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On the direct relation between REDOR and DIPSHIFT experiments in solid-state NMR

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ABSTRACT

Rotational-echo double resonance (REDOR) and Dipolar-coupling chemical-shift correlation (DIPSHIFT) are commonly used experiments to probe heteronuclear dipole-dipole couplings between isolated pairs of spin-$\frac{1}{2}$ nuclei in magic-angle-spinning (MAS) solid-state NMR. Their widespread use is due to their robustness to experimental imperfections and a straightforward interpretation of data. Both of these experiments use rotor-synchronised π pulses to recouple the heteronuclear dipole-dipole couplings, and the observed intensity of resonances is modulated by a recoupled phase factor depending on the position or duration of the recoupling pulses. Several modifications to both of these experiments have been proposed, for example, the development of DIPSHIFT which employs strategies that mimic the multi-rotor-period nature of REDOR. We show here that REDOR and DIPSHIFT are in fact alternate implementations of the same experiment. The overt similarity in the design of REDOR and DIPSHIFT is also reflected in their theoretical description. Dipolar dephasing curves in REDOR are obtained by increasing the recoupling duration whilst keeping the position of the pulses constant, which results in a dephasing factor that is a function of only the dephasing time. DIPSHIFT, on the other hand, is a constant-time version of REDOR; the dipolar dephasing is a function of the position of the pulses with respect to the rotor period. We discuss the advantages and disadvantages of each implementation and suggest domains of applicability for these sequences.

1. Introduction

Estimating dipole-dipole couplings between selected heteronuclear spin-pairs and hence, distances between them, is a common application of magic-angle-spinning (MAS) NMR in material and biomolecular sciences [1–6]. Rotational Echo DOuble Resonance (REDOR) and DIPolar chemical SHIFT correlation (DIPSHIFT) are the two most commonly used techniques when both the nuclei under study are spin-$\frac{1}{2}$[7–10]. Both these experiments are used in a form nearly identical to their original implementations even after more than three decades of development in the field [7,8]. This is due to the ease of implementation, robustness to experimental imperfections, and a single-variable fit of the peak-intensity modulation profile to the effective dipole-dipole coupling. Since the effective dipole-dipole couplings can be partially or completely averaged due to motion, these experiments can be used directly to extract motional order parameters when distances between nuclei are known [11–20]. The basic recoupling block in both these experiments consists of rotor-synchronised π pulses. In REDOR, the recoupling block consists of two π pulses per rotor period (commonly placed at 0.5 τ_r and 1.0 τ_r or 0.25 τ_r and 0.75 τ_r, τ_r being the rotor period), and the number of rotor periods during which this block is applied is incremented. This results in a modulation of intensity in resonances due to an additional phase factor which is a function of (a) the heteronuclear dipole-dipole coupling and (b) the duration of the recoupling block. On the other hand, in DIPSHIFT, the recoupling block consists of a single rotor period with a π pulse, whose position is changed. This results in a modulation of intensity that is dependent on the heteronuclear dipole-dipole coupling and additionally dependent on the position of the pulse.

Hong et al. [9] and Cobo et al. [21] have implemented multi-rotor-period versions of DIPSHIFT, with the number of recoupling blocks as a variable for the interpretation of the intensity modulations in terms of the dipole-dipole couplings. The method suggested by Cobo et al. retains the effects of T_2' and was shown to be very sensitive for detecting molecular motions in the regime, where the exchange process has a time scale of the order of the inverse of the dipole-dipole coupling [22]. We have recently shown

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that a change in the position of the first π pulse whilst keeping all relative pulse positions the same, results in scaling of the effective dipole-dipole coupling in the REDOR experiment [23]. These developments have led to a peculiar situation that the same three variables (dipole-dipole coupling, number of rotor periods, and position of the pulses in a rotor period) are now used to describe these two seemingly different experiments. The similarities in the experimental implementation of these sequences and their theoretical description point towards there being a more than co-incidental relationship between them. In this article, we show that both these experiments can be described by the same pulse sequence but with different experimental realisations. Such a description is powerful in the sense that it opens up the application of DIPSHIFT to cases which were traditionally considered the domain of REDOR, and *vice versa*. The existing implementations of REDOR are restricted to measuring low to moderate dipole-dipole couplings (irrespective of MAS frequencies), whilst DIPSHIFT has been extensively used to measure large dipole-dipole couplings. Further, experimental considerations restrict the use of DIPSHIFT to slow to moderate MAS regime, and multi-rotor (more than 1 τ_r) versions of DIPSHIFT have been implemented at MAS frequencies below 20 kHz. These have long been considered their respective domains of applicability. In addition to our previous results that show the use of REDOR to estimate large dipole-dipole couplings (>20 kHz) in the fast MAS frequency regimes [23], we show here that DIPSHIFT can be used to estimate small dipolar couplings (~1 kHz) at slow to moderate MAS frequencies as well. We discuss the advantages and disadvantages of these experiments and shed light on possible domains of applicability for these experiments. Experimental results are presented by measuring one-bond C-N dipole-dipole coupling in alanine using DIPSHIFT at a MAS frequency of 20 kHz and the tripeptide f-MLF to study one-bond C-N couplings at MAS of 18.181 kHz. The utility of the new DIPSHIFT experiment is demonstrated by using it to determine ^{13}C-^{1}H dipole-dipole couplings in alanine and ^{13}C-^{15}N dipolar couplings in MLF at a MAS frequency of 62.5 kHz.

