Published in final edited form as:

J Magn Reson. 2011 July ; 211(1): 18–24. doi:10.1016/j.jmr.2011.03.013.

Frequency-Selective Heteronuclear Dephasing and Selective Carbonyl Labeling to Deconvolute Crowded Spectra of Membrane Proteins By Magic Angle Spinning NMR

Nathaniel J. Traaseth¹ and Gianluigi Veglia^{1,2,*}

¹ Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, Minnesota 55445

² Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55445

Abstract

We present a new method that combines carbonyl-selective labeling with frequency-selective heteronuclear recoupling to resolve the spectral overlap of magic angle spinning (MAS) NMR spectra of membrane proteins in fluid lipid membranes with broad lines and high redundancy in the primary sequence. We implemented this approach in both heteronuclear ^{15}N - $^{13}\text{C}^{\alpha}$ and homonuclear ^{13}C - ^{13}C dipolar assisted rotational resonance (DARR) correlation experiments. We demonstrate its efficacy for the membrane protein phospholamban reconstituted in fluid PC/PE/PA lipid bilayers. The main advantage of this method is to discriminate overlapped $^{13}\text{C}^{\alpha}$ resonances by strategically labeling the preceding residue. This method is highly complementary to $^{13}\text{C}'_{i-1}$ - $^{15}\text{N}_i$ - $^{13}\text{C}^{\alpha}_i$ and $^{13}\text{C}^{\alpha}_{i-1}$ - $^{15}\text{N}_{i-1}$ - $^{13}\text{C}'_i$ experiments to discriminate inter-residue spin systems at a minimal cost to signal-to-noise.

Keywords

solid-state NMR; membrane proteins; magic angle spinning; phospholamban; REDOR; frequency-selective dipolar recoupling; heteronuclear recoupling

Introduction

Magic angle spinning (MAS) and oriented NMR experiments have been widely employed to determine the structure, topology, and dynamics of biomacromolecules [1–3]. These methods are particularly suited for integral membrane proteins, whose association with lipid vesicles limits the use of liquid-state NMR techniques. In the past years, there has been an outburst of MAS methodologies for resonance assignments and distance measurements in membrane protein samples [1, 4–13]. Unlike oriented solid-state NMR (SSNMR), which requires a high-degree of orientation with respect to the magnetic field [14–21], MAS sample preparations are more straightforward, making this technique popular in membrane protein structural biology. The strategy to obtain structural parameters for membrane protein structures by MAS is similar to that defined for liquid-state NMR, with the first step

© 2011 Elsevier Inc. All rights reserved.

*Corresponding Author: Gianluigi Veglia, 6-155 Jackson Hall, 321 Church St SE, Minneapolis, MN 55455, Phone: (612) 625-0758, Fax: (612) 625-2163, vegli001@umn.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

involving the tedious and lengthy process of resonance assignments followed by distance and torsion angle measurements. The NMR experiments carried out on micro-crystalline membrane protein samples give highly resolved NMR spectra [22–25], although the lack of a fluid membrane protein environment is a concern, and risks (as with X-ray crystallography) to offer conformational snapshots that may not reflect the physiological conditions of proteins in membranes.

In contrast, the membrane protein samples obtained in fluid lipid membranes [26] display significant line broadening and overlap of resonances, particularly in transmembrane (TM) segments, composed of highly redundant hydrophobic amino acids types (Leu, Ala, Gly, Val, Ile, and Phe) [27]. The helical nature of the majority of membrane proteins, the redundancy of the residue-types, and the line broadening caused by static and dynamic disorder limit the resolution in the ^{15}N and ^{13}C spectra. Only in selected cases do the linewidths enable sequence-specific assignments for non-crystalline uniformly ^{13}C and ^{15}N samples [28–31]. A number of approaches have been proposed to address these challenges: (1) main-chain assignment [32] to identify specific residues based on $^{13}\text{C}/^{13}\text{C}$ correlation spectra [33], (2) solid-phase peptide synthesis that introduces a minimal number of labeled residues at a time [34–38], and (3) selective [25, 39] or reverse biosynthetic labeling [40–43] to achieve *pair-wise* distributions. The second and third methods substantially reduce the number of resonances in the spectrum, making sequential assignments possible. Nevertheless, these labeling approaches used in combination with multiple bond transfers pulse sequences have been optimized for protein fibers, crystalline proteins, or lipid-depleted membrane protein samples and do not perform well for membrane proteins functionally reconstituted in fluid lipid membranes.

