Supplementary Information (SI) for Journal of Materials Chemistry B.

Supporting Information

Table S1: Sampling ranges and window divisions in WTM-eABF calculations. The range of each window is given in Å, with the corresponding simulation time (μs) listed below.

Membrane	RhoVRs	win01	win02	win03	win04	win05
POPC	RVR1	[10, 55] 4.0	[-15, 15] 5.6	[-55, -10] 4.0	-	-
	SRV1	[10, 55] 4.0	[-15, 15] 4.0	[-55, -10] 2.9	-	-
	RVR1-component	[10, 43] 1.5	[-15, 15] 2.0	[-43, -10] 1.0	-	-
	SRV1-component	[10, 43] 1.5	[-15, 15] 2.0	[-43, -10] 0.5	-	-
	RVR1-protonated	[10, 50] 1.5	[-15, 15] 3.0	[-50, -10] 1.7	-	-
POPC:CHL	RVR1	[13, 43] 3.0	[-15, 15] 5.6	[-43, -13] 1.0	[36, 50] 0.5	[-50, -36] 0.5
	SRV1	[41, 13] 1.5	[-15, 15] 5.4	[-41, -13] 1.0	[36, 50] 0.5	[-50, -36] 0.5
	RVR1-component	[10, 43] 1.0	[-15, 15] 1.0	[-43, -10] 1.0	-	-
	SRV1-component	[10, 43] 1.0	[-15, 15] 2.0	[-43, -10] 1.0	-	-
	RVR1-protonated	[10, 50] 2.0	[-15, 15] 3.8	[-50, -10] 1.8	-	-

