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Supporting Information

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Dual Acquisition Magic-Angle Spinning Solid-State NMR-Spectroscopy: Simultaneous Acquisition of Multidimensional Spectra of Biomacromolecules**

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Experimental Section

NMR Spectroscopy

The ^{13}C and ^{15}N RF carrier frequencies were centered at 100.4 and 121.6 ppm respectively. The 90^{o} pulse length for ^{1}H, ^{13}C and ^{15}N were 2.5, 5.5 and 5.5 μs, respectively. During conventional CP (^{1}H-^{13}C or ^{1}H-^{15}N) and SIM-CP (^{1}H-^{13}C-^{15}N CP), ^{13}C and ^{15}N RF amplitudes were set to 36.7 kHz, whereas ^{1}H RF amplitude was linearly ramped from 80% to 100 % with the center of the slope set at 45 kHz. Based on the 1D calibration spectra (Figures 2S-4S), the Hartmann-Hahn contact time for ^{1}H-^{13}C and ^{1}H-^{15}N CP were respectively set to 400 and 600 μs for ubiquitin, and 300 and 500 μs for PLN. For SIM-CP contact time was set to 400 μs for ubiquitin and 300 μs for PLN. For specific-CP from ^{15}N to $^{13}C\alpha$ (or ^{13}CO), the ^{13}C offset was shifted to 70 (or 177) ppm. During specific-CP, the ^{15}N RF amplitude was set to $(5/2)\cdot\omega_r$ (=20 kHz), whereas ^{13}C RF amplitude was set to $(3/2)\cdot\omega_r$ (= 12 kHz) and $(7/2)\cdot\omega_r$ (= 28 kHz) for $^{13}C\alpha$ and ^{13}CO specific-CP, respectively. The specific-CP was implemented with an adiabatic ramp (Δ~1.6 kHz and β=0.5 kHz) on ^{13}C, and the contact times for $^{13}C\alpha$ and ^{13}CO transfers were set to 3.2 and 4.5 ms, respectively. For heteronuclear decoupling (CW or TPPM), ^{1}H RF amplitude was set to 100 kHz. No RF heating was detected for both microcrystalline or membrane protein preparations. For the DARR experiment, a mixing time of 40 ms was used with ^{1}H RF amplitude set to 8.33 kHz (ω_r). For DQ-SQ experiment, States-mode acquisition[1] of t_1' dimension was achieved by 0^{o} and 45^{o} overall phase shift of DQ excitation sequence. The 360^{o} pulse length during SPC5 was set to 24 μs, with a total SPC5 mixing time of 480 μs corresponding to four rotor periods, and a z-filter of 120 μs was used. A recycle delay of 2s was used in all experiments. All of the spectra were recorded using a dwell time of 10 μs in the direct dimension and ^{13}C acquisition time of 20 ms for both t_2' and t_2". The indirect acquisition parameters of conventional method are identical to DUMAS method, given by: $dw(t_1')$=30μs, $ni(t_1')$=70, $nt(t_1')$=100 for ubiquitin and 200 for PLN, and t_{1max}'=2.1 ms; dw(t1")=240 μs, $ni(t_1'')$ = 35, $nt(t_1")$=200 for ubiquitin and 400 for PLN, and t_{1max}"=8.2ms. In the DUMAS scheme, τ was set to 2 ms. The ^{13}C spectra were referenced with respect to CH_2 resonance of adamantane at 40.48 ppm and indirectly to ^{15}N using relative gyromagnetic ratio of ^{15}N and ^{13}C[2].

Table1: Indirect dimension acquisition parameters for the DUMAS scheme. The constant c is an integer number that is used to equalize the experiments (number of experiments = $nt(t_1)\cdot ni(t_1)$) for the two parallel acquisitions.

