Supporting information

Spatial distribution of PEO-PPO-PEO block copolymer and PEO homopolymer in lipid bilayers

Mihee Kim[a], Frank Heinrich[b,c], Greg Haugstad[d,*], Guichuan Yu[e], Guangcui Yuan[c,f], Sushil K. Satija[c], Wenjia Zhang[a], Hannah S. Seo[a], Joseph M. Metzger[g], Samira M. Azarin[a], Timothy P. Lodge[a,h], Benjamin J. Hackel[a], Frank S. Bates[a,*]

[a]Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, MN 55455; [b]Department of Physics, Carnegie Mellon University, Pittsburgh, PA 15213; [c]National Institute of Standards and Technology Center for Neutron Research, Gaithersburg, MD 20899; [d]Characterization Facility, University of Minnesota, Minneapolis, MN 55455; [e]Informatics Institute, University of Minnesota, Minneapolis, MN 55455; [f]Department of Physics, Georgetown University, Washington, DC 20057; [g]Department of Integrative Biology and Physiology, University of Minnesota, Minneapolis, MN 55455; [h]Department of Chemistry, University of Minnesota, Minneapolis, MN 55455

*To whom correspondence should be addressed. Email: haugs001@umn.edu, bates001@umn.edu

Content: 13 pages

SI Text

8 Figures, S1-S8

4 Tables, S1-S4

SI Text

Additional explanation regarding the dynamic light scattering (DLS) data presented in Figure 2a. The *y*-intercept of correlation function $g^{(1)}(\tau)$ is affected by the spatial coherence factor, which depends on the aperture size of the photodetector and other instrumental factors.[1] As the sizes of the polymer single chains measured in this work are small, we used a large aperture size to obtain enough intensity, which could have resulted in low *y*-intercept values.