An elegant way to resolve complex spectra in a uniformly labeled background is to use rotational echo double resonance (REDOR) difference spectroscopy [44–46]. This experiment is analogous to the spin-echo difference spectroscopy developed for solution NMR [47], which has been applied to deconvolute spectra containing three different isotopically labeled species [48, 49]. Similar approaches have been utilized in solid-state NMR to measure short- and long-range distances between atoms. Since MAS causes *I-S* dipolar couplings to average to zero over a complete rotor cycle, 180° recoupling pulses on the *I*-spin applied during the rotor cycle reintroduce the dipolar couplings. By subtracting the spectra obtained with and without recoupling pulses, only *S* resonances that have a measurable dipolar coupling with *I* spins are observed. Significant efforts have been made to achieve frequency-selective REDOR [44] and TEDOR [50] experiments for spectral filtering, heteronuclear magnetization transfer, as well as distance/dihedral angle measurements [7, 24, 51–60]. One of these methods, the frequency-selective dipolar recoupling (FDR), has been implemented in 1D spectra showing applicability to model amino acid compounds [57, 58]. This sequence uses 90° pulses instead of 180° recoupling pulses to *I* spins that makes the average Hamiltonian offset dependent [57].

Here, we combined the FDR approach with carbonyl-selective labeling to obtain unambiguous assignments in $^{15}\text{N}/^{13}\text{C}^\alpha$ and $^{13}\text{C}^\alpha/^{13}\text{C}^\text{X}$ 2D spectra. This new approach complements the existing experiments for sequential assignment (^{15}N - ^{13}C , ^{15}N - $^{13}\text{C}^\alpha$, $^{13}\text{C}^\alpha$ - ^{15}N - ^{13}C , $^{13}\text{C}'$ - ^{15}N - $^{13}\text{C}^\alpha$, etc.) of crowded MAS spectra of membrane proteins. When extended in the third dimension, this approach will increase the spectral resolution for membrane proteins with high primary sequence redundancy, allowing the measurement of inter-residue dipolar correlations.

Results

Figure 1 shows heteronuclear and homonuclear pulse sequences used to correlate ^{15}N - $^{13}\text{C}^\alpha$ (t_1 - t_2) and ^{15}N - $^{13}\text{C}^\alpha$ - $^{13}\text{C}^\chi$ (t_1 - t_2 - t_3) with the FDR block. Following cross-polarization from ^1H to ^{15}N , the FDR train of 90° pulses is applied after each rotor cycle (125 μsec) at the $^{13}\text{C}'$ frequency (177 ppm). In the middle of rotor cycles, 180° pulses are applied to the ^{15}N channel in order to refocus chemical shift evolution, and allow for dipolar recoupling to occur between $^{13}\text{C}'$ and ^{15}N [57, 58]. The pulses utilized MLEV-4 [61] phasing cycling to ^{15}N (S spins) and XY-4 [62] to ^{13}C (I spins).

