Biophysical Journal, Volume *119*

Supplemental Information

Membrane Interactions of Cy3 and Cy5 Fluorophores and Their Effects on Membrane-Protein Dynamics

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Contact time with lipid bilayers

For simulations with PC and PE bilayers, the fluorophores are attracted to the headgroup layer, while the fluorophores spend most of the time diffusing in the bulk solution in the case of PS:PC bilayer. Measuring the time that the fluorophores make zero contacts to the lipids, it is shown that Cy3 and Cy5 spend ∼25% of the time in the bulk solution with PC membrane; ∼11–19% with PE; and ∼63% with PS:PC, respectively (Table S1).

	Time interacting with membrane (%)			Time in bulk solution (%)		
	PC	PE	PS:PC	PC	PE	PS:PC
Cy3	61.9	81.0	28.5 (16.6/21.8)*	25.2	11.2	62.9
Cy5	57.6	72.0	28.6 (17.3/22.3)*	24.7	18.9	62.8

Table S1: Summary of fluorophore interaction with the membrane. Time interacting with membrane is defined by the portion of simulation time (1000 ns in total) in which the fluorophore makes more than 20 contacts with lipids ($C > 20$, see main text for definition of C). Time in bulk solution is defined by portion of simulation time in which the fluorphore makes zero contacts with the lipids.
* First number in the parenthesis indicates contact time with PS lipids, second number indicates contact time with PC lipids.

Convergence of HMMM simulations

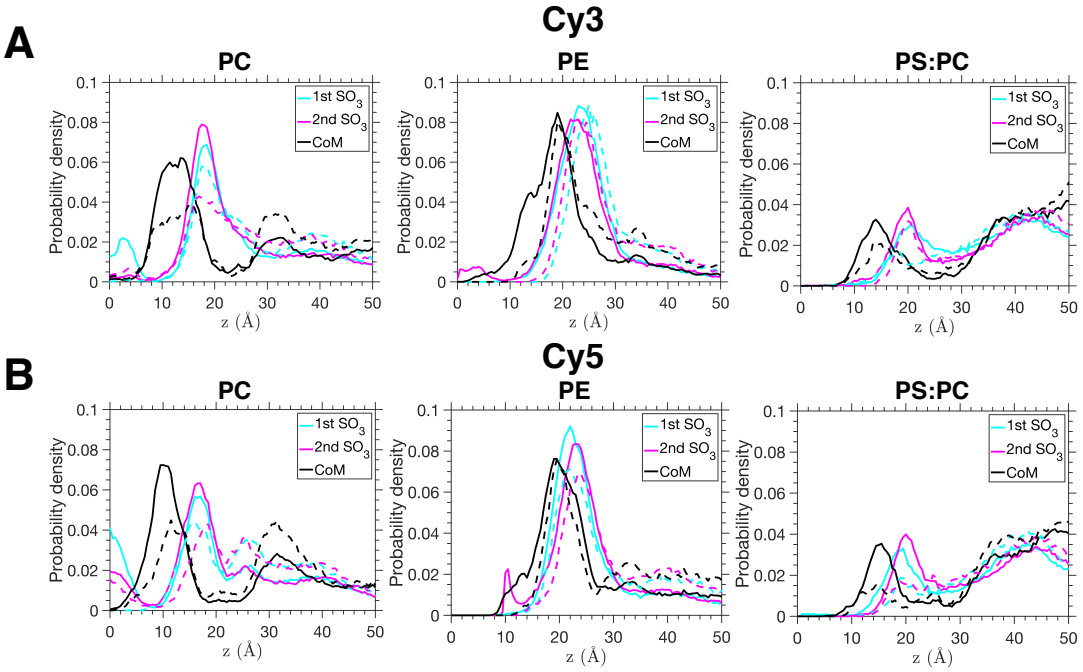

Figure S1: Convergence of the samplings in HMMM membrane binding simulations. The probability density of the z-position of the two SO_3 groups and the center of mass of (A) Cy3 and (B) Cy5 are shown in cyan, magenta, and black lines (membrane midplane at z = 0). Solid lines represent the samplings from the entire simulation sets (5×200 ns=1μs) for each fluorophore. Dashed lines represent the samplings from the first half of the simulation sets (5×100 ns=500 ns).

