Supporting Information

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SI Text

Reconstitution of AfAQP into Proteoliposomes for Activation Energy Studies. Reconstitution of AfAQP is similar to that described earlier for AQP1 (1). Ten milligrams of *Escherichia coli* lipids was sonicated to partial clarity and *n*-octyl glucopyranoside (OG) was added to a final concentration of 1.2%. AfAQP in 1.2% OG was added to the OG-saturated liposomes and incubated on ice for 30 min. This mixture was diluted into 25 mL of reconstitution buffer (100 mM NaCl, 20 mM Tris-HCl, pH 7.4), and proteoliposomes were collected by centrifugation at 100,000 × *g* for 1 h. The proteoliposomes were resuspended in the same buffer at 10,000 × *g* for 10 min to remove aggregates, and the supernatant was spun down at 100,000 × *g* for 1, h and the pellet containing the proteoliposomes was resuspended in 300 μL of above buffer and used for transport studies. Water permeability was measured by established methods as previously described (1, 2).

AfAQP Sequence. mtmtlakrftaevvgtfilvffgpgaavitlmiangadkpnefnigigalgglgdwfaigmafalaiaav iyslgrisgahinpavtialwsigrfpgrevvpyivaqfigaalgsllflacvgpaaatv gglgatapfp gigygqailt eaigtfllmlvimgvavderappgfaglvigltvggiitt ignitgssln partfgpylg dslmginlwq yfpiyvigpivgavaaawlynylake.

(accession no: O28846)

1. Zeidel ML, Ambudkar SV, Smith BL, Agre P (1992) Reconstituion of functional water channels in liposomes containing purified red cell chip28 protein. *Biochemistry* 31:7436–7440.
2. Mamonov AB, Coalson RD, Zeidel ML, Mathai JC (2007) Water and deuterium oxide permeability through aquaporin 1: MD predictions and experimental verification. *J Gen Physiol* 130:111–116.

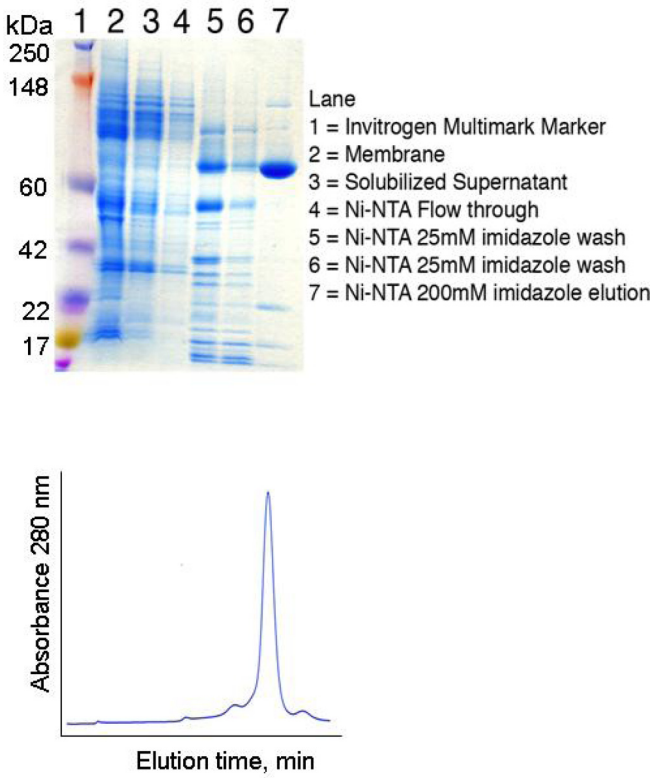

Fig. S1. Purification of AfAQP. At various stages of AfAQP, purification aliquots were taken and protein was separated on a SDS/PAGE and visualized by Coomassie staining. AfAQP (lane 7) is enriched severalfold after metal affinity chromatography, and it runs mostly as a tetramer. Similar to other aquaporins, its apparent molecular weight is smaller than the predicted molecular weight of 25 kDa for monomer. AfAQP is further purified by size-exclusion chromatography on a Superdex 200 column; a representative tracing is shown in the lower frame.

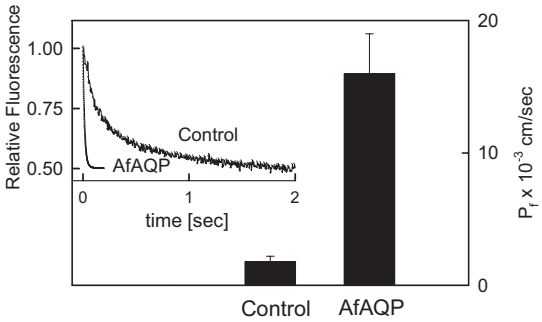

Fig. S2. Water permeability of AfAQP. Proteoliposomes containing AfAQP show a more than tenfold increase in water permeability compared with control liposomes lacking AfAQP. (*Inset*) The time trace of water efflux measured as a decrease in relative fluorescence. Protreoliposomes containing AfAQP equilibrate in millisecond time scale compared with control liposomes upon abrupt exposure to a doubling of external osmolarity.

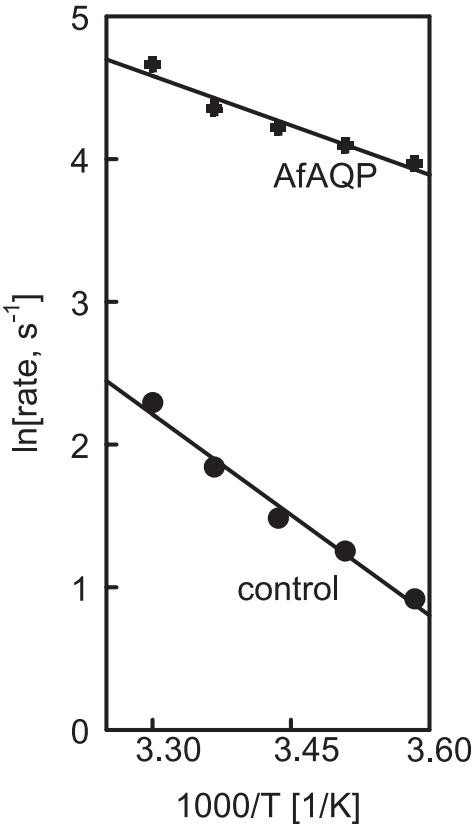

Fig. S3. Temperature kinetics of AfAQP. Rate of water efflux was measured at various temperatures, and from the slope of rate vs. inverse temperature (absolute) the activation energy was computed. AfAQP (top line) shows an activation energy of 4.5 kcal/mole and liposomes without AfAQP gave an activation energy of 9.5 kcal/mole.