

## Communication

# Time displacement rotational echo double resonance: Heteronuclear dipolar recoupling with suppression of homonuclear interaction under fast magic-angle spinning

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## ABSTRACT

We have developed a novel variant of REDOR which is applicable to multiple-spin systems without proton decoupling. The pulse sequence is constructed based on a systematic time displacement of the  $\pi$  pulses of the conventional REDOR sequence. This so-called time displacement REDOR (td-REDOR) is insensitive to the effect of homonuclear dipole–dipole interaction when the higher order effects are negligible. The validity of td-REDOR has been verified experimentally by the P-31{C-13} measurements on glyphosate at a spinning frequency of 25 kHz. The experimental dephasing curve is in favorable agreement with the simulation data without considering the homonuclear dipole–dipole interactions.

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## 1. Introduction

Solid-state NMR spectroscopy has been well recognized as a powerful technique for the structural elucidation of biological solids [1,2]. Under the high-resolution condition provided by magic-angle spinning (MAS), recoupling sequences are used to restore the desirable anisotropic interactions for structural determination [3,4]. In particular, many heteronuclear dipole–dipole recoupling sequences have been developed to probe the structural information in biological samples, such as internuclear distances and dihedral angles. The most successful pulse sequence to date for this purpose is the rotational echo double resonance (REDOR) technique [5,6]. REDOR has the advantages that it is very robust with respect to pulse imperfection and that the amplitude of the corresponding average Hamiltonian is relatively large [7]. Recently, REDOR has been applied in the regime of fast spinning for better resolution and sensitivity [8–10]. To date, the version of REDOR proposed by Anderson et al., where the rotor synchronized pulses are alternating between the dephasing and observe channels, remains to be the method of choice for the study of biological samples [11]. However, the rotor-synchronized  $\pi$  pulses in each channel may recouple the homonuclear dipole–dipole interactions [12], which is detrimental for the study of uniformly labeled samples. Consequently, several pulse sequences have been developed based on the pulse symmetry rules [13–15] so that the heteronu-

clear dipolar recoupling can be achieved with active suppression of homonuclear dipole–dipole interactions [16–24]. However, they are not as prevailing as REDOR at the moderate MAS regime because of the stringent requirement in proton decoupling [12,25].

As inspired by the time displacement symmetry discussed in the technique of PITHIRDS [26], we demonstrate in this work that the suppression of homonuclear dipole–dipole interaction can be achieved in REDOR by time displacements of the  $\pi$  pulses. This novel pulse sequence is referred to as the time displacement REDOR (td-REDOR) in the subsequent discussion. Simulation and experimental data show that the homonuclear dipolar interaction is suppressed in td-REDOR. The experimental dephasing curve obtained for glyphosate ( $\text{HOOC}(\text{CH}_2)\text{NH}(\text{CH}_2)\text{PO}_3\text{H}_2$ ) is in favorable agreement with the simulation based on its X-ray crystal structure [27].

## 2. Experimental method

[2- $^{13}\text{C}$ ,  $^{15}\text{N}$ ] glyphosate and [U- $^{13}\text{C}$ ,  $^{15}\text{N}$ ]-L-alanine were used as received from Isotec. All NMR experiments were carried out at  $^{31}\text{P}$ ,  $^{13}\text{C}$ , and  $^1\text{H}$  frequencies of 121.5, 75.5, and 300.1 MHz, respectively, on a Bruker DSX300 NMR spectrometer equipped with a commercial 2.5-mm probe. The measurements were carried out at ambient temperature. The sample was confined to the middle one-third of the rotor volume using Teflon spacers. The condition of MAS was carried out at a frequency of 25 kHz and its variation was limited to  $\pm 2$  Hz using a commercial pneumatic control unit (Bruker, MAS II). During the contact time (2.5 ms) of the cross polarization (CP), the  $^1\text{H}$  rf field was set to 50 kHz and that of  $^{31}\text{P}$

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was adiabatically ramped through the Hartmann–Hahn matching condition [28,29]. Recycle delay was set to 4 s. During the REDOR recoupling period, the  $^{31}\text{P}$  and  $^{13}\text{C}$   $\pi$  pulses were set to 4 and 6.6  $\mu\text{s}$ , respectively. The tachometer signals for pulse sequence synchronization were filtered with a home-built phase-locked loop circuit. The rf field of XiX proton decoupling was set to 100 kHz during the acquisition period [30]. Typically, for each spectrum of glyphosate, a total of 32 transients were accumulated.

