Electronic Supplementary Material (ESI) for Faraday Discussions.

Lipid distributions and transleaflet cholesterol migration near heterogeneous surfaces in asymmetric bilayers

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Table S1: System configurations.

System	PDB	Number of lipids						Water	Ions	Length
		Upper leaflet			Lower leaflet					
		CHOL	POPC	DPSM	CHOL	POPE	POPS			
Lipids	n/a	78	57	57	78	84	43	2735	43	20 μs x 3
Lipids*[a]	n/a	39	57	57	39	84	43	2999	43	20 μs x 3
CNT	n/a	78	57	57	78	82	43	6305	43	20 μs x 3
AQP1	1J4N	78	57	57	78	82	43	6980	31	20 μs x 3
KcsA	1R3J	78	57	57	78	96	48	6131	180	20 μs x 3
OmpX	1QJ9	78	57	57	78	85	43	5644	45	20 μs x 3
OmpF	3POX	78	57	57	78	91	46	6732	236	20 μs x 3
WALP23	n/a	78	57	57	78	84	43	3387	43	20 μs x 3
AQP1 (AA)[b]	1J4N	78	57	57 (SSM)	78	82	43	28205	35	1 μs x 3

[a] System containing approximately half the number of cholesterol molecules.

[b] Atomistic simulations initiated after back mapping the final configurations of the AQP1 CG systems.

Table S2: Cholesterol flip rates (flips/μs) and leaflet partitioning.

System	Replica 1	Replica 2	Replica 3	Avg ± sdev	Avg C_{ul}/C_{ll} ± sdev[a]
Lipids	0.8 ± 0.3	0.8 ± 0.2	0.8 ± 0.3	0.8 ± 0.02	1.337 ± 0.002
Lipids*	1.5 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.03	1.61 ± 0.03
CNT	0.7 ± 0.3	0.6 ± 0.3	0.7 ± 0.3	0.7 ± 0.02	1.338 ± 0.007
AQP1	0.7 ± 0.3	0.7 ± 0.3	0.6 ± 0.3	0.7 ± 0.02	1.39 ± 0.02
KcsA	0.8 ± 0.4	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.01	1.25 ± 0.03
OmpX	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.01	1.27 ± 0.01
OmpF	0.9 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	0.9 ± 0.01	1.43 ± 0.01
WALP23	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.01	1.32 ± 0.01

[a] Cholesterol ratio between the upper and lower leaflets.

