Supplementary Information: Liquid-liquid phase coexistence in lipid membranes observed by natural abundance ^{1}H–^{13}C solid-state NMR

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Sample preparation

The phospholipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) were purchased from Avanti Polar Lipids, while cholesterol and ethanol were purchased from Sigma-Aldrich. Each DPPC/DOPC/cholesterol sample was prepared by first mixing ethanol equimolar solutions of DPPC, DOPC and cholesterol in the right amounts and then rapidly evaporating the solvent in a 1.5 ml EPPENDORF tube under a N_2 gas stream. During the evaporation (which took no more than 1-2 min) the tube was immersed in a water bath at 55 ^{o}C and under sonication. After evaporation observed with the naked eye, the dried lipid mixtures were left overnight under vacuum. The resulting lipid film (about 20 mg in total) was then hydrated such that samples had 40 %wt of deuterium oxide in total weight. For hydrating the systems the EPPENDORF tubes were placed in an EPPENDORF THERMOMIXER set to 55 ^{o}C and water was added. The mixture was then centrifuged, heated and mixed manually with a thin metal tip repeatedly, as many times necessary to attain an homogeneous sample. Each sample was then centrifuged from the EPPENDORF tube to a KEL-F insert for solid-state NMR 4 mm rotors with a sample volume of 25 μl.

Solid-state Nuclear Magnetic Resonance

All experiments were performed using a Bruker Avance III 400 spectrometer operating at a ^{1}H Larmor frequency of 400.03 MHz equipped with a standard 4 mm CP-MAS HXY probe. The temperature was calibrated with a methanol sample spinning at the MAS frequency used [1]. The composition of each sample was determined by performing ^{13}C direct polarisation experiments (^{13}C 90^{o} followed by acquisition under SPINAL64 ^{1}H decoupling [2]) and fitting gaussians to the spectra in order to determine area fractions between components. The fits and calculations are shown in figures S5-S8 for all samples.

For describing the set up of the R-PDLF experiments we define the pulse sequence parameters according to Figures 1c and 2c of the original reference for the R-PDLF experiment [3]. The MAS frequency used was 5.15 kHz and t_1 increments used were six 180^{o} pulses for each of the two R18$_1^7$ blocks. The refocused-INEPT [4, 5] delays τ_1 and τ_2 were multiples of the MAS rotation period, namely 1.94 ms and 0.97 ms, respectively. The RF pulses used had the following nutation frequencies: 46.35 kHz (R18$_1^7$ pulses), 63.45 kHz (^{13}C 90^{o} and 180^{o} pulses) and 50 kHz (SPINAL64 ^{1}H decoupling [2]). A recycle delay of 5 s was used. The total acquisition time for each scan in the direct dimension was 0.1 s using a spectral width of 200 ppm (a total number of 256 scans was used). For a description of the processing of data see e.g. [6].

For a given C–H bond its order parameter magnitude, $|S_{\mathrm{CH}}|$, relates to its R-PDLF dipolar splitting by

$$\Delta\nu^{\mathrm{R-PDLF}} = 0.315 d_{\mathrm{CH}}^{\mathrm{max}} |S_{\mathrm{CH}}| \tag{1}$$

where 0.315 is a scaling factor specific of the dipolar recoupling sequence used and $d_{\mathrm{CH}}^{\mathrm{max}}$ the rigid dipolar coupling constant equal to 21.5 kHz [3].

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[1] A. L. V. Geet, Anal. Chem. **42**, 679 (1970).
[2] B. Fung, A. Khitrin, and K. Ermolaev, J. Mag. Res. **142**, 97 (2000).
[3] S. V. Dvinskikh, H. Zimmermann, A. Maliniak, and D. Sandström, J. Mar. Res. **168**, 194 (2004).
[4] G. Morris and R. Freeman, J. Am. Chem. Soc. **101**, 760 (1979).
[5] D. Burum and R. Ernst, J. Mag. Res. **39**, 163 (1980).
[6] T. M. Ferreira, B. Medronho, R. W. Martin, and D. Topgaard, Phys. Chem. Chem. Phys. **10**, 6033 (2008).

