Determination of Structural Topology of a Membrane Protein using Polarization Optimized Experiments (POE) for Static and MAS Solid-State NMR Spectroscopy

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SUPPLEMENTARY INFORMATION

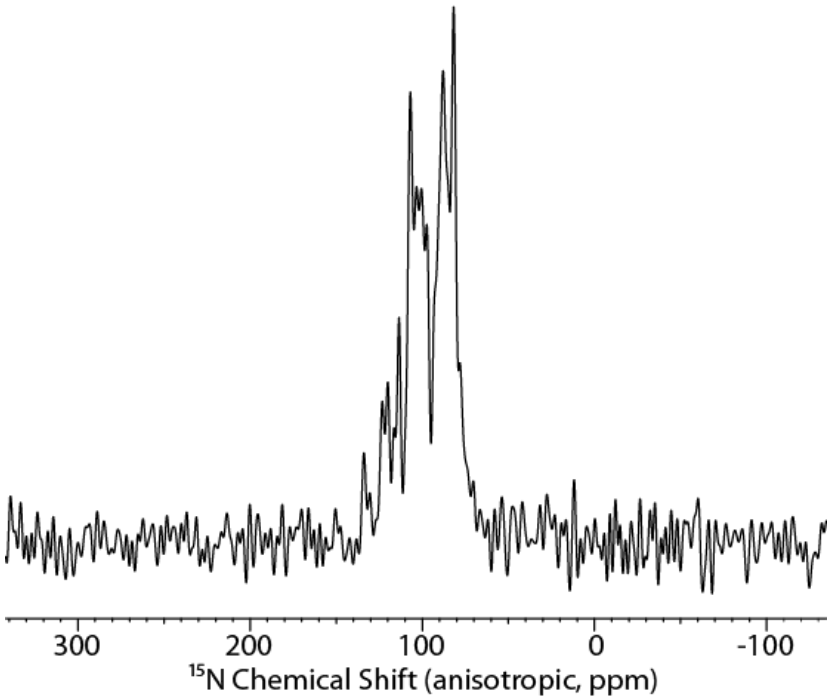

Figure S1

1D-cross polarization ^{15}N spectrum of U^{15}N-SLN in oriented DMPC/POPC/D6PC bicelles (184 transients, Fourier transformed without window function)

