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Supporting Material

Lipid polymorphism induced by surfactant peptide SP-B1-25

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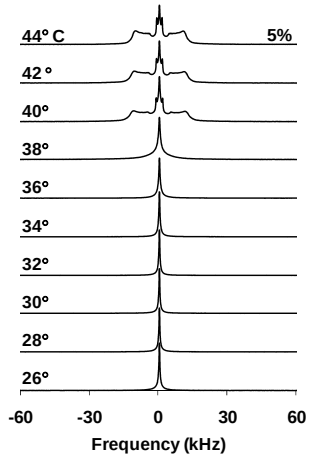
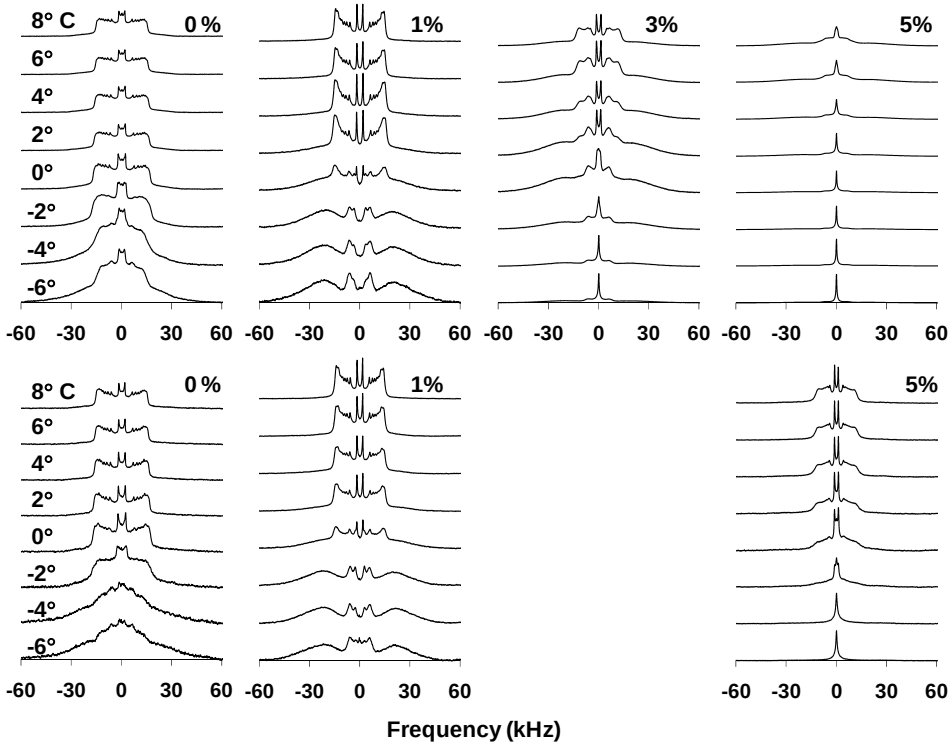
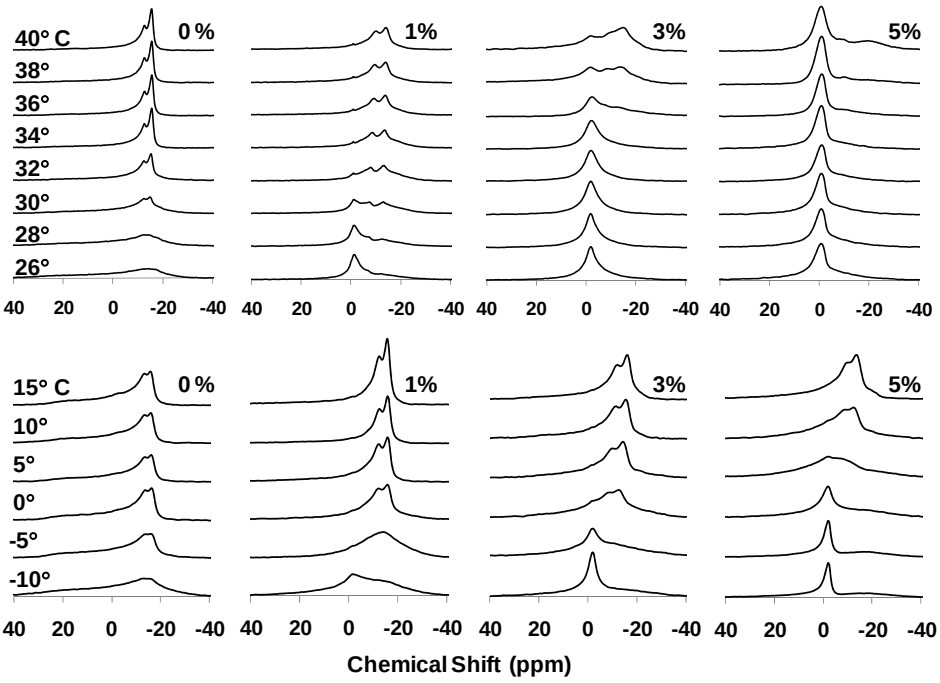


