Supporting Information

Structures of the Excited States of Phospholamban and Shifts in their Populations upon Phosphorylation

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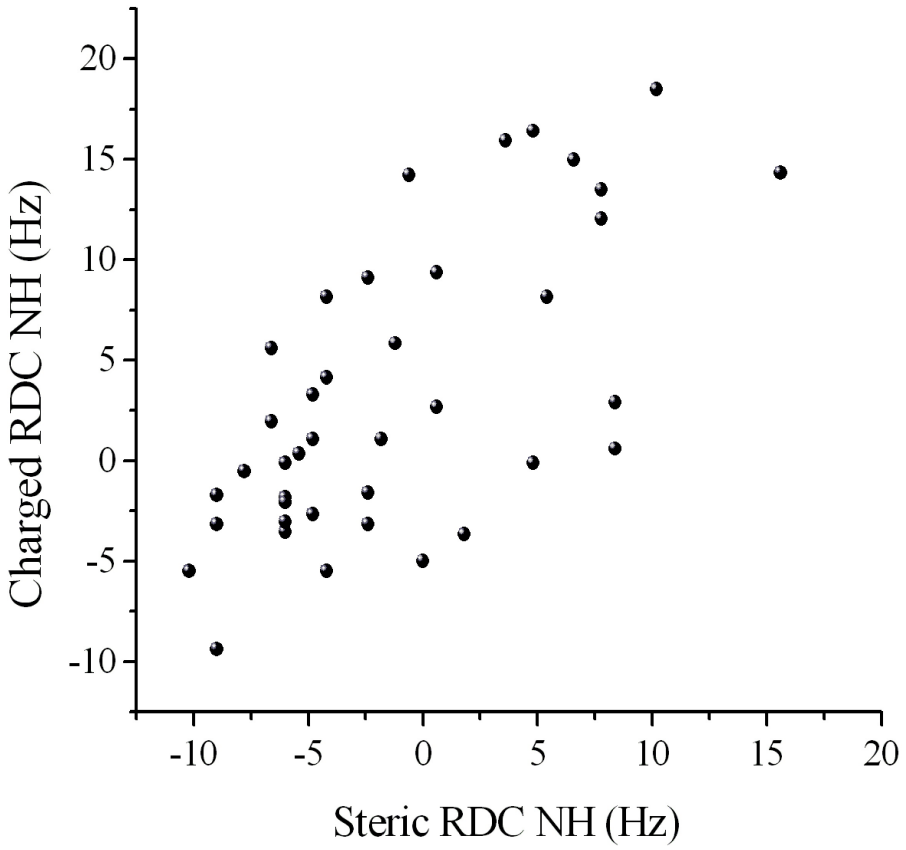

Figure S1. Correlation between the RDCs for AFA-PLN measured in neutral and charged gels (see also Tables S1 and S2). The Q factor between the two sets of RDCs is 1.13 (RMSD 5.39 Hz).

