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Journal of Magnetic Resonance 168 (2004) 358–365
JMR Journal of Magnetic Resonance
www.elsevier.com/locate/jmr

# Compensation for pulse imperfections in rotational-echo double-resonance NMR by composite pulses and EXORCYCLE

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Received 17 January 2004; revised 2 March 2004
Available online 22 April 2004

**Abstract**

We describe a simple method to compensate for pulse-angle errors in rotational-echo double resonance (REDOR) experiments for determining heteronuclear distances in solids. By using composite 180° pulses on the unobserved dephasing spin and EXORCYCLE for the single π pulse on the observed channel, the REDOR curve becomes much less sensitive to pulse-angle errors. Both improvements are demonstrated by experiments on the model compound, $^{15}$N, $^{13}$Cα -labeled *N-t*-BOC-glycine, and are confirmed by numerical simulations. The advantage of EXORCYCLE is also shown analytically using the product operator formalism. The proposed simple schemes compensate for unavoidable pulse-angle errors that arise, for example, from radiofrequency field inhomogeneity. They also make REDOR experiments more accurate and robust for low-sensitivity samples where direct pulse-length calibration is difficult.


*Keywords:* REDOR; Pulse imperfections; Composite pulses; EXORCYCLE; Dipolar recoupling

## 1. Introduction

Rotational-echo double resonance (REDOR) is the most widely used technique for measuring heteronuclear dipolar couplings between two spin-1/2 nuclei in solids under magic angle spinning (MAS) [1]. It recouples the heteronuclear dipolar interaction of interest by a train of π pulses spaced half a rotor period apart applied to either of the two nuclear spins. In one version of REDOR, a single π pulse is applied on the observed channel, X, in the middle of the recoupling period to refocus the chemical shift, while all other π pulses are applied on the unobserved channel, Y. Two experiments are conducted, one with the Y-pulses on ($S$), which induces dipolar dephasing, and the other with the Y-pulses off ($S_0$), which gives a full echo. The ratio $\Delta S/S_0 = (S_0 - S)/S_0$ is a function of the recoupled dipolar interaction $\omega_d$ and the total REDOR mixing time $\tau$ according to $\Delta S/S_0 = 1 - \langle\cos(\omega_d\tau)\rangle$, where $\langle\cdots\rangle$ denotes powder averaging.

To measure long heteronuclear distances or weak couplings, long REDOR mixing times of tens of milliseconds and a hundred or more π pulses are often necessary. The imperfections of the many π pulses accumulate, which can result from inhomogeneity of the radiofrequency (rf) fields, resonance offsets on both channels, and rf amplitude fluctuations over time. In particular, rf inhomogeneity can be quite significant: it has been shown that rf fields at the ends of the coil can be as low as 60% of the rf fields at the center of the coil [2]. The most widely used method to compensate for pulse imperfections is the XY-8 family of phase cycles [3]. This phase cycling scheme compensates for small pulse-length imperfections and resonance offset effects and has been applied both to the unobserved Y-channel pulses and to the X-channel pulses. In the latter case, the two π pulses in each rotor period are alternated between the observed channel and the dephasing channel [4]. Despite the improvement by XY-8, the effect of pulse imperfections remains, manifested as incomplete dephasing $S/S_0$, or equivalently an underestimate of $\Delta S/S_0$. In fact, sometimes an empirical scaling factor $\lambda$ had to be added to the REDOR

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doi:10.1016/j.jmr.2004.03.025

equation $\Delta S/S_0 = \lambda[1 - \langle\cos(\omega_d\tau)\rangle]$ to obtain accurate distances [5]. This amplitude scaling in principle does not interfere with the extraction of the dipolar oscillation frequency, as long as one measures the REDOR curve $\Delta S/S_0$ to its first maximum. However, in applications where long distances are sought for, it is often unrealistic to measure $\Delta S/S_0$ to its first maximum due to sensitivity and hardware limitations [6–8]. Instead, the dipolar dephasing is measured only in the initial linear regime. Without the $\Delta S/S_0$ maximum, the reduction of the initial slope due to pulse imperfections could be misinterpreted as a decreased dipolar coupling, resulting in an overestimate of the distance. The effects of pulse imperfection may be more severe in the single X-pulse REDOR, since the advantage of XY-8 phase cycling is lost. However, the multiple X-pulse REDOR is undesirable in $^{13}$C-detected experiments on uniformly $^{13}$C-labeled molecules, since homonuclear $^{13}$C–$^{13}$C $J$-coupling creates phase twists under many refocusing pulses [9].

Recently, two methods were introduced to correct for pulse-angle errors in REDOR. Chan and Eckert proposed a scheme where instead of turning off the Y-channel π pulses for the $S_0$ experiment, they leave these pulses on. To ensure echo formation, they apply an additional π pulse on the Y-channel simultaneously with the single X π pulse in the middle of the REDOR period [10]. If the Y-channel π pulses are exact, then a full echo is formed. However, if the Y-pulses are not perfect, then the new $S_0'$ is smaller than the true echo $S_0$ obtained without any Y-channel pulses. The difference between $S_0$ and $S_0'$ is then used to correct for the observed dephasing, by adding a scaled $(S_0 - S_0')/S_0$ to $\Delta S/S_0$, to obtain more ideal dephasing. Weldeghiorghis and Schaefer [11] proposed an alternative scheme where the ''dummy'' echo experiment $S_0'$ consists of the same number of Y-pulses as the dephasing experiment S, but the two pulses in each rotor period are centered around the middle of the rotor period, $nT_r/2$. Again, pulse-angle errors manifest as $S_0'$ intensities that deviate from the true echo $S_0$. This correction scheme was demonstrated for both the single X-pulse REDOR experiment and for the alternating π-pulse REDOR.