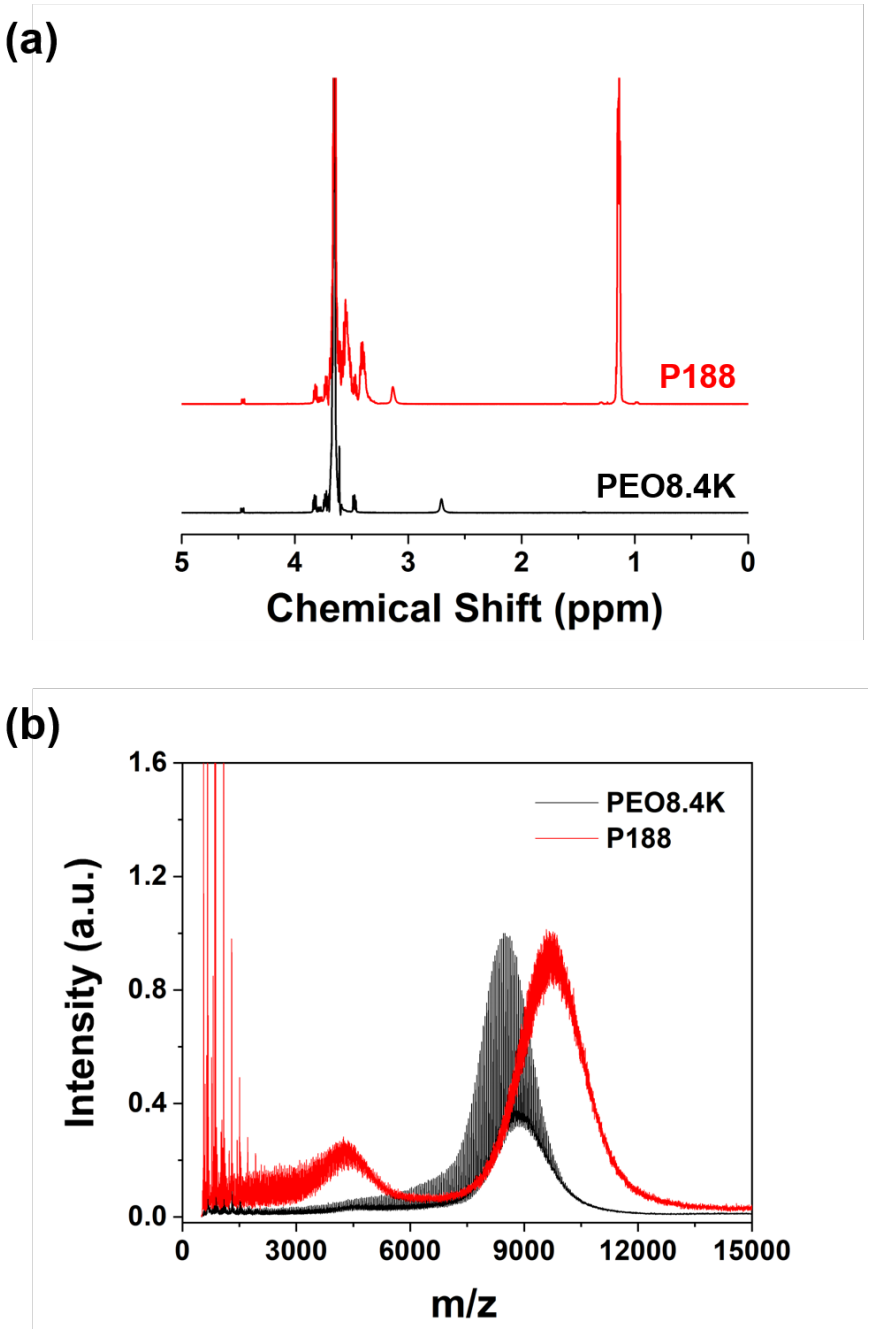

Figure S1. (a) ^{1}H NMR spectra of P188 and PEO8.4K, (b) MALDI-TOF mass spectra of P188 and PEO8.4K. M_n of P188 = 8700 g/mol, $Đ$ = 1.09, M_n of PEO8.4K = 8400 g/mol, $Đ$ = 1.05.

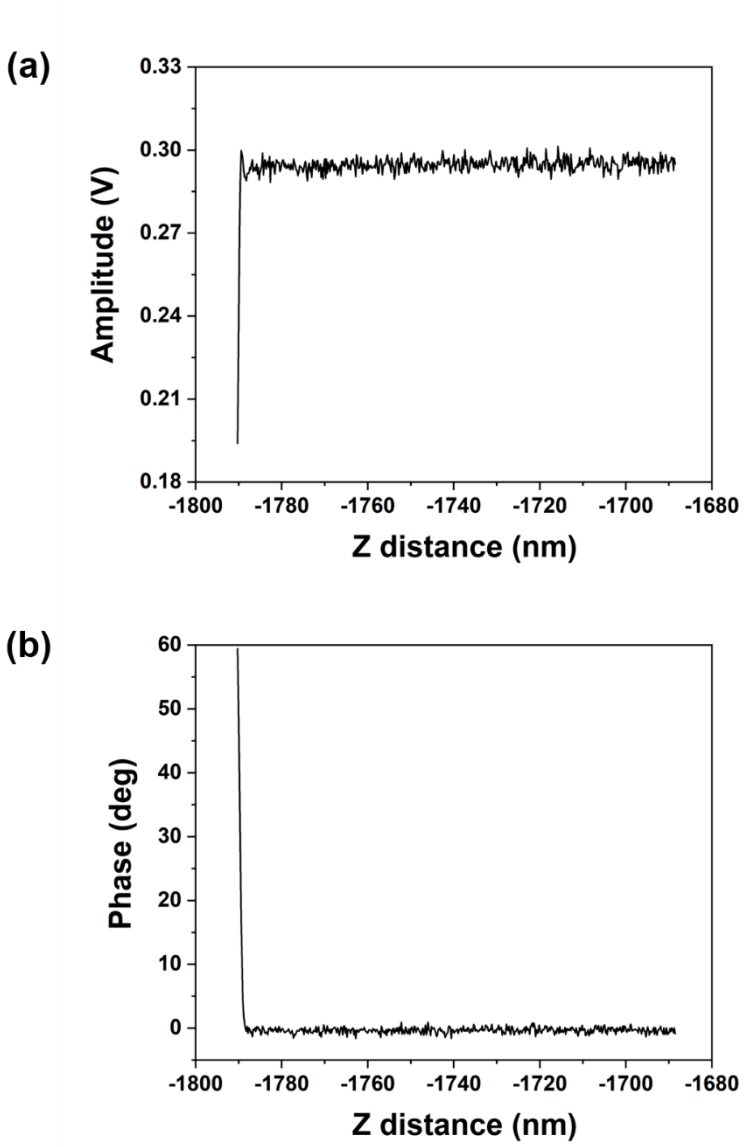

Figure S2. Representative curves of (a) amplitude vs. Z scanner displacement and (b) phase vs. Z scanner displacement.

