Supporting Information

^{17}O NMR spectroscopy as a tool to study hydrogen bonding of cholesterol in lipid bilayers

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Experimental details

Materials

1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and egg sphingomyelin (eSM) were purchased from Avanti Polar Lipids inc. and stored in chloroform at -20°C. ^{17}O enriched cholesterol was synthesised as described previously and stored at room temperature.[1]

Preparation of bilayers

Lipid bilayers were made with 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and egg sphingomyelin (eSM). Lipids were added as a chloroform solution to the ^{17}O cholesterol at a 50:50 mol % ratio. The chloroform was removed by nitrogen stream to produce thin lipid films. Remaining chloroform and water were removed by leaving samples in the lyopholiser for 12 hours. Samples were hydrated with ^{17}O depleted water at a 20:1 water:lipid mole ratio (65 wt %). Thorough hydration was ensured by rapid heat cycling of the samples 15 times using liquid nitrogen and hot air. Samples were stored at 4°C until use.

Preparation of cholesterol monohydrate

Preparation of ^{17}O enriched cholesterol monohydrate was achieved by recrystallisation of ^{17}O cholesterol from a solution of 95% ethanol 5% ^{17}O depleted water. The crystals were filtered and washed with ^{17}O depleted water. Crystals were stored under water to prevent dehydration. Images of the crystals were taken Nikon Eclipse TE2000-E microscope at 40X magnification.

Solution state NMR spectroscopy

Solution state NMR was carried out on a Bruker Avance II spectrometer with a 9.4 T magnet. Samples were run at a concentration of 35mM. Pulse length was 5.33 μs, data was acquired over 150 ms and the recycle delay was 200 ms. The filter width used was 250 kHz and no more than 10232 scans were taken.

Solid state NMR spectroscopy

Solid-state NMR was carried out using Bruker Avance III spectrometers equipped 20.0 T wide-bore magnets. Samples were loaded into 3.2 mm rotors and were spun at the magic angle with a speed of 20 kHz. Experiments were carried out at the UK 850 MHz Solid-State NMR Facility based at the University of Warwick.

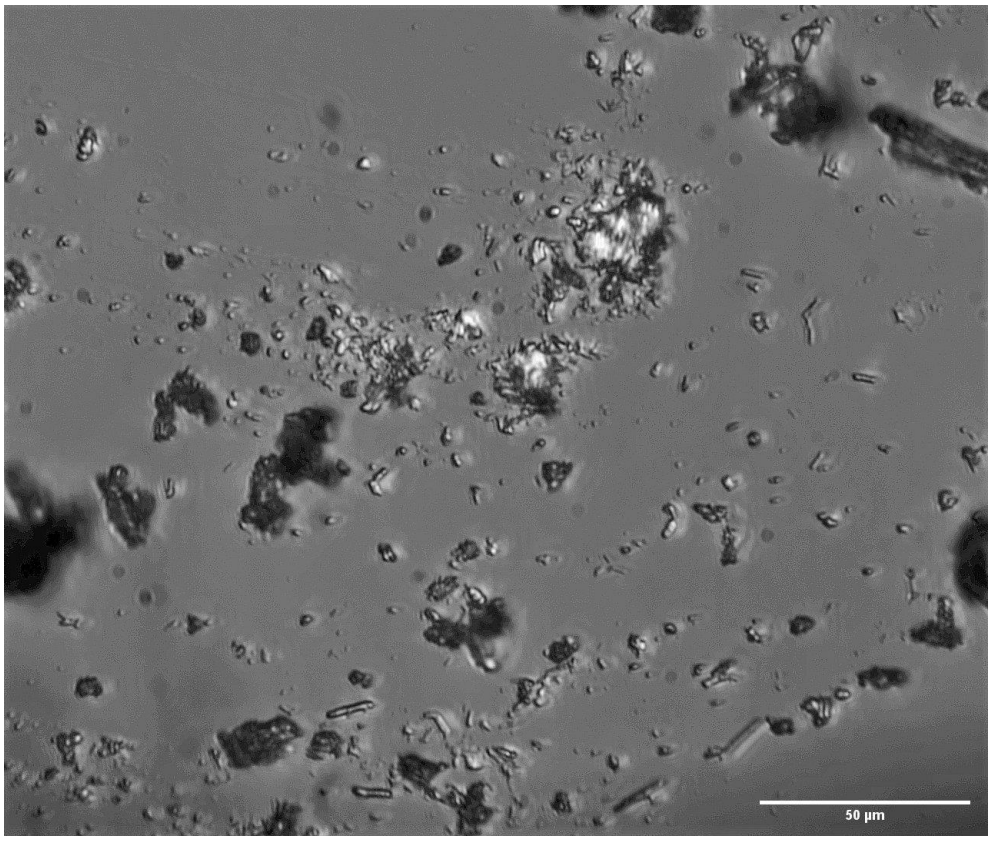
^{1}H -NMR spectroscopy ^{1}H-MAS spectra were applied using a 1-pulse sequence with of 4 scans and a filter width of 240 MHz. The pulse length was 2 μs with an acquisition time of 655 ms and a 1s recycle delay. Data was processed with an exponential line broadening of 10Hz.