These correction methods are empirical and have been found to cause either under-compensation or over-compensation of $\Delta S/S_0$ [10,11]. Neither approach compensates completely for pulse-angle errors on the observed X-channel. Moreover, these correction approaches require an additional experiment $S_0'$ to be conducted for a number of mixing times. Given the low-sensitivity of many biological samples, this is quite costly. Thus, a more direct compensation approach that yields more accurate REDOR dephasing without more experiments would be highly desirable. In this work, we show that phase cycling of the single π pulse on the observed channel by the EXORCYCLE and the use of composite pulses on the indirect channel greatly reduce the effect of pulse angle imperfections. We focus on the single X-π-pulse version of REDOR, which is more widely applicable to highly labeled molecules [9] and amenable to perfect compensation regardless of the pulse length. We show by experiment, product operator analysis and numerical simulations the improvement of the accuracy of REDOR dephasing by these two compensation schemes. This simple and robust approach can be readily incorporated into the REDOR pulse sequence without requiring additional experimental time.

## 2. Results and discussion

### 2.1. X-channel pulse-angle error: EXORCYCLE

We consider a REDOR experiment with a single $\beta$-angle pulse on the observed spin $^{13}$C (X) and multiple π-pulses on the unobserved spin $^{15}$N (Y). The effect of the $^{13}$C pulse-angle error on the $^{13}$C–$^{15}$N REDOR curve can be calculated analytically using the product operator formalism. The detected magnetization depends on the phase of the $\beta$-pulse relative to the initial magnetization obtained from CP. We assume that this initial magnetization is along the $x$ direction at the beginning of the REDOR period. For simplicity we assume that the $^{13}$C signal is on resonance so that there is no isotropic shift evolution. Adding resonance offset does not change the conclusion of the REDOR signal $S/S_0$ in the presence of EXORCYCLE (see Eq. (7)). The $^{13}$C magnetization evolves under the recoupled C–N dipolar interaction, $H_d \propto C_zN_z$, for $n/2$ rotor periods, then is subjected to a $^{13}$C pulse with a flip angle of $\beta$. It is reconverted to observable magnetization by the sign-inverted dipolar Hamiltonian $-C_zN_z$ active for the same $n/2$ rotor periods. If the $\beta$-pulse is along the $x$ direction, then the spin evolution can be written as:

$$\begin{aligned} C_x &\xrightarrow{C_zN_z} C_x\langle\cos n\Theta\rangle + 2C_yN_z\langle\sin n\Theta\rangle \\ &\xrightarrow{\beta_xC} C_x\langle\cos n\Theta\rangle + 2C_yN_z\cos\beta\langle\sin n\Theta\rangle \\ &\xrightarrow{-C_zN_z} C_x\langle\cos^2 n\Theta\rangle + C_x\cos\beta\langle\sin^2 n\Theta\rangle \\ &= C_x\left(\frac{1+\langle\cos 2n\Theta\rangle}{2} + \cos\beta\frac{1-\langle\cos 2n\Theta\rangle}{2}\right). \end{aligned} \quad (1)$$

In the above derivation, only those product operator terms that contribute to the final signals are kept. $\Theta$ is the phase accumulated due to the recoupled C–N dipolar interaction for half a rotor period, $\Theta = \int_0^{T_r/2}\omega_d(t)\,dt$. The $^{15}$N-spin dephased $^{13}$C signal, $S^x$, can be simplified as:

$$S^x = C_X\left[\tfrac{1}{2}(1+\cos\beta) + \tfrac{1}{2}(1-\cos\beta)\langle\cos(2n\Theta)\rangle\right]. \quad (2)$$

If the $\beta$ pulse is applied along the $y$-axis, then the observed signal becomes:

$$S^y = C_X\left[\tfrac{1}{2}(1+\cos\beta)+\tfrac{1}{2}(\cos\beta-1)\langle\cos(2n\Theta)\rangle\right]. \quad (3)$$

The REDOR reference signal $S_0$, measured with the $^{15}$N $\pi$ pulses off, corresponds to the dipolar phase $\Theta$ being set to 0. This leads to:

$$S_0^x = C_X\left[\tfrac{1}{2}(1+\cos\beta)+\tfrac{1}{2}(1-\cos\beta)\right] = C_X, \quad (4)$$

$$S_0^y = C_X\left[\tfrac{1}{2}(1+\cos\beta)+\tfrac{1}{2}(\cos\beta-1)\right] = C_X\cos\beta. \quad (5)$$

When the $^{13}$C $\beta$-pulse phase follows the CP phase, the REDOR dephasing $S/S_0$ is:

$$\frac{S}{S_0} = \frac{S^x}{S_0^x} = \tfrac{1}{2}(1+\cos\beta)+\tfrac{1}{2}(1-\cos\beta)\langle\cos(2n\Theta)\rangle. \quad (6)$$

This REDOR dephasing depends on the flip angle $\beta$ of the $^{13}$C pulse and is thus susceptible to flip-angle errors.

