Supplementary data in support of "Glycerol modulates water permeation through *Escherichia coli* aquaglyceroporin GlpF"

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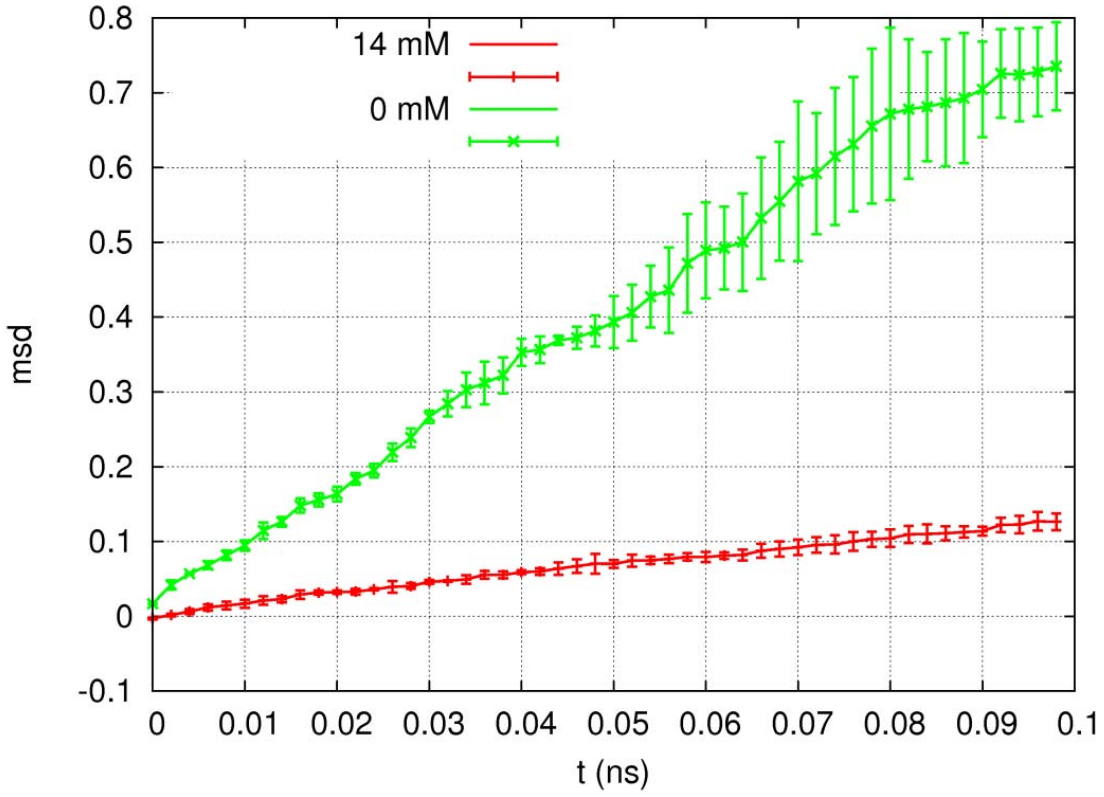

Fig. S1. The mean square displacement (msd) of waters in the conducting pore of GlpF, $\left\langle \delta n^2(t) \right\rangle$, as defined in Refs. (1, 2). The mean and standard deviation were taken over three sets of 10 ns trajectories (70 to 80 ns, 80 to 90 ns, and 90 to 100 ns) from the 100 ns equilibrium MD run for each system. In each set, the 10 ns MD trajectory was divided into 100 segments of 0.1 ns each, over which the displacements of the waters in the pore were included in the relevant statistics. The osmotic permeation rate of water, $p_f = v_W D_n$, is related to the slope of an msd curve, $D_n = \left\langle \delta n^2(t) \right\rangle / 2t$ (1, 2). Here $v_W = 3 \times 10^{-23} cm^3$ is the average volume occupied by one water molecule.

