

A BIOMIMETIC MEMBRANE

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of priority of Singapore patent application No. 10201506438Q filed on 14 August 2015, the content of which is incorporated herein by reference in its entirety for all purposes.

TECHNICAL FIELD

[0002] Various embodiments relate to a biomimetic membrane and a method of preparing the biomimetic membrane.

BACKGROUND

[0003] Water is the most essential component for life. Faced with scarcity of fresh water in many areas of the world, water purification has become a promising industry with a variety of techniques applied, such as multi-effect distillation (MED), multistage flash distillation (MSF), and reverse osmosis (RO) membrane desalination.

[0004] Among the various technologies, biomimetic membrane desalination has been proposed to be the most prospective technique in the future. As studied in the last two decades, biological membranes have evolved as the most effective way for water transport across an osmotic pressure gradient via a transmembrane protein such as aquaporin (Aqp). The aquaporins are usually bound in phospholipid cellular membranes, where water passes through the protein channel and ions are rejected. Permeability of up to thirty billion water molecules per aquaporin per second and rejection of more than 99 % ions have been achieved. Water permeability of 960 L/m²h was estimated to be achieved by a biomimetic membrane consisting of lipid/aquaporin with molar ratio of 2000:1, which is more than two orders higher than state-of-art membranes.

[0005] In the last couple of years, aquaporin-incorporated proteoliposomes and aquaporin-incorporated proteopolymersomes have been intensively studied and developed to immobilize in the porous substrates and aquaporin-based biomimetic membranes have been fabricated using a variety of strategies. These biomimetic membranes may be achieved by fusion of the proteoliposomes or proteopolymersomes onto the nano-sized porous substrates

or nanofiltration membranes, and also by embedding intact proteoliposomes or proteopolymersomes via interfacial polymerizations or electrostatic interactions.

[0006] The scale of the fabricated biomimetic membranes ranges from several square millimeters to hundreds of square centimeters depending on fabrication strategies. Most of the membranes possess enhanced water permeability and maintain fairly good salt rejection compared to those without aquaporin incorporation. However, proteoliposomes or proteopolymersomes are involved in the fabrication process in all the above-mentioned biomimetic membranes. There is no report on successful membrane integration of aquaporin without using a lipid or a copolymer.

[0007] The aquaporin-incorporated vesicles, i.e., proteoliposomes and proteopolymersomes, may be formed by sophisticated biological-like methods of film hydration method with detergent-assisted aquaporin incorporation via complicated processes, such as vortex, cycles of freeze-thaw, extrusion and dialysis. The methods are time-consuming and takes up materials, which may limit their further engineering applications and scale up. Besides, although the idea of the aquaporin-based biomimetic membrane has been successfully demonstrated, its unique advantages over conventional membranes are not so prominent due to several incompatible technical requirement, such as increase of aquaporin contribution vs. increased weakness of the membrane and ionic leakage as well as increased mechanical strength vs. decreased stability/activity of aquaporins.

[0008] In view of the above, there exists a need for an improved biomimetic membrane and method to prepare the biomimetic membrane that overcomes or at least alleviates one or more of the above-mentioned problems.

SUMMARY

[0009] In a first aspect, a method of preparing a biomimetic membrane is provided. The method comprises

- a) depositing a first mixture comprising a first polyelectrolyte having a charge and a transmembrane protein on a surface of a support;
- b) depositing a second mixture comprising a second polyelectrolyte and a cross-linking agent on the first mixture which is deposited on the surface of the

support, the second polyelectrolyte having a charge opposite to the charge on the first polyelectrolyte; and

- c) cross-linking the second polyelectrolyte with the cross-linking agent to obtain the biomimetic membrane.

5 [0010] In a second aspect, a biomimetic membrane prepared by a method according to the first aspect is provided.

[0011] In a third aspect, a biomimetic membrane is provided. The membrane comprises a polyelectrolyte rejection layer having a transmembrane protein dispersed therein, wherein the transmembrane protein is not contained in a vesicle.

10 [0012] In a fourth aspect, use of a biomimetic membrane according to the second aspect or the third aspect in nanofiltration is provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The invention will be better understood with reference to the detailed description
15 when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0014] **FIG. 1** is a schematic diagram of aquaporin Z (AqpZ)-hydrogel membrane preparation according to embodiments. Poly(allylamine hydrochloride) (PAH) and AqpZ with *n*-octyl- β -D-glucoside (OG) are dissolved in the aqueous solution to form precursor A,
20 while vinylsulfonic acid sodium salt (VSS) and cross-linker N, N'-methylenebis(acrylamide) in the aqueous solution to form precursor B. Precursor A is deposited onto the negative charged polyacrylonitrile (PAN) substrate for about 10 seconds with additional 20 seconds of spinning to spin off the excess solution followed by precursor B in the same process via spin coating. Thereafter, the composite membrane containing both precursor A and B is
25 immediately subject to UV irradiation to form the cross-linked semi- interpenetrate network (semi-IPN) hydrogel membrane.

[0015] **FIG. 2A** is a scanning electron microscopy (SEM) image of the surface of the pristine PAN substrate before sodium hydroxide (NaOH) treatment. Scale bar in the figure denotes 5 μ m.

30 [0016] **FIG. 2B** is a SEM image of the surface of negative-charged PAN substrate after treatment. Scale bar in the figure denotes 5 μ m.

[0017] **FIG. 2C** is a SEM image of the surface of the hydrogel membrane without AqpZ embedment. Scale bar in the figure denotes 5 μm .

[0018] **FIG. 2D** is a SEM image of the surface of the AqpZ-hydrogel membrane. Scale bar in the figure denotes 5 μm .

5 [0019] **FIG. 3A** shows normalized stopped flow curves of DOPC, UV-DOPC, AqpZ-DOPC, UV-(AqpZ-DOPC), (UV-AqpZ)-DOPC, and mAqpZ-DOPC.

[0020] **FIG. 3B** shows water permeability of DOPC, UV-DOPC, AqpZ-DOPC, UV-(AqpZ-DOPC), (UV-AqpZ)-DOPC, and mAqpZ-DOPC.

[0021] **FIG. 4** shows water flux and salt rejection of the hydrogel membrane with
10 different weight ratio of AqpZ embedded by the dead-end filtration measurement. Nominal AqpZ concentration refers to the AqpZ weight concentration in the PAH precursor solution. 0 mg/mL indicates the hydrogel membrane without any AqpZ embedment. The error bars present one standard deviation.

[0022] **FIG. 5** is a schematic illustration of structures of conventional aquaporin-based
15 membranes (Case 1), the AqpZ-hydrogel membrane with AqpZ embedment in present studies (Case 2), and the ideal AqpZ-hydrogel membrane proposed to be studied and achieved (Case 3). The images are not drawn strictly to scale.

[0023] **FIG. 6** is a graph showing water flux and salt rejection of different membranes by
20 dead-end filtration measurement: the hydrogel membrane without AqpZ or mutant (hydrogel membr.), AqpZ-hydrogel membrane with 0.20 mg/mL AqpZ in the precursor of PAH solution (AqpZ-hydrogel membr.), and mAqpZ-hydrogel membrane with 0.20 mg/mL mutant AqpZ in the precursor of PAH solution (mAqpZ-hydrogel membr.). The error bars present the standard deviation.