In the EXORCYCLE scheme [12], the $^{13}$C $\beta$-pulse is 90° out of phase from the CP spin-lock direction in every other scan. This inverts the initial magnetization, thus the receiver phase is correspondingly inverted in every other scan. The phase-cycled REDOR dephasing, combining Eqs. (2)–(5), is:

$$\frac{S}{S_0} = \frac{S^x - S^y}{S_0^x - S_0^y} = \frac{(1-\cos\beta)\langle\cos 2n\Theta\rangle}{1-\cos\beta} = \langle\cos 2n\Theta\rangle. \quad (7)$$

The dephasing is now independent of the pulse angle $\beta$ and depends only on the dipolar phase $\Theta$. It has the same expression as the ideal REDOR dephasing in the absence of X-pulse imperfection. The only price paid for the incorrect pulse angle is a reduction of the signal intensity by a factor of $(1-\cos\beta)/2$.

Eq. (6) shows that, in the absence of EXORCYCLE, the deviation of $S/S_0$ from the ideal value increases as $\beta$ deviates increasingly from 180°. For example, when $\beta = 150°$, $S/S_0 = 0.067 + 0.933\langle\cos 2n\Theta\rangle$; when $\beta = 90°$, $S/S_0 = 0.5 + 0.5\langle\cos 2n\Theta\rangle$. Since the minimum intensity of the ideal REDOR curve is approximately 0, these indicate that the flip-angle dependent $S/S_0$ curve will be scaled down and shifted up with a new minimum value of approximately $(1+\cos\beta)/2$. Fig. 2 shows the pulse-angle dependence of this $S/S_0$ minimum. Due to this upward shift and the amplitude scaling, the initial decay of the REDOR curve is less steep than the ideal curve. This is particularly detrimental to the measurement of weak couplings, where the actual minimum often occurs at mixing times too long to be practically accessible, and where the initial slope is the main indicator of the dipolar coupling strength.

The analytical prediction of the effect of the observed-channel flip-angle error is confirmed by numerical simulations using SIMPSON. Fig. 3C shows the uncompensated REDOR $S/S_0$ curves for $\beta$ angles of 180°, 150°, and 120°. Indeed, the $^{13}$C pulse-angle errors move the REDOR $S/S_0$ curve upward, but the oscillation frequency remains unchanged. Experimental corroboration of this effect is shown in Fig. 3A. We chose a well

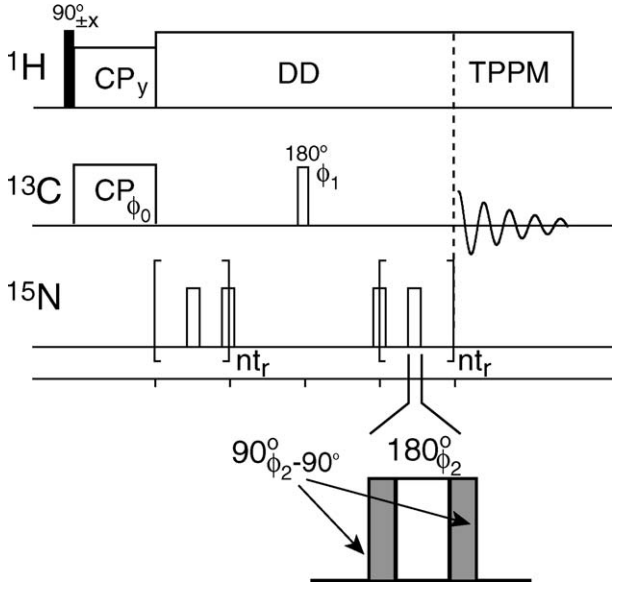


Fig. 1. $^{13}$C-detected C–N REDOR pulse sequence with pulse-angle compensations. For the composite 180° pulses on the $^{15}$N channel, the central 180° pulse was phase-cycled using XY-16, while the phase of the flanking 90° pulses was shifted by 90° from the 180°-pulse phase. $\phi_0 = xxyy\bar{x}\bar{x}\bar{y}\bar{y}$, $\phi_1 = xxyy\bar{x}\bar{x}\bar{y}\bar{y}$, $yy\bar{x}\bar{x}\bar{y}\bar{y}xx$, $\bar{x}\bar{x}\bar{y}\bar{y}xxyy$, $\bar{y}\bar{y}xxyy\bar{x}\bar{x}$, $\phi_2$ is incremented within each scan according to XY-16, and the receiver phase is $x\bar{x}y\bar{y}\bar{x}x\bar{y}y$, $\bar{x}x\bar{y}yx\bar{x}y\bar{y}$.

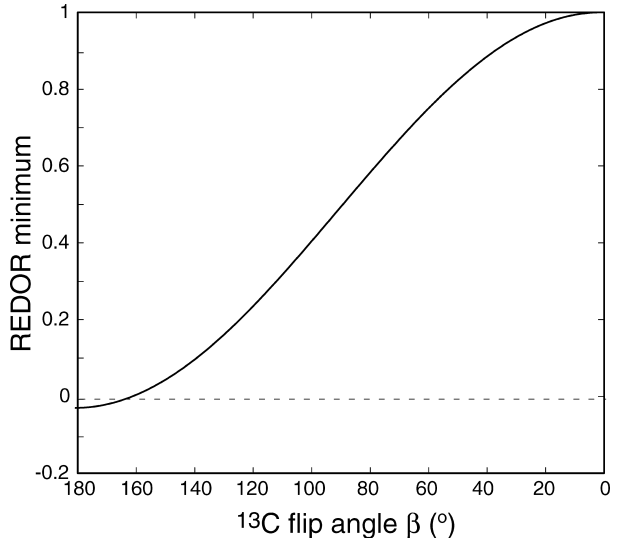


Fig. 2. Dependence of the REDOR $S/S_0$ minimum value on the observed-channel flip angle $\beta$. As $\beta$ increasingly deviates from 180°, the REDOR minimum increases.