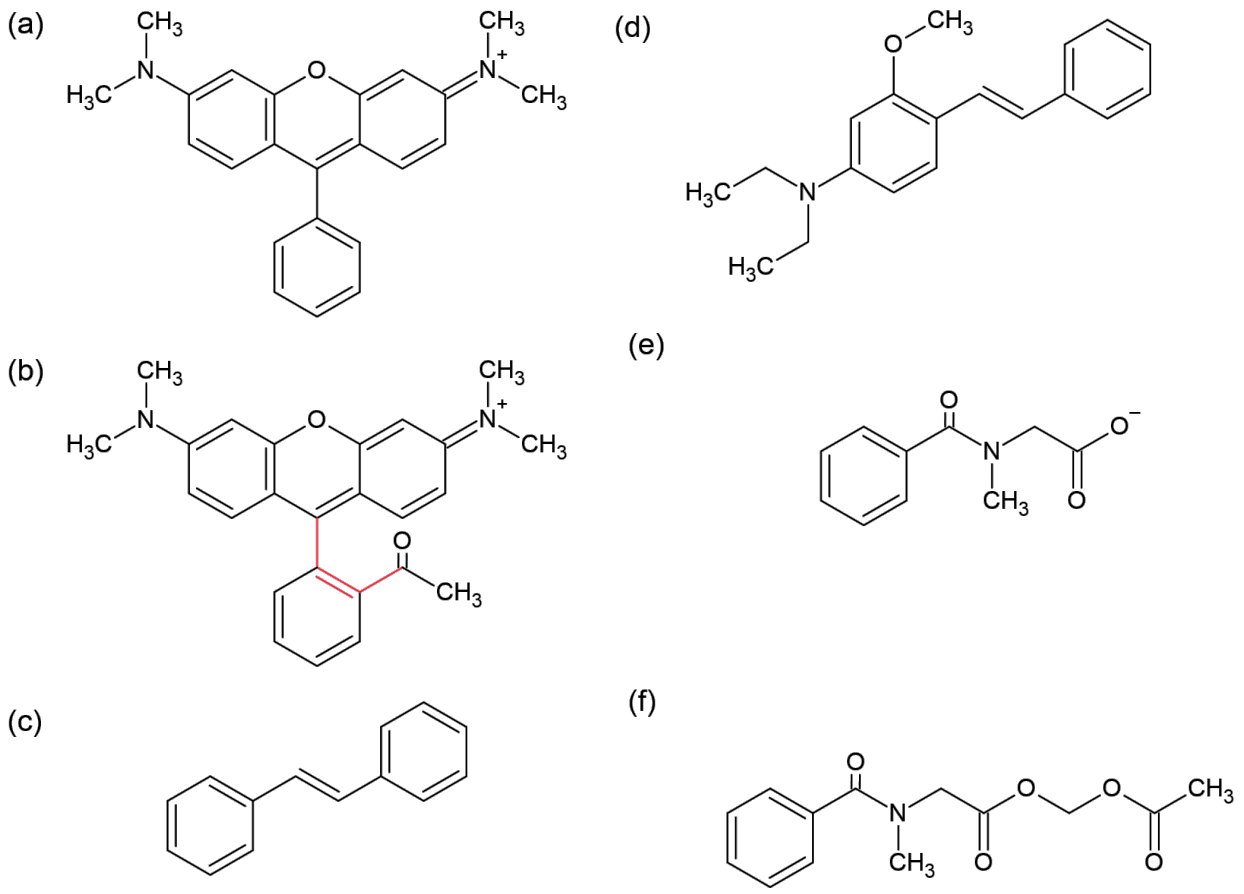

Figure S1: Fragments of RhoVRs employed in their force field parameter optimization (see Methods). Fragment (b) is used to parameterize a dihedral term (highlighted in red) not present in the other components.

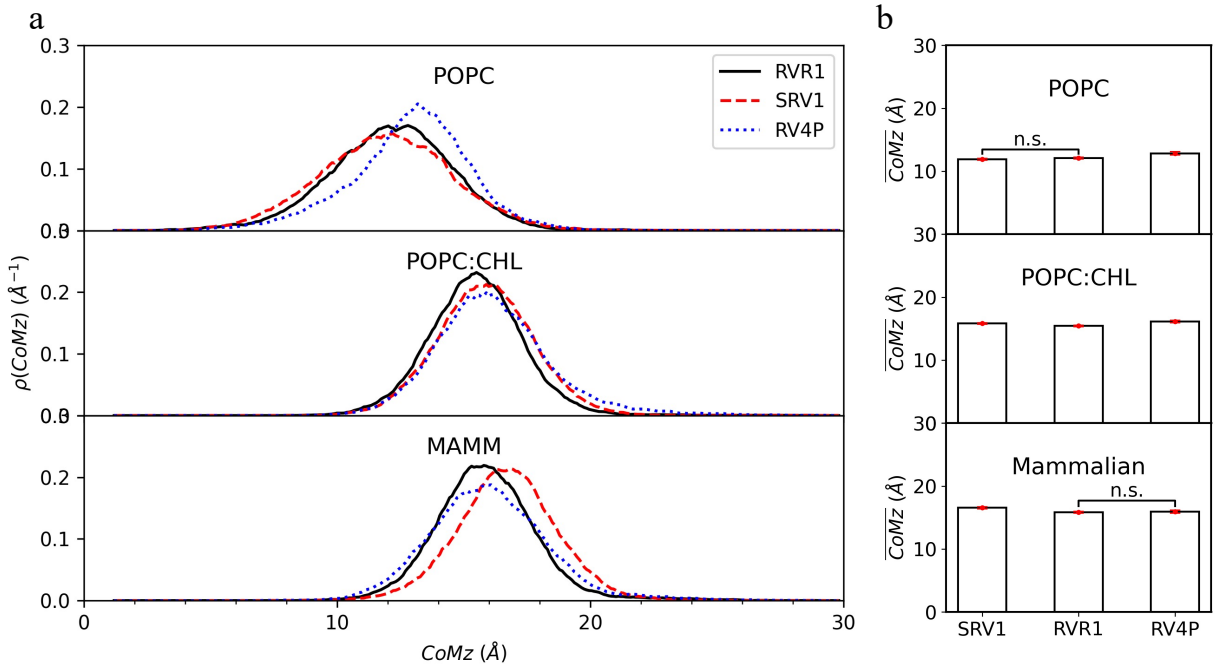

Figure S2: Center of mass of RhoVRs (CoMz) in equilibrium MD simulations. (a) Probability density distribution of CoMz (ρ(CoMz)). (b) Average CoMz ($\overline{\text{CoMz}}$) of RhoVR dyes with the standard error of the mean shown as error bars. Statistical significance analyzed by Welch's t-test yields $P < 0.05$ for all pairs of RhoVR dyes in a given lipid environment except for those labeled as *n.s.* (not significant).

