Influence of the Headgroup on the Interaction of Poly(ethylene oxide)-Poly(propylene oxide) Block Copolymers with Lipid Bilayers

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Table S1. Polymer Characterization.

	$M_{n,1}$[a] (g/mol)	$M_{n,2}$[b] (g/mol)	$M_{n,3}$[c] (g/mol)	$Đ$[b]	$Đ$[c]	$w_{PEO,1}$[a]	$w_{PEO,2}$[d]	N_{PO}[e]	N_{EO}[e]
F68	8,400	8,200	7,900	1.01	1.04	0.80	0.81	27	75
F108	14,600	15,900	14,700	1.01	1.04	0.80	0.82	49	148
F127	12,600	13,200	10,800	1.01	1.07	0.70	0.72	64	108
P103	4,950	5,200	4,600	1.02	1.14	0.30	0.33	60	19
P105	6,500	6,500	5,700	1.02	1.12	0.50	0.50	56	37
P84	4,200	4,200	3,600	1.03	1.05	0.40	0.42	42	20
$tPPO_{14}$-PEO_{46}	-	2,900	3,000	1.03	1.07	-	0.70	14	46
$tPPO_{29}$-PEO_{68}	-	4,700	4,900	1.04	1.10	-	0.63	29	68

[a]Specified by supplier. [b]Determined by MALDI mass spectroscopy. [c]Determined by SEC. Weight-average *dn*/*dc* was used with *dn*/*dc* = 0.068 for PEO and 0.087 for PPO. [d]Determined from molar ratios by ^{1}H NMR spectroscopy. [e]Calculated from number-average molecular weight ($M_{n,2}$) and weight fraction of PEO ($w_{PEO,2}$). For triblock Pluronics, N_{EO} represents the number of repeat units of EO in one PEO block.

Table S2. Summary of polymer binding to 5 mM POPC liposomes in D_2O, to POPC liposomes in 150 mM NaCl D_2O solution, and to POPG liposomes in 150 mM NaCl D_2O solution at 27 °C.

Polymer	Concentration (mg/mL)	D_{bound}[a] (10^{-11} m^2/s)	D_{free}[b] (10^{-11} m^2/s)	f_{bound}[c] (%)
POPC liposomes in D_2O				
$tPPO_{14}$-PEO_{46}	1	0.5*	11.7	0.5±0.1
$tPPO_{29}$-PEO_{68}	1	0.6	9.2	1.9±0.1
P84	1	0.4	10.2	1.0±0.1
F68	1	0.5*	6.8	0.10±0.02
P103	0.2	0.5*	9.7	12.5±1.3
P105	0.2	0.4	8.7	9.4±0.6
F127	0.2	0.5	5.9	18.2±0.7
F108	1	0.5*	4.9	0.9±0.3
POPC liposomes in 150mM NaCl D_2O solution				
$tPPO_{14}$-PEO_{46}	1	0.5*	11.7	0.3±0.1
$tPPO_{29}$-PEO_{68}	1	0.6	9.3	3.1±0.1
P84	1	0.5*	10.1	0.8±0.1
F68	1	0.5*	7.2	0.12±0.06
P103	0.2	0.5*	10.1	14.5±1.5
P105	0.2	0.5*	8.6	11.2±0.5
F127	0.2	0.4	6.1	23.4±0.1
F108	1	0.5*	4.9	2.0±0.4
POPG liposomes in 150mM NaCl D_2O solution				
$tPPO_{14}$-PEO_{46}	1	0.5	11.4	1.6±0.1
$tPPO_{29}$-PEO_{68}	1	0.5	9.3	20.2±0.6
P84	1	0.5	9.8	7.2±1.3
F68	1	0.5*	7.3	0.2±0.1
P103	0.2	0.4	10.1	43.4±1.6
P105	0.2	0.4	8.7	48.3±1.6
F127	0.2	0.4	5.8	52.8±1.1
F108	1	0.4	4.9	17.5±1.1

[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 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 polymer without the presence of liposome. 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 2.

