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Supplemental Information

Surface-Binding to Cardiolipin Nanodomains Triggers Cytochrome *c* Pro-apoptotic Peroxidase Activity via Localized Dynamics

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Table S1. Related to Figure 3 and S4. Chemical shift assignments of cyt c bound to 50 mol-% TOCL and 50 mol-% DOPC containing membrane. The ^{13}C and ^{15}N chemical shifts are referenced to aqueous DSS and ammonia, respectively.

Res. Num.	Type	N	C'	C^{α}	C^{β}	C^{γ}	$C^{\gamma 2}$	C^{δ}	$C^{\delta 2}$
2	Asp	124.6*		52.7*					
3	Val	124.1*		66.2*					
6	Gly	108.1*	174.4	46.5*					
9	Ile	119.7	177.1	64.4	37.7	27.9	18.2	14.6	
10	Phe	121.1	179.2	62.5	39.9				
11	Val	122.6	178.0	66.6*	32.4	23.5*	21.7		
13	Lys	112.1*		57.0*					
14	Cys	115.3*		54.6*					
15	Ala	124.5	179.2	55.4	20.8				
19	Thr	115.7	176.8	61.3	72.5	23.8			
20	Val	112.1*	179.0	62.3	35.1	24.0	18.8		
23	Gly	118.8	175.1	45.7					
24	Gly	108.9*	173.1	45.2*					
27	Lys	126.4*		55.4*					
31	Asn	127.9*		56.5*					
34	Gly	115.4	174.3	46.2					
40	Thr	108.7*	176.9	61.3	68.5	23.8			
41	Gly	109.5*	175.0	45.6*					
42	Gln	113.2*		54.5*					
43	Ala	126.0	176.4	51.7*	18.9*				
47	Thr	124.3	171.1	61.0	67.2	22.2			
48	Tyr	127.2	178.6	57.6	41.3				
49	Thr	113.2	176.1	62.0	70.9	22.4			
50	Asp	123.4	178.1	57.1	40.6[a]				
51	Ala	120.1	180.4	55.1*	19.2*				
52	Asn	117.8*		55.1*					
56	Gly	104.3*	175.0	46.8*					
59	Trp	129.6*		57.1*					
63	Thr	109.0	177.7	62.9	68.9	24.6			
64	Leu	122.1	178.3	57.9*	42.0	27.3		26.3	23.0*
68	Leu	111.1*		55.5*					
70	Asn	104.9*		52.5*					
74	Tyr	121.9	176.9	61.3	40.8				
75	Ile	115.6	171.3	59.7*	38.6	28.9	19.4*	13.8	
76	Pro		179.5	63.3	32.3	28.3		51.2	
77	Gly	112.9	176.7	45.0					
78	Thr	115.2	173.8	62.0*	70.9*	22.7			
81	Ile	137.4		61.2	35.5	26.9	18.0	11.4	
82	Phe	127.2*		60.0*					
83	Ala		177.5	55.3	19.6				
84	Gly	100.7	171.9	43.0					
85	Ile	121.1		59.1*	40.3	26.4	18.2*	13.7	
89	Thr	111.2		65.2*	68.1*	22.8			
90	Glu	118.2	178.7	59.3	30.5	37.7			
91	Arg	117.4	177.6	61.2	30.9				
92	Glu	117.3	180.4	59.3	29.5				
93	Asp	123.8	177.6	57.9	39.3				
94	Leu	121.0	178.4	57.8	41.0	27.2		26.8	23.0
95	Ile	119.4	176.4	66.0	37.5	31.8	17.9	14.6	
96	Ala	123.0	181.1	54.8*	17.6*				
102	Thr	102.6*		62.4*					
103	Asn	118.0*		52.6*					

*resonances in isolated regions assigned based on the closest correspondence in solution NMR shifts.
[a] ambiguous assignment.

Table S2. Related to Figures 3-5 and S3-S6. A summary of SSNMR samples, experiments, and experimental parameters.