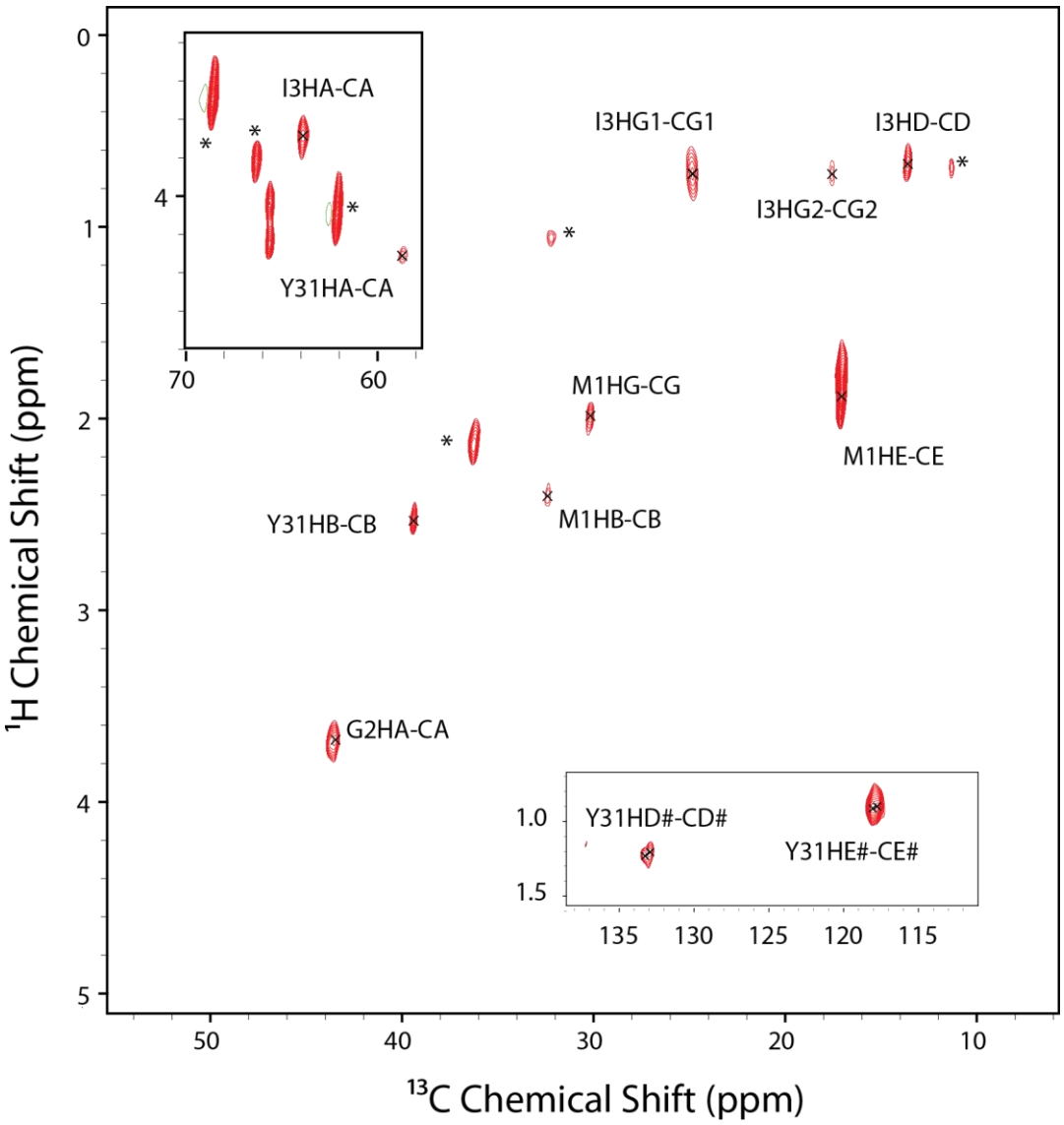

Figure S2

Refocused-INEPT spectrum of SLN at 298.15 K. Residues 1-3 and 31 are assigned using this spectrum. Lipid peaks are marked by an asterix.