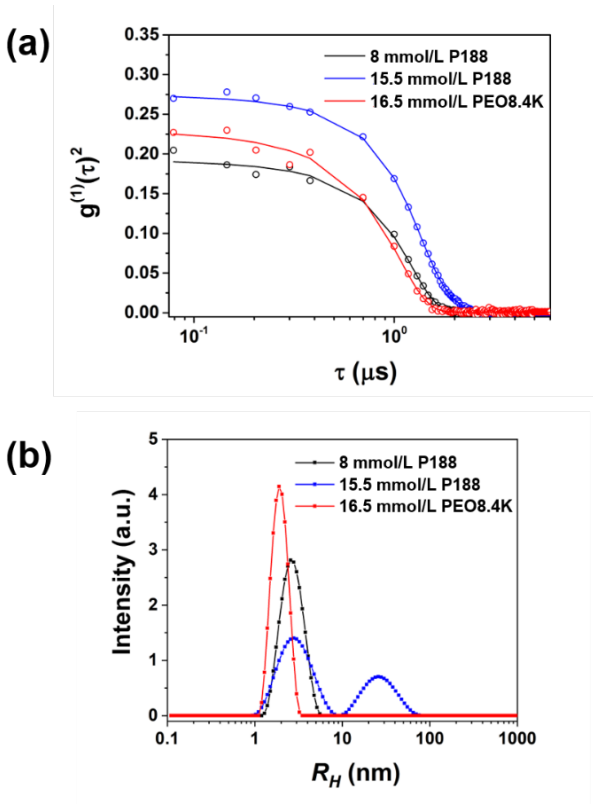

Figure S3. (a) Autocorrelation function obtained from dynamic light scattering (DLS) at 37 °C. Solid lines are the fit by REPES algorithm (see SI for comment regarding $g^{(1)}(\tau)^2$). (b) Size distributions of hydrodynamic radius (R_H) obtained by DLS.

