

Gumbart et al., <http://www.jgp.org/cgi/content/full/jgp.200810062/DC1>

Supplemental Materials and Methods

The cumulative water “current” for the simulation of the full-plug deletion mutant, shown in Fig. S2 A, increases fairly steadily, but with a few dormant periods. The distribution of water occupancies of the pore region is also shown. The region was defined as a box of dimensions $10 \times 10 \times 6 \text{ \AA}^3$. The distribution is slightly bimodal, with peaks around 0–1 water molecules and 4–5 water molecules. This suggests that the pore ring acts as a hydrophobic pore, although the distribution may also be a byproduct of the motion of the pore ring residues, which flip in and out of the central pore. In Fig. S2 B, one can see that water occupancy of the pore ring is anti-correlated with protein occupancy of the same region, also suggesting that steric effects give rise to the observed distribution.

We also examined the water occupancy of the pore and its density for two additional simulations with an

enforced pore radius of 3.5 \AA , one in which the pore ring residues were free to move and one in which they were held rigid. Water density was calculated by assuming a cylindrical pore with area $\pi r^2 L_{\text{pore}}$, where $r = 3.5 \text{ \AA}$ and L_{pore} is taken to be 7 \AA , slightly larger than the 6 \AA box defined above because occupancy was counted even when only a water’s oxygen was inside the pore. In the case of the free pore ring, shown in Fig. S3 A, the water density is ~ 0.6 times the bulk density of water (53.67 mol/liter) on average (Beckstein, O., and M.S.P. Sansom. 2003. *Proc. Natl. Acad. Sci. USA.* 100:7063–7068). As seen in Fig. S3 B, the water density decreases to an average of 0.5 when the pore ring residues are fixed. A vapor-like state for the pore is observed when the pore ring residues are fixed (indicated by the bimodal distribution of occupancies in Fig. S3 D), but not when the residues are free (see Fig. S3 C).

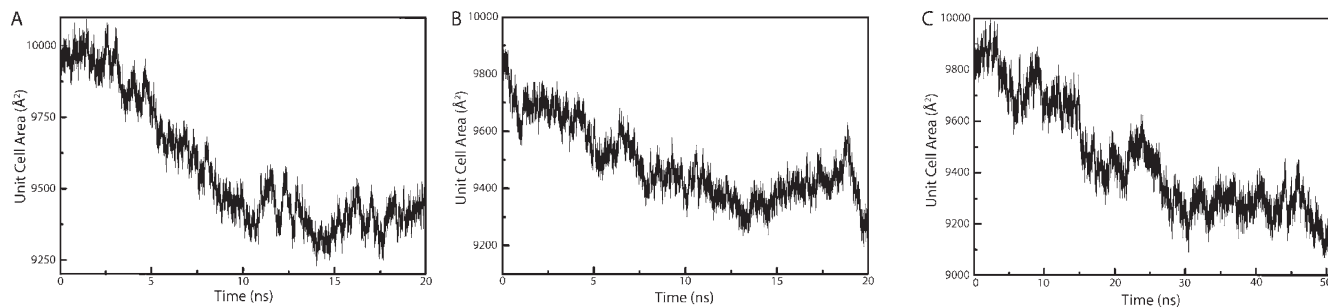


Figure S1. Simulated unit cell area as a function of time. The area is provided for the CP simulations of (A) native SecY, (B) the half-plug deletion mutant, and (C) the full-plug deletion mutant.

