Supporting Information for:

Influence of Cholesterol and Bilayer Curvature on the Interaction of PPO-PEO Block Copolymers with Liposomes

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Table S1. Summary of liposome radii and the percentage of liposome surface area within multilamellar liposomes measured from cryo-TEM.

Cholesterol Molar Percentage (%)	Total Counts[a]	Mean (nm)[b]	Standard Deviation (nm)	% of Liposome Surface Area within Multilamellar Liposomes
0	791	49	17	17
10	856	50	19	17
30	525	46	21	19

[a] The liposomes were extruded through a polycarbonate membrane with 50 nm pore radius. Non-spherical liposomes were excluded.
[b] The radius of a multilamellar liposome was measured at the outermost layer.

Table S2. Summary of polymer binding to 5 mM POPC/cholesterol liposomes extruded through a polycarbonate membrane with 50 nm pore radius in D_2O at 27 °C at different cholesterol molar percentages.

Cholesterol Molar Percentage (%)	D_{bound}[a] ($\times 10^{-11}$ m^2/s)	D_{free}[b] ($\times 10^{-11}$ m^2/s)	f_{bound}[c] (%)
F127 0.2 mg/mL			
0	0.5	5.9	18.2±0.7
1	0.4	6.0	17.5±0.9
2	0.4	6.0	15.4±0.7
5	0.5	5.9	10.3±0.7
10	0.5	5.9	5.5±0.6
20	0.6*	5.9	1.4±0.3
30	0.6*	5.9	0.7±0.1
P103 0.2 mg/mL			
0	0.5*	9.7	12.5±1.3
1	0.5*	9.8	11.3±1.4
2	0.5*	9.8	10.9±1.3
5	0.5*	9.8	5.5±0.7
10	0.5*	9.8	2.4±0.4
20	0.5*	9.8	1.4±0.2
30	0.5*	9.8	1.1±0.1
tPPO$_{14}$-PEO$_{46}$ 1 mg/mL			
0	0.5*	11.7	0.52±0.07
1	0.5*	11.6	0.30±0.01
2	0.5*	11.6	0.32±0.05
5	0.5*	11.6	0.20±0.03
10	0.5*	11.6	0.10±0.03
20	0.5*	11.6	0.11±0.03
30	0.5*	11.6	0.03±0.04
F68 1 mg/mL			
0	0.5*	6.8	0.10±0.02

[a] Obtained from the final slope of the echo decay curves of the polymer in the presence of liposomes.

*Estimated from the liposome diffusion coefficients measured by PFG-NMR using the ^{1}H signal of the three methyl groups from the choline group of POPC as the characterization peak, due to noise in the final slope of the polymer echo decay curves.

[b] Obtained from the linear fit of the echo decay curves of the polymer without liposomes. Only the data points before 5.0×10^{10} s/m^2 $\gamma^2G^2\delta^2(\Delta-\delta/3)$ were used for the linear fit to ensure a strong signal, since the signal of polymer decays very rapidly.

[c] Obtained from the average and standard deviation of the fitting results at Δ = 300, 500, and 700 ms based on eq 4.