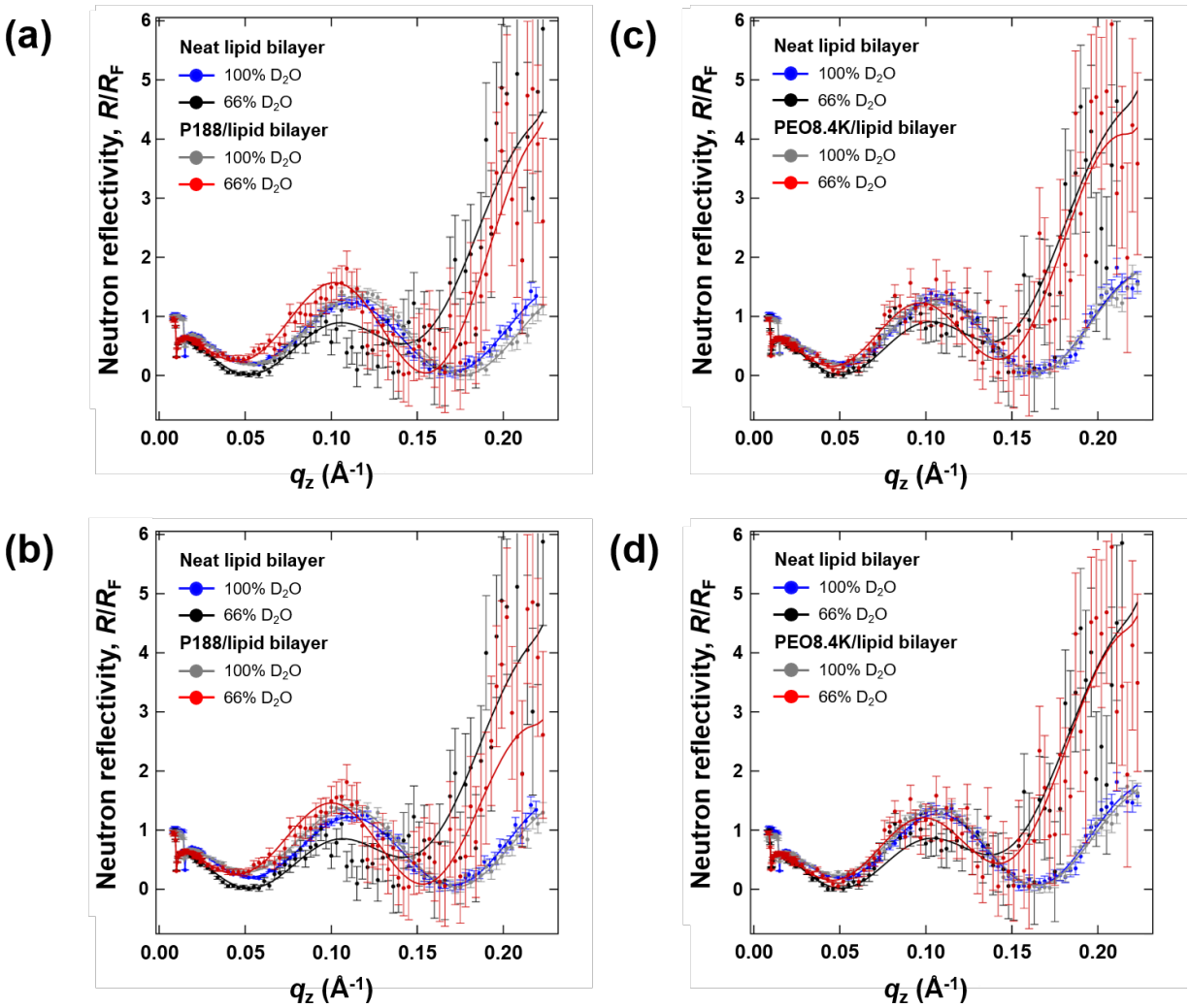

Figure S4. Neutron reflectivity (NR) data (symbols) and best model fits (solid curves) normalized to the Fresnel reflectivity R_F (i.e., the reflectivity of a neat Si/buffer interface without interfacial roughness). Error bars represent 68% confidence intervals for the measured reflectivity based on Poisson statistics. The NR profiles of neat lipid bilayers were measured in 100% D_2O (blue) and 66% D_2O (black) before polymer incubation. The neat bilayer NR profiles shown in (a) and (b) are identical and the corresponding data in (c) and (d) are identical as well. The NR profiles of P188-incubated bilayers were measured consecutively in 100% D_2O ("D_2O-1"), 66% D_2O, and 100% D_2O ("D_2O-2"); The measurement for PEO-incubated bilayers followed the same protocol. (a) NR profiles of a P188-incubated lipid bilayer measured in "D_2O-1"(grey) or 66% D_2O (red). (b) NR profiles of a P188-incubated lipid bilayer measured in "D_2O-2"(grey) or 66% D_2O (red). The data for P188-incubated lipid bilayer in 66% D_2O is identical to the corresponding data shown in (a). (c) NR profiles of a PEO8.4K-incubated lipid bilayer measured in "D_2O-1"(grey) or 66% D_2O (red). (d) NR profiles of a PEO8.4K-incubated lipid bilayer measured in "D_2O-2"(grey) or 66% D_2O (red). The data for PEO8.4K-incubated lipid bilayer in 66% D_2O is identical to the corresponding data shown in (c). The data shown in (a-d) correspond to the data presented in Figure S4a, 3a, S4c, and 3c, respectively.

