Biophysical Journal, Volume *113*

Supplemental Information

Divide & Conquer: Surfactant Protein SP-C and Cholesterol Modulate Phase Segregation in Lung Surfactant

Nuria Roldan, Jesús Pérez-Gil, Michael R. Morrow, and Begoña García-Álvarez

Supplementary material

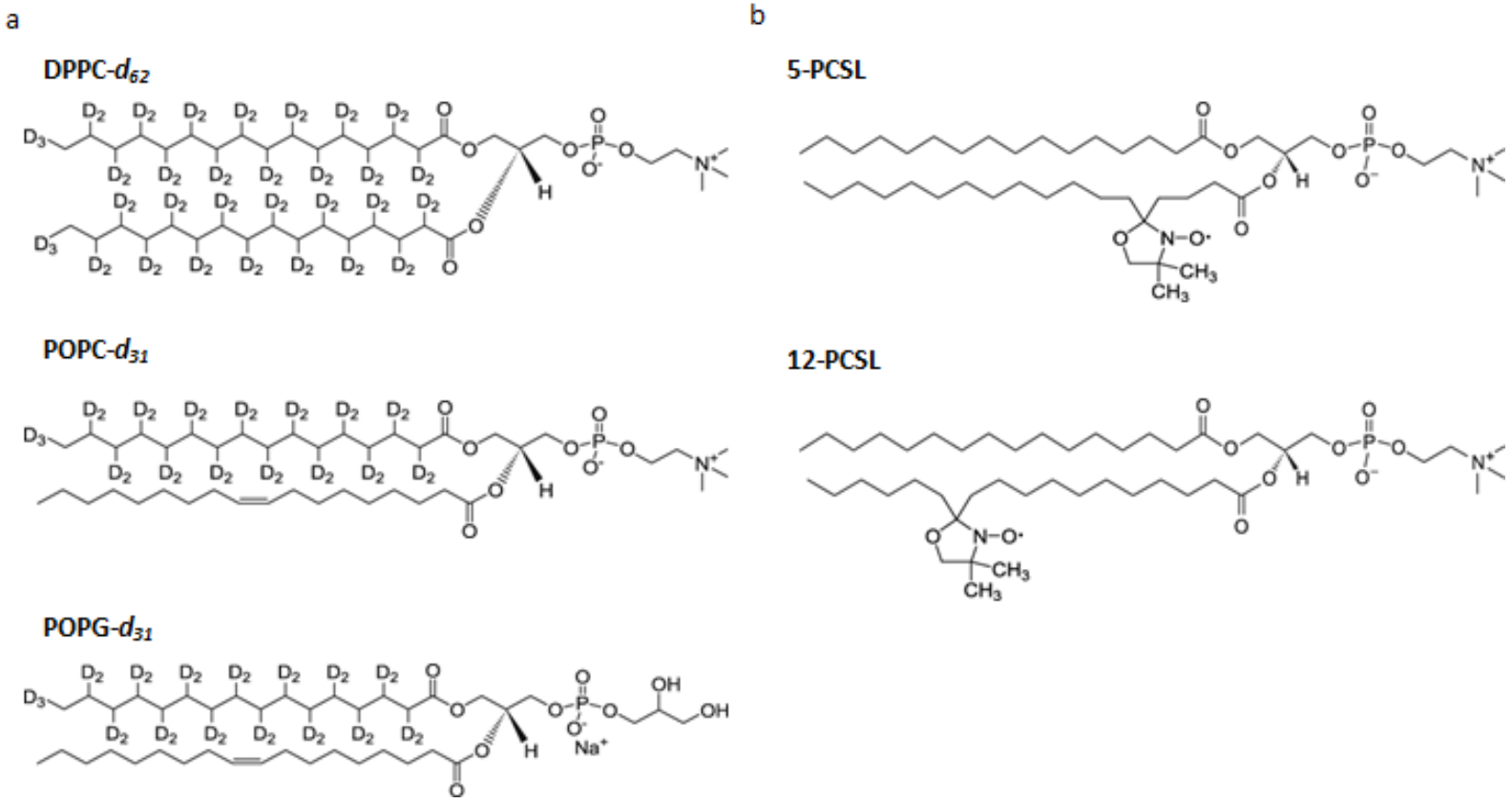
Divide and conquer: Phase segregation in Lung Surfactant is modulated by Surfactant Protein SP-C and Cholesterol

Nuria Roldan[1], Jesús Pérez-Gil[1], Michael R. Morrow[2], Begoña García-Álvarez[1]*

