



Improved magnetization transfer in solid-state NMR with fast magic angle spinning

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ABSTRACT

The efficiency of magnetization transfer between different spins S such as chemically inequivalent carbon-13 nuclei in solid samples that are spinning at high frequencies about the magic angle can be enhanced by a phase-alternated recoupling irradiation scheme (PARIS). Dipolar recoupling is assisted by a radio-frequency (rf) field applied to the abundant I (proton) spins. In contrast to rotary resonance-based recoupling schemes, the new method does not depend critically on the rf amplitude, which need not be matched with the spinning frequency. Modest rf amplitudes suffice to bring about efficient magnetization transfer even at high spinning speeds, thus avoiding excessive sample heating. The new method compensates efficiently for rf field inhomogeneity, so that the full sample volume is used more effectively.

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1. Introduction

Efficient magnetization transfer between spins S such as carbon-13 is a prerequisite for the assignment of solid-state NMR spectra of isotopically enriched molecules of biological interest. Once the assignment is completed, the structures of molecules, supramolecular assemblies, fibril aggregates, and crystal lattices can be determined from the strengths of dipolar interactions. Magnetization transfer can be brought about either via through-bond scalar $J(^{13}\text{C}-^{13}\text{C})$ couplings or via through-space dipolar $D(^{13}\text{C}-^{13}\text{C})$ interactions, provided the latter are ‘recoupled’, i.e., provided their averaging by magic angle spinning (MAS) can be effectively cancelled. It has been shown that fast magic-angle spinning (MAS) with frequencies ν_{rot} on the order of 30 kHz and beyond can be safely applied to microcrystalline proteins [1] and peptides [2], thus allowing one to obtain homonuclear two-dimensional (2D) correlation spectra with improved signal-to-noise ratios [1]. At low MAS frequencies, larger rotor volumes can be used, and magnetization transfer between spins S can occur spontaneously (although often inefficiently) by so-called proton-driven spin diffusion (PDS), i.e., without any rf irradiation in the mixing interval τ_m . At high spinning frequencies, magnetization transfer between spins S and S' must be ‘driven’ or ‘promoted’ by recoupling the heteronuclear $I-S$ dipolar Hamiltonian. The resulting ‘dipolar broadening’ of the S spin spectra allows for a compensation of the energy mismatch involved in $S-S'$ flip-flop processes ($\alpha_S\beta_{S'} \leftrightarrow \beta_S\alpha_{S'}$) by the dipolar reservoir. However, the mixing schemes used in fast MAS experiments, like total through-bond correlation spectroscopy (TOBSY) [3], dipolar recoupling enhancement through ampli-

tude modulation (DREAM) [4], or cosine-modulated adiabatic recoupling CMAR [2], either require high ^{13}C rf field strengths on the order of 100 kHz [2,3] or high ^1H rf amplitudes for decoupling [3,4]. Intense rf fields can lead to significant sample heating and may damage fragile biological solids.

It is well known that magnetization exchange between spins S and S' can be assisted by rotary resonance recoupling (R^3) [5–11], i.e., by applying an rf field with an amplitude adjusted to the spinning frequency, $\nu_1^r = n\nu_{rot}$. The use of ratios $n = 1/2, 1, 2$ leads to the restoration of different anisotropic spin interactions. For $n = 1$, $^{13}\text{C}-^{13}\text{C}$ spin exchange is promoted through recoupling of both homo- and heteronuclear dipolar interactions. However, the efficiency of rotary resonance recoupling is critically dependent on the homogeneity of the rf field [7], which may lead to non-uniform $^{13}\text{C}-^{13}\text{C}$ spin exchange across the sample [9]. This drawback of R^3 -based experiments can be avoided by using a restricted sample volume, albeit at the expense of sensitivity.

In this work, we present a robust phase-alternated recoupling irradiation scheme (PARIS) for promoting efficient magnetization exchange under fast MAS. This scheme uses an rf field applied only to the protons I with a moderate constant amplitude ν_1^r (see Fig. 1). The phase is reversed from $+x$ to $-x$ after a duration τ_p . In this work, we shall use $\tau_p = N\tau_{rot}$ with $N = 1/2$ or 2. No rf irradiation need to be applied to the S spins in the mixing interval τ_m thus avoiding losses of the ^{13}C magnetization in the presence of rf irradiation. The PARIS scheme bears some analogies with our earlier phase-alternated irradiation method for broad-band rotary resonance recoupling (B^2R^3) [9], but does not require fulfilling any rotary resonance condition to promote efficient magnetization transfer. Furthermore, the new scheme exploits modulation sidebands [12] which originate from interference of the sample spinning and the phase-alternated rf irradiation. In contrast to

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the phase-alternated version of the recently introduced mixed rotational and rotary resonance (MIRROR) scheme [13], the PARIS method uses only multiples of half the rotor period, is independent of the rf amplitude ν_1^l , and can promote broadband magnetization exchange between S spins over a wide range of isotropic chemical shifts.

This paper is organized as follows. Section 2 describes the theoretical framework of dipolar recoupling in the presence of phase-alternated irradiation. In Section 3 we present experimental evidence of the improved efficiency of magnetization exchange between spins S and S' that can be achieved with the new PARIS method, compared with recoupling by proton-driven spin diffusion (PDS) and by dipolar assisted rotational resonance (DARR) [8]. Numerical simulations take into consideration up to eight abundant I spins.

2. Theory

Using average hamiltonian theory (AHT), we shall show that, in contrast to continuous wave (CW) irradiation, phase-alternated irradiation with $\tau_p = N\tau_{rot}$ with $N = 1/2$ can lead to recoupling of the heteronuclear dipolar Hamiltonian even if none of the rotary resonance conditions are fulfilled. Such a recoupled dipolar Hamiltonian can promote the exchange of longitudinal magnetization between spins S and S' .

