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Communication

High-resolution ^{1}H homonuclear dipolar recoupling NMR spectra of biological solids at MAS rates up to 67 kHz

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ABSTRACT

Two-dimensional ^{1}H homonuclear correlation NMR spectra of solids of biological interest have been recorded at high magnetic fields (14.1 and 18.8 T) and MAS rates up to 67 kHz, using RN_n^v symmetry-based homonuclear recoupling and CRAMPS decoupling; this method affords exceptional spectral resolution and is well suited to probe ^{1}H–^{1}H proximities in powdered solids.

^{1}H NMR spectroscopy is an extremely powerful and now routine tool for studying the molecular structure and dynamics in liquids. In contrast, investigating solids by ^{1}H NMR still presents considerable challenges because the strong ^{1}H–^{1}H dipolar coupling (dominant interaction in rigid solids) homogeneously broadens the proton resonances up to a few tens of kHz. The ^{1}H homonuclear dipolar interaction may be partially averaged out using NMR techniques developed since the sixties [1–7], which rely on two strategies: (i) periodic radio-frequency (rf) multiple-pulse sequences, acting on the spin part of the interaction; (ii) magic-angle spinning (MAS) to average the spatial part of the dipolar interaction. Both come together in the so-called Combined Rotation and Multiple-Pulse Spectroscopy (CRAMPS) [6]. Recent technological developments in NMR probes (MAS up to 70 kHz) and spectrometer consoles (fast electronics) contributed to a considerable improvement in the quality and resolution of ^{1}H NMR spectra.

Double-quantum (DQ) homonuclear recoupling MAS NMR methods are among the most useful techniques available to chemists. Such recoupling techniques encode important structural information by restoring the through-space dipole–dipole couplings, which depend on the inverse cube of the distances between interacting nuclei. DQ experiments are used to estimate torsional angle [8–11], filter signals of mobile or isolated spin species [12], providing also a route to the excitation of higher coherence orders [13,14]. The availability of CRAMPS decoupling techniques capable of performing well at very fast MAS, opens new perspectives in ^{1}H NMR spectroscopy, providing improved resolution in 2D double-quantum–single-quantum (DQ–SQ) correlation experiments. Recently, we have shown that DUMBO and PMLG ^{1}H homonuclear decoupling perform well at a spinning rate of 35 kHz [15]. At the same time, Vega et al. extended the MAS rate to 65 kHz [16].

Symmetry-based CN sequences, such as $C9_1^4$ [17] and POST-$C7_2^1$ [18], have been employed with, respectively, PMLG [7] and DUMBO [5]. However, the use of such sequences is mainly confined to moderate MAS rates (ν_R = 10–15 kHz), because the recoupling part of the sequence requires very large rf fields. Recently, we have combined CRAMPS decoupling and back-to-back (BABA) DQ recoupling at ν_R = 35 kHz [19]. Although, BABA imposes less MAS rate

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constrains, it suffers from low efficiency because it is not a 'γ-encoded' recoupling sequence [20,21]. Thus, methods are needed for a more efficient recoupling of double-quantum coherence at very fast sample spinning.

Here we wish to show that RN_n^ν symmetry sequences allow efficient ^{1}H–^{1}H DQ recoupling at MAS rates up to 67 kHz. Although RN_n^ν sequences have already been used to recouple ^{13}C–^{13}C dipolar interactions at MAS rates <22 kHz [22–24], they have not been employed to reintroduce ^{1}H–^{1}H dipolar couplings at ultra-fast spinning rates. The method is illustrated on two solids of biological interest, amino-acid tyrosine hydrochloride (Tyr) and tri-peptide glutathione in its reduced form (GSH), at external magnetic fields (B_0) of 14.1 and 18.8 T. ^{1}H homonuclear decoupling (DUMBO) is employed in the DQ (t_1) and SQ (t_2) dimensions using the pulse sequence depicted in Fig. 1.

Although many CN_n^ν and RN_n^ν symmetries are suitable for double-quantum recoupling, the latter present some advantages and will be used here [20,22]. Table 1 depicts the RN_n^ν symmetries available for DQ recoupling at high spinning speed.

