Structure, Volume *28*

Supplemental Information

Structural Basis for Glycerol Efflux and Selectivity of Human Aquaporin 7

Sofia W. de Maré, Raminta Venskutonytė, Sandra Eltschkner, Bert L. de Groot, and Karin Lindkvist-Petersson

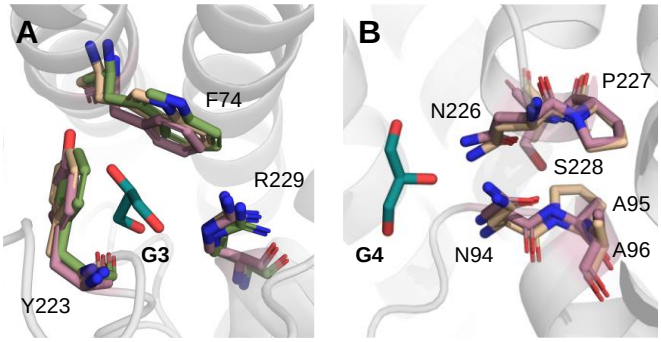

Figure S1. Superposition of AQP7, GlpF (pdbid: 1fx8) and PfAQP (pdbid: 3c02), related to Figure 1. (**A**) The ar/R selectivity filter consisting of Phe74, Tyr223 and Arg 229 in AQP7. In GlpF and PfAQP the phenylalanine is replaced by tryptophans and the tyrosine is replaced by phenylalanines. (**B**) The NPA-motifs consisting of Asn94, Ala95, Ala96 and Asn226, Pro227, Ser228 in AQP7. Residues are shown as sticks, AQP7 is in pink, GlpF in wheat, PfAQP in green and glycerol molecules in teal with AQP7 backbone represented by cartoon drawing in gray.

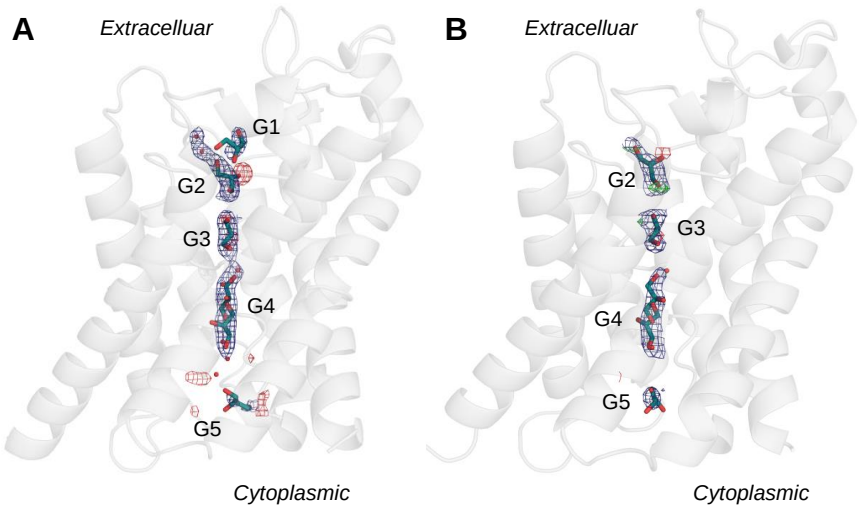

Figure S2. Glycerol and water molecules in the conducting pore of AQP7 with corresponding electron density, related to Figure 2. (**A**) Five glycerol molecules (G1-G5) were modeled into the density of the 1.9 Å structure. (**B**) Four glycerol molecules (G2-G5) were modeled in the 2.2 Å structure. Glycerol molecules are shown as sticks (teal), water molecules as spheres (red) in cartoon representation of AQP7 (gray). 2Fo-Fc (blue) and FoFc (green/red) electron density maps contoured at 1 sigma and 3 sigma, respectively.

