Supporting Material

Population shift between the open and closed states changes the water permeability of an aquaporin Z mutant

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Materials and Methods:

Modeling and MD simulations**:** Simulations were performed for AqpZ triple mutant F43W/H174G/T183F tetramers embedded in palmitoyloleyl phosphatidylcholine (POPC) lipid bilayers. The AqpZ triple mutant tetramer from the crystal structure (PDB ID: 3NK5) were incorporated into POPC bilayers. The initial POPC equilibrated structure was downloaded from the Tieleman group, http://moose.bio.ucalgary.ca/index.php?page=Structures_and_Topologies. For the purpose of protein insertion, a hole was created in the centre of pre- equilibrated POPC lipid bilayers with the size of 1.2 nm*1.2 nm. The triple mutant tetramer was placed into the hole by aligning the center of the mass of the protein with the geometric center of the hole. The system consists of 4 mutant monomers, 330 POPC and 23172 TIP3P(1) water molecules in a simulation box size of approximately 12nm*12nm*9nm. MD simulations were carried out using GROMACS version 4.5.4 (2) with the CHARMM 27 force field (3-4). The systems were initially minimized and equilibrated for 1 ns with protein heavy atoms restrained and then subjected to longer NPT (T = 300 K, P = 1 atm) simulations. The system was coupled to external heat bath T =300k with the implementation of velocity rescaling algorithm(5). The system pressure was maintained at 1 atm with Berendsen anisotropic coupling scheme(6). Totally, 2*120 ns and 6*50 ns simulation has been carried out with different random initial velocities. In all simulations the periodic boundary conditions were applied and the particle-mesh Ewald method (7) with real space cutoff of 0.9 nm for electrostatic potential calculation. The Lennard-Jones interactions were switched off beyond the range of 1.2 nm. An integration step of 2 fs was used, and the simulated structures of the system were recorded every 1 ps.

Initially 2*120 ns simulations have been carried out starting from the same equilibrated system with different random initial velocities. In these two simulations, some distinct phenomena have been observed only once or twice, such as the channel opening events. To get a better statistics of those events, another 6 simulations starting from the same equilibrated system have been performed with each of them lasting 50 ns. The water channel profiles and the minimum radius of channels were calculated by the HOLE program(8).

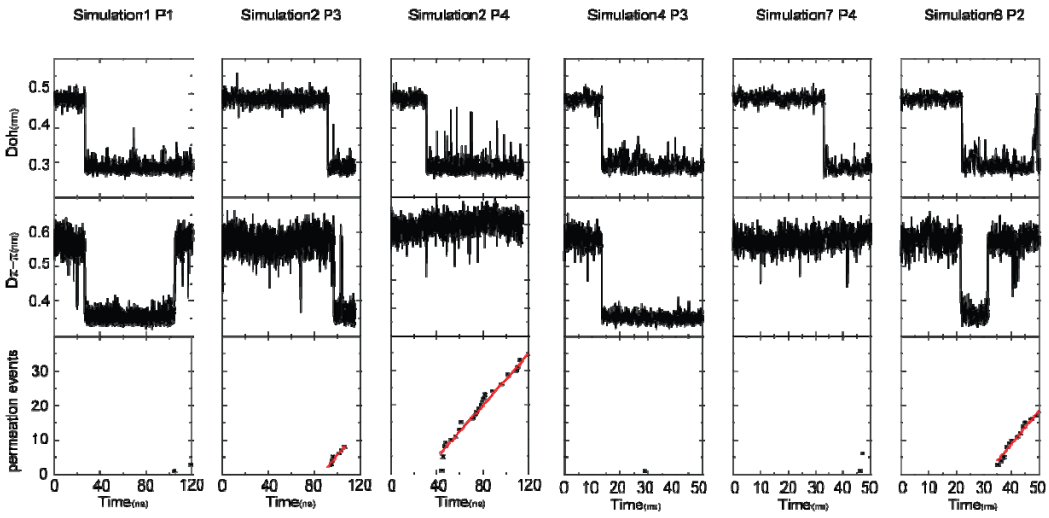

Figure S1 Doh as function of time (top panels); Distance between F183 and W43 ring as function of time (mid panels); Water permeation event as function of time (lower panels).

In the 5th column, the channel is open for around 15 ns, and the recorded permeation events is 6. Compared to Column 3, which is open for 80 ns , the permeation is about 35. Their permeation rates are very close.