Parameter	t_1'	t_1''
dw (dwell time)	$dw(t_1')$	$dw(t_1'')$
ni (number of increments)	$ni(t_1')$	$ni(t_1'') = \left(\frac{1}{c}\right)\cdot ni(t_1')$
t_{1max} (max. of t_1)	$t_{1max}' = dw(t_1')\cdot ni(t_{1max}')$	$t_{1max}'' = dw(t_1'')\cdot ni(t_{1max}'')$
nt (number of transients)	$nt(t_1')$	$nt(t_1'') = c\cdot nt(t_1')$

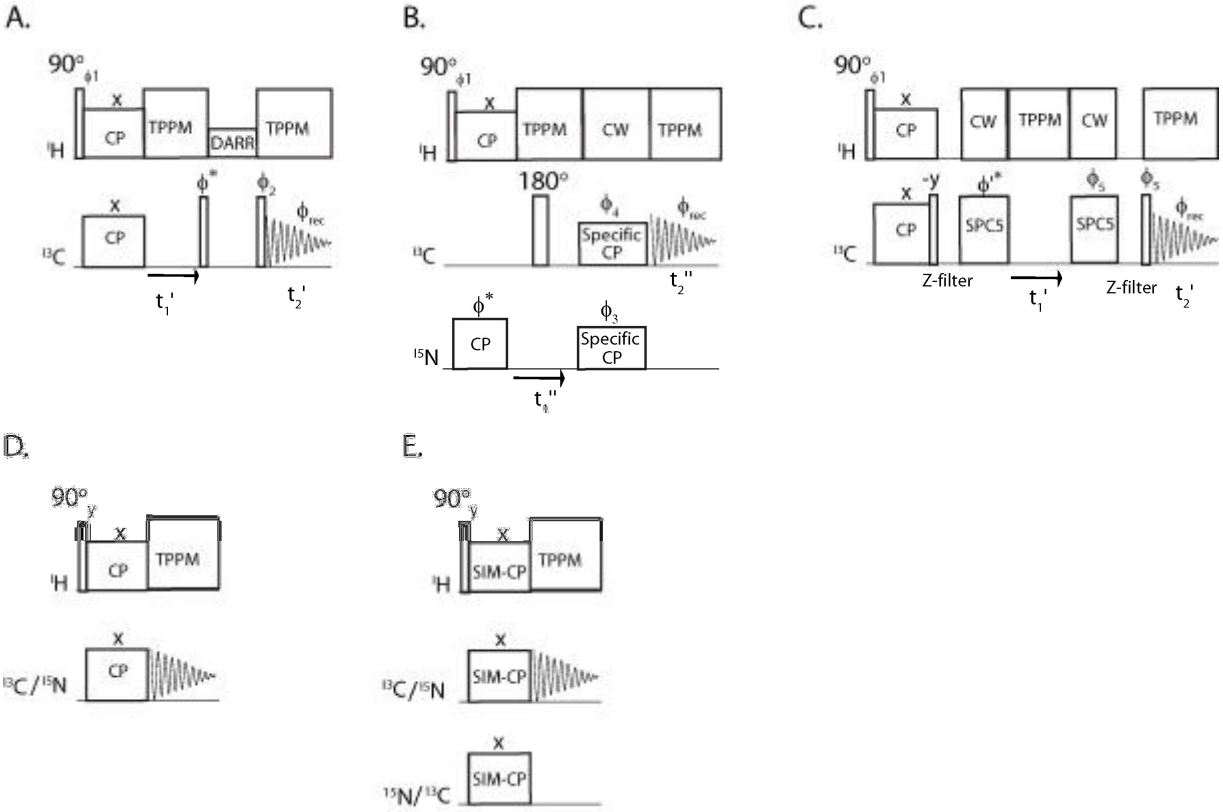

Figure1S: 2D Pulse sequences of conventional DARR (A), NCA or NCO (B), and DQSQ (C). The (t_1', t_2') and (t_1", t_2") are in accordance with the notations used in DUMAS scheme of Figure 1. The phase cycle $\phi_1 = (y)_4, (-y)_4$; $\phi_2 = (x, -y, -x, y)_2$; $\phi_3 = (x, x, -x, -x)_2$; $\phi_4 = (x, -y)_4$; $\phi_5 = (x, y, -x, -y)_2$; ϕ_{rec} = x, -y, -x, y, -x, y, x, -y. The phases ϕ* and ϕ'* are used for States-mode acquisition in the indirect dimensions. For NCA and NCO experiments, the offset of ^{13}C during specific-CP is shifted to Cα and CO regions respectively. (D) 1D pulse sequence used for optimizing the ^{1}H-^{13}C and ^{1}H-^{15}N CP parameters, ^{13}C and ^{15}N are detected in separate experiments. (E) 1D pulse sequence used for optimizing SIM-CP parameters, ^{13}C and ^{15}N are detected in separate experiments.

