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Computing osmotic permeabilities of aquaporins AQP4, AQP5, and GlpF from near-equilibrium simulations

Thierry O. Wambo, Roberto A. Rodriguez, Liao Y. Chen *

Department of Physics, University of Texas at San Antonio, San Antonio, TX 78249, USA

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ABSTRACT

Measuring or computing the single-channel permeability of aquaporins/aquaglyceroporins (AQPs) has long been a challenge. The measured values scatter over an order of magnitude but the corresponding Arrhenius activation energies converge in the current literature. Osmotic flux through an AQP was simulated as water current forced through the channel by kilobar hydraulic pressure or theoretically approximated as single-file diffusion. In this paper, we report large scale simulations of osmotic current under sub M gradient through three AQPs (water channels AQP4 and AQP5 and glycerol-water channel GlpF) using the mature particle mesh Ewald technique (PME) for which the established force fields have been optimized with known accuracy. These simulations were implemented with hybrid periodic boundary conditions devised to avoid the artifactitious mixing across the membrane in a regular PME simulation. The computed single-channel permeabilities at 5 °C and 25 °C are in agreement with recently refined experiments on GlpF. The Arrhenius activation energies extracted from our simulations for all the three AQPs agree with the *in vitro* measurements. The single-file diffusion approximations from our large-scale simulations are consistent with the current literature on smaller systems. From these unambiguous agreements among the *in vitro* and *in silico* studies, we observe the quantitative accuracy of the all-atom force fields of the current literature for water-channel biology. We also observe that AQP4, that is particularly rich in the central nervous system, is more efficient in water conduction and more temperature-sensitive than other water-only channels (excluding glycerol channels that also conduct water when not inhibited by glycerol).

1. Introduction

Aquaporins/aquaglyceroporins (AQPs) [1–16], the water/glycerol channel proteins, are fundamental and ubiquitous in living organisms. Naturally, these membrane proteins have been investigated in a great many experimental and theoretical-computational studies, *e.g.*, Refs. [4,16–34] on GlpF and AQPZ expressed in *E. coli*, Refs. [8,35–77] on AQP4, and Refs. [38,70,78–86] on AQP5. An essential task in the computational studies of aquaporins is to compute the channel permeability as the ratio between the osmotic current and the osmolyte concentration gradient that induces the water flux through the AQP channel, in direct parallel to the experimental measurements. Due to technical difficulties in the numerical implementation of osmotic flux induced by a sub M concentration gradient, a few studies [87,88] have been accomplished to substitutionally compute the permeability from the water flux induced by kilobar hydraulic pressure. Due to the large pressure gradient across the membrane, these studies leave open the question about far-out-of-equilibrium effects that are absent under physiological conditions. Many computational studies (*e.g.* Refs. [17,22,50,51,57,79,89–91]) have been conducted on the basis of the theoretical approximation of single-file diffusion [88,91,92]. The accuracy of these single-file diffusion approximations has not been ascertained because the *in vitro* measurements of single-channel permeability have proven to be very challenging as well. In fact, the experimental data on a given AQP scatter over the range of an order of magnitude. Recent experimental investigations have given us some converging data on some AQPs including GlpF [16] and AQP4 [37]. In contrast to the scatter of the absolute values of permeabilities, certainty has been consistent in the *in vitro* measurements of the Arrhenius activation barrier which is rather independent of the membrane-protein expression levels and of the temperatures of the experiments. AQP1, AQP5, AQPZ and GlpF (if not inhibited by glycerol [17]) were all measured to have an Arrhenius activation barrier around 3 kcal/mol. Interestingly, AQP4 was measured to have about 5 kcal/mol, indicating this particular water channel is far more temperature-sensitive than others. (Namely, AQP4's permeability increases more than other aquaporins when the temperature is elevated.) However, theoretical-computational studies predicted <3 kcal/mol for all cases including AQP4 in the current literature.

In this paper, we present a computational study of two water-specific channels (AQP4 and AQP5) and one water-glycerol channel (GlpF) at

* Corresponding author at: Department of Physics, University of Texas at San Antonio, One UTSA Circle, San Antonio, TX 78249, USA.
E-mail address: Liao.Chen@utsa.edu (L.Y. Chen).

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two temperatures (5 °C and 25 °C) in direct correspondence to the *in vitro* experiments. We conducted large-scale simulations of all-atom model systems in which the signal-to-noise ratios were sufficiently high to achieve unambiguous accuracy. We computed the osmotic permeability under near-physiological conditions directly as the osmotic water-current divided by the osmolyte-concentration gradient, achieving close agreement with the latest refined experimental data. We also conducted single-file diffusion approximations in all six cases (three AQPs at two temperatures) which are consistent with the current literature on smaller systems, indicating single-file diffusion approximations are not quantitatively accurate. From the temperature-dependence of the permeabilities, we extracted the Arrhenius activation barriers for the three AQPs that are all in excellent agreement with the *in vitro* results. From the differentiation between the dynamic characteristics of AQP4 and AQP5, we gained atomistic insights (structures and fluctuations) about why AQP4 is preferable for maintaining hydrohomeostasis of the central nervous system.

2. Methods

Our main objective is to achieve clear signal-to-noise ratios in direct computations of the water flux induced by a sub M osmolyte concentration gradient across the membrane. To achieve this goal, we build model systems four times as large as those in the current literature. Each model system consists of four tetramers (16 water channels) of either of the three proteins embedded in a large patch of lipid bilayer representing the cellular membrane. We compute the osmotic permeability under near-physiological conditions directly as the osmotic water-current divided by the osmolyte-concentration gradient. We employ a hybrid periodic boundary conditions (PBC) for a particle mesh Ewald (PME) implementation with the CHARMM force field [93]. In the hybrid PBC scheme, images of the system volume (cell) are arranged periodically in all three dimensions as in a usual PBC setup, but a rigid plane/wall parallel to the membrane is placed at the top/bottom of the system volume to eliminate the artifactitious mixing between the aqueous volumes on the two sides of the membrane that is intrinsic in the usual PBC scheme. In this manner, we are able to maintain, for a sufficient period of time, a constant osmotic flux through 16 aquaporin channels induced by a sub M concentration gradient across the membrane. In fact, during the simulation time of 40 ns, the osmolyte concentration gradient decreased by <0.5% in all cases.