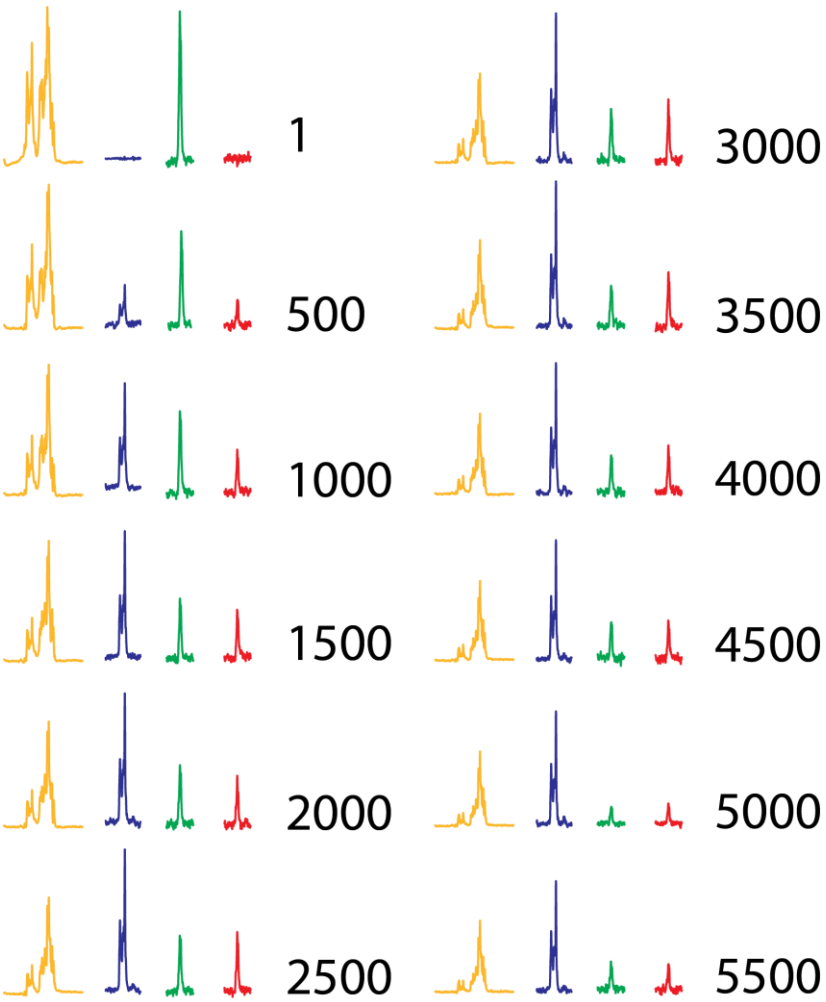

Figure S3

Dependence of polarization available for the 4 pathways during MEIOSIS experiment on the contact time for the first SPECIFIC-CP step (N-Cα transfer) for $U^{13}C^{15}N$-sarcolipin in lipid bilayers. In orange (lane 1), residual ^{13}C polarization after NCα transfer (used for ^{13}C-^{13}C DARR experiment) in blue (lane 2) ^{15}N polarization after the NCα transfer (used for the N(CA)CX experiment); in green (lane 3), residual ^{15}N polarization after the NCα transfer (used for the NCO experiment) and in red (lane 4), ^{13}C polarization coming via ^{15}N (used in the CA(N)CO experiment).

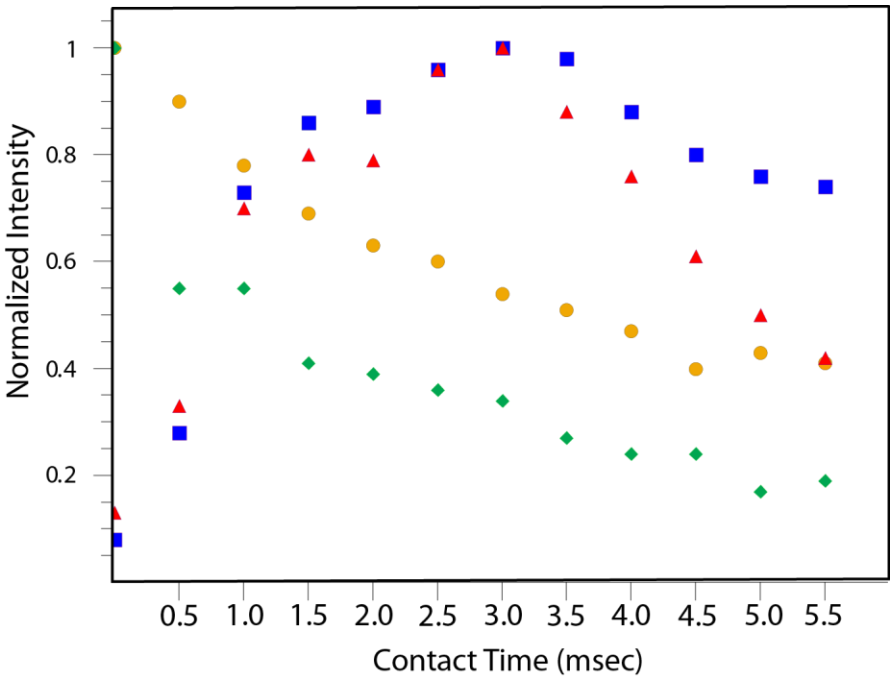

Figure S4

Integrated intensities of the spectra for the four pathways in MEIOSIS (normalized to the maximum in each of the pathways), color coded as shown in Figure S3. The maximum for ^{15}N-^{13}C and ^{13}C-^{15}N polarization transfer occurs at the contact time of 3.0msec and the relative intensity at this point for residual ^{13}C polarization is 54% and residual ^{15}N polarization is 34%.

Setting up 2D-DUMAS and 2D MEIOSIS Experiments

On the indirect evolution in ^{15}N and ^{13}C dimensions:

The evolution time for ^{15}N and ^{13}C are independent for 2D-DUMAS and 2D MEIOSIS and this gives the flexibility to choose any spectral width and any evolution time. For 2D-MEIOSIS, the DARR mixing time for ^{13}C-^{13}C DARR and N(CA)CX time is the same. The t1 evolution time for N(CA)CX and NCO will be the same and ^{13}C-^{13}C DARR and CA(N)CO will be the same.

