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Performance of RINEPT is Amplified by Dipolar Couplings Under Ultrafast MAS Conditions

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Abstract

The refocused *insensitive nuclei enhanced by polarization transfer* (RINEPT) technique is commonly used for heteronuclear polarization transfer in solution and solid-state NMR spectroscopy. Suppression of dipolar couplings, either by fast molecular motions in solution or by a combination of MAS and multiple pulse sequences in solids, enables the polarization transfer via scalar couplings. However, the presence of unsuppressed dipolar couplings could alter the functioning of RINEPT, particularly under fast/ultrafast MAS conditions. In this study, we demonstrate, through experiments on rigid solids complemented by numerical simulations, that the polarization transfer efficiency of RINEPT is dependent on the MAS frequency. In addition, we show that heteronuclear dipolar coupling is the dominant factor in the polarization transfer, which is strengthened by the presence of ¹H-¹H dipolar couplings. In fact, the simultaneous presence of homonuclear and heteronuclear dipolar couplings is the premise for the polarization transfer by RINEPT, whereas the scalar coupling plays an insignificant role under ultrafast MAS conditions on rigid solids. Our results additionally reveal that the polarization transfer efficiency decreases with the increasing duration of RF pulses used in the RINEPT sequence.

Keywords

Ultrafast MAS; Polarization Transfer; RINEPT; Solid-State NMR; Dipolar Couplings

Introduction

While magic angle spinning (MAS) solid-state NMR spectroscopy has been highly valuable for the atomic-level characterization of a variety of non-soluble and non-crystalline solids [1–3], recent technical advances have swiftly expanded its range of applications [4–9]. In particular, the development of ultrafast MAS techniques has initiated numerous exciting

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discoveries [10–23]. One of the most obvious advantages of performing NMR experiments at ultrafast MAS speeds is the considerable improvement in spectral resolution, especially in proton-detected experiments [14, 21, 24–33]. Due to the efficient averaging of ^1H - ^1H dipolar couplings by ultrafast MAS, and with appropriate deuteration and the utilization of proton-detection, ultrafast MAS NMR spectroscopy is becoming a powerful approach in the structural studies of biomolecules [25, 26, 29, 32, 34]. It is reported that the line width of ^{13}C peaks decreases with increasing MAS frequency in uniformly ^{13}C -labeled samples [35]. In addition, the use of ultrafast MAS frequencies makes it feasible to successfully implement pulse sequences with a low radio-frequency (RF) power for heteronuclear cross-polarization (CP) [36–38] and heteronuclear decoupling [17, 35, 39–43]; this is a significant benefit for heat-sensitive samples such as membrane proteins and hydrated proteins with high salt content [44]. It has been experimentally demonstrated that low-power double-quantum (DQ) CP ($w_{1\text{H}}+w_{1\text{C}}=nw_{\text{r}}$) and second-order CP ($w_{1\text{H}}=w_{1\text{C}}$) yield high magnetization transfer efficiency comparable to the traditional high-power zero-quantum CP ($w_{1\text{H}}-w_{1\text{C}}=nw_{\text{r}}$) [38]; low-power CP can additionally be used as a filter for selective heteronuclear polarization transfer under ultrafast MAS conditions [37, 38, 45–48]. Moreover, recent ultrafast MAS studies have shown that low-power RF irradiation can also be effectively used to achieve higher proton decoupling efficiency than the traditional high-power decoupling sequences such as two-pulse phase modulation (TPPM), X-inverse-X (XiX), small phase incremental alternation with 64 steps (SPINAL-64), phase-inverted super-cycled sequence for attenuation of rotary resonance (PISSARRO) [17, 39, 40, 42, 43, 49].

The RINEPT (refocused insensitive nuclei enhanced by polarization transfer) pulse sequence is one of the most widely used techniques to enhance the NMR sensitivity of low- γ nuclei on the basis of heteronuclear scalar couplings in solution NMR [50, 51], and also in solid-state NMR studies [52]. INEPT and refocused-INEPT (RINEPT) pulse sequences have been frequently utilized in the development of numerous sophisticated RF pulse sequences for solution NMR studies [53], and their theoretical analyses are well understood [54, 55]. RINEPT experiments are also utilized in solid-state NMR studies on oriented lipid bilayers containing a membrane protein [56, 57]. The presence of strong ^1H - ^1H dipolar couplings in rigid solids often results in the failure of RINEPT polarization transfer from protons due to the fast decay of proton transverse magnetization, which necessitates the use of a combination of a homonuclear dipolar decoupling sequence and MAS [10, 58]. Since the loss of transverse magnetization is dominated by ^1H - ^1H dipolar couplings, the corresponding T_2 values and the RINEPT efficiency are expected to increase with the MAS frequency. Thus, the use of ultrafast MAS has enabled the development of through-bond heteronuclear correlation experiments on solids [10, 11, 13, 14]. A recent study by Yarger and co-workers pointed out that the polarization transfer mechanism at a spinning speed of 60 kHz appears to be primarily dipolar in nature [52]. Here, we report a systematic investigation of the polarization transfer efficiency of the RINEPT sequence under ultrafast MAS conditions. Experimental results obtained from powder samples of uniformly- ^{13}C -labeled glycine and $^{13}\text{C}_\alpha$ -leucine are presented, and are corroborated with numerical simulations. We first examined the ^1H to ^{13}C polarization transfer efficiency of RINEPT at different spinning speeds, followed by a thorough investigation of the contributions by

heteronuclear dipolar couplings, scalar couplings and homonuclear dipolar couplings to the polarization transfer mechanism. We also report on the dependence of RINEPT's efficiency on the duration of RF pulses used in the sequence.