2. Results and discussion

The pulse sequence in Fig. 1a is an adaptation of the REDOR scheme used to measure arbitrary dipole-dipole couplings, including large couplings (viz. single bond ^{15}N-^{1}H and ^{13}C-^{1}H) [23]. This sequence has a constant displacement of all the π-pulses from their position in the original REDOR experiment, and results in the dipole-dipole coupling being scaled by a factor that is proportional to this displacement (*vide infra*). According to the nomenclature devised to describe such experiments [23], this sequence is notated $m\mathcal{R}_{\epsilon}^{0.5+\epsilon}$. $m\mathcal{R}$ indicates that the sequence corresponds to the mirror-symmetric version of REDOR where the mirror-symmetry is with respect to the central π pulse. The super- and sub-scripts indicate that the π pulses are centred at times $\epsilon\tau_r$ and $(0.5+\epsilon)\tau_r$ from the start of dipolar evolution, where ϵ is given as a fraction of the rotor period. This ensures that all the pulses are separated by 0.5 τ_r. Since all durations are measured from the centre of the (finite) π pulse (τ_π), the value of ϵ in Fig. 1a is:

$$\epsilon = \frac{\tau_0 + 0.5\tau_r - 0.5\tau_\pi}{\tau_r} \tag{1}$$

The sequence reduces to the original REDOR scheme when ϵ is an odd integer multiple of 0.25. In this implementation, τ_0 can take all values between 0.5 τ_r + 0.5 τ_π and 0.75 τ_r + 0.5 τ_π to span the ϵ range of 1.0–1.25. The central π pulse on the I channel refocusses the I spin chemical shift, and a simultaneous π pulse on the S channel ensures that evolution of heteronuclear dipole-dipole couplings is not affected. Note that the π pulses on either side of the central rotor period are arranged in a mirror-symmetric manner. Incrementing the value of n, in multiples of 2, will increase the recoupling duration resulting in a REDOR dephasing profile as shown in Fig. 1b.

The first-order effective Hamiltonian for the $m\mathcal{R}_{\epsilon}^{0.5+\epsilon}$ scheme used here is:

$$\bar{\mathcal{H}}_{DD}^{(1)} = \kappa\, b_{IS}\left[\frac{\sqrt{2}}{\pi}\kappa_\phi \cos(\gamma)\sin(2\beta)2\hat{I}_z\hat{S}_z\right] \tag{2}$$

where $\kappa = \sin(2\pi\epsilon)$ is the scaling factor for the dipole-dipole coupling constant (b_{IS}) dependent on the position of the first pulse (given by ϵ). The term in the square braces is constant for a given crystallite orientation (γ and β angle) and is dependent on the length of the π pulses with respect to the rotor period ($\kappa_\phi = \cos(\pi\phi/2)/(1-\phi^2)$ with, $\phi = 2\tau_\pi/\tau_r$). I spin signal (F) observed after $n\tau_r$ evolution under the Hamiltonian $\bar{\mathcal{H}}_D$, powder averaged over all crystallite orientations is

$$F(n\tau_r, \epsilon) = Tr\{\rho I_x\} \tag{3}$$

where the density matrix ρ is

$$\rho(n\tau_r, \epsilon) = \int_0^{2\pi} d\gamma \int_0^{\pi} d\beta \sin\beta e^{\left(-i\bar{\mathcal{H}}_D^{(1)}n\tau_r\right)} I_x e^{\left(i\bar{\mathcal{H}}_D^{(1)}n\tau_r\right)} \tag{4}$$

When $\kappa < 1$, the dipolar dephasing is slower than that in the REDOR experiment, as shown in Fig. 1b. This fact can be used to measure strong dipole-dipole couplings under slow and fast magic-angle spinning conditions, as the slower build-up allows one to sample a larger number of points in the initial part of the dephasing curve [23]. This sequence has a distinct advantage over strategies such as shifted-REDOR [16,24], as the radiofrequency (RF) requirements for this experiment are identical to the original REDOR implementation, whilst those for shifted-REDOR are higher.

The three important variables in Eq. 3 are the heteronuclear dipole-dipole coupling constant b_{IS}, the scaling factor κ, and the duration of evolution $n\tau_r$. In a REDOR-type experiment, the value of κ is kept constant (by keeping τ_0 fixed) and the dephased signal $F(n\tau_r, \epsilon)$ is monitored stroboscopically at the end of successive recoupling blocks by increasing n in multiples of 2. The effective dipole-dipole coupling between the two spins is *scaled* by the term $\sin(2\pi\epsilon)$, and changing ϵ using Eq. (1) gives different REDOR $\Delta S/S_0$ profiles for the same spin system as shown in Fig. 1b. The DIPSHIFT profile (Fig. 1d), on the other hand, is obtained *with the same sequence* (Fig. 1a) by varying the value of τ_0 from 0.5 τ_π to 0.5 τ_r + 0.5 τ_π, corresponding to ϵ values of 0.5–1.0. This arises from the fact that κ is sinusoidal in nature and its magnitude rises to 1 and falls back to 0 every 0.5τ_r from the start of the evolution duration. DIPSHIFT profile is obtained by varying the ϵ parameter to scale the coupling from 0 to 1 and back to 0 using the Eq. (1), after fixing the value of n. The congruence between these experiments is clearly brought out by Fig. 1c, which shows the intensity of a resonance as a function of ϵ and n. The REDOR dephasing profiles correspond to vertical slices whereas the DIPSHIFT dephasing profiles correspond to horizontal slices. The dephasing time is constant in DIPSHIFT, and is normally kept as one rotor period. In our implementation, this rotor period is divided into 0.5 τ_r before and after the central refocusing block. The multi-rotor DIPSHIFT experiment, where the recoupling time is $n\tau_r$ with $n > 1$, can also be put into an appropriate context within this pulse scheme (Fig. 1a). In such conditions, the signal $F(n\tau_r, \epsilon)$ is recoupled over $n\tau_r$ leading to a larger dephasing of signal. This has been previously termed as an amplification of the dipole-dipole coupling [9]. Fig. 1d shows the dephasing profiles obtained for the same dipole-dipole coupling when the value of n is varied in the DIPSHIFT experiment.