center-packed $^{15}$N, $^{13}$C$\alpha$-labeled *N*-*t*-BOC-glycine sample so that the $^{13}$C nutation angle can be precisely calibrated. The one-bond C$\alpha$–N distance gives a dipolar coupling of 950 Hz, which is preserved in all three curves with $^{13}$C $\beta$ angles of 180°, 150°, and 120°. However, the curves for the wrong pulse angles shift up. The slight difference in the degree of vertical shift between the experiment and the simulation most likely results from the

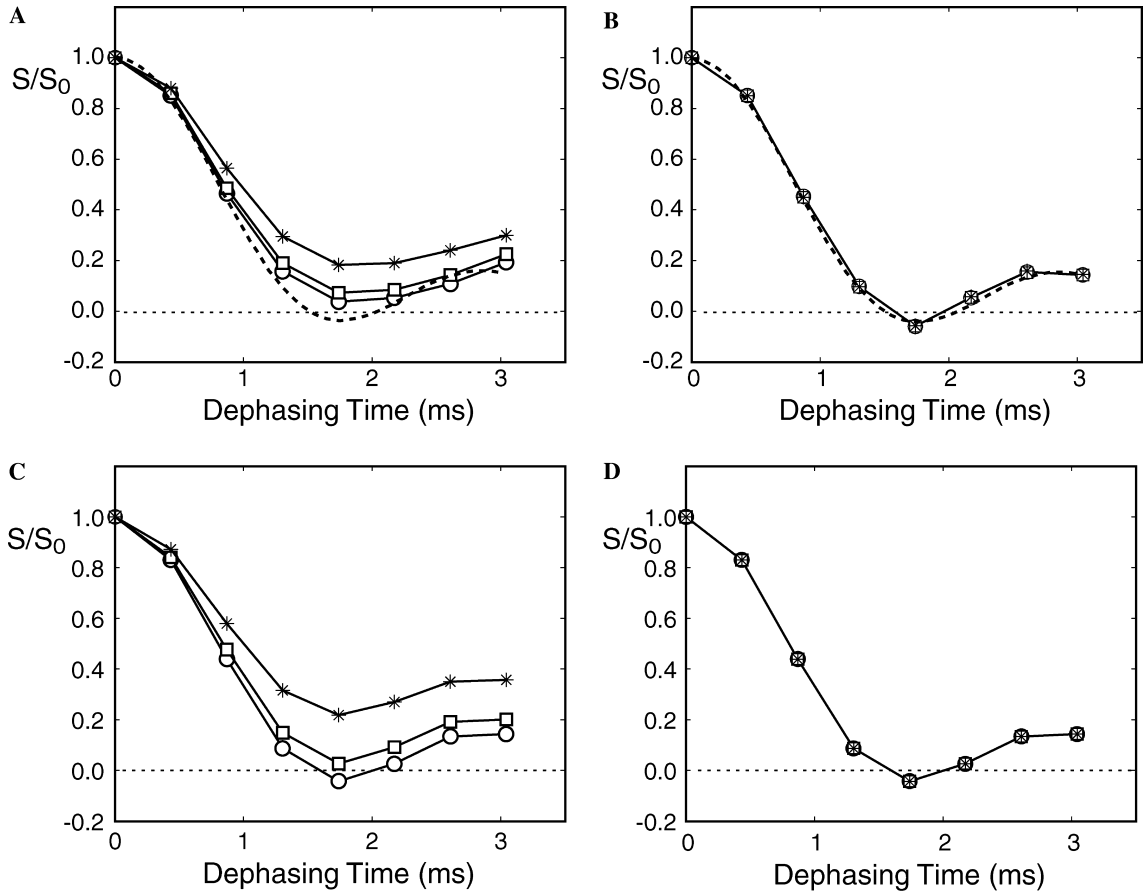


Fig. 3. $^{13}$C-detected C–N REDOR dephasing curves as a function of the $^{13}$C flip angles for $^{13}$Cα, $^{15}$N-labeled *N*-*t*-BOC-Gly. (A,B) Experimental REDOR curves obtained without (A) and with (B) EXORCYCLE on the $^{13}$C channel. The flip angles are 180° (circles), 150° (squares), and 120° (stars). (C,D) SIMPSON simulations of the REDOR curves without (C) and with (D) EXORCYCLE. The dashed curve in (A,B) indicates the ideal REDOR curve for a coupling of 950 Hz and the horizontal dotted line guides the eye for $S/S_0 = 0$.

small degree of rf field inhomogeneity that is present even in this center-packed sample.

Incorporation of the EXORCYCLE completely removed the $\beta$-angle dependence. This is shown both experimentally (Fig. 3B) and by numerical simulations (Fig. 3D). The curves for pulse angles of 180°, 150°, and 120° are indistinguishable within experimental uncertainty.

