Supplementary Information

Translocation of a single Arg_9 peptide across a DOPC/DOPG(4:1) model membrane using the weighted ensemble method

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I. THE NUMBER OF HYDROGEN BONDS BETWEEN ARG_9 AND THE LIPIDS MOLECULES AND THE CONFORMATIONAL CHANGE OF ARG_9 DURING THE TRANSLOCATION

We counted hydrogen bonds between Arg_9 and the lipid molecules during the WE simulations. Fig. S1 denotes the number of hydrogen bonds calculated in VMD [1]. The blue line is the number of the hydrogen bond between Arg_9 and the lipids, while the green line is between Arg_9 and the phosphate group. On the other hand, the red line denotes the hydrogen bond between Arg_9 and water. Each point in the figure corresponds to an averaged value over 10 iterations. The red line shows a rapid increase when Arg_9 reaches the bottom of the membrane. The figure shows that the average number of hydrogen bonds between Arg_9 and the lipids (or water) was about 20. We suggest in the main text that the water flow and the orientation angle of Arg_9 are vital factors that control the translocation of Arg_9.

Fig. S2 shows the conformational change of Arg_9s during the WE simulation calculated in the Timeline in VMD [1]. The third Arg_9 is the one that showed the translocation across the membrane. The other three Arg_9s showed the conformation of the random coil during the translocation, while the third one maintained "turn" conformation during the whole WE simulation.

II. THE POTENTIAL OF MEAN FORCE(PMF) PROFILES (RAW DATA)

We have obtained five pmf profiles based on our five WE simulation data at different bin boundaries. Fig. S3 shows the raw data from each WE simulation. Next, we must combine these profiles to connect (Fig. 6 in the main text).

III. COMPARISON OF THE ORIENTATION ANGLE OF ARG_9 AT THE LAST PART OF THE TRANSLOCATION

In addition to the WE5 simulation, we performed two more simulations (WE5a and WE5b) to compare the orientation angle of Arg_9 during the last part of the translocation to the bottom of the membrane. We used the same system set up in the WE5 simulation for the WE5a and WE5b simulations. They have the same boundary and bin size. Table S1

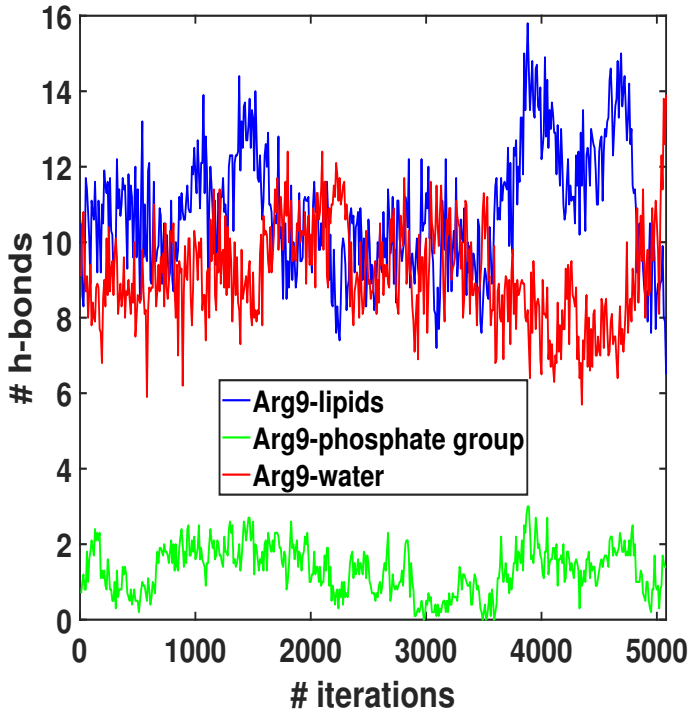


FIG. S1: The number of hydrogen bonds (blue: between Arg_9 and the lipids molecules, green: between Arg_9 and the phosphate group, red: between Arg_9 and water) vs. the number of iterations

denotes a list of three WE simulations performed at the end of translocation, including the WE5 simulation in the main text. Fig. S4 shows the penetration depth vs. the orientation angle from the WE5, WE5a, and WE5b simulations. The figure shows a strong correlation between the penetration depth and the orientation angle. Arg_9 can penetrate rapidly with a smaller orientation angle. Fig. S5 presents free energy profiles from the WE5, WE5a, and WE5b simulations and shows that a smaller angle is energetically favorable for translocating Arg_9 across the membrane.

