Supporting information for: Depletion with cyclodextrin reveals two populations of cholesterol in model lipid membranes

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Bilayer area per molecule

All data used to determine the average area per molecule in a bilayer as a function of the mole fraction of cholesterol, $a_{\text{avg}}(\chi_C)$, are given in Tables S1-S5. d_{pp} is the electron density peak-to-peak distance as determined by X-ray diffraction. d_{H1} is the distance from the peak of the electron density profile to the interface of the hydrocarbon region of the bilayer as determined by the joint analysis of X-ray and neutron scattering data. A_{UC} is the area per unit cell as determined by neutron scattering or the joint analysis of X-ray and neutron scattering data. V_L, V_H, and V_C are the volumes of a PC-lipid, a PC-headgroup, and cholesterol in a bilayer, respectively.

Data and uncertainties from references S1, S2, and S3 were interpreted from graphs. Uncertainties in S4, S5, and S6 were reported as less than 2%; we treated the uncertainty as ±2%.

Table S1: Literature d_{pp} and A_{UC}, and calculated a_{avg} values for DMPC.

χ_C	Lit. d_{pp} (Å)	Lit. A_{UC} (Å^2)	Ref.	Calc. a_{avg} (Å^2)
0.00	34.9 ± 0.7	59.8 ± 1.2	S5	59.8 ± 1.2
0.00	35.3 ± 0.3		S2	59.4 ± 1.9
0.00	35.9 ± 0.1		S1	58.1 ± 1.3
0.09	38.4 ± 0.1		S1	51.8 ± 1.9
0.10	38.3 ± 0.5		S2	51.8 ± 2.6
0.17	40.3 ± 0.1		S1	47.6 ± 1.6
0.20	41.5 ± 0.4		S2	45.4 ± 1.8
0.23	41.6 ± 0.1		S1	44.9 ± 1.4
0.29	42.5 ± 0.1		S1	43.2 ± 1.4
0.30	41.8 ± 0.5		S2	44.0 ± 1.9
0.36	43.2 ± 0.1		S1	41.8 ± 1.3
0.38	43.3 ± 0.1		S1	41.6 ± 1.3
0.41	43.3 ± 0.1		S1	41.4 ± 1.2
0.44	43.3 ± 0.1		S1	41.2 ± 1.2

Table S2: Literature d_{pp} and A_{UC}, and calculated a_{avg} values for SOPC.

χ_C	Lit. d_{pp} (Å)	Lit. A_{UC} (Å^2)	Ref.	Calc. a_{avg} (Å^2)
0.00	38.7 ± 0.8		S6	65.6 ± 3.7
0.00	38.6 ± 0.8	65.5 ± 1.3	S5	65.5 ± 1.3
0.00	39.0 ± 0.1		S1	65.0 ± 1.3
0.00	39.2 ± 0.3		S2	64.6 ± 1.8
0.09	40.8 ± 0.1		S1	59.4 ± 1.9
0.10	40.3 ± 0.5		S2	60.1 ± 2.7
0.14	41.7 ± 0.1		S1	56.7 ± 1.7
0.20	42.1 ± 0.5		S2	54.7 ± 2.3
0.20	42.1 ± 0.8		S6	54.7 ± 3.1
0.20	42.7 ± 0.1		S1	53.7 ± 1.6
0.28	43.8 ± 0.1		S1	50.4 ± 1.5
0.30	43.6 ± 0.3		S2	50.3 ± 1.7
0.38	44.8 ± 0.1		S1	47.1 ± 1.3
0.40	43.9 ± 0.5		S2	47.9 ± 1.9
0.44	44.6 ± 0.1		S1	46.2 ± 1.3
0.46	44.6 ± 0.1		S1	45.8 ± 1.3
0.47	44.6 ± 0.1		S1	45.6 ± 1.3
0.50	44.2 ± 0.5		S2	45.5 ± 1.8

Table S3: Literature d_{pp} and A_{UC}, and calculated a_{avg} values for DOPC.

