Supporting Information

Efficient and Robust Heteronuclear Cross-Polarization for High-Speed-Spinning Biological Solid-State NMR Spectroscopy

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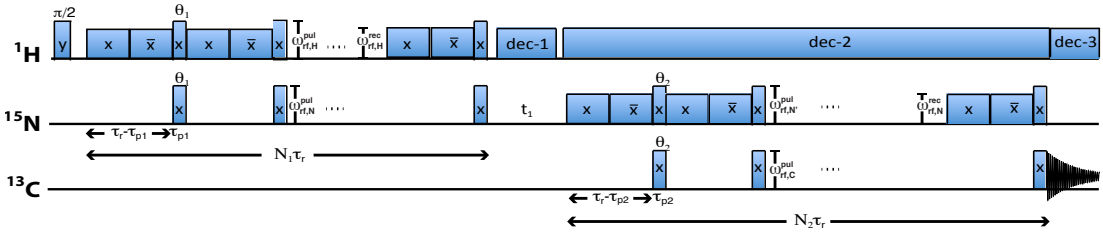

Figure S1. Detailed 2D pulse sequence for NCA/NCO experiments outlining typical ^{1}H→^{15}N (before the t_1 period) and ^{15}N→^{13}C (after the t_1 period) elements using RESPIRATIONCP. The initial $(\pi/2)_y$ pulse generates *x*-phase coherence on the ^{1}H spins, which in the first element is transformed to *x*-phase coherence on the ^{15}N spins, which subsequently is transferred to *x*-phase coherence on the ^{13}C spins. Each *RESPIRATION*CP element contains the element in Fig. 1a repeated N_1 and N_2 rotor periods for the ^{1}H→^{15}N and ^{15}N→^{13}C coherence transfers, respectively, with each rotor period contain one small-flip-angle pulse on each channel (upon irradiation in several rotor periods these takes the form of a RESPIRATION pulse[1] on each channel interleaved with phase-alternated CW irradiation on one of the rf channels for dipolar recoupling). For each rotor period the length of the CW irradiation (half of it with phase *x*, half of it with phase –*x*) correspond to a rotor period minus the length of the small flip-angle pulse (θ).

1. Experiments

All experiments were performed on a Bruker Avance 400 (9.4 T, 400 MHz for ^{1}H) wide-bore NMR spectrometer equipped with a standard triple-resonance 2.5 mm MAS probe. The spectra were acquired using 30 kHz sample spinning, 3 s repetition delay, 122 kHz decoupling during ^{15}N→^{13}C coherence transfer (marked "dec-2" in Fig. S1; although the experiments also works without decoupling, this provided the most representative case relative to standard experiments in the field), and 139 kHz decoupling during acquisition (marked "dec-3 in Fig. S1). The experiments in Figs. 1d and 1e used 1024 scans, with the standard CP spectrum (red) in Fig. 1d recorded using ^{1}H-^{15}N cross-polarization with rf field strengths of 70 (average) and 40 kHz on ^{1}H and ^{15}N, respectively, with a 80-100% ramp[2] at the ^{1}H channel during the 350 μs contact time. The *RESPIRATION*CP spectrum (red) in Fig. 1d was recorded using 60 kHz rf field strength on the ^{1}H channel for recoupling ($\omega_{rf,H}^{rec}/(2\pi)$ in the notation of Fig. S1), and also with 60 kHz rf field strength for each of the 3.5 μs ($\theta \approx 75^{\circ}$) RESPIRATION

pulses[1] on both the ^{1}H and ^{15}N rf channels (one pulse per rotor period of flip-angle θ_1 and amplitude $\omega_{rf,H}^{pul}/(2\pi) = \omega_{rf,N}^{pul}/(2\pi)$=60 kHz as illustrated for the ^{1}H→^{15}N element in Fig. S1). The mixing time was *N*=6 rotor periods (N_1 in Fig. S1), corresponding to 200 μs. The ^{15}N→^{13}C coherence transfers in Fig. 1e were recorded using a carefully optimized adiabatic variant[3] of the DCP experiment[4] (a tangential shape with β=-500, Δ=2300 was applied on the ^{15}N channel; similar shapes were tried for ^{13}C channel also but without improving the efficiency) with average rf field strengths of 35 and 65 kHz on the ^{15}N and ^{13}C rf channels during the 3 ms mixing time. For a volume restricted sample (sample extending over the central 2 mm in the axial dimension), the adiabatic DCP experiment provided 64% transfer relative to a corresponding direct-transfer ^{1}H-^{13}C CP/MAS experiment, a number which is reduced to 40% for a full rotor (sample extending over 8 mm in the axial dimension; this dimension was used for all experiments presented). The *RESPIRATION*CP experiment for the ^{15}N-^{13}C transfer used phase-alternated CW irradiation on the ^{15}N rf channel with amplitude of 30 kHz, while the RESPIRATION pulses on the ^{15}N and ^{13}C rf channels used 58 kHz (0.9 μs duration; i.e., $\theta_2 \approx 19^{\circ}$ using the notation in Fig. S1). The mixing time was *N*=60 rotor periods (N_2 in Fig. S1), corresponding to 2 ms.

