Constant-time 2D and 3D through-bond correlation NMR spectroscopy of solids under 60 kHz MAS

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Establishing connectivity and proximity of nuclei is an important step in elucidating the structure and dynamics of molecules in solids using magic angle spinning (MAS) NMR spectroscopy. Although recent studies have successfully demonstrated the feasibility of proton-detected multidimensional solid-state NMR experiments under ultrafast-MAS frequencies and obtaining high-resolution spectral lines of protons, assignment of proton resonances is a major challenge. In this study, we first re-visit and demonstrate the feasibility of 2D constant-time uniform-sign cross-peak correlation (CTUC-COSY) NMR experiment on rigid solids under ultrafast-MAS conditions, where the sensitivity of the experiment is enhanced by the reduced spin-spin relaxation rate and the use of low radio-frequency power for heteronuclear decoupling during the evolution intervals of the pulse sequence. In addition, we experimentally demonstrate the performance of a proton-detected pulse sequence to obtain a 3D $^1\text{H}/^{13}\text{C}/^1\text{H}$ chemical shift correlation spectrum by incorporating an additional cross-polarization period in the CTUC-COSY pulse sequence to enable proton chemical shift evolution and proton detection in the incrementable t_1 and t_3 periods, respectively. In addition to through-space and through-bond $^{13}\text{C}/^1\text{H}$ and $^{13}\text{C}/^{13}\text{C}$ chemical shift correlations, the 3D $^1\text{H}/^{13}\text{C}/^1\text{H}$ experiment also provides a COSY-type $^1\text{H}/^1\text{H}$ chemical shift correlation spectrum, where only the chemical shifts of those protons, which are bonded to two neighboring carbons, are correlated. By extracting 2D F1/F3 slices ($^1\text{H}/^1\text{H}$ chemical shift correlation spectrum) at different ^{13}C chemical shift frequencies from the 3D $^1\text{H}/^{13}\text{C}/^1\text{H}$ spectrum, resonances of proton atoms located close to a specific carbon atom can be identified. Overall, the through-bond and through-space homonuclear/heteronuclear proximities determined from the 3D $^1\text{H}/^{13}\text{C}/^1\text{H}$ experiment would be useful to study the structure and dynamics of a variety of chemical and biological solids. © 2016 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4940029>]

INTRODUCTION

Solid-state NMR spectroscopy has been a powerful tool in providing piercing insights into atomic-resolution molecular structures and dynamics of a variety of non-soluble and non-crystalline materials.^{1–6} In particular, the scalar coupling (i.e., J -coupling) signifies the through-bond connectivity of atoms, whereas the dipole-dipole interaction provides through-space interatomic distance constraints.^{7,8} Therefore, a combination of through-bond and through-space correlation experiments could be used to determine molecular structures and inter-molecular assemblies. In spite of the development of a diverse set of dipolar-coupling driven experiments and their application to determine heteronuclear/homonuclear connectivity and distances,^{9–23} relatively few experiments^{24–32} make use of scalar coupling for structural studies due to its smaller magnitude, when compared to dipolar coupling, in solids. For example, the dipolar coupling between single bonded ^{13}C – ^{13}C pair is around 2 kHz, whereas the scalar coupling is only around 55 Hz. However, unlike the dipolar coupling, the scalar coupling is unique in that it is insensitive to molecular dynamics and therefore can be used to establish

a through-bond correlation for the assignment of chemical shift resonances.²⁸ In addition, because the scalar coupling is not averaged out by magic-angle-spinning (MAS), there is no need for a recoupling sequence. Therefore, scalar-coupling-based methods can be excellent alternative to the dipolar coupling based experiments and are crucial for unambiguous sequential assignments of resonances in solids.^{26,30} However, a scalar coupling based experiment generally requires a long transverse magnetization evolution, which is not desirable due to spin-spin relaxation (T_2) loss of magnetization. For example, in a ^{13}C – ^{13}C J -coupling-based refocused-INEPT (refocused insensitive nuclei enhanced by polarization transfer) experiment, the transverse ^{13}C magnetization has to be evolved under ^{13}C – ^{13}C scalar couplings for about 15–25 ms to refocus the anti-phase coherence,^{33,34} whereas the dipolar coupling based experiments often utilize flip-flop spin diffusion process where the T_2 relaxation effect is absent. Therefore, the use of approaches to slow down the T_2 process will greatly benefit J -based experiments. In particular, for rigid solids, the signal sensitivity is often compromised to a great extent by the fast T_2 relaxation. Fortunately, ultrafast-MAS is one of the simplest and easiest way to significantly slow down the T_2 process, because it is efficient in suppressing the line-broadening interactions including homonuclear and heteronuclear dipolar

