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Hyperfast Water Transport through Biomimetic Nanochannels from Peptide-attached (pR)-pillar[5]arene

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Abstract

Synthetic water channels offer great promise to replace natural aquaporins (AQPs) for making new-generation biomimetic membranes for water treatment. However, the water permeability of the current synthetic water channels was still far below that of AQPs. Here, peptide-attached (pR)-pillar[5]arene (pR-PH) channels are reported to mimic the high permeability of AQPs. It is demonstrated that the pR-PH channels with an open pore can transport water smoothly and efficiently. The pR-PH channels are competitive with AQPs in terms of water permeability and are much superior to diastereomer peptide-attached (pS)-pillar[5]arene (pS-PH) and other reported synthetic water channels. The exceptional water-transport properties of the pR-PH channels are further demonstrated in a composite polymeric membrane that incorporated the nano-channels into the top selective layer. This membrane gives a significantly improved water flux while retaining high salt rejection. Our results establish a

tangible foundation for developing highly efficient artificial water channel-based biomimetic membrane for water purification applications.

Main Text

Growing water scarcity worldwide has driven the development of synthetic membranes as a mainstream process for water production. However, membrane technology is challenged by the need to reduce its energy consumption. A membrane involves controlled nanostructures to impart permselectivity when subject to a driving force, which provides exciting opportunities for novel materials and manufacturing.^[1] In recent years aquaporins (AQPs), natural water channels, have attracted attention because of their astonishing water-transport properties.^[2] Incorporating AQPs into synthetic membranes for low-energy desalination and water purification has been initiated.^[3] However, practical utilization of AQP-based biomimetic membranes still faces many challenges that include the high production cost of AQPs, the potentially poor stability of biological proteins and difficulties in scaling-up the fabrication.

A possible way forward is to replace AQPs with biomimetic water channels that are designed directly based on synthetic chemistry.^[4] To date, a series of synthetic water channels have been developed to mimic the permeability and selectivity of AQPs.^[5] However, water permeability of these channels was found not to be competitive with that of AQPs (Table S1).^[6] Among various artificial water channels, pillar[5]arene derivatives have been identified as excellent templates for forming tubular structures via functionalization that may exhibit particular water-transport ability.^[5c,5g] Note that pillar[5]arene possesses an intriguing characteristic of planar chirality resulted from the rotation of the benzene units around the methylene bridges.^[7] After incorporating the bulky and rigid substituents into the pillar[5]arene scaffold, the rotation of the units can be inhibited and two stable isomers (pS and pR) should form (**Figure 1A**), which are allowed to be isolated.^[8] Chirality has been

proven to have the possibility to influence the channel properties.^[5h] In particular, the chiral substituents can induce the enantiomers pS- and pR-pillar[5]arenes into two diastereomers which may have different chemical and physic properties. Nevertheless, the impact of these two isomers from pillar[5]arene on the channel performance has not yet been explored in the open literature.

In the present work we synthesized peptide-attached pillar[5]arene and isolated for the first time two diastereomers, peptide-attached (pR)- and (pS)-pillar[5]arenes, designated as pR-PM and pS-PM, respectively (Figure 1B). These two compounds were analyzed by NMR and mass spectroscopy [Figure 1C and see the supporting information (SI)]. Circular dichroism (CD) spectroscopy and UV-vis spectroscopy were also used to characterize the structures of the pR-PM and the pS-PM. In the CD spectra (Figure 1D) a positive Cotton effect for the pR-PM and a negative Cotton effect for the pS-PM were observed; this agrees with previously reported results for the pillar[5]arene derivatives.^[8a,8b] The observed Cotton effect can be attributed to the planar chirality of the pillar[5]arene. Both the pR-PM and the pS-PM showed a special UV-vis absorption band at 293 nm corresponding to π - π^* transition of the aryl fragments of the pillar[5]arenes (Figure 1E).

Figure 1. Structures and characterization of diastereomers from peptide-attached pillar[5]arene. A) The pS and the pR-isomers of planar chiral pillar[5]arene. B) Chemical structures of the pS-PM and the pR-PM, and their corresponding hydrolysates (the pS-PH and the pR-PH). Each peptide chain was composed of three L-phenylalanine. C) Partial ^1H NMR spectra of the pS-PM, the pR-PM and their mixture (molar ratio of the pS-PM/pR-PM was 2/1) in DMSO- d_6 at 298 K. D, E) The CD and UV-vis spectra of the pS-PM, pR-PM, pS-PH and pR-PH in THF at 298 K (0.02 mM).

The methyl groups in the pS-PM and the pR-PM were easily removed via hydrolysis that resulted in the corresponding compounds of the pS-PH and the pR-PH with free carboxylic acids, which are expected to facilitate water transport (Figure 1B). The UV-vis and CD spectra (Figure 1, D and E) of the pS-PH and the pR-PH confirmed that the conditions employed for hydrolysis did not result in any change in the configurations. The ^1H NMR spectra (Figure S1) showed that the pS-PH and the pR-PH were stable and the interconversion between the pS and the pR did not occur even at high temperature. In addition, the ^1H NMR spectrum of the pS-PH (see SI) was consistent with that of the peptide-appended pillar[5]arene (compound 1b) reported previously by Hou's group.^[9] Note that the proton signals of the pR-PH were not detected in the ^1H NMR spectrum of compound 1b, implying that the pR-PH was not detected.^[10]

Stopped-flow light scattering, a common technique, has been widely applied to study the water-transport properties of AQPs, carbon nanotubes and synthetic water channels.^[5i,11] To investigate the water transport behavior of the pS-PH and the pR-PH, each compound was incorporated into phosphatidylcholine/phosphatidylserine (PC/PS) liposomes. The water permeability of these liposomes then was measured using the stopped-flow light-scattering technique by exposing the liposomes to a hypertonic solution (**Figure 2A** and **Figure S2**). The outwardly hypertonic conditions led to an increase in the light-scattering due to shrinking of the liposomes (**Figure S3**). It was difficult to determine the contribution of the pS-PH to the water transport via a comparison of the light-scattering curves for the liposomes with and without the pS-PH channels (**Figure 2B** and **Figure S3A**), which suggests that there is no water transport activity in the pS-PH channels. In contrast, the incorporation of pR-PH channels into the liposomes increased their shrinkage rate dramatically in comparison to the control liposomes (**Figure 2C** and **Figure S3B**). Moreover, the net water permeability, which was determined by excluding the lipid background permeability, increased linearly with the loading of the pR-PH channels (**Figure 2D** and **Figure S3C**). When the channel-to-lipid molar ratio was 0.005, the net water permeability at 10 °C was as high as $108.3 \pm 19.3 \mu\text{m/s}$, which is 300 times greater than peptide-appended pillar[5]arene channels ($0.35 \mu\text{m/s}$) under identical conditions.^[5g] Interestingly, the water permeability of the pR-PH channels was comparable to that of aquaporin Z (AqpZ) (**Figure S5**), which is consistent with previously reported results.^[12] The dramatically enhanced water permeability of the liposomes containing the pR-PH channels indicates the excellent water-transport activity of the pR-PH channels. The activation energies extracted from Arrhenius plots (**Figure 2E**) showed that the addition of the pR-PH channels into the liposomes reduced the activation energy ($11.64 \pm 0.68 \text{ kcal/mol}$ for the control) to $7.77 \pm 1.06 \text{ kcal/mol}$, implying that water transport across the pR-PH incorporated liposomes is channel-mediated.