^{17}O -NMR spectroscopy All ^{17}O spectra were acquired with a filter width of 240 MHz and a 100 ms recycle delay. Chemical shift was externally referenced to $H_2{}^{17}O$. For anhydrous cholesterol a double frequency sweep with a spin echo acquisition (dfsspinecho) was used. This sequence consisted of a 2 ms double frequency sweep, followed by a 1.4 μs 90° pulse, then a 2.8 μs 180° pulse. The FID was acquired for 4.1ms and spectra were acquired over 32768 scans. Static spectra of cholesterol in bilayers were acquired using a basic one pulse sequence, with a pulse length of 2 μs. The FID was acquired in 4 ms and 4096 scans were performed. For the 17O MAS spectra of cholesterol in bilayers a double frequency sweep pulse sequence with a 90° pulse (dfs90sel) was used to better capture the narrower spectra. The sequence consisted of a 2 ms double frequency sweep followed by a 1.33 μs pulse. Data was acquired for 65 ms. Spectra were acquired for 16348 scans. ^{17}O spectra were processed with 200 Hz exponential line broadening.

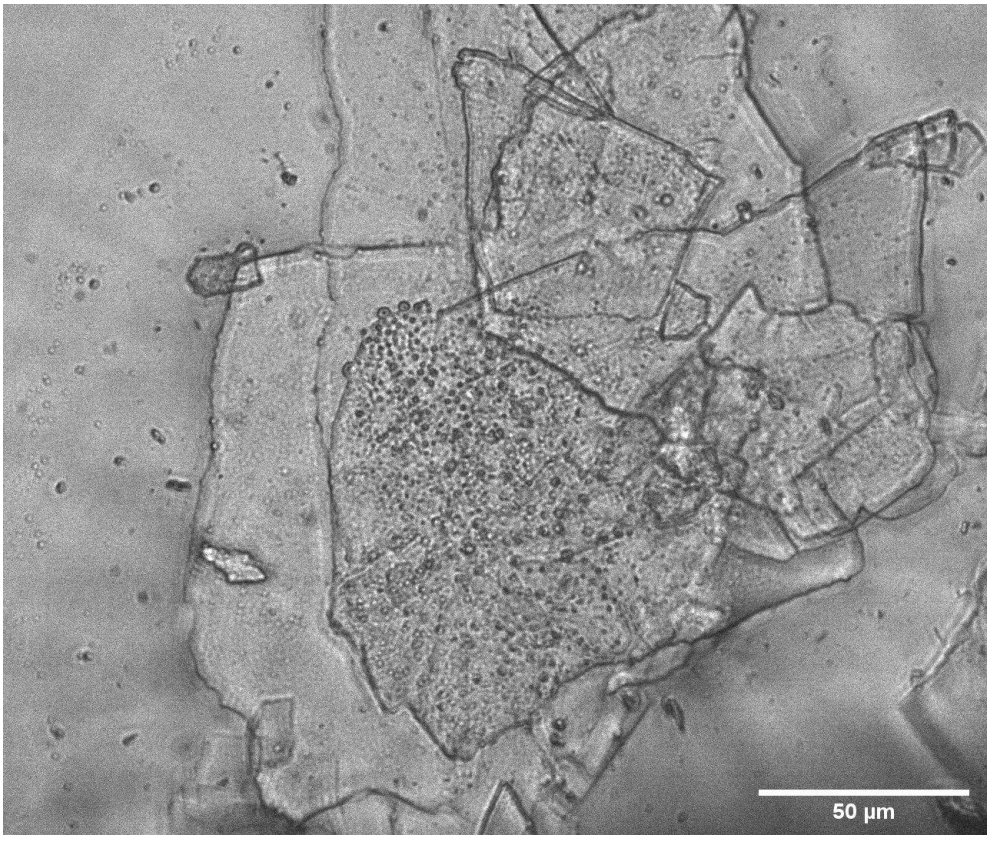
Fitting Solid-state NMR for anhydrous cholesterol and static spectra of cholesterol contained in bilayers were fitting using TopSpin 4.0.6 using the Solids NMR fitting methodology. Fitting of cholesterol in bilayers and cholesterol monohydrate under MAS regime was carried out using OriginPro 2018.

