1016/j.desal.2006.03.530.
- [10] D. Li, Y. Yan, H. Wang, *Recent advances in polymer and polymer composite membranes for reverse and forward osmosis processes*, Prog. Polym. Sci. 61 (2016) 104–155.
doi:10.1016/j.progpolymsci.2016.03.003.
- [11] C.Y. Tang, Y. Zhao, R. Wang, C. Hélix-Nielsen, A.G. Fane, *Desalination by biomimetic aquaporin membranes: Review of status and prospects*, Desalination. 308 (2013) 34–40.
doi:10.1016/j.desal.2012.07.007.
- [12] C. Tang, Z. Wang, I. Petrinic, A.G. Fane, C. Hélix-Nielsen, *Biomimetic aquaporin membranes coming of age*, Desalination. 368 (2014) 89–105.
doi:10.1016/j.desal.2015.04.026.
- [13] X. Li, S. Chou, R. Wang, L. Shi, W. Fang, G. Chaitra, C.Y. Tang, J. Torres, X. Hu, A.G. Fane, *Nature gives the best solution for desalination: Aquaporin-based hollow fiber composite membrane with superior performance*, J. Memb. Sci. 494 (2015) 68–77.
doi:10.1016/j.memsci.2015.07.040.
- [14] P.A. Pedersen, F.B. Bjørkskov, S. Alvisse, C. Hélix-Nielsen, *From channel proteins to industrial biomimetic membrane technology*, Faraday Discuss. 209 (2018) 287–301.
doi:10.1039/c8fd00061a.
- [15] C. Helix-Nielsen, *Biomimetic Membranes for Sensor and Separation Applications*, 2015.
doi:10.1017/CBO9781107415324.004.
- [16] C.G. Palivan, R. Goers, A. Najer, X. Zhang, A. Car, W. Meier, *Bioinspired polymer vesicles and membranes for biological and medical applications*, Chem. Soc. Rev. 45 (2016) 377–

411. doi:10.1039/C5CS00569H.
- [17] M.P. Grzelakowski, *Novel Polymers and Process for Making Membranes*, WO 2015/144725 A1, 2015.
- [18] F. Itel, A. Najer, C.G. Palivan, W. Meier, *Dynamics of Membrane Proteins within Synthetic Polymer Membranes with Large Hydrophobic Mismatch*, (2015). doi:10.1021/acs.nanolett.5b00699.
- [19] M. Kumar, M. Grzelakowski, J. Zilles, M. Clark, W. Meier, *Highly permeable polymeric membranes based on the incorporation of the functional water channel protein Aquaporin Z*, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 20719–24. doi:10.1073/pnas.0708762104.
- [20] H. Wang, T.S. Chung, Y.W. Tong, K. Jeyaseelan, A. Armugam, Z. Chen, M. Hong, W. Meier, *Highly permeable and selective pore-spanning biomimetic membrane embedded with Aquaporin Z*, Small. 8 (2012) 1185–1190. doi:10.1002/smll.201102120.
- [21] M. Grzelakowski, M.F. Cherenet, Y. xiao Shen, M. Kumar, *A framework for accurate evaluation of the promise of aquaporin based biomimetic membranes*, J. Memb. Sci. 479 (2015) 223–231. doi:10.1016/j.memsci.2015.01.023.
- [22] M.P. Grzelakowski, *Novel Polymers and Process for Making Membranes*, WO 2015/144725, 2018.
- [23] D. Wu, M. Spulber, F. Itel, M. Chami, T. Pfohl, C.G. Palivan, W. Meier, *Effect of molecular parameters on the architecture and membrane properties of 3D assemblies of amphiphilic copolymers*, Macromolecules. 47 (2014) 5060–5069. doi:10.1021/ma500511r.
- [24] N. Muhammad, T. Dworeck, M. Fioroni, U. Schwaneberg, *Engineering of the E. coli Outer Membrane Protein FhuA to overcome the Hydrophobic Mismatch in Thick Polymeric Membranes*, Journal of Nanobiotechnology 9:8 (2011) 1–9.