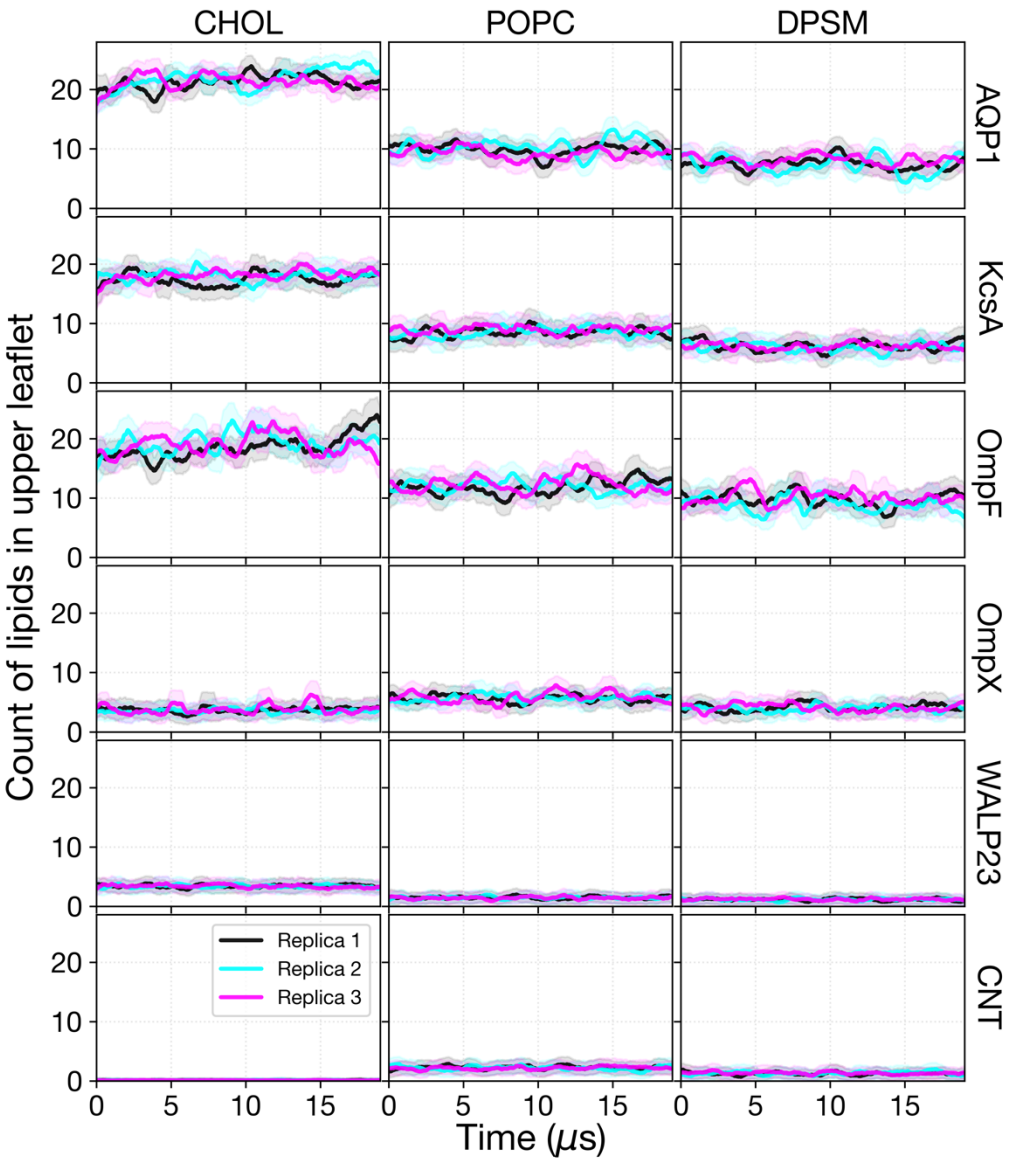

Fig. S1 Counts of upper leaflet lipids around surfaces. Average number of CHOL, POPC, and DPSM lipids in the upper leaflet within a 0.7 nm radius around the surface of the proteins or CNT.

