Evolution of CPMAS under fast magic-angle-spinning at 100 kHz and beyond

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Abstract

This article describes recent trends of high-field solid-state NMR (SSNMR) experiments for small organic molecules and biomolecules using ^{13}C and ^{15}N CPMAS under ultra-fast MAS at a spinning speed (ν_R) of 80-100 kHz. First, we illustrate major differences between a modern low-power RF scheme using UFMAS in an ultra-high field and a traditional CPMAS scheme using a moderate sample spinning in a lower field. Features and sensitivity advantage of a low-power RF scheme using UFMAS and a small sample coil are summarized for CPMAS-based experiments. Our 1D ^{13}C CPMAS experiments for uniformly ^{13}C - and ^{15}N -labeled alanine demonstrated that the sensitivity per given sample amount obtained at ν_R of 100 kHz and a ^1H NMR frequency (ν_H) of 750.1 MHz is ~ 10 fold higher than that of a traditional CPMAS experiment obtained at ν_R of 20 kHz and ν_H of 400.2 MHz. A comparison of different decoupling schemes in CPMAS at ν_R of 100 kHz for the same sample demonstrated that low-power WALTZ-16 decoupling unexpectedly displayed superior performance over traditional low-power schemes designed for SSNMR such as TPPM and XiX in a range of decoupling field strengths of 5-20 kHz. Excellent ^1H decoupling performance of WALTZ-16 was confirmed on a protein microcrystal sample of GB1 at ν_R of 80 kHz. We also discuss the feasibility of a SSNMR microanalysis of a GB1 protein sample in a scale of 1 nmol to 80 nmol by ^1H -detected 2D $^{15}\text{N}/^1\text{H}$ SSNMR by a synergetic use of a high field, a low-power RF scheme, a paramagnetic-assisted condensed data collection (PACC), and UFMAS.

Graphic abstract

Introduction

High-resolution ^{13}C solid-state NMR (SSNMR) using a CPMAS scheme has marked a milestone of 40 years since the scheme was originally introduced by Schaefer and Stejskal for characterization of polymers and other organic systems at a spinning speed of 3 kHz.¹ The work not only demonstrated a creative integration of earlier inventions of cross polarization (CP)² and magic-angle-spinning (MAS),³ but also involved development of hardware such as a spinning system (see **Figure 1**; a photograph courtesy of Prof. Schaefer). With enormous progress after this discovery, high-resolution SSNMR spectroscopy is generally considered as a matured field. Nevertheless, recent advances in SSNMR have markedly improved its capabilities. In particular, novel developments for fast MAS systems have increased the available spinning speed from 30 kHz to 100 kHz or higher over the past decade.⁴⁻¹¹ Engineering needs for ultra-fast MAS (UFMAS) at 80 kHz or higher demand a small MAS rotor having a diameter of 1 mm or less (see **Figure 2**). Because of its very restricted sample volume ($\leq 1\ \mu\text{L}$), there was a natural skepticism about the sensitivity and feasibility of biomolecular SSNMR using such a UFMAS system in the NMR community. Nevertheless, recent studies have demonstrated that SSNMR using UFMAS at 80-100 kHz in a high field offers a practical tool for structural biology.¹¹⁻¹³ These findings signify a crucial development in biological SSNMR as a production of a protein and other biological sample in a large quantity is often prohibitive for systems of biological interest. The new development was prompted by a series of sensitivity enhancement approaches that are compatible with UFMAS. These approaches include ^1H indirect detection,^{5,14-18} paramagnetic assisted condensed data collection (PACC),^{6,19-22} usage of a high field,^{12,13,23-25} and deuteration and other unique isotope labeling schemes.^{12,18,26,27} Importantly, sensitivity enhancement factors by these approaches can be compounded since many of these methods can be integrated together without any major interferences. Thus, despite a limited sample volume, recent studies using UFMAS indicate unparalleled mass-sensitivity (i.e. sensitivity per sample amount) for a 3D SSNMR analysis of a mass-limited protein sample.^{12,13} Moreover, unlike dynamic nuclear polarization (DNP) approach,²⁸ UFMAS-based methods offer an analysis of a biological sample in a hydrated state. Another important aspect in SSNMR spectroscopy using UFMAS is that a

drastic change in the spin physics allows one to explore new types of pulse sequences such as low-power ^1H decoupling^{14,15,29,30} and low-power cross-polarization sequences.^{6,8,31}