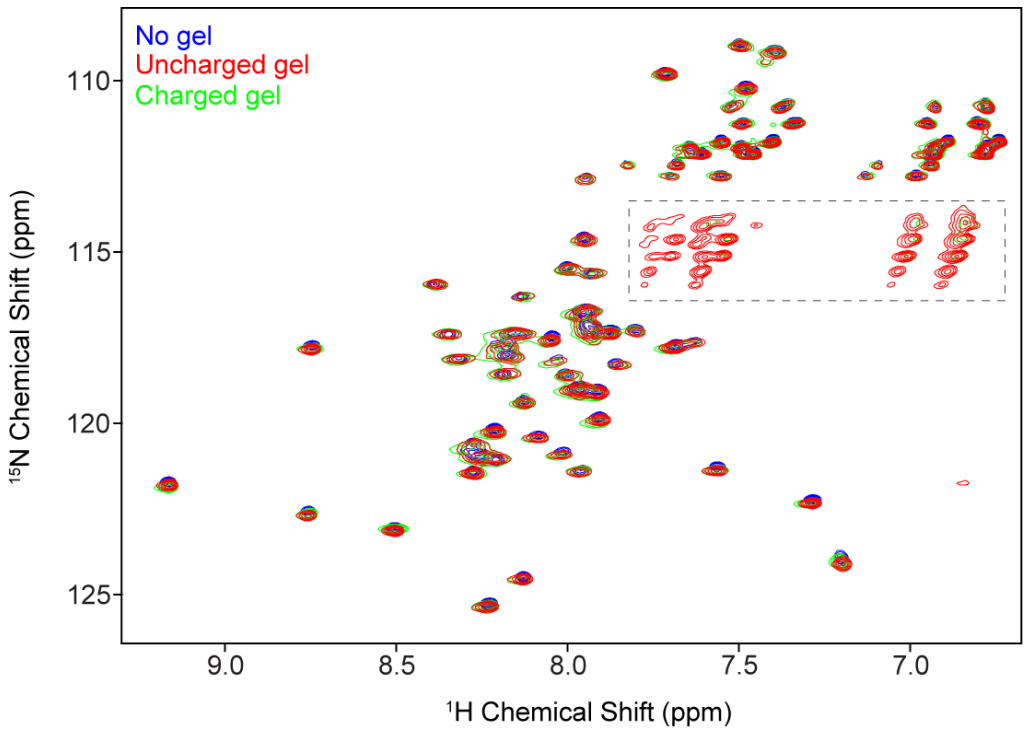

Figure S2. ^{1}H-^{15}N HSQC spectra of AFA-PLN in DPC micelles. The spectra recorded in isotropic solution (blue) and in charged (green) and uncharged (red) gels are overlaid.

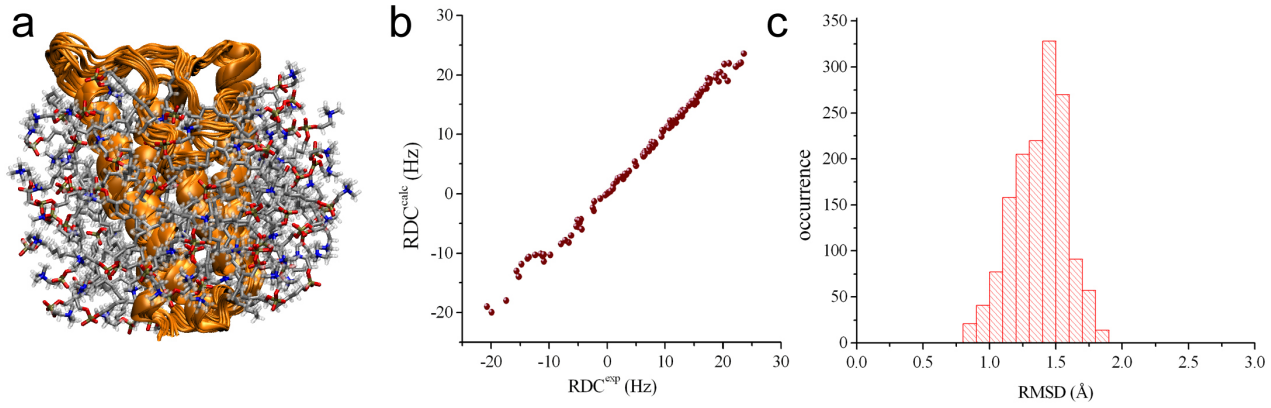

Figure S3. Summary of the determination of the structural ensemble of DsdB in micelle determined by adopting the same approach used for PLN. The molecular dynamics simulations were started from an NMR structure[1] obtained by NOE, PRE, RDC and dihedral restraints (PDB code: 2K73) and generated a micelle-bound conformation by following the protocol described in the Methods. We then determined the micelle-bound DsdB structural ensemble by using molecular dynamics simulations (see Methods) with replica-averaged RDC restraints; we employed ^{15}N-^{1}H RDC measured in positively charged stretched gels. A) Structural ensemble of DsdB; protein structures are shown by orange ribbons, DPC lipids by sticks. B) Comparison between calculated and experimental ^{15}N-^{1}H RDC (Q Factor 0.075, RMSD 0.83 Hz). C) Distribution of Cα-RMSD values from the PDB structure 2K73 for the conformations in the ensemble shown in panel (A).