Supplementary Figures

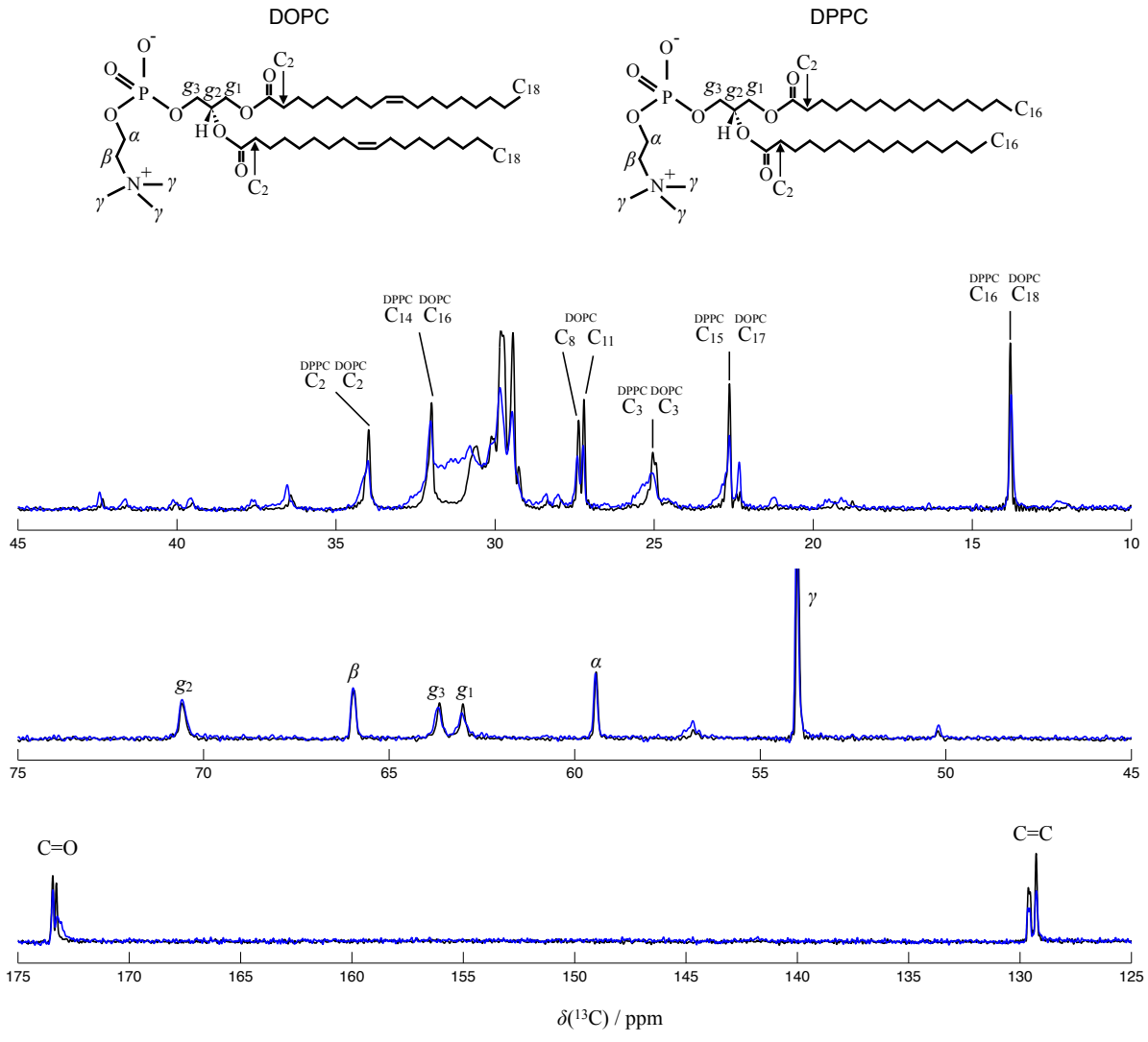


FIG. 1: Molecular structures of DPPC and DOPC with carbon labels and full ^{13}C spectrum for the samples with DPPC/DOPC/cholesterol compositions 32:57:11 (black) and 44:37:19 (blue).