IV. AN ADDITIONAL EQUILIBRIUM SIMULATION WITHOUT THE WE METHOD

After finishing the WE simulations (from the WE1 to WE5), we ran an additional equilibrium simulation for 1 μs to see conformational changes of Arg_9s and the lipid molecules. The equilibrium simulation method was similar to the previous ones[2, 3]. Fig. S6 presents

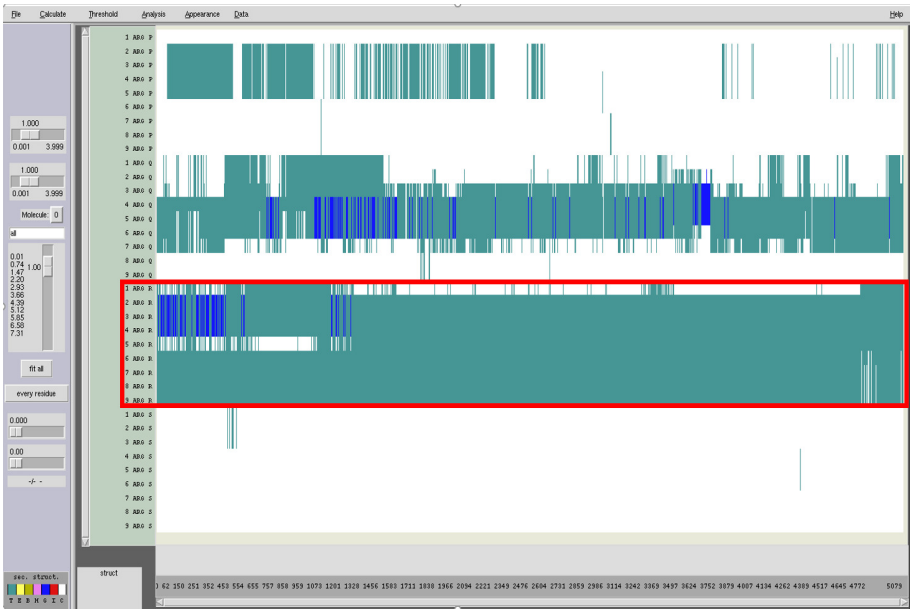


FIG. S2: The conformational change of Arg_9s during the WE simulation. We used the Timeline in VMD [1]. The third Arg_9 is the one that showed the translocation across the membrane

TABLE S1: A list of three WE simulations performed at the last part of the translocation

simulation no.	bin boundaries	bin size (the smallest)	# iterations
WE5	[−45.0 Å, −33.0 Å]	0.10 Å	196
WE5a	[−45.0 Å, −33.0 Å]	0.10 Å	315
WE5b	[−45.0 Å, −33.0 Å]	0.10 Å	390

snapshots at a few different times during the equilibrium simulation. During the simulation, Arg_9 moved back to the membrane's middle with a few lipid molecules. As mentioned in the main text, six lipid molecules moved along with Arg_9 during the WE simulations. Four stayed in the lower leaflet until the end of the equilibrium simulation, while two moved back to the upper leaflet when Arg_9 moved back to the middle of the membrane. Since the equilibrium simulation started, it took about 45 ns for two lipids to move back to the upper leaflet. The water flow and the movement of Arg_9 affect the phospholipid(PL) translocation. The water pore was closed after about 70 ns, as shown in Fig. S7. Fig. S7 presents the total number of water molecules translocated across the membrane during the first 100 ns

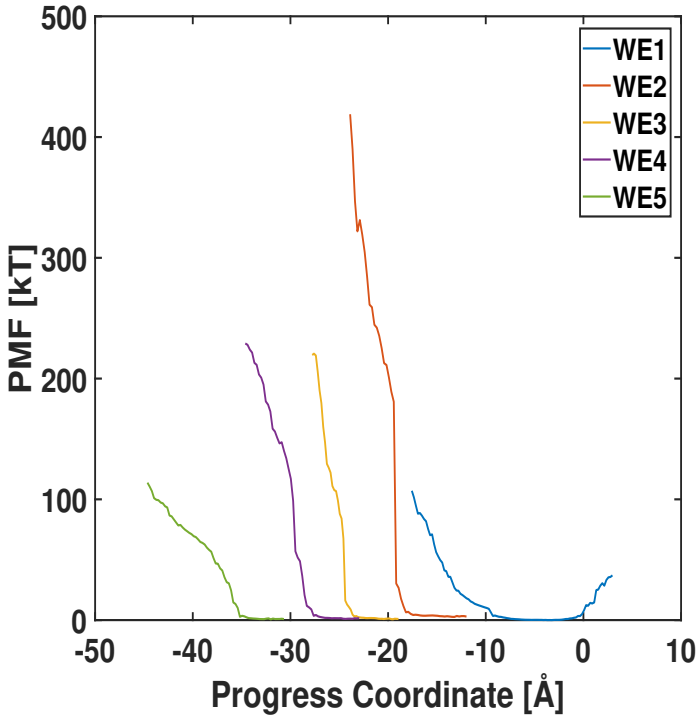


FIG. S3: Comparison of free energy profiles(raw data) along the progress coordinate.

simulation. Before closing the water pore ($\sim$ 70 ns), the upward water flux was 1.5 (water molecules/ns), while the downward water flux was 1.2. The upward water flux is slightly higher than the downward water flux due to the upward movement of Arg_9.

