Supplementary Materials

Label	Length (ns)	Description
sim6	1.4	repeat of sim1 at 150 mM ion concentration
sim7	7.5	relaxation continuing from sim6
sim8	4.6	equilibration of alanine/leucine helix
sim9	0.5	constant velocity pulling of plug
sim10	1.0	SMD simulation of deca-alanine with plug removed
sim11	3.6	relaxation continuing from sim1a

Table 1: Summary of further simulations performed. Simulations sim0 through sim5 are described in the main text.

Simulations at higher ion concentration

The ability of the translocon to block the flows of ions before, during, and after translocation is important for maintaining membrane integrity. All simulations described in the main text were performed at an ion concentration of 50 mM (except for sim5 which was performed at a concentration of 100 mM). However, the rather small number of ions in the simulated volume (5 Na^+, 21 Cl^- ions) implies poor statistics for ion conduction events. Therefore, we carried out simulations at a higher ion concentration, namely 150 mM, that corresponds to 25 Na^+ and 41 Cl^- in the simulated volume.

We repeated sim1, pulling deca-alanine through the translocon, at this higher ion concentration while keeping all other parameters the same (sim6, see Table S1). After 1.4 ns, we again allowed the system to relax. We ran the corresponding simulation sim7 for longer than its counterpart, sim3, allowing it to continue for 7.5 ns instead of 3.6 ns. We analyzed each simulation, tracking the positions of all ions throughout. We conclude that even at 150 mM, no ions crossed the pore ring. Supplementary Fig. 1 shows a comparison of the initial ion distribution for sim1 and sim6.

Equilibration of helix AL19

The helix AL19 (see text) was constructed by mutating the residues of another known helix. In order to test its stability, we equilibrated it in a water box of approximate dimensions $50 \times 50 \times 75$ Å^3. The total atom count for the system was 19,708, and the simulation, sim8, was run for 4.6 ns. It does not take more than 250 ps to reveal that the N-terminus of the helix is not stable; the first five residues unfold almost immediately. However, after this, the rest of the helix remains stable for the entire simulation length. Supplementary Fig. 2 shows the system at both the beginning and end of sim8.

Constant velocity simulations examining the separate roles of the pore ring and plug

In order to measure separately the effects of the pore ring and the plug, we performed two further simulations. We first carried out a constant velocity simulation, sim9, pulling the plug out of the pore in the -z direction with the same velocity as used in sim1, sim2, and sim6 (.05 Å/ps). The forces were applied to the C_α atoms of residues 58 through 62 of the plug with a spring constant of 5 kcal/(mol Å^2). Again, the center of mass of the lipid head groups was restrained in the z direction with a force constant of 7 kcal/(mol Å^2). Since there was no translocating polypeptide here, the simulation was run for only 0.5 ns, enough time to remove the plug from the pore into the solvent. A plot of force *vs.* position is shown in Fig. 3. Based on this, one can see that the maximum force encountered in the simulation is approximately 1750 pN.

To better measure the barrier presented by the pore ring, we again simulated the pulling of deca-alanine across the channel. However, this time, we used a state of SecY with the plug outside of the pore (akin to that resulting from sim9). In this simulation, sim10, all conditions otherwise were those of sim1. The simulation was run for approximately 0.8 ns (not 1.4 ns as in sim1 since we no longer were interested in what happens after deca-alanine leaves the pore ring). Again, we measured the force as compared to position for deca-alanine, shown in Fig. 4. Here, we see that the largest force necessary to pull deca-alanine through the pore ring of SecYEβ was 1800 pN. From this it appears that the pore ring and plug independently present similar barriers to translocation.