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couplings. As experimentally demonstrated, proton T_2 linearly increased^{35,36} and ^{13}C linewidth decreased^{36,37} with the spinning speed.

Moreover, the use of ultrafast-MAS has significantly reduced the demand on radio-frequency (RF) power to accomplish an efficient heteronuclear decoupling; studies have demonstrated that even a low power continuous-wave (CW) RF is sufficient for heteronuclear decoupling under ultrafast-MAS conditions.^{38,39} Instead, under a MAS speed of 25 kHz, a 150 kHz SPINAL decoupling⁴⁰ has to be applied to effectively decouple protons during the ^{13}C – ^{13}C scalar coupling evolution period.^{30,41} Such high power RF irradiation is not desirable for heat-sensitive samples like membrane proteins.^{42–44} Therefore, in principle, it is easier to implement through-bond ^{13}C – ^{13}C correlation experiments under ultrafast-MAS for sequential resonance assignment purposes. In addition to the suppression of ^1H – ^1H dipolar couplings, ultrafast-MAS dramatically enhances spectral resolution of protons.^{45–49} Because of this reason and due to the enhanced signal-to-noise ratio rendered by proton detection, the development of proton-based solid-state NMR techniques has been the main focus of recent ultrafast-MAS studies.^{50–60} Recent studies from our laboratory have demonstrated the feasibility of recoupling proton-proton dipolar couplings,^{14,61–63} proton-based multidimensional experiments,^{64–67} spectral-editing techniques,^{68,69} and proton-proton distance measurements⁶⁹ under ultrafast-MAS conditions, whereas effective use of deuteration has also very recently been shown to be useful in proton-proton distance measurements by Reif *et al.*^{70,71} We have also shown the role of dipolar couplings that control the efficiency of refocused-INEPT pulse sequence under ultrafast-MAS.⁷² While the recent proton-based ultrafast-MAS studies have successfully demonstrated the feasibility of using well-resolved proton chemical shift resonances, there is a need for methods to assign proton resonances.

In this study, we first revisit the constant-time uniform-sign cross-peak correlation (CTUC-COSY) experiment under ultrafast-MAS (Fig. 1(a)) to obtain a high-resolution through-bond $^{13}\text{C}/^{13}\text{C}$ chemical shift correlation spectrum. Following this, we demonstrate the performance of a 3D $^1\text{H}/^{13}\text{C}/^1\text{H}$

chemical shift correlation experiment by incorporating proton chemical shift evolution into an incrementable t_1 period and a second cross-polarization (CP) period to enable proton detection in the t_3 period (Fig. 1(b)). Using this 3D experiment, abundant through-space and through-bond homonuclear/heteronuclear correlation information can be extracted for structural studies on solids. Moreover, a 2D $^1\text{H}/^1\text{H}$ COSY-type spectrum, correlating the isotropic chemical shifts of protons bonded to neighboring carbons, can be extracted from the 3D spectrum for identifying the proximity of neighboring protons.

EXPERIMENTAL

Samples

Uniformly ^{13}C , ^{15}N labeled L-alanine was purchased from Isotec (Champaign, IL), while uniformly ^{13}C -labeled L-isoleucine sample was bought from Cambridge Isotope Laboratory (Andover, MA). All samples were used as received without any further purification.