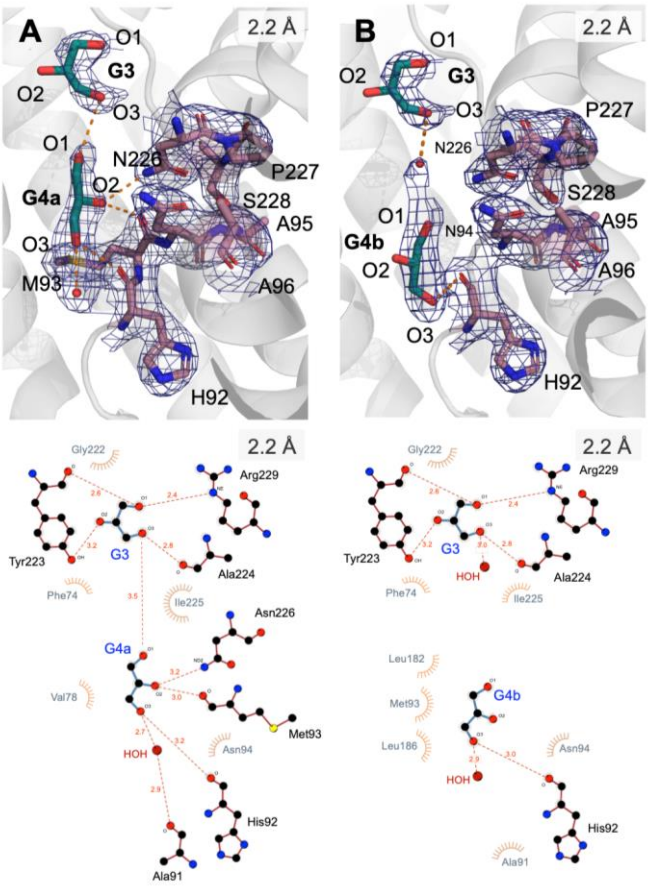

Figure S3. The hydrogen bond network of glycerol and water molecules in the conducting pore of AQP7 in the lower-resolution structure, related to Figure 2. (**A-B**) Glycerol G4 in two alternative positions in the NPA-region of the lower-resolution structure. Stick-representation of the residues (pink) and glycerol (teal) with the backbone represented by cartoon drawing (gray). Dashed lines indicate hydrogen bonds within 3.3 Å. Lower panels show the hydrogen bond network of water and glycerol in 2D representation. 2Fo-Fc electron density maps contoured at 1 sigma.

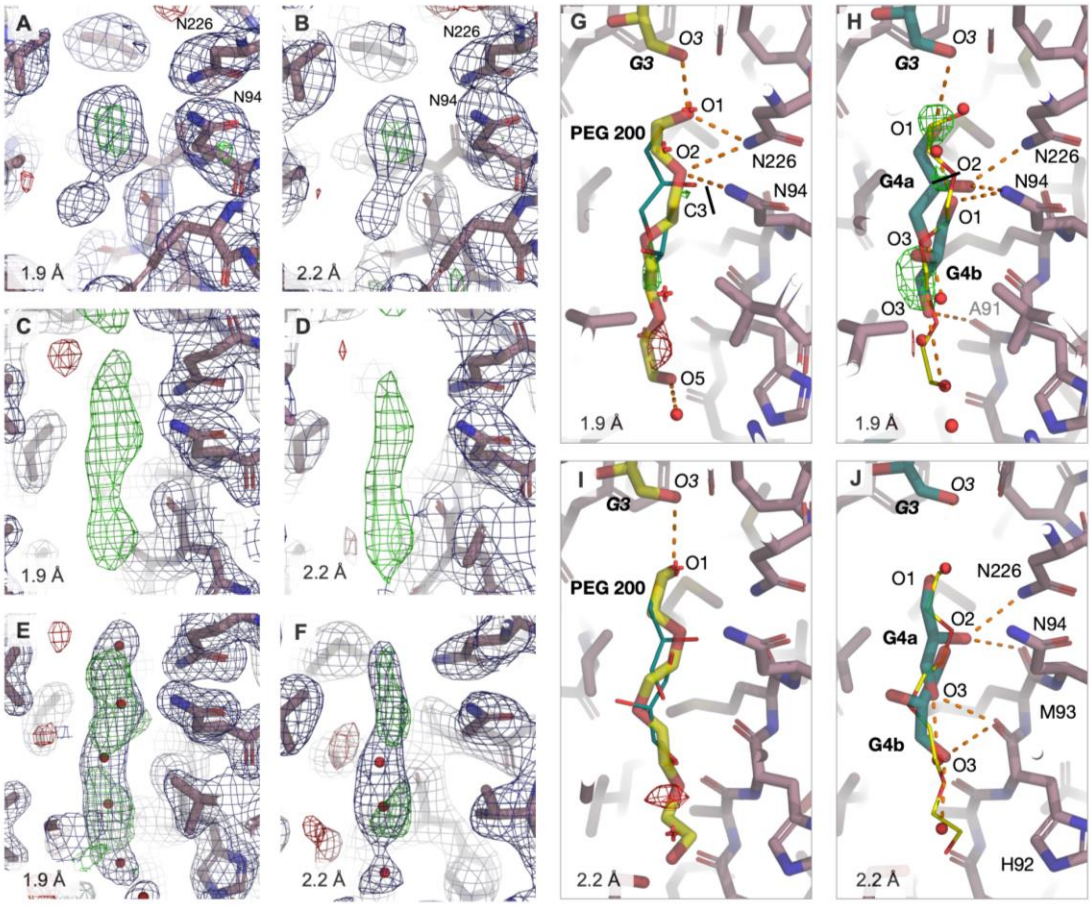

Figure S4. Comparison of two alternative glycerol molecules versus PEG 200 in the pore, related to STAR Methods. (**A-F**) Electron densities in the NPA region for both data sets (1.9 Å and 2.2 Å) after molecular replacement with Phaser-MR (**A** and **B**), after structure rebuilding with AutoBuild (**C** and **D**), and after initial refinement with phenix.refine with water molecules automatically added during the refinement (**E** and **F**). Protein is shown as sticks (pink) and water molecules as spheres (red). 2Fo-Fc (blue) and Fo-Fc (green/red) electron density maps contoured at 1 sigma and 3 sigma, respectively. (**G-H**) PEG 200 and glycerol modeled in the 1.9 Å structure. Potential hydrogen bonds shown as dashed lines (orange) and Fo-Fc electron density map (green/red) contoured at 2.2 sigma. (**G**) Structure refined with PEG 200. The PEG 200 molecule in the selectivity filter shown as sticks (yellow). Glycerol and water molecules are superimposed, represented by lines (teal) and non-bounded wire (red), respectively.

