Electronic Supplementary Material (ESI) for Soft Matter.

Electronic Supplementary Information for:

Adsorption and Fusion of Hybrid Lipid/Polymer Vesicles onto 2D and 3D Surfaces

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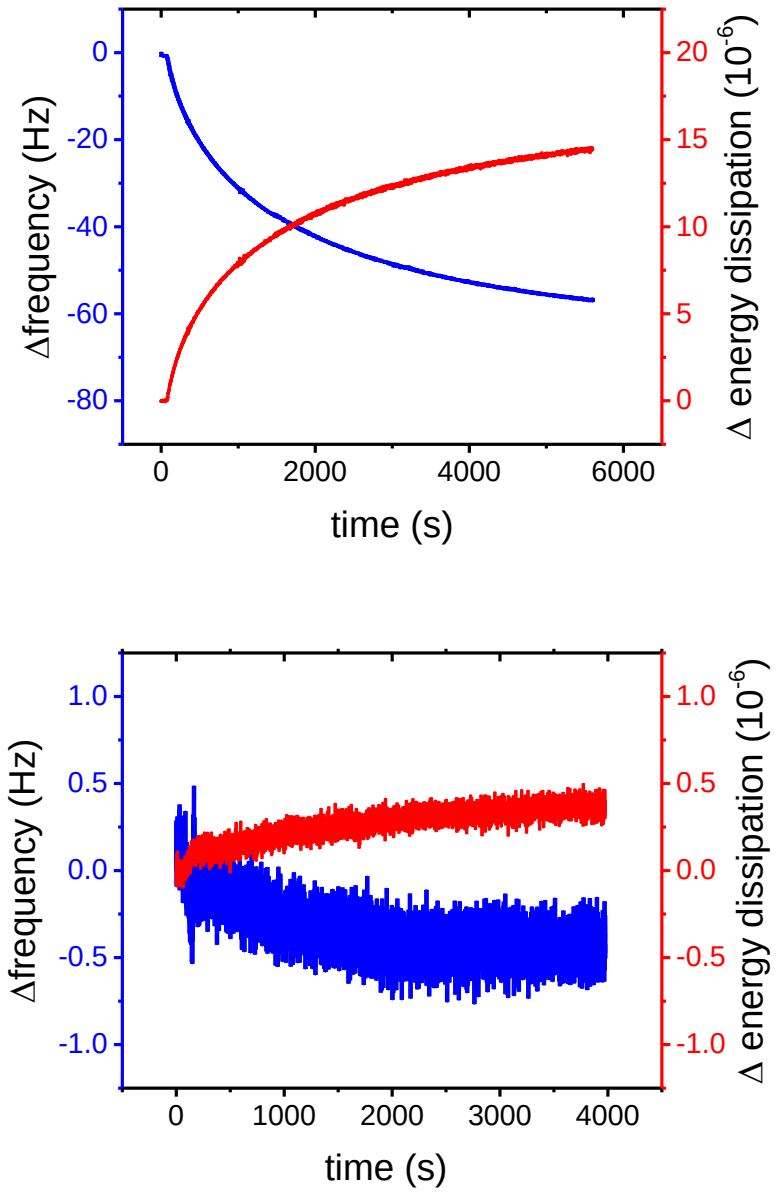

Figure S1 - Quartz crystal microbalance with dissipation (QCM-D) monitoring (n=3; i.e. 3rd overtone) of vesicles interacting with plasma-cleaned borosilicate glass substrates. Vesicles are comprised of DOPC and 50 mol% $EO_{22}Bd_{37}$ at 25 °C (TOP) or 100% $EO_{22}Bd_{37}$ at 40°C (BOTTOM). The baseline buffer measurement was stabilized at the desired temperature (25 °C or 40 °C, respectively) for at least 15 min and the vesicle solutions (0.1 mg/mL in each case) were injected.

Cleaning Effects. We observed a striking difference between the interaction of lipid-only and polymer-only vesicles with plasma-treated surfaces. While both lipid and polymer vesicles interact with chemically-treated surfaces, polymer-only vesicles did not interact with plasma-treated surfaces at all and indicated that the variable interaction of polymer vesicles with cover glass depends on the cleaning treatment of the glass substrates. The striking difference between the interaction of polymer vesicles with plasma- treated surfaces (Figure S2) and chemically-treated surfaces (Figure 1) is puzzling, and warrants a brief discussion of the effect these cleaning procedures have on glass.