χ_C	Lit. d_{pp} (Å)	Lit. A_{UC} (Å^2)	Ref.	Calc. a_{avg} (Å^2)
0.00		68.5 ± 2.3	S3	68.5 ± 2.3
0.00	36.6 ± 0.1		S1	67.9 ± 1.4
0.00	36.7 ± 0.3		S2	67.6 ± 1.9
0.00	36.9 ± 0.7		S6	67.2 ± 3.5
0.00	36.8 ± 0.7	67.4 ± 1.3	S4	67.4 ± 1.3
0.00		65.9 ± 1.3	S7	65.9 ± 1.3
0.09	38.0 ± 0.1		S1	62.7 ± 1.9
0.10	37.2 ± 0.5		S2	64.1 ± 2.9
0.14	38.7 ± 0.1		S1	60.1 ± 1.8
0.17		71.5 ± 2.4	S3	59.3 ± 2.0
0.17		70.8 ± 1.4	S7	58.8 ± 1.2
0.20	39.0 ± 0.5		S2	58.2 ± 2.5
0.20	40.9 ± 0.8		S6	54.9 ± 3.0
0.25	40.2 ± 0.1		S1	55.0 ± 1.5
0.29		76.2 ± 1.8	S7	54.1 ± 1.3
0.29		76.0 ± 2.9	S3	53.9 ± 2.0
0.30	39.9 ± 0.3		S2	54.4 ± 1.8
0.33	41.2 ± 0.1		S1	51.7 ± 1.4
0.34		79.3 ± 4.2	S3	53.1 ± 2.8
0.38		81.1 ± 3.0	S3	50.3 ± 1.9
0.38	41.6 ± 0.1		S1	50.1 ± 1.3
0.38		79.2 ± 2.4[a]	S7	49.1 ± 1.5
0.39	41.6 ± 0.1		S1	49.9 ± 1.3
0.40	40.4 ± 0.5		S2	51.5 ± 2.1
0.40	41.6 ± 0.1		S1	49.7 ± 1.3
0.44		86.2 ± 1.9	S3	48.3 ± 1.1
0.45		83.9 ± 2.7[a]	S7	46.1 ± 1.5
0.50		91.9 ± 4.1	S3	46.0 ± 2.1

[a] The authors reported signatures of pauci-lamellar vesicles in this sample.

Table S4: Literature d_{H1} values.

Lipid	d_{H1} (Å)	Ref.
DMPC	4.61 ± 0.09^a	S5
SOPC	4.35 ± 0.09^a	S5
DOPC	3.9 ± 0.1^a	S4

[a] There are no published values for the χ_C dependence of d_{H1} for these phospholipids, so we estimated uncertainties at ±5% for all $\chi_C > 0$.

Table S5: Literature V_L, V_H, and V_C values.

Lipid	V_L (Å^3)	V_H (Å^3)	V_C (Å^3)
DMPC ($\chi_C < 0.24$)	1099.6 ± 0.5^a	325 ± 6^c	565.1 ± 3.4^a
DMPC ($\chi_C \geq 0.24$)	1076.8 ± 1.3^a	325 ± 6^c	637.5 ± 1.8^a
SOPC	1309.5 ± 1.3^b	325 ± 6^c	630 ± 10^a
DOPC	1302.2 ± 0.4^a	325 ± 6^c	632.9 ± 0.9^a

[a] Reference S8. If not reported, the uncertainty was obtained by refitting published data. [b] Reference S9. [c] References S10 and S11. Uncertainty estimated from the spread of published values.