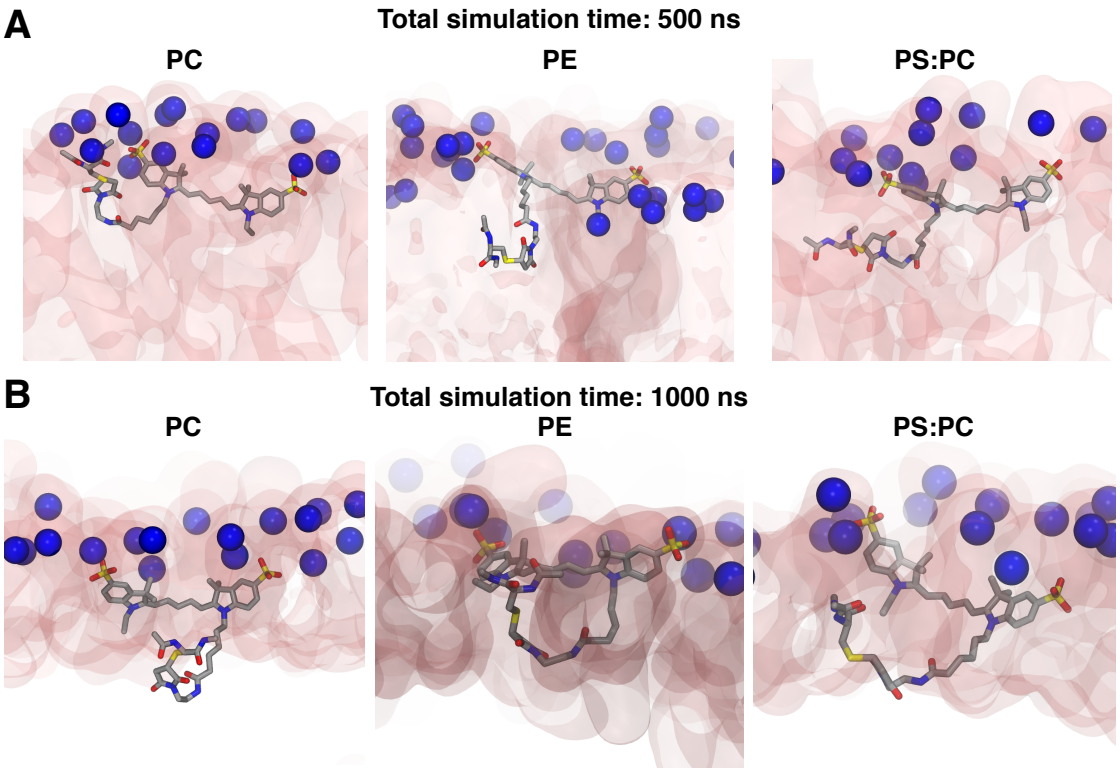

Figure S2: Representative membrane-bound configurations in HMMM simulations. Representative configurations are obtained by clustering all membrane-bound configurations in the HMMM simulations. (A) Centroid structures of a Cy5 bounding to PC, PE, or PS:PC HMMM membranes, obtained from the first half of the simulation sets (total simulation time: 500 ns). (B) Centroid structures of membrane-bound Cy5, obtained from the entire simulation sets (total simulation time: 1000 ns). The Cy5 molecule is shown in licorice representation. Lipids are shown in pink transparent surface. The nitrogen atoms in the lipid headgroups are shown in blue spheres.

Convergence of full-tailed lipid packing

To assess the convergence of lipid packing after the conversion from HMMM membranes to full-tailed membranes, the area per lipid for each system during the initial 15-ns equilibration is shown in Fig. S3. The area per lipid is calculated by dividing the lateral area (X-Y) of the simulation box by the number of lipids in each leaflet. At the end of the equilibration, the average area per lipid (POPC: 65.4±1.1 Å^2, POPE: 58.8±1.0 Å^2, POPS/POPC: 62.7±0.8 Å^2) is consistent with experimental values (POPC: 64.3 Å^2 [1], POPE: 58 Å^2 [2], POPS/POPC: $(64.3 + 62.7)/2 = 63.5$ Å^2 [3]).

