

New and Notable

Teaching Old Coefficients New Tricks: New Insight into the Meaning of the Osmotic and Diffusive Permeation Coefficients

Oliver Beckstein*

Structural Bioinformatics and Computational Biochemistry Unit, Department of Biochemistry, University of Oxford, Oxford, OX1 3QU, United Kingdom and Johns Hopkins University, School of Medicine, Department of Physiology, Baltimore, Maryland 21205

In living cells specialized water channels, the glycerol-aquaporins, are responsible for rapid passive transport of water through the membrane. Crystal structures showed that these protein pores have diameters comparable to the size of a single water molecule (around 0.3 nm) and lengths around 2 to 3 nm. Despite being strongly confined, water moves rapidly through these and other narrow peptide pores such as gramicidin. Understanding how water behaves in confinement has not been easy, not the least because there is no experimental technique available to unambiguously measure water flux through a single water pore that would be comparable to single channel patch clamp recordings on ion channels. Thus, if we want to understand how a physiological function such as the water flux derives from the structure, namely the geometry and chemical character of the water pathway, we can do this only with models that relate macroscopic transport coefficients to microscopic properties of the pore.

Although there is no satisfactory microscopic theory for water transport across all the pore dimensions relevant for biology (1,2) it is clear that the single most important parameter for water permeation is the radius of the pore.

Biological, water-permeable pores have radii ranging from 0.14 nm (gramicidin A, K⁺-channel selectivity filter) through ~0.8 nm (ligand gated ion channels in the open state) to around 10 nm (the open region of the nuclear pore complex) (3). Transport through pores that are wide compared to the typical interaction and correlation lengths in liquid water ($\gg 1$ nm or more than ~4 water diameters) can be described well by continuum theories. There is also progress being made on narrow pores that can only accommodate a 1D chain of water molecules; here the important simplification is that water molecules cannot pass each other. However, as soon as we enter the “multi-pass” regime of $R > 2r_{\text{water}} \approx 0.28$ nm no theories are available.

The molecular dynamics simulations by Portella and de Groot (4) in this issue hint at what a new theory for the transition from the no-pass to the multi-pass regime could look like. In these simulations water can move freely through “toy model” pores of variable radius, length, and surface character. The pores are based on the architecture of the gramicidin A channel and embedded in a membrane mimetic. Computer simulations afford full control over all parameters of the simulation, including many at the atomic level that are not accessible to experimentalists in the real world. The authors use this to good effect; for instance, the degree of hydrophilicity of a pore is adjusted by simply editing the backbone dipole moment.

The simulations measure the two experimentally accessible permeation coefficients that characterize water transport. The osmotic permeability coefficient p_f expresses the observation that osmotic water flux $J_w = p_f \Delta c_{\text{solute}}$ is driven by a concentration difference of an impermeable solute between two compartments separated by a semipermeable membrane. For single-file pores, p_f was shown to be proportional to the rate of translocation of the whole n -water chain by one hop. From a physiological point of view such a collective hop is the important observable: one

water molecule enters the pore on one side, another one exits on the other and the net effect is the movement of one water molecule across the membrane. The second observable is the diffusive permeability coefficient p_d . It is proportional to the number of translocation events of individual water molecules through the pore per unit time and thus primarily characterizes the pore-water interaction. It can be measured by following dilute isotope-tagged water molecules in solution that interact in the same way as the untagged ones.

Because it takes $n + 1$ individual hops along the chain to achieve an individual translocation event there exists a relationship between the measurable permeabilities and the number of water molecules n in the single file chain,

$$\frac{p_f}{p_d} = n + 1. \quad (1)$$

Equation 1 has been used successfully to determine the channel length of single-file pores such as gramicidin A from osmotic measurements (2); the length is simply calculated as n times the average water-water distance. However, the theoretical value of the p_f/p_d ratio for wider molecular pores than single file ones has not been established unequivocally and Alan Finkelstein’s remark from 1987 is still true: “There is presently no satisfactory physical theory for diffusion and flow through pores with radii only a few times larger than that of the solvent molecule.” (2) One of the many intriguing findings of Portella and de Groot (4) is that a more general, although (at the moment) phenomenological, relation,

$$\frac{p_f}{p_d} = q(R)(\langle n \rangle + 1), \quad (2)$$

seems to hold for any water filled molecular pore (at least up to a radius of ~0.6 nm). Here $q(R)$ is the number of effective single file columns that fit into the pore of radius R ; for a true single file pore $q(R) = 1$ holds. Equation 2

Submitted August 29, 2008, and accepted for publication October 9, 2008.