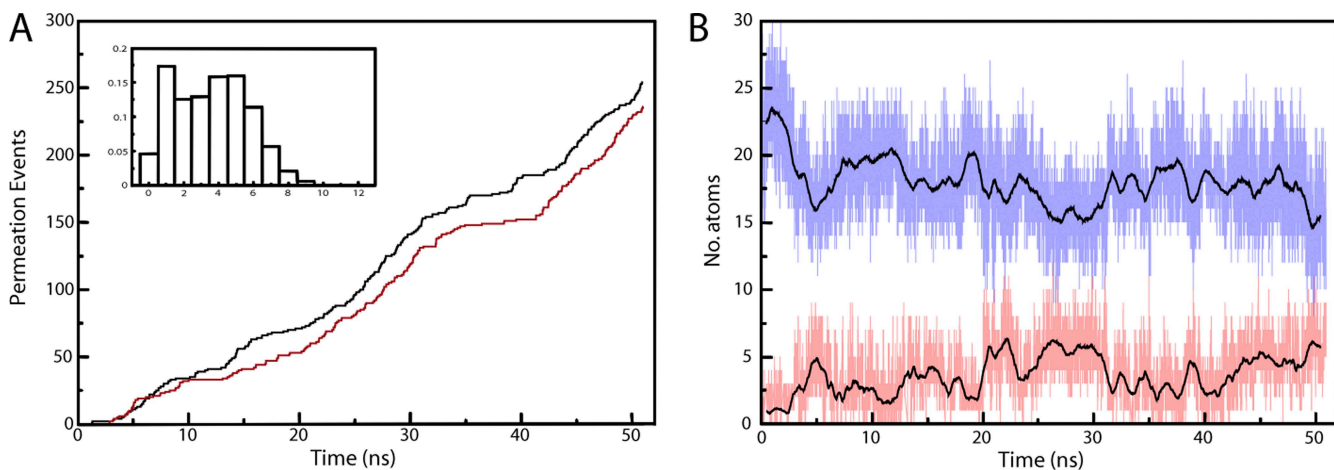


Figure S2. Occupancy of the pore ring. (A) Cumulative permeation events for the full-plug deletion mutant. Water molecules going up through the channel are shown in red and those going down are shown in black. The inset shows the normalized distribution of occupancy of the pore ring region by water molecules. (B) Pore occupancy over time. Shown in red and blue are the occupancies of the pore ring for water and protein heavy atoms, respectively. The black curve denotes a running average.