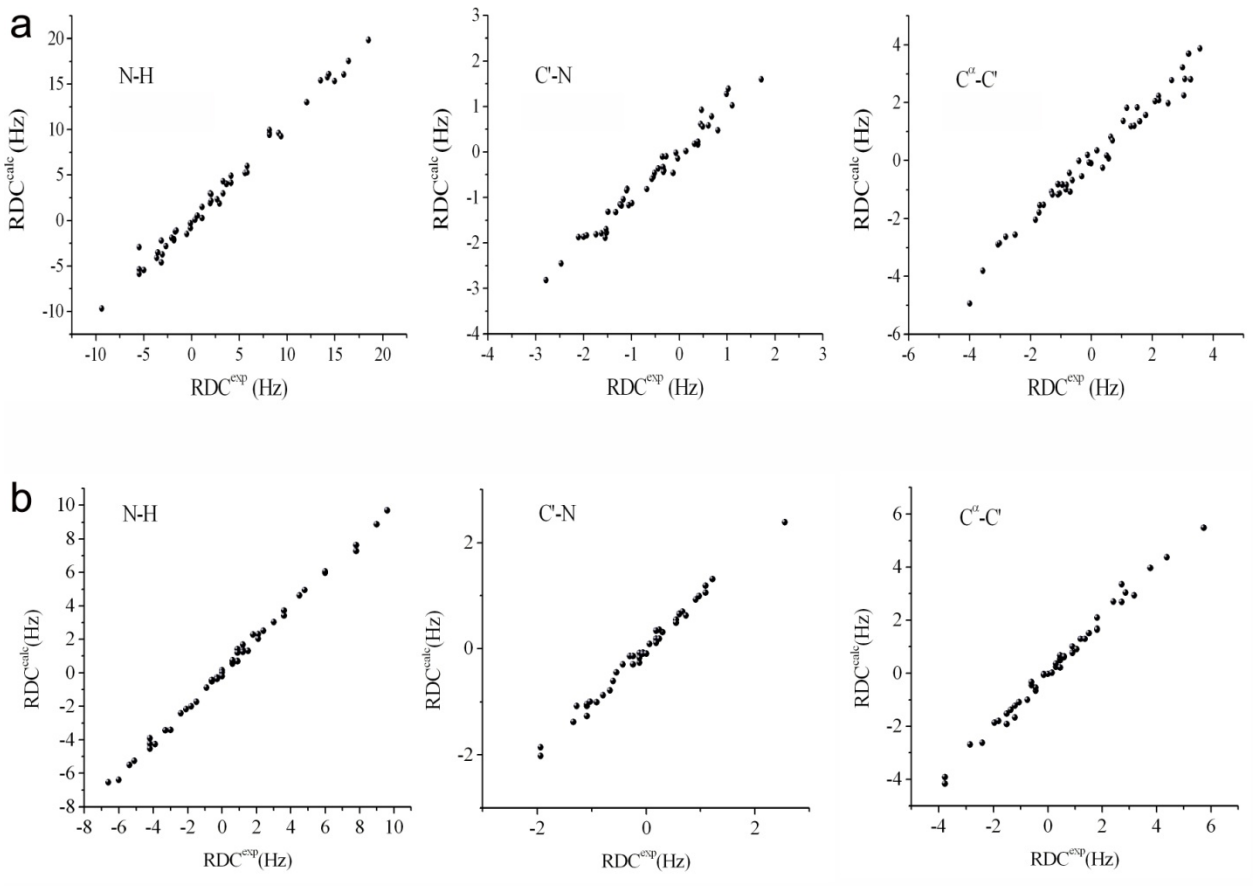

Figure S4. Correlation between experimental and calculated RDC values: (a) PLN, and (b) pS16-PLN. Results are shown for the RDCs used as restraints (neutral gel in Tables S1 and S2); those not used as restraints (charged gel in Tables S1 and S2) are shown in Fig. S4. Standard Deviations in panel A are (from the left) 0.27Hz (Q = 0.107), 0.26Hz (Q = 0.153), 0.28Hz (Q = 0.131). Standard Deviations in panel B are (from the left) 0.22Hz (Q = 0.057), 0.18Hz (Q = 0.122), 0.20Hz (Q = 0.101).