Figure 2. Water-transport measurements of the channels in liposomes. A) Schematic illustration of a liposome with channels and water transport through the channels. B) Stopped-flow light-scattering curves of liposomes containing the pS-PH channels with different channel-to-lipid molar ratios (CLRs) at 25 °C. The liposomes were exposed to a hypertonic solution of 400 mM sucrose. C) Stopped-flow light-scattering curves of liposomes containing the pR-PH channels with different CLRs at 25 °C. The liposomes were exposed to a hypertonic solution of 400 mM sucrose. D) The net water permeability of the pR-PH channels in liposomes with different CLRs measured under hypertonic conditions at 25 °C. The net water permeability was obtained by excluding the lipid background permeability. Data are mean $\pm$ s.d. ($n = 4$ independent experiments). E) Arrhenius plots for calculating the activation energy. The rate constants (k) of the liposome containing the pR-PH (CLR = 0.01) and the control liposome were measured by exposing them to a hypertonic solution of 400 mM sucrose at different temperatures. The activation energies were derived from the slope of the fitted lines. F) The single-channel water permeability of the pR-PH channels (calculation method was described in SI). Data are mean $\pm$ s.d. ($n = 3$ independent experiments).

To make a better comparison with other water channels, the single-channel water permeability was calculated by employing the UV-vis and CD techniques to determine the insertion number of the pR-PH channels into the liposomes (Figure S4 and see SI for details). The single-channel water permeability reached $3.9 \times 10^{-14} \text{ cm}^3/\text{s}$, corresponding to 1.3×10^9 water molecules/s (Figure 2F), which is similar to that of the AQPs and is well above that of other artificial water channels reported in literature (Table S1). In addition, the insertion ability of pS-PH into liposomes by utilizing the CD technique was also evaluated. At CLR = 0.005, the

pR-PH and the pS-PH channels presented similar insertion efficiency (52.7 ± 6.76 % for pS-PH channels, 49.20 ± 4.53 % for pR-PH channels) under identical conditions, implying that the huge difference in water permeability of the pR-PH and the pS-PH channels was not caused by their insertion ability.

We also measured the water permeability during the swelling experiments by exposing the liposomes into hypotonic solutions,^[5e,5g] which led to a decrease in the light-scattering due to the swelling of the liposomes (Figure S6A). Unlike the peptide-appended pillar[5]arene channels,^[5g] incorporating the pR-PH channels showed water-permeability values in both the swelling and shrinking experiments of the same order-of-magnitude (Figure S6B), which implies stabilization of the water-transport activity of the pR-PH channels in the lipid bilayer membrane environment. In contrast, the pS-PH channels showed no observable increase in the water permeability, which was consistent with the shrinking experiments for these channels.

To understand the structure/activity relationships of the channels for the pS-PH and the pR-PH, molecular dynamics (MD) simulations of two systems, in which the pS-PH and the pR-PH channels were embedded individually in the lipid bilayer, were carried out (**Figure 3**, A and B, and Figure S7, see SI for details). The large values of root-mean-square-deviation (RMSD) for the pR-PH revealed a significant structural deviation from the initial structures that resulted from the considerable flexibility of the peptide chain in the lipid bilayer membrane (Figure S8A and Figure S9). When compared to the pR-PH, the larger RMSD values deduced from the MD simulations indicated that the pS-PH experienced even more structural changes (Figure S8B and Figure S11). To investigate changes in their water-transport behavior with time, the number of water molecules permeating through the pR-PH and the pS-PH channels was calculated (Figure 3, C and D). The last 200 ns production

trajectories were used to calculate the water permeability by using the collective diffusion model, since the RMSD of the backbone heavy atoms showed that the structures of the pR-PH and the pS-PH converged to an equilibrium state after 100 ns (Figure S8). Five parallel simulations of the pR-PH yielded similar water-permeability values. The average permeability was $1.9 \pm 0.6 \times 10^{-14} \text{ cm}^3/\text{s}$, which agrees well with the experimental results. In contrast to the pR-PH, the permeability of the pS-PH exhibited considerable variability with values ranging from $4.9 \times 10^{-16} \text{ cm}^3/\text{s}$ to $2.4 \times 10^{-14} \text{ cm}^3/\text{s}$. The structural basis underlying the poor permeability of the pS-PH then was investigated. As shown in Figure 3E and Figure S12, the phenylalanine side chain blocked the channel and thus impeded the permeation of water molecules. The simulations of the pS-PH system were initiated with a perfect unblocked water channel; however, the side chain of the phenylalanine adjacent to the pillar[5]arene ring displayed an evident propensity subsequently to rotate into the pore (Movie S1). In contrast to the pS-PH, the pR-PH maintained an open channel through which water molecules permeated smoothly in a single-file (Movie S2 and Figure S13).

Figure 3. Molecular dynamics (MD) simulations of the pR-PH and the pS-PH channels in a lipid membrane. A) The initial structures of the pR-PH and the pS-PH. The structures are colored by atom type, blue is nitrogen, red is oxygen, cyan is carbon. B) Initial setup of MD

simulations in a 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer. Water molecules are shown as a cyan surface. Phosphorus atoms of POPC are shown as orange spheres and hydrophobic tails are shown as tan lines. To highlight the structural features of the channels, the five identical chains are colored differently. C, D) Accumulated permeation events of water molecules through the pR-PH (C) and the pS-PH (D) as a function of the time for the production run. E) Permeability and structural properties of the pS-PH. Run1 was selected to illustrate how the side-chain of phenylalanine blocked the pS-PH pore. The side-chains are shown as a Van der Waals (VDW) representation.

To characterize the structural differences between the pS-PH and the pR-PH, a principal component analysis was carried out on the structures sampled from ten trajectories. As shown in Figure S14, the pS-PH and the pR-PH are distinct molecules and have almost no overlap in their free energy landscapes. The free energy minima of the pR-PH were closely positioned; moreover, the widths of these minima were rather small. In contrast, the pS-PH exhibited much broader free energy local minima that were dispersively distributed; moreover, the widths of the minima were much larger. The free energy contours of the pS-PH and the pR-PH suggested that the structures of the pS-PH were much more diverse than those of the pR-PH. The well-organized structure of the pR-PH ensured that its pore remained intact and unobstructed while the disordered structure of the pS-PH indicated an unstable pore that can be blocked by the phenylalanine of the flexible peptides.

The transport of pR-PH channels for metal ions was investigated by creating a pH gradient across the membranes of the large unilamellar vesicles. The fluorescent probe HPTS (8-hydroxypyrene-1,3,6-trisulfonic acid) as a pH-sensitive probe was used to measure the transport of alkali metal ions.^[5c,13] As shown in Figure S15, after introduction of pR-PH channels, an obvious increase in relative fluorescence intensity was observed. The result illustrated that the pR-PH channels were capable of transporting these alkaline metal ions, similar to other pillar[5]arene-based channels.^[5g,14] This is believed to be related to the large pore diameter of the pR-PH channels (Figure S10), which makes the pR-PH channels unable

to completely exclude these ions.^[6] The transport activity decreased in the order of $\text{Cs}^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+ \approx \text{Li}^+$.

Furthermore, to evaluate the relative salt rejection of the pR-PH channels, we also measured the reflection coefficient based on the stopped-flow light-scattering experiments by employing NaCl and sucrose as osmolytes (Figure S16).^[5g,15] Sucrose was selected as the reference solute due to its relatively large molecular size. The reflection coefficient for NaCl was approximately 1 (Figure S16), thereby probably indicating a relatively high rejection for salt.^[5h,15-16] The high reflection coefficient, in combination with high single-channel water permeability, suggests that the pR-PH channels can allow water molecules to pass much faster than salt, exhibiting a high water selectivity. These promising results suggest that the pR-PH channels have the potential to be incorporated into polymeric membranes for water-purification applications.