S1: Microscopy Images of anhydrous cholesterol and cholesterol monohydrate

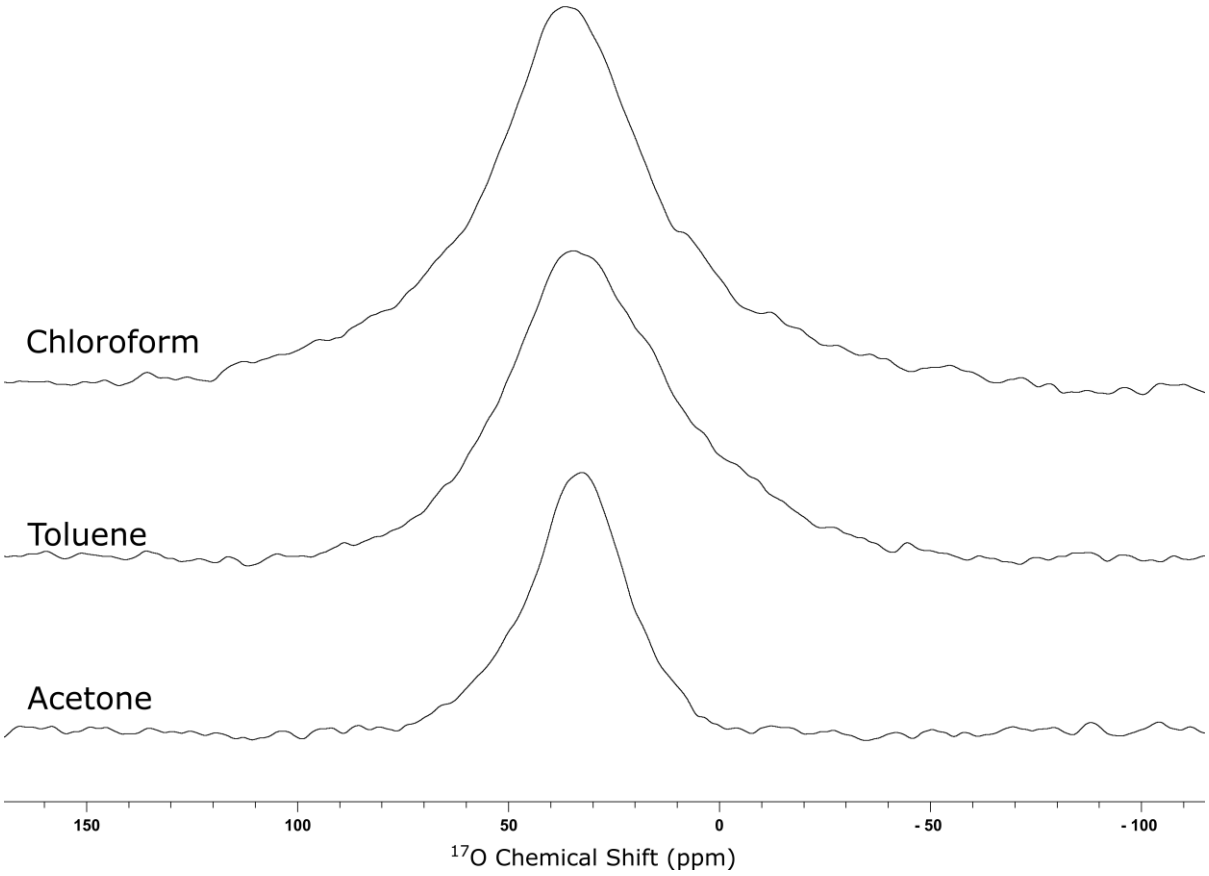
Anhydrous needles



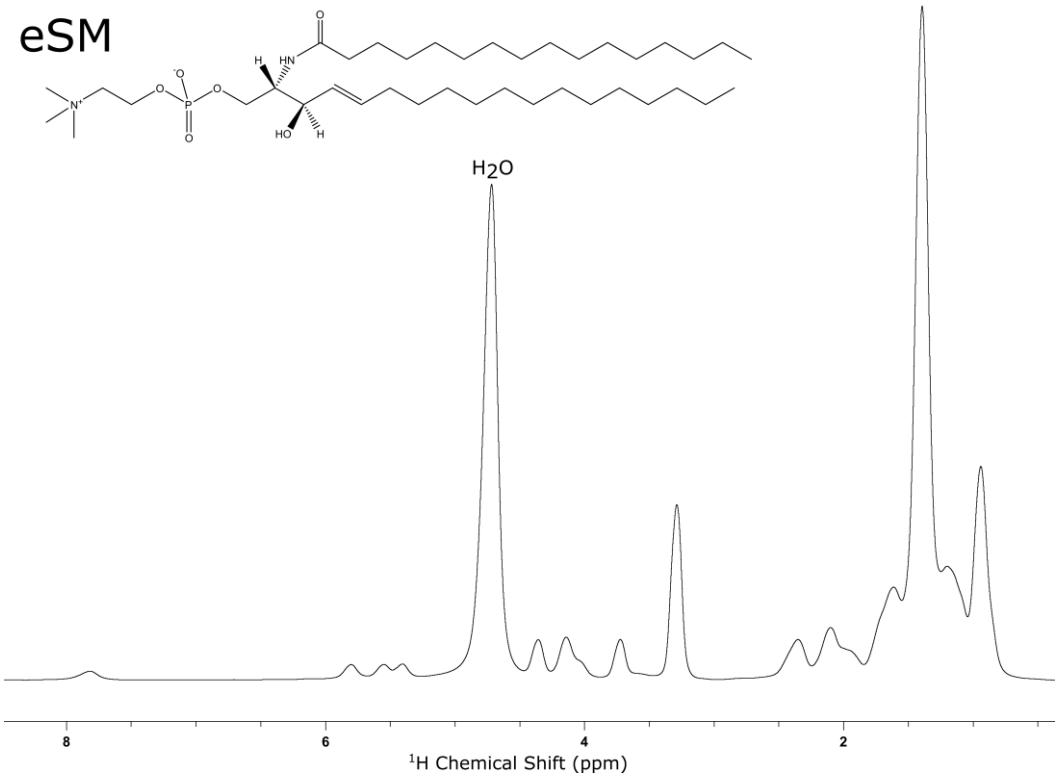
Monohydrate Plates

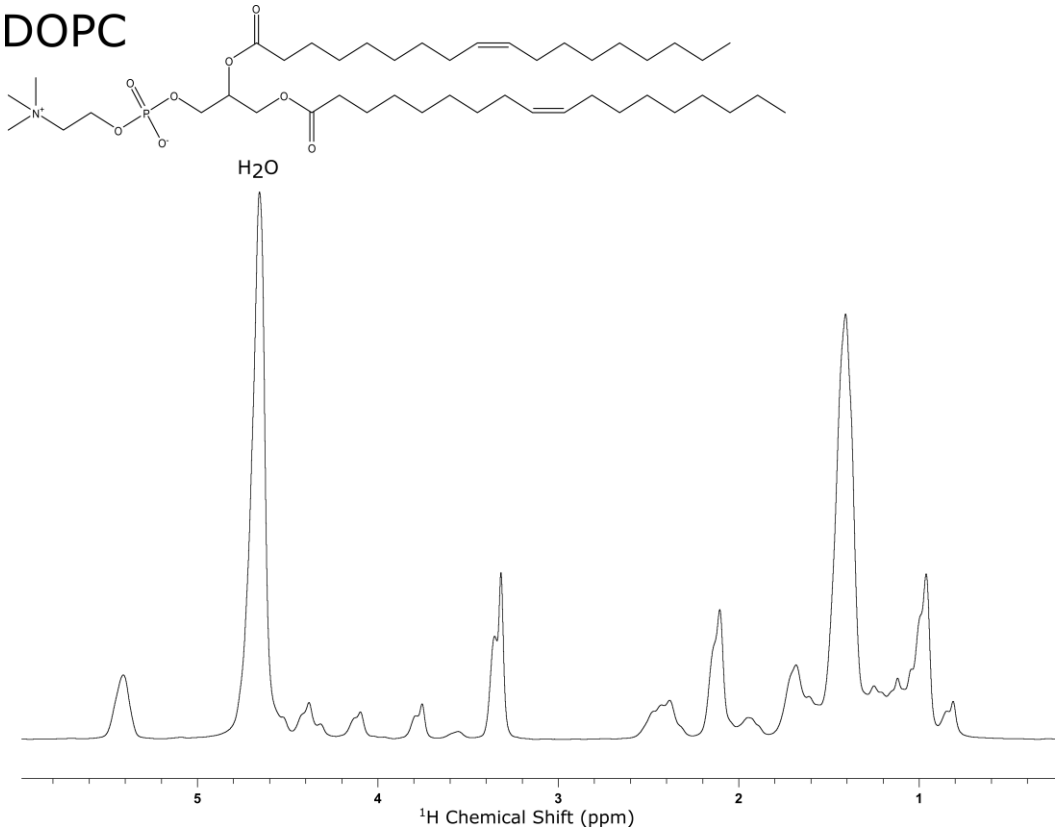


S2: Solution State ^{17}O NMR spectra of enriched cholesterol