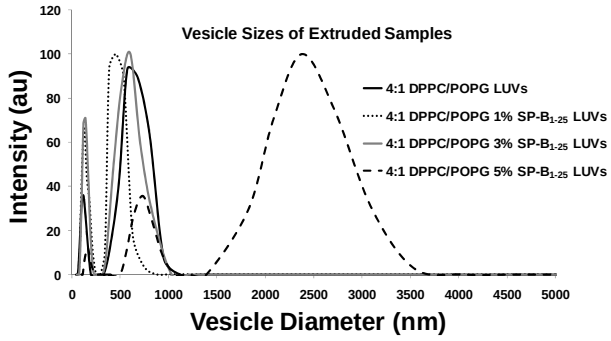
Supplementary Figure 1. Deuterium NMR spectra as a function of temperature for 4:1 DPPC-d_{62}/POPG MLVs with 5% SP-$B_{1\text{-}25}$. An isotropic peak persists until ~ 44°C.

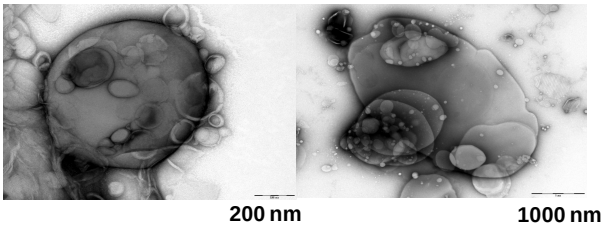


Supplementary Figure 2. Deuterium NMR spectra as a function of temperature for (top) 3:1 POPC-d_{31}/POPG MLVs and (bottom) 3:1 POPC/POPG-d_{31} MLVs with SP-$B_{1\text{-}25}$ added at the indicated molar percentages. The temperatures were taken from -6 °C to 8 °C to allow us to monitor transitions around the melting temperatures of POPC and POPG.