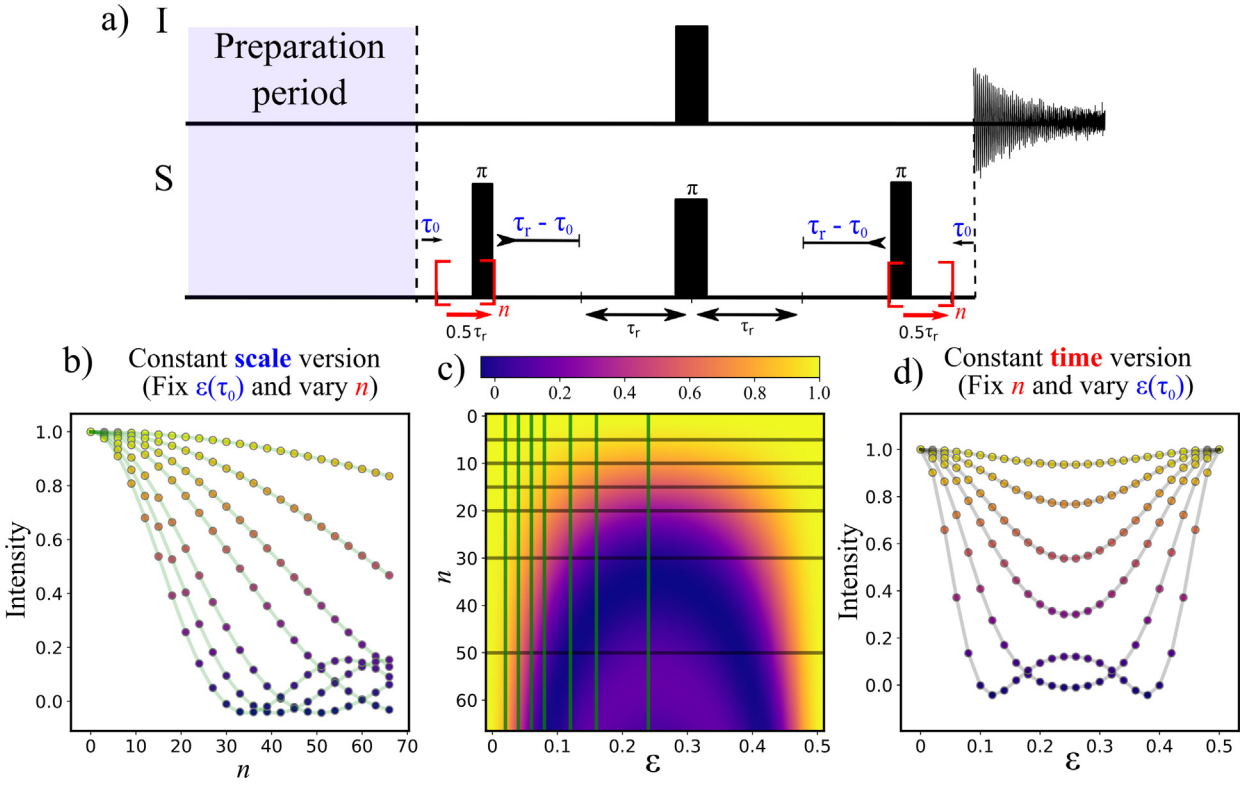

Fig. 1. (a) Pulse sequence for recoupling heteronuclear dipole-dipole couplings. Simulated dephasing profile obtained by (b) incrementing the value of n in multiples of 2 after fixing τ_0 (corresponding to REDOR). Each dephasing curve represents a single value of ϵ and dephasing increases as a function of ϵ (c) Intensity as a function of ϵ (related to τ_0 as given in Eq. (1)) and n with slices along n plotted in (b) and along ϵ in (d). (d) Intensity variation as a function of ϵ for a fixed n (corresponding to DIPSHIFT). Each dephasing curve represents a single value of n and dephasing increases as a function of n. The gradient in the colour of the contours plot in (c) are mapped onto individual points in (b) and (d). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

It is instructive to compare the differences between the original DIPSHIFT implementation and the one described above. Here, the delay between the two consecutive π pulses is always 0.5 τ_r, whilst in the original implementation, this delay is different for each point in the dephasing profile. The first-order average Hamiltonian for the DIPSHIFT experiment described in [9], with ideal π pulses, is as shown in Eq. 5 (see Supporting Information for the Hamiltonian with finite pulses).