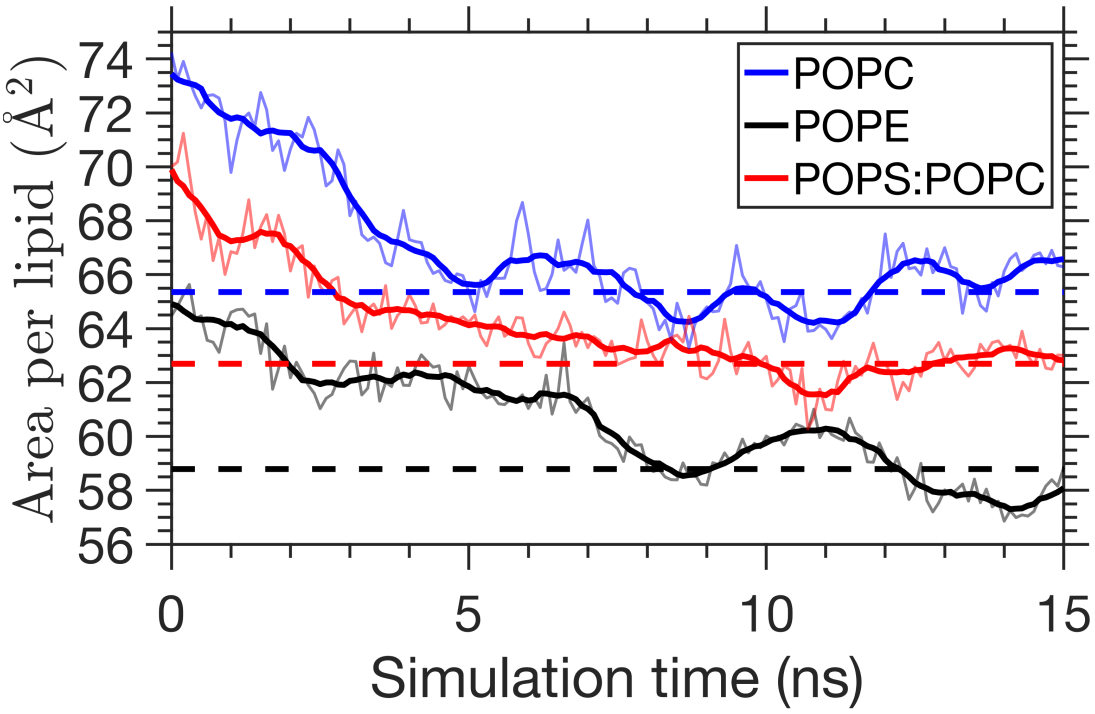

Figure S3: Convergence of area per lipid after the conversion of HMMM membrane to full-tailed membrane. The area per lipid for POPC, POPE, and POPS/POPC mixture membrane is shown in blue, black, and red, respectively. The average area per lipid over the last 7 ns is shown by the horizontal dashed lines.

Convergence of free energy calculations

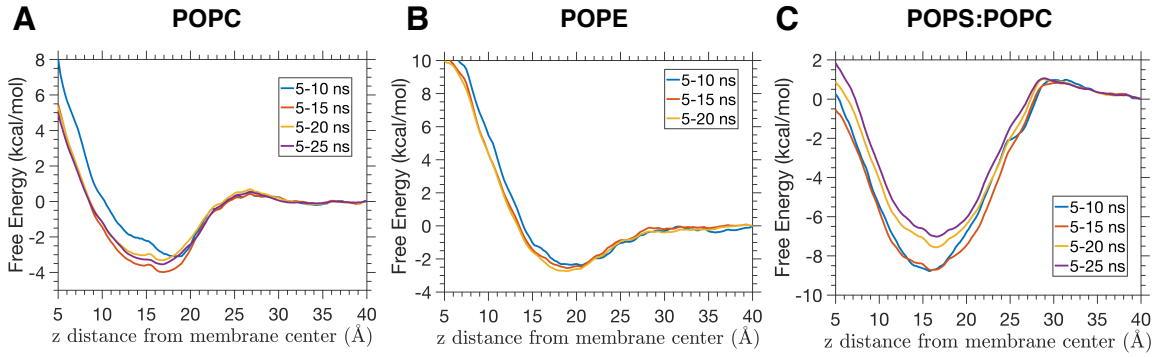

Figure S4: Convergence of BEUS free energy calculations. The membrane-binding free energy is calculated with different blocks of the BEUS samplings in each window (5–10 ns, 5–15 ns, 5–20 ns, and 5–25 ns, discarding first 5 ns) for (A) POPC, (B) POPE, and (C) POPS:POPC mixture membrane.

