SYNTHETIC CHANNELS

FIELD OF THE INVENTION

[0001] The present invention relates generally to the fields of bioengineering, material science and chemistry. In particular, the present invention relates to synthetic channels and uses thereof in water purification.

BACKGROUND

[0002] Water scarcity is a global problem. Reverse osmosis is a costly and energetic water purification technology. Biomimetic membranes embedded with aquaporin (AQP) or artificial water channels (AWC) had been developed to meet the industry needs. Although aquaporin exhibits high water permeability, its long-term stability is a limiting factor.

[0003] Fresh water scarcity currently affects over 4 billion people worldwide and is a growing challenge faced by humanity. High population, industrialization, deforestation and climate change led to depletion and contamination of natural water resources, all of which increase the demand for fresh water. Fresh water sources are becoming scarce and competing demands such as industrial needs, domestic use for the increasing population will make water a scarce commodity in the future. The effects of climate change will exacerbate this problem. Approximately half of the world population suffers from water scarcity or lack of clean drinking water.

[0004] Currently, reverse osmosis (RO) membrane and membrane distillation technologies are used to produce ~100 million tons of desalinated water per day from seawater. However, the high energy consumption and overall cost of the existing technologies necessitate the search for better alternatives. In this regard, the aquaporins (AQPs) have been proposed as active components for the fabrication of desalination reverse osmosis bio-assisted membranes. Aquaporins are transmembrane proteins that allow high water permeability (about 10^9 H_2O s^{-1}) across lipid membranes, while completely rejecting ions and protons. The main structural feature of the aquaporin hydrophobic interior is a narrow 3 Å diameter pore, composed of a key asparagine-proline-alanine (NPA) motif, which enables the transport of water molecules in a single file. Unfortunately, development of aquaporin-based membranes is limited by the long-term protein stability issues under harsh pressure and salinity reverse osmosis (RO) conditions.

[0005] Thus, there is a need for technologies with improved properties for water purification.

SUMMARY

[0006] In one aspect, the present disclosure refers to an oligourea foldamer comprising one or more sequences selected from the group consisting of SEQ ID NO: 1 (Ac-$L^UE^UL^UK^UP^UL^UE^UL^UK^UA^U$-$NH_2$) and SEQ ID NO: 2 (Ac-$L^UE^UL^UK^UA^UL^UE^UL^UK^UA^U$-$NH_2$).

[0007] In another aspect, the present disclosure refers to a compound comprising oligourea foldamers as disclosed herein.

[0008] In yet another aspect, the present disclosure refers to a permeable membrane comprising the oligourea foldamer as disclosed herein or the compound as disclosed herein.

[0009] In a further aspect, the present disclosure refers to a method of purifying water, the method comprising allowing water to pass through a filtration device comprising the permeable membrane as disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0011] **Fig. 1** shows an overview of the molecular design and biophysical characterization of oligourea foldamers. Fig. 1A shows helical-wheel representations of designed H2 variants showing side-chain distribution of oligourea residues in pentad. Polar and charged residues, and uncharged residues are depicted as filled and unfilled circles, respectively. Fig. 1B shows the sequences of synthesized amphipathic oligourea foldamers H2OC1, H2OC2, H2OC3 and H2OC4. Highlighted residues represent the point mutation to H2OC1. Fig. 1C shows circular dichroism (CD) spectra of oligourea foldamers show a peak at ~202 nm indicating their helical conformation. Fig. 1D shows CD spectra of H2OC2 in water exhibit concentration-dependent increase of molar ellipticity at ~202 nm. Fig.1E and Fig. 1F show negative-stained transmission electron micrographs of H2OC1 (Fig. 1E) and H2OC2 (Fig. 1F) revealed self-assembled morphologies at 200 μM in 10 mM HEPES buffer, pH 7.0. They showed fibre-like networks packed in large, knotted bundles (H2OC2, diameter ranging from 12.4 to 40.0 nm) and distinct fibres (H2OC1, diameter 5.5±0.6 nm). Fig. 1G and Fig. 1H show electrospray ionization (ESI) mass spectrometry profiles of H2OC1 (Fig. 1G) and H2OC2 (Fig. 1H), respectively. The ESI spectra reveal distribution of heterogeneous multimeric species of oligoureas. The isotopic peak at m/z value of 1856.76 of H2OC1 indicates pentameric species ([5]4+). Other isotopic peaks of multimers are also identified.

[0012] **Fig. 2** shows an overview of biophysical properties of foldamer insertion into lipid vesicles. Fig. 2A shows 31P static solid-state NMR experiments performed at 303K on PC/PS control vesicles and PC/PS vesicles incorporating H2OC1 and H2OC2. Fig. 2B shows ^{2}H static solid-state NMR experiments performed at 303K. Fig. 2C shows a line graph depicting thermal variation of the first spectral moment M1 of PC lipids as measured by ^{2}H solid state NMR. Fig. 2D shows a graph depicting C-^{2}H order parameter as a function of labelled lipid carbon position.

[0013] **Fig. 3** shows the results of measurements of water and ion transport through oligourea foldamers. Fig. 3A Schematic representation of water permeability measurement through foldamer channels in presence of phosphatidylcholine/phosphatidylserine (PC/PS) vesicles while mixing with hypertonic osmolyte (600 mM sucrose). Fig. 3B shows the results of stopped-flow analysis of oligourea foldamers, which shows water permeation through foldamer channels in PC/PS (4:1) vesicles. Fig. 3C shows light scattering traces of H2OC2 embedded in PC/PS vesicles with different molar ratio. This shows an increasing water permeability with increased channel concentration while mixing with hypertonic osmolyte (600 mM sucrose). Curves were fitted with stretched function "$y = c+a \exp(-Kx^{b})$" to obtain exponential coefficients (K). Fig. 3D shows results indicating that water permeability through H2OC2 channel measured at different channel densities exhibited a jump in permeability when lipid/channel molar ratio reached 100:1 to 50:1. This indicates a possible cooperativity. Fig. 3E shows a schematic representation of HPTS based fluorescence assay under high ionic gradients (100 mM NaCl and KCl). HPTS=8-hydroxypyrene-1,3,6-trisulfonic acid. Fig. 3F and Fig. 3G shows salt rejections by H2OC1 and H2OC2 with respect to natural ionophore gA and foldamers H2OC3 and H2OC4 as evidenced from curve. gA= gramicidin A. Fig. 3H shows a schematic representation of chloride-selective ion transport by SPQ dye based on fluorescence assay. Fig. 3I shows results indicating chloride-selective ion transport. Results obtained by SPQ-based quenching assays show rejection of chloride ion by H2OC1, H2OC2 and H2OC3. Concentration-dependent fluorescence quenching observed upon addition of H2OC4 indicating chloride transport by foldamer H2OC4.

[0014] **Fig. 4** shows images of the crystal structure revealing quaternary structure of foldamer H2OC1. Fig. 4A is an image of the crystal structure of H2OC1 showing a helical conformation of the monomer with a distinct hydrophobic and hydrophilic surfaces. Fig. 4B is an image of crystal packing showing two distinct channels (Figs. 4A and 4B). In this structure, channel B is formed in the interspace of four channel A assemblies. Both channels are packed through hydrophobic interactions of exterior surfaces. In the channel assembly, eight monomeric oligourea helices per turn are packed laterally. The neighbouring helices are in an antiparallel orientation within the superhelical structure. Interior of both channels are composed of

hydrophilic residues, creating a charged nanopore, while hydrophobic residues line the exterior. Fig. 4C shows images of the crystal structure with water molecules that are H-bonded with the foldamer, shown as spheres, in the pores of channels A and B. Water molecules that do not form any H-bonding with foldamer helices are shown as well. Only selected water molecules are displayed. Fig. 4D shows water molecules in the pore form a H-bonding network with charged residues, Glu^{U} and Lys^{U}. Hydrogen bonds show O-O and N-O distances of 2.7 and 3.6 Å, respectively. Images were created using PyMOL.

[0015] **Fig. 5** shows data of molecular dynamics simulations of H2OC1 oligourea channel in POPC:POPS (4:1 ratio) lipid bilayer. Fig. 5A shows that the H2OC1 oligourea channel composed of 8 helical and 16 kinked helices embedded in lipid bilayer together with simulation snapshots: i) prior to any simulation, ii) after 200 ns of equilibration with position restraints on helical foldamer alpha carbon atoms, iii) after 600 ns of the production run without any restraints. Surface representation of the helices are shown in dark grey. The 16 kinked helices are shown in light grey stick representation. Spheres correspond to phospholipid head groups. Water, ions and lipid headgroups were omitted for figure clarity. Fig 5B shows the control system of the H2OC1 oligourea channel composed of 8 helices alone together with simulation snapshots: iv) after 200 ns of equilibration with position restraints on alpha carbon atoms, v) after 600 ns of the production run without any restraints. Fig. 5C shows the root mean-square-deviation (RMSD) of alpha carbon atoms from 8 helices calculated with respect to the experimental structure. Fig. 5D and Fig. 5E show the number of water molecules (Fig. 5D) and number of ions (Fig. 5E) passing through both channel types (shown in Fig. 5A and Fig. 5B) over the last 200 ns of the trajectory are shown. Fig. 5F and Fig. 5G show the average channel radius profile across the membrane normal (the two radius profile tracings in each represent the standard deviation limits) together with snapshots of the averaged density of the solvent within the pore (light grey, traversing the membrane) are shown for the two cases, namely, with (Fig. 5(F)) and without kinked (Fig. 5(G)) helices. The radius profile as well as solvent density were averaged over the last 200 ns of the production run.

[0016] **Fig. 6** shows graphs depicting the solid phase synthesis of oligourea H2OC1. Fig. 6A and Fig. 6B show the chemical structure and analytical HPLC profile of purified H2OC1 foldamer (tR = 5.85 min). Fig. 6C shows the electrospray ionization mass spectrum of H2OC1 foldamer (pure). Expected M.W. 1484.88 Da; observed M.W. 1485.25 Da ($[M+H]^{+}$), 743.19 Da ($[M+2H]^{2+}$), 495.82 Da ($[M+3H]^{3+}$).

[0017] **Fig. 7** shows graphs depicting the solid phase synthesis of oligourea H2OC2. Fig. 7A and Fig. 7B show the chemical structure and analytical HPLC profile of purified H2OC2

foldamer (tR = 6.39 min). Fig. 7C shows the electrospray ionization mass spectrum of H2OC2 foldamer. Expected M.W. 1458.84 Da; observed M.W. 1459.99 Da ($[M+H]^{+}$), 730.00 Da ($[M+2H]^{2+}$), 486.99 Da ($[M+3H]^{3+}$).