In proteins, the covalent $^{13}\text{C}'$ - ^{15}N peptide bond is ~ 1.3 Å, whereas the two-bond distance is 2.5 Å, giving maximum dipolar couplings of 1.4 and 0.2 kHz, respectively. The theoretical average Hamiltonian dephasing curves for the two distances are plotted in Figure 2 using analytical Bessel functions [63]. Note that the dephasing of FDR scales $1/\sqrt{2}$ with respect to the REDOR experiment [58]. From the theoretical curves, it is evident that ^{15}N magnetization with one-bond $^{13}\text{C}'$ dipolar couplings is dephased after 1.5 – 2.0 msec ($\Delta S/S_0 > 0.9$), whereas the magnetization for the two-bond couplings is not dephased ($\Delta S/S_0 < 0.1$). Following the FDR block, ^{15}N chemical shift is evolved during t_1 under ^1H TPPM decoupling [64]. The next step uses SPECIFIC-CP [65, 66] to transfer magnetization from ^{15}N to $^{13}\text{C}^\alpha$. Therefore, the FDR selectivity filter combined with the ^{15}N - $^{13}\text{C}^\alpha$ transfer gives a 2D heteronuclear correlation spectrum of ^{15}N - $^{13}\text{C}^\alpha$ filtered by the preceding $^{13}\text{C}'$ (Figure 1A). When 90° pulses are applied to $^{13}\text{C}'$, the magnetization of the ^{15}N spins covalently attached to $^{13}\text{C}'$ will be selectively dephased (filtered-out), while that of the ^{15}N spins attached to $^{12}\text{C}'$ will be retained. Subtracting the two experiments results in a spectrum similar to that obtained from the $^{13}\text{C}'$ - ^{15}N - $^{13}\text{C}^\alpha$ pulse sequence with double SPECIFIC-CP transfers [28, 67, 68].

The results from the FDR- ^{15}N - $^{13}\text{C}^\alpha$ 2D spectra on microcrystalline NAVL are shown in Figure 3. For these experiments, NAVL was labeled [$\text{U-}^{13}\text{C}$, ^{15}N]-Leu/Val with the acetyl group at natural ^{13}C abundance. The MAS rate was set to 8 kHz and used 2 msec of dipolar dephasing. The experiment with no ^{13}C 90° pulses (reference spectrum) gives two peaks from the two ^{15}N - $^{13}\text{C}^\alpha$ bonds in NAVL (Figure 3B). In contrast, the experiment with dephasing gives one intense resonance that is 85% of the intensity of the reference spectrum and one that was reduced to 12% intensity (Figure 3A). The difference spectrum (dephased - reference spectra) gives one intense peak that was assigned to the ^{15}N resonance covalently bonded to $^{13}\text{C}'$ (i.e., the valyl peak). These experiments demonstrate the usefulness of the FDR scheme to discriminate between one- and two-bond $^{13}\text{C}'$ - ^{15}N dipolar couplings in heteronuclear multidimensional correlation experiments.

Filtering one- and two-bond $^{13}\text{C}'$ - ^{15}N dipolar couplings can also be achieved using the $^{13}\text{C}'$ - ^{15}N - $^{13}\text{C}^\alpha$ experiment [28, 67, 68]. This experiment utilizes two SPECIFIC-CP [65] elements to transfer magnetization from $^{13}\text{C}'$ to ^{15}N and then from ^{15}N to $^{13}\text{C}^\alpha$. To compare the two approaches, we optimized all parameters in each experiment and directly tested the efficiencies with respect to the ^1H - ^{13}C cross-polarization and ^{15}N - $^{13}\text{C}^\alpha$ experiments using NAVL (Figure 4). The integrated intensities from these spectra are shown in Table 1. We found that the FDR approach gives 36–41% (for 2.0 and 1.5 msec dephasing times) of the signal with respect to the ^1H - ^{13}C cross-polarization experiment for the peak at 61.0 ppm (resonance not dephased), while the largest peak in the $^{13}\text{C}'$ - ^{15}N - $^{13}\text{C}^\alpha$ experiment (peak at 56.3 ppm) gives 22% intensity retention. Relative to the ^{15}N - $^{13}\text{C}^\alpha$ spectrum, FDR gives 68–76%, while the $^{13}\text{C}'$ - ^{15}N - $^{13}\text{C}^\alpha$ experiment gives 49% efficiency, an improvement in signal-to-noise of 38–55%. Another added benefit of our method is the relative ease of setting up the FDR experiment, which requires calibration of 90° and 180° pulses, while the ^{13}C

${}^{\nu}\text{-}^{15}\text{N}\text{-}^{13}\text{C}^{\alpha}$ experiment requires an additional SPECIFIC-CP transfer, which is sensitive to instrumental instabilities [88].