A supported lipid membrane with pR-PH channels was fabricated via liposome fusion on a porous polyethersulfone (PES) substrate (**Figure 4B**), which is shown schematically in Figure 4A (see SI for details). The porous substrate was covered by the liposomes incorporated with synthetic channels, which would start to rupture and fuse upon reaching a critical vesicular coverage on the underlying support.^[17] After a hydraulic pressure was applied to promote the complete fusion of liposomes, a lipid-layer membrane would form on the support where the synthetic channels incorporated were aligned vertically alongside the lipids. The substrate had a relatively smooth and moderately hydrophilic surface, which is desirable for the spread of the liposomes and minimizing the disruption of the lipid layer.

The scanning electron microscopy (SEM) images showed that the porous substrate was covered by the liposomes containing the pR-PH channels to form a lipid film (Figure 4, C and

D). A pS-PH-based membrane was also prepared as a control using the same method (Figure 4E). Figure 4F shows that the supported lipid membrane with the pR-PH channels had a significantly higher water flux of $\sim 30 \text{ L/m}^2/\text{h}$ (LMH) in comparison to $\sim 17 \text{ LMH}$ for the control **with pS-PH channels**. Both membranes exhibited a **similarly** high Na_2SO_4 rejection ($\sim 88\%$). These results clearly suggested that the increased water flux came from the highly permeable pR-PH channels rather than any defects in the lipid film. The pR-PH channels incorporated into the liposomes remained active even when they were immobilized on the polymer surface. In comparison, AQPs possessed a poor stability and even lost their activity completely once they were immobilized on a support.^[12a,18] In addition, the effect of the pR-PH loading on the membrane performance was evaluated (Figure 4G). The water flux and Na_2SO_4 rejection of the supported lipid membranes continued to increase when more pR-PH channels were incorporated into the liposomes, which explained well the increased salt rejection that accompanied the increased water flux. The permselectivity trade-off of a selective membrane has been well-documented to describe the difficulty of simultaneously enhancing the permeability and the selectivity of a membrane.^[19] However, our results show that synthetic water channels offer great promise to circumvent this trade-off limitation.

Figure 4. Fabrication and characterization of PES-supported lipid membranes with synthetic water channels. A) Schematic illustration of the fabrication process of supported lipid membranes. B) Digital photo of PES-supported lipid membrane containing channels with an effective membrane area of 17.3 cm². C) SEM image of a pristine PES membrane substrate with a small pore size (top-view). D) SEM image of resultant lipid membrane after liposome fusion on the substrate under hydraulic pressure (top-view). E) Schematic illustration of water transport through the pR-PH and the pS-PH channels in lipid membranes. F) Water flux and salt rejection of the membranes with the pR-PH and pS-PH channels, respectively. The CLR was 0.01. G) Water flux and salt rejection of membranes with the pR-PH at different CLR values. The pristine PES membrane substrate had a water permeability of 120 ± 15 LMH/bar with no salt rejection. The molecular weight cut-off (MWCO) of the PES substrate is 28 kDa. All membranes were tested with 100 ppm Na₂SO₄ under 1 bar. Data are mean ± s.d. (*n* = 3 independent experiments).

In conclusion, we developed for the first time peptide-attached (pR)-pillar[5]arene (pR-PH) as new-generation synthetic water channels. In contrast to the diastereomer peptide-attached (pS)-pillar[5]arene (pS-PH) with an unstable pore, the pR-PH holds an open and suitable pore that can allow water molecules to pass fast in a single-file. To the best of our knowledge, the water permeability (up to a billion water molecules/s) of pR-PH channels is extremely high, comparable to that of natural water channels. In addition, the supported lipid membranes

incorporated with the pR-PH channels exhibit dramatically improved water flux and Na₂SO₄ rejection, thereby making the newly-developed artificial water channels a promising material for fabricating new-generation highly permeable membranes for water purification.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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New-generation synthetic water channels are created by introducing peptide-attached pillar[5]arene with two diastereomers, peptide-attached (pR)- and (pS)-pillar[5]arenes, designated as pR-PH and pS-PH, respectively. The pR-PH channels with open pore can allow water molecules to pass fast while pS-PH channels are easily blocked by side chain and lose the permeation ability of water molecules.

Keyword: synthetic water nanochannels, pillar[5]arene, diastereomer, water permeability, pore

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Hyperfast Water Transport through Biomimetic Nanochannels from Peptide-attached (pR)-pillar[5]arene

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Supporting Information

Hyperfast Water Transport through Biomimetic Nanochannels from Peptide-attached (pR)-pillar[5]arene

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Reference

1. Materials and Methods

1.1 Materials

Dichloromethane (CH₂Cl₂) was distilled over CaH₂ under a N₂ atmosphere. Trifluoroacetic acid (TFA), 1,4-dimethoxybenzene, paraformaldehyde, boron tribromide (BBr₃), ethyl bromoacetate, 4-Dimethylaminopyridine (DMAP), anhydrous DMF and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) were purchased from Sigma Aldrich. L- α -Phosphatidylcholine (chicken egg, PC) and L- α -phosphatidylserine (porcine brain, sodium salt, PS) were purchased from Avanti Polar Lipids. All amino acids, *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium tetrafluoroborate *N*-oxide (*N*-TBTU) and *N*-Ethyl-*N*'-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) were purchased from GL biochem (Shanghai) Ltd. Water purified by a Milli-Q system (18 M Ω cm) was used. Polyethersulfone (PES, E6020P) was purchased from BASF for the support membrane fabrication. Other materials were used as provided by supplier. The peptides were synthesized according to the previous synthesis method using *N*-TBTU as the condensation reagent.^[1]

1.2 Instruments

The ¹H and ¹³C NMR spectra were recorded on a 500 MHz Bruker DRX NMR, BrukerAvance III 400 (400MHz) (100 MHz) or Bruker AV-300 (300 MHz) spectrometer. Low resolution mass spectra (LR-MS) were obtained on a ThermoFinnigan LCQ Fleet MS. The matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) spectra were obtained on a JMS-S3000 SpiralTOF (JEOL Ltd., Japan) at an accelerating potential of 20 kV in the positive spiral mode. The LR-MS and MALDI-TOF-MS were reported in units of mass per

charge ratio (m/z). Ultraviolet–visible (UV–vis) spectra were measured on a Cary Varian 5000 UV-vis spectrometer using 1-cm quartz cuvettes. Circular dichroism (CD) spectroscopy was conducted with a Jasco J-1500 CD spectrometer in a 1-cm path length cuvette at room temperature.

1.3 Synthetic procedures of planar-chiral (pS)- and (pR)-pillar[5]arene containing peptides (pS-PH and pR-PH)

1.3.1 Synthesis of 1,4-dimethoxypillar[5]arene (DMP5)

The DMP5 was prepared by employing trifluoroacetic acid as a catalyst. Trifluoroacetic acid (15 mL) was added to a solution of 1,4-dimethoxybenzene (4.15 g, 30 mmol) and freshly ground paraformaldehyde (0.9 g, 30 mmol) in 1,2-dichloroethane (285 mL), and the mixture was refluxed at 90 °C for 3 h. After cooling to room temperature, the solution was poured into

methanol (400 mL) and the resulting precipitate was collected by filtration and then dissolved in CHCl_3 (70 mL). Acetone (70 mL) was added to give a precipitate and the solid was washed with acetone to obtain the DMP5 as a white solid (3.25 g). Yield: 69%. ^1H NMR (500 MHz, CDCl_3): δ = 6.90 (s, 10H), 3.77 (s, 10H), 3.75 (s, 30H). LR-MS (EI): Calcd. for $\text{C}_{45}\text{H}_{51}\text{O}_{10}$ $[\text{M}+\text{H}]^+$: 751.35. Found: 751.31.

