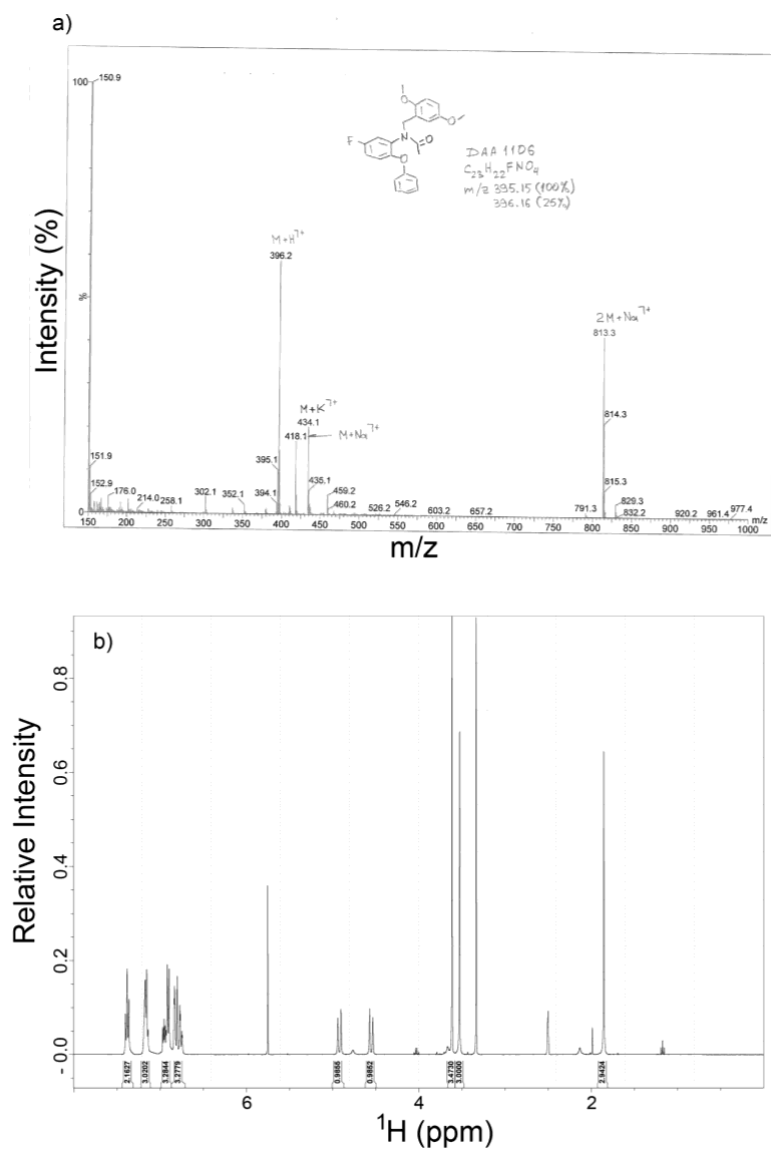## Supplementary Figures

a)

b)

Figure S1. Characterization of DAA1106. (a) ESI-MS spectrum. (b) 1D ^{1}H NMR spectrum.