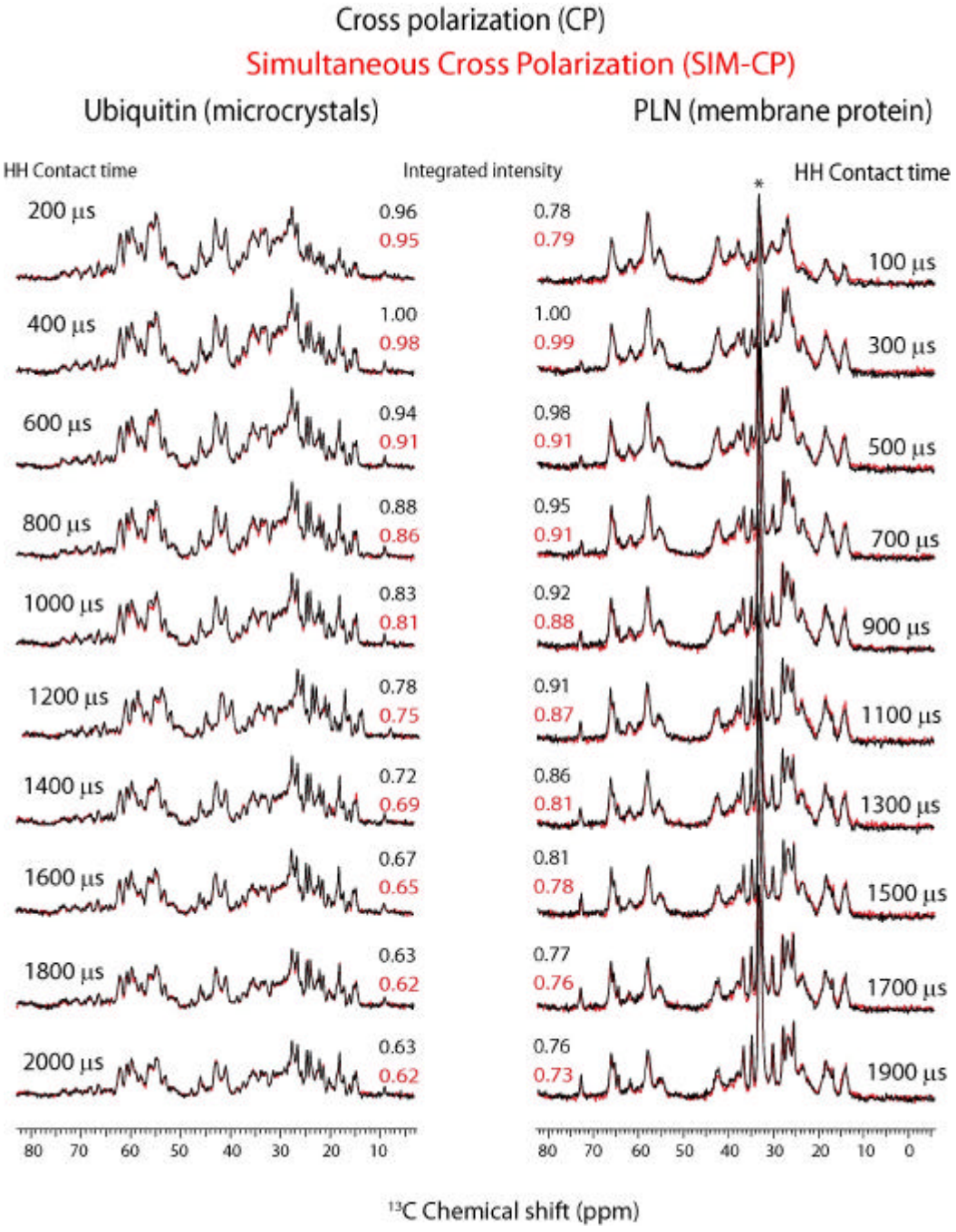

Figure 2S: ^{1}H-^{13}C CP (black) and SIM-CP (red) spectra recorded at various Hartmann-Hahn (HH) contact times using the CP and SIM-CP pulse sequences shown in figure 1S (D) and (E), respectively. Ubiquitin and PLN spectra were recorded using 32 and 64 scans respectively. All the spectra of ubiquitin and PLN are drawn at the same noise level. The integrated intensity between 5 and 80 ppm was measured for each spectrum and normalized with respect to ^{1}H-^{13}C CP spectrum with maximum intensity. The integrated intensity is very similar for CP and SIM-CP spectra at all contact times. Also note that the maximum intensity of CP and SIM-CP occur at the same contact time which qualitatively indicates similar spin dynamics of CP and SIM-CP.