Numerical simulations were carried out using SPINEVOLUTION (version 3.4.2) [31]. The maximum time step over which the Hamiltonian is approximated to be time-independent was set to 1  $\mu\text{s}$ . Typically, a powder averaging scheme containing 320 REPULSION angles ( $\alpha$  and  $\beta$ ) [32] and 18  $\gamma$  angles was chosen. Relaxation effects were ignored. The geometry and NMR parameters of the spin systems were set with reference to glyphosate [27] and L-alanine [33].

### 3. Results and discussion

The td-REDOR consists of three fundamental blocks, of which each block comprises a rotor-synchronized  $\pi$  pulse every rotor period with different time displacements with respect to the rotor period. The average Hamiltonian affected by the time displacement has been obtained by Tycko [26]:

$$\bar{H} = R(M\tau_R - \tau, 0)e^{im(\gamma + \omega_R\tau)}\bar{H}R(M\tau_R - \tau, 0)^{-1} \quad (1)$$

where  $\bar{H}$  is the average Hamiltonian without the time displacement,  $\tau$  is the time displacement,  $R(t, t')$  is the net rotation made by the pulse sequence from time  $t$  to  $t'$ ,  $M\tau_R$  is the period of the pulse sequence, and  $\bar{H}$  is the resultant average Hamiltonian after the time displacement. Previously, it has been shown that proton decoupling during the REDOR period could be omitted for short dephasing time under the conditions of strong rf fields and fast spinning [34]. Fig. 1 shows the pulse sequences used in this study, where proton decoupling is not applied during the REDOR period. The REDOR signal ( $S$ ) is generated by the sequence shown in Fig. 1a, whereas the reference signal ( $S_0$ ) is obtained by eliminating the rf pulses in the dephasing channel (Fig. 1b). For the concatenated pulse blocks A, B, and C of the observe ( $S$ ) and dephasing channels ( $I$ ), the time displacements are  $(0, \tau_R/3, 2\tau_R/3)$  and  $(\tau_R/2, 5\tau_R/6, \tau_R/6)$ , respectively. Note that the third time displacement of the  $I$  channel is obtained by calculating the shifting of the “second”  $\pi$  pulse in block C:  $3\tau_R/6$ .

$2 + 2\tau_R/3 - 2\tau_R = \tau_R/6$ . As a result, the average Hamiltonians of the homonuclear dipolar interaction for both channels become nullified to the lowest order:

$$\bar{H}_{II} = \bar{H}_{SS} = \frac{\bar{H}_A + \bar{H}_B + \bar{H}_C}{3} = 0 \quad (2)$$

because

$$e^{im\gamma} + e^{im(\gamma+2\pi/3)} + e^{im(\gamma+4\pi/3)} = 0 \quad (3)$$

for the observe channel and

$$e^{im(\gamma+\pi)} + e^{im(\gamma+5\pi/3)} + e^{im(\gamma+\pi/3)} = 0 \quad (4)$$

for the dephasing channel. On the other hand, because the  $\pi$  pulses of the two channels are interchangeable for REDOR-like sequences, the time displacements in td-REDOR may be taken as 0,  $\tau_R/3$ , and  $\tau_R/6$  for the heteronuclear dipolar interaction (Fig. S1 of Supporting Information). Consequently, one can immediately derive that the average Hamiltonian for the heteronuclear dipolar interaction will not be zero because

$$e^{im\gamma} + e^{im(\gamma+2\pi/3)} + e^{im(\gamma+\pi/3)} \neq 0 \quad (5)$$

To explicitly derive the average Hamiltonian for td-REDOR, the high-field truncated Hamiltonian for the heteronuclear dipolar interaction between spins  $I$  and  $S$  under MAS is calculated as:

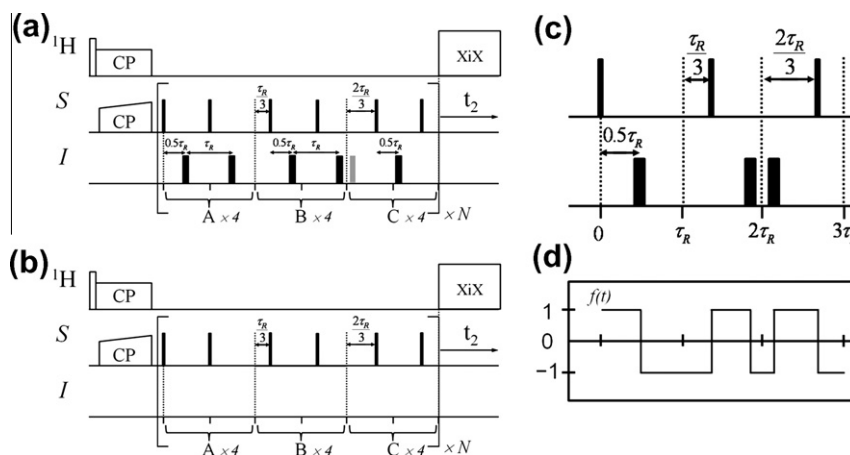
$$H_{IS}(t) = -\frac{1}{2}b_{IS}\{\sin^2\beta\cos[2(\gamma + \omega_R t)] - \sqrt{2}\sin 2\beta\cos(\gamma + \omega_R t)\}2I_SZ \quad (6)$$

where the Euler angles  $\beta$  and  $\gamma$  delineate the relative orientation of the principal axis of the dipole vector and the rotor-fixed frame,  $b_{IS}$  is the dipolar coupling constant which depends on the  $I$ – $S$  distance and gyromagnetic ratios of  $I$  and  $S$  spins:

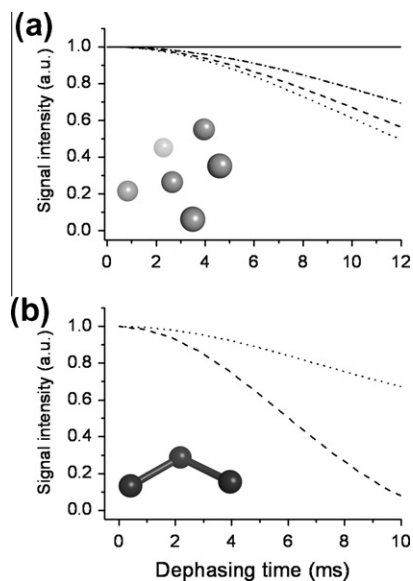
$$b_{IS} = -\left(\frac{\mu_0}{4\pi}\right)\frac{\gamma_I\gamma_S\hbar}{r_{IS}^3} \quad (7)$$

For a  $\pi$ -train sequence like REDOR with ideal-pulse approximation, the time dependence of the Hamiltonian can be expressed as [8]:

$$\tilde{H}_{IS}(t) = f(t) \cdot H_{IS}(t) \quad (8)$$



**Fig. 1.** Pulse sequences used in this study. The corresponding signals measured by the pulse sequences (a) and (b) are denoted as  $S$  and  $S_0$ , respectively. The normalized difference signals,  $(S_0 - S)/S_0$ , as a function of the dephasing time constitute the dephasing curve. The sequences in (a) and (b) would reduce to those for regular REDOR when the time displacements are removed. The open rectangles preceding the CP periods denote  $\pi/2$  pulses. All the filled rectangles represent hard  $\pi$  pulses. The grayish rectangle represents the shifted  $\pi$  pulse, which is positioned at  $\tau_R/6$  with respect to the initial time point of the block C. In our instrumental setup, the  $\pi$  pulses in the  $^{13}\text{C}$  channel are longer than those in the  $^{31}\text{P}$  channel. Phase cycling of the  $\pi$  pulses follows the XY-8 scheme in each block [36]. (c) The simplest version of td-REDOR with a cycle period equal to  $3\tau_R$ . (d) The modulation of  $f(t)$  by the pulse sequence in (c) as discussed in the main text.



