Electronic Supplementary Information

Regulation of Aquaporin-3 Water Permeability by Hyaluronan

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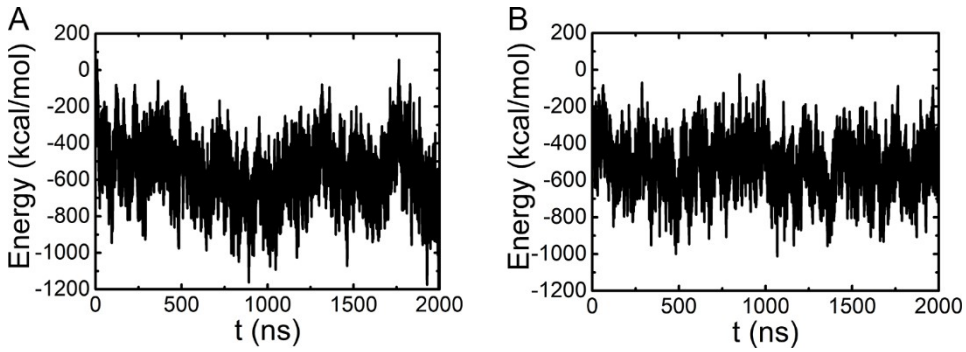

Fig. S1 Variation of interaction energies of HA and AQP3-membrane along the 2-μs trajectories for (A) large molecular HA, and (B) small molecular HA.

Key residues. As described in Fig. S2, we analyzed the key residues of AQP3 responsible for the adsorption in the last 100 ns trajectory of parallel simulations. Residues R50, H129, F130, A131, D132, V137, S138, G139, P140, N141, Y150, H154, A234, T237 and T238 constitute the adsorbed residues of AQP3 for large molecular HA. Residues F136, S138, G139, P140, Y150, G153, H154, T237, T238, G239 and Q240 constitute the adsorbed residues of AQP3 for small molecular HA. These results can infer that the adsorption behavior of HA on AQP3 surface is mainly related to the hydrophilic residues.

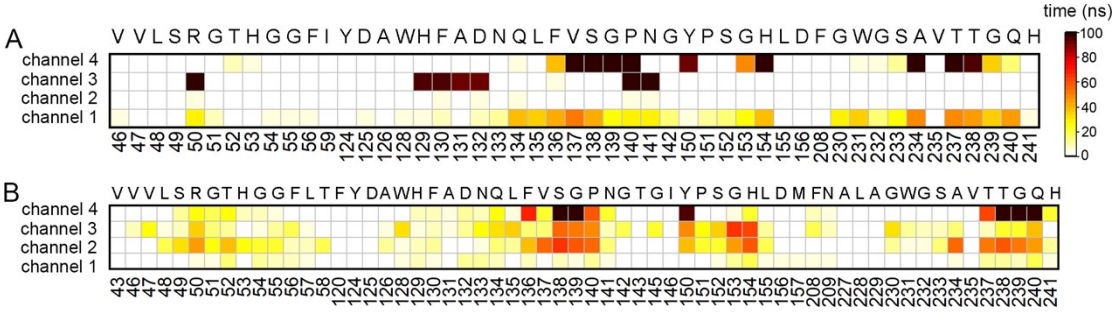

Fig. S2 Residues of AQP3 interacting with (A) large and (B) small molecular HA polymers are colored in terms of the residence time of HA molecules on the surface of AQP3, obtained from the last 100 ns of each 1-μs parallel MD trajectory.

Analysis of residue H154. Extracellular loop C plays a significant role in regulating the permeability of AQP3. According to the previous study,[1,2] the residue H154 on loop C has been regarded as one of important residues related to the gating of AQP3. For the open state of AQP3, the backbone of H154 forms a H-bond with the backbone of S152, and the H154 side-chain forms a H-bond with the side-chain of H129. The break of these H-bond network or mutation of H154 can decrease the water permeability.[1,2] In our present study, both small and large HA molecules have been found to interact with H154. In addition, H-bonds have been found between H154 and HA molecules (see Fig. S3 and S4), which can weaken the original H-bond network and hence affect the gating function of H154. Therefore, we suggested that the interaction between HA molecules and H154 can hamper the function of the latter, which can decrease the water permeability.