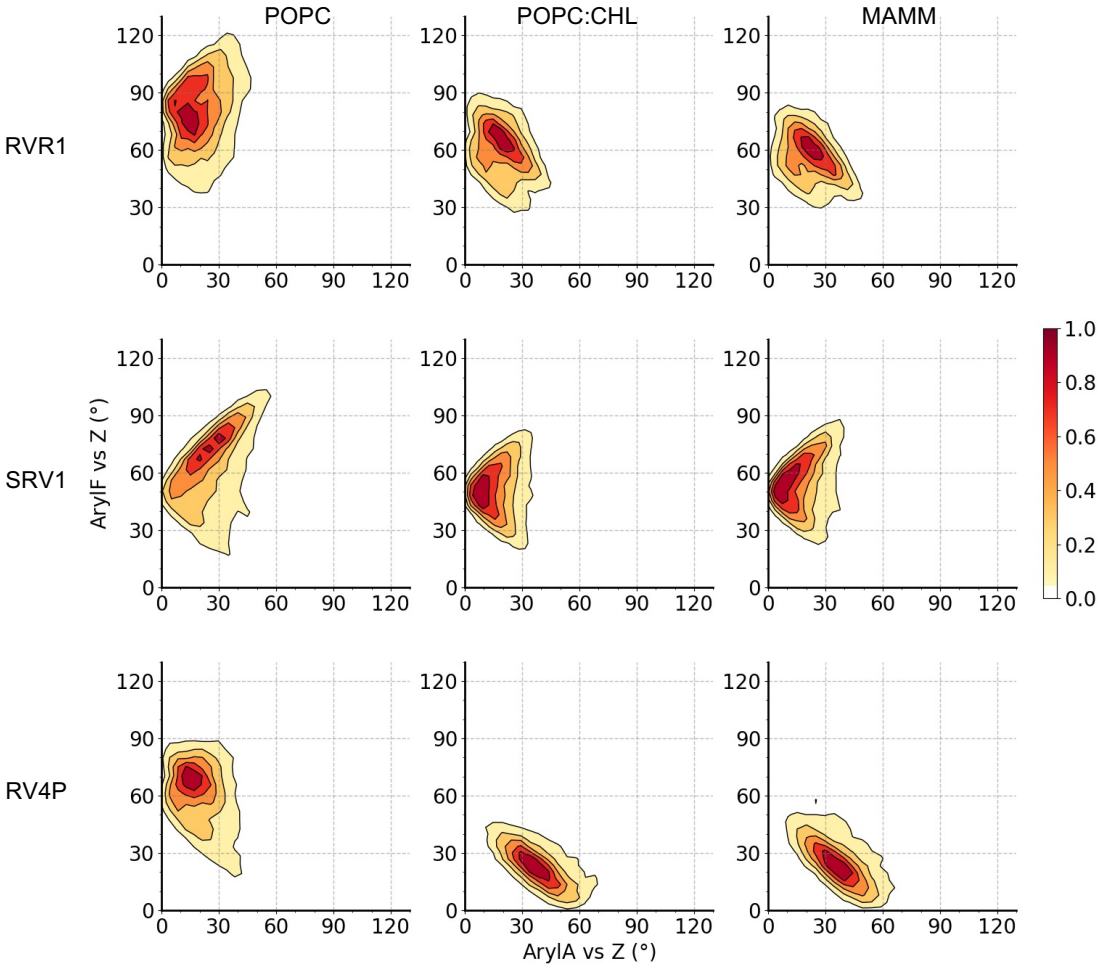

Figure S3: Contour plots depicting the tilt angles of $\overrightarrow{ArylA}$ and $\overrightarrow{ArylF}$ for RhoVR 1, RhoVR 1-4', and SPIRIT RhoVR 1 in equilibrium MD simulations.