1.3.2 Synthesis of pillar[5]arene (P5)

Boron tribromide (13.2 mL, 140 mmol) was added slowly to the solution of the DMP5 (3.00 g, 4 mmol) in dry CH_2Cl_2 (100 mL). The resulting mixture was stirred at room temperature for 72 h. Then ice water (100 mL) was added slowly and the mixture was stirred for another 36 h at room temperature. The precipitate was filtered and washed with water to obtain an oyster-white solid (2.4 g). Yield: 98%. ^1H NMR (300 MHz, CD_3COCD_3): δ = 7.98 (s, 10H), 6.68 (s, 10H), 3.60 (s, 10H). LR-MS (EI): Calcd. for $\text{C}_{35}\text{H}_{34}\text{NO}_{10}$ $[\text{M}+\text{NH}_4]^+$: 628.22. Found: 628.26.

1.3.3 Synthesis of EP5

K_2CO_3 (7.0 g) was added to a solution of the P5 (1.4 g, 2.3 mmol) in acetonitrile (60 mL). The mixture was stirred for 1 h at room temperature, then KI (80 mg) and ethyl bromoacetate (5 mL, 45 mmol) were added. The mixture was heated to reflux under a nitrogen atmosphere for 24 h. After cooling to room temperature, the solution was filtered and washed with CHCl_3 . The filtrate was concentrated under vacuum, and the residue was dissolved in CHCl_3 (15 mL). A white solid was formed by slow diffusion of methanol into the CHCl_3 solution. The solid was collected by filtration, washed with methanol, and dried under vacuum to give a white product (2.74 g).

Yield:81%. ^1H NMR (300 MHz, CDCl_3): δ = 7.04 (s, 10H), 4.54 (q, J =15 Hz, 20H), 4.09 (m, J =6 Hz, 20H), 3.85 (s, 10H), 0.96 (m, J =6 Hz, 30H).

1.3.4 Synthesis of AP5

A 20% aqueous sodium hydroxide solution (60 mL) was added to the solution of the EP5 (2.7 g, 1.8 mmol) in THF (100 mL). The solution was heated to reflux for 15 h. After cooling, the solution was concentrated, diluted with water (100 mL), and acidified with HCl. The white precipitate was collected by filtration, washed with water, and dried under vacuum to give a white solid (1.9 g). Yield:87%. ^1H NMR (300 MHz, CD_3SOCD_3): δ = 7.03 (s, 10H), 4.64 (d, J =18 Hz, 10H), 4.41 (d, J =15 Hz, 10H), 3.72 (s, 10H). LR-MS (EI): Calcd. for $\text{C}_{55}\text{H}_{49}\text{O}_{30}$ $[\text{M}-\text{H}]^-$: 1189.23. Found: 1189.33.

1.3.5 Synthesis of pS-PM and pR-PM

$\text{NH}_2\text{-L-Phe-L-Phe-L-Phe-OMe}$ (3.26 g, 6.8 mmol), DMAP (3.5 g, 28 mmol) and EDC (1.34 g, 7 mmol) were added to the solution of the AP5 (0.44 g, 0.37 mmol) in anhydrous DMF (45 mL). The mixture was stirred at 55 °C for 48 h under a nitrogen atmosphere. After cooling, the solution was poured into an aqueous HCl solution (2%, 200 mL). The resulting precipitate was collected by filtration and washed with water. Then the precipitate was dissolved in acetone. After filtration and washing thoroughly with acetone, the insoluble solid was collected and subjected to column chromatography on silica gel ($\text{CH}_2\text{Cl}_2\text{:CH}_3\text{OH}$ =10:1) to give pR-PM (0.10 g, yield:4.7%), the filtrate was evaporated and subjected to column chromatography on silica gel ($\text{CH}_2\text{Cl}_2\text{:CH}_3\text{OH}$ =20:1) to give pS-PM (0.35 g, yield: 16.5%).

pS-PM. ^1H NMR (300 MHz, CD_3SOCD_3): δ = 8.47-8.44 (br, 20H), 7.56 (br, 10H), 7.15-6.96 (m, 150H), 6.73 (s, 10H), 4.74-4.66 (m, 20H). 4.54-4.44 (m, 10H), 4.35 (d, J =15 Hz, 10H), 4.20 (d, J =15 Hz, 10H), 3.50 (s, 40H), 2.98-2.77 (m, 60H). ^{13}C NMR (100 MHz, CD_3SOCD_3): δ =172.0, 171.4, 171.2, 167.9, 149.0, 137.7, 137.6, 137.3, 129.6, 129.4, 128.7, 128.4, 127.0, 126.7, 115.0, 54.1, 54.0, 53.6, 52.2, 38.6, 38.2, 37.2. MALD-TOF-MS: Calcd. for $\text{C}_{335}\text{H}_{340}\text{N}_{30}\text{O}_{60}\text{Na}$ $[\text{M}+\text{Na}]^+$: 5768.4463. Found: 5768.5095.

^1H NMR spectrum of pS-PM

^{13}C NMR spectrum of pS-PM

MALDI-TOF-MS spectrum of pS-PM

pR-PM. ^1H NMR (300 MHz, CD_3SOCD_3): δ = 8.50-8.40 (br, 20H), 7.89 (br, 10H), 7.22-6.95 (m, 150H), 6.60 (s, 10H), 4.67 (br, 20H), 4.49 (br, 10H), 4.20-4.09 (br, 20H), 3.52 (s, 40H), 2.95-2.78 (m, 60H). ^{13}C NMR (100 MHz, CD_3SOCD_3): δ =172.0, 171.4, 171.2, 167.9, 149.0, 137.7, 137.6, 137.3, 129.6, 129.4, 128.7, 128.4, 127.0, 126.7, 126.7, 115.0, 67.7, 54.2, 54.1, 53.6, 52.2, 38.5,

38.2, 37.2. MALD-TOF-MS: Calcd. for $C_{335}H_{340}N_{30}O_{60}Na$ $[M+Na]^+$: 5768.4463. Found: 5768.5073.

1H NMR spectrum of pR-PM

^{13}C NMR spectrum of pR-PM

MALDI-TOF-MS spectrum of pR-PM

1.3.6 Synthesis of pS-PH and pR-PH

Lithium hydroxide monohydrate (30 mg, 0.70 mmol) and water (2.5 mL) were added to a solution of the pS-PM or the pR-PM (80 mg, 0.014 mmol) in THF (7.5 mL). The mixture was stirred at room temperature for 20 h. Then the solution was concentrated under reduced pressure and poured into water (30 mL). After acidifying with an aqueous HCl solution (2%), the resulting precipitate was filtered, washed with water, and dried under a vacuum to give a white product.

pS-PH. Yield: 93%. ^1H NMR (300 MHz, CD_3SOCD_3): δ = 8.43 (br, 10H), 8.31 (br, 10H), 7.54 (br, 10H), 7.19-6.95 (m, 150H), 6.71 (s, 10H), 4.71 (br, 20H), 4.48 (br, 10H), 4.35 (br, 10H), 4.16 (br, 10H), 3.51 (s, 10H), 3.00-2.80 (m, 60H). ^{13}C NMR (100 MHz, CD_3SOCD_3): δ = 173.1, 171.3, 170.9, 168.1, 148.9, 137.6, 136.8, 129.6, 129.4, 128.6, 128.4, 126.8, 114.9, 67.6, 54.2, 53.1, 38.2, 38.1, 37.3. MALD-TOF-MS: Calcd. for $\text{C}_{325}\text{H}_{320}\text{N}_{30}\text{O}_{60}\text{Na}$ $[\text{M}+\text{Na}]^+$: 5628.2898. Found: 5628.2896.