A clash between C3 of PEG 200 and Asn94 is indicated by black line. (**H**) Structure refined with glycerol and water molecules. Glycerol molecules shown as sticks (teal) and water molecules as spheres (red). PEG 200 is superimposed, represented by lines (yellow). A clash between G4b and adjacent water molecule is indicated by black line. (**I-J**) PEG 200 and glycerol modeled in the 2.2 Å structure. Potential hydrogen bonds within 3.3 Å shown as dashed lines (orange) and Fo-Fc electron density map (green/red) contoured at 2.4 sigma. (**I**) Structure refined with PEG 200. The PEG 200 molecule in the selectivity filter shown as sticks (yellow). Glycerol and water molecules are superimposed, represented by lines (teal) and non-bounded wire (red), respectively. (**J**) Structure refined with glycerol and water molecules. Glycerol molecules shown as sticks (teal) and water molecules as spheres (red). PEG 200 is superimposed, represented by lines (yellow).

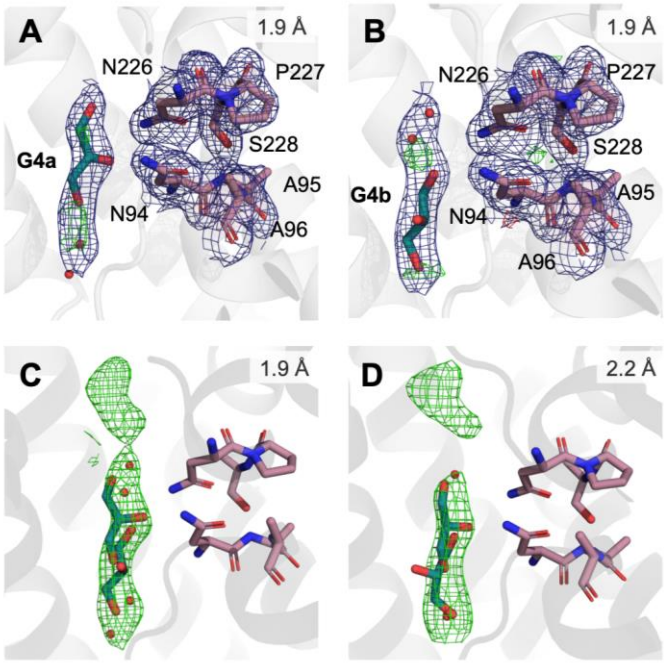

Figure S5. Electron density in the NAA/NPS-region of the 1.9 Å structure (A and B) and omit maps for both 1.9 Å and 2.2 Å structures, related to Figure 2 and Figure 3. (**A**) Alternative A of G4 (G4a). (**B**) Alternative B of G4 (G4b). Cartoon representation in gray with residues and glycerol as sticks (pink and teal, respectively). Water molecules represented by spheres (red). 2Fo-Fc (blue) and Fo-Fc (green/red) electron density maps contoured at 1 sigma and 3 sigma, respectively. (**C**) Fo-Fc omit map for the 1.9 Å structure. (**D**) Fo-Fc omit map for the 2.2 Å structure. Glycerol omit maps were generated in phenix.refine using simulated annealing and contoured at 3 sigma, colored green/red (negative density absent in both structures).

Table S1. Possible hydrogen bonding of PEG 200 and glycerol, related to STAR Methods.

Structure	PEG 200	Protein	Distance (Å)	Glycerol	Protein	Distance (Å)
				O1a	O3 G3*	3.2
	O1	O3 G3*	2.6	O2a	ND2 N226	3.0
	O1	ND2 N226	2.8	O2a	ND2 N94	2.6
	O2	ND2 N226	3.1	O3a	HOH	2.4
1.9 Å	O2	ND2 N94	3.1	O1b	HOH	2.2
	O5	HOH	3.0	O1b	ND2 N94	3.3
				O3b	O A91	3.2
				O3b	HOH	3.1
				O1a	O3 G3*	3.5
				O2a	ND2 N226	3.2
				O2a	O M93	3.0
2.2 Å	O1	O3 G3*	3.2	O3a	O H92	3.2
				O3a	HOH	2.7
				O3b	O H92	3.0
				O3b	HOH	2.9

*Glycerol molecule in the selectivity filter