NMR experiments

All NMR experiments were performed on an Agilent VNMRs 600 MHz solid-state NMR spectrometer under 60 kHz MAS using a triple-resonance 1.2 mm MAS probe (Agilent) operating at 599.8 MHz for ^1H and 150.8 MHz for ^{13}C . The 90° pulse length was $1.4\ \mu\text{s}$ on both ^1H and ^{13}C RF channels. Ramped-CP⁷³ was used for transferring magnetization from ^1H to ^{13}C and from ^{13}C to ^1H . 110 kHz of $\omega_{1\text{H}}$ (20% ramp) was matched to $\omega_{1\text{C}}$ (170 kHz) $-w_r$ (60 kHz) which is the 1st lower side band of the Hartmann-Hahn matching condition. The 2D and 3D CTUC-based NMR pulse sequences used in this study are shown in Fig. 1. The through-bond 2D $^{13}\text{C}/^{13}\text{C}$ chemical shift correlation pulse sequence is the same as reported in the literature,³⁰ which is modified on the basis of a double-quantum-filtered (DQF) COSY experiment.^{74,75} Two π pulses are added in the sequence as refocusing pulses in order to get uniform-sign in-phase cross peaks for the doublet components in solids as in a liquid-state DQF COSY experiment.⁷⁶ A constant time (CT) evolution is

FIG. 1. Schematics of radio-frequency pulse sequences used in this study for (a) through-bond 2D $^{13}\text{C}/^{13}\text{C}$ constant-time uniform-sign cross-peak (CTUC) COSY and (b) 3D $^1\text{H}/^{13}\text{C}/^1\text{H}$ chemical shift correlation experiments. The solid and blank rectangles indicate the 90° and 180° pulses, respectively. Low-power continuous-wave RF decoupling was applied to protons during the constant time intervals and the z-filter periods. Phase cycling schemes for the ^{13}C pulses can be found elsewhere.³⁰ A rotor-synchronized z-filter for a duration of ~ 1 ms before the ^{13}C signal detection (a), or second CP (b), was utilized to remove the residual magnetization on the transverse plane. ^{13}C decoupling can also be used during t_1 and t_3 to further enhance the proton spectral resolution.

applied in the indirect ^{13}C chemical shift evolution period (i.e., t_1 and t_2 in Figs. 1(a) and 1(b), respectively) for improving the resolution and sensitivity, which was originally introduced in solution NMR to achieve proton decoupling in the indirect dimension of a homonuclear correlation experiment.^{77,78} For the 3D experiment given in Fig. 1(b), proton chemical shift evolution is introduced right after the first ^1H 90° pulse. The contact time for the first CP is optimized to achieve the best overall signal enhancement for all carbons. After the first CP, the ^{13}C magnetization is flipped to the z-direction for storage, while phase-alternating HORROR sequence⁷⁹ is used to remove residual ^1H magnetization after first CP. Following this, the ^{13}C magnetization is flipped to the transverse plane for constant time chemical shift evolution modulated by ^{13}C – ^{13}C J -couplings, while the ^{13}C – ^{13}C dipolar couplings are completely averaged out by ultrafast-MAS and ^1H – ^{13}C dipolar couplings are suppressed by a low power CW RF irradiation. Finally, the transverse ^{13}C magnetization is transferred to protons using a short contact-time CP for detection. If needed, ^{13}C decoupling could be applied during t_1 and t_3 periods to further enhance the proton spectral resolution. For both

pulse sequences given in Fig. 1, a low power (~ 20 kHz) CW irradiation was used for heteronuclear decoupling during the constant time intervals as well as during the z-filter periods.

RESULTS AND DISCUSSION

The through-bond COSY experiment has always been a valuable experiment both in solution and solid-state NMR for unambiguous sequential signal assignment, where the correlations between only those nuclei that are one or two bonds apart are visible in the spectrum. The 2D $^{13}\text{C}/^{13}\text{C}$ COSY experiments enable sequential assignment, while 2D $^1\text{H}/^1\text{H}$ COSY-type experiment is useful for identifying proton spins that are close to each other, especially when there is extensive overlap of proton peaks. In this study, we first revisit and demonstrate the feasibility of a through-bond 2D $^{13}\text{C}/^{13}\text{C}$ CTUC COSY experiment³⁰ under ultrafast MAS. Furthermore, as demonstrated below, by utilizing the magnetization evolution under through-bond ^{13}C – ^{13}C J -coupling as a filter, chemical shift correlation between