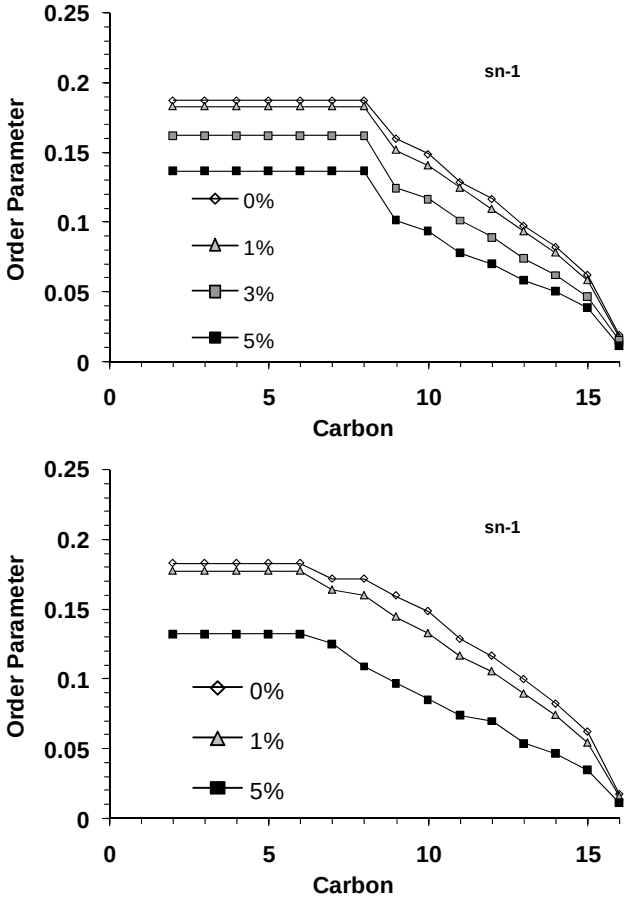


Supplementary Figure 3. Phosphorus NMR spectra as a function of temperature for (top) 4:1 DPPC-d_{62}/POPG MLVs and (bottom) 3:1 POPC-d_{31}/POPG MLVs.





Supplementary Figure 4. (Top) DLS of 4:1 DPPC/POPG LUVs with 0-5% SP-B_{1-25}. (Bottom) EM micrographs of (left) 4:1 DPPC/POPG MLVs and (right) 4:1 DPPC/POPG MLVs containing 5 mol% SP-B_{1-25}.



Supplementary Figure 5. Order parameter profiles for the *sn*-1chain of (top) POPC-d_{31} in 3:1 POPC-d_{31}/POPG and (bottom) POPG-d_{31} in 3:1 POPC/POPG-d_{31} MLVs at 44 ºC with SP-$B_{1\text{-}25}$ at the indicated molar percentages.

DETAILED MATERIALS AND METHODS

Synthesis of SP-$B_{1\text{-}25}$: SP-$B_{1\text{-}25}$, (FPIPLPYCWLCRALIKRIQAMIPKG) was synthesized via automated solid-phase peptide synthesis on a Wang resin (ABI 430, ICBR, UF), cleaved with King's reagent and ether precipitated. Crude product was purified by RP-HPLC using a C18 Vydac column (Grace, Deerfield, IL) with a water/acetonitrile gradient (containing 0.3% TFA). Fractions corresponding to SP-$B_{1\text{-}25}$ were collected and purity of the product was verified by mass spectrometry with a mass to charge ratio (m/z) of 2928. Dried peptide was dissolved in methanol to a stock concentration of approximately 1 mM, and aliquots were analyzed by amino acid analysis for a more accurate determination of concentration (Molecular Structure Facility, UC Davis).

Preparation of Peptide:Lipid Samples: POPC, DPPC, POPG, POPC-d_{31}, DPPC-d_{62} and POPG-d_{31} were purchased as chloroform solutions (Avanti Polar Lipids, Alabaster, AL) and concentrations were verified by phosphate analysis (*1*) (Bioassay Systems, Hayward, CA). Appropriate volumes of lipid chloroform solutions were mixed to give final lipid molar ratios of 4:1 DPPC/POPG and 3:1 POPC/POPG. For peptide containing samples, SP-$B_{1\text{-}25}$ in methanol was added to the lipid chloroform solutions resulting in P/L ratios

ranging from <1:1000 to 1:20. Samples were dried under a stream of nitrogen while in a water bath at >45°C; the resulting films were suspended in warm cyclohexane (>45 °C), flash frozen in nitrogen, and lyophilized overnight to remove residual solvent.