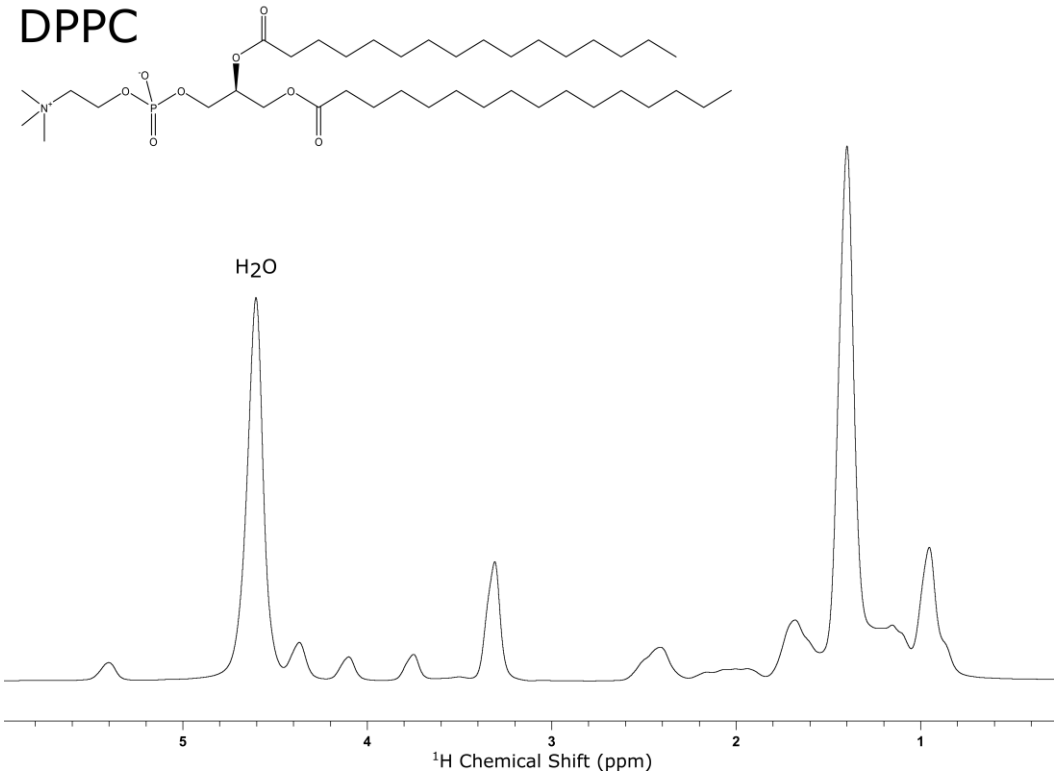


S3: ^{1}H NMR of bilayers containing ^{17}O enriched cholesterol

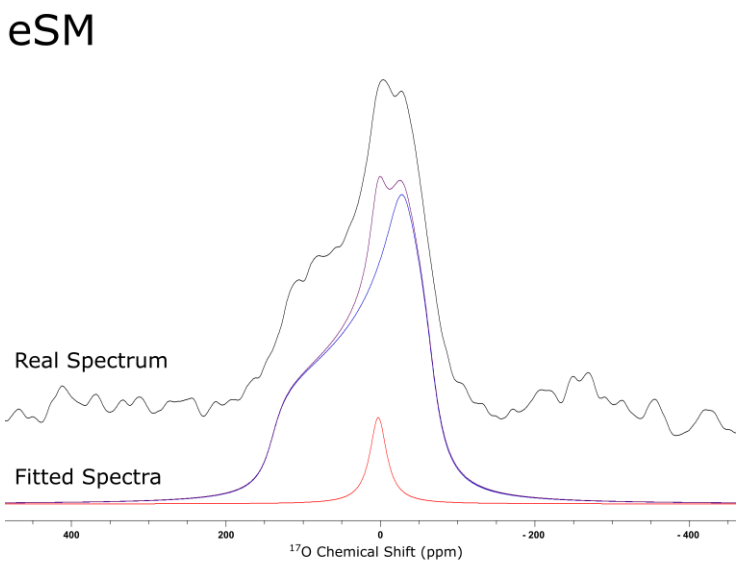


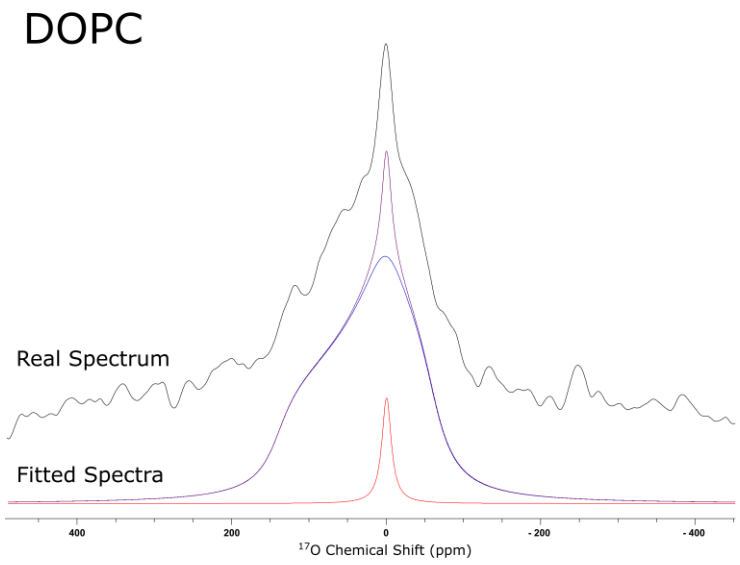


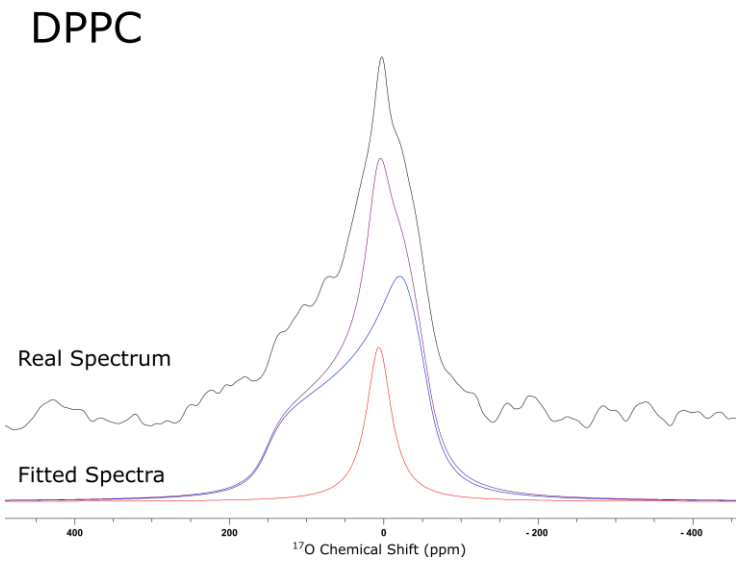
DPPC



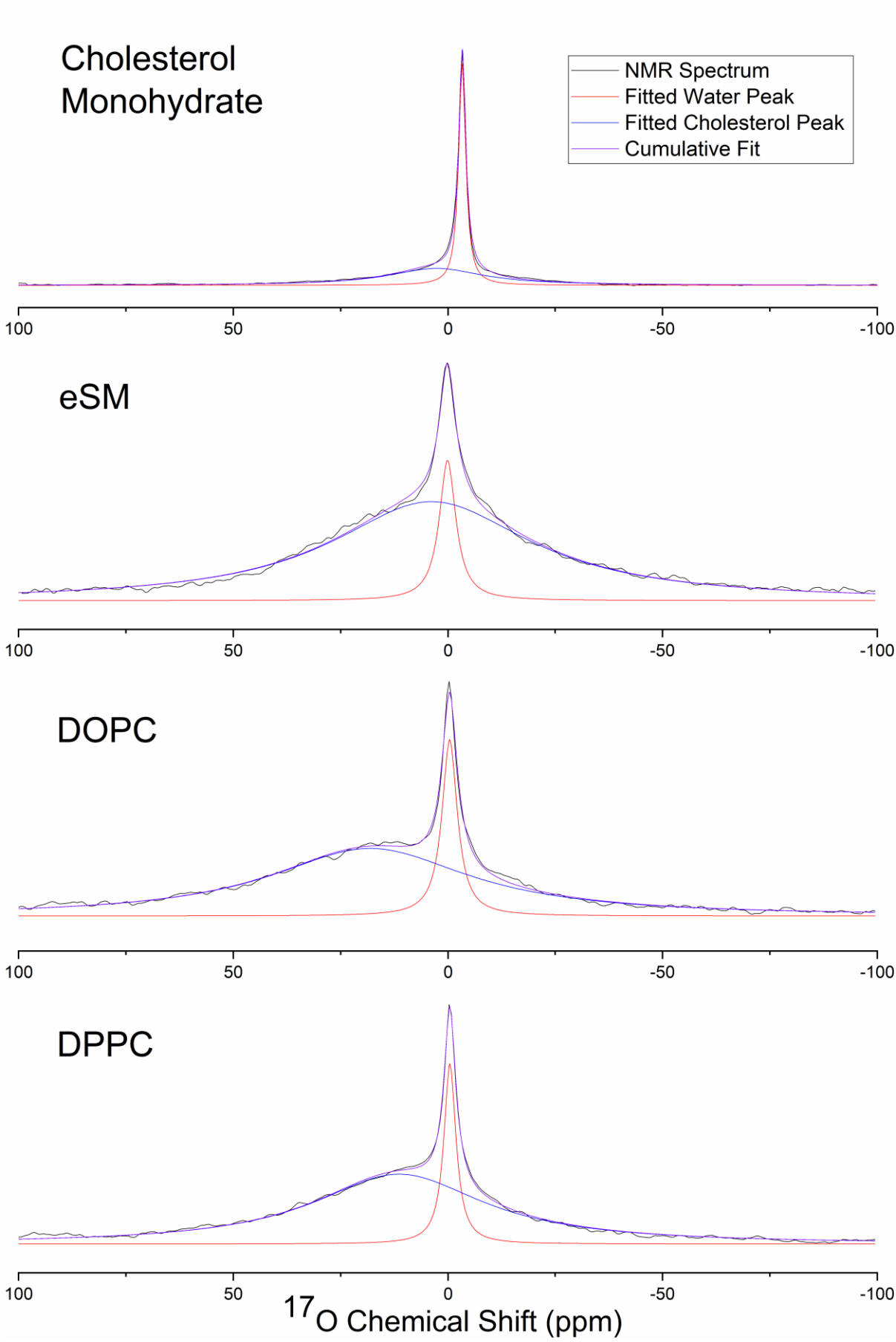