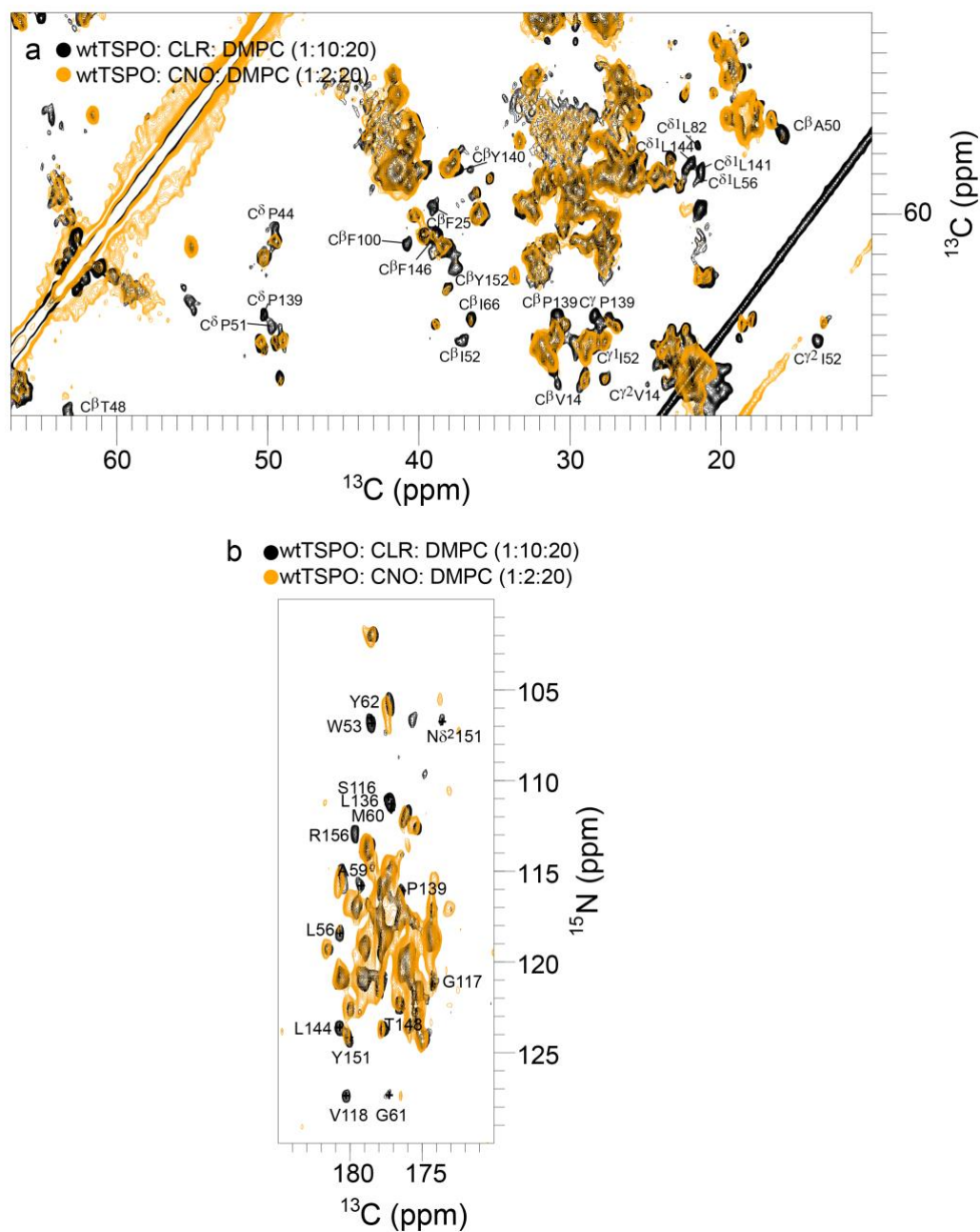
Figure S2. Interaction of wt mTSPO with the paramagnetic cholesterol analogue 25-doxyl-cholesterol (CNO). (a,b) Superposition of two-dimensional PDSD (a) and NCO (b) spectra of mTSPO in complex with DAA1106 in the presence of a 10-fold excess of diamagnetic cholesterol (CLR; black) and the presence of a 2-fold excess of CNO (orange) over protein. Cross-peaks strongly broadened by paramagnetic CNO are labeled by residue name.
