

Supporting Information

Accelerated ^{13}C detection by concentrating the NMR sample in biphasic solvent system.

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SI Table 1: Parameter table: Acquisition and processing parameters for all the experiments Figure wise

TD=Time domain data size (For 2D TD2/TD1)

DS=dummy scan

NS=Number scan

SW=spectral width (For 2D SW2/SW1)

AQ=acquisition time(For 2D aq(t2)/aq(t1))

RG=Receiver gain

O1P=frequency offset (For 2D O2P/O1P)

D1=relaxation Delay

SI=zero filling (For 2D SI F2/F1)

WDW=Window function

LB=Line broadening

Pulse sequence for 2D mentioned with Figure

AEOSP=all experiment on same parameters

SS=slice selective

SP=single pulse

SL=Single Layer

	TD	DS/NS	SW(p pm)	AQ(s)	RG	O1P	D1(s)	SI	WDW	LB	
Fig. 2 400 MHz	65536	8/160	238	1.36	203	100	2	131072	EM	1	AEOSP
Fig 3 800 MHz	65536	8/256	238	0.681	203	100	2	131072	EM	1	AEOSP
Fig 4 400 MHz	22386	8/8	14	2	80. 6	4.69	3	65536	EM	0.3	AEOSP
Fig 5 800 MHz	65786	8/8	20.55	2	10	4.69	3	131072	EM	0.3	AEOSP
Fig 6 800 MHz	65536	4/360	238	0.681	203	100	2	131072	EM	1	AEOSP
Fig 7 HSQC ETGP 800 MHz	2048/25 6	16/2	10/16 5	0.127 /0.00 4	203	5/74. 6	1.8	4k/512	QSINE	1/0. 3	SSB 2 AEOSP
Fig S1 400 MHz	22386	1	14	2	80. 6	4.69	3	65536	EM	0.3	AEOSP

Fig S2 400 MHz	22386	8/8	14	2	80. 6	4.69	3	65536	EM	0.3	AEOSP
Fig S3 400 MHz	65536	4/400	238	1.36	203	100	2	131072	EM	1	AEOSP
Fig S4 HSQC ETGP 400 MHz	1200/19 2	8/2	8/145	0.187 /0.00 65	203	4/75	1.8	2k/512	QSINE	1/0. 3	SSB 2 AEOSP
Fig S5 TOCS Y 800 MHz	3072/16 0	8/8	8/8	0.240 /0.01 24	64	4/4	1.5	4k/256	SINE	1/0. 3	SSB 2 AEOSP
Fig S6 COSY 400 MHz	2048/25 6	16/1	8/8	0.320 /0.04	203	4/4	1.5	4k/512	SINE	1/0. 3	SSB 0 AEOSP

Sample preparation: For strychnine sample 25 mg was dissolved in 1 ml CDCl₃ of which 0.2 ml was transferred to each of the four NMR tubes for the 4 cm standard (5 mm o.d.) sample, 3 c.m. single layer sample, and the two biphasic samples respectively. For cholesterol 35.5 mg was dissolved in 1.4 ml CDCl₃ of which 0.2 ml was transferred to each of the five NMR tube for the 4 cm standard (5 mm o.d.) sample, shigemi tube, 3 mm o.d. tube, and the two biphasic samples respectively. Similarly a second cholesterol sample was also prepared.