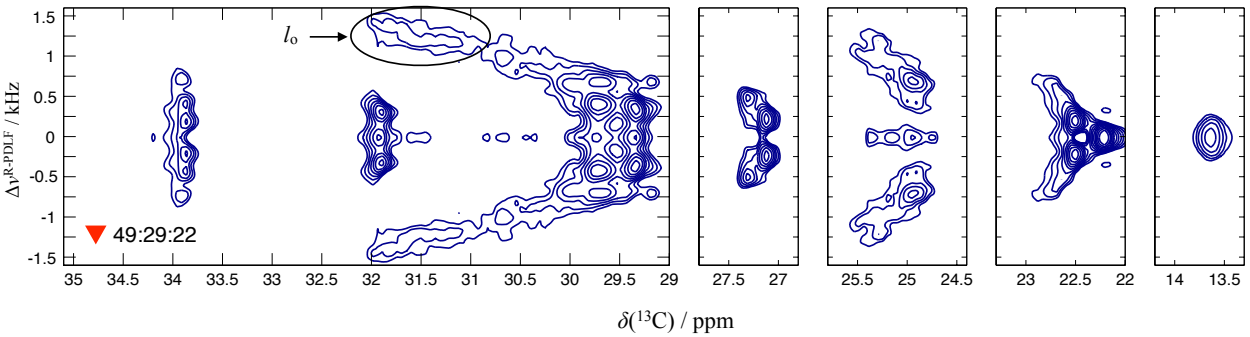


FIG. 2: Regions of the ^{1}H–^{13}C R-PDLF spectrum for the sample with DPPC/DOPC/cholesterol composition 49:29:22. Spectra acquired using a MAS frequency of 5.15 kHz and SPINAL64 decoupling at an RF frequency of 50 KHz.