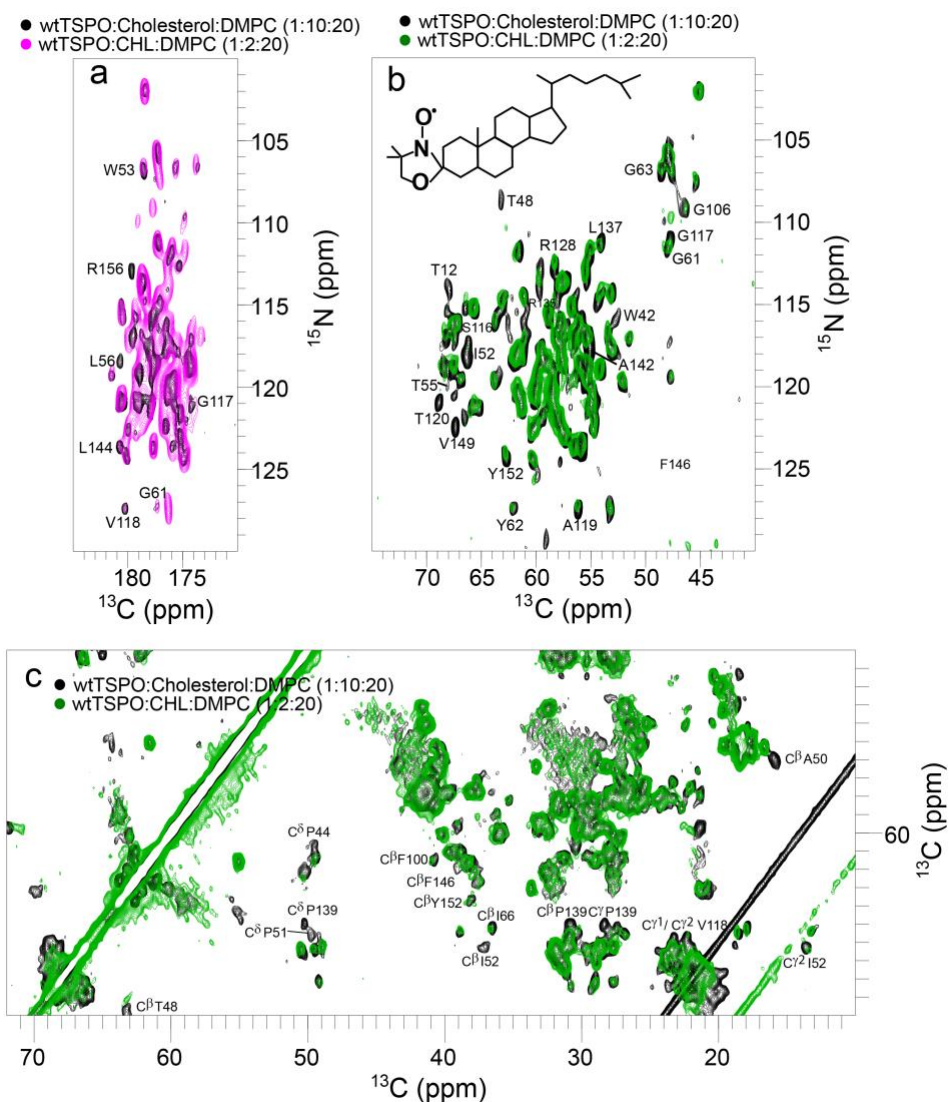
Figure S3. Interaction of mTSPO with the paramagnetic cholesterol analogue 3-beta-doxyl-5-alpha-cholestane (CHL). Superposition of two-dimensional NCO (a), NCA (b) and PDSD (c) spectra of mTSPO in complex with DAA1106 in the presence of a 10-fold excess of diamagnetic cholesterol (black) and the presence of a 2-fold excess of CHL (pink/green) over protein. Cross-peaks strongly broadened by the paramagnetic CHL are labeled by residue/atom name.
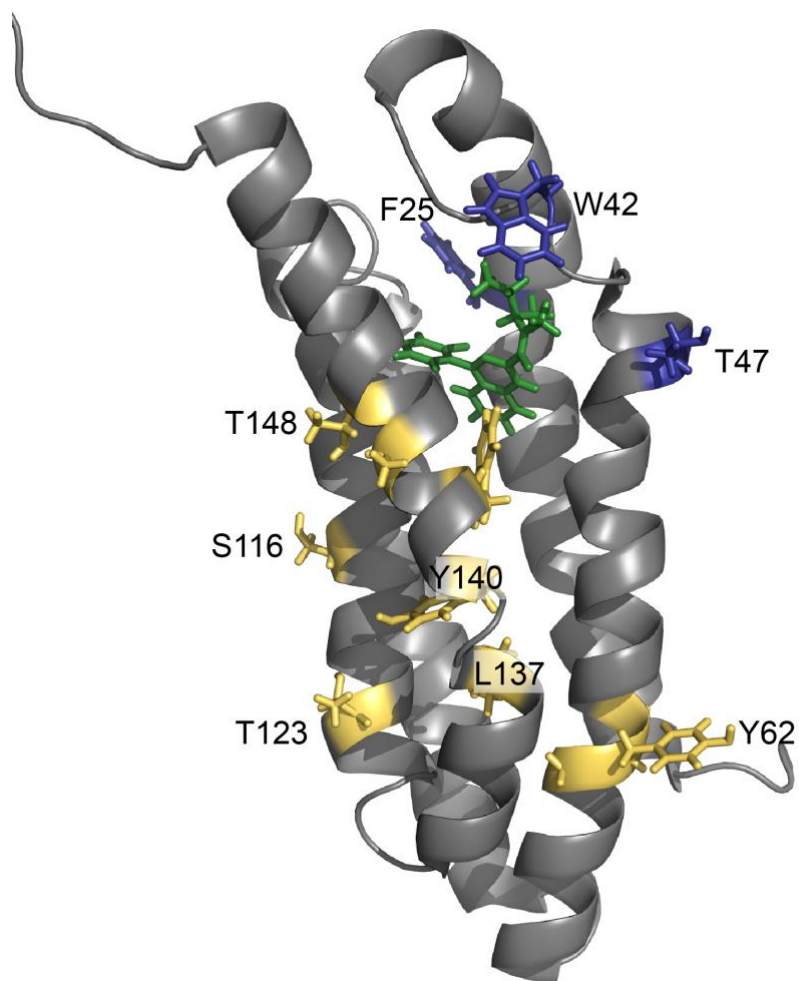
Figure S4. Mapping of residues strongly broadened by CHL (CNO) are highlighted in mTSPO 3D structure in blue (orange). This is an enlarged view of the inset shown in Fig. 3b.