Water contacts with Cy5

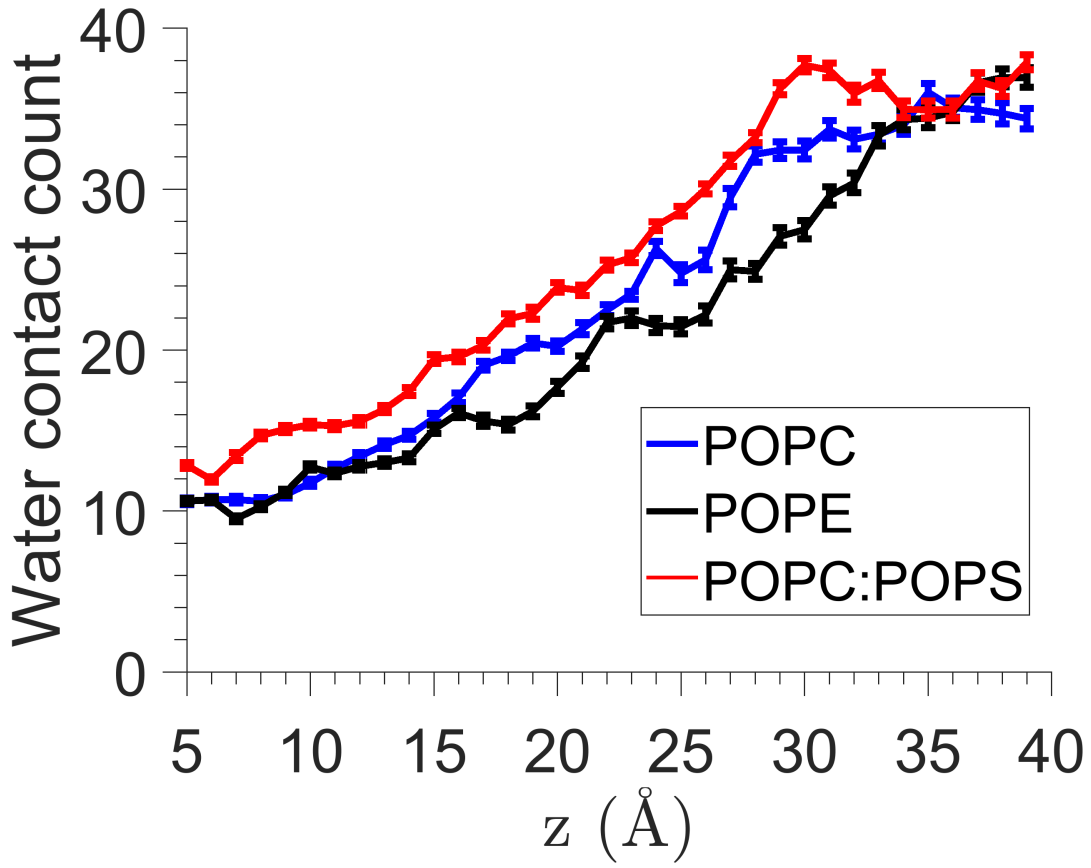

Figure S5: Water contact count with Cy5 during membrane insertion. Mean water contact counts are calculated for each window (center of mass separation between Cy5 and membrane projected on the z axis, z) in the BEUS simulations for different lipid compositions. Any water molecule within 3.5 Å of any heavy atoms of a Cy5 molecule is considered to be in contact. Standard errors of the mean water contact counts in each window are reported by the error bars.

