Homonuclear dipolar recoupling of arbitrary pairs in multi-spin systems under magic angle spinning: A double-frequency-selective ZQ-SEASHORE experiment

Takeshi Kobayashi  
*Ames Laboratory*, takeshi@AMESLAB.GOV

Zhuoran Wang  
*Iowa State University and Ames Laboratory*, zrwang@Iastate.edu

Marek Pruski  
*Iowa State University and Ames Laboratory*, mpruski@Iastate.edu

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Homonuclear dipolar recoupling of arbitrary pairs in multi-spin systems under magic angle spinning: a double-frequency-selective ZQ-SEASHORE experiment

Takeshi Kobayashi,a,* Zhuoran Wang,a,b and Marek Pruski,a,b,*

a U.S. DOE Ames Laboratory, Iowa State University, Ames, Iowa 50011-3020, United States

b Department of Chemistry, Iowa State University, Ames, Iowa 50011-3020, United States

AUTHOR INFORMATION

Corresponding Author

T. Kobayashi: Ames Laboratory, Iowa State University, 229 Spedding Hall, Ames, IA 50011-3020, USA. Phone: +1 515 294 6823. E-mail address: takeshi@iastate.edu.

M. Pruski: Ames Laboratory, Iowa State University, 230 Spedding Hall, Ames, IA 50011-3020, USA. Phone: +1 515 294 2017. E-mail address: mpruski@iastate.edu.
Abstract

We describe a useful method for measuring the internuclear distances within arbitrarily selected pairs of like nuclei in dipolar-coupled multi-spin systems. The method uses a combination of the zero-quantum shift-evolution-assisted selective homonuclear recoupling (ZQ-SEASHORE) technique developed by Hu and Tycko \cite{Hu2009} and double-frequency-selective radio-frequency pulse. The double-frequency-selective pulse inverts polarizations of two spins simultaneously, and thus applications of the method presented here are only limited by the spectral resolution, and not by the number of interacting spins. Our experiments demonstrate the validity of the method and present analytical expressions for the dephasing curve.

KEYWORDS Solid-state NMR, homonuclear recoupling, internuclear distance, multi-spin effect, double-frequency-selective pulse
1. Introduction

Measurements of magnetic dipole-dipole couplings between nuclear spins provide quantitative constraints on internuclear distances which are very useful for determining three-dimensional (3D) structures of molecules and their arrangements, especially in materials that do not have a long-range molecular order and are not amenable to X-ray crystallography, such as polymers [1-3], biomolecules [4-11] and other organic [12-14] and inorganic [15, 16] solids. In particular, the ability to measure arbitrary carbon-carbon internuclear distances can be instrumental in resolving the NMR-based 3D structures of many organic materials [17-27].

The NMR measurement of internuclear distance, or to be exact, the quantification of dipole-dipole coupling, relies on “dipolar recoupling” which restores, at least partially, the dipolar couplings that are averaged out by magic-angle spinning (MAS) [28-39]. For sensitivity reasons, homonuclear dipolar recoupling experiments with low-abundance nuclei such as $^{13}$C and $^{15}$N usually require isotopic labeling; however, in the universally labeled samples the interactions of interest are often obscured by multi-spin effects [35, 40, 41]. Generally, dipole-dipole coupling is quantified by analyzing the build-up or dephasing behaviors of the correlated signals by assuming an isolated two-spin system. However, the two-spin approximation describes only the short-term behavior of the recoupled spins, because for longer recoupling times the buildup curves are damped by interference with couplings to other spins, generation of higher-order coherences and experimental imperfections [40]. Another challenge is the so-called dipolar truncation [38]. When two homonuclear spin pairs share a common spin, their dipolar Hamiltonians do not commute with each other and dipolar truncation is observed, where the stronger coupling to a closer spin significantly attenuates dipolar coupling with the weakly coupled third spin, thereby prohibiting the measurement of structurally important long-range
Selective isotope enrichment can be applied to constrain the multi-spin effects, but this approach is often more time consuming and expensive than uniform labeling.