### 2.2. Y-channel pulse-angle error: composite pulses

We now consider the effect of the indirect-channel pulse-angle error on the REDOR curve. The multiple π pulses on the indirect channel are already phase-cycled according to the XY-16 scheme [3,13]. However, XY-16 does not completely compensate for large pulse-angle errors. Fig. 4A demonstrates experimentally that wrong $^{15}$N pulse angles both lift the REDOR curve and reduce the oscillation frequency. The measured $S/S_0$ minimum values are −0.05, 0.07, and 0.33 for 180°, 150°, and 120° pulses, respectively. Moreover, the apparent coupling strengths decrease with decreasing pulse angles. For exact 180° pulses, the coupling is 950 Hz; but for 150° and 120° pulses, the apparent couplings decrease to 750 and 550 Hz, respectively, if no amplitude scaling is applied in the simulation to correct for the vertical lift of the $S/S_0$ curve. In other words, the measured couplings deviate from the true coupling by as much as 42%. Amplitude scaling in the simulation alleviated the coupling reduction only partially, yielding dipolar couplings of 800 and 650 Hz for the 150° and 120° pulses, respectively.

It is well known that imperfect inversion of *z*-polarization can be compensated by composite pulses of the type $90°_x\ 180°_y\ 90°_x$, where the phase of the flanking π/2 pulses is shifted by 90° from the phase of the central π pulse [14,15]. When we implemented the composite pulses on the $^{15}$N channel, both the frequency and the amplitude of oscillation improved significantly and approached the ideal REDOR curve (Fig. 4B). In fact, the REDOR curve for the 150° composite-pulse experiment is nearly identical to that for the 180° experiment. Again, SIMPSON simulations (Fig. 4D) show good agreement with the experiments.

The use of composite pulses on the dephasing channel has been previously demonstrated for $^{2}$H–$^{13}$C REDOR

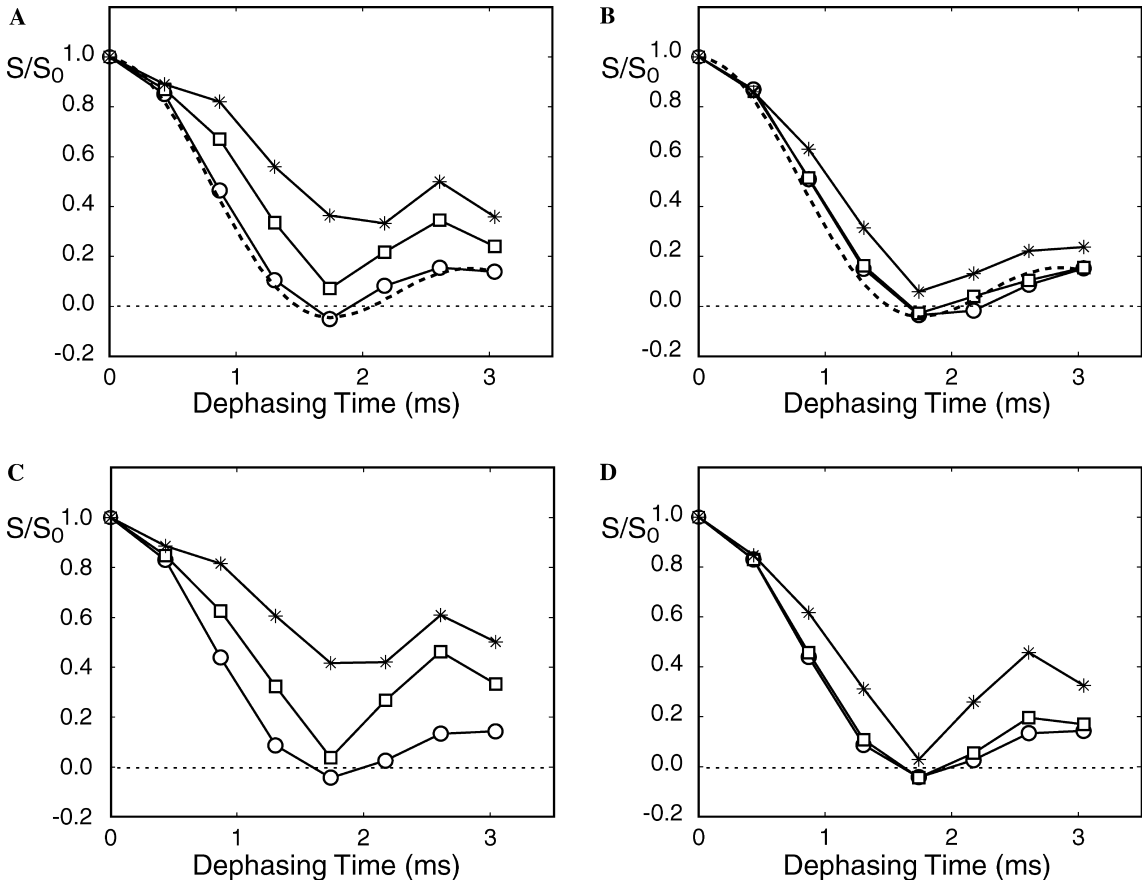


Fig. 4. $^{13}$C-detected C–N REDOR dephasing curves as a function of $^{15}$N flip angles for $^{13}$C$\alpha$, $^{15}$N-labeled *N-t*-BOC-Gly. The flip angles are 180° (circles), 150° (squares), and 120° (stars). (A,B) Experimental REDOR curves obtained without (A) and with (B) $^{15}$N composite pulses. (C,D) SIMPSON simulations without (C) and with (D) composite pulses. The dashed curve in (A,B) indicates the ideal REDOR curve for a coupling of 950 Hz and the horizontal dotted line guides the eye for $S/S_0 = 0$.

experiments [16,17]. There the composite pulses compensated for pulse imperfections resulting from the large $^{2}$H quadrupolar coupling.