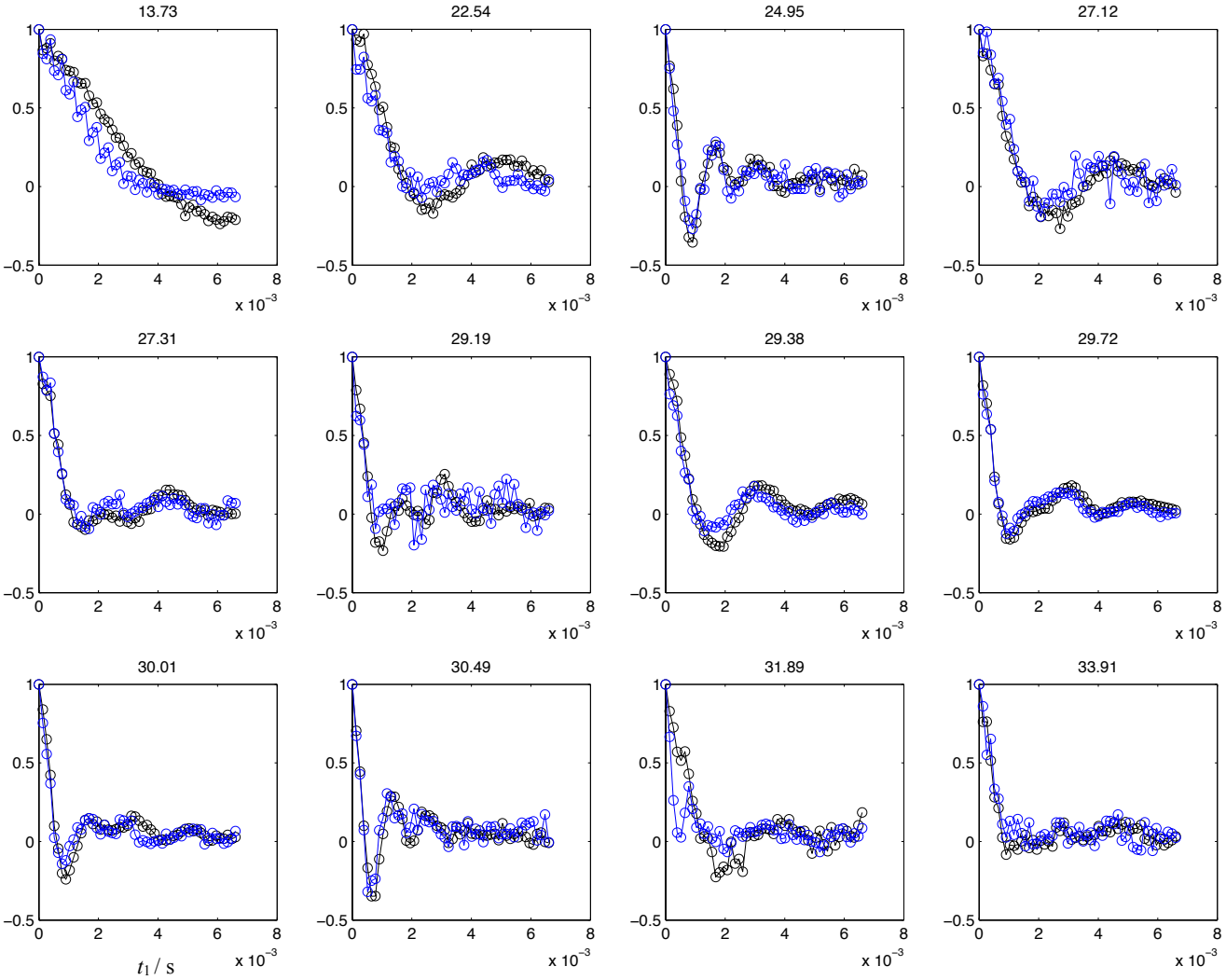


FIG. 3: Time dependence over the dipolar coupling dimension of the R-PDLF experiments for samples with DPPC/DOPC/cholesterol composition 32:57:11 (black) and 44:37:19 (blue) at the ^{13}C chemical shifts indicated on top of each plot.