Three possibilities were considered: the effect of substrate pre-treatment on i) solution pH, ii) surface chemistry, and iii) surface morphology. The favorable interaction between PEO and silica particles in aqueous solutions with pH <10 is well-documented.[1] It may be argued that plasma treated glass could increase the pH above the threshold that would prevent adsorption. However, the pH of buffer solutions in contact with plasma-treated glass was essentially unchanged from the starting value of 7. Furthermore, plasma treated silica substrates were rinsed liberally with buffer prior to QCM-D experiments, which would effectively dilute any pH changes due to surface ionization. Both plasma and chemically-treated surfaces were highly hydrophilic, as indicated by water contact angles in each case <3°. In addition to the surface wettability measurements, we have also collected high resolution XPS spectra and measured AFM surface roughness. While different from the as-received and water-rinsed cover glass, the high resolution XPS spectra of the C 1s, O 1s, and Si 2p regions for the chemically-etched and plasma-treated surfaces were remarkably similar (see Figure S4), with no significant differences that might account for the differences in polymer vesicle interaction. X-ray photoelectron spectroscopy[2] of clean glass cover slips revealed virtually the same amount and oxidation state of oxygen and silicon (Figures S3 and S4). On the other hand, there was slightly less adventitious carbon on plasma-treated surfaces relative to chemically-treated surfaces (Table S1). These differences do not appear to affect the formation of supported *lipid* bilayers (compare Figure S2A), and it is not clear if they are related to differences in polymer adsorption. Our results echo previous reports that caution against directly comparing experiments involving adsorption behavior on silica that has been cleaned in different ways.[3] Nevertheless, except in the case of the 100% polymer vesicles, our LSCM and AFM observations of hybrid vesicle interactions on chemically etched surfaces are in good agreement with our QCM-D observations on plasma-treated glass.

Surface roughness of cover glass was measured after various surface treatments by atomic force microscopy. The roughness of four 10×10 μm areas and four 2.5×2.5 μm areas were measured and analyzed using surface roughness measurement tools (MFP3D, version 04_08, Asylum Research Inc.). While both the average roughness, Ra, and root mean square roughness, Rq, of the plasma-treated and chemically-etched surfaces are comparable for the 10×10 um^2 samples at the 90% confidence level, the smaller sample areas (2.5×2.5 μm^2) revealed that there is considerably more variability in the chemically-etched surfaces (Table S2). In addition, slight pitting of the chemically-etched surfaces was observed (Figure S5). Surface roughness has been invoked to explain differences in lipid vesicle interaction with surfaces,[4] but those differences were not observed by us for lipid bilayers, and it is still not clear how roughness differences prevent or otherwise affect the interaction of the 100 mol% vesicles. Nevertheless, it is important to point out that special care and attention may be needed when preparing substrates for the formation of supported hybrid lipid/polymer assemblies.