[0018] **Fig. 8** shows graphs depicting the solid phase synthesis of oligourea H2OC3. Fig. 8A shows the chemical structure of H2OC3 foldamer. Fig. 8B shows ESI-MS spectrum of H2OC3 foldamer. Expected M.W. 1454.00 Da; observed M.W. 1455.13 Da ($[M+H]^{+}$), 728.08 Da ($[M+2H]^{2+}$), 485.73 Da ($[M+3H]^{3+}$). (Inset) analytical HPLC profile of purified H2OC3 foldamer is shown (t_R = 5.77 min).

[0019] **Fig. 9** shows graphs depicting the solid phase synthesis of oligourea H2OC4. Fig. 9A and Fig. 9B show the chemical structure and analytical HPLC profile of purified H2OC4 foldamer (t_R = 7.54 min). Fig. 9C shows the electrospray ionization mass spectrum of H2OC4 foldamer. Expected M.W. 1611.03 Da; observed M.W. 1612.04 Da ($[M+H]^{+}$), 806.04 Da ($[M+2H]^{2+}$), 537.69 Da ($[M+3H]^{3+}$).

[0020] **Fig. 10** shows the results of a variable-concentration CD analysis of foldamers. Foldamer H2OC1 (Fig. 10A), H2OC3 (Fig. 10B) and H2OC4 (Fig. 10C) are shown to exhibit concentration-dependent increase of molar ellipticity at 202 nm in pure water. Fig. 10D shows the results of a CD analysis of H2OC1 and H2OC2 at pH 4.4 exhibiting helical conformation.

[0021] **Fig. 11** shows the results of the biophysical characterization of H2OC3 and H2OC4 foldamers. Fig. 11A shows a TEM image of H2OC3 showing spherical morphology with 24.3±8.7 nm in diameter. Fig. 11B shows the native electrospray ionization (ESI) mass spectrometry profile of H2OC3 showing a major peak distribution at 1819.27 indicating pentameric [5^{4+}] species. Fig. 11C show a TEM image of H2OC4 showing long filament like self-assembly with a width of 132.1±24.4 nm. Inset exhibiting close view of a discrete filament.

[0022] **Fig. 12** shows histograms depicting the hydrodynamic radius of foldamer embedded PC/PS (4:1) lipid vesicles. Histogram plot of foldamers embedded in lipid vesicles was determined by dynamic light scattering (DLS).

[0023] **Fig. 13** shows the stopped flow data of foldamer embedded PC/PS (4:1) lipid vesicles. Light scattering traces of H2OC1, H2OC2, H2OC3, H2OC4 and gA embedded in PC/PS (4:1) vesicles with molar ratio 1:100 (foldamer:lipid) show an increasing water permeability compared to blank (without foldamer) while mixing with a hypertonic osmolyte (600 mM sucrose). Curves were fitted with stretched function $y= c+a \exp(-Kx^{b})$ to obtain exponential coefficients (K).

[0024] **Fig. 14** shows a histogram depicting the water permeability of H2OC2 at different PC/PS molar ratio. H2OC2. shows increased water permeability until PC/PS molar ratio reach 4:1 followed by decreasing water permeability with increased PS concentration.

[0025] **Fig. 15** shows a histogram depicting the water permeability of H2OC2 in presence of different osmolytes. H2OC2 showed water permeability in presence of sucrose, glycine and glucose in PC/PS (4:1) lipid vesicles.

[0026] **Fig. 16** shows graphs depicting data indicating that foldamers reject divalent ion transport. Results of HPTS assays show that H2OC1 and H2OC2 did not transport divalent ions Mg^{2+} and Ca^{2+} ($MgCl_2$ and $CaCl_2$) in PC/PS (4:1) lipid vesicles.

[0027] **Fig. 17** shows the results of the analysis of proton transport of H2OC1 and H2OC2 by patch-clamp technique in PC/PS (4:1) lipid. Single channel current traces and I-V plots of H2OC2 (Fig. 17A) and H2OC1 (Fig. 17B) recorded in symmetrical solutions (cis chamber = trans chamber = 0.1 M HCl solution) are shown.

[0028] **Fig. 18** shows the results of the analysis of proton transport of H2OC1 and H2OC2 by patch-clamp technique in DOPC lipid. Single channel current traces and I-V plots of H2OC2 (Fig. 18A) and H2OC1 (Fig. 18B) recorded in symmetrical solutions (cis chamber = trans chamber = 0.1 M HCl solution) are shown.

[0029] **Fig. 19** shows the results of ion transport analysis of H2OC1 by patch-clamp technique in DOPC lipid. Single channel current traces and I-V plots of H2OC1 recorded in (Fig. 19A) symmetrical solutions (cis chamber = trans chamber = 1 M KCl solution) and (Fig. 19B) unsymmetrical solutions (cis chamber = 1 M KCl solution, trans chamber = 1 M NaCl solution) in DOPC lipid are shown.

[0030] **Fig. 20** shows the results of ion transport analysis of H2OC2 by patch-clamp technique in DOPC lipid. Single channel current traces and I-V plots of H2OC2 recorded in (Fig. 20A) symmetrical solutions (cis chamber = trans chamber = 1 M KCl solution) and (Fig. 20B) unsymmetrical solutions (cis chamber = 1 M KCl solution, trans chamber = 1 M NaCl solution) using DOPC lipid are shown.

[0031] **Fig. 21** shows the results of ion transport analysis of H2OC1 and H2OC2 by patch-clamp technique in PC/PS (4:1) lipid. Single channel current traces and I-V plots of (Fig. 21A) H2OC1 and (Fig. 21B) H2OC2 recorded in symmetrical solution (cis chamber = trans chamber = 1 M KCl) using PC/PS (4:1) lipid are shown.

[0032] **Fig. 22** shows images depicting the structural comparison of H2OC1 and H2 crystal structures. Dashed lines showing intramolecular H-bonding. Nitrogen atoms and oxygen atoms are coloured blue and red, respectively. All canonical helical intramolecular H-bonds are observed except at the C-terminal region of H2OC1 helices.

[0033] **Fig. 23** shows crystal structures showing water filled channels in H2OC1. Crystal form shown with all water molecules (top) and selected water molecules only (bottom). Water

molecules are shown as spheres. Carbon, nitrogen and oxygen atoms are coloured white, blue and red, respectively.

[0034] **Fig. 24** shows crystal structures showing water wires in the H2OC1 channel. A pattern of water wires observed in the superhelix of channel A and channel B, indicated by ball and stick model. Water molecules are shown as red spheres. Water molecules that do not form any H-bonds with foldamer helices are represented as pink spheres. Water-water H-bonding marked with yellow dashed lines. Only selected water molecules shown here. Carbon, nitrogen and oxygen atoms are coloured white, blue and red, respectively.

[0035] **Fig. 25** shows stick-and-ball as well as ribbon 3D models of a H2OC1 channel with kinked helix. (top, left) A distorted helix filling the void space in between the neighbouring channels highlighted with magenta colour in H2OC1. It has a kink region near Pro^{U} and Lys^{U}. (top, right) the distorted helix binds to the channels. The distorted helix is shown in the ribbon model and channels in the stick model. (bottom, right) channel structure shown in surface model.

[0036] **Fig. 26** shows images depicting the H2OC1 and H2OC2 nanostructures. Results of TEM analysis of H2OC1 (Fig. 26A) and H2OC2 (Fig. 26B) under conditions (10 mM HEPES pH 7.5, 25% isopropanol) similar to those of crystallization are shown. Both show long fibres with different fibre widths of 20.674±2.53 and 9.31±0.52 nm, respectively. Inset images shows that H2OC2 forms a fibre bundle, whereas H2OC1 shows a distinct fibre assembly with a larger diameter.

[0037] **Fig. 27** shows line graphs showing molecular dynamics simulations of H2OC1 oligourea channel in POPC:POPS (4:1 ratio) lipid bilayer. Fig. 27A shows the permeation pathway length (membrane normal axis) over last 200 ns of the simulation time, while Fig. 27B shows the number hydrogen bonds between water molecules and side chains of two glutamic acids of H2OC1 helical oligoureas.

[0038] **Fig. 28** shows a scheme of the solid-phase synthesis and cleavage of the oligourea foldamers. Solid phase synthesis of oligourea foldamers was performed in a microwave reactor (CEM Discover) using the protocol described earlier (Douat-Casassus *et al.* Organic Letters 2012 and Collie *et al*. Nat. Chem., 2015). NovaPEG Rink amide resin (shown as spheres in the image) was used for the synthesis.

DETAILED DESCRIPTION

[0039] Biomimetic membranes incorporating artificial water channels (AWC) are being developed for industrial water purification. Achieving high water permeation with salt rejection

remains a challenge to be overcome while designing artificial water channels. Natural porins are the perfect examples of selective water transport through the cell membrane.

[0040] Oligourea foldamers form predictable, helical structures that can be used to create similar biomimetic porin-like architectures. Disclosed herein is the synthesis of amphipathic, helical oligourea foldamers that self-assemble to generate artificial channel-type nanostructures. Two of these foldamers (H2OC1, H2OC2) allow water permeability of up to 88.7 μm/s across lipid membranes while providing almost total salt rejection. Solid-state NMR experiments suggest proper insertion of foldamers into lipid vesicles, without perturbing the lipid phase, thermotropism and internal dynamics.

[0041] The H2OC1 crystal structure shows that oligourea helices pack together by hydrophobic and salt bridge interactions to build two channel-like assemblies. These channels differ in their hydrogen bonding patterns and result in hydrophilic pores of diameter 4.8 and 6.4 Å, respectively. Molecular dynamics simulation supports the experimental data on transport properties and indicates that H2OC1 can form a stable channel in lipid membranes. The selectivity of water and ions through foldamer assemblies is regulated at the sequence level. Ease of design, synthesis, purification, proteolytic stability, and microbial resistance of oligourea foldamers are added advantages. Thus, the information obtained and disclosed herein has been used to develop novel artificial water channels for water purification applications.

[0042] In summary, the present disclosure refers to the design of synthetic, self-assembling, helical oligourea foldamers that form artificial water channels. Results of solid-state NMR experiments and molecular dynamics simulation indicate proper insertion of foldamers into lipid vesicles, without perturbations of the lipid phase, thermotropism and internal dynamics. Furthermore, oligomerization of the amphipathic foldamers is shown to lead to channel-type nanostructures in lipid membranes with selective, high water transport and ion rejection. X-ray crystal structure analysis shows a narrow (4.8 Å) hydrophilic pore in the superhelical assembly of a selected water transporting oligourea foldamer. Short helical amphipathic oligourea foldamers were designed with distinct hydrophobic and hydrophilic faces. Two of the foldamers (H2OC1 and H2OC2) obtained by solid phase synthesis self-assembled into channel-type nanostructures that show efficient water permeability across lipid membranes, while rejecting ions. A self-assembled quaternary structure with two superhelical scaffolds results in the formation of 4.8 and 6.4 Å inner pores that were observed in the crystal packing of H2OC1. Both pores have hydrophilic interior and hydrophobic exterior surfaces. The findings disclosed herein demonstrate the channel forming ability of oligourea foldamers in lipid bilayers and the use of such artificial water channels in water purification applications.