Experiments and Simulations

Samples

Uniformly ^{13}C -labeled glycine and $^{13}\text{C}_\alpha$ -labeled L-leucine samples were purchased from Cambridge Isotope Laboratory (Andover, MA) and used as received without any further purification.

Solid-State NMR

All NMR experiments were performed on an Agilent VNMRs 600MHz solid-state NMR spectrometer operating at 599.8 MHz frequency for ^1H and 150.8 MHz for ^{13}C , and equipped with a 1.2 mm triple-resonance ultrafast MAS probe. The RINEPT pulse sequence used in this study is very similar to that used in solution NMR experiments [59], with each τ delay synchronized with the MAS rotor period, as shown in Figure 1. The 90° pulse width was 1.2 μs on both ^1H and ^{13}C RF channels. Proton decoupling during ^{13}C signal acquisition was achieved using the SPINAL-64 sequence [60] with an RF field strength of about 45 kHz. The ^{13}C chemical shift was externally referenced to adamantane by setting the downfield ^{13}C resonance signal to 38.5 ppm.

Spin Dynamics Simulations

All simulations were carried out using the SPINEVOLUTION software [61]. Three spin-1/2 nuclei were used for simulations of the CH_2 group of a uniformly ^{13}C -labeled glycine. The ^1H - ^1H and ^{13}C - ^1H dipolar couplings were calculated according to the atomic coordinates used in the simulation, and the ^{13}C - ^1H scalar couplings were set to 145 Hz. The scalar coupling between protons was set to zero in all simulations. For the CH group, we adopted the C_αH group of leucine amino acid, and included two other proton spins with the coordinates of the neighboring CH_2 group in the leucine molecule.

Results and Discussion

In this study, we systematically investigated the heteronuclear polarization transfer efficiency of the RINEPT RF pulse sequence at different MAS speeds as well as the influence of both heteronuclear and homonuclear dipolar couplings and scalar couplings. Results obtained from experiments and spin dynamics simulations on the CH_2 group of glycine and the C_αH group of leucine are discussed below.

Polarization transfer efficiency of RINEPT is MAS frequency-dependent

Compared to previous RINEPT experiments in solid-state NMR [10, 13], no ^1H - ^1H decoupling was used during both the evolution (τ_1) and refocusing (τ_2) periods in the RINEPT experiments presented in this study. Therefore, the suppression of homonuclear ^1H - ^1H dipolar couplings is solely due to MAS. The lifetime of the transverse magnetization in RINEPT, which is determined by both the residual dipolar couplings under

MAS and the spinning speed, greatly influences the heteronuclear polarization transfer efficiency of the pulse sequence. Therefore, it would be interesting to observe the polarization transfer efficiency of the RINEPT sequence as a function of MAS speed. RINEPT experiments were carried out on powder samples of U- ^{13}C -labeled glycine and $^{13}\text{C}_\alpha$ -labeled leucine at 40, 50 and 60 kHz MAS to measure the transfer of ^1H magnetization to ^{13}C nuclei. Since the ^{13}C spectra acquired with 40 kHz MAS speed exhibited very poor signal-to-noise ratios, only the results obtained from 50 and 60 kHz MAS are shown in Figure 2. The experimental results reveal a number of interesting features of the RINEPT sequence under ultrafast MAS. Firstly, the observed rates of polarization transfer are very fast as compared to that obtained from the regular J -based RINEPT sequence. The polarization transfer reaches the maximum around 0.1 ms (Figure 2). Secondly, the rate of polarization transfer is dependent on the spinning speed of the sample: about 7- and 4-fold- decreases in the RINEPT efficiency were observed respectively for the CH_2 group of glycine and the C_αH group of leucine for a decrease in the MAS rate from 60 to 50 kHz. These experimental observations suggest that the mechanism of polarization transfer from ^1H to ^{13}C nuclei operating under ultrafast MAS on rigid solids should be very different from that in liquids or semi-solids. While ultrafast MAS (like 50 or 60 kHz) is expected to average the inhomogeneous dipolar couplings between ^1H and ^{13}C nuclei, the presence of very strong ^1H - ^1H dipolar couplings in rigid solids plays an important role in the efficiency of the RINEPT sequence, as qualitatively explained below. Since ultrafast MAS does not fully suppress the ^1H - ^1H dipolar couplings, the interference between the ^1H - ^1H and ^1H - ^{13}C dipolar couplings prevents the complete averaging of ^1H - ^{13}C dipolar couplings by MAS. In other words, the effective Hamiltonian - comprising homo- and hetero-nuclear dipolar couplings - does not commute with itself at different times of the rotor period. Therefore, the presence of residual dipolar couplings enables the above-mentioned spinning speed dependent fast polarization transfer from ^1H to ^{13}C nuclei. The experimentally observed RINEPT polarization transfer efficiency is higher at 60 kHz than at 50 kHz; the difference is larger for the CH_2 group (7-fold) than that for the CH group (4-fold) (Figure 2). This difference could be attributed to the strong ^1H - ^1H dipolar couplings associated with the CH_2 group as discussed below using results obtained from numerical simulations.