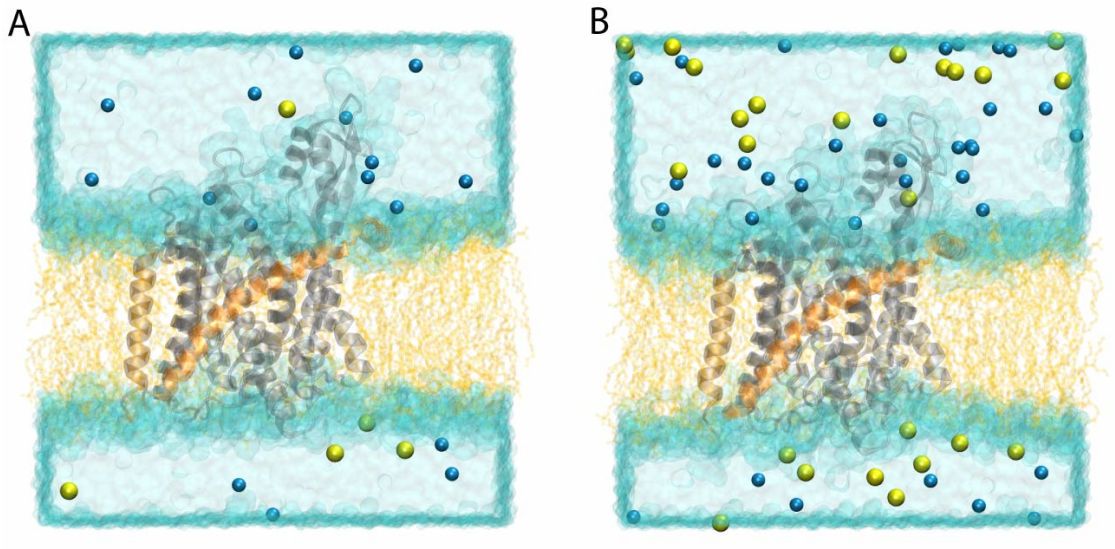

Figure 1: Distribution of ions in the simulated system. The entire simulated system is shown in a side view with some lipids removed for better clarity. The protein is shown in a transparent representation with SecY, SecE, and Secβ colored in grey, orange, and ochre, respectively. The lipids are shown in a yellow licorice representation and are also transparent; the surrounding water is shown in a blue transparent surface representation. The ions are shown as spheres with yellow representing sodium and blue chloride ions. (A) System used for simulation sim1 (see Table 1). (B) System used for simulation sim6 (see Supplementary Table 1).

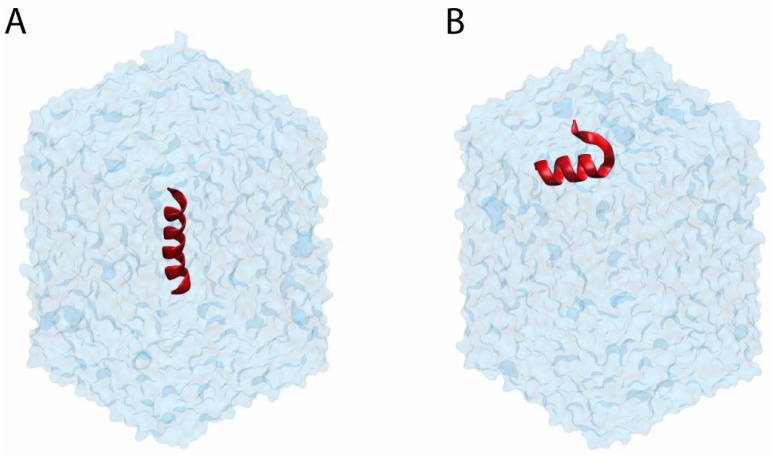

Figure 2: Equilibration of AL19. The helix AL19 is shown in cartoon representation, colored red, with the surrounding water box shown in transparent blue surface representation. (A) Helix AL19 at 0 ns. (B) Helix AL19 at 4.6 ns.