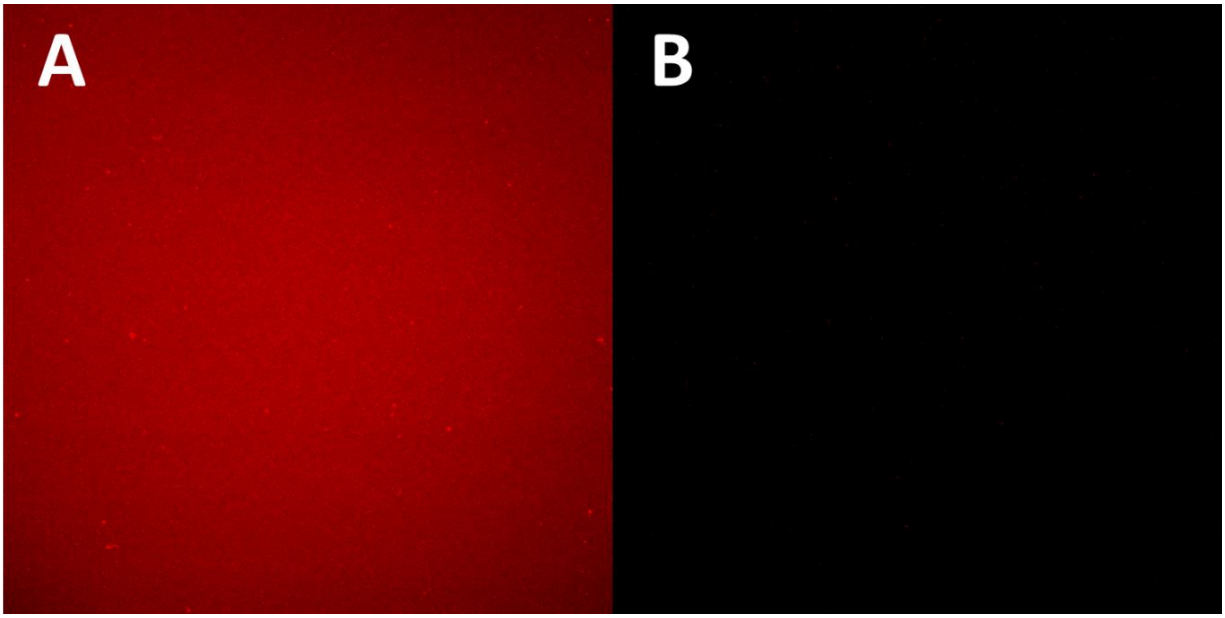

Figure S2. Laser scanning confocal micrographs of plasma-treated borosilicate glass cover slips after incubating with 0.1 mg/mL DOPC (A) and 0.1 mg/mL $EO_{22}Bd_{37}$ (B), each with ~0.5% TR-DHPE, under identical imaging conditions. The average fluorescence intensity of the sample containing polymer only was 1% of the lipid only sample, demonstrating the substantial difference in propensity to form supported bilayers and adsorbed vesicle films. Image size in each case is 300 × 300 μm.