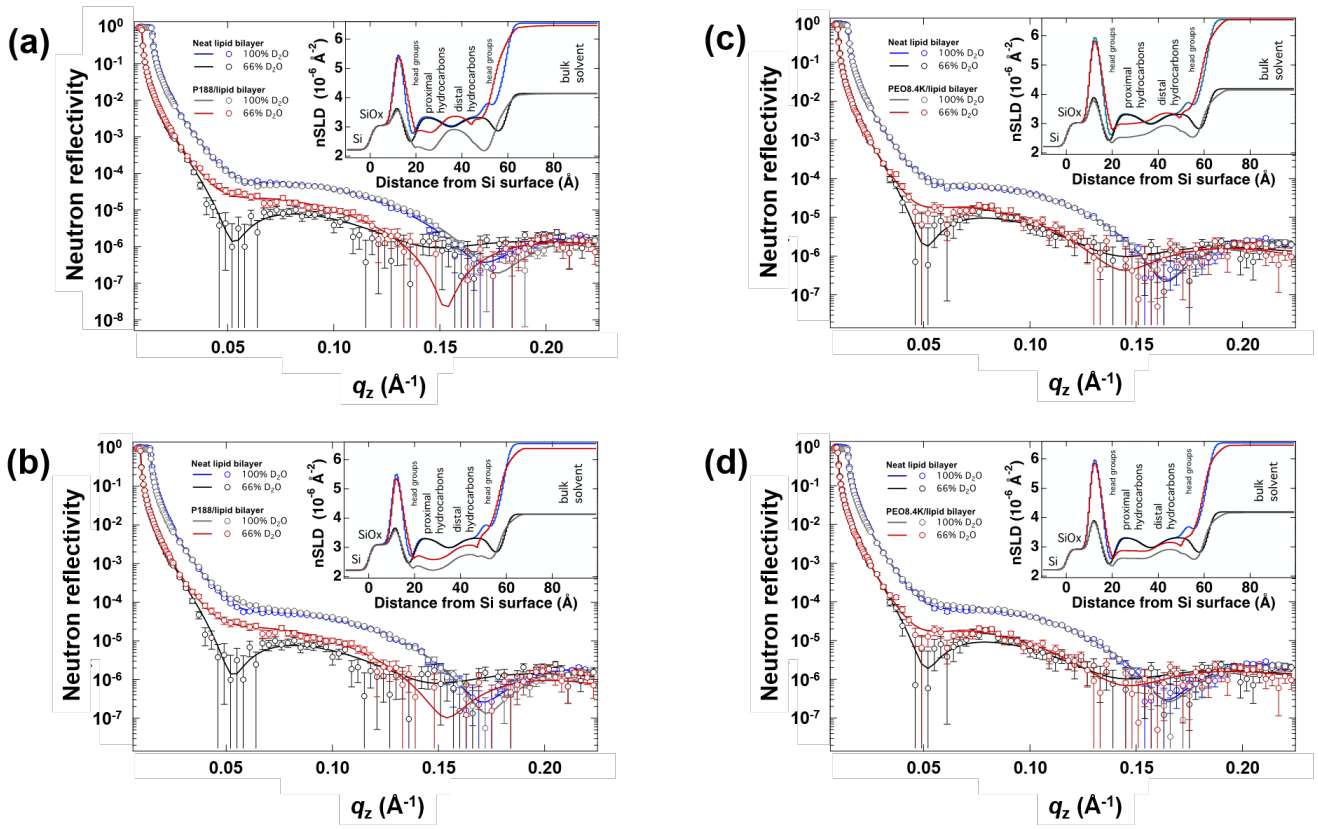

Figure S5. Neutron reflectivity (NR) data (symbols) and best model fits (solid curves) along with the scattering length density (SLD) profiles corresponding to each reflectivity curve with matching color (insets). The data presented in each panel corresponds to that in Figure S4. Error bars represent 68% confidence intervals for the measured reflectivity based on Poisson statistics. The NR profiles of neat lipid bilayers were measured in 100% D_2O (blue) and 66% D_2O (black) before polymer incubation. The neat bilayer NR profiles shown in (a) and (b) are identical and the corresponding data in (c) and (d) are identical as well. The NR profiles of P188-incubated bilayers were measured consecutively in 100% D_2O ("D_2O-1"), 66% D_2O, and 100% D_2O ("D_2O-2"); The measurement for PEO-incubated bilayers followed the same protocol. (a) NR profiles of a P188-incubated lipid bilayer measured in "D_2O-1"(grey) or 66% D_2O (red). (b) NR profiles of a P188-incubated lipid bilayer measured in "D_2O-2"(grey) or 66% D_2O (red). The data for P188-incubated lipid bilayer in 66% D_2O is identical to the corresponding data shown in (a). (c) NR profiles of a PEO8.4K-incubated lipid bilayer measured in "D_2O-1"(grey) or 66% D_2O (red). (d) NR profiles of a PEO8.4K-incubated lipid bilayer measured in "D_2O-2"(grey) or 66% D_2O (red). The data for PEO8.4K-incubated lipid bilayer in 66% D_2O is identical to the corresponding data shown in (c). The data shown in (a-d) correspond to the data presented in Figure S4a, 3a, S4c, and 3c, respectively.