¹H NMR spectrum of pS-PH

¹³C NMR spectrum of pS-PH

MALDI-TOF-MS spectrum of pS-PH

pR-PH. Yield: 92%. ^1H NMR (300 MHz, CD_3SOCD_3): δ = 8.35 (br, 20H), 7.88 (br, 10H), 7.19-6.94 (m, 150H), 6.59 (s, 10H), 4.64 (m, 20H). 4.45 (br, 10H), 4.20-4.10 (br, 20H), 3.01-2.76 (m, 60H). ^{13}C NMR (100 MHz, CD_3SOCD_3): δ = 173.1, 171.3, 171.2, 167.9, 148.9, 137.7, 137.6, 129.6, 129.5, 128.6, 128.4, 126.9, 126.7, 115.0, 67.6, 54.1, 53.6, 38.5, 38.2, 37.3. MALD-TOF-MS: Calcd. for $\text{C}_{325}\text{H}_{320}\text{N}_{30}\text{O}_{60}\text{Na}$ $[\text{M}+\text{Na}]^+$: 5628.2898. Found: 5628.2880.

^1H NMR spectrum of pR-PH

^{13}C NMR spectrum of pR-PH

MALDI-TOF-MS spectrum of pR-PH

1.4 Liposome preparation and stopped-flow measurements

The liposomes were prepared by the film rehydration method similar to published procedures.^[2]

The pR-PH or pS-PH channels in a chloroform/methanol mixture (v/v=1/1) were added to a 2 μmol PC/PS mixture with a molar ratio of 4/1. The solvent was slowly distilled on a rotary

evaporator and subsequently dried under high vacuum to remove the residual solvent. The dried film was rehydrated with 1 mL buffer containing 10 mM Hepes (pH=7), 100 mM NaCl and 0.01% NaN₃. The suspension was further incubated with stirring at 4 °C overnight and then extruded through a 0.2 µm track-etched membrane 21 times (Whatman, UK). The size of the liposomes was measured to be 160 ± 15 nm using a Nano Zetasizer (NanoZS, Malvern Instruments Limited, UK).

The stopped-flow measurements were performed with a stopped flow apparatus (SX-20, Applied Photophysics) at a given temperature. The liposome solution was mixed rapidly with hypertonic osmolyte (400 mM sucrose) in the same buffer to induce the liposomes to shrink due to the osmotic gradient. The changes in the light scattering were recorded at a wavelength of 500 nm. The stopped-flow data were fit to a single exponential function to obtain the rate constant (k). The water permeability of the liposomes (P_f) was calculated using the following equation:^[3]

$$P_f = \frac{k}{S_o / V_o \times V_w \times \Delta_{osm}} \quad (1)$$

where k is the rate constant, S_o / V_o is the ratio of the initial surface area to the volume of the liposomes, V_w is the partial molar volume of water (18 cm³ mol⁻¹), Δ_{osm} is the osmolarity difference. The osmolarity was measured with a freezing-point osmometer (Model 3250, Advanced Instruments. Inc.).

1.5 Channel insertion efficiency and the calculation of the single-channel water permeability

The liposomes with and without the pR-PH channels were first prepared in the phosphate buffer (10 mM sodium phosphate, pH=6.4, 100 mM NaCl) using the film-rehydration method described

previously, containing 2 mM PC/PS lipids with a molar ratio of 4/1. The resulting liposomes were further extruded through a 0.2 μm track-etched membrane 21 times (Whatman, UK) to obtain unilamellar liposomes. The water permeability of the liposomes was studied by the stopped-flow measurement. The liposomes were exposed to a hypertonic osmolyte (400 mM sucrose). The net water permeability for the pR-PH in the liposome (CLR = 0.005) was calculated to be $255.9 \pm 26.4 \mu\text{m/s}$.

1.5.1 The insertion efficiency of the channels: 500 μL pR-PH channel solutions (from 0 μM to 30 μM), in phosphate buffer containing 10 mM sodium phosphate (pH=6.4), 100 mM NaCl and 8% n-octyl- β -D-glucoside (OG), were mixed with 500 μL control liposomes. The resulting solutions (final concentration of channels: from 0 μM to 15 μM) were scanned on a UV-Vis or CD spectrophotometer. We noted that the special UV-Vis absorbance of the channels at 293 nm and CD absorbance at 306 nm increased with the concentration linearly to generate a standard curve (Figure S4). For liposomes containing the channels used for water-transport studies, 500 μL liposomes were mixed with 500 μL of the same phosphate buffer containing 8% OG. The insertion efficiency of the channels was evaluated based on the special UV-vis absorbance of the channels at 292 nm or CD absorbance at 306 nm.

1.5.2 The insertion efficiency of the lipids: PC/PS lipid solutions (from 0 mM to 1.5 mM) with a molar ratio of 4/1 were prepared in phosphate buffer containing 10 mM sodium phosphate (pH=6.4), 100 mM NaCl and 4% n-octyl- β -D-glucoside (OG). The solutions were scanned on a UV-vis spectrophotometer. The UV-vis absorbance of the lipid solutions at 280 nm increased in proportion to the concentration thereby generating a calibration curve. When the extruded liposomes (initially 1 mM) were measured at a wavelength of 280 nm, the concentration

estimated based on the calibration curve was 0.96 mM. Hence, the insertion efficiency of the lipids was 96%.

1.5.3 Calculation of the single-channel water permeability: The single-channel permeability was calculated based on the insertion efficiency of the pR-PH channel and lipid. When the channel-to-lipid molar ratio (CLR) was 0.005, the radius (r) of the liposome was 80 nm. If the bilayer thickness is assumed to be 5 nm, the sum of the outer and inner surface areas was $4\pi \times r^2 + 4\pi \times (r-5)^2 = 151110 \text{ nm}^2$. The average cross-sectional area of a lipid in average was 0.7 nm^2 (the cross-sectional areas of the PC and the PS were $\sim 0.7 \text{ nm}^2$), and that of the pR-PH channel was estimated as 4.5 nm^2 (Figure S4E). The initial molar ratio of channels/lipids was 1/200. If 96% of the lipids and 48% of the pR-PH channels are assumed to remain in the purified vesicles, the real molar ratio of channels/lipids was 1/400. The insertion number of the channels was 523 per vesicle. If the overall net permeability by the channels in the liposomes was $255.9 \text{ } \mu\text{m/s}$, the single-channel permeability was $3.9 \times 10^{-14} \text{ cm}^3/\text{s}$, or 1.3×10^9 water molecules/s.