Figure	NMR sample	Expt.	NS	Temp	MAS	Field	RD	^{1}H 90	TPPM decoupling	t_1 evol.	t_2 evol.	Mix.	H-C/N CP	C-N CP	τ_1, τ_2	Exp. Time
				(K)	(kHz)	T	(s)	(µs)	(kHz)	(ms)	(ms)	(ms)	(ms)	(ms)	(ms)	(h)
2b	DLcyt c/TOCLDOPC	^{31}P	64	275*	10	15.6	5	7 (^{31}P)			36					0.1
2b	n.a. cyt c/TOCLDOPC	^{31}P	18432	282*	0	14.1	4	9.7 (^{31}P)	85 (CW)		21.2					20.5
2b	TOCLDOPC	^{31}P	18432	282*	0	14.1	4	9.7 (^{31}P)	85 (CW)		21.2					20.5
2c	DLcyt c/TLCLDOPC	CCP	1024	265*	8	14.1	2	3	83.3		27.7					0.58
2d	DLcyt c/TLCLDOPC	HH-CP-HETCOR	128	265*	8	14.1	2	3	83.3	6.2	27.7	0	1			4.3
2d, S3d	DLcyt c/TLCLDOPC	HH-CP-HETCOR	128	265*	8	14.1	2	3	83.3	6.2	27.7	50	1			4.4
3b, 4c, S8c	DLcyt c/TOCLDOPC	DARR	64	252	10	15.6	2	2.8	89	5	18	25	1			18.1
4a, S5, S8a	DLcyt c/TOCLDOPC	NCA	704	264	10	15.6	1.5	2.8	89	10	13.7		1.4	3.8		24
4a	DLcyt c/TOCLDOPC	NCA	704	252	10	15.6	1.5	2.8	83.3	10	13.7		1.4	3.8		24
4a	DLcyt c/TOCLDOPC	NCA	656	240	10	15.6	1.5	2.76	90.6	10	13.7		1.4	3.8		22.6
4c	DLcyt c/TOCLDOPC	DARR	64	240	10	15.6	2	2.76	83.3	5	18	25	1			18.1
4c	DLcyt c/TOCLDOPC	DARR	64	264	10	15.6	2	2.8	83.3	5	18	25	1			18.1
5a	DLcyt c/TLCLDOPC	INEPT	256	285*	10	14.1	2	3.15	84	10.4	46.1				1.8,1.0	7.5
5b, S6f	DLcyt c/TLCLDOPC	INEPT	2048	285*	10	14.1	2	3.15	79.4		46.1				1.8, 1.0	1.18
5b, S6f	DLcyt c/TLCLDOPC	INEPT	2048	265*	8	14.1	2	3.05	82		46.1				1.5, 0.75	1.18
S3a	TOCLDOPC	INEPT	128	282	10	15.6	2	2.85	87.7	20	36.9				1.6, 0.8	22
S3b	n.a. cyt c/TOCLDOPC	INEPT	64	282	10	15.6	2	2.9	86	20	36.9				1.8, 0.8	11
S3c	DLcyt c/TOCLDOPC	INEPT	128	282	10	15.6	2	2.8	73.5	5.1	36.9				1.6, 0.8	7.5
S4	DLcyt c/TOCLDOPC	NCACX	176	252	10	15.6	1.7	2.8	89	5, 2.9	13.6 (t_3)	25	1.4	3.8		138
S4	DLcyt c/TOCLDOPC	NCOCX	176	252	10	15.6	1.7	2.8	89	5, 3.2	13.6 (t_3)	25	1.4	3.8		116.5
S6a	DLcyt c/TOCLDOPC	NCP	2048	282	10	15.6	1.5	2.79	89		16.6		1.4			0.9
S6a	DLcyt c/TOCLDOPC	NCP	176	264	10	15.6	1.5	2.79	89		16.6		1.4			0.075
S6a	DLcyt c/TOCLDOPC	NCP	1024	252	10	15.6	2	2.9	70		16.6		1			0.58
S6a	DLcyt c/TOCLDOPC	NCP	176	249	10	15.6	1.5	2.8	89		16.6		1.4			0.075
S6a	DLcyt c/TOCLDOPC	NCP	176	245	10	15.6	1.5	2.8	89		16.6		1.4			0.075
S6a	DLcyt c/TOCLDOPC	NCP	176	240	10	15.6	1.5	2.76	90		16.6		1.4			0.075
S6b	DLcyt c/TOCLDOPC	SPECIFIC CP	176	264	10	15.6	1.5	2.8	89		13.7		1.4	3.8		0.075
S6b	DLcyt c/TOCLDOPC	SPECIFIC CP	176	252	10	15.6	1.5	2.8	89		13.7		1.4	3.8		0.075
S6b	DLcyt c/TOCLDOPC	SPECIFIC CP	176	249	10	15.6	1.5	2.8	89		13.7		1.4	3.8		0.075
S6b	DLcyt c/TOCLDOPC	SPECIFIC CP	176	245	10	15.6	1.5	2.8	89		13.7		1.4	3.8		0.075
S6b	DLcyt c/TOCLDOPC	SPECIFIC CP	176	240	10	15.6	1.5	2.76	90		13.7		1.4	3.8		0.075
S6c	DLcyt c/TOCLDOPC	INEPT	256	282	10	15.6	2	2.85	71.6		36.9				1.6, 0.8	0.15
S6c	DLcyt c/TOCLDOPC	INEPT	4096	264	10	15.6	2	2.79	83.3		36.9				1.4, 1.0	2.3
S6c	DLcyt c/TOCLDOPC	INEPT	2048	252	10	15.6	2	2.79	83.3		36.9				1.4, 1.0	1.2
S6c	DLcyt c/TOCLDOPC	INEPT	2048	249	10	15.6	2	2.8	89		36.9				1.4, 1.0	1.2
S6c	DLcyt c/TOCLDOPC	INEPT	2048	245	10	15.6	2	2.8	89		36.9				1.4, 1.0	1.2
S6c	DLcyt c/TOCLDOPC	INEPT	3072	240	10	15.6	2	2.76	90		36.9				1.4, 1.0	1.2
S6d	DLcyt c/TOCLDOPC	^{1}H MAS	32	264	10	15.6	2	2.79			68.2					0.017
S6d	DLcyt c/TOCLDOPC	^{1}H MAS	32	252	10	15.6	2	2.79			68.2					0.017
S6d	DLcyt c/TOCLDOPC	^{1}H MAS	32	249	10	15.6	2	2.8			68.2					0.017
S6d	DLcyt c/TOCLDOPC	^{1}H MAS	32	245	10	15.6	2	2.8	89		68.2					0.017
S6d	DLcyt c/TOCLDOPC	^{1}H MAS	32	240	10	15.6	2	2.76			68.2					0.017
S6d	DLcyt c/TOCLDOPC	^{1}H MAS	32	282	10	15.6	2	2.8			68.2					0.017
S6e	DLcyt c/TLCLDOPC	NCP	2048	268*	8	14.1	2	3.05	82		25.7		1.2			1.18
S6e	DLcyt c/TLCLDOPC	NCP	2048	265*	8	14.1	2	3.05	82		25.7		1.2			1.18
S6f	DLcyt c/TLCLDOPC	INEPT	2048	285*	10	14.1	2	3.15	82		46.1				1.8, 1.0	1.18
S6g	DLcyt c/TLCLDOPC	^{1}H MAS	32	285*	10	14.1	2	3.15			255.7					0.017
S6g	DLcyt c/TLCLDOPC	^{1}H MAS	32	268*	8	14.1	2	3.05			255.7					0.017
S6g	DLcyt c/TLCLDOPC	^{1}H MAS	32	265*	8	14.1	2	3.05			255.7					0.017
S7	DLcyt c/TLCLDOPC	TOBSY	128	285*	10	14.1	2	3.15	83	6.3	33.9				1.8, 1.0	39
S8a	DLcyt c/TLCLDOPC	NCA	512	265*	8	14.1	2	3.05	83	10.7	20.5		1.2	5.4		17.5
S8c	DLcyt c/TLCLDOPC	DARR	256	265*	8	14.1	2	3.05	82	7	28.3	25	1			72
															Total	580.1

