Formation and Characterization of Supported Lipid Bilayers Composed of Phosphatidylethanolamine and Phosphatidylglycerol by Vesicle Fusion, a Simple but Relevant Model for Bacterial Membranes

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Supporting Information

Supporting Information Table S1

	SLD (10^{-6} Å^{-2})		*Volume (Å^3)*
Lipid component	H_2O	D_2O	L_α
PG head	2.5[a]	3.2[a]	288[b]
PE head	2.5[a]	3.5[a]	269[c]
PO tail	-0.3[a]	-0.3[a]	934[d]
25% PG head	2.5[e]	3.4[e]	274[e]
10% PG head	2.5[e]	3.5[e]	271[e]

a: calculated from the molecular component volumes and nuclear scattering lengths.

b: from ref [1].

c: from ref [2].

d: derived from ref [3].

e: calculated from the nominal lipid composition using the component volumes and SLDs.

Supporting Information TableS2

		Thickness (Å)	*SLD × 10^{-6} ($Å^{-2}$)*	*Solvent penetration (vol%)*	*Roughness (Å)*	*MMA** ($Å^2$)*
	Si		2.07*			
	Sio_2	7.0 ± 0.5	3.41*	5 ± 3	4.0 ± 0.5	
	Solvent	4.0 ± 0.5	0*	100*	4.0 ± 0.5	
50 ℃	Inner head	5.5 ± 0.5	2.5/3.4*	17 ± 3	3.0 ± 0.2	60.0 ± 11
	Tail	31.0 ± 0.5	-0.3*	0 ± 1	4.0 ± 0.2	60.0 ± 4
	Outer head	5.5 ± 0.5	2.5/3.4*	17 ± 3	4.0 ± 0.2	60.0 ± 11
	Backing				3.0 ± 0.2	
37 ℃	Inner head	5.5 ± 0.5	2.5/3.4*	17 ± 3	3.0 ± 0.2	60.0 ± 11
	Tail	31.0 ± 0.5	-0.3*	0 ± 1	4.0 ± 0.2	60.0 ± 4
	Outer head	5.5 ± 0.5	2.5/3.4*	17 ± 3	4.0 ± 0.2	60.0 ± 11
	Backing				3.0 ± 0.2	
25 ℃	Inner head	5.5 ± 0.5	2.5/3.4*	16 ± 3	3.0 ± 0.2	59.0 ± 11
	Tail	32.0 ± 0.5	-0.3*	1 ± 1	5.0 ± 0.2	59.0 ± 4
	Outer head	5.5 ± 0.5	2.5/3.4*	16 ± 3	5.0 ± 0.2	59.0 ± 11
	Backing				3.0 ± 0.2	

* Values kept constant during the fitting process.

**Using the molecular volumes for the tails (934 $Å^3$[3]) and head for POPE (269 $Å^3$[2]) and POPG (288 $Å^3$[1]) .The mean molecular area (*MMA*) was calculated according to equation 1.