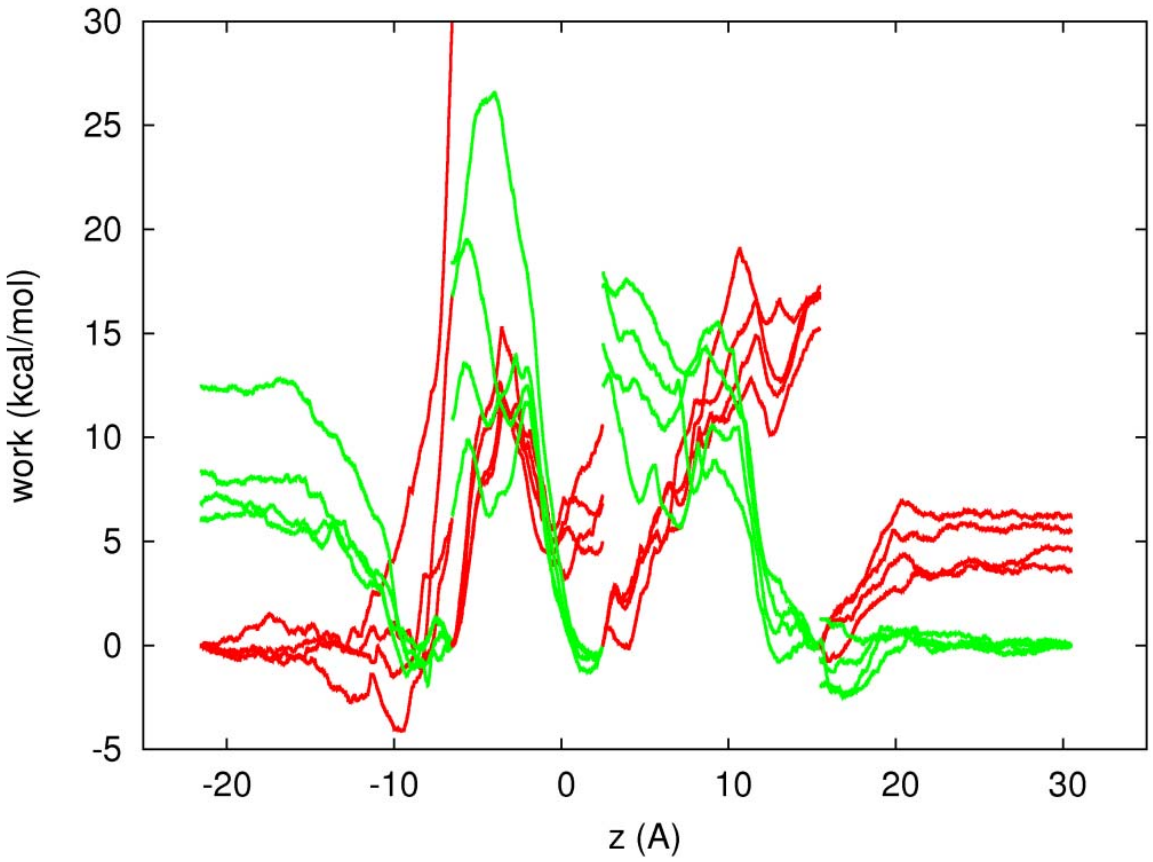

Fig. S2. Work done to the system as a function of the glycerol's center-of-mass z-coordinate along the forward (red) and reverse (green) pulling paths in SMD simulation Set #1. From the periplasm to the entry vestibule of GlpF, $-21.5\text{Å} < z < -6.5\text{Å}$, the glycerol center-of-mass was pulled along a straight line. Through the single-file channel of GlpF, $-6.5\text{Å} < z < 15.5\text{Å}$, the glycerol center-of-mass was pulled along the z-direction while its x- and y-degrees of freedom were not restricted. From the GlpF exit to the cytoplasm, $15.5\text{Å} < z < 30.5\text{Å}$, the pulling was along a straight line. The pulling speed was $2.5\text{Å} / ns$. Long time equilibrations (4 ns each) were done at every end of each segment, $z = -21.5\text{Å}, -6.5\text{Å}, 4.5\text{Å}, 14.5\text{Å},$ and 30.5Å, respectively, so that the SMD pulling was between equilibrium states at the two ends of each segment.