FIG. 2. (a) 2D $^{13}\text{C}/^{13}\text{C}$ through-bond correlation spectrum of a uniformly ^{13}C , ^{15}N -labeled L-alanine powder sample obtained using the CTUC COSY experiment (Fig. 1(a)) under 60 kHz MAS. 2D F2/F1 ($^{13}\text{C}/^1\text{H}$) (b), F2/F3 ($^{13}\text{C}/^1\text{H}$) (c), and F1/F3 ($^1\text{H}/^1\text{H}$) (d) chemical shift correlation spectra obtained through skyline projection from the 3D $^1\text{H}/^{13}\text{C}/^1\text{H}$ chemical shift correlation experiment (Fig. 1(b)). The τ delay in the pulse sequence was set to 3 ms for J -coupling evolution.

FIG. 3. 2D $^1\text{H}/^1\text{H}$ chemical shift correlation (F1/F3) spectra sliced at the ^{13}C chemical shift frequency of (a) CH_3 , (b) CH , and (c) COOH groups. The COSY-type correlations among protons are indicated with dashed lines in blue color.

those protons that are bonded to neighboring carbons can also be obtained.

The performances of the pulse sequences given in Fig. 1 were first demonstrated on a uniformly ^{13}C , ^{15}N -labeled L-alanine powder sample, and the results are given in Fig. 2. Fig. 2(a) is the through-bond $^{13}\text{C}/^{13}\text{C}$ chemical shift correlation spectrum obtained using the pulse sequence shown in Fig. 1(a), where only bonded ^{13}C – ^{13}C pairs are correlated as indicated; in contrast, there is no correlation between COOH and CH_3 groups due to the rather weak ^{13}C – ^{13}C scalar coupling between the carbons of these two groups. In this rigid solid sample, ^{13}C – ^{13}C dipolar couplings are completely averaged out by ultrafast-MAS, while ^{13}C – ^1H heteronuclear dipolar couplings are efficiently suppressed by the low-power CW decoupling of protons under ultrafast MAS. Therefore, the constant-time chemical shift evolution in t_1 is only modulated by through-bond ^{13}C – ^{13}C J couplings. It is worth mentioning here that a low-power RF field strength (~ 20 kHz) is quite sufficient for efficient heteronuclear ^{13}C – ^1H decoupling as previously reported,³⁸ in contrast to the very high-power

decoupling (~ 150 kHz) used for experiments under 25 kHz MAS.³⁰

The 3D $^1\text{H}/^{13}\text{C}/^1\text{H}$ chemical shift correlation experiment (Fig. 1(b)) was performed on alanine. The 2D projections extracted from the 3D $^1\text{H}/^{13}\text{C}/^1\text{H}$ spectrum are given in Fig. 2. The 2D F2/F1 ($^{13}\text{C}/^1\text{H}$) spectrum (Fig. 2(b)) exhibits a total correlation of all ^1H and ^{13}C nuclei because of the use of a long contact time for the first CP. However, it can be used to probe the heteronuclear proximity by adjusting the contact time of the first CP. On the other hand, only a pair of cross peaks between CH_3 and CH groups is observed in the 2D F2/F3 ($^{13}\text{C}/^1\text{H}$) spectrum (Fig. 2(c)) because of the use of a short contact-time for the second CP before proton detection. Both spectra (shown in Figs. 2(b) and 2(c)) exhibit through-bond magnetization exchange between carbons of CH and CH_3 groups. Such a pair of cross peaks indicates covalent bonding of two corresponding ^{13}C atoms as well as the proximity of protons bonded to these specific ^{13}C atoms. As expected, there is a ^{13}C – ^1H cross peak between carbon of COOH and proton of the CH group in Fig. 2(c), because there