One of the solutions to the abovementioned dilemma is the use of frequency selective techniques, where the dipole-dipole coupling is reintroduced only within the specific spin pairs of interest [34, 38, 39, 42-45]. For example, Nielsen and co-workers developed the triple oscillating field technique (TOFU) [38], and demonstrated selective dipolar recoupling unperturbed by dipolar truncation, using uniformly $^{13}$C, $^{15}$N-labelled L-alanine and L-threonine samples. The experimentally observed dipolar dephasing curves for directly bonded spins, C$_\alpha$ and carboxyl carbon (C$'$), showed good agreement with the analytical Fresnel curves for isolated spin pairs; however, for spins separated by more than one bond (C$_\beta$–C$'$ and C$_\gamma$–C$'$) the fits were not nearly as accurate. Levitt and co-workers [43], followed by Brown and co-workers [45], used ‘off-magic-angle’ spinning to introduce so-called residual $^{13}$C-$^{13}$C dipolar couplings (RDCs). The RDCs modulated homonuclear $J$-coupling patterns observed in rotor-synchronized spin-echo experiments, allowed for accurate estimation of individual dipolar couplings and the corresponding distances. However, this experiment necessitates the use of switched-angle MAS probe for the resolution and long-range distance sensitivity to be retained. Paravastu and Tycko developed a homonuclear dipolar recoupling method, named shift-evolution-assisted selective homonuclear recoupling (SEASHORE) [34], in which rotor-synchronized periods of broad-band double-quantum (DQ) recoupling are followed by rotor-synchronized periods of chemical shift evolution. Arbitrary $^{13}$C-$^{13}$C pairs can be selected by adjusting the lengths of the chemical evolution periods, as demonstrated by accurate measurements of polarization transfers and internuclear distances between C$_\beta$-C$_\gamma$, C$_\alpha$-C$_\gamma$ and CO-C$_\gamma$ spin pairs in $^{13}$C-labeled L-valine. Practical challenges included experimental setup, achieving pure DQ recoupling at relevant
ranges of MAS frequencies and chemical shifts, and damping of dipolar oscillations due to the imperfect $^1$H decoupling inhomogeneity of radio-frequency (RF) fields or inhomogeneous broadening of $^{13}$C lines. In a subsequent study [44], Hu and Tycko developed a related sequence, termed ZQ-SEASHORE, consisting of alternating periods of zero-quantum (ZQ) homonuclear recoupling (using finite-pulse radio-frequency-driven recoupling, fpRFDR [46]) and chemical shift precession to produce secular ZQ couplings, which could be subsequently manipulated with $\pi$ pulses to achieve the desired selectivity. In particular, they demonstrated that the constant-time adaptation of this sequence could be used for measuring internuclear distances under fast MAS (40 kHz) and at high magnetic fields, which is desirable for achieving better spectral resolution and minimizing the damping influence of $T_2$ relaxation. However, the ZQ-SEASHORE approach selects the spin pair to be recoupled 'indirectly', by inverting the third spin, which complicates its use in samples with large numbers of inequivalent sites. Indeed, the applications of ZQ-SEASHORE were demonstrated on $^{13}$C-labeled L-alanine and L-threonine, each exhibiting only three distinct $^{13}$C NMR resonances (note that L-threonine has four carbons but the chemical shifts of $C_\alpha$ and $C_\beta$ are close to each other, and thus experience the $\pi$ rotation simultaneously by a single frequency-selective pulse).

In this study, we propose to overcome the abovementioned ‘three spin’ constraint of the ZQ-SEASHORE experiment by implementing a double-frequency-selective $\pi$ pulse [47]. We further demonstrate that this approach can be used to quantify the dipole-dipole couplings between arbitrarily chosen spins within the multi-spin system, without the ill effects of dipolar truncation. An analytical treatment is developed for fitting the experimentally obtained dipolar dephasing curves.

2. Samples and methods
To suppress the contribution of intermolecular dipole-dipole interactions, an L-histidine·HCl·H₂O sample containing 25% of uniformly $^{13}$C/$^{15}$N-labeled molecules was prepared by recrystallization of a mixture of labeled and naturally abundant L-histidine from a hydrochloric acid solution (pH = 4) [48]. The recrystallized sample was ground by a mortar pestle, and packed in a 1.6 mm zirconia MAS rotor.