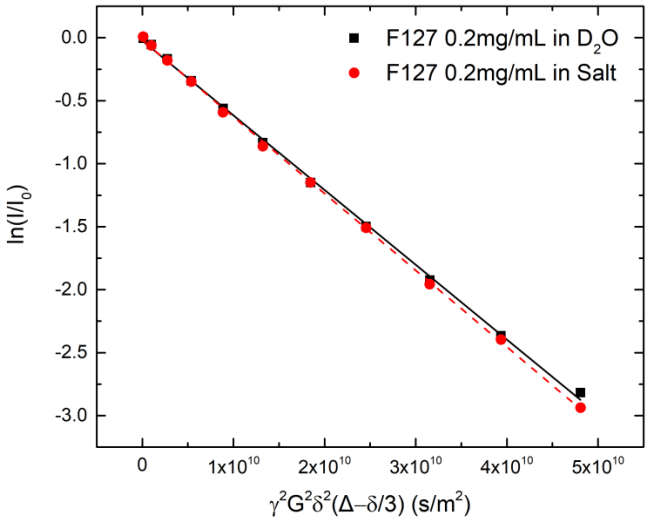
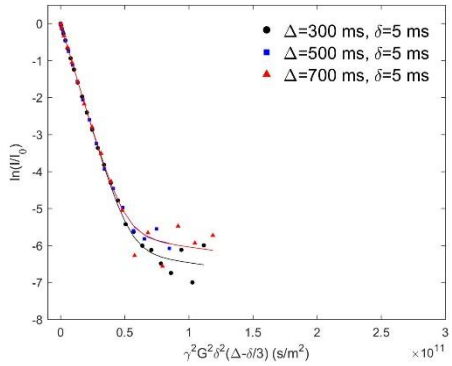
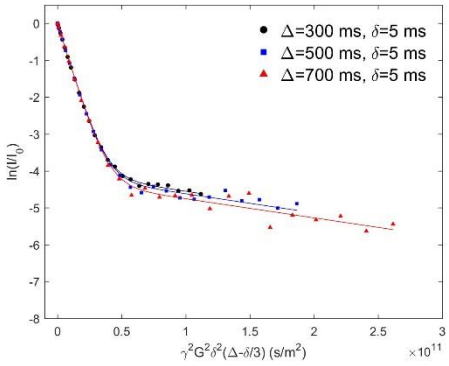


Figure S1. Echo decay curves of 0.2 mg/mL F127 in D_2O (black squares) and in 150 mM NaCl D_2O solution (red circle). The black and red lines are linear fits to the data, which give D = 5.9×10^{-11} m^2/s for the polymer in water, and 6.1×10^{-11} m^2/s for the polymer in the salt solution.

(a)


Δ=300 ms, δ=5 ms
Δ=500 ms, δ=5 ms
Δ=700 ms, δ=5 ms
ln(I/I0)
γ²G²δ²(Δ-δ/3) (s/m²)
×10¹¹

(h)


Δ=300 ms, δ=5 ms
Δ=500 ms, δ=5 ms
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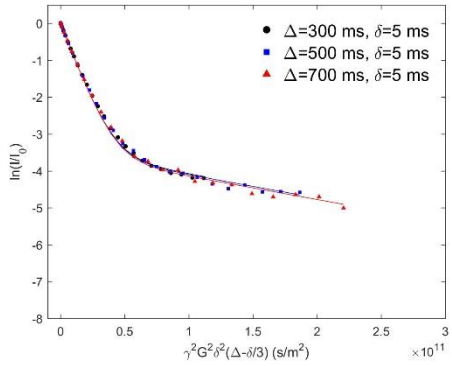
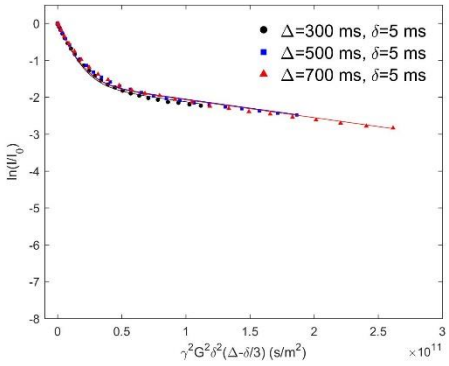
ln(I/I0)
γ²G²δ²(Δ-δ/3) (s/m²)
×10¹¹

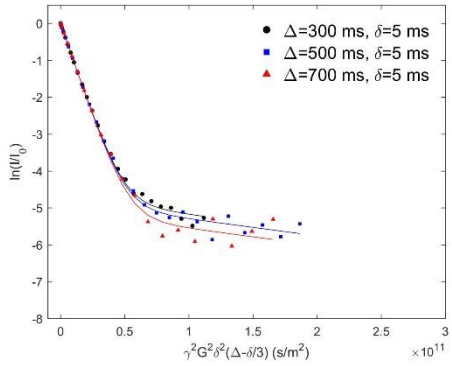
(b)


