Supporting Information

Determining Cholesterol Binding to Membrane Proteins by Cholesterol ^{13}C Labeling in Yeast and Dynamic Nuclear Polarization NMR

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Table S1. Experimental parameters for the 2D ^{13}C-^{13}C correlation NMR spectra.

Experiment	**2D DQF-CC with DNP**	**2D CC with DNP**	**2D CC**	**INADEQUATE**
Samples	1, 2, 3	1	1	1
Temperature	109 - 112 K	111 K	250 K	111 K
MAS rate	13.33 kHz	13 kHz	12 kHz	13 kHz
^{13}C-^{13}C mixing sequence	CORD	CORD	CORD	SPC5
Mixing time	300, 400, 500 ms	30, 100, 300, 500 ms	300 ms	0.6 ms
^{13}C carrier frequency	98 ppm	98 ppm	100 ppm	41 ppm
Number of t_1 points	450	330 - 440	280	208
$\omega_1/2\pi$ spectral width	40 kHz	39 kHz	30.1 kHz	62.5 kHz
Maximum t_1 time	5.0 - 6.0 ms	4.2 - 5.6 ms	4.6 ms	1.7 ms
Number of t_2 points	2048	1600	1600	2048
$\omega_2/2\pi$ spectral width	62.5 kHz	50 kHz	50 kHz	62.5 kHz
t_2 acquisition time	16.4 ms	16 ms	16 ms	16.4 ms
^{1}H rf field during t_2	100 kHz	100 kHz	83.3 kHz	100 kHz
Recycle delay	2.5 - 3.0 s	2.5 s	1.5 s	2.5
Number of scans	32 or 112	32	320	64

Samples:
1 IFGA-M2T in POPC/POPG
2 G34, I39-labeled M2W(22-64) in POPC/POPG
3 G34, I39-labeled M2W(22-64) in VMS

Table S2. Measured M2-cholesterol cross peaks and the distance restraints input into HADDOCK.

Atom Protein	Atom CHOL	Samples	Experiments						
			A, H	B	C	D	E	F	G
I39/I42 Cγ1	C17	1	X	X	X	X	X		
I35/I39 Cγ1	C24	1	X	X	X	X			
I39/I42 Cα	C13	1	X						
I39/I42 Cβ	C21	1					X		
I39/I42 Cβ	C19	1					X		
I39/I42 Cβ	C13	1					X		
I39/I42 Cδ	C19	1					X		
I39/I42 Cα	C18	1							X
I39 Cγ1	C13	2						X	
I39 Cβ	C15	2, 3						X	
I39 Cβ	C21	2, 3						X	
I39 Cγ2	C17	3						X	
F47 Cδε	C9	1			X	X			
F47 Cδε	C3	1		X					

Experiments:
A 30 ms 2D CC spectra at 111 K
B 100 ms 2D CC spectra at 111 K
C 300 ms 2D CC spectra at 111 K
D 500 ms 2D CC spectra at 111 K
E 300 ms & 500 ms 2D DQF-CC spectra at 111 K
F 400 ms 2D DQF-CC spectra at 111 K
G 300 ms 2D CC spectra at 250 K
H 0.6 ms dipolar INADEQUATE spectrum at 111 K

Samples:
1 IFGA-M2T in POPC/POPG
2 G34, I39-labeled M2W(22-61) in POPC/POPG
3 G34, I39-labeled M2W(22-61) in VMS

Table S3. Distance restraints input to HADDOCK and output distances from the top docked structural model.

Protein Atom	CHOL Atom	d_{lower} (Å)	d_{upper} (Å)	d_{docked}(Å)
Ambiguous restraints				
I39 Cγ1	C17	0	5	5.0
I42 Cγ1	C17	0	5	6.0
I35 Cγ1	C24	0	5	7.4
I39 Cγ1	C24	0	5	3.3
I39 Cα	C13	0	5	5.2
I42 Cα	C13	0	5	5.8
I39 Cβ	C21	0	8	5.0
I42 Cβ	C21	0	8	4.9
I39 Cδ	C19	0	8	10.6
I42 Cδ	C19	0	8	6.8
I39 Cα	C18	0	7	4.2
I42 Cα	C18	0	7	4.6
I39 Cβ	C13	0	8	5.3
I42 Cβ	C13	0	8	4.7
Unambiguous restraints				
I39 Cγ1	C13	0	8	5.9
I39 Cβ	C15	0	8	5.2
I39 Cβ	C21	0	8	5.0
I39 Cγ2	C17	0	8	3.7
I35 Cα	C25†	8.2	9.2	8.0
L36 Cα	C25†	6.6	7.6	7.7
I39 Cα	C25†	6.6	7.6	6.5
L40 Cα	C25†	8.7	9.7	10.0
I39 Cγ2	C26†	0	8	6.7
I39 Cα	C26†	0	8	7.2
I39 Cα	C27†	0	8	7.6
F47 Cγ, δ, ε	C3	0	7	4.4*
F47 Cγ, δ, ε	C9	0	8	6.3*

* Distances measured from F47 Cγ

† Restraints from ^{13}C-^{19}F or ^{13}C-^{13}C experiments reported previously[1]

Table S4. Relative vector orientation restraints input into HADDOCK and the output angles from the docked structural model. Angle restraints are based on simulations of experimental ^{2}H NMR spectra reported previously.[1]

Vectors	Atoms	Input Angle	Output Angle
Bilayer normal Cholesterol Z axis	$I35_D$ Cα - $W41_D$ Cα C13 - C3	11 ± 5	20˚
Bilayer normal Cholesterol X axis	$I35_D$ Cα - $W41_D$ Cα C10 - C19	79 ± 15	77˚

Table S5. HADDOCK parameters and orientation angles from the top 10 HADDOCK structures as determined by agreement with cholesterol β and γ angles obtained from ^{2}H NMR spectra.[1] The HADDOCK score is reported in arbitrary units.

HADDOCK score	HADDOCK rank	PRODIGY score (kcal/mol)	PRODIGY rank	β	γ	RSS (β, γ) (degrees)^2	RSS Rank
-14.18	2	-7.43	67	20.1°	16.0°	125	1
-12.64	6	-6.88	179	21.8°	16.9°	130	2
2.39	81	-7.44	64	22.8°	13.8°	131	3
8.67	136	-7.38	83	23.5°	8.2°	143	4
17.13	182	-6.93	169	23.6°	10.4°	155	5
13.97	167	-7.37	86	24.6°	7.2°	167	6
1.17	75	-7.39	79	25.0°	7.7°	191	7
4.47	95	-7.39	79	25.5°	7.9°	201	8
-10.26	10	-7.39	79	25.6°	11.6°	213	9
20.14	194	-7.28	106	25.6°	9.3°	214	10