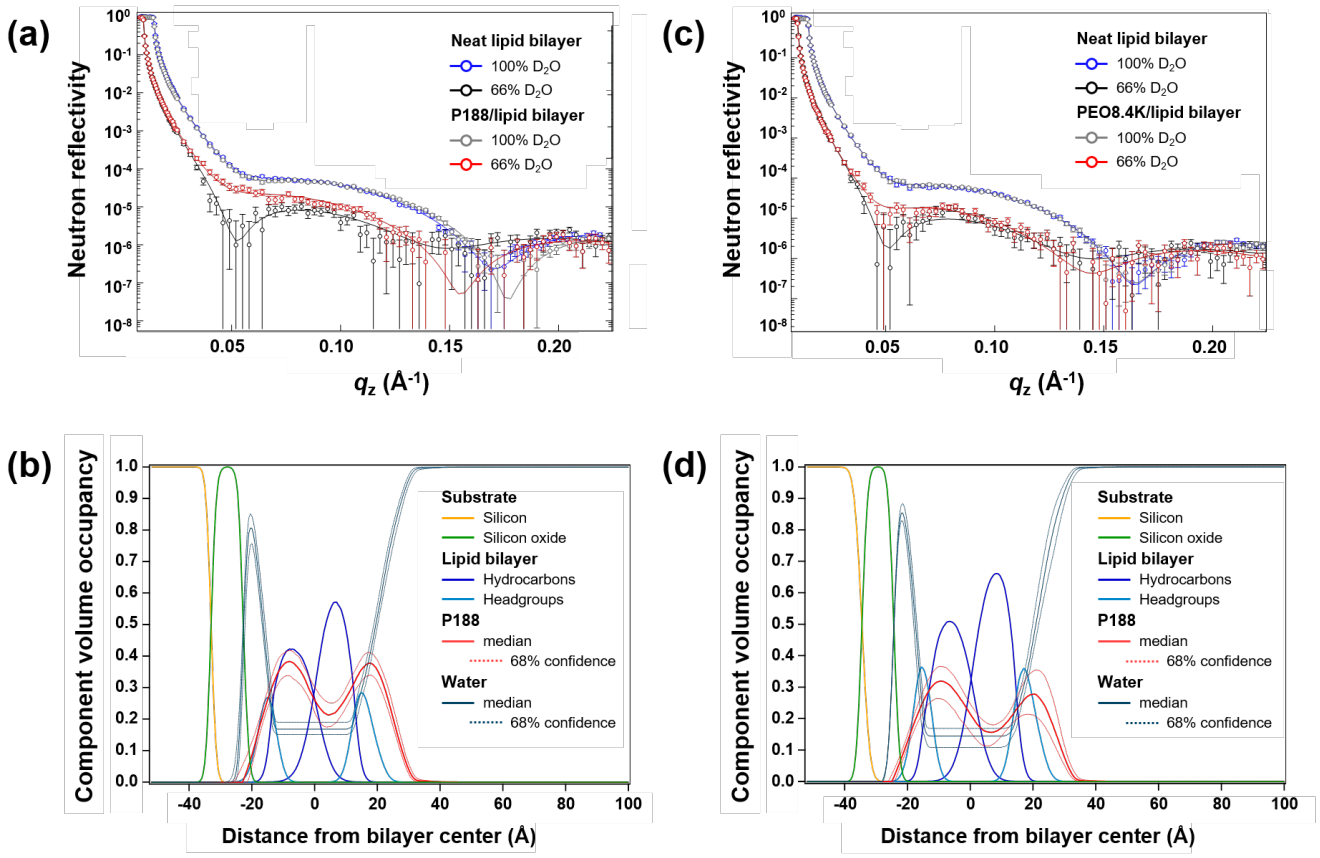

Figure S6. (a,c) Neutron reflectivity (NR) data (symbols) and best model fits (solid curves) of lipid bilayer before and after incubation with (a) P188 or (c) PEO8.4K in 100% D_2O ("D_2O-1") and 66% D_2O. Error bars represent 68% confidence intervals for the measured reflectivity based on Poisson statistics. (b,d) Component volume occupancy (CVO) profiles of (b) P188 and (d) PEO8.4K, obtained from NR fitting. The median polymer envelope is shown with 68% confidence intervals.

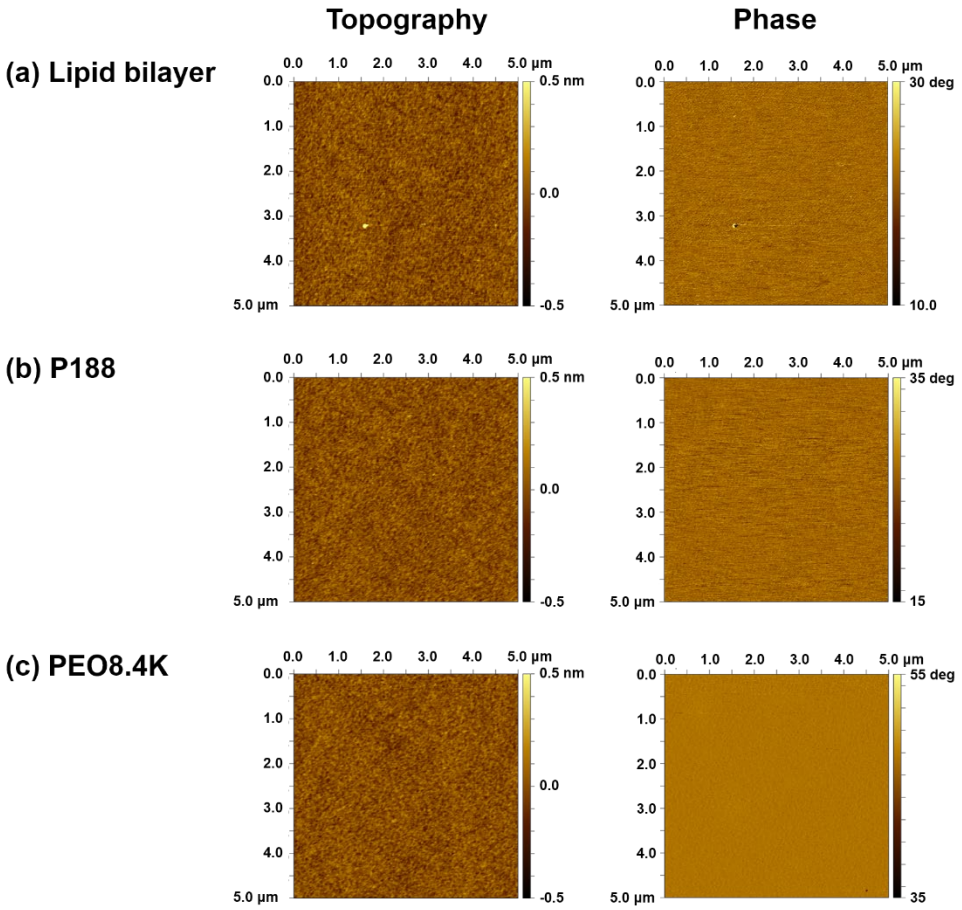

Figure S7. Atomic force microscopy (AFM) topography and phase images (5µm × 5 µm, 512 × 512 pixels) of (a) neat lipid bilayer, (b) P188-incubated bilayer, and (c) PEO8.4K-incubated bilayer.