Δ=300 ms, δ=5 ms
Δ=500 ms, δ=5 ms
Δ=700 ms, δ=5 ms
ln(I/I0)
γ²G²δ²(Δ-δ/3) (s/m²)
×10¹¹

(i)


Δ=300 ms, δ=5 ms
Δ=500 ms, δ=5 ms
Δ=700 ms, δ=5 ms
ln(I/I0)
γ²G²δ²(Δ-δ/3) (s/m²)
×10¹¹

(c)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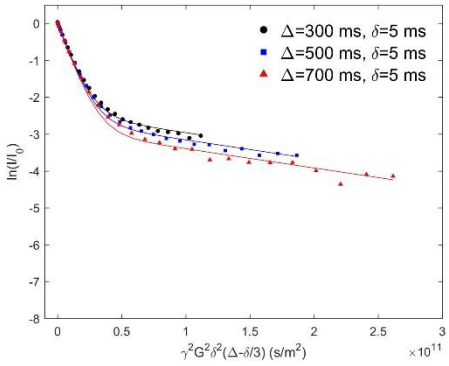
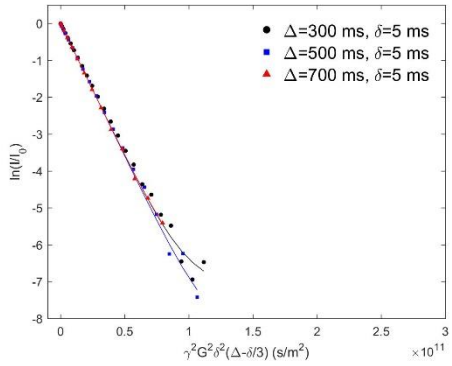
γ²G²δ²(Δ-δ/3) (s/m²)
×10¹¹

(j)


Δ=300 ms, δ=5 ms
Δ=500 ms, δ=5 ms
Δ=700 ms, δ=5 ms
ln(I/I0)
γ²G²δ²(Δ-δ/3) (s/m²)
×10¹¹

(d)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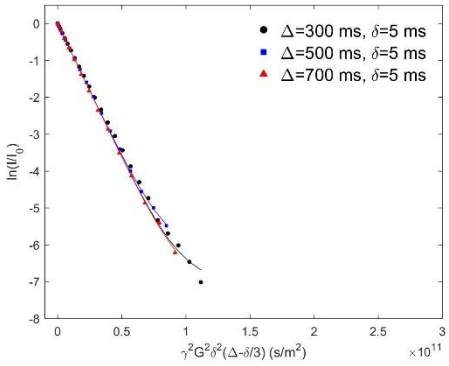
γ²G²δ²(Δ-δ/3) (s/m²)
×10¹¹

(k)


Δ=300 ms, δ=5 ms
Δ=500 ms, δ=5 ms
Δ=700 ms, δ=5 ms
ln(I/I0)
γ²G²δ²(Δ-δ/3) (s/m²)
×10¹¹