*Temperature read from the spectrometer without further calibration

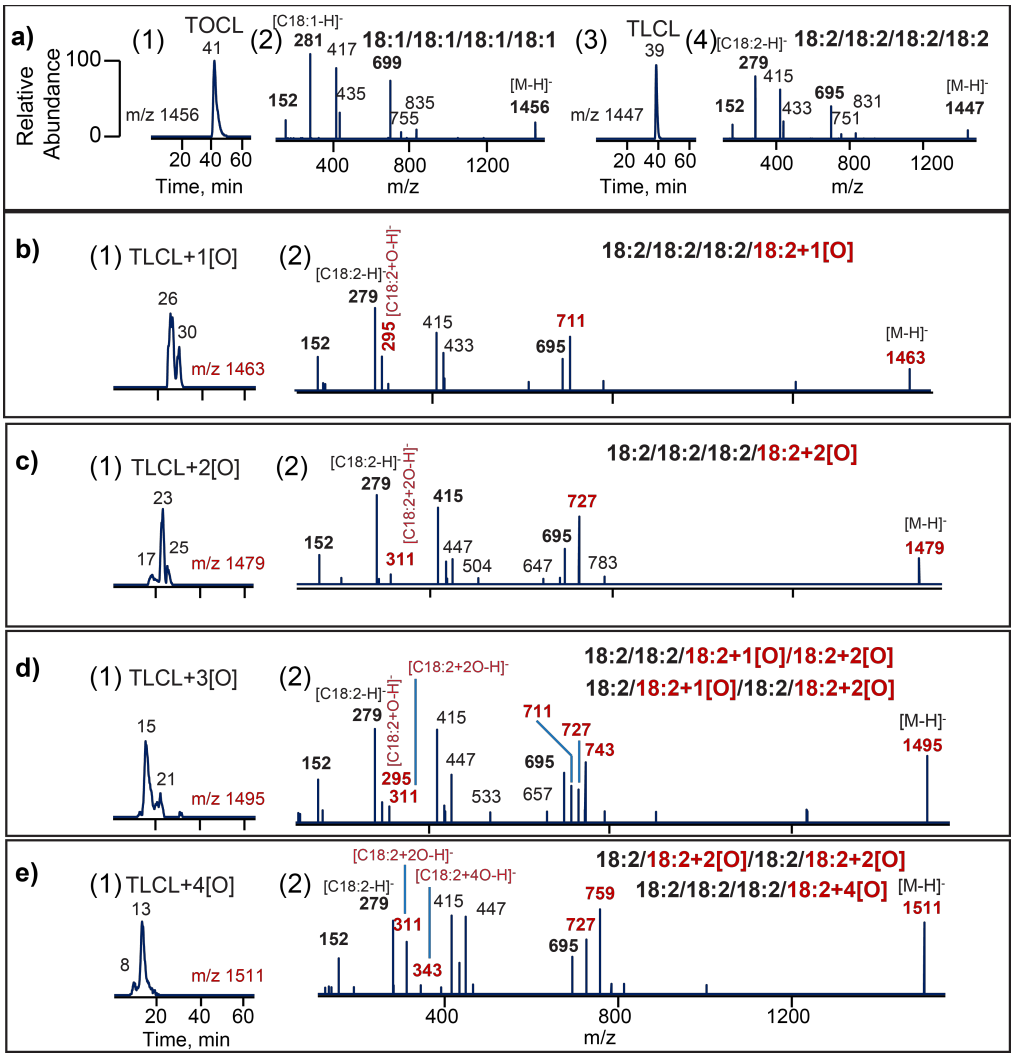

Figure S1. Related to Figure 1. Typical LC-ESI-MS profiles and MS/MS spectra of CL molecular species. a) (1) and (3) LC-MS profiles of intact TOCL and TLCL, respectively; (2) and (4) MS/MS spectra of intact TOCL and TLCL, respectively. Typical ions formed during the MS/MS fragmentation of CL include fragments of diacylglycerolphosphate (a, b, a+56, or b+136), monoacylglycerolphosphate and fatty acid carboxylate anions. For example, MS/MS fragmentation of TLCL (RT 39 min) resulted in the formation of the fragments: diacylglycerol phosphatidate (*a=b m/z 695, a+56= m/z 751; a+136= m/z 831*), monoacylglycerol phosphatidate fragment (*m/z 415*) and linoleic acid carboxylate anion (*m/z 279*) leading to the identification of the symmetric structure of TLCL (C18:2/C18:2/C18:2/C18:2). The same three most intense peaks corresponded to free oleate (*m/z 281*), monoacylglycerol phosphatidate (*m/z 417*), and diacylglycerol phosphatidate (*m/z 699*) for TOCL (C18:1/C18:1/C18:1/C18:1). **b) - e)** LC-MS profiles and MS/MS spectra for oxygenated TLCL molecular species at m/z 1463, 1479, 1495 and 1511, respectively, which reflect oxygenation products containing one, two, three, and four oxygen atoms as indicated. The location of the oxidative modifications among the four C18:2 acyl chains is represented with the "18:2/18:2/18:2/18:2+1[O]" labels in each panel. MS/MS analysis of CL containing one oxygen (m/z 1463.9599) revealed the appearance of two diacylglycerol phosphatidate fragments m/z 695 (*a*), 711 (*b*), linoleic acid carboxylate anion (m/z 279) and its oxygenated derivatives (m/z 295). The fragment at m/z 695 originated from non-oxidized fragment of TLCL, whereas ion with m/z 711 represents oxidized fragment of TLCL. Detailed MS/MS analysis showed that the product with m/z 1463 corresponds to four different oxygenated CL molecular species observed at different retention times (26 and 30 min). MS/MS fragmentation of oxidized fragment (m/z 711) revealed the formation of monoacylglycerol phosphatidate (m/z 415) and oxygenated carboxylate anion of linoleic acid (m/z 295). MS/MS of the parent ion with m/z 295 revealed two products, containing hydroxy-groups at positions C9 or C13 with acyl chains 18:2/18:2/18:2/9-HODE; 18:2/18:2/18:2/13-HODE (retention time 26.3 min) and two products, containing epoxy-group 18:2/18:2/18:2/9,10-EpOME; 18:2/18:2/18:2/12,13-EpOME (30.2 min), respectively.