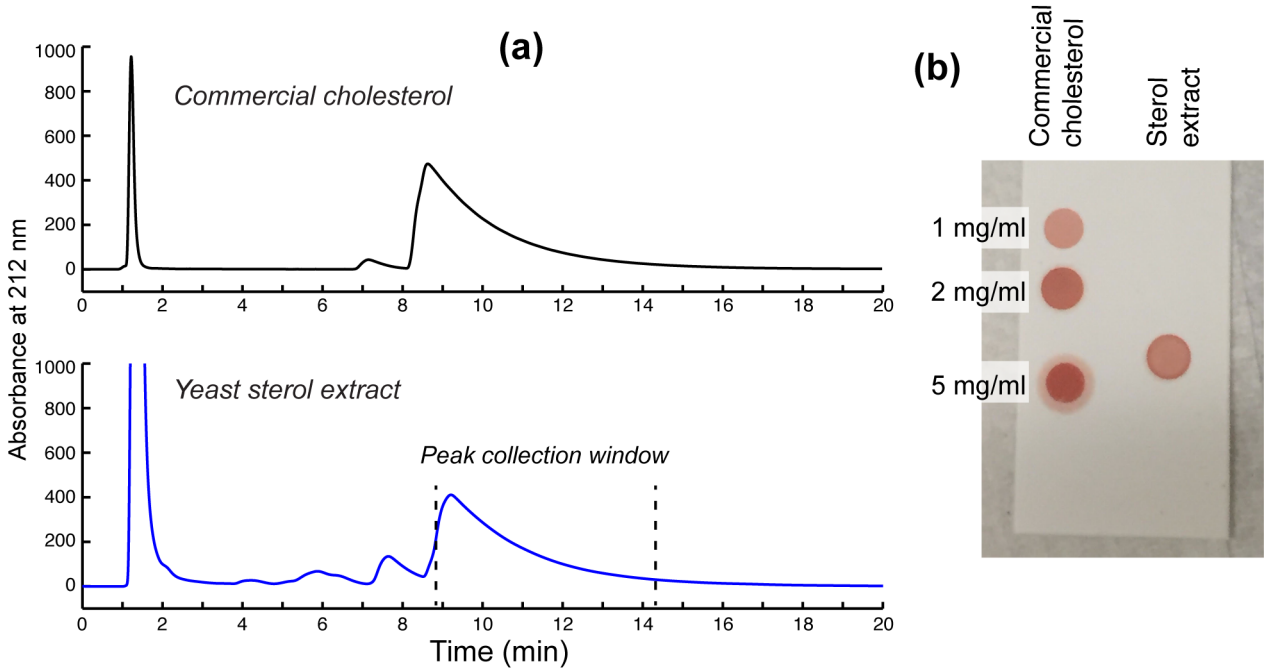

Figure S1. Purification and analysis of yeast-produced cholesterol. (a) HPLC traces of commercial cholesterol and the yeast sterol extract. Approximately 0.5 mg of commercial cholesterol was used at 5 mg/ml. HPLC separation used a Vydac 5 μm, 4.6 mm x 150 mm C18 column under isocratic conditions of 30% solvent A (90% EtOH, 5% *i*-PrOH, 4.6% MeOH) and 70% solvent B (acetonitrile). (b) Spots on a TLC plate used to estimate the concentration of the yeast sterol extract. Cholesterol was spotted from an acetone solution, and the plate was developed using a ferric chloride stain (50% w/v ferric chloride, 90% v/v water, 5% v/v glacial acetic acid, 5% v/v sulfuric acid).

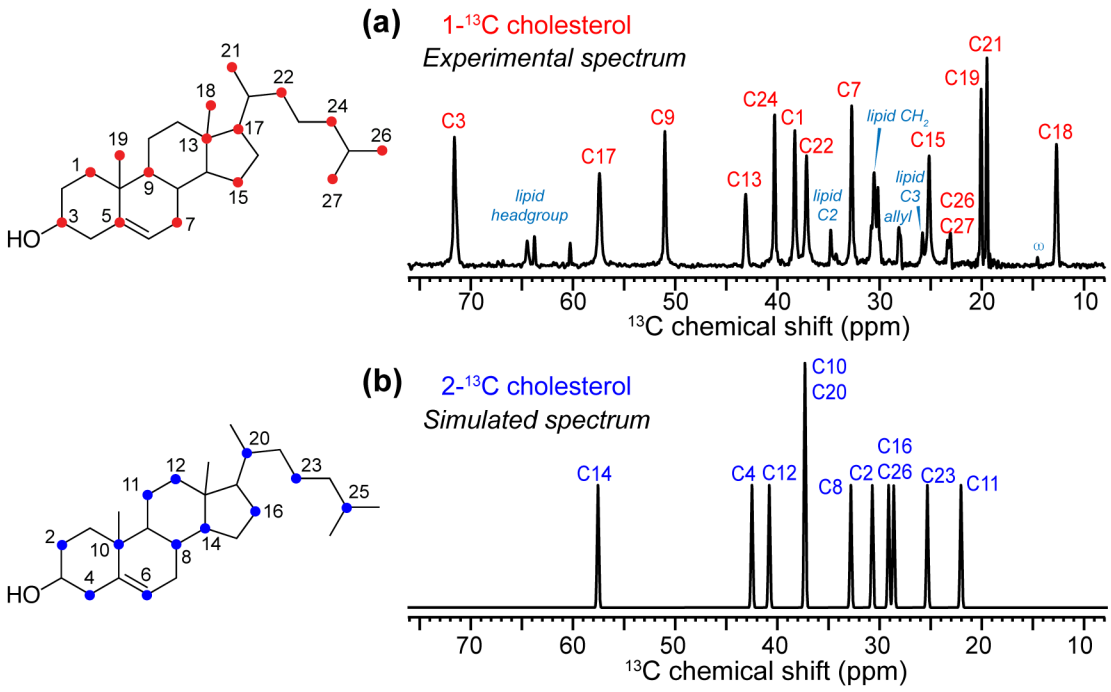

Figure S2. Comparison of the ^{13}C chemical shifts of 1-^{13}C and 2-^{13}C labeled CHOL. (a) Experimental ^{13}C CP spectrum of 1-^{13}C CHOL in liquid-crystalline POPC/POPG bilayers, measured at 275 K. (b) Simulated spectrum of 2-^{13}C CHOL, using chemical shifts from the literature[2] and the software Nmrglue.[3]

