Comment on “Enhanced water permeability and tunable ion selectivity in subnanometer carbon nanotube porins”

Andreas Horner and Peter Pohl

Tunuguntla et al. (Reports, 25 August 2017, p. 792) report that permeation of single-file water occurs faster through carbon nanotubes than through aquaporins. We show that this conclusion violates fundamental thermodynamic laws: Because of its much lower activation energy, aquaporin-mediated water transport must be orders of magnitude faster. Leakage at the nanotube-membrane interface may explain the discrepancy.

Tunuguntla et al. mistakenly concluded that single-file water flow through narrow carbon nanotubes is responsible for the observed acceleration of osmotic vesicle deflation upon nanotube insertion into vesicular lipid membranes (1). Calculation of the unitary nanotube water permeability \( p_t = 6.8 \times 10^{-13} \text{ cm}^2 \text{ s}^{-1} \) was performed by estimating the integral permeability \( P_l \) of the lipid vesicle and taking into account the total number of carbon nanotubes per vesicle. The latter was estimated by measuring the total proton conductivity across the vesicular membrane (pH 7.5 inside versus pH 6.9 outside) and using a previously published value for the proton conductivity of a single nanotube.

These results leave open the possibility that instead of being channeled through the nanotube, water moves through defects at the nanotube-bilayer interface (Fig. 1). To exclude this possibility, experiments with a molecule that could occlude the tubes would be required. In the absence of such an inhibitor, measurements of the Gibbs activation energy barrier \( \Delta G_t \) constitute the traditional way of showing the presence of an aqueous pore: \( \Delta G_t \sim 5 \text{ kcal/mol} \) represents the hallmark for water movement through protein water channels (2). This value is close to the activation energy for the self-diffusion of water (3). When plotting vesicular water permeability as a function of \( 1/T \), where \( T \) is absolute temperature, Tunuguntla et al. found \( \Delta G_t = 24.1 \text{ kcal/mol} \). Such a large \( \Delta G_t \) value is incompatible with diffusion through a water-conducting pore.

To bolster the fact that \( p_t \) and \( \Delta G_t \) are intricately linked in single-file transport, we transform \( p_t \) into the “hopping rate” \( r \) with which the water chain moves forward or backward (4):

\[
r = \frac{N_A}{V_w} \exp \left( -\frac{\Delta G_t}{k_B T} \right) \tag{1}
\]

where \( V_w \) is the molecular volume of water and \( N_A \) is Avogadro’s constant. The water molecule loses two of its four neighbors when entering the pore. This lifts the water molecule from its energetic ground state to a state of higher energy. Moieties of wall-lining residues in proteinaceous channels may act as surrogates for the lost waters (5). Yet constraints in both abundance and strength of the newly formed hydrogen bonds serve to keep the energy difference between bulk and intraluminal water molecules at ambient temperatures on the order of the thermal energy \( k_B T \), where \( k_B \) is the Boltzmann constant. Equilibration between the individual jumps is ensured by the short hydrogen bond lifetime, which is ~2 ps in neat water. A much longer lifetime for pore waters (as sometimes observed for waters of hydration) seems doubtful because they retain bulk diffusibility (6). The theory of water permeation through nanotubes describes an analogous situation with imaginary water-binding sites (4).

We thus may apply transition state theory and describe the hopping of the water file by linking \( r \) to \( \Delta G_t \):

\[
r = v_0 \exp \left( -\frac{\Delta G_t}{k_B T} \right) \tag{2}
\]

where \( v_0 = 10^{13} \text{ s}^{-1} \) is the universal transition state theory attempt frequency. Using Eqs. 1 and 2 to calculate \( p_t \) as

\[
p_t = v_0 V_w \frac{N_A}{N_A} \exp \left( -\frac{\Delta G_t}{k_B T} \right) \tag{3}
\]

we find a satisfactory match to experimentally obtained \( p_t \) values for aquaporin-1, aquaporin-2, the bacterial potassium channel KcsA, and the pore-forming peptide gramicidin A (Table 1). In contrast, the large activation energy of 24.1 kcal/mol, now reported for carbon nanotubes (2), corresponds to a \( p_t \) value 15 orders of magnitude smaller than the \( p_t \) value derived from the rate of vesicle deflation.