$$\bar{\mathcal{H}}_{DD}^{(1)} = \frac{b_{IS}}{2\pi}\Big[\sqrt{2}\sin(2\beta)\sin(\pi\epsilon)\cos(\pi\epsilon-\gamma) + \frac{1}{2}\sin^2(\beta)\sin(2\pi\epsilon)\cos(2\pi\epsilon-2\gamma)\Big]2\hat{I}_z\hat{S}_z \quad (5)$$

This implies that whilst each point in the original DIPSHIFT experiment has a different relative contribution from the first and second Fourier components of the heteronuclear dipole-dipole coupling, the newly proposed sequence - described by Eq. 2 - recouples only a well-defined fraction of the first Fourier component at each point. The symmetry of the recoupled Hamiltonian around the central point also means that only one half of the traditional DIPSHIFT profile needs to be sampled. The ability to implement a frequency-selective DIPSHIFT experiment using selective π-pulses in the central rotor period is an additional feature of the new DIPSHIFT experiment. We note here that Echelmeyer et al. have previously explored a constant-time implementation of REDOR in the context of robust data-fitting [25]. Its relationship to DIPSHIFT was however, missed.

The value of n in a DIPSHIFT experiment described in this manner can be arbitrarily large, and is only limited by the T_2' values of the signals being monitored, thus, allowing measurement of weak couplings. We demonstrate this by recording DIPSHIFT experiment to measure the N-Cα dipole-dipole couplings in a sample of U-^{13}C-^{15}N-Alanine (~880 Hz). Results from these experiments are shown in Fig. 2. Fig. 2a–i shows the DIPSHIFT-type dephasing profiles for various n, ranging from 10 to 50 in steps of 5, and Fig. 2j–l shows the REDOR dephasing profiles for ϵ values of 0.25, 0.125 and 0.085 over a period of 4 ms. All the experimental curves were fit with intensity scaled simulated grids with dipole-dipole coupling range of 600–1200 Hz as described previously [23]. The consistent fits to the data obtained suggest that this DIPSHIFT experiment can easily be used to study a full range of dipole-dipole couplings, and that such a fit is identical to the ones obtained using REDOR experiments. Trends in solid-state NMR literature suggest that the DIPSHIFT experiment is suitable for the measurement of large dipole-dipole couplings at slow to moderate MAS frequencies, whilst REDOR performs better with low to moderate dipole-dipole couplings and can be applied at fast MAS frequencies as well. Contrary to these expectations, Fig. 2 shows that the two experiments are essentially identical and are expected to give identical fits for dipole-dipole couplings in practice.

One of the biggest bottlenecks in the application of both REDOR and DIPSHIFT sequences is the finite pulse width of the π pulses. Since both of these experiments rely on having two π pulses per rotor period, there is an inherent limit to the length of the pulses that can be used. With increasing MAS frequencies or decreasing RF amplitudes of the pulses, the pulses themselves take up a significant part of the rotor period. In the proposed sequence, a total of one rotor period is split into τ_0 and $\tau_r - \tau_0$, and is placed on either side of the recoupling blocks, which is 0.5 τ_r. In practical situations, this is unlikely to cause significant signal decay due to it being part of the overall echo. The recoupling block is designed such that the

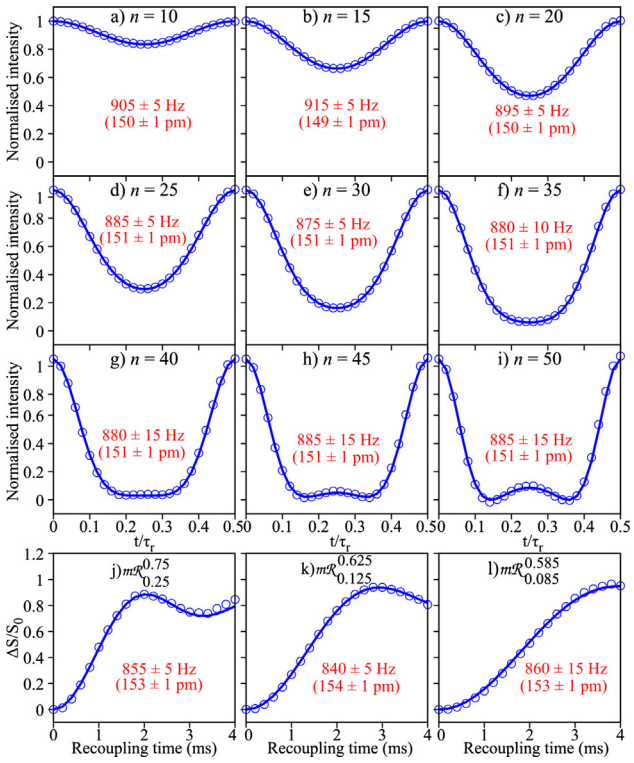

Fig. 2. Experimental data from the pulse sequence in Fig. 1 for DIPSHIFT amplification over (a) 10, (b) 15, (c) 20, (d) 25, (e) 30, (f) 35, (g) 40, (h) 45 and (I) 50 rotor periods. REDOR curves from the pulse sequence in Fig. 1 with scaling values of (j) 1.0, (k) 0.71 and (l) 0.51. Error of 1 pm in distance corresponds to 15 Hz in dipole strength. The fitted value of the dipole-dipole coupling for the each of the dephasing profiles is indicated in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pulse comes at the end of the rotor block. Unlike in the original implementation of DIPSHIFT, the π pulse width does not affect the sampling of data points as their positions in the recoupling blocks are fixed [11]. This design provides an advantage of using pulses as long as 0.5 τ_r in the DIPSHIFT block, practically removing the high RF requirements for the π pulses for moderate spinning frequencies.