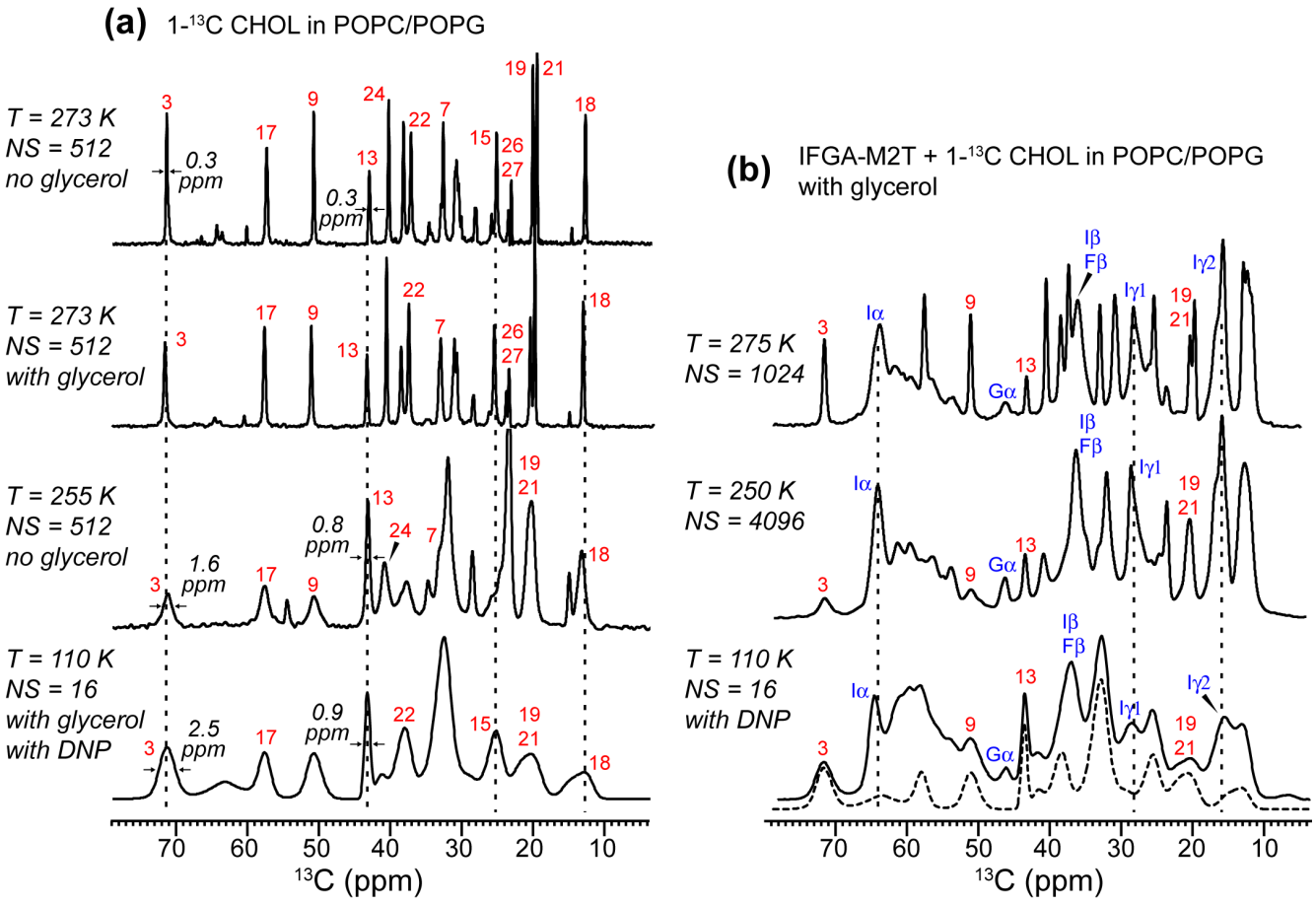

Figure S3. Temperature dependence of the ^{13}C spectra of M2 and 1-^{13}C CHOL-containing membranes. (a) ^{13}C spectra of 1-^{13}C CHOL in POPC/POPG bilayers as a function of temperature. At 273 K the membrane is liquid crystalline; at 255 K the membrane is in the gel phase, and at 110 K the spectrum was measured with microwave irradiation. The full widths at half-maximum show that the membrane phase transition is the main causes of the line broadening, and the degree of broadening varies for different ^{13}C signals. The spectrum measured at 273 K with glycerol added shows that glycerol does not perturb the cholesterol ^{13}C chemical shifts, but the spectrum shows slightly lower intensities compared to the glycerol-free sample (top) due to paramagnetic relaxation enhancement by the biradical AMUPOL in the sample.[4] (b) ^{13}C spectra of IFGA-M2T and 1-^{13}C CHOL in POPC/POPG bilayers. Dotted spectrum reproduces the CHOL-only spectrum on the left to distinguish the spectral envelopes of CHOL and M2.

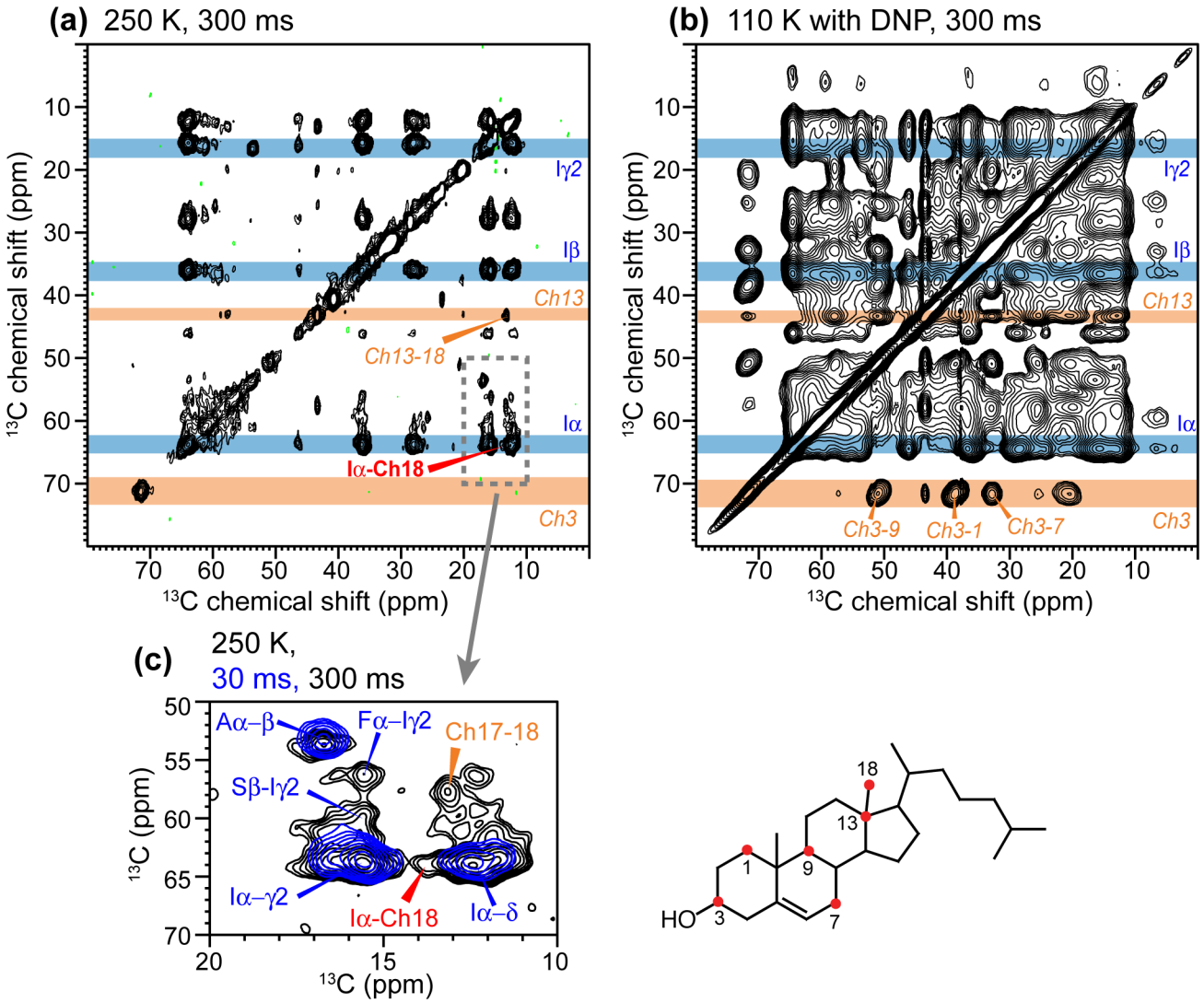

Figure S4. 2D ^{13}C-^{13}C correlation spectra of IFGA-M2T with 1-^{13}C CHOL in POPC/POPG membranes. The spectra were measured with a long ^{13}C spin diffusion mixing time of 300 ms. (a) 250 K spectrum measured without DNP. (b) 110 K spectrum measured with DNP. (c) An expanded view of the dashed-box region in (a) shows a cross peak between protein Ile and CHOL Ch18. This peak is absent in the 30 ms 2D spectrum (blue), indicating that it is a long-range contact. The high-temperature spectrum shows relatively narrow linewidths but few strong peaks of the protein and cholesterol due to their abundant motion in the membrane at this temperature and the general low sensitivity. The 110 K spectrum measured with DNP shows broader lines but higher sensitivity and many CHOL-CHOL and protein-CHOL cross peaks, thus allowing assignment of intermolecular contacts. Representative ω_1 cross sections for the protein (blue) and cholesterol (orange) chemical shifts are highlighted.