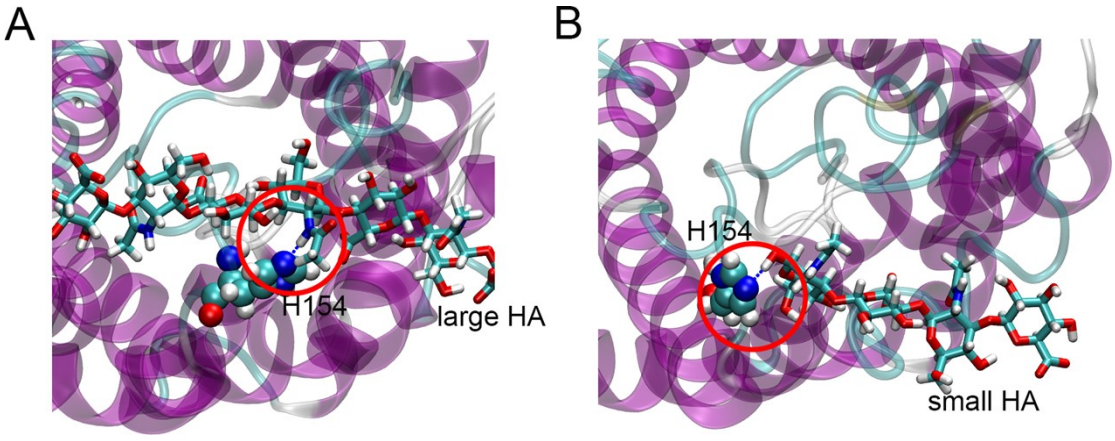

Fig. S3 Structure details of the binding mode between (A) large and (B) small HA molecules with residue H154 on AQP3. The H-bonds are shown within red circle.

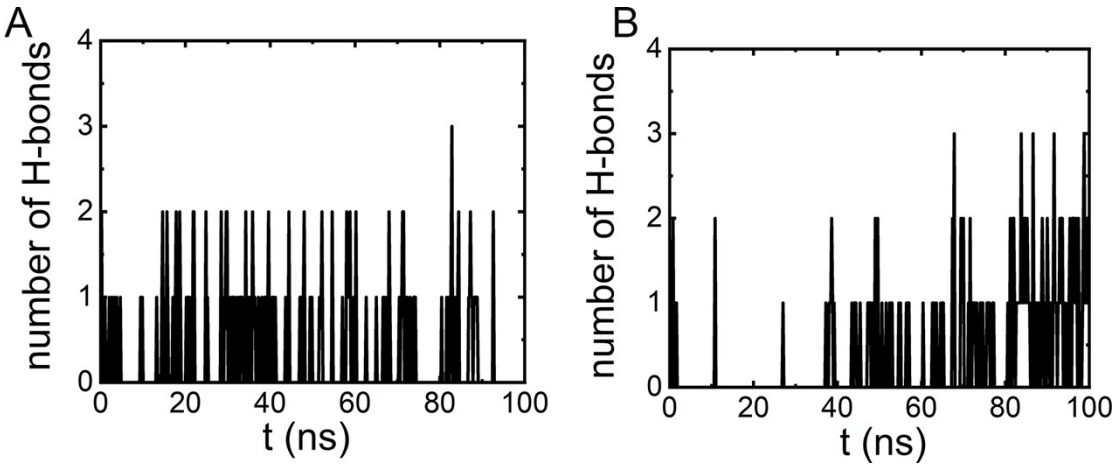

Fig. S4 Number of H-bonds between (A) large and (B) small HA molecules with residue H154 of AQP3, obtained from the last 100 ns of each 2-μs MD trajectory.

The number of water molecules in the cytoplasm environment. As shown in Fig. S3, we found that the number of water molecules in the cytoplasm decreases in the absence of HA, while that remains almost constant in the presence of HA. In addition, we found that its fluctuation is larger for the large molecular HA than the smaller one.

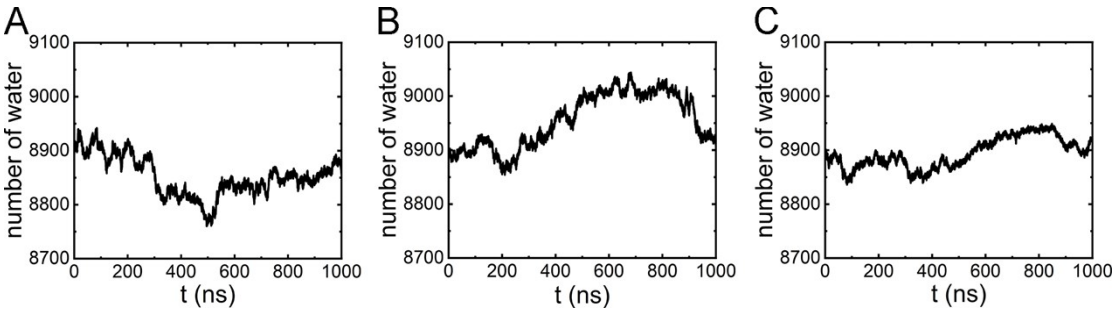

Fig. S5 Variation of the number of water molecules in the cytoplasm environment for (A) no HA, (B) large molecular HA, and (C) small molecular HA.