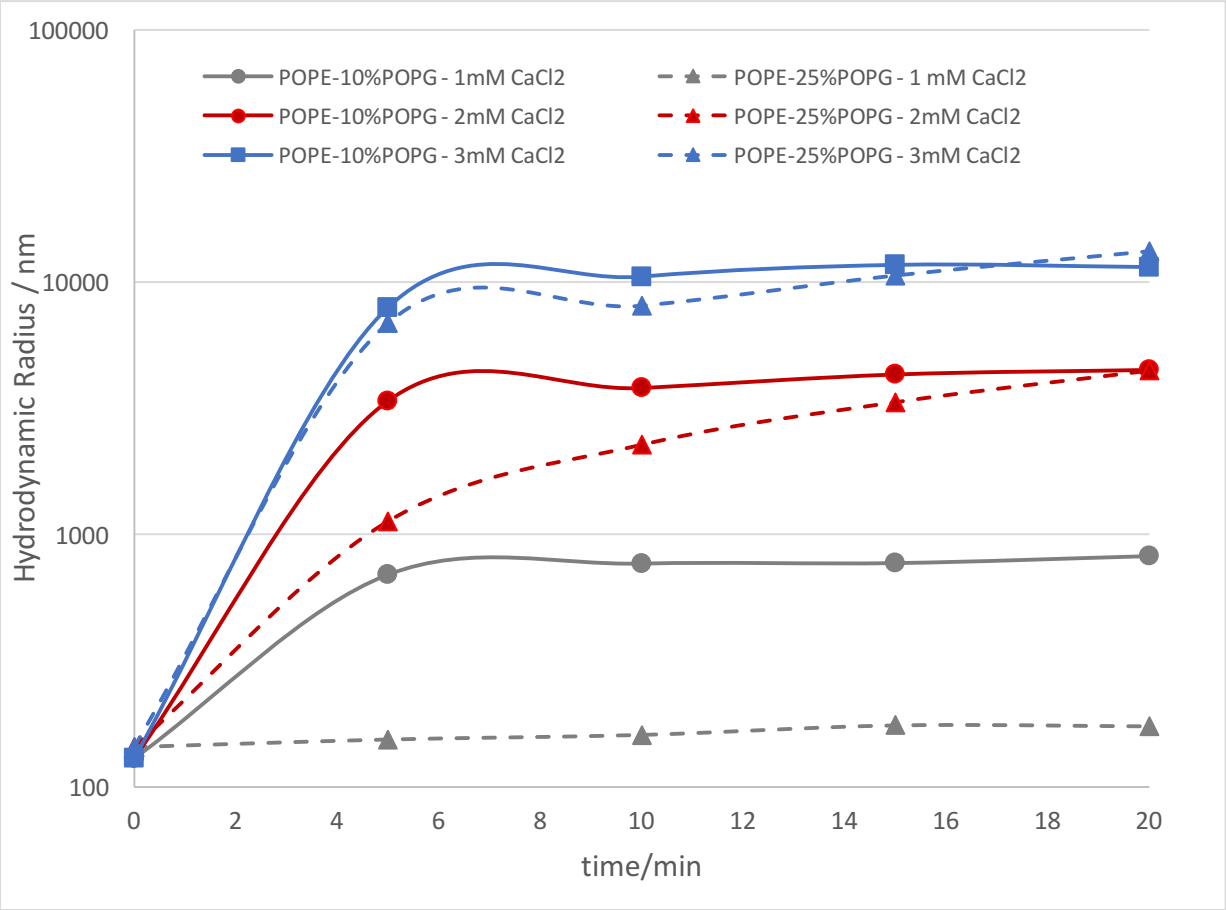

Figure S1. DLS study: 2 different vesicle preparations were made for each composition yielding hydrodynamic radii of 160 +/- 40 nm or 155 +/- 2 nm for POPE:POPG with 10 and 25 mol% POPG respectively. The hydrodynamic radius was followed as a function of time as soon as the vesicles were mixed with $CaCl_2$. Aggregation took place as soon as the vesicles were put in presence of calcium, and stable aggregates were formed for 10 mol% PG while no stable radii was obtained during the time of the measurements. Independent duplicates showed the same trend.