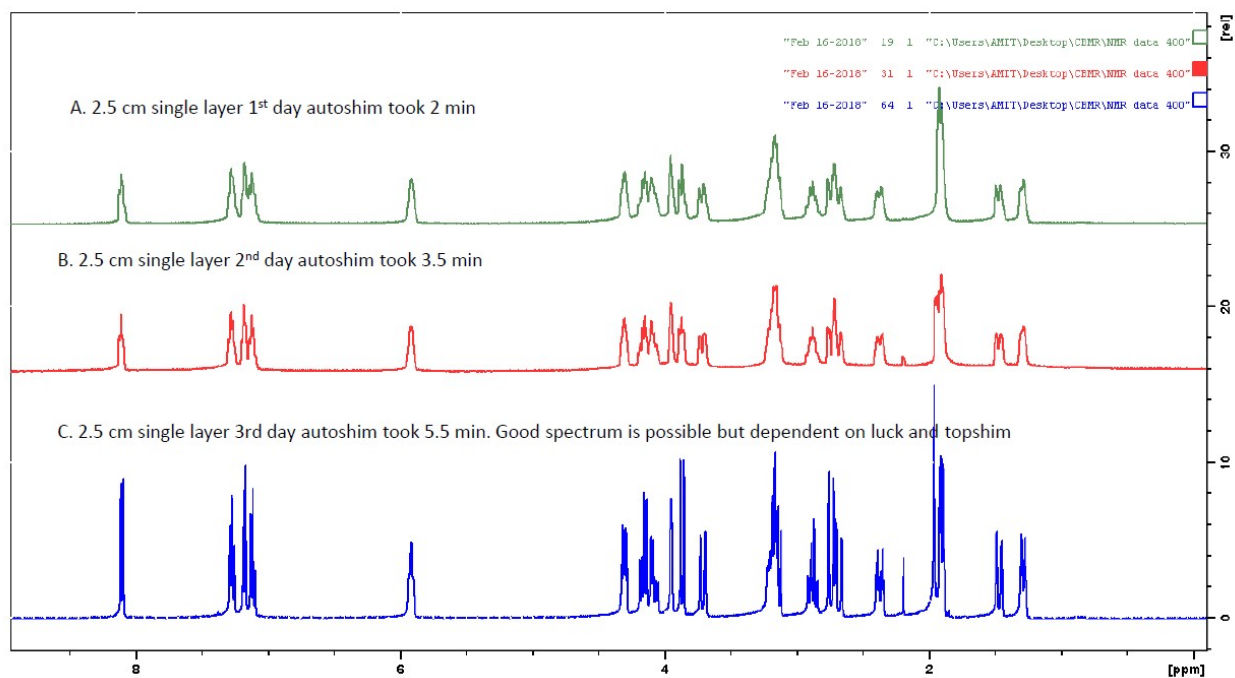


Figure S1. Investigation of autoshimming (topshim in bruker spectrometers) in small samples. The sample is 5 mg strychnine in CDCl_3 . (A) Result of autoshim on 2.5 cm small sample on the 1st day. The procedure took 2 minutes and resulted in a poor ^1H spectrum. (B) Result of autoshim on the same sample on the 2nd day. The procedure took 3.5 minutes and resulted in a poor ^1H spectrum. (C) Result of autoshim on the same sample on the 3rd day. The procedure took 5.5 minutes and resulted in a reasonably good spectrum than the 1st two attempts.

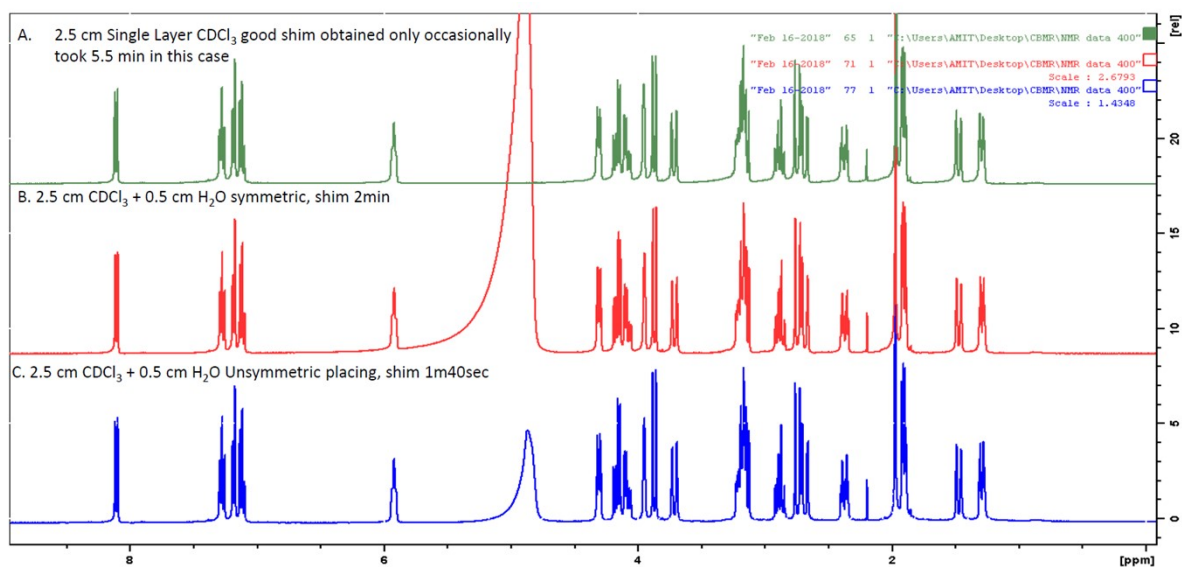


Figure S2. The comparison of shimming for biphasic (these are 3 cm sample) samples and single layer 2.5 cm CDCl_3 sample. (A) Result of occasionally obtained good autoshim on 2.5 cm small sample taking 5.5 minutes (from S1C) (B) Result of autoshim on DLIS-S 2.5 cm CDCl_3 +0.5 cm D_2O taking 2 minutes (C) Result of autoshim on DLIS-U 2.5 cm CDCl_3 +0.5 cm D_2O taking 1 minute and 40 seconds. As the sample height increases, the distance to the air-liquid and solid-liquid interface also increases from the coil region resulting in less interference from field inhomogeneity.