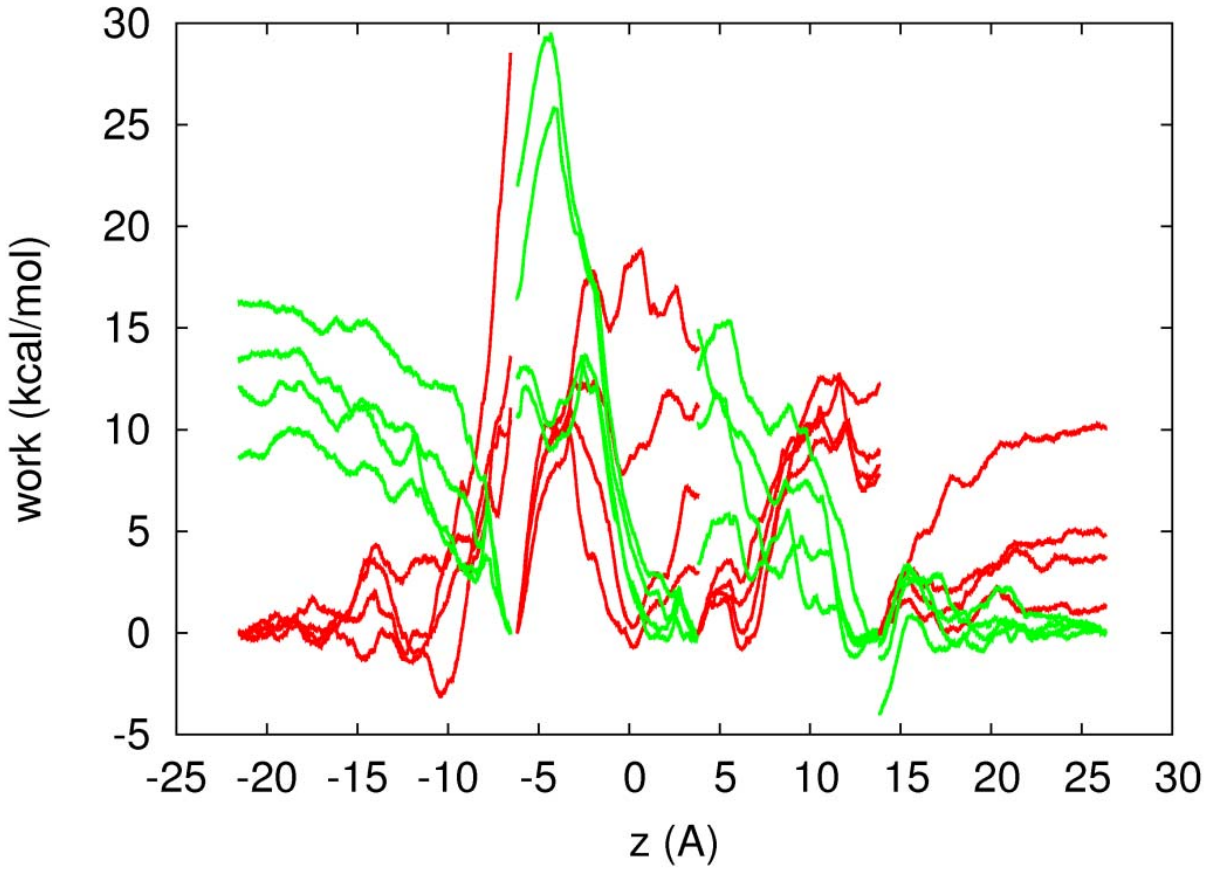

Fig. S3. Work done to the system as a function of the glycerol's center-of-mass z-coordinate along the forward (red) and reverse (green) pulling paths in SMD simulation Set #2. From the periplasm to the entry vestibule of GlpF, $-21.5\mathring{A} < z < -6.5\mathring{A}$, the glycerol center-of-mass was pulled along a straight line. Through the single-file channel of GlpF, $-6.5\mathring{A} < z < 14.5\mathring{A}$, the glycerol center-of-mass was pulled along the z-direction while its x- and y-degrees of freedom were not restricted. From the GlpF exit to the cytoplasm, $14.5\mathring{A} < z < 26.5\mathring{A}$, the pulling was along a straight line. The pulling speed was $2.5\mathring{A}/ns$. Long time equilibrations (4 ns each) were done at every end of each segment, $z = -21.5\mathring{A}, -6.5\mathring{A}, 4.5\mathring{A}, 14.5\mathring{A},$ and $26.5\mathring{A}$, respectively, so that the SMD pulling was between equilibrium states at the two ends of each segment.