We consider a heteronuclear pair I (^1H) and S (^{13}C). For simplicity, we neglect the isotropic and anisotropic chemical shielding interactions of both spins. The heteronuclear dipolar interaction is described by

$$H^S(t) = D(t)I_z S_z \quad (1)$$

The spatial term $D(t)$ is modulated by MAS with the angular frequencies $\omega_{rot} = 2\pi\nu_{rot}$ and $2\omega_{rot}$:

$$D(t) = d^S [g_1 \cos(\omega_{rot}t + \gamma) + g_2 \cos(2\omega_{rot}t + 2\gamma)] \quad (2)$$

where d^S is the heteronuclear coupling constant,

$$d^S = \left(\frac{\mu_0}{4\pi}\right) \frac{\gamma_I \gamma_S \hbar}{r_{IS}^3} \quad (3)$$

The gyromagnetic ratios of the nuclei I and S are denoted by γ_I and γ_S , μ_0 is the magnetic permeability of vacuum and r_{IS} is the distance between the two nuclei. The coefficients g_1 and g_2 are

$$\begin{aligned} g_1 &= \frac{3}{2} \sin(2\theta_m) \sin(2\beta), \\ g_2 &= -\frac{3}{2} \sin^2(\theta_m) \sin^2 \beta, \end{aligned} \quad (4)$$

where θ_m denotes the magic angle, and β and γ are Euler angles describing the orientation of the internuclear vector \mathbf{r}_{IS} with respect to the rotor frame.

We shall first consider CW irradiation with an amplitude $\omega_1^l = 2\pi\nu_1^l$ applied to the I spins. The Hamiltonian in the doubly-rotating frame (DRF) is

$$H_{DRF}^{IS}(t) = -\omega_1^l I_x + H^S(t). \quad (5)$$

In the tilted doubly-rotating frame (TDRF) the Hamiltonian is [14,15]

$$H_{TDRF}^{IS}(t) = \exp(-i\frac{\pi}{2}\hat{I}_y)H_{DRF}^{IS}(t) = \omega_1^l I_z + \exp(-i\frac{\pi}{2}\hat{I}_y)H^S(t) \quad (6)$$

where the Liouville operator \hat{L} is defined by $\hat{L}O = LOL^{-1}$. In a second TDRF rotating about the z -axis of the first TDRF with the angular frequency ω_1^l , the Hamiltonian has the form

$$\tilde{H}^S(t) = \exp(i\omega_1^l \hat{I}_z t)H_{TDRF}^{IS}(t) - \omega_1^l I_z \quad (7)$$

From Eqs. (5) and (6) we obtain finally

$$\tilde{H}^S(t) = D(t)S_z [I_x \cos(\omega_1^l t) - I_y \sin(\omega_1^l t)] \quad (8)$$

The zeroth-order average Hamiltonian [16] is

$$\bar{H}^{(0)IS} = \frac{1}{\tau_{rot}} \int_0^{\tau_{rot}} \tilde{H}^S(t') dt' \quad (9)$$

where τ_{rot} is the rotor period. This integral leads to an average Hamiltonian where the dominant terms are inversely proportional to $(\omega_{rot} - \omega_1^l)\tau_{rot}$. If the R^3 condition $\omega_1^l = \omega_{rot}$ is fulfilled ($n = 1$), the recoupled heteronuclear dipolar Hamiltonian for a particular crystallite is described by the Euler angles (β, γ)

$$\bar{H}_{DARR}^{(0)IS} \cong \frac{1}{2} d^S g_1 S_z [I_x \cos \gamma + I_y \sin \gamma] \quad (10)$$

It can be shown that $\bar{H}_{DARR}^{(0)IS}$ vanishes rapidly when neither of the R^3 conditions $n = 1, 2$ are fulfilled (*vide infra*). For phase-alternated irradiation in the manner of PARIS, the zeroth-order heteronuclear Hamiltonian has the general form

$$\bar{H}_{PARIS, N=1/2}^{(0)IS} = \frac{1}{\tau_{rot}} \left\{ \int_0^{\tau_{rot}/2} \tilde{H}^S(t') dt' + \int_{\tau_{rot}/2}^{\tau_{rot}} e^{i\pi \hat{I}_x} \tilde{H}^S(t') dt' \right\} \quad (11)$$

where the Liouville operator $\exp(i\pi \hat{I}_x)$ describes the phase-alternation. After lengthy but straightforward algebraic computations, the zeroth-order average Hamiltonian may be cast in the following form

$$\begin{aligned} \bar{H}_{PARIS, N=1/2}^{(0)IS} &\cong d^S S_z I_x \left\{ \frac{1}{2} g_1 \left[\frac{1}{(\omega_{rot} + \omega_1^l)\tau_{rot}} [\sin(\omega_1^l \tau_{rot} + \gamma) - \sin \gamma] \right. \right. \\ &\quad \left. \left. + \frac{1}{(\omega_{rot} - \omega_1^l)\tau_{rot}} [\sin(-\omega_1^l \tau_{rot} + \gamma) - \sin \gamma] \right] \right. \\ &\quad \left. + \frac{1}{2} g_2 \left[\frac{1}{(2\omega_{rot} + \omega_1^l)\tau_{rot}} [\sin(\omega_1^l \tau_{rot} + 2\gamma) - \sin 2\gamma] \right. \right. \\ &\quad \left. \left. + \frac{1}{(2\omega_{rot} - \omega_1^l)\tau_{rot}} [\sin(-\omega_1^l \tau_{rot} + 2\gamma) - \sin 2\gamma] \right] \right\} \\ &+ d^S S_z I_y \left\{ \frac{1}{2} g_1 \left[\frac{1}{(\omega_{rot} + \omega_1^l)\tau_{rot}} \left[-\cos(\omega_1^l \tau_{rot} + \gamma) - 2 \cos\left(\frac{\omega_1^l \tau_{rot}}{2} + \gamma\right) - \cos \gamma \right] \right. \right. \\ &\quad \left. \left. + \frac{1}{(\omega_{rot} - \omega_1^l)\tau_{rot}} \left[\cos(-\omega_1^l \tau_{rot} + \gamma) + 2 \cos\left(-\frac{\omega_1^l \tau_{rot}}{2} + \gamma\right) + \cos \gamma \right] \right] \right. \\ &\quad \left. + \frac{1}{2} g_2 \left[\frac{1}{(2\omega_{rot} + \omega_1^l)\tau_{rot}} \left[-\cos(\omega_1^l \tau_{rot} + 2\gamma) + 2 \cos\left(\frac{\omega_1^l \tau_{rot}}{2} + 2\gamma\right) - \cos 2\gamma \right] \right. \right. \\ &\quad \left. \left. + \frac{1}{(2\omega_{rot} - \omega_1^l)\tau_{rot}} \left[\cos(-\omega_1^l \tau_{rot} + 2\gamma) - 2 \cos\left(-\frac{\omega_1^l \tau_{rot}}{2} + 2\gamma\right) + \cos 2\gamma \right] \right] \right\} \end{aligned} \quad (12)$$

