MEMBRANES

Enhanced water permeability and tunable ion selectivity in subnanometer carbon nanotube porins

Ramya H. Tunuguntla,1,2 Robert Y. Henley,1,3,4 Yun-Chiao Yao,1,3 Tuan Anh Pham,4 Meni Wanunu,2 Aleksandr Noy1,3,*

Fast water transport through carbon nanotube pores has raised the possibility to use them in the next generation of water treatment technologies. We report that water permeability in 0.8-nanometer-diameter carbon nanotube porins (CNTPs), which confine water down to a single-file chain, exceeds that of biological water transporters and of wider CNT pores by an order of magnitude. Intermolecular hydrogen-bond rearrangement, required for entry into the nanotube, dominates the energy barrier and can be manipulated to enhance water transport rates. CNTPs block anion transport, even at salinities that exceed seawater levels, and their ion selectivity can be tuned to configure them into switchable ionic diodes. These properties make CNTPs a promising material for developing membrane separation technologies.

Increasing demands for fresh water pose a global threat to sustainable development, resulting in water scarcity for 4 billion people (I, 2). Current water purification technologies (3) can benefit from the development of membranes with specialized pores that mimic highly efficient and water selective biological proteins such as Aquaporin-1 (AQP1). The key structural feature that enables efficient transport in AQP1 is its hydrophobic and narrow 0.3-nm-diameter channel, which forces water to translocate in a single-file arrangement (4). The limited success of efforts to create scalable AQP-based membranes (5) motivated researchers to develop bioinspired artificial water channels (6, 7). Computer simulations (8–10) and experimental studies of water transport through carbon nanotubes (CNTs) with >1 nm diameters (11–13) reported enhanced water flux in these channels. However, these nanotubes have failed to match the transport efficiency of AQP channels and have disappointed ion selectivity that vanished at higher salinities (14).

We investigated water and ion transport through carbon nanotube porins (CNTPs), ~10-nm-long CNT segments that spontaneously insert into a lipid membrane to form transmembrane channels (15). We compared transport through narrow 0.8-nm-diameter CNTPs (nCNTPs), which force water into a one-dimensional (1D) wire configuration, to transport through wider 1.5-nm-diameter CNTPs (wCNTPs) that maintain a bulklike water arrangement. We measured CNT water permeability in a stopped-flow apparatus by exposing large unilamellar vesicles (LUVs) with CNTPs embedded in their walls (Fig. 1A) to a hypertonic buffer solution containing pyranine osmolyte. This charged membrane-impermeable osmolyte is too large to enter nCNTPs and wCNTPs. Osmotic pressure induced water efflux, and the resulting vesicle shrinkage was observed as increased light scattering from the sample (16) (Fig. 1, B and C) (see fig. S1 for controls). From this data, we extracted the true water permeability, Pw, of the vesicles over a range of applied osmotic pressures (fig. S2) (see the supplementary materials (SM) for details of the analysis and controls (17)). Addition of CNTPs to the membrane increased its permeability, with overall water permeabilities increasing linearly with CNTP concentration (fig. S3) for both wCNTPs and nCNTPs. Single-channel CNTP permeabilities, Ṗw_CNTPs (Table 1), reached 5.9 ± 1.3 × 10^−12 cm^3/s and 6.8 ± 1.4 × 10^−13 cm^3/s for the wCNTPs and nCNTPs, respectively, with the permeability of nCNTPs exceeding that of wCNTPs by a factor of 11 and that of AQP1 by a factor of 6. nCNTP permeability also agrees closely with the value of 5.1 × 10^−13 cm^3/s calculated from molecular dynamics (MD) simulations for CNT pores of the same diameter (19).

To provide an unbiased microscopic description of water dynamics inside CNTPs, we performed first-principles MD simulations based on density functional theory (see SM for details). Whereas the structure and hydrogen-bonding (H-bonding) pattern inside wCNTPs resembled bulk water (Fig. 2, A and B, and fig. S16), water in nCNTPs adopted a single-file configuration (Fig. 2C and fig. S16), with a 1D H-bonding pattern that was akin to AQP channels (18).