In this article, we discuss the present status and a prospect of biomolecular SSNMR using CPMAS under UFMAS. We review how basic building blocks of a CPMAS scheme such as CP and ^1H decoupling have been evolved under UFMAS. Then, we discuss an alternative ^1H low-power decoupling scheme using composite-pulse decoupling schemes such as WALTZ-16, which were previously ineffective for ^1H decoupling in SSNMR. We also demonstrate that a SSNMR microanalysis of proteins in a 1-100 nmol scale is feasible through discussing the sensitivity and resolution of ^1H -detected 2D $^1\text{H}/^{15}\text{N}$ SSNMR experiment for a GB1 microcrystalline sample under UFMAS.

Material and Methods

Unless otherwise mentioned, all the SSNMR experiments were performed on a Bruker Avance III 750 MHz spectrometer at the UIC Center for Structural Biology using a JEOL 0.75-mm or 1-mm $^1\text{H}/^{13}\text{C}/^{15}\text{N}/^2\text{H}$ quad-resonance MAS probe. The experiment in Figure 4b was performed on a Bruker Avance III 400MHz spectrometer using a homebuilt 2.5-mm $^1\text{H}/^{13}\text{C}/^{15}\text{N}$ CPMAS probe. The sample temperatures were maintained at $\sim 15^\circ\text{C}$ by applying a cooled VT air at -10°C in order to compensate for the temperature increase by sample spinning. Figure 3 shows (a) a CPMAS pulse sequence for the UFMAS experiment and (b) the sequence for the conventional CPMAS experiment in Figure 4b. In the 1D ^{13}C CPMAS experiments using UFMAS, ^{13}C spin polarization was prepared with double-quantum adiabatic cross polarization (DQ-CP) using an amplitude-modulated shaped pulse with an upward tangential ramp for the ^{13}C channel and a rectangular pulse for the ^1H channel.⁸ The ^{13}C RF field strength was swept from 55 kHz to 95 kHz with the average rf field at $\sim 3\nu_{\text{R}}/4$ while the ^1H RF field amplitude was set kept constant at 25 kHz ($\sim \nu_{\text{R}}/4$), where the sum of the average RF fields was matched to ν_{R} for DQ-CP, where ν_{R} denotes a spinning speed. The ^{13}C signals were acquired under low-power WALTZ-16 ^1H decoupling³² at 5 kHz in Figure 4a. The same CP sequence was used for Figures 4-6 with different low-power ^1H -decoupling sequences^{8,10,15,30}, as specified in the figure captions. In the conventional 1D ^{13}C CPMAS experiment in Figure 3b, ^{13}C signals were acquired during high-power ^1H TPPM decoupling with a

^1H field strength of 85 kHz after the initial CP polarization transfer. For cross polarization, an adiabatic CP scheme was used with an amplitude-modulated shaped pulse with an upward tangential ramp for the ^{13}C channel and a rectangular pulse for the ^1H channel. The other experimental conditions are in the caption. For TPPM³³ and XiX,^{34,35} the phases of the two pulses were modulated following $\phi, -\phi$, where ϕ was set to 10° and 90° for TPPM and XiX, respectively. “High phase” conditions for TPPM ($60^\circ < \phi < 90^\circ$)³⁶ were not considered in this work. For SPINAL-64,^{32,33} the phase was cycled as $Q\bar{Q}Q\bar{Q}Q\bar{Q}Q\bar{Q}$,^{37,38} where for each Q unit, the pulse phases were modulated following, $\phi, -\phi, \phi+\alpha, -\phi-\alpha, \phi+2\alpha, -\phi-2\alpha, \phi+\alpha, -\phi-\alpha$ and for $\bar{Q}$, all the phases for Q were inverted. Here, ϕ was set to 10° while α was set to 5° after optimization.