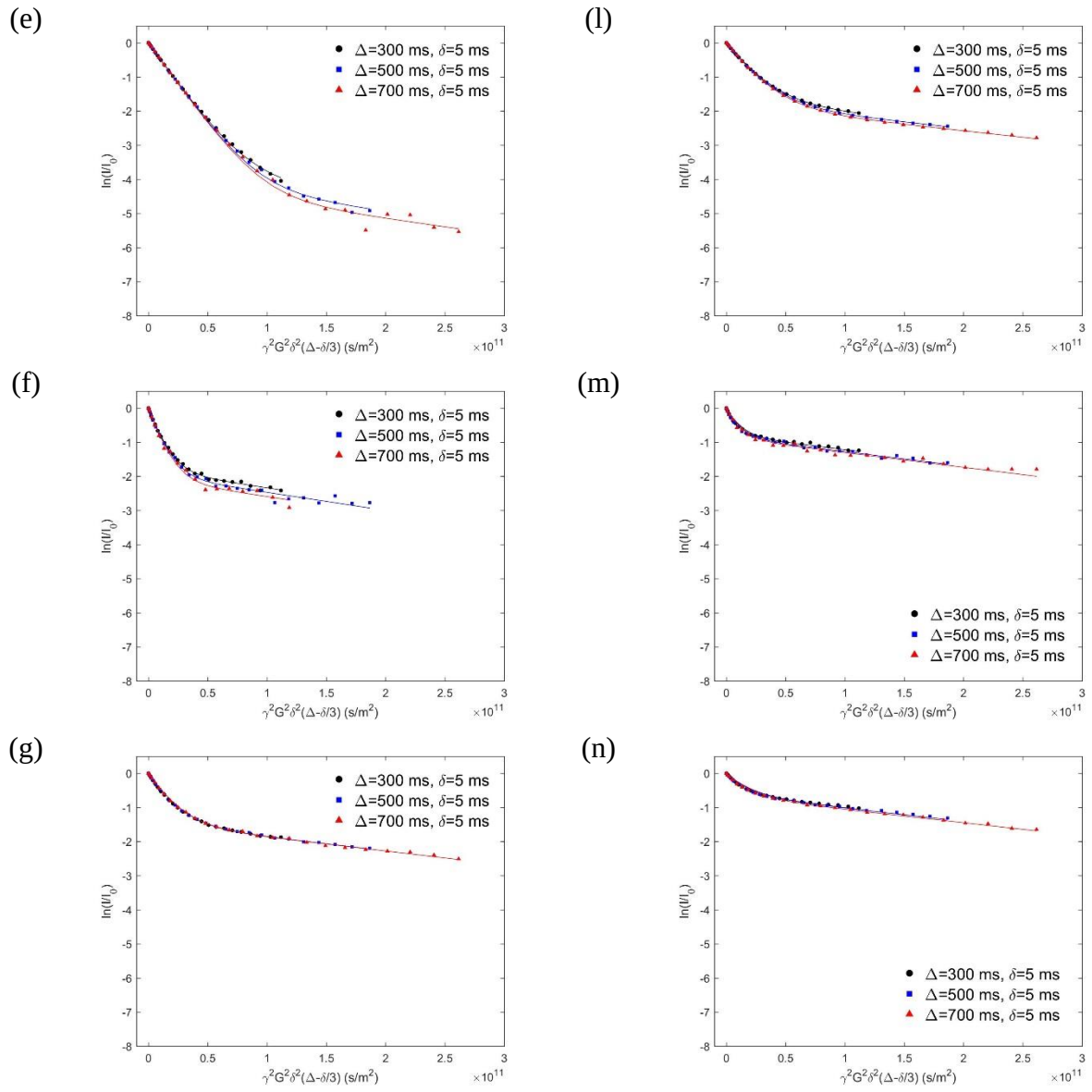


Figure S2. Experimental and fitted echo decay curves of the protons from PEO of 1 mg/mL (a) $tPPO_{14}$-PEO_{46}, (b) $tPPO_{29}$-PEO_{68}, (c) P84, (d) F68, (e) F108, 0.2 mg/mL (f) P103, and (g) F127 in the presence of 5 mM POPC liposome solution in 150 mM NaCl D_2O solution at 27 °C with Δ = 300, 500, 700 ms and with fixed δ = 5 ms; (h) – (n) are corresponding echo decays curves of the polymers in the presence of 5 mM POPG liposome solution in 150 mM NaCl D_2O solution. The value of f_{bound} in eq 2 was fit to the data.

Table S3. Summary of polymer binding of 0.2 mg/mL F127 to 5 mM POPC/POPG liposomes at various POPG molar percentage in the lipid bilayer in 150 mM NaCl D_2O solution at 27 °C.

POPG mol%	D_{bound}[a] **(10^{-11} m^2/s)**	D_{free}[b] **(10^{-11} m^2/s)**	f_{bound}[c] **(%)**
0	0.4	6.1	23.4±0.1
20	0.5	5.9	21.3±2.6
40	0.4	5.9	26.2±1.0
60	0.4	5.9	35.6±1.1
80	0.4	5.9	46.3±1.0
100	0.4	5.8	52.8±1.1

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

[b] Obtained from the linear fit of the echo decay curves of polymer without the presence of liposome. 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 2.