What contributes to the greater water flux through a nCNTP, as compared to an AQP channel, when both structures similarly confine water in single file? Pore-lining residues in aquaporins can donate or accept H bonds to incoming water molecules; thus, transport rates are limited by kinetics of H-bond breakage, molecular reorientation, and H-bond reformation (19). In contrast, CNTP walls, which cannot form H bonds, permit virtually unimpeded water flow (fig. S17) (9).

When we repeated our measurements at pH 3.0, where the anionic carbohydrate groups (COO−) on the CNTP rim became protonated, we observed significant increases in water flux (Fig. 1D and Table 1), with nCNTPs and wCNTPs showing five- and fourfold enhanced permeabilities. This behavior parallels findings in a simulation study where addition of four COO− groups to the rims of a 1.1-nm-diameter CNT reduced the water flux threefold due to enhanced rim-water interactions (20).

We also measured water transport rates in CNTPs over a temperature range of 10° to 50°C and extracted the corresponding activation energies (Fig. 1, F and G, and fig. S6 for LUV and AQP-1 Arrhenius plots). The activation energy (Ea) penalty of 24.1 kcal/mol at pH 7.8 for nCNTPs decreased twofold at pH 3.0, highlighting the importance of rim-water interactions for transport efficiency. The remaining energy barrier at pH 3.0 should then originate from the H-bonding configuration of water molecules as they transition from bulk to single file inside the nCNTP. Our simulations show that the average number of H bonds drops from 3.9 in the bulk to 1.8 inside the nCNTP (Fig. 2D), which, given the single H-bond energy of 5.1 kcal/mol (21), implies an energy penalty of 10.7 kcal/mole, in excellent agreement with the value of 10.6 kcal/mole measured at pH 3.0. Activation energy measurements for the wCNTPs did not show a large change upon rim neutralization (Fig. 1G), suggesting that rim-water interactions do not play a substantial role in water transport through wider channels. The combination of high water permeability and relatively large activation energy observed in CNTPs suggests an important role for collective motion of water molecules in the transport mechanism (9, 22).

Compounds that either strengthen (kosmotropes) or break down (chaotropes) the intermolecular H-bonding network in water (23) altered the permeability of nCNTPs (Fig. 1E). In nCNTPs, addition of kosmotropic glucose and trehalose decreased water permeability 14- and 9.3-fold, while chaotrope agents, polyethylene glycol (PEG) and urea, increased it 2- and 1.6-fold, respectively. Therefore, weakening interactions between water molecules facilitates their rearrangement into a single-file configuration. In wCNTPs, chaotropes also enhanced water permeability by 11-fold (PEG) and 13-fold (urea); however, equivalent concentrations of kosmotropes did not alter permeabilities in wCNTPs, possibly due to a less stringent H-bonding rearrangement requirement in these wider pores. We also observed permeability enhancements in the presence of chaotropic PEG4 at pH 3 (fig. S5), indicating that the enhancement mechanism is not related to the chaotrope interactions with the CNTP rim.

We calculated the effective water diffusion coefficients, Dw, in CNTPs from their osmotic permeabilities (Table 1) (see SM for details). To minimize contribution from rim interactions, we

*These authors contributed equally to this work. †Corresponding author. Email: noy1@lanl.gov

1Biology and Biotechnology Division, Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA. 2Physics Department, Northeastern University, Boston, MA 02115, USA. 3School of Natural Sciences, University of California Merced, Merced, CA 95343, USA. 4Materials Science Division, Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA.


1Department, Northeastern University, Boston, MA 02115, USA. 2Physics Division, Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA. 4Materials Science Division, Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA.