25 DETAILED DESCRIPTION

[0024] Various embodiments refer in a first aspect to a method of preparing a biomimetic membrane. The term “biomimetic” is used herein to describe a human-made process, substance or material that imitate or mimic properties of processes, substances or materials found in nature, while the term “membrane” refers to a semi-permeable material that
30 selectively allows certain species to pass through it while retaining others within or on the material. Accordingly, the term “biomimetic membrane” refers to a semi-permeable material

which imitates or mimics properties of living cell membranes, and which selectively allows certain species to pass through it while retaining others within or on the material.

[0025] As demonstrated herein, incorporation of transmembrane proteins such as aquaporin (Aqp) into a membrane has exhibited extremely high permeability and selectivity for ions, which may potentially be used in applications in water purification and seawater desalination. Advantageously, the biomimetic membrane disclosed herein may be prepared without using a vesicle, such as lipid or polymer vesicles, thereby avoiding use of complicated biological processes relating to incorporation of transmembrane proteins such as aquaporin into vesicles, which in turn translates into reduction in process and material costs as well as ease of process control.

[0026] In various embodiments, incorporation of Aqp in the membrane has been shown to retain functionality of the membrane as demonstrated by good salt rejection performances, while water flux of the membrane may increase significantly by more than 40 % compared to that without using Aqp. The membrane may be further optimized by aligning Aqp incorporated in the membrane to increase water flux performance. It has great potential for substantially reducing cost of the membrane fabrication and facilitates adoption of methods disclosed herein towards various engineering applications such as nanofiltration.

[0027] With the above in mind, various embodiments relate to a method of preparing a biomimetic membrane, the method comprising depositing a first mixture comprising a first polyelectrolyte having a charge and a transmembrane protein on a surface of a support.

[0028] The term “polyelectrolyte” as used herein refers to a macromolecule carrying a charge, and may include polymers that have either cationic or anionic groups chemically bonded to a polymer chain. Polymers having cationic groups chemically bonded to a polymer chain may have a positive net charge, and may be termed as a polycation, while polymers having anionic groups chemically bonded to a polymer chain may have a negative net charge, and may be termed as a polyanion.

[0029] Suitable polyanions may include anionic polyelectrolytes which have high solubility in aqueous solution or which have low steric hindrance. Examples of polyanions include, but are not limited to, poly(styrene sulfonic acid), poly(acrylic acid), poly(methacrylic acid), poly(maleic acid), poly(itaconic acid), sulfated poly(vinyl alcohol), poly(vinylsulfonic acid), poly(acrylic acid-co-maleic acid), poly(styrene sulfonic acid-co-maleic acid), poly(ethylene-co-acrylic acid), poly(phosphoric acid), poly(silicic acid),

hectorite, bentonite, alginic acid, pectic acid, xanthan, gum arabic, dextran sulfate, carboxy methyl dextran, carboxy methyl cellulose, cellulose sulfate, cellulose xanthogenate, starch sulfate, starch phosphate, lignosulfonate, polygalacturonic acid, polyglucuronic acid, polyguluronic acid, polymannuronic acid, chondroitin sulfate, heparin, heparan sulfate, hyaluronic acid, dermatan sulfate, keratan sulfate; poly-(L)-glutamic acid, poly-(L)-aspartic acid, acidic gelatins (A-gelatins); starch, amylose, amylopectin, cellulose, guar, guar gum, pullulan, dextran, chitin or chitosan derivatives having the following functional groups: carboxymethyl, carboxyethyl, carboxypropyl, 2-carboxyvinyl, 2-hydroxy-3-carboxypropyl, 1,3-dicarboxyisopropyl, sulfomethyl, 2-sulfoethyl, 3-sulfopropyl, 4-sulfobutyl, 5-sulfopentyl, 2-hydroxy-3-sulfopropyl, 2,2-disulfoethyl, 2-carboxy-2-sulfoethyl, maleate, succinate, phthalate, glutarate, aromatic and aliphatic dicarboxylates, xanthogenate, sulfate, phosphate, 2,3-dicarboxy, N,N-di(phosphatomethyl)aminoethyl, N-alkyl-N-phosphatomethylaminoethyl, and combinations thereof.

[0030] Examples of polycations include, but are not limited to, poly(aniline); poly(pyrrole); poly(alkylenimines); poly-(4-vinylpyridine); poly(vinylamine); poly(2-vinylpyridine), poly(2-methyl-5-vinylpyridine), poly(4-vinyl-N-C1-C18-alkylpyridinium salt), poly(2-vinyl-N-C1-C18-alkylpyridinium salt), polyallylamine, aminoacetylated polyvinyl alcohol; poly-(L)-lysine, poly-(L)-arginine, poly(ornithine), basic gelatins (B-gelatins), chitin or chitosan derivatives having the following functional groups: 2-aminoethyl, 3-aminopropyl, 2-dimethylaminoethyl, 2-diethylaminoethyl, 2-diisopropylaminoethyl, 2-dibutylaminoethyl, 3-diethylamino-2-hydroxypropyl, N-ethyl-N-methylaminoethyl, 2-diethylhexylaminoethyl, 2-hydroxy-2-diethylaminoethyl, 2-hydroxy-3-trimethylammonionopropyl, 2-hydroxy-3-triethylammonionopropyl, 3-trimethylammonionopropyl, 2-hydroxy-3-pyridiniumpropyl, S,S-dialkylthioniumalkyl, and combinations thereof.

[0031] Besides the first polyelectrolyte, a transmembrane protein is also present in the first mixture. The first polyelectrolyte and the transmembrane protein may be dissolved and/or dispersed in an aqueous solution such as water to form the first mixture. The term “transmembrane protein” is generally understood by a person skilled in the art to refer to a membrane protein that at least partially spans a biological membrane. In some embodiments, a transmembrane protein is a membrane protein that spans a biological membrane. By

incorporating a transmembrane protein in a non-biological membrane as disclosed herein, extremely high permeability and selectivity for ions have been demonstrated.

[0032] Examples of transmembrane protein include, but are not limited to, aquaporins, aquaglyceroporins, and other channel proteins or analogues such as ion channel proteins and analogues.

[0033] In various embodiments, the transmembrane protein comprises an aquaporin (AQP). The term "aquaporin" as used herein refers to any functional water channel, which may be selected from the group consisting of Aqp 4, Aqp 1, Aqp Z, SoPIP2; 1 and monomeric, dimeric, tetrameric and higher oligomers as well as functional variants thereof including mutated, conjugated and truncated versions of the primary sequence, e.g. engineered variants of specific aquaporins that are optimised for heterologous expression.

[0034] In specific embodiments, the aquaporin is AqpZ, which refers to *E. coli* aquaporin Z such as that used in the examples.

[0035] The transmembrane protein may be protected with a surfactant. The surfactant, otherwise termed herein as a detergent, may be used to solubilize the transmembrane protein without loss of biological activity.