[0043] Thus, disclosed herein are artificial water channels obtained using sequence-defined proteinomimetic foldamers that are used as scaffolds that self-assemble into precise quaternary nanostructures with intrinsic porosity (i.e. self-assembled foldamer-based porin-like channels).

[0044] 17-21 N,N'-linked oligoureas are a class of synthetic peptidomimetic foldamers which have been demonstrated to form well-defined helical structures in both organic and aqueous environment akin to regular peptide helices.

[0045] Amphipathic sequences with a patch of hydrophobic side chains at the surface of the helix have been shown to self-assemble into various quaternary structures including nanotubular

[0046] structures. These oligourea sequences were synthesized using, for example, solid-phase techniques akin to peptide synthesis, from enantiopure activated monomers prepared from amino acid derivatives (building blocks, BB). The building blocks are protected on one side as azides and activated at the other extremity as succinimidyl carbamates (e.g., N_3-Leu^U-OSu). In contrast to natural peptides, these foldamers are more resistant to proteolytic degradation due to their oligourea (i.e. non amide) backbone. These different properties make them candidates for synthesizing artificial water channels.

[0047] Disclosed herein is the design and synthesis of amphiphilic oligourea foldamers, which are able to form transmembrane channels exhibiting high water permeability rejecting all ions. These non-natural oligomers are shown herein to form tuneable helical scaffolds self-assembled in porin-like nanostructures. The state of oligomerization was investigated by mass spectrometry and transmission electron microscopy (TEM). The molecular insertion into lipid vesicles was investigated by solid-state NMR spectroscopy. Their water and ion permeability properties were further characterised in lipid vesicles. Also, shown herein are their three-dimensional structures elucidated by X-ray crystallography. The high-water permeability coupled with resistance to proteolytic degradation makes these novel synthetic foldamers possible candidates for industrial water purification applications. The designed and synthesized helical amphipathic oligourea foldamers can be used to generate artificial porins, as a new class of artificial water channels. The chemical synthesis can be scaled up with sequence-based tunability of their properties. They self-assemble to form channel-type structures that exhibit selective water permeability across lipid bilayers. The crystal packing of one foldamer shows a superhelical assembly resulting in the formation of a porin-like superstructure with a hydrophilic pore of 4.8 Å diameter. The resistance of oligoureas to proteolytic degradation could offer long-term stability indicating their suitability for water purification.

Design and synthesis

[0048] To design transmembrane water channels, H2OC1-H2OC4 scaffolds were designed based on a 10-mer showing an extended superhelical structure with two right-handed intertwined superhelices that form a channel in aqueous conditions with hydrophilic interior and a pore diameter of about 17 Å due to its global amphipathicity. The oligourea sequence, composed of two pentad repeats, has been designed to form an amphipathic helix with an extended hydrophobic face using the following principles. At positions 'a' and 'c': four leucine-type urea residues (Leu^U) (two in each pentad repeat); at position 'e': proline-type (Pro^U) residue in the first pentad and alanine-type (Ala^U) in the second pentad (Fig. 1A). These hydrophobic residues at the exterior surface assist in forming a dense and precise packing during self-assembly of H2. Additionally, the side chains of four charged residues (two glutamate-type side chains (Glu^U) at position 'b' and two lysine-type side chains (Lys^U) at position 'd') point into the lumen and form a highly charged interior of the channel. Without being bound by theory, it is thought that the electrostatic interactions and salt-bridges play a major role in the formation of intricate H-bonding network among proximal and distal oligourea residues and contribute to stabilize the overall packing.

[0049] A series of close H2 analogues were designed and synthesised, and structural and functional analyses were performed (Fig. 1, Fig. 6 to 9 and Table 2). In the first analogue, H2OC1, the N-terminal isopropyl group was replaced by an acetyl group and the C-terminal methyl urea was replaced by a simple urea termination to reduce the hydrophobicity (Figures 1A-1B). To understand the local constraining effect of Pro^U, a second analogue H2OC2 was prepared in which Pro^U is replaced with Ala^U at position 'e' (Figure 1A; 1B). It is shown that Pro^U does not interfere significantly with the helicity of oligoureas, unlike Pro in peptide chains. In H2OC3, two Glu^U were replaced with the less hydrophilic Asn^U at the 'b' sites of pentads (Figure 1A;1B). Such substitutions were made to reduce the interaction with water in the polar channel and facilitate faster water flow. To enhance the penetration into lipid bilayers, the analogue H2OC4 was designed by replacing Ala^U and Pro^U residues with Phe^U at position 'e' in both pentads of H2OC1 (Figure 1A;1B). Such aromatic Phe-type residues are thought to favourably interact with fatty acyl chains and lipid head groups. All oligourea foldamers were synthesized on a solid support using microwave assistance (Fig. 34; Table 2; and Figs. 6 to 9) and purified according to previously established procedures.

[0050] The specific structures described herein are the result of the amino acid sequence of foldamers claimed herein. It is further shown by the data disclosed herein that the specific structures of the foldamers disclosed herein define the transport functional properties of the foldamers, and the membranes comprising the foldamers disclosed herein. It is therefore shown

herein that the claimed foldamers exhibit unique structural properties over foldamers disclosed in the art.

Biophysical characterization of foldamers

[0051] The secondary structure and self-assembly of the foldamers were first investigated by circular dichroism (CD). All spectra showed a maximum at ~202 nm (Figs. 1C, 1D and 10), indicating helical conformation of H2OC1 to H2OC4 analogues at 100 μM in water. These analogues were shown to exhibit helicity even at lower concentrations. Concentration-dependent circular dichroism experiments of foldamers showed increased molar ellipticity with increasing foldamer concentration, indicating self-assembly in water (Figs. 1D and 10). Negative-staining TEM studies of H2OC1 and H2OC2 showed the formation of extensive fibre networks in aqueous condition (Figs. 1E; 1F). H2OC2 showed large fibre bundles with knots (with diameters ranging from about 12 to about 40 nm) consisting of protofilaments with diameter of 3.7 ± 0.8 nm, whereas H2OC1 showed untangled fibres with a diameter of 5.5 ± 0.6 nm (Figs. 1E to 1F). The TEM observations support their fibre-like self-assembly. In contrast, H2OC3 showed vesicle-like morphology with about 24 to 33 nm diameter (Fig. 11A). H2OC4 formed longer filaments with about 132 to 156 nm width (Fig. 11C). The stoichiometry of oligourea foldamer self-assemblies was next investigated by mass spectrometry. ESI-MS analyses revealed the presence of discrete multimeric species ranging from pentamer to nonamer of H2OC1 and H2OC2 in aqueous condition (Fig. 1G; 1H). In contrast, H2OC3 showed a predominant pentameric species (Fig. 11B). The TEM and ESI-MS results are thus consistent with H2OC1, H2OC2 and H2OC4 forming helical tubes as previously reported.

Molecular insertion of foldamer channels into lipid vesicles

[0052] To investigate whether H2OC1 and H2OC2 foldamers have the molecular properties to be stably inserted into lipid membranes, solid-state NMR spectroscopy was employed to measure biophysical parameters in a lipid environment. Lipid vesicles composed of a mixture of phosphatidylcholine (PC) and phosphatidylserine (PS) (molar ratio of 4/1) were reconstituted in the presence of H2OC1 and H2OC2 at a molecule-to-lipid ratio of 1/20.

[0053] To monitor the impact of foldamer insertion into the lipid vesicles, ^{31}P solid-state NMR was first used to probe the lipid phase and membrane curvature effect. PC/PS vesicles exhibited a ^{31}P spectral pattern characteristic of a lamellar lipid phase. ^{31}P experiments carried out for vesicles containing H2OC1 and H2OC2 showed a comparable asymmetric spectral profile and a similar chemical shift anisotropy (Fig. 2A), indicating that the lipid phase and the membrane curvature of the vesicles were not significantly impacted by the molecular insertion of the foldamers. To monitor the insertion of foldamers into the hydrophobic part of the lipid

bilayer, ^{2}H solid-state NMR was used on PC/PS vesicles for which the PC acyl chains were deuterated. Comparable ^{2}H quadrupolar couplings were detected for control vesicles and vesicles incorporating H2OC1 and H2OC2 (Fig. 2B). Since ^{2}H quadrupolar couplings are related to the molecular orientation and the dynamics of the C-^{2}H bonds, this indicates that the foldamer insertion has a negligible effect on the internal lipid dynamic at the level of the acyl chain. Lipid thermotropism was evaluated by monitoring the $_{2}H$ first spectral moment as a function of the sample temperature (Fig. 2C). It was observed that the melting temperature (T_m = 285 K) of PC/PS vesicles stayed the same in the presence of the H2OC1 and H2OC2 foldamers. Using spectral deconvolution, individual 2H quadrupolar splittings were employed to derive the lipid order parameters along the PC acyl chain (Fig. 2D) that span half of a bilayer. Control PC/PS vesicles exhibited a characteristic profile of SCD order parameters with a plateau for carbons near the membrane surface and having a high structural order, followed a decreased of order parameters towards the mobile methyl carbon C^{14}. A similar profile was observed for vesicles incorporating H2OC1 and H2OC2, with a slight increase of SCD order parameters in presence of the foldamers, suggesting that their insertion slightly increases lipid acyl chain ordering. Taken together, solid-state NMR experiments indicate a proper and stable insertion of foldamers into lipid vesicles, without perturbations of the lipid phase, thermotropism and internal dynamics.

Water permeability of foldamer Porin-type channels

[0054] The water permeability in lipid bilayers was measured using a stopped-flow method to capture fast kinetics on the millisecond time scale of water efflux from lipid vesicles led by osmotic pressure difference was determined by monitoring the change in the light scattering intensity of the vesicles (Fig. 3A). Water permeability was quantified by the shrinkage mode, measured in PC/PS (4:1) lipid vesicles. The foldamer-embedded vesicles were exposed to hypertonic osmolytes (sucrose and NaCl) (Fig. 3A; 3B). All four foldamers showed significant water permeability in the presence of sucrose or competitive ionic NaCl used as osmolytes (Figs. 3B and 12 to 13). H2OC2 showed the highest water permeability compared to other sequences (5- to 7-fold increase in water transport at 1:50 ratio over the control vesicles without H2OC2 foldamer) (Fig. 3B). Additionally, these foldamer channels showed comparable water permeability to that of gramicidin A (gA), a natural ionophore, which transports water and cations across the membranes (Figs. 3B, 12, and 13). Due to the high water permeability of H2OC2, this foldamer was studied more detail.