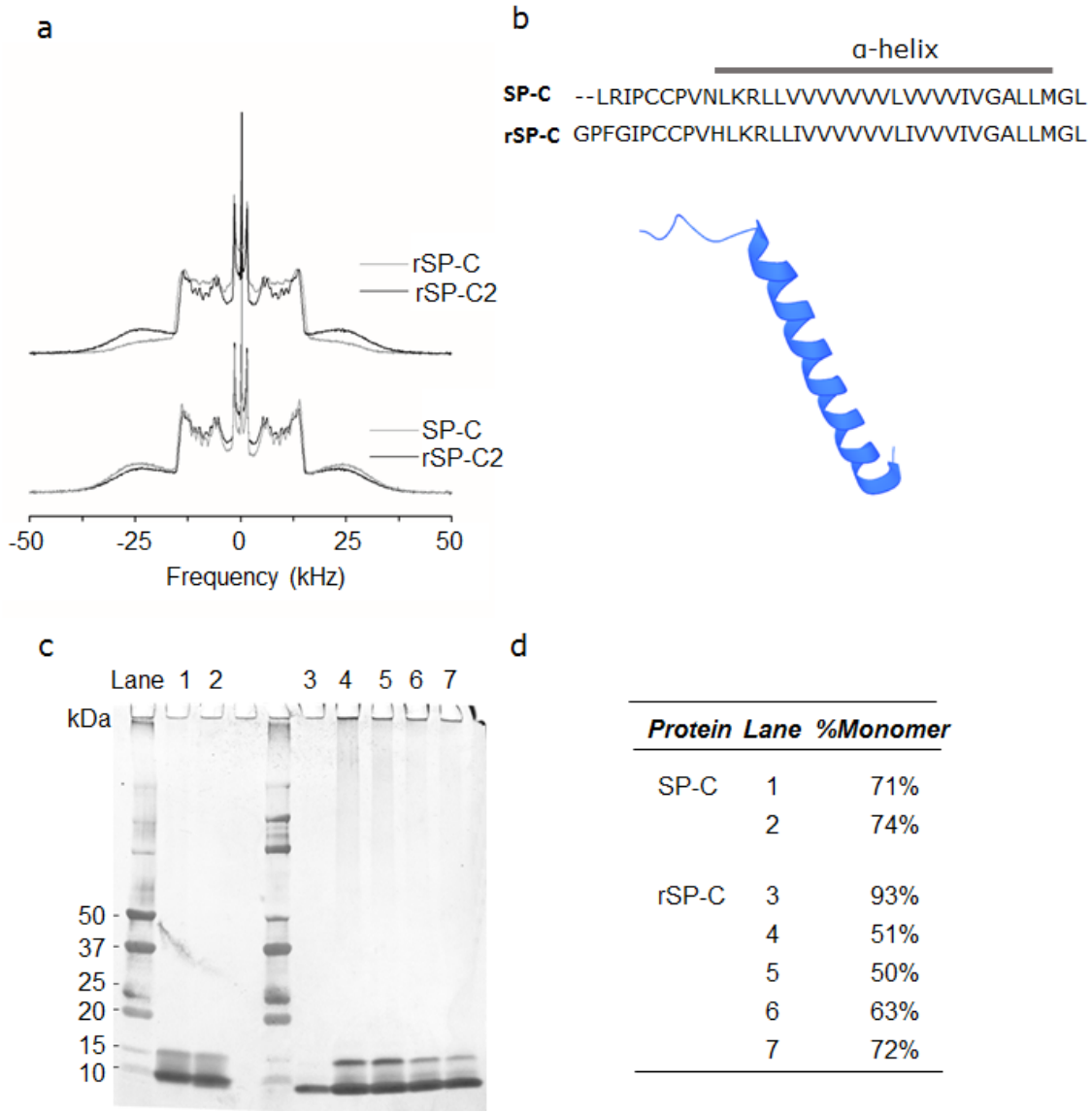
[1]Dept. of Biochemistry, Faculty of Biology, and Research Institute "Hospital 12 de Octubre", Complutense University, 28040 Madrid, Spain

[2]Dept. of Physics and Physical Oceanography, Memorial University of Newfoundland, St. John's, Newfoundland, Canada