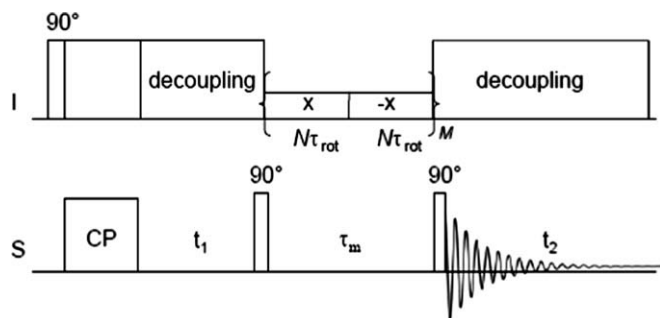


Fig. 1. Sequence for two-dimensional homonuclear correlation where the transfer of longitudinal magnetization between spins S and S' in the mixing interval τ_m is promoted by applying a phase-alternated recoupling irradiation scheme (PARIS) to the (proton) spins I . In this work, $N = 1/2$ or 2 is used to promote efficient magnetization transfer, while M determines the overall duration of the mixing time τ_m .

For the particular case of $\omega_1^I = \omega_{rot}$ ($n = 1$), this Hamiltonian becomes

$$\overline{H}_{PARIS, N=1/2}^{(0)IS, n=1} \cong d^S S_z \left[\frac{1}{2} g_1 I_x \cos \gamma + \frac{2}{3\pi} g_2 I_y \cos 2\gamma \right] \quad (13)$$

This equation leads to a characteristic lineshape of a dipolar recoupled I - S pair, which is significantly different from the DARR lineshape (*vide infra*). Moreover, in contrast to DARR recoupling, the efficiency of PARIS recoupling decays slowly when moving away from R^3 conditions. This opens new possibilities for efficient dipolar recoupling independently of any rotary resonance.

3. Results and discussion

3.1. Improving S - S' spin exchange at rotary resonance with $\nu_1^I = \nu_{rot}$

The AHT treatment presented above shows that the recoupled zeroth-order heteronuclear I - S Hamiltonian has a different form in the presence of CW or phase-alternated irradiation. This leads to a different extent of spectral overlap of the dipolar broadened resonance lines of the spins S and S' and thus to a different compensation of the energy imbalance of chemically inequivalent ^{13}C spins. Multi-spin proton-proton interactions are difficult to handle analytically so that it is not easy to mimic the behaviour of proton baths. Bearing in mind the relevance of these multi-spin interactions for heteronuclear decoupling and recoupling [17,18], we simulated the dipolar-broadened resonance signals of the C^α and C^β carbons of L-alanine by taking into account the eight nearest protons in the crystal structure. Fig. 2 shows significant differences in the S spin lineshapes simulated for CW and phase-alternated irradiation of the I spins, with a more compact form in the latter case. This should in turn manifest itself by a more efficient S - S' magnetization exchange. Indeed, assuming a perfectly homogeneous rf field ν_1^I , numerical simulations reveal that magnetization exchange is enhanced by phase-alternated irradiation (Fig. 2 bottom). This is consistent with the steeper initial slopes of the experimental build-up curves recorded with a limited sample volume.

3.2. Compensating for the rf field inhomogeneity

In the case of a fully packed rotor, rotary resonance recoupling is affected by the unavoidable inhomogeneity of the rf field [19,20]. To show how the PARIS method compensates for this drawback, we compared ^{13}C spectra of L-alanine recorded either with a fully packed 2.5 mm rotor or with a restricted volume in the middle of the rotor. As shown in Fig. 3 for continuous-wave irradiation,

rotary resonance recoupling does not occur uniformly over the whole sample. Indeed, simulations of the lineshapes reveal that up to 40% of the spin do not fulfill the $n = 1$ R^3 condition with sufficient accuracy to promote magnetization transfer. Consequently, the S - S' spin exchange is not uniform, which severely penalizes the