**Fig. 2.** Numerical simulations of the  $S_0$  signals calculated for td-REDOR. The geometries were taken from the crystal structures of glyphosate and L-alanine. Only isotropic chemical shifts and the homonuclear dipole-dipole interactions of the spin systems were taken into consideration. Other simulation parameters were matched to the experimental settings. (a) Spin systems containing 2–6 phosphorus nuclei. The largest  $^{31}\text{P}$ - $^{31}\text{P}$  dipolar coupling constant is 295 Hz. The solid line denotes the  $S_0$  signal of td-REDOR calculated for six phosphorus spins. Other lines correspond to the  $S_0$  signals of REDOR (i.e. without time displacement): (...) 6 P; (---) 4 P; (----) 2 P. All  $^{31}\text{P}$  chemical shifts were set to 0 ppm. (b) Spin system of  $\text{C}'\text{-C}^\alpha\text{-C}^\beta$ . The dashed and dotted lines represent the  $S_0$  signals of  $\text{C}^\alpha$  and  $\text{C}^\beta$ , respectively. The chemical shifts were set to 180, 50, 20 ppm for  $\text{C}'$ ,  $\text{C}^\alpha$  and  $\text{C}^\beta$ , respectively. The transmitter frequency was put in the middle of the chemical shifts of  $\text{C}^\alpha$  and  $\text{C}^\beta$ .

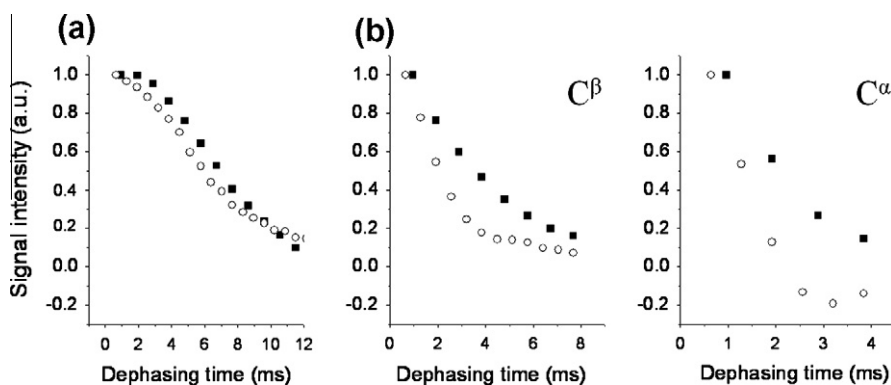
where  $f(t)$  toggles between  $\pm 1$  by the ideal  $\pi$  pulses applied in both channels. Hence, the lowest-order average Hamiltonian is obtained as:

$$\overline{\overline{H}}_{IS} = \frac{1}{\tau_c} \int_0^{\tau_c} \tilde{H}_{IS}(t) dt \quad (9)$$

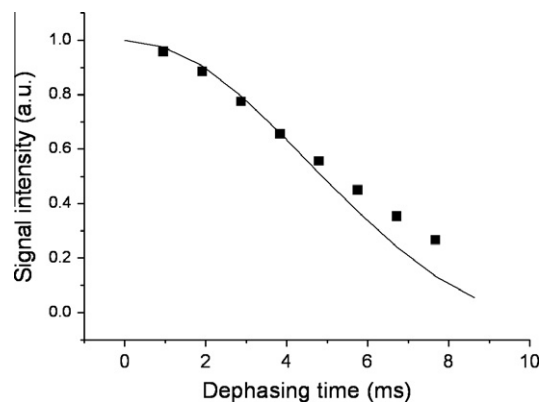
For the simplest version of td-REDOR shown in Fig. 1c, where the cyclic period  $\tau_c$  is equal to  $3\tau_R$ , one can explicitly obtain the lowest-order average Hamiltonian as:

$$\overline{\overline{H}}_{IS} = -\sqrt{2}b_{IS} \sin 2\beta (a \cos \gamma + b \sin \gamma) 2I_z S_z \quad (10)$$

where the numerical values of  $a$  and  $b$  are calculated as 0.368 and 0.212, respectively. Note that the Hamiltonian of td-REDOR has



