SI Appendix

Protein Modeling. Coordinates for aquaporin monomers in the junctional form ($AQP0_J$) and non-junctional form ($AQP0_{NJ}$) were obtained from PDB entries 2B6O and 2B6P/1TM8, respectively (1, 2). VMD (3) was used to add atoms missing from the PDB structures, including hydrogens, the gamma oxygen in the Ser6 residue of $AQP0_J$, and the Pro36 residue of $AQP0_{NJ}$. Pro36 was introduced into $AQP0_{NJ}$ by aligning residues Ala35 and Gly37 with the corresponding residues in $AQP0_J$ and modeling the Ala35-Pro36-Gly37 sequence into $AQP0_{NJ}$, retaining the original coordinates of the two flanking residues of the sequence. Tetrameric forms of $AQP0_J$ and $AQP0_{NJ}$ ($AQP0^4_J$ and $AQP0^4_{NJ}$, respectively) were generated by applying the transformation matrices in the PDB files to protein monomers and all resolved water molecules. All titratable amino acids were modeled assuming standard protonation states at pH = 6.5.

Membrane Embedding. Initially $AQP0^4_J$ was embedded into a pre-equilibrated, fully hydrated 16:0/18:1c9-palmitoyloleoyl-phosphoethanolamine (POPE) lipid bilayer. Prerequisite equilibration of the pure bilayer was performed at constant pressure (P = 1 bar) and temperature (T = 300 K) for 60 ns using a system of 340 lipid molecules and 10,305 waters. An equilibrated membrane resulted after approximately 20 ns of simulation with an average area of 51.8 ± 0.1 Å^2. The pre-equilibrated bilayer was replicated laterally to accommodate $AQP0^4_J$ and all resolved water molecules, such that the final system included 400 lipid molecules. To ensure overall neutrality, 32 counterions were added using GROMACS (4, 5). A 5 Å wide spacing between lipid and protein was imposed in order to avoid initially unfavorable protein lipid contacts. Initially, the system measured 151.4 × 151.6 × 80.3 Å^3. POPE-embedded $AQP0^4_J$ was minimized using GROMACS (4, 5) with protein atoms fixed and then equilibrated under *NPT* conditions (N = 134,683 atoms, P = 1 bar, T = 310 K). A long equilibration time of ~30 ns was imposed in order to ensure equilibration of the bilayer and appropriate establishment of protein-lipid contacts. This was followed by 170 ns of production *NPT* simulation with P = 1 bar and T = 310 K.

$AQP0^4_{NJ}$ was substituted into pre-equilibrated $AQP0^4_J$/POPE, replacing protein and experimentally resolved waters of $AQP0^4_J$ with those of $AQP0^4_{NJ}$. $AQP0^4_{NJ}$ was oriented by superimposing protein C_α atoms of residues 5–235 onto those of $AQP0^4_J$. The non-cleaved C-terminus (residues 240 to 263) present in $AQP0^4_{NJ}$ protrudes more into the water than the cleaved C-terminus of $AQP0^4_J$ and we therefore added another 200 water molecules. In order to ensure overall neutrality the number of negative counter charges was adjusted from 32 for $AQP0^4_J$ to 20 for $AQP0^4_{NJ}$. Lipid-embedded $AQP0^4_{NJ}$ (N = 136,863 atoms) underwent an initial equilibration period of 2 ns, followed by 200 ns of production *NPT* simulation with P = 1 bar and T = 310 K.

Membrane Junction Modeling. To construct the simulated octameric junction ($AQP0^8_J$), $AQP0^4_J$, pre-equilibrated in a POPE bilayer was duplicated and one copy was rotated 180° along one of the two in-plane axes of the bilayer. Subsequently, the two copies were fused together. The intermembrane spacing was dictated by superimposing all protein C_α atoms of the two copies of $AQP0^4_J$ onto the C_α atoms of the native junction as specified in PDB entry 2B6O (1). Overlapping water molecules within the intermembrane region were deleted.

Protein and crystal water molecules were fixed during an initial minimization and during subsequent equilibration, involving ~0.4 ns of *NPT* simulation (N = 225,089 atoms, P = 1 bar,

T = 310 K). Protein and crystal water molecules were then released and another minimization was performed, followed by 3 ns of simulation in which harmonic potentials restrained the center of mass of each tetramer to the overall center of mass of the octamer such that the two tetramers maintained their mutual contacts. Finally, 110 ns of production *NPT* simulation was performed with P = 1 bar and T = 310 K.

Permeability Computation. We computed the osmotic permeability constant (p_f) from our equilibrium simulations using the method of Zhu et al. (6). Specifically, we defined a collective diffusion coordinate $n(t)$ capturing the cumulative displacement along the channel axis (z) of all water molecules in the lumen of one monomer. We computed $n(t)$ as the time integral of the instantaneous displacement $\mathrm{d}n(t) = 1/L(t)\sum_i z_i(t) - z_i(t+\delta t)$, where $L(t)$ is the instantaneous length of the lumen (taken for each monomer as the region between Arg187:N_ε and Ala65:O; see Fig. 2*A* and Table 1), $z_i(t)$ and $z_i(t+\delta t)$ are the z coordinates of the i^{th} water molecule at two successive time steps, and the sum is over all water molecules found in the lumen at time t. We then computed $p_f = v_w D_n$, where the diffusion coefficient D_n is derived from $\langle n^2(t) \rangle = 2D_n t$, and v_w is the volume of a single water molecule. This approach has been shown to produce accurate results for other aquaporins (7, 8, 9).