Uniformly ^{13}C - and ^{15}N -labeled L-alanine (U ^{13}C -, ^{15}N -labeled L-Ala) was purchased from Sigma Aldrich/Isotec and was used without further treatments. A lysine-reverse-labeled GB1 microcrystal sample used for Figure 10 was prepared as described in ref. 13. The reverse labeling was introduced to demonstrate a spectral editing method in our previous studies.¹³ A uniformly ^{13}C - and ^{15}N -labeled GB1 sample for Figure 9 was prepared as described in ref. ³⁹ The sample was precipitated in a deuterated crystallization solution with D_2O for other experiments (H/D exchange). A sodium phosphate buffer of the sample was exchanged with equal volume of the sodium phosphate buffer prepared in D_2O to suppress the H_2O content and precipitated as microcrystals as described in ref. 13 using 2-methyl-2,4-pentane- d_{12} -diol and 2-propanol- d_8 (Sigma Aldrich) as the crystallization solution. Approximately 80 % of the amide protons were retained in the sample. For both the GB1 samples, the microcrystalline sample of ~ 0.5 mg (dry protein weight) was packed in a 1-mm rotor using a home-made packing tool using centrifugation.

Results and Discussion

Figure 2 shows three MAS rotors to be used in this study (left: 0.75-mm rotor; middle: 1-mm rotor; right: 2.5-mm rotor). Although a 2.5-mm rotor has been used for fast MAS experiments at ~ 30 kHz, it is clear that the sample amount that can be accommodated in the 0.75-mm or 1-mm rotor is far smaller than that for the 2.5-mm rotor. Hence, it is reasonable that many in the NMR community were originally skeptical about the feasibility of multi-dimensional protein SSNMR experiments under UFMAS using such a small rotor. Figure 4

shows 1D ^{13}C CPMAS spectra of U ^{13}C -, ^{15}N -labeled L-Ala obtained by (a) a low-power scheme in Figure 3a under UFMAS at ν_{R} of 100 kHz and at a ^1H NMR frequency of 750.1 MHz ($B_0 = 17.6$ T) and (b) a tradition scheme in Figure 3b at ν_{R} of 20 kHz and at a ^1H NMR frequency of 400.2 MHz ($B_0 = 9.4$ T). The data in Figure 4a were obtained with the 0.75-mm CPMAS probe while the data in (b) were obtained with the 2.5-mm home-built CPMAS probe. Clearly, the spectral resolution obtained under low-power decoupling and UFMAS in (a) is higher than that in (b) obtained under high-power decoupling in a lower field. Line broadening due to ^{13}C - ^{13}C J couplings is scaled down in units of ppm in a higher field. Nevertheless, it is not trivial that low-power ^1H decoupling at ~ 5 kHz could successfully remove ^{13}C - ^1H dipolar couplings in (a), considering that broadening due to residual dipolar couplings is clearly visible in (b) obtained with high-power ^1H decoupling at 85 kHz. The results suggest that since fast MAS over 80 kHz eliminates broadening due to ^{13}C - ^1H dipolar couplings to large extent, residual dipolar couplings can be sufficiently eliminated by ^1H RF decoupling at a minimal power. To our surprise, ^1H low-power decoupling by WALTZ-16 used here is more effective under UFMAS than traditional low-power decoupling schemes used in SSNMR such as TPPM and XiX, as summarized below. WALTZ and other composite-pulse ^1H -decoupling sequences have not been useful for SSNMR for their long cycle times for coherent averaging of dipolar couplings. One more crucial difference between UFMAS and traditional MAS approaches in Figure 4 is an available sample volume. Under UFMAS at 80-100 kHz, the engineering needs limit sample amount to less than 1 mg. For example, in Figure 4a, only 0.3 mg of U ^{13}C -, ^{15}N -labeled L-Ala was accommodated in a 0.75-mm MAS rotor with a sample volume of 0.3 μL while 5.9 mg of U ^{13}C -, ^{15}N -labeled L-Ala was placed in a 2.5-mm rotor for (b) as a mixture with 5.9 mg of adamantane. Despite 20-fold difference in the sample size, the sensitivity in (a) was found to be approximately 50-60% of that in (b). It is noteworthy that the same number of scans (4 scans), recycle delays (3 s), and acquisition time (36 ms) were used for (a, b). Thus, the sensitivity per given sample amount (mass sensitivity) in (a) is 10–12 times higher than that in (b), which was obtained by a traditional SSNMR approach using a lower field instrument. The excellent mass-sensitivity in the preliminary data in (a) is partly attributed to an efficient probe circuit of the 0.75-mm UFMAS probe equipped with a micro sample coil. As highlighted in this example, a combination of a UFMAS probe and an ultra-high field has offered as much as 12-fold enhancement of the mass sensitivity. Because a use of the higher field should offer only 3-fold sensitivity gain theoretically, the results signify the importance of