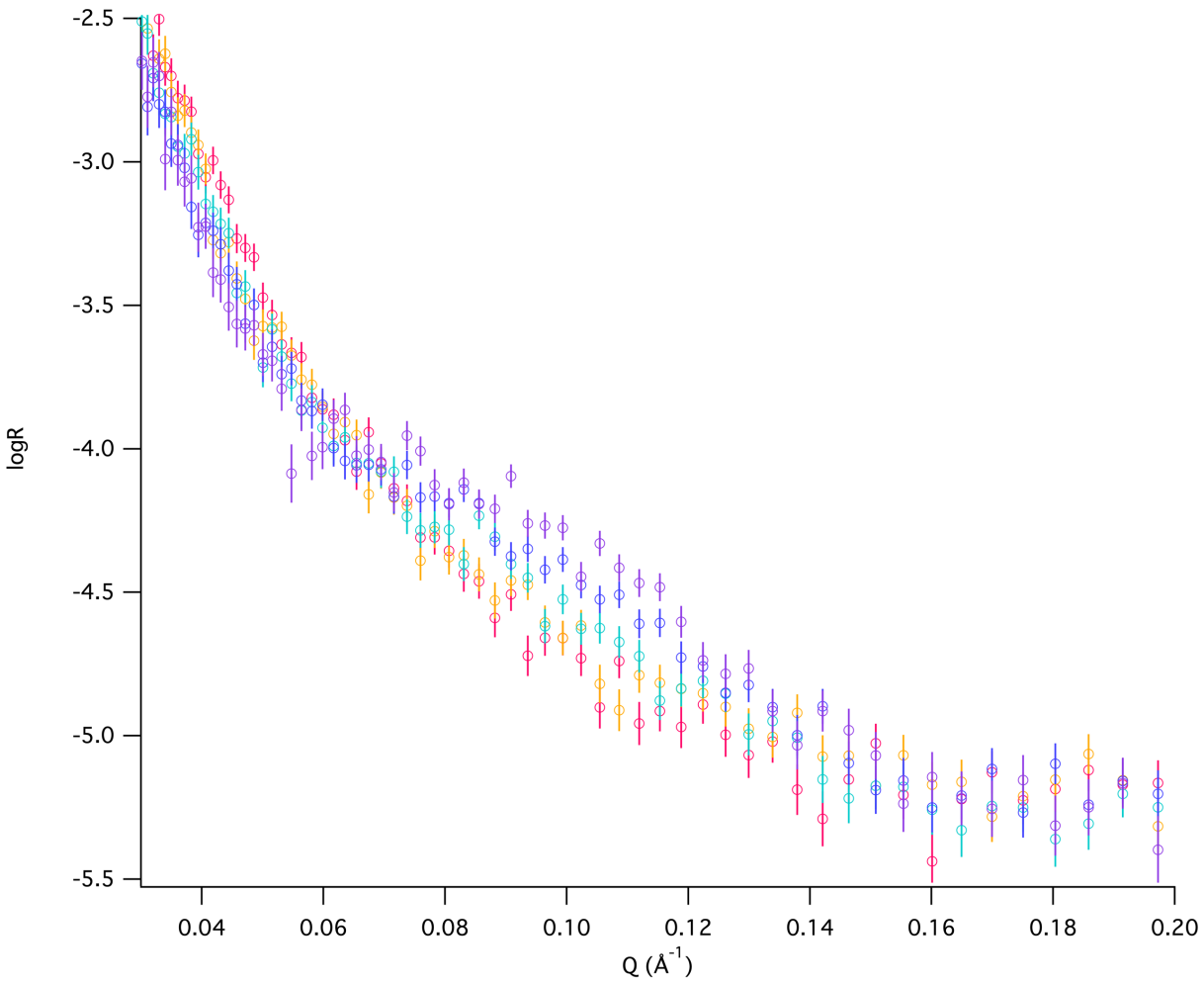

Figure S2. NR data for the kinetics of deposition of 25 mol% PG with 2 mM $CaCl_2$ at 37 °C. The low coverage and bad statistics prevented any meaningful fitting of the data.