1.6 Ion transport experiments with HPTS fluorescence assay

The preparation of vesicles with HPTS (pH sensitive dye) was as follow: 20 mg Egg yolk L- α -phosphatidylcholine (EYPC) was dissolved in 2 mL CHCl_3 , the solvent was evaporated under a rotary evaporator and the formed thin film was dried under high vacuum for 4 h. The lipid was then hydrated with 1 mL buffer (10 mM sodium phosphate, pH=6.4, 100 mM NaCl) containing 0.1 mM HPTS (pyranine, 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt) for 40 minutes. After hydration, this suspension was submitted to 5 freeze-thaw cycles (liquid nitrogen, water at 40°C) and further extruded through a $0.1 \text{ } \mu\text{m}$ track-etched membrane 21 times (Whatman, UK) to obtain large unilamellar vesicles (LUVs). The vesicles were purified by Sephadex G-50 to

remove unencapsulated HPTS using the same buffer solution without HPTS as elute and diluted with the elute to yield 8 mM lipid stock solution. The stock solution was kept under 4°C and used within 24 hours.

The HPTS-containing vesicle suspension (50 μ L, 8 mM) was added to a buffer solution (1.95 mL, 10 mM sodium phosphate, pH = 7.4, 100 mM MCl, where M= Li⁺, Na⁺, K⁺, Rb⁺, Cs⁺) to form a pH gradient for ion-transport study. And then 20 μ L DMSO solution of pR-PH channels (10 μ M) was injected into the suspension under gentle stirring. Upon the addition of channels, the emission of HPTS at 510 nm was immediately monitored (excitations at both 460 and 403 nm) for 300 s. Maximal changes in dye fluorescence emission were achieved by complete lysis of the liposomes with 40 μ L Triton X-100 solution (detergent, 5%). The final transport trace was obtained as a ratio of I_{460}/I_{403} and normalized to 100% of transport.

1.7 Molecular dynamics (MD) simulations

In order to setup initial structures for the molecular dynamics simulations, the pS-PH and pR-PH were built manually based on the following four assumptions (as shown in Figure S7): (1) the structures contain a tubular framework composed by a pillar[5]arene and ten peptide chains; (2) the height of the structures should be similar to the thickness of the lipid; (3) the side chains of phenylalanine project outwards; (4) there are hydrogen bonds between adjacent backbones. The proposed structures are the ideal configurations of the channels. On the one hand, the tubular structures stabilized by hydrogen bonds are dimensionally adapted for water permeation; on the other hand, the matching height and the protruding hydrophobic side chains can accommodate the lipid tails well. The two systems then were inserted in a 60 Å $\times$ 60 Å 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) membrane patch. Water and lipid molecules within 3 Å of

the channel were deleted and there were no water molecules in the channel (Figure 3B). To characterize the movement of water molecules in the channel under equilibrium conditions, no osmotic or hydrostatic difference was established across the channel. The final systems contained no ions and were electro-neutral. The visual molecular dynamics (VMD) 1.9.3^[4] was used to build the two solvated systems.

The Nanoscale Molecular Dynamics (NAMD) 2.11^[5] was employed to carry out the molecular dynamics simulations. The CHARMM36 force field^[6] was used to depict water, POPC and peptide while the topology and parameters of the pillar[5]arene were obtained from the CHARMM General Force Field (CGenFF) website.^[7] The combination of CHARMM36 and CGenFF is a commonly used method to depict topologies and parameters of complexes.^[2a,8] All simulations were performed using periodic boundary conditions. The particle-mesh Ewald method^[9] with a cubic grid of 1 Å was used for the computation of the electrostatic forces. The Lennard Jones potentials were cut off at 12 Å. The TIP3P water model^[10] was used. The temperature was kept constant at 303 K using Langevin dynamics with a damping coefficient of 1 ps.

The membrane patch was not pre-equilibrated and the structures of the channels were constructed manually. Therefore, several steps were taken to remove the initial conflicts and equilibrate the two systems. After 10000 steps of minimization, the first 1 ns MD simulations were carried out in the canonical (NVT) ensemble with all water molecules, atoms of all hydrophilic heads of POPC and all atoms of the channels fixed, only the hydrophobic tails of POPC being allowed to move. The aim of the first MD simulations is melting of the lipid tails and filling of the artificial voids between the channels and membrane. In the first stage of the MD simulations, we used 1 fs time step without the SHAKE constraint.^[11] Then, 6 ns MD

simulations were carried out with the atoms of the channel backbone constrained with a spring constant of 2 kcal/mol Å². The O-C-N-H dihedrals were kept at 180° because the trans form is preferred overwhelmingly in most peptide bonds. The SHAKE algorithm was imposed on the bonds involving hydrogen atoms for which a 2 fs time step was used. The ensemble was shifted to the NPT and the Nosé-Hoover Langevin method^[12] was used to control the fluctuations in the barostat. The constraints on the channel were partially removed in the following MD simulations for which only the carbon atoms of the backbones were restrained. The MD simulations lasted until the thickness of the membrane and the area per lipid were stable and close to the experimental values. After minimization and equilibration with the constrained channel, the lipids were well-packed around the channel while the water molecules did not enter the membrane and the channel. We continued to release the harmonic constraints of the channel while retaining only the dihedral restraint. The dimension of the system in the x-y plane was kept constant while the fluctuation of the z-axis was allowed. A 300 ns production run was carried out after 4 ns further equilibration. In the production run all the restraints were released. For the pS-PH and pR-PH, 5 replicate simulations were run in parallel.

1.7.1 Calculation of the diffusion permeability (p_d) and the osmotic permeability (p_f)

When the solutions on the two sides of the membrane have different concentrations of osmotically active molecules, water can move from the lower concentration side to the higher concentration side when water channels are present. When there is no hydrostatic pressure imposed on the two sides and the solution is dilute, the net water flux is linearly proportional to the concentration difference of the osmotically active molecules:

$$J_w = p_f \Delta C_s \quad (2)$$

where J_w (mol/s) is the flux of water, ΔC_s (mol/cm³) is the difference in concentration and p_f (cm³/s) is the osmotic permeability of the channel.

In contrast, when no solute concentration exists, no net water flux is expected in equilibrium. To study the diffusion of water, radioactively labeled water molecules can be used as tracers to track the movement of water molecules through the channels. When the solutions on the two sides of a membrane have different concentrations of tagged water, the flux of the tracers is linearly proportional to the difference of the tracer concentrations:

$$J_t = p_d \Delta C_t \quad (3)$$

where J_t (mol/s) is the tracer flux, ΔC_t (mol/cm³) is the tracer concentration difference and p_d (cm³/s) is the diffusion permeability of the channel. Based on the definition of J_t , we can calculate its value based on the number of tagged water molecules that permeate through the channel in one direction. Let n_0 be the total number of water molecules experiencing complete transport from one side to the other. Ideally, the number of water molecules permeating in either direction should be identical. Due to the limited time scale of the MD simulations, the average number of water molecules permeating in both directions was considered as n_0 . We assumed that the concentration of the tracers on one side was C_t and that the other side had no tracer. Hence, the relationship between the total number of water molecules and the tagged molecules is given by the following equation:

$$n_t = (C_t / C_w) n_0 \quad (4)$$

where C_w is the concentration of water, which is a constant (0.055 mol/cm³). The relationship between J_t and n_t is given by the following equation:

$$J_t = (n_t / t) / N_A \quad (5)$$

where t (s) is the time during which the number of water molecules has been calculated, and N_A is Avogadro's number. Thus, when t is given, p_d can be calculated by n_0 as follows:

$$p_d = \frac{J_t}{\Delta C_t} = \frac{(C_t / C_w) n_0 / t / N_A}{C_t} = \frac{n_0}{t C_w N_A} \quad (6)$$

In our study, there was no pressure gradient or osmotic concentration gradient across the POPC membrane, which makes the direct calculation of p_f impossible. We calculated p_f according to the collective diffusion model provided by Zhu and co-workers.^[13] The collective coordinate n was introduced to describe the translocation of water molecules.