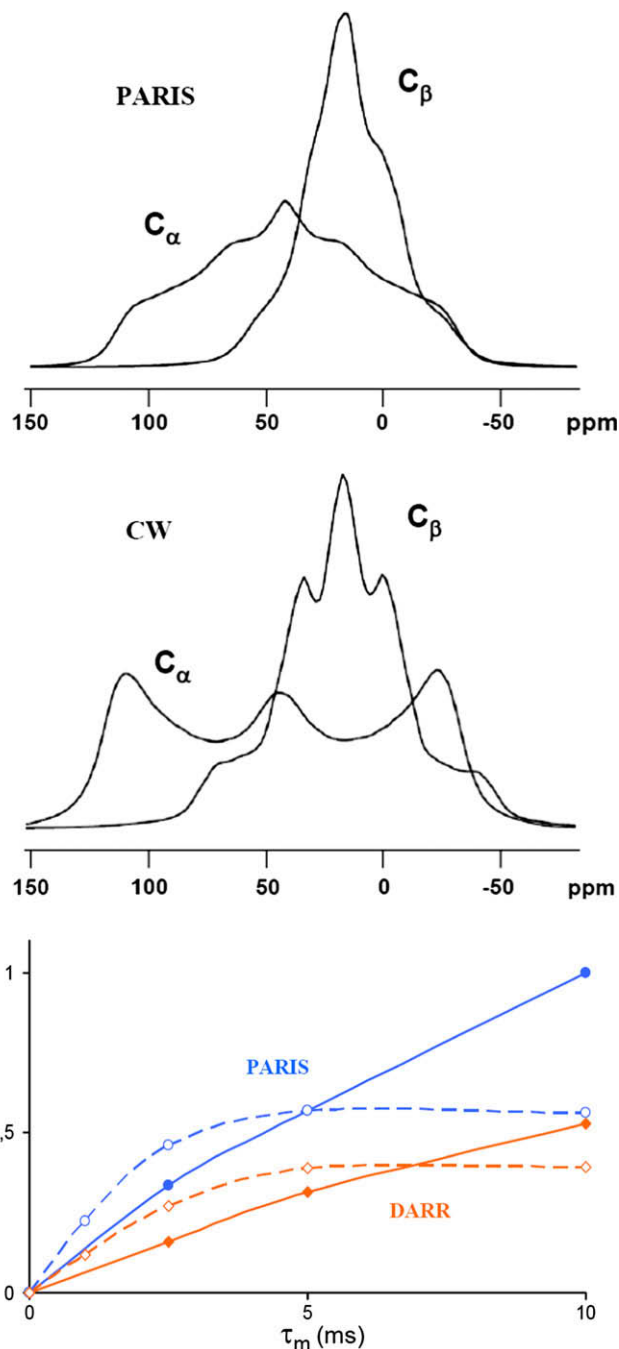


Fig. 2. (top) Simulations of the dipolar broadening of the C^αH and C^βH_3 signals of L-alanine in the presence of PARIS proton irradiation with $\nu_1^I = \nu_{rot} = 30$ kHz ($n = 1$) and $\tau_p = \tau_{rot}/2$ ($N = 1/2$). (middle) Same with CW proton irradiation. (bottom) Experimental (continuous lines) and simulated (dashed lines) build up-curves of the $\text{C}^\alpha/\text{C}^\beta$ cross-peak as a function of the mixing time τ_m in the presence of PARIS proton irradiation ($N = 1/2$) (blue circles) and DARR proton irradiation (red lozenges), both with $\nu_1^I = \nu_{rot} = 30$ kHz ($n = 1$). The intensities of all experimental cross-peaks were normalised to the highest intensity, while the simulated PARIS peak height was scaled to agree with the experimental PARIS peak height at $\tau_m = 5$ ms. The simulated build-up curves were extracted from simulations of full 2D experiments taking into account all six spins in the $\text{C}^\alpha\text{H}-\text{C}^\beta\text{H}_3$ fragment of L-alanine. Powder averaging included 3660 (α, β)-pairs and 12 γ angles.

sensitivity of ^{13}C - ^{13}C correlation experiments. This stands in contrast to the PARIS method which fully compensates for the rf field inhomogeneity. This is proven by the fact that the lineshapes recorded with two differently packed samples are virtually identical, and by the excellent agreement with the simulated lineshapes of the dipolar broadened resonances in the aliphatic region of the C^α and C^β carbons. This proves the two main advantages of the PARIS approach: (i) it leads to a more efficient S - S' magnetization exchange and (ii) it benefits from immunity to the inhomogeneity of the rf field.

3.3. Promoting efficient dipolar recoupling and magnetization exchange at low rf amplitudes without fulfilling any rotary resonance conditions

If one uses CW irradiation to promote magnetization transfer by rotary resonance recoupling, this requires higher rf amplitudes with increasing spinning frequencies ν_{rot} . This constitutes a major drawback, since high rf amplitudes can be harmful for heat-sensitive samples. This can be avoided by using phase-alternated irradiation with a weak rf field amplitude $\nu_1' < \nu_{rot}$ well below the $n = 1$ condition. Indeed, as shown in Figs. 4 and 5, the efficiency of dipolar

recoupling is essentially independent of the rf amplitude at $N = 1/2, 1, 3/2, \dots$ over the whole range $5 < \nu_1' < 140$ kHz. This allows one to use moderate rf amplitudes even at high spinning speeds.

The efficiency of magnetization transfer for different recoupling conditions is shown in Fig. 6 by comparing the build-up curves of $\text{C}^\alpha/\text{C}^\beta$, $\text{C}'/\text{C}^\alpha$ and C'/C^β cross-peaks in uniformly labeled L-alanine recorded as a function of the mixing time τ_m . This confirms the dramatic improvement of magnetization transfer that can be achieved with PARIS compared to DARR, even when using moderate rf field amplitudes.

The different durations $\tau_p = N\tau_{rot}$ used in Fig. 6a–c deserve further explanation. In Fig. 6a, phase-alternated irradiation with $\tau_p = \tau_{rot}/2$ ($N = 1/2$) leads to an efficient magnetization transfer between aliphatic carbons S and S' having a small difference $\Delta\nu_{iso}$ in isotropic chemical shifts. This difference must be compared with the heteronuclear dipolar broadening terms D_{IS} and $D_{IS'}$. When $\Delta\nu_{iso} > D_{IS}$ or $D_{IS'}$, it is difficult to recouple 'distant' resonances such as those of carboxyl and aliphatic carbons, which have large differences $\Delta\nu_{iso}$. For this purpose, one can exploit the interference between the spinning frequency ν_{rot} and the rf cycle frequency $\nu_c = 1/(2\tau_p)$. The resulting modulation sidebands (ms) occur at multiples of the mismatch frequency [12]