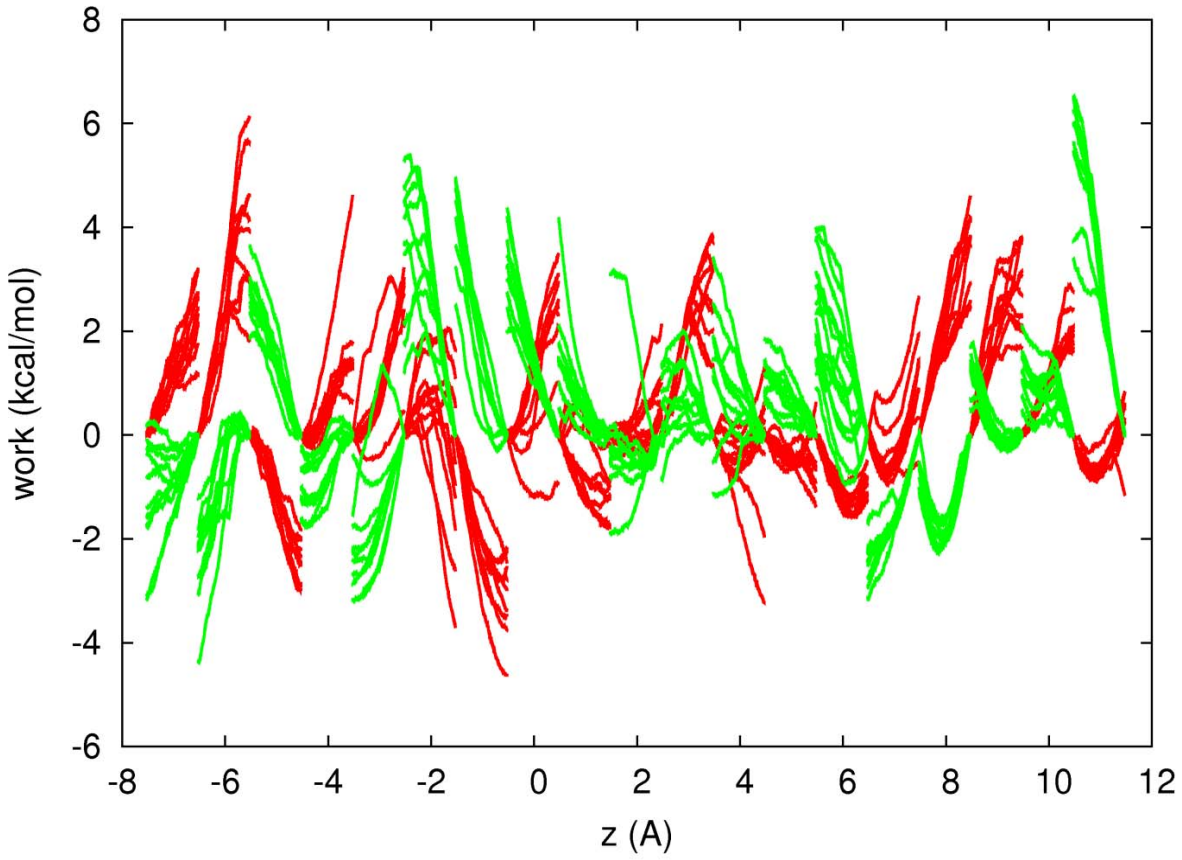

Fig. S4. Work done to the system as a function of the glycerol's center-of-mass z-coordinate along the forward (red) and reverse (green) pulling paths in SMD simulation Set #3. The conditions were identical to those of Fig. S2 except that the channel region ($-7.5\text{Å} < z < 11.5\text{Å}$) was divided into 19 segments of 1Å each.