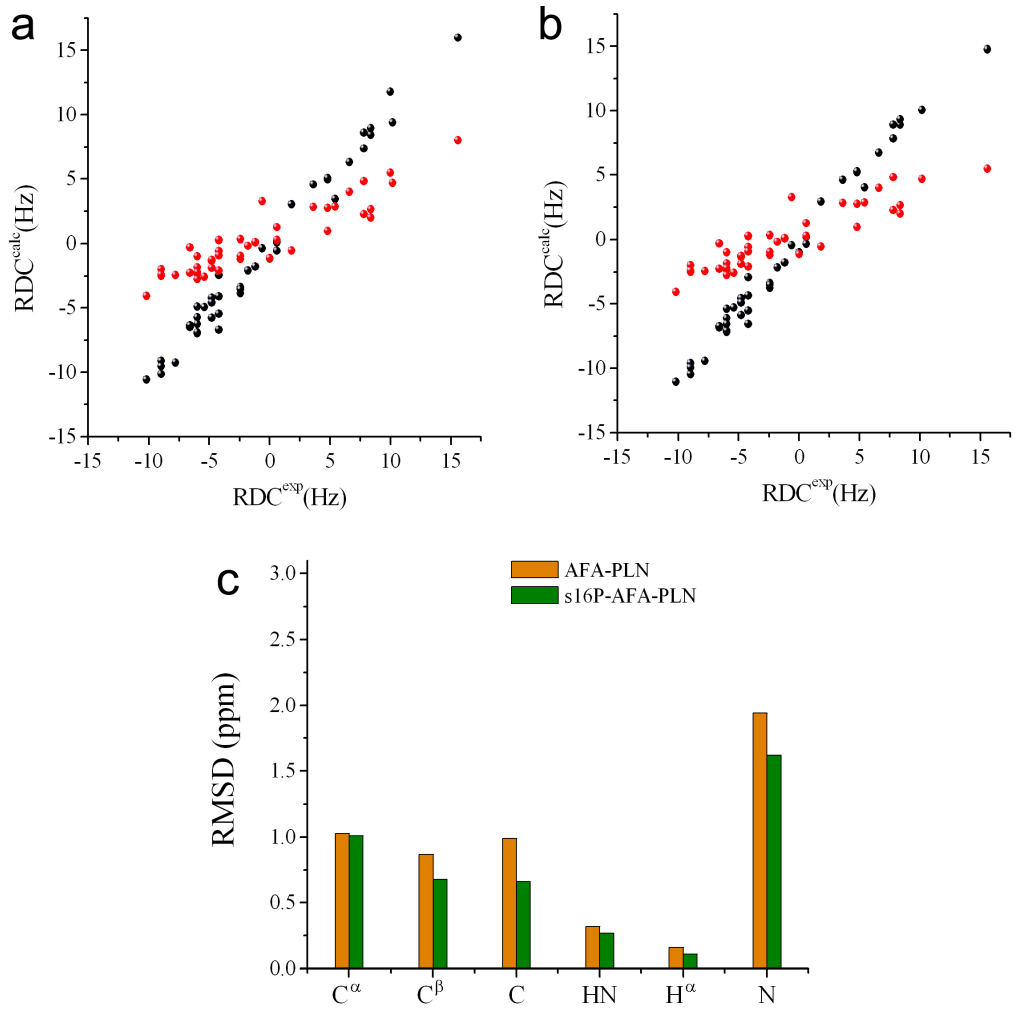

Figure S5. Validation of the PLN and pS16-PLN structural ensembles with NMR data not used in the structure determination. (a,b) Comparison between measured and back-calculated RDC values recorded in negatively charged gels (Tables S1 and S2). Black and red dots refer to back-calculations performed with a structural-based alignment method[2,3] and a SVD fitting[4], respectively. (a) PLN. Q factors: 0.15 and 0.65 for structure-based and SVD alignment methods, respectively. Standard deviations: 0.93Hz and 1.04Hz for structure-based and SVD alignment methods, respectively. (b) pS16-PLN. Q factors: 0.15 and 0.68 for structure-based and SVD alignment methods, respectively. Standard deviations: 0.81Hz and 1.00Hz for structure-based and SVD alignment methods, respectively. (c) Standard deviations between measured chemical shift values and those back-calculated on the PLN and pS16-PLN ensembles by using the Sparta+ program[5]. Orange and green bars refer to PLN and pS16-PLN, respectively. The standard errors of Sparta+ are shown in blue.