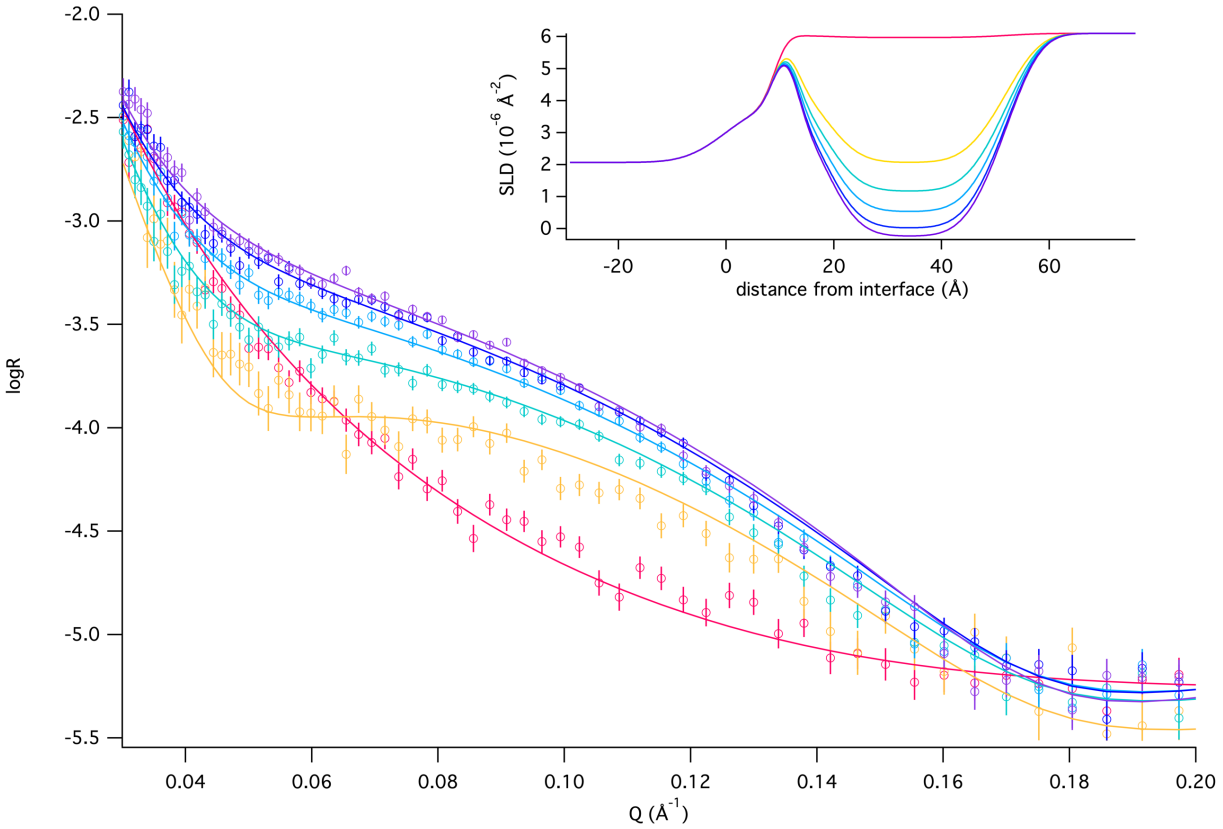

Figure S3. NR data and best fits for the kinetics of deposition of 25 mol% PG with 2 mM CaCl2 at 50 °C. The inset shows the corresponding SLD profiles for the best fits. The fitted coverage is shown as the blue curve in Figure 3B in the main text.

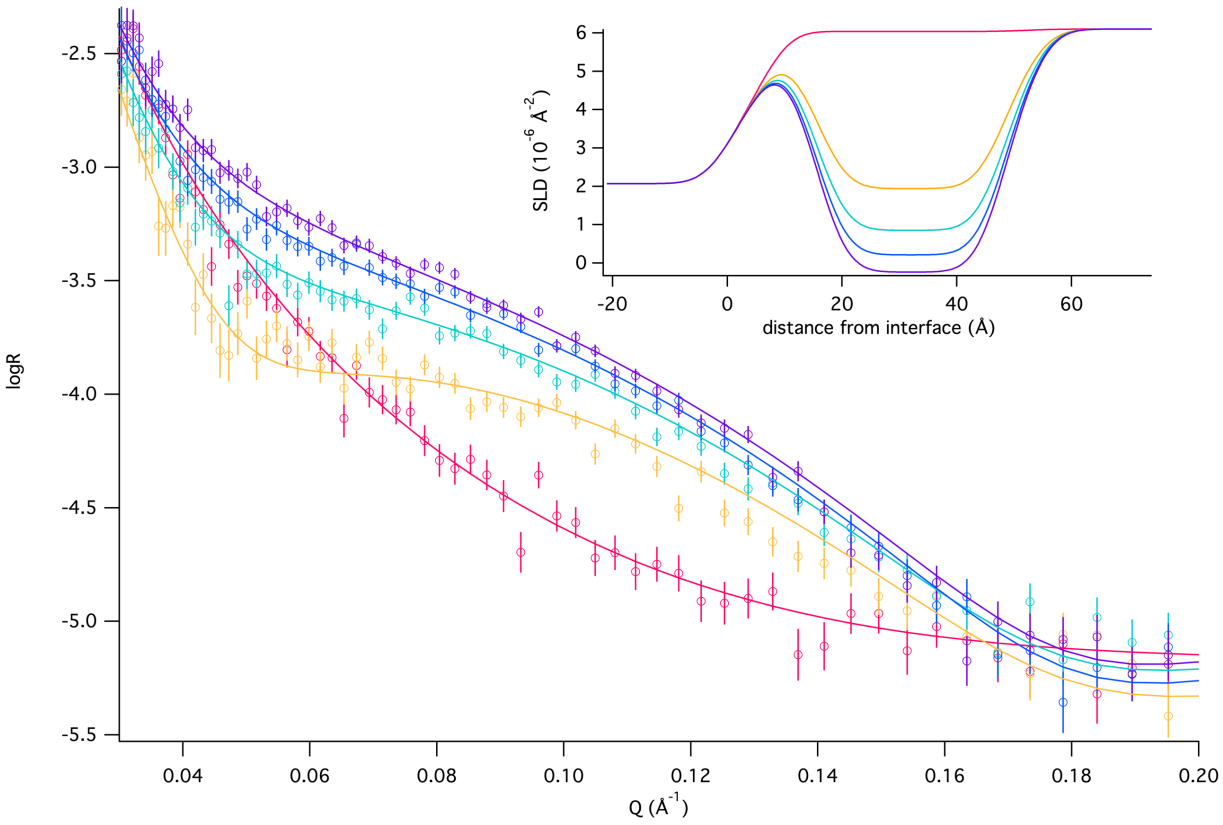

Figure S4: NR data and best fits for the kinetics of deposition of 25 mol% PG with 3 mM CaCl2 at 50 °C and inset showing the corresponding SLD profiles for the best fits. This is a duplicate experiment of the experiment shown in Figure 3A in the main text. The fitted coverage is shown as the green curve in Figure 3B in the main text.