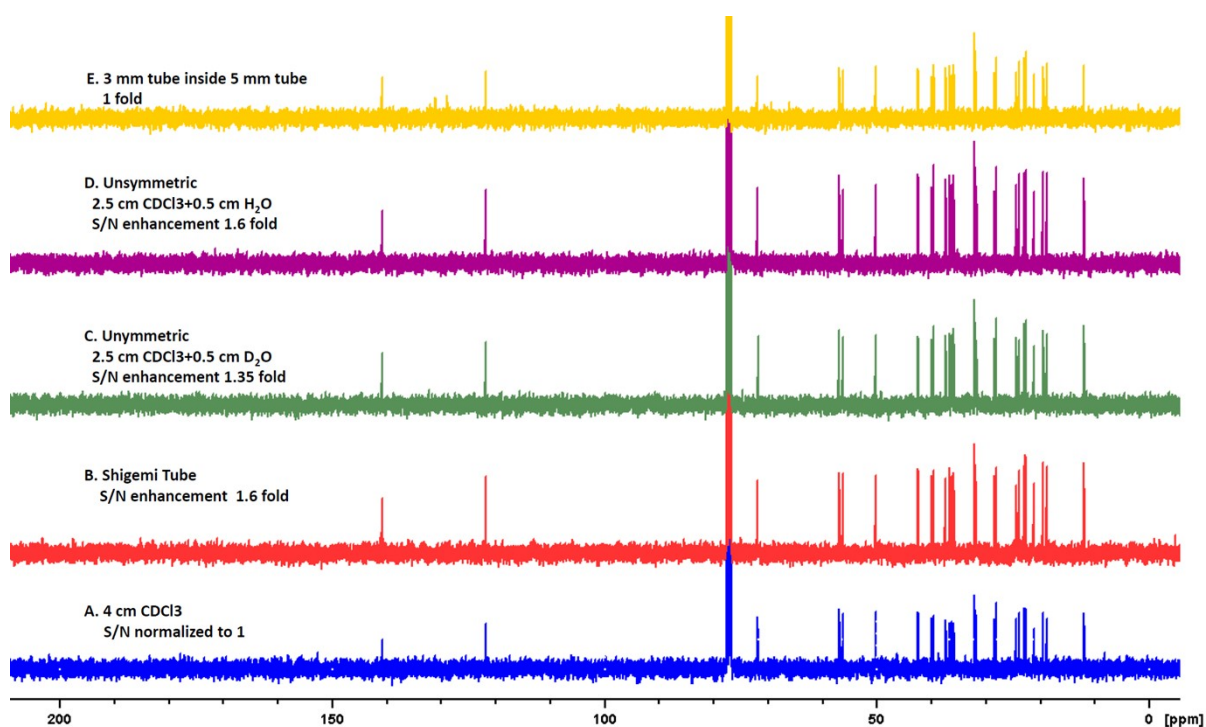


Figure S3. displays the comparison of ^{13}C NMR (NOE enhanced ^1H decoupled) spectra on a sample of cholesterol (5.1 mg) from 400 MHz spectrometer at the same noise level for various sample sizes (A) standard sample (4 cm), (B) Shigemi tube, (C) biphasic-S 3 cm (2.5 cm $\text{CDCl}_3 + 0.5$ cm D_2O), (D) biphasic-S 3 cm (2.5 cm $\text{CDCl}_3 + 0.5$ cm H_2O), and (E) 3 mm o.d. tube samples respectively. The SNR enhancement factors were 1.6 (shigemi tube), 1.35 ($\text{CDCl}_3/\text{D}_2\text{O}$), 1.6 ($\text{CDCl}_3/\text{H}_2\text{O}$), and 1.1 (3 mm tube) fold respectively relative to that of 4 cm standard sample. All spectra were acquired with the same acquisition and processing parameters. All experiments were carried out at 400 MHz using BBFO probe.

