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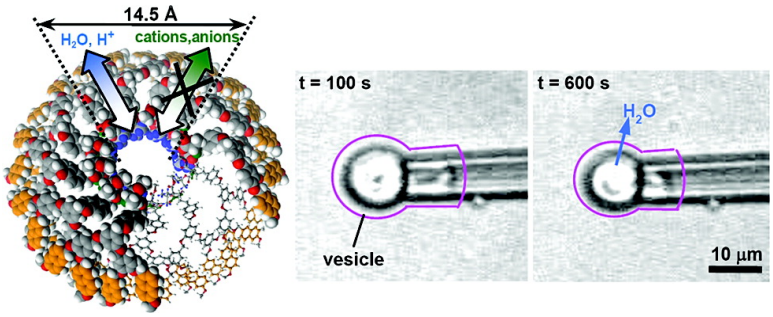


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# Selective Transport of Water Mediated by Porous Dendritic Dipeptides

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Synthetic channels and pores have been of increasing interest for their applications as antimicrobial agents, drug delivery, catalysts, and sensors.[1] Furthermore, the development of synthetic channels and pores that mimic porous proteins should contribute to a better understanding of the structure and function of natural transmembrane channels.[2] Here, we report a dendritic dipeptide that self-assembles into thermally stable helical pores. The helical pores are stable in phospholipid membranes and selectively transport water.

There has been little progress in the area of synthetic water channels, pores, and transporters.[1] Natural channels such as Aquaporin selectively transport water through primarily hydrophobic pores.[2,3] Recently, water was shown to transport through hydrophobic nanotubes very efficiently.[4] Hydrophobic pore sizes of 13−20 Å transport water faster than diffusion models.[5] Protons are also translocated through hydrophobic pores via a Grotthuss-type mechanism.[6]

We have previously reported the dendritic dipeptide, (4-3,4-3,5)-12G2-$CH_2$-Boc-L-Tyr-L-Ala-OMe, that self-assembles into helical pores in bulk, in solution, and in vesicles.[7] This dendritic dipeptide self-assembled into helical pores with a column diameter ($D_{col}$) of 71.3 Å and pore diameter ($D_{pore}$) of 12.8 ± 1.2 Å. These helical pores suffered from low thermal stability since at 22 °C they were in dynamic equilibrium with the dendritic dipeptide. Further studies led to improvements in the stability of the helical pores by more complex strategies.[8] We decided to attempt the stabilization of helical pores through simple $\pi$-stacking interactions on the dendron periphery by replacing the benzyl group with a naphthyl group. This generated (6Nf-3,4-3,5)12G2-$CH_2$-Boc-L-Tyr-L-Ala-OMe (**1**).[9]

In the bulk state, **1** formed helical pores similar to previously reported dendritic dipeptides[7,8] with $D_{col}$ = 82.3 Å and $D_{pore}$ = 14.5 ± 1.5 Å (Figure 1) in the bulk state. $D_{pore}$ is within the range of sizes required to facilitate transport of water and protons.[4,5] Wide-angle X-ray diffraction fiber patterns (Figure 1b and Supporting Information) showed strong 3.5 Å $\pi$-stacking interactions due to the naphthyl groups not seen in previous dendritic dipeptides.[7,8] Modifying the outer periphery from benzyl to naphthyl increased the melting temperature ($T_m$) in bulk from 95 to 139 °C. Furthermore, **1** self-assembled into helical pores in organic solvents that mimic the aliphatic region of phospholipid membranes (Figure 2a). The $T_m$ in solution and the molecular ellipticity were also higher compared to the benzyl dendritic dipeptide previously reported ($T_m$ = 40 vs 22 °C).[7] The enhanced stability of the pores formed from

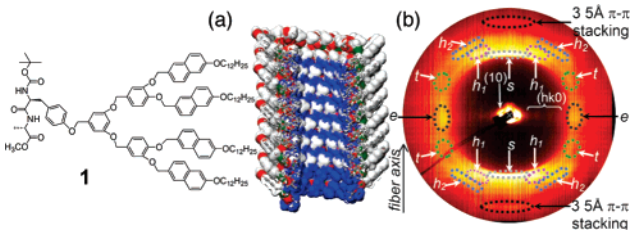


***Figure 1.*** (6Nf-3,4-3,5)12G2-$CH_2$-Boc-L-Tyr-L-Ala-OMe (**1**). (a) Cross section of the helical pore assembled from **1**. Color code: −$CH_3$ of the protective group of Tyr, blue; −$CH_3$ of the methyl ester of Ala, white; C, gray; O, red; N−H, green. (b) Wide angle XRD of an oriented fiber of the helical pores assembled from **1** (assignments in Supporting Information Figure SF4).