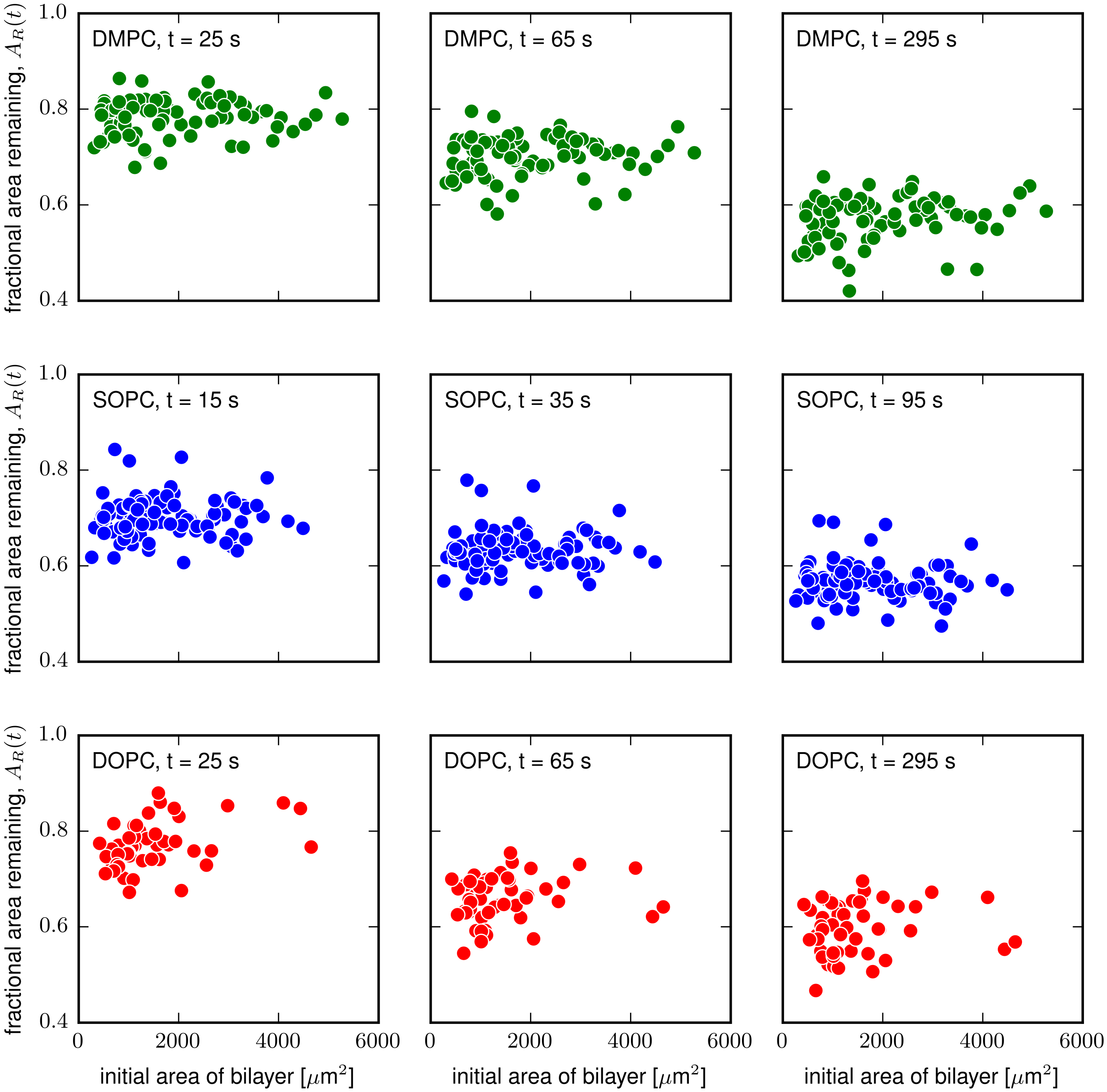

Figure S1: There is no apparent correlation between the initial area of the supported lipid bilayer and the fraction of bilayer area remaining as a function of time after mβCD is added, $A_R(t)$, for the systems we studied.