To investigate the role of spin-spin relaxation time (T_2) on this large difference between the polarization transfer efficiencies of RINEPT at two different spinning speeds, the ^1H and ^{13}C T_2 values were measured, and are listed in Table 1. Although faster MAS can suppress the spin-spin relaxation processes, the difference in the experimentally measured T_2 values (Table 1) between 50 and 60 kHz MAS is only moderate. The RINEPT ^{13}C signal intensity obtained at 60 kHz is much larger than that at 50 kHz even if a correction factor for T_2 is taken into account; which can be performed by multiplying the experimental data by the factor $\exp(2\pi/T_2^{\text{H}})\exp(2\pi/T_2^{\text{C}})$ (results not included). Therefore, the difference in the spin-spin relaxation alone cannot explain the observed spinning speed dependent efficiency of RINEPT. Therefore, to better understand the MAS-dependent efficiency of the RINEPT sequence, spin dynamics simulations were carried out on a three-spin-1/2 system mimicking the CH_2 group of glycine, and the results are shown in Figure 3(A and B). The simulated results shown in Figure 3(A and B) clearly reveal the advantage of the higher spinning speed for the polarization transfer; simulated results for 60 and 100 kHz are compared in Figure S1

of the Supporting Information. However, when the magnitude of the ^1H - ^1H dipolar coupling is reduced, as shown in Figure 3(C and D), the polarization transfer at the lower spinning speed is better than that obtained at the higher spinning speed. There are two main discrepancies between the experimental (Figure 2) and simulated (Figure 3) results. First, the simulations show that the maximum transfer occurs around 0.2 ms, rather than at 0.1 ms as determined by experiments (Figure 2). Second, unlike the experimental results shown in Figure 2, the simulations show a moderate MAS rate dependent difference in the polarization transfer (Figures 3 and S1). These differences between the simulated and experimental results may be attributed to the fact that T_2 effects are not included in the simulation.

Effect of ^1H - ^{13}C dipolar coupling on RINEPT

Spin dynamics simulations were carried out to understand the effect of ^{13}C - ^1H dipolar, ^1H - ^1H dipolar and ^{13}C - ^1H scalar couplings on the polarization transfer efficiency of RINEPT. As mentioned above, a three-spin CH_2 model was used in simulations. The ^1H to ^{13}C magnetization transfer by RINEPT was calculated as a function of rotor-synchronized τ delays for various ^{13}C - ^1H dipolar coupling magnitudes, and the corresponding results are given in Figure 4. It is apparent from Figure 4 that for short τ_1 and τ_2 values, the polarization transfer efficiency of RINEPT increases with increasing ^{13}C - ^1H dipolar coupling value. For a constant τ_2 value, the rates of magnetization transfer (or the build-up rates) are similar when the ^{13}C - ^1H dipolar coupling strength is above 60% of the rigid-limit value. On the other hand, for a constant evolution time (τ_1), the transfer efficiency as a function of the refocusing delay (τ_2) gradually increases with increasing ^{13}C - ^1H dipolar coupling strength. As also shown in Figure 4, the stronger the dipolar coupling value, the faster are the maximum and decay of the transferred ^{13}C signal intensity. Notably, there is almost no polarization transfer when the ^{13}C - ^1H dipolar coupling is set to zero. These results indicate that the polarization transfer by RINEPT originates from the dipolar couplings, rather than from the scalar coupling, in rigid solids under fast MAS conditions.

Effect of ^1H - ^1H dipolar coupling on RINEPT

The effect of ^1H - ^1H dipolar couplings on the polarization transfer efficiency of the RINEPT sequence under ultrafast MAS was investigated using spin dynamics simulations; the simulated results are shown in Figure 5. For a constant τ_2 value, the RINEPT efficiency is similar to that shown in Figure 4A; where the efficiencies are similar when the ^1H - ^1H dipolar coupling is above 60% of the rigid-limit value. In contrast, for a constant τ_1 value, the transfer efficiency builds up at a faster rate with increasing ^1H - ^1H dipolar coupling and reaches a maximum at a shorter evolution time. In addition, there is almost no polarization transfer when the ^1H - ^1H dipolar coupling was set to zero. It must be noted that the small amplitude oscillations seen in Figure 5 for small (or no) ^1H - ^1H dipolar couplings may be due to the interference of the spinning speed with the ^1H - ^{13}C dipolar coupling, and that these oscillations disappear at a higher spinning speed (Figure S1). Based on the results presented in Figures 4 and 5, it can be concluded that an efficient polarization transfer by RINEPT requires the simultaneous presence of homonuclear and heteronuclear dipolar couplings.