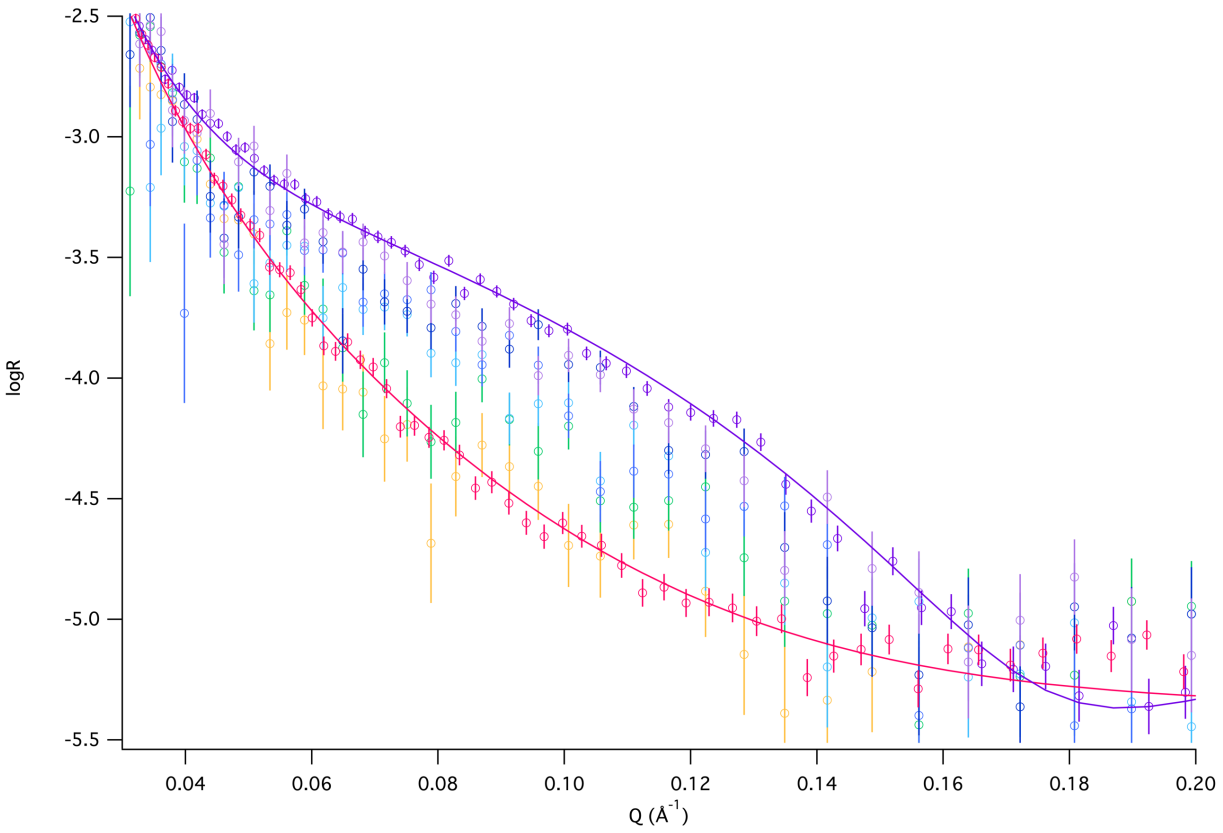

Figure S5. NR data and best fits for the kinetics of deposition of 10 mol% PG with 3 mM CaCl2 at 50 °C. The kinetic NR data show that full coverage (95 ± 1 vol%) is reached within the first 10 min of incubation (purple curve). Due to fast bilayer formation and bad statistics the kinetic data cannot be fitted. The fitted coverage is shown as the purple curve in Figure 3B in the main text.