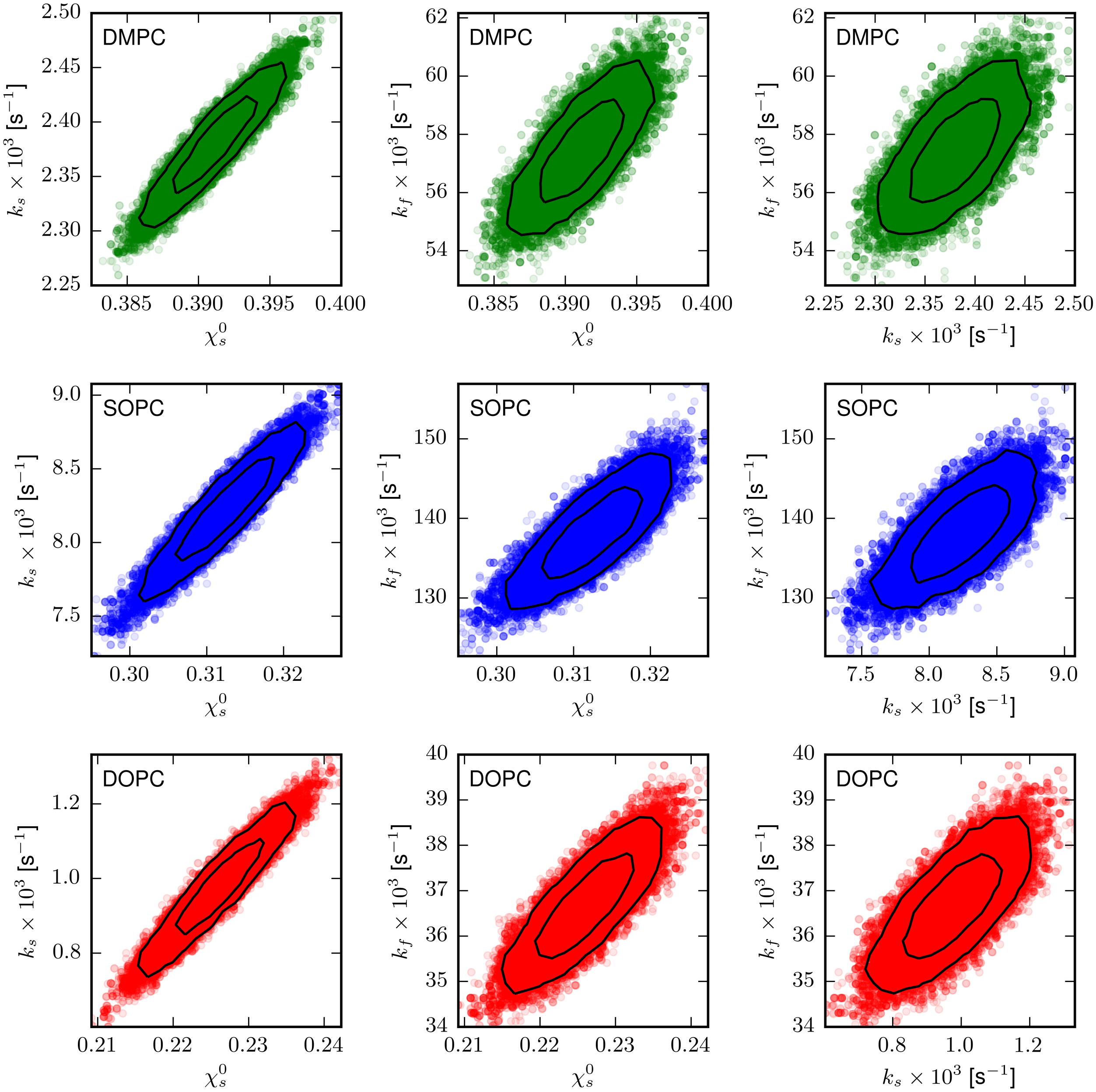

Figure S2: Samples from the posterior distribution of fitting parameters corresponding to the two cholesterol population model (see Fig 4, Table 2, and Eq. 10). Contours correspond to one and two standard deviations of the marginalized posterior samples.

