Supplementary Materials

Pulling the separated gate helices

In order to further understand the effect of the membrane on lateral gate opening, the four helices comprising the gate, specifically 2b and 3 (residues 75-130) and 7 and 8 (residues 256 to 338), were separated from the protein and simulated alone in a lipid bilayer (simulation sim4, see Table 1). The helices were then pulled apart in exactly the same manner as the gate was opened (see Methods). The forces, shown in Fig. S1, are very large, indicating that the membrane resists pulling helices through it.

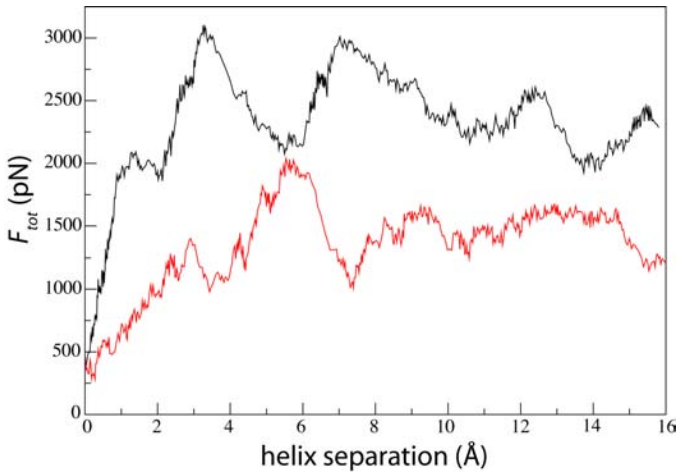

Figure S1: Force needed to separate helices in membrane as a function of separation distance. The force required for the four helices alone (sim4) is shown in red as compared to the force required for all of SecYEβ (sim1a, see also Fig. 4).

Loosening of the plug

After opening the lateral gate for 3 ns (at 3 Å/ns), the gate was held in this intermediate opening for another 3 ns in sim1h. This simulation allowed us to investigate the behavior of the plug and determine if it was effectively separated from the rest of the protein. The root mean-square deviation (RMSD) for the plug is shown in Fig. S2.

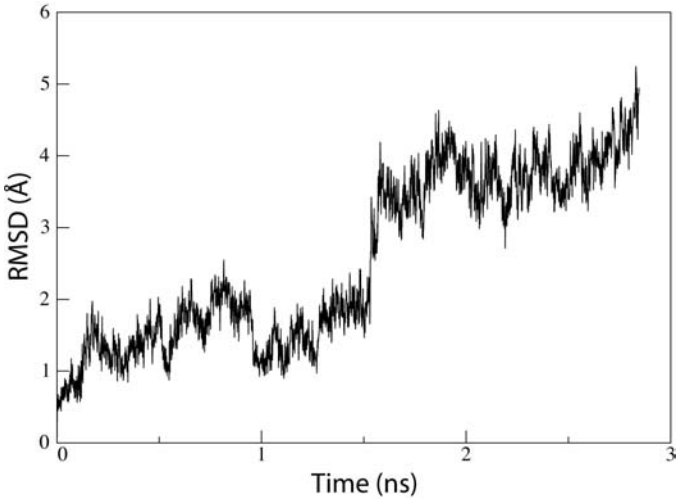

Figure S2: RMSD for the plug (residues 55 to 65 of SecY). The RMSD was calculated for the backbone atoms of the plug after aligning the rest of SecY at each picosecond.

Effects of surface tension

Since it is known that simulations of membranes with zero surface tension can lead to over-compressed bilayers (*1*, *2*), we wanted to understand if this also played a significant role in our simulations of gate opening. Therefore, gate opening for SecYEβ (simulation sim1a) was repeated at constant surface tensions of 20 dyn/cm and 50 dyn/cm (simulations sim1a-20 and sim1a-50, respectively). The resulting force profiles shown in Fig. S3 show little change in magnitude compared to the original force profile (Fig. 4 in the main text). Therefore, surface tension on the scale of tens of dyn/cm appears to only minimally affect lateral gate opening.

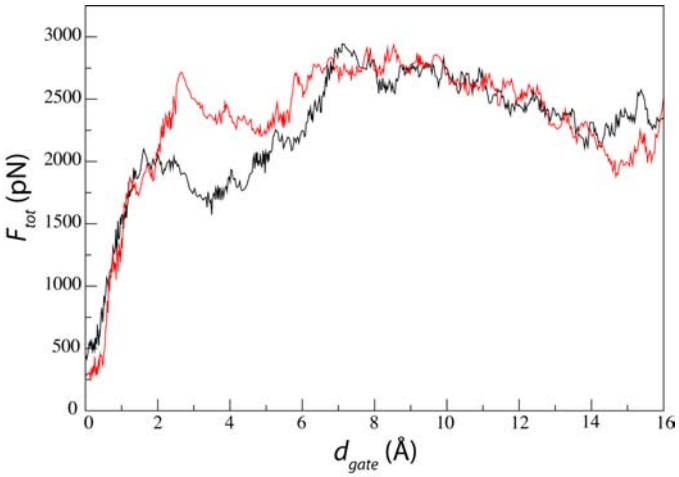

Figure S3: Effect of surface tension on the force needed to open the lateral gate. The force is shown as a function of gate opening. The black curve represents the simulation performed at a constant surface tension of 50 dyn/cm (sim1a-50) and the red curve represents the simulation at 20 dyn/cm (sim1a-20).