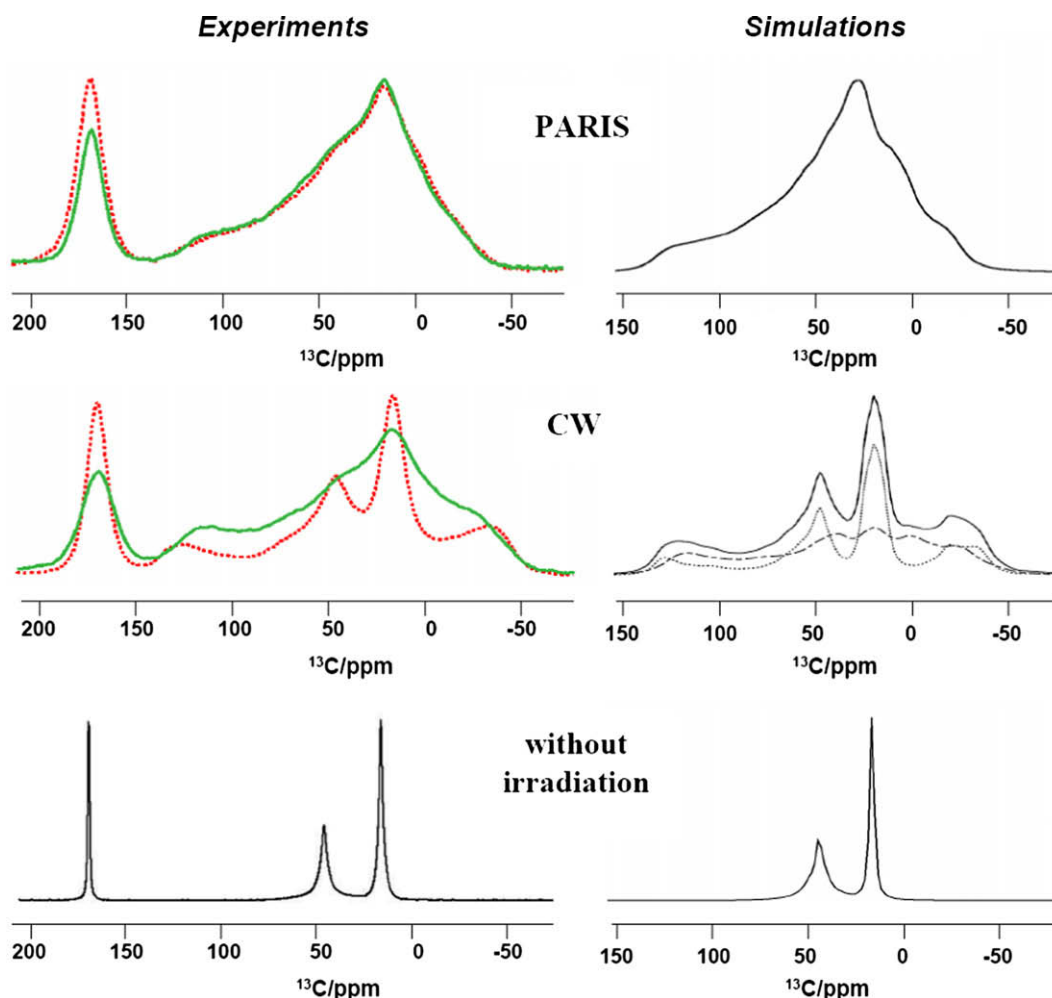


Fig. 3. (top) Experimental one-dimensional ^{13}C spectrum of L-alanine recorded with PARIS irradiation of the I (proton) spins during the observation of the S (carbon-13) signal to illustrate the effects of dipolar broadening through recoupling. The proton rf field amplitude was $\nu_1' = \nu_{rot}$ and the pulse duration $\tau_p = \tau_{rot}/2$ ($N = 1/2$). (Red dotted curve): Fully packed 2.5 mm rotor with 12 μl sample; (green solid curve) restricted volume with approximately 2 μl sample. (middle) Same with continuous-wave (CW) irradiation of the protons. In this case, the rf inhomogeneity leads to less efficient recoupling, particularly with a fully-packed sample. (bottom) Same without any proton irradiation. (right) Sum of simulated C^αH and C^βH_3 signals using PARIS (top) and CW (middle) proton irradiation and without any proton irradiation (bottom). The lineshapes for the fully packed rotor with CW irradiation were simulated by assuming two components in the sample, one (dashed line) fulfilling the $n = 1$ rotary resonance condition $\nu_1' = \nu_{rot}$, the other (dotted line) with $\nu_1' = \nu_{rot} + 3.5$ kHz. All signals have been simulated by taking into account the eight nearest protons in the crystal of L-alanine.

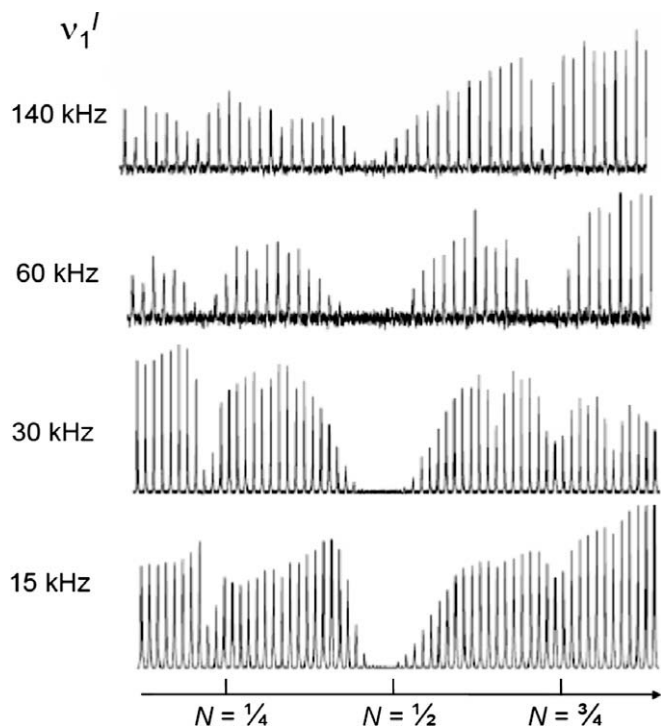


Fig. 4. Experimental peak intensity of the $C^\alpha H$ signal of L-alanine recorded at $v_{rot} = 30$ kHz with PARIS irradiation of the I (proton) spins as a function of the pulse length $\tau_p = N\tau_{rot}$ and the proton rf field amplitude v_1^I . Note the broad 'dips' centred at $\tau_p = \tau_{rot}/2$ ($N = 1/2$) which indicate that dipolar broadening (and therefore recoupling) is effective over a broad range of rf amplitudes.