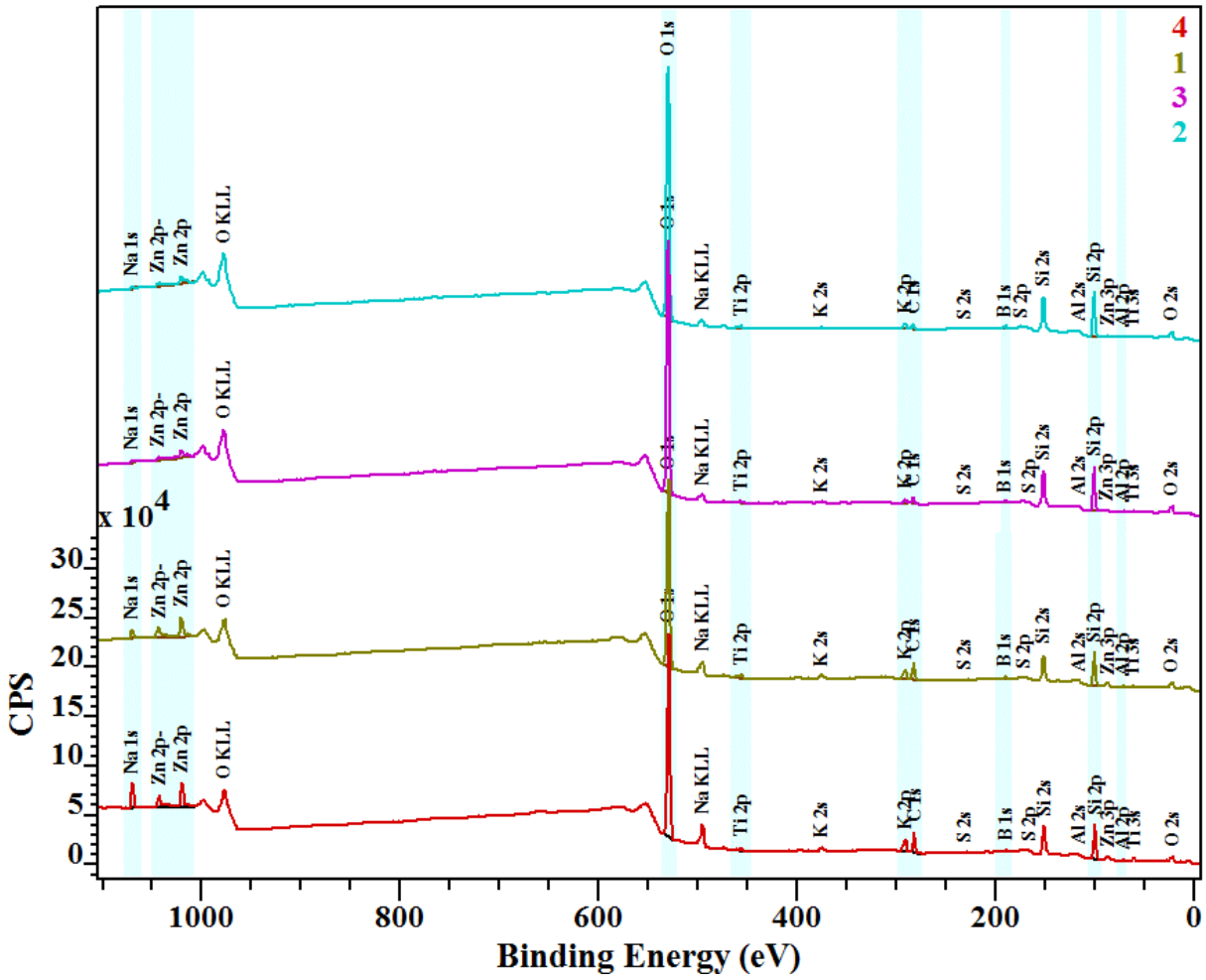

Figure S3. X-ray photoelectron spectroscopy survey scans of as-received glass cover slips used for bilayer formation (4, red), rinsed with deionized water and dried under N_2 (1, brown), chemically-etched with H_2O_2/HCl solution at 70 °C for 30 min (magenta, 3), and chemically-etched as (3) and then plasma-treated (2, blue).