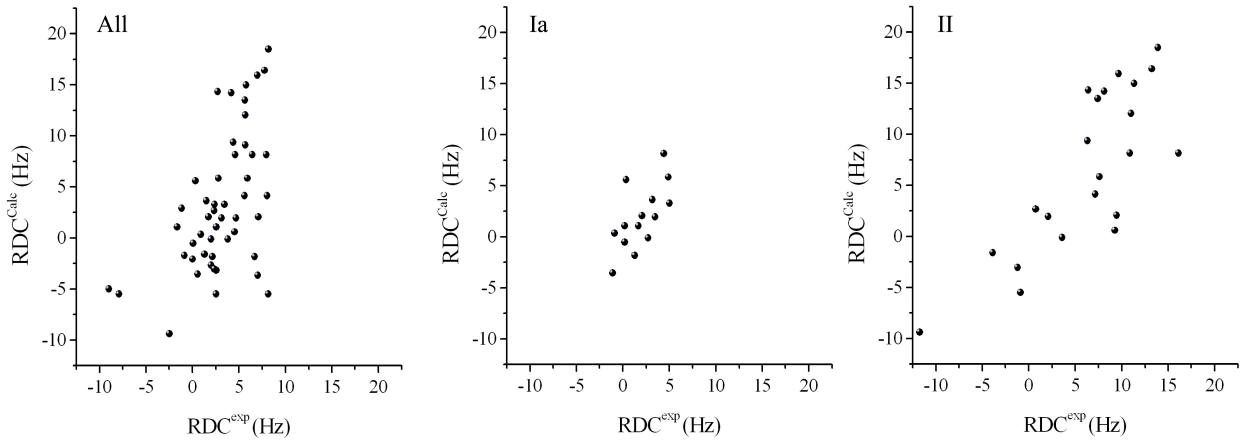

Figure S6. Results of the SVD fit to individual helices (i.e. domain Ia and domain II). The Q factors are 1.18 for the entire protein (SD 5.707 Hz), 0.83 for domain Ia (SD 2.884 Hz) and 0.53 (SD 5.44 Hz) for domain II.