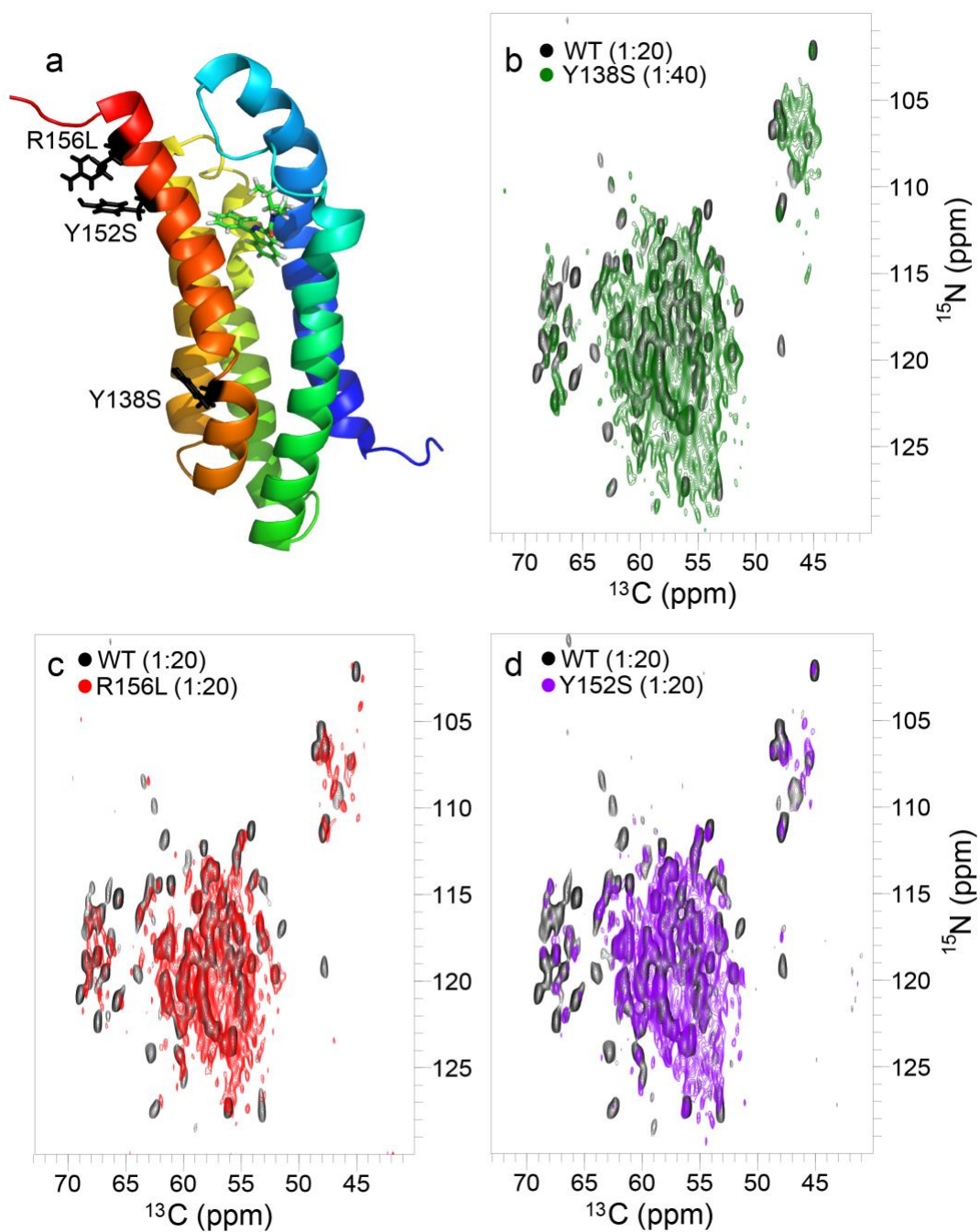
Figure S5. Two-dimensional NCA spectra of mTSPO mutants. (a) Sites of mutation in the 3D structure of mTSPO (PDB id: 2MGY). (b-d): Spectra of Y138S, R156L and Y152S, respectively. In black, the NCA spectrum of wild-type mTSPO is shown. Protein:DMPC molar ratios are indicated.