$$dn(t) = \sum_i^M \frac{z_i(t+dt) - z_i(t)}{L} \quad (7)$$

where L is the length of channel in the z -direction. M represents the water molecules in the channel at time t . When $n = 0$ at $t = 0$ and L is a constant, the value of $n(t)$ was uniquely determined by integrating $dn(t)$. To define a suitable L , we calculated the positions of the phosphorus atoms in an equilibrated POPC membrane in which the channel was embedded and the components were extracted along the z -axis. The two distributions of z components in the upper layer and lower layer were constructed, respectively, and the difference between the two values corresponding to the maximum peaks was used to estimate the thickness of the hydrophobic tail. Due to the disordered structures of the channels, the thickness of the hydrophobic tail (22 Å) was used to represent the length of the channels. On the other hand, the translocation of water molecules from one side of the POPC membrane to the other side is a complete permeation event. Based on this definition, when a water molecule crosses the channel in the $+z$ -direction, it contributes $n + 1$. When a water molecular permeates through the channel in the $-z$ -direction, it contributes $n - 1$. Note that n quantifies the net number of water molecules

permeating through the water channels and $n(t)$ is the number of permeation events at any instant of time. Based on the Einstein relation:

$$\langle n^2(t) \rangle = 2D_n t = 2 \frac{P_f}{v_w} t \quad (8)$$

where $\langle n^2(t) \rangle$ is the mean-square-displacement (MSD) of n , D_n is the one-dimensional diffusion coefficient of n , and v_w is the volume of a single water molecule ($3 \times 10^{-23} \text{ cm}^3$). The permeability values were calculated for each replicate MD simulation for which only the last 200 ns of the trajectory was considered.

1.8 Principle component analysis

Principle component analysis (PCA) on Cartesian coordinates was performed and the first two principal components were selected to construct the free energy landscape. PCA is an efficient method to reduce the dimensionality.^[14] The free energy was calculated based on the following formula:

$$\Delta G_{i,j} = -k_B T \left(\ln \frac{p_{i,j}}{p_{max}} \right) \quad (9)$$

here $p_{i,j}$ is the probability distribution of all the simulation data (coordinates) projected onto the first two principal vectors and p_{max} is the maximum of the distribution, which is subtracted to guarantee that the lowest free energy is zero.

1.9 Fabrication of Supported lipid membrane with channels

1.9.1 Fabrication of the PES supports: PES flakes were dried at 50 °C for 24 h prior to polymer solution preparation. A certain amount of PES and LiCl were dissolved in 1-methyl-2-pyrrolidone (NMP) that was stirred at 55°C for 2 days until the mixture became homogenous and transparent. The final concentrations of the PES and LiCl in the mixture were 19 wt% and 3 wt%, respectively. To fabricate a flat sheet support, a casting knife set at a blade height of 150 µm was used to cast and spread the homogenous solution as a uniform film on a glass plate, which was subsequently immersed into a water bath at 23 °C. The support membrane was immersed in tap water for 12 h to remove the residual solvent and then transferred to DI water for further use.

1.9.2 Preparation of the channel-incorporated liposome suspension: First the pR-PH or the pS-PH channels and the PC (3.9 mg) were dissolved in CHCl₃/MeOH (v/v=1/1). The solvent was removed under a rotary evaporator and subsequently dried under high vacuum, and the resulting film was rehydrated with 1 mL phosphate buffer (10 mM sodium phosphate, PH=7.4, 100 mM NaCl). After incubation at 4 °C overnight, the suspension was extruded through a 0.4 µm track-etched membrane 15 times and diluted with the same buffer to produce a liposome suspension containing the pR-PH or the pS-PH (the lipid concentration = 0.5 mg/mL). The fresh liposome solution was directly used to fabricate the channel-based membrane.

1.9.3 Preparation of the PES supported lipid membrane with water channels: The PES support membrane was cut and sealed in a dead-end membrane cell with an effective area of 17.3 cm². A 2 mL liposome solution (the lipid concentration = 0.5 mg/mL) was transferred into the membrane cell to cover the entire membrane surface and pressurized at 1 bar with compressed nitrogen gas. To ensure full coverage of the lipid film on the support, another 2 mL of the liposome solution was added to the cell and pressurized at 1 bar. After the solution was completely filtered through the membrane, DI water then was added to the cell and pressurized

to promote the complete fusion of the liposomes on the membrane surface until the permeate flux became steady.

1.9.4 Membrane characterization

Scanning electron microscopy (SEM) images of the membranes were acquired by a field-emission scanning-electron microscope (JSM-7600F, JEOL). All membranes were dehydrated by freeze-drying and coated with platinum prior to SEM characterization. The molecular weight cut-off (MWCO) of the PES support was determined by examining the rejection of dextrans with molecular weights ranging from 6000 to 500,000 Daltons by size-exclusion chromatography.

1.9.5 Performance evaluation of the supported lipid membranes

After pressurizing the liposomes on the membrane surface with DI water, a 100 ppm Na₂SO₄ solution was added to the dead-end cell to test the water flux and salt rejection of the as-prepared supported lipid membranes. As the supported lipid membranes have high water permeability, applying a high pressure will produce a very high water flux, which induces a significant concentration polarization during rejection tests. In addition, a very high water flow will disrupt the structure of bilayers constructed on a porous support because of the low mechanical strength of bilayer membrane. Therefore, the Na₂SO₄ solution was pressurized under 1 bar with vigorous stirring to minimize the concentration polarization. The temperature of the feed solution was kept constant at 23 ± 1 °C. The water flux was calculated based on the gravimetric measurement of the permeate collected over a certain time that was normalized with the effective membrane area. The salt rejection was determined based on the difference between the measured conductivity of feed and permeate solutions.

2. Supporting Figures and Tables

Figure S1. A) Partial ^1H NMR spectra of the pR-PH and the pS-PH in $\text{DMSO-}d_6$ (2.0 mM) before and after heating at 80 °C for 8 h. No change in ^1H NMR spectra before and after heating was found, indicating that rotation of the unites in pillar[5]arene was inhibited due to the introduction of bulky peptide chains. B) The ^1H NMR spectra of pS-PH and pR-PH in $\text{DMSO-}d_6$ (2.0 mM) at 25 °C and 80 °C. The proton signals of the pR-PH were not observed in the ^1H NMR spectrum of the pS-PH even at 80 °C, implying that the interconversion between pS-PH and pR-PH did not occur.

Figure S2. Schematic representation of the stopped-flow light-scattering test under hypertonic conditions. The liposome solution was abruptly mixed with a hypertonic solution containing 200 mM NaCl or 400 mM solutes in equal volumes. Under hypertonic conditions, a reduction in liposome volume due to water efflux leads to an increase in the light scattering signal.

Figure S3. Water-transport measurements of channels in liposomes at 10 °C. A, B)

Representative stopped-flow light-scattering curves of liposomes containing the pS-PH (A) and the pR-PH (B) channels with different channel-to-lipid molar ratios (CLRs). The liposomes were exposed to a hypertonic solution of 400 mM sucrose. C) The net water permeability of pR-PH in liposomes with different CLRs measured under hypertonic conditions. The net water permeability was obtained by excluding the lipid background permeability ($46.83 \pm 4.16 \mu\text{m/s}$ at 10 °C). Data are mean $\pm$ s.d. ($n = 4$ independent experiments).