[0055] The effect of different PC/PS ratios in vesicles on water permeability of H2OC2 was evaluated (Fig. 14). No detectable water permeability was observed in the presence of pure PC. However, with increasing PS concentration, the permeability increased until PC/PS ratio reached

4:1. Further increase in PS concentration for H2OC2 resulted in decreased water permeability (Fig. 14). Subsequently, H2OC2 and PC/PS vesicles were mixed to obtain foldamer/lipid molar ratios from 1:1000 to 1:50 in 10 mM HEPES buffer, pH 7.0. The light scattering intensity increased with exposure to an equal volume of hypertonic osmolyte (600 mM sucrose) with the increasing foldamer ratio (Figs. 3C; 2D). This indicates that increasing the molar ratio results in effective increase in incorporation of foldamers into the vesicles, leading to enhanced water permeation. The calculated water permeability (Pf) values of H2OC2 vary from 10.5 μm/s (foldamer/lipid ratio 1:400), 23.2 μm/s (at 1:200) to 88.7 μm/s (at 1:50) (Fig. 3C; 3D), close or improved compared to water permeability observed with gramicidin A (gA) channels. H2OC2 also showed water permeability in the presence of other osmolytes such as glycine and glucose (Fig. 15). Furthermore, the water permeability at pH 4.5 was lower than that at pH 7.0.

Proton/ Ion transport properties of foldamers

[0056] High, selective water transport is a key feature for an artificial water channel (AWC). Another important feature that is equally important for water purification is its ability to reject ions transport. Therefore, the ion transport properties of selected foldamers disclosed herein was tested in PC/PS (4:1) vesicles using standard fluorescence based HPTS assay (Figure 3E). The HPTS loaded vesicles were incubated with the foldamers for 5 minutes before the experiments. A base pulse (20 μl of 0.5 N NaOH) was given at 50 seconds of the measurement to increase the external pH from 7.0 to 8.0. The natural ionophore gA, a monovalent cation selective channel was used as a positive control (Figs. 3F;3G). The changes in fluorescence intensity showed that H2OC3 (36%) and H2OC4 (25%) channels can moderately transport Na^+ and K^+ ions. Ion transport facilitated by the foldamers was shown to be much lower than that of gA (Figs. 3F; 3G). No detectable ion transport activity was observed for H2OC1 (7%) and H2OC2 (8%) in the presence of NaCl and KCl (Figs. 3F; 3G). In addition, H2OC1 and H2OC2 foldamers were able to reject the divalent ions (Fig. 16). This indicates a strong salt rejection ability of these two foldamers, which are therefore suitable for water purification applications. Thus, in one example, the foldamers and membranes disclosed herein are selectively ion permeable, wherein the ions can be, but are not limited to, Na^+, K^+, Mg^{2+}, Ca^{2+}, NO_3^-, and Cl^-. In another example, the foldamers, and membranes disclosed herein, have the ability to reject salt, if and when present in the solution to be purified. Examples of salts are, but not limited to, NaCl, $NaNO_3$, $MgCl_2$, $CaCl_2$, and KCl.

[0057] The chloride permeation through H2OC1 and H2OC2 channels was further investigated using chloride-selective dye 6-methoxy- N-(3-sulfopropyl) quinolinium (SPQ) trapped in PC/PS (4:1) vesicles containing intravesicular $NaNO_3$ and extravesicular NaCl (Fig.

3H). Oligourea H2OC1, H2OC2 and H2OC3 did not show any detectable chloride transport (Fig. 3I). However, concentration-dependent quenching of fluorescence intensity was observed for H2OC4 due to the influx of Cl^- ions (Fig. 3I). Collectively, these assays confirm the ion rejection properties of H2OC1 and H2OC2.

[0058] The proton transport activity of oligourea foldamers was evaluated by the patch-clamp experiments in PC:PS (4:1) and DOPC vesicles (Figs. 17 to 21). H2OC2 showed a low proton conductance rate (γH^+ =25.8 pS) than H2OC1 (γH^+ =7.4 pS) in PC:PS lipid (Fig. 17). The conductance values were at the same level when it came to DOPC lipid as shown in Fig. 18. Moreover, H2OC1 presents a better channel shape, longer retention time and higher channel opening probability at both lipid conditions. Along with the water transport activity, it is thought that protons are transferred overall along water wires. Patch clamp single channel current traces and I-V plots of H2OC1 and H2OC2 recorded in symmetrical solutions (cis chamber = trans chamber = 1 M KCl solution) and asymmetrical solutions (cis chamber = 1 M KCl solution, trans chamber = 1 M NaCl solution) using DOPC and PC/PS (4:1) lipids showed no obvious channel activity confirming the previously observed lack of ion transport ability by H2OC1 (Figure 19 and 21A) and H2OC2 (Figs. 20 and 21B).

[0059] Thus, in one example, the oligourea foldamer is water permeable. In another example, the oligourea foldamer is selectively ion permeable or ion impermeable.

Self-assembled channel structure of foldamer

[0060] An X-ray crystal structure of foldamer H2OC1 at a high-resolution of 1.2 Å was determined in order to provide atomic-scale details of channel formation via self-assembly. (Figs. 4, 22 to 24, and Table 3 to 4). The monomers exhibited the design-imposed amphipathic helical conformation consisting of distinct hydrophobic and hydrophilic faces (Figs. 1A and 4A), similar to those formed from H2 (Fig. 22 and Table 4). Crystal packing revealed oligourea helices forming a self-assembled superhelical channel-type assembly (Fig. 4B). Two distinct channels can be identified in the crystal structure of H2OC1, namely channels A and B with an external diameter of 29 Å each (Fig. 4B). These channels show apparent pore diameters of 4.8 and 6.4 Å, respectively. In channel B, the electron density maps for C-terminal Lys^{U9}, and Ala^{U10} residues are not 'visible' leading to overestimation of its pore size. In both channels, the exterior surface is composed of the hydrophobic residues Leu^U, Ala^U, Pro^U located at positions 'a', 'c' and 'e' in the helical-wheel representation. The pore of the channels is lined by the hydrophilic residues Glu^U and Lys^U situated at positions 'b' and 'd' (Fig. 4B). Eight oligourea helices per turn associate laterally in a right-handed, staggered, antiparallel orientation by hydrophobic and electrostatic interactions within the superhelical structure. The intricate H-bonding network of

salt-bridges between neighbouring oligourea helices greatly contributes to stabilize the pore structure. The hydrophilic pore is hydrated with both H-bonded (to the Glu^U and Lys^U side chains) and mobile water molecules (Figs. 4C, 4D, and 23 to 24). A single turn (23 Å) can span across phospholipid membranes. Crystal packing also revealed the presence of distorted helices that fill the void spaces between channel assemblies. These helices with a kink near the Pro^U and Lys^U residues are situated externally, defining four corners surrounding both channel assemblies (Fig. 25). The well-defined self-assembled helical tubular structure of H2OC1 in the crystal (Fig. 4B) suggests a model of interaction between the hydrophobic face of amphipathic foldamer helices and the hydrophobic component of lipid membranes compatible with the formation of pore in the lipid membranes. Without being bound by theory, it is thought that water transport across the membranes occurs via the H-bonding networks between water molecules and the hydrophilic residues in the pore wall (Figs. 4C; 4D). TEM analysis of H2OC1 under conditions similar to crystallization conditions showed extended fibre assembly with external diameter of about 20.6 nm (Fig. 26A). These fibres may be formed of five- to six-channel units across its diameter. In contrast, H2OC2 fibre bundles are about 9.3 nm in diameter (Fig. 26).

[0061] Thus, in one example, an oligourea foldamer is described. In one example, the oligourea foldamer self-assembles. In another example, the oligourea foldamer comprises or consists of one or more sequences, which can be, but are not limited to SEQ ID NO: 1 (Ac-$L^UE^UL^UK^UP^UL^UE^UL^UK^UA^U$-$NH_2$) and SEQ ID NO: 2 (Ac-$L^UE^UL^UK^UA^UL^UE^UL^UK^UA^U$-$NH_2$). In one example, the oligourea foldamer sequence is that of SEQ ID NO: 1. In another example, the oligourea foldamer sequence is that of SEQ ID NO: 2. In another example, there is described a compound comprising oligourea foldamers as disclosed herein.

[0062] In another example, the oligourea foldamers self-assemble into a three-dimensional nanostructure under aqueous conditions. In yet another example, the three-dimensional nanostructure is a helix or a pore. In one example, the pore has an internal diameter of between 4.5 Å to 6.5 Å.

All-atom molecular dynamics (MD) simulations

[0063] To gain molecular insights into the dynamics of H2OC1 channel in the lipid bilayer, explicitly solvated, all atom MD simulations were performed. The experimental crystal structure of the H2OC1 channel composed of 8 monomeric helices was used, as well as 16 kinked H2OC1 oligourea helices positioned on the side of the channel. This construct was embedded in a POPC: POPS (4:1 ratio; POPC: 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, POPS: palmitoyl-oleoyl phosphatidylserine) lipid bilayer (Fig. 5Ai). After 200 nanoseconds of the equilibration simulation with restrained alpha carbon positions of all helical oligoureas, only 12 kinked

peptides were observed that were positioned between lipid head groups of the membrane remained bound to the channel (Fig. 5Aii), while those above or below dissociated. After 600 ns of unrestrained production run, the channel retained its supra helical conformation (Fig. 5Aiii) with kinked peptides bound. A control system was also built, which corresponded to H2OC1 channel composed only of 8 monomeric helices in the same lipid environment (Fig. 5Biv). After 200 nanoseconds of equilibration and 600 nanoseconds of the production, the supra helix collapsed resembling a closed and cylindrical conformation (Fig. 5Bv). This is indicated by higher root-mean-square deviation (RMSD) of alpha carbons for the system without kinked helices (Fig. 5D). Although water molecules were found to permeate through both channels, the H2OC1 channel with kinked peptides could transport better water molecules (Fig. 5D). The longer permeation pathway of H2OC1 channel with kinked helices allowed formation of more hydrogen bonds between two glutamate-type residues in each oligourea with water molecules (Fig. 27A-B). On the other hand, a small fraction of ions could enter both channels, in agreement with experimental observations (Fig. 2E; 2I). Averaged radius profiles as well as averaged density of solvent in both channels is shown in Figs. 5F-5G. Although both channels have a radius in the membrane centre of mass corresponding to about 0.2 nm, the H2OC1 channel with kinked peptides showed a longer permeation pathway. In summary, computer simulations showed that kinked peptides can stabilise the H2OC1 channel, which allows more water molecules to permeate through the membrane.