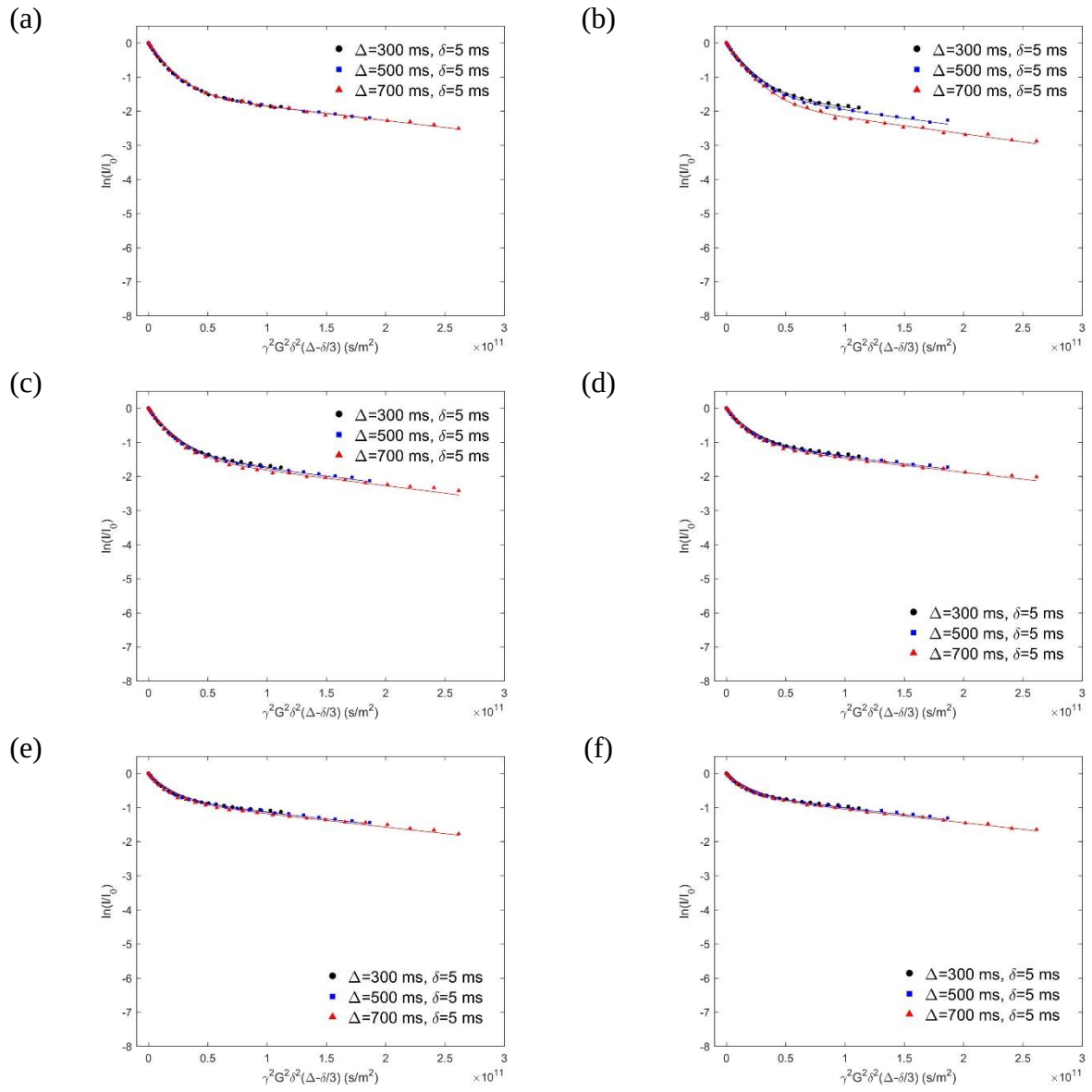


Figure S3. Experimental and fitted echo decay curves of the protons from PEO of 0.2 mg/mL F127 in the presence of 5 mM POPC/POPG liposomes with (a) 0%, (b) 20%, (c) 40%, (d) 60%, (e) 80%, and (f) 100% POPG in 150 mM NaCl D_2O solution at 27 °C with Δ = 300, 500, 700 ms and with fixed δ = 5 ms. The value of f_{bound} in eq 2 was fit to the data.