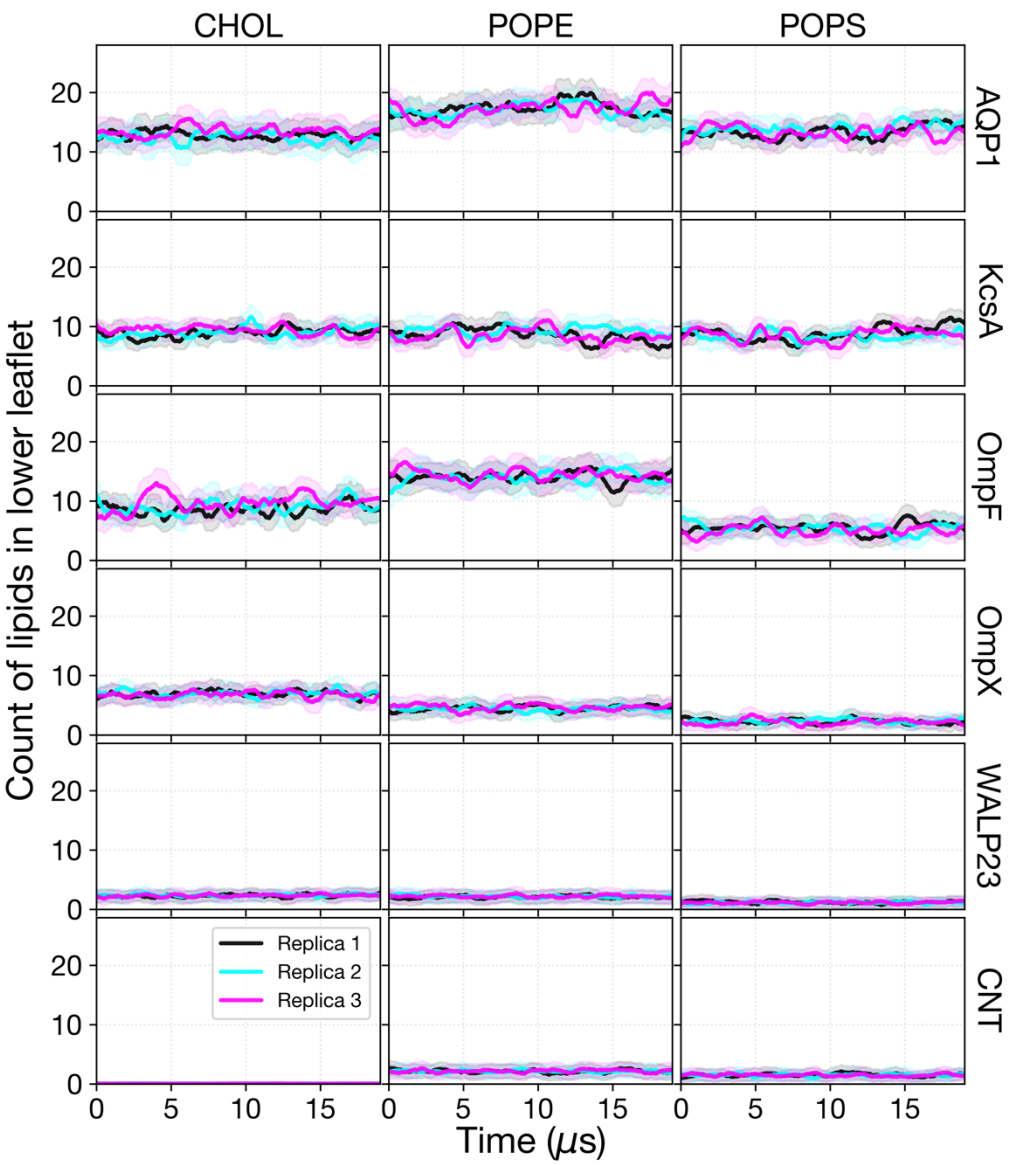

Fig. S2 Counts of lower leaflet lipids around surfaces. Average number of CHOL, POPC, and DPSM lipids in the lower leaflet within a 0.7 nm radius around the surface of the proteins or CNT.

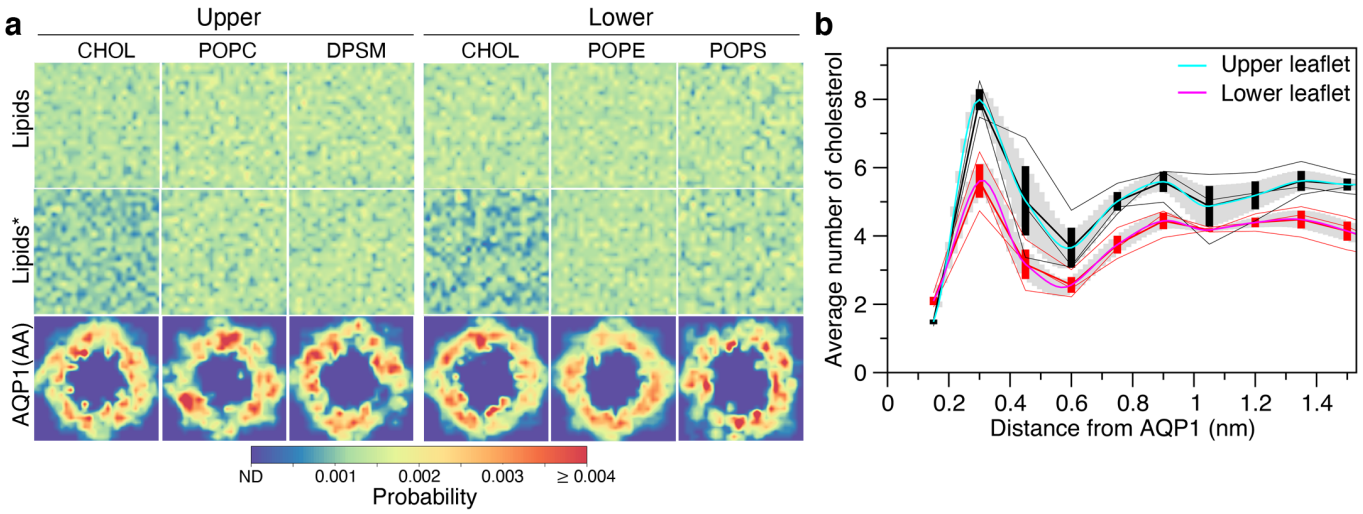

Fig. S3 Lipid distributions. a Probability density of CHOL, POPC, DPSM, POPE, and POPS in the upper and lower leaflets. **b** Cholesterol count as a function of distance from the AQP1 surface in the AA model. The number of cholesterol molecules were counted as a function of nearest distance from the protein to cholesterol oxygen (O3) atom for upper and lower leaflets separately. Data for individual replicas are shown in black and red for the upper and lower leaflets, respectively, with spline fits of the replica averages in cyan and magenta.