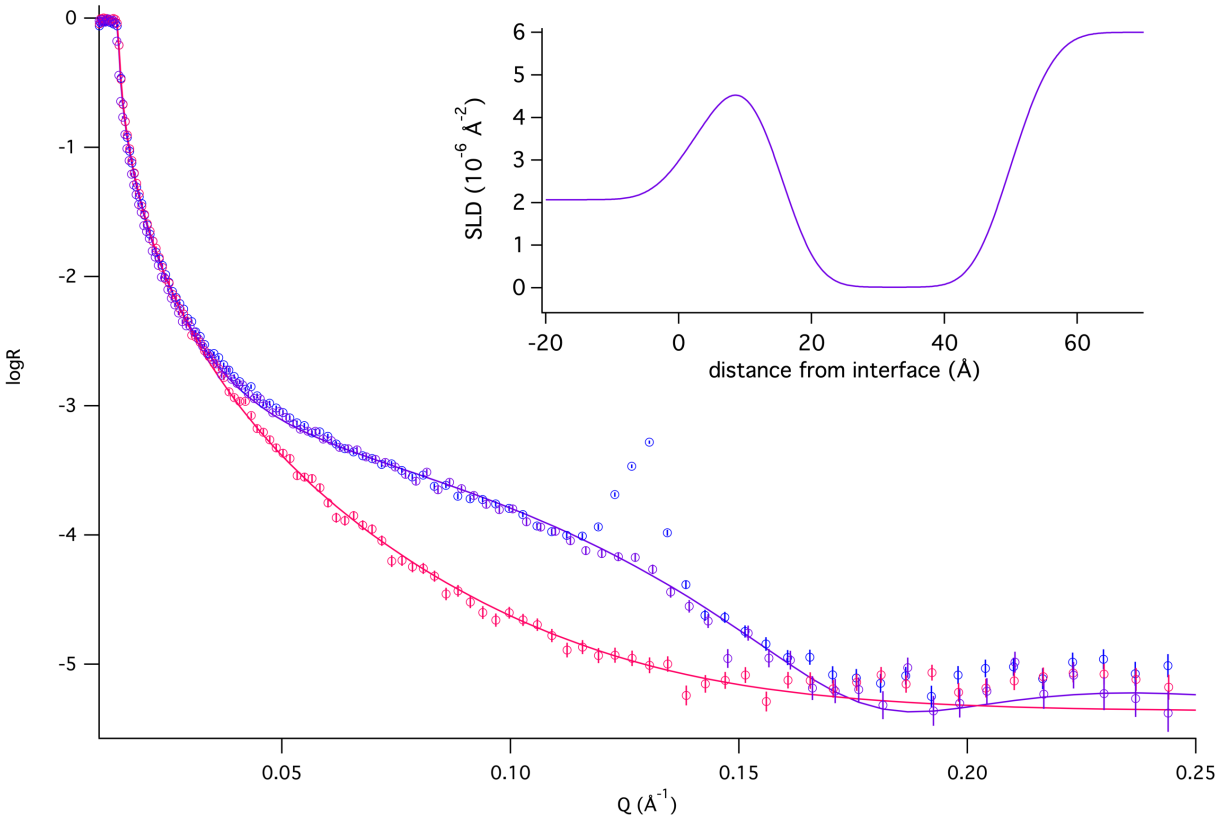

Figure S6, NR data and best fits for 10 mol% PG formed at 50 °C in 3 mM $CaCl_2$. Due to prolonged exposure to lipid vesicles, a Bragg peak appeared after rinsing with D_2O. When opening the solid-liquid fluid cell we noticed that there was impaired flow in the cell which led to incomplete rinsing and excessive vesicle incubation. The inset shows the SLD profile for the best fit shown in the figure. The lipid core is 32 ± 1 Å thick while the heads are 5.5 ± 1 Å.