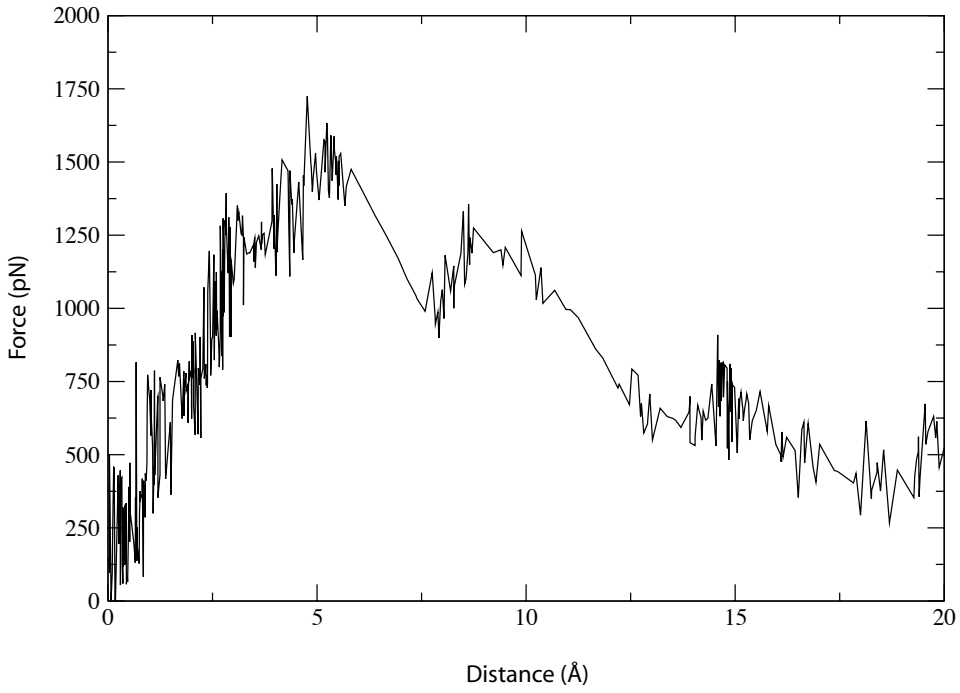

Figure 3: Force profile for plug removal. Force *vs.* position is shown for the plug in simulation sim9. Data points were taken every 100 fs during the simulation and then averaged over every picosecond to produce the plot.