Hydrogen bonds of interhelical loops

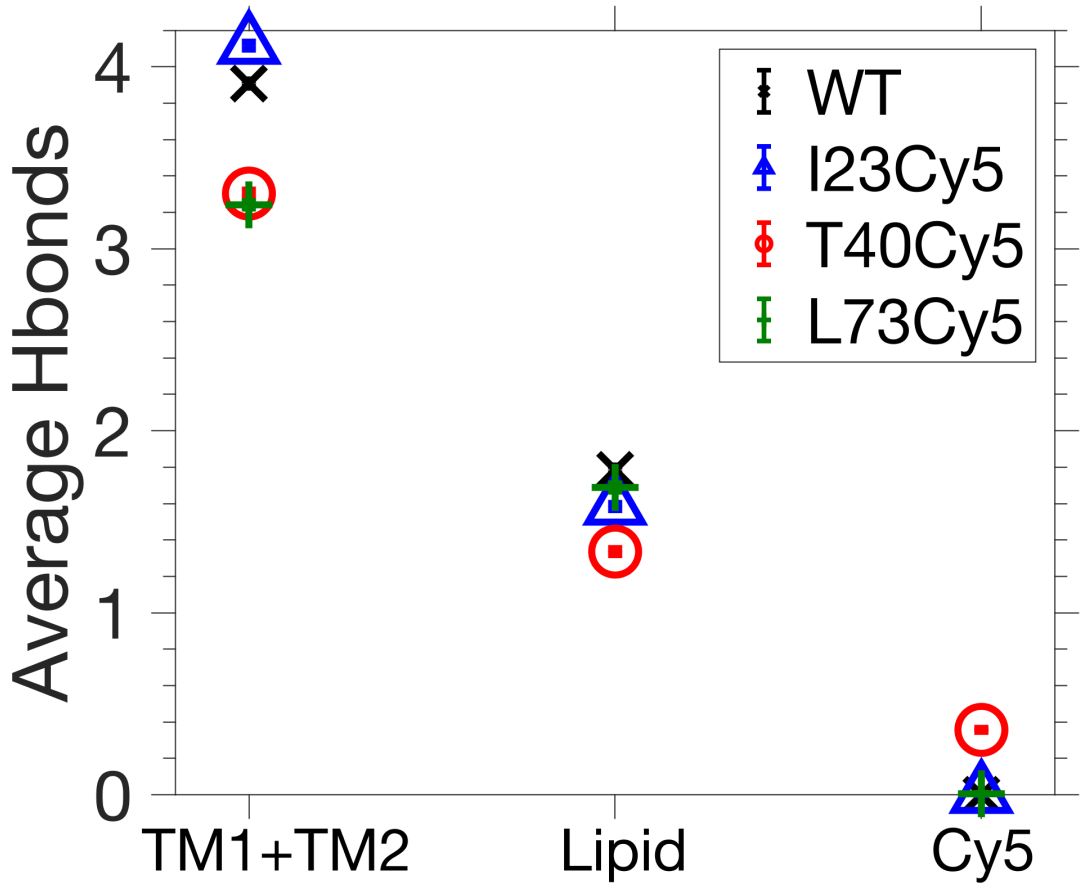

Figure S6: Hydrogen bonds formed between the interhelical loops of MscL and its TM helices, lipids, or fluorophores. The number of hydrogen bonds is averaged over the last 75 ns of the two independent trajectories of each system. Standard errors of the average hydrogen bond are reported by the error bars, which are all less than 0.05.

References

[1] Kučerka, N., M.-P. Nieh, and J. Katsaras, 2011. Fluid phase lipid areas and bilayer thicknesses of commonly used phosphatidylcholines as a function of temperature. *Biochim. Biophys. Acta, Biomembr.* 1808:2761–2771.

[2] Kučerka, N., B. van Oosten, J. Pan, F. A. Heberle, T. A. Harroun, and J. Katsaras, 2015. Molecular structures of fluid phosphatidylethanolamine bilayers obtained from simulation-to-experiment comparisons and experimental scattering density profiles. *J. Phys. Chem. B* 119:1947–1956.

[3] Pan, J., X. Cheng, L. Monticelli, F. A. Heberle, N. Kučerka, D. P. Tieleman, and J. Katsaras, 2014. The molecular structure of a phosphatidylserine bilayer determined by scattering and molecular dynamics simulations. *Soft Mat.* 10:3716–3725.