As shown from our experimental results in Figure 6, the intensity of the ^{13}C peak observed around 176 ppm for the carboxyl group is very weak as compared to that of the CH_2 group in the uniformly ^{13}C -labeled glycine. Since very short evolution ($\tau_1 = 0.1$ ms) and refocusing ($\tau_2 = 0.1$ ms) delays were used in the RINEPT sequence, the appearance of the weak ^{13}C carboxyl peak must be due to the transfer of magnetization from ^1H to ^{13}C via the remote ^1H - ^{13}C dipolar couplings, and not due to ^1H - ^{13}C scalar couplings. This observation implies that the ^1H to ^{13}C polarization transfer is dominated by the dipolar couplings between the directly bonded ^{13}C and ^1H nuclei. This could be confirmed by spin dynamics simulations as shown in Figure 6(B and C), where the effects of remote ^1H - ^1H and ^{13}C - ^1H dipolar couplings are examined. For comparison, we included another proton spin, which is located at the position of the NH_2 group in glycine, to specifically examine the role of ^1H - ^1H and ^{13}C - ^1H dipolar couplings associated with this remote proton spin. For a constant τ_2 value, when the remote ^1H - ^1H dipolar coupling was set to zero (while the ^1H - ^1H dipolar coupling between the CH_2 protons is still present), the RINEPT buildup curve for the four-spin system is exactly the same as the three-spin system (as shown in Figure 6(B and C)). This result suggests that the weak ^{13}C - ^1H dipolar coupling with the remote proton spin alone does not change the polarization transfer process observed for the CH_2 group (or for the two-spin C-H system discussed above). However, when ^1H - ^1H dipolar coupling with the remote proton spin is turned on, and with or without the weak ^{13}C - ^1H dipolar coupling with the remote proton spin, the four-spin system has the same RINEPT buildup rate as the three-spin system, but the efficiency begins to decay slightly earlier than the three-spin system. Likewise, for a constant τ_1 value, the four-spin system has very similar RINEPT buildup curve as the three-spin system, as shown in Figure 6(B and C). All in all, these results suggest that the remote protons do not play any significant role in the polarization transfer by the RINEPT sequence.

Furthermore, as mentioned above, the polarization transfer requires the mediation of ^1H - ^1H dipolar couplings, as the ^{13}C - ^1H dipolar coupling alone is insufficient to enable the polarization transfer. Hence, for an isolated CH spin system, if there are no other protons, the RINEPT polarization transfer will only depend on the scalar couplings, rendering the transfer efficiency very low at short evolution times under ultrafast MAS conditions.

Effect of ^1H - ^{13}C scalar coupling on RINEPT

Although under ultrafast MAS, the dipolar coupling based polarization transfer has a higher efficiency than the scalar-coupling-based transfer, it is still necessary to investigate whether the scalar coupling affects the polarization transfer process. As shown in Figure 7(A and B) for the CH_2 group, where there are strong ^{13}C - ^1H and ^1H - ^1H dipolar couplings, the weak ^{13}C - ^1H scalar couplings obviously have no influence in the polarization transfer process for short evolution times. Scalar couplings, however, could contribute to the polarization process at long evolution times (>1 ms). On the other hand, for the weak ^1H - ^1H dipolar coupling system, the dipolar-coupling-based RINEPT transfer efficiency is much lower, which allows the scalar coupling to play an obvious role even for a short evolution time as shown in Figure 7(C and D). It is important to note that it is necessary to include some nearby proton spins in the spin dynamics simulations for the ^{13}CH group in order to observe the dipolar-coupling-based polarization transfer, which again confirms that ^{13}C - ^1H

heteronuclear dipolar coupling alone is not sufficient to enable polarization transfer, and further suggests that the interference between the Hamiltonians of homonuclear and heteronuclear dipolar couplings is indispensable for the polarization transfer to occur. Overall, scalar couplings essentially have no effect on the dipolar-coupling-based RINEPT polarization transfer in rigid solids under ultrafast MAS conditions.

Effect of the pulse width on RINEPT efficiency

Although a very high RF field can be generated for ultrafast MAS probes, there are several disadvantages with the use of such very high fields. The RF-field induced sample heating can denature heat-sensitive samples like membrane proteins. In addition, the use of paramagnetic doping to shorten the spin-lattice relaxation times can enhance sample heating if high power RF fields are used. Therefore, it is important to examine the efficiency of the RINEPT sequence with respect to the RF field strength. For this purpose, we have carried out experiments and simulations to determine the role of the RF field strength – by using different pulse widths for the pulses used in the RINEPT sequence – and the results are shown in Figure 8. The results show that the RINEPT efficiency decreases with decreasing RF field strength. It is worth noting that the transfer efficiency almost reaches the maximum when the 90° pulse duration is 1 μ s, which means that higher RF fields beyond 250 kHz may not offer additional benefits for the RINEPT experiments.

Conclusion

INEPT and refocused-INEPT (or RINEPT) are the simplest and most robust forms of scalar-coupling-based polarization transfer pulse sequence schemes that have been well utilized in the development of a plethora of multidimensional heteronuclear NMR techniques, which are commonly used in the structural studies of biomolecules in solution. Although RINEPT pulse sequences have been used in static and MAS solid-state NMR experiments, an in-depth investigation of the mechanism of the polarization transfer has been lacking, particularly under ultrafast MAS conditions. In this study, we have reported a systematic investigation of the polarization transfer efficiency of the RINEPT sequence under ultrafast MAS through experiments on powder model samples and spin dynamics simulations. Further theoretical analysis to fully understand the nature of the effective Hamiltonians operating during the evolution and refocusing delays of the RINEPT would provide better insights into the polarization transfer mechanism. Our results reveal that the RINEPT efficiency is dependent on the MAS speed and the T_2 relaxation time. In addition, we have shown that the heteronuclear polarization transfer under ultrafast MAS completely originates from homonuclear and heteronuclear dipolar couplings, and scalar couplings play an insignificant role when a short evolution time is used in RINEPT. Whereas the scalar coupling becomes significant only when a longer evolution time is used, the RINEPT efficiency is much lower than that observed for the dipolar coupling based polarization transfer at a short evolution time. Further, we have demonstrated that the RINEPT's efficiency increases with increasing RF field strength in the sequence, but the maximum efficiency is achieved when a 1 μ s 90° pulse length was utilized, a particularly important observation for studies on heat sensitive samples like membrane proteins. We believe that the findings reported in this study would pave the way for the development of