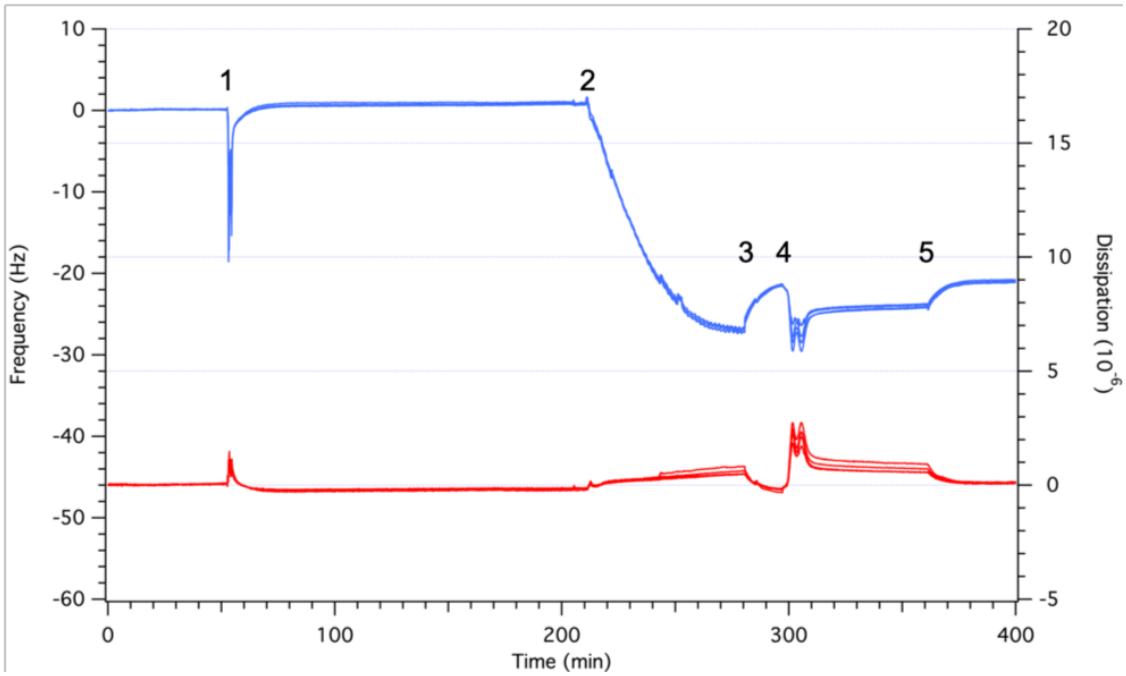

Figure S7. QCM-D signals for formation of lipid bilayers with 10 mol% PG in D_2O. Events marked with consecutive numbers mark 1: 3 mM $CaCl_2$. 2: lipids at 0.1 mg/mL. 3: pump stopped. 4: D_2O rinse for 30 min (peak from removal of $CaCl_2$). 5: pump stopped. While the pump is on (at high flow rate; 1 mL/min), it creates a pressure which appears like added mass. When the pump is off the bilayer frequency stabilizes at -25 Hz.