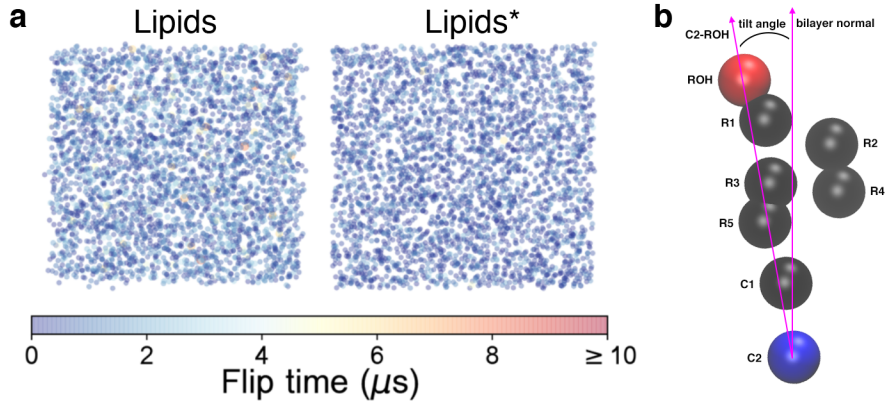

Fig. S4 Cholesterol flip events and times in the lipids only systems. a Each spot represents a cholesterol flip event. The time between flip events are indicated by spot color and size (larger points reflect longer flip times). **b** Schematic of the MARTINI cholesterol model illustrating the tilt angle that was used as a determinant of flip events (see Methods).

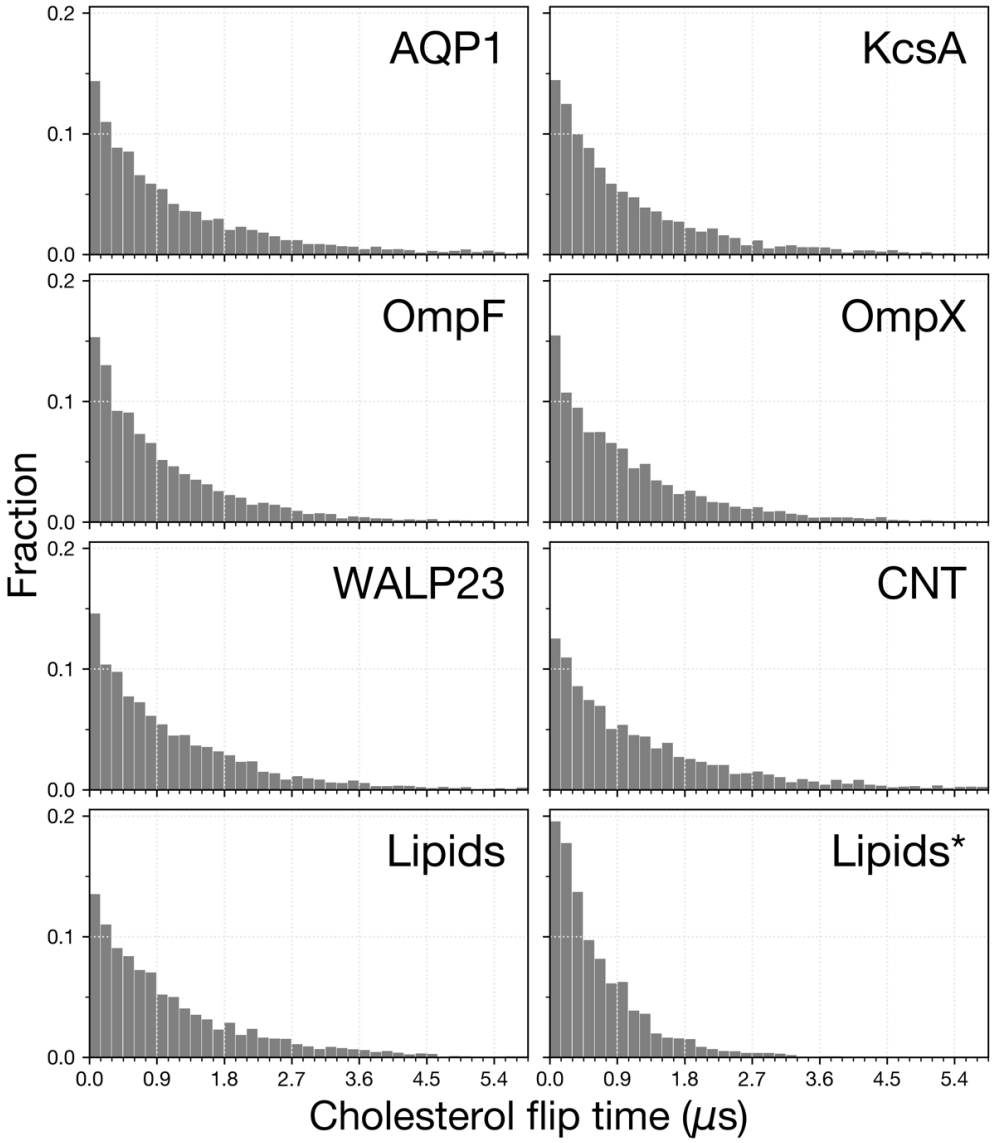

Fig. S5 Cholesterol flip time distributions. A bin width of 0.15 μs was used.