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Broadband Homonuclear Correlation Spectroscopy Driven by Combined R_{2n}^V Sequences under Fast Magic Angle Spinning for NMR Structural Analysis of Organic and Biological Solids

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Abstract

We recently described a family of experiments for R_{2n}^V Driven Spin Diffusion (RDSD) spectroscopy suitable for homonuclear correlation experiments under fast MAS conditions (*J. Am. Chem. Soc.*, 133, **2011**, 3943). In these RDSD experiments, since the broadened second-order rotational resonance conditions are dominated by the radio frequency field strength and the phase shifts, as well as the size of reintroduced dipolar couplings, the different R_{2n}^V sequences display unique polarization transfer behaviors and different recoupling frequency bandwidths. Herein, we present a series of modified R_{2n}^V sequences, dubbed *CO*mbined *R*_{2n}^V-*DR*iven (CORD), that yield broadband homonuclear dipolar recoupling and give rise to uniform distribution of cross peak intensities across the entire correlation spectrum. We report NMR experiments and numerical simulations demonstrating that these CORD spin diffusion sequences are suitable for broadband recoupling at a wide range of magnetic fields and MAS frequencies, including fast-MAS conditions ($\nu_r = 40$ kHz and above). Since these CORD sequences are largely insensitive to dipolar truncation, they are well suited for the determination of long-range distance constraints, which are indispensable for the structural characterization of a broad range of systems. Using U-¹³C-alanine and U-¹³C,¹⁵N-histidine, we show that under fast-MAS conditions, the CORD sequences display polarization transfer efficiencies within broadband frequency regions that are generally higher than those offered by other existing spin diffusion pulse schemes. A 89-residue U-¹³C,¹⁵N-dynein light chain (LC8) protein has also been used to demonstrate that the CORD sequences exhibit uniformly high cross peak intensities across the entire chemical shift range.

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Keywords

R_{2n}^V -Driven Spin Diffusion (RDSD); COmbined R_{2n}^V -Driven Spin Diffusion (CORD SD); symmetry sequences; solid-state NMR; fast MAS; broadband homonuclear correlation spectroscopy

INTRODUCTION

In recent decades, solid-state NMR (SS-NMR) spectroscopy has emerged as a powerful method for probing molecular structure and dynamics in inorganic materials, polymers, and biological solids.[1-10] Magic angle spinning (MAS) technique [11, 12] is widely used in SS-NMR for obtaining narrow spectral lines, and with the breakthroughs in MAS technologies starting in the late 1990's, MAS frequencies of the order of $\nu_r = 40$ -110 kHz are now accessible to solid-state NMR spectroscopists.[13-15] Under these MAS frequencies, both the resolution and sensitivity per unit mass can be enhanced greatly due to: (i) the efficient suppression of various anisotropic NMR interactions, (ii) the increased coupling of the magnetization with the coil due to smaller rotor diameter, (iii) the related higher radio frequency (rf)-fields that allow for a better control of the magnetization, and (iv) the access to the proton detection in solids under high resolution.[16-21] With these advantages, detailed structural and dynamics analysis of very large proteins and protein assemblies containing either uniform or dilute isotopic labels has become feasible. Numerous SS-NMR sequences have been developed for recoupling anisotropic interactions, and with the aid of these methods structural and dynamics information can be obtained.[22-37] However, the majority of the existent recoupling techniques cannot work well at MAS frequencies above 30 kHz.

The conventional spin-diffusion-based recoupling techniques, including proton-driven spin diffusion (PDSD),[38, 39] RF-assisted diffusion (RAD),[40] and dipolar-assisted rotational resonance (DARR),[33, 41] have lead to extensive ^{13}C (or ^{15}N) applications for resonance assignments, determination of structural constraints, and quantitative SS-NMR measurements under the MAS frequencies of 30 kHz and lower.[42-58] These second-order rotary-resonance-recoupling (R^3) techniques do not severely suffer from dipolar truncation and are particularly advantageous for structural determination in uniformly isotopically enriched systems. However, their performances deteriorate rapidly with increased MAS frequencies and in practice they do not work above $\nu_r = 25$ -30 kHz, where the polarization transfer rates are more dependent on the R^3 conditions, which cannot be effectively attained by conventional spin diffusion methods. Recently, several pulse sequences have been developed with modified ^1H rf field ($\nu_{1\text{H}}$) irradiation to obtain ^{13}C - ^{13}C spin diffusion spectra at MAS frequencies of 30 kHz and above, including the mixed rotational and rotary resonance (MIRROR),[59] phase-alternated recoupling irradiation (PARIS) and its variants, [60, 61] and R_{2n}^V -symmetry driven spin diffusion (RDSD) [62] techniques. Within these pulse schemes, phase- or amplitude-modulated rf field irradiation is applied on protons in a periodic fashion, and the second order R^3 conditions, $\Delta\nu_{\text{iso}} = \nu_{1\text{H}} \pm n\nu_r$, are largely broadened resulting in more efficient polarization transfers at MAS frequencies faster than 30 kHz.[59, 62]