The experimental data from the tripeptide f-MLF over a recoupling period of 30 τ_r corresponding to a recoupling time of 1650 μs are shown in Fig. 3. Results from C_α-N and C′-N are presented in Fig. 3a–c, and Fig. 3d–f, respectively, for the three

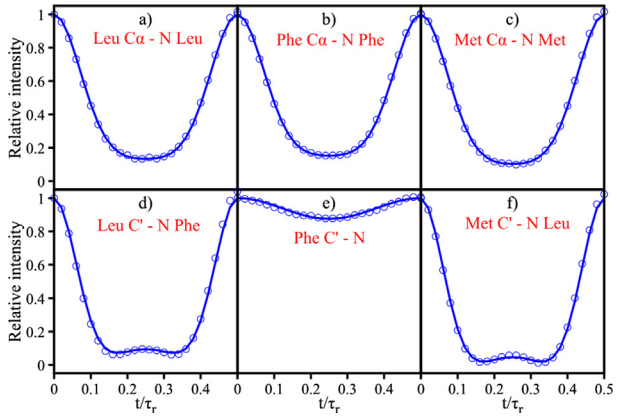

Fig. 3. Experimental data from all the one-bond C-N couplings of MLF tripeptide with a recoupling duration of 30 rotor periods at a MAS-frequency of 18.181 kHz (1650 μs).

amino-acid residues of MLF. We can clearly see from Fig. 3 that C′-N coupling is stronger than C_α-N due to the presence of partial double-bond character. The estimates of the coupling and distance measured here are compared with the X-ray data in Table 1[26].

Systematic deviations from the theoretical DIPSHIFT (the original and new implementations) as well as REDOR dephasing profiles are seen with methods of preparation of I-spin polarisation as shown in Fig. 4a. These deviations can be reproduced in simulations for the proposed and the original DIPSHIFT sequences (Fig. 4b,c), and are attributable to the crystallite-dependencies of the preparation period. Similar deviations were reported by Saalwachter and co-workers in the context of the DIPSHIFT experiment [27]. In all implementations of DIPSHIFT and REDOR, we have used an additional parameter that adjusts for these systematic deviations in intensity to fit these data precisely. The total magnitude of these deviations in all cases of practical interest is ∼10% or lower, but this value can increase to >15% when very short contact times are used. The RODEO-DIPSHIFT experiment compensates for this by using a dedicated period to redistribute magnetization evenly over the entire powder using RODEO-CP [28] at very slow MAS frequencies. Whether REDOR and DIPSHIFT can be further modified to compensate for this effect for applications involing moderate or fast MAS frequencies is an open question. Note that these deviations are likely to be substantially smaller when INEPT is used to transfer polarisation in solids, especially at fast MAS frequencies, as the isotropic part of the scalar coupling is the major contributor in this step.

The DIPSHIFT experiment is, under the conditions described here, the method of choice to measure dipole-dipole couplings irrespective of their strength. The major reason for this is that REDOR relies on comparing a dephasing profile (termed S) to a control sequence, which is effectively a T_2' profile (termed S_0) [8]. The control for the DIPSHIFT profile lies in its initial and final points which are a part of the sequence. Thus, in practice, DIPSHIFT experiments are likely to require lesser time than REDOR experiments. However, when the S_0 profiles can be approximated with a mono-exponentially decaying function, one can reduce the number of points along the S_0 profile in REDOR as well, and the time difference between the two experiments will be minimal. The constant-time nature of DIPSHIFT also means that no specific precautions need to be taken to ensure constant duty cycles during the course of an experiment. The REDOR dephasing profile can only be sampled at the end of 2 rotor-periods, i.e. stroboscopically. The sampling in a DIPSHIFT experiment is limited only by the time resolution of the hardware. On our spectrometer, a pulse or a delay can be defined in units of 50 ns, which is also the time increment in which τ_0 can be changed. This means that at 20 kHz MAS ($\tau_r = 50$ μs), as many as 500 points can be obtained in the DIPSHIFT dephasing profile without increasing the RF requirements. Practically, far fewer points are required to accurately fit a dephasing profile, and we have chosen to collect 26 points in our experiments. In samples with limited signal-to-noise ratios, the number of points to sample can be even lower, and these points should be chosen such that the scaling factor for each of them is different. For both of these experiments, the T_2' values of the resonances being monitored dictate the lower limit of the dipole-dipole coupling that can be measured. It may be advantageous to use slow-moderate MAS frequencies whilst measuring weak couplings (up to 5 kHz) which avoids the use of large n values (corresponding to $2n\pi$ pulses). We note that for measuring small dipole-dipole couplings, symmetry-based recoupling sequences such as rPDLF [29] or DROSS [30] can also be good alternatives to REDOR and DIPSHIFT.