Keep in mind that spectral width for most uniformly labeled proteins will be 20-25ppm for ^{15}N and 180-200ppm for ^{13}C. Such a difference between spectral widths is, however, not a requirement for any of the POE experiments in MAS-ssNMR. For example, these experiments will work just as well in the case of partially/specifically labeled samples, where the ^{13}C spectral widths will be much smaller than 180-200ppm)

Of consideration here is the choice of setup depending on sample conditions. We discuss the cases below:

1. **High sensitivity for ^{15}N-edited but poor sensitivity for ^{13}C-edited experiment** (typically this would be in situations where DARR mixing > 150ms, partial ^{13}C labeling or ^{13}C-single quantum-double quantum correlation experiment):

In this case one would ideally like to acquire a ^{13}C-edited experiment (eg. DARR) with a large number of scans and a^{15}N-edited experiment (eg. NCA) with shorter number of scans. Let's say, DARR is being acquired with 'x' scans and 'y' indirect increments. While you can run this DARR experiment, you can acquire during the second acquisition:

a. Two independent ^{15}N-edited experiments with y/2 increments each. (eg. NCA with x scans and y/2 increments and NCO with x scans and y/2 increments)
b. NCACX (or NCOCX) with 2 different mixing times, each with x scans and y/2 increments
c. ^{15}N and ^{13}C T_2 relaxation can be quite different in the same protein and this must be accounted for wile setting up t_1 evolution times. As the dwell time for ^{15}N evolution (τ_N) is independent of the dwell time during ^{13}C evolution (τ_C), you can shorten your dwell time ($\tau_N \leq 10\tau_C$ for most proteins), and acquire:
   i. y/6 increments for NCA and then repeat this experiment 6 times (6x scans with t_1 evolution of y*τ_N/6)
   ii. y/4 increments and repeat 4 times (4x scans, y*τ_N/4 t_1 evolution)
   iii. y/3 increments and repeat 3 times (3x scans, y*τ_N/3 t_1 evolution)

d. You are not restricted to the same experiment for the different options in (d). You can acquire NCA, NCACX with different mixing times, NCO or NCOCX with different mixing times all in the same experiment.

2. **High sensitivity for ^{13}C-edited but poor sensitivity for ^{15}N-edited experiment** (typically this would be in situations SPECIFIC-CP transfers are inefficient – membrane proteins, disordered aggregates, etc.):
   a. All of the points in (1) above still apply.
   b. Let's say, the t1 evolution parameters are the same as before. You can reduce the number of transients and do the entire experiment (DARR with x/2 scans and y increments, NCA with x/2 scans and y increments). Then repeat the experiment after changing the ^{13}C-edited block (to a different mixing time, or a different experiment altogether) and keeping the ^{15}N-edited block the same. The multiple ^{15}N-edited experiments thus acquired can be added up later to augment the SNR.
   c. Point (c) from Case 1 will help augment SNR in this case for the ^{15}N edited experiment

The 2D-MEIOSIS experiment will also be beneficial for membrane proteins and fibrils where the NCA transfers are inefficient and N(CA)CX experiment requires a lengthy acquisition. Note that:

a. DARR spectrum is from residual ^{13}C polarization, which is 54%. Hence, if you need to acquire a traditional DARR with the same SNR as that of MEIOSIS-DARR, you would need ~0.25x the experimental time. Also, only the cross-peaks corresponding the transfer of magnetization from side chains to Cα will be observed as the Cα to N transfer occurs before the DARR mixing element.
b. NCO is from residual ^{15}N-magnetization, which is 30% of what one would get if you wanted to obtain an NCO spectrum. So, one would need ~0.09x the experimental time for a conventional NCO with same SNR as that of MEIOSIS-NCO
c. NCA and CA(N)CO are from direct ^{15}N and ^{13}C polarization respectively. The total experimental times need to acquire these spectra independently with the same SNR as that in a MEIOSIS experiment are thus, 0.9x and 1.0x, respectively.
d. Trasseth and co-workers (Banigan et al. 2013) described how residual polarization can be used for resonance assignment in combination with selective labeling schemes. In addition to the residual-NCO pathway suggested in this experiment, MEIOSIS provides the CA(N)CO pathway that can be used to supplement such a strategy.

This experiment is ideal if one wants to set up 3D experiments as it will allow a quick determination of relative dispersion in each dimension, choosing the appropriate mixing times as well as help choosing an appropriate 3D experiment that gives the best chance

of assigning a majority of the residues. That is why we chose to implement this experiment on SLN before setting up the 3D experiments.