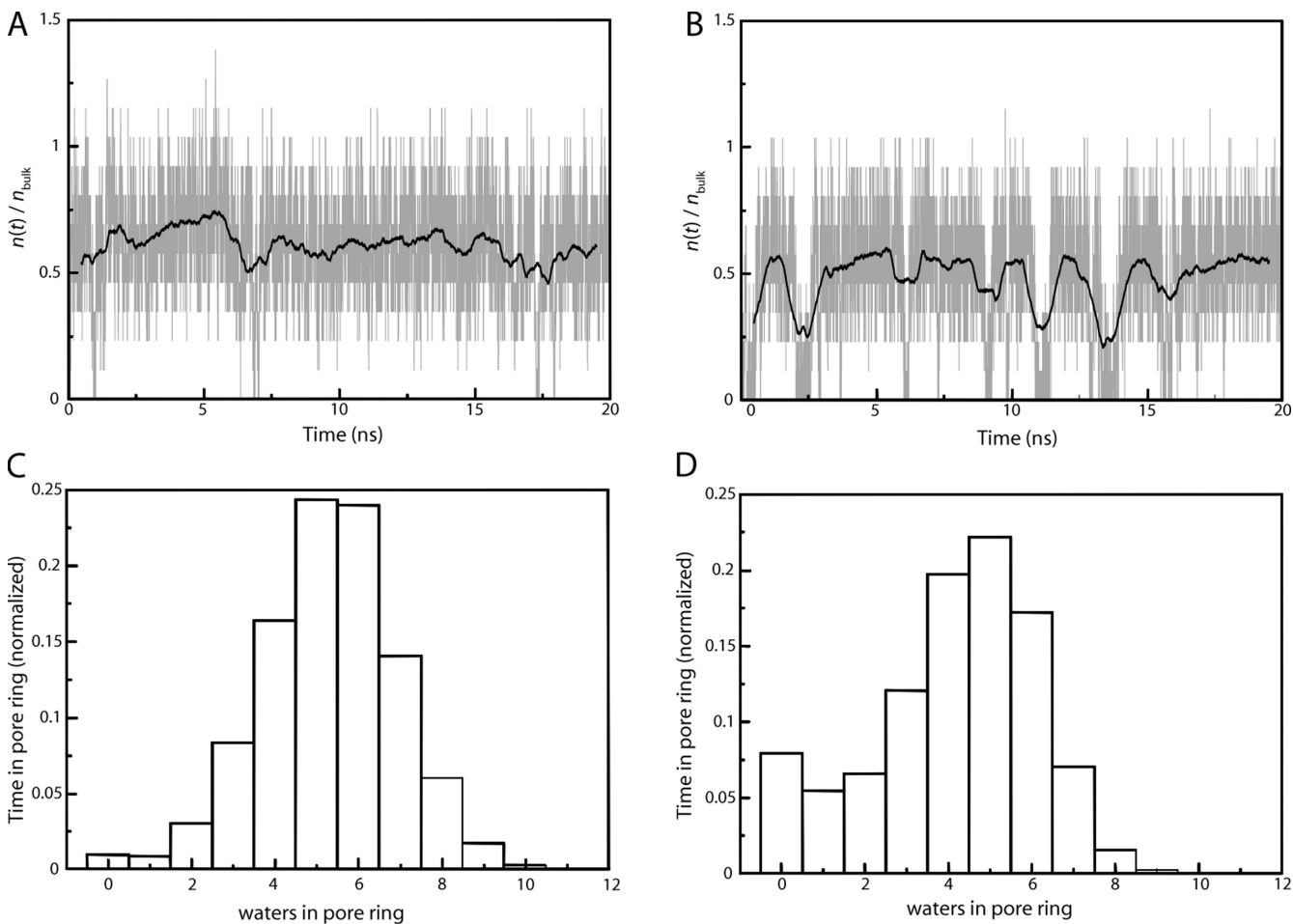


Figure S3. Water density and occupancy in the pore. (A and B) Water density ($n(t)/n_{bulk}$) is shown for simulations of the plug-SecE-locked mutant with an enforced pore radius of 3.5 Å with the pore ring residues (A) free to move and (B) held fixed. (C and D) Normalized distributions of water occupancy of the pore for the same simulations as in A and B, respectively.