We also applied the FDR- $^{15}\text{N}\text{-}^{13}\text{C}^{\alpha}$ experiment to the integral membrane protein phospholamban (PLN). The monomeric mutant of PLN (AFA-PLN, C36A/C41F/C46A) was synthesized by solid-phase Fmoc chemistry with residues 30–33 labeled [$\text{U-}^{13}\text{C}, {}^{15}\text{N}$], resulting in four $^{15}\text{N}\text{-}^{13}\text{C}^{\alpha}$ bonds and three $^{13}\text{C}'\text{-}^{15}\text{N}$ peptide bonds. The protein was reconstituted in 8/1/1 (w/w/w) egg phosphatidylcholine/phosphatidylethanolamine/phosphatidic acid (PC/PE/PA) lipid bilayers at a lipid/protein ratio of 60/1. This ratio is higher than the typical membrane protein samples utilized for MAS spectroscopy and is necessary to maintain the protein structural and functional integrity [69, 70]. We acquired 1D (Figure 5) and 2D spectra (Figure 6) of the reference and FDR sequence. From these spectra, it is clear that the most upfield peak in the reference spectrum is the only signal that does not change in intensity (Figures 5A and 5B, respectively). This is more apparent from the difference spectrum in Figure 5C, which shows only three peaks. The 2D version of the sequence is shown in Figure 6 with a comparison to a standard $^{15}\text{N}/^{13}\text{C}^{\alpha}$ spectrum obtained with SPECIFIC-CP. It is straightforward to assign the missing peak to Asn³⁰. The incomplete dephasing of the signals in the FDR experiment can result from a small offset of the ^{13}C transmitter from the carbonyl carbons, isotopic dilution in the sample, or protein dynamics that can scale the dipolar couplings.

The 3D version of the FDR- $^{15}\text{N}\text{-}^{13}\text{C}^{\alpha}$ -DARR pulse sequence is shown in Figure 1B. Prior to t_2 evolution, this pulse sequence is identical to the 2D version reported in Figure 1A. Following SPECIFIC-CP, $^{13}\text{C}^{\alpha}$ chemical shifts are evolved during t_2 , and then allowed to mix under the dipolar assisted rotational resonance (DARR) condition. This gives a 3D spectrum of $^{15}\text{N}\text{-}^{13}\text{C}^{\alpha}\text{-}^{13}\text{C}^{\text{X}}$ ($t_1\text{-}t_2\text{-}t_3$). *The advantage of this method is to discriminate overlapped C^{α} resonances by strategically labeling the preceding residue (e.g., $^{13}\text{C}'$).* The 2D spectra without evolution of ^{15}N is shown in Figure 7, and demonstrates that the FDR pulse element filters out more than 80% of the signals from the reference spectrum.

Discussion

Sequence-specific assignments in MAS spectra of membrane proteins in fluid lipid membranes are a challenging endeavor. Several SSNMR studies on membrane proteins have reported residue-specific assignments that fail to give the atomic resolution provided for in crystalline or synthetically labeled samples. To address these problems, we propose an approach that is based on carbonyl-selective labeling combined with frequency-selective dipolar recoupling experiments and SPECIFIC-CP. These experiments will be useful to obtain specific assignments, while retaining better signal to noise (~68–76%) with respect to a 2D $^{15}\text{N}\text{-}^{13}\text{C}^{\alpha}$ experiment using SPECIFIC-CP than the $^{13}\text{C}'\text{-}^{15}\text{N}\text{-}^{13}\text{C}^{\alpha}$ experiment (~49%).