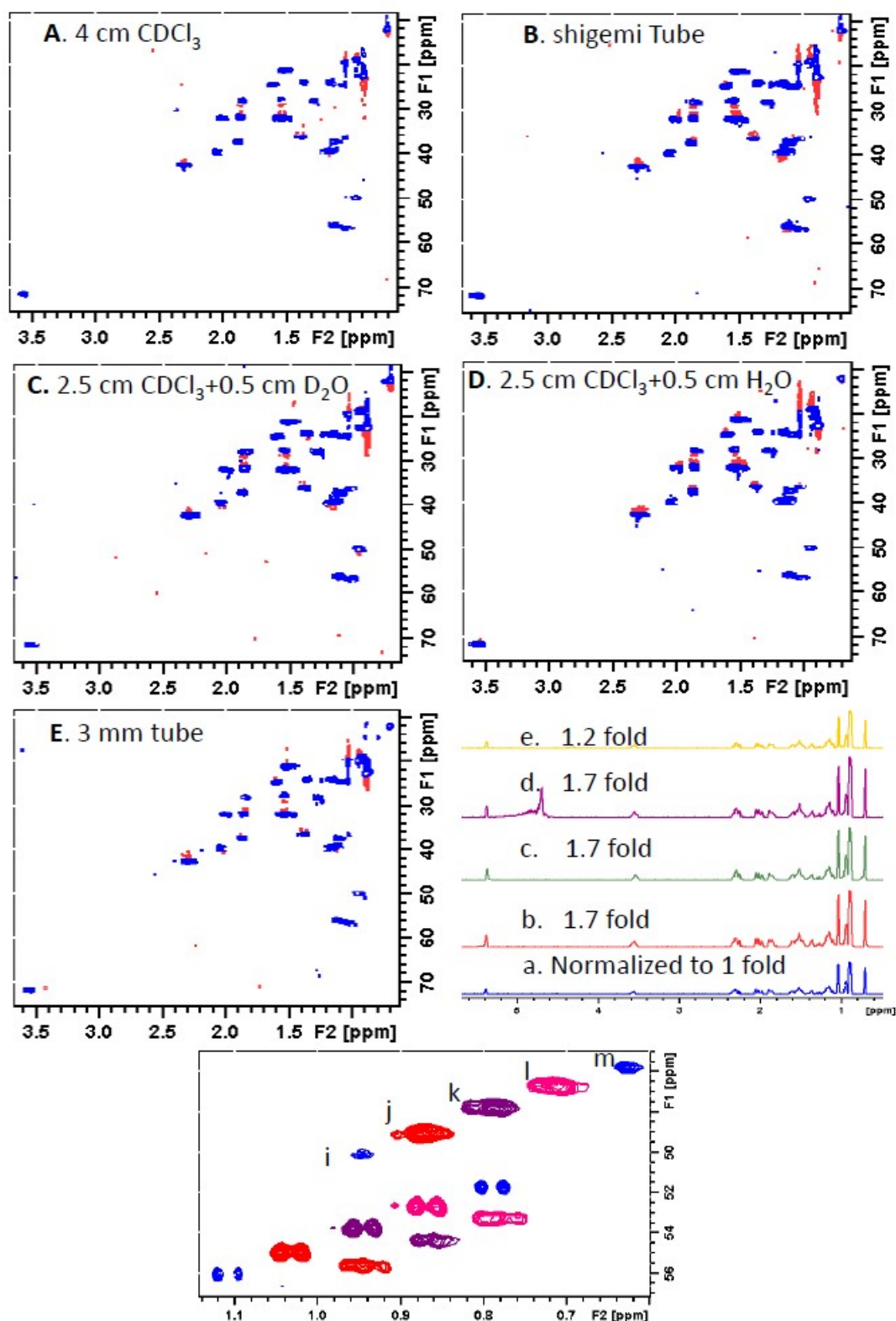


Figure S4. 2D ^1H - ^{13}C HSQC spectra recorded on a sample of cholesterol (5.1 mg) using different sample types (A) standard sample-4 cm, (B) Shigemi tube, (C) biphasic-S 3 cm (2.5 cm $\text{CDCl}_3 + 0.5$ cm D_2O), (D) biphasic-S 3 cm (2.5 cm $\text{CDCl}_3 + 0.5$ cm H_2O), and (E) 3 mm o.d. tube respectively. All 2D ^1H - ^{13}C HSQC spectra were recorded with the same acquisition and processing parameters. Signal to noise enhancement for the different sample types relative to the standard sample- 4 cm are shown

in the projections a, b, c, d, and e which are extracted from panels A-E respectively. The SNR enhancement was 1.7 fold for each of the shigemi tube, biphasic S-CDCl₃/D₂O, and biphasic S-CDCl₃/H₂O samples respectively relative the standard 4 cm sample. 3 mm o.d. tube displayed lower enhancement 1.2 fold compared to standard sample. All experiments were carried out at 400 MHz using BBFO probe. The experimental parameters and processing parameters are mentioned in SI table 1.

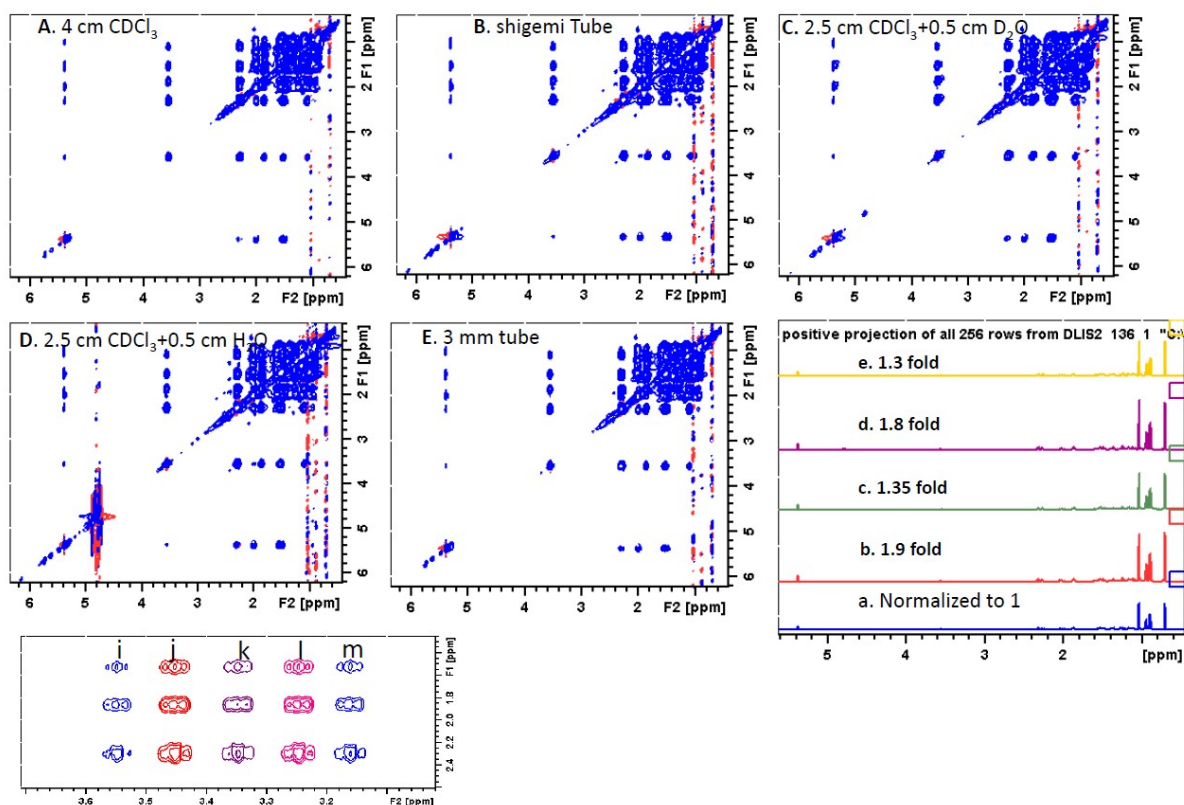


Figure S5. 2D ¹H-¹H TOCSY spectra on a sample of cholesterol (5.1mg) for different sample types (A) standard sample-4 cm, (B) Shigemi tube, (C) biphasic-S 3 cm (2.5 cm CDCl₃+0.5 cm D₂O), (D) biphasic-S 3 cm (2.5 cm CDCl₃+0.5 cm H₂O), and (E) 3 mm o.d. tube respectively. All spectra were recorded with the same acquisition and processing parameters. The projections a-e are extracted from panels A-E and compares the enhancement in SNR for the different sample types relative to the standard sample- 4 cm. Shigemi tube shows highest SNR enhancement (1.9 fold) followed by biphasic-S CDCl₃/H₂O (1.8 fold), and biphasic-S CDCl₃/D₂O sample (1.35 fold) respectively. 3 mm o.d. tube sample displayed 1.3 fold gain in SNR compared to the standard sample (4 cm CDCl₃). All experiments were carried out at 800 MHz using TCI cryoprobe. The experimental parameters and processing parameters are mentioned in SI table 1.