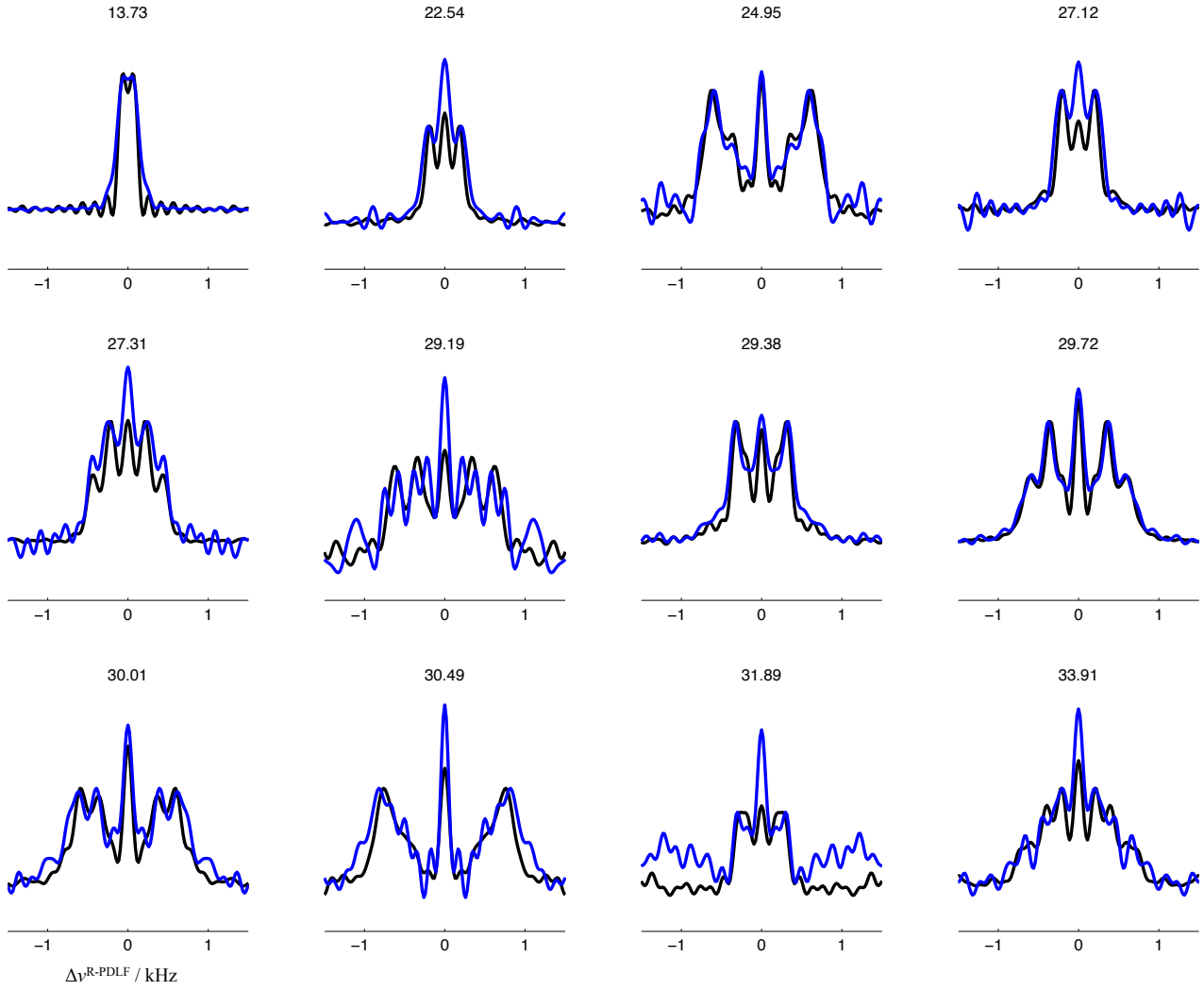
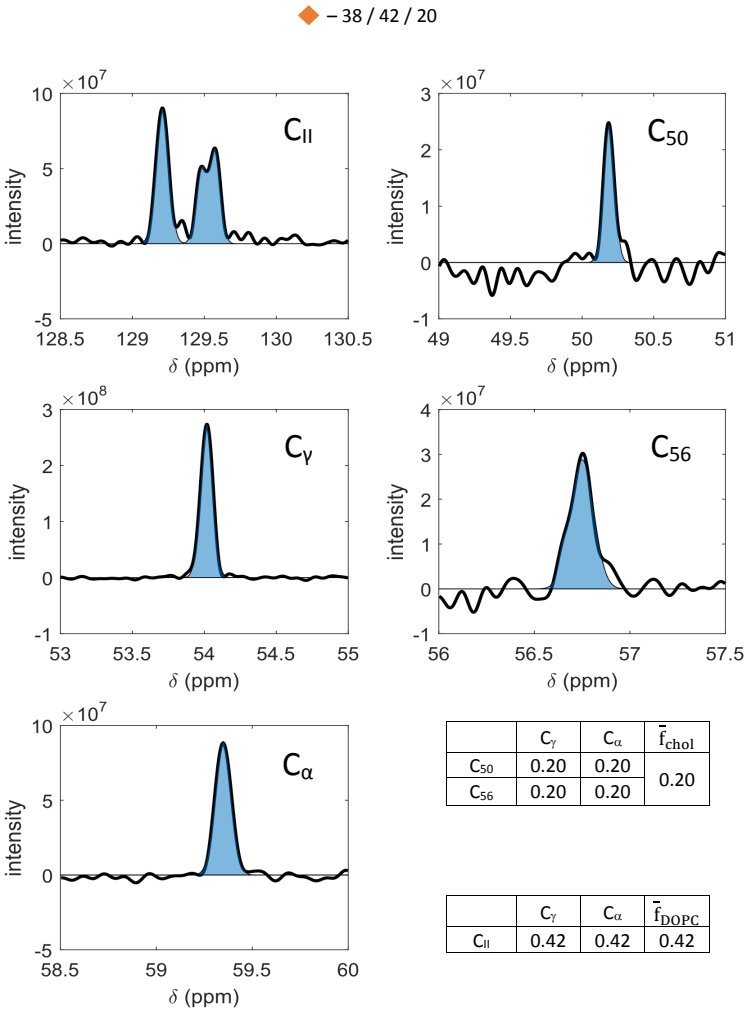