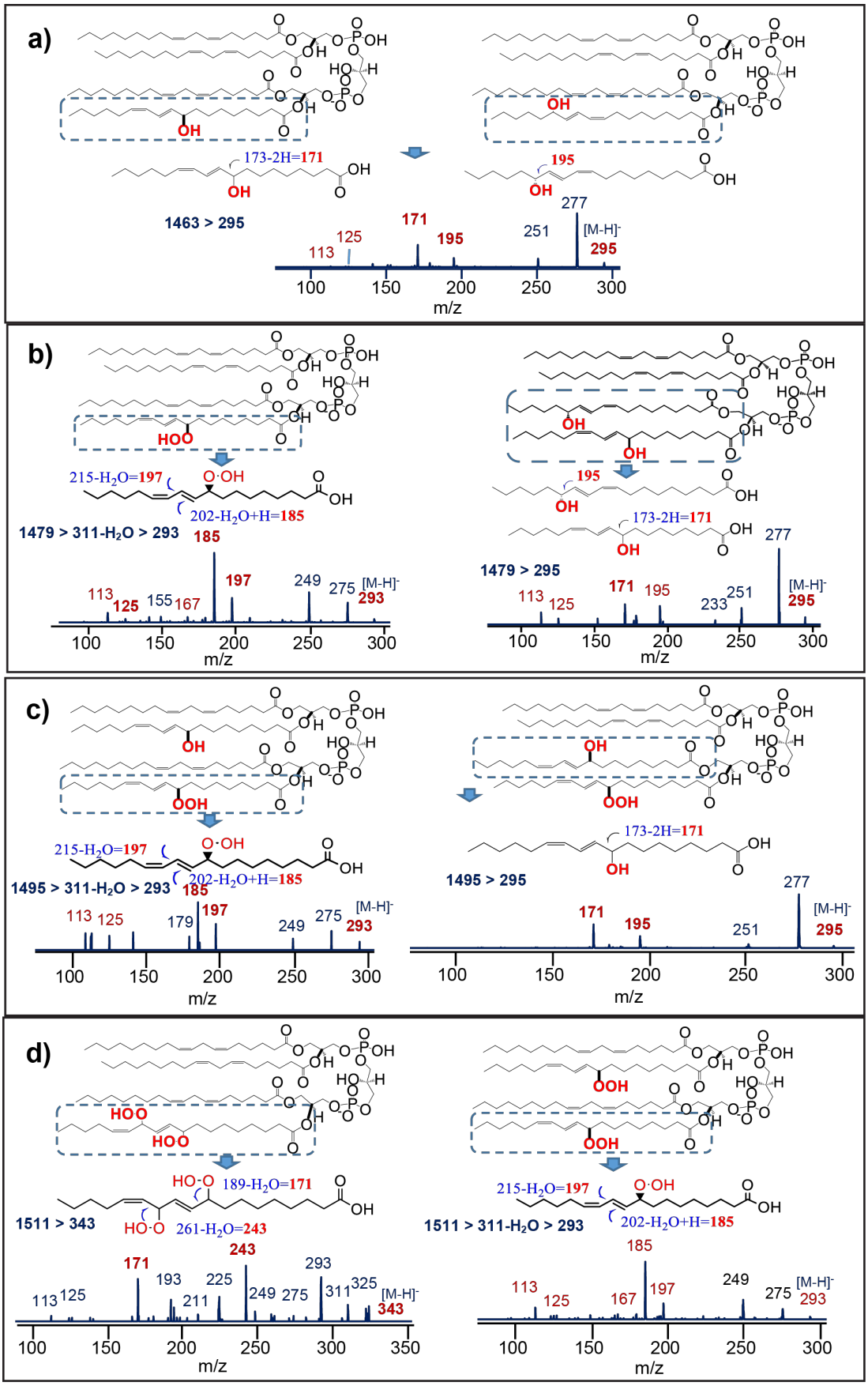
a)
OH
173-2H=171
OH
1463 > 295
171
125
113
195
251
277
[M-H]-
295
100
150
200
250
300
m/z
OH
195
OH
b)
HOO
215-H2O=197
O·OH
202-H2O+H=185
1479 > 311-H2O > 293
185
113
125
155
167
197
249
275
[M-H]-
293
100
150
200
250
300
m/z
OH
OH
195
OH
173-2H=171
OH
277
1479 > 295
113
125
171
195
233
251
[M-H]-
295
100
150
200
250
300
m/z
c)
OH
OOH
215-H2O=197
O·OH
202-H2O+H=185
1495 > 311-H2O > 293
185
113
125
179
197
249
275
[M-H]-
293
100
150
200
250
300
m/z
OH
OOH
173-2H=171
OH
1495 > 295
277
171
195
251
[M-H]-
295
100
150
200
250
300
m/z
d)
HOO
HOO
HO·O
189-H2O=171
1511 > 343
HO·O
261-H2O=243
243
171
113
125
193
211
225
249
275
293
311
325
[M-H]-
343
100
150
200
250
300
350
m/z
OOH
OOH
215-H2O=197
O·OH
202-H2O+H=185
1511 > 311-H2O > 293
185
113
125
167
197
249
275
[M-H]-
293
100
150
200
250
300
m/z