Selective labeling strategies have become useful to reduce the complexity of spectra and the assignment process in general. A common procedure is to grow bacteria and overexpressing target proteins in unlabeled growth media enriched with one or more ^{13}C and ^{15}N amino acids [71]. Alternatively, one can use reverse labeling [40], where unlabeled amino acids are added to the isotopically enriched growth media. Both give a significant reduction in spectral complexity. More advanced strategies involve the use of labeled metabolic precursors (Reviewed in [71]). This approach has been primarily used for reducing the number of $^{13}\text{C}\text{-}^{13}\text{C}$ scalar couplings [41, 43, 72], while maintaining a significant amount of ^{15}N and ^{13}C labels in the protein. The most robust protocols have been developed for labeling $^{13}\text{C}'$, $^{13}\text{C}^{\alpha}$, and $^{13}\text{C}^{\beta}$ with the goal of performing ^{13}C solution NMR relaxation studies without the interference of covalently attached ^{13}C nuclei [72, 73]. In particular, $^{13}\text{C}'$ labels can be incorporated in several different residues without the need to reduce the

labeling of the $^{13}\text{C}^\alpha$ (reverse labeling + labeled amino acids). The ideal use of our approach from a sensitivity perspective is to label all $^{13}\text{C}^\alpha$ in the protein and incorporate $^{13}\text{C}'$ labels in several different residue types. This can be accomplished using 2- ^{13}C -glucose [73] and supplementing the growth medium with [U- ^{13}C] labeled leucine. This application would allow $i+1$ residues with respect to leucine to be filtered out in the FDR-NC $^\alpha$ experiment, and clearly identified in the difference spectrum, which would simplify the assignment. Further advancements will be to combine carbonyl-selective labeling with combinatorial approaches used to assign overlapped spectra in solution NMR [74–79]. In particular, one can apply the FDR approach to $^{13}\text{C}^\alpha$ and $^{13}\text{C}^\beta$ atoms to achieve frequency selective filters for assignment, or can use any of the labeling strategies discussed above to achieve pair-wise $^{13}\text{C}'$ - ^{15}N labels to simplify the spectrum.

Conclusions

The approach presented here is a sensitive complement to the sequential $^{13}\text{C}'$ - ^{15}N - $^{13}\text{C}^\alpha$ experiments [28, 67, 68]. In particular, our method gives 38–55% more signal than the $^{13}\text{C}'$ - ^{15}N - $^{13}\text{C}^\alpha$ experiment. It allows one to select $^{12}\text{C}'$ - ^{15}N - $^{13}\text{C}^\alpha$ patterns while filtering out the $^{13}\text{C}'$ - ^{15}N - $^{13}\text{C}^\alpha$ patterns, with a sensitivity ~68–76% of the standard ^{15}N - $^{13}\text{C}^\alpha$ spectrum obtained with SPECIFIC-CP. In addition, our method allows for the possibility of identifying chemically identical proteins with differential labeling in a single sample. Frequency selective methods combined with novel isotope incorporation such as the method described in this paper and others [1, 41, 68, 80–83] will be useful to making sequence-specific assignments in fluid membrane protein samples and other samples with non-crystalline linewidths.

Experimental

Sample Preparation

N-acetyl-valyl-leucine was synthesized using solid-phase synthesis from Fmoc-[U- ^{13}C , ^{15}N]-Leu and Fmoc-[U- ^{13}C , ^{15}N]-Val (Sigma-Aldrich, Isotec). The N-terminus of valine was acetylated by acetic anhydride at natural ^{13}C abundance. AFA-PLN was labeled at residues 30–33 with 99% [U- ^{13}C , ^{15}N]-Asn, Leu, Phe, and Ile (Sigma-Aldrich, Isotec) using solid-phase synthesis as described previously [16]. Labeling was checked by solution NMR in dodecylphosphocholine micelles [70] and mass-spectrometry. PLN (~2 mg) was solubilized in 500 μl trifluoroethanol and added to 15 mg egg PC/PE/PA lipids (8/1/1, w/w/w; Avanti Polar Lipids, Alabaster, AL) in chloroform. The solvent was dried under a stream of nitrogen gas and placed in a vacuum dessicator overnight to remove residual organics. Vesicles were prepared by adding 2 ml buffer (20 mM HEPES pH 7.0, 100 mM potassium chloride, 10 mM dithiothreitol, and 3 mM sodium azide) to the lipid/protein and flash frozen 3–4 times in liquid nitrogen. Samples were then spun for 30 min at 200,000 $\times$ g in a benchtop ultracentrifuge. The pellet was transferred to a 3.2 mm thin-walled rotor with sample spacers (Agilent, Fort Collins, CO) to avoid dehydration.