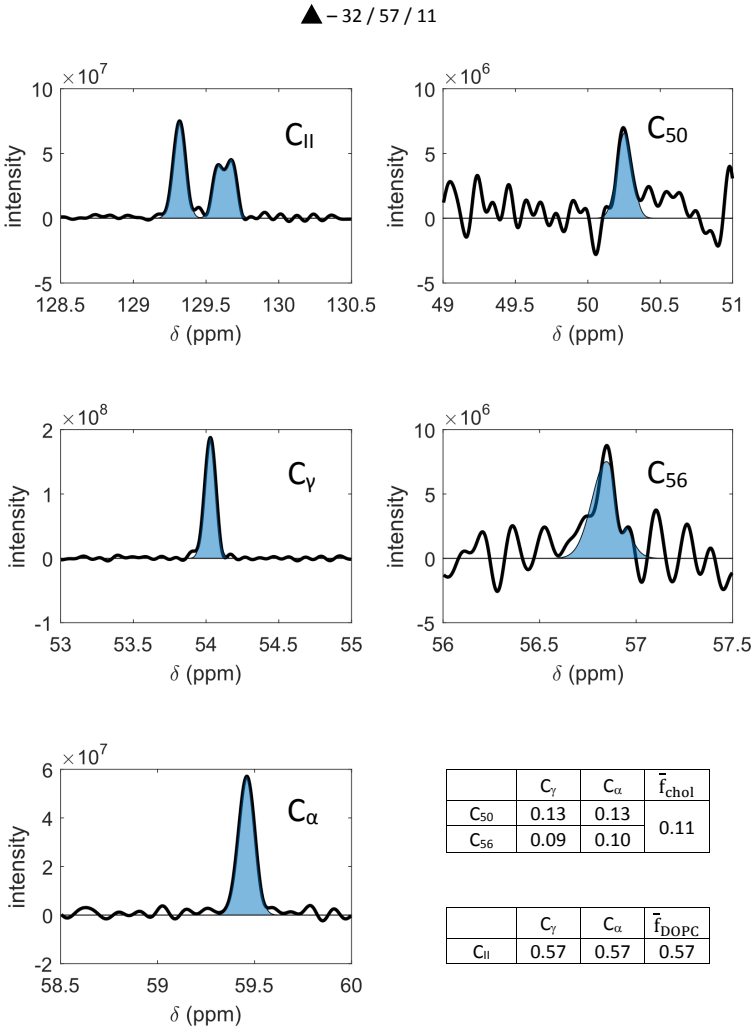
FIG. 4: Dipolar slices of the R-PDLF spectra for samples with DPPC/DOPC/cholesterol composition 32:57:11 (black) and 44:37:19 (blue) at the ^{13}C chemical shifts indicated on top of each plot (i.e. the Fourier transforms of the time domain curves shown above).