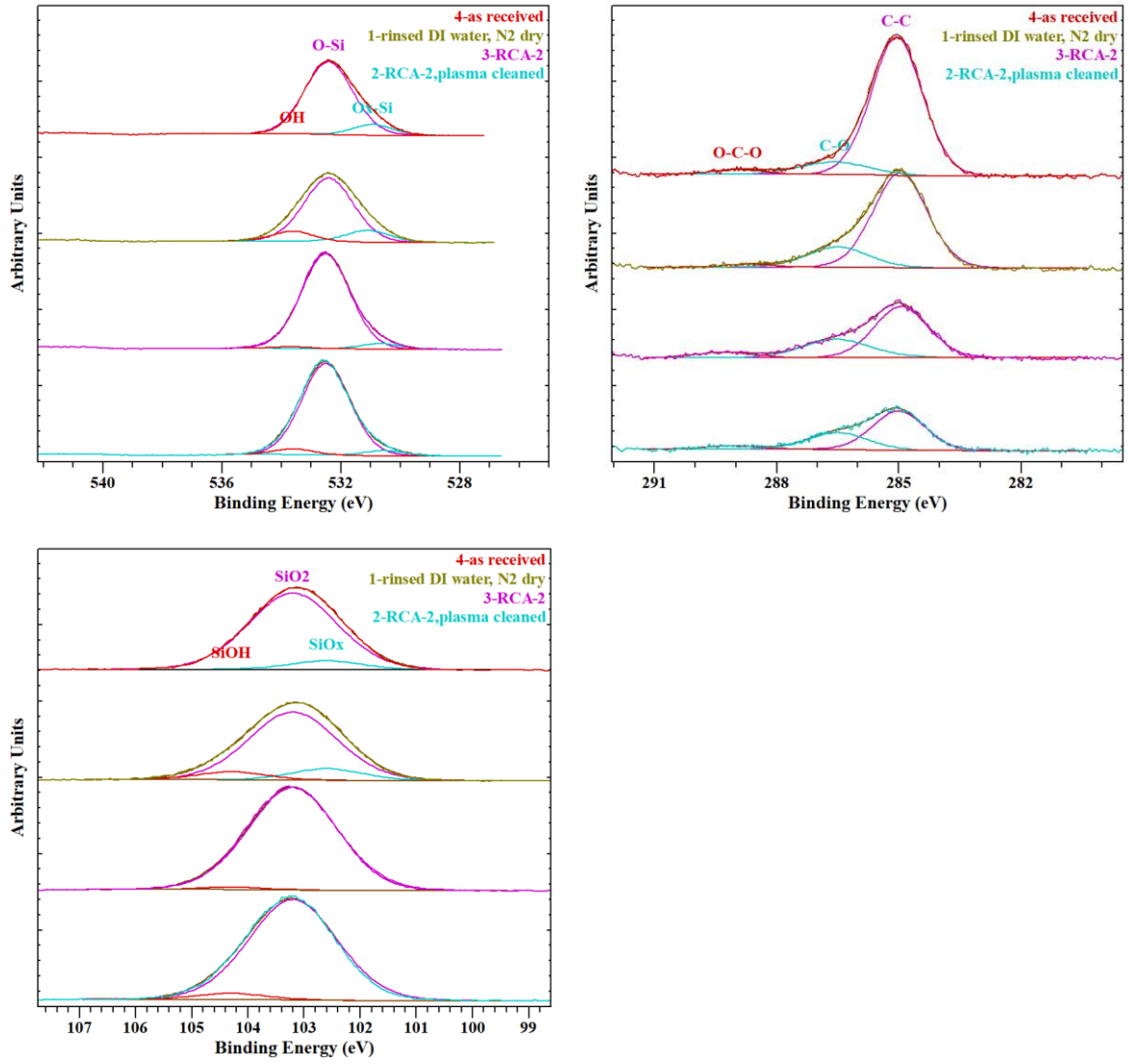

Figure S4. High resolution XPS spectra of the O 1s (top-left) K 2p and C 1s (top-right) and Si 2p (bottom-left) regions of borosilicate cover glass (1) rinsed with deionized water; (2) RF-plasma treated; (3) chemically etched with HCl/H_2O_2; or (4) as-received.

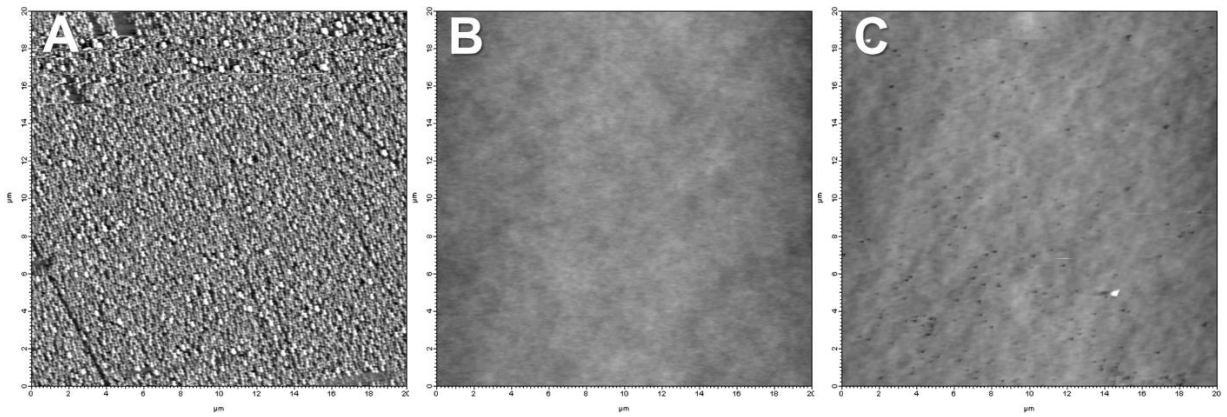

Figure S5. Atomic force micrographs of as-received (A); plasma-treated (B) and chemically-etched (C) borosilicate cover glass surfaces. Each image is 20×20 μm^2.