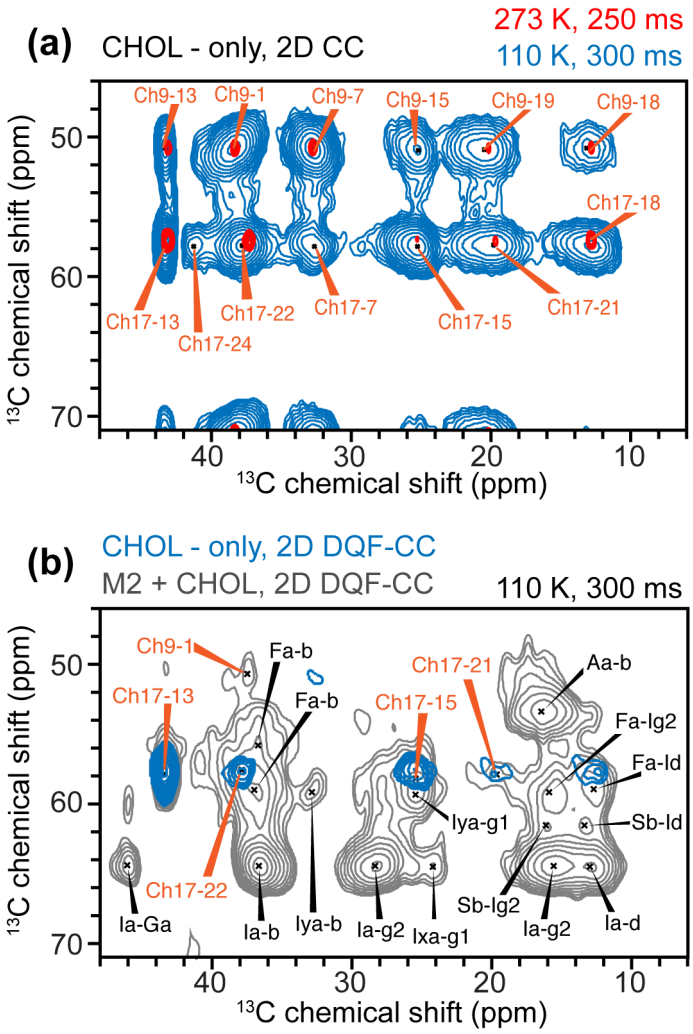

Figure S5. Representative 2D ^{13}C-^{12}C correlation spectra to illustrate resonance assignment. (a) Overlay of spectra of CHOL-containing POPC/POPG membranes measured at 273 K (red) and at 110 K with DNP (blue). No chemical shift perturbation is seen, therefore assigned CHOL ^{13}C chemical shifts at high temperature can be transferred to the cryogenic-temperature spectra. (b) Overlay of DQF-CC spectra of lipid membranes with only CHOL (blue) versus with both M2 and CHOL (grey). The cholesterol ^{13}C chemical shifts obtained from (a) simplify the assignment of the protein-protein cross peaks.

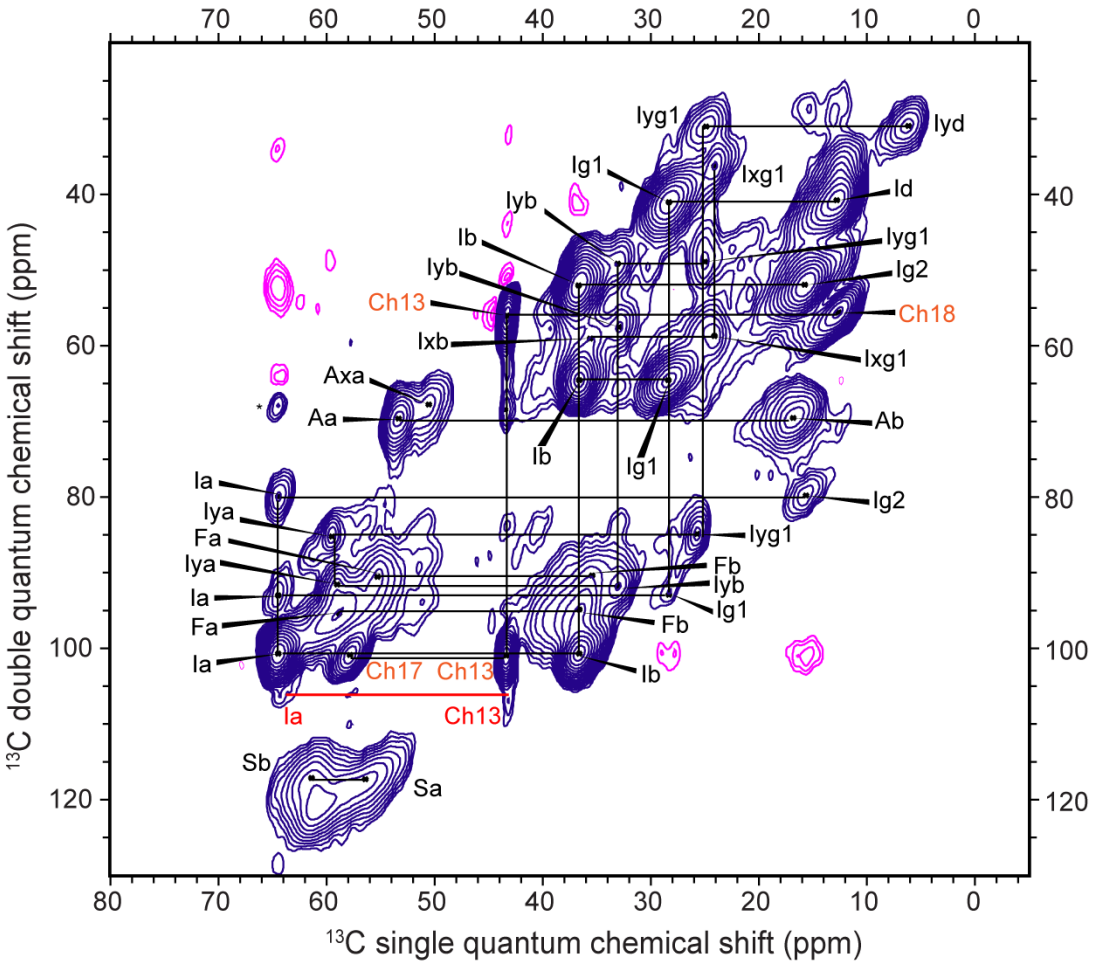

Figure S6. 2D dipolar INADEQUATE spectrum of IFGA-M2T with 1-^{13}C CHOL in POPC/POPG membranes for amino-acid type assignment of the protein. The spectrum was measured under 13 kHz MAS at ~110 K with DNP. A weak Ile Cα-Ch13 cross peak is observed. Cholesterol cross peaks are assigned in orange, protein cross peaks in black, and CHOL-M2 cross peaks in red.