[0064] The present disclosure shows the design and synthesis of oligourea foldamers that form superhelical water channels in aqueous condition stabilized by hydrophobic and electrostatic interactions. Oligourea foldamers H2OC1 and H2OC2 showed water permeability with high salt rejection in liposomes. In contrast, H2OC3 and H2OC4 showed permeability to ions along with water in liposomes. A high-resolution crystal structure of H2OC1 revealed that salt-bridges facilitate intricate H-bonding network among proximal and distal oligourea residues to stabilize the packing that was confirmed by the formation of fibres of the same dimension that were observed by TEM. Solid-state NMR data indicated stable insertion of foldamers without perturbing the lipid vesicles. The design of the amphiphilic foldamers resulted in the formation of self-assembled tertiary structures conferring a hydrophobic exterior and hydrophilic interior lumen filled with H-bonded water clusters. This results in high water permeability across lipid membranes, indicating the use of these foldamers in the production of artificial water channel (AWC) membranes for water purification applications.

Structural differences

[0065] Both the H2OC1 and H2OC2 N-terminal are blocked by small, acetyl group. In addition, in H2OC1 and H2OC2, the C-terminal has an amide group. Both these changes affect the oligomerization and pore formation. In H2OC2, additionally, Pro^U is replaced by Ala^U.

[0066] Transmission electron microscopy (TEM) structures show that H2OC2 forms large fiber bundles with knots (diameter ranging from 12.4 to 39.3 nm) compared to H2OC1, which forms untangled fibers of diameter 5.5 ± 0.6 nm.

[0067] Crystal structures of H2OC1 shows its oligomerization status. H2OC1 forms only one right-handed superhelix with 8 oligourea helices per superhelical turn. H2OC1 assembly shows two types of hydrophilic pores with internal diameters of 4.8 Å and 6.4 Å.

Functional characterization of H2OC1 and H2OC2

[0068] The results indicate that both H2OC1 and H2OC2 exhibit high water permeability with high salt rejection across lipid membranes. Using fabricated test membranes, use of H2OC1 and H2OC2 for water purification has been shown.

[0069] The structural changes at the N- and C-termini of the claimed peptides led to changes in the self-assembly and channel formation. The pore size of channels formed by H2OC1 are smaller (4.8 – 6.4 Å) compared to 17 Å pore formed by other peptides know in the art. Based on the similarity in functional properties and, without being bound by theory, it is thought that H2OC2 forms channels with similar pore size as H2OC1. Collectively, this indicates the use of the claimed peptides suitable for water purification.

[0070] Oligourea foldamer-embedded membranes can be used in for water filtration by reverse osmosis or forward osmosis methods, as well as sea water reverse osmosis (SWRO) membrane preparation and performance evaluation.

Uses

[0071] Reverse osmosis (RO) is a membrane purification method. The currently available seawater reverse osmosis (SWRO) membranes require around 3.5 kWh/m^3 of electricity for routine commercial desalination projects. The process has pre-treatment stage prior to the second pass reverse osmosis and yields drinking water at 50% recovery rate based on the feed water. One strategy to reduce the energy cost is to increase the water permeability rate. But increased water permeability tends to reduce salt rejection by membranes. The commercial thin film composite (TFC) seawater reverse osmosis (SWRO) membranes currently in use have best water permeance of between 1 to 2 $Lm^{-2}h^{-1}bar^{-1}$ with ~99% NaCl rejection. Therefore, novel strategies to develop high-performance seawater reverse osmosis (SWRO) membranes that require lower operational energy are necessary.

[0072] Compared to conventional thin film composite (TFC) membranes, test membranes incorporating a selective layer of oligourea foldamer-embedded liposomes on the PSF membranes exhibited enhanced water permeability with ~99% salt rejection. As indicated in Table 1, the test membranes have about 3 $Lm^{-2}h^{-1}bar^{-1}$ water permeability. This indicates that the energy use can be significantly reduced with such membranes. Techniques exist to develop seawater reverse osmosis (SWRO) spiral wound modules and hollow fibre modules, which are the two commonly used membranes. Hence this method combines the advantages of high water permeability, salt rejection, and good scalability for seawater reverse osmosis (SWRO) membrane fabrication. Further, it has application in the reduction of energy used for desalination.

[0073] Membrane modules can be fabricated as (a) hollow fibre modules, and (b) as spiral wound modules. These can be of diameters in the range of 2.5-inch and 8-inch (with one meter length), where the 8-inch membrane modules have the seawater reverse osmosis (SWRO) capacity to produce water at commercially meaningful rates of about 100 m^3/day (= 100,000 L/day).

[0074] Another process for water filtration is by forward osmosis (FO) using membranes, which requires less pressure to be applied. This can be applied in industries where water content must be removed without loss of other components present in the solution, including ions. Additional uses include: (a) concentrating fruit juices, (b) purification of brackish water, and (c) in pharmaceutical industries for concentrating products after solution phase manufacturing steps. For such applications, one can use membrane modules incorporating a selective layer of liposome-embedded aquaporin proteins, including those disclosed herein.

Test membrane performance

[0075] A polysulfone (PSF) flat sheet, seawater reverse osmosis (SWRO) test membrane was prepared with its polyamide selective layer incorporated with the foldamer-containing liposomes by spraying to a uniform thickness. H2OC1 and H2OC2 membranes showed enhanced water permeability of 3.19 ± 0.01 $Lm^{-2}h^{-1}bar^{-1}$ and 3.09 ± 0.02 $Lm^{-2}h^{-1}bar^{-1}$, respectively, compared to the test membrane coated with liposome lacking foldamers (2.89 ± 0.01 $Lm^{-2}h^{-1}bar^{-1}$) (Table 1). This indicates that the increased water permeability is due to the incorporated foldamer channels. In addition, this data taken together with the disclosure in the present specification, indicates that oligomers comprising the short foldamer molecules disclosed herein possess one or more of the characteristics selected from, for example, high water permeability, high salt rejection, sequence-based functional tunability, convenient design and chemical synthesis, proteolytic stability, and ease of scalability. Furthermore, both membranes containing foldamer channels showed high

NaCl rejection (>98.76 %). These results shown the feasibility and performance of incorporating H2OC1 and H2OC2 membranes in the fabrication of artificial channel-based biomimetic membrane for brackish reverse osmosis desalination.

[0076] Thus, in one example, there is disclosed a permeable membrane comprising the oligourea foldamer disclosed herein or the compound disclosed herein. In another example, the membrane is water permeable and/or selectively ion permeable. In another example, the membrane is ion impermeable.

[0077] Also envisioned herein is a method of purifying water using the membranes and methods disclosed herein. In one example, the membranes disclosed herein can be used for test membrane studies, as follows: Biomimetic membranes can be fabricated with the foldamer-embedded liposomes as a water selective (ion rejecting) layer on top of an ultrafiltration membrane. In one example, the test membrane was a polysulfone (PSF) flat sheet, SWRO test membrane - prepared with its polyamide selective layer incorporated with the foldamer-containing liposomes by spraying to a uniform thickness. H2OC1 and H2OC2 test membranes showed enhanced water permeability over the controls without the foldamer incorporation. Techniques exist to develop SWRO spiral wound modules and hollow fiber modules, which are the two commonly used membranes. Hence, this method combines the advantages of high water permeability, salt rejection, and good scalability for SWRO membrane fabrication. Further, it has the potential to reduce energy used for desalination.

[0078] An exemplary method of the fabrication of the membranes disclosed herein follows: Membrane modules can be fabricated as (a) hollow fiber modules, and (b) as spiral wound modules. These can be of diameters in the range of 2.5-inch and 8-inch (with one meter length), where the 8-inch membrane modules have the SWRO capacity to produce water at commercially meaningful rates of about 100 m^3/day (roughly 100,000 L/day).

[0079] Production of recombinant aquaporin at scale can be expensive, and the protein can be prone to degradation by microbial proteases. The oligourea foldamers disclosed herein are resistant to proteases and are therefore less prone to degradation by microbial proteases. The oligourea foldamers disclosed herein can produced using large-scale synthesis methods and utilised in water filtration membranes produced at commercial scale.

[0080] The invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and

expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[0081] As used in this application, the singular form "a," "an," and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a genetic marker" includes a plurality of genetic markers, including mixtures and combinations thereof.

[0082] As used herein, the term "about", in the context of concentrations of components of the formulations, typically means +/- 5% of the stated value, more typically +/- 4% of the stated value, more typically +/- 3% of the stated value, more typically, +/- 2% of the stated value, even more typically +/- 1% of the stated value, and even more typically +/- 0.5% of the stated value.

[0083] Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[0084] Certain embodiments may also be described broadly and generically herein. Each of the narrower species and sub-generic groupings falling within the generic disclosure also form part of the disclosure. This includes the generic description of the embodiments 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.

[0085] The invention has been described broadly and generically herein. Each of the narrower species and sub-generic 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.

[0086] 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.

TABLES

[0087] Table 1. Water flux and salt rejection of flat sheet membranes embedded with H2OC1 and H2OC2. A polysulfone (PSF), seawater reverse osmosis (SWRO) flat sheet test membrane (42 cm^2) was coated with foldamer-containing liposomes (foldamer/lipid ratio 1:50). This was compared to a control membrane and a membrane with liposome only coating on the membrane surface (PC/PS 4:1) embedded with foldamer channels were fabricated and used for seawater reverse osmosis (SWRO) test. The control membranes were coated with liposomes lacking the foldamers. Membrane performance was tested for 1 hour with 35 g/l NaCl solution (NaCl concentration 35 000 ppm) at an operating pressure of 50 bar, and a cross-flow velocity of 10cm/s.