Comparison of DUMAS-DARR-NCA and Conventional DARR and NCA experiments

In the comparison given in Table S1 and Fig S5, the DUMAS-DARR–NCA is acquired in the following manner:

1. 32 transients co-added for each t1 increment.
2. 128 total increments for ^{13}C giving a total evolution time of 4.1ms
3. 8 experiments of NCA repeated with 16 increments each giving total evolution time of 4.8ms for ^{15}N

Table S1: Time savings for DUMAS experiments

Conventional 2D			**2D DUMAS**		
Expt.	Time	Relative SNR	Expt.	Time	Relative SNR
DARR	4.1 hrs	1	DUMAS-DARR	4.1 hrs	1
NCA	4.1 hrs	1	DUMA-NCA		0.8
	Total Time = 8 hrs			**Total Time = 4.1 hrs**	

Conventional 3D			**3D DUMAS**		
Expt.	Time	Relative SNR	Expt.	Time	Relative SNR
CANCO	12 days*	1	DUMAS-CANCO	12 days	1
NCACX	11 days*	1	DUMA-NCACX		1
	Total Time = 23 days*			**Total Time = 12 days**	

*Estimated time required. We performed only the DUMAS-3D experiments. For a side-by-side comparison of spectra acquired using 3D-DUMAS and conventional 3D pulse sequences (polycrystalline ubiquitin), see (Gopinath and Veglia 2012)

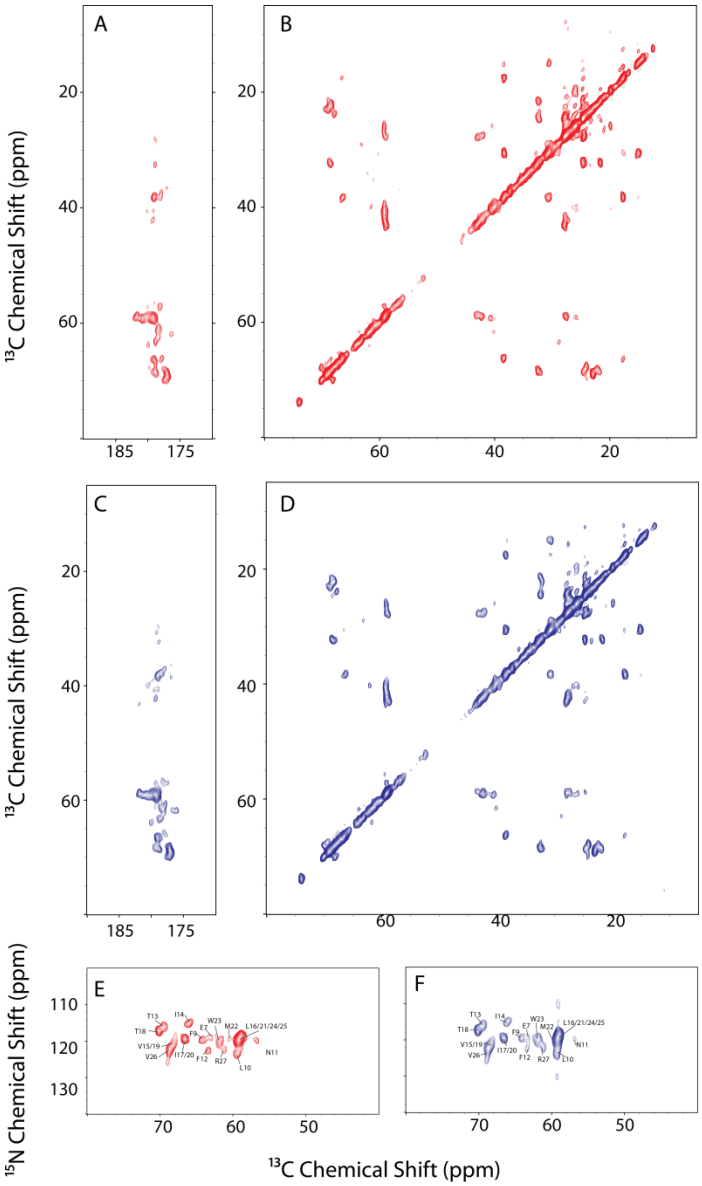

Figure S5

Comparison of spectra acquired using:

(A) DUMAS-DARR (C' region)
(B) DUMAS-DARR (Cα-Cβ region)
(C) Conventional-DARR (C' region)
(D) Conventional DARR (Cα–Cβ region)
(E) DUMAS-NCA
(F) Conventional NCA

Comparison of MEIOSIS-(DARR-NCACX-CANCO-NCO) with conventional experiments

In the comparison given in Figure S6 and Table S2, the experimental setup was as follows:

1. 64 transients for each t1 increment (due to an 8 step-phase cycle, this corresponds to 128 transients when compared to conventional experiment which is generally obtained with only a 4-step phase cycle)
2. 128 t1 increments, giving ^{13}C evolution time of 4.1ms
4. 8 experiments of NCA repeated with 16 increments each giving total evolution time of 4.8ms for ^{15}N

Table S2: Time Savings for 2D-MEIOSIS experiments

Conventional Experiment			**2D-MEIOSIS**		
Expt.	Time	Relative SNR	Expt.	Time	Relative SNR
DARR	2.0 hrs	1	MEIOSIS-DARR	9.15 hrs	1
N(CA)CX	9.15 hrs	1	MEIOSIS-N(CA)CX		0.8
NCO	1.2 hrs	1	MEIOSIS-NCO		0.8
CA(N)CO	9.15 hrs	1	MEIOSIS-CA(N)CO		1
	Total Time = 21.2 hrs			**Total Time = 9.15 hrs**	

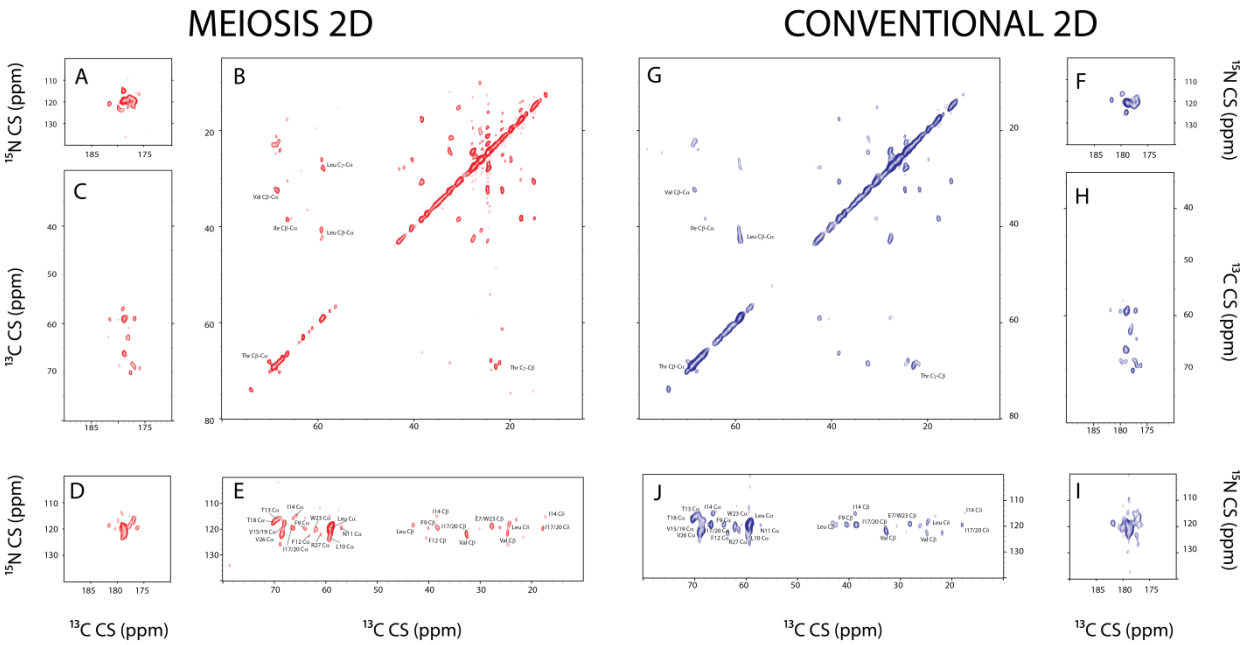

Figure S6

Comparison of spectra acquired using:

(A) MEIOISIS-NCO (residual ^{15}N pathway)
(B) MEIOSIS-DARR (residual ^{13}C pathway)
(C) MEIOSIS-CANCO
(D) MEIOSIS-NCACX (C' region)
(E) MEIOSIS-NCACX (Cα-Cβ region)
(F) Conventional-NCO
(G) Conventional -DARR
(H) Conventional -CANCO
(I) Conventional -NCACX (C' region)
(J) Conventional -NCACX (Cα-Cβ region)

A-E are acquired simultaneously using 2D-MEIOSIS (Fig 1B in the main text)

Table S3: Isotropic Chemical Shift Assignments in Lipid Bilayers (MAS-ssNMR). NA=Not Assigned.