**These authors contributed equally to this work. †Corresponding author. Email: noy1@lanl.gov
Fig. 1. Water transport through CNTPs. (A) A cartoon showing an LUV with embedded CNTPs (not to scale). (Inset) Water molecules escape the interior of the LUV through the CNTP to relieve an applied osmotic gradient. (B and C) Light-scattering traces recorded after subjecting lipid vesicles containing 1.5 nm wCNTPs (B), and 0.8 nm nCNTPs (C) to varying osmotic gradients. (D) Water permeability values (pyranine osmolyte, N = 5) measured for nCNTPs and wCNTPs at two pH values (blue and red bars) compared to the water permeability of AQP1 protein measured at pH 7.8, and water permeability of nCNTPs measured (N =3) at pH 7.8 with 0.6 M NaCl osmolyte (gray bar). Water permeability of AQP1 measured in our experiments, $1.19 \times 10^{-13}$ cm$^3$/s, matches the literature values (31). nCNTP permeability measured using NaCl osmolyte is greater than values measured using pyranine because of the chaotropic nature of NaCl (32). (E) Water permeability of CNTPs measured at pH 7.8 in the presence of 150 mM concentrations of chaotropes PEG and urea, as well as kosmotropes glucose and trehalose (N =3). Dashed lines are guides to the eye that indicate permeability levels measured without additives. (F and G) Arrhenius plots of the water permeability for nCNTPs (F) and wCNTPs (G), with corresponding activation energies indicated on the graph (N = 3).

<table>
<thead>
<tr>
<th>Channel</th>
<th>pH</th>
<th>Pore diam. (nm)</th>
<th>Pore length (nm)</th>
<th>Water permeability, $P_w$ (cm$^3$/s)</th>
<th>Diffusion coefficient, $D_w$ (cm$^2$/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(H$_2$O molec/s)</td>
<td>Experiment</td>
</tr>
<tr>
<td>wCNTP</td>
<td>7.8</td>
<td>1.35</td>
<td>13.4</td>
<td>$5.9 \pm 1.3 \times 10^{-14}$</td>
<td>$1.9 \times 10^9$</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>1.35</td>
<td></td>
<td>$2.2 \pm 0.05 \times 10^{-13}$</td>
<td>$7.3 \times 10^9$</td>
</tr>
<tr>
<td>nCNTP</td>
<td>7.8</td>
<td>0.68</td>
<td>10.6</td>
<td>$6.8 \pm 1.4 \times 10^{-13}$</td>
<td>$2.3 \times 10^{10}$</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>0.68</td>
<td></td>
<td>$3.4 \pm 0.04 \times 10^{-12}$</td>
<td>$1.1 \times 10^{11}$</td>
</tr>
<tr>
<td>AQP1</td>
<td>7.0</td>
<td>0.28</td>
<td>2.24</td>
<td>$1.17 \times 10^{-13}$ (18)</td>
<td>$3.9 \times 10^5$ (18)</td>
</tr>
</tbody>
</table>
focused on the $D_w$ values measured at pH 3. $D_w$ measured for the wCNTPs ($2.1 \pm 0.2 \times 10^{-5}$ cm$^2$/s) correlates well with the bulk value of $2.3 \times 10^{-5}$ cm$^2$/s (24). The higher $D_w$ measured in nCNTPs ($4.4 \pm 0.2 \times 10^{-5}$ cm$^2$/s) is also close to the value predicted (19) for 0.8-nm-diameter CNTs ($\sim 3.5 \times 10^{-5}$ cm$^2$/s).

We also compared our results with $D_w$ determined from our simulations by fitting the computed mean square displacement (MSD) to the power law form (Fig. 2E). These fits indicate that water diffusion mechanism in CNTPs deviates significantly from Fickian type in the bulk, where the power law coefficient $\alpha = 1$, and more so in nCNTPs, where $\alpha$ reaches the value of 1.81. Furthermore, the fitted $D_w$ ratio of 2.9 (nCNTP:wCNTP) is in reasonable agreement with the ratio of 2.1 obtained in our experiments, also consistent with a further reduction of the number of H bonds within nCNTPs.