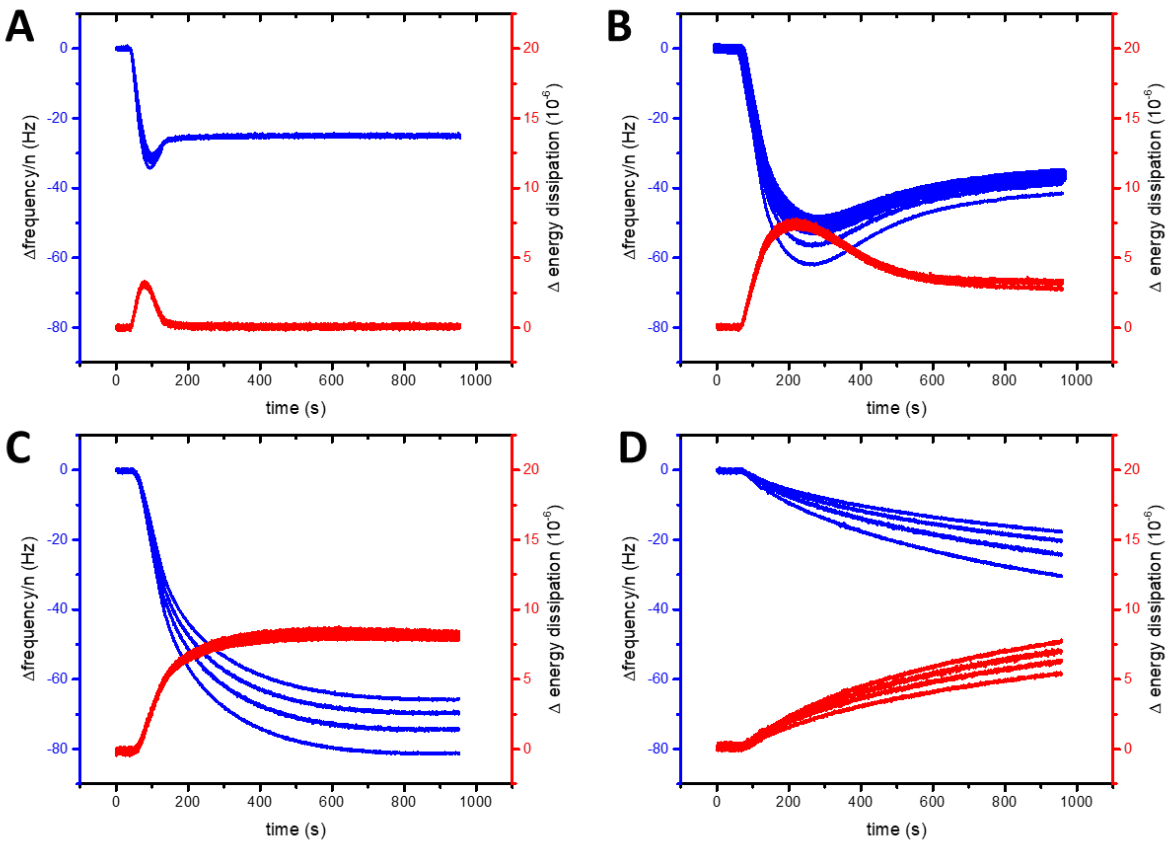

Figure S6. Quartz crystal microbalance with dissipation (QCM-D) monitoring for overtones 3, 5, 7, and 9 of hybrid lipid/polymer film formation on plasma-cleaned borosilicate glass substrates. The baseline buffer measurement was stabilized and the vesicle solution (0.1 mg/mL) of either pure DOPC (A), 10 (B), 25 (C), 50 (D), and 100 (E) mol% of $EO_{22}Bd_{37}$ was injected.

Table S1. Atomic concentration of borosilicate glass cover slips after various surface treatments.

	Atomic Concentration [%]								
	Al	**B**	**C**	**K**	**Na**	**O**	**Si**	**Ti**	**Zn**
as received	1.4	8.3	12	2.7	1.9	47	25	0.6	1.2
rinsed w/DI water	1.6	9.6	11	2.2	0.9	48	25	0.7	1.1
RCA-2	1.2	8.2	5.1	1.1	0.2	55	29	0.4	0.5
RCA-2, plasma cleaned	1.3	7.8	3.3	1.4	0.2	56	29	0.5	0.5

Table S2. Quantification of core level peak fits for various surface conditions.