Shown in Fig. 1 is the all-atom model system of GlpF (PDB code: 1FX8 [30]) which consists of four GlpF tetramers (16 individual monomers/channels) embedded in a patch of phosphatidylethanolamine (POPE C16:0C18:1) lipid bilayer with a layer of saline of higher concentration on the top side ($z > 0$) and a layer of saline of 150 mM on the bottom side ($z < 0$). The Cartesian coordinates are set up so that the xy-plane is parallel to the lipid bilayer. The usual PBCs are implemented in all three dimensions but the interfaces parallel to the xy-plane between the system and its images along the z-direction are impenetrable preventing the artificial mixing between the two sides of the lipid bilayer. An atom approaching the top and the bottom interfaces will be elastically reflected, namely, the z-component of its velocity will be inverted but the xy-components will remain unaltered. The images along the xy-directions are treated in the usual way of PBC.

In order to lower computing costs, we first built a small patch (1/4 of the membrane area of the large system) with one tetramer embedded in the lipid bilayer which is sandwiched between two layers of 150 mM saline. We equilibrated the system for 100 ns with equilibrium molecular dynamics (MD) under constant temperature and constant pressure. We replicated the fully equilibrated system thrice in the xy-plane and patched them together to form the large system that has four times the membrane area of the small system. Then we added additional NaCl to the top side of the system to established an osmolyte concentration gradient across the membrane. In similar manners, we built up the model systems of AQP4-M1 (PDB code: 3GD8 [8]) and AQP5 (PDB code: 3D9S [82]), which are illustrated in Figs. S1 and S2 of the supplemental information (SI). Conducting 40 ns MD runs for each of the six systems (three systems at two different temperatures), we counted the number of waters on the top side ($z > 0$) as a function of time and used linear regression to extract the osmotic flux across the membrane through the aquaporin water pores. For the purpose of

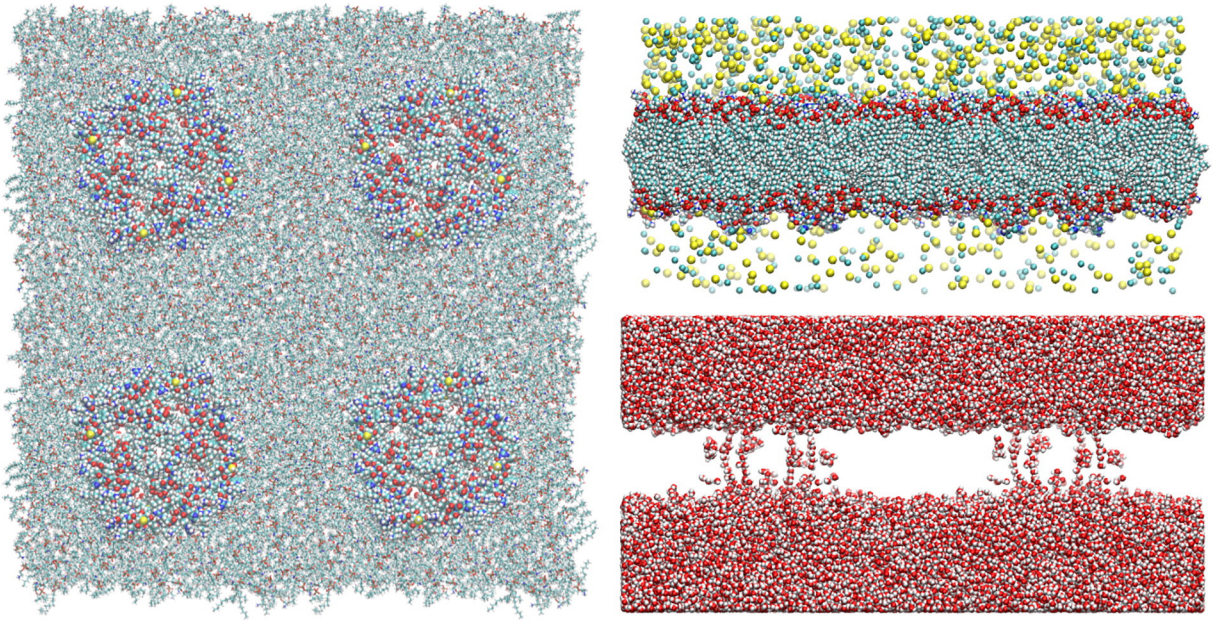

Fig. 1. Model system of GlpF at 5 °C. Shown in the left panel are the top view of four GlpF tetramers (16 individual water channels/monomers) embedded in a patch of POPE lipid bilayer. The proteins are shown as spheres and the lipids as licorices, all colored by atom names. Shown in the right panels are the side views of the GlpF model system. Top, lipids, proteins, and ions (Na^+ and Cl^-) are all shown as spheres colored by atom names. Bottom, waters are shown as spheres colored by atom names. Colors by atom names: C, cyan; N, blue; H, white; O, red; Na^+, light yellow; Cl^-, green; S, yellow; P, red. The fully equilibrated system of GlpF has the dimensions of 228A × 229A × 112A. It consists of 601,548 atoms. This and other model systems were built and the molecular graphics were rendered with VMD [94].