Membrane (Flat sheet SWRO)	**Permeability, A (LMH/bar)**	**Salt Rejection, R (%)**
Control (without PC/PS)	2.70 ± 0.07	99.19 ± 0.10
Blank PC/PS	2.89 ± 0.02	98.89 ± 0.33
H2OC1-PC/PS	3.20 ± 0.02	98.76 ± 0.28
H2OC2-PC/PS	3.09 ± 0.03	99.00 ± 0.13

[0088] Table 2. Oligourea foldamer sequences

Code	Foldamer sequence	Expected Mw (Da)	Observed M.W. (Da)
H2OC1	Ac-Leu^U Glu^U Leu^U Lys^U Pro^U Leu^U Glu^U Leu^U Lys^U Ala^U-NH_2	1484.88	1485.25 Da ($[M+H]^+$), 743.19 Da ($[M+2H]^{2+}$), 495.82 Da ($[M+3H]^{3+}$)

H2OC2	Ac-Leu^{U} Glu^{U} Leu^{U} Lys^{U} Ala^{U} Leu^{U} Glu^{U} Leu^{U} Lys^{U} Ala^{U}-NH_2	1458.84	1459.99 Da ($[M+H]^{+}$), 730.00 Da ($[M+2H]^{2+}$), 486.99 Da ($[M+3H]^{3+}$)
H2OC3	Ac-Leu^{U} Asn^{U} Leu^{U} Lys^{U} Pro^{U} Leu^{U} Asn^{U} Leu^{U} Lys^{U} Ala^{U}-NH_2	1454.00	1455.13 Da ($[M+H]^{+}$), 728.08 Da ($[M+2H]^{2+}$), 485.73 Da ($[M+3H]^{3+}$)
H2OC4	Ac-Leu^{U} Glu^{U} Leu^{U} Lys^{U} Phe^{U} Leu^{U} Glu^{U} Leu^{U} Lys^{U} Phe^{U}-NH_2	1611.03	1612.04 Da ($[M+H]^{+}$), 806.04 Da ($[M+2H]^{2+}$), 537.69 Da ($[M+3H]^{3+}$)

[0089] Table 3. Data collection and refinement statistics for X-ray crystal structure of foldamer H2OC1.

Crystallization condition	0.1 M HEPES pH 7.5, 0.2 M ammonium acetate, 15% v/v isopropanol
Data collection	
Space group	$P4_1$
a, b, c (Å)	39.83, 39.83, 36.46
α, β, γ (°)	90.00, 90.00, 90.00
Resolution (Å)	39.83 – 1.20 (1.26 – 1.20)
R_{meas} (%)	12.8 (42.3)
I / σ	8.9 (4.1)
Reflections (total)	109117
Reflections (unique)	17988
Completeness (%)	99.5 (96.9)
Redundancy	6.1 (5.2)
Refinement	
Resolution (Å)	39.83 – 1.20
R_{work} / R_{free} (%)	15.1 / 18.6
Atoms	515
Waters	30
Overall B-factor (Å^2)	19.17
R.m.s. deviations	
Bond-lengths (Å)	0.019

Bond-angles (°)	2.796
CCDC code	2211250

[0090] Table 4. Comparison of primary sequences and self-assembled structures of H2OC1, H2, and other H2 analogues.

	H2	**H2' ($P6_1$)**	**H2' ($P4_122$)**	**H2OC1**
N-terminal	isopropyl	isopropyl	isopropyl	acetyl
C-terminal	methylamine	amide	amide	amide
Intra helical H-bonding	22	21	19	17
Helix length (Å)	22.9	23.5	23.4	23.0
Packing	12 helices/turn	12 helices/turn	8 helices/turn	8 helices/turn
Pore size (Å)	17	11	5	6

[0091] Table 5. Comparison between Aquaporin and oligourea foldamers

	Aquaporin	**Oligourea Foldamers**
1	High water selectivity with high salt rejection	High water selectivity with high salt rejection
2	Protein – with peptide bonds	Non-natural oligourea bonds
3	Susceptible to proteolytic/microbial degradation	Resistant to proteolysis/microbes
4	Can be denatured (e.g., by excess heat)	Not denatured, stable molecules
5	Tedious production of recombinant protein – subject to batch-to-batch variations	Ease of chemical synthesis – better control, cost effective
6	Larger footprint per AQP molecule on a membrane	Smaller molecules – can be packed to higher density
7	Fine tuning – protein engineering is difficult	Amenable for fine tuning by sequence alterations
8	Established methods to fabricate biomimetic membranes using AQP-embedded liposomes	Similar methods can be used for oligourea foldamer membranes

EXPERIMENTAL SECTION

Materials

[0092] All reagents for the synthesis of foldamers were purchased from Sigma-Aldrich unless stated otherwise. Lipids were purchased from Avanti-polar lipids. Gramicidin A, 8-hydroxypyrene-1,3,6-trisulfonate (HPTS), [6-Methoxy-N-(3-sulfopropyl)quinolinium] (SPQ) were obtained from Sigma-Aldrich for fluorescence-based assays. Milli-Q water was used to prepare all buffers and in biophysical experiments (CD, TEM, crystallization).

Synthesis of building blocks and oligourea foldamers

[0093] Oligourea foldamers were synthesized by standard solid phase peptide synthesis methods using azide chemistry. Synthesis of appropriate azidoalkyl succinimidyl carbamate monomers was carried out by solution phase chemistry methods published earlier.1–3 NovaPEG Rink amide resin (0.45 mmol/g) was used to synthesize the foldamers. The synthesized foldamers were purified by C18-RP HPLC and characterized by mass spectrometry and analytical RP-HPLC.

Solid phase synthesis of full oligourea

[0094] The compounds H2OC1, H2OC2, H2OC3 and H2OC4 were synthesized according to previously reported procedures1,3 in a polypropylene SPE tube in microwave reactor (CEM Discover) on NovaPEG Rink amide resin (NovaBiochem substitution 0.45 mmol/g) using variable scales (0.050-0.075 mmol). The resin was swelled in dichloromethane (DCM) (3 mL) and in dimethylformamide (DMF) (3 mL) for 2 hours. Activated N3-BB (150 µmol, 1.5 equiv.) and DIEA (40 µL, 300 µmol, 3 equiv.) were dissolved in DMF (2 mL) and the solution was added to the reaction vessel. N_2 purge and bubbling in the vessel were performed. All microwave experiments were conducted at atmospheric pressure. The vessel was then placed inside the microwave reactor and irradiated (70°C, 25 W, 20 minutes). After 20 minutes, the resin was filtered and washed with DMF (4×3 mL). This coupling step was repeated a second time. The resin was washed with a mixture of 1,4-dioxane:H_2O (i.e. reduction solvent, 7:3 v/v, 4×3 mL). Then, 1M PMe3 solution in THF (0.75 mL, 750 µmol, 10 equiv.) and reduction solvent (3 mL) was added. The Staudinger reaction was performed to reduce the azide group under the microwave irradiation (70°C, 25 W, 15 minutes). After the reaction, the resin was filtered and washed with reduction solvent (3x3 mL) and DMF (3x3 mL). The deprotection of Teoc group was performed for 2h at RT in 1M TBAF solution (3 mL, 20 equiv. per Teoc) and DMF (1.5 mL). The deprotection reaction was repeated once. All steps were monitored by chloranil test. After reduction of the last residue of the sequence, acetic anhydride (76 µL, 10 eq) and DIEA (267 µL, 2000 µmol, 20 equiv.) in DMF were added to the resin to perform the N-terminal

capping under microwave irradiation (70 °C, 25 W, 10 minutes). After the reaction, the resin was filtered and washed with DMF (4×3 mL).

[0095] Before the cleavage, the resin was moved to the syringe with filter, and washed with DMF (4×3 mL) and DCM (5×2 mL). The cleavage was performed under acidic conditions, with trifluoracetic acid TFA (95%)/TIS (2.5%) /H_2O (2.5%) (total volume 4 mL) for 4 hours. Resulting filtrates were evaporated. Oligourea was precipitated in diethyl ether, then dried. The crude solid was dissolved in acetonitrile/deionized water ACN:DW=1:9 and lyophilized. Purity was checked by analytical HPLC (C18 column, method: 10-100%B/10 min, A: Milli-Q H_2O+0.1% TFA, B: ACN+0.1% TFA, flow=1mL/min, T=50 °C, λ=214 nm). The product was purified by prep C18-RP-HPLC (Macherey-Nagel Nucleodur 100-5 C18ec column, method: 40-100% B; 20 min, A: Milli-Q H_2O+0.1% TFA, B: ACN+0.1% TFA, flow 20 mL/min, λ=214 nm). The counterion was exchanged by lyophilizing powder with HCl solution (1. 0.05 N $HCl_{(aq)}$, 2. 0.1N $HCl_{(aq)}$, 3. MQ H_2O).

Methods

Circular dichroism (CD)

[0096] CD experiments of foldamers were carried out using a J-1100 Circular Dichroism spectrophotometer. Experiments were measured in a quartz cuvette with path length of 1 mm in water at foldamer concentration of 100 μM. Concentration-dependent CD measurements of H2OC1 and H2OC2 was carried out in the range of 12.5 μM to 200 μM. Data were collected at 20°C at 190-260 nm wavelength at 0.2 interval at a speed of 50 nm/min with three accumulations. Data were plotted using Origin Pro after subtracting from blank.

Transmission electron microscopy (TEM)

[0097] For TEM visualization, foldamer samples at a concentration of 200 μM in 10 mM HEPES pH 7.0 were incubated at room temperature for 3 days before the experiments. A 5 μL of sample was deposited on glow discharge carbon coated copper grid (200 mesh) and incubated for 2 minutes. Excess sample was soaked with filter paper followed by negative staining two times with 2% uranyl acetate and dried with filter paper. The grid was observed under electron microscope (JEOL 1400Flash TEM).

Mass spectrometry analysis

[0098] ESI-MS was performed using a Synapt-G2Si (Waters, UK) instrument. Foldamers were analysed in 10 mM HEPES pH 7.0 at a concentration of 200 μM with a pump flow rate 300 μL min-1 and source voltage 3kVA. The samples were eluted using methanol in the presence of 0.1% formic acid. The data analyses were performed using MassLynx and MaxEnt 1 software. Data were plotted using Origin pro 8.

Size measurement by dynamic light scattering (DLS)

[0099] The size of vesicles and foldamers embedded vesicles were determined using dynamic light scattering in Dynapro Wyatt technology. The vesicle samples were centrifuged at 12000 rpm for 20 minutes before the measurements. The measurements data were averaged in triplicates.

Water transport assay by stopped-flow method

[00100] The samples were prepared by film rehydration method. L-α-phosphatidylcholine (PC)/ L-α-phosphatidylserine (PS) (4:1) were mixed in methanol and chloroform (1:1) in round bottom flask and evaporated under reduced pressure using rotavapor overnight. The resulting thin lipid film was rehydrated with buffer (10 mM HEPES pH 7.0) and mixed well to detach the lipids from the glass surface with occasional vortexing. The mixture was further subjected to 9 freeze-thaw cycles (liq. N_2 1 minute and heating dry bath 55°C for 2 minutes) and extruded through 0.2 μM membrane filter. The large unilamellar vesicles (LUVs) obtained by this method contained 5 mM lipids and stored at 4°C until measurement done. The particle size of LUV was confirmed using DynaPro dynamic light scattering (DLS). The water permeability was measured on a stopped-flow instrument (Chirascan circular dichroism spectrometer, Applied photophysics, UK). PC/PS (4:1) lipid vesicles (100 μL, 5 mM in 10 mM HEPES 7.0) and foldamer (5 μL, 1mM foldamer stock in double distilled water) were mixed at molar ratio of 100 and equilibrated for 5 minutes before the measurements. The vesicles were exposed to hypertonic osmolytes (600 mM sucrose) leading to shrinkage of vesicles due to water efflux. The abrupt decrease of vesicles size was monitored by increased light scattering intensity. The faster size change with foldamer-incorporated vesicles compared to non-incorporated vesicles indicates a higher water permeability in the former. The changes in light scattering with time due to water efflux from vesicles with or without channels can determine water permeability (Pf in cm^3/s).