### 2.3. Combined $^{13}C$ and $^{15}N$ pulse-angle errors

Since rf inhomogeneity across a fully packed rotor affects both the X- and Y-channels in a given sample, the $^{13}$C and $^{15}$N flip angles would simultaneously deviate from 180°. Fig. 5 demonstrates the effect of simultaneous flip-angle errors on the directly observed $^{13}$C channel and the indirect $^{15}$N channel. In the absence of compensating schemes other than XY-16 phase cycling on the $^{15}$N channel, the imperfections of the REDOR curve are approximately the sum of the effects of individual pulse-angle errors. The REDOR dephasing amplitude is reduced more significantly than for each error source alone and the oscillation frequency decreases (Figs. 5A and C). Incorporation of EXORCYCLE on $^{13}$C and composite pulses on $^{15}$N corrected most of the error down to pulse angles of 120° (Figs. 5B and D). However, with a flip angle of 90° on both channels, even these compensating schemes no longer save the situation.

The observed REDOR imperfections induced by the double-channel pulse-angle errors are consistent with a recent study of the accuracy of REDOR for different sample positions in the rotor [2]. It was observed that when a sample is moved to a position that corresponds to 60% of the rf nutation frequency, the REDOR oscillation frequency decreases to less than 30% of the correct value. In that work, no distinction was made between rf inhomogeneity on the observed channel and the unobserved channel. Thus, the pulse imperfections were the combined result of both channels. A nutation frequency of 60% of the full frequency corresponds to a pulse angle of 108°. This is analogous to our experimental demonstration in Fig. 5A for a flip angle of 120° for both spins (stars). Even with this gross pulse-angle error, the application of EXORCYCLE and composite pulses corrected most of the error in the REDOR curve, as shown in Fig. 5B.

### 2.4. Effect of $^{1}H$ decoupling on composite pulses

The application of composite pulses on the indirect channel increases the fraction of the rotor period when the pulses are on. If the $^{15}$N $\pi$-pulse length is 10 μs, then

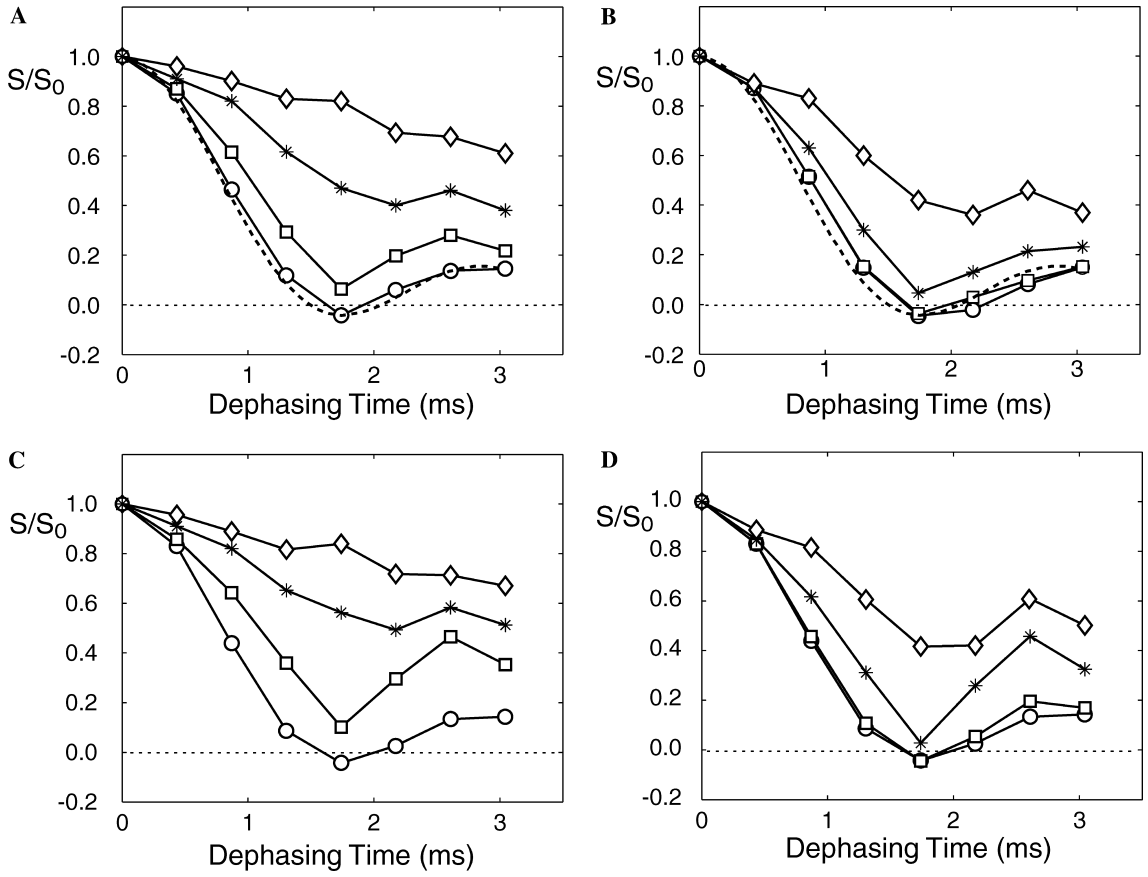


Fig. 5. $^{13}$C-detected C–N REDOR dephasing as a function of simultaneous $^{15}$N and $^{13}$C pulse-angle errors. The pulse angles are 180° (circles), 150° (squares), 120° (stars), and 90° (diamonds). (A) Experimental REDOR curves obtained without $^{15}$N composite pulses nor $^{13}$C EXORCYCLE. (B) Experimental REDOR curves obtained with both $^{15}$N composite pulses and $^{13}$C EXORCYCLE. (C,D) SIMPSON simulations for (A) and (B), respectively. The dashed curve in (A,B) indicates the ideal REDOR curve for a coupling of 950 Hz, and the horizontal dotted line guides the eye for $S/S_0 = 0$.

the total pulse time in each rotor period for the composite-pulse sequence is 40 μs. For spinning speeds below 10 kHz, the rf duty cycle is less than 40%. Previous experiments [18] and simulations [19] have shown that this duty cycle does not cause significant changes in the measured REDOR dipolar coupling.