Table S1. Acquisition parameters of solid-state NMR experiments recorded for $^{13}C,^{15}N$-labeled mTSPO proteins reconstituted into DMPC liposomes.

Frequency (MHz)	Experiment	Acquisition details: Complex points; t_{max} (ms); SW	Scans	Spinning Speed (kHz)
1. mTSPO/DAA1106 in DMPC (protein-to-lipid molar ratio of 1:20)				
2. G87V-mTSPO/DAA1106 in DMPC (protein-to-lipid molar ratio of 1:20)				
950	2D NCA $^1H/^{15}N$ Ramp CP (500μs) $^{15}N/^{13}C$ Tang CP (3800us)	$\omega_1(^{13}C)$: 1792; 12.5; 299 $\omega_2(^{15}N)$: 80; 12.9; 32	1. 144 2. 256	19
950	2D NCO $^1H/^{15}N$ Ramp CP (500μs) $^{15}N/^{13}C$ Tang CP (4200us)	$\omega_1(^{13}C)$: 1792; 12.5; 299 $\omega_2(^{15}N)$: 80; 12.9; 32	160	19
950	2D PDSD $^1H/^{13}C$ Ramp CP (500μs) 20ms mixing	$\omega_1(^{13}C)$: 1880; 15; 262 $\omega_2(^{13}C)$: 1260; 12; 220	1. 112 2. 96	11
R156L-mTSPO/DAA1106 in DMPC (1:20)				
950	2D NCA $^1H/^{15}N$ Ramp CP (500μs) $^{15}N/^{13}C$ Tang CP (1500us)	$\omega_1(^{13}C)$: 1792; 12.5; 299 $\omega_2(^{15}N)$: 80; 12.9; 32	416	19
950	2D PDSD $^1H/^{13}C$ Ramp CP (400μs) 20ms mixing	$\omega_1(^{13}C)$: 1618; 13; 262 $\omega_2(^{13}C)$: 1260; 12; 220	96	12.5
1. Y138S-mTSPO/DAA1106 in DMPC (1:40)				
2. Y138S-mTSPO/DAA1106 : 3-beta-doxyl-5-alpha-cholestane (CHL) in DMPC (1:2:40)				
850	2D NCA $^1H/^{15}N$ Ramp CP (800μs) $^{15}N/^{13}C$ Tang CP (3600us)	$\omega_1(^{13}C)$: 1178; 12; 230 $\omega_2(^{15}N)$: 82; 14; 34	1. 640 2. 672	19
850	2D PDSD $^1H/^{13}C$ Ramp CP (500μs) 20ms mixing	$\omega_1(^{13}C)$: 1536; 14.3; 262 $\omega_2(^{13}C)$: 1280; 12; 220	1. 220 2. 152	11
G87V-mTSPO/DAA1106: 3-beta-doxyl-5-alpha-cholestane (CHL) in DMPC (1:2:20)				
850	2D NCA $^1H/^{15}N$ Ramp CP (400μs) $^{15}N/^{13}C$ Ramp CP (1400us)	$\omega_1(^{13}C)$: 1216; 11; 260 $\omega_2(^{15}N)$: 72; 13; 32	448	19
850	2D NCO $^1H/^{15}N$ Ramp CP (400μs) $^{15}N/^{13}C$ Ramp CP (1100us)	$\omega_1(^{13}C)$: 1216; 11; 260 $\omega_2(^{15}N)$: 72; 13; 32	384	19
850	2D PDSD $^1H/^{13}C$ Ramp CP (400μs) 20ms mixing	$\omega_1(^{13}C)$: 1530; 14.2; 250 $\omega_2(^{13}C)$: 1280; 12; 250	144	11