Results and Discussion of Raman Spectroscopy

Raman spectroscopy was employed to probe possible hydrogen bonding between the polymers and the POPG headgroups. The spectra of pure polymer F108 and pure liposome samples in the region of 750 – 1750 cm^{-1} are shown in Figure S4a and S4b, respectively. The intensity of these two spectra were adjusted to equalize the maximum intensity of the peak due to water bending vibrations. The peaks were assigned to different vibrational modes of the various bonds.[1–9] In Figure S4a, the strongest peak appears at ~1550 – 1750 cm^{-1}, which is due to the bending vibration (δ) of water molecules. The peaks at 1400 – 1500 cm^{-1} and at 1200 – 1350 cm^{-1} are attributed to the bending and twisting (*t*) vibrations of methylene groups in the polymer backbone, respectively. The peak observed at ~ 1140 cm^{-1} originates from the superposition of C-O-C asymmetric stretching (ν_a), C-C stretching, and CH_2 wagging (*w*). The shoulder to its left (lower wavenumber) is due to combined C-O-C symmetric stretching (ν_s) and CH_2 rocking (*r*). The broad peak from 800 to 1000 cm^{-1} is due to coupled C-O-C symmetric stretching with CH_2 rocking. In Figure S4b, two strong peaks evident in the region of 1200 - 1500 cm^{-1} correspond to CH_2 twisting and bending vibrations as POPG lipids contain relatively long alkyl chains. The broad peak from 1000 to 1200 cm^{-1} is attributed to the superposition of symmetric stretching of O-P-O with C-C stretching. Note that the concentration of polymers and liposomes for Raman measurements is much higher than that used in the PFG-NMR measurements because Raman scattering produces weak signals, especially in aqueous solution.

To probe potential hydrogen bonding formed between POPG headgroups and polymers, the peak containing C-O-C asymmetric stretching vibrations at ~ 1140 cm^{-1} was monitored as an indicator since it is anticipated that hydrogen bonding would affect the ether oxygen of C-O vibrations of the polymers. An appreciable peak shift or change in intensity would indicate a significant amount of hydrogen bond formation. Note that the stretching vibrations of the O-H bonds in the POPG headgroups could also be affected by hydrogen bond formation. However, it is not feasible to use this peak as an indicator because it is covered by the extremely strong peak

of stretching vibrations of water molecules in the solution, which is at ~3400 cm^{-1} (not shown in the spectra). The spectrum of a polymer-liposome mixture is shown in Figure S4c and a direct superposition of the spectra of pure polymers (Figure S4a) and pure liposomes (Figure S4b) is shown in Figure S4d as a control, which represents the case of no polymer-liposome interactions. The intensity of the two spectra were also adjusted to keep maximum intensity of the water bending peak the same as that in Figure S4a and S4b. Comparing Figure S4c and S4d, no significant peak shift or intensity change was observed for the C-O-C asymmetric stretching vibrations at ~ 1140 cm^{-1} or even the full range of the spectra. This could mean there is not a significant amount of hydrogen bond formation between the POPG headgroups and polymers. However, the resolution of the Raman measurements may not be adequate to resolve the effects of the delicate hydrogen bonds for the following two reasons. First, the peak associated with the C-O-C asymmetric stretching vibrations (*i.e.*, the indicator of hydrogen bonding) is not well resolved due to overlap with the peaks of other vibrations (*i.e.*, C-C stretching and CH_2 wagging). Second, the maximum percentage of the ether oxygen atoms of the polymers in solution bound to POPG liposomes is estimated to be approximately 14%. This means at most 14% of the C-O-C stretching signal can contribute to a peak shift or intensity change due to hydrogen bonding. This estimate is based on the assumptions that (1) all ether oxygen from both PPO and PEO in the bound polymers form hydrogen bonds with lipid headgroups, and (2) all hydroxyl groups in lipid headgroups can potentially participate in hydrogen bonding. In all likelihood the 14% estimate is considerably higher than the actual level of hydrogen bonding, and accordingly the associated Raman signal may not be detectable.