Within spin diffusion spectroscopy under MAS frequencies of 30 kHz and above, the polarization transfer efficiency between ^{13}C spin pairs is more sensitive to their frequency differences, $\Delta\nu_{\text{iso}}$. As shown in our recent RDSD report,[62] each R_{2n}^V symmetry sequence exhibits different polarization transfer behaviors with distinct dependencies on $\Delta\nu_{\text{iso}}$ because the recoupled bands are dominated not only by the reintroduced dipolar couplings, but also by the amplitude, $\nu_{1\text{H}}$, and the phase shifts of the applied rf field. The R_{21}^1

sequence (PARIS) is most efficient for accomplishing spin diffusion among the carbons with small $\Delta\nu_{\text{iso}}$ values, i.e. aliphatic-to-aliphatic, while the $R2_2^2$ sequence shows highest polarization transfer efficiencies for coupled spins with relatively large $\Delta\nu_{\text{iso}}$ value, i.e., aliphatic-to-carbonyl. The $R2_2^1$ sequence displays high transfer efficiency and uniform excitation for both aliphatic-to-aliphatic and aliphatic-to-carbonyl carbon regions, but the transfer efficiency for the ^{13}C spin pairs with $\Delta\delta_{\text{iso}} = 60\text{-}90$ ppm is much lower, in accordance with the numerically simulated ^{13}C - ^{13}C transfer profiles.

To achieve broadband ^{13}C - ^{13}C correlation spectroscopy at MAS frequencies of 30 kHz and above, several spin-diffusion schemes with super phase-cycling have been reported recently, including PARIS_{xy} [63] and SHANGHAI [64]. In this work, we present an alternative approach to broadband spin diffusion under fast-MAS conditions: a series of modified $R2_n^V$ sequences dubbed *CO*mbined *R2_n^V*-Driven (CORD). We demonstrate that CORD sequences yield broadband homonuclear dipolar recoupling and give rise to uniform distribution of cross peak intensities across the entire correlation spectrum spanning a ^{13}C chemical shift range of 200 ppm. The variations in rf field strengths and phase shifts in these super phase-cycled CORD schemes and sequences derived from CORD assure significant broadening of the second-order R^3 conditions, which helps driving ^{13}C - ^{13}C spin diffusion in a truly broad frequency range, even for the spin systems with sparse proton content. We report here SS-NMR experiments and numerical simulations demonstrating that these CORD spin diffusion sequences are suitable for broadband recoupling over a wide range of magnetic fields and MAS frequencies including MAS frequencies of 40 kHz and above. The comparison to the recent spin-diffusion schemes (PARIS_{xy} ($m = 2$) and SHANGHAI) and to the fpRFDR [37] and DREAM [32] sequences is also included, both through numerical simulations and experimental measurements. We present experimental results on model systems $\text{U-}^{13}\text{C}$, ^{15}N -alanine and $\text{U-}^{13}\text{C}$, ^{15}N -histidine, as well as on a protein, $\text{U-}^{13}\text{C}$, ^{15}N -dynein light chain (LC8) protein. These findings indicate that at $\nu_r = 40$ kHz, the basic CORD sequence and the super-cycled CORD schemes display polarization transfer efficiencies that are generally higher than those attained by other existing spin diffusion pulse schemes, which give rise to uniformly high cross peak intensities across the entire ^{13}C chemical shift range. Furthermore, these CORD sequences can also be used for the determination of long-range distance constraints because they are generally insensitive to dipolar truncation, in contrast with DREAM and fpRFDR techniques, and which is particularly important in uniformly labeled biological samples. Thus CORD-based sequences are well suited for truly broadband spin diffusion spectroscopy at MAS frequencies of 30 kHz and faster.

EXPERIMENTS AND METHODS

Materials

$\text{U-}^{13}\text{C}$, ^{15}N -labeled L-alanine (Ala) and L-histidine (His) were purchased from Cambridge Isotope Laboratory. Ala powder sample was packed into the 1.8 mm MAS rotor for subsequent SS-NMR experiments without any further purification or re-crystallization. His sample was doped with 0.1 mol% CuCl_2 and re-crystallized before packing into MAS rotor. $\text{U-}^{13}\text{C}$, ^{15}N -enriched dynein light chain protein (LC8) was expressed in *E.coli* and purified as described previously.[65] This LC8 sample was prepared by controlled precipitation, via slow addition of a solution of 30% PEG-3350 to the solution of 11 mg of LC8 (30 mg/ml). Prior to the precipitation step, both PEG-3350 and LC8 were dissolved in 10 mM MES buffer (10 mM MgCl_2 , pH 6.0) and doped with 50 mM EDTA-Cu(II).