Figure S4. The insertion efficiency of pR-PH channels into liposomes. A, C) The UV-vis spectra (A) and CD spectra (C) of the pR-PH channel solutions (from 0 μM to 15 μM), in phosphate buffer containing 10 mM sodium phosphate (pH=6.4), 100 mM NaCl and 4% n-octyl- β -D-glucoside (OG) and 1 mM extruded control liposomes. B, D) The calibration curves of the UV-vis (B) and CD (D) absorbance as a function of concentration for the pR-PH channels. E) Size of a hypothetical liposome and surface area of the PC/PS lipids and pR-PH channels. F) The insertion efficiency and number of the pR-PH channels in the liposomes at CLR = 0.005 (the radius of liposome is 80 nm and the theoretical channel number is 1014 per liposome). Data are mean $\pm$ s.d. ($n = 3$ independent experiments)

Figure S5. Water transport measurements of AqpZ in PC/PS liposomes (CLR = 0.005) at 25 °C. The AqpZ was reconstituted into liposomes (PC/PS = 4/1) in a buffer (10 mM Hepes, pH=7, 100 mM NaCl and 0.01% NaN₃) based on our previous study.^[15] A, B) Representative stopped-flow light-scattering curves of liposomes containing AqpZ after exposure to a hypertonic solution of 200 mM NaCl or 400 mM sucrose. C) The net water permeability of AqpZ in the liposomes was obtained from an exponential fit of the stopped-flow light-scattering results. Data are mean $\pm$ s.d. ($n = 3$ independent experiments)

Figure S6. Swelling experiments of the liposomes. A) Representative stopped-flow light-scattering curves of liposomes containing the pR-PH channels (CLR =0, 0.0025) at 25 °C. The liposomes were exposed to a hypotonic solution (the same buffer without 100 mM NaCl). B) The net water permeability of the pR-PH in the liposomes (CLR =0.0025, 0.005) in both the swelling and shrinking modes at 25 °C. For the swelling experiments the liposomes were exposed to a hypotonic solution (lacking 100 mM NaCl in the buffer used to form liposomes). For the shrinking experiments the liposomes were exposed to a hypertonic solution of 200 mM NaCl. Data are mean $\pm$ s.d. ($n = 3$ independent experiments)

Figure S7. The initial structures of the pR-PH and the pS-PH. A) the initial structure of the pR-PH with the length labeled for clarity; the hydrogen atoms are not shown. B) top view of a channel formed by the backbones of the pR-PH. C) Inter-chain hydrogen bonds of the pR-PH. D-F) the initial structure (D), channel (E) and hydrogen bonds (F) of the pS-PH, respectively, in the same way as the pR-PH. **G) Chirality of phenylalanine residues.** One chain of pR-PH was selected and all the phenylalanine residues in this chain were shown in CPK model. The selected phenylalanine residues were displayed according to Fischer projection with the carboxylic acid on top and side chain on the bottom. All the amine groups are located on the left sides of chiral carbons and these molecules are all L-phenylalanine. The structures are colored by atom type: blue is nitrogen, red is oxygen, cyan is carbon and white is hydrogen.

Figure S8. A, B) Time evolution of the root-mean-square-deviation (RMSD) for the pR-PH (A) and the pS-PH (B) relative to their initial structures. When the RMSD was calculated, only the backbone heavy atoms were considered with the side chains of phenylalanine and hydrogen atoms omitted. Each panel has five independent trajectories plotted in red, blue, black, green and cyan, respectively.

Figure S9. A) Last frames of the pR-PH. The structures are colored by chains and the chains are shown in licorice representation. B) Pore profiles of the pR-PH. The residues forming the pores are shown in transparent licorice representations. Pore profiles were prepared with the program HOLE and Simple AMBER VDW radii were used.

Figure S10. Pore diameter of the pR-PH. The averaged structures of the pR-PH were used to calculate the pore sizes. The structures of the pR-PH extracted from the last 100 ns in 100 ps intervals were averaged and 1000 frames were used in total for each run. The diameter was calculated by the program HOLE.

Figure S11. A) Last frames of the pS-PH. B) Pore profiles of the pS-PH. The side chains blocking the channels are colored by their corresponding chains and shown in opaque licorice representation.

Figure S12. Permeability and structural properties of the pS-PH. A, B) Run 3 (A) and Run 5 (B) were selected to illustrate how the side chain of phenylalanine blocked the pore of the pS-PH. The benzene rings are shown as a VDW representation.

Figure S13. Water molecules permeating through the pR-PH.

Figure S14. Free energy landscapes of the pR-PH and the pS-PH along the first two principal components. Only the backbone carbon atoms of the appended peptides were selected to extract coordinates to obtain the covariance matrix. By diagonalizing the covariance matrix, the eigenvectors and eigenvalues were calculated. The first two eigenvectors (PCA1 and PCA2) were used as the reaction coordinate to construct the free energy landscapes of the pS-PH and the pR-PH.

Figure S15. Normalized ion transport activities of pR-PH channels assessed by the HPTS assay.

The LUVs contained 100 mM NaCl buffered at pH 6.4 with 10 mM sodium phosphate and pH sensitive dye HPTS (0.1 mM). Outside of the vesicles was 10 mM sodium phosphate solution buffered at pH 7.4 containing 100 mM MCl (M= Li⁺, Na⁺, K⁺, Rb⁺, Cs⁺). DMSO was added as a control.

Figure S16. The net water permeability of the pR-PH channels in the liposomes (CLR = 0.005) using different solutes as osmolytes. The liposomes were abruptly exposed to a hypertonic solution of 200 mM NaCl or 400 mM sucrose at 25 °C. All the osmolarity difference values were measured with a freezing-point osmometer (Model 3250, Advanced Instruments. Inc.). Reflection coefficients of the pR-PH channels in liposomes were calculated as the ratio of the net water permeability in the presence of NaCl to that in the presence of sucrose as osmolyte. Sucrose was selected as the reference solute (reflection coefficient = 1). Data are mean $\pm$ s.d. (n = 3 independent experiments)

Table S1. Comparison of the pR-PH channels with AQPs and other artificial water channels.

Channel	Net permeability ($\mu\text{m/s}$)	Single channel permeability
Aquaporin 1 – AQP1 ^[16]	443	$11.7 \times 10^{-14} \text{ cm}^3/\text{s}$, 4×10^9 water molecules/s/channel
Dendritic dipeptide, (4-3,4-3,5)-12G2-CH2-Boc-L-Tyr-L-Ala-OMe [17]	No report	No reported
I-quartet Channels, Alkylureidoimidazoles ^[2b,18]	1.0-4.0	1.5×10^6 water molecules/s/channel
Tubular pores from arylene-ethynylene macrocycle ^[19]	7	4.9×10^7 water molecules/s/channel
Bola-amphiphile bis-triazole (TCT) ^[20]	No report	No report
Hydrazide appended Pillar[5]arenes ^[21]	8.6×10^{-6}	40 water molecules/s/channel
Peptide-appended Pillar[5]arenes ^[2a]	0.03-0.35 (shrinking), 30 (swelling)	3.5×10^8 (swelling) and 3.7×10^6 water molecules/s/channel (shrinking)
Carboxylic acid triarylamine ^[22]	17	No calculation
Aquaporin Z – AqpZ (current work)	346	$3.1 \times 10^{-14} \text{ cm}^3/\text{s}$, 1.0×10^9 water molecules/s/channel
pR-PH channel (current work)	130-783	$3.93 \times 10^{-14} \text{ cm}^3/\text{s}$, 1.31×10^9 water molecules/s/channel

Movie S1. Movie illustrating how the side chain of the pS-PH block water permeation.

Movie S2. Water molecules permeating through the pore of the pR-PH.

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