*Corresponding Author

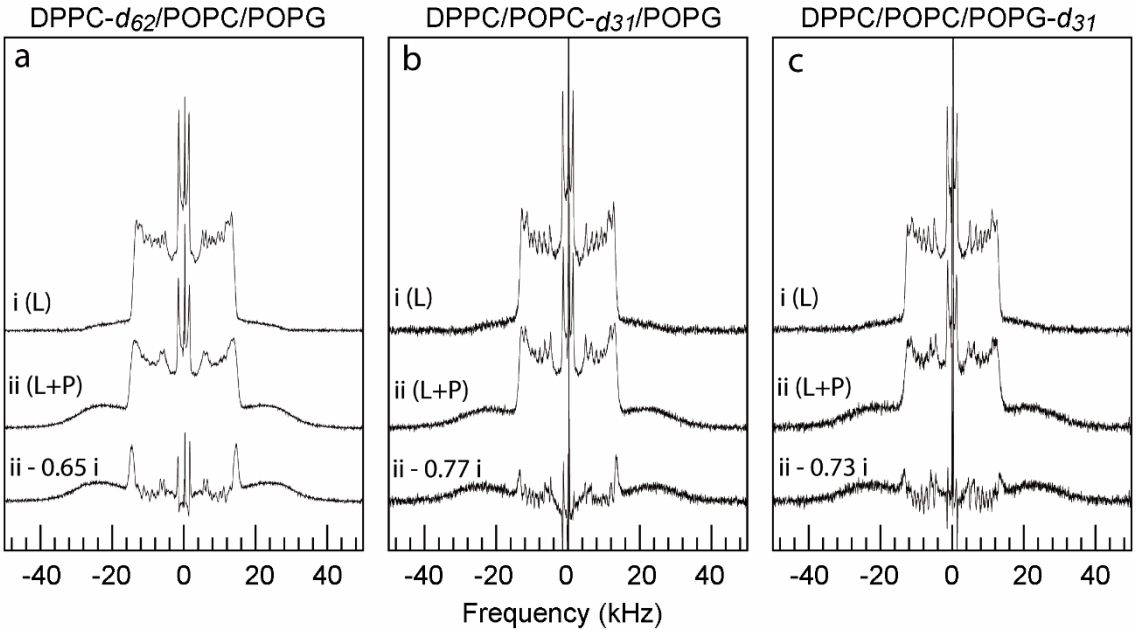


Supplementary Figure 1. a) Schematic representation of deuterium (D)-labelled lipids employed in this study. b) Scheme of the spin-labelled probes used for ESR experiments.

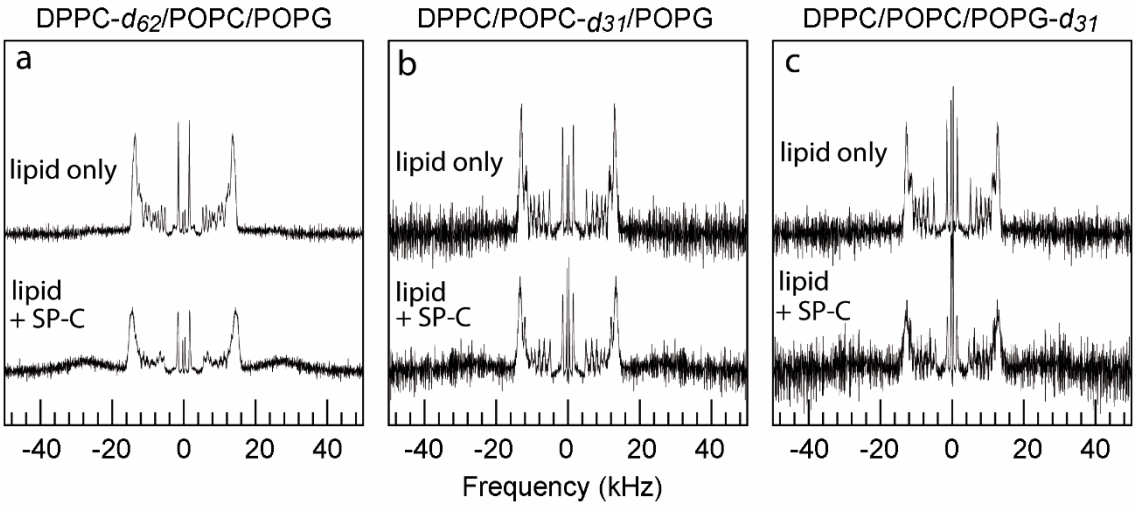


Protein	*Lane*	*%Monomer*
SP-C	1	71%
	2	74%
rSP-C	3	93%
	4	51%
	5	50%
	6	63%
	7	72%