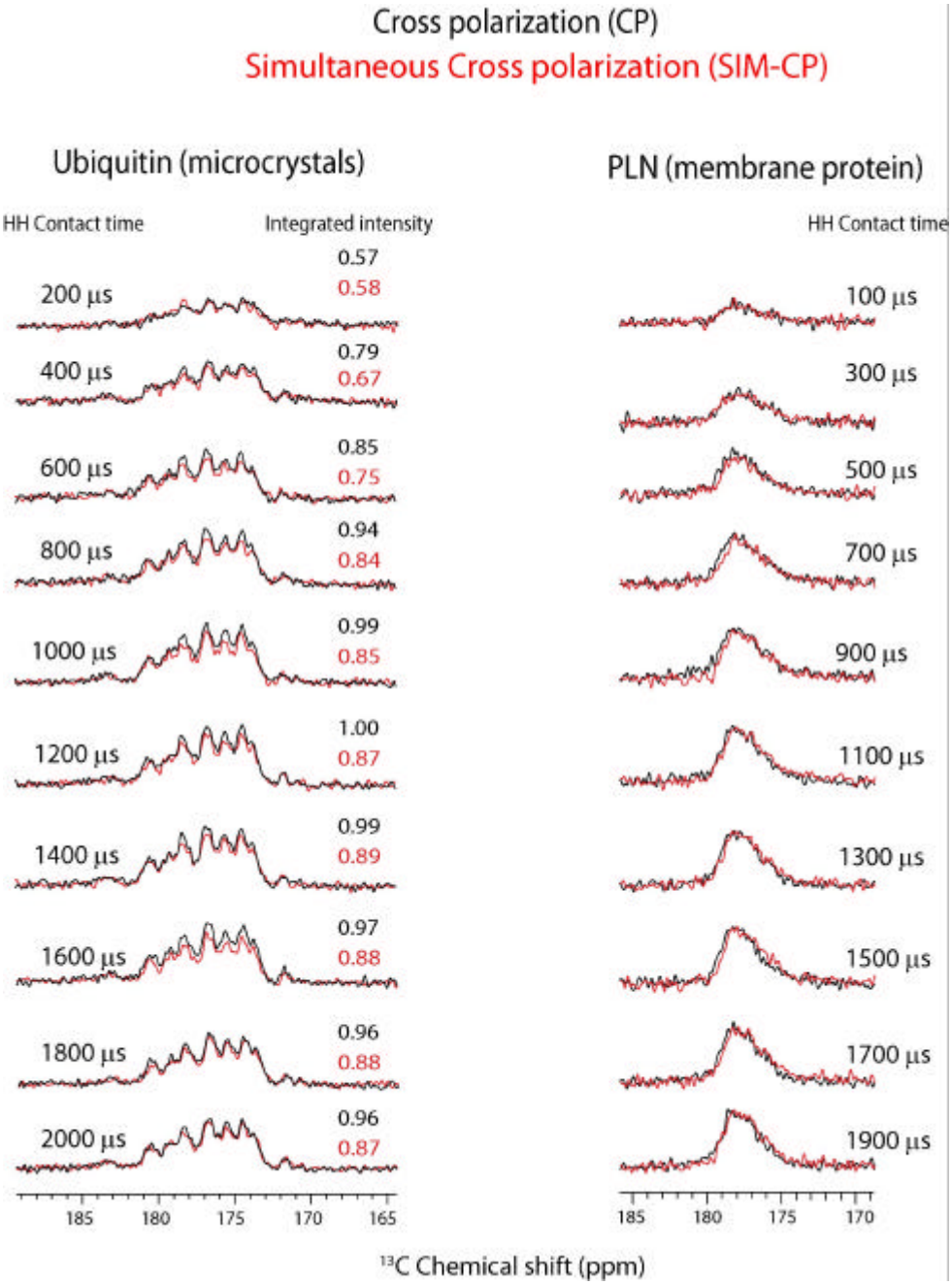

Figure 3S: ^{13}CO region of ^{1}H-^{13}C CP (black) and SIM-CP (red) spectra recorded at various HH contact times using the CP and SIM-CP pulse sequences shown in figures 1S panels D and E. All the spectra of ubiquitin and PLN are drawn at the same noise level. The integrated intensity between 170 and 182 ppm was measured for each spectrum and normalized with respect to CP spectrum of maximum intensity. The integrated intensity at various mixing times is 10-15 % higher for CP compared to SIM-CP spectra. For PLN the loss of sensitivity of SIM-CP is less than 5 %.