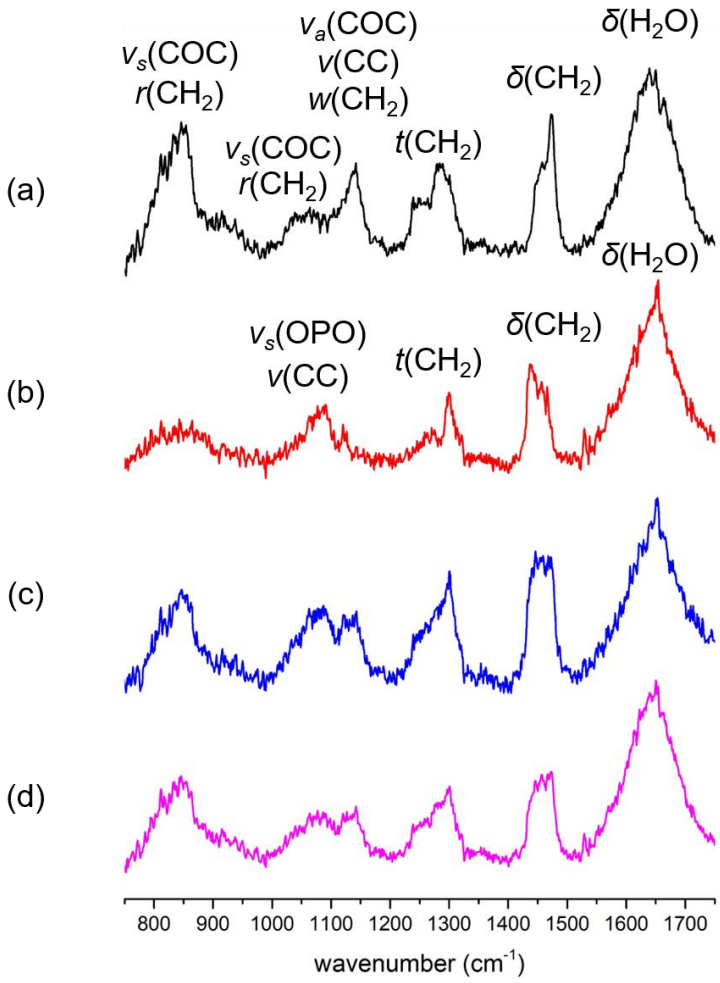


Figure S4. Raman spectra of (a) 20 mg/mL F108, (b) 30 mM POPG liposomes, and (c) polymer-liposome mixture of 20 mg/mL F108 and 30 mM POPG liposomes in H_2O. (d) Superposition of pure polymers and pure liposomes. δ: bending; ν: stretching; t: twisting; r: rocking; w: wagging. The subscripts "s" and "a" stands for "symmetric" and "asymmetric", respectively.

References

(1) Papke, B. L.; Ratner, M. A.; Shriver, D. F. Vibrational Spectroscopy and Structure of Polymer Electrolytes, Poly(Ethylene Oxide) Complexes of Alkali Metal Salts. *J. Phys. Chem. Solids* **1981**, *42*, 493–500.

(2) Chaurasia, S. K.; Singh, R. K.; Chandra, S. Ion–Polymer Complexation and Ion-Pair Formation in a Polymer Electrolyte PEO:LiPF6 Containing an Ionic Liquid Having Same Anion: A Raman Study. *Vib. Spectrosc.* **2013**, *68*, 190–195.

(3) Matsui, Y.; Kubota, T.; Tadokoro, H.; Yoshihara, T. Raman Spectra of Polyethers. *J. Polym. Sci. Part A Gen. Pap.* **1965**, *3*, 2275–2288.

(4) Yoshihara, T.; Tadokoro, H.; Murahashi, S. Normal Vibrations of the Polymer Molecules of Helical Conformation. IV. Polyethylene Oxide and Polyethylene‐D4 Oxide. *J. Chem. Phys.* **1964**, *41*, 2902–2911.

(5) Machida, K.; Miyazawa, T. Infrared and Raman Spectra of Polyethyleneglycol Dimethylethers in the Liquid State. *Spectrochim. Acta* **1964**, *20*, 1865–1873.

(6) Carrier, D.; Pézolet, M. Raman Spectroscopic Study of the Interaction of Poly-L-Lysine with Dipalmitoylphosphatidylglycerol Bilayers. *Biophys. J.* **1984**, *46*, 497–506.

(7) Spiker, R. C.; Levin, I. W. Raman Spectra and Vibrational Assignments for Dipalmitoyl Phosphatidylcholine and Structurally Related Molecules. *Biochim. Biophys. Acta - Lipids Lipid Metab.* **1975**, *388*, 361–373.

(8) Lis, L. J.; Kauffman, J. W.; Shriver, D. F. Raman Spectroscopic Detection and Examination of the Interaction of Amino Acids, Polypeptides and Proteins with the Phosphatidylcholine Lamellar Structure. *Biochim. Biophys. Acta - Biomembr.* **1976**, *436*, 513–522.

(9) Lippert, J. L.; Peticolas, W. L. Raman Active Vibrations in Long-Chain Fatty Acids and Phospholipid Sonicates. *Biochim. Biophys. Acta - Biomembr.* **1972**, *282*, 8–17.