However, the increased $^{15}$N pulse duration may affect the accuracy of REDOR through its interference with $^{1}$H decoupling if strong $^{1}$H decoupling power is not available. Fig. 6 shows the minimum $S/S_0$ value for the one-bond $^{15}$N–$^{13}$Cα coupling as a function of the ratio of the $^{1}$H and $^{15}$N field strengths, $\zeta = \omega_{1,H}/\omega_{1,N}$. The mixing time was fixed at 1.73 ms. The $^{1}$H decoupling field strength was kept constant at 62 kHz while the $^{15}$N field strength varied from 18.1 to 54.3 kHz. At 1.73 ms, for the one-bond C–N dipolar coupling, the ideal REDOR curve should reach a minimum value of −0.03.

Fig. 6 shows that, regardless of the type of π-pulses applied, the closer the $^{1}$H and $^{15}$N field strengths, the more imperfect or higher the minimum $S/S_0$ value. In other words, the REDOR curve is distorted and shifted up when the $^{1}$H decoupling power is low. Below $\zeta = 1.8$, the non-composite-pulse experiment exhibits lower $S/S_0$ values and thus more ideal REDOR behavior. This is understandable, since when the $^{1}$H decoupling field is weak, residual $^{1}$H–$^{15}$N dipolar coupling that interferes with C–N dipolar recoupling is more severe for the composite-pulse experiment due to its longer pulse overlap. When the field strength ratio exceeds $\zeta = 1.8$, the composite pulses yield more complete dephasing. At $\zeta = 3$, the $S/S_0$ value is about 0, nearly identical to the ideal value. Therefore, strong $^{1}$H decoupling of at least twice the $^{15}$N field strength is required for the $^{15}$N composite-pulse compensation to have a beneficial effect. If we use a 40% duty cycle as the maximum $^{15}$N pulse duration in each rotor period, and require the $^{1}$H decoupling field strength to be at least twice the $^{15}$N field strength, then we arrive at the requirement of $\omega_{1,H} = 10\omega_r$ for the composite-pulse REDOR. This is readily achievable for spinning speeds of 10 kHz or less on standard 4 mm MAS probes.

The observed dependence of REDOR dephasing on the ratio of the $^{1}$H and $^{15}$N power levels differs from a recent study of the effect of $^{1}$H–$^{13}$C dipolar decoupling on REDOR. In that study, the initial REDOR dephasing was found to be relatively insensitive to the $^{1}$H decoupling power relative to the observed channel pulse

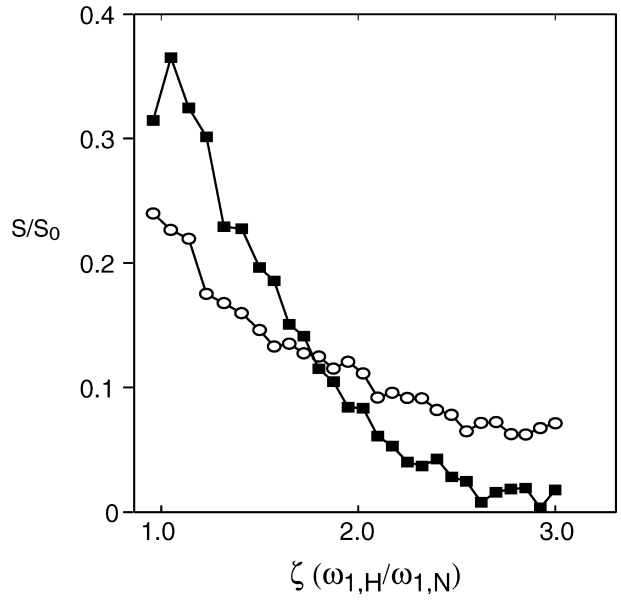


Fig. 6. Effect of $^{1}$H decoupling during REDOR: minimum $S/S_0$ as a function of the ratio of the $^{1}$H and $^{15}$N field strength $\zeta = \omega_{1,H}/\omega_{1,N}$. The ideal value of $S/S_0$ for the chosen REDOR recoupling time of 1.73 ms is −0.03. Open circles: without $^{15}$N composite pulses. Filled squares: with $^{15}$N composite pulses.

field strength [20]. This is understandable, since the X pulses are active in both the $S_0$ and $S$ experiments, so that interference with $^{1}$H decoupling is partially canceled. In contrast, the Y-pulses are active only in the $S$ experiment. Weak $^{1}$H decoupling relative to the Y-pulse field strength reduces the $z$-coherence of the Y-spin, thus affecting the REDOR dephasing $S/S_0$.