In cases where the complexity of motion cannot be characterised by a single order parameter, an asymmetric dipolar tensor

Table 1
Dipole-dipole couplings measured from DIPSHIFT experiments on MLF and a comparison with the X-ray crystallography data on f-MLF-OMe [26]. Error of 1 pm in distance corresponds to 15 Hz in dipole-dipole coupling constant.

Spin-pair	X-ray (pm)	SSNMR at 18.2 kHz		SSNMR at 62.5 kHz			
		b_{CN} (Hz)	Dist. (pm)	b_{CN} (Hz)	Dist. (pm)	b_{CN} (Hz)	Dist. (pm)
Leu C_α-N Leu	146	958	147	960	147	960	147
Phe C_α-N Phe	147	962	147	960	147	960	147
Met C_α-N Met	146	954	147	960	147	960	147
Leu C′-N Phe	133	1178	137	1180	137	1180	137
Met C′-N Leu	134	1188	137	1160	137	1120	140
Phe C′-N Phe	245	230	237	240	234	260	228

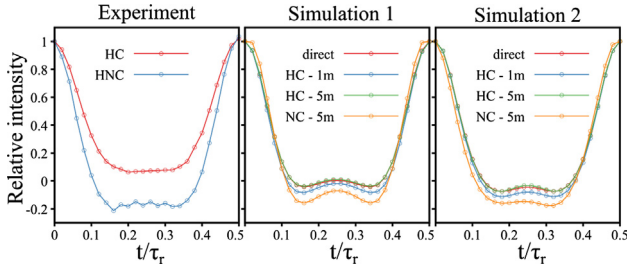

Fig. 4. Deviations arising in the original and the proposed DIPSHIFT experiments due to the method of preparation of magnetisation. (a) Experimental data from MLF sample at a MAS of 18.18 kHz with magnetisation prepared by cross-polarisation from ^{1}H to ^{13}C (red) with a mixing period of 2 ms ramp; ^{1}H to ^{15}N (blue) with a mixing period of 1.5 ms (linear ramp) followed by ^{15}N to ^{13}C mixing period of 6.2 ms (tangential ramp). Ramp pulse with an amplitude variation of 70–100 and tangent pulse with 80–100 percent were used. (b, c) Simulations demonstrating the effect with various preparation methods for magnetisation for the proposed DIPSHIFT sequence (b) and the original DIPSHIFT sequence (c). The label 'direct' denotes that the initial state is C_x, HC-1 m and HC-5 m denote that the initial state was prepared by a cross-polarisation transfer from ^{1}H to C with a contact time of 1 ms and 5 ms, respectively. NC-5 m denotes that the initial state was prepared via a double-CP experiment with ^{1}H to ^{13}C contact time of 5 ms and ^{15}N to ^{13}C contact time of 5 ms. Ideal π pulses were used in simulations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

can be used to characterize motion in rigidly connected nuclei [31]. REDOR was shown to be sensitive to this asymmetry parameter and exploited to understand motion by biomolecules by Schanda and co-workers [32]. The DIPSHIFT and REDOR experiments reported here are equally sensitive to the asymmetry parameter that describes such a motion. This parameter is encoded in the deviations from the expected intensity of each point along the dephasing curve when compared to the case with no asymmetry. As seen in Supporting Information Fig. 1, it is best to sample the initial part of the build-up curve to measure the dipole-dipole coupling in both DIPSHIFT and REDOR, whereas for information on the asymmetry parameter, the points around the second oscillation must be sampled in both these experiments.

The application of DIPSHIFT at fast MAS frequencies follows directly from the discussion above. Fig. 5 shows the application of DIPSHIFT to measure ^{13}C-^{1}H dipole-dipole couplings at a MAS frequency of 60 kHz in a sample of ^{13}C-labelled alanine that is deuterated at the Hβ and H_N positions. Even at this spinning frequency, 26 points can be comfortable recorded, without the use of prohibitively high RF amplitudes. The estimated dipole-dipole couplings match the ones expected based on the known single-bond distance in alanine. We can also measure the comparatively weak ^{13}C-^{15}N couplings in MLF at this MAS frequency, as shown in Fig. 6. The data are in close agreement with those recorded at slow-moderate MAS frequencies. These experiments can be easily adapted into sequences that utilise ^{1}H detection, and will be of immediate use for measuring dynamics in proteins at these MAS frequencies.

3. Simulations and experimental details

3.1. Numerical simulations

All the simulations were performed with SIMPSON 4.2.1 [33,34]. ^{13}C-^{15}N or ^{13}C-^{1}H two-spin systems were used for the calculations described in this article. Powder averaging was performed using the REPULSION scheme [35] with 320 crystallites and 64 gamma angles. RF amplitude and other pulse sequence parameters were kept the same as those used in the experiments, unless stated otherwise. For fitting the dipole-dipolar couplings, a grid of recoupling profiles was generated by varying the coupling strength from 200 Hz to 1300 Hz for ^{13}C-^{15}N and 18–22 kHz for ^{13}C-^{1}H, at the appropriate MAS frequencies. The intensities in the simulated profiles were multiplied with a correction factor c, ranging from 0.85 to 1.15. The deviations in the DIPSHIFT and REDOR profiles increase with increasing dephasing and hence, Eq. 6 was used to scale the intensities for each point. This gives a correction for intensity that is proportional to amount of dephasing. The complete 2-D grid with varying dipole-dipole coupling strength and intensity correction factor was used to fit the experimental profiles and the best fit based on RMSD is reported.