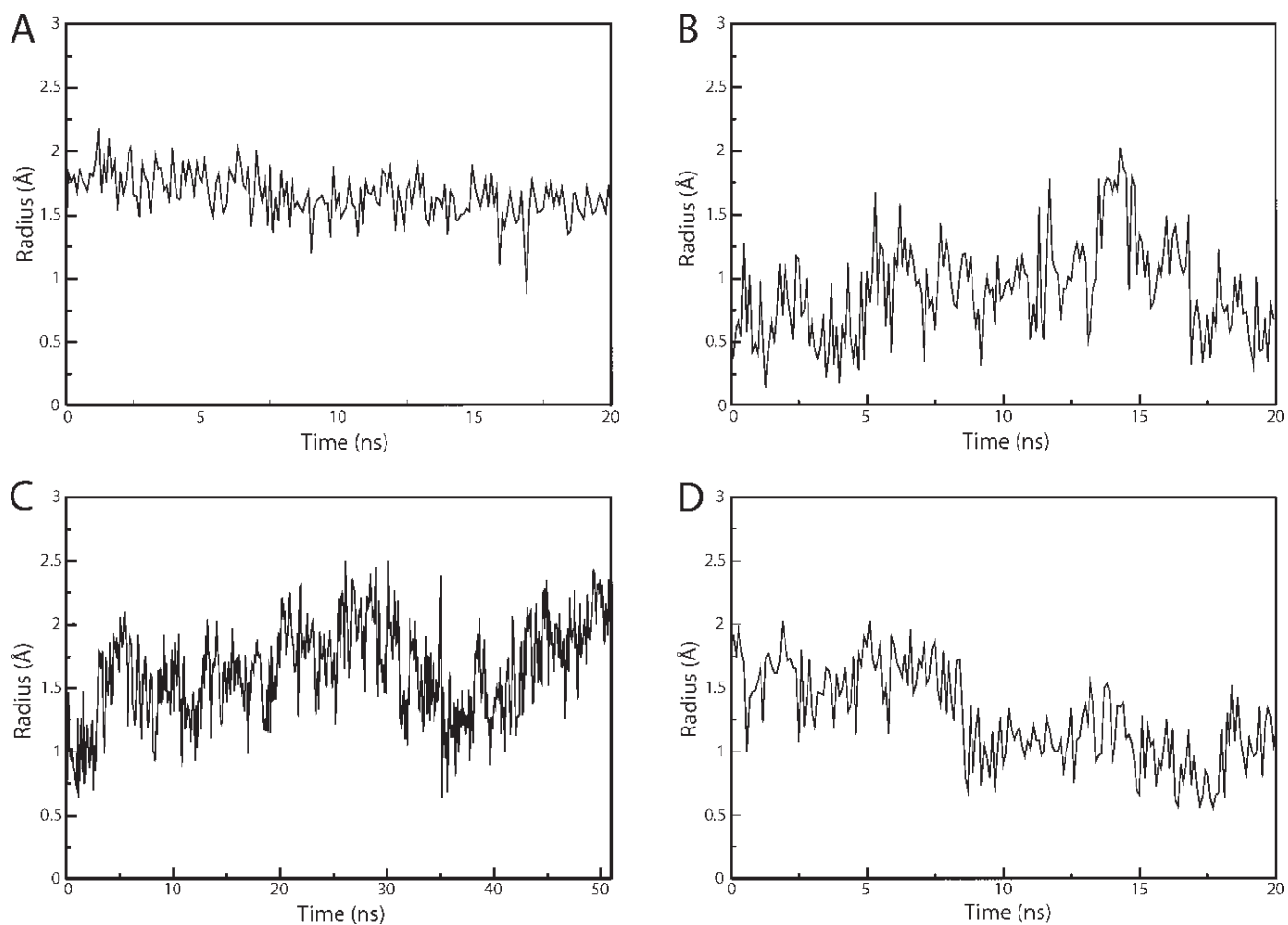


Figure S4. Minimum pore radius as a function of time for (A) native SecY, (B) the half-plug deletion mutant, (C) the full-plug deletion mutant, and (D) the plug-SecE-locked mutant. The radius was calculated with the program HOLE (Smart, O., J. Goodfellow, and B. Wallace. 1993. *Biophys. J.* 65:2455–2460; Smart, O.S., J.G. Neduvellil, X. Wang, B.A. Wallace, and M.S.P. Sansom. 1996. *J. Mol. Graph.* 14:354–360), which places spheres along the pore to estimate its size. However, because the pore is not uniform in shape, this method can underestimate the actual area available to solvent.

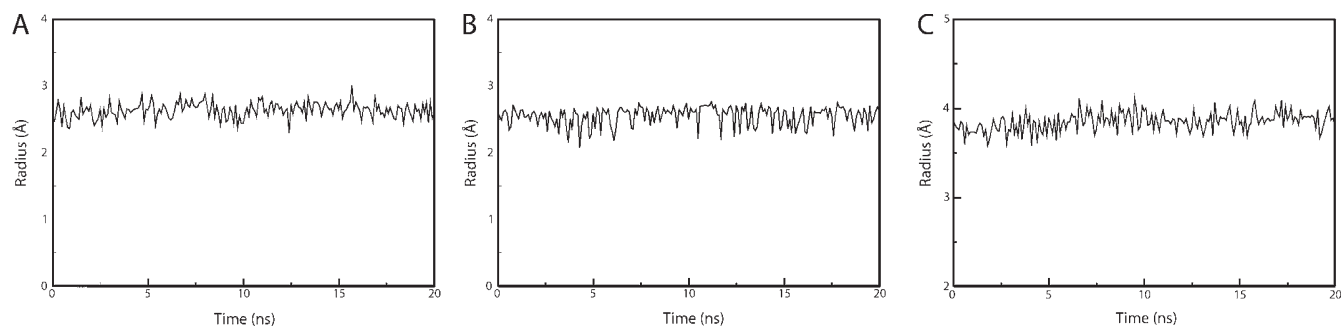


Figure S5. Minimum pore radius as a function of time for the plug-SecE-locked mutant with an enforced radius of (A) 3.5 Å (pore ring residues free), (B) 3.5 Å (pore ring residues fixed), and (C) 5 Å (pore ring residues free). It is clear from this figure that HOLE underestimates the enforced radii by ~ 1 Å.