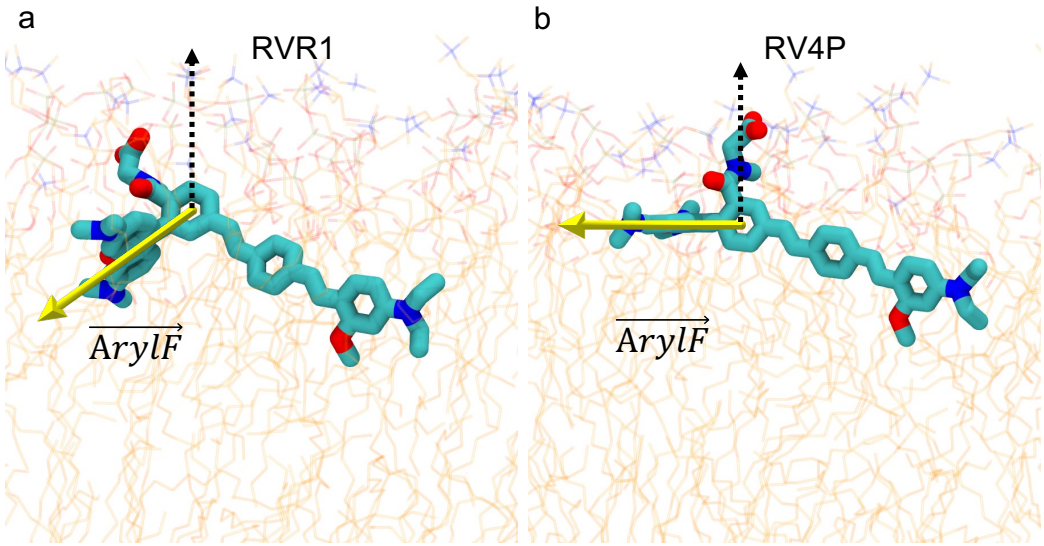

Figure S4: Representative snapshots from equilibrium MD simulations in pure POPC showing the tilting of $\overrightarrow{ArylF}$ in RhoVR 1 (a) and RhoVR 1-4' (b). The tilt angles of $\overrightarrow{ArylF}$ are $\sim$120° and $\sim$90° in (a) and (b), respectively.