Figure S2. Related to Figure 1. Structural analysis of oxygenated CL species by tandem MS/MS. LC-MS^3 spectra of TLCL containing one **(a)**, two **(b)**, three **(c)** and four **(d)** oxygens. Possible structures are shown. The characteristic fragments that are used for identification are shown in bold red. Numbers in red color are the major fragments that formed during fragmentation closed to functional group (OOH, OH etc). **(b)** The predominant CL products, eluted at RT 22.9 min, were 18:2/18:2/18:2/9-HpODE and 18:2/18:2/18:2/13-HpODE. The product eluted at RT 17.4 min contained hydroxy-groups in two acyl chains of CL and corresponded to 18:2/18:2/9-HODE/13-HODE. Product at RT 25.2 min contained epoxy/hydroxy- groups and corresponded to 18:2/18:2/9-HODE/12,13-EpOME and 18:2/18:2/9,10-EpOME/13-HODE species (not shown). **(c)** Oxidative modifications occurred in two acyl chains (*18:2/18:2/18:2+1[O]/18:2+2[O]*), resulting in the diacylglycerol phosphatidate fragments m/z 695(a) and 743(b). MS/MS fragmentation of oxidized fragment (m/z 743) showed monoacylglycerol phosphatidate (m/z 431) with carboxylate anion of linoleic acid containing hydroperoxy-group (m/z 311) and monoacylglycerol phosphatidate (m/z 447) with oxidized carboxylate anion of linoleic acid (m/z 295). Two other oxygenated acyl chains (*18:2/18:2+1[O]/18:2/18:2+2[O]*) were also observed and corresponded to oxidized diacylglycerol phosphatidate fragments m/z 711(a), 727(b), containing one and two oxygens. **(d)** Products with oxidatively modifications in one acyl chain and two chains were detected. In the former, the product containing four oxygens (*18:2/18:2/18:2/18:2+4[O]*) was identified as diacylglycerol phosphatidate fragments m/z 695(a) and 759(b). MS/MS fragmentation of the oxidized fragment (m/z 759) identified monoacylglycerol phosphatidate (m/z 415) with oxygenated carboxylate anion of linoleic acid (m/z 343) and monoacylglycerol phosphatidate (m/z 479) with non-oxidized carboxylate anion of linoleic acid (m/z 279). Further, during fragmentation of CL containing four oxygens, the formation of different acyl chains containing hydroperoxy-groups was found (*18:2/18:2+2[O]/18:2/18:2+2[O]*) with the corresponding oxidized diacylglycerol phosphatidate fragments at m/z 727(a), 727(b). MS/MS fragmentation of the oxidized fragment (m/z 727) confirmed the presence of monoacylglycerol phosphatidate (m/z 415) with oxygenated carboxylate anion of linoleic acid (m/z 311) and monoacylglycerol phosphatidate (m/z 447) with non-oxidized carboxylate anion of linoleic acid (m/z 279).