- 514 [25] M. Spulber, D. Tvermoes, R. Górecki, F. Haugsted, ***Vesicle Incorporating Transmembrane***
 515 ***Protein***, WO 2019/081371 A1, 2019.
- 516 [26] Y. Zhao, C. Qiu, X. Li, A. Vararattanavech, W. Shen, J. Torres, C. Hélix-Nielsen, R. Wang,
 517 X. Hu, A.G. Fane, C.Y. Tang, ***Synthesis of robust and high-performance aquaporin-based***
 518 ***biomimetic membranes by interfacial polymerization-membrane preparation and RO***
 519 ***performance characterization***, J. Memb. Sci. 423–424 (2012) 422–428.
 520 doi:10.1016/j.memsci.2012.08.039.
- 521 [27] R. Sengur-Tasdemir, B. Sayinli, G.M. Urper, H.E. Tutuncu, N. Gul-Karaguler, E. Ates-
 522 Genceli, V. V. Tarabara, I. Koyuncu, ***Hollow fiber nanofiltration membranes with***
 523 ***integrated aquaporin Z***, New J. Chem. 42 (2018) 17769–17778. doi:10.1039/C8NJ04367A.
- 524 [28] X. Li, C.H. Loh, R. Wang, W. Widjajanti, J. Torres, ***Fabrication of a robust high-***
 525 ***performance FO membrane by optimizing substrate structure and incorporating***
 526 ***aquaporin into selective layer***, J. Memb. Sci. 525 (2016) 257–268.
 527 doi:10.1016/j.memsci.2016.10.051.
- 528 [29] D.E. Discher, A. Eisenberg, ***Polymer Vesicles***, Science 297 (2002) 967–973
- 529 [30] P.H.H. Duong, T.S. Chung, K. Jeyaseelan, A. Armugam, Z. Chen, J. Yang, M. Hong, ***Planar***
 530 ***biomimetic aquaporin-incorporated triblock copolymer membranes on porous alumina***
 531 ***supports for nanofiltration***, J. Memb. Sci. 409–410 (2012) 34–43.
 532 doi:10.1016/j.memsci.2012.03.004.
- 533 [31] P.S. Zhong, T.S. Chung, K. Jeyaseelan, A. Armugam, ***Aquaporin-embedded biomimetic***
 534 ***membranes for nanofiltration***, J. Memb. Sci. 407–408 (2012) 27–33.
 535 doi:10.1016/j.memsci.2012.03.033.
- 536 [32] L. Xia, M.F. Andersen, C.H. Nielsen, J.R. McCutcheon, ***Novel Commercial Aquaporin Flat-***
 537 ***sheet Membrane for Forward Osmosis***, Ind. Eng. Chem. Res. (2017) 11919–11925.

- doi:10.1021/acs.iecr.7b02368.
- [33] W. Xie, F. He, B. Wang, T.-S. Chung, K. Jeyaseelan, A. Armugam, Y.W. Tong, *An aquaporin-based vesicle-embedded polymeric membrane for low energy water filtration*, J. Mater. Chem. A. 1 (2013) 7592. doi:10.1039/c3ta10731k.
- [34] D. Li, Y. Yan, H. Wang, *Recent advances in polymer and polymer composite membranes for reverse and forward osmosis processes*, Prog. Polym. Sci. (2016). doi:10.1016/j.progpolymsci.2016.03.003.
- [35] A. Wagner, K. Vorauer-Uhl, *Liposome Technology for Industrial Purposes*, J. Drug Deliv. 2011 (2011) 1–9. doi:10.1155/2011/591325.
- [36] X. Sui, P. Kujala, G.J. Janssen, E. De Jong, I.S. Zuhorn, J.C.M. Van Hest, *Robust formation of biodegradable polymersomes by direct hydration*, Polym. Chem. 6 (2015) 691–696. doi:10.1039/c4py01288g.
- [37] M. Spulber, K. Gerstandt, *Diblock Copolymer Vesicles and Separation Membranes Comprising Aquaporin Water Channels and Methods of Making and Using Them*, WO 2018/141985 A1, 2018.
- [38] A. Horner, P. Pohl, *Single-file transport of water through membrane channels*, Faraday Discuss. 209 (2018) 9–33. doi:10.1039/c8fd00122g.
- [39] S. Qi, W. Fang, W. Siti, W. Widjajanti, X. Hu, R. Wang, *Polymersomes-based high-performance reverse osmosis membrane for desalination*, J. Memb. Sci. 555 (2018) 177–184. doi:10.1016/j.memsci.2018.03.052.
- [40] F.B. Bjørkskov, S.L. Krabbe, C.N. Nurup, J.W. Missel, M. Spulber, J. Bomholt, K. Molbaek, C. Helix-Nielsen, K. Gotfryd, P. Gourdon, P.A. Pedersen, *Purification and functional comparison of nine human Aquaporins produced in Saccharomyces cerevisiae for the purpose of biophysical characterization*, Sci. Rep. 7 (2017).

doi:10.1038/s41598-017-17095-6.

[41] H.X. Gan, H. Zhou, Q. Lin, Y.W. Tong, *Quantification of Aquaporin-Z reconstituted into vesicles for biomimetic membrane fabrication*, Sci. Rep. 7 (2017) 1–13. doi:10.1038/s41598-017-11723-x.

[42] M. Chau, H. Chu, W. Light, *Dry High Flux Semipermeable Membranes*, WO 91/08828, 1991.

[43] T. Tadros, *Colloid and interface aspects of pharmaceutical science*, Elsevier B.V., 2014. doi:10.1016/B978-0-444-62614-1.00002-8.

[44] V. Pata, F. Ahmed, D.E. Discher, N. Dan, *Membrane Solubilization by Detergent : Resistance Conferred by Thickness*, Langmuir. 20 (2004) 3888–3893. doi:10.1021/la035734e.

[45] S. Pispas, *Vesicular structures in mixed block copolymer / surfactant solutions*, Soft Matter. 7 (2011) 8697–8701. doi:10.1039/c1sm05584d.

[46] A.S. Verkman, A.K. Mitra, *Structure and function of aquaporin water channels*, Am.. J. Physiol. Ren. Physiol. 278 (2000) F13–F28. doi:10.1002/mus.20766.

[47] X.-H. Ma, Z. Yao, Z. Yang, H. Guo, Z. Xu, C.Y. Tang, M. Elimelech, *Nanofoaming of Polyamide Desalination Membranes To Tune Permeability and Selectivity*, Environ. Sci. & Technol. Lett. 5 (2018) 123–130. doi:10.1021/acs.estlett.8b00016.