probe development in an ultra-high field for biomolecular SSNMR. As described below, additional sensitivity enhancement methods are available for biomolecular SSNMR. Thus, the high sensitivity in (a) clearly indicates that multi-dimensional biomolecular SSNMR using this probe is a reasonable option even with a traditional ^{13}C -detected SSNMR method in a high field.

Figure 5 compares ^{13}C CPMAS spectra of the U ^{13}C -, ^{15}N -labeled L-Ala sample obtained with (a) low-power WALTZ-16 ^1H decoupling, (b) low-power cw ^1H decoupling^{14,15,29} at $\nu_{\text{dec}} = 5$ kHz, and (c) no decoupling; all the data were collected at ν_{R} of 100 kHz in a high field of 17.6 T. It is interesting to find that the resolution is reasonable for non-protonated $^{13}\text{CO}_2^-$ peak at ~ 180 ppm even without any decoupling in (c). However, in (c) signals were broader for the protonated $^{13}\text{C}_\alpha$ and $^{13}\text{C}_\beta$ peaks, which were observed at ~ 50 and ~ 20 ppm, respectively. This finding suggests that averaging of ^{13}C - ^1H dipolar couplings only by UFMAS is *not* sufficient even at 100 kHz. Low-power cw decoupling at 5 kHz considerably eliminated the residual broadening in (b). Nevertheless, it is clear that WALTZ-16 low-power decoupling offered much better resolution in (a) than the cw decoupling. In particular, the difference between cw and WALTZ-16 sequences is notable for $^{13}\text{C}_\beta$, for which the ^1H offset effect is not negligible with the ^1H carrier frequency set at 3.5 ppm off from the $^1\text{H}_\beta$ resonance at ~ 1.9 ppm. Although WALTZ-16 and other composite-pulse ^1H -decoupling sequences have not been effective in SSNMR, fast averaging of ^1H -X and ^1H - ^1H dipolar couplings by UFMAS and a resultant extended lifetime of ^1H spin states have changed the situation drastically. The results outline the important requirements for ^1H low-power decoupling under UFMAS at 100 kHz, including considerations of off-resonance effects.

Next, we compare various low-power ^1H -decoupling schemes for UFMAS applications. Figure 6 shows ^1H RF field strength (ν_{dec}) dependence of ^{13}C signal intensities for (a) ^{13}CO , (b) $^{13}\text{C}_\alpha$ (CH) and (c) $^{13}\text{C}_\beta$ (CH_3) in ^{13}C CPMAS spectra of U ^{13}C -, ^{15}N -labeled L-Ala at ν_{R} of 100 kHz obtained with different decoupling schemes. Five decoupling schemes to be compared were low-power WALTZ-16 (red square), TPPM³⁰ (green square), XiX⁴⁰ (blue X), SPINAL-64³⁸ (orange circle), and cw (magenta triangle) decoupling sequences. Although other sophisticated TPPM- or XiX-based variants were reported to be useful for low-power ^1H -decoupling sequences,^{41,42} the listed here represents a widely used subset with varied number of adjustable parameters such as rf strengths, phase and flip angle; thus, they are suited for a comparison with the WALTZ-16 scheme.