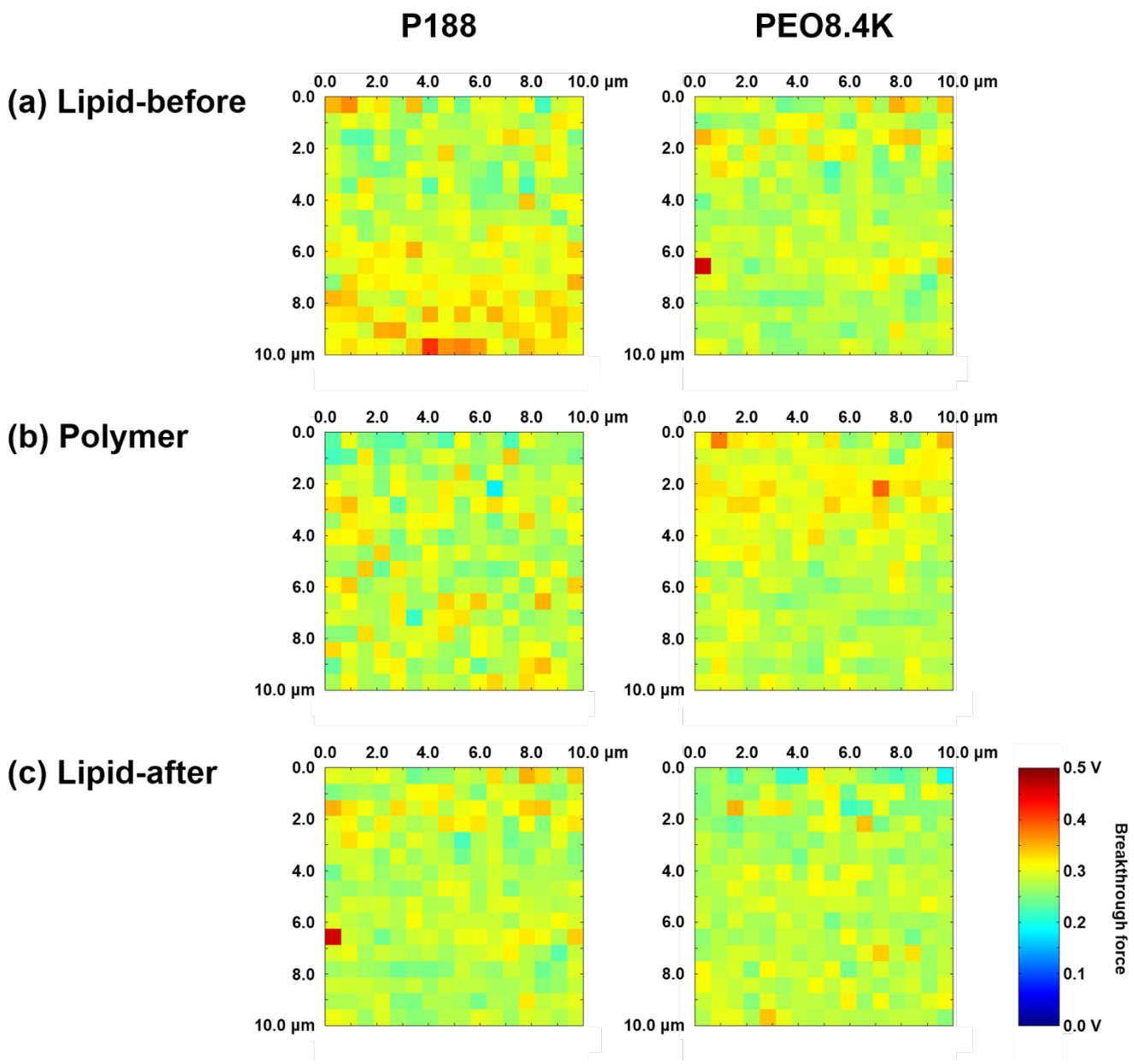

Figure S8. Breakthrough force mapping (10 µm × 10 µm) of lipid bilayer before and after incubation with P188 or PEO8.4K. The data corresponds to the histograms presented in Figure 5a-b. Note that the data presented in the bottom left panel and that in the top right panel are from the same lipid bilayer sample measured back-to-back, so the two data are identical.

Table S1. Fit parameters and derived quantities for P188-incubated lipid bilayer from simultaneous fitting of the NR curves measured in two different contrasts ("D_2O-2" and 66% D_2O). Corresponding NR curves are presented in Figure 3a. The values indicate median fit values with 68% confidence limits.

	Neat lipid bilayer	P188/lipid bilayer
Fit parameters		
SLD of silicon (10^{-6} Å^{-2})	2.07 (fix)	
Thickness of silicon oxide (Å)	10.2 ± 0.3	
SLD of silicon oxide (10^{-6} Å^{-2})	3.0 ± 0.1	
Thickness of sub-membrane space (Å)	3.0 ± 0.3	
Thickness of substrate-proximal headgroups (Å)	7±1	
Thickness per hydrophobic lipid tail (Å)	14.6 ± 0.4	Change: -2.0 ± 0.4
Thickness of substrate-distal headgroups (Å)	9.56 (fix)	
Bilayer completeness (%)	99 ± 1	93 ± 3
Derived quantities		
Polymer volume surface density (Å^3/ Å^2)	-	12.7 ± 0.7
Fraction of polymer outside of bilayer (%)	-	13 ± 3
Fraction of polymer in headgroups (%)		32 ± 3
Fraction of polymer in tail region (%)		53 ± 6
Fraction of polymer in submembrane space (%)		2 ± 1
Fit quality, χ^2	1.9	

Table S2. Fit parameters and derived quantities for P188-incubated lipid bilayer from simultaneous fitting of the NR curves measured in two different contrasts ("D_2O-1" and 66% D_2O). Corresponding NR curves are presented in Figure S3a. The values indicate median fit values with 68% confidence limits.