	Concentration (% of the peak fit)		
	O 1s: (O-Si)	**O 1s: (Ox-Si)**	**O 1s: O-H**
4-as received	89	11	0.0
1-rinsed DI water, N2 dry	77	13	10
3-RCA-2	94	5.0	1.0
2-RCA-2,plasma cleaned	91	4.1	4.9
	Si 2p: (SiO2)	**Si 2p: (SiOx)**	**Si 2p: (Si-OH)**
4-as received	91	8.9	0.1
1-rinsed DI water, N2 dry	81	11	8
3-RCA-2	98	0	2
2-RCA-2, plasma cleaned	95	0	5
	C 1s: (C-C)	**C 1s: (C-O)**	**C 1s: (O-C-O)**
4-as received	86	11	3
1-rinsed DI water, N2 dry	78	19	2
3-RCA-2	63	29	8
2-RCA-2, plasma cleaned	63	31	6

Table S3. Surface roughness for cover glass after surface treatment.

	$10 \times 10\ \mu m^2$		$2.5 \times 2.5\ \mu m^2$	
Sample	**Rq (nm)**	**Ra (nm)**	**Rq (nm)**	**Ra (nm)**
As received	5.2 ± 0.3	4.1 ± 0.2	9.6 ± 0.2	7.7 ± 0.2
RF plasma	0.32 ± 0.02	0.26 ± 0.01	0.168 ± 0.006	0.126 ± 0.004
H_2O_2/HCl	0.30 ± 0.03	0.24 ± 0.02	0.179 ± 0.030	0.139 ± 0.020

Root mean square roughness, Rq, and roughness average, Ra, measured using AFM analysis software (MFP3D, version 04_08, Asylum Research Inc.). 90% confidence intervals calculated using student t-scores for n=4 individual measurements.

Table S4. Vesicle size distributions by dynamic light scattering.

Sample	DOPC (mol%)	$EO_{22}Bd_{37}$ (mol%)	DLS Size (nm)[a]
0%	100	0	194 ± 6
25%	75	25	184 ± 6
50%	50	50	188 ± 4
75%	75	25	182 ± 5
100%	0	100	202 ± 8
Average	-	-	190 ± 8[b]

[a] Z-average diameter from cumulants analysis ± standard deviation from at least three measurements. [b] Z-average diameter of vesicles comprised of 0, 25, 50, 75, 100 mol% polymer.

References.

1. (a) Stuart, M. A. C.; Tamai, H. *Langmuir* **1988,** *4,* 1184-1188. (b) Rubio, J.; Kitchener, J. A. *Journal of Colloid and Interface Science* **1976,** *57,* 132-142. (c) Joppien, G. R. *The Journal of Physical Chemistry* **1978,** *82,* 2210-2215. (d) Malmsten, M.; Linse, P.; Cosgrove, T. *Macromolecules* **1992,** *25,* 2474-2481. (e) Killmann, E.; Maier, H.; Baker, J. A. *Colloids and Surfaces* **1988,** *31,* 51-71. (f) Cosgrove, T. *J. Chem. Soc.-Faraday Trans.* **1990,** *86,* 1323-1332. (g) Vanderbeek, G. P.; Stuart, M. A. C.; Cosgrove, T. *Langmuir* **1991,** *7,* 327-334. (h) Killmann, E.; Maier, H.; Kaniut, P.; Gutling, N. *Colloids and Surfaces* **1985,** *15,* 261-276. (i) Char, K.; Frank, C. W.; Gast, A. P. *Langmuir* **1990,** *6,* 767-770. (j) Char, K.; Gast, A. P.; Frank, C. W. *Langmuir* **1988,** *4,* 989-998.
2. X-ray photoelectron spectroscopy (XPS) was performed with a Kratos AXIS Supra. Base pressures were less than 5×10^{-9} torr. X-ray excitation utilized monochromatic Al Kα (1486.7 eV) operating at 75 W. Analysis locations were an elliptical area of 300 × 700 microns. Survey spectra were recorded at 160 eV pass energy, 500 meV step sizes, with 200 millisecond dwell times. Charge neutralization was performed using low energy electrons from a filament above the sample.
3. de Vos, W. M.; Cattoz, B.; Avery, M. P.; Cosgrove, T.; Prescott, S. W. *Langmuir* **2014,** *30,* 8425-8431.
4. Kumar, A.; Dahl, V.; Kleinen, J.; Gambaryan-Roisman, T.; Venzmer, J. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2017,** *521,* 302-311.