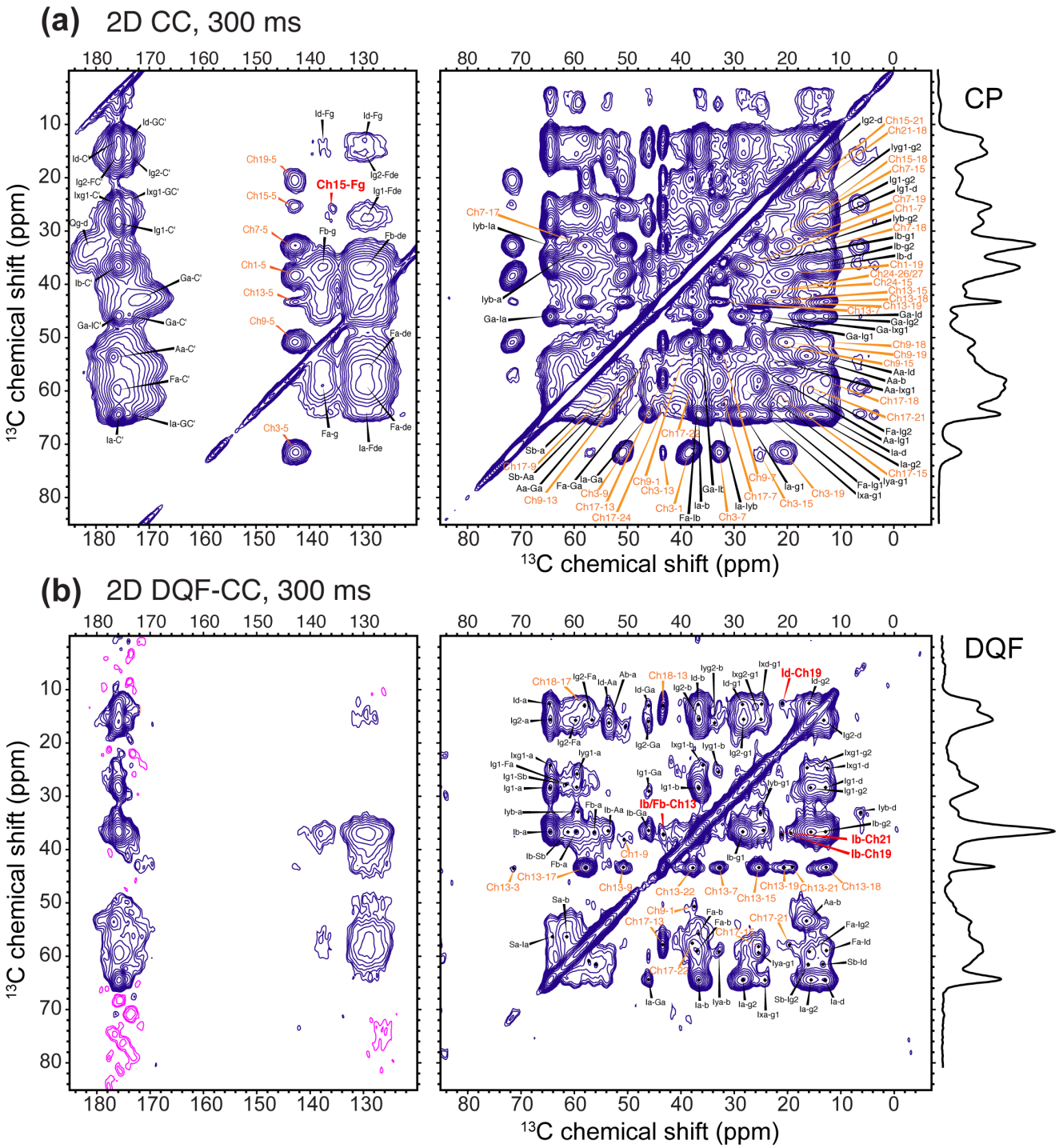

Figure S7. Full assignment of 300 ms 2D ^{13}C-^{13}C correlation spectra of IFGA-M2T and 1-^{13}C-CHOL in POPC/POPG membranes, measured at 110 K with DNP sensitivity enhancement. (a) 2D CC spectrum. (b) 2D DQF-CC spectrum. Cholesterol cross peaks are assigned in orange, protein cross peaks in black, and CHOL-M2 cross peaks in red.

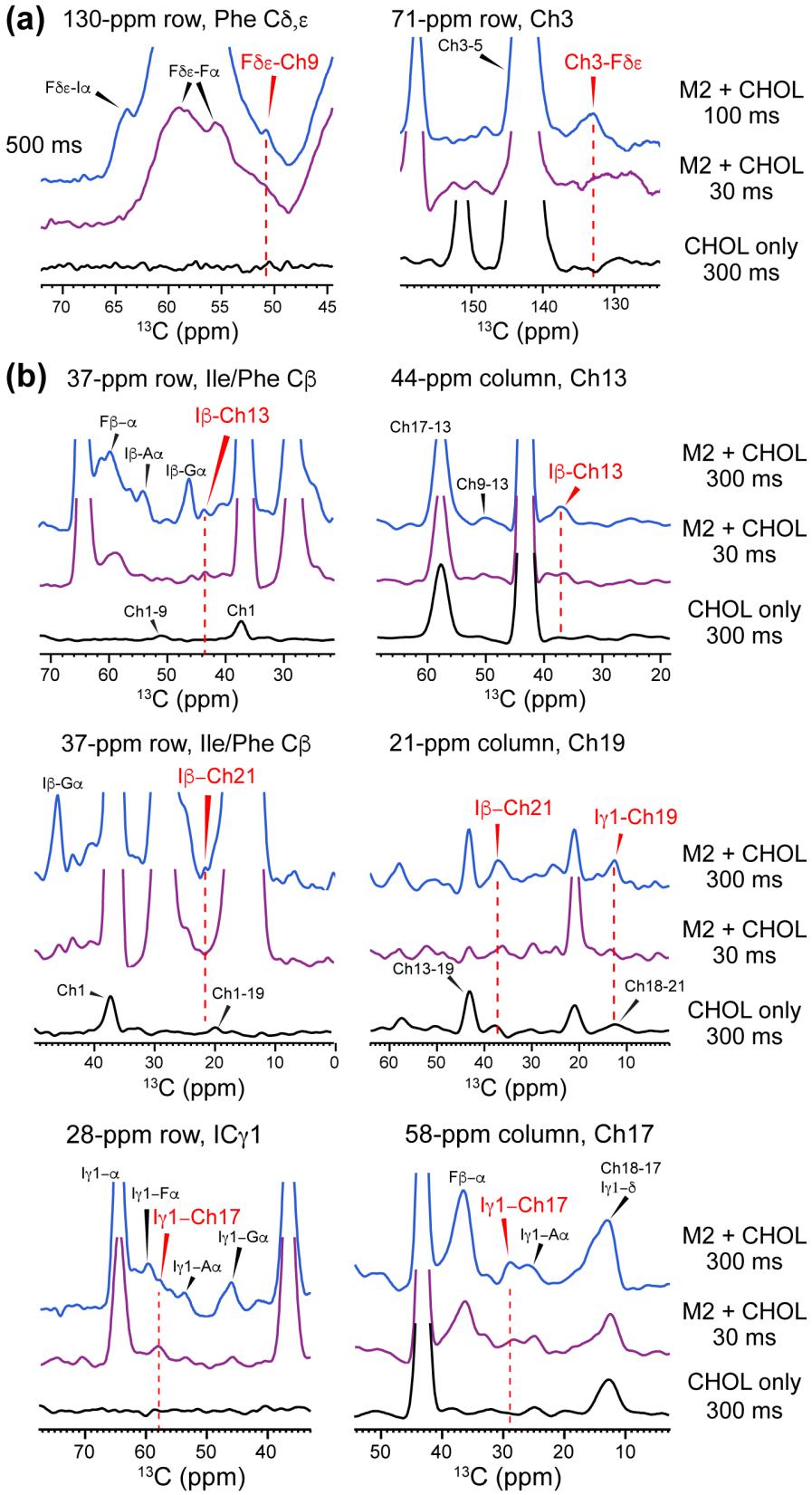

Figure S8. 1D ^{13}C cross sections extracted from the DNP-enhanced 2D CC and DQF-CC spectra in Fig. 3. M2-containing spectra with 100-500 ms mixing are shown in blue and spectra with 30 ms mixing are shown in purple. Protein-free spectra with 300 ms mixing are shown in black.