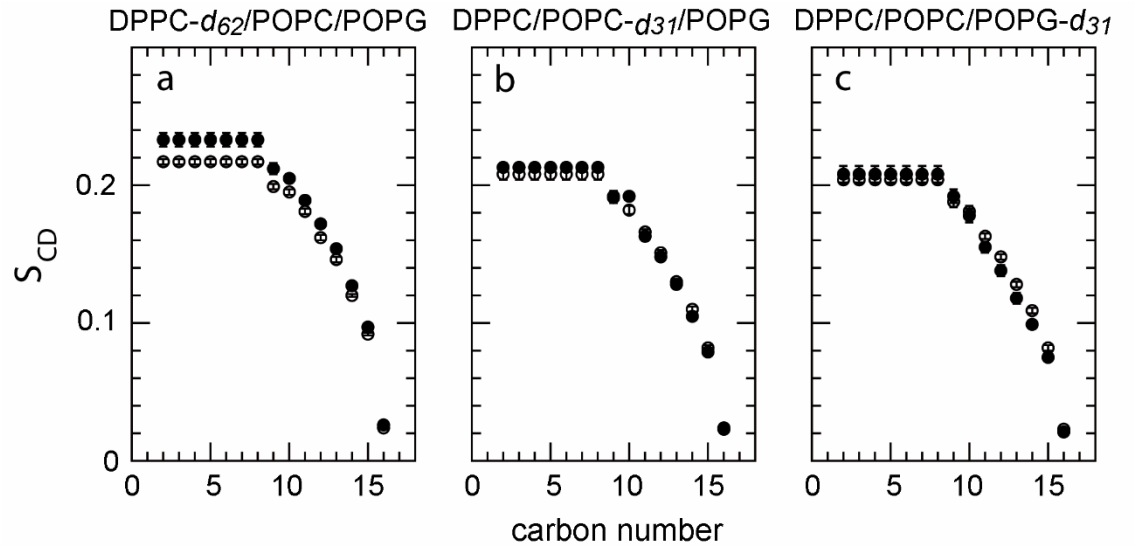
Supplementary Figure 2. a) Powder spectra of DPPC-d_{62}/POPC/POPG at 37°C incorporating different protein stocks. Top spectra represent the difference between two rSP-C stocks, in particular the one presented in Fig. 1 (rSP-C, grey line), and a different one (rSP-C2, black line). Bottom spectra illustrate the similarities between the spectra containing native SP-C presented in Fig. 1 (SP-C, grey line) and the second stock of rSP-C tested (rSP-C2, black line). b) Amino acid sequence of native SP-C and the recombinant human form rSP-C with the helical stretch highlighted. The 3D structure of native SP-C is shown below (PDB 1SPF). c) Silver stained SDS electrophoresis gradient gel (4%-20% polyacrylamide) showing the running pattern of different SP-C and rSP-C batches. Lanes 1 and 2 correspond to two different SP-C stocks and 3-7, to five rSP-C batches. d) %monomer calculated for each protein form by a densitometry analysis.



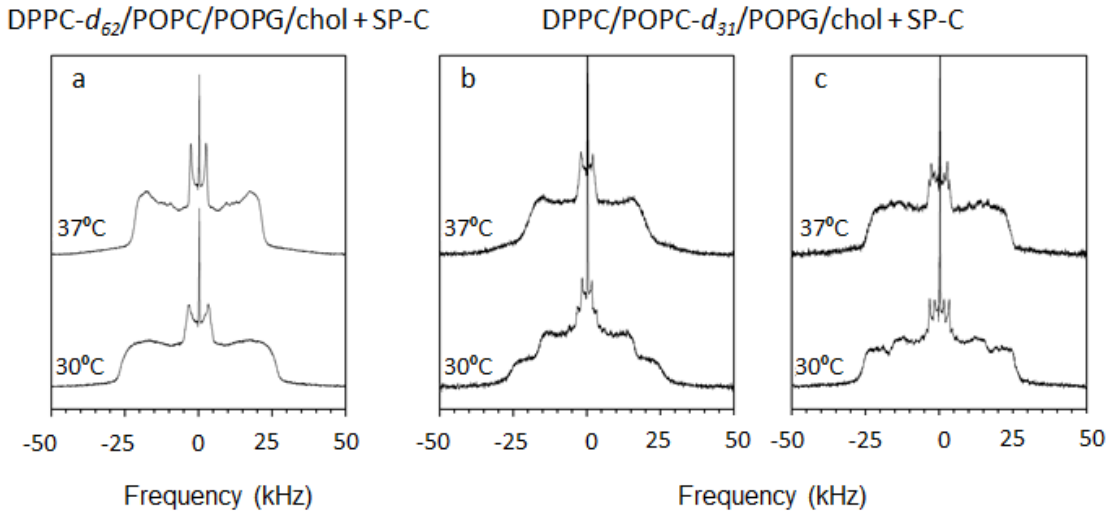
Supplementary Figure 3. Representation of the subtraction method employed to determine the amount of lipid forming part of the SP-C-induced ordered phase. The spectrum corresponding to lipid-only samples, i (L), was subtracted from that of protein-containing liposomes, ii (L+P) in a proportion (x) that eliminated the fluid region of the lipid-protein sample. Resulting spectra were obtained as: ii – x i. Some spectral components of the fluid phase remain in the subtracted spectrum since the fluid phase spectra with and without SP-C are not identical. Spectral subtractions are shown for the three lipid systems studied: DPPC-d_{62}/POPC/POPG (a), DPPC/POPC-d_{31}/POPG (b) and DPPC/POPC/POPG-d_{31} (c).