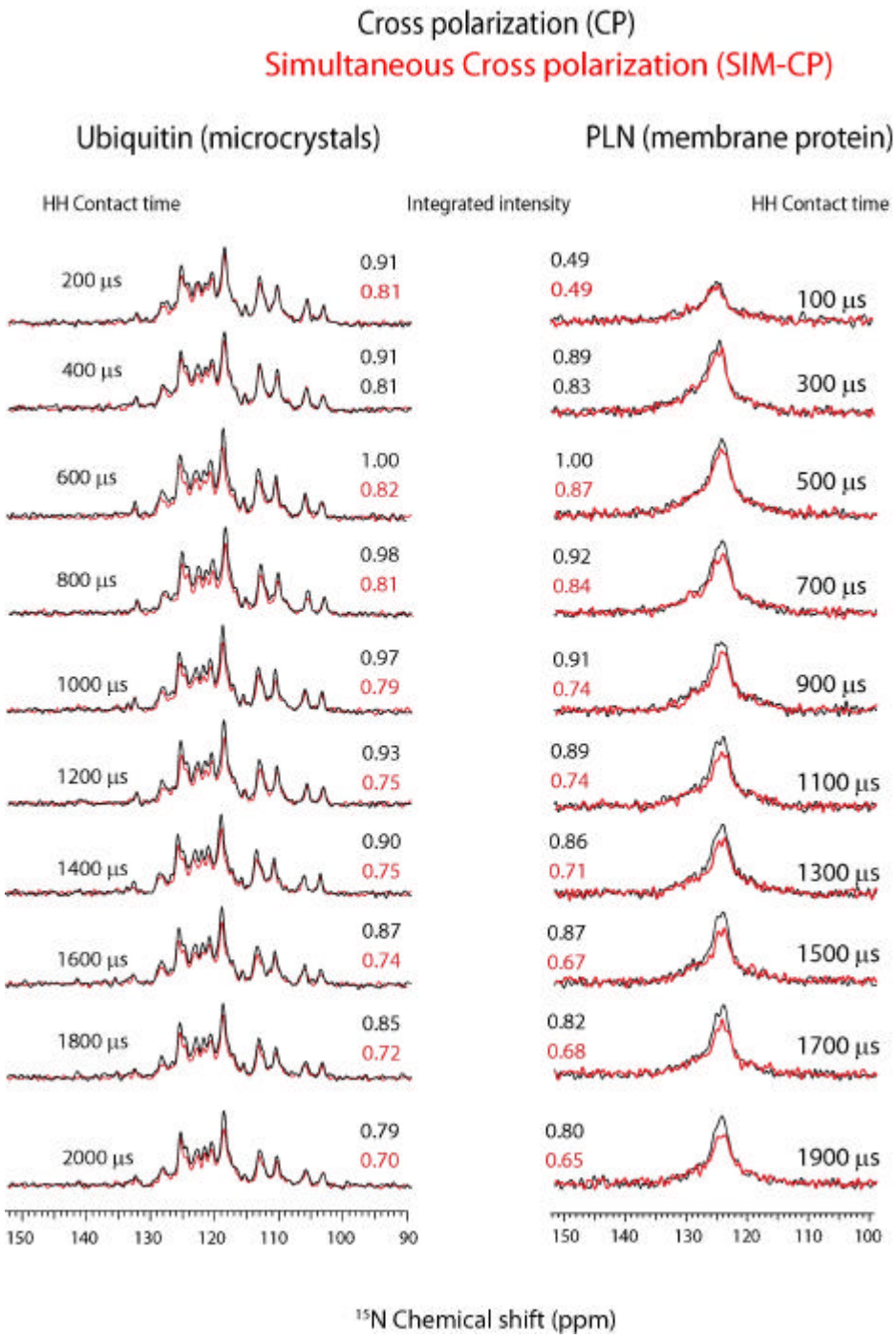

Figure 4S: ^{1}H-^{15}N CP (black) and SIM-CP (red) spectra recorded at various contact times using the CP and SIM-CP pulse sequences shown in Figure 1S (D) and (E), respectively. Each spectrum was recorded in 64 scans. All the spectra of ubiquitin and PLN are drawn at the same noise level. The integrated intensity between 100 and 135 ppm was measured for each spectrum and normalized with respect to CP spectrum at 600 µs. The integrated intensity at various mixing times is 12-23 % higher for CP compared to SIM-CP spectra.