Solid-State NMR Spectroscopy

Experiments were conducted on a 14.1 T Varian Infinity-Plus and a 19.97 T Bruker AVIII SS-NMR spectrometers, with ^1H and ^{13}C Larmor frequencies of 599.8/850.4 and

150.8/213.8 MHz, respectively. A 1.8 mm triple-resonance HCN MAS probe developed in the Samoson laboratory was used for the experiments performed at 14.1 T on Ala and His samples, and all spectra were recorded at the MAS frequency of $\nu_r = 40$ kHz, controlled to within ± 5 Hz using a Varian MAS controller. The typical 90° pulse lengths were then of 2.8 (^1H) and 4.1 μs (^{13}C). A Bruker 1.3 mm triple-resonance HCN MAS probe was used for the experiments at 19.97 T. For these experiments, Cu(II)-EDTA doped LC8 protein sample was packed into the rotor and spun at the MAS frequency of $\nu_r = 40$ kHz, controlled to within ± 10 Hz using a Bruker MAS controller. The typical 90° pulse lengths were then of 1.4 (^1H) and 3.0 μs (^{13}C).

To reduce sample heating during fast MAS, nitrogen gas was used for cooling, resulting in a final sample temperature of 0°C for the LC8 sample, and 20°C for Ala and His samples. Low-power TPPM ^1H decoupling with a rf field strength of 11 kHz was used during t_1 and t_2 periods. The $^1\text{H} \rightarrow ^{13}\text{C}$ cross polarization was performed with a ^1H rf field of 55 kHz and a linear amplitude ramp (80-100%) on ^{13}C with the center of the ramp Hartmann-Hahn matched to the first spinning sideband. During the mixing period, τ_{mix} , different composite pulse irradiations were applied on protons, and a series of corresponding 2D NMR spectra were recorded. The rf field strengths on the ^1H channel during the mixing time were set to their nominal value of $\nu_{1\text{H}} = 40$ and 20 kHz for R2_1^V and R2_2^V symmetry sequences, respectively. Appropriate rf field strengths, corresponding to the $n = 1$ R^3 condition, were used for PARIS_{xy} and SHANGHAI sequences, i.e., $\nu_{1\text{H}} = \nu_r$. All 2D NMR data were processed with NMR-Pipe software [66] in a Mac environment using a standard protocol, including zero-filling to twice the number of points in the indirect dimension, Fourier transform, and phase correction in both dimensions.

Numerical Simulations

All numerical simulations were performed using the SIMPSON [67] and SPIN-EVOLUTION [68] software packages. 168 REPULSION angles (α , β) and 64 γ angles were used to generate a powder average. The atomic coordinates for the model spin systems employed in the simulations were taken from the SS-NMR structure of the leucine residue in the *N*-f-MLF-OH tri-peptide (PDB ID 1Q7O).[51] These atomic coordinates are generally regarded as a valid representation for spin systems of other amino acids, including Ala and His. The one-bond dipolar coupling constants for ^1H - ^{13}C and ^{13}C - ^{13}C were set as 22,690 and 2,251 Hz, respectively. Throughout the simulations, all possible pairwise dipolar couplings were taken into account. J couplings were ignored as their effects are negligible given their small sizes. Other parameters used in simulations were the same as in the corresponding experiments.

RESULTS AND DISCUSSION

Bandwidth and Polarization Transfer Efficiency in CORD Sequences: Numerical Simulations

The pulse sequence for homonuclear CORD spin diffusion experiments is shown in Figure 1a. As illustrated in Figure 1b-d, during the mixing period, a series of combined R2_n^V symmetry pulses are applied on the ^1H spins; these include the basic CORD (Figure 1b), CORD_{xix} (Figure 1c) and CORD_{xy4} (Figure 1d). The basic CORD sequence shown in Figure 1b is composed of four types of R2_n^V symmetry pulses, R2_1^1 , R2_1^2 , R2_2^1 , and R2_2^2 . The order of these R2_n^V symmetry pulses does not influence the outcome of a particular experiment and can be set arbitrarily. As in our recent report, the nominal rf field strengths are set at $\nu_{1\text{H}} = \nu_r$ and $\nu_{1\text{H}} = \nu_r/2$ for R2_1^V and R2_2^V sequences, respectively. Various super-cycled CORD versions can be derived as well, including $\text{CORD}_0\text{CORD}_{180}$ denoted as CORD_{xix} (Figure 1c), $\text{CORD}_0\text{CORD}_{90}\text{CORD}_{180}\text{CORD}_{270}$ denoted as CORD_{xy4} (Figure

1d), CORD₀CORD₉₀ denoted as CORD_{xy} (not shown), and CORD₀CORD₉₀CORD₁₈₀CORD₂₇₀CORD₀CORD₂₇₀CORD₁₈₀CORD₉₀ denoted as CORD_{xy8} (not shown).