Res No	RES TYPE	Cα (ppm)	N (ppm)	Cβ (ppm)	Cγ1 (ppm)	C' (ppm)	Cγ2 (ppm)	Cδ/Cε (ppm)
1	M	NA	NA	34.4	32.2	NA	NA	19.1
2	G	45.5	NA	NA	NA	NA	NA	
3	I	65.9	NA	NA	26.84	NA	19.6	15.6
4	N	52.7	NA	40.6	NA	NA		
5	T	NA	NA	NA	NA	NA		
6	R	NA	NA	NA	NA	NA		
7	E	61.9	119.1	28.6	38.4	177.5		179
8	L	57.8	118.4	39.5	24.8	177.7		26.9
9	F	63.3	118.6	37.3	NA	178.9		
10	L	58.2	122.3	40	24.8	178.7		26.9
11	N	55.1	118.4	41.5	NA	177.1		
12	F	63.4	118.5	36.4	NA	176.3		
13	T	68.8	115.4	68.8	22.2	177.6		
14	I	65.5	114.4	37.6	NA	176.4	16.6	
15	V	67.9	121.1	31.7	30	177.6	20.8	
16	L	58.1	119.2	39.5	NA	178.3		26.9
17	I	66.1	118.4	37.4	NA	176.2	16.9	14.1
18	T	69.4	116.1	67.1	21.3	176.1		
19	V	67.3	120.2	31.3	30	177.6	20.8	
20	I	65.7	118.3	37.3	NA	177.8		
21	L	58	118.4	39.1	24.8	179.9		26.9
22	M	60.4	120.3	33.4	34	181.1		
23	W	61.6	121	28	NA	178.1		
24	L	57.8	118.3	42.3	24.8	181		26.9
25	L	57.7	118.3	39.6	24.8	179.1		26.9
26	V	68.1	123	31	23.8	176.1		
27	R	60.2	120.2	30.4	NA	175.3		
28	S	61.8	113.2	64.0	NA	NA		
29	Y	60.8	118.3	39.5	NA	NA		
30	Q	59.3	118.6	29.7	NA	NA		
31	Y	60.7	NA	41.4	NA	NA		

Table S4: Assignments for Chemical Shift Anisotropy and Dipolar Couplings in Lipid Bicelles (O-ssNMR). NA= Not Assigned, DO=Shifts scaled by conformational dynamics.

Res No	RES TYPE	NCSA (ppm)	NHDC (kHz)	HCSA (ppm)
1	M	NA	NA	NA
2	G	NA	NA	NA
3	I	NA	NA	NA
4	N	NA	NA	NA
5	T	NA	NA	NA
6	R	56.1	8.6	NA
7	E	57	7.2	NA
8	L	73.8	4	NA
9	F	82.5	6	NA
10	L	64.5	9.8	NA
11	N	58.6	8.1	5
12	F	73.2	4	5.2
13	T	83.4	8.9	4.7
14	I	42.6	8.3	3.6
15	V	59.1	4.3	1.8
16	L	87	6.2	5.1
17	I	75.9	10.3	5.6
18	T	51.8	5.4	2.7
19	V	67	4.1	3.2
20	I	88.2	7.8	5.3
21	L	57.5	10.3	7
22	M	53.1	4.1	1.3
23	W	81	5.4	4.7
24	L	75.3	8.9	2.7
25	L	47.4	6.8	5.1
26	V	76.9	5.2	5.5
27	R	81.6	10.3	2.3
28	S	53.5	6.9	NA
29	Y	DO	DO	NA
30	Q	DO	DO	NA
31	Y	DO	DO	NA

References

Banigan JR, Gayen A, Traaseth NJ (2013) Combination of (15)N reverse labeling and afterglow spectroscopy for assigning membrane protein spectra by magic-angle-spinning solid-state NMR: application to the multidrug resistance protein EmrE. Journal of biomolecular NMR 55:391–9. doi: 10.1007/s10858-013-9724-z

Gopinath T, Veglia G (2012) 3D DUMAS: simultaneous acquisition of three-dimensional magic angle spinning solid-state NMR experiments of proteins. J Magn Reson 220:79–84. doi: 10.1016/j.jmr.2012.04.006