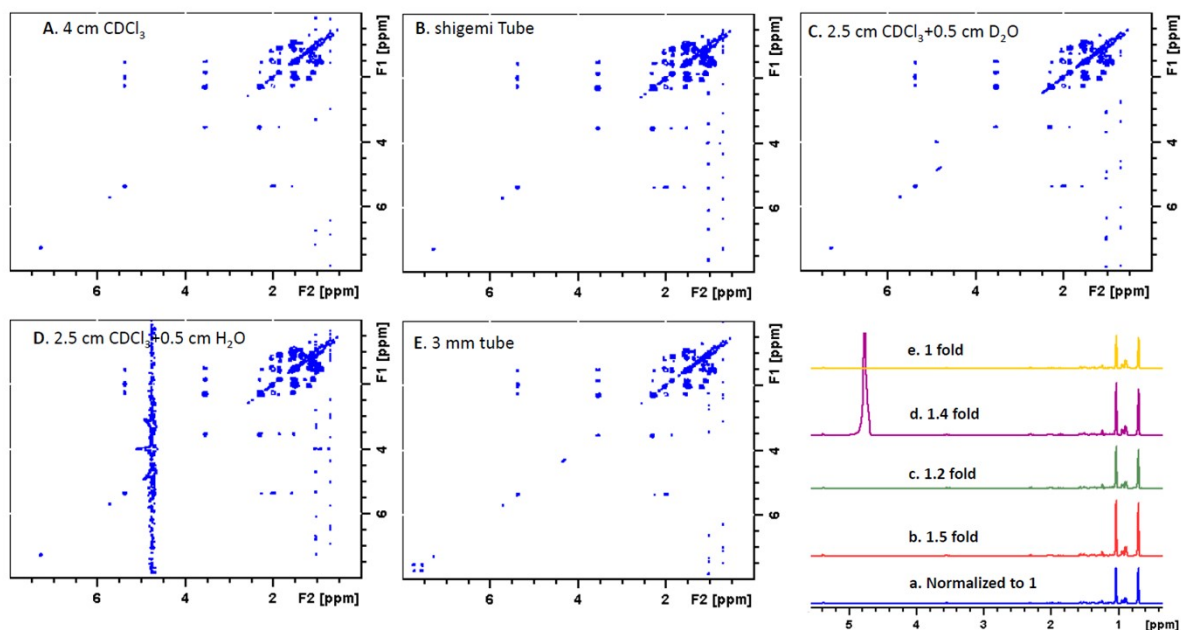


Figure S6. 2D ^1H - ^1H COSY spectra on a sample of cholesterol (5.1 mg) on different sample types (A) standard sample-4 cm, (B) Shigemi tube, (C) biphasic-S 3 cm (2.5 cm CDCl_3 +0.5 cm D_2O), (D) biphasic-S 3 cm (2.5 cm CDCl_3 +0.5 cm H_2O), and (E) 3 mm o.d. tube respectively. The projections a, b, c, d, and e are extracted from the panels A-E respectively and compares the improved sensitivity for the different sample types relative to the standard sample- 4 cm. Highest SNR enhancement is observed for Shigemi tube (1.5 fold) followed by biphasic-S $\text{CDCl}_3/\text{D}_2\text{O}$ (1.2 fold), and biphasic-S $\text{CDCl}_3/\text{H}_2\text{O}$ sample (1.4 fold) respectively. Small diameter tube 3 mm o.d. displayed same SNR as the standard sample 4 cm CDCl_3 in 5 mm o.d. tube. All spectra were recorded with the same acquisition and processing parameters. The experimental parameters and processing parameters are mentioned in SI table 1