Protons play important roles in all varieties of 2nd-order ¹³C-¹³C spin diffusion experiments, where the ¹³C-¹³C polarization transfers can be driven by the recoupled ¹H-¹³C hetero- and ¹H-¹H homonuclear dipolar couplings. Under MAS frequencies below 30 kHz, even the residual dipolar couplings can easily drive ¹³C-¹³C polarization transfers over a very broad frequency range. However, at MAS frequencies of 30 kHz and above, the first-order rotational resonance (R²) conditions, $\Delta\nu_{\text{iso}} = \pm n\nu_r$, cannot be fulfilled, and the general PDS technique fails. Under such high MAS frequencies, the homonuclear polarization transfers mostly depend on the second-order rotary resonance recoupling (R³) conditions, $\Delta\nu_{\text{iso}} = \nu_{\text{IH}} \pm n\nu_r$, and broadband recoupling would be reached when the broadened R³ conditions are met, $\nu_{\text{IH}} \pm n\nu_r - K_{\text{sc}}\nu_{\text{DD}}$ and $\nu_{\text{IH}} \pm n\nu_r + K_{\text{sc}}\nu_{\text{DD}}$, which can be accomplished by various recoupling sequences applied on protons. K_{sc} is the effective recoupling scaling factor, and ν_{DD} the ¹H-¹³C dipolar coupling constant. In Figure 2, the simulated dependence of the ¹³C-¹³C polarization transfer efficiency is plotted versus the resonance frequency difference, $\Delta\nu_{\text{iso}}$, for R₂¹ (basic PARIS) and R₂² symmetry sequences at the MAS frequency of $\nu_r = 40$ kHz and magnetic field of 14.1 T. For the spin systems containing a small amount of protons (i.e. C₂H in (a, c)), only ¹³C spin pairs belonging to very narrow frequency bands can be recoupled. However, it can be seen that the frequency bands are greatly broadened when spin systems contain more protons (i.e. C₂H₂ in (b, d)). When the rf pulse irradiation is of R₂¹ symmetry (PARIS), only aliphatic-to-aliphatic correlations are observed, while aliphatic-to-aromatic and aliphatic-to-carbonyl correlations are very weak or absent. With R₂² sequence, broadband recoupling is attained, but the transfer efficiency for the ¹³C spin pairs with $\Delta\delta_{\text{iso}} \approx 60\text{-}90$ ppm (corresponding to aliphatic-to-aromatic correlations) is much lower than that for other regions, which would result in the missing cross peaks in the corresponding ¹³C-¹³C spin diffusion spectra.

In contrast, the ¹³C-¹³C recoupling bandwidths attained in the CORD schemes are expected to be much broader, and be the composite of the individual R₂ⁿ symmetry sequences. Moreover, the phase shifts introduced to the π pulses in the super-cycled CORD schemes are also expected to contribute to broadening the R³ conditions that determine the ¹³C-¹³C polarization transfers, in the same way as the improvements introduced by the PARIS_{xy} sequence over PARIS.

Figure 3 demonstrates the simulated dependence of the ¹³C-¹³C polarization transfer efficiency on the frequency difference for CORD, CORD_{xy4}, PARIS_{xy}, SHANGHAI, DREAM and fpRFDR sequences, using the same C₂H₂ spin system model as in Figure 2b and 2d. As shown in Figure 3c, the phase-cycled PARIS_{xy} sequence exhibits much broader recoupling band-width compared with the basic PARIS sequence, in spite of the low efficiency of the aliphatic-to-aromatic and aliphatic-to-carbonyl polarization transfers. The modified PARIS_{xy} sequence with additional phase cycles, dubbed SHANGHAI, gives rise to a more broadband recoupling profile compared with PARIS_{xy} when standard rf field condition is used ($\nu_{\text{IH}} = \nu_r$). Nevertheless, none of these sequences exhibits truly broadband behavior. In contrast, recoupling by both the basic CORD and the super phase-cycled CORD_{xy4} is fully broadbanded, covering all the possible correlations among ¹³C spins within the 0-200 ppm chemical shift range (Figure 3a and 3b). Notably, the numerical simulations reveal that in the super phase-cycled CORD_{xy4} the ¹³C-¹³C polarization transfer is much more uniform across the entire frequency (chemical shift) difference range than in the individual R₂ⁿ, PARIS_{xy} or SHANGHAI sequences, and this is also the case for other versions of phase-cycled CORD sequences (see Figure S1 of the Supporting Information). As discussed above, the basic CORD scheme is composed of four R₂ⁿ symmetry sequences

that can be introduced in any order, such as $R2_1^1 R2_2^1 R2_1^2 R2_2^2$. Regardless of the order in which the individual $R2_n$ elements are arranged, the composite CORD sequences give rise to broadband polarization transfer (Figure S1).

Besides the spin-diffusion-based sequences, DREAM[32] and fpRFDR[65] also work well under fast MAS conditions, and high transfer efficiency can be easily attained provided sufficiently short mixing time is used. However, both sequences have limitations in the regimes where CORD is advantageous. For example, in the DREAM sequence, the polarization transfer efficiency is very sensitive to the isotropic chemical shift difference between the coupled spin pairs, and the recoupling frequency band is very limited (< 100 ppm), as seen in Figure 3e. Therefore, it is not possible to establish broadband polarization transfer across the entire spectrum in a single DREAM experiment. Both DREAM and fpRFDR sequences are very sensitive to dipolar truncation restricting their utility to intra-residue resonance assignments or acquisition of long-range constraints only in sparsely (but not uniformly) labeled systems. Furthermore, both DREAM and fpRFDR sequences suffer the sensitivity loss from various relaxation processes characterized by the respective time constants, including T_1^{RF} , $T_{1\rho}$, and T_1^{ZQ} . For the spin-diffusion-based experiments, the strength of the detected signal is mostly dependent on the ^{13}C spin-lattice relaxation times, and generally ^{13}C T_1 are of the order of seconds, therefore permitting determination of long-distance structural constraints with long mixing times of up to seconds.