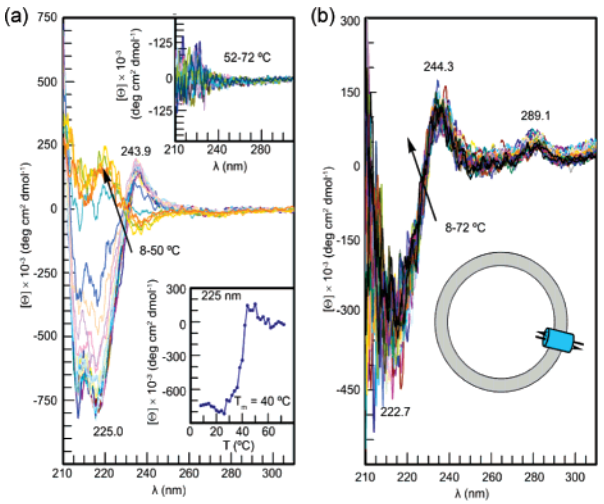


***Figure 2.*** CD of **1** in (a) 2.5 × $10^{-5}$ M in cyclohexane/THF 98.7/1.3 v/v; (b) in vesicles (2 mM lipid) in 10 mM phosphate buffer saline (PBS). Arrows indicate trends upon increasing temperature. The bottom insets show the molecular ellipticity as a function of temperature at the wavelength that the intensity of the Cotton effect is maximum. No CD signal is observed for vesicles without **1** or for **1** in PBS (details in Supporting Information Figure SF8).

**1** allowed their improved assembly into phospholipid membranes (Figure 2b). The helical pores showed CD spectra when incorporated into vesicles similar to their solution spectra. Additionally, the helical pores did not disassemble at high temperatures. This improved stability is due to enhanced $\pi$-stacking interactions on their periphery and higher concentration of **1** in the vesicle membrane.

Further confirmation that the helical pores were reconstituted into phospholipid vesicles came from proton translocation studies

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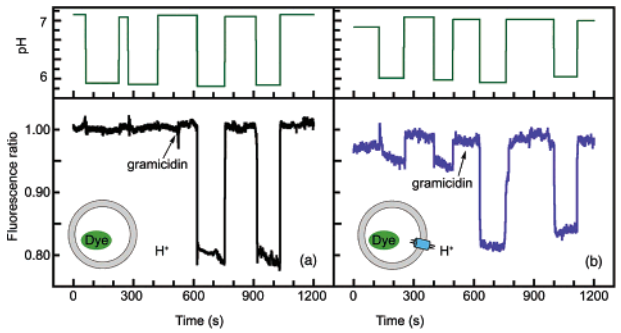


***Figure 3.*** Fluorescence assays showing proton transport in pores assembled from **1** reconstructed in phospholipid vesicles (Supporting Information). (a) Vesicles containing only the membrane-impermeable pH-sensitive fluorescent dye. (b) Vesicles containing both the pH-sensitive fluorescent dye and **1**. Arrows indicate the addition of excess gramicidin (5 $\mu$L of DMSO solution) that assesses the total amount of dye in vesicles and the absence of pores assembled from **1** in some vesicles. The pH jumps inside vesicles (bottom) were indicated by the change of fluorescence ratio $I_{647}/I_{670}$ created by pH changes outside vesicles (shown on top in green). Details in Supporting Information.