The experiments in Fig. 2g-j used the following parameters. The rf field strengths for the different sequences were varied in a range of [-10,10] kHz relative to the optimum values. For ^{1}H→^{15}N transfers, the rf field strengths on the ^{1}H channel (Fig. 2g) were varied around 70 kHz in case of ramped CP (red) and around 60 kHz for the RESPIRATIONCP (blue). On the ^{15}N channel (Fig. 2h) the center is 40 kHz for the ramped CP (Red) and 60 kHz for *RESPIRATIONCP* (blue). In Figs. 2i and 2j respectively, the rf field strengths on ^{15}N and ^{13}C were varied during the ^{15}N→^{13}C transfer centered around 35 and 30 kHz on ^{15}N for the adiabatic DCP and *RESPIRATION*CP, respectively, and around 65 and 58 kHz on the ^{13}C channel. The remaining experimental parameters were kept same as in Fig. 1.

In Fig. 3, the resonance-offset dependencies are shown by both simulations and experimental results demonstrating the performances of the various pulse sequences over a range of 20 kHz. The relevant protein/peptide chemical shift ranges in kHz for 400 and 800 MHz spectrometer conditions are shown by the bar and dashed bar respectively. At 800 MHz these ranges (in kHz) are [-2, 2], [-2.5, 2.5], [-1.4, 1.4] and [-1.65, 1.65], respectively, for the ^{1}H, ^{13}Cα, ^{13}C' and ^{15}N.

The two 2D experiments in Fig. 4 were recorded with the same optimized parameters as in Fig. 1 using 64 increments in the indirect dimension and with 772 scans per increment.

2. Numerical simulations

All numerical simulations were conducted using the open-source SIMPSON simulation program[5-7] using typical amide ^{1}H, ^{13}Cα, and amide ^{15}N chemical shielding tensors and heteronuclear dipolar and *J* couplings as given in the SIMMOL paper.[8] The simulations used 20 to 144 REPULSION α,β crystallite angles[9] and 5-18 γ-angles distributed using γ-COMPPUTE.[10] All simulations presented correspond to 9.4 T (400 MHz resonance frequency for ^{1}H) static field and $\omega_r/2\pi$=30 kHz sample spinning.

The *RESPIRATION*CP simulations in Fig. 1c use phase-alternated CW irradiation with amplitude $\omega_{rf}=2\omega_r$ at the ^{1}H channel for ^{1}H→^{15}N step and at the ^{13}C channel for the ^{15}N →^{13}C step, 2 μs interleaving rf pulses of amplitude 120 kHz (one pulse of flip-angle $\theta \approx 90^\circ$ on each channel per rotor period; virtually identical results are obtained using substantially lower rf amplitudes on these pulses, e.g., 75 kHz ($\theta \approx 54^\circ$) as used in the Fig. 3 simulations). The CP and DCP simulations in Fig. 1c uses constant amplitude rf irradiation with amplitude $\omega_{rf}=2.5\omega_r$ and $\omega_{rf}=1.5\omega_r$ on ^{1}H and ^{15}N, respectively, for the ^{1}H→^{15}N transfer and $\omega_{rf}=1.5\omega_r$ and $\omega_{rf}=2.5\omega_r$ on ^{15}N and ^{13}C, respectively, for the ^{15}N →^{13}C transfer.

The *RESPIRATION*CP simulations in Figs. 2a-2d use phase-alternated CW irradiation on the ^{1}H channel for the ^{1}H→^{15}N transfer (*N*=7 rotor period mixing) and on the ^{13}C channel for the ^{15}N →^{13}C transfer (*N*=68 rotor period mixing). The RESPIRATION pulses (one pulse on each channel per rotor pulse) were of duration 2 μs implying that the vertical axes in Figs. 2a and 2b correspond to flip angles of $\theta = 0^\circ$ to $\theta = 180^\circ$). The DCP simulations in Figs. 2e and 2f use mixing times corresponding to 5 and 44 rotor periods, respectively.

The *RESPIRATION*CP simulation in Fig. 3a uses CW irradiation ($\omega_{rf}=2\omega_r$) on the ^{1}H channel (*N*=6 rotor period mixing) and 75 kHz rf field strength for the 2 μs rf pulses (i.e., $\theta \approx 54^\circ$; we note that the broadbandedness in the ^{15}N dimension increases with increasing power on these pulses). For Fig. 3d the CW irradiation ($\omega_{rf}=2\omega_r$) is on the ^{13}C rf channel (*N*=68 rotor period mixing), while in Fig. 3f the CW irradiation is on the ^{15}N channel – in both cases 2 μs rf pulses of 75 kHz amplitude is used (i.e., $\theta \approx 54^\circ$).

Acknowledgements

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