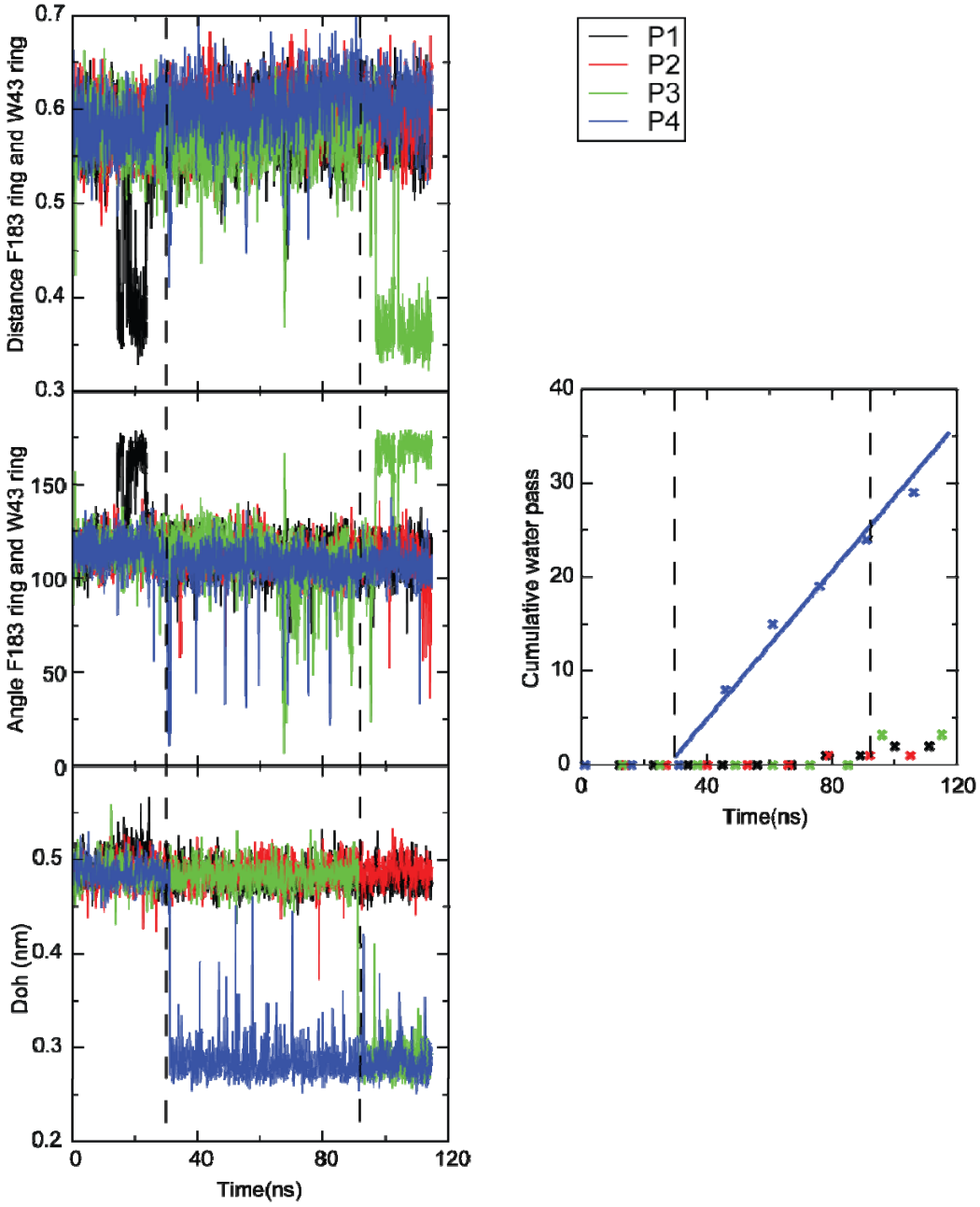

Figure S2. Distance between F183 and W43 ring as function of time (left panel top); angle between F183 and W43 ring as function of time (left panel middle); Doh as function of time (left panel down); water permeation event as function of time (right panel). P1-4 denote 4 single channels in the simulated tetramer.

We investigated this by monitoring the distance (D_{WF}) between the centers of mass of W43 methylindole ring and F183 toluene ring together with the angle (χ_{WF}) between the two ring-plane normal vectors as functions of time and this is shown in Fig. S2. The time evolutions of

these two quantities are found to have interesting relationship with the water permeation events.

In trajectory 2 P1 and P2 channels remained closed with D_{oh} larger than 0.45 nm, consistent with our previous study. During the simulation, in P1 between 18 ns and 23 ns, W43 and F183 side chain rings aligned together as indicated by a small D_{WF} around 0.35 nm and almost a parallel placement of the rings (χ_{WF} around 180º) which suggests a stacked π-π interaction. For P3, around t=95 ns D_{oh} decreased below 0.45 nm; there was a finite number of water permeation events. Shortly after, around t=96 ns, a stable ring stacking was observed with D_{WF} around 0.35 nm and χ_{WF} around 180º, no more water permeation events happened in this channel. For P4 after 30 ns D_{oh} became small and no ring stacking was observed throughout the simulation, water passed the channel constantly, in a typical open state.

SUPPORTING REFERENCES

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