Unexpectedly, the low-power WALTZ-16 scheme, which was originally developed for ^1H J-decoupling in solution NMR, performed better than low-power TPPM, SPINAL-64, and XiX schemes, which were developed specifically for SSNMR. In the low-power decoupling regime using ν_{dec} of 5-30 kHz, the best performance for WALTZ-16 was observed at ν_{dec} of 5-20 kHz. Although the WALTZ-16 sequence performed best at 10 kHz, it is also practical to employ decoupling at 5 kHz as shown in Figure 4 since the excellent performance at a lower RF power is ideal for applications to heat-sensitive biomolecules. In Figure 7, we compared magnified spectral regions of (top) $^{13}\text{CO}_2^-$, (middle) ^{13}CH , and (bottom) $^{13}\text{CH}_3$ in the ^{13}C CPMAS spectra of U- ^{13}C -, ^{15}N -labeled L-Ala obtained with (a) WALTZ-16, (b) TPPM, (c) XiX, (d) SPINAL-64, (e) cw decoupling at ν_{dec} of 10 kHz and (f) no decoupling. Clearly, all the spectra by modern SSNMR decoupling schemes in (b-d) show good resolution, yet WALTZ-16 outperformed or showed a comparable performance to all of the sequences. In Figures 6-7, the pulse width for each decoupling sequence was individually optimized for each ν_{dec} value. Figure 8 shows a flip-angle setting dependence of the signal intensities of $^{13}\text{C}_\alpha$ for the same CPMAS experiments with ν_{dec} of 5 kHz. Empirically, XiX, TPPM, and WALTZ-16 sequences should have optimum pulse flip angles close to $\sim 360^\circ$, $\sim 180^\circ$, and $\sim 90^\circ$, respectively, where we used a 90° -pulse width for the adjustment for the WALTZ-16 sequence. From the data in Figure 8, we found optimum flip angles of 324° , 162° , and 90° for XiX, TPPM, and WALTZ-16 sequences, respectively. To our surprise, the WALTZ-16 sequence performed better than or comparably to the other sequences over a wide range of flip angles from 55° to 360° . This excellent performance over a wide-range of flip angle means the ease of optimization. This is an important factor for an application in ultra-high field SSNMR as a machine time is often limited at a shared high-field spectrometer. In contrast, SPINAL-64 sequence, which performed comparably to WALTZ-16, generally requires a more careful adjustment of the flip angle in addition to adjustments of two phase-switch angles (ϕ , α) for the best performance. We confirmed that a similar performance of WALTZ-16 was obtained from the experiments under UFMAS at 80 kHz as will be discussed below. Thus, the WALTZ-16 sequence offers optimum resolution under UFMAS with minimal adjustment efforts for ^1H low-power decoupling. Although degradation of decoupling performance due to an offset effect could be a concern in an ultra-high field, our preliminary analysis showed that the decoupling performance of the WALTZ-16 sequence at ν_{dec} of 5

kHz was nearly unchanged when the ^1H carrier frequency was shifted within ± 6 kHz (± 8 ppm) at ν_R of 100 kHz in a static field of 17.6 T. Figure 9 shows a comparison of ^{13}C CPMAS spectra of a uniformly ^{13}C - and ^{15}N -labeled GB1 microcrystalline sample obtained at ν_R of 80 kHz with (a) WALTZ-16, (b) SPINAL-64, and (c) cw ^1H decoupling sequences at ν_{dec} of 10 kHz. The data indicate excellent decoupling performance of the low-power WALTZ-16 scheme for the hydrated protein sample. The obtained resolution by the WALTZ scheme is comparable to or slightly better than that of SPINAL-64, including that for the CH_2 resonances, which was not investigated for the L-Ala sample. These results illustrate excellent prospect of low-power ^1H composite-pulse decoupling and UFMAS approach for ^{13}C CPMAS-based experiments and their biological applications in an ultra-high field. Although a further study is needed to elucidate the decoupling mechanism under UFMAS, the first successful application of WALTZ-16 for low-power ^1H decoupling in SSNMR may prompt development of other composite-pulse based sequences for ^1H decoupling in SSNMR.