	C_γ	C_α	$\bar{f}_{chol}$
C_{50}	0.20	0.20	0.20
C_{56}	0.20	0.20	

	C_γ	C_α	$\bar{f}_{DOPC}$
C_{II}	0.42	0.42	0.42

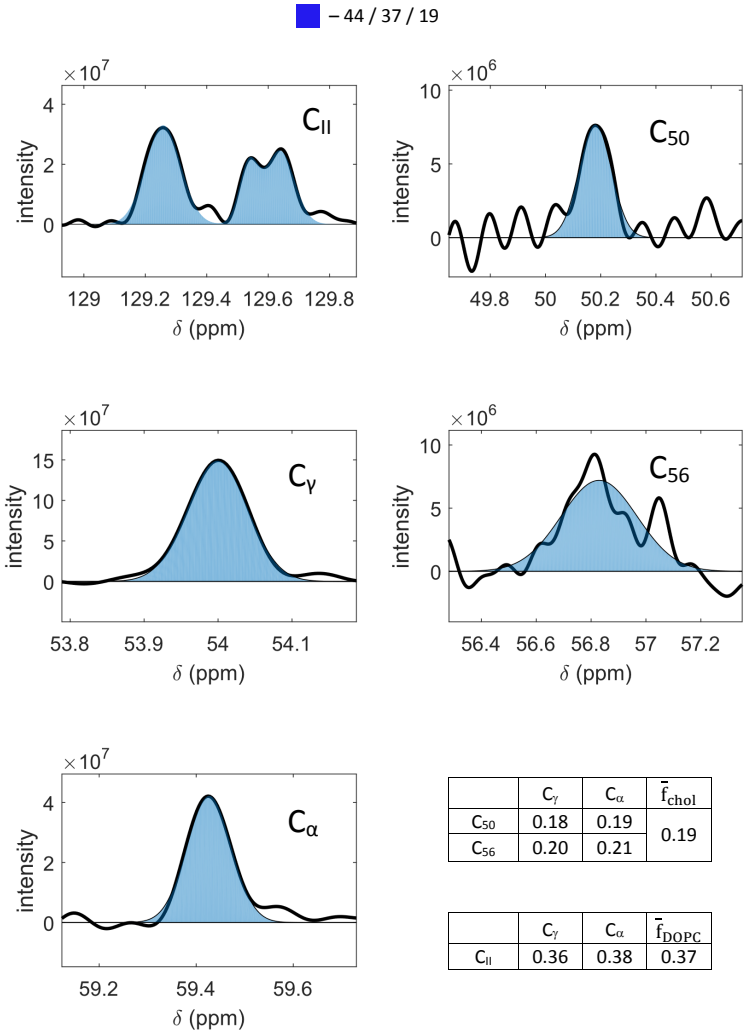
FIG. 5: Regions of the ^{13}C direct polarization spectrum for sample 38:42:20. The composition was determined by using the areas of the fits shown for each carbon indicated. The upper table shows the mol fraction of cholesterol as determined from using the γ and α carbons of the phospholipids and using two distinct carbons for cholesterol with ^{13}C chemical shifts at approx. 50 and 56.5 ppm. The bottom table shows the fraction of DOPC by using the fraction of cholesterol calculated above and the areas from the γ, α, and the carbons connected by double bonds in the DOPC acyl chains within 129-130 ppm.



	C_γ	C_α	$\bar{f}_{chol}$
C_{50}	0.13	0.13	0.11
C_{56}	0.09	0.10	

	C_γ	C_α	$\bar{f}_{DOPC}$
C_{II}	0.57	0.57	0.57

FIG. 6: Regions of the ^{13}C direct polarization spectrum for sample 32:57:11. The composition was determined by using the areas of the fits shown for each carbon indicated. The upper table shows the mol fraction of cholesterol as determined from using the γ and α carbons of the phospholipids and using two distinct carbons for cholesterol with ^{13}C chemical shifts at approx. 50 and 56.5 ppm. The bottom table shows the fraction of DOPC by using the fraction of cholesterol calculated above and the areas from the γ, α, and the carbons connected by double bonds in the DOPC acyl chains within 129-130 ppm.