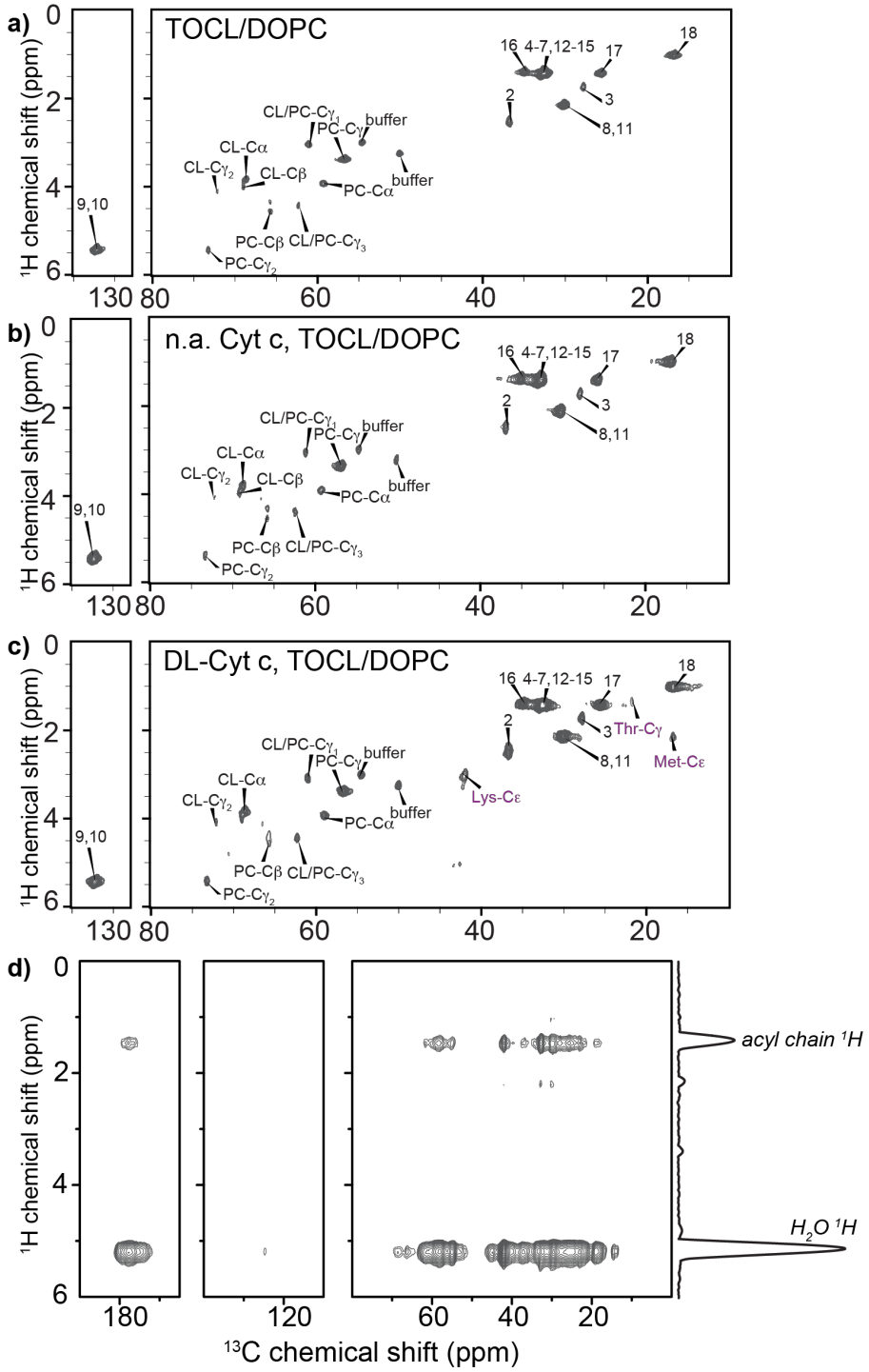

Figure S3. Related to Figure 2. Comparison of 2D ^{1}H-^{13}C INEPT ssNMR spectra of the membrane lipids in the absence and presence of cyt c. (a) TOCL/DOPC (1:1) lipid vesicles; (b) TOCL/DOPC (1:1) vesicles with natural abundant (n.a.) cyt c bound, at a 1:25 protein:lipid (P/L) molar ratio. (c) TOCL/DOPC (1:1) vesicles with bound ^{13}C, ^{15}N doubly labeled (DL) cyt c at 1:25 P/L molar ratio. All spectra acquired at 282 K, 10 kHz MAS, and a magnetic field of 15.6 T. d) ^{1}H-^{13}C cross polarization heteronuclear correlation (HETCOR) spectrum with 50 ms ^{1}H-^{1}H diffusion time. ^{1}H dimension projections are shown to the right, with peaks for the lipid acyl chain and mobile H_2O marked. The spectrum was acquired on DL-cyt c bound to TLCL/DOPC (1:1) liposomes at a P/L = 1:25, a magnetic field of 14.1 T, MAS rate of 8 kHz, and temperature of 265 K. Detailed experimental parameters are in Table S2.