Comparison of relaxing structure and closed structure

While gate opening was used as a measure of relaxation, we wanted to also determine if the structure was relaxing approximately to its original closed state. Therefore, we calculated the RMSD of the structure as compared to the closed structure.

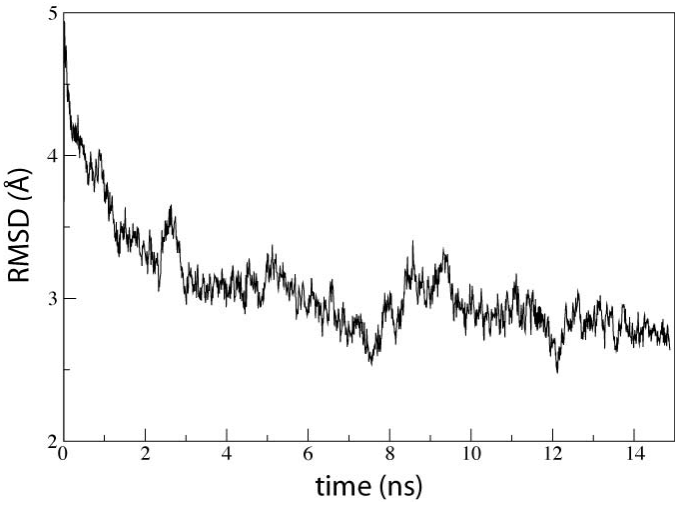

Figure S4: RMSD of SecYEβ during simulation sim1c. The RMSD was calculated for the backbone of the relaxing structure as compared to the closed structure used as the starting point for sim1a.

Effect of bilayer size

To examine if finite size effects played a role in the forces measured for gate opening, we repeated gate opening for SecYEβ (simulation sim1a) with a larger bilayer containing 515 lipids (as opposed to 251 before). As shown in the comparison of force profiles below (Fig. S5), the shape and magnitude of the force curve was reproduced in this simulation, indicating that the size of the bilayer did not affect the results of gate opening.

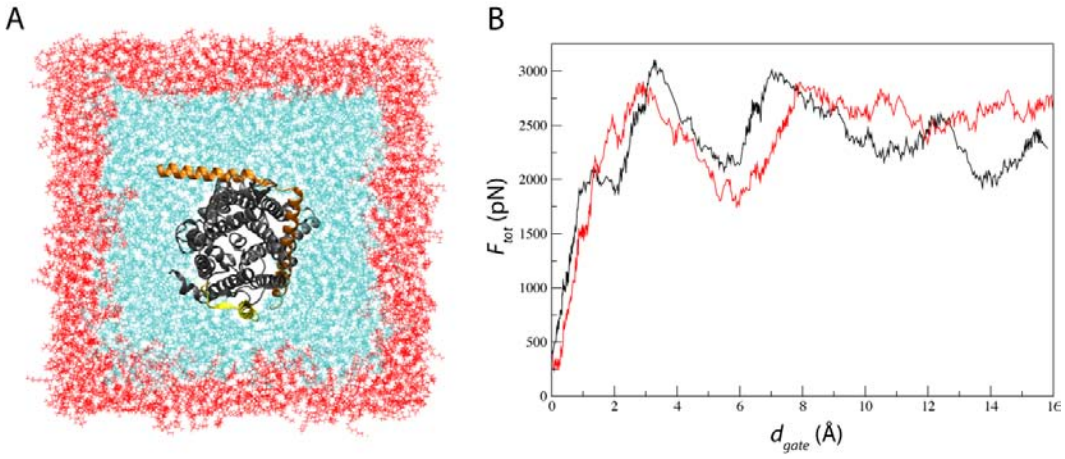

Figure S5: Effect of doubling bilayer size. *(A)* Membrane/protein system viewed from the cytoplasmic side. SecYEβ is shown in grey, orange, and yellow, respectively, while the original lipids are shown in light blue and the added lipids in red. *(B)* Force profile for gate opening. In black is the force profile for the original system (sim1a) and in red is that for the new system (sim1a-515).