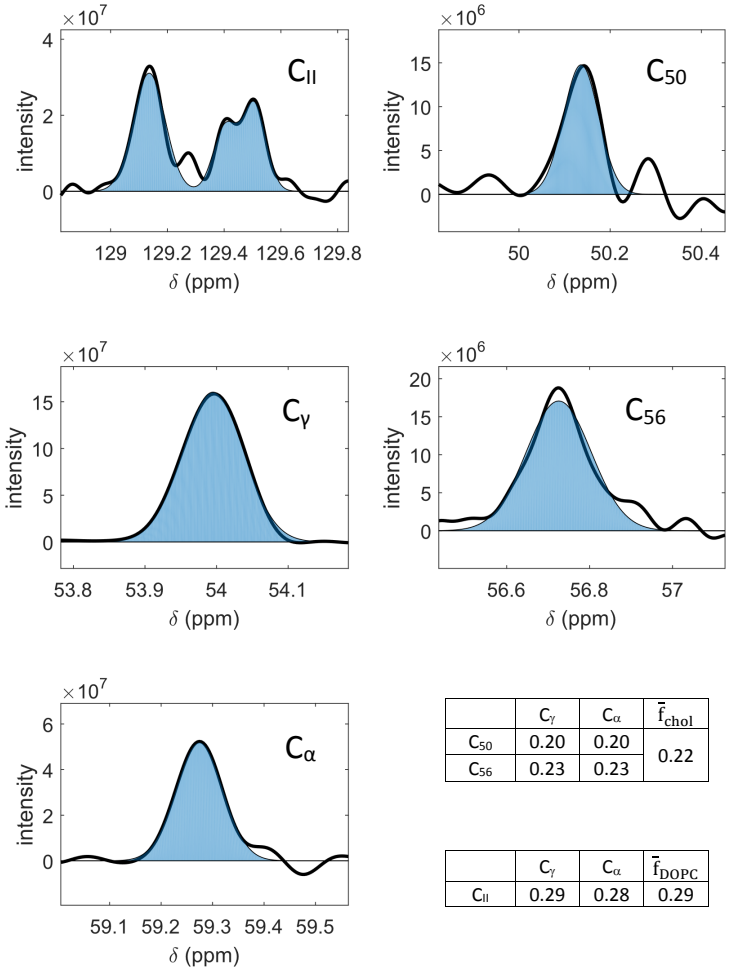


	C_γ	C_α	$\bar{f}_{chol}$
C_{50}	0.18	0.19	0.19
C_{56}	0.20	0.21	

	C_γ	C_α	$\bar{f}_{DOPC}$
C_{II}	0.36	0.38	0.37

FIG. 7: Regions of the ^{13}C direct polarization spectrum for sample 44:37:19. The composition was determined by using the areas of the fits shown for each carbon indicated. The upper table shows the mol fraction of cholesterol as determined from using the γ and α carbons of the phospholipids and using two distinct carbons for cholesterol with ^{13}C chemical shifts at approx. 50 and 56.5 ppm. The bottom table shows the fraction of DOPC by using the fraction of cholesterol calculated above and the areas from the γ, α, and the carbons connected by double bonds in the DOPC acyl chains within 129-130 ppm.

▼ – 49 / 29 / 22



	C_γ	C_α	$\bar{f}_{chol}$
C_{50}	0.20	0.20	0.22
C_{56}	0.23	0.23	

	C_γ	C_α	$\bar{f}_{DOPC}$
C_{II}	0.29	0.28	0.29

FIG. 8: Regions of the ^{13}C direct polarization spectrum for sample 49:29:22. The composition was determined by using the areas of the fits shown for each carbon indicated. The upper table shows the mol fraction of cholesterol as determined from using the γ and α carbons of the phospholipids and using two distinct carbons for cholesterol with ^{13}C chemical shifts at approx. 50 and 56.5 ppm. The bottom table shows the fraction of DOPC by using the fraction of cholesterol calculated above and the areas from the γ, α, and the carbons connected by double bonds in the DOPC acyl chains within 129-130 ppm.