[0036] In various embodiments, the surfactant is selected from the group consisting of n-octyl- β -D-glucoside, n-octanoylsucrose, n-nonanoylsucrose, n-decanoylsucrose, n-undecanoylsucrose, n-dodecanoylsucrose, n-heptyl- β -D-glucoside, n-heptyl- β -D-maltoside, n-heptyl- β -D-maltopyranoside, n-heptyl- β -D-glucopyranoside, n-heptyl- β -D-thioglucoside, n-heptyl- β -D-thiomaltoside, n-heptyl- β -D-thiomaltopyranoside, n-heptyl- β -D-thioglucopyranoside, n-octyl- β -D-maltoside, n-octyl- β -D-maltopyranoside, n-octyl- β -D-glucopyranoside, n-octyl- β -D-thioglucoside, n-octyl- β -D-thiomaltoside, n-octyl- β -D-thiomaltopyranoside, n-octyl- β -D-thioglucopyranoside, n-nonyl- β -D-glucoside, n-nonyl- β -D-maltoside, n-nonyl- β -D-maltopyranoside, n-nonyl- β -D-glucopyranoside, n-nonyl- β -D-thioglucoside, n-nonyl- β -D-thiomaltoside, n-nonyl- β -D-thiomaltopyranoside, n-nonyl- β -D-thioglucopyranoside, n-decyl- β -D-glucoside, n-decyl- β -D-maltoside, n-decyl- β -D-maltopyranoside, n-decyl- β -D-glucopyranoside, n-decyl- β -D-thioglucoside, n-decyl- β -D-thiomaltoside, n-decyl- β -D-thiomaltopyranoside, n-decyl- β -D-thioglucopyranoside, n-undecyl- β -D-glucoside, n-undecyl- β -D-maltoside, n-undecyl- β -D-maltopyranoside, n-undecyl- β -D-glucopyranoside, n-undecyl- β -D-thioglucoside, n-undecyl- β -D-thiomaltoside, n-undecyl- β -D-thiomaltopyranoside, n-undecyl- β -D-thioglucopyranoside, n-dodecyl- β -D-

glucoside, n-dodecyl- β -D-maltoside, n-dodecyl- β -D-maltopyranoside, n-dodecyl- β -D-glucopyranoside, n-dodecyl- β -D-thioglucoside, n-dodecyl- β -D-thiomaltoside, n-dodecyl- β -D-thiomaltopyranoside, n-dodecyl- β -D-thioglucopyranoside, and any combination thereof.

[0037] In specific embodiments, the surfactant is n-octyl- β -D-glucoside.

5 [0038] Amount of the first polyelectrolyte in the first mixture may be in the range of about 4 wt% to about 8 wt%, such as about 4 wt% to about 6 wt%, about 4 wt% to about 5 wt%, or about 6 wt% to about 8 wt%. In specific embodiments, amount of the first polyelectrolyte in the first mixture is about 4 wt%.

[0039] Amount of the transmembrane protein in the first mixture may be in the range of
10 about 0.01 mg/mL to about 0.2 mg/mL, such as about 0.01 mg/mL to about 0.15 mg/mL, about 0.01 mg/mL to about 0.1 mg/mL, about 0.1 mg/mL to about 0.2 mg/mL, or about 0.15 mg/mL to about 0.2 mg/mL. Advantageously, it has been found by the inventors that incorporating an amount of about 0.2 mg/mL of transmembrane protein in the first mixture has resulted in more than 40 % increase in water flux of the resulting membrane, as compared
15 to a membrane that does not contain any transmembrane protein.

[0040] The first mixture comprising the first polyelectrolyte having a charge and the transmembrane protein are deposited on a surface of a support, which may be carried out using any suitable methods such as, but not limited to, spin-coating, spray coating, dip coating, and roll coating. The support may be a polymeric support, and may be formed of a
20 polymer suitable for use in a membrane. For example, the support may comprise a polymer selected from the group consisting of polyacrylonitrile (PAN), polysulfone (PSF), polyvinylidene fluoride (PVDF), polypropylene, copolymers thereof, and combinations thereof. In specific embodiments, the support comprises polyacrylonitrile.

[0041] The surface of the support having the first mixture deposited thereon may possess a
25 charge, such as a positive charge or a negative charge, prior to deposition of the first mixture. The charge on the surface may function to increase electrostatic interaction between the deposited first mixture comprising the first polyelectrolyte, as well as to enhance hydrophilicity of the support surface in cases where the charge is a negative charge.

[0042] In various embodiments, the charge is a negative charge. To confer negative
30 charges to a support comprising or formed completely of polyacrylonitrile, for example, the support may be modified using an alkali solution such as sodium hydroxide, so as to hydrolyze surface -CN groups present on the support to carboxyl groups which are

negatively charged. In addition to the above, pores which may be present on a surface of the support may be rendered more pronounced by hydrolysis of the $-CN$ groups and repulsion between carboxyl groups formed by the hydrolysis.

[0043] In various embodiments, the surface of the support having the first mixture deposited thereon is porous. Size of the pores may, for example, be in the range of about 80 nm to about 150 nm, such as about 100 nm to about 150 nm, or about 80 nm to about 100 nm. In depositing the first mixture on the surface of the support, substantially all, or all of the surface of the support may be covered by the first mixture.

[0044] After depositing the first mixture on a surface of the support, the method of preparing a biomimetic membrane disclosed herein comprises depositing a second mixture comprising a second polyelectrolyte and a cross-linking agent on the first mixture which is deposited on the surface of the support, wherein the second polyelectrolyte has a charge opposite to the charge on the first polyelectrolyte. The second polyelectrolyte and the cross-linking agent may be dissolved and/or dispersed in an aqueous solution such as water to form the second mixture. The deposition may be carried out by any suitable method such as, but not limited to, spin-coating, spray coating, dip coating, and roll coating.

[0045] As mentioned above, a polyelectrolyte may include polymers that have either cationic or anionic groups chemically bonded to a polymer chain, which may respectively be termed a polycation or a polyanion.

[0046] In various embodiments, the first polyelectrolyte and the second electrolyte are independently a polyanion or a polycation. For example, the first polyelectrolyte may be a polyanion, and the second polyelectrolyte may be a polycation. In further examples, the first polyelectrolyte may be a polycation, and the second polyelectrolyte may be a polyanion. Suitable polyanions and polycations have already been described above.

[0047] In some embodiments, the first polyelectrolyte is a polycation. For example, the first polyelectrolyte may comprise or be formed entirely of poly(allylamine hydrochloride) (PAH). The poly(allylamine hydrochloride) may have an average molecular weight in the range of about 120,000 to about 200,000, such as about 150,000 to about 200,000, or about 120,000 to about 150,000, although poly(allylamine hydrochloride) with other molecular weight values may also be used.

[0048] The second polyelectrolyte has a charge opposite to the charge on the first polyelectrolyte. Accordingly, in embodiments wherein the first polyelectrolyte is a

polycation, the second polyelectrolyte may be a polyanion. By virtue of their opposite charges, the first polyelectrolyte and the second polyelectrolyte may self-assemble to form a polyelectrolyte hydrogel. Advantageously, the cationic polyelectrolyte and counterions of anionic polyelectrolyte may work in tandem to provide extra salt rejection due to Donnan exclusion effect as well as strong and favorable interfacial interactions.

[0049] In various embodiments, the second polyelectrolyte may comprise or be formed entirely of poly(vinylsulfonic acid), wherein the poly(vinylsulfonic acid) is obtainable by polymerizing a vinylsulfonic acid sodium salt solution. The vinylsulfonic acid sodium salt and the cross-linking agent may be present, such that molar ratio of the cross-linking agent to the vinylsulfonic acid sodium salt in the second mixture is in the range of about 2 % to about 3 %, such as about 2.5 % to about 3 %, about 2 % to about 2.5 %, or about 2.4 % to about 2.6 %.