Here, $R12_2^5$ and $R14_4^5$ symmetry sequences are used for the excitation and reconversion of DQ coherences. The former is attractive for ^{1}H applications at MAS rates up to 67 kHz because it spans over only two rotor periods, minimising the effect of relaxation losses. $R14_4^5$ has the advantage of requiring lower rf fields (*ca.* 117 kHz at 67 kHz MAS) than the $R12_2^5$ sequence (*ca.* 201 kHz at 67 kHz), although it spans over four rotor periods (Fig. S1, see supporting information). A comparison between the intensities of DQ-filtered spectra for $R12_2^5$, $R14_4^5$ and BABA homonuclear recoupling schemes is shown in Fig. 2 for GSH. In the 0–4 ppm region, the intensities of the resonances recorded with $R12_2^5$ and $R14_4^5$ are three times higher than those measured with BABA-4, for the same excitation time (four rotor periods). Similar results were observed onglycine (see Fig. S3).

The ^{1}H–^{1}H DQ–SQ spectra of Tyr recorded with $R14_4^5$ and CRAMPS decoupling is much better resolved than the spectrum acquired without CRAMPS (Fig. 3). In the former, ten out of the twelve (Fig. 3c) ^{1}H resonances, including the chemically distinct aromatic protons, are assigned. The NH_3 resonances are unresolved. All intra- and inter-molecular (nearest neighbour) connections between protons present in the crystal structure of Tyr may be derived from the DQ cross-peaks in Fig. 3b. For example, aromatic protons inter-nuclear distances of 2.76 Å for H8···H10, and 2.84 Å for H7···H11, correspond to inter-molecular interactions between aromatic rings and are observed as off-diagonal DQ–SQ cross-peaks appearing at DQ chemical shifts, respectively, δ_{DQ} 12.1 and 12.6 ppm. The intra-molecular H8···H11 contacts are observed at δ_{DQ} 10.6 ppm. The CH_2 diastereostopic protons, H3 and H4, resonating at *ca.* 4.35 and 4.55, respectively, may be distinguished because H3 is closer to CH (H12) and Ar–H (H10) protons than the H4 protons (intra-molecular H3···H12 and inter-molecular H3···H10 cross-peaks at, respectively, δ_{DQ} 6.9 and 10.9 ppm). H6 and H2 are also in close spatial proximity (*ca.* 2.22 Å, δ_{DQ} 22.5 ppm). The full assignment of the remaining DQ–SQ ^{1}H···^{1}H cross-peaks is given in Table S1 (see supporting information).

The ^{1}H–^{1}H DQ–SQ CRAMPS spectra of GSH were recorded using the $R12_2^5$ recoupling sequence, at 14.4 T, MAS at 67 kHz (Fig. 4c), and 18.8 T, MAS at 30 kHz (Fig. 4a). These spectra are much better resolved than the spectra recorded without CRAMPS. However, it stands out that the ultimate resolution, at least in the particular case of GSH, is determined more by the very high external magnetic field than ultra-fast sample spinning. A detailed assignment of the seventeen ^{1}H resonances is not attempted here, although most part of the peaks are individualised in the 2D spectrum of Fig. 4a. Three distinct spectral regions are present. The aliphatic protons resonate at 0–5 ppm; peaks in the 8–10 ppm region are attributed to NH protons, as revealed by ^{1}H–^{14}N HMQC experi-

Table 1
RN_n^ν symmetries for γ-encoded homonuclear DQ recoupling, with selection of {l, m, λ, μ} = {2, ±1, 2, ±2} or {2, ±1, 2, ∓2} terms, and suppression of all CSA terms. *J*-coupling is allowed. Solutions with $n \leqslant 4$ and requiring rf < 200 kHz at a MAS 65 kHz are shown. The rf field and the scaling factors for the recoupled DQ terms are also given.

RN_n^ν	rf field (kHz)	Scaling factor
$R12_2^1$, $R12_2^5$	195	0.173
$R14_4^2$, $R14_4^5$	113	0.165
$R18_4^2$, $R18_4^7$	146	0.171
$R22_4^2$, $R22_4^9$	178	0.173