[00101] To obtain net osmotic permeability, the $Pf_{(blank)}$ value of blank vesicles must be deducted from $Pf_{(channel)}$. The changes of light scattering intensity were recorded at 572 nm wavelength and fitted the curves with stretched exponential function using Chirascan software and plotted using Origin 8.5 software.

$$y = A \exp(-kx^b) + y0$$

where y= change in light scattering; k= exponential coefficient depicting change in light scattering; x= time and b= constant factor. The osmotic permeability was calculated using following equation.

$$Pf = k/[(S/V_0) \times Vw \times \Delta osm]$$

S and V_0 are the initial surface area and volume of the vesicles, respectively; Vw is the molar volume of water, and Δosm is the osmolarity difference.

Measurements of ion transport activity

Preparation of PC/PS (4:1) LUV

[00102] PC/PS lipids were mixed at 4:1 molar ratio in methanol and chloroform (1:1) in round bottom flask and evaporated under reduced pressure using rotavapor overnight. The film was hydrated with buffer (10 mM HEPES, 100 mM NaCl pH 7.0) having pH-sensitive 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS, 1 mM) dye. The mixture was further subjected to 9 freeze-thaw cycles (liq. N_2 1 minutes and heating dry bath 55 °C for 2 minutes) and extruded through 0.2 μM polycarbonate membrane filter to yield homogeneous suspension of LUVs. The unencapsulated HPTS dye was removed by size-exclusion chromatography using Sephadex G-50 column and obtained 5 mM lipid stock at the end and stored at 4 °C until measurement done.

Ion transport activity by HPTS assay

[00103] These HPTS containing PC/PS (4:1) vesicles (40 μL, 5 mM in 10 mM HEPES, 100 mM NaCl pH 7.0) was added to the buffer (1.95 mL, 10 mM HEPES, 100 mM NaCl, pH 7.0). The foldamers (10 μL, 1mM foldamer stock in double distilled water) and vesicles was equilibrated for 5 minutes before the experiments. A base pulse (20 μL, 0.5M NaOH) was added at 50 s of the measurement to increase the pH from 7 to 8. The emission was monitored at 510 nm with excitations at both 403 and 460 nm simultaneously for 300 s using fluorescence spectrophotometer. The vesicles were lysed at the end of the measurements by adding 10% Triton X-100 to obtain maximum fluorescence emission. For data analysis and comparison, time (X-axis) was normalized between the point of foldamer addition (i.e. t = 50 s was normalized to t = 0 s) and end point of experiment (i.e. t = 300 s was normalized to t = 250 s). The data was plotted as a ratiometric value of I460/I403 and normalized (IF). IF= It-I0/(I∞-I0), where It and I0 are the ratiometric values of I460/I403 before addition of Triton X-100 and I∞ is the ratiometric values right after addition of Triton X-100.

SPQ assay for chloride-selective transport

[00104] PC/PS lipids were mixed at 4:1 molar ratio followed by addition with methanol and chloroform (1:1) in round bottom flask and evaporated under reduced pressure using rotavapor. After drying the resulting film for overnight at room temperature, the film was rehydrated 200 mM $NaNO_3$ containing chloride selective SPQ dye (1 mM). The purified vesicles were prepared as mentioned above. The SPQ containing vesicle suspensions (40 μL, 200 mM NaNO3) was added to 200 mM NaCl (1.95 mL). The foldamers (10 μL, 1 mM foldamer stock in double distilled water) was added at 50 seconds of the experiments. The emission was measured at

430 nm with excitations at 360 nm for 300 s using fluorescence spectrophotometer. The vesicles were lysed at the end of the measurements by adding 10% Triton X-100 to obtain maximum change in dye fluorescence emission. For data analysis and comparison, time (X-axis) was normalized between the point of foldamer addition (i.e. t = 50 seconds was normalized to t = 0 seconds) and end point of experiment (i.e. t = 300 seconds was normalized to t = 250 seconds). The data was normalized using the equation IF= It-I0/(I0-I∞), where I0 is fluorescence intensity just before addition of compound at 50 seconds, It is fluorescence intensity at time t and I∞ is fluorescence intensity after addition of Triton X-100 after an additional 250 seconds.

X-ray crystallography

[00105] For crystallization experiments, a lyophilized powder of H2OC1 was dissolved in double distilled water to a final concentration of 10 mg/ml. Crystallization trials were performed using sparse-matrix screening kits (Cation suite (Nextal) and Proplex (Molecular Dimensions)) in standard 96-well sitting-drops. Crystals obtained by this method were then optimized in 24-well plates with hanging drops typically composed of 0.5 μL foldamer solution and 0.5 μL crystallization reagent. Crystallization experiments were performed at room temperature as well as at 16C°. Diffraction quality crystals of H2OC1 were obtained from a crystallization reagent composed of 0.1 M HEPES pH 7.5, 0.2 M ammonium acetate plus 15% v/v isopropanol within three days at room temperature. Before data collection, crystals were soaked in the crystallization solution supplemented with 25% glycerol as a cryo-protectant and frozen in liquid nitrogen. X-ray diffraction data were collected on beamline 23-ID-D at the Advanced Photon Source, Argonne National Laboratory. Diffraction data were integrated and scaled using XDS7 and CCP49 to a final resolution of 1.2 Å. The structure was solved by molecular replacement using the crystal structure of H2' as a search model using Phaser8 from the CCP4 suite. Geometric restraints were generated using PRODRG with model building and restrained refinement performed in Coot and Refmac5, respectively. B-factors were refined isotropically for water molecules and anisotropically for all other non-solvent atoms. Data collection and refinement statistics can be found in Table 3. The structure has been deposited in the CCDC with accession code 2211250.

[00106] For the measurement of ion selectivity ratio (P_{Na+}/P_{K+}), the following method was used: KCl buffer (1 M, 10 mM HEPES, 10 mM Tris) was used in the *cis* chamber and the *trans* chamber was still filled in NaCl buffer (1 M, 10 mM HEPES, 10 mM Tris). The obtained data was fitted with the following Goldman-Hodgkin-Katz equation for calculation: $\varepsilon_{rev}=RT/F\times In(P_{Na+}/P_{K+})$, R=universal gas constant (8.314 $J\cdot K^{-1}\cdot mol^{-1}$), T = 300 K, F = Faraday's constant (96485 $C\cdot mol^{-1}$), P is the permeability of sample for ions. ε_{rev} was determined by fitting

using a linear equation of y=a+bx where –intercept/slope (-a/b) is the reverse potential value (ε_{rev}).

Solid-state NMR

[00107] For solid-state NMR experiments, liposome samples were prepared. Lipids (DMPC-d54 and POPS (4/1 molar ratio, 10 mg total lipids) were mixed and dissolved in $CHCl_3$. H2OC1 and H2OC2 were dissolved in $CHCl_3$ / methanol (2:1 volume) and added to the lipid mixture at a ratio of 1/20 (molecule/lipid molar ratio). A control sample, only constituted by lipids, was also prepared. The solvent was evaporated under a stream of compressed air. The residual lipid film was dispersed in 1 ml of milliQ-filtered water and freeze-dried overnight. The resulting powder was suspended into 100 µL of deuterium-depleted water to obtain a hydration level of ~90 %. After shaking into a vortex mixer, samples were frozen in liquid nitrogen for few seconds, heated at 45 °C for 10 minutes in a water bath and shaken again for better sample homogeneity; this freeze-thaw-shaking cycle was repeated 3 times and the resulting milky dispersion transferred into a 4 mm diameter Zirconium rotor of 80 µL (Cortecnet, Les Ulis, France). Spectral moments First moments were calculated using a homemade routine, NMRdepacker (Buchoux S., unpublished). Orientational order parameters (SCD) were calculated for each temperature by using a simulation program for wide line spectra developed in FORTRAN code by Erick Dufourc and implemented in a user-friendly graphical interface (Microsoft.NET) for Windows platforms by Arnaud Grélard. For ^2H static wide-line solid-state NMR experiments, a quadrupolar spin-echo sequence was applied at a ^2H frequency of 76.8 MHz on a 500 MHz (11.7 T) Bruker Avance III NMR spectrometer, with a 90° pulse of 3.5 µs, an echo delay of 50 µs, a recycle delay of 2 seconds, a spectral width of 500 kHz and 256 scans for each temperature. Spectra were acquired at different temperatures, ranging from 273K to 318K.

[00108] The temperature of the sample was stabilized for 20 minutes prior to the acquisition. For ^{31}P static wide-line solid-state NMR, a Hahn-echo sequence was applied at a 31P frequency of 161.9 MHz on a 400 MHz (9.4 T) Bruker Avance III HD NMR spectrometer, with a 90° pulse of 8 µs, an echo delay of 40 µs, a recycle delay of 5 seconds, a spectral width of 400 ppm and 512 scans for each temperature. Spectra were acquired at different temperatures, ranging from 273K to 318K. The temperature of the sample was stabilized for 20 minutes prior to the acquisition.

Measurements of water permeability at pH 6.4

[00109] Liposomes were prepared using a phosphatidylcholine (PC) and phosphatidylserine (PS) (phosphatidylcholine, Avanti Polar lipids). A PC/PS mixture with a molar ratio of 4/1 was dissolved in chloroform/methanol mixture (v/v=1/1). The solvent was removed under vacuum

and then rehydrated with PBS buffer (10 mM, pH=6.4) for 40 minutes. After hydration, the suspension was submitted to 5-10 freeze-thaw cycles (liquid nitrogen, water at 25°C).

[00110] Monodisperse unilamellar vesicles were obtained after extruding through 100 nm track-etched filters (Whatman, UK) for 21 times and diluted with HEPES buffer solution to give 11 mM lipid stock solution (considering all the lipids have been incorporated). The average radius of the vesicles was determined by using Zetasizer Nano (Malvern) dynamic light scattering experiment. 10 μL of 1 mM of compound was injected in the corresponding hypertonic osmolyte (which contains 100 μL of 11 mM lipid vesicles) to get a 5 μM final concentration in 2 mL total volume. Two times at least of repeated experiments were carried out to obtain the average permeability.