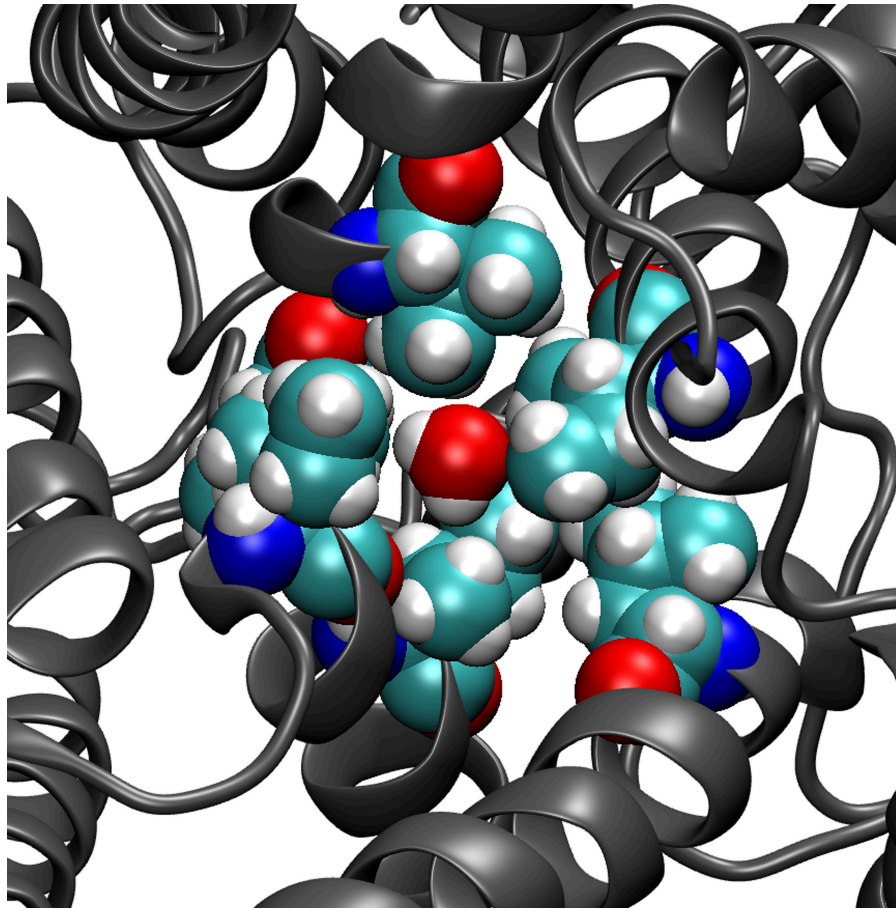


Figure S6. Size of the equilibrated pore ring opening. The pore ring residues, colored by atom type, are shown in a space-filling representation along with a single water molecule at the center; oxygen atoms are shown in red, carbon in cyan, nitrogen in blue, and hydrogen in white. SecY is shown in gray. The pore ring, after the addition of hydrogens and equilibration, may accommodate at most a single water molecule in its opening.