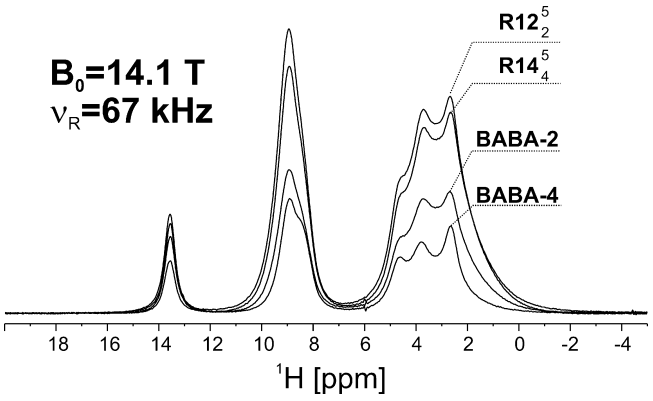

Fig. 2. Comparison of the intensities of DQ recoupling on GSH, using four rotor periods during the RN_n^ν or BABA-4 excitation/reconversion blocks. BABA-2 was performed using only two rotor periods (*ca.* 30 μs). MAS rate 67 kHz, Larmor frequency 600 MHz (B_0 = 14.1 T, Bruker wide-bore NMR spectrometer).

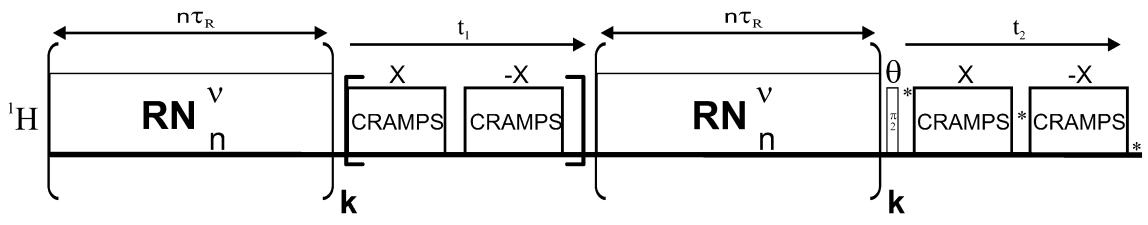

Fig. 1. Pulse sequence for 2D ^{1}H–^{1}H DQ homonuclear recoupling experiments. $R12_2^5$ and $R14_4^5$ symmetries are used for DQ excitation/reconversion (k = 1). Here, we use the CRAMPS decoupling scheme DUMBO during the t_1 and t_2 evolutions. For experimental details regarding DUMBO decoupling the reader is referred to Ref. [15]. The flip angle of the read pulse is $\theta = 90°$. RN_n^ν recoupling blocks are of the form $[R_\phi R'_{-\phi}]^{N/2}$ where $\phi = \nu\frac{\pi}{N}$ (the rf phase in degrees) and the basic element R is a 180° flip angle. Therefore, for sequences employing a complete $R12_2^5$ recoupling six pairs of building blocks of the type $[R_{75°}R'_{-75°}]^6$ were employed, which gives a total of 12 R pulses spanning over two rotor periods during the excitation and reconversion blocks because n = 2. In the same way, $R14_4^5$ recoupling employs a $[R_{64.29°}R'_{-64.29°}]^7$ building block spanning over four rotor periods (n = 4). The nutation frequency (ν_1) for any of the RN sequences is detailed in Table 1 and may be calculated using the expression $\nu_1 = (N/2n)^*\nu_R$.