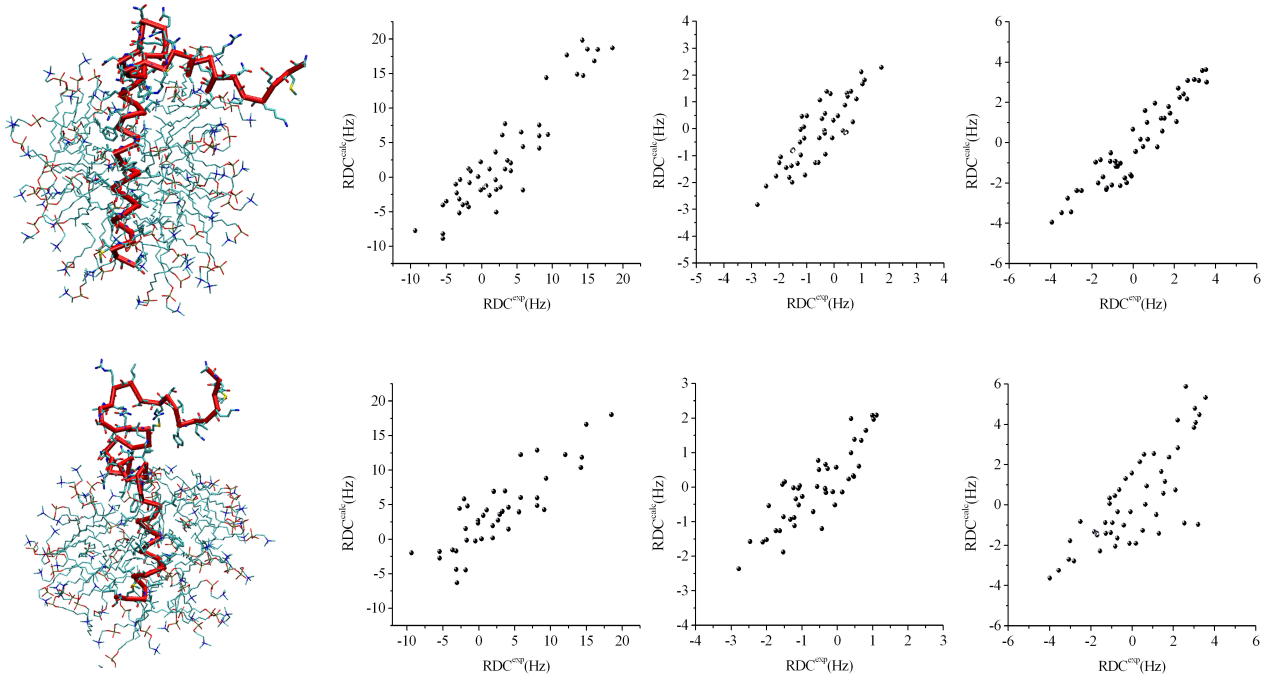

Figure S7. Results of the calculations carried out by applying the RDC restraints to single conformations of PLN. Top and bottom conformations were extracted as the centroids of the T and R basins (Figure 5), respectively. The agreement between experimental and calculated RDC values (of individual conformations) was optimized by single-replica restrained MD simulations, i.e. by following the protocol described in the methods section. Standard Deviations in the top panel are (from the left) 2.99 Hz (Q = 0.409), 0.775 Hz (Q = 0.633), 0.721 Hz (Q = 0.372). Standard Deviations in panel B are (from the left) 6.72 Hz (Q = 0.918), 0.839 Hz (Q = 0.719), 1.47 Hz (Q = 0.758).

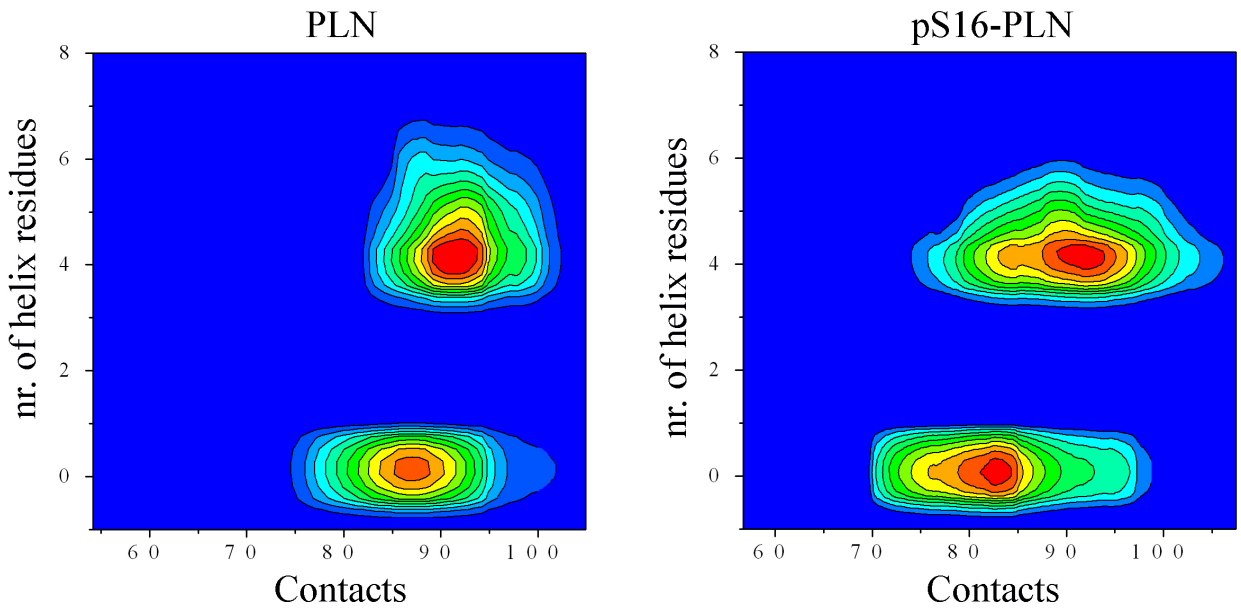

Figure S8. Free energy landscapes of PLN and pS16-PLN obtained by the same molecular dynamics protocol used in Fig. 4 in the main text but without RDC restraints. These results demonstrate that the conformationally excited states identified for PLN and pS16-PLN are not metastable states in the force field used in the simulations, but they arise from the information provided by the RDC restraints.