Solid-state NMR experiments were performed on a Varian NMR system spectrometer, equipped with a 1.6 mm triple-resonance MAS probe operated at 14.1 T. The modified pulse sequence used for $^{13}$C-$^{13}$C and $^{15}$N-$^{15}$N distance measurements is shown in Fig. 1a (the pulse program is given in the Supporting Information). In short, the $^{13}$C/$^{15}$N magnetizations, created by cross polarization from $^1$H spins, are driven by combinations of rotor-synchronized blocks of $\pi$ pulses shown in Fig. 1b. The ZQ-SEASHORE dephasing sequence, shaded in grey in Fig.1a, comprises 6$k$ segments, each consisting of a period of ZQ homonuclear recoupling, achieved by the abovementioned fpRFDR sequence [37, 46] with XY-8 phases [49] (block B), which is followed by a chemical shift evolution period ($n\tau_R$) to produce a truncated ZQ dipole-dipole Hamiltonian containing only secular terms. The length of each $\pi$ pulse is equal to $\tau_R/3$ (where $\tau_R$ denotes the rotor period) to maximize the scaling factor [50]. The underlying theoretical treatments and additional experimental details associated with ZQ-SEASHORE and fpRFDR sequences can be found in references [44] and [46], respectively. The key new feature is the double-frequency-selective inversion [51], achieved by inserting the rotor-synchronized cosine-modulated Gaussian $\pi$ pulse (Fig. 1c) in the middle of the sequence, which selects spin pairs instead of a single spin and isolates them from other spins.

The additional ABC blocks of $\pi$ pulses, which are separated by chemical shift precession periods $3n\tau_R$, serve to minimize the effects of $T_2$ relaxation and incomplete proton decoupling, by
preserving the constant-time length of the entire sequence, while using the same number of pulses and keeping $n$ constant [52]. This specific version of the constant-time approach, called PITHIRDS-CT, was described and demonstrated experimentally by Tycko [37], who showed that the supersequence of blocks A, B and C with cyclic displacements of $\pi$ pulses as illustrated in Fig. 1b does not produce any net dipolar recoupling under fast MAS. Note that the total length of the ZQ-SEASHORE blocks is given by $2[(l-k)(8\tau_R+8\tau_R+8\tau_R+3n\tau_R)+3k(8\tau_R+n\tau_R)] = 2l(24\tau_R+3n)\tau_R$, and thus the total duration of the sequence is kept constant, once $l$ and $n$ are chosen as described in the paragraph below. Finally, SPINAL-64 $^1$H decoupling was applied throughout the entire sequence [53].

Despite the complexity of the pulse sequence, the experiment is relatively straightforward: the selected $^{13}$C signal, typically the stronger of the two in a chosen pair, is simply measured as a function of the homonuclear dipolar recoupling time, given by $\tau_{rec} = 2 \times 3k \times 8\tau_R = 48k\tau_{rec}$ (see Fig. 1). To accomplish this, $k$ is advanced between 0 and $k_{max}$ ($\leq l$) in increments of $\Delta k$, until significant dipolar dephasing is achieved, in a manner similar to the well-known REDOR experiment. The increment $\Delta k$ is chosen such that the number of measured datapoints $(1 + k_{max}/\Delta k)$ allows to accurately simulate the decay of the selected $^{13}$C signal and estimate the internuclear distance (note that we used 7 or 8 points in our experiments, see Fig. 3). It is advisable to use $l = k_{max}$, whereas $n$ has to be sufficient for all non-selected spins to dephase under the sequence in Fig. 1a with $k = 0$. The optimization of $n$ and $l$ ($k_{max}$) can be quickly done in an iterative manner.