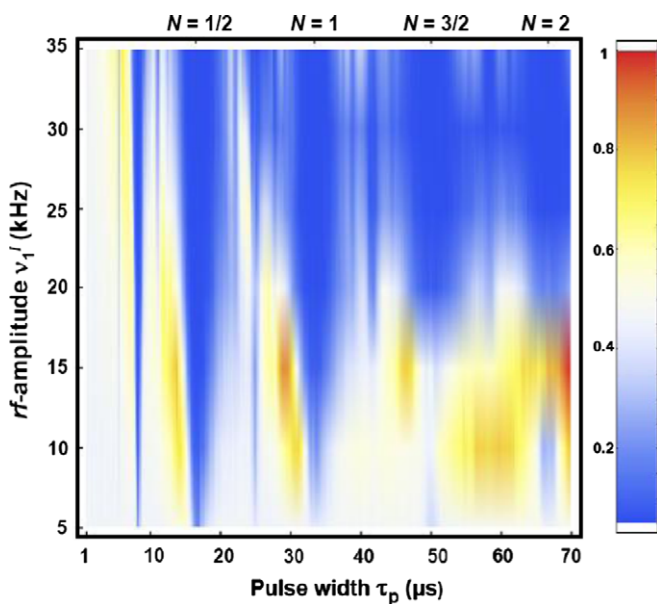


Fig. 5. Two-dimensional contour plot showing the experimental peak intensity of the $C^\alpha H$ signal of L-alanine with PARIS irradiation measured as in Fig. 4 but with small increments $\Delta v_1^I = 5$ kHz and $\Delta \tau_p = 0.2$ μ s. Note that the signal amplitude for $\tau_p = \tau_{rot}/2$ ($N = 1/2$) is almost completely independent of the rf amplitude v_1^I all the way down to 5 kHz.

$$v_{ms} = K(\pm v_{rot} \mp 2v_c) \quad (14)$$

Note that v_{ms} is independent of the rf amplitude v_1^I . If $2v_c$ matches a sum or difference of the spinning frequency and the chemical shift difference, i.e., if $2v_c = v_{rot} \pm \Delta v_{iso}$, then v_{ms} equals Δv_{iso} and the

first-order ($K = 1$) modulation sidebands overlap with the spectrally distant resonances. In analogy to rotational resonance [21], this can compensate for the energy imbalance involved in $S-S'$ flip-flop processes, and thus promote magnetization transfer between arbitrarily distant resonances at high spinning speeds. It is worth pointing out that the modulation sidebands can cover a broad range of spectral frequencies, depending on the magnitude of the recoupled dipolar interactions, which greatly alleviates the need to fulfill the matching condition exactly. Indeed, simulations for $v_{rot} = 30$ kHz and $v_1^I = 20$ kHz show that the first-order modulation sidebands ($K = 1$) of the $C^\alpha H$ signal in L-alanine spread over a width of about 4 kHz. This breadth will be even larger for signals of rigid

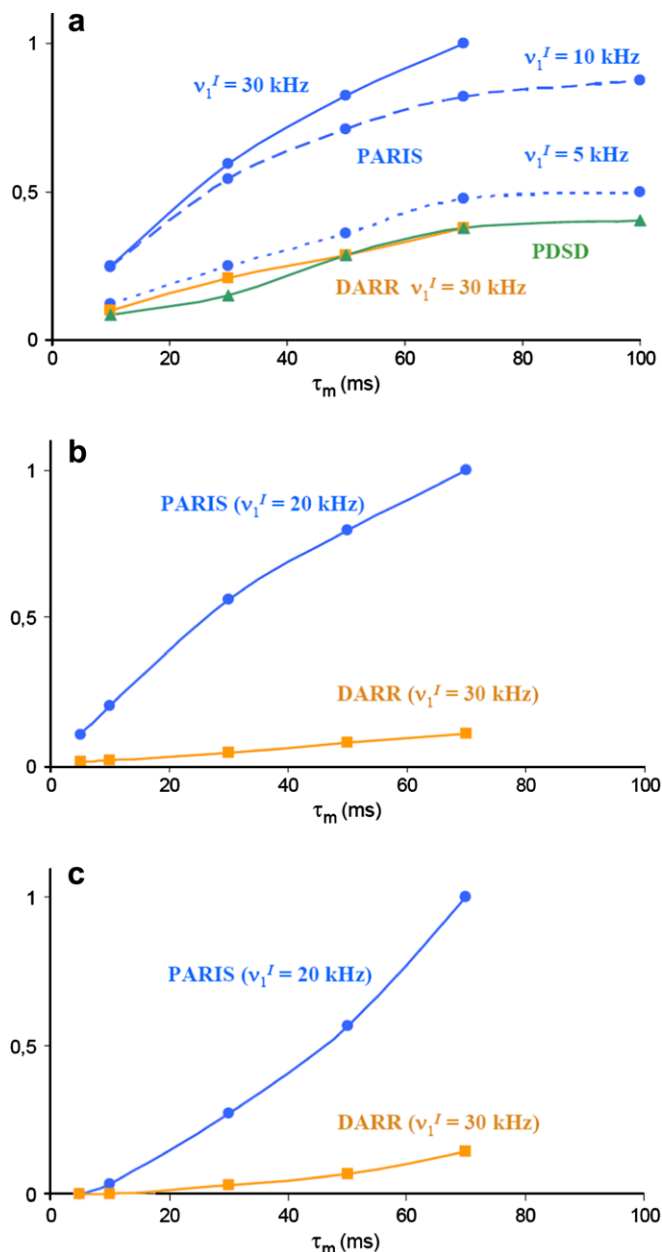


Fig. 6. (a) Build-up curves of C^α/C^β cross-peak amplitudes in L-alanine recorded with $v_{rot} = 30$ kHz and different proton rf amplitudes with dotted, dashed and solid lines for $v_1^I = 5, 10$ and 30 kHz. Comparison of PARIS proton irradiation (blue filled circles), DARR (orange filled squares) and PSDS (green filled triangles). (b) Build-up curves for C^α/C^α cross-peaks with proton rf amplitudes $v_1^I = 20$ and 30 kHz for PARIS and DARR, respectively. (c) Same for C^α/C^β cross-peaks. The PARIS build-up curves in (a) were recorded with $\tau_p = \tau_{rot}/2$ ($N = 1/2$), while (b) and (c) were recorded with $\tau_p = 2\tau_{rot}$ ($N = 2$).