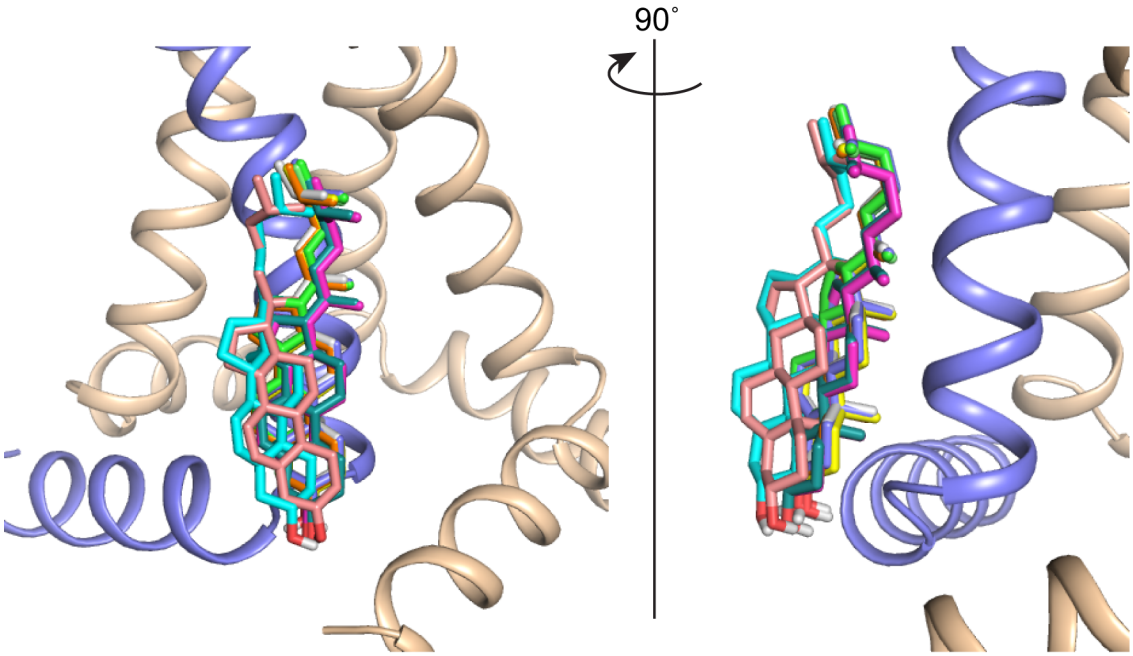

Figure S9. Top ten HADDOCK docked M2-CHOL structures. The ten structures were chosen by their agreement with the experimentally measured cholesterol orientation angles from the ^{2}H NMR spectra.[1] The ensemble of structures show β angles of 20 – 26° and γ angles of 7 – 17°, in good agreement with the measured β angle of 11˚ and γ angle of 10˚.

References

(1) Elkins, M. R.; Williams, J. K.; Gelenter, M. D.; Dai, P.; Kwon, B.; Sergeyev, I. V.; Pentelute, B. L.; Hong, M., *Proc. Natl. Acad. Sci. U. S. A.* **2017**, Cholesterol-binding site of the influenza M2 protein in lipid bilayers from solid-state NMR, *114*, 12946-12951.

(2) Kalinowski, H.-O.; Berger, S.; Braun, S. *13C-NMR-Spektroskopie*; Thieme-Verlag: Stuttgart, 1984.

(3) Helmus, J. J.; Jaroniec, C. P., *J. Biomol. NMR* **2013**, Nmrglue: an open source Python package for the analysis of multidimensional NMR data, *55*, 355-367.

(4) Liao, S. Y.; Lee, M.; Wang, T.; Sergeyev, I. V.; Hong, M., *J. Biomol. NMR* **2016**, Efficient DNP NMR of membrane proteins: sample preparation protocols, sensitivity, and radical location, *64*, 223-237.

Appendix A. HADDOCK parameter file. The PDB text for the M2 2L0J structure has been omitted for brevity. Renumbering of the residues in 2L0J is required for HADDOCK submission; in this case residue numbers for chain A were added to 100, chain B added to 200, chain C to 300, and chain D to 400. For example, L26 is chain A is renumbered to L126, while L26 in chain D is renumbered to L426.