[0050] The cross-linking agent may be selected from the group consisting of N, N'-methylenebis(acrylamide), divinylbenzene, N,N'-ethylenebis(acrylamide), N,N'-propylenebis(acrylamide), N,N'-butamethylenebis(acrylamide), N,N'-diallylacrylamide, N,N'-hexamethylenebisacrylamide, triallylisocyanurate, 1,4-diacryloylpiperazine-1,1,1-trimethylolpropane diallyl ether, triethylene glycol divinyl ether, diallyl maleate, bis(acryloylamido)methane, ethyleneglycoldimethacrylate, diethyleneglycoldimethacrylate, 3-(acryloyloxy)-2-hydroxypropyl methacrylate, and combinations thereof.

[0051] In specific embodiments, the cross-linking agent is N, N'-methylenebis(acrylamide).

[0052] Following deposition of the second mixture on the first mixture which is deposited on the surface of the support, a composite film of two layers may result, with the first mixture constituting a first layer on the support, and the second mixture constituting a second layer on the support. In some embodiments, deposition of the second mixture on the first mixture may for example be carried out while the first mixture remains substantially in a liquid form, such that the first mixture and the second mixture inter-disperse to form a single layer.

[0053] By cross-linking the second polyelectrolyte with the cross-linking agent, a biomimetic membrane may be obtained. The cross-linking may be carried out to improve on the mechanical strength of the membrane and its durability. In various embodiments, cross-linking the second polyelectrolyte with the cross-linking agent comprises irradiating the second mixture with ultraviolet light.

[0054] In carrying out the cross-linking reaction, the first polyelectrolyte may interpenetrate across the cross-linked matrix formed by the second polyelectrolyte and the cross-linking agent, while embedding the transmembrane proteins which provide water channels for enhanced water flux in the network. Meanwhile, the strong anionic polyelectrolytes and counterions of cationic polyelectrolytes provide extra salt rejection due to Donnan exclusion effect, in particular for divalent ions, as well as strong and favourable interfacial interactions. In use, the swollen hydrophilic network also allows water molecules to pass through, which further improves water flux through the membrane.

[0055] Various embodiments refer in a second aspect to a biomimetic membrane prepared by a method according to the first aspect. Various embodiments refer in a further aspect to a biomimetic membrane comprising a polyelectrolyte rejection layer having a transmembrane protein dispersed therein, wherein the transmembrane protein is not contained in a vesicle.

[0056] Examples of transmembrane protein which may be used have already been discussed above. In various embodiments, the transmembrane protein comprises an aquaporin, which may, for example, be AqpZ.

[0057] The transmembrane protein may be protected with a surfactant. Examples of suitable surfactant have already been discussed above. In specific embodiments, the surfactant is n-octyl- β -D-glucoside.

[0058] As mentioned above, a membrane functions like a filter medium to permit a component separation by selectively controlling passage of the components from one side of the membrane to the other side. The polyelectrolyte rejection layer may act as a selective layer for rejecting dissolved compounds, and which may be arranged and/or attached onto a support that provides mechanical strength to the membrane. Advantageously, the polyelectrolyte rejection layer may be formed on an existing membrane which acts as a support for the biomimetic membrane, so as to enhance performance such as salt rejection level of the membrane.

[0059] Examples of suitable support that may be used have already been discussed above. In various embodiments, the support comprises or is formed entirely of polyacrylonitrile.

[0060] The biomimetic membrane disclosed herein may be used in nanofiltration (NF), which may be classified in the category between ultrafiltration (UF) and reverse osmosis (RO), and refers generally to a filtration technology involving use of a pressure driven membrane with pore size in the range of about 0.5 nm to 5 nm in diameter. The membranes

may be used in diverse fields such as, but not limited to, water industry for water softening, color removal, heavy metal recovery, food industry, as well as pharmaceutical and biomedical industries.

[0061] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0062] Other embodiments are within the following claims and non-limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

EXPERIMENTAL SECTION

[0063] A facile approach is disclosed herein to prepare a vesicle-free aquaporin-embedded hydrogel membrane for the first time targeting nanofiltration applications by combining both unique advantages of the aquaporin and polyelectrolyte hydrogel. In other words, the process to prepare aquaporin-based biomimetic membrane disclosed herein does not require use of lipid or polymer vesicles. The expressed aquaporin units are directly used for the first time to embed into the hydrogel membrane targeting membrane filtration applications, completely skipping the process of the proteoliposomes and proteopolymersomes. The aquaporin-embedded hydrogel membranes have shown significantly enhanced water flux and the aquaporin is proved to stay functional in the matrices.

[0064] As demonstrated herein, aquaporin may be effectively incorporated into membranes without using lipids or block copolymers, leaving out the biological process during the preparation and provides more concise process and easy membrane scaling-up. The highly flexible alkyl backbones and the semi-IPN hydrogel network provide favourable environments for aquaporin to be immobilized in. Meanwhile, the cross-linking ensures the mechanical strength of the membrane and durability. On the other hand, the strong anionic polyelectrolytes and counterions of cationic polyelectrolytes provide extra salt rejection due to Donnan exclusion effect as well as strong and favourable interfacial interactions.

[0065] In various embodiments, aquaporin Z (AqpZ) capped with detergent was directly deposited in the semi-interpenetrated hydrogel by subsequently spin coating of AqpZ-dispersed poly(allylamine hydrochloride) (PAH) solution and vinylsulfonic acid sodium salt solution mixed with cross-linker of N, N'-methylenebis(acrylamide) followed by UV cross-linking on the surface-modified polyacrylonitrile (PAN) substrate. Stopped flow spectrometry was used to prove the functionality of AqpZ after UV treatment in the fabrication process. The aquaporin-embedded hydrogel membranes were characterized by contact angle and scanning electron microscopy.

[0066] In various embodiments, the aquaporins are randomly dispersed in the membrane with irregular orientations. Advantageously, the aquaporins may be induced to orientate in specific orientations, therefore membrane performance such as water flux and salt rejection may be further enhanced.

[0067] Aquaporin/PAH-PVSS hydrogel membranes described here demonstrated and proved the feasibility, principles and basic fabrication processes towards high performance membranes. The methods and principles described herein can be extended using other polymers materials and aquaporin-like natural or synthetic water/ion channels using the same approach.

[0068] The novel strategy and facile preparation process disclosed herein provide a new opportunity for engineering application and scale-up of the biomimetic membrane. Meanwhile, it also helps to cut the cost of the biomimetic membrane in future industrial applications.

[0069] **Example 1: Chemicals and materials**

[0070] AqpZ and its mutant were expressed and purified in term of the previously reported procedures. 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) in chloroform solution (Avanti Polar Lipids, Alabaster, USA), PBS buffer (Fisher Scientific), *n*-octyl- β -D-glucoside (OG) (Calbiochem[®], Singapore) were used to prepare liposomes and AqpZ/mutant-incorporated proteoliposomes. Polyacrylonitrile (PAN) with molecular weight of 150,000 (International Laboratory, USA), lithium chloride (LiCl) (Chemicals Testing and Calibration Laboratory, Singapore), dimethylformamide (DMF) (Merck Chemicals, Singapore) and sodium hydroxide (Fisher Scientific) were used to cast the PAN substrate film and for its surface treatment. PAH with molecular weight of 120,000-200,000 (Polyscience Inc., USA), vinylsulfonic acid sodium salt solution (VSS, 25 wt.% solution, Sigma Aldrich, Singapore),

N, N'-methylenebis(acrylamide) (Sigma Aldrich, Singapore), and sodium sulfate (Merck Chemicals, Singapore) were used for hydrogel membrane fabrication and salt rejection measurement. Milli-Q water (Millipore, Integral 10, USA) with a resistivity of 18.2 MΩ was used for membrane fabrication and measurement except otherwise stated.