**Fig. 3.** Experimental  $S_0$  signals acquired for (a) glyphosate and (b) L-alanine using the techniques of td-REDOR (filled squares) and REDOR (open circles). The observe channels in (a) and (b) correspond to  $^{31}\text{P}$  and  $^{13}\text{C}$ , respectively. The rf fields of  $^{31}\text{P}$  and  $^{13}\text{C}$  were both set to 125 kHz. The time resolutions of the td-REDOR and REDOR experiments are different because of the different cycle times.



**Fig. 4.** Experimental data of  $^{31}\text{P}\{^{13}\text{C}\}$  td-REDOR obtained for glyphosate. The experimental data (squares) is in good agreement with the numerical simulation data (solid line) calculated for a spin system containing one  $^{31}\text{P}$  and seven  $^{13}\text{C}$  spins.

the same spin operator as REDOR. Nevertheless, the orientation dependencies are slightly different for the two sequences because REDOR depends on  $\sin \gamma$  only, whereas td-REDOR depends on the linear combination of  $\sin \gamma$  and  $\cos \gamma$ .

To evaluate the practicability of our approach, a series of simulations have been carried out based on the crystal structures of glyphosate and L-alanine. The results obtained for glyphosate are very encouraging, where no dephasing is observed for the  $S_0$  signal over a long period. That is, the suppression of homonuclear dipole-dipole interaction by the strategy of time displacement is well demonstrated (Fig. 2a). If there is no time displacement for the  $\pi$  pulses, the  $S_0$  signal would decay significantly due to the finite-pulse effect [35]. On the other hand, simulations carried out for a three-spin system ( $\text{C}'\text{-C}^\alpha\text{-C}^\beta$ ) indicate that considerable dephasing of the  $S_0$  signals would occur for the carbons (Fig. 2b). The dephasing of the  $S_0$  signal of  $\text{C}^\alpha$  is particularly strong. These observations are not unexpected because of the significant one-bond dipolar couplings among the carbons and the large chemical shift difference between  $\text{C}'$  and  $\text{C}^\alpha$ . The dephasing of the  $S_0$  signals is due to the higher order effects because the extent of dephasing is less severe under the conditions of ultra-fast MAS (Fig. S2 of Supporting Information).

Fig. 3a shows the experimental  $S_0$  signal of td-REDOR acquired for glyphosate. The significant decay of the  $S_0$  signal is in contrast to what predicted in our simulations shown in Fig. 2. Additional simulations show that the decay might be attributed to insufficient proton decoupling (Fig. S3 of Supporting Information). Nevertheless, a 10% of improvement is observed for td-REDOR compared

with the conventional REDOR. Fig. 3b shows the data obtained for  $[U-^{13}C, ^{15}N]$ -L-alanine. As predicted in our simulations, the decay of the  $C^\alpha$  signal is faster than that of  $C^\beta$  because  $C^\alpha$  is more susceptible to inadequate proton decoupling (Fig. S4 of Supporting Information). In any case, the suppression of homonuclear dipolar interaction provided by the cyclic time displacement is well illustrated experimentally. Fig. 4 shows the  $^{31}P\{^{13}C\}$  td-REDOR data acquired for glyphosate. The simulation data, for which only one phosphorus spin is included, agree favorably with the experimental data. Our results suggest that the homonuclear dipole–dipole interaction among the phosphorus spins, where the homonuclear  $^{31}P$  second moment is about  $4.2 \times 10^6/s^2$ , does not interfere significantly with the heteronuclear dephasing curve in the td-REDOR measurements.

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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.2011.10.003](https://doi.org/10.1016/j.jmr.2011.10.003).

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