Bilayer closure

In order to examine how quickly lipids would close in on an artificial pore in a membrane, a patch of membrane with an approximately 20-Å-diameter solvated hole created in the middle was simulated. It was found that the hole shrank to approximately 8 Å within 2.4 ns, shown in Fig. S6. The rapid closure is reasonable since the hydrophobic core of membrane is exposed to water with a surface area determined by the radius of the hole, clearly an energetically unfavorable state. However, at the open gate of SecY (see main text), lipids are not in such an energetically unfavorable position; the hydrophobic tails were found to be stabilized in part by interacting with the hydrophobic gating helices. Furthermore, there is an energetic penalty for one or two lipids (the number limited by the

width of the gate) to enter the solvated and largely polar channel, greatly reducing their propensity to do so.

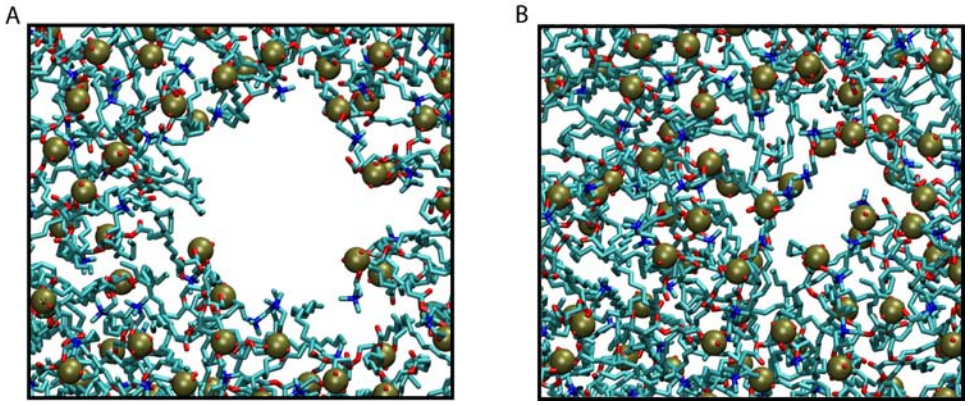

Figure S6: Bilayer closure on an artificial pore. Shown is a close-up view of a bilayer with a hole punched out and then solvated. Lipids are shown in a licorice representation with carbon atoms in cyan, nitrogen in blue, and oxygen in red. The phosphate atoms of the lipid head groups are shown in tan as large spheres. The bilayer is shown at *(A)* 20 Å diameter and 0 ns and at *(B)* 8 Å diameter and 2.4 ns.

Additional runs

Simulations sim1a and sim3a, namely opening the lateral gate of SecYEβ and SecYE$\beta\Delta$plug, were repeated in order to examine the reproducibility of the results. Plots of F_{tot} versus d_{gate} for each of these is shown in Fig. S7. One primary difference between this plot and that in Fig. 4 is the initial peak between 2 and 5 Å which is not as pronounced in the repeated run as in the original simulation. As this peak was originally attributed to SecE, the decrease seen here supports the conclusion that SecE is not relevant to lateral gating in general. Otherwise, the decrease in force when the plug is removed is duplicated here as well as the magnitudes of the forces involved.

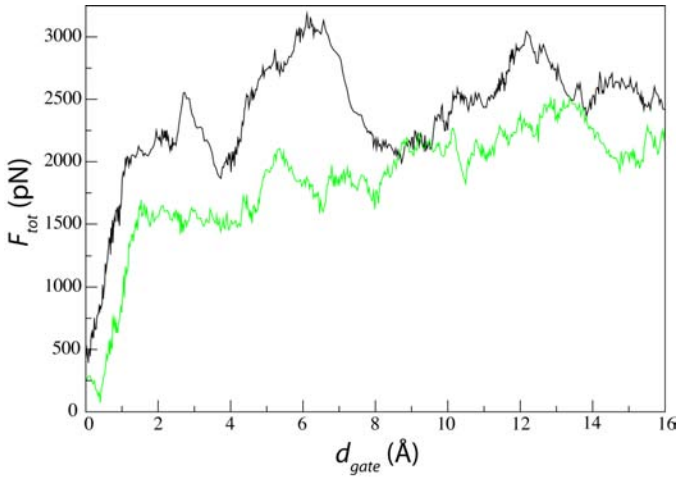

Figure S7: Force required to open the lateral gate as a function of gate opening. Shown are two repeated simulations for sim1a (SecYEβ, black) and sim3a (SecYE$\beta\Delta$plug, green).

References

1. Feller, S. E. and Pastor, R. W. (1999) Constant surface tension simulations of lipid bilayers: The sensitivity of surface areas and compressibilities. *J. Chem. Phys. 111*, 1281–1287.

2. Gullingsrud, J. and Schulten, K. (2004) Lipid bilayer pressure profiles and mechanosensitive channel gating. *Biophys. J. 86*, 3496–3509.