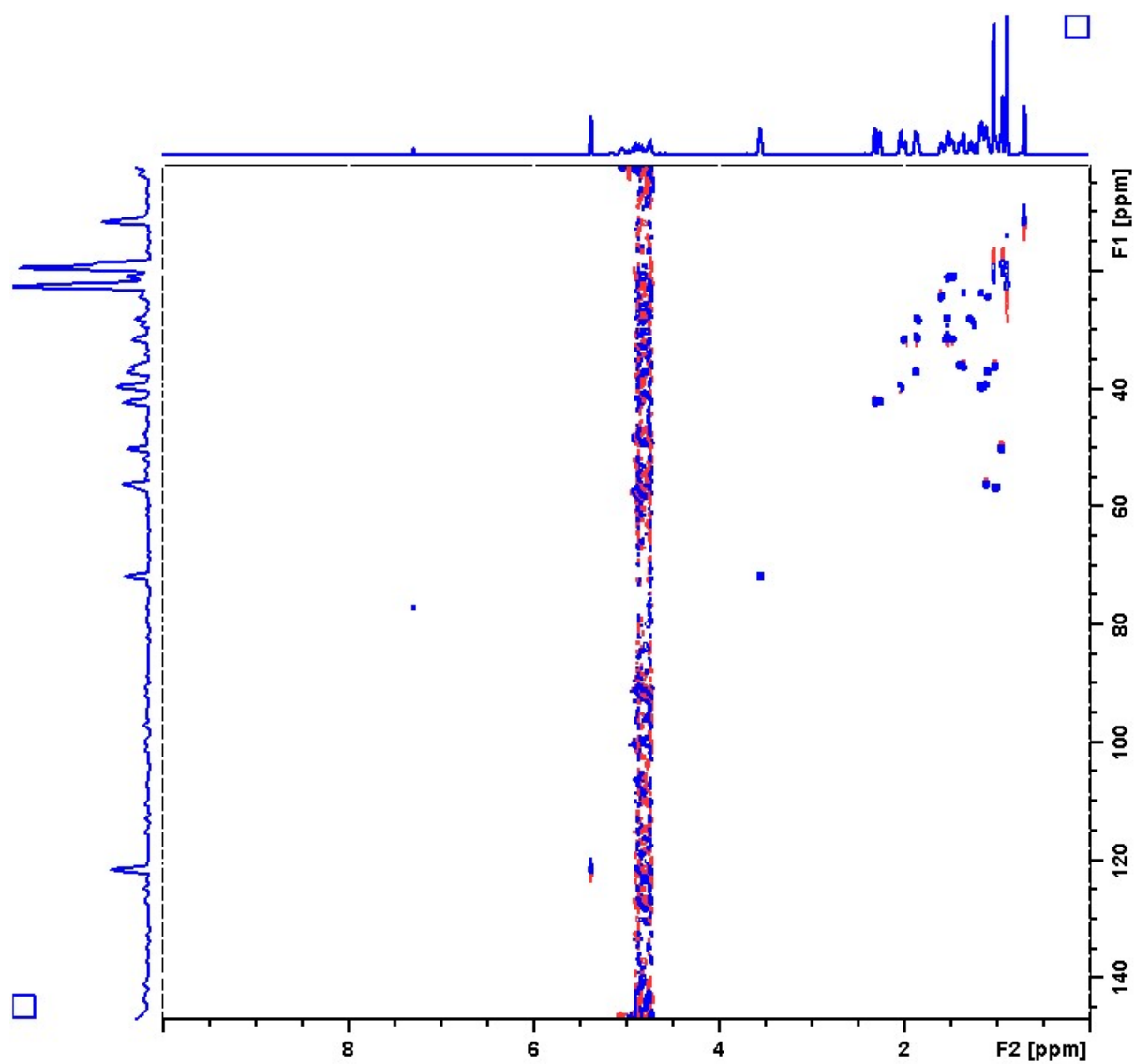


Fig S7. 800 MHz full hsqc spectrum of S-CDCl₃/H₂O symmetric cholesterol sample (5.1 mg)

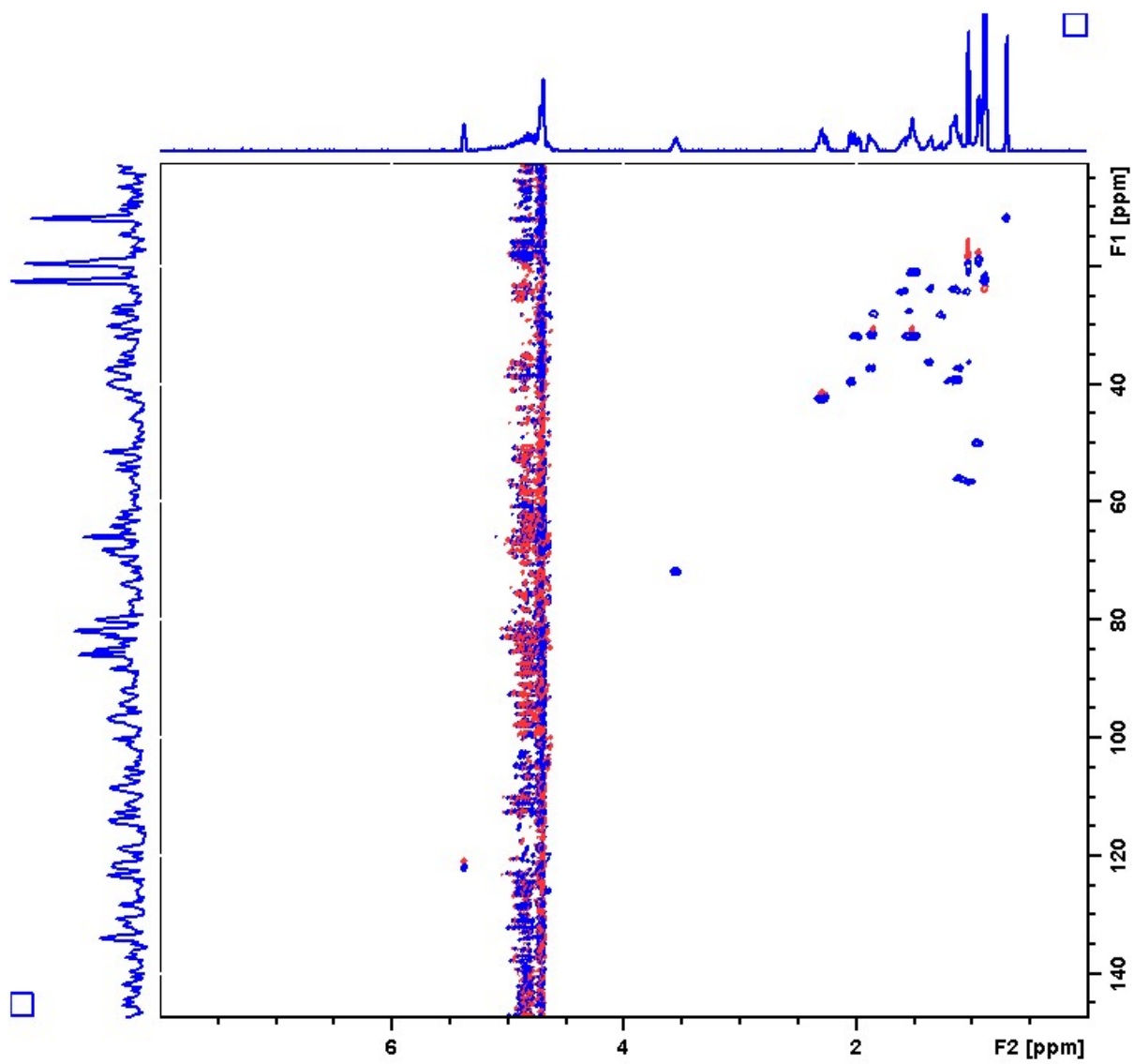


Fig S8. 400 MHz full hsqc spectrum of S-CDCl₃/H₂O cholesterol sample (5.1 mg)