*Correspondence: oliver.beckstein@bioch.ox.ac.uk

Editor: Peter Tieleman.

© 2009 by the Biophysical Society
0006-3495/09/02/0763/02 \$2.00

doi: 10.1016/j.bpj.2008.10.048

relaxes two important constraints that were required to derive Eq. 1: The “no-pass” and the “no-vacancy” condition. It seems that we can interpret flow through wider pores as consisting of effective 1D chains of water molecules. Second, by identifying the ratio of the permeabilities with the average occupancy of the single file it also becomes possible to apply permeability measurements in a meaningful way to hydrophobic pores that may only be partially occupied.

Computer simulations indicated that sufficiently hydrophobic pores would be, depending on the degree of hydrophobicity, filled with water vapor, or fluctuate between liquid filled and vapor filled states. Although plausible on physical grounds—liquid water at ambient conditions is close to its liquid-vapor phase coexistence (5) and thus any influence such as a surface or a change in chemical potential may tilt the balance toward the vapor state—it is not clear that such liquid-vapor oscillations actually exist in nature. This work, however, suggests how to experimentally measure such behavior. By measuring $p_f/p_d - 1$ for a narrow pore one determines the average pore occupancy; choosing R so small that $q = 1$ simplifies the considerations. Because the liquid-vapor equilibrium is easily shifted in either direction by a change in pressure or temperature (6) one should only have to repeat the measure-

ments under different conditions and look for a sudden change in $\langle n(T, P) \rangle$.

It should, however, also be kept in mind that simulations ultimately need to find justification in experiments (and experiments need a model, based on theory and simulation). Before Eq. 2 can be really used to interpret measurements it will require some additional experimental validation. This may be quite a formidable task that needs to be addressed by theoreticians and experimentalists together, similar to the ongoing attempts to explain the dependence of p_f on the pore length from experiments (7) and simulations (8).

Even so, the work of Portella and de Groot should already inspire the formulation of new theories and clarify the meaning of the experimental observables. For instance, together with the insight that water permeation can be described by a simple collective coordinate (9) it lets us qualitatively interpret p_f and p_d in a slightly different light: p_d is related to the probability that a distinguishable water molecule completes a translocation whereas p_f measures the translocation of indistinguishable particles. It seems that we may be able to learn more about the microscopic water structure in a pore from macroscopic measurements than thought previously.

Discussions with Elizabeth Denning are gratefully acknowledged. The author was supported

by a Junior Research Fellowship at Merton College, Oxford.

REFERENCES

1. Zeuthen, T., and N. MacAulay. 2002. Passive water transport in biological pores. In *Molecular Mechanisms of Water Transport Across Biological Membranes*, Vol. 215, International Review of Cytology. T. Zeuthen and W. D. Stein, editors. Elsevier, New York, 203–230.
2. Finkelstein, A. 1987. *Water Movement Through Lipid Bilayers, Pores, and Plasma Membranes. Theory and Reality*. John Wiley & Sons, New York.
3. Hille, B. 2001. *Ion Channels of Excitable Membranes*, 3rd ed. Sinauer Associates, Sunderland, MA.
4. Portella, G., and B. L. de Groot. 2008. Determinants of water permeability through nanoscopic hydrophilic channels. *Biophys. J.* 92:925–938.
5. Chandler, D. 2005. Interfaces and the driving force of hydrophobic assembly. *Nature*. 437:640–647.
6. Brovchenko, I., A. Geiger, and A. Oleinikova. 2001. Phase equilibria of water in cylindrical nanopores. *Phys. Chem. Chem. Phys.* 3: 1567–1569.
7. Saparov, S. M., J. R. Pfeifer, L. Al-Momani, G. Portella, B. L. de Groot, et al.. 2006. Mobility of a one-dimensional confined file of water molecules as a function of file length. *Phys. Rev. Lett.* 96:148101.
8. Portella, G., P. Pohl, and B. L. de Groot. 2007. Invariance of single-file water permeability in gramicidin-like peptidic pores as function of pore length. *Biophys. J.* 92: 3930–3937.
9. Zhu, F. Q., E. Tajkhorshid, and K. Schulten. 2004. Collective diffusion model for water permeation through microscopic channels. *Phys. Rev. Lett.* 93:224501.