CD experiments: CD spectra were acquired at 45 °C on an Aviv Model 215 (Lakewood, NJ) using a 195-260 nm wavelength range, a 1 nm step size and averaging of 50 scans. Samples for CD analysis were prepared by hydrating, at 50 °C, a portion of the lyophilized peptide-lipid powders in 1 mL 10 mM HEPES buffer, pH 7.4, with 140 mM NaCl and 1 mM EDTA, to achieve a final concentration of 36 μM SP-$B_{1\text{-}25}$. Hydrated samples were then passed through 5 freeze thaw cycles with vortex mixing to achieve homogeneity. Immediately prior to CD analysis, the hydrated multilamellar vesicle (MLV) samples were extruded through 100 nm filters (Avanti Polar Lipids, Alabaster, AL) 15-25 times to form LUVs. Extrusion was performed at above the T_m of all the lipid mixtures (>45 °C). Background contributions from the buffer and LUVs were removed by subtracting appropriate controls. Control spectra of 40 μM SP-$B_{1\text{-}25}$ in methanol were also collected.

DSC analysis: Thermograms were collected on a VP-DSC microcalorimeter (Microcal Inc, LLC Northampton, MA). Experiments were conducted over a range of 10-70° C at a scan rate of 1° C/min and run in triplicate. Samples for DSC were prepared by solubilizing 4 mg of peptide-lipid powder in 2 mL 10 mM HEPES buffer, pH 7.4, with 140 mM NaCl, 1 mM EDTA to achieve a 2.5 mM lipid concentration. Hydration proceeded as described above. Prior to DSC measurement, LUV samples were prepared as described above and degassed.

Solid state NMR analysis: ^{31}P and ^{2}H NMR data were collected on a 500 MHz Bruker DRX system (Billerica, MA) using a standard 5 mm BBO probe. For the ^{31}P NMR experiments, data were collected using a Bloch decay (to minimize T_2 effects due to lipid dynamics); 25 kHz proton decoupling was employed during acquisition to remove dipolar couplings. ^{31}P Spectra were acquired with 512-1024 scans and a 5 second recycle delay between scans to minimize RF sample heating. The ^{31}P B_1 field was 31.25 kHz (8 μs 90°pulse). For the ^{2}H NMR experiments, data were collected using a quad echo sequence (90°-τ-90°-τ-acq with τ = 30 μs) with a B_1 field of 40 kHz (6.25 μs 90°pulse). ^{2}H spectra were acquired with 1024 or 2048 scans and a 0.5 second recycle delay between scans. For each solid-state NMR sample, ~20 mg of peptide-lipid powder was placed in a 5 mm diameter NMR tube and 200 μL of buffer containing 10mM HEPES, pH 7.4, 140mM NaCl, and 1mM EDTA in ^{2}H depleted water (Cambridge Isotopes, Andover MA) was added. Samples with final lipid ratios of 4:1 DPPC-d_{62}/POPG, 4:1 DPPC/POPG-d_{31}, 3:1 POPC-d_{31}/POPG, and 3:1 POPC/POPG-d_{31} were prepared. The hydrated dispersions (in the NMR tubes) were subjected to 5 freeze-thaw cycles with gentle vortexing to form MLVs.

DePaking of NMR data was accomplished with previously published algorithms which simultaneously dePake and determine macroscopic ordering in partially aligned lipid spectra using Tikonov regularization (*2, 3*). ^{31}P NMR spectra were referenced to phosphate buffer prior to dePaking and dePaked spectra were quantitated by fitting the

two peaks with Lorentzian line shapes**.** Assignments of ^{2}H resonances were made based on Petrache, *et al.* (*4*).

Lipid acyl chain order parameters (*5*) were determined by measuring the quadrupolar splitting ($\Delta\nu_Q$) for deuterium atoms at various positions along the acyl chain and determining their order parameters, S_{CD} (equation 1).

$$\Delta\upsilon_{Q} = \frac{3}{4}\frac{e^2qQ}{h}(3\cos^2\theta - 1)S_{CD} \qquad (1)$$