$$Int_{corr}(t1) = (Int(t1) - 1.0) * c + 1.0 \quad (6)$$

3.2. Experimental details

Experiments were performed on U-^{13}C-^{15}N-alanine and U-^{13}C-^{15}-MLF tripeptide samples with 2.5 mm Trigamma MAS probe on 700 MHz (^{1}H Larmor frequency) Bruker Avance-III spectrometer. The samples were obtained from Cambridge isotopes and were used without any further modification. Experiments were implemented with a recycle delay of 3.0 s and 32 scans for signal averaging. Uninterrupted XY-8 phase-cycling was applied on ^{15}N channel over the complete evolution period and EXOR-CYCLE was used on the ^{13}C channel for detection [36]. Note that the central π pulse on the channel subjected to REDOR π pulses was included as a part of the XY8-cycle to avoid large stretches of uncompensated π pulses. Experiments on alanine were performed at a MAS frequency of 20 kHz with the recoupling ^{15}N π pulse duration of 9.8 μs and central π pulse duration of 9.8 μs. t_1 was varied in the steps of 1.0 μs over a duration of 25 μs (0.5 τ_r) to sample 26 points. Experiments on MLF were performed at a MAS frequency 18.181 kHz corresponding to a τ_r of 55 μs and 26 points were sampled with steps of 1.1 μs. Cross-polarisation parameters were individually optimised to ensure maximum transfer in both C_α and C′ while measuring distances on C_α-N and C′-N respectively. For all the experiments, 90 kHz rCW^{ApA} decoupling was applied on ^{1}H during acquisition and 90 kHz CW decoupling was applied during the DIPSHIFT and REDOR recoupling blocks [37]. Experiments at 62.5 kHz MAS frequency were performed with the same pulse-sequence on deuterated alanine (with only Hα) and f-MLF samples. For ^{13}C-^{1}H experiments, π pulses of

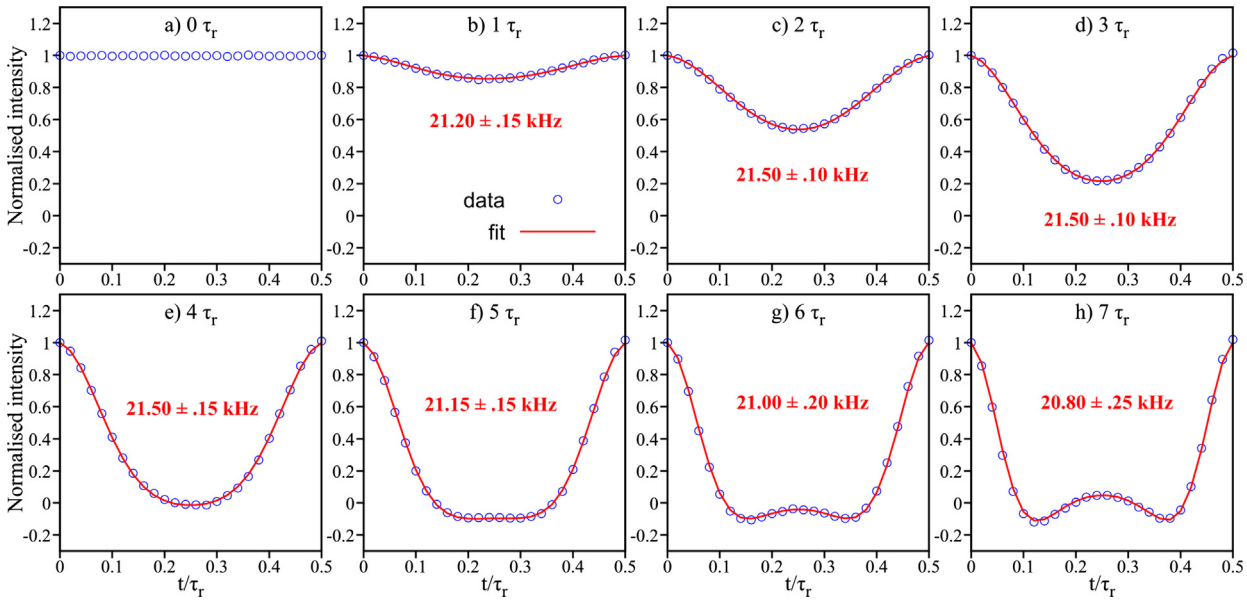

Fig. 5. DIPSHIFT experiments recorded at an MAS frequency of 62.5 kHz to measure Cα-Hα distance in a sample of alanine which was deuterated at the Hβ and H_N positions. (a–h) DIPSHIFT experiments with increasing duration of the recoupling block for the same sample, resulting in a larger dephasing. For a 1 bond ^{13}C-^{1}H dipole coupling, a dephasing duration of ∼6–7 rotor periods gives the typical 'W'-shaped DIPSHIFT profile.