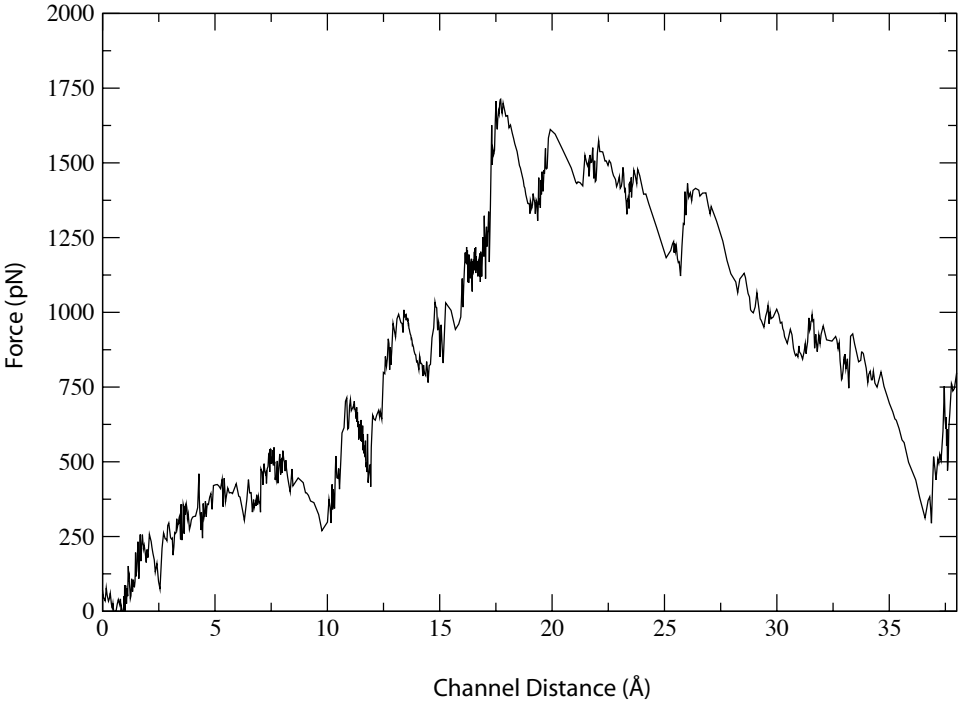

Figure 4: Force profile for simulation sim10. Force *vs.* position is shown for deca-alanine. Data points were taken every 100 fs during the simulation and then averaged over every picosecond to produce the plot.

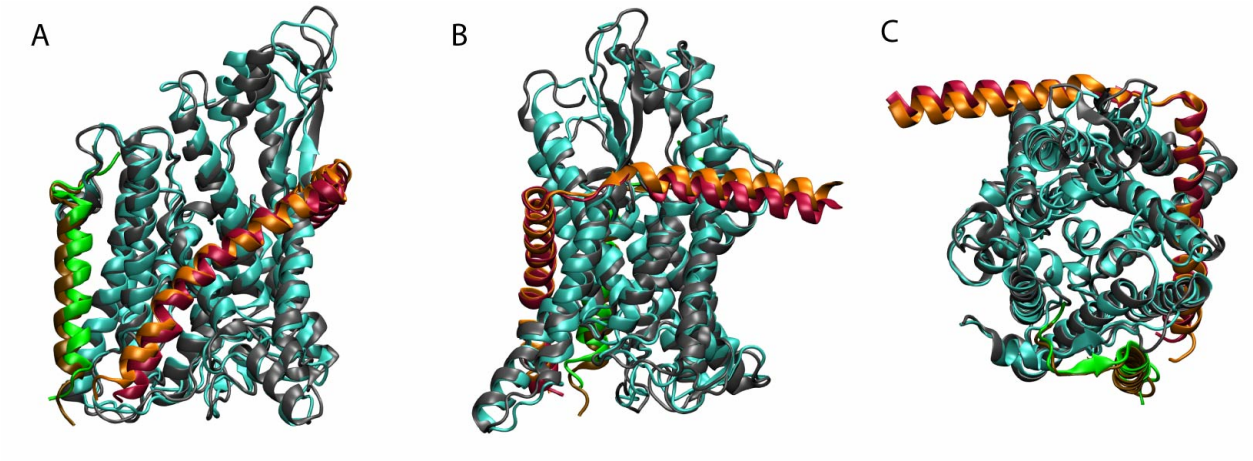

Figure 5: Structure comparison between the cyrstal structure and the equilibrated structure. The original crystal structure (shown in cartoon representation with cyan, red, and green for SecY, SecE, and Secβ, respectively) is shown in a best-fit alignment with the structure resulting from the end of the equilibration sim0 (see main text). The equilibrated structure of SecYEβ is shown in the same representation as in Figure S1. (A) Side view of the two structures. (B) Side view rotated by 90 degrees as compared to that shown in A. (C) Top view of the compared structures.