multidimensional solid-state NMR techniques centered upon the dipolar-coupling-based RINEPT sequence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Mehring, M.; Mehring, M.; Mehring, M.; Mehring, M. High resolution NMR spectroscopy in solids. Springer-Verlag; Berlin: 1976.
2. Schmidt-Rohr, K.; Spiess, HW. Multidimensional solid-state NMR and polymers. Academic Press; London: 1994.
3. Ramamoorthy, A. NMR spectroscopy of biological solids. CRC Press; 2010.
4. Tycko R. NMR at Low and Ultralow Temperatures. *Acc Chem Res.* 2013; 46:1923–1932. [PubMed: 23470028]
5. Bernd R. Ultra-high resolution in MAS solid-state NMR of perdeuterated proteins: Implications for structure and dynamics. *J Magn Reson.* 2012; 216:1–12. [PubMed: 22280934]
6. Asami S, Reif B. Proton-Detected Solid-State NMR Spectroscopy at Aliphatic Sites: Application to Crystalline Systems. *Acc Chem Res.* 2013; 46:2089–2097. [PubMed: 23745638]
7. Tang M, Comellas G, Rienstra CM. Advanced Solid-State NMR Approaches for Structure Determination of Membrane Proteins and Amyloid Fibrils. *Acc Chem Res.* 2013; 46:2080–2088. [PubMed: 23659727]
8. Watt ED, Rienstra CM. Recent Advances in Solid-State Nuclear Magnetic Resonance Techniques to Quantify Biomolecular Dynamics. *Anal Chem.* 2014; 86:58–64. [PubMed: 24313950]
9. Tycko R. Molecular structure of amyloid fibrils: insights from solid-state NMR. *Q Rev Biophys.* 2006; 39:1–55. [PubMed: 16772049]
10. Mao K, Pruski M. Directly and indirectly detected through-bond heteronuclear correlation solid-state NMR spectroscopy under fast MAS. *J Magn Reson.* 2009; 201:165–174. [PubMed: 19833538]
11. Mao K, Wiench JW, Lin VSY, Pruski M. Indirectly detected through-bond chemical shift correlation NMR spectroscopy in solids under fast MAS: Studies of organic-inorganic hybrid materials. *J Magn Reson.* 2009; 196:92–95. [PubMed: 18955001]
12. Mao K, Pruski M. Homonuclear dipolar decoupling under fast MAS: Resolution patterns and simple optimization strategy. *J Magn Reson.* 2010; 203:144–149. [PubMed: 20093059]
13. Althaus SM, Mao K, Stringer JA, Kobayashi T, Pruski M. Indirectly detected heteronuclear correlation solid-state NMR spectroscopy of naturally abundant ^{15}N nuclei. *Solid State Nucl Magn Reson.* 2013 in press.
14. Kobayashi T, Mao K, Paluch P, Nowak-Król A, Sniechowska J, Nishiyama Y, Gryko DT, Potrzebowski MJ, Pruski M. Study of Intermolecular Interactions in the Corrole Matrix by Solid-State NMR under 100 kHz MAS and Theoretical Calculations. *Angew Chem.* 2013; 125:14358–14361.
15. Parthasarathy S, Nishiyama Y, Ishii Y. Sensitivity and Resolution Enhanced Solid-State NMR for Paramagnetic Systems and Biomolecules under Very Fast Magic Angle Spinning. *Acc Chem Res.* 2013; 46:2127–2135. [PubMed: 23889329]