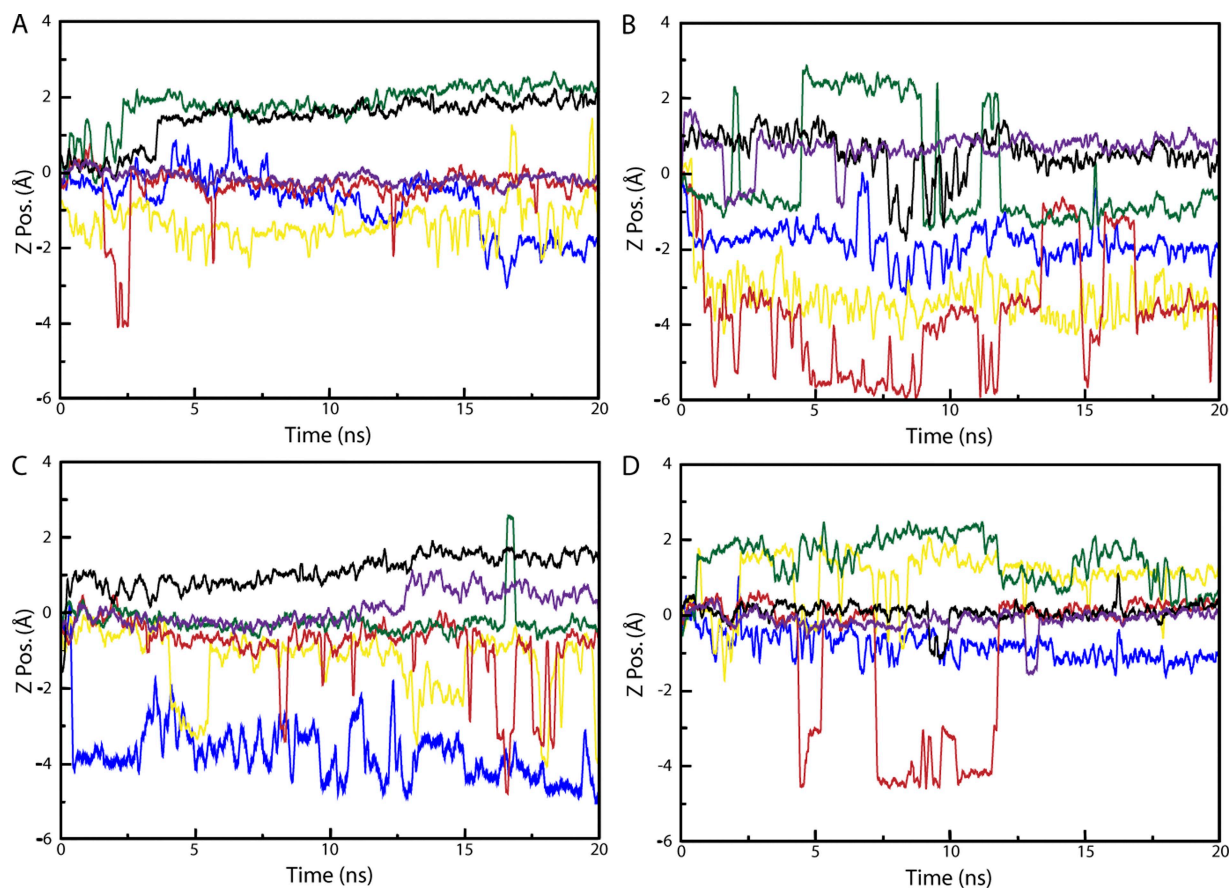


Figure S7. Fluctuations of pore ring residues. The z position of each pore ring residue over time is plotted, normalized at $t = 0$, for native SecY (A), its half-plug deletion mutant (B), its full-plug deletion mutant (C), and for native SecY with the plug transparent to water (D). Black corresponds to Ile⁷⁵, purple to Val⁷⁹, blue to Ile¹⁷⁰, yellow to Ile¹⁷⁴, red to Ile²⁶⁰, and green to Leu⁴⁰⁶. Although the residues of the half-plug deletion mutant apparently fluctuate most, it should be noted that the residues of the full-plug deletion mutant have a greater net deviation from the starting structure (see Fig. 2), indicating that water permeation depends on both distortion of the pore ring residues and their fluctuations.