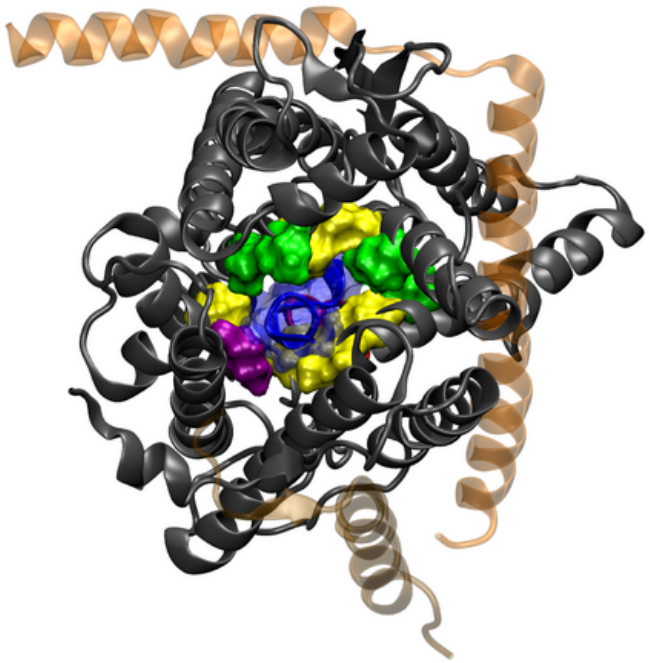

Figure 6: Pore ring during translocation of deca-alanine. Shown in the same representation and orientation as in Figure 3B (see main text) is the translocation during simulation sim1 (see Table 1) at t=0.8 ns. In addition to the main pore ring residues, also present and highlighted here in green surface representation, are residues Leu^{261}, Ile^{257}, Ile^{410}, and Val^{178}. Shown in purple surface representation is residue Ile^{78}.