16. Wickramasinghe NP, Shaibat MA, Jones CR, Casabianca LB, de Dios AC, Harwood JS, Ishii Y. Progress in ^{13}C and ^1H solid-state nuclear magnetic resonance for paramagnetic systems under very fast magic angle spinning. *J Chem Phys*. 2008; 128:052210. [PubMed: 18266415]
17. Kotecha M, Wickramasinghe NP, Ishii Y. Efficient low-power heteronuclear decoupling in ^{13}C high-resolution solid-state NMR under fast magic angle spinning. *Magn Reson Chem*. 2007; 45:S221–S230.
18. Wickramasinghe NP, Shaibat MA, Ishii Y. Elucidating Connectivity and Metal-Binding Structures of Unlabeled Paramagnetic Complexes by ^{13}C and ^1H Solid-State NMR under Fast Magic Angle Spinning. *J Phys Chem B*. 2007; 111:9693–9696. [PubMed: 17661508]
19. Nishiyama Y, Lu X, Trébosc J, Lafon O, Gan Z, Madhu PK, Amoureux JP. Practical choice of ^1H – ^1H decoupling schemes in through-bond ^1H – $\{X\}$ HMQC experiments at ultra-fast MAS. *J Magn Reson*. 2012; 214:151–158. [PubMed: 22130518]
20. Paluch P, Pawlak T, Amoureux JP, Potrzebowski MJ. Simple and accurate determination of X-H distances under ultra-fast MAS NMR. *J Magn Reson*. 2013; 233:56–63. [PubMed: 23727588]
21. Ye YQ, Malon M, Martineau C, Taulelle F, Nishiyama Y. Rapid measurement of multidimensional ^1H solid-state NMR Spectra at ultra-fast MAS frequencies. *J Magn Reson*. 2014; 239:75–80. [PubMed: 24424008]
22. Nishiyama Y, Endo Y, Nemoto T, Utsumi H, Yamauchi K, Hioka K, Asakura T. Very fast magic angle spinning ^1H – ^{14}N 2D solid-state NMR: Sub-micro-liter sample data collection in a few minutes. *J Magn Reson*. 2011; 208:44–48. [PubMed: 21035366]
23. Nishiyama Y, Malon M, Gan Z, Endo Y, Nemoto T. Proton–nitrogen-14 overtone two-dimensional correlation NMR spectroscopy of solid-sample at very fast magic angle sample spinning. *J Magn Reson*. 2013; 230:160–164. [PubMed: 23542742]
24. Lu X, Lafon O, Trébosc J, Thankamony ASL, Nishiyama Y, Gan Z, Madhu PK, Amoureux JP. Detailed analysis of the TIMES and TIMES0 high-resolution MAS methods for high-resolution proton NMR. *J Magn Reson*. 2012; 223:219–227. [PubMed: 22985982]
25. Chevelkov V, van Rossum BJ, Castellani F, Rehbein K, Diehl A, Hohwy M, Steuernagel S, Engelke F, Oschkinat H, Reif B. ^1H Detection in MAS Solid-State NMR Spectroscopy of Biomacromolecules Employing Pulsed Field Gradients for Residual Solvent Suppression[†]. *J Am Chem Soc*. 2003; 125:7788–7789. [PubMed: 12822982]
26. Chevelkov V, Rehbein K, Diehl A, Reif B. Ultrahigh Resolution in Proton Solid-State NMR Spectroscopy at High Levels of Deuteration. *Angew Chem Int-Edit*. 2006; 45:3878–3881.
27. Linser R, Fink U, Reif B. Proton-detected scalar coupling based assignment strategies in MAS solid-state NMR spectroscopy applied to perdeuterated proteins. *J Magn Reson*. 2008; 193:89–93. [PubMed: 18462963]
28. Linser R, Dasari M, Hiller M, Higman V, Fink U, Lopez del Amo J-M, Markovic S, Handel L, Kessler B, Schmieder P, Oesterhelt D, Oschkinat H, Reif B. Proton-Detected Solid-State NMR Spectroscopy of Fibrillar and Membrane Proteins. *Angew Chem Int-Edit*. 2011; 50:4508–4512.
29. Zhou DH, Shah G, Cormos M, Mullen C, Sandoz D, Rienstra CM. Proton-Detected Solid-State NMR Spectroscopy of Fully Protonated Proteins at 40 kHz Magic-Angle Spinning. *J Am Chem Soc*. 2007; 129:11791–11801. [PubMed: 17725352]
30. Zhou DH, Shea JJ, Nieuwkoop AJ, Franks WT, Wylie BJ, Mullen C, Sandoz D, Rienstra CM. Solid-State Protein-Structure Determination with Proton-Detected Triple-Resonance 3D Magic-Angle-Spinning NMR Spectroscopy. *Angew Chem Int-Edit*. 2007; 46:8380–8383.
31. Zhou DH, Rienstra CM. High-performance solvent suppression for proton detected solid-state NMR. *J Magn Reson*. 2008; 192:167–172. [PubMed: 18276175]
32. Zhou DH, Shah G, Mullen C, Sandoz D, Rienstra CM. Proton-Detected Solid-State NMR Spectroscopy of Natural-Abundance Peptide and Protein Pharmaceuticals. *Angew Chem*. 2009; 121:1279–1282.
33. Zhou D, Nieuwkoop A, Berthold D, Comellas G, Sperling L, Tang M, Shah G, Brea E, Lemkau L, Rienstra C. Solid-state NMR analysis of membrane proteins and protein aggregates by proton detected spectroscopy. *J Biomol NMR*. 2012; 54:291–305. [PubMed: 22986689]