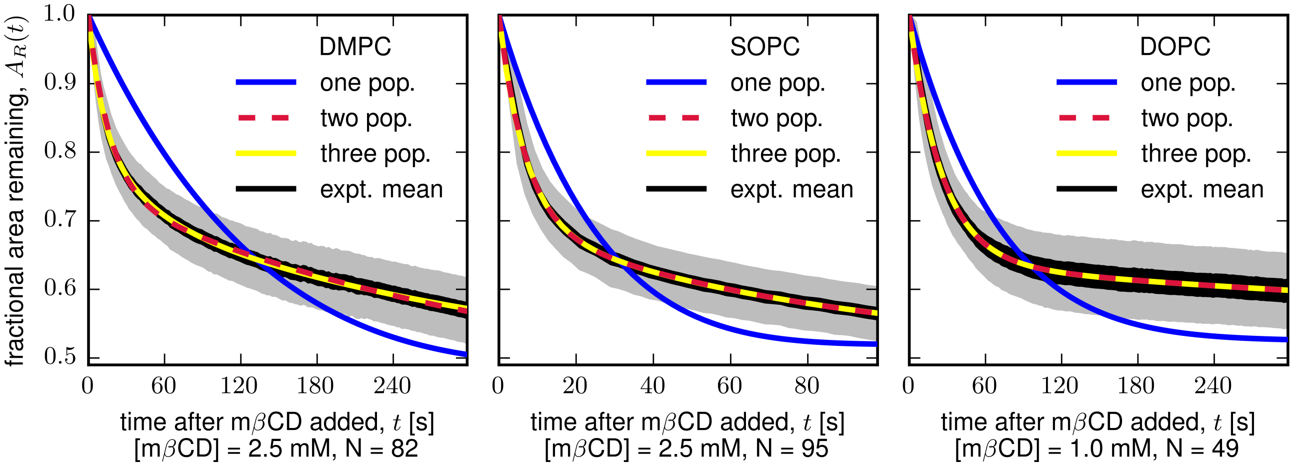

Figure S3: The fraction of bilayer area remaining as a function of time, $A_R(t)$ after addition of mβCD. The black region depicts our experimental mean ± two standard errors, and the gray region depicts the standard deviation of our data. The model with two cholesterol populations (three fitting parameters) describes our data well (crimson dotted line, main text eq. 10). The model with one cholesterol population (one fitting parameter, main text eq. 6) underfits our data (blue line). The model with three cholesterol populations (five fitting parameters, SI eq. 1) is unwarranted by our data (yellow line).

The equation of fit for the three population fit is

$$A_R(t) = \left(1 - \chi_C(0) + \chi_1 e^{-k_1 t} + \chi_2 e^{-k_2 t} + (\chi_C(0) - \chi_1 - \chi_2) e^{-k_3 t}\right) \times \frac{a_{\text{avg}}(\chi_C[t])}{a_{\text{avg}}(\chi_C[0])}, \qquad (1)$$

where χ_1, χ_2, k_1, k_2, and k_3 are the five fitting parameters.

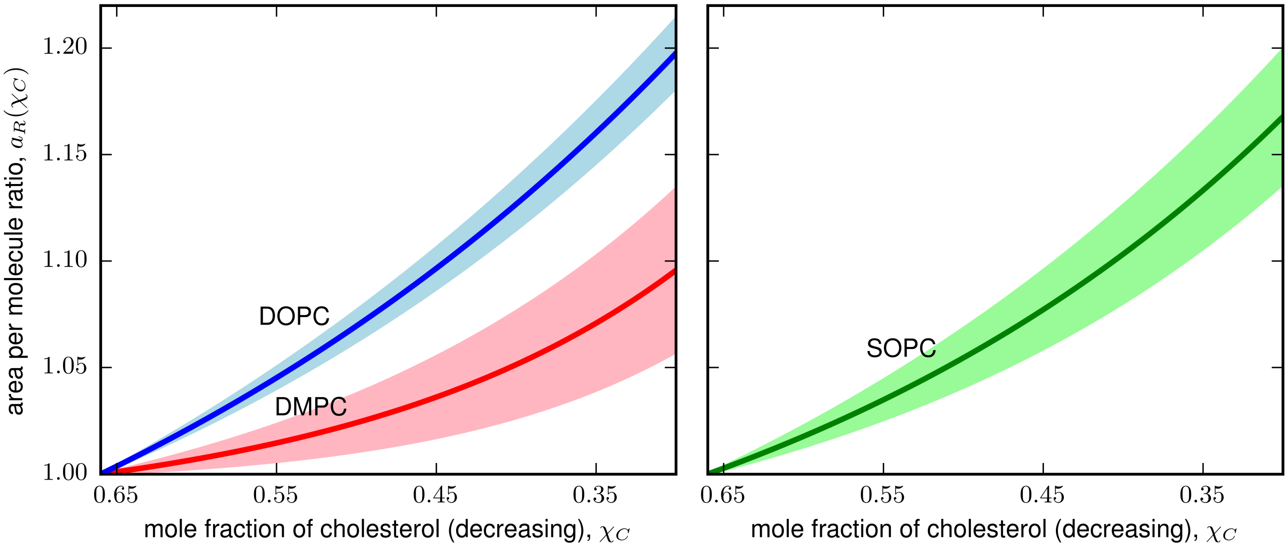

Figure S4: The average area per molecule ratio as a function of the mole fraction of cholesterol, $a_R(\chi_C)$ (solid lines) and the uncertainty in these values (one standard deviation, encompassed by the shaded area overlaying each line). Eq. 1 of the main text shows how the observable of our experiments, the fraction of bilayer area remaining as a function of time $A_R(t)$, is proportional to the ratio of the average area per molecule in the bilayer at time t and the average area per molecule in the bilayer at time $t = 0$. This figure shows that over the regime of $\chi_C > 0.3$ in which our experiment takes place, uncertainty resulting from $a_R(\chi_C)$ is small.

We define the average area per molecule ratio as follows:

$$a_R(\chi_C) = \frac{a_{\text{avg}}(\chi_C)}{a_{\text{avg}}(\chi_C = 0.66)}. \tag{2}$$

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