FIG. 4. Through-bond 2D $^{13}\text{C}/^{13}\text{C}$ CTUC COSY spectrum of a uniformly ^{13}C -labeled L-isoleucine powder sample obtained under 60 kHz MAS: the full spectrum (a) and the expanded dashed-red-line rectangle region (b). The molecular structure of isoleucine is shown at the top. The τ delay was set to 2 ms for the J -coupling evolution. An exponential line broadening of 100 Hz was applied in both dimensions of the 2D spectrum. The green dashed circles (right) indicate cross peaks between two low-field CH_3 groups and the bonded CH_β group.

is through-bond magnetization exchange between carbons of COOH and CH groups, while only the proton signal of CH group is detected in the t_3 period due to the short contact-time used in the second CP. Therefore, the 2D F2/F3 ($^{13}\text{C}/^1\text{H}$) spectrum actually provides ^{13}C – ^{13}C and ^1H – ^{13}C bonding information, where there is a symmetrical correlation pattern as indicated in Fig. 2(c), and the ^{13}C – ^{13}C and ^{13}C – ^1H bonded pairs are indicated by their chemical shift values along ^{13}C and ^1H dimensions. In addition, as shown in the 2D F1/F3 ($^1\text{H}/^1\text{H}$) spectrum (Fig. 2(d)), we observed a pair of cross peaks among the neighboring protons from CH and CH_3 groups. Indeed, only protons from the two neighboring carbons exhibit a symmetric correlation pattern as indicated, similar to the COSY-type correlation, because the $^1\text{H}/^1\text{H}$ correlation arises from the through-bond correlation of the related bonded carbons. In contrast, there is only a single cross peak corresponding to the correlation between protons of CH and COOH groups due to the absence of proton signal from the COOH group in the detection (t_3) period.

Valuable information about proton-proton proximity could be extracted from 2D $^1\text{H}/^1\text{H}$ (F1/F3) chemical shift correlation spectra sliced at different ^{13}C chemical shift values in the 3D spectrum (as shown in Fig. 3), because each 2D slice provides a correlation among only those protons that are relevant to the chosen carbons. Since CH and CH_3 group are bonded, we could see a pair of COSY-type proton correlation peaks in the 2D F1/F3 spectrum sliced at the ^{13}C chemical shift frequency of CH_3 (Fig. 3(a)) or CH (Fig. 3(b)) group. The other cross peaks in Figs. 3(a) and 3(b) arise from the initial proton magnetization of NH_3^+ group to carbons of CH and CH_3 groups due to the long contact-time used for the first CP, or via the J -coupling-based magnetization exchange between CH and COOH groups. In contrast, there is no COSY-type correlation observed in the 2D $^1\text{H}/^1\text{H}$ (F1/F3) spectrum sliced

at the ^{13}C chemical shift frequency of the COOH group (Fig. 3(c)). But there are J -coupling based magnetization exchange between carbons of COOH and CH groups, so there is a cross peak between protons of the CH and COOH group; the other two cross peaks come from the proton magnetization mixing during the first long contact-time CP.

The performances of the pulse sequences are also demonstrated on a uniformly ^{13}C -labeled L-isoleucine sample. The through-bond 2D $^{13}\text{C}/^{13}\text{C}$ chemical shift correlation spectrum is given in Fig. 4, where the sequential backbone ^{13}C resonances can be easily assigned. In particular, there are three ^{13}C peaks for the CH_3 group in the up-field region, which arise from the different tautomers (*trans* and *gauche*).⁸⁰ Through this COSY-type 2D $^{13}\text{C}/^{13}\text{C}$ correlation experiment, the two low-field CH_3 peaks are assigned to the CH_3 group bonded to the CH_β group, while the high-field one is assigned to the CH_3 group bonded to the CH_2 group as shown in Fig. 4(b).