Lastly, we present an example of a SSNMR protein microanalysis by sensitivity and resolution enhancements by ^1H -detected SSNMR under UFMAS from our previous studies.¹³ Figure 10 compares ^1H -detected 2D $^1\text{H}/^{15}\text{N}$ correlation spectra obtained at spinning speeds of (a) 80 kHz and (b) 50 kHz for a lysine reverse-labeled GB1 microcrystalline sample (~ 0.5 mg or ~ 80 nmol), which was uniformly ^{13}C - and ^{15}N -labeled except for 6 lysine residues. Although the spectrum collected at ν_R of 50 kHz in (b) shows a modest resolution, it is clear that the resolution is greatly enhanced in (a) by UFMAS at 80 kHz. The signals are well resolved for a majority of residues in the 2D spectrum obtained at 80 kHz. The experimental time was only 4.5 min with the aid of sensitivity enhancements by a combined use of fast recycling with the PACC method^{6,8,22} (200 ms per scan), a high field (17.6 T), and ^1H detection.¹³⁻¹⁵ It should be noted that the PACC method generally requires a low-power RF scheme and very fast MAS to minimize a risk of a probe arcing and sample degradation in a fast recycling.⁶ The average signal-to-noise ratio (S/N) of the resolved signals in (a) was ~ 20 . Thus, the detection limit of the protein sample at S/N of ~ 5 is approximately ~ 1 nmol (~ 6 μg) within an experimental time of 24 h. We also recently demonstrated that main-chain sequential assignments¹³ and side chain assignments¹² by ^1H detected 3D SSNMR are feasible for as little as ~ 10 nmol of isotope labeled protein samples. These results suggest the effectiveness of a microanalysis using SSNMR in the UFMAS approach. Further enhancements of

mass-sensitivity for biomolecular SSNMR are most likely to be feasible using UFMAS at 100 kHz and beyond and an ultra-high field.

Conclusion

Although NMR spectroscopy is generally considered to be a matured research field, recent development of novel SSNMR instrumentations and methods has provided tremendous enhancements in sensitivity and resolution for biomolecular SSNMR. Here, we presented up to 12-fold mass-sensitivity improvements by ^{13}C -detected CPMAS, compared with the sensitivity obtained by a traditional ^{13}C CPMAS method in a lower field. The results indicate that a required sample for SSNMR measurements can be drastically scaled down by a synergistic use of an ultra-high field, UFMAS, and an efficient CPMAS probe equipped with a micro coil. We also reported excellent performance of WALTZ-16 low-power ^1H -decoupling sequence under UFMAS; the sequence was not suited for ^1H decoupling in traditional SSNMR experiments. Equally importantly, our data clearly showed sensitivity advantage of ^1H indirect detection 2D method for a SSNMR micro-analysis of a protein sample under UFMAS. It will be feasible to reach the detection limit of sub-nmol of proteins by further sensitivity enhancement using modified polarization transfer schemes⁴³ and non-uniform sampling.⁴⁴ It should be also noted that we successfully implemented frequency-selective REDOR experiments at ν_R of 80kHz.¹³ Based on these results, it is most likely that CPMAS and REDOR will enjoy further evolution under UFMAS at ν_R of 100 kHz or higher.

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Figure 1. Spinning system for the original CPMAS experiment.¹ The photograph provided courtesy of Prof. Jacob Schaefer at the Washington University in St. Louis. “The rotor was suspended from two phosphor-bronze wires held to the "stator" by teflon tape. A pipette brought the drive air in. A rotor would only last through 2 or 3 stop-start cycles. It took Ed (Stejskal) a day to make a rotor,” according to a note from Prof. Schaefer.