Another important consideration is the dependence of the rf field strength on the performance of the CORD sequences. The rf field strengths for the $R2_n^v$ elements in CORD are typically defined according to their symmetry properties. However, the rf field strength of each $R2_n^v$ block can also be set to an arbitrary value different from its nominal requirement ($v_{1H} - r$ or $0.5 \cdot v_r$). Figure 4 shows the simulated dependence of the ^{13}C - ^{13}C polarization transfer efficiency on the rf field strength and the frequency difference, Δv_{iso} , for various recoupling schemes: CORD, CORD_{xix} , CORD_{xy4} , and CORD_{xy8} , PARIS_{xy} and SHANGHAI. The MAS frequency was 40 kHz and the magnetic field was 14.1 T. In these simulations, the C_2H_2 four-spin model was utilized, and the variable rf field strengths of v_{1H} and $v_{1H}/2$ were used for $R2_1^v$ and $R2_2^v$ sequences, respectively. It is clear that broadband polarization transfers can be easily attained by a series of CORD sequences, even with mismatched rf fields, in contrast with the PARIS_{xy} sequence. Notably, SHANGHAI sequence is more broadbanded than PARIS_{xy} , but the transfer efficiency is lower than that by the CORD sequences. The super phase-cycling of the CORD sequences improves the ^{13}C - ^{13}C polarization transfer efficiency greatly, following the order: $\text{CORD}_{xy8} > \text{CORD}_{xy4} > \text{CORD}_{xix} > \text{CORD}$. Interestingly, for CORD_{xy8} , the positions of the recoupling bands are only very weakly (if at all) dependent on the rf field strength, and the polarization transfer efficiency should thus be largely insensitive to the rf-inhomogeneity. Another relevant observation emerging from the simulations is that due to the numerous resonance conditions, broadband recoupling can even be achieved in spin systems with low amount of protons, such as the C_2H spin system (Figure S2), especially for CORD_{xy4} and CORD_{xy8} schemes. This property may be advantageous when using partly deuterated samples, and experiments are necessary to verify this prediction made on the basis of computational results. It should be noted that the standard rf field settings imposed by the $R2_n^v$ symmetries were used for all the following SS-NMR experiments and simulations in the present report, while further explorations of other recoupling conditions will be the subject of future studies.

Another important consideration is the performance of these various methods at the MAS frequencies higher than 40 kHz. As discussed above, CORD sequences perform very well at the spinning frequencies of 40 kHz, but it is more challenging to attain broadband recoupling at frequencies of 60 kHz and higher (the regime where ^1H detection becomes

useful). As demonstrated in Figure S3 of the Supporting Information, the super phase-cycled $\text{CORD}_{\text{xy}4}$ and $\text{CORD}_{\text{xy}8}$ sequences exhibit truly broadband polarization transfer at the MAS frequency of 60 kHz, where both PARIS_{xy} and SHANGHAI sequences cannot work well. This finding indicates that the amplitude and phase modulation in phase-cycled CORD sequences permit to fulfill the broadened second-order rotary resonance conditions, opening venues for ^{13}C - ^{13}C spin-diffusion-based correlation spectroscopy in the “ultrafast” MAS regime.

Experimental Results

Guided by the above numerical simulations, we have hence first performed a series of 2D ^{13}C - ^{13}C spin diffusion experiments on U- ^{13}C , ^{15}N -alanine at 14.1 T. The sample was spun at the MAS frequency of 40 kHz, and the various composite pulse irradiation sequences were applied during the mixing period. The results are summarized in Figure 5. An example of 2D ^{13}C - ^{13}C CORD correlation spectrum acquired with the mixing time of $\tau_{\text{mix}} = 150$ ms is given in Figure 5a. It can be seen that, even with the basic CORD sequence, all the expected correlations are present in the spectrum. Figure 5b shows the comparison of the three 1D traces extracted along the F_1 dimension from the corresponding 2D correlation spectra obtained with various recoupling sequences, plotted on the same scale. PARIS_{xy} sequence exhibits the best transfer efficiency for the $\text{C}_\alpha \leftrightarrow \text{C}_\beta$ correlations, but very weak polarization transfer for the $\text{C}_\alpha/\text{C}_\text{O}$ and $\text{C}_\beta/\text{C}_\text{O}$ regions characterized by large chemical shift differences. Notably, the polarization transfers by both CORD series and the SHANGHAI sequences are broadband, even though the $\text{C}_\alpha \leftrightarrow \text{C}_\beta$ transfer rates are slower than that attained by PARIS_{xy} , which is consistent with the simulations shown in Figure 3. Moreover, the ^{13}C - ^{13}C polarization transfer efficiencies by CORD sequences are similar to or higher than that by SHANGHAI in both aliphatic-to-aliphatic and aliphatic-to-carbonyl regions. All the CORD sequences display broadband recoupling with similar polarization transfer behaviors, and super phasecycled CORD sequences are only slightly better, which might be due to the abundance of protons ($N_{\text{H}}/N_{\text{C}} > 1.5$).