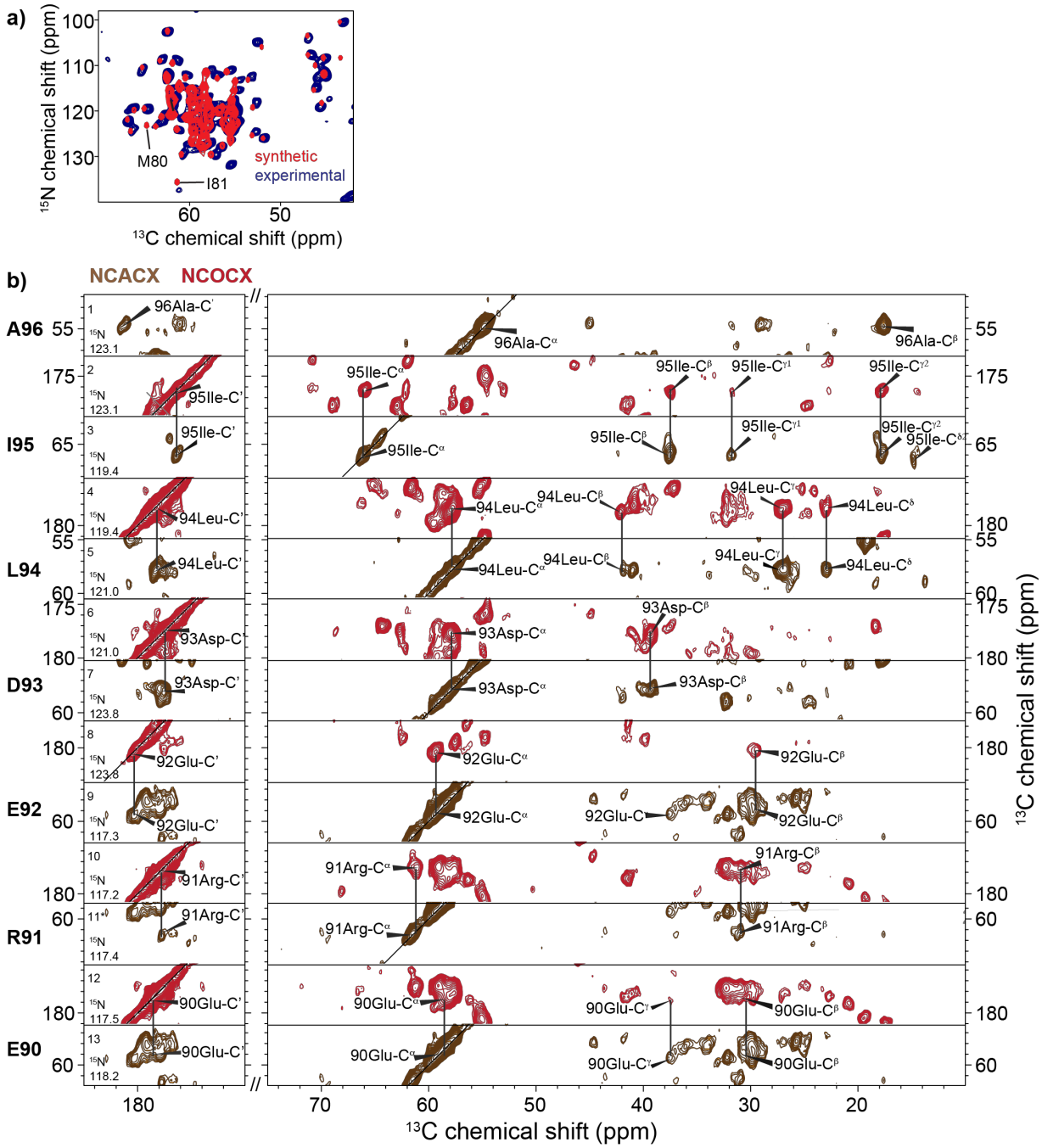

Figure S4. Related to Figure 3 and Table S2. Chemical shift assignments of cyt c in its membrane bound state. (a) An overlay of a synthetic NCA spectrum (red) based on cyt c solution NMR shifts and an experimental NCA spectrum (navy) of cyt c bound to 50 mol-% TOCL and 50 mol-% DOPC LUVs at a P/L molar ratio of 1:25. Note the remarkable similarity for most peak positions, with few exceptions. Black peak labels mark heme-ligating residue M80 and its neighbor I81, both of which show perturbations upon membrane binding. The experimental NCA ssNMR spectrum was acquired at 264 K, 10 kHz MAS, and a field strength of 15.6 T. The assignments made through direct comparison of the two spectra are listed and labeled with asterisk (*) in Table S1. (b) Backbone walk of E90-A96 in the C terminal helix using 3D NCACX and 3D NCOCX spectra**.** The spectra were acquired on uniformly ^{13}C, ^{15}N labeled cyt c bound to TOCL/DOPC (1:1) LUVs at a 25:1 P/L ratio. The sample was spun at 10 kHz MAS, temperature of 252 K, and a magnetic field strength of 15.6 T. Refer to Table S2 for more experimental parameters.

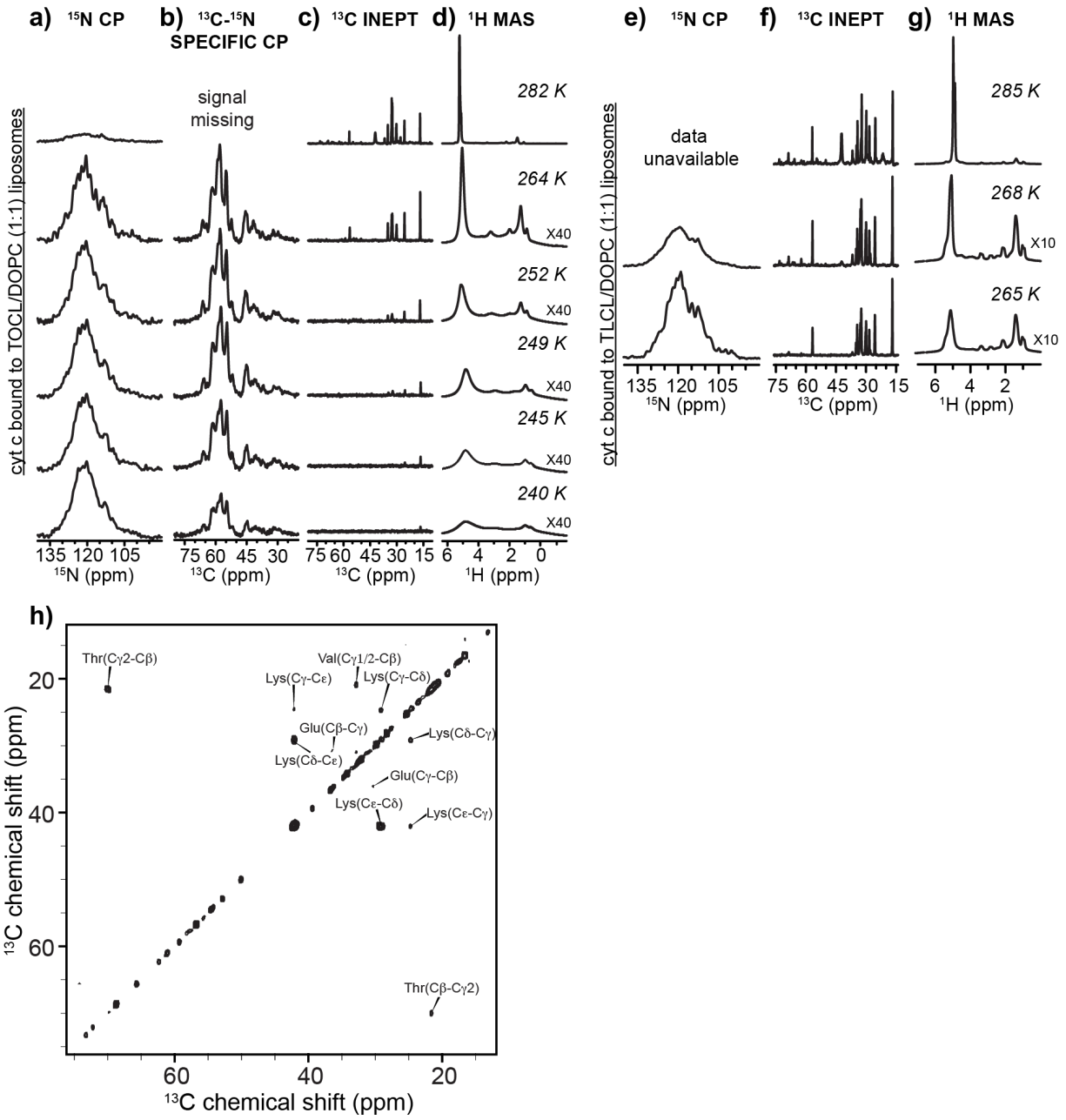

Figure S5. Related to Figure 4 and 5. Dynamics changes in samples containing cyt c bound to TOCL/DOPC (1:1) and TLCL/DOPC (1:1) liposomes and dynamic residues of the protein/lipid complexes, investigated by different dynamics-based spectral editing (DYSE) ssNMR spectroscopy. The P/L ratio is 1:25 in both samples. (a) and (e) 1D ^{1}H-^{15}N cross polarization (CP) spectra of protein backbone amide; (b) 1D double CP (DCP) spectra of the protein C$^{\alpha}$ carbons; (c) and (f) 1D ^{1}H-^{13}C INEPT spectra of highly mobile components, mostly the lipids; (d) and (g) 1D ^{1}H single pulse spectra. The spectra in the same panel are normalized to the same number of scans. The spectra of cyt c bound to TOCL/DOPC (1:1) were acquired at MAS rate of 10 kHz, and a magnetic field of 15.6 T. The spectra at 285 K for cyt c bound to TLCL/DOPC (1:1) were acquired at 10 kHz, 14.1 T with the rest acquired at 8 kHz, 14.1 T. Temperatures are indicated in the spectra. (h) 2D ^{13}C-^{13}C INEPT TOBSY spectrum of cyt c bound to TLCL/DOPC (1:1) membrane. The MAS ssNMR measurement was done at 285 K, magnetic field of 14.1 T, and 10 kHz MAS.