G87V-mTSPO/DAA1106: 3-beta-doxyl-5-alpha-cholestane (CHL) in DMPC (1:0.5:50)				
950	2D NCA $^1H/^{15}N$ Ramp CP (400μs)	$\omega_1(^{13}C)$: 1792; 12.5; 299	512	19

	^{15}N/^{13}C Tang CP (1400us)	ω_2(^{15}N): 80; 12.9; 32		
950	2D NCO ^{1}H/^{15}N Ramp CP (400μs) ^{15}N/^{13}C Tang CP (1200us)	ω_1(^{13}C): 1792; 12.5; 299 ω_2(^{15}N): 80; 12.9; 32	512	19
950	2D PDSD ^{1}H/^{13}C Ramp CP (400μs) 20ms mixing	ω_1(^{13}C): 1880; 15; 262 ω_2(^{13}C): 1260; 12; 220	168	12.5
1. Y152S-mTSPO/DAA1106 : DMPC (1:20) 2. Y152S-mTSPO/DAA1106 : DMPC : Cholesterol (1:20:10) 3. mTSPO/DAA1106:Cholesterol:DMPC (1:10:20)				
950	2D NCA ^{1}H/^{15}N Ramp CP (500μs) ^{15}N/^{13}C Tang CP (3800 μs)	ω_1(^{13}C): 1792; 12.5; 299 ω_2(^{15}N): 80; 12.9; 32	1. 640 2. 640 3. 144	19
950	2D NCO ^{1}H/^{15}N Ramp CP (500μs) ^{15}N/^{13}C Tang CP (4200 μs)	ω_1(^{13}C): 1792; 12.5; 299 ω_2(^{15}N): 80; 12.9; 32	1. 384 2. 384 3. 160	19
950	2D PDSD ^{1}H/^{13}C Ramp CP (500μs) 20 ms mixing	ω_1(^{13}C): 1880; 15; 262 ω_2(^{13}C): 1260; 12; 220	1. 96 2. 120 3. 128	11
G87V-mTSPO/DAA1106 : Cholesterol in DMPC (1:10:20)				
850	2D NCA ^{1}H/^{15}N Ramp CP (500μs) ^{15}N/^{13}C Ramp CP (4000us)	ω_1(^{13}C): 1216; 11; 260 ω_2(^{15}N): 72; 13; 32	480	20
850	2D NCO ^{1}H/^{15}N Ramp CP (500μs) ^{15}N/^{13}C Ramp CP (3200us)	ω_1(^{13}C): 1216; 11; 260 ω_2(^{15}N): 72; 13; 32	416	20
850	2D PDSD ^{1}H/^{13}C Ramp CP (400μs) 20ms mixing	ω_1(^{13}C): 1488; 15; 233 ω_2(^{13}C): 1200; 12; 233	160	11
1. mTSPO/DAA1106 : 3-beta-doxyl-5-alpha-cholestane (CHL) in DMPC (1:2:20) 2. mTSPO/DAA1106 : 25-doxyl-cholesterol (CNO) in DMPC (1:2:20)				
850	2D NCA ^{1}H/^{15}N Ramp CP (500μs) ^{15}N/^{13}C Ramp CP (1300/1600us)	ω_1(^{13}C): 1178; 12; 230 ω_2(^{15}N): 82; 14; 34	1. 384 2. 256	19
850	2D NCO ^{1}H/^{15}N Ramp CP (500μs) ^{15}N/^{13}C Ramp CP (1200us)	ω_1(^{13}C): 1280; 13.4; 222 ω_2(^{15}N): 82; 14; 34	1. 384 2. 256	19
850	2D PDSD ^{1}H/^{13}C Ramp CP (400μs) 20ms mixing	ω_1(^{13}C): 1536; 14.3; 250 ω_2(^{13}C): 1280; 12; 250	1. 136 2. 120	11