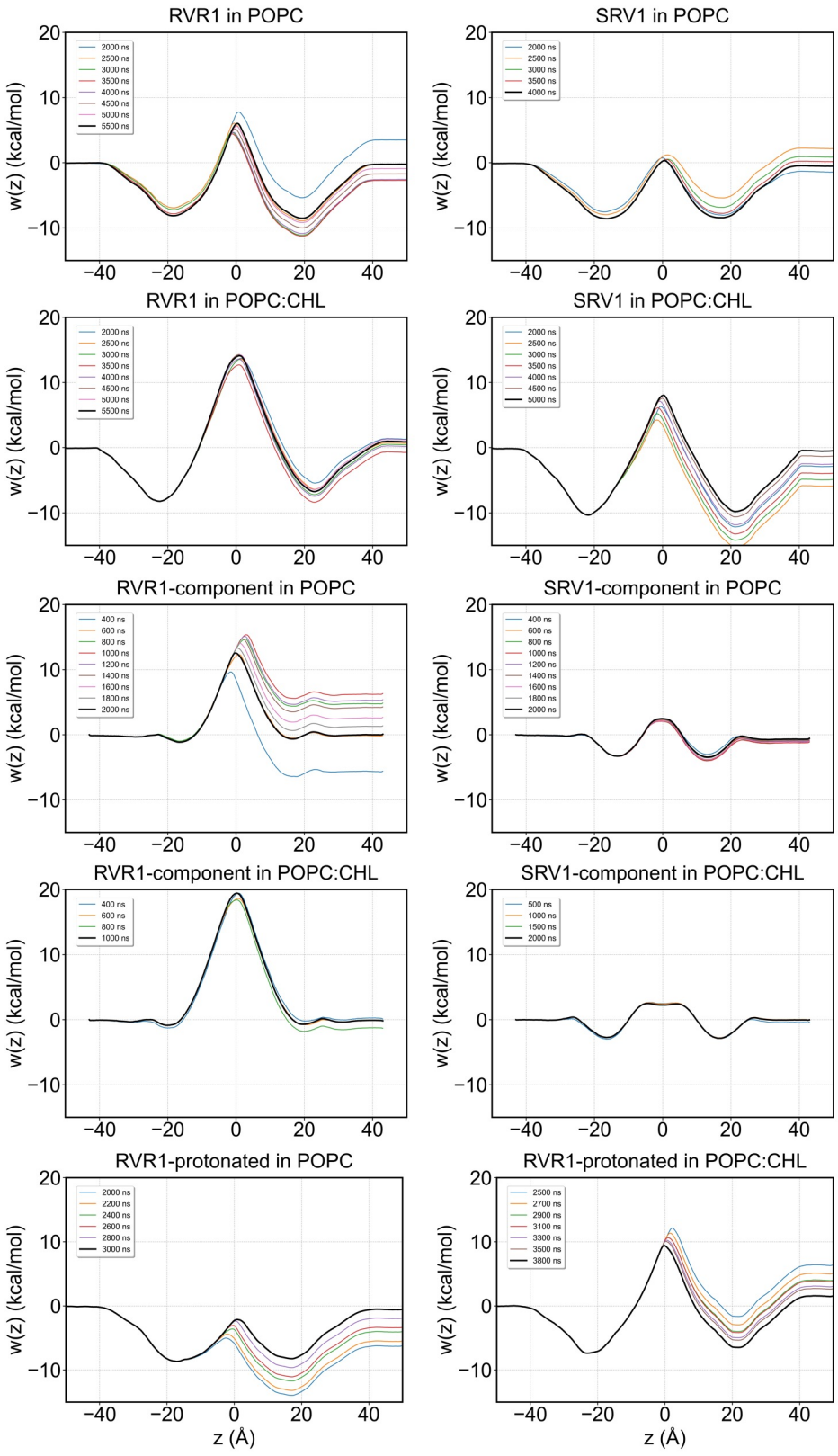

Figure S5: Convergence of unsymmetrized free-energy profiles of RhoVR 1, SPIRIT RhoVR 1 and their components in POPC and POPC:CHL bilayers. Each panel displays PMF profiles calculated at different simulation lengths (per window) with the final profile highlighted in black.