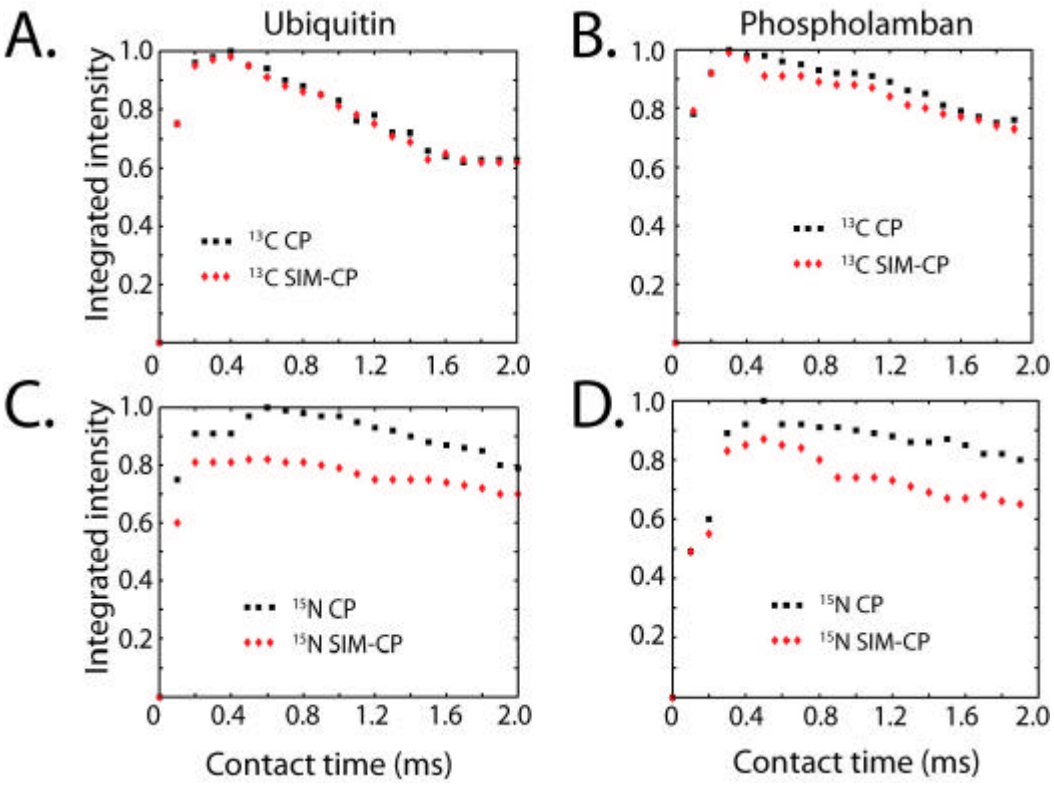

Figure 5S: Comparison of CP and SIM-CP polarization transfer as a function of contact time. The integrated intensities of aliphatic ^{13}C spectra from figure 2S are plotted in A and B, whereas the ^{15}N intensities of figure 4S are plotted in C and D. For ^{13}C, the intensities are nearly identical for CP and SIM-CP, while for ^{15}N, the intensities are 10-20% lower for SIM-CP compared to CP. However, qualitatively the polarization enhancement is similar for CP and SIM-CP. Note that for phospholamban the lipid peaks also contribute to the resultant intensities.