In a pair of interlocked junctional monomers, the flow of water through each lumen is coupled to the flow of water through the intermembrane cavity. Over sufficiently long time periods, this coupling will reduce the diffusion of water through each region, and hence the computed osmotic permeability of each region, resulting in an underestimation of the osmotic permeabilities of the lumens and the cavity. We verified, however, that over the time period of the simulations presented here, the flows through the two lumens exhibited no detectable correlation with one another, such that the effect on our estimates of the lumenal permeabilities should be very small. Similarly, the water flow through each lumen exhibited little correlation with water flow through the cavity. Even though the restricting effect of the adjacent lumens biases our estimated value for the cavity permeability toward a lower value than the actual permeability of an unrestricted cavity, this biasing effect should be minor.

The diffusive permeability constant (p_d) was calculated as $p_d = v_w k_o$, where k_o is the average rate at which water molecules permeate the lumen of a monomer in one direction.

Figure S1. Sequence analysis of AQP0

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                      23     149
                       |      |
AQP0  Human          LFYVF  ATYDE   P30301 H. sapiens
      Sheep          LFYVF  ATYDE   Q6J8I9 O. aries
      Cow            LFYVF  ATYDE   P06624 B. taurus
      Mouse          LFYVF  ATYDE   P51180 M. musculus
      Chicken        LLYTL  ASFDD   P28238 G. gallus
      Frog           MFYVF  ATYDE   Q52KZ5 X. laevis
      Zebrafish      MFFVF  AVTDE   Q6DEI6 D. rerio
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AQP6 Human        GLYVF  ASTDS   Q13520 H. sapiens
AQP1 Human        TLFVF  ATTDR   P29972 H. sapiens
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The lumen-protruding residues Tyr23 and Tyr149 (bovine AQP0 numbering) are conserved in slow-conducting aquaporins. Tyrosine 23 is conserved in all known mammalian, avian, and amphibian AQP0s, and tyrosine 149 is conserved in most. The anion-selective channel AQP6, which also exhibits anomalously slow water conduction, conserves Tyr23 as well. Tyr23 and Tyr149 are not conserved in fish AQP0 nor in a prototypical fast-conducting aquaporin, AQP1. Swiss-Prot/TrEMBL accession numbers follow each sequence (http://www.expasy.org).
Note that human, bovine and ovine AQP0 numbering are equivalent; AQP0 from each species has 263 residues and sequence alignment of these AQP0s has no gaps.

Derivation of Cavity Pressures and the Cavity Pressure Difference. The AQP0 junction can be approximated as four head-to-head interlocked monomers (see Figure S1) each contributing a junctional channel through which water permeates when crossing the junction. Driven by the pressure difference $\Delta P \equiv P_1 - P_2 > 0$ where P_1 and P_2 are pressures at the two ends of the junctional channel, each water flow is given by $J = p_f \Delta P/(RT)$ where R and T are the gas constant and temperature. p_f is the overall osmotic permeability constant of the junctional channel $1/p_f = 2/p_{f,\mathrm{lum}} + 1/p_{f,\mathrm{cav}}$ encountered as water flows across three regions; two luminal (*lum*) and one cavity (*cav*) region. This implies a total pressure difference $\Delta P = \left(2/p_{f,\mathrm{lum}} + 1/p_{f,\mathrm{cav}}\right) J$ across the interlocked monomers (and the junction). With the pressure at the two ends of the intermonomeric cavity defined as $P_{1,\mathrm{cav}}$ and $P_{2,\mathrm{cav}}$ where $P_{1,\mathrm{cav}} > P_{2,\mathrm{cav}}$, the pressure difference across the cavity is $\Delta P_{\mathrm{cav}} \equiv P_{1,\mathrm{cav}} - P_{2,\mathrm{cav}} = 1/p_{f,\mathrm{cav}} J$ (see Figure S1). Since steady state water flux is same everywhere in the channel $J \equiv J_{1,\mathrm{lum}} = J_{\mathrm{cav}} = J_{2,\mathrm{lum}}$ we obtain:

$$RT(J_{1,\mathrm{lum}} - J_{\mathrm{cav}}) = p_{f,\mathrm{lum}}\left(P_{1,\mathrm{cav}} - P_1\right) - p_{f,\mathrm{cav}}\left(P_{2,\mathrm{cav}} - P_{1,\mathrm{cav}}\right) = 0$$

and

$$RT(J_{\mathrm{cav}} - J_{2,\mathrm{lum}}) = p_{f,\mathrm{cav}}\left(P_{2,\mathrm{cav}} - P_{1,\mathrm{cav}}\right) - p_{f,\mathrm{lum}}\left(P_2 - P_{2,\mathrm{cav}}\right) = 0.$$

Solving for $P_{1,\mathrm{cav}}$ and $P_{2,\mathrm{cav}}$ we have:

$$P_{1,\mathrm{cav}} = P_1 - (P_1 - P_2)\frac{p_{f,\mathrm{rel}}}{2p_{f,\mathrm{rel}} + 1} \text{ and } P_{2,\mathrm{cav}} = P_2 - (P_2 - P_1)\frac{p_{f,\mathrm{rel}}}{2p_{f,\mathrm{rel}} + 1}, \text{ with } p_{f,\mathrm{rel}} = \frac{p_{f,\mathrm{cav}}}{P_{f,\mathrm{lum}}}.$$

and for the cavity pressure difference:

$$\Delta P_{\mathrm{cav}} = P_{1,\mathrm{cav}} - P_{2,\mathrm{cav}} = \left(P_1 - P_2\right)\left(\frac{1/p_{f,\mathrm{cav}}}{2/p_{f,\mathrm{lum}} + 1/p_{f,\mathrm{cav}}}\right) = \Delta P\left(\frac{1}{2p_{f,\mathrm{rel}} + 1}\right).$$

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