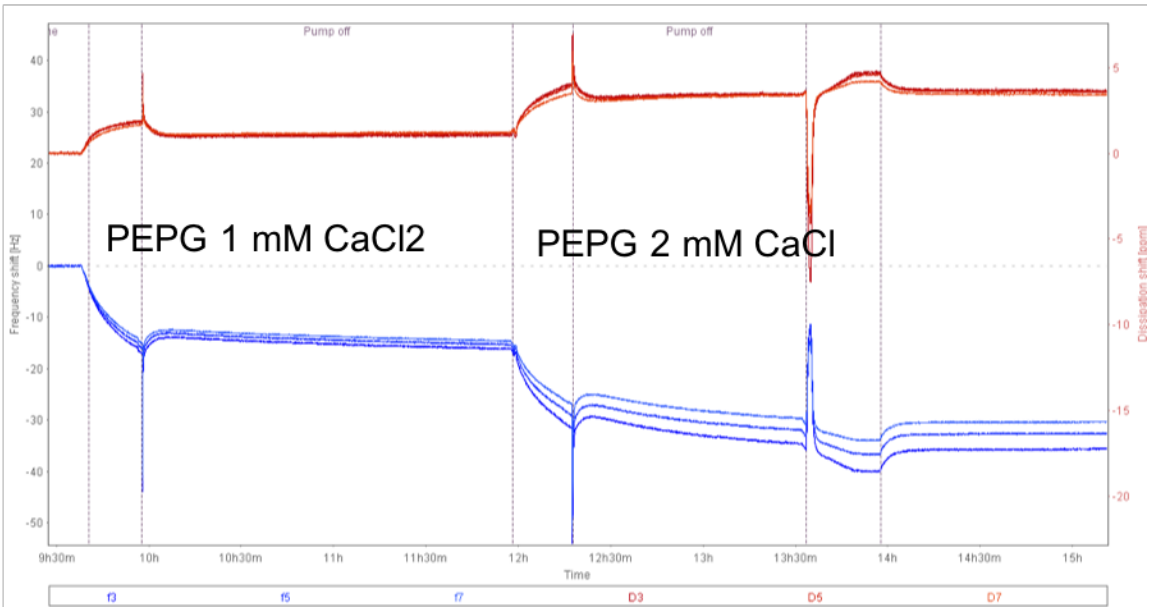

Figure S8: QCM-D data collected for 25 mol% PG at 37 °C and 1 mM $CaCl_2$ which gave insignificant lipid adsorption on the Si crystal in the NR setup (red curve in Figure 2 in the main text), however some adsorption occurred on the QCM-D sensor ($\Delta f = \sim$ -15 Hz). This experiment clearly shows that a discontinued flow leads to a plateau in the QCM-D signals and no further lipid adsorption. Subsequently, adding a lipid solution prepared in 2 mM $CaCl_2$ led to typical QCM-D signals that are considered as full bilayer coverage in the QCM-D but insufficient coverage on the NR crystal.

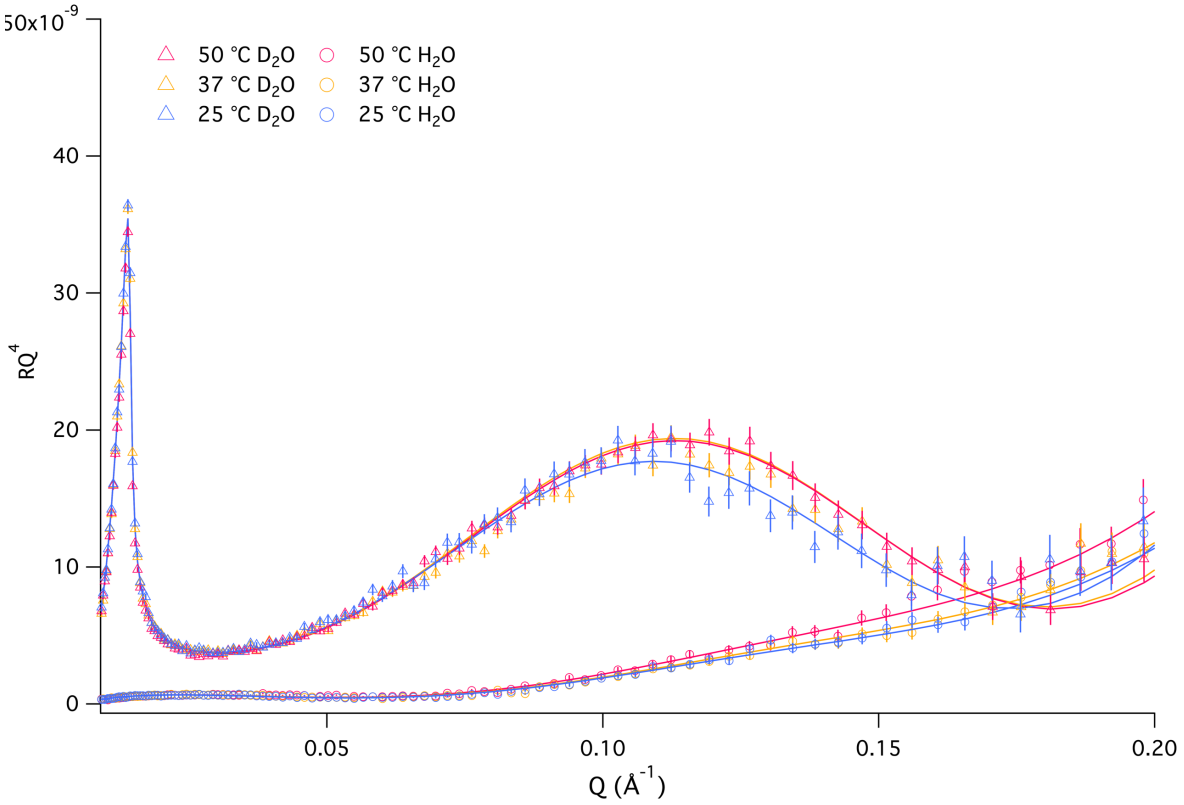

Figure S9: Same data as in Figure 4 but plotted as RQ^4 *vs* Q to highlight the quality of the fits.

References

1. Kucerka, N., et al., *Scattering Density Profile Model of POPG Bilayers As Determined by Molecular Dynamics Simulations and Small-Angle Neutron and X-ray Scattering Experiments*. Journal of Physical Chemistry B, 2012. **116**(1): p. 232-239.
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