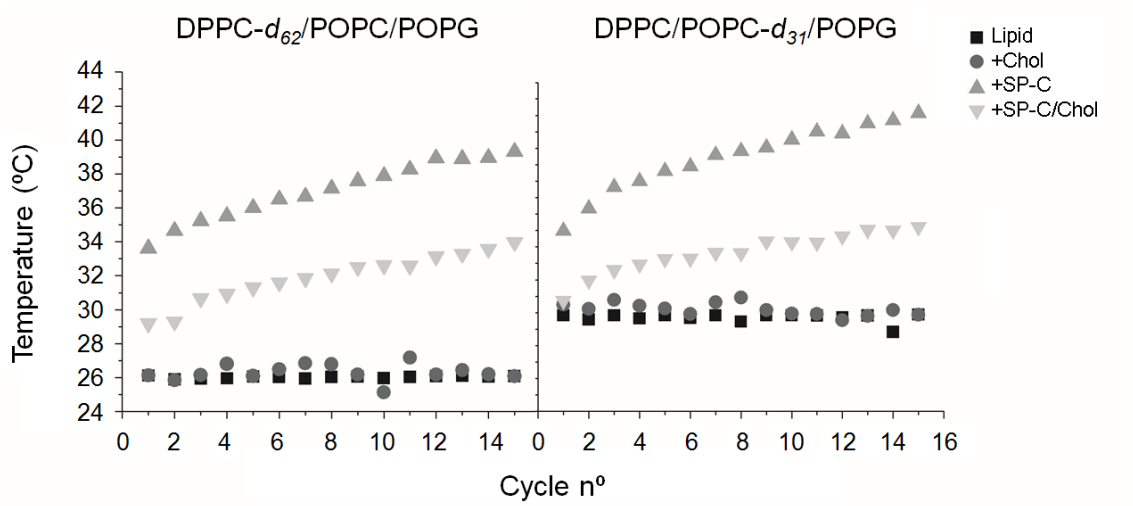
Supplementary Figure 4. DePaked spectra for the liquid crystalline phase of each spectrum in the presence and absence of SP-C and no cholesterol. Almost no differences in the splitting distributions are observable between the lipid only and SP-C-associated spectrum. DePaking is shown for the three lipid systems studied: DPPC-d_{62}/POPC/POPG (a), DPPC/POPC-d_{31}/POPG (b) and DPPC/POPC/POPG-d_{31} (c).