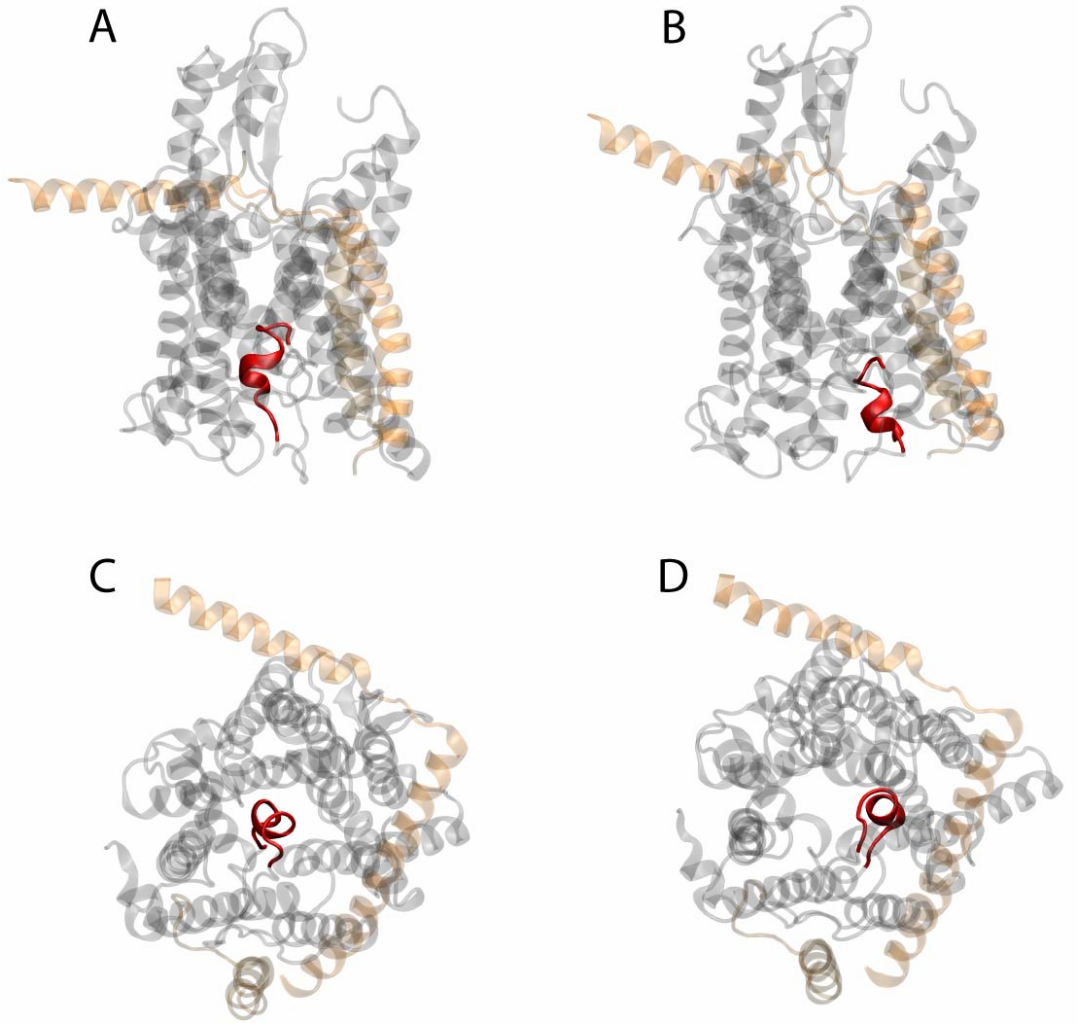

Figure 7: Plug movement in simulation sim2 (see main text). SecYEβ is shown in a transparent cartoon representation with the same coloring as in Figure S1. The plug is highlighted in red and is not transparent. (A) Channel state at t=0 ns. Shown is a side view of the channel. (B) Channel state at t=1.9 ns. The same view of the channel as in A is shown. (C) Channel state at t=0 ns, shown from the top. (D) Channel state at t=1.9 ns, also shown from the top.

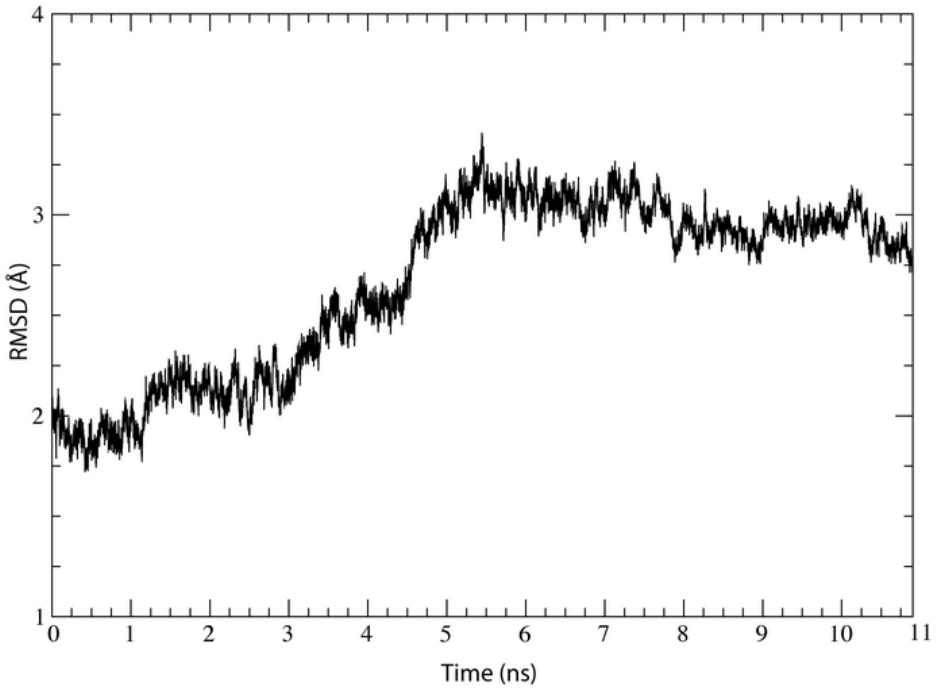

Figure 8: Deformation of SecYEβ during a translocation cycle. Shown is the RMSD for sim1a and sim11 (see Table 1 in the main text as well as Supplementary Table 1), calculated as in Fig. 7 in the main text. Sim1a ends and sim11 begins at 7.4 ns.