5 [0071] **Example 2: PAN substrate preparation and surface modification**

[0072] PAN substrates were prepared as follows. Briefly, PAN (18 wt.%) and LiCl (2 wt.%) were dissolved in DMF in a sealed bottle at 60 °C with mild stirring for at least 24 hours. The polymer solution was cooled to the room temperature (23 °C) before use. A casting knife with a gate height of 175 μm was used to spread the polymer solution onto a
10 clean glass plate. The plate was immediately immersed into a coagulant bath of the tap water at the room temperature. The nascent substrates were washed and kept in water for more than one day to remove the trace solvent and additives.

[0073] The substrates were cut into circular shape with the diameter of 60 mm before post treatment. The circular samples were then further modified to impart the negative charges on
15 the surface by soaking in 1.5 M NaOH solution at 45 °C for two hours. The modification was carried out to increase the electrostatic interaction between the deposited PAH and substrate surface as well as to enhance the substrate hydrophilicity. The samples were rinsed with water to remove the excess NaOH and kept in water before use.

[0074] **Example 3: Hydrogel membrane fabrication**

20 [0075] The hydrogel membrane fabrication process is as shown in **FIG. 1**. Briefly, aqueous PAH solution with weight ratio of 8 % was prepared and stirred for more than one day in order to ensure complete dissolution. Certain amount of cross-linker, N, N'-methylenebis(acrylamide), was added to 25 wt.% of VSS solution to make cross-linker-to-monomer molar ratio of 2.5 %.

25 [0076] For the vesicle-free AqpZ-embedded hydrogel membrane (short for AqpZ-hydrogel membrane), different amount of AqpZ stock solution was added into 8 wt. % PAH solution with additional water to make final PAH concentration of 4 wt. %. The AqpZ-PAH solution was mixed and stirred for 30 minutes before spin coating onto the treated PAN substrates. The AqpZ-PAH solution and VSS solution with 2.5 % cross-linker were spin
30 coated onto the negative charged surface (the “surface” refers to the top side of polymer solution facing the air at casting step and immediate direct contact with the coagulant solution during the substrate formation in this chapter) of the treated PAN substrate at a speed of

2,000 rpm with additional 20 seconds of spinning after each solution deposition (Spin coater, SPIN-3600D, MIDAS System, Korea).

[0077] The composite membrane was immediately incubated in the UV chamber with irradiation intensity of 51 mW/cm² for 150 seconds to form the cross-linked semi-interpenetrate network (semi-IPN) hydrogel membrane (Programmable UV Flood Curing Lamp, Incure F200P, USA). The UV treated hydrogel membrane was soaked in water again to remove unreacted precursors. The AqpZ-hydrogel membrane was then soaked in water until the membrane characterization and measurements. The control hydrogel membrane was prepared in the same process except using 4 wt.% of PAH solution instead of the AqpZ-PAH solution.

[0078] **Example 4: Preparation and permeability characterization of liposomes and proteoliposomes**

[0079] DOPC liposomes and proteoliposomes were prepared by a film hydration method as follows.

[0080] Briefly, DOPC chloroform solution was dried by slowly passing the pure nitrogen stream and then the dry lipid films were kept in vacuum for at least eight hours. The dried lipid films were then hydrated in PBS buffer solution. After agitating for 10 minutes and subject to three freeze-thaw cycles, the solution was extruded 21 times through a polycarbonate membrane with a mean pore size of 200 nm by a mini-extruder (Avanti Polar Lipids, Alabaster, USA). The final concentration of the DOPC liposome solution was 2.0 mg/mL.

[0081] For AqpZ-incorporated proteoliposomes, a certain amount of the AqpZ stock solution in terms of nominal lipid-to-protein ratio (LPR) of 200 was added to the DOPC liposomes buffer solution containing 1 % OG detergent. The AqpZ/DOPC solution was incubated for about one hour and then dialyzed against PBS solution for more than four hours for three times to completely remove the OG. The proteoliposome solution was then extruded through the polycarbonate membrane with 200 nm mean pore size for many times again. Certain amount of the DOPC liposomes and proteoliposomes were exposed to UV irradiation at the same conditions and time intervals as in the hydrogel membrane fabrication.

[0082] Size of the liposomes was measured by a zetasizer Nano ZS (Malvern, UK) at 296 K. Water permeability of liposomes and proteoliposomes were performed using a stopped flow spectrometer (SX20 Stopped Flow Spectrometer, Applied Photophysics) at room

temperature. The vesicles in PBS were rapidly mixed with an equal volume of a hypertonic solution of 400 mM sucrose in PBS, inducing water efflux out of vesicles as a result of osmotic pressure difference between intravesicular and extravesicular. The change curves in light scattering caused by vesicles shrinkage were recorded at 90 ° from the incident monochromatic light with emission wavelength of 500 nm in the stopped flow spectrometer. The initial rate of vesicle shrinkage (k) was determined by fitting the averaged curve of more than three measurements to an exponential rise equation. The osmotic water permeability, P_f ($\mu\text{m/s}$), was calculated by using Equation. (1):

$$[0083] \quad P_f = \frac{k}{(S/V_0) \times V_w \times \Delta_{osm}} \quad (1)$$

[0084] where S/V_0 is the initial surface-to-volume ratio of the vesicles, V_w is the molar volume of water ($18 \text{ cm}^3 \text{ mol}^{-1}$), and Δ_{osm} is the osmolarity difference across the bilayer which is the osmotic driving force for the shrinkage of vesicles.

[0085] **Example 5: Membrane characterization and performance measurements**

[0086] Surface properties of the hydrogel membranes and PAN substrates were characterized by contact angle (FTA32, First Ten Angstroms Inc., USA) and scanning electron microscopy (JSM 6360, JEOL, Japan). The samples were frozen in liquid nitrogen and applied to freeze dry in the freeze-dryer chamber (Martin Christ Alpha 2-4 LD plus, Germany) for more than 12 hours before subject to contact angle and SEM measurements. The contact angle of the sample surface was obtained by measuring and averaging over at least three different areas of the surface. SEM images were obtained from the samples with a uniform gold coating sputtered before the measurement.

[0087] Water flux and salt rejection of the samples were measured using fresh water and a 400 ppm Na_2SO_4 solution as the feed respectively under 0.1 MPa pressure in a stirred dead-end filtration cell.

[0088] Water flux, J_w (LMH), was calculated according to Equation. (2):

$$[0089] \quad (2)$$

[0090] where Δw (kg) is the increase of permeate weight over a certain period of time, Δt (h) is the time interval, S (m^2) is the effective membrane area measured.

[0091] Salt rejection, R (%) was calculated according to Equation. (3):

$$[0092] \quad R = \frac{c_f - c_p}{c_f} \times 100\% \quad (3)$$

[0093] where C_f and C_p are the salt concentration in the feed and permeate, respectively. Effective area of the membranes was 12.57 cm^2 . All measurements were carried out at room temperature (295 K – 296 K).

[0094] **Example 6: Substrates and membranes characterization**

5 [0095] The PAN substrate was prepared by non-solvent induced phase separation process. Morphology of the pristine substrate surface is presented by SEM image as shown in **FIG. 2A**. The surface has a few pores ranging hundreds of nanometers as shown in the image and is quite hydrophilic according to the contact angle of $34.2 \pm 0.8^\circ$ as listed in **TABLE 1**.