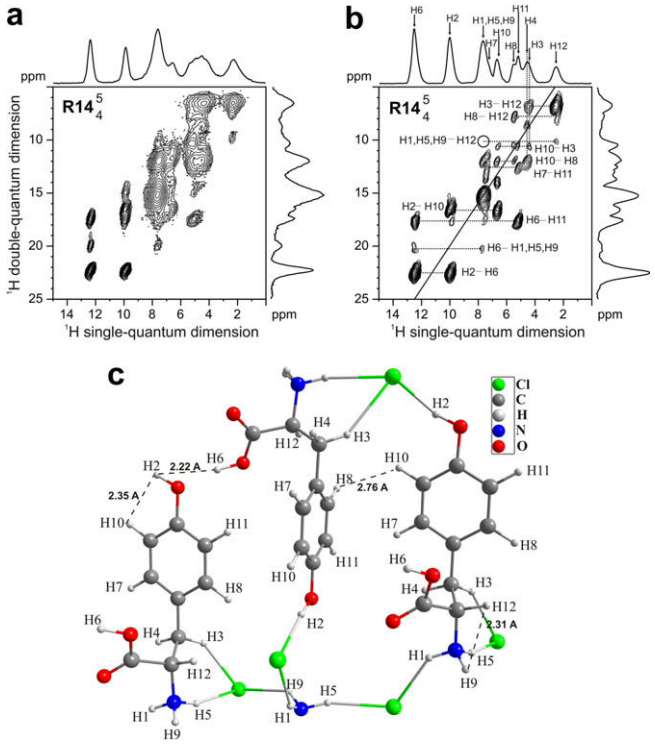

Fig. 3. 2D ^{1}H–^{1}H DQ–SQ spectra of Tyr, recorded at MAS 67 kHz using ν_1 = 117 kHz and Larmor frequency of 600 MHz (B_0 = 14.1 T, Bruker wide-bore NMR spectrometer), using the $R14_4^5$ symmetry for ^{1}H–^{1}H recoupling. (a) No CRAMPS used, (b) DUMBO decoupling in both dimensions (DUMBO shape pulse length = 15 μs; Decoupling power = 198 kHz), (c) schematic representation of the solid-state structure of Tyr.

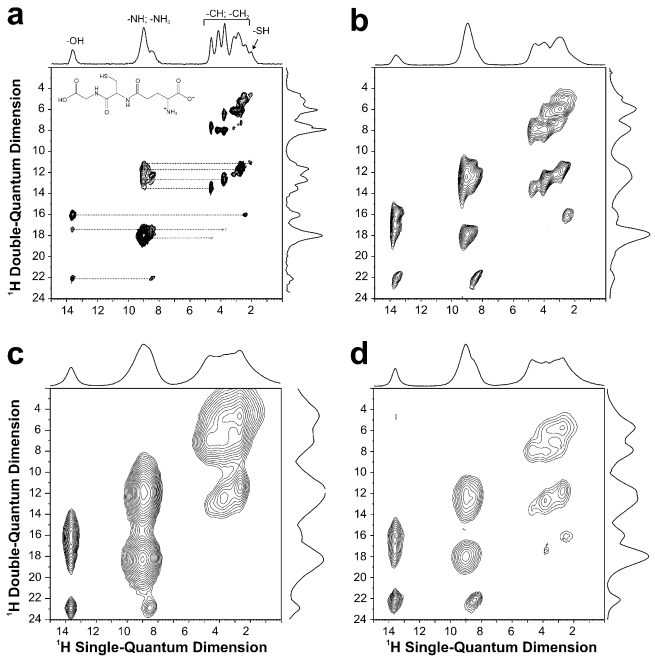

Fig. 4. 2D ^{1}H–^{1}H DQ–SQ spectra of GSH using the $R12_2^5$ symmetry acquired (a and c) with DUMBO decoupling during t_1 and t_2 according to Fig. 1 (DUMBO shape pulse length = 16 and 15 μs; decoupling power = 150 and 198 kHz for (a and c), respectively) and (b and d) without CRAMPS decoupling. 2D spectra were recorded at MAS rates of 35 kHz using ν_1 = 105 kHz and Larmor frequency of 800 MHz (B_0 = 18.8 T, Bruker standard-bore NMR spectrometer) for (a and d) and at MAS rates of 67 kHz using ν_1 = 201 kHz and Larmor frequency of 600 MHz (B_0 = 14.1 T, Bruker wide-bore NMR spectrometer) for (c and d).

ments (Fig. S2, see supporting information); and the peak at *ca.* 13.6 ppm is assigned to hydrogen-bonded OH groups.

In conclusion, it has been shown that symmetry-based RN_n^ν $R12_2^5$, $R14_4^5$ pulse sequences combined with CRAMPS afford high-resolution 2D ^{1}H–^{1}H γ-encoded DQ homonuclear correlation spectra, at ultra-fast MAS rates, on samples of biological interest. At 67 kHz MAS, the $R14_4^5$ sequence is the more favourable symmetry, requiring rf fields lower than $R12_2^5$, making it more attractive for studying heat sensitive biomolecules.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmr.2009.04.004.

DQ build up of $R14_4^5$ and $R12_2^5$ recoupling sequences performed at 14.1 T and ν_R = 67 kHz (Fig. S1); 2D ^{1}H–^{14}N dipolar-HMQC spectrum of GDH (Fig. S2); comparison of the intensities of the DQ-filtered spectra for glycine using and BABA-2 recorded at 18.8 T and ν_R = 35 kHz (Fig. S3); assignment of the ^{1}H NMR Tyr peaks (Table S1) are available in the online.

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