[00111] The stop-flow light scattering experiments were performed on a stopped-flow instrument (SFM3000+ MOS450. Bio-Logic SAS, Claix, France) and recored at a wavelength of 345 nm, under 600 mM sucrose as osmolytes (Δosm=600 mOsmol/kg respectively). The water permeability tests were conducted on a stopped-flow instrument (SFM3000+MOS450, Bio-Logic SAS, Claix, France). The abrupt change of the vesicle size under osmotic pressure leads to variation in the light scattering at 90° according to the Rayleigh-Gans theory applied to this system and could be fitted in the form of the sum of two exponential function

$$(y=at+b+\sum_{i=1}^{N=2} c_i e^{-k_i t}).$$

by using Biokine software. The osmotic permeability (Pf) was calculated by following the equation:

$$Pf=k/[(S/V_0) \times Vw \times \Delta osm],$$

where k is the exponential coefficient of the change in the light scattering; S and V_0 are the initial surface area and volume of the vesicles, respectively; Vw is the molar volume of water, and Δosm is the osmolarity difference.

[00112] The water transport ability of H2OC1 and H2OC2 was evaluated by stopped-flow assay using PC/PS (4:1) lipid vesicles at pH 6.4 (10 mM PBS). As shown in Figure 1, under the hypertonic condition driven by sucrose osmolyte, the net permeabilities were showing a decreasing trend with the increasing concentration of sucrose. H2OC1 showed a slightly higher water permeabilities than H2OC2 at high osmotic pressure.

Single channel current measurement using planar lipid bilayer workstation

For measuring proton transport in the unit of pS

[00113] A mixture of phosphatidylcholine (PC) and phosphatidylserine (PS) (PC:PS=4:1), or DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine, Avanti Polar lipids, 25 mg/ml in CHCl3) was dried under N2 gas for 1 hour and then re-dissolved in decane. Salt bridges (KCl/Agar) were

placed in the chambers filled with 0.1 M HCl solution, attaching electrodes (Ag/AgCl) which were placed in KCl solution (1 M). Planar lipid bilayers were formed by brushing 0.2 μL of lipid containing n-decane solution around the aperture, and a stable bilayer were obtained with a capacitance value ranging from 80 to 150 pF without applying any voltage. Single channel current traces were tested repeatedly for capacitance and resistance at different voltage. The obtained data was collected by ClampFit 10.3.1.5, and then analysed in Origin, fitting using a linear equation of "y=a+bx" where slope b is the conductance value (γ), unit: nS.

For measuring ion transport in the unit of pS

[00114] A solution of DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine, Avanti Polar lipids, 25 mg/ml in $CHCl_3$) was dried under N_2 gas for 1 hour and then redissolved in n-decane. Salt bridges (KCl/Agar) were placed in the chambers filled with KCl buffer (1 M, 10 mM HEPES, 10 mM Tris), attaching electrodes (Ag/AgCl) which were placed in KCl solution (1 M). Planar lipid bilayers were formed by brushing 0.2 μL of lipid-containing n-decane solution around the aperture, and a stable bilayer were obtained with a capacitance value ranging from 80 to 150 pF without applying any voltage. Single channel current traces were tested repeatedly for capacitance and resistance at different voltage. The obtained data was collected by ClampFit 10.3.1.5, and then analysed in Origin, fitting using a linear equation of "y=a+bx" where slope b is the conductance value (γ), unit: nS.

[00115] For the measurement of ion selectivity ratio (PNa+/PK+): KCl buffer (1 M, 10 mM HEPES, 10 mM Tris) was used in the cis chamber and the trans chamber was still filled in NaCl buffer (1 M, 10 mM HEPES, 10 mM Tris). The obtained data was fitted with the following Goldman-Hodgkin-Katz equation for calculation: $\varepsilon_{rev}=RT/F\times In(P_{Na+}/P_{K+})$, R=universal gas constant (8.314 J·K-1·mol-1), T = 300 K, F = Faraday's constant (96485 C·mol-1), P is the permeability of sample for ions. ε_{rev} was determined by fitting using a linear equation of "y=a+bx", where – intercept/slope (-a/b) is the reverse potential value (ε_{rev}).

All-atom MD Simulations

[00116] All-atom simulations were performed using the GROMACS 2018.3 simulation package utilizing the CHARMM36m force field with the TIP3P water model. The experimental structure of suprahelical H2OC1 oligourea channel composed of 8 helical monomers and 16 kinked H2OC1 helices was extracted from (Fig. 25). Topologies were generated using CHARMM-GUI ligand reader and modeler.24 According to experimental procedures, the N-terminus was acetylated (CH3CO-) while the C-terminus was amidated (-CONH2) with all residues in a charged state at neutral pH. Two systems were built: i) 8 H2OC1 monomeric oligourea peptides with 16 kinked H2OC1 helices and ii) 8 H2OC1 monomeric oligourea

peptides alone as a control. Both systems were minimized in vacuum using steepest descents algorithm for 5,000 steps. A symmetrical lipid bilayer composition corresponded to 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS) in 4:1 lipid ratio, respectively. The model bilayer was generated using CHARMM-GUI membrane builder. The box size was ~10×10×12 nm^3. Subsequently, either i) or ii) construct was placed in the middle of the membrane. Any lipid within 0.3 nm of the channel were removed ensuring no lipids are inside of the channel. Approximately 30,000 TIP3P water molecules were added to the box and 150 mM NaCl salt was added to the box on top of neutralizing overall system charge. The energy minimization was performed using steepest descent minimization algorithm with a 0.1 energy step size. The system was equilibrated in the NVT and NPT ensemble for 200 ns with position restraints applied to helical H2OC1 peptides backbone atoms and a force constant of 1000 kJ mol^{-1} nm^{-2}. Unrestrained production run was set to 600 ns in the NPT ensemble. A temperature of 310 K was maintained using the velocity rescaling thermostat with additional stochastic term using a time constant of 1 ps. Pressure was maintained semi-isotropically at 1 atm using Parrinello-Rahman barostat and a time constant of 5 ps. All bonds which involved hydrogens were constrained using the LINCS. Equations of motion were integrated using leap-frog algorithm with a time step of 2 fs. Long-range electrostatic interactions were described using the particle mesh Ewald method. The short-range electrostatic cut-off used was 1.2 nm and the short-range van der Waals cut-off was also 1.2 nm. Periodic boundaries conditions were applied in all directions.

[00117] Simulations were performed on an in-house Linux cluster composed of 8 nodes containing 2 GPUs (Nvidia GeForce RTX 2080 Ti) and 24 CPUs (Intel® Xeon® Gold 5118 CPU @ 2.3 GHz). All simulations snapshots as well as averaged solvent density of each channel were performed using VMD.23 Averaged radius profile, number of water molecules or ions in the channel as well as permeation length were calculated using Channel Annotation Package (CHAP) tool.25 Root mean square deviation (RMSD) was calculated for alpha carbon atoms of 8 helical oligourea monomers alone.

Test membranes

[00118] Methods used for the test membrane study are, for example, as follows: A polysulfone (PSF) membrane substrate was prepared using a pilot-scale casting machine according to methods known in the art. To incorporate oligourea foldamers that act as artificial water channels (AWC) into the polyamide selective layer of membranes, a spray-assisted interfacial polymerization (IP) technique was used. In brief, a substrate of 20×13 cm^2 was soaked in a 2% (w/v) m-phenylenediamine (MPD) solution for 2 minutes and the excess MPD was removed

using an air knife. An automated spraying machine was used to evenly distribute channel-containing liposomes on the substrate surface before being immersed into TMC (0.1 w/v%) solution.

[00119] The membrane separation performance was evaluated using a high-pressure cross-flow reverse osmosis setup equipped with four units of membrane cells (Sterli-tech CF042D, effective area of 42 cm^2). To achieve stable water flux, each membrane coupon was compacted using ultrapure water at 50 bar for 1 hour. NaCl was then added to the feed tank to achieve 35,000 ppm concentration to measure rejection performance at cross-flow velocity of 10 cm/s and temperature of 23°C. For each type of membrane, four independent coupons were tested to obtain the deviation in membrane performance. The membrane pure water permeability coefficient (A) and solute rejection (R) were determined using Eqns (1-2):

$$A = \frac{J_w}{\Delta P} \qquad (Eqn\ 1)$$

$$R - 1 - \frac{C_p}{C_f} \qquad (Eqn\ 2)$$

where Jw is the pure water flux measured using ultrapure water feed; ΔP is the transmembrane pressure; Cf and Cp are the solute concentrations in the feed and permeate, respectively.

SEQUENCES

SEQ ID NO:	**Sequence** (shown C to N terminal)	**Description**
1	Ac-$L^UE^UL^UK^UP^UL^UE^UL^UK^UA^U$-$NH_2$	H2OC1
2	Ac-$L^UE^UL^UK^UA^UL^UE^UL^UK^UA^U$-$NH_2$	H2OC2
3	Ac-$L^UN^UL^UK^UP^UL^UN^UL^UK^UA^U$-$NH_2$	H2OC3
4	Ac-$L^UE^UL^UK^UF^UL^UE^UL^UK^UF^U$-$NH_2$	H2OC4

CLAIMS

1. An oligourea foldamer comprising one or more sequences selected from the group consisting of SEQ ID NO: 1 (Ac-$L^UE^UL^UK^UP^UL^UE^UL^UK^UA^U$-$NH_2$) and SEQ ID NO: 2 (Ac-$L^UE^UL^UK^UA^UL^UE^UL^UK^UA^U$-$NH_2$).

2. The oligourea foldamer of claim 1, wherein the sequence is SEQ ID NO: 1.

3. The oligourea foldamer of claim 1, wherein the sequence is SEQ ID NO: 2.

4. The oligourea foldamer of any one of the preceding claims, wherein the oligourea foldamer is water permeable.

5. The oligourea foldamer of any one of the preceding claims, wherein the oligourea foldamer rejects 99% of ions.

6. A compound comprising oligourea foldamers of any one of claims 1 to 5.

7. The compound according to claim 6, wherein the oligourea foldamers self-assemble into a three-dimensional nanostructure under aqueous conditions.

8. The compound according to claim 7, wherein the three-dimensional nanostructure is a helix or a pore.

9. The compound according to claim 8, wherein the three-dimensional nanostructure has a pore size with an internal diameter of between 4.5 Å to 6.5 Å.

10. A permeable membrane comprising the oligourea foldamer of any one of claims 1 to 5 or the compound of claims 6 to 9.

11. A method of purifying water, the method comprising allowing water to pass through a filtration device comprising the permeable membrane according to claim 10.

12. The method according to claim 11, wherein the permeable membrane is water permeable and rejects 99% of salt present.