We have also examined the performance of the CORD series of sequences on His sample spun at 40 kHz. Figure 6 shows the comparison of 2D ^{13}C - ^{13}C correlation spectra recorded at 14.1 T by CORD (a) and PARIS_{xy} (b) sequences, with a mixing time of $\tau_{\text{mix}} = 150$ ms. All ^{13}C - ^{13}C cross peaks for carbons separated by one to five bonds can be observed within 2D CORD spectrum. With the PARIS_{xy} rf field irradiation, the polarization transfer among ^{13}C spin pairs is more sensitive to the frequency difference, $\Delta \nu_{\text{iso}}$, which results in missing cross peaks. Detailed comparisons of all cross peaks recorded by the various composite pulse sequences, including CORD, $\text{CORD}_{\text{xy}4}$, PARIS_{xy} and SHANGHAI , are presented in Figure 7. The results clearly show that CORD and $\text{CORD}_{\text{xy}4}$ sequences give the highest transfer efficiencies for most of the ^{13}C frequency regions, indicating that they are suitable for broadband homonuclear recoupling at MAS frequency of 40 kHz. PARIS_{xy} sequence shows the highest transfer efficiencies for ^{13}C spin pairs with small $\Delta \nu_{\text{iso}}$, but for spin pairs characterized by larger $\Delta \nu_{\text{iso}}$, the performance is much worse than for the other three sequences. Compared to the original PARIS_{xy} scheme, SHANGHAI sequence can drive ^{13}C - ^{13}C polarization transfer across a much broader frequency range. However, its transfer efficiency is lower than for both CORD and CORD derivative sequences throughout the entire carbon region except for C_α -to- C_β correlations (see Figure S4 for the traces extracted along the F_1 indirect dimension of the 2D ^{13}C - ^{13}C correlation spectra). The results on the two model compounds indicate that phase- and amplitude-modulated CORD pulse schemes are advantageous for broadband ^{13}C - ^{13}C spin diffusion experiments at MAS frequencies of 40 kHz and potentially higher.

We have also examined the performances of super phase-cycled CORD sequences for obtaining broadband ^{13}C - ^{13}C correlation spectra in proteins, using U- ^{13}C , ^{15}N -LC8 protein.

Figure 8 displays the 2D ^{13}C - ^{13}C correlation spectra of LC8 acquired with CORD_{xy4} , PARIS_{xy} and SHANGHAI sequences at 14.1 T. The sample was spun at the MAS frequency of 40 kHz; and the mixing time of 150 ms was used for all NMR spectra. As demonstrated above on model compounds, PARIS_{xy} sequence shows low polarization transfer efficiency for carbon pairs with large $\Delta \nu_{\text{iso}}$ values, and therefore the aliphatic-to-carbonyl and aliphatic-to-aromatic correlations are missing in the spectrum, as illustrated in Figures 8b and S5. With the SHANGHAI sequence, broadband ^{13}C - ^{13}C transfer can be achieved, but the transfer efficiency is lower than that attained by CORD_{xy4} for most ^{13}C spin pairs. As can be appreciated, CORD_{xy4} sequence exhibits uniformly broadband transfers giving rise to ^{13}C - ^{13}C correlations through the entire carbon frequency range, including the aliphatic-to-aromatic cross peaks (Figure S5) missing in PARIS_{xy} and the aliphatic-to-aliphatic cross peaks (albeit the transfer efficiency is slightly lower compared to PARIS_{xy}).

To compare the performance of CORD with that of other commonly used homonuclear recoupling sequences, we have collected a series of 2D ^{13}C - ^{13}C CORD_{xy4} , DREAM and fpRFDR spectra with various mixing times, at 19.97 T and the MAS frequency of 40 kHz. The results are summarized in Figures 9, 10 and 11. Consistent with our results at 14.1 T, with the CORD sequences, ^{13}C - ^{13}C polarization transfers can be easily attained across the entire spectrum including the aromatic region with a mixing time of 150 ms (Figure 9a). Importantly, at a longer mixing time of 500 ms, numerous multiple-bond correlations can be observed, as shown in Figure 9b. This observation indicates that CORD sequences are well suited for recoding medium- and long-range constraints in the regime of long mixing times.

As shown in Figure 10, even though ^{13}C - ^{13}C correlations in aliphatic-to-aliphatic region can be readily obtained with the DREAM sequence, the correlations in aromatic and carbonyl regions are largely missing, which is consistent with the simulations shown in Figure 3. Furthermore, there are no new cross peaks in the DREAM spectrum acquired with long mixing time of 8 ms due to the effect of the dipolar truncation. In this 8-ms DREAM spectrum, some cross peaks actually disappear (e.g., the 35-to-18 ppm region) because of the fast signal decay due to the sensitivity to various relaxation times.

