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Communication

Adiabatic Rotor-Echo-Short-Pulse-Irradiation mediated cross-polarization

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ABSTRACT

We present a new dipolar recoupling method for efficient and robust heteronuclear polarization transfer in solid-state NMR under magic-angle-spinning (MAS) conditions. The method combines the recent $^{\mathrm{RESPIRATION}}$CP method with a modulation of the amplitude of the rotor-synchronized pulses at one of the involved rf channels through the recoupling condition. In this manner, it is possible to achieve high transfer efficiencies while maintaining robustness towards rf-field inhomogeneities and resonance offsets. The performance of the so-called adiabatic-$^{\mathrm{RESPIRATION}}$CP experiment is demonstrated numerically and experimentally using uniformly ^{13}C,^{15}N-labeled samples of alanine and ubiquitin. In particular for cases with relatively high rf inhomogeneity, the scheme offers advantages over the commonly used dipolar recoupling pulse sequences.

Dipolar recoupling techniques are the basic building blocks of magic-angle-spinning (MAS) solid-state NMR experiments aimed at providing detailed information about structure and dynamics of, e.g., organic and biological molecules [1–7]. Dipolar recoupling enables homo- or heteronuclear coherence transfers needed for resonance assignments and establishment of unambiguous structural restraints [8–11]. Probably the most important, and most widely applied, recoupling technique is the MAS adaption of the cross-polarization (CP) experiment [12,13] and variants of this experiment [14–25]. The fundamental multi-dimensional solid-state NMR experiments for addressing biological samples include several CP elements for polarization transfer between high-γ and low-γ nuclei (e.g., ^{1}H → ^{15}N and ^{1}H → ^{13}C transfers) or between different low-γ nuclei (e.g., ^{15}N → ^{13}C transfer). In the latter case, the experiment is often referred to as double cross-polarization (DCP) [14] which in the original form or in refined variants [18,19,22] has proven indispensable for sequence-specific ^{15}N and ^{13}C resonance assignment of proteins based on HNCA or HNCO type of experiments [26].

Ever since the first uses of CP recoupling experiments under MAS there has been substantial interest in improvements of the basic experiment. The standard CP experiment consists of CW irradiation on the two involved rf channels matched to the modified Hartmann–Hahn conditions under MAS. In particular for applications involving transfer across small dipole–dipole couplings (e.g., for low-γ nuclei), the CP experiments are quite sensitive to rf mismatch/inhomogeneity and resonance offsets. This has lead to ramped [15], adiabatic [17,18], phase-alternated [23], and amplitude-modulated variants [16,20,21] all displaying improvements in various areas of application. Recently, an improved experiment, the so-called $^{\mathrm{RESPIRATION}}$CP experiment [25], changing focus from the prevailing γ-encoding [27] Hartmann–Hahn match design to experiments with simultaneous recoupling of several Fourier components of the MAS-modulated dipolar coupling was introduced. The method combines phase-alternated rf irradiation on one channel with so-called Rotor Echo Short Pulse IrradiaTiON (RESPIRATION) pulses [24] on both rf channels to achieve efficient dipolar recoupling. A schematic representation of the basic $^{\mathrm{RESPIRATION}}$CP pulse-sequence element [25], inspired from recent optimal-control CP experiments [24], is shown in Fig. 1A. The $^{\mathrm{RESPIRATION}}$CP experiment significantly improved the robustness of the CP/DCP experiment with respect to rf mismatch/inhomogeneity and resonance offset broadbandedness on the channel with phase-alternating rf irradiation.

It is well-established that the efficiency of recoupling experiments such as CP can be significantly improved through an adiabatic sweep through the recoupling condition enhancing the ideal transfer efficiency of γ-encoded recoupling sequences from 0.73 to approach almost unity [28,29]. In this Communication, we demonstrate how a modulation of the RESPIRATION pulse amplitudes, as shown schematically in Fig. 1B, may be used to improve the efficiency of the $^{\mathrm{RESPIRATION}}$CP experiment to values