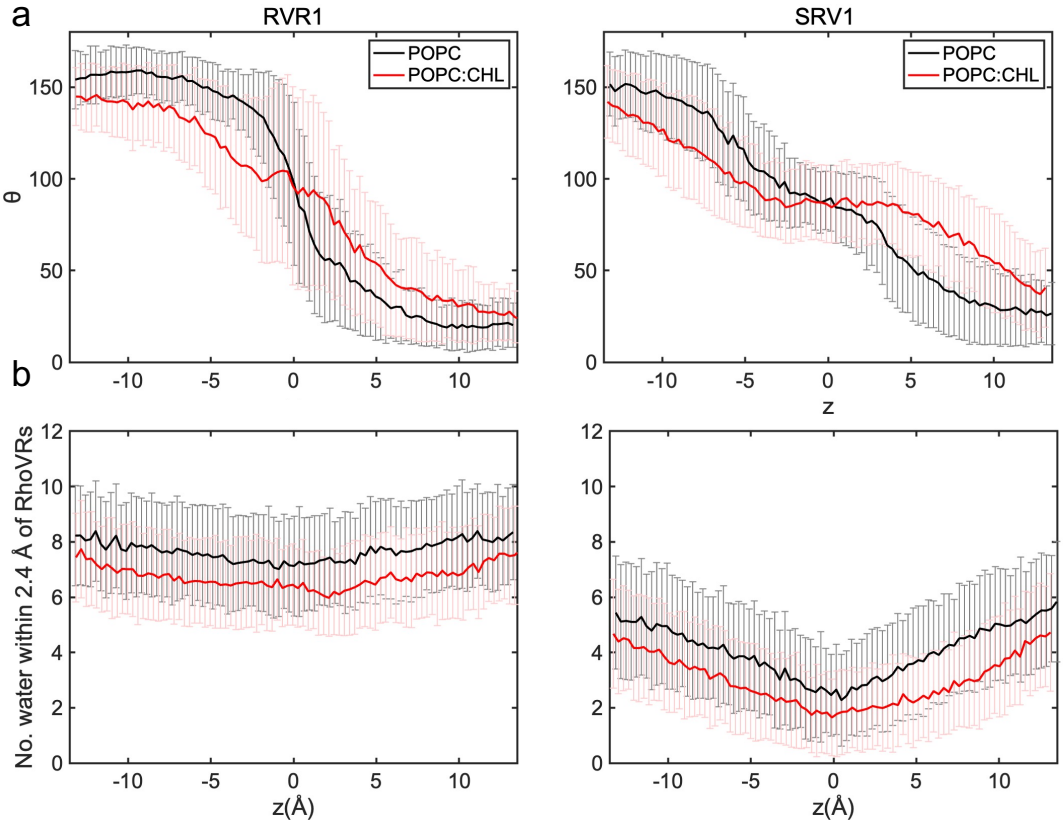

Figure S6: Orientation and solvation of RhoVRs during membrane permeation. (a) RhoVR Orientation as a function of dye location in a given membrane. (b) Average number of water within 2.4 Å of RhoVRs. All panels were computed using the WTM-eABF trajectories with -15 Å $< z <$ 15 Å.