	Neat lipid bilayer	P188/lipid bilayer
Fit parameters		
SLD of silicon (10^{-6} Å^{-2})	2.07 (fix)	
Thickness of silicon oxide (Å)	10.3 ± 0.3	
SLD of silicon oxide (10^{-6} Å^{-2})	2.9 ± 0.1	
Thickness of sub-membrane space (Å)	3.1 ± 0.3	
Thickness of substrate-proximal headgroups (Å)	7.2 ± 0.3	
Thickness per hydrophobic lipid tail (Å)	14.4 ± 0.2	Change: -2.8 ± 0.3
Thickness of substrate-proximal headgroups (Å)	9.56 (fix)	
Bilayer completeness (%)	99 ± 1	83 ± 2
Derived quantities		
Polymer volume surface density (Å^3/ Å^2)	-	14 ± 1
Fraction of polymer outside of bilayer (%)	-	13 ± 3
Fraction of polymer in headgroups (%)	-	36 ± 2
Fraction of polymer in tail region (%)	-	50 ± 7
Fraction of polymer in submembrane space (%)	-	2 ± 1
Fit quality, χ^2	1.4	

Table S3. Fit parameters and derived quantities for PEO8.4K-incubated lipid bilayer from simultaneous fitting of the NR curves measured in two different contrasts ("D_2O-2" and 66% D_2O). Corresponding NR curves are presented in Figure 3c. The values indicate median fit values with 68% confidence limits.

	Neat lipid bilayer	PEO8.4K/lipid bilayer
Fit parameters		
SLD of silicon (10^{-6} Å^{-2})	2.07 (fix)	
Thickness of silicon oxide (Å)	10.2 ± 0.3	
SLD of silicon oxide (10^{-6} Å^{-2})	2.8 ± 0.1	
Thickness of sub-membrane space (Å)	4.3 ± 0.5	
Thickness of substrate-proximal headgroups (Å)	7 ± 1	
Thickness per hydrophobic lipid tail (Å)	15.0 ± 0.6	Change: -2.3 ± 0.4
Thickness of substrate-distal headgroups (Å)	9.56 (fix)	
Bilayer completeness (%)	99 ± 1	95 ± 3
Derived quantities		
Polymer volume surface density (Å^3/ Å^2)	-	10 ± 1
Fraction of polymer outside of bilayer (%)	-	18 ± 5
Fraction of polymer in headgroups (%)	-	37 ± 3
Fraction of polymer in tail region (%)	-	43 ± 8
Fraction of polymer in submembrane space (%)	-	3 ± 2
Fit quality, χ^2	1.8	

Table S4. Fit parameters and derived quantities for PEO8.4K-incubated lipid bilayer from simultaneous fitting of the NR curves measured in two different contrasts ("D_2O-1" and 66% D_2O). Corresponding NR curves are presented in Figure S3c. The values indicate median fit values with 68% confidence limits.

	Neat lipid bilayer	PEO8.4K/lipid bilayer
Fit parameters		
SLD of silicon (10^{-6} Å^{-2})	2.07 (fix)	
Thickness of silicon oxide (Å)	10.3 ± 0.3	
SLD of silicon oxide (10^{-6} Å^{-2})	2.8 ± 0.1	
Thickness of sub-membrane space (Å)	4.4 ± 0.4	
Thickness of substrate-proximal headgroups (Å)	7.2 ± 0.3	
Thickness per hydrophobic lipid tail (Å)	15.2 ± 0.4	Change: -2.4 ± 0.4
Thickness of substrate-distal headgroups (Å)	9.56 (fix)	
Bilayer completeness (%)	99 ± 1	88 ± 3
Derived quantities		
Polymer volume surface density (Å^3/ Å^2)	-	11.9 ± 0.9
Fraction of polymer outside of bilayer (%)	-	18 ± 4
Fraction of polymer in headgroups (%)	-	36 ± 3
Fraction of polymer in tail region (%)	-	44 ± 7
Fraction of polymer in submembrane space (%)	-	4 ± 2
Fit quality, χ^2	1.5	

(1) Hassan, P. A.; Rana, S.; Verma, G. Making Sense of Brownian Motion: Colloid Characterization by Dynamic Light Scattering. *Langmuir* **2015**, *31*, 3-12.