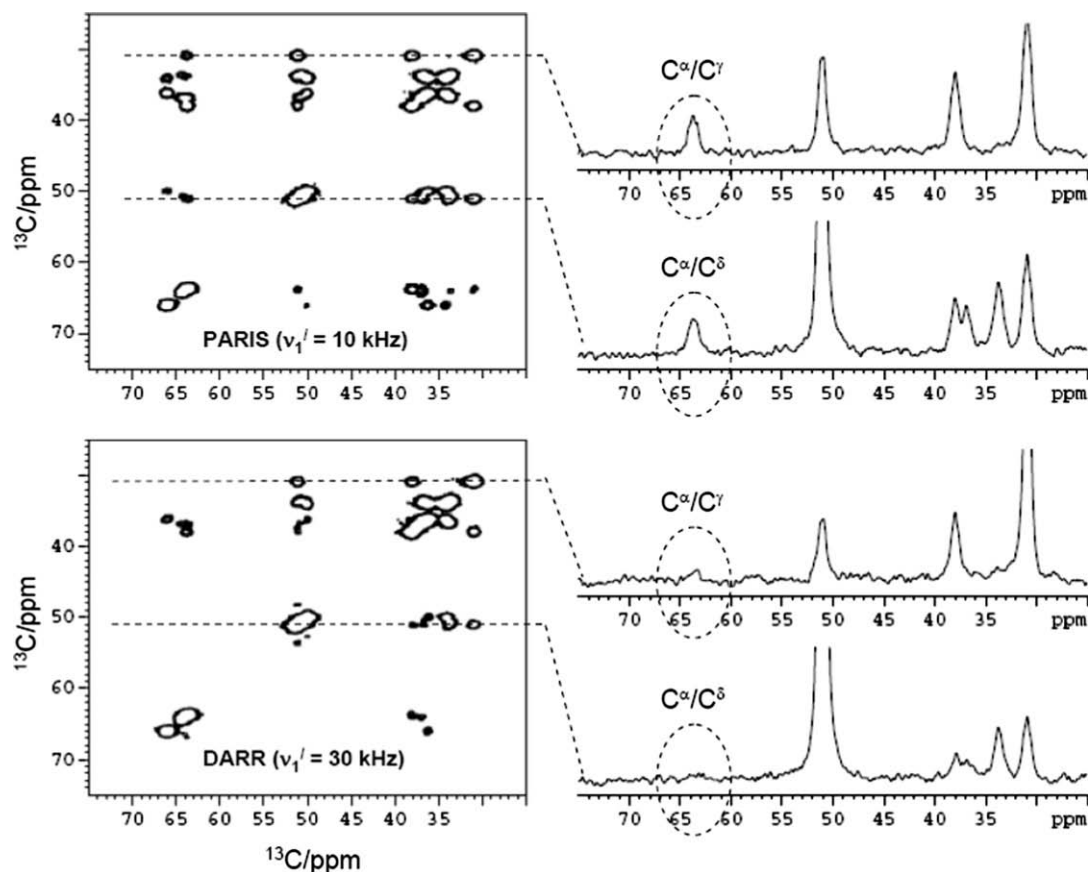


Fig. 7. Aliphatic regions of 2D ^{13}C correlation spectra of uniformly enriched arginine recorded with $\nu_{rot} = 30$ kHz. (top) PARIS and (bottom) DARR proton irradiation in the mixing interval $\tau_m = 30$ ms. The proton rf amplitudes were $\nu_1' = 10$ kHz for PARIS with $\tau_p = \tau_{rot}/2$ ($N = 1/2$), and $\nu_1' = 30$ kHz for DARR experiments. Note the enhanced cross-peaks and attenuated diagonal peaks in the PARIS experiment despite the weak rf amplitude.