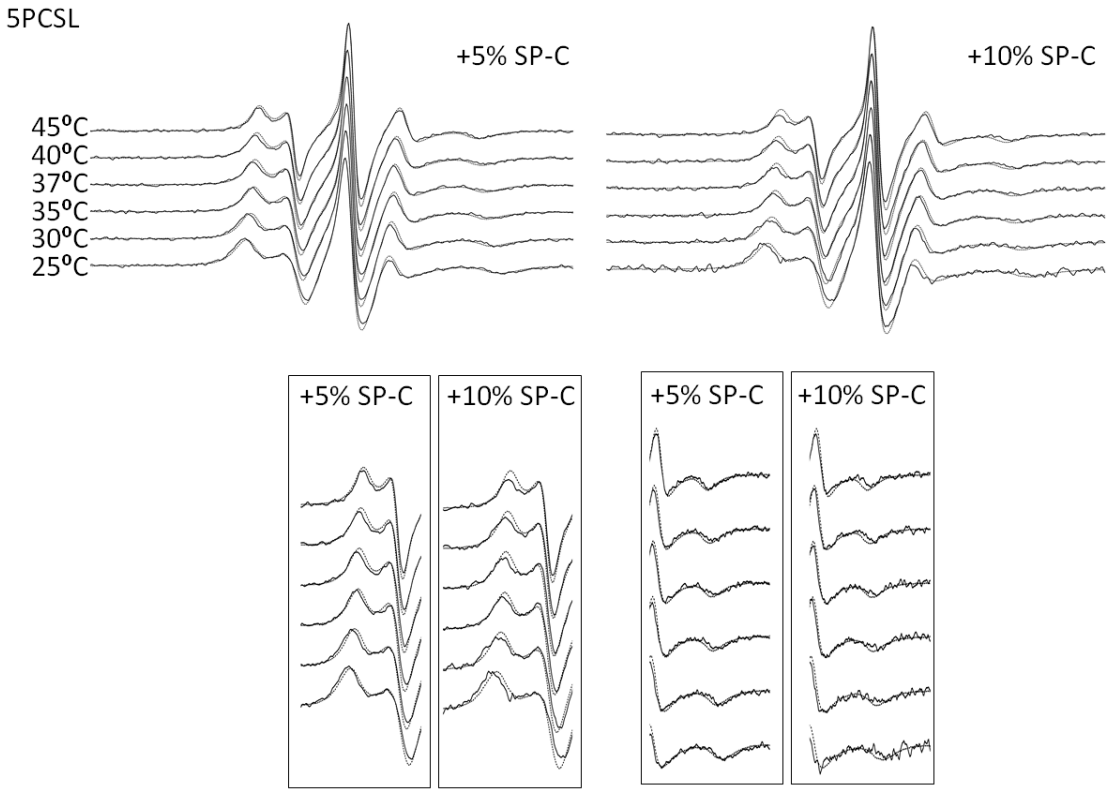
Supplementary Figure 5. Order parameter plots obtained from the dePaked spectra shown in Supplementary Figure 4. Filled symbols represent SP-C-containing liposomes and empty symbols correspond to lipid-only vesicles. The assignments for DPPC doublets were performed as described elsewhere (72). POPC and POPG doublets were assigned using the protocol in (73). The three lipid systems studied are represented: DPPC-d_{62}/POPC/POPG (a), DPPC/POPC-d_{31}/POPG (b) and DPPC/POPC/POPG-d_{31} (c).



Supplementary Figure 6. (a) Powder ^{2}H NMR spectra of DPPC-d_{62}/POPC/POPG incorporating 10 wt% cholesterol and 5 wt% (1 mol%) SP-C at 30^{o}C. The spectra show lipid reorientations too slow to be axially symmetric on the experiment timescale. No phase separation is observed. (b,c) ^{2}H NMR spectra of multilamellar vesicles made of DPPC/POPC-d_{31}/POPG + 10 wt% Cholesterol and 5 wt% (1 mol%) SP-C. Each panel represents an independent experiment performed with a newly prepared sample incorporating different stocks of SP-C. Spectra at 30^{o}C are characteristic of a two-component phase segregation that is not apparent in the corresponding spectra at 37^{o}C. Differences found between replicates are likely due to slight different cholesterol concentrations found between the lipid stocks used to prepare these samples.



Supplementary Figure 7. Melting temperature of DPPC-d_{62} and POPC-d_{31}-containing systems as determined from DSC thermograms. Calculated T_m is represented with respect to each heating cycle from 15 to 60°C. As observed, for the lipid alone systems, the melting temperature changed by 4°C depending on the incorporation of DPPC-d_{62} or POPC-d_{31}. No effect of cholesterol was observed. The incorporation of SP-C raised the melting temperature abruptly reducing the gap between the two lipid systems and increasing the differences between SP-C and SP-C/Chol-containing liposomes. Over the number of cycles tested, the observed transition temperature for samples containing protein continued to increase with each cycle.



Supplementary Figure 8. ESR spectra of DPPC/POPC/POPG vesicles incorporating no protein (dotted line), 5 wt% (1 mol%) or 10 wt% (2 mol%) SP-C (solid lines) labelled with 1 mol% of the probe 5PSCL at different temperatures. Spectra below correspond to magnifications showing a detailed view of the low and high-field spectra for vesicles with no protein (dotted line), and samples containing either 5 wt% (1 mol%) SP-C or 10 wt% (2 mol%) SP-C (solid lines). The total scan range was 100G.