## 3. Conclusion

The effect of pulse-angle errors due to rf field inhomogeneity and miscalibration on the REDOR curve differs for the observed and unobserved spins. For the observed spin, this causes an upward lift of the $S/S_0$ curve or a scaling of the $\Delta S/S_0$ amplitude, but the frequency of oscillation is unaffected. For the unobserved spin, the error is manifested as a combination of amplitude scaling and changes in the oscillation frequency. We have demonstrated here that the observed-channel pulse-angle error is completely compensated by applying the EXORCYCLE with respect to the CP pulse phase, while the unobserved-channel pulse-angle error is compensated to a large extent by applying composite pulses of the type $90°_x\ 180°_y\ 90°_x$. These simple schemes compensate for flip angles as small as 120°, but lose effectiveness for larger angle errors.

The pulse-angle compensation schemes recommended here for REDOR are easy to apply and promise to improve the accuracy of the REDOR technique. It should be especially beneficial to low-sensitivity samples where direct pulse length calibration is difficult and sensitivity considerations make it unrealistic to achieve center packing and thus high rf homogeneity.

## 4. Experimental

### 4.1. Sample

$^{13}$Cα, $^{15}$N-labeled *N-t*-BOC glycine was purchased from Cambridge Isotope Laboratory (Andover, MA). The sample was re-crystallized from ethyl acetate. A total of 13 mg of the sample was center-packed into a 4-mm MAS rotor using a Teflon block at the bottom of the rotor.

### 4.2. NMR Experiments

All NMR experiments were carried out on a Bruker DSX-400 spectrometer (Karlsruhe, Germany) operating at 9.4 T (100.70 MHz for $^{13}$C and 40.58 MHz for $^{15}$N), using a triple-resonance 4 mm MAS probe. The pulse sequence for the $^{13}$C-detected C–N REDOR experiment is shown in Fig. 1. It consists of a cross polarization (CP) step from $^{1}$H to $^{13}$C to create transverse $^{13}$C magnetization. The $^{13}$C magnetization evolves under the recoupled C–N dipolar interaction. The recoupling was achieved by applying two 180° pulses per rotor period on the $^{15}$N channel except for the middle of the period, when the 180° pulse was applied on the $^{13}$C channel to refocus the $^{13}$C chemical shift. $^{1}$H continuous-wave decoupling was used during the REDOR period and TPPM decoupling was used during detection. The $S$ and $S_0$ spectra were acquired with the $^{15}$N pulses on and off, respectively. All experiments were carried out at a spinning speed of 4.6 kHz. The REDOR mixing time was incremented rotor-synchronously to a maximum of 3.0 ms. The $^{13}$Cα signal of *N-t*-BOC-glycine was placed on resonance.

For experiments to study $^{13}$C flip-angle effects, $^{15}$N and $^{1}$H field strengths of 34.3 and 71.4 kHz were used, respectively. The correct $^{13}$C 180° pulse length was 14.6 μs. To generate 150° and 120° $^{13}$C pulses, we kept the pulse duration the same but changed the power level. This ensures that the influence of $^{1}$H decoupling is the same among the experiments. For each REDOR experiment, 32 scans were added with a recycle delay of 2.5 s.

The $^{15}$N flip-angle experiments were carried out with $^{13}$C and $^{1}$H field strengths of 18.5 and 71.4 kHz, respectively. Similar to the $^{13}$C flip-angle experiments, we kept the $^{15}$N pulse duration constant but changed the field strengths. The calibrated 180° pulse length was 20.6 μs, corresponding to an rf field of 24.3 kHz. The $^{15}$N power level was then varied to achieve 150° and 120° nutation angles.

For the composite-pulse experiments, each 180° pulse was sandwiched between two 90° pulses of appropriate

phases. For a 180° pulse of phase x (y), the phase of the 90° pulses was −y (x). The middle 180° pulse of the composite pulses was phase-cycled in the XY-16 fashion and the phase of the 90° pulse was correspondingly varied.

The experiments involving simultaneous $^{13}C$ and $^{15}N$ flip-angle errors were conducted under a $^{1}H$ decoupling field of 71.4 kHz. The $^{13}C$ and $^{15}N$ 180° pulse lengths were calibrated for field strengths of 34.3 kHz (14.6 μs) and 24.3 kHz (20.6 μs), respectively. The pulse power was then varied while keeping the pulse length fixed.

### 4.3. Simulations

The REDOR curves for various combinations of pulse-angle errors were simulated using the SIMPSON program [21]. The parameters used for the simulation are the following. The C–N one-bond dipolar coupling is 950 Hz. The common molecular frame is defined with its $z$-axis along the Cα–N bond and the $x$-axis in the peptide plane normal to the $z$-axis. Thus, the Euler angles rotating the C–N vector into the common frame are all 0. The $^{13}C$ chemical shift tensor has an isotropic shift $\delta_{iso}$ of 0, an anisotropy parameter $\delta_{aniso} = 26$ ppm, and an asymmetry parameter $\eta = 0.73$. The Euler angles rotating the Cα chemical shift tensor into the common frame are $\alpha_{PC} = 80°$, $\beta_{PC} = 35°$, and $\gamma_{PC} = 0°$. For the $^{15}N$ chemical shift tensor, $\delta_{iso} = 0$, $\delta_{aniso} = 106$ ppm, and $\eta = 0.27$. The Euler angles are $\alpha_{PC} = -20°$, $\beta_{PC} = 137°$, and $\gamma_{PC} = 0°$ [22–24].

## Acknowledgment

M.H. thanks the Sloan Foundation for a research fellowship. This work is supported by the National Science Foundation (MCB-93398).

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