For saturated C-D bonds, the quadrupolar coupling, $\frac{3}{4}\frac{e^2qQ}{h}$, is 167 kHz in the static limit (*6*). If the bilayers adopt random orientations with respect to the magnetic field, the resulting spectra of perdeuterated lipid acyl chains are a superposition of axially symmetric powder patterns, arising from each deuterated position, whose intensities follow the well-established distribution function $p(\theta) \propto \sin(\theta)$ where θ is the angle between the bilayer normal and the magnetic field. The spectra can be deconvoluted using a standard inversion (dePaking) procedure (*2*). For samples in which the lipid bilayers align to some degree in the magnetic field, assuming the magnetic field leads to an ellipsoidal deformation of the MLVs, the probability distribution function becomes (*3*):

$$p_{(E)}(\theta) \propto \sin(\theta)[1-(1-\kappa_E)\cos^2\theta]^{-2} \qquad (2)$$

where κ_E is square of the ratio of the long to short axes of the ellipsoids. Deconvolution is accomplished using an iterative procedure which simultaneously determines κ_E and dePakes the spectrum. Since our lipid samples showed some degree of alignment in the magnetic field, the latter procedure was utilized. Both ^{31}P and ^{2}H experiments exhibited the same degree of distortion as evidenced by comparable kappa values, further supporting our interpretation that distortion of the lineshapes can be attributed to alignment of the lipids rather than experimental differences (i.e. Bloch decay vs. echo experiments).

Dynamic Light Scattering: Dynamic light scattering measurements were performed using a Brookhaven 90Plus/BI-MAS ZetaPALS spectrometer with BI-9000AT Digital Autocorrelator and 9KDLSW data acquisition software. The instrument was operated at a wavelength of 659 nm over a temperature range of 25-45 ºC. A 100 µL sample volume in a disposable cuvette was used for each measurement. Samples were prepared as above and contained a 1 mM suspension of 4:1 DPPC/POPG MLVs in 10 mM HEPES buffer, pH 7.4, with 140 mM NaCl, 1 mM EDTA.

TEM analysis: TEM images of 4:1 DPPC/POPG MLVs were captured using a Hitachi H-7000 transmission electron microscope operated at 75 kV with a Soft-Imaging System MegaViewIII and AnalySIS digital camera (Lakewood, Colorado). The microscope has a maximum resolution at 0.2 nm with a magnification range of 110 to 600,000X. Samples were prepared as above and contained a 1 mM suspension of 4:1 DPPC/POPG MLVs. Just prior to TEM measurements, sample grids were prepared by negative staining. For

each grid, a drop of the equilibrated sample was absorbed on a 400-mesh Formvar-coated copper grid for 2 min at room temperature. Excess sample was wicked away with filter paper and the grid was allowed to dry for 2 min. A drop of deionized water was added to the grid to wash away any excess salt from the buffer solution. One drop of 2% uranyl acetate was then added to the grid and allowed to stain for 2 min, after which any excess uranyl acetate was wicked away and the sample was allowed to dry for 2 min.

References

1. Chen, P. S., T. Y. Toribara and H. Warner. 1956. Microdetermination of phosphorus. *Anal Chem* 28:1756-1758.
2. Sternin, E., M. Bloom and A. L. Mackay. 1983. De-pake-ing of nmr-spectra. *J Magn Reson* 55:274-282.
3. Sternin, E., H. Schafer, I. V. PolozovK. Gawrisch. 2001. Simultaneous determination of orientational and order parameter distributions from nmr spectra of partially oriented model membranes. *J Magn Reson* 149:110-113.
4. Petrache, H. I., S. W. Dodd and M. F. Brown. 2000. Area per lipid and acyl length distributions in fluid phosphatidylcholines determined by (2)h nmr spectroscopy. *Biophys J* 79:3172-3192.
5. Seelig, J. and A. Seelig. 1980. Lipid conformation in model membranes and biological membranes. *Q Rev Biophys* 13:19-61.
6. Burnett, L. J. and B. H. Muller. 1971. Deuteron quadrupole coupling constants in 3 solid deuterated paraffin hydrocarbons-c2d6, c4d10, c6d14. *J Chem Phys* 55:5829-&.