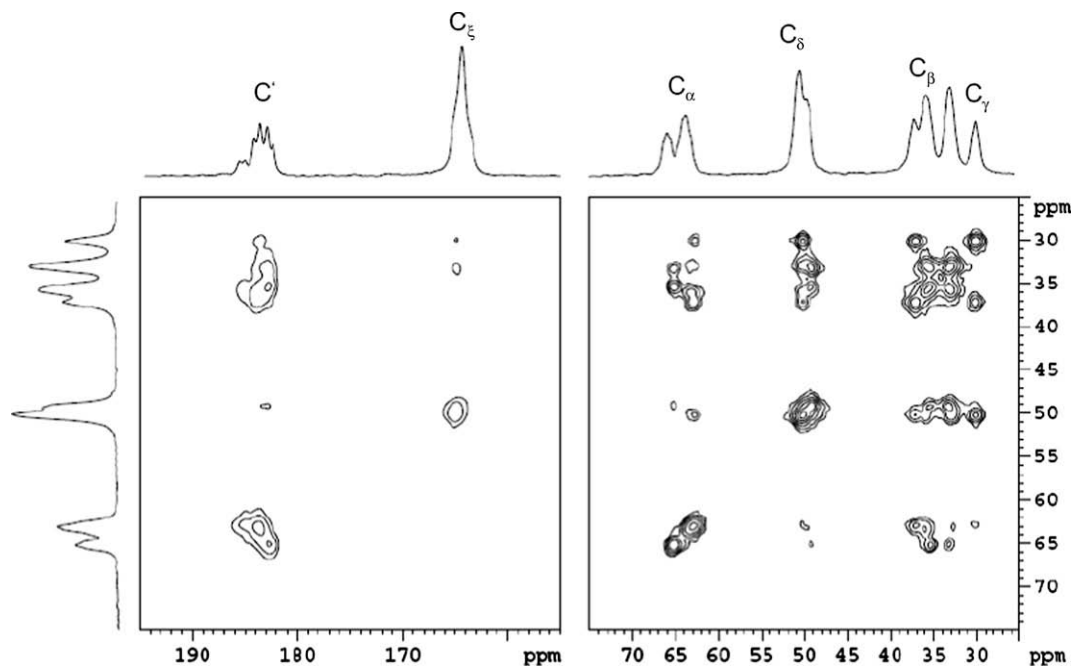


Fig. 8. (left) Carboxylic/aliphatic cross-peak region and (right) aliphatic/aliphatic diagonal region of a 2D ^{13}C correlation spectrum of uniformly enriched arginine recorded with $\nu_{rot} = 30$ kHz, PARIS proton irradiation with $\nu_1' = 30$ kHz and $\tau_p = 2\tau_{rot}$ ($N = 2$) during a mixing interval $\tau_m = 90$ ms. Note the presence of three-bond C'/C' , C'/C^δ , C'/C^ϵ and four-bond C'/C^δ correlation peaks which indicates that there are no dipolar truncation effects.

$C^\beta H_2$ groups with large homo- and heteronuclear dipolar couplings. On the other hand, exact matching is more critical for methyl

groups, where the dipolar couplings are small due to fast rotation. To promote magnetization exchange at $\nu_{rot} = 30$ kHz between C'

and aliphatic C^α and C^β carbons in L-alanine (Fig. 6b and c), we used $N = 2$ ($1/\tau_p = 15$ kHz) which, according to Eq. (13), allows one to position the modulation sidebands associated with the aliphatic C^α and C^β carbons at a distance of 15 kHz from the isotropic shifts of the parent signals. While this does not exactly match the frequency differences $\Delta v_{\text{iso}}(C'-C^\alpha) = 12.2$ kHz or $\Delta v_{\text{iso}}(C'-C^\beta) = 15.8$ kHz, the modulation sidebands associated with the aliphatic C^α and C^β carbons are sufficiently broad to overlap significantly with the distant C' resonance when using an rf amplitude $v_1' = 20$ kHz. This leads to a considerable improvement of the magnetization transfer driven by PARIS compared to DARR. Further simulations reveal that not only the width but also the shape and intensity of the modulation sidebands depend on the rf amplitude, the proton offset, the heteronuclear and homonuclear dipolar couplings, as well as on the carbon and proton chemical shift anisotropies.

Fig. 7 shows two-dimensional ^{13}C – ^{13}C correlation spectra of the aliphatic C^α , C^β , C^γ , and C^δ resonances in a fully ^{13}C enriched powder sample of L-arginine. Note the good performance of PARIS with a low rf amplitude $v_1' = 10$ kHz $\ll v_{\text{rot}} = 30$ kHz, compared with DARR with $v_1' = v_{\text{rot}} = 30$ kHz. Besides the expected increase of the intensity of one-bond correlations, two- and three-bond C^α/C^γ and C^α/C^δ correlations also clearly appear in PARIS-driven magnetization exchange. These cross-peaks are hardly visible in the DARR experiment in spite of the three-fold higher rf field amplitude and nine-fold higher rf power.

Finally, as shown in Fig. 8, one can take advantage of the broadband recoupling capability of the PARIS scheme, due to the improved spectral overlap between dipolar-broadened resonance signals of the aliphatic carbons and to the modulation sidebands that become effective with higher rf amplitudes. This leads to efficient magnetization exchange *simultaneously* between nearby aliphatic carbons *and* between distant carboxylic and aliphatic signals. This compares favorably with earlier recoupling schemes for homonuclear correlation experiments in fast spinning samples [1–2,13].

4. Experimental

All experiments were performed on a Bruker Avance II spectrometer operating at $B_0 = 9.4$ T (400.2 MHz for protons), equipped with a triple resonance MAS probe with 2.5 mm rotors spinning at $v_{\text{rot}} = 30$ kHz. Polycrystalline powders of uniformly ^{13}C , ^{15}N -labeled L-alanine and L-arginine were used without further purification. In all experiments, standard cross-polarization (CP) was used to enhance the initial S spin (carbon-13) magnetization. All numerical simulations were performed using SPINEVOLUTION [22].

5. Conclusions

We have introduced a simple approach for efficient dipolar recoupling in solids that are rapidly spinning at the magic angle.

Contrary to previous rotary resonance-based recoupling schemes such as DARR where S – S' magnetization exchange is also promoted by rf irradiation applied to the I (proton) spins, the PARIS method need not fulfill any rotary resonance condition. It has been shown that phase-alternated irradiation compensates efficiently for the rf field inhomogeneity and allows one to use moderate rf amplitudes, particularly at high spinning speeds. These features were exploited to promote magnetization exchange in correlation experiments with fast MAS. At $v_{\text{rot}} = 30$ kHz and a proton resonance frequency of 400 MHz, recoupling with pulse lengths $\tau_p = \tau_{\text{rot}}/2$ ($N = 1/2$) effectively promotes magnetization exchange between aliphatic carbons which span a limited range of isotropic shifts, while larger pulse lengths, i.e., $\tau_p = 2\tau_{\text{rot}}$ ($N = 2$) allow one to correlate efficiently spectrally distant resonances such as carboxylic and aliphatic carbons. The robustness of the new experiment in promoting efficient dipolar recoupling and magnetization exchange at fast MAS, combined with its simplicity, should lead to a wide range of applications for the structure determination of biomolecules and other materials by solid state NMR spectroscopy.

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