The experimental parameters are given in figure captions using the following symbols; $\nu_R = (\tau_R)^{-1}$ denotes the MAS rate, $\nu_{RF}(X)$ the magnitude of the RF magnetic field applied to X spins, $\tau_{CP}$ the cross-polarization time, and $\tau_{RD}$ the recycle delay. The $^{13}$C chemical shifts are referenced to TMS at 0 ppm. The $^{15}$N chemical shift is referenced to CH$_3$NO at 0 ppm.
3. Results and discussion

3.1. Dipolar dephasing experiments for selected spin pairs. We first demonstrated the performance of simultaneous two-spin inversion for selecting the desired spin pairs. Figs. 2a and d show the $^{13}$C and $^{15}$N CPMAS spectra of uniformly $^{13}$C/$^{15}$N-labeled L-histidine·HCl·H$_2$O,
obtained with the conventional $^{13}$C{$^1$H} and $^{15}$N{$^1$H} CPMAS experiments. The 2 ms cosine-modulated Gaussian pulse (Fig. 1c), applied in the middle of the sequence shown in Fig. 1a with $k = 0$, selectively inverted the $C_{\alpha}^{-}C_{\gamma}$ and $C'^{-}C_{\gamma}$ spin pairs (Figs. 2b and c), despite the difference in the resonance frequency between $C_{\gamma}$ and $C_{\delta2}/C_{\epsilon1}$ being only 1.2~1.5 kHz. In the $^{15}$N NMR spectrum, the resonance frequencies of $N_{\delta1}$ and $N_{\epsilon2}$ differ by just ~480 Hz (Fig. 2d); nevertheless, the $N_{\alpha}^{-}N_{\delta1}$ and $N_{\alpha}^{-}N_{\epsilon2}$ spin pairs were cleanly selected as well (Figs. 2e and f). These spectra demonstrate the competence of the double-frequency-selective approach in selecting arbitrary spin pairs for the measurement of homonuclear dipole-dipole couplings, regardless of the number of coupled spins in the system. Note that there are substantial differences between signal intensities within the selected spins, i.e., $C' > C_{\gamma} > C_{\alpha}$ and $N_{\alpha} > N_{\delta1}, N_{\epsilon2}$, which arise from the differences in $T_2$ relaxation. To increase the accuracy, the measurements of dipolar dephasing described in the following section were made using the stronger signals within each pair.

![Fig. 2. $^{13}$C{$^1$H} (a) and $^{15}$N{$^1$H} (d) CPMAS spectra of the L-histidine·HCl·H2O sample and demonstrations of double-frequency-selective inversion (b, c, e, and f). Spectra were obtained](image-url)
using $\nu_R = 40$ kHz, $\nu_{RF}(^1H) = 100$ kHz during $\pi/2$ pulse, $\nu_{RF}(^1H) = 50$ kHz during CP, $\nu_{RF}(^1H) = 150$ kHz during SPINAL-64 decoupling, $\nu_{RF}(^{13}C) = 90$ kHz during CP, $\nu_{RF}(^{13}C) = 60$ kHz during pre-saturation and fpRFDR $\pi$ pulses, $\nu_{RF}(^{15}N) = 90$ kHz during CP, $\nu_{RF}(^{15}N) = 60$ kHz during pre-saturation and fpRFDR $\pi$ pulses, $\tau_{CP} = 1$ ms for $^1\text{H}$$-^{13}\text{C}$ and 2 ms for $^1\text{H}$$-^{15}\text{N}$, and $\tau_{RD} = 4$ s. The double-frequency-selective spectra were obtained using the sequence in Fig. 1a with 2 ms cosine-modulated Gaussian $\pi$ pulses and the following parameters: $l = 7$, $k = 0$, $n = 7$ for $C\alpha$$-C\gamma$ (b); $l = 7$, $k = 0$, $n = 8$ for $C'$$-C\gamma$ (c); and $l = 24$, $k = 0$, $n = 16$ for $N\alpha$$-N\delta_1$ (e) and $N\alpha$$-N\epsilon_2$ (f).