Figure 2. A picture of three MAS rotors used for this study in a comparison with a metric ruler. (Left) JEOL 0.75-mm rotor, (middle) JEOL 1-mm rotor, and (right) Varian 2.5-mm rotor.

Figure 3. Pulse sequences of (a) a low-power CP scheme suited for a UFMAS condition and (b) a high-power CP scheme used for a conventional CPMAS.

Figure 4. A comparison of ^{13}C CPMAS spectra of U ^{13}C -, ^{15}N -labeled L-Ala obtained with (a) a low-power decoupling scheme under UFMAS at 100 kHz in a high static magnetic field at 17.6 T and (b) a high-power decoupling under MAS at 20 kHz in a lower field at 9.4 T. The data were obtained with (a) JEOL 0.75-mm quad-resonance CPMAS probe and (b) a home-built 2.5-mm triple-resonance CPMAS probe equipped with a Varian 2.5-mm spinning module. In (a), ^{13}C signals were prepared with an adiabatic DQ-CP sequence in which the ^1H RF field strength was set to ~ 25 kHz while the ^{13}C RF field strength was ramped from 55 kHz to 95 kHz. The ^{13}C signals were acquired under low-power WALTZ-16 ^1H decoupling at 5 kHz with a 90-pulse width of 50 μs . In (b), ^{13}C signals were prepared with an adiabatic CP sequence in which the ^1H RF field strength was set to ~ 75 kHz while the ^{13}C RF field strength was ramped from 40 kHz to 70 kHz. The signals were acquired under high-power ^1H TPPM decoupling at 85 kHz. The spectra were processed without any window functions. The sample amount is only 0.3 mg in (a) while that is 5.9 mg in (b); the sample in (b) was mixed with 5.9 mg of adamantane.

Figure 5. A comparison of ^{13}C CPMAS spectra of $\text{U-}^{13}\text{C}$, ^{15}N -labeled L-Ala obtained under UFMAS at 100 kHz in a high static magnetic field at 17.6 T using (a) a low-power WALTZ-16 decoupling, (b) a cw low-power decoupling, and (c) no decoupling. The decoupling field strength was 5 kHz in (a-b). Even by averaging under UFMAS at 100 kHz, there is notable broadening for protonated carbons in (c).

Figure 6. ^1H RF decoupling field strength (ν_{dec}) dependence of signal intensities for (a) ^{13}CO , (b) ^{13}CH , (c) $^{13}\text{CH}_3$ in ^{13}C CPMAS spectra of U- ^{13}C , ^{15}N -labeled L-Ala under UFMAS at 100 kHz. The signal intensities were compared for five ^1H low-power decoupling schemes: WALTZ-16 (■), XiX (◆), TPPM (x), SPINAL-64 (●), and CW (△). The signal intensities were normalized by the maximum signal intensity observed by TPPM for each ^{13}C species among different ν_{dec} values. The pulse widths of WALTZ-16, XiX, TPPM, and SPINAL-64 were optimized for each ^{13}C species following the protocol in Figure 8. The error ranges in the normalized intensities are approximately ± 0.006 , ± 0.008 , and ± 0.004 for CO_2^- , CH, and CH_3 , respectively. For all the ^{13}C species, the WALTZ-16 sequence performed best at 5-15 kHz.

Figure 7. A comparison of ^{13}C CPMAS spectral patterns for (top) ^{13}CO , (middle) ^{13}CH , (bottom) $^{13}\text{CH}_3$ of U- ^{13}C , ^{15}N -labeled L-Ala obtained under UFMAS at 100 kHz with (a) WALTZ-16, (b) TPPM, (c) XiX, (d) SPINAL-64, (e) cw low-power ^1H -decoupling sequences and (f) no decoupling. The decoupling field strengths in (a-e) were 10 kHz. Dotted cyan bars are eye guides for a comparison of the signal intensities. The pulse width was individually optimized based on the $^{13}\text{C}_\alpha$ signal intensity for each sequence of WALTZ-16, TPPM, XiX and SPINAL-64 as shown in Figure 8.