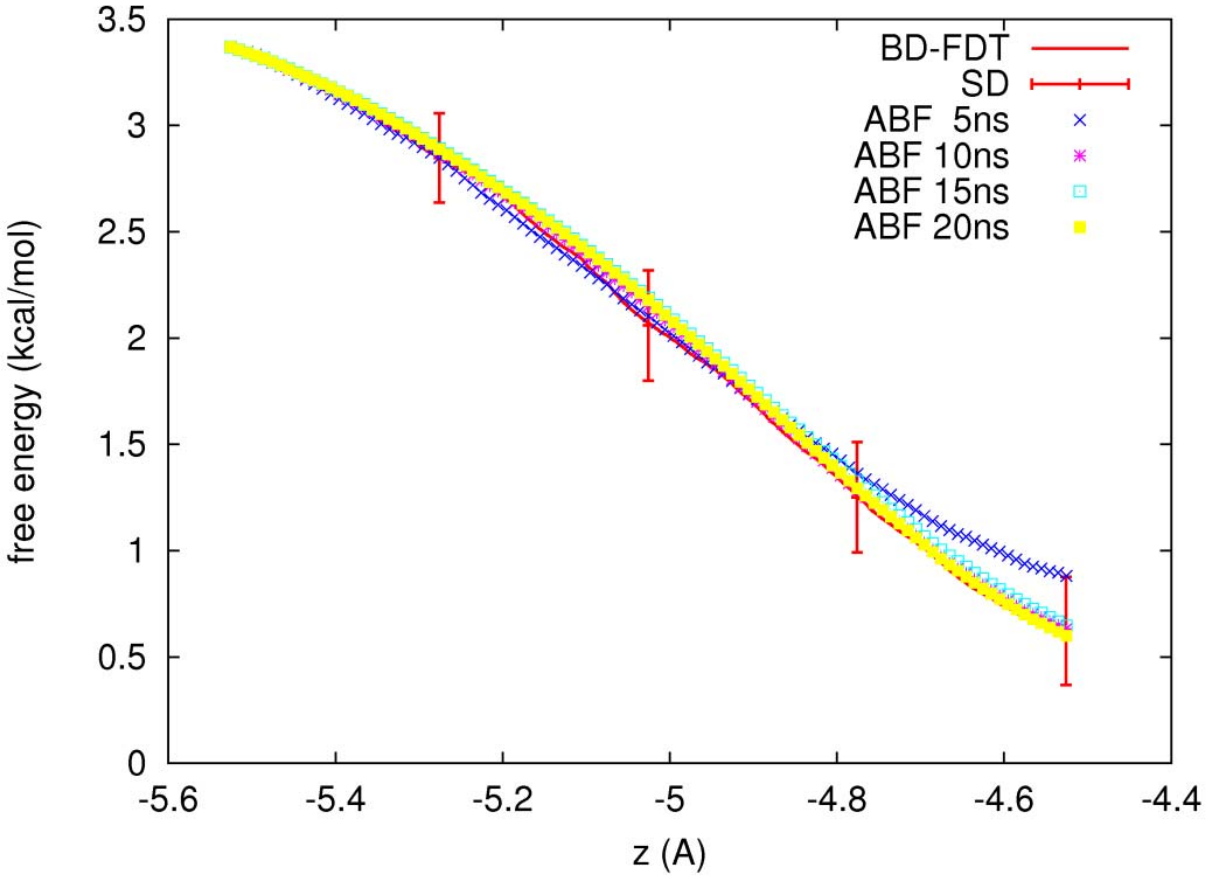

Fig. S5. Convergence of free-energy computation using the ABF method (3, 4), a mature approach of equilibrium MD. The sampling was over a window of $1\mathring{A}$ in the z-coordinate of the center of mass of glycerol. The lengths of simulations are indicated to show the convergence behavior of the ABF approach. The BD-FDT (5) results and ABF results for 15 ns and for 20 ns overlap almost completely, suggesting the accuracy of both approaches.