The 3D $^1\text{H}/^{13}\text{C}/^1\text{H}$ chemical shift correlation experiment was also performed on the U- ^{13}C -L-isoleucine sample; the 2D projections obtained from the 3D spectrum are given in Fig. 5. Due to the severe overlap of aliphatic protons peaks, only two peaks are resolved as given in Fig. 5(a) (the amino proton peak is not shown), where the low-field peak is assigned to the proton of the CH_α group, while the high-field peak is assigned to protons from CH_2 , CH_3 , and CH_β groups.⁸¹ As expected, there is a pair of COSY-type correlation peaks in the 2D F1/F3 ($^1\text{H}/^1\text{H}$) spectrum (Fig. 5(a)) due to the chemical bonding between CH_α and CH_β groups. The 2D F1/F2 ($^1\text{H}/^{13}\text{C}$) spectrum shown in Fig. 5(b) gives a total correlation among all protons and carbons due to the magnetization mixing during the long contact-time CP as well as the J -coupling-based ^{13}C magnetization exchange. In contrast, the 2D F3/F2 ($^1\text{H}/^{13}\text{C}$) spectrum shown in Fig. 5(c) is a 2D $^{13}\text{C}/^1\text{H}$ HETCOR (heteronuclear correlation) spectrum

FIG. 5. 2D projections extracted from the 3D $^1\text{H}/^{13}\text{C}/^1\text{H}$ spectrum of a powder sample of U- ^{13}C -L-isoleucine under 60 kHz MAS: (a) $^1\text{H}/^1\text{H}$ (F1/F3), (b) $^1\text{H}/^{13}\text{C}$ (F1/F2), and (c) $^{13}\text{C}/^1\text{H}$ (F3/F2) chemical shift correlation spectra shown with 1D projections at the top. τ was set to 2 ms for the J -coupling evolution. The cross peaks indicated by green circles arise from different polarization transfer mechanisms as explained in the main text. An exponential broadening of 100 and 20 Hz was applied on ^{13}C and ^1H dimensions, respectively, when processing the 3D spectrum. The spectral resolution on the ^{13}C dimension is not as good as the one shown in Fig. 4 as we only acquired 32 complex points with a dwell time of 27.7 μs along the F2 dimension.

FIG. 6. 2D $^1\text{H}/^1\text{H}$ chemical shift correlation (F1/F3) spectra sliced at the ^{13}C chemical shift frequency of (a) CH_2 , (b) CH_α , and (c) CH_β groups. The COSY-type correlations among protons are indicated with dashed blue lines.

where there are symmetrical ^{13}C – ^1H cross peaks between the two neighboring aliphatic groups like in the case of alanine (Fig. 2(c)), such as CH_β and CH_α groups, CH_β and CH_2 groups, CH_β and $^2\text{CH}_3$ groups, and CH_2 and $^1\text{CH}_3$ groups. However, they are not as clear as the case of alanine (Fig. 2(c)) due to a severe overlap of proton peaks. An interesting difference is also noticed in the cross peak (indicated by green dashed circles) between the carbonyl carbon and the aliphatic proton in 2D F1/F2 and F3/F2 spectra. In the 2D F1/F2 ($^1\text{H}/^{13}\text{C}$) spectrum, this cross peak is due to a long contact time CP used to transfer magnetization from ^1H to ^{13}C , and it suggests that the ^{13}C polarization of COOH mainly comes from protons resonating at a chemical shift of ~ 0.9 ppm, corresponding to the protons of CH_2 , CH_3 , and CH_β groups. In contrast, in the 2D F3/F2 ($^1\text{H}/^{13}\text{C}$) spectrum, this cross peak arises from the J -coupling-based ^{13}C magnetization exchange between COOH and CH_α groups, as the final detected proton signals directly come from the bonded carbons due to the use of a short contact-time CP.

Similarly, by taking 2D F1/F3 slices at different ^{13}C chemical shift values, we could obtain 2D $^1\text{H}/^1\text{H}$ chemical shift correlation spectra among protons bonded to neighboring carbons as shown in Fig. 6. By taking the slice at the chemical shift of CH_2 group, we did not observe any cross peaks, because all the protons of the chemical groups bonded to CH_2 group ($^1\text{CH}_3$ and CH_β) are all overlapped within the peak ~ 0.9 ppm. In contrast, the 2D F1/F3 slices extracted at the ^{13}C chemical shift frequencies of CH_α and CH_β show symmetric COSY-type correlations arising from through-bond magnetization exchange between carbons of CH_α and CH_β groups. The 2D $^1\text{H}/^1\text{H}$ correlation of CH_β with CH_2 and CH_3 is all overlapped with the peak ~ 0.9 ppm, and therefore no cross peaks among them could be observed.