Figure 8. Flip-angle dependence of signal intensities of the $^{13}\text{C}_\alpha$ peak in a ^{13}C CPMAS spectrum of $\text{U-}^{13}\text{C}$, ^{15}N -labeled L-Ala under UFMAS at 100 kHz for WALTZ-16 (■), XiX (◆), TPPM (×), SPINAL-64 (●) at ν_{dec} of 10 kHz. The intensities were normalized to the maximum intensity obtained by WALTZ-16 when the flip angle (θ) was set to 90° . The data for XiX are not displayed for $\theta \leq 180^\circ$, where the normalized intensities are less than 0.5. For XiX, the flip angle optimization was performed up to 450° ; the decoupling efficiency was found to be optimum at $315\text{--}360^\circ$. The range of the errors is approximately ± 0.008 .

Figure 9. A comparison of ^{13}C CPMAS spectra of a $\text{U-}^{13}\text{C}$, ^{15}N -labeled GB1 microcrystal sample obtained under UFMAS at 80 kHz in a magnetic field at 17.6 T using (a) WALTZ-16 decoupling, (b) SPINAL-64, and (c) cw low-power decoupling schemes. The dotted lines are eye guides. The decoupling field strength was 10 kHz in (a-c). ^{13}C signals were prepared with an adiabatic DQ-CP sequence in which the ^1H RF field strength was set to 30 kHz while the ^{13}C RF field strength was ramped from 36 kHz to 60 kHz.

Figure 10. A comparison of 2D $^{15}\text{N}/^1\text{H}$ correlation spectra collected under MAS at (a) 80 kHz and (b) 50 kHz for the lysine-reverse-labeled GB1 microcrystal sample (0.5 mg or ~ 80 nmol) with (c) 1D slices along the ^1H dimension. The total experimental time was 4.5 min each. The pulse sequence is comprised by two adiabatic CP schemes with the contact times of 1.5 ms.¹³ The spin polarization was transferred from ^1H to ^{15}N by the first adiabatic CP; then the ^{15}N signal was monitored in the t_1 period. After the t_1 period, the polarization was transferred back from ^{15}N to ^1H spins by the second adiabatic CP scheme for ^1H detection. For the data in (a), during the first adiabatic CP period, the ^{15}N RF field strength was ramped up from 18.6 kHz to 31.0 kHz with the average rf field at $\sim \nu_{\text{R}}/3$ while the ^1H RF field was kept constant at 55 kHz ($\sim 2\nu_{\text{R}}/3$). For the second adiabatic CP period, the ^{15}N RF field was ramped down from 33.3 kHz to 19.9 kHz with the average rf field at $\sim \nu_{\text{R}}/3$ while the ^1H RF field was kept at 55 kHz ($\sim 2\nu_{\text{R}}/3$). For the data in (b), during the first adiabatic CP period, the ^{15}N RF field was ramped from 10.6 kHz to 17.7 kHz with the average rf field at $\sim 2\nu_{\text{R}}/7$, while the ^1H RF field strength was kept at 35 kHz ($\sim 5\nu_{\text{R}}/7$). In the second adiabatic CP period, the ^{15}N RF field was ramped from 9.7 kHz to 16.1 kHz with the average rf field at $\sim \nu_{\text{R}}/4$ while the ^1H RF field amplitude was kept at 37 kHz ($\sim 3\nu_{\text{R}}/4$). The cooling N_2 gas temperature was set to -18°C for experiment under 80 kHz spinning and 18°C for experiment under 50 kHz spinning so that the GB1 sample temperature was approximately 30°C for the both cases. The data were collected with 16 scans for each t_1 period; the recycle delay was set as 0.2 s. (c) 1D slices of the 2D NH spectra for the spinning speeds of 80 kHz (red) and 50 kHz (blue). For a comparison with the data at 50 kHz MAS, a low-power SPINAL-64 decoupling sequence was used here. For a comparison of the sensitivity and resolution, the slices in (c) are displayed in the same scale. The spectra were modified from the data in ref. 13.

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