As illustrated in Figure 11, with fpRFDR sequence with xy-16 phase steps,[37, 69] broadband ^{13}C - ^{13}C correlation spectrum can be obtained with a mixing time of 2.4 ms, although many fewer cross peaks are seen than in the CORD spectrum. Similar to the results observed in the DREAM experiment, when the fpRFDR mixing time is increased to 9.6 ms, very few new peaks appear in the fpRFDR spectrum and instead some cross peaks disappear. This may be due to the compounded effects of (i) dipolar truncation, (ii) signal decay due to relaxation, and (iii) insufficient super-cycling.[70]

Taken together, the numerical simulations and the experimental results on two model systems and a protein discussed in this report indicate that CORD series of sequences are advantageous for spin diffusion experiments at MAS frequencies of 40 kHz and above, compared with the previously developed methods. The uniform broadband and efficient polarization transfers across the entire frequency range, as well as lack of sensitivity to dipolar truncation, makes CORD series of sequences well suited for various applications in ^{13}C - ^{13}C correlation spectroscopy in biological systems, including resonance assignments and measurement of distance restraints for protein structure calculation.

CONCLUSIONS

In summary, a series of combined R2_n^V -symmetry (CORD) sequences have been presented for obtaining broadbanded ^{13}C - ^{13}C homonuclear correlation spectra under MAS frequencies of 40 kHz and above. Since the broadened second-order rotational resonance conditions are

determined by the rf field strength, the phase shifts and the size of reintroduced dipolar couplings, the individual $R2_n^V$ sequences display different polarization transfer behaviors and different recoupling band-widths. On the contrary, the combination of rf fields and phase shifts in the super phase-cycled composite CORD pulse schemes results in efficient broadening of the second-order rotary resonance conditions, and produces truly broadbanded ^{13}C - ^{13}C spin diffusion. Compared to the original PARIS_{xy} and SHANGHAI sequences, the super phase-cycled CORD schemes display higher transfer efficiency throughout the entire ^{13}C spectrum. That CORD sequences are largely insensitive to dipolar truncation makes them appropriate for recording long-range distance constraints in uniformly labeled systems. The CORD series of sequences are therefore well suited for broadbanded one- and multi-bond homonuclear correlation spectroscopy under a wide range of MAS frequencies up to 40 kHz and above, even at moderate or low magnetic fields, and the CORD_{xy4} and CORD_{xy8} sequences with super phase cycling are expected to work best for the uniform broadband correlation spectroscopy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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HIGHLIGHTS

- We present *COMBINED* R_{2n}^V -Driven (CORD) sequences for spin diffusion at fast MAS ($\nu_r = 40$ kHz).
- We show that CORD sequences exhibit broadband homonuclear dipolar recoupling.
- CORD sequences display uniform cross peak intensities across the entire spectrum.
- CORD sequences are advantageous for homonuclear spectroscopy in biological solids.

Figure 1.

(a) The general pulse sequence for 2D ^{13}C - ^{13}C CORD homonuclear correlation experiments. An rf irradiation consisting of a series of composite pulses is applied on proton spins during the mixing time, τ_{mix} , with the irradiation sequences corresponding to: (b) basic CORD; (c) CORD_{xiX} ; (d) CORD_{xy4} . These irradiation schemes are composed of rotor-synchronized R2_n^V -type symmetry sequences: R2_1^1 ($\nu_{1\text{H}} = \nu_r$), R2_1^2 ($\nu_{1\text{H}} = \nu_r$), R2_2^1 ($\nu_{1\text{H}} = \nu_r/2$), R2_2^2 ($\nu_{1\text{H}} = \nu_r/2$).

Figure 2.

Simulated dependence of the ^{13}C - ^{13}C polarization transfer efficiency versus the isotropic chemical shift difference for R2_1^1 (basic PARIS) and R2_1^1 symmetry sequences for C_2H_n spin systems containing $n = 1$ proton (a, c) and $n = 2$ protons (b, d). The transfer efficiency is defined as $2I_{2Z}/(I_{1Z} + I_{2Z})$, where I_{1Z} is the initial polarization on nucleus 1, and I_{2Z} is the polarization on spin 2 after the polarization transfer. The MAS frequency is $\nu_r = 40$ kHz, and the mixing time is $\tau_{\text{mix}} = 150$ ms. The $\nu_{1\text{H}}$ rf-field amplitude is equal to its nominal value of 20 (c,d) and 40 (a,b) kHz. The ^{13}C - ^{13}C polarization transfer efficiency was simulated over the frequency difference of $\Delta\nu_{\text{iso}} = \pm 50$ kHz at the magnetic field of 14.1 T.

Figure 3.