[0096] **TABLE 1:** Contact angle results of the surfaces of the pristine PAN substrate,
10 NaOH-treated PAN substrate, hydrogel membrane, and AqpZ-embedded hydrogel membrane. The error bars present the standard deviations.

Sample	pristine PAN substrate	treated PAN substrate	hydrogel membrane	AqpZ-hydrogel membrane
Contact angle ($^\circ$)	34.2 ± 0.8	32.4 ± 2.2	18.1 ± 1.1	18.7 ± 0.7

[0097] The NaOH post-treatment for PAN substrate is applied to make the surface negative charged by the hydrolysis of $-\text{CN}$ groups on the surface to form negatively charged
15 carboxyl groups. The SEM image of the treated substrate surface is much more porous with pore size around 100 nm compared to the pristine one (**FIG. 2B**). It is highly possible that NaOH hydrolysis process changes the surface structures and makes pores much pronounced by $-\text{CN}$ hydrolysis and repulsion between carboxyl groups. The hydrophilicity of the treated surface has almost the same and just minor increase as indicated by 1.8° decrease of the
20 contact angle in **TABLE 1**. When the hydrogel active layer formed on the substrate, the surface has no obvious pores observed (**FIG. 2C**). It indicates that the hydrogel active layer fully covers the substrate and spans over the substrate pores. Besides, the huge decrease in contact angle by 14.3° also proves the successful formation of the hydrogel membrane.

[0098] The semi-IPN structure of the hydrogel layer presents as the linear PAH polymer
25 chains interpenetrating across the cross-linked poly(vinylsulfonic acid sodium salt) (PVSS) matrix cross-linked by N, N'-methylenebis(acrylamide). With the AqpZ embedment, the AqpZ-hydrogel membrane shows similar surface hydrophilicity and morphologies with no obvious pores to the hydrogel membrane observed by contact angle and SEM images (**TABLE 1** and **FIG. 2D**).

30 [0099] **Example 7: UV treatment effect on AqpZ**

[00100] AqpZ is quite stable under different circumstances. UV treatment effect on the activities of AqpZ, which is a key factor in the fabrication and functionality of the hydrogel membranes, has however, not been investigated. Herein, the stopped flow was applied to compare the activities of AqpZ in the proteoliposomes before and after the UV treatment.

5 The original solutions of AqpZ with OG detergent, DOPC liposome, and AqpZ-incorporated DOPC proteoliposome, referred as AqpZ-DOPC, were spread respectively on the glass petri dish surfaces to get a solution layer as thin as possible and subject to UV irradiation using the same conditions as in the AqpZ-hydrogel membrane fabrication process.

[00101] As disclosed herein, the original solution with AqpZ protected by OG detergent
10 was applied to UV irradiation before reconstitution into DOPC liposomes to form AqpZ proteoliposomes referred as (UV-AqpZ)-DOPC and AqpZ proteoliposomes with original AqpZ solution (no UV applied on the stock solution) was post-treated under UV irradiation in the same conditions referred as UV-(AqpZ-DOPC), respectively.

[00102] To better evaluate the results, pristine DOPC liposome referred as DOPC, UV
15 irradiated DOPC liposomes referred as UV-DOPC, and mutant AqpZ (mediated by the 189th amino acid, arginine) incorporated DOPC proteoliposomes referred as mAqpZ-DOPC were also prepared and compared via the stopped flow. The main advantage of using the mutant was to maintain the same structures of proteoliposomes and AqpZ-hydrogel membrane but with a loss in the aquaporin functionality.

20 [00103] The normalized light scattering curves of stopped flow and water permeability calculated based on stopped flow and Equation. (1) are presented in **FIG. 3A** and **3B**. The overlap of the DOPC and UV-DOPC curves together with the equivalent permeability indicates the UV treatment has no significant effect on the DOPC bilayer. The 3 to 4 % decrease in the permeability of (UV-AqpZ)-DOPC and UV-(AqpZ-DOPC) compared to
25 AqpZ-DOPC is negligible when considering more than 300% increase in the permeability of AqpZ-DOPC than DOPC (without AqpZ). Also the curves of the AqpZ-DOPC, (UV-AqpZ)-DOPC and UV-(AqpZ-DOPC) curves show almost overlapping in **FIG. 3A**. Eliminating the possibility of reduced AqpZ functionality and increased permeability of DOPC bilayer simultaneously caused by UV irradiation, it draws conclusion that AqpZ maintains
30 functionality after UV treatment. The equivalent results of (UV-AqpZ)-DOPC and UV-(AqpZ-DOPC) in **FIG. 3A** and **3B** also indicate that AqpZ protected both by detergent and by lipid bilayer have the similar tolerance under the UV treatment. The mutant AqpZ-

incorporated proteoliposomes (mAqpZ-DOPC) show about 28% increase in the water permeability compared to DOPC and only less than 40% of that of AqpZ-DOPC, which agrees with water flux results of the hydrogel membranes in the following section.

[00104] **Example 8: Performance and structure analysis of the AqpZ-hydrogel membranes**

[00105] AqpZ-hydrogel membranes were fabricated as the positive-charged linear PAH chains interpenetrating across the cross-linked PVSS matrix with AqpZs embedded in the network. The polyelectrolytes of PAH and PVSS provide rejection to ions and the swollen hydrophilic network allows water molecules to pass through. The embedded AqpZs provide additional water channel inside the network and facilitate the water transport across the active hydrogel layer.

[00106] **FIG. 4** shows the water flux and salt rejection results of the AqpZ-hydrogel membranes with different amounts of AqpZ embedded as well as the hydrogel membrane. The water flux of the hydrogel membrane without any AqpZ embedment (shown as 0 mg/mL in **FIG. 4**) was around 6.76 LMH while the rejection reached to 93.1 % using 400 ppm Na₂SO₄ as the feed. The PAN substrate had no salt rejection with pore size ranging around 100 nm (**FIG. 2B**) at more than two magnitudes larger than the radius of ions molecules. High rejection of the hydrogel membrane indicates successful deposition of the PAH/PVSS semi-IPN hydrogel layer on the substrate. The AqpZ-hydrogel membranes exhibited equivalent salt rejections but with increasing water flux while increasing the AqpZ concentration in the PAH precursor solution during the fabrication process. When 0.20 mg/mL AqpZ was added to the AqpZ/PAH precursor solution in the process, the flux reached up to 9.70 LMH, which was more than 40 % increase compared to the control membrane (0 mg/mL).

[00107] In conventional aquaporin-based membranes, aquaporin-incorporated proteoliposomes or proteopolymersomes are prepared by film hydration method before integrating into the membrane. The aquaporin-incorporated vesicles are embedded in the membrane with intact vesicular structures as depicted in Case 1 of **FIG. 5**. The curvature of the vesicles reduces the functional efficiency of the aquaporins as the projected area of the vesicular bilayer is much smaller than the vesicular surface area. Another drawback of using vesicles is that the water molecules need to pass twice through the vesicular bilayer to get through the membrane, which also reduces the membrane efficiency.