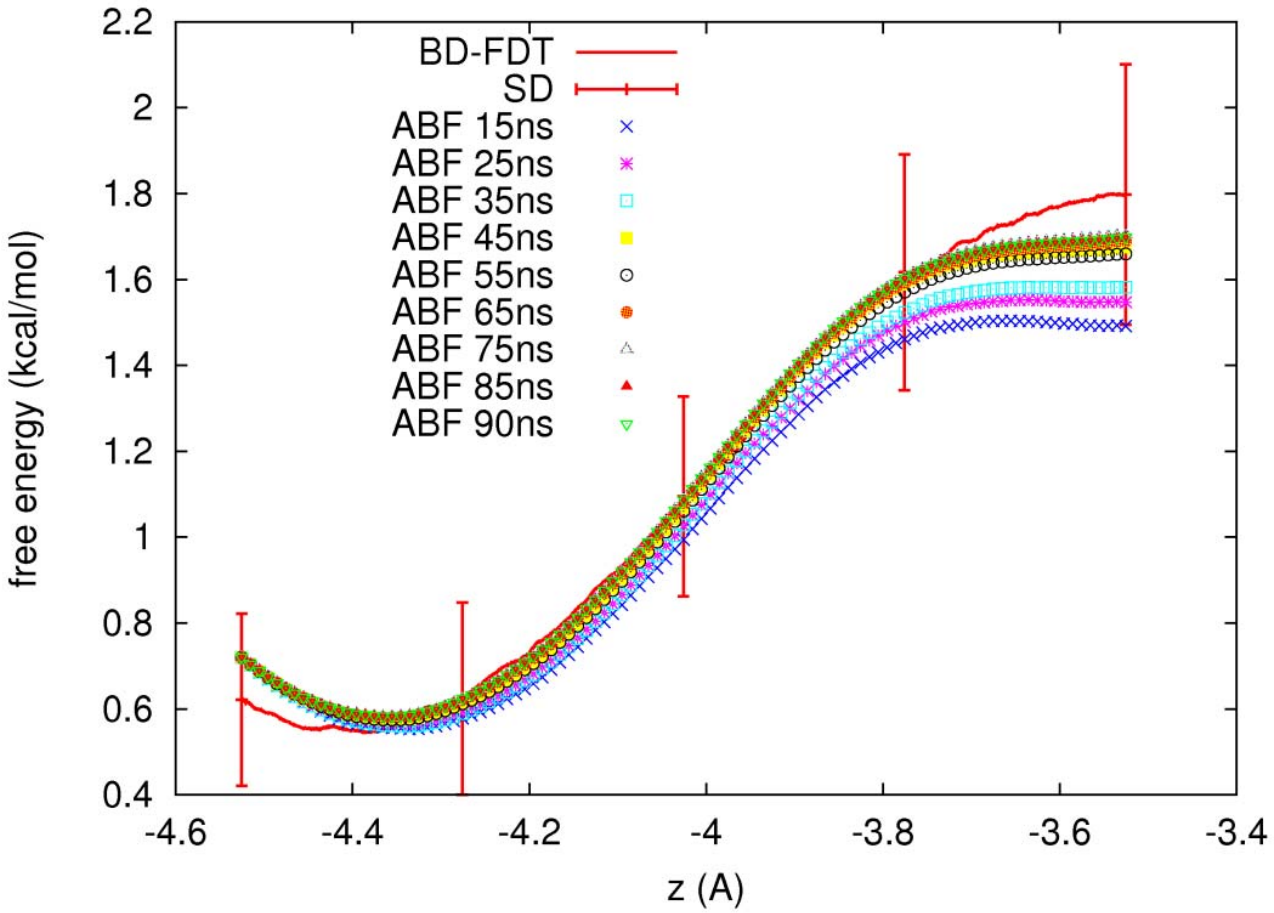

Fig. S6. Convergence of free-energy computation using the ABF method (3, 4). The lengths of simulations are indicated to show the convergence behavior of the ABF approach. The results for 75 ns, 85 ns, and 90 ns overlap almost completely and all fall within the error bars of the nonequilibrium BD-FDT approach (5), suggesting the accuracy of both approaches.

References

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5. Chen LY (2011) Exploring the free-energy landscapes of biological systems with steered molecular dynamics. *Physical Chemistry Chemical Physics* 13(13):6176-6183.