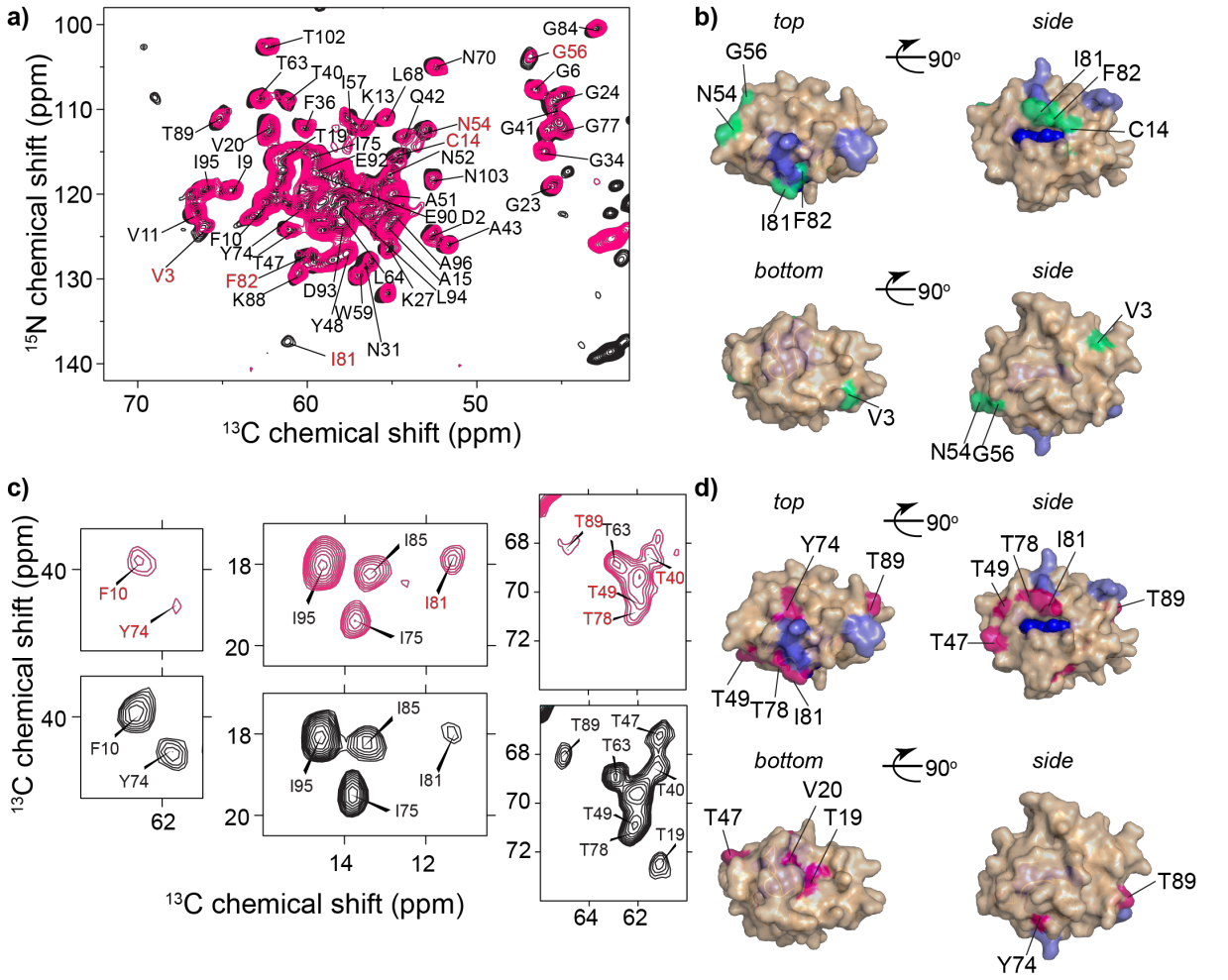

Figure S6. Related to Figure 5. Dynamic perturbations in cyt c backbone and sidechains upon binding to 50 mol-% TLCL and 50 mol-% DOPC containing membrane in comparison to binding to 50 mol-% TOCL and 50 mol-% DOPC containing membrane. P/L molar ratio is kept at 1:25. (a) NCA spectral comparison between cyt c bound to TOCL- (black) and TLCL- (hot pink) containing vesicles. Residues with identifiable perturbations are labeled in red. (b) Mapping of these NCA-based backbone perturbations (green) on the cyt c structure. The binding site known as "site A" is shown in slate blue and the heme is shown in blue. (c) Selected expansions showing spectral perturbations of cyt c bound to 50 mol-% TOCL- (in black) and TLCL- containing membrane (hot pink) in DARR experiments. Strongly changing residues are labeled in red. (d) Residues with apparent dynamic changes identified by comparing ^{13}C-^{13}C DARR spectra in c) and their mapping on its structure (hot pink; right). NCA and DARR spectra of cyt c bound to TLCL/DOPC (1:1) were acquired at 8 kHz MAS, temperature of 265 K, and a magnetic field of 14.1 T. The corresponding spectra of cyt c bound to TOCL/DOPC (1:1) were acquired at 10 kHz, 264 K, and 15.6 T.