[1] W. Humphrey, A. Dalke, and K. Schulten, J. Molec. Graphics **14**, 33 (1996).

[2] S. Choe, AIP Advances **10**, 105103 (2020).

[3] S. Choe, Membranes **11**, 974 (2021).

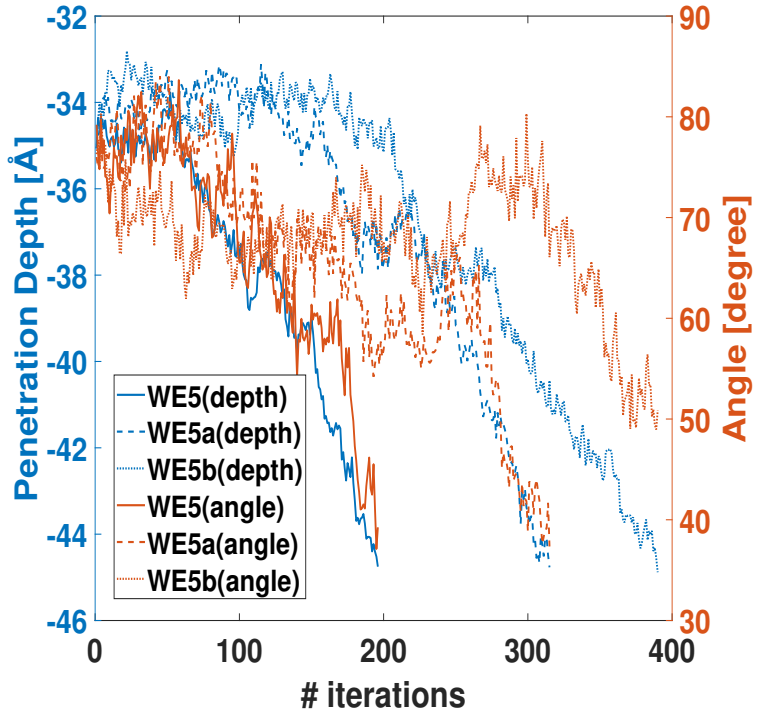


FIG. S4: The penetration depth vs. the orientation angle

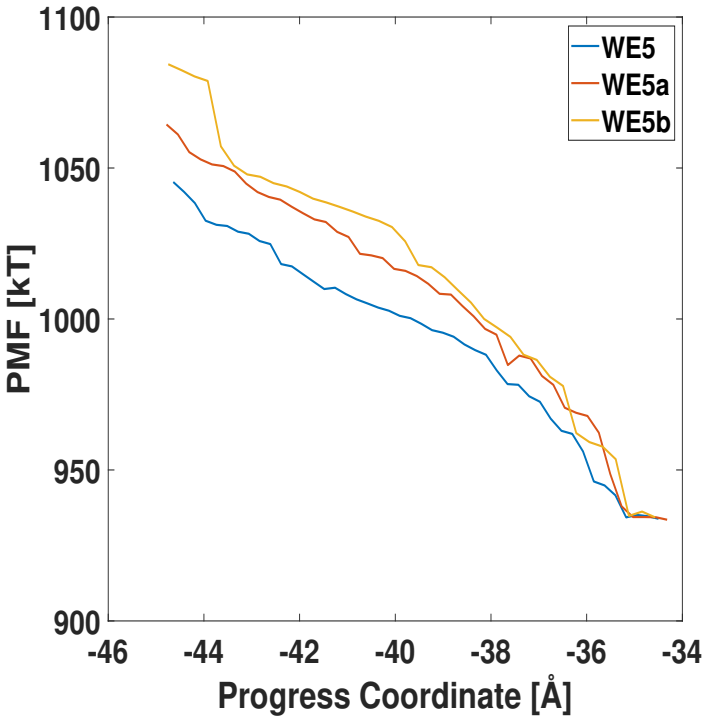
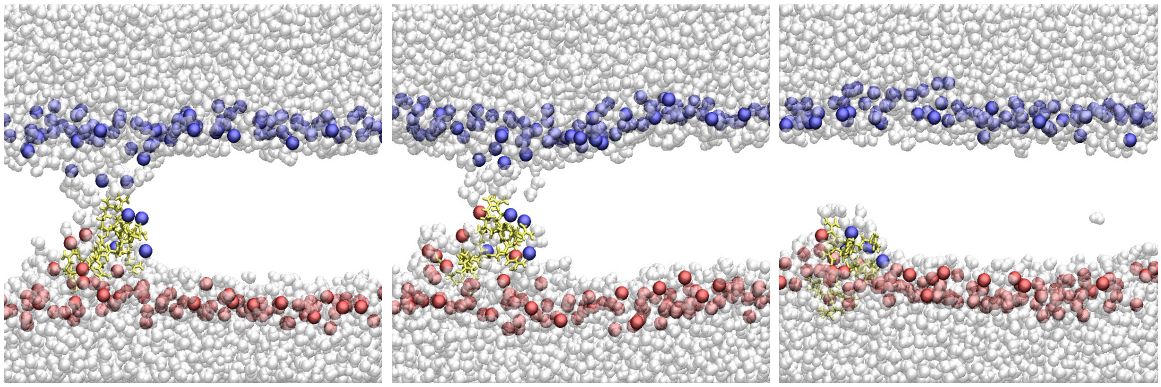
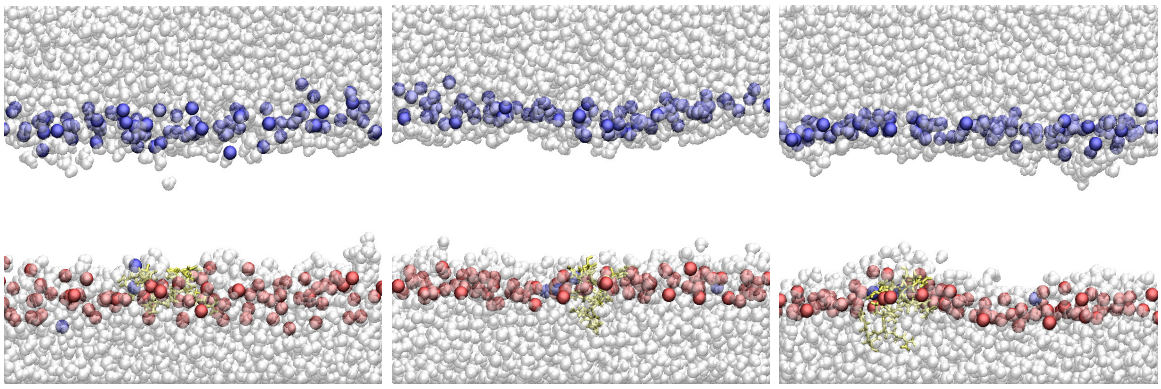


FIG. S5: Comparison of free energy between the WE5, WE5a, and WE5b simulations.