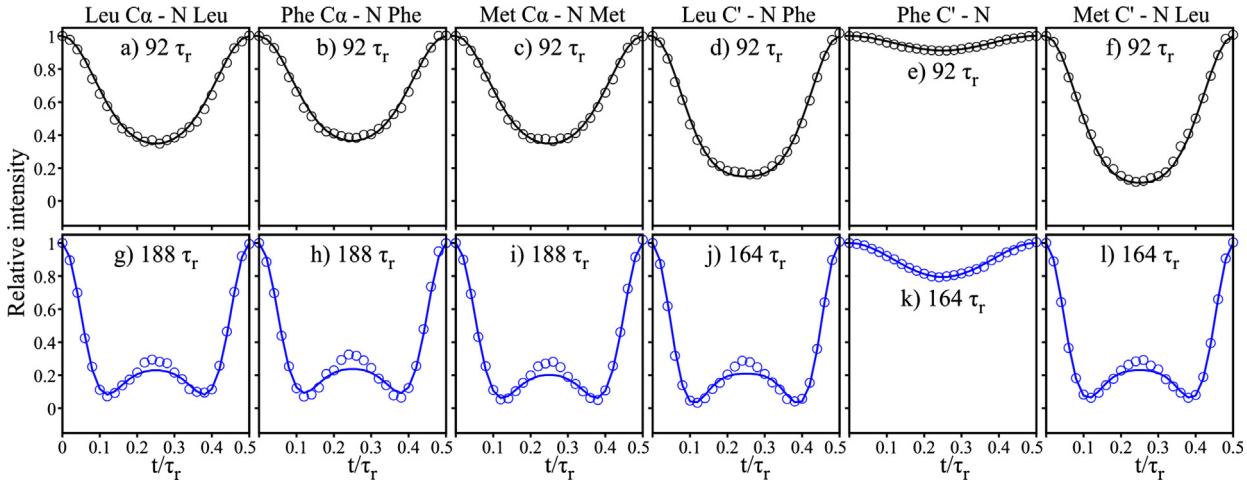

Fig. 6. DIPSHIFT experiments recorded at a MAS frequency of 62.5 kHz to measure ^{13}C-^{15}N distances in a sample of f-MLF. Cα-N and C′-N distances for a recoupling duration of (a–f) 92 τ_r (1472 μs), (g–i) 188 τ_r (3008 μs) for Cα-N and (j–l) 164 τ_r (2624 μs) for C′-N.

200 kHz on ^{1}H and 100 kHz on ^{13}C were applied during recoupling and rCW^{ApA} decoupling with rf amplitude of 10 kHz with a cycle time of 384 μs (24 τ_r) was applied during direct detection [38]. For experiments on ^{13}C-^{15}N second order cross-polarisation with ^{1}H rf amplitude of 40 kHz and ^{13}C rf amplitude of 25 kHz and rCW^{ApA} decoupling of 10 kHz for recoupling and direct detection were used [39]. The π pulse duration of 6.6 μs on ^{13}C and ^{15}N were used for recoupling pulses with a recycle delay of 5.0 s. The pulse-sequence used for measuring ^{13}C-^{1}H distances is provided in the Supporting Information.

All experimental data were processed with NMRPipe and intensities were obtained by integrating the area under the respective peak [40]. Figures were made using Matplotlib 2.2, Gnuplot 5.0, and Inkscape 0.92.

4. Conclusion

We have shown here that the DIPSHIFT and REDOR experiments are alternate realisations of the same experimental scheme. In theory, these two experiments are equivalent, i.e. they are described by the same effective Hamiltonian and have the same dependence on experimental parameters. The difference is only in the choice of the experimental variable which is varied; in the case of REDOR, it is the number of recoupling blocks, whilst in the case of DIPSHIFT, it is the position of a π pulse with respect to the start of the dipolar evolution period. Practically, the DIPSHIFT experiment is expected to be the method of choice to measure dipole-dipole couplings irrespective of their strength, as it is expected to take less time due to the in-built control sequence. Our implementation of this sequence also allows the use of DIPSHIFT with recoupling over an arbitrarily large number of recoupling blocks, limited only by the T_2' of the resonance that is monitored. This in turn allowed us to record the relatively small N-Cα and N-C′ couplings (∼1 kHz) using DIPSHIFT at a moderate MAS frequency of 20 kHz and below. This implementation is unlikely to result in differences in the estimated dipole-dipole couplings values as the fitting routines use data-sets simulated using the appropriate pulse sequences. It will, however, reduce RF

requiements as well as allow frequency-selective DIPSHIFT experiments to be recorded in a manner analogous to the frequency-selective REDOR experiment.

A logical extension of this argument is the implementation of the proposed DIPSHIFT at fast MAS frequencies, which was demonstrated for the estimation of ^{13}C-^{1}H and ^{13}C-^{15}N dipole-dipole couplings at a MAS frequency of 62.5 kHz. We expect these sequences to be of immediate utility in determining motion and order-parameters, as well as distances at fast MAS frequencies. However, we note that under fast MAS frequencies, it becomes experimentally demanding for both REDOR and DIPSHIFT to measure weak couplings (measuring a dipole-dipole-dipole coupling of 1 kHz would require a recoupling duration of about 2.5 ms corresponding to about 160 rotor periods at 62.5 kHz MAS and thus, requiring 320 π pulses with high RF amplitudes). Further, measuring strong couplings, like one-bond ^{15}N-^{1}H and ^{13}C-^{1}H in fully protonated solids, with these sequences, brings with them a litany of issues related to the application of homonuclear decoupling pulse sequences on ^{1}H and the placement of π pulses with respect to them. These and other issues will be dealt with in a separate article, currently under preparation.

Declaration of Competing Interest

There are no conflicts to declare.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jmr.2019.07.050.

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