[00108] For the AqpZ-hydrogel membrane, no vesicles are involved and the water molecules pass the aquaporins freely as depicted in Case 2 of **FIG. 5**. In this case, the increase of the water flux is attributed to the high permeability and selectivity of active AqpZ embedded in the hydrogel rejection layer. Considering that the water permeability of AqpZ-incorporated proteoliposomes shows more than three times higher than the control liposomes in the previous section, only more than 40 % increase in the flux of the AqpZ-hydrogel membrane is observed. It is possibly due to the structures of the hydrogel layers and different manners of AqpZ inside the hydrogel matrix. In this case, there is no addition force or process to array AqpZs. They are believed to stay randomly distributed in the semi-IPN hydrogel matrix, where they can either “stand”, “lay down” or “lean” with a certain degree of slope. The water flux is not optimized due to the random distribution of AqpZs. AqpZ “standing” in the hydrogel matrix with its selective channel perpendicular to the substrate surface and channel-to-channel alignment promotes the water molecules passing through the protein channel more effectively with the shortest path way in the hydrogel matrix. For the AqpZ “leaning” in the hydrogel matrix, the water molecules pass through the protein channel with reduced efficiency and undergo longer path way through the hydrogel matrix. But the AqpZ “laying down” in the hydrogel matrix serves as barriers to the water molecules and the water molecules are rejected or need to bypass the AqpZ, which reduces water flux of the membranes. Another reason is that the low deposition efficiency of AqpZs in the fabrication process and a significant portion of AqpZs precursor were spinned off due to moderately weak affinity to the composite precursor membrane and dense packing of AqpZ leading to large weight and density. As a result, the water flux has only increased by more than 40 %.

[00109] In an ideal case (Case 3 of **FIG. 5**), all the AqpZs protected by the detergents “stand” in the hydrogel rejection layer with their selective channels perpendicular to the substrate surface and channel-to-channel alignment, hence water could pass through the protein channels more effectively with maximized flux and through the shortest path way of the water molecules. This is an ideal case, while it will be achieved by additional effort such as electrostatic force, fine nano-fabrication, or special substances to make the AqpZs well-regulated alignments.

[00110] One key advantage of using the hydrogel membrane to embed aquaporins is the excellent flexibility of the semi-IPN hydrogel membrane with good mechanical strength. The 2.5 % of cross-linking ratio gives the nano-sized porous network of the polyelectrolyte

hydrogel, which has good size match with the detergent-protected AqpZs. Besides, the strong polyelectrolyte of PVSS and counterions provided by PAH give the hydrogel layer excellent salt repulsion mainly by Donnan exclusion effect and the flexible alkyl backbones of both PAH and PVSS provide favorable environments for aquaporin incorporation and functionality. It partially solves the technical incompatibility issues of the aquaporin-incorporated membranes, providing both increased mechanical strength of the membrane and flexibility of the membrane for stability/activity of the aquaporins.

[00111] **Example 9: Performance comparison with mutant AqpZ hydrogel membranes**

[00112] To further prove the enhanced water flux of AqpZ-hydrogel membranes by the functionality of AqpZs, mutant AqpZ that have the same structures as the wild type of AqpZ in its tetrameric state but show great reduced functionality by deactivation of the water channel are used as a control. The mutant AqpZ substituting for wild type of AqpZ is applied in the membrane fabrication process with the same conditions to form mutant AqpZ-embedded hydrogel membrane (mAqpZ-hydrogel membrane).

[00113] **FIG. 6** shows the water flux and salt rejection of the mAqpZ-hydrogel membrane and AqpZ-hydrogel membrane at the protein concentration of 0.20 mg/mL in the precursor solution of protein/PAH as well as the hydrogel membrane as a control. All the three membranes show similar salt rejections which are controlled mainly by the polyelectrolytes in the hydrogel membranes. The mAqpZ-hydrogel membrane has a water flux of 13.6 % higher than the hydrogel membrane, which agrees well with 28 % increase of the permeability data by the stopped flow measurement in accordance with the structural argument of the AqpZ-embedded hydrogel membrane. More than 40 % increase in the water flux of the AqpZ-hydrogel membrane compared to only 13.6 % increase in the water flux of the mAqpZ-hydrogel membrane evidences the AqpZ functionality because other factors are set the same. It proves that AqpZ protein acts as the active water channel in the AqpZ-hydrogel membrane.

[00114] A novel vesicle-free AqpZ-embedded hydrogel composite membrane has been fabricated and characterized. The contact angle and SEM results show the successfully deposition and formation of the hydrogel layer on the modified PAN substrate. The excellent water flux and high salt rejection of the hydrogel membrane prove good functionality for nanofiltration applications. AqpZ has been successfully embedded in the hydrogel layer as

the individual water channel unit without assistance of the vesicular bilayer and the AqpZ-embedded hydrogel membrane has significant enhanced performance of water flux increasing by more than 40 % due to the embedment of AqpZs. The mutant AqpZ control experiment proves the water flux increase of the AqpZ-hydrogel membrane comes from the functionality of AqpZ in the hydrogel layer. The structures of the hydrogel membrane and AqpZ manners inside the rejection layer have been discussed. It shows high potential of cutting the cost of the aquaporin-based membrane fabrication and facilitating the engineering applications. By fine design and controlled aquaporin array inside the hydrogel membrane, the aquaporin-embedded hydrogel membrane with much more outstanding performance is believed to be developed in the near future.

[00115] While the present invention has been particularly shown and described with reference to exemplary embodiments thereof, it will be understood by those of ordinary skill in the art that various changes in form and details may be made therein without departing from the spirit and scope of the present invention as defined by the following claims.

CLAIMS

1. A method of preparing a biomimetic membrane, wherein a vesicle is not used in preparing the biomimetic membrane, the method comprising
 - 5 a) depositing a first mixture comprising a first polyelectrolyte having a charge and a transmembrane protein on a surface of a support;
 - b) depositing a second mixture comprising a second polyelectrolyte and a cross-linking agent on the first mixture which is deposited on the surface of the support, the second polyelectrolyte having a charge opposite to the charge on

10 the first polyelectrolyte; and
 - c) cross-linking the second polyelectrolyte with the cross-linking agent to obtain the biomimetic membrane.
2. The method according to claim 1, wherein the transmembrane protein comprises an

15 aquaporin.
3. The method according to claim 2, wherein the aquaporin is AqpZ.
4. The method according to any one of claims 1 to 3, wherein the transmembrane protein

20 is protected with a surfactant.
5. The method according to claim 4, wherein the surfactant is selected from the group consisting of n-octyl- β -D-glucoside, n-octanoylsucrose, n-nonanoylsucrose, n-decanoylsucrose, n-undecanoylsucrose, n-dodecanoylsucrose, n-heptyl- β -D-glucoside, n-heptyl- β -D-maltoside, n-heptyl- β -D-maltopyranoside, n-heptyl- β -D-glucopyranoside, n-heptyl- β -D-thioglucoside, n-heptyl- β -D-thiomaltoside, n-heptyl- β -D-thiomaltopyranoside, n-heptyl- β -D-thioglucopyranoside, n-octyl- β -D-maltoside, n-octyl- β -D-maltopyranoside, n-octyl- β -D-glucopyranoside, n-octyl- β -D-thioglucoside, n-octyl- β -D-thiomaltoside, n-octyl- β -D-thiomaltopyranoside, n-octyl- β -D-thioglucopyranoside, n-nonyl- β -D-glucoside, n-nonyl- β -D-maltoside, n-nonyl- β -D-maltopyranoside, n-nonyl- β -D-glucopyranoside, n-nonyl- β -D-thioglucoside, n-nonyl- β -D-thiomaltoside, n-nonyl- β -D-thiomaltopyranoside, n-nonyl- β -D-

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thioglucopyranoside, n-decyl- β -D-glucoside, n-decyl- β -D-maltoside, n-decyl- β -D-maltopyranoside, n-decyl- β -D-glucopyranoside, n-decyl- β -thioglucoside, n-decyl- β -D-thiomaltoside, n-decyl- β -D-thiomaltopyranoside, n-decyl- β -D-thioglucopyranoside, n-undecyl- β -D-glucoside, n-undecyl- β -D-maltoside, n-undecyl- β -D-maltopyranoside, n-undecyl- β -D-glucopyranoside, n-undecyl- β -D-thioglucoside, n-undecyl- β -D-thiomaltoside, n-undecyl- β -D-thiomaltopyranoside, n-undecyl- β -D-thioglucopyranoside, n-dodecyl- β -D-glucoside, n-dodecyl- β -D-maltoside, n-dodecyl- β -D-maltopyranoside, n-dodecyl- β -D-glucopyranoside, n-dodecyl- β -D-thioglucoside, n-dodecyl- β -D-thiomaltoside, n-dodecyl- β -D-thiomaltopyranoside, n-dodecyl- β -D-thioglucopyranoside, and any combination thereof.