(a) 20 ns (b) 40 ns (c) 60 ns



(d) 600 ns (e) 800 ns (f) 1 μs

FIG. S6: Snapshots at (a) 20 ns (b) 40 ns (c) 60 ns (d) 600 ns (e) 800 ns, and (f) 1 μs during the additional equilibrium simulation (yellow: Arg_9, white: water, blue & red : phosphorus atoms of the upper leaflet & lower leaflet, respectively).

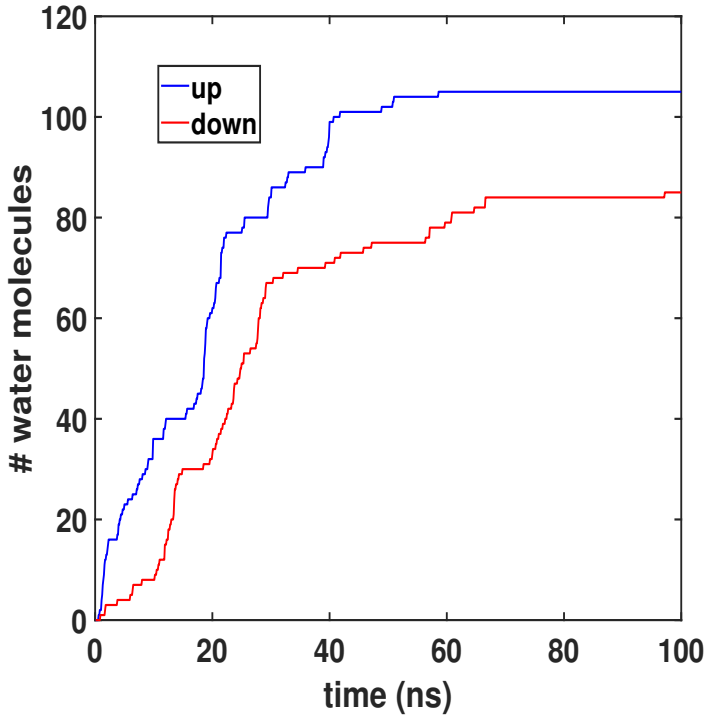


FIG. S7: A total accumulated number of water molecules translocated across the membrane (the moving-up & the moving-down) vs. equilibrium simulation time