Extending Table 1 to other channels may reveal additional discrepancies. First, it is important to exclude channels that may accommodate more than one water molecule in their cross section, because widening of the channel is likely to increase \( p_t \) (7), whereas \( \Delta G_t \) cannot adopt values that are smaller than those measured for the self-diffusion of water (3). Second, it is important to keep in mind that direct \( p_t \) measurements are subject to large inherent technical difficulties: They may be hampered by stagnant water layers in the membrane vicinity or uncertainties in the actual channel density (6). Water-conducting ion channels, such as the bacterial potassium channel KcsA, may be discounted when deriving the channel number from conductivity measurements (8), as this procedure does not account for water transport by electrically silent channels (i.e., by channels that are in their inactivated state) (9). Other obstacles arise when the precise analytical solution (5) that allows calculation of \( p_t \) from the time course of vesicle volume decrease is substituted for a more simple but inaccurate expression (1, 9). Such miscalculations may partly explain the dependence of the vesicular osmotic permeability \( P_o \) on the osmotic gradient (Figure S2).

**Fig. 1. Comparison of the water pathways across membranes with embedded carbon nanotubes and reconstituted aquaporins.** (A) Conceivably, water leaks at the interface between the nanotubes and lipid membrane. (B) Altering the position of the nanotubes in the membrane by neutralizing the charged carboxylate groups at their ends (red to black) alters leak size and thus membrane water permeability. (C) Aquaporins offer aqueous pores that efficiently channel water. Protein-lipid interactions prevent any water from passing along the outer channel wall.
in (1)). Alternative methods confirm that $P_f$ is independent of the osmotic gradient (8, 10), thereby supporting this conclusion.

The bilayer may be permeated not only by water, but also by whole nanotubes (II). The first step is the transient protonation of one of their carboxylated ends, which facilitates membrane insertion of the nanotube. Water and ions may pass along the interface between lipids and the nanotube (Fig. 1). Permanent removal of the charge by switching pH from 7.8 to 3.0 should increase the probability of nanotube partition into the bilayer. $\Delta G^f_\text{f}$ drops from 24.1 kcal/mol at pH 7.8 to only 10.6 kcal/mol at pH 3.0. This suggests that at acidic pH, the nanotube may bury both ends within the bilayer. Lipid packing defects along the whole length of the nanotube represent a water pathway that offers less resistance than an unperturbed bilayer. At elevated temperatures, the pH difference vanishes because the thermal energy partly compensates for the additional expense in Born energy that is required for membrane partitioning of a charged moiety.

We conclude that the large $\Delta G^f_\text{f}$ is incompatible with the presence of water-filled channels. Consequently, water leaks at the nanotube-bilayer interface, which is supported by the reported pH dependence of the water flux.

**REFERENCES AND NOTES**


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**Table 1. The unitary pore water permeability $p_f$ and the Gibbs activation energy barrier $\Delta G^f_\text{f}$ are intricately linked (Eq. 3).**

<table>
<thead>
<tr>
<th>Membrane pore</th>
<th>$p_f$ (cm$^3$ s$^{-1}$) (measured)</th>
<th>$\Delta G^f_\text{f}$ (kcal/mol) (measured)</th>
<th>$r$ (s$^{-1}$) (calculated from $\Delta G^f_\text{f}$)</th>
<th>$p_{f,\text{cal}}$ (cm$^3$ s$^{-1}$) (calculated from $\Delta G^f_\text{f}$)</th>
<th>$P_{f,\text{cal}}/P_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquaporin-1</td>
<td>$5.3 \times 10^{-13}$ (5)</td>
<td>3.1 (12)</td>
<td>$5.3 \times 10^{10}$</td>
<td>$1.6 \times 10^{-12}$</td>
<td>3.0</td>
</tr>
<tr>
<td>Aquaporin-Z</td>
<td>$2.9 \times 10^{-13}$ (5)</td>
<td>4.0 (13)</td>
<td>$1.2 \times 10^{10}$</td>
<td>$3.5 \times 10^{-13}$</td>
<td>1.2</td>
</tr>
<tr>
<td>KcsA</td>
<td>$5.3 \times 10^{-14}$ (5)</td>
<td>5.1 (8)</td>
<td>$1.8 \times 10^{9}$</td>
<td>$5.4 \times 10^{-14}$</td>
<td>1.0</td>
</tr>
<tr>
<td>Gramicidin A</td>
<td>$1.6 \times 10^{-14}$ (14)</td>
<td>6.1 (15)</td>
<td>$3.3 \times 10^{8}$</td>
<td>$1 \times 10^{-14}$</td>
<td>0.6</td>
</tr>
<tr>
<td>Carbon nanotubes</td>
<td>$6.8 \times 10^{-13}$ (1)</td>
<td>24.1 (1)</td>
<td>$2.1 \times 10^{-5}$</td>
<td>$6.2 \times 10^{-28}$</td>
<td>$9.1 \times 10^{-16}$</td>
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