TECHNICAL COMMENT

MEMBRANES

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 $p_f = 6.8 \times 10^{-13}$ cm^3 s^{-1} was performed by estimating the integral permeability P_f 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 $\Delta G^{\ddagger}_t$ constitute the traditional way of showing the presence of an aqueous pore: $\Delta G^{\ddagger}_t \sim 5$ 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 $\Delta G^{\ddagger}_t = 24.1$ kcal/mol. Such a large $\Delta G^{\ddagger}_t$ value is incompatible with diffusion through a water-conducting pore.

To bolster the fact that p_f and $\Delta G^{\ddagger}_t$ are intricately linked in single-file transport, we transform p_f into the "hopping rate" r with which the water chain moves forward or backward (*4*):

$$r = p_f \frac{N_A}{V_w} \quad (1)$$

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_BT, 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 $\Delta G^{\ddagger}_t$:

$$r = \nu_0 \exp\left(\frac{-\Delta G^{\ddagger}_t}{k_B T}\right) \quad (2)$$

where $\nu_0 \approx 10^{13}$ s^{-1} is the universal transition state theory attempt frequency. Using Eqs. 1 and 2 to calculate p_f as

$$p_f = \frac{\nu_0 V_w}{N_A} \exp\left(\frac{-\Delta G^{\ddagger}_t}{k_B T}\right) \quad (3)$$

we find a satisfactory match to experimentally obtained p_f values for aquaporin-1, aquaporin-Z, 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 (*1*), corresponds to a p_f value 15 orders of magnitude smaller than the p_f 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_f (*7*), whereas $\Delta G^{\ddagger}_t$ 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_f 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 miscounted 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_f 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_f on the osmotic gradient [figure S2

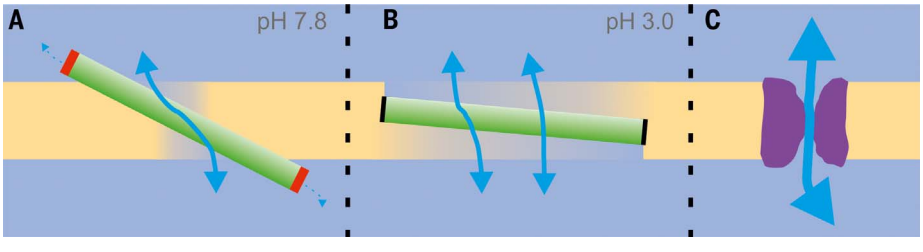

Fig. 1. Comparison of the water pathways across membranes with embedded carbon nanotubes and reconstituted aquaporins. (**A**) Conceivably, water leaks at the interface between the nanotubes and lipid membrane. (**B**) Altering the position of the nanotubes in the membrane by neutralizing the charged 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.

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

Membrane pore	p_f (cm^3 s^{-1}) (measured)	$\Delta G^{\ddagger}_t$ (kcal/mol) (measured)	r (s^{-1}) (calculated from $\Delta G^{\ddagger}_t$)	$p_{f,c}$ (cm^3 s^{-1}) (calculated from $\Delta G^{\ddagger}_t$)	$p_{f,c}/p_f$
Aquaporin-1	5.3×10^{-13} (*5*)	3.1 (*12*)	5.3×10^{10}	1.6×10^{-12}	3.0
Aquaporin-Z	2.9×10^{-13} (*5*)	4.0 (*13*)	1.2×10^{10}	3.5×10^{-13}	1.2
KcsA	5.3×10^{-14} (*5*)	5.1 (*8*)	1.8×10^{9}	5.4×10^{-14}	1.0
Gramicidin A	1.6×10^{-14} (*14*)	6.1 (*15*)	3.3×10^{8}	1×10^{-14}	0.6
Carbon nanotubes	6.8×10^{-13} (*1*)	24.1 (*1*)	2.1×10^{-5}	6.2×10^{-28}	9.1×10^{-16}

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 (*11*). 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 in the bilayer. $\Delta G^{\ddagger}_t$ 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 portioning of a charged moiety.

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

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