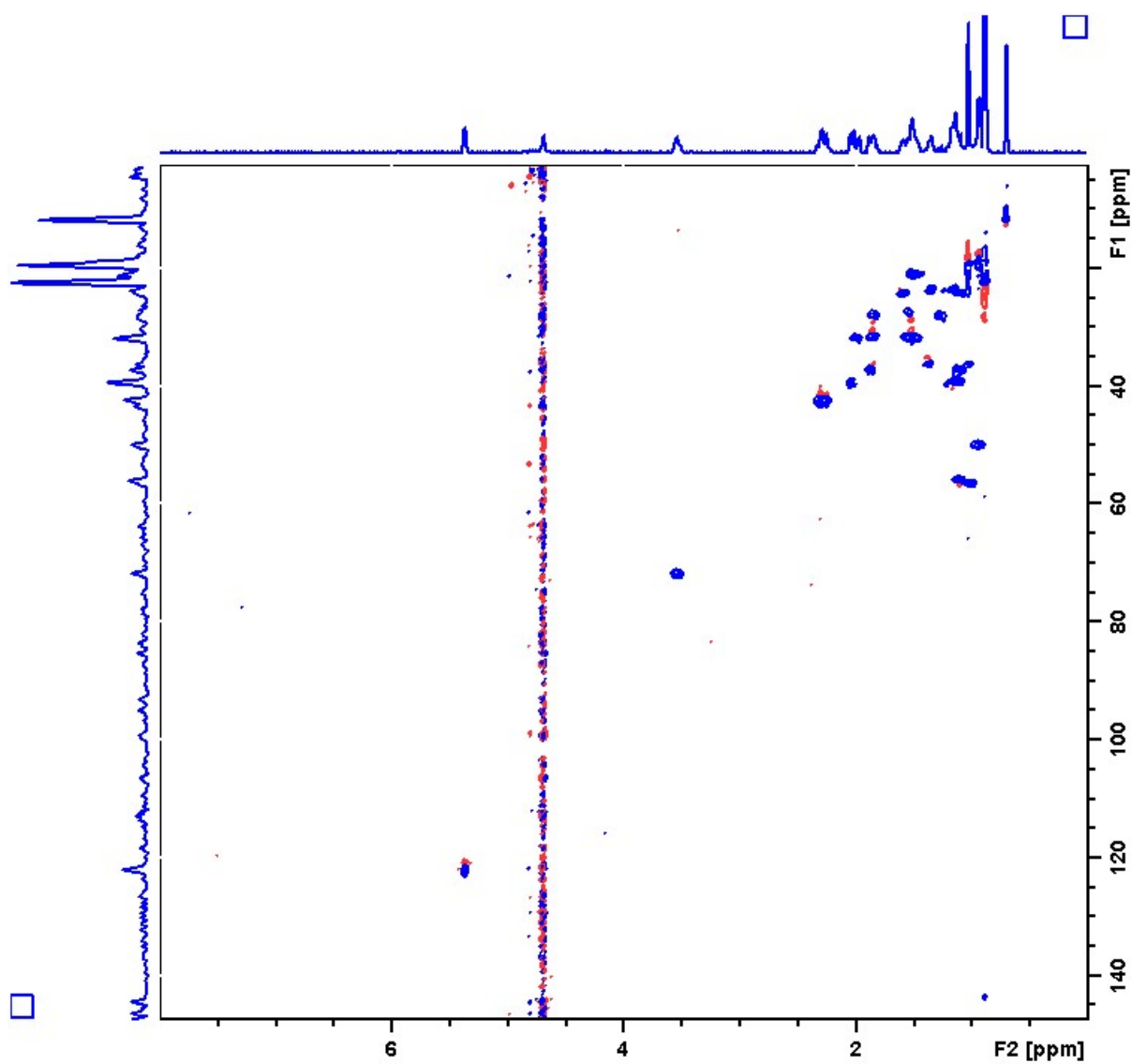


Fig S9. 400 MHz full hsqc spectrum of U-CDCl₃/H₂O cholesterol sample (5.1 mg). Small intensity of water peak is evident when compared to Figures S8 and S9 as the Unsymmetrical positioning pushes the water layer away from the receiver coil region.