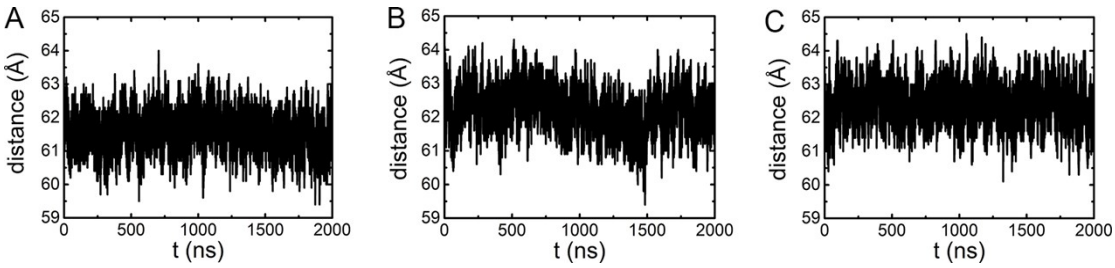

Fig. S6 Variation of distance between the two membranes along the 2-μs trajectories for (A) no HA, (B) large molecular HA, and (C) small molecular HA.

Effects of salt ions. In order to reveal the effects of salt ions on the HA molecules in regulating water permeation, we have performed a new simulation with small molecular HA for 1 μs, in which the 150 mM KCl has been removed. The key residues of AQP3 responsible for the adsorption of HA molecules have been determined. As shown in Fig. S7, the absence of ions does not affect the longstanding interaction of the HA molecules with the AQP3 surface. In addition, we analyzed the variation of the number of water molecules in the cytoplasm environment (see Fig. S8). The fluctuation remains within 200 water molecules (see Fig. S8), which is in agreement with that in the 150 mM KCl environment (see Fig. 5 and S5). In addition, the rate of transmembrane diffusion of water in the absence of ions along the last 400 ns trajectory has been calculated, namely, 29.67 and 29.80 water molecules per nanosecond for entering and exporting the cell, respectively. These values are similar to that in the presence of ions for small molecular HA (see Table 1). Put together, we concluded that the ions have no obvious effects on the HA molecules in regulating water permeation.

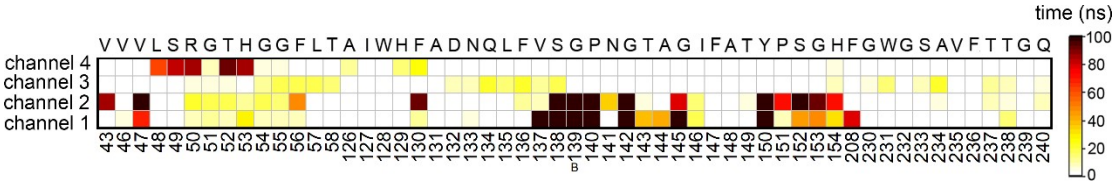

Fig. S7 Residues of AQP3 interacting with small molecular HA polymers in the absence of additional ions are colored in terms of the residence time of HA molecules on the surface of AQP3, obtained from the last 100 ns of the 1-μs MD trajectory.

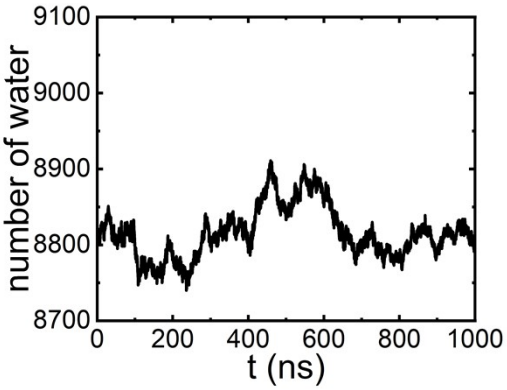

Fig. S8 Variation of the number of water molecules in the cytoplasm environment for small molecular HA without additional ions.

Reference

1. M. Zelenina, A. A. Bondar, S. Zelenin, A. Aperia, Nickel and Extracellular Acidification Inhibit the Water Permeability of Human Aquaporin-3 in Lung Epithelial Cells. *J. Biol. Chem.* 2003, **278**, 30037–30043.
2. A. de Almeida, A. P. Martins, A. F. Mósca, H. J. Wijma, C. Prista, G. Soveral, A. Casini, Exploring the Gating Mechanisms of Aquaporin-3: New Clues for the Design of Inhibitors? *Mol. Biosyst.* 2016, **12**, 1564–1573.