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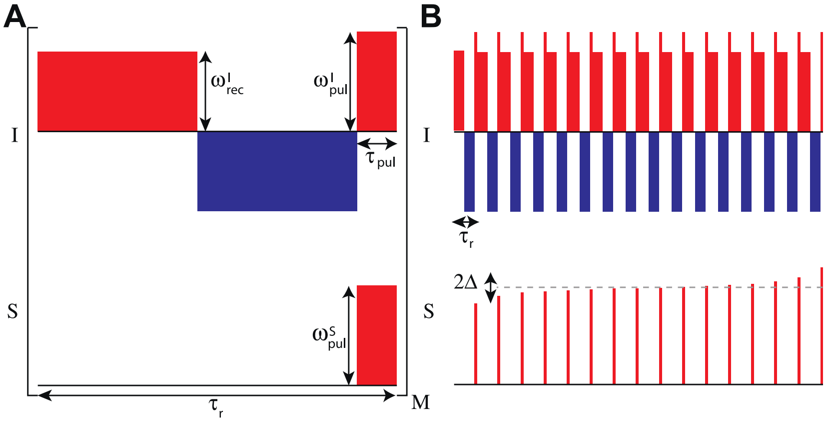

Fig. 1. (A and B) Schematic representation of the $^{\text{RESPIRATION}}$CP and adiabatic-$^{\text{RESPIRATION}}$CP pulse sequences, respectively. For $^{\text{RESPIRATION}}$CP a basic element spanning a rotor period $\tau_r = \frac{2\pi}{\omega_r}$ is repeated M times. The element consists of two phase-alternated pulses on the I-spin rf channel, both employing an rf field strength of ω^{I}_{rec} for a duration of $\tau = \frac{\tau_r - \tau_{pul}}{2}$. These are followed by interleaving rf pulses of duration τ_{pul} and rf field strength ω^{S}_{pul} and ω^{I}_{pul} on the S- and I-spin rf channels, respectively. For the adiabatic version in (B), the effective amplitude on the S-spin rf channel is modulated throughout the sequence by 2Δ with mean amplitude indicated by a broken line. Red and blue indicate pulses of phase x and $-x$, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

higher than 0.75. By modulating the rf-field amplitude of the rf pulses on the S-spin channel, an adiabatic passage through the recoupling condition can be implemented with a theoretical transfer efficiency approaching 100% even in powder samples.

The effective zero-quantum Hamiltonian in a double-rotating Zeeman and rf interaction/toggling frame for the $^{\text{RESPIRATION}}$CP pulse sequence have been presented by Jain et al. [25] and verified by Floquet theory using same parameters for the two phase-alternated pulses ($\omega^{I}_{rec} = 2{*}\omega_r$). In a tilted coordinate system where the z-axis is aligned with the field direction of the rf pulses, the first-order effective heteronuclear dipolar-coupling Hamiltonian is given by

$$\overline{\widetilde{H}}_{IS} = \frac{b_{IS}}{8}\sin^2(\beta_{PR})\cos(2\gamma_{PR})(2\widehat{I}_x\widehat{S}_x + 2\widehat{I}_y\widehat{S}_y) + \frac{8}{6\pi}\frac{b_{IS}}{2\sqrt{2}} \times \sin(2\beta_{PR})\cos(\gamma_{PR})(2\widehat{I}_y\widehat{S}_x - 2\widehat{I}_x\widehat{S}_y). \tag{1}$$

By rotating the Hamiltonian around the operator $-\widehat{S}_z$ with a crystallite dependent function $f(\gamma_{PR}, \beta_{PR}) = \tan^{-1}(\frac{32\sin(2\beta_{PR})\cos(\gamma_{PR})}{6\pi\sqrt{2}\sin^2(\beta_{PR})\cos(2\gamma_{PR})})$, the effective dipolar Hamiltonian may be cast as

$$\overline{\widetilde{H}}_{IS} = d_{eff}(2\widehat{I}_x\widehat{S}_x + 2\widehat{I}_y\widehat{S}_y), \tag{2}$$

where $d_{eff} = \sqrt{(\frac{b_{IS}}{8}\sin^2(\beta_{PR})\cos(2\gamma_{PR}))^2 + (\frac{8}{6\pi}\frac{b_{IS}}{2\sqrt{2}}\sin(2\beta_{PR})\cos(\gamma_{PR}))^2}$ describes the orientation-dependent magnitude of the coupling.