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    pdbdata = 'HETATM    1 O03  CLR A   1       5.627  10.237  17.927  1.00  0.00           O \nHETATM    2 C01  CLR A
1       5.730   8.470  21.289  1.00  0.00           C \nHETATM    3 C02  CLR A   1       6.253   8.960  19.863  1.00  0.00
C \nHETATM    4 C03  CLR A   1       5.187   9.682  19.218  1.00  0.00           C \nHETATM    5 C04  CLR A   1
4.579  10.805  19.999  1.00  0.00           C \nHETATM    6 C05  CLR A   1       4.168  10.341  21.391  1.00  0.00           C
\nHETATM    7 C06  CLR A   1       2.969  10.769  21.968  1.00  0.00           C \nHETATM    8 C07  CLR A   1       2.590
10.578  23.292  1.00  0.00           C \nHETATM    9 C08  CLR A   1       3.668  10.034  24.209  1.00  0.00           C
\nHETATM   10 C09  CLR A   1       4.601   9.022  23.428  1.00  0.00           C \nHETATM   11 C10  CLR A   1       5.260
9.635  22.172  1.00  0.00           C \nHETATM   12 C11  CLR A   1       5.670   8.393  24.345  1.00  0.00           C
\nHETATM   13 C12  CLR A   1       5.079   7.824  25.669  1.00  0.00           C \nHETATM   14 C13  CLR A   1       4.354
8.848  26.450  1.00  0.00           C \nHETATM   15 C14  CLR A   1       3.240   9.311  25.465  1.00  0.00           C
\nHETATM   16 C15  CLR A   1       2.167  10.001  26.348  1.00  0.00           C \nHETATM   17 C16  CLR A   1       2.198
9.285  27.672  1.00  0.00           C \nHETATM   18 C17  CLR A   1       3.436   8.207  27.570  1.00  0.00           C
\nHETATM   19 C18  CLR A   1       5.160   9.927  26.959  1.00  0.00           C \nHETATM   20 C19  CLR A   1       6.378
10.685  22.545  1.00  0.00           C \nHETATM   21 C20  CLR A   1       4.008   7.914  28.962  1.00  0.00           C
\nHETATM   22 C21  CLR A   1       5.179   7.063  28.928  1.00  0.00           C \nHETATM   23 C22  CLR A   1       2.821
7.242  29.777  1.00  0.00           C \nHETATM   24 C23  CLR A   1       3.231   6.889  31.169  1.00  0.00           C
\nHETATM   25 C24  CLR A   1       2.005   6.379  32.120  1.00  0.00           C \nHETATM   26 C25  CLR A   1       1.494
5.108  31.780  1.00  0.00           C \nHETATM   27 C26  CLR A   1       2.678   4.227  32.425  1.00  0.00           C
\nHETATM   28 C27  CLR A   1       0.457   4.877  32.765  1.00  0.00           C \nHETATM   29 H01  CLR A   1       5.583
11.195  17.958  1.00  0.00           H  \nEND\n',
   ),
  ),
  tbldata = 'assign  (segid P and resid 339 and name CG1)\n        (segid L and resid 1 and name C17) 5.0 5.0 0\nassign
(segid P and resid 342 and name CG1) \n        (segid L and resid 1 and name C17) 5.0 5.0 0\nassign  (segid P and resid 335
and name CG1)\n        (segid L and resid 1 and name C24) 5.0 5.0 0\nassign  (segid P and resid 339 and name CG1)\n
(segid L and resid 1 and name C24) 5.0 5.0 0\nassign  (segid P and resid 339 and name CA)\n        (segid L and resid 1 and
name C13) 5.0 5.0 0\nassign  (segid P and resid 342 and name CA)\n        (segid L and resid 1 and name C13) 5.0 5.0
0\nassign  (segid P and resid 339 and name CB)\n        (segid L and resid 1 and name C21) 8.0 8.0 0\nassign  (segid P and
resid 342 and name CB)\n        (segid L and resid 1 and name C21) 8.0 8.0 0\nassign  (segid P and resid 339 and name
CD1)\n        (segid L and resid 1 and name C19) 8.0 8.0 0\nassign  (segid P and resid 342 and name CD1)\n        (segid L
and resid 1 and name C19) 8.0 8.0 0\nassign  (segid P and resid 339 and name CA)\n        (segid L and resid 1 and name
C18) 7.0 7.0 0\nassign  (segid P and resid 342 and name CA)\n        (segid L and resid 1 and name C18) 7.0 7.0 0\nassign
(segid P and resid 339 and name CB)\n        (segid L and resid 1 and name C13) 8.0 8.0 0\nassign  (segid P and resid 342
and name CB)\n        (segid L and resid 1 and name C13) 8.0 8.0 0',
  unambigtbldata = 'assign  (segid P and resid 339 and name CG1)\n        (segid L and resid 1 and name C13) 8.0 8.0
0\nassign  (segid P and resid 339 and name CB)\n        (segid L and resid 1 and name C15) 8.0 8.0 0\nassign  (segid P and
resid 339 and name CB)\n        (segid L and resid 1 and name C21) 8.0 8.0 0\nassign  (segid P and resid 339 and name
CG2)\n        (segid L and resid 1 and name C17) 8.0 8.0 0\nassign  (segid P and resid 335 and name CA)\n        (segid L and
resid 1 and name C25) 8.7 0.5 0.5\nassign  (segid P and resid 336 and name CA)\n        (segid L and resid 1 and name C25)
7.1 0.5 0.5\nassign  (segid P and resid 339 and name CA)\n        (segid L and resid 1 and name C25) 7.1 0.5 0.5\nassign
(segid P and resid 340 and name CA)\n        (segid L and resid 1 and name C25) 9.2 0.5 0.5\nassign  (segid P and resid 339
and name CG2)\n        (segid L and resid 1 and name C26) 8.0 8.0 0\nassign  (segid P and resid 339 and name CA)\n
(segid L and resid 1 and name C26) 8.0 8.0 0\nassign  (segid P and resid 339 and name CA)\n        (segid L and resid 1 and
name C27) 8.0 8.0 0\nassign  (segid P and resid 347 and (name CD1 or name CD2 or name CE1 or name CE2 or name
CG))\n\t\t(segid L and resid 1 and name C03) 7.0 7.0 0\nassign  (segid P and resid 347 and (name CD1 or name CD2 or
name CE1 or name CE2 or name CG))\n\t\t(segid L and resid 1 and name C09) 8.0 8.0 0',
  rdc1 = RDCParameters (
```

```
    choice = 'VANGLE',
    r = 0.4,
    d = 8.0,
    constants = ExtStageConstants (
      firstit = 0,
      lastit = 2,
      stages = StageConstants (
        hot = 0.1,
        cool1 = 1.0,
        cool2 = 1.0,
        cool3 = 1.0,
      ),
    ),
    ini_bor = StageConstants (
      hot = 1.0,
      cool1 = 10.0,
      cool2 = 40.0,
      cool3 = 40.0,
    ),
    fin_bor = StageConstants (
      hot = 10.0,
      cool1 = 40.0,
      cool2 = 40.0,
      cool3 = 40.0,
    ),
    ini_cen = StageConstants (
      hot = 0.25,
      cool1 = 2.5,
      cool2 = 10.0,
      cool3 = 10.0,
    ),
    fin_cen = StageConstants (
      hot = 2.5,
      cool1 = 10.0,
      cool2 = 10.0,
      cool3 = 10.0,
    ),
    rdcdata = 'assign (segid P and resid 435 and name CA)\n    (segid P and resid 441 and name CA)\n    (segid L and resid 1
and name C13)\n    (segid L and resid 1 and name C03) 0 0 11 5\nassign (segid P and resid 435 and name CA)\n    (segid P
and resid 441 and name CA)\n    (segid L and resid 1 and name C10)\n    (segid L and resid 1 and name C19) 0 0 79.2 15',
  ),
  rdc2 = RDCParameters (
    choice = 'NO',
    r = 0.4,
    d = 8.0,
    constants = ExtStageConstants (
      firstit = 0,
      lastit = 2,
      stages = StageConstants (
        hot = 0.1,
        cool1 = 1.0,
        cool2 = 1.0,
        cool3 = 1.0,
      ),
    ),
    ini_bor = StageConstants (
      hot = 1.0,
      cool1 = 10.0,
```

```
    cool2 = 40.0,
    cool3 = 40.0,
   ),
   fin_bor = StageConstants (
    hot = 10.0,
    cool1 = 40.0,
    cool2 = 40.0,
    cool3 = 40.0,
   ),
   ini_cen = StageConstants (
    hot = 0.25,
    cool1 = 2.5,
    cool2 = 10.0,
    cool3 = 10.0,
   ),
   fin_cen = StageConstants (
    hot = 2.5,
    cool1 = 10.0,
    cool2 = 10.0,
    cool3 = 10.0,
   ),
  ),
 ),
 rdc3 = RDCParameters (
  choice = 'NO',
  r = 0.4,
  d = 8.0,
  constants = ExtStageConstants (
   firstit = 0,
   lastit = 2,
   stages = StageConstants (
    hot = 0.1,
    cool1 = 1.0,
    cool2 = 1.0,
    cool3 = 1.0,
   ),
  ),
  ini_bor = StageConstants (
   hot = 1.0,
   cool1 = 10.0,
   cool2 = 40.0,
   cool3 = 40.0,
  ),
  fin_bor = StageConstants (
   hot = 10.0,
   cool1 = 40.0,
   cool2 = 40.0,
   cool3 = 40.0,
  ),
  ini_cen = StageConstants (
   hot = 0.25,
   cool1 = 2.5,
   cool2 = 10.0,
   cool3 = 10.0,
  ),
  fin_cen = StageConstants (
   hot = 2.5,
   cool1 = 10.0,
   cool2 = 10.0,
```