Table S1.
Measured RDCs for AFA-PLN (in Hz)

	Neutral gel						Charged gel
	NH		NCO		CαCO		
	value	error	value	error	value	error	NH
					-1.04	0.31	
3	5.84	1.74	-0.44	0.37	-0.63	0.30	-1.20
4	-0.12	0.69	0.99	0.33	1.30	0.23	-6.00
5	1.95	0.36	-1.55	0.18	-0.32	0.33	-6.60
6	8.15	1.50	1.11	0.24	-1.10	0.26	-4.20
7	-0.49	1.14	-0.35	0.19	1.41	0.40	-7.79
8	0.37	1.39	-0.99	0.25	1.05	0.55	-5.40
9	-1.83	1.25	-1.09	0.60	0.37	0.40	-6.00
10	3.65		0.47	0.33	-0.96	0.52	
11	5.60	0.66	-1.33	0.31	2.21	0.28	-6.60
12	1.10	1.34	-1.17	0.20	0.18	0.37	-4.80
13	3.29		1.72	0.21	-1.72	0.36	
14	1.10	1.33	0.45	0.26	2.64	0.41	-1.80
15	2.07		-0.27	0.25	1.59	0.30	
16	-3.53	0.50	-1.21	0.25	2.22	0.25	-6.00
17	2.92	2.15	-0.54	0.19	-1.84	0.26	8.39
18	4.14	1.05	-1.63	0.33	0.57	0.15	-4.20
19	-5.48	0.86	-0.68	0.13	1.17	0.20	-10.19
20	-3.16	0.59	-1.24	0.22	-0.41	0.17	-8.99
21	-4.99	0.65	-1.22	0.14			0.00
22					-0.01	0.41	
23	-2.68	0.26	-0.32	0.64	0.64	0.36	-4.80
24	-1.70	1.84	-0.52	0.37	-2.82	0.32	-8.99
25	-3.65	0.25	0.14	0.30	-0.82	0.34	1.80
26	-1.83	0.66	-0.13	0.45	-0.72	0.31	
27	-2.07	0.99	-0.34	0.33	-0.84	0.35	-6.00
28	-3.16	0.61	-1.10	0.28	2.53	0.61	-2.40
29	-5.48	1.60	-0.57	0.52	-1.57	0.53	-4.20
30	9.13	1.04	-1.49	0.55	-1.31	0.40	-2.40
31	3.29	1.08	0.61	0.37	1.51	1.38	-4.80
32	-3.04	1.65	1.03	0.54	3.26	0.74	-6.00
33	8.15				-3.07	1.29	
34	14.24	5.90	-1.52	0.92	-0.08	0.53	-0.60
35	-1.58	1.38	0.81	0.39	3.20	1.04	-2.40
36	4.14	1.12	-2.79	0.41	-3.57	0.85	-4.20
37	16.43	1.64	0.68	0.90	-1.28	0.73	4.80
38	14.97	3.61	0.39	0.50	1.79	0.67	6.60
39	1.95	0.19	-2.00	0.68	-1.10	0.37	
40	2.07				-4.00	0.70	
41	8.15	3.12			-0.13	0.45	5.40

42	-0.12	2.99	0.49	0.39	3.56	0.55	4.80
43	9.37	2.11	-2.11	0.41	3.04	0.54	0.60
44	15.94	1.39	-2.47	0.58	-0.70	0.60	3.60
45	0.61	4.20	0.39	0.48	0.51	0.46	8.39
46	-5.48	4.48	-0.07	0.26	3.00	0.50	
47	5.84	0.05	-1.74	0.33	0.70	0.74	
48	18.50	0.86	0.32	0.54	-1.70	0.58	10.19
49	12.05	4.21	-0.03	0.44	2.10	0.48	7.79
50	2.68	2.18	-1.94	0.34	3.08	0.66	0.60
51	13.51	5.93	-1.06	0.78	-2.51	0.54	7.79
52	14.36	4.35	-0.33	0.61	-3.02	0.24	15.59
53	-9.37	2.31	-1.53	0.39			-8.99