To evaluate their ion selectivity, we measured ion transport through individual nCNTPs using a modified suspended lipid bilayer platform (Fig. 3A).
and figs. S7 to S10) in which we burst a vesicle containing single or multiple CNTPs onto an ~200-nm-diameter silicon nitride nanopore (see SM). Unitary nCNTP conductance of 69 ± 6 pS observed in these experiments (Fig. 3, B and C) matches values that we observed by monitoring stepwise spontaneous nCNTP insertions into lipid bilayer (figs. S10 and S11). We then explored the relationship between nCNTP conductance and electrolyte (KCl) concentration in bilayers containing multiple nCNTPs. At pH 7.5, nCNTP conductance increased monotonically up to 250 mM, thereafter beginning to saturate (Fig. 3D). Single-file biological ion channels display similar behavior, where interactions of ions with the charged residues lead to Michaelis-Menten–type ion transport kinetics (25). Similarly, for moderate-to-high electrolyte concentrations, interactions of potassium ions with the anionic carboxylic groups on the nCNTP rim result in Michaelis-Menten transport kinetics (Fig. 3D). At low salt concentrations, we observed a C^{1/22} dependence (Fig. 3D, inset) that is different from the C^{1/2} scaling reported for larger 7- to 28-nm-diameter CNTs at low concentrations (26), although similar to the C^{1/2} dependence recently reported for very long 1.3- to 1.5-nm-diameter CNTs (27).

Reversal potential measurements, in which both sides of the nCNTP are exposed to different salt concentrations, allowed us to determine nCNTP ion selectivity. These measurements, performed at pH 7.5 (Fig. 4A) (see figs. S12 to S14 for controls), showed that nCNTPs almost exclusively conduct cations, with ~0.99 permselectivity, corresponding to K+ :Cl− ion selectivity greater than 184:1 at typical seawater salinity level of 0.6 M. Thus, in our nCNTPs, the negatively charged rims create a “good co-ion (Cl−) exclusion” condition, which is expected to produce our reported C^{1/22} conductance scaling (28). Furthermore, permselectivity measurements at different electrolyte concentrations show that strong anion exclusion in nCNTPs persists at up to 1 M salinity levels (Fig. 4C and Table 2), only diminishing at very high salt concentrations (~2 M), in contrast to previous studies where ion selectivity of 1.6-nm-diameter CNTs begins to drop at ~30 mM ionic strength (14). This strong ion selectivity also allowed us to use osmotic pressure from 0.6 M NaCl to induce water transport in nCNTPs (Fig. 1D and fig. S4), underscoring the potential of these narrow nanotubes for water desalination applications.

We can explore the mechanism of ion selectivity in nCNTPs further by measuring their ion conductance and permselectivity at pH 3.0, where the rims are uncharged (Fig. 3D). At pH 3.0, nCNTP conductance did not exhibit saturation but remained proportional to ion concentration (Fig. 3D, inset), indicating the removal of the rate-limiting step of counterion binding to the charged rim. Moreover, reversal potential measurements for the nCNTPs (Fig. 4B) at pH 3.0 showed a weak preference to anions over cations, in strong contrast to our observations at pH 7.5 (Fig. 4C).

Lastly, we exploited the nCNTP’s tunable ion selectivity to demonstrate an ionic diode. When

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**Table 2.** Permselectivities (mean ± SD) and corresponding ion selectivity ratios measured for nCNTPs at pH 7.5 and 3.0 at varying KCl concentrations.