An adiabatic passage through the recoupling condition can be fulfilled by applying a time-dependent field along the tilted zero-quantum z-axis to Eq. (2) [28]. From the expression of d_{eff}, it is evident that the powder-averaged transfer efficiency $E(\tau_{exc}) = \frac{1}{8\pi^2}\int_0^{2\pi} d\alpha_{PR}\int_0^{\pi}\sin\beta_{PR}d\beta_{PR}\int_0^{2\pi} d\gamma_{PR}\sin^2(d_{eff}\tau_{exc})$ is dependent on the γ_{PR} crystallite angle as opposed to traditional γ_{PR}-encoded sequences. However, for the adiabatic transfer this has the same effect as the β_{PR} crystallite angle dependence and will just give an additional spread in the magnitude of the coupling without appreciable effects on the overall transfer efficiency. Aimed at short sweep times, a tangential function $\chi(t)$ for the applied rf field has been employed for adiabatic-$^{\text{RESPIRATION}}$CP as illustrated in Fig. 1B, where the rf-field strength on S-spin is changed throughout the entire sequence. The tangential function is described by

$$\chi(t) = |d_{est}|\tan(\alpha(0.5\tau - t)), \tag{3}$$

where τ describes the time for the entire sequence and $\alpha = \frac{2}{\tau}\tan^{-1}(\frac{\Delta}{d_{est}})$ denotes the angular velocity with $\Delta = |\chi(t)|$ being the amplitude depth of the sweep. $|d_{est}|$ is a parameter defining the shape of the adiabatic sweep. The size of these parameters follows earlier recommendations for the dipolar recoupling enhanced by amplitude modulation (DREAM) scheme [30] with $\frac{\Delta}{2\pi} \approx 1$ kHz for directly bonded ^{15}N–^{13}C$_{\alpha}$ spins. However, as the sweep is not applied on top of a continuous wave irradiated field but for adiabatic-$^{\text{RESPIRATION}}$CP in pulsed discrete steps, the parameter Δ is scaled by the ratio $\frac{\tau_r}{\tau_{pul}}$ between the rotor period τ_r and the pulse width τ_{pul} hereby getting the same effective flip angle as for a continuous wave field.

Fig. 2 shows numerical simulations comparing the original $^{\text{RESPIRATION}}$CP experiment (A and C) with the novel adiabatic-$^{\text{RESPIRATION}}$CP (B and D) experiment ^{15}N → ^{13}C coherence transfer with respect to variations in the rf field and resonance offset. The simulations were conducted using the open-source SIMPSON simulation program [31] with typical peptide spin-system parameters [32] (for detailed information about simulation parameters, see Supporting material). The duration of the interleaved pulses were $\tau_{pul} = 7.0$ µs and a spinning speed at 18.181 kHz was assumed. A comparison of the coherence-transfer efficiency contour plots in Fig. 2A and B, shows that the adiabatic variant is less sensitive towards rf mismatch/inhomogeneity between the two rf channels. Simultaneously, it provides higher maximum transfer efficiency, with efficiencies above 80% obtained for a mixing time of 5.5 ms compared to the maximum of 72% at 2.3 ms mixing time for the standard experiment. From Fig. 2C and D, one can see that the chemical-shift-offset robustness neither on ^{13}C nor on ^{15}N is influenced by introducing an adiabatic shape on top of the pulse amplitudes for the adiabatic-$^{\text{RESPIRATION}}$CP. We note that the offset range of $^{\text{RESPIRATION}}$CP depends on the spinning frequency: faster spinning leads to a broader band widths, being most relevant for the rf channel with RESPIRATION pulses only (cf. Fig. 2) [25].

All experimental data was acquired at 18.181 kHz spinning frequency at a 9.4 T (400 MHz for ^{1}H) Bruker Avance-III spectrometer using a 3.2 mm triple-resonance MAS probe. The data have been scaled following this procedure: (1) determination of the ^{1}H → ^{15}N CP transfer efficiency $\epsilon_{\text{HN}} = \frac{1}{10}\frac{\eta^{\text{HN}}}{\eta^{\text{N}}}$ obtained by comparing the ^{1}H → ^{15}N CP signal η^{HN} with ten times the signal intensity from direct ^{15}N pulse experiment η^{N} for the same number of scans. (2) Determination of the ^{15}N → ^{13}C transfer efficiency by comparing the ^{1}H → ^{15}N → ^{13}C$_{\alpha}$ signal η^{HNC} with four times the signal intensity from a direct ^{13}C pulse experiment η^{C}. From these intensities, the ^{15}N → ^{13}C$_{\alpha}$ coherence transfer efficiency can be evaluated by