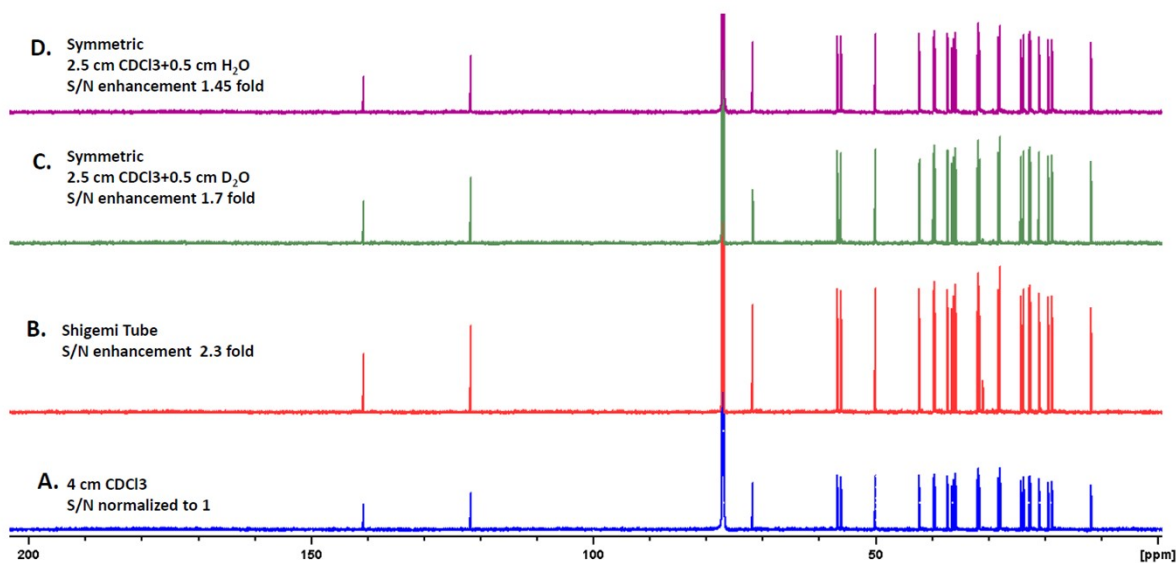


Figure S10. displays the comparison of ^{13}C NMR spectra on second sample of cholesterol (5.9 mg) from 800 MHz at the same noise level for- (A) standard sample (4 cm), (B) Shigemi tube, (C) biphasic-S 3 cm (2.5 cm CDCl₃+0.5 cm D₂O), (D) biphasic-S 3 cm (2.5 cm CDCl₃+0.5 cm H₂O), and (E) 3 mm o.d. tube samples respectively. The spectra from Shigemi tube display SNR enhancement by a factor of 2.3 fold relative to the standard 4 cm sample. The biphasic-S CDCl₃/D₂O and biphasic-S CDCl₃/H₂O in 3C and 3D display SNR enhancement by a factor of 1.7 and 1.45 fold, respectively, relative to that from the standard sample. 3 mm o.d. tube displayed lower sensitivity 0.87 fold relative to the standard sample. The experimental parameters and processing parameters are kept same as in Figure 6 of the main manuscript and detailed in SI table 1.

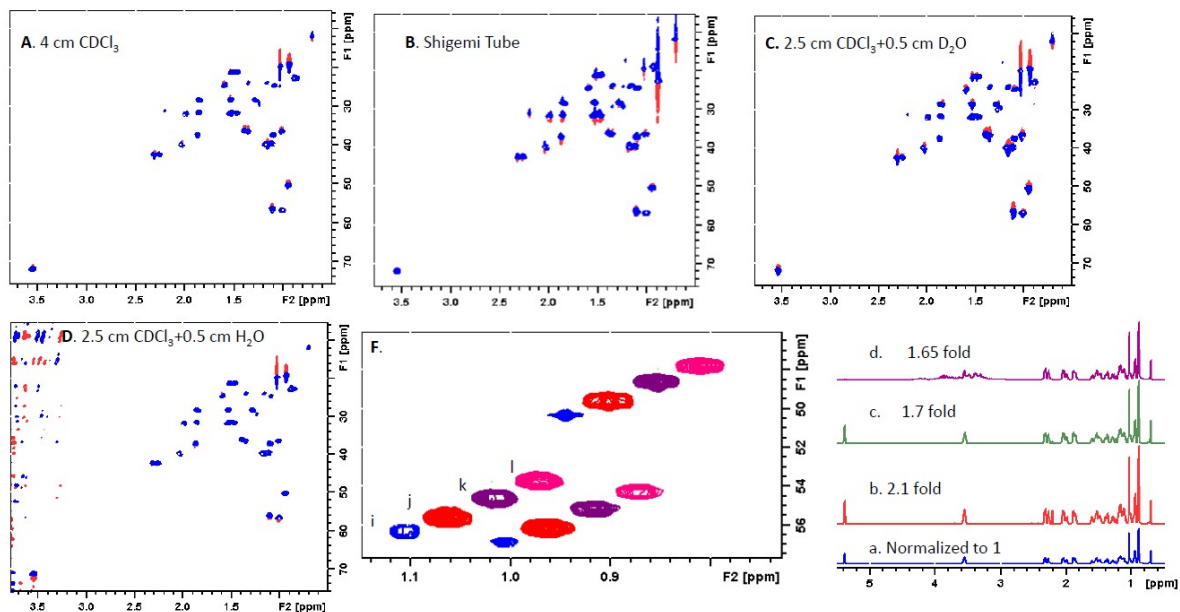


Figure S11. 2D ^1H - ^{13}C HSQC spectra at 800 MHz on second sample of cholesterol (5.9 mg) on different sample types (A) standard sample-4 cm, (B) shigemi tube, (C) biphasic-S 3 cm (2.5 cm CDCl_3 +0.5 cm D_2O), (D) biphasic-S 3 cm (2.5 cm CDCl_3 +0.5 cm H_2O), and (E) 3 mm o.d. tube respectively. All 2D ^1H - ^{13}C HSQC spectrum was recorded with the same acquisition and processing parameters. The projections a, b, c, d, and e compares the enhancement in signal to noise ratio for the different sample types relative to the standard sample- 4 cm. The enhancement is highest for shigemi tube (2.1 fold) followed by biphasic $\text{CDCl}_3/\text{D}_2\text{O}$ (1.7 fold), and biphasic $\text{CDCl}_3/\text{H}_2\text{O}$ sample (1.65) respectively. Small diameter tube 3 mm o.d. has lower sensitivity (0.85 fold) than standard sample. Panel F display comparison of the expanded and shifted cross-peaks plotted on same contour levels for standard- 4 cm (i), shigemi tube, biphasic-S ($\text{CDCl}_3/\text{D}_2\text{O}$), biphasic-S ($\text{CDCl}_3/\text{H}_2\text{O}$), and 3 mm o.d. tube samples respectively. The experimental parameters and processing parameters are kept same as in Figure 7 of the main manuscript and detailed in SI table 1.