<table>
<thead>
<tr>
<th>[KCl]</th>
<th>Permselectivity</th>
<th>Selectivity ratio (K+/Cl−)</th>
<th>Permselectivity</th>
<th>Selectivity ratio (Cl−/K+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 M</td>
<td>0.826 ± 0.007</td>
<td>10.2</td>
<td>−0.354 ± 0.022</td>
<td>2.2</td>
</tr>
<tr>
<td>1 M</td>
<td>0.976 ± 0.026</td>
<td>80</td>
<td>−0.617 ± 0.069</td>
<td>4.9</td>
</tr>
<tr>
<td>0.6 M</td>
<td>0.989 ± 0.027</td>
<td>184</td>
<td>−0.457 ± 0.067</td>
<td>2.9</td>
</tr>
<tr>
<td>0.1 M</td>
<td>1.031 ± 0.037</td>
<td>∞</td>
<td>−0.836 ± 0.062</td>
<td>15</td>
</tr>
</tbody>
</table>
both sides of the nCNTP were exposed to 100 mM KCl solutions at pH 7.5, we observed a resistor-like linear current-voltage (I-V) characteristic (Fig. 4D). However, when one side of the bilayer was acidified to pH 3.0, the nCNTP exhibited a highly rectifying diode-like behavior that indicates unidirectional ion transport, similar to rectification behavior observed in CNT membranes (29). Switching the pH gradient direction reversed the direction of the diode conduction (Fig. 4D). Transition between resistor and diode behavior was robust, could be switched repeatedly, and was observed even in 1 M KCl (Fig. S15), pointing to potential applications of nCNTPs in dynamically reconfigurable ionic circuits (30).

Our results indicate that sub-nm confinement, which forces water molecules into a single-chain configuration, plays a critical role in enabling ultrafast water transport and selective ion transport in narrow CNTPs. High water permeability and ion selectivity of these channels, even at high salinity, coupled with the potential to tune their transport properties, present opportunities to develop ultra-permeable membranes for water purification applications, industrial separations, or flexible reconfigurable nanofluidic circuits.

REFERENCES AND NOTES

17. Materials and methods are available as supplementary materials.


ACKNOWLEDGMENTS

We thank P. Waduge, Northeastern University, for fabrication of the solid-state nanopore chips; Y. Zhang, Lawrence Livermore National Laboratory (LLNL) for help with graphics used in Figs. 1 and 3; and V. Freiger, Technion, Israel, for helpful discussions. T. A. P. acknowledges support from the Lawrence Fellowship Program. Computational resources were provided by the LLNL Institutional Computing Grand Challenge Program. This work was supported by the U.S. Department of Energy, Office of Basic Energy Sciences, Division of Materials Sciences and Engineering. Work at LLNL was performed under the auspices of the U.S. Department of Energy under contract DE-AC52-07NA27344. Work at the Molecular Foundry at Lawrence Berkeley National Laboratory was supported by the Office of Science, Office of Basic Energy Sciences, of the U.S. Department of Energy under contract DE-AC02-05CH11231. Solid-state nanopore fabrication at Northeastern was funded by National Science Foundation (grant EFMA-1542707). A.N. is an inventor on U.S. Patent Application Ser. No. 15/503,983, submitted by Lawrence Livermore National Security, LLC, which covers carbon nanotube pores. A.N. holds shares in Porifera, Inc., a company that commercializes carbon nanotube membrane technology.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/357/6353/792/suppl/DC1

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Supplementary Text
Figs. S1 to S17
Table S1
References (35–68)

16 March 2017; accepted 11 July 2017
10.1126/science.aan2438
Enhanced water permeability and tunable ion selectivity in subnanometer carbon nanotube porins
Ramya H. Tunuguntla, Robert Y. Henley, Yun-Chiao Yao, Tuan Anh Pham, Meni Wanunu and Aleksandr Noy

Science 357 (6353), 792-796.
DOI: 10.1126/science.aan2438

Go with the flow
Enhanced water transport occurs in a number of narrow pore channels, such as biological aquaporins. Tunuguntla et al. thoroughly characterized molecular transport through narrow carbon nanotubes (CNTs) (see the Perspective by Siwy and Fornasiero). In contrast to previous studies, the authors focused on water and ion transport through relatively short (∼10-nm) fragments of CNTs embedded in lipid bilayer membranes. Strong confinement generated highly accelerated water flow compared with that observed in biological water transporters. A key factor in the transport rate was the tunable rearrangement of intermolecular hydrogen bonding. Furthermore, by changing the charges at the mouth of the nanotube, the authors were able to alter the ion selectivity. Science, this issue p. 792; see also p. 753