Table S2
Measured RDCs for pS16-PLN (in Hz)

	Neutral gel						Charged gel
	NH value	error	NCO value	error	CαCO value	error	NH
3			0.67	2.58	-4.98	1.46	-1.20
4	-3.00	1.70	0.61	1.36	-2.41	1.47	-8.99
5	0.60	0.00	-0.91	0.54	0.00	0.82	-5.40
6	3.60	0.84	0.73	0.67	-1.51	1.22	1.80
7	1.50	0.42	-0.30	0.61	0.30	0.78	-7.19
8	-1.50	0.43	0.06	0.90	1.51	0.97	-7.19
9	2.40	0.00	-0.24	1.18	-0.15	1.03	-1.20
10					0.45	1.20	
11	-2.10	1.28	-0.12	2.42	-1.21	2.53	-6.60
12	-0.60	1.70	0.55	1.00	2.41	1.07	-8.39
13	-0.90	2.97	0.30	0.66	0.45	0.80	0.00
14	1.80	0.00	-1.34	1.45	0.90	1.32	0.60
15	0.00		1.09	2.29	2.86	1.51	
16	0.90	0.43	-0.06	2.42	1.21	1.21	-1.20
17	6.00	2.54	2.55	2.55	-1.21	1.21	10.19
18	1.20	1.70	-1.09	1.39	-0.45	0.65	0.60
19	1.20	2.54	0.61	0.61	-0.15	0.48	-3.00
20	-0.60	0.00	-0.06	0.51	-1.96	0.64	1.20
21	0.60	0.85	-0.67	0.59	-1.06	0.54	-1.80
22							
23	1.20	0.00	-0.12	5.13	-1.36	1.99	-4.80
24	-2.40	4.25			-3.77	1.75	-6.60
25	-4.20	1.69	-0.12	1.18	1.81	1.32	-5.40
26	-4.20	x	-0.12	0.96	1.81	2.06	
27	-3.90	2.13	0.55	1.42	-1.51	0.98	-4.20
28	-4.20				-0.75	1.18	-7.19
29	0.90	0.43	0.18	1.40			-11.39
30	-0.30	0.42	-1.09	1.33	-3.77	1.04	-1.20
31	-3.30	1.28	0.91	0.57	0.15	1.31	-8.39
32	-6.60	1.70	-1.28	1.13	4.37	2.60	-8.99
33	-5.40		0.24	0.68			7.19
34	0.60				3.77	1.67	
35	-1.80	0.85	-0.24	1.05	-0.45	1.13	-1.80
36	-6.00	2.54	-0.43	0.95	5.73	1.91	-6.00
37	2.10	5.51	-1.94	1.38	-0.60	1.50	12.59
38	4.50	1.27	0.18	1.21	0.60	1.52	7.79
39	0.00	2.55			0.90	1.50	-6.00
40	2.10	2.97	-0.55	0.81	0.45	1.00	3.00

41	6.00	3.40					
42	3.00	1.70	0.18	0.89	-1.81	0.98	5.40
43	-0.30	1.27	-1.94	0.93	1.81	1.12	-1.80
44	4.80	0.85	0.24	1.10	0.30	1.01	6.60
45	7.80	1.69	1.09	0.87	0.45	1.02	10.79
46	0.00	0.85	-0.61	0.94	1.06	1.07	1.20
47	3.60	0.85	-1.09	0.83	3.17	0.98	1.20
48	9.60	0.01	0.00	1.16	-2.86	1.34	12.59
49	7.80	0.01	1.22	1.02	2.71	1.48	6.60
50	0.90	2.12	-1.03	0.80	1.36	0.99	-0.60
51	0.60	3.39	0.18	1.19	2.71	1.15	8.99
52	9.00	1.71	0.97	1.07	-0.60	0.99	13.19
53	-5.10	0.42	-0.79	0.62	0.60	0.45	-8.39

References

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