The experimentally measured dipolar dephasing data obtained by selecting $C\alpha$$-C\gamma$ or $C'$$-C\gamma$ spin pairs are shown as filled circles in Figs. 3a and b, respectively. The black lines correspond to signal decays simulated by the SIMPSON program [54] for the pulse sequence in Fig. 1 and a set of chosen internuclear distances, assuming the presence of only ideal isolated spin pairs. Also shown, in red, are the corresponding SIMPSON simulations involving all six dipole-coupled $^{13}\text{C}$ spins in L-histidine·HCl·H$_2$O. The parameters for the six-spin system were generated from the crystal structure data [55] using the SPINACH program [56]. The intermolecular contacts were omitted based on 25 % dilution and generally longer distances.
Fig. 3. Dipolar dephasing data for uniformly $^{13}\text{C}/^{15}\text{N}$-labeled L-histidine·HCl·H$_2$O (filled circles), experimentally obtained using the sequence in Fig. 1. The measurements used $k = 0, 1, 2, ..., 7$ (i.e., $k_{\text{max}} = 7, \Delta k = 1$), $n = 7$ and $l = 7$ (a), $k = 0, 1, 2, ..., 7, n = 8$ and $l = 7$ (b), $k = 0, 4, 8, ..., 24, n = 16$ and $l = 24$ (c, d). Other experimental conditions were the same as described in the caption to Fig. 2. Black and red lines represent the simulated dipolar decay curves for two-spin and six-spin systems, respectively, using the same parameters as those used in the experiments. The two-spin simulations were performed for various distances, as indicated in the figures. The multi-spin simulations were carried using the intramolecular distances determined for all six carbons in L-histidine·HCl·H$_2$O by X-ray crystallography.
The best two-spin fits corresponded to a Cα–Cγ distance of 2.4 ± 0.1 Å and C′–Cγ distance of 3.0 ± 0.1 Å, both being in very good agreement with the crystal structure values of 2.54 Å and 3.02 Å [55]. This high accuracy, achieved despite the multi-bond separations and the presence of more strongly coupled neighbors, confirmed that dipolar dephasing of the selected spin pairs is not influenced by the multi-spin effects such as dipolar truncation and spin relay. This was further confirmed by the agreement between the experimental result and the six-spin simulations by SIMPSON.

Note that ideal two-spin simulations yielded different profiles of the dephasing curves for the same corresponding internuclear distances between the Cα–Cγ and C′–Cγ spin pairs (2.7 Å and 2.8 Å). This resulted from different experimental conditions (n = 7 and 8 for Cα–Cγ and C′–Cγ, respectively, see Fig. 2 caption), as well as the difference of scaling factors, which depend on the separation of isotropic chemical shifts (Δω) between two spins. The effect of Δω on the scaling factor will be discussed later.

Figs. 3c and d show measurements of the dephasing of Nα signals resulting from dipolar coupling with Nδ1 and Nε2, respectively. The observed signal decays are significantly slower than for 13C–13C pairs, because of the smaller gyromagnetic ratio of 15N nuclei and longer internuclear distances. The estimated Nα–Nδ1 distance (3.8 ± 0.1 Å) agrees well with the crystal structure, while the measured Nα–Nε2 distance (3.7 ± 0.1 Å) is much smaller than the XRD value of 4.8 Å. This inconsistency can be reasonably attributed to intermolecular dipole-dipole interactions, which depend solely on the arrangement of labeled neighbors with respect to the selected spin pair. In crystalline L-histidine·HCl·H2O, each molecule is surrounded by 6 nearest neighbors. Fig. 4 depicts the strongest intermolecular interactions influencing the measurements of the circled labeled molecule, with the cut-off distance set at 6 Å. Note that for the studied 13C–13C pairs, the
closest intermolecular sites are located much farther than the intramolecular neighbors, 3.8 Å vs 2.5 Å for C\textsubscript{\alpha}–C\textsubscript{\gamma} and 4.8 Å vs 3.0 Å for C\textsuperscript{′}–C\textsubscript{\gamma} (Figs. 4a and b). In addition, the sample used in our study contains only 25 % of uniformly labeled molecules, which further neutralized the intermolecular influence. However, this influence is notably stronger in the case of the N\textsubscript{\alpha}–N\textsubscript{\epsilon2} pair (Fig. 4d). Here, the shortest intermolecular N\textsubscript{\alpha}–N\textsubscript{\epsilon2} distance (4.2 Å) can be less than the intramolecular one (4.8 Å), thereby affecting the observed dephasing. In addition, N\textsubscript{\alpha} may have another N\textsubscript{\alpha} neighbor at 4.3 Å, in which case \(\Delta \omega = 0\) and the non-secular term of the effective Hamiltonian is not truncated [44], causing additional dephasing and underestimation of the N\textsubscript{\alpha}–N\textsubscript{\epsilon2} distance.

![Spatial distributions of studied spin pairs in L-histidine·HCl·H\textsubscript{2}O. Only selected neighbors are shown.](image)

**Fig. 4.** The spatial distributions of studied spin pairs in L-histidine·HCl·H\textsubscript{2}O. Only selected neighbors are shown.
3.2. Fitting experiment data to analytical expression. For a pair of spins with angular momenta $I_s$ and $I_r$ spun under MAS in a high magnetic field, the homonuclear dipolar Hamiltonian in the rotating frame can be written as

$$H_{sr}^D(t) = \omega_{sr}^D(t)(3I_{sz} - I_s \cdot I_r) = \hbar \sum_{m=-2}^{2} e^{i\omega_R t_{i}} C_m(\beta)(3I_{sz} - I_s \cdot I_r),$$

where $\hbar = \frac{\mu_s \gamma^2 \hbar}{4\pi r_{sr}^3}$ (in rad/s), $r_{sr}$ is the interspin distance, $I_{sz}$ and $I_{rz}$ are the z-components of $I_s$ and $I_r$, $\omega_R = 2\pi v_R$, and $\beta$ and $\gamma$ are the Euler angles relating the principal axis frame of the dipolar interaction to the rotor-fixed frame. The coefficients $C_m(\beta)$ are expressed as

$$C_1(\beta) = C_{-1}(\beta) = -\frac{1}{2\sqrt{2}} \sin(2\beta)$$

and

$$C_2(\beta) = C_{-2}(\beta) = \frac{1}{4} \sin^2(\beta) [57].$$

In the ZQ-SEASHORE technique developed by Hu and Tycko[44], the flip-flop (non-secular) terms of the recoupled dipolar interaction Hamiltonian are attenuated during the rotor-synchronized chemical shift evolution periods, leaving an effective Hamiltonian containing only secular terms,

$$U_{\text{trans.}}(N) \approx \exp(-iNrt_R \sum_{i<j} 2a_{ij} I_i I_j) \exp(-iNn_t R \sum_i \omega_i I_i),$$

where $r_t_R$ and $n_t_R$ are the lengths of the dipolar recoupling (i.e. B block) and chemical shift evolution periods, $N$ represents the total number of cycles in the ZQ-SEASHORE block (note that in our experiments, $p = 8$ and $N = 6k$, see Figs.1a and b), $\omega_i$ is the resonance frequency of spin $i$ relative to the RF carrier frequency, and $a_{ij}$ is the scaled dipole-dipole coupling (in rad/s) between spins $i$ and $j$. If the double-selective inversion pulse is applied to spins $s$ and $r$ in the middle of the ZQ-SEASHORE sequence, the evolution operator becomes
which indicates that the spins \(s\) and \(r\) are isolated from the rest of the spin system, remain coupled to each other and do not evolve under the chemical shift. In another words, the effective Hamiltonian for spins \(s\) and \(r\) is given by

\[
H_{\text{eff}}^s = 2a_s^* l_s^* \hat{I}_r.
\] (4)

In our experiment based on the PITHIRDS-CT technique, the fpRFDR sequence was used with \(\pi\) pulse length equal to \(\tau_R/3\), which results in [37, 52]

\[
a_s^* = \frac{b_{sr}}{2\pi} \left\{ \frac{27\sqrt{3}}{32} \left[ C_1(\beta)e^{\gamma\tau} + C_{-1}(\beta)e^{-\gamma\tau} \right] - \frac{27\sqrt{3}}{40} \left[ C_2(\beta)e^{2\gamma\tau} + C_{-2}(\beta)e^{-2\gamma\tau} \right] \right\}.
\] (5)

To calculate the powder averaged dephasing curve, Eq. 5 is summed over all sample orientations, which leads to the normalized dephased signal intensity \(S(\tau)\) as a function of the recoupling time \(\tau\),

\[
S(\tau) = \frac{1}{4\pi} \int_0^{2\pi} \int_0^{\pi} \sin(\beta)\cos(a_s^* \tau) d\beta d\gamma.
\] (6)

In Fig.5, we compare the dephasing curves obtained using Eq. 6 with those simulated using SIMPSON for several values of \(b_{sr}\) and \(\Delta\omega\). In most examples shown, Eq. 6 accurately described the dephasing. Discrepancies were only observed when \(\Delta\omega\) was very large (25 kHz, which corresponds to 167 ppm for \(^{13}\text{C}\) at 14.1 T) or \(b_{sr}/2\pi\) was very small (25 Hz, which corresponds to \(^{13}\text{C}-^{13}\text{C}\) distance of almost 7 Å). The former is attributable to the effect of offset on the fpRFDR pulse sequence, which is unaccounted for in Eqs. 5 and 6. In the case of large \(\Delta\omega\), the deviation
occurs at longer recoupling time, when \( S(\tau) < \sim 0.8 \); nevertheless, an accurate fit to Eq. 6 can be obtained using the data corresponding to \( S(\tau) > \sim 0.8 \). For \( b_{sr}/2\pi = 25 \) Hz, however, small discrepancies can be also seen for short \( \tau \), in which case our strategy would underestimate the dipole-dipole coupling. In this case, fitting the entire dataset may provide better accuracy, regardless of \( \Delta \omega \).

![Fig. 5](image)

**Fig. 5.** Dipolar dephasing curves simulated using SIMPSON for spin pairs with dipole-dipole couplings \( b_{sr}/2\pi = 250 \) Hz (a), 100 Hz (b), 50 Hz (c) and 25 Hz (d), and chemical shift separations \( \Delta \omega = 5 \) kHz (○), 15 kHz (◇), and 25 kHz (□), compared to those obtained from analytical expressions given by Eq. 6 (thin line) and Eq. 7 (bold line).

To further simplify the analysis, we can introduce a small angle approximation, \( \cos(\theta) \approx 1 - \theta^2/2 \), and use Eqs. 5 and 6 to derive a simple analytical expression for the ZQ dephasing curve in the limit of short \( \tau \):
Indeed, at short dephasing times, corresponding to $S(\tau) > 0.8$, the fits obtained from Eq. 7 (thick gray lines in Fig. 5) match very well with those derived from full analytical expression. Accordingly, fitting the experimental data to Eq. 7 accurately estimates the $C_{\alpha}-C_{\gamma}$ (2.54 Å) and $C'-C_{\gamma}$ (3.02 Å) distances to be $2.65 \pm 0.1$ Å and $3.06 \pm 0.1$ Å, respectively. On the other hand, fitting using Eq. 7 yields a less accurate value for the $^{15}N_{\alpha}-^{15}N_{\delta_1}$ distance, 3.39 Å instead of 3.80 Å, most likely due to the influence of non-secular contributions to the dipolar Hamiltonian, which are unaccounted for in the analytical analysis. We finally note that Eq. 7 can be useful in a special case of multi-spin systems containing equivalent spins, such as $s$, $r_1$ and $r_2$, where $r_1$ and $r_2$ have the same chemical shifts and $r_{sr_1} = r_{sr_2} = r_{sr}$. In such a case, the short-term dephasing is given by $b_{sr}^2 = 2b_{s}\alpha^2$ [58]. Clearly, Eq. 7 can only provide a rough distance estimate, and simulations using Eq. 6 or, better yet, SIMPSON, are required to obtain more accurate results.

4. Conclusion

We implemented a double-frequency-selective inversion pulse into the ZQ-SEASHORE sequence to increase its flexibility in measuring distances between arbitrary spin pairs in complex molecules. Our experiments indeed confirmed that this method provides accurate results for weakly coupled $^{13}C-^{13}C$ and $^{15}N-^{15}N$ pairs without interference from neighboring spins. We also offered simple analytical expressions to fit the dipolar dephasing data and compared the results with rigorous numerical simulations. The examples demonstrated in this study were limited to lower-$\gamma$ nuclei such as $^{13}C$ and $^{15}N$ because their relatively small homonuclear dipolar couplings can be well averaged out by MAS at 40 kHz. Recent advances in fast MAS technology may enable
the use of similar pulse sequences to recouple homonuclear dipole-dipole interactions between $^1$H nuclei.

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6. References