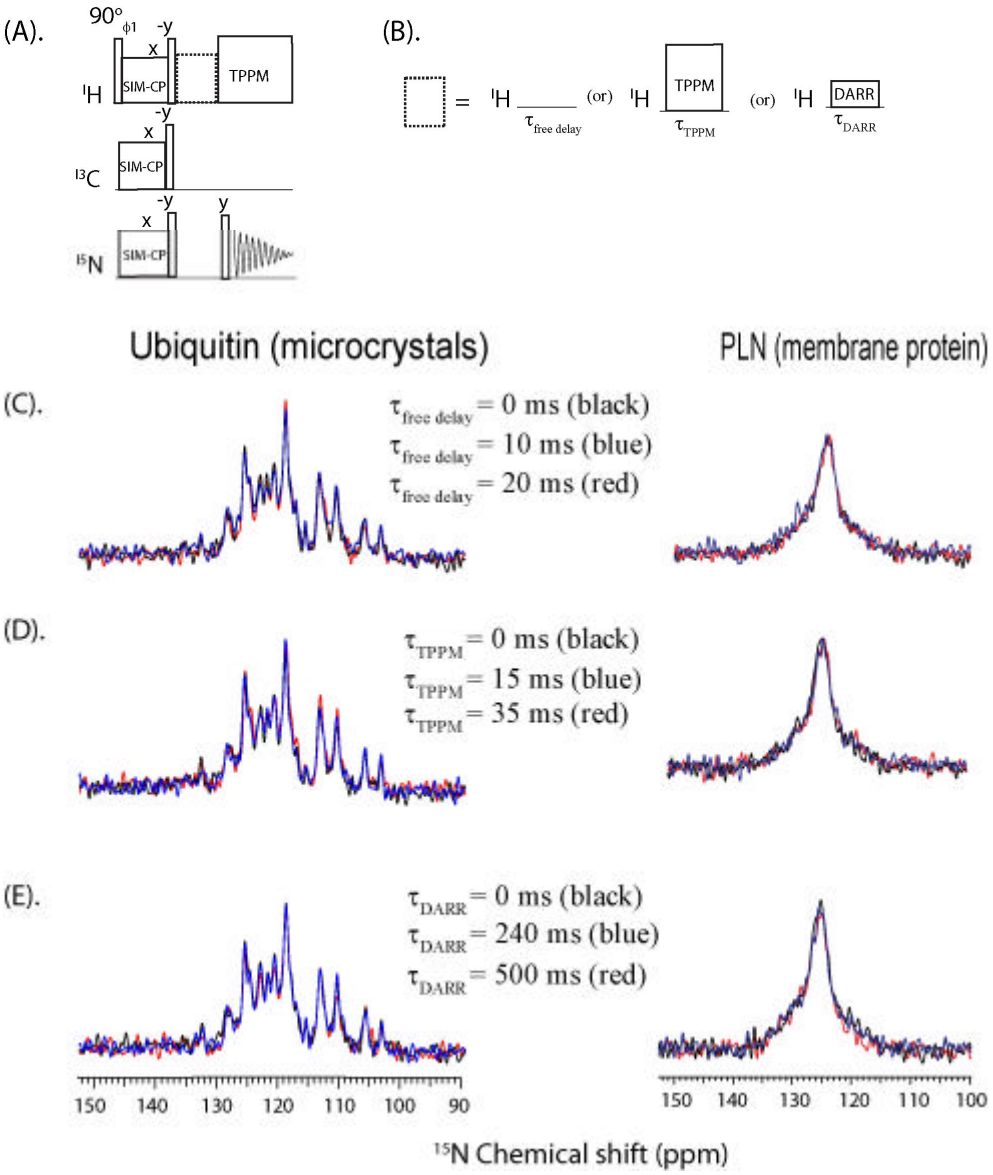

Figure 6S: (A) Pulse sequence to test the effects of ^{15}N longitudinal relaxation in the DUMAS scheme. After SIM-CP the ^{15}N magnetization is stored along z-axis, then a free evolution delay or TPPM decoupling or DARR mixing (B) is applied with various time periods. Finally, a 90^{o} pulse tilts the z-magnetization in to transverse plane followed by acquisition. (C) ^{15}N spectra at 0 ms (black), 10 ms (blue) and 20 ms (red) delay period. (D) ^{15}N spectra at 0 ms (black), 15 ms (blue) and 35 ms (red) TPPM decoupling periods. (E) ^{15}N spectra at 0 ms (black), 240 ms (blue) and 500 ms (red) DARR mixing periods. From these spectra it is clear that under DUMAS experimental conditions, ^{15}N magnetization can be stored for several milliseconds without loss of sensitivity.

[1] D. J. States, R. A. Haberkorn, D. J. Ruben, *J. Magn. Res.* **1982**, *48*, 286-292.

[2] C. R. Morcombe, K. W. Zilm, *J. Magn. Res.* **2003**, *162*, 479-486.