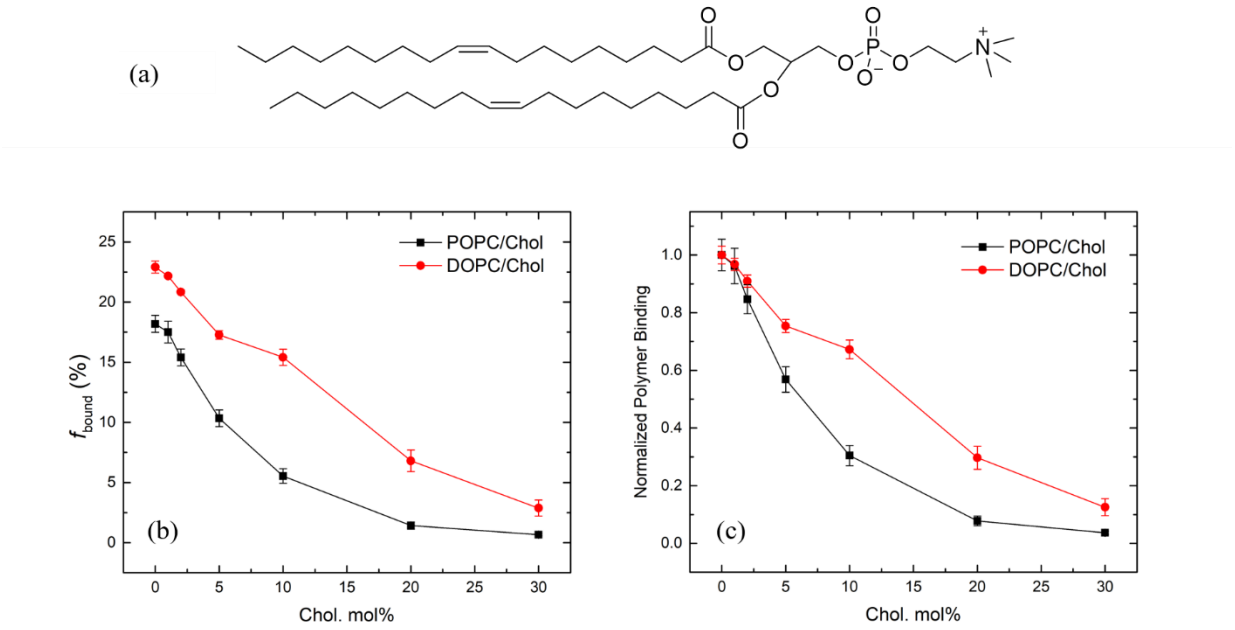

Figure S1. (a) Chemical structure of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC). (b) Polymer binding percentage and (c) normalized polymer binding of 0.2 mg/mL F127 to 5 mM DOPC/cholesterol liposomes (red) and POPC/cholesterol liposomes (black) in D_2O at 27 °C as a function of cholesterol molar percentage.

POPC and DOPC were selected as two representative phospholipids that contain one and two mono-unsaturated hydrocarbon chains, respectively. Despite the fact that the saturated chain of POPC only contains 16 carbons, which is two carbons shorter than the mono-unsaturated chains of DOPC (i.e., 18 carbons), we assume that this has little effect compared to the number of monosaturated chains of lipids on polymer binding and membrane bending modulus.

Phospholipids with fully saturated hydrocarbon chains (e.g., DMPC) were not selected for this comparison because fully saturated lipids have relatively high phase transition temperatures, which would require temperatures above 27 °C used for the NMR measurements in order to access the fluid phase of the lipid membranes.

Table S3. Summary of polymer binding to 5 mM POPC liposomes with different curvature in D_2O at 27 °C.

Extrusion Pore Radius (nm)	R_h[a] (nm)	κ[b] (nm^{-1})	D_{bound}[c] ($\times 10^{-11}$ m^2/s)	D_{free}[d] ($\times 10^{-11}$ m^2/s)	f_{bound}[e] (%)
F127 0.2 mg/mL					
25	42	0.024	0.6	5.9	28.5±0.9
50	69	0.014	0.5	5.9	18.2±0.7
100	109	0.009	0.4	5.9	16.6±0.6
P103 0.2 mg/mL					
25	42	0.024	0.6	9.5	18.9±2.5
50	69	0.014	0.5*	9.7	12.5±1.3
100	109	0.009	0.5*	9.7	11.7±1.0
$tPPO_{14}$-PEO_{46} 1 mg/mL					
25	42	0.024	0.6*	11.7	0.8±0.1
50	69	0.014	0.5*	11.7	0.5±0.1
100	109	0.009	0.5*	11.6	0.45±0.04
F68 1 mg/mL					
25	42	0.024	0.6*	6.8	0.16±0.03
50	69	0.014	0.5*	6.8	0.10±0.02
100	109	0.009	0.5*	6.8	0.04±0.01

[a] Obtained from DLS, as listed in Table 2.

[b] Calculated from R_h obtained from DLS ($\kappa = 1/R$, using R_h for R).

[c] Obtained from the final slope of the echo decay curves of the polymer in the presence of liposomes.

*Estimated from the liposome diffusion coefficients measured by PFG-NMR using the ^1H signal of the three methyl groups from the choline group of POPC as the characterization peak due to noise in the final slope of the polymer echo decay curves.

[d] Obtained from the linear fit of the echo decay curves of the polymer without liposomes. Only the data points before 5.0×10^{10} s/m^2 $\gamma^2G^2\delta^2(\Delta-\delta/3)$ were used for the linear fit to ensure a strong signal, since the signal of polymer decays very rapidly.

[e] Obtained from the average and standard deviation of the fitting results at Δ = 300, 500, and 700 ms based on eq 4.

Table S4. Binding of F127 to 5 mM POPC/cholesterol liposomes extruded through a polycarbonate membrane with 25 nm pore radius in D_2O at 27 °C.