S4: Static ^{17}O-NMR of cholesterol in bilayers with fitted simulated spectra







S5: MAS ^{17}O-NMR of cholesterol in bilayers and cholesterol monohydrate with fitted simulated spectra



S6: Solid State ^{17}O-NMR fitting parameters- samples with observable quadrupolar component (Cholesterol peak)

Sample	MAS speed	δ(iso)/ ppm	δ(11)/ ppm	δ(22)/ ppm	δ(33)/ ppm	Cq/MHz	η (quad)	Euler α	Euler β	Euler γ
eSM	Static	15.93	140.67	-27.34	-65.54	0.395	0	-189	0	-527
DOPC	Static	26.81	140.25	0.83	-60.62	0.932	0	-191	0	-396
DPPC	Static	25.41	151.97	-21.72	-54.03	0.001	0	-3	0	499
Anhydrous cholesterol	20kHz	34.59	301.45	9.49	-207.18	9.113	0.76	0	0	0

S7: Solid State ^{17}O-NMR fitting parameters- samples under MAS regime with no observable quadrupolar/CSA component.

Sample	Cholesterol Chemical shift/ppm	Cholesterol FWHM/kHZ	Water Chemical shift/ppm	Water FWHM/kHz
Cholesterol Monohydrate	2.74 ± 0.29	3.09 ± 0.10	-3.30 ± 0.00	0.23 ± 0.00
eSM	3.82 ± 0.16	6.54 ± 0.11	0.12 ± 0.03	0.54 ± 0.01
DOPC	18.13 ± 0.32	6.54 ± 0.11	-0.37 ± 0.05	0.50 ± 0.02
DPPC	11.42 ± 0.15	5.59 ± 0.06	-0.43 ± 0.01	0.40 ± 0.00

References

1 C. De La Calle Arregui, J. A. Purdie, C. A. Haslam, R. V. Law and J. M. Sanderson, *Chem. Phys. Lipids*, 2016, **195**, 58–62.