For the 2D CTUC COSY experiment, it is important to evaluate the evolution time τ for an optimum performance. In principle, the evolution time is generally set as $\frac{1}{4}J_{\text{CC}}$, where J_{CC} is the scalar coupling for the two directly bonded carbons, e.g., C1–C2. Under this condition, the intensity of cross peaks between C1 and C2 is expected to reach a maximum. However, the loss of transverse magnetization due to T_2 relaxation, a variation in ^{13}C – ^{13}C scalar couplings (30–60 Hz), and the presence of scalar couplings to multiple nearby carbons can complicate the overall J coupling evolution, and therefore

an optimum value for τ could deviate from the theoretical value.^{28,76} Indeed, the choice of τ value is a trade-off between spectral resolution along the indirect ^{13}C chemical shift dimension and the peak intensities when multiple types of spin systems are present. As demonstrated in this study, a τ duration between 2 and 3 ms is sufficient to observe all the through-bond ^{13}C – ^{13}C cross peaks in a rigid solid under ultrafast-MAS. Furthermore, the successful implementation of the 3D $^1\text{H}/^{13}\text{C}/^1\text{H}$ experiment actually relies on the proton spectral resolution. Spin dilution by deuteration or increasing the MAS speed could enhance the observed proton spectral resolution, which would be helpful to obtain accurate $^1\text{H}/^1\text{H}$ correlation information. Recent studies have reported ~ 0.9 ppm linewidth for ^1H resonances even for microcrystalline biological sample under 80 kHz MAS,⁴⁹ while it can be reduced to ~ 20 Hz with an optimum level of deuteration in combination.⁸² If sample and experimental conditions can be optimized to obtain high resolution proton spectra, then the 3D $^1\text{H}/^{13}\text{C}/^1\text{H}$ experiment could be reduced to 2D $^1\text{H}/^1\text{H}$ correlation experiment by ignoring the indirect ^{13}C dimension. In such case, a COSY-type $^1\text{H}/^1\text{H}$ chemical shift correlation spectrum could be easily obtained. Furthermore, the 2D F1/F3 ($^1\text{H}/^1\text{H}$) slices taken at different ^{13}C chemical shift values could also be used for proton resonance assignment, as they only contain the information of the protons close to the carbons at the chosen chemical shift value.

CONCLUSION

In this study, we have successfully demonstrated the application of through-bond ^{13}C – ^{13}C CTUC COSY experiment under ultrafast-MAS conditions. The use of ultrafast-MAS enables a low RF power (~ 20 kHz) proton decoupling during the constant time J coupling evolution, which is beneficial for heat-sensitive samples, such as membrane proteins or other salt-containing samples. In contrast, a strong RF field strength up to 150 kHz often needs to be applied for a CTUC COSY experiment under 25 kHz MAS. Furthermore, the ultrafast-MAS significantly increased the T_2 relaxation time, which remarkably reduced the signal loss during the J coupling evolution period. Although T_2 relaxation could still reduce the signal sensitivity for rigid solids, a shortened J coupling evolution time (2–3 ms) is

sufficient for the observation of through-bond correlations as demonstrated in this study. Finally, by incorporating the chemical shift evolution into the CTUC-COSY pulse sequence, a three 3D $^1\text{H}/^{13}\text{C}/^1\text{H}$ chemical shift correlation experiment is demonstrated, which enables the determination of the proximity between carbon and nearby protons. The 2D $^1\text{H}/^1\text{H}$ chemical shift correlation spectra extracted at different ^{13}C chemical shift frequencies provide information about the proximity of protons close to each of the chosen carbons, which could also be used for proton resonance assignment. Therefore, the proposed 3D method can also be used to simplify the generally crowded spectra of crystalline solids. However, line-broadening due to conformational heterogeneity, as observed for biological solids such as membrane proteins embedded in fluid lipid bilayers, may limit the application of the proposed technique.

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