Simulated dependence of the ^{13}C - ^{13}C polarization transfer efficiency versus the isotropic chemical shift difference for various composite rf pulse irradiations: (a) CORD, (b) CORD_{xy4}, (c) PARIS_{xy}, (d) SHANGHAI, (e) DREAM, and (f) fpRFDR. A C_2H_2 spin model (the same as in Figure 2b and 2d) was used for all the simulations. The MAS frequency is $\nu_r = 40$ kHz, and the mixing time is $\tau_{\text{mix}} = 150$ ms for (a-d), and 3 ms for (e) and (f). ^{13}C - ^{13}C polarization transfer efficiency was simulated over the frequency difference of $\Delta\nu_{\text{iso}} = \pm 50$ kHz at the magnetic field of 14.1 T.

Figure 4.

Simulated dependence of the ^{13}C - ^{13}C polarization transfer efficiency as a function of the rf field strength, ν_{1H} , and the resonant frequency difference, $\Delta \nu_{iso}$, for various composite rf pulse irradiations: (a) CORD, (b) CORD_{xix}, (c) CORD_{xy4}, (d) CORD_{xy8}, (e) PARIS_{xy} and (f) SHANGHAI. A C_2H_2 spin model was used for ^{13}C - ^{13}C spin diffusion simulations at MAS frequency of $\nu_r = 40$ kHz and magnetic field of 14.1 T, with a mixing time of $\tau_{\text{mix}} = 150$ ms.

Figure 5.

(a) 2D ^{13}C - ^{13}C CORD correlation spectra recorded on U- ^{13}C , ^{15}N -alanine spun at 14.1 T, with the MAS frequency of $\nu_{\text{r}} = 40$ kHz, the mixing period of $\tau_{\text{mix}} = 150$ ms and the rf fields of $\nu_{\text{1H}} = 20$ and 40 kHz. The first contour is set at $10 \times \sigma$ (σ is the noise rmsd) with the multiplier of 1.2. In (b), 1D traces extracted along the F_1 dimension of the 2D ^{13}C - ^{13}C correlation spectra are shown, together with 1D traces extracted from homonuclear correlation spectra recorded by other composite pulse sequences: CORD (red), CORD_{mix} (blue), CORD_{xy4} (black), PARIS_{xy} (green) and SHANGHAI (brown)

Figure 6. 2D ^{13}C - ^{13}C correlation NMR spectra of U- ^{13}C , ^{15}N -enriched histidine spun at the MAS frequency of $\nu_r = 40$ kHz recorded at 14.1 T with CORD (a) and PARIS_{xy} (b) sequences, with the mixing time was $\tau_{\text{mix}} = 150$ ms. The 1D traces extracted along the F_1 dimension are shown to illustrate the polarization transfer efficiency. The first contour in all spectra is set at $10 \times \sigma$ with the multiplier of 1.2.

Figure 7.

1D traces along F_1 showing ^{13}C - ^{13}C cross peaks extracted from 2D spin diffusion correlation NMR spectra of $\text{U-}^{13}\text{C}$, ^{15}N -enriched histidine recorded at 14.1 T with various composite pulse sequences: CORD (red), CORD_{xy4} (blue), PARIS_{xy} (purple), and SHANGHAI (black). The spinning frequency of $\nu_r = 40$ kHz and the mixing time of $\tau_{\text{mix}} = 150$ ms were used for all spin diffusion experiments. The diagonal auto-correlation peaks are not shown.

Figure 8. 2D ^{13}C - ^{13}C correlation NMR spectra of U- ^{13}C , ^{15}N -LC8 protein spun at the MAS frequency of $\nu_r = 40$ kHz recorded at 14.1 T with CORD $_{xy4}$ (a), PARIS $_{xy}$ (b) and SHANGHAI (c) sequences. The mixing time was $\tau_{\text{mix}} = 150$ ms. The first contour in all spectra is set at $5 \times \sigma$ with the multiplier of 1.2.

Figure 9. 2D CORD_{xy4} ^{13}C - ^{13}C correlation NMR spectra of U- ^{13}C , ^{15}N -LC8 protein spun at the MAS frequency of $\nu_r = 40$ kHz and the magnetic field of 19.97 T, recorded with the mixing times of (a) 150 and (b) 500 ms. The first contour in all spectra is set at $5 \times \sigma$ with the multiplier of 1.2.

Figure 10.

2D DREAM ^{13}C - ^{13}C correlation NMR spectra of $\text{U-}^{13}\text{C}$, ^{15}N -LC8 protein spun at the MAS frequency of $\nu_r = 40$ kHz and the magnetic field of 19.97 T, recorded with the mixing times of (a) 4 and (b) 8 ms. High power ^1H decoupling with the rf field strength of 180 kHz was applied during DREAM mixing period. The first contour in all spectra is set at $5 \times \sigma$ with the multiplier of 1.2.

Figure 11. 2D fpRFDR_{xy16} ^{13}C - ^{13}C correlation NMR spectra of U- ^{13}C , ^{15}N -LC8 protein spun at the MAS frequency of $\nu_r = 40$ kHz and the magnetic field of 19.97 T, recorded with the mixing times of (a) 2.4 and (b) 9.6 ms. No ^1H decoupling was applied during the RFDR mixing period. The first contour in all spectra is set at $5 \times \sigma$ with the multiplier of 1.2.