6. The method according to claim 4 or 5, wherein the surfactant is n-octyl- β -D-glucoside.
7. The method according to any one of claims 1 to 6, wherein the support comprises a polymer selected from the group consisting of polyacrylonitrile (PAN), polysulfone (PSF), polyvinylidene fluoride (PVDF), polypropylene, copolymers thereof, and combinations thereof.
8. The method according to any one of claims 1 to 7, wherein the support comprises polyacrylonitrile.
9. The method according to any one of claims 1 to 8, wherein the surface of the support possesses a charge prior to having the first mixture deposited thereon.
10. The method according to claim 9, wherein the charge is a negative charge.
11. The method according to any one of claims 1 to 10, wherein the support is a membrane.

12. The method according to any one of claims 1 to 11, wherein the first polyelectrolyte and the second polyelectrolyte are independently

(i) a polyanion selected from the group consisting of poly(vinylsulfonic acid),
 5 poly(styrene sulfonic acid), poly(acrylic acid), poly(methacrylic acid), poly(maleic acid), poly(itaconic acid), sulfated poly(vinyl alcohol), poly(vinylsulfonic acid), poly(acrylic acid-co-maleic acid), poly(styrene sulfonic acid-co-maleic acid), poly(ethylene-co-acrylic acid), poly(phosphoric acid), poly(silicic acid), hectorite, bentonite, alginic acid, pectic acid, xanthan, gum arabic, dextran sulfate, carboxy
 10 methyl dextran, carboxy methyl cellulose, cellulose sulfate, cellulose xanthogenate, starch sulfate, starch phosphate, lignosulfonate, polygalacturonic acid, polyglucuronic acid, polyguluronic acid, polymannuronic acid, chondroitin sulfate, heparin, heparan sulfate, hyaluronic acid, dermatan sulfate, keratan sulfate; poly-(L)-glutamic acid, poly-(L)-aspartic acid, acidic gelatins (A-gelatins), starch, amylose, amylopectin,
 15 cellulose, guar, guar gum, pullulan, dextran, chitin or chitosan derivatives having the following functional groups: carboxymethyl, carboxyethyl, carboxypropyl, 2-carboxyvinyl, 2-hydroxy-3-carboxypropyl, 1,3-dicarboxyisopropyl, sulfomethyl, 2-sulfoethyl, 3-sulfopropyl, 4-sulfobutyl, 5-sulfopentyl, 2-hydroxy-3-sulfopropyl, 2,2-disulfoethyl, 2-carboxy-2-sulfoethyl, maleate, succinate, phthalate, glutarate, aromatic
 20 and aliphatic dicarboxylates, xanthogenate, sulfate, phosphate, 2,3-dicarboxy, N,N-di(phosphatomethyl)aminoethyl, N-alkyl-N-phosphatomethylaminoethyl, salts thereof, and combinations thereof, or

(ii) a polycation selected from the group consisting of polyallylamine, poly(aniline);
 25 poly(pyrrole); poly(alkylenimines); poly-(4-vinylpyridine); poly(vinylamine); poly(2-vinylpyridine), poly(2-methyl-5-vinylpyridine), poly(4-vinyl-N-C1-C18-alkylpyridinium salt), poly(2-vinyl-N-C1-C18-alkylpyridinium salt), aminoacetylated polyvinyl alcohol; poly-(L)-lysine, poly-(L)-arginine, poly(ornithine), basic gelatins (B-gelatins), chitin or chitosan derivatives having the following functional groups: 2-aminoethyl, 3-aminopropyl, 2-dimethylaminoethyl, 2-diethylaminoethyl, 2-diisopropylaminoethyl, 2-dibutylaminoethyl, 3-diethylamino-2-hydroxypropyl, N-ethyl-N-methylaminoethyl, 2-diethylhexylaminoethyl, 2-hydroxy-2-diethylaminoethyl, 2-hydroxy-3-trimethylammonionopropyl, 2-hydroxy-3-

triethylammonionopropyl, 3-trimethylammonionopropyl, 2-hydroxy-3-pyridiniumpropyl, S,S-dialkylthioniumalkyl, salts thereof, and combinations thereof.

13. The method according to any one of claims 1 to 12, wherein the first polyelectrolyte is a polycation.
14. The method according to any one of claims 1 to 13, wherein the first polyelectrolyte comprises poly(allylamine hydrochloride).
15. The method according to claim 14, wherein the poly(allylamine hydrochloride) has a molecular weight in the range of about 120,000 to about 200,000.
16. The method according to any one of claims 1 to 15, wherein amount of the first polyelectrolyte in the first mixture is in the range of about 4 wt% to about 8 wt%.
17. The method according to any one of claims 1 to 16, wherein amount of the transmembrane protein in the first mixture is in the range of about 0.01 mg/mL to about 0.2 mg/mL.
18. The method according to any one of claims 1 to 17, wherein the second polyelectrolyte is a polyanion.
19. The method according to any one of claims 1 to 18, wherein the second polyelectrolyte comprises poly(vinylsulfonic acid).
20. The method according to claim 19, wherein the poly(vinylsulfonic acid) is obtainable by polymerizing a vinylsulfonic acid sodium salt solution.
21. The method according to claim 20, wherein molar ratio of the cross-linking agent to the vinylsulfonic acid sodium salt in the second mixture is in the range of about 2 % to about 3 %.

22. The method according to any one of claims 1 to 21, wherein the cross-linking agent is selected from the group consisting of N, N'-methylenebis(acrylamide), divinylbenzene, N,N'-ethylenebis(acrylamide), N,N'-propylenebis(acrylamide), N,N'-butamethylenebis(acrylamide), N,N'-diallylacrylamide, N,N'-hexamethylenebisacrylamide, triallylisocyanurate, 1,4-diacryloylpiperazine-1,1,1-trimethylolpropane diallyl ether, triethylene glycol divinyl ether, diallyl maleate, bis(acryloylamido)methane, ethyleneglycoldimethacrylate, diethyleneglycoldimethacrylate, 3-(acryloyloxy)-2-hydroxypropyl methacrylate, and combinations thereof.
23. The method according to any one of claims 1 to 22, wherein the cross-linking agent is N, N'-methylenebis(acrylamide).
24. The method according to any one of claims 1 to 23, wherein cross-linking the second polyelectrolyte with the cross-linking agent comprises irradiating the second mixture with ultraviolet light.