Cholesterol Molar Percentage (%)	D_{bound}[a] ($\times 10^{-11}$ m^2/s)	D_{free}[b] ($\times 10^{-11}$ m^2/s)	f_{bound}[c] (%)
0	0.6	5.9	28.5±0.9
1	0.7	5.9	22.9±0.2
2	0.7	5.9	22.9±0.4
5	0.7	5.9	18.2±0.5
10	0.7	5.9	9.6±0.7
20	0.6*	5.9	1.9±0.1
30	0.6*	5.9	0.6±0.2

[a] Obtained from the final slope of the echo decay curves of the polymer in the presence of liposomes.

*Estimated from the liposome diffusion coefficients measured by PFG-NMR using the ^{1}H signal of the three methyl groups from the choline group of POPC as the characterization peak due to noisy data at the final slope of polymer echo decay curves.

[b] Obtained from the linear fit of the echo decay curves of the polymer without liposomes. Only the data points before 5.0×10^{10} s/m^2 $\gamma^2G^2\delta^2(\Delta-\delta/3)$ were used for the linear fit to ensure a strong signal since the signal of polymer decays very rapidly.

[c] Obtained from the average and standard deviation of the fitting results at Δ = 300, 500, and 700 ms based on eq 4.

Table S5. Fitting results of the decay parameter *A*.

Polymer Species	Bilayer Curvature (κ, nm^{-1})	Parameter *A**
F127	0.015	0.13±0.01
F127	0.020	0.15±0.01
P103	0.015	0.12±0.02
$tPPO_{14}$-PEO_{46}	0.015	0.16±0.03

*A single exponential decay was applied to fit the data. Errors of the data points (as shown by the error bars in Figure 7) were taken into account by weighting the fitting, using $w_i = \frac{1}{\sigma_i^2}$, where σ_i is the error of each data point.

Derivation of equilibrium binding constant *K* as a function of cholesterol content

$$K = e^{-\frac{\Delta G^\circ}{RT}} \tag{S1}$$

For a specific polymer, define G°_{free} as the free energy of a polymer in water (*i.e.*, unbound state), $G^\circ_{bound,lipid}$ as the free energy of a polymer bound to a liposome composed of 100 mol% POPC, and $G^\circ_{bound,chol}$ as the free energy of a polymer bound to a liposome composed of 100 mol% cholesterol (a hypothetical limit). Assuming ideal mixing of POPC and cholesterol in the liposome, the free energy change of the polymer bound to a liposome composed of *x* mol% cholesterol and (1−*x*) mol% POPC can be written as:

$$\Delta G^\circ = (1-x)\cdot G^\circ_{bound,lipid} + x\cdot G^\circ_{bound,chol} - G^\circ_{free} \tag{S2}$$

Rearrange the above equation, we have:

$$\Delta G^\circ = \left(G^\circ_{bound,chol} - G^\circ_{bound,lipid}\right)\cdot x + G^\circ_{bound,lipid} - G^\circ_{free} \tag{S3}$$

Therefore, *K* can be written as:

$$K = e^{-\frac{G^\circ_{bound,lipid} - G^\circ_{free}}{RT}} \cdot e^{-\frac{\left(G^\circ_{bound,chol} - G^\circ_{bound,lipid}\right)}{RT}\cdot x} \tag{S4}$$

Since $e^{-\frac{G^\circ_{bound,lipid} - G^\circ_{free}}{RT}}$ is a constant, *K* after normalization can be written as:

$$K/K(0) = e^{-\frac{\left(G^\circ_{bound,chol} - G^\circ_{bound,lipid}\right)}{RT}\cdot x} \tag{S5}$$

Therefore, Figure 7 can be fitted to a single exponential decay model $y = e^{-Ax}$, where

$$A = \frac{\left(G^\circ_{bound,chol} - G^\circ_{bound,lipid}\right)}{RT} \tag{S6}$$

Now assume that for two different polymers with different hydrophilic/hydrophobic balance, the extra cost to bind the more hydrophilic polymer to the lipid is the same as the extra cost to bind to pure cholesterol. In this case, *A* is the same for all three polymers. Similarly, we can assume that for a specific polymer interacting with two different lipid bilayer curvatures, the extra cost of binding due to the curvature change is the same for the polymer bound to pure lipid and to pure

cholesterol, and thereby *A* is the same for two different curvatures. This assumption is therefore sufficient to explain the collapse of the curves in Figures 4c, 6b, and 7.