Solid-State NMR Spectroscopy

NMR experiments were performed using a VNMRs spectrometer operating at a ^1H frequency of 600 MHz, with a 3.2 mm bioMAS probe [84], an MAS spinning rate of 8 kHz (ν_R), and a temperature of 20 $^\circ\text{C}$. All experiments utilized cross-polarization from ^1H (61 kHz) to ^{15}N (45 kHz) satisfying the $n = 2$ condition for 1.0 msec (PLN) or 2.0 msec (NAVL). The FDR sequence used 5.5 μsec 90 $^\circ$ pulses (45.5 kHz) applied to $^{13}\text{C}'$ and 11 μsec 180 $^\circ$ pulses (45.5 kHz) centered on the ^{15}N resonances. The ^{13}C pulses were phase-cycled using XY-4 [62], while those to ^{15}N were cycled using MLEV-4 [61]. A total of 12 (1.5 msec) or 16 (2.0 msec) rotor periods were employed to achieve dipolar dephasing from $^{13}\text{C}'$. The ^{15}N dimension had spectral width of 2 kHz and was evolved for 12 and 50

increments for PLN and NAVL experiments, respectively. Transfers from ^{15}N to $^{13}\text{C}^{\alpha}$ were achieved by moving the ^{13}C carrier frequency to $^{13}\text{C}^{\alpha}$ (59.2 ppm) and performing SPECIFIC-CP [65], which is related to the double cross-polarization experiment [66]. Spin-lock was applied to ^{15}N at $\frac{5}{2} \frac{\omega_R}{2\pi}$ (20 kHz) and $^{13}\text{C}^{\alpha}$ at $\frac{3}{2} \frac{\omega_R}{2\pi}$ (12 kHz) or $^{13}\text{C}'$ at $\frac{7}{2} \frac{\omega_R}{2\pi}$ (28 kHz). The SPECIFIC-CP time was 5–6 msec utilizing either a linear ($\pm 10\%$ γB_1) or adiabatic ramp ($\frac{\Delta}{2\pi} = \sim 2$ kHz, $\frac{\beta}{2\pi} = \sim 0.6$ kHz) [89] to $^{13}\text{C}^{\alpha}$ or $^{13}\text{C}'$ for reducing the sensitivity to instrumental instability [88]. The adiabatic ramp was used only to acquire spectra in Figure 4 in order to achieve the optimal transfers for the $^{13}\text{C}'$ - ^{15}N - $^{13}\text{C}^{\alpha}$ experiment (two SPECIFIC-CP transfers). The direct ^{13}C spectral width was 100 kHz and was acquired for 20 msec under TPPM [64] ^1H decoupling at 100 kHz. For the NAVL 2D experiments, 8 scans were collected with a recycle delay of 4 sec. The ^{13}C - ^{13}C FDR experiment used an indirect ^{13}C spectral width of 16 kHz, 50 increments, and a DARR [85] mixing time of 100 msec set to $n = 1$ rotary resonance condition. The 2D FDR- ^{15}N - $^{13}\text{C}^{\alpha}$ PLN experiment used 2,048 scans with a recycle delay of 2 sec. The 1D FDR experiment used 20,000 scans with a recycle delay of 2 sec. The CH_2 resonance of adamantane was referenced to 40.48 ppm [86] and indirectly to ^{15}N using the relative frequency ratio between ^{15}N and ^{13}C of $\Xi = 0.402979946$ [87].