34. Paulson EK, Morcombe CR, Gaponenko V, Dancheck B, Byrd RA, Zilm KW. Sensitive High Resolution Inverse Detection NMR Spectroscopy of Proteins in the Solid State. *J Am Chem Soc.* 2003; 125:15831–15836. [PubMed: 14677974]
35. Ernst M, Samoson A, Meier BH. Low-power decoupling in fast magic-angle spinning NMR. *Chem Phys Lett.* 2001; 348:293–302.
36. Lewandowski JR, De Paëpe G, Griffin RG. Proton Assisted Insensitive Nuclei Cross Polarization. *J Am Chem Soc.* 2007; 129:728–729. [PubMed: 17243786]
37. Lange A, Scholz I, Manolikas T, Ernst M, Meier BH. Low-power cross polarization in fast magic-angle spinning NMR experiments. *Chem Phys Lett.* 2009; 468:100–105.
38. Laage S, Sachleben JR, Steuernagel S, Pierattelli R, Pintacuda G, Emsley L. Fast acquisition of multi-dimensional spectra in solid-state NMR enabled by ultra-fast MAS. *J Magn Reson.* 2009; 196:133–141. [PubMed: 19028122]
39. Mithu VS, Paul S, Kurur ND, Madhu PK. Heteronuclear dipolar decoupling in solid-state nuclear magnetic resonance under ultra-high magic-angle spinning. *J Magn Reson.* 2011; 209:359–363. [PubMed: 21354839]
40. Weingarth M, Trébosc J, Amoureux JP, Bodenhausen G, Tekely P. Efficiency at high spinning frequencies of heteronuclear decoupling methods designed to quench rotary resonance. *Solid State Nucl Magn Reson.* 2011; 40:21–26. [PubMed: 21498056]
41. Agarwal V, Tuhem T, Reinhold A, Past J, Samoson A, Ernst M, Meier BH. Amplitude-modulated low-power decoupling sequences for fast magic-angle spinning NMR. *Chem Phys Lett.* 2013; 583:1–7.
42. Ernst M, Samoson A, Meier BH. Low-power XiX decoupling in MAS NMR experiments. *J Magn Reson.* 2003; 163:332–339. [PubMed: 12914849]
43. Weingarth M, Bodenhausen G, Tekely P. Low-power decoupling at high spinning frequencies in high static fields. *J Magn Reson.* 2009; 199:238–241. [PubMed: 19467891]
44. Shekar SC, Lee DK, Ramamoorthy A. An Experimental Strategy to Dramatically Reduce the RF Power Used in Cross Polarization Solid-State NMR Spectroscopy. *J Am Chem Soc.* 2001; 123:7467–7468. [PubMed: 11472196]
45. Laage, Sgn; Marchetti, A.; Sein, J.; Pierattelli, R.; Sass, HJ.; Grzesiek, S.; Lesage, A.; Pintacuda, G.; Emsley, L. Band-Selective 1H – ^{13}C Cross-Polarization in Fast Magic Angle Spinning Solid-State NMR Spectroscopy. *J Am Chem Soc.* 2008; 130:17216–17217. [PubMed: 19053413]
46. Vijayan V, Demers JP, Biernat J, Mandelkow E, Becker S, Lange A. Low-Power Solid-State NMR Experiments for Resonance Assignment under Fast Magic-Angle Spinning. *ChemPhysChem.* 2009; 10:2205–2208. [PubMed: 19603450]
47. Demers JP, Vijayan V, Becker S, Lange A. Tailored low-power cross-polarization under fast magic-angle spinning. *J Magn Reson.* 2010; 205:216–223. [PubMed: 20570194]
48. Webber AL, Pell AJ, Barbet-Massin E, Knight MJ, Bertini I, Felli IC, Pierattelli R, Emsley L, Lesage A, Pintacuda G. Combination of DQ and ZQ Coherences for Sensitive Through-Bond NMR Correlation Experiments in Biosolids under Ultra-Fast MAS. *ChemPhysChem.* 2012; 13:2405–2411. [PubMed: 22528552]
49. Weingarth M, Tekely P, Bodenhausen G. Efficient heteronuclear decoupling by quenching rotary resonance in solid-state NMR. *Chem Phys Lett.* 2008; 466:247–251.
50. Pegg DT, Doddrell DM, Brooks WM, Robin Bendall M. Proton polarization transfer enhancement for a nucleus with arbitrary spin quantum number from n scalar coupled protons for arbitrary preparation times. *J Magn Reson.* 1981; 44:32–40.
51. Morris GA. Sensitivity enhancement in nitrogen-15 NMR: polarization transfer using the INEPT pulse sequence. *J Am Chem Soc.* 1980; 102:428–429.
52. Holland GP, Cherry BR, Jenkins JE, Yarger JL. Proton-detected heteronuclear single quantum correlation NMR spectroscopy in rigid solids with ultra-fast MAS. *J Magn Reson.* 2010; 202:64–71. [PubMed: 19857977]
53. Ernst, RR.; Bodenhausen, G.; Wokaun, A. Principles of nuclear magnetic resonance in one and two dimensions. Clarendon Press Oxford; London: 1987.

54. Ramamoorthy A, Chandrakumar N. Comparison of the coherence-transfer efficiencies of laboratory- and rotating-frame experiments. *Journal of Magnetic Resonance* (1969). 1992; 100:60–68.
55. Griesinger C, Gemperle C, Sørensen OW. Symmetry in coherence transfer. *Mol Phys.* 1987; 62:295–332.
56. Dürr UHN, Yamamoto K, Im SC, Waskell L, Ramamoorthy A. Solid-State NMR Reveals Structural and Dynamical Properties of a Membrane-Anchored Electron-Carrier Protein, Cytochrome b5. *J Am Chem Soc.* 2007; 129:6670–6671. [PubMed: 17488074]
57. Yamamoto K, Dürr UHN, Xu J, Im SC, Waskell L, Ramamoorthy A. Dynamic Interaction Between Membrane-Bound Full-Length Cytochrome P450 and Cytochrome b5 Observed by Solid-State NMR Spectroscopy. *Sci Rep.* 2013; 3:2538. [PubMed: 23985776]
58. Elena B, Lesage A, Steuernagel S, Böckmann A, Emsley L. Proton to Carbon-13 INEPT in Solid-State NMR Spectroscopy. *J Am Chem Soc.* 2005; 127:17296–17302. [PubMed: 16332079]
59. Sørensen OW, Ernst RR. Elimination of spectral distortion in polarization transfer experiments. Improvements and comparison of techniques. *J Magn Reson.* 1983; 51:477–489.
60. Fung BM, Khitrin AK, Ermolaev K. An Improved Broadband Decoupling Sequence for Liquid Crystals and Solids. *J Magn Reson.* 2000; 142:97–101. [PubMed: 10617439]
61. Veshkort M, Griffin RG. SPINEVOLUTION: A powerful tool for the simulation of solid and liquid state NMR experiments. *J Magn Reson.* 2006; 178:248–282. [PubMed: 16338152]

Highlights

- RINEPT's efficiency depends on the MAS frequency.
- Polarization transfer by ^1H -undecoupled RINEPT depends on the dipolar couplings.
- Scalar coupling plays an insignificant role under ultrafast MAS.