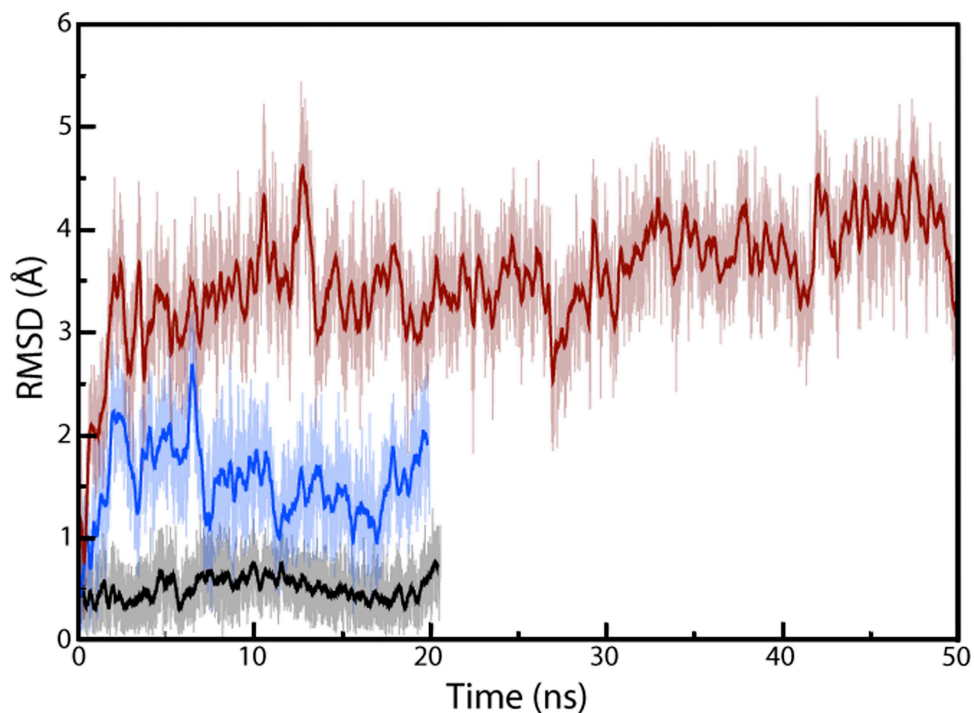


Figure S8. Center-of-mass motion of the plug. The center-of-mass of the plugs of native SecY (black), its half-plug deletion mutant (blue), and its full-plug deletion mutant (red) were tracked as a function of time. The transparent curves represent original data, and the solid lines are running averages.

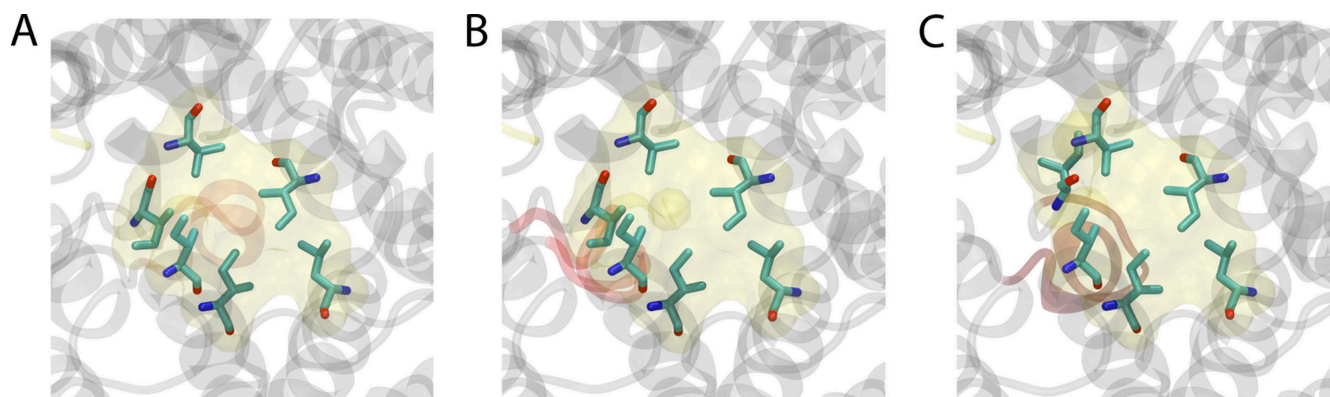


Figure S9. Comparison of SecYE β crystal structures. In all panels, SecY is shown in transparent gray, viewed from the cytoplasmic side. The yellow pore ring surface is transparent with individual residues shown in stick representation; the plug, in red, is behind the pore ring. Shown are (A) native SecY, (B) the half-plug deletion mutant, and (C) the full-plug deletion mutant.