Acknowledgments

We thank Dan Mullen for synthesizing NAVL and PLN and T. Gopinath, Raffaello Verardi, and Martin Gustavsson for helpful discussions. This work was supported by the National Institute of Health (GM64742 to G.V.).

ABBREVIATIONS

SSNMR	solid-state NMR
MAS	magic angle spinning
PLN	phospholamban
FDR	frequency-selective dipolar recoupling
DARR	Dipolar Assisted Rotational Resonance

References

1. Baldus M. Correlation experiments for assignment and structure elucidation of immobilized polypeptides under magic angle spinning. *Prog Nucl Magn Reson Spectrosc.* 2002; 41:1–47.
2. Opella SJ, Ma C, Marassi FM. Nuclear magnetic resonance of membrane-associated peptides and proteins. *Methods Enzymol.* 2001; 339:285–313. [PubMed: 11462817]
3. Watts A, Ulrich AS, Middleton DA. Membrane protein structure: the contribution and potential of novel solid state NMR approaches. *Mol Membr Biol.* 1995; 12:233–246. [PubMed: 8520624]
4. Nielsen AB, Straaso LA, Nieuwkoop AJ, Rienstra CM, Bjerring M, Nielsen NC. Broadband Heteronuclear Solid-State NMR Experiments by Exponentially Modulated Dipolar Recoupling without Decoupling. *J Phys Chem Lett.* 2010; 1:1952–1956. [PubMed: 20689682]
5. Lewandowski JR, De Paepe G, Griffin RG. Proton assisted insensitive nuclei cross polarization. *J Am Chem Soc.* 2007; 129:728–729. [PubMed: 17243786]
6. Jaroniec CP, Filip C, Griffin RG. 3D TEDOR NMR experiments for the simultaneous measurement of multiple carbon-nitrogen distances in uniformly (^{13}C), (^{15}N)-labeled solids. *J Am Chem Soc.* 2002; 124:10728–10742. [PubMed: 12207528]

7. Oyler NA, Tycko R. Conformational constraints in solid-state NMR of uniformly labeled polypeptides from double single-quantum-filtered rotational echo double resonance. *Magn Reson Chem.* 2007; 45(Suppl 1):S101–6. [PubMed: 18157809]
8. Bayro MJ, Huber M, Ramachandran R, Davenport TC, Meier BH, Ernst M, Griffin RG. Dipolar truncation in magic-angle spinning NMR recoupling experiments. *J Chem Phys.* 2009; 130:114506. [PubMed: 19317544]
9. Verel R, Meier BH. Polarization-transfer methods in solid-state magic-angle-spinning NMR: adiabatic CN pulse sequences. *Chemphyschem.* 2004; 5:851–862. [PubMed: 15253311]
10. Bertini I, Emsley L, Lelli M, Luchinat C, Mao J, Pintacuda G. Ultrafast MAS solid-state NMR permits extensive ¹³C and ¹H detection in paramagnetic metalloproteins. *J Am Chem Soc.* 2010; 132:5558–5559. [PubMed: 20356036]
11. Franks WT, Wylie BJ, Schmidt HL, Nieuwkoop AJ, Mayrhofer RM, Shah GJ, Graesser DT, Rienstra CM. Dipole tensor-based atomic-resolution structure determination of a nanocrystalline protein by solid-state NMR. *Proc Natl Acad Sci U S A.* 2008; 105:4621–4626. [PubMed: 18344321]
12. Zhou DH, Kloepper KD, Winter KA, Rienstra CM. Band-selective ¹³C homonuclear 3D spectroscopy for solid proteins at high field with rotor-synchronized soft pulses. *J Biomol NMR.* 2006; 34:245–257. [PubMed: 16645815]
13. Nadaud PS, Helmus JJ, Hofer N, Jaroniec CP. Long-range structural restraints in spin-labeled proteins probed by solid-state nuclear magnetic resonance spectroscopy. *J Am Chem Soc.* 2007; 129:7502–7503. [PubMed: 17530852]
14. De Angelis AA