Figure 1.

A radio-frequency pulse sequence for RINEPT experiments used in this study, where 90° and 180° pulses are indicated in solid and blank rectangles, respectively. The τ_1 and τ_2 delays, during which the transverse magnetization evolves under heteronuclear (dipolar and scalar) couplings, are varied in our experimental study as discussed in the main text. Both τ_1 and τ_2 were synchronized with the MAS rotor period.

Figure 2.

The transfer of magnetization from ^1H to ^{13}C nuclei by the RINEPT pulse sequence as a function of τ_1 with $\tau_2 = 0.1$ ms (A, C) and τ_2 with $\tau_1 = 0.1$ ms (B, D) at 50 (red) and 60 (black) kHz MAS. Experimental results were obtained from the $^{13}\text{CH}_2$ group of a uniformly ^{13}C -labeled glycine (A and B) and $^{13}\text{C}\alpha$ -labeled L-leucine (C and D) powder samples. For each data point, spectra were acquired with 16 scans and a recycle delay of 5s.

Figure 3.

Simulated RINEPT efficiency for the $^{13}\text{CH}_2$ group of glycine as a function of rotor-synchronized τ_1 with $\tau_2=0.1\text{ms}$ (A, C) and rotor-synchronized τ_2 with $\tau_1=0.1\text{ms}$ (B, D) at different spinning speed as indicated. Three spins including one carbon and two protons were considered for simulations. The ^1H - ^1H dipolar couplings calculated by the SPINEVOLUTION software is about 21.3 kHz in (A, B) and 4.26 kHz in (C, D).

Figure 4.

Simulated RINEPT efficiency for the CH₂ group of glycine at 60 kHz MAS as a function of rotor-synchronized τ_1 with $\tau_2=0.1$ ms (A) and rotor-synchronized τ_2 with $\tau_1=0.1$ ms (B) for different heteronuclear ^1H - ^{13}C dipolar coupling values as indicated; both ^{13}C - ^1H dipolar couplings in the CH₂ group were set to be equal and varied simultaneously. Three spins (two protons and one carbon) were considered in the simulations. The ^{13}C - ^1H rigid-limit dipolar coupling value calculated by the SPINEVOLUTION software is about 23.3 kHz (that is the indicated 100%; the indicated 0% means the ^{13}C - ^1H dipolar coupling is 0 Hz). The ^1H - ^1H dipolar coupling was 21.3 kHz and the ^{13}C - ^1H scalar coupling was 145 Hz in all the simulations.

Figure 5.

Simulated RINEPT efficiency for the CH_2 group of glycine at 60 kHz MAS as a function of rotor-synchronized τ_1 with $\tau_2 = 0.1$ ms (A) and rotor-synchronized τ_2 with $\tau_1 = 0.1$ ms (B) for different ^1H - ^1H dipolar coupling values as indicated. Three spins (two protons and one carbon) were considered in the simulations. The ^1H - ^1H dipolar coupling value calculated by the SPINEVOLUTION software is about 21.3 kHz (that is the indicated 100%; the indicated 0% means the H-H dipolar coupling is 0 Hz). The ^1H - ^{13}C dipolar coupling was 23.3 kHz and the ^{13}C - ^1H scalar coupling was 145 Hz in all the simulations.

Figure 6.

(A) ^{13}C RINEPT spectrum of uniformly ^{13}C -labeled glycine with $\tau_1 = \tau_2 = 0.1$ ms acquired with 16 scans and a recycle delay of 5 s under 60 kHz MAS. (B) Simulated RINEPT efficiency for the CH₂ group of glycine at 60 kHz MAS as a function of rotor-synchronized τ_1 with $\tau_2 = 0.1$ ms and (C) rotor-synchronized τ_2 with $\tau_1 = 0.1$ ms under different number of spins and dipolar couplings as indicated.

Figure 7.

Simulated RINEPT efficiency for CH_2 (A, B) and CH (C, D) groups at 60 kHz MAS as a function of rotor-synchronized τ_1 with $\tau_2 = 0.1$ ms (A, C) and rotor-synchronized τ_2 with $\tau_1 = 0.1$ ms (B, D) with (red) and without (black) the 145 Hz ^{13}C - 1H scalar coupling. For the CH group, we adopted the $C_\alpha H$ group from leucine, and included another two remote proton spins with the coordinates of the neighboring CH_2 group in leucine.

Figure 8.

(A) Simulated and (B) experimental RINEPT polarization transfer efficiency as a function of the length of the 90° pulse with $\tau_1 = \tau_2 = 0.1$ ms at 60 kHz MAS. Experimental data were obtained from uniformly ^{13}C -labeled-glycine powder sample as mentioned in the text.

Table 1

Spin-spin relaxation times (T_2) of ^1H and ^{13}C nuclei measured for the $^{13}\text{CH}_2$ group of glycine and $^{13}\text{C}_\alpha\text{H}$ group of L-leucine under ultrafast MAS conditions.

MAS speed	Gly ^1H T_2	Gly ^{13}C T_2	Leu ^1H T_2	Leu ^{13}C T_2
50 kHz	0.29 ms	0.47 ms	0.38 ms	1.74 ms
60 kHz	0.44 ms	0.56 ms	0.56 ms	2.04 ms