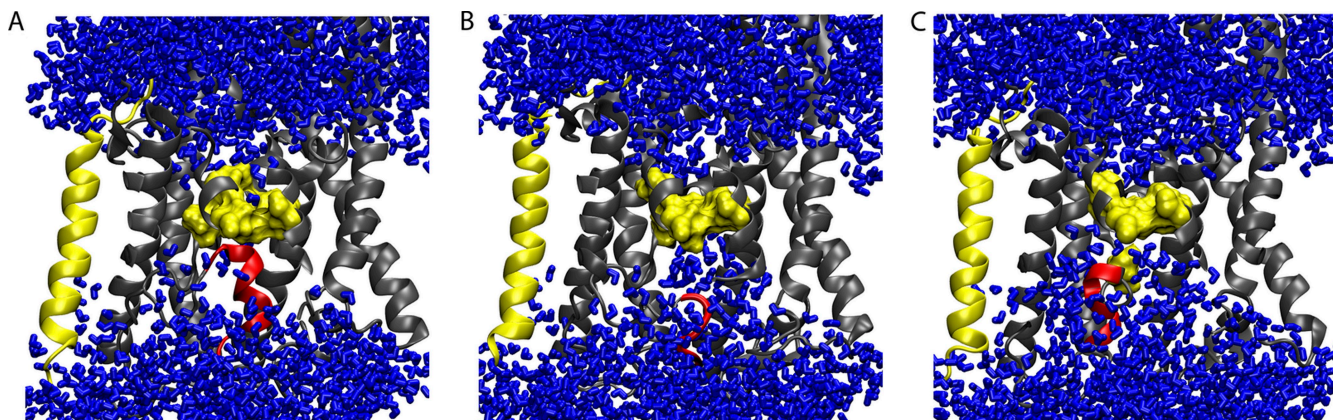


Figure S10. Water intrusion into the pore. SecY is shown in gray, partially cut to show the pore interior. Secβ is shown in yellow, the plug in red, and the pore ring also in yellow; the water is shown in blue. The extent to which water enters the pore shortly after beginning equilibration is illustrated for (A) native SecY, (B) the half-plug deletion mutant, and (C) the full-plug deletion mutant.

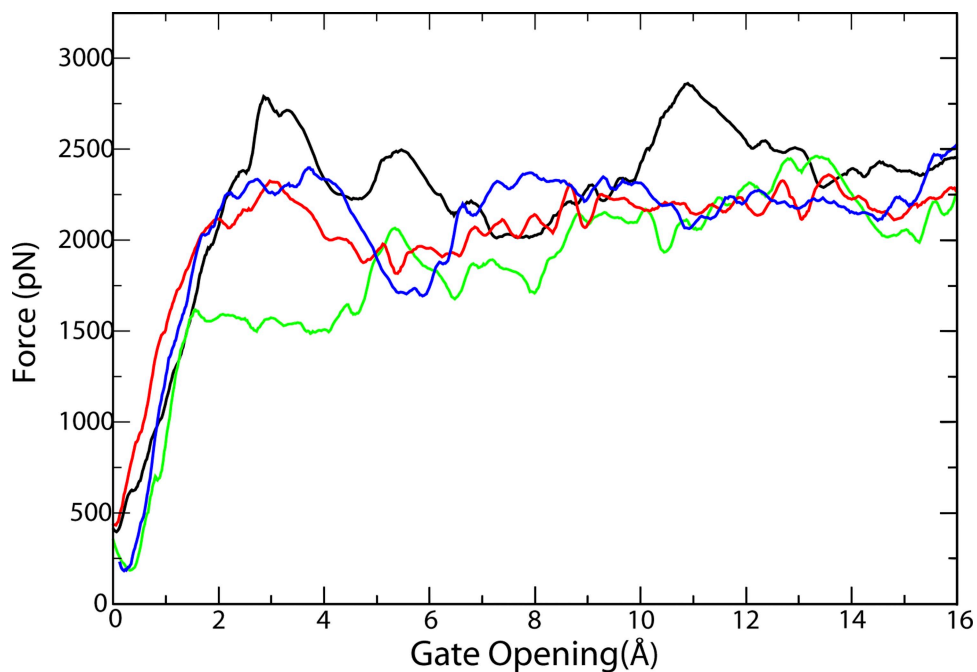


Figure S11. Lateral gate opening of plug deletion mutants. The force required to open the lateral gate is shown as a function of gate opening for four different simulations. The black and green lines are from two independent simulations of SecYEβΔplug in Gumbart and Schulten (2007. *Biochemistry*. 46:11147–11157) (Fig. S7). Shown in blue and red are the forces required for the full-plug and half-plug deletion mutants, respectively, from this study.