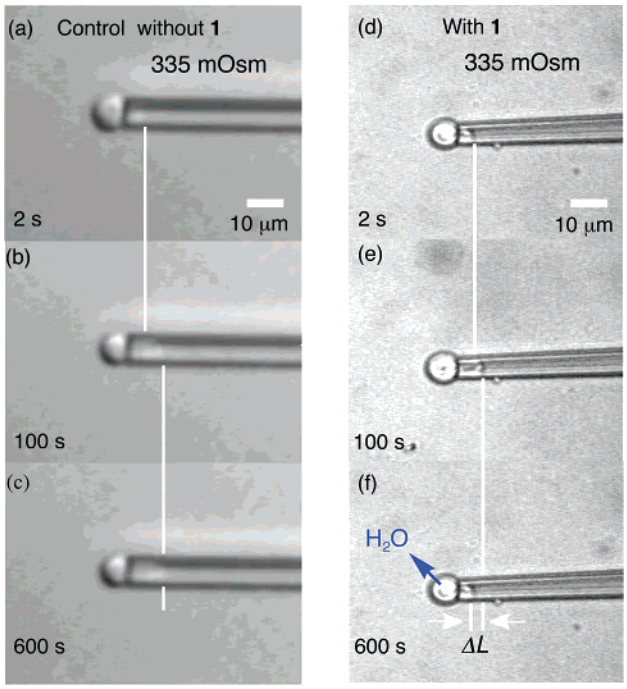


***Figure 4.*** Size transformations driven by changing the osmotic pressure of a single GUV without (a−c) and with **1** (d−f) and imaged in phase contrast video microscopy. Vesicles were formed in 285 mOsm PBS and placed in 335 mOsm external hypertonic solution and recorded over 20 min (shown at 2, 100, and 600 s). No further changes were observed after 600 s. Lines represent decrease in vesicle projection length ($\Delta L$) (details in Supporting Information).

(Figure 3). Transport across phospholipid vesicles was monitored using a pH-sensitive fluorescent dye.[7,10] Vesicles that had **1** transported protons, while vesicles without **1** did not. The transport of protons through water-filled hydrophobic pores occurs through a Grotthuss mechanism.[6] Furthermore, no measurable transport of $Li^+$, $Na^+$, or $Cl^-$ was observed by multinuclear NMR studies (Supporting Information).[11]

Water transport via the pores assembled from **1** incorporated in giant unilamellar vesicles (GUV) was studied through optical microscopy. GUVs ranging in size of 3−10 $\mu$m were captured with a micropipet and individually studied (Figure 4).[12]

Vesicles were formed in 285 mOsm PBS. After capture of the GUV with a micropipet, it was transferred to 335 mOsm hypertonic PBS. Transfer of the GUVs into the hypertonic PBS created a pressure difference that increased the length ($\Delta L$) of the vesicle in the pipet (Figure 4a−c). In vesicles without **1**, no further change was observed. In vesicles that contained **1**, a decrease of the vesicle's length in the pipet was observed, indicating a reduction in the vesicle size (Figure 4d−f). The reduction in size of the vesicle is due to transport of water out of the vesicle, thus balancing the osmotic pressure. Similarly, vesicles that were transferred to 235 mOsm hypotonic PBS exhibited opposite effects via swelling of the vesicles that contained **1** (Supporting Information Figure SF12).

Dynamic light scattering (DLS) was also utilized to monitor the size of the vesicles. Small unilamellar vesicles (SUV, 150 nm) without pores did not reduce their size, while SUVs with **1** exhibited an average reduction size of 8.4 ± 3.2 nm when transferred to 385 mOsm hypertonic solutions from PBS.

In summary, we have demonstrated a simple and general method to stabilize pores self-assembled from dendritic dipeptides through enhanced $\pi$-stacking interactions on their periphery. **1** was shown to form thermally stable helical pores in bulk, solution, and vesicles. Transport studies showed that **1** facilitated the transport of protons, while excluding cationic and anionic transport. Although the translocation of protons indicates transport of water, additional water transport studies were performed by optical microscopy and DLS. Therefore, the pores of **1** can be envisioned as a primitive mimic of Aquaporin that transports water but does not reject protons. Dendritic pores that selectively transport Na and K cations will be reported.

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**Supporting Information Available:** Experimental section containing materials, techniques, synthesis, structural and retrostructural analysis, vesicle preparation, transport studies, phase contrast video microscopy, and DLS. This material is available free of charge via the Internet at http://pubs.acs.org.

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