```
    cool3 = 10.0,
   ),
  ),
 ),
 rdc4 = RDCParameters (
  choice = 'NO',
  r = 0.4,
  d = 8.0,
  constants = ExtStageConstants (
   firstit = 0,
   lastit = 2,
   stages = StageConstants (
    hot = 0.1,
    cool1 = 1.0,
    cool2 = 1.0,
    cool3 = 1.0,
   ),
  ),
  ini_bor = StageConstants (
   hot = 1.0,
   cool1 = 10.0,
   cool2 = 40.0,
   cool3 = 40.0,
  ),
  fin_bor = StageConstants (
   hot = 10.0,
   cool1 = 40.0,
   cool2 = 40.0,
   cool3 = 40.0,
  ),
  ini_cen = StageConstants (
   hot = 0.25,
   cool1 = 2.5,
   cool2 = 10.0,
   cool3 = 10.0,
  ),
  fin_cen = StageConstants (
   hot = 2.5,
   cool1 = 10.0,
   cool2 = 10.0,
   cool3 = 10.0,
  ),
 ),
 rdc5 = RDCParameters (
  choice = 'NO',
  r = 0.4,
  d = 8.0,
  constants = ExtStageConstants (
   firstit = 0,
   lastit = 2,
   stages = StageConstants (
    hot = 0.1,
    cool1 = 1.0,
    cool2 = 1.0,
    cool3 = 1.0,
   ),
  ),
  ini_bor = StageConstants (
   hot = 1.0,
```

```
    cool1 = 10.0,
    cool2 = 40.0,
    cool3 = 40.0,
   ),
   fin_bor = StageConstants (
    hot = 10.0,
    cool1 = 40.0,
    cool2 = 40.0,
    cool3 = 40.0,
   ),
   ini_cen = StageConstants (
    hot = 0.25,
    cool1 = 2.5,
    cool2 = 10.0,
    cool3 = 10.0,
   ),
   fin_cen = StageConstants (
    hot = 2.5,
    cool1 = 10.0,
    cool2 = 10.0,
    cool3 = 10.0,
   ),
  ),
  pcs1 = PCSParameters (
   choice = 'NO',
   constants = ExtStageConstants (
    firstit = 0,
    lastit = 2,
    stages = StageConstants (
     hot = 100.0,
     cool1 = 100.0,
     cool2 = 100.0,
     cool3 = 1000.0,
    ),
   ),
   r = 1000.0,
   d = 10000.0,
  ),
  pcs2 = PCSParameters (
   choice = 'NO',
   constants = ExtStageConstants (
    firstit = 0,
    lastit = 2,
    stages = StageConstants (
     hot = 100.0,
     cool1 = 100.0,
     cool2 = 100.0,
     cool3 = 1000.0,
    ),
   ),
   r = 1000.0,
   d = 10000.0,
  ),
  pcs3 = PCSParameters (
   choice = 'NO',
   constants = ExtStageConstants (
    firstit = 0,
    lastit = 2,
```

```
    stages = StageConstants (
      hot = 100.0,
      cool1 = 100.0,
      cool2 = 100.0,
      cool3 = 1000.0,
    ),
  ),
  r = 1000.0,
  d = 10000.0,
),
pcs4 = PCSParameters (
  choice = 'NO',
  constants = ExtStageConstants (
    firstit = 0,
    lastit = 2,
    stages = StageConstants (
      hot = 100.0,
      cool1 = 100.0,
      cool2 = 100.0,
      cool3 = 1000.0,
    ),
  ),
  r = 1000.0,
  d = 10000.0,
),
pcs5 = PCSParameters (
  choice = 'NO',
  constants = ExtStageConstants (
    firstit = 0,
    lastit = 2,
    stages = StageConstants (
      hot = 100.0,
      cool1 = 100.0,
      cool2 = 100.0,
      cool3 = 1000.0,
    ),
  ),
  r = 1000.0,
  d = 10000.0,
),
pcs6 = PCSParameters (
  choice = 'NO',
  constants = ExtStageConstants (
    firstit = 0,
    lastit = 2,
    stages = StageConstants (
      hot = 100.0,
      cool1 = 100.0,
      cool2 = 100.0,
      cool3 = 1000.0,
    ),
  ),
  r = 1000.0,
  d = 10000.0,
),
pcs7 = PCSParameters (
  choice = 'NO',
  constants = ExtStageConstants (
```

```
    firstit = 0,
    lastit = 2,
    stages = StageConstants (
      hot = 100.0,
      cool1 = 100.0,
      cool2 = 100.0,
      cool3 = 1000.0,
    ),
  ),
  r = 1000.0,
  d = 10000.0,
),
pcs8 = PCSParameters (
  choice = 'NO',
  constants = ExtStageConstants (
    firstit = 0,
    lastit = 2,
    stages = StageConstants (
      hot = 100.0,
      cool1 = 100.0,
      cool2 = 100.0,
      cool3 = 1000.0,
    ),
  ),
  r = 1000.0,
  d = 10000.0,
),
pcs9 = PCSParameters (
  choice = 'NO',
  constants = ExtStageConstants (
    firstit = 0,
    lastit = 2,
    stages = StageConstants (
      hot = 100.0,
      cool1 = 100.0,
      cool2 = 100.0,
      cool3 = 1000.0,
    ),
  ),
  r = 1000.0,
  d = 10000.0,
),
pcs10 = PCSParameters (
  choice = 'NO',
  constants = ExtStageConstants (
    firstit = 0,
    lastit = 2,
    stages = StageConstants (
      hot = 100.0,
      cool1 = 100.0,
      cool2 = 100.0,
      cool3 = 1000.0,
    ),
  ),
  r = 1000.0,
  d = 10000.0,
),
dan1 = DANIParameters (
```

```
  choice = 'NO',
  constants = ExtStageConstants (
    firstit = 0,
    lastit = 2,
    stages = StageConstants (
      hot = 1.0,
      cool1 = 5.0,
      cool2 = 10.0,
      cool3 = 10.0,
    ),
  ),
  tc = 9.771,
  anis = 1.557,
  r = 0.455,
  wh = 599.91,
  wn = 60.82,
),
dan2 = DANIParameters (
  choice = 'NO',
  constants = ExtStageConstants (
    firstit = 0,
    lastit = 1,
    stages = StageConstants (
      hot = 1.0,
      cool1 = 5.0,
      cool2 = 10.0,
      cool3 = 10.0,
    ),
  ),
  tc = 9.84,
  anis = -1.35,
  r = 0.308,
  wh = 599.91,
  wn = 60.82,
),
dan3 = DANIParameters (
  choice = 'NO',
  constants = ExtStageConstants (
    firstit = 1,
    lastit = 1,
    stages = StageConstants (
      hot = 1.0,
      cool1 = 5.0,
      cool2 = 10.0,
      cool3 = 10.0,
    ),
  ),
  tc = 9.84,
  anis = -1.35,
  r = 0.308,
  wh = 599.91,
  wn = 60.82,
),
dan4 = DANIParameters (
  choice = 'NO',
  constants = ExtStageConstants (
    firstit = 0,
    lastit = 2,
```

```
      stages = StageConstants (
        hot = 1.0,
        cool1 = 5.0,
        cool2 = 10.0,
        cool3 = 10.0,
      ),
    ),
    tc = 9.84,
    anis = -1.35,
    r = 0.308,
    wh = 599.91,
    wn = 60.82,
  ),
  dan5 = DANIParameters (
    choice = 'NO',
    constants = ExtStageConstants (
      firstit = 0,
      lastit = 2,
      stages = StageConstants (
        hot = 1.0,
        cool1 = 5.0,
        cool2 = 10.0,
        cool3 = 10.0,
      ),
    ),
    tc = 9.84,
    anis = -1.35,
    r = 0.308,
    wh = 599.91,
    wn = 60.82,
  ),
  username = 'melk@mit.edu',
)
```