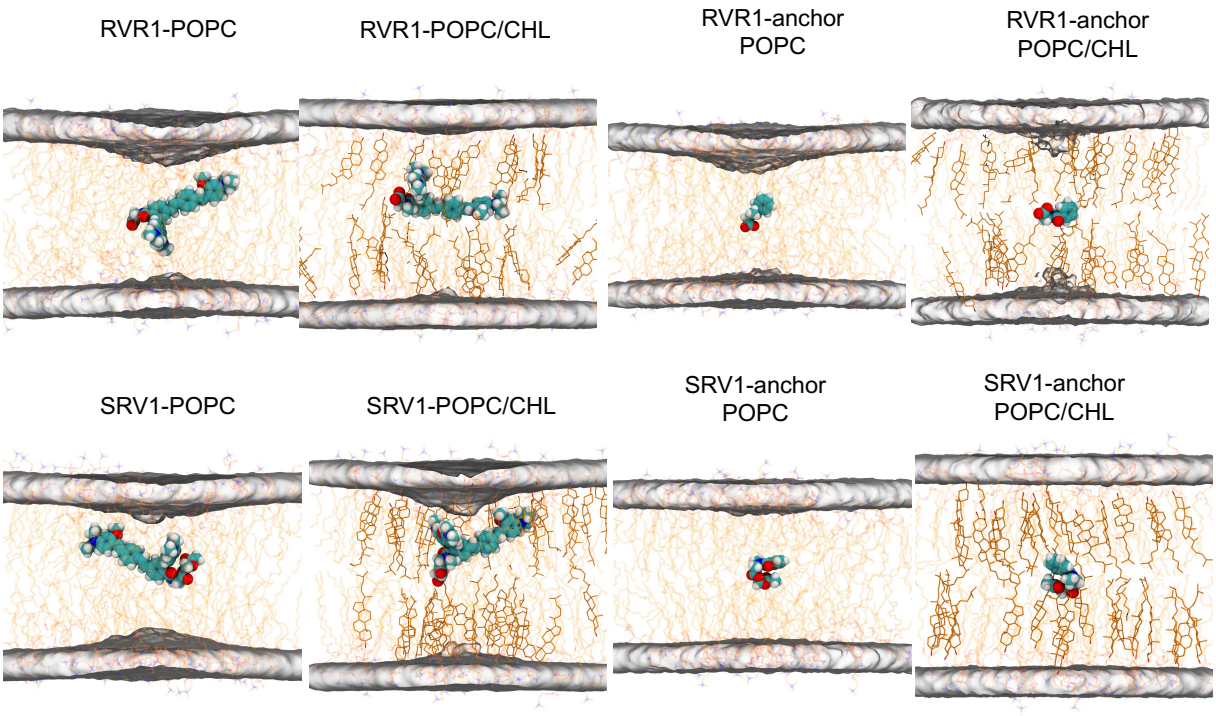

Figure S7: Occupancy of phosphorus atoms calculated from the WTM-eABF trajectories of a given permeant with $-5\,\text{Å} < z < 5$ Å. The isosurfaces with an isovalue of 3% occupancy are superimposed with representative permeant conformations obtained from root mean square deviation (RMSD)-based clustering analysis.

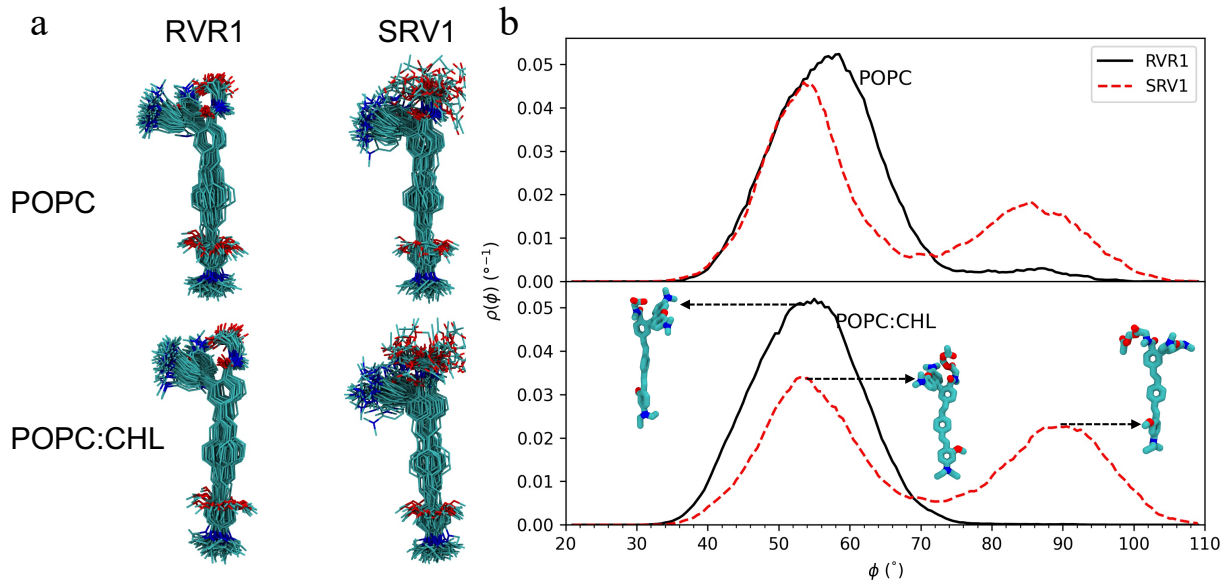

Figure S8: (a) Configurations of RhoVR 1 and SPIRIT RhoVR 1 extracted from WTM-eABF trajectories with $-15\,\text{Å} < z < 15\,\text{Å}$. Following alignment by minimizing RMSD, simulation snapshots are taken every 100 ns, resulting in approximately 50 superimposed configurations for each VSD. (b) Probability density distributions of the angle φ between $\overrightarrow{ArylF}$ and $\overrightarrow{ArylA}$ computed from the same trajectories analyzed in (a).