Proton-detected 3D $^1\text{H}/^{13}\text{C}/^1\text{H}$ correlation experiment for structural analysis in rigid solids under ultrafast-MAS above 60 kHz

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A proton-detected 3D $^1\text{H}/^{13}\text{C}/^1\text{H}$ chemical shift correlation experiment is proposed for the assignment of chemical shift resonances, identification of ^{13}C - ^1H connectivities, and proximities of ^{13}C - ^1H and ^1H - ^1H nuclei under ultrafast magic-angle-spinning (ultrafast-MAS) conditions. Ultrafast-MAS is used to suppress all anisotropic interactions including ^1H - ^1H dipolar couplings, while the finite-pulse radio frequency driven dipolar recoupling (fp-RFDR) pulse sequence is used to recouple dipolar couplings among protons and the insensitive nuclei enhanced by polarization transfer technique is used to transfer magnetization between heteronuclear spins. The 3D experiment eliminates signals from non-carbon-bonded protons and non-proton-bonded carbons to enhance spectral resolution. The 2D (F1/F3) $^1\text{H}/^1\text{H}$ and 2D $^{13}\text{C}/^1\text{H}$ (F2/F3) chemical shift correlation spectra extracted from the 3D spectrum enable the identification of ^1H - ^1H proximity and ^{13}C - ^1H connectivity. In addition, the 2D (F1/F2) $^1\text{H}/^{13}\text{C}$ chemical shift correlation spectrum, incorporated with proton magnetization exchange via the fp-RFDR recoupling of ^1H - ^1H dipolar couplings, enables the measurement of proximities between ^{13}C and even the remote non-carbon-bonded protons. The 3D experiment also gives three-spin proximities of ^1H - ^1H - ^{13}C chains. Experimental results obtained from powder samples of L-alanine and L-histidine- H_2O -HCl demonstrate the efficiency of the 3D experiment. © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4933373>]

INTRODUCTION

Although solid-state NMR has become a valuable technique in providing atomic-resolution structural and dynamic insights into a variety of non-crystalline and non-soluble molecular systems,^{1–9} its poor sensitivity and demand for a large sample quantity continue to restrict its widespread applications.¹⁰ However, with the remarkable improvements in proton spectral resolution enabled by recent developments in ultrafast-MAS technology, sensitivity related difficulties are largely overcome by utilizing the advantages of very high abundance and large gyromagnetic ratio of protons by proton detection.¹¹ Indeed, proton-detected NMR methodology has become an attractive avenue in the development of multi-dimensional solid-state NMR spectroscopy.^{12–24} Ultrafast magic-angle-spinning (Ultrafast-MAS) has also enabled the use of low power radio frequency (RF) fields for cross-polarization (CP) and heteronuclear decoupling,^{25–31} which are significant benefits for solid-state NMR studies on heat-sensitive samples and also for experiments requiring long mixing or decoupling periods.

One of the most commonly used NMR-based structural studies strategy employs internuclear correlations, which are easily obtained from homonuclear or heteronuclear multidimensional NMR experiments. While two-dimensional correlation experiments provide two-spin correlations, higher dimensional

NMR experiments can give insights into more complex spin connectivities/proximities. The nuclear spin connectivity/proximity can be achieved via either through-space (using NOE or coherent dipolar coupling) or through-bond (using scalar or J coupling) depending on the magnetization transfer process used in the correlation experiments. Although, because of the strong ^1H - ^1H dipolar coupling networks, $^1\text{H}/^1\text{H}$ connectivity/proximity is mostly obtained via through-space interactions in solids, heteronuclear connectivity can be obtained via either through-bond or through-space interactions using the insensitive nuclei enhanced by polarization transfer (INEPT).

Among all the approaches used for heteronuclear polarization transfer, the INEPT experiment is commonly used for signal enhancement of low- γ nuclei in solution NMR spectroscopy.^{32,33} On the other hand, the presence of strong ^1H - ^1H dipolar couplings in solids results in a fast decay of magnetization due to a short spin-spin relaxation time (T_2), and therefore the inefficiency of magnetization transfer via J couplings in solids. To overcome this difficulty, homonuclear ^1H - ^1H decoupling pulse sequences, such as frequency-switched Lee-Goldburg (FSLG),^{34,35} PMLG (phase-modulated Lee-Goldburg),³⁶ or DUMBO (decoupling using mind-boggling optimization),^{37,38} are employed in the evolution periods of INEPT^{39–41} to enable J coupling based transfer of magnetization. Interestingly, it was recently demonstrated that the heteronuclear polarization transfer occurs mainly through dipolar couplings instead of J coupling under ultrafast-MAS,^{42,43} where the premise for the polarization transfer is

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the simultaneous presence of ^1H - ^1H homonuclear and ^{13}C - ^1H heteronuclear dipolar couplings. It has been demonstrated that the quaternary ^{13}C magnetization can be suppressed, due to weak non-bonded ^{13}C - ^1H dipolar couplings and absence of nearby ^1H - ^1H dipolar couplings, when INEPT is used for $^1\text{H} \rightarrow ^{13}\text{C}$ polarization transfer under ultrafast-MAS.⁴³ Therefore, it is possible to obtain a $^{13}\text{C}/^1\text{H}$ heteronuclear chemical shift correlation (HETCOR) spectrum only for the directly ^1H - ^{13}C bonded pairs of nuclei in solids by INEPT based $^1\text{H} \rightarrow ^{13}\text{C}$ polarization transfer as demonstrated in the literature,⁴² although the polarization transfer is primarily due to unaveraged dipolar couplings present in solids.

In this study, we propose a proton-detected 3D pulse sequence (Fig. 1) that combines a finite-pulse radio frequency driven dipolar recoupling (fp-RFDR)⁴⁴⁻⁴⁷ and an INEPT-based polarization transfer to obtain three-spin isotropic chemical shift correlation of ^1H and ^{13}C nuclei under ultrafast-MAS conditions. In spite of a very small sample quantity used in ultrafast-MAS experiments, higher sensitivity rendered by ^1H detection enables 3D experiments to be completed within a reasonable time, and the higher dimension gives additional resolution enhancement. For simplicity, we refer this 3D fp-RFDR incorporated INEPT-based heteronuclear single-quantum correlation (HSQC) experiment as RFDR-HSQC. From this 3D RFDR-HSQC experiment, 2D $^1\text{H}/^1\text{H}$ (F1/F3) and 2D $^{13}\text{C}/^1\text{H}$ (F2/F3) chemical shift correlation spectra can be easily extracted for chemical shift resonance assignments. More importantly, the 2D $^1\text{H}/^{13}\text{C}$ (F1/F2) chemical shift correlation spectrum, mediated by ^1H - ^1H magnetization exchange via the fp-RFDR-recoupled dipolar couplings among protons, could be used to determine the proximities of non-bonded ^{13}C and ^1H nuclei. The 3D

experiment also gives significant insights into the ^1H - ^1H - ^{13}C three-spin correlations/proximities. As demonstrated by the experimental results, the use of INEPT suppresses non-carbon-bonded ^1H resonances and thus enhances the spectral resolution. The sensitivity enhancement rendered by proton-detection^{11,15} significantly reduces the measurement time of the 3D $^1\text{H}/^{13}\text{C}/^1\text{H}$ chemical shift correlation experiment.

EXPERIMENTAL

Samples

Uniformly ^{13}C , ^{15}N -labeled L-alanine was purchased from Isotec (Champaign, IL) and uniformly ^{13}C , ^{15}N -labeled L-histidine $\cdot \text{HCl} \cdot \text{H}_2\text{O}$ was bought from Acros Organics (Morris Plains, NJ). All samples were used as received without any further purification.

Solid-state NMR

The 3D RFDR-HSQC pulse sequence along with phase cyclings is shown in Fig. 1. First, the ^1H magnetization is prepared in the transverse plane for isotropic chemical shift evolution during the t_1 period, and then it is flipped to the z -axis for magnetization exchange through the recoupled ^1H - ^1H dipolar couplings during fp-RFDR. After fp-RFDR, the transverse ^1H magnetization is transferred to ^{13}C through the INEPT sequence. After ^{13}C chemical shift evolution during the t_2 period, the ^{13}C magnetization is transferred back to ^1H for detection using INEPT-based sequence. 3D experiments on uniformly ^{13}C , ^{15}N labeled L-alanine and L-histidine $\cdot \text{HCl} \cdot \text{H}_2\text{O}$ were performed using an Agilent/Varian VNMRs 600 MHz solid-state NMR spectrometer operating at 599.8 MHz for ^1H and 150.8 MHz for ^{13}C nuclei, respectively, and a 1.2 mm HXY triple-resonance MAS probe. The spinning speed of the sample was 60 kHz MAS. The 90° pulse lengths were 1.4 μs for both ^{13}C and ^1H nuclei. 16 t_1 and 36 t_2 increments with 16 scans were used to acquire 3D spectra. XY 4 ₁₄ phase cycling scheme was used for fp-RFDR, which has been demonstrated to provide the best performance for recoupling ^1H - ^1H dipolar couplings under ultrafast-MAS and overcoming the effects of chemical shift offset and RF field inhomogeneity.^{45,46} For comparison, a regular 2D $^1\text{H}/^1\text{H}$ correlation experiment^{45,46} was also performed on both alanine and histidine samples, where a total proton correlation was achieved using a fp-RFDR mixing time of 1 ms and 3 ms, respectively.

RESULTS AND DISCUSSION

The performance of the 3D RFDR-HSQC pulse sequence is demonstrated on uniformly ^{13}C , ^{15}N labeled L-alanine and uniformly ^{13}C , ^{15}N -labeled L-histidine $\cdot \text{HCl} \cdot \text{H}_2\text{O}$ powder samples using 600 NMR spectrometers and at spinning speeds of 60 kHz and above. The 3D experimental results are presented as 2D spectra (obtained through skyline projection) in Figs. 2 and 3 for L-alanine and in Figs. 4-7 for L-histidine $\cdot \text{HCl} \cdot \text{H}_2\text{O}$.

FIG. 1. Pulse sequence for the 3D RFDR-HSQC $^1\text{H}/^{13}\text{C}/^1\text{H}$ chemical shift correlation experiment. Solid and blank rectangles represent the 90° and 180° pulses, respectively. Finite-pulse-RFDR with an XY 4 ₁₄ phase cycling (XYXY XYXYXYXY XYXY) is used for ^1H - ^1H dipolar recoupling under ultrafast-MAS. INEPT-based HSQC pulse sequence is used for $^1\text{H} \rightarrow ^{13}\text{C}$ and $^{13}\text{C} \rightarrow ^1\text{H}$ magnetization transfers as well as to enable ^{13}C chemical shift evolution. Phase cyclings used for RF pulses in the sequence and the receiver are given at the bottom. Low RF power continuous-wave ^{13}C heteronuclear decoupling could also be used during the t_1 and t_3 periods or ^1H heteronuclear decoupling during the t_2 period to obtain a better spectral resolution if necessary.

FIG. 2. 1D and 2D NMR spectra of U- ^{13}C - ^{15}N -L-alanine under 60 kHz MAS. (a) A single pulse proton spectrum. (b) ^{13}C CPMAS spectrum obtained with double quantum CP.²⁸ (c) 2D $^{13}\text{C}/^1\text{H}$ correlation spectrum (F2/F3) extracted from the 3D RFDR-HSQC spectrum that was obtained using a fp-RFDR mixing time of 1 ms, 16 t_1 , 36 t_2 , and 16 scans. In the INEPT sequence of the 3D experiment, the evolution delays (τ_1 and τ_2) were both set to 0.5 ms.

3D RFDR-HSQC suppresses non-carbon-bonded ^1H resonances

Although faster spinning speed (up to 110 kHz) has been shown to effectively suppress ^1H - ^1H dipolar couplings and enable proton-detected multidimensional experiments with reasonably well-resolved proton spectral lines,^{48–51} there is a need for approaches to further enhance the spectral resolution. There are two ways to achieve higher spectral resolution. One way is to further reduce the linewidth and the other way is to make the spectrum less crowded by filtering or higher dimensional experiments. In spite of the contributions

from ultrafast-MAS to the former mechanism, the observed spectral resolution in solids is rather limited compared to that in solution. Line-broadening from anisotropic-bulk magnetic-susceptibility⁵² and conformational heterogeneity cannot be suppressed even at infinite MAS rate. On the other hand, ultrafast-MAS enables the use of the latter approach, i.e., higher dimensionality, thanks to the higher sensitivity rendered by ^1H detection. The involvement of low- γ nuclei is an obvious way to resolve the overlapping proton peaks in a 2D $^1\text{H}/^1\text{H}$ correlation spectrum as demonstrated in the recently reported 3D proton-detected ultrafast-MAS NMR experiments.^{20,53–55} In addition, the use of spectral editing can

FIG. 3. 2D $^1\text{H}/^1\text{H}$ homonuclear correlation and $^{13}\text{C}/^1\text{H}$ heteronuclear correlation spectra. ((a) and (b)) 2D $^1\text{H}/^1\text{H}$ (F1/F3) homonuclear correlation spectra extracted from the 3D spectra that were obtained without (a) or with (b) fp-RFDR during the mixing time. (c) A regular 2D $^1\text{H}/^1\text{H}$ fp-RFDR correlation spectrum. ((d) and (e)) 2D fp-RFDR mediated $^1\text{H}/^{13}\text{C}$ (F1/F2) correlation spectra extracted from the 3D spectra that were obtained without (d) or with (e) fp-RFDR during the mixing time. The fp-RFDR mixing time was $t_{\text{mix}} = 1$ ms, and the time delays in the INEPT sequence were $\tau_1 = \tau_2 = 0.5$ ms.

FIG. 4. 60 kHz MAS 1D and 2D spectra of L-histidine-HCl-H₂O. (a) A single pulse proton spectrum. (b) ¹³C CPMAS spectrum obtained with double quantum CP.²⁸ (c) Schematic molecular structure of L-histidine-HCl-H₂O. (d) 2D ¹³C/¹H chemical shift correlation spectrum (F2/F3) extracted from the 3D RFDR-HSQC spectrum that was acquired with a 3 ms fp-RFDR mixing time and using 0.15 ms for the τ_1 and τ_2 delays in INEPT.

further enhance the spectral resolution as demonstrated by the 3D RFDR-HSQC pulse sequence. The magnetization transfer from ¹H to ¹³C in 3D RFDR-HSQC can be used to selectively suppress certain resonances based on the magnitude of ¹H-¹³C dipolar couplings, particularly the non-carbon-bonded proton resonances can be removed as demonstrated in this study. The 2D ¹³C/¹H (F2/F3) spectrum, extracted from the 3D RFDR-HSQC spectrum, that correlates the chemical shifts of ¹³C and ¹H nuclei is shown in Fig. 2(c). The 2D spectrum displays well-resolved CH and CH₃ resonances from alanine while the non-carbon-bonded ¹H resonance from the NH₃⁺ group is suppressed by using the INEPT sequence to transfer proton magnetization to carbon-13. Similarly, the non-hydrogen-bonded ¹³C resonances can also be suppressed.

As demonstrated previously,^{42,43} the INEPT-based ¹H to ¹³C polarization transfer under ultrafast-MAS requires the simultaneous presence of ¹H-¹H and ¹³C-¹H dipolar couplings. As a result, the non-carbon-bonded ¹H and non-proton-bonded ¹³C resonances are suppressed or extremely weak due to their weak ¹³C-¹H dipolar couplings; the absence of nearby ¹H-¹H homonuclear dipolar couplings also assists the suppression of non-hydrogen-bonded ¹³C resonances. It may be noted that a short contact time (50 ~ 100 μ s) CP can also avoid the non-bonded ¹H-¹³C polarization transfer and could also be used instead of INEPT as their polarization transfer efficiencies are comparable.⁴²

3D RFDR-HSQC reveals the proximity of hydrogen and carbon atoms

The 3D RFDR-HSQC experiment cannot only identify the bonded ¹H and ¹³C nuclei as mentioned above but it can also be used to reveal the proximities between homonuclei (i.e., protons in this case) and heteronuclei (for example, ¹H and ¹³C or ¹H and ¹⁵N nuclei). As demonstrated in this study, the homonuclear proximities can be obtained from the 2D ¹H/¹H (F1/F3) spectrum extracted from the 3D RFDR-

HSQC spectrum, whereas the heteronuclei proximities can be determined from the 2D ¹³C/¹H (F1/F2) chemical shift correlation. Obviously, the ¹H/¹H chemical shift correlation (F1/F3) spectrum provides information about ¹H-¹H proximity due to the magnetization exchange via the recoupled dipolar couplings among protons, which, in principle, is similar to the previously demonstrated 2D ¹H/¹H fp-RFDR chemical shift correlation experiment.^{45,46} However, the suppression of non-carbon-bonded protons and non-proton-bonded ¹³C resonances can render higher resolution in the 3D RFDR-HSQC spectrum as compared to the 2D ¹H/¹H correlation spectrum. This is experimentally demonstrated as shown in Fig. 3. The 2D F1/F3 spectrum shows only diagonal peaks (self-correlation of resonances from chemically equivalent proton nuclei) corresponding to the aliphatic protons in the absence of RFDR (i.e., mixing time, $t_{\text{mix}} = 0$) (Fig. 3(a)). But, when fp-RFDR is introduced in the mixing time, the magnetization exchange among protons results in cross peaks between proton resonances (Fig. 3(b)). However, due to the absence of non-carbon-bonded proton chemical shift evolution in the F3 dimension, the cross peaks between aliphatic and non-carbon-bonded protons only appear below the diagonal lines in the 2D F1/F3 spectrum (Fig. 3(b)), as compared to the regular 2D ¹H/¹H chemical shift correlation spectrum shown in Fig. 3(c). Similarly, when t_{mix} is zero, the 2D F1/F2 spectrum shown in Fig. 3(d) is exactly the same as the 2D F2/F3 spectrum, which directly provides the connectivity between ¹³C and ¹H nuclei as discussed above. However, when fp-RFDR is introduced in the mixing time of the 3D experiment, cross peaks between carbon and remote protons are also observed as shown in Fig. 3(e). Indeed, the cross peaks between the ¹³CH₃ resonance and ¹H resonances of CH and NH₃⁺ groups, and that between the ¹³CH resonance and ¹H resonances of CH₃ and NH₃⁺ groups, resulted from the fp-RFDR enabled magnetization exchange among protons. Therefore, the 3D experiment can be potentially used to measure distance between a carbon and many protons and also between protons.

FIG. 5. 3D RFDR-HSQC spectrum of L-histidine·HCl·H₂O. 2D ¹³C/¹H chemical shift correlation spectrum (F2/F3) extracted from the 3D RFDR-HSQC spectrum that was acquired with a fp-RFDR mixing time of 0.457 ms (a) and 0.914 ms (b) and using 0.5 ms for τ_1 and τ_2 in INEPT. These two experiments were performed on a 600 MHz JEOL ECZ600R solid-state NMR spectrometer using a 1.0 mm double-resonance ultrafast-MAS probe (JEOL RESONANCE, Inc.) at a spinning speed of 70 kHz.

More importantly, the 3D experimental results clearly give us a set of three spin ¹H-¹H-¹³C proximities/correlations, where the ¹H/¹H correlation is determined by the fp-RFDR mixing time, and the ¹H-¹³C connectivity is provided by the INEPT-based polarization transfer.

3D RFDR-HSQC experiment on L-histidine·HCl·H₂O

The performance of the 3D RFDR-HSQC pulse sequence was further examined through experiments performed on a uniformly ¹³C,¹⁵N-labeled L-histidine·HCl·H₂O powder sample. The ¹H and ¹³C spectra under 60 kHz MAS are shown in Fig. 4 along with the molecular structure of histidine·HCl·H₂O. Similar to the spectrum of alanine (Fig. 2), the 2D ¹³C/¹H (F2/F3) chemical shift correlation spectrum provides the ¹³C-¹H bonding information. All the quaternary ¹³C resonances and non-carbon-bonded ¹H resonances are absent in the 2D F2/F3 spectrum. It is also worth mentioning that the C2-H2 correlation peak is relatively weak compared to other peaks. This is because the C₂H₂ group experiences relatively a faster T_2 relaxation compared to nuclei in other chemical groups, which results in the fast decay and therefore the loss of transverse magnetization during INEPT.

In fact, the evolution delays were 150 μ s ($\tau_1 = \tau_2 = 0.15$ ms) for the INEPT-based polarization transfer. When the evolution time was 500 μ s, both C1-H1 and C2-H2 correlation peaks disappeared, as shown in Fig. 5, even though a short fp-RFDR mixing time was used (0.457 or 0.914 ms). Therefore, it is very important to optimize the evolution time (τ_1 and τ_2) periods in INEPT before performing the 3D experiment in order to achieve overall maximum polarization transfer efficiency, as was discussed in our previous work.⁴³ If T_2 is too short and results in a significant loss of signal intensity, CP may be used as an alternative to INEPT for polarization transfer in the 3D experiment. However, the contact time of CP has to be sufficiently short to avoid the remote ¹H to ¹³C polarization transfer, which is generally around 50 μ s for a uniformly ¹³C-labeled polypeptides.⁵⁶ On the other hand, spectral editing is also possible by effectively utilizing the short T_2 of protons to eliminate resonances from certain chemical groups in rigid solids or from certain species in a heterogeneous mixture. It is worth noting that the CP contact-time for bonded ¹H-¹³C polarization transfer could

be increased with further increasing the spinning speed due to better suppression of anisotropic ¹H-¹H dipolar couplings at higher spinning speeds.²³ Besides, the ¹³C and ¹H spin-spin relaxation times increase significantly with the increasing spinning speed. Therefore, higher spinning speed (above 60 kHz) would weaken the T_2 relaxation and thus can improve the signal sensitivity.^{42,43,48}

As mentioned above, the non-carbon-bonded proton signals can be greatly suppressed or removed by the INEPT-based polarization transfer. As shown in Fig. 6, after the two-step ¹H $\rightarrow$ ¹³C and ¹³C $\rightarrow$ ¹H polarization transfers through INEPT, all the non-carbon-bonded proton signals (such as H10 and H7) are completely removed whereas residual antiphase signals can be seen for H9 and H8. Such low intensity antiphase non-carbon-bonded proton signals were also observed in 1D ¹³C-detected INEPT experiments.^{42,43} However, these antiphase signals do not affect the results obtained in the 3D experiment, as they are completely removed when the t_1 and t_2 evolution times are not zero. Interestingly, the H5 and H3 peaks are very well resolved due to the complete removal of signals from H7 in the 1D spectrum obtained using the 3D RFDR-HSQC pulse sequence by setting t_1 and t_2 to zero, whereas the peaks of H5, H3, and H7 protons are completely overlapped in a single pulse 1D ¹H spectrum as shown in Fig. 4(a). Therefore, these experimental results demonstrate that the 3D RFDR-HSQC sequence can be used to resolve proton peaks that are overlapped with non-carbon-bonded proton peaks.

FIG. 6. 1D ¹H spectrum obtained using the 3D pulse sequence resolves the overlapping ¹H peaks. 60 kHz MAS ¹H NMR spectrum of L-histidine·HCl·H₂O obtained using the 3D RFDR-HSQC sequence with $t_1 = t_2 = 0$. All non-carbon-bonded proton peaks are filtered out or greatly suppressed. The red arrows indicate the residual antiphase signals from H9 and H8.

FIG. 7. Two-dimensional $^1\text{H}/^1\text{H}$ homonuclear correlation and $^{13}\text{C}/^1\text{H}$ heteronuclear correlation spectra. (a) 2D $^1\text{H}/^1\text{H}$ chemical shift correlation spectra extracted from the 3D spectrum. (b) A regular 2D $^1\text{H}/^1\text{H}$ correlation spectrum using fp-RFDR. (c) fp-RFDR mediated $^1\text{H}/^{13}\text{C}$ chemical shift correlation spectrum extracted from the 3D spectrum. A 3 ms fp-RFDR mixing time was used for all experiments. The cross peaks between C3 and other protons are indicated in blue.

The 2D F2/F3 ($^{13}\text{C}/^1\text{H}$), F1/F3 ($^1\text{H}/^1\text{H}$), and F1/F2 ($^1\text{H}/^{13}\text{C}$) spectra extracted from the 3D spectrum further enable one to probe $^1\text{H}-^1\text{H}$ and $^{13}\text{C}-^1\text{H}$ proximities as shown in Fig. 7. Similar to the 2D $^1\text{H}/^1\text{H}$ experiment,^{45,46} a 3 ms fp-RFDR mixing time is sufficient to accomplish a total correlation of all proton resonances as shown in Fig. 7(a). Even though the non-carbon-bonded proton resonances are completely removed in the final 3D spectrum, their chemical shift evolution is encoded in t_1 period before they are removed by the INEPT sequence. As a result, the cross peaks between aliphatic and all other protons could still be observed in the $^1\text{H}/^1\text{H}$ (F1/F3) spectrum, although the spectral pattern is not symmetrical with respect to the diagonal (Fig. 7(a)) unlike the regular 2D $^1\text{H}/^1\text{H}$ spectrum shown in Fig. 7(b). It is worth noting that due to the suppression of non-carbon-bonded H7 signals, H5 and H3 signals are better resolved in the 2D $^1\text{H}/^1\text{H}$ (F1/F3) correlation spectrum (Fig. 7(a)) as well as their correlation signals with other protons, in contrast to the regular 2D $^1\text{H}/^1\text{H}$ spectrum given in Fig. 7(b). In the fp-RFDR mediated $^1\text{H}/^{13}\text{C}$ (F1/F2) correlation spectrum, cross peaks between non-bonded $^{13}\text{C}-^1\text{H}$ pairs appear due to the magnetization exchange among protons before the $^1\text{H} \rightarrow ^{13}\text{C}$ polarization transfer period in the 3D experiment. In fact, with a 3 ms fp-RFDR mixing time, the proton-bonded carbons are correlated with all protons (Fig. 7(c)). For example, C3 correlates with all the protons as indicated in Fig. 7(c), because H3 has magnetization exchange with all the protons through recoupled $^1\text{H}-^1\text{H}$ dipolar couplings with a 3 ms fp-RFDR mixing. Indeed, by controlling the magnetization exchange between H3 and other protons using the fp-RFDR mixing time, the proximity between H3-bonded C3 and the other protons can be obtained. The cross peaks between C2 and other protons are relatively weak in intensity due to the fast T_2 relaxation as mentioned above.

CONCLUSION

In this study, the performance of a 3D $^1\text{H}/^{13}\text{C}/^1\text{H}$ pulse sequence that correlates the chemical shifts of ^1H and ^{13}C nuclei is experimentally demonstrated under ultrafast magic-angle-spinning (ultrafast-MAS) conditions. The experimental

results demonstrate that the INEPT-based transfer of magnetization between dipolar coupled heteronuclei can be used to enhance spectral resolution by eliminating resonances from non-carbon-bonded protons and non-proton-bonded carbons. Though the dipolar couplings present in rigid solids are used in INEPT, heteronuclear scalar couplings can also be used in 3D experiments on semi-solids, dynamic chemical groups, or heterogeneous mixture containing rigid and mobile solids. By incorporating fp-RFDR to enable magnetization exchange among protons, the 3D RFDR-HSQC sequence can be used to determine the proximities of homonuclear and heteronuclear nuclei. As demonstrated by the experimental results, the combination of the three different 2D spectra extracted from a single 3D spectrum enables $^1\text{H}-^1\text{H}-^{13}\text{C}$ proximities/correlations to be obtained, which would be valuable in the structural studies of solids. Our results also show that a 3D spectrum with a desired extent of spectral resolution and correlation with remote protons can be obtained by appropriately selecting the fp-RFDR mixing time and INEPT delay times. Overall, the proposed proton-detected 3D pulse sequence is easy to set up and can be used to extract abundant information about molecular structures under ultrafast-MAS conditions. We believe that the 3D NMR experiment when combined with proton dilution by deuteration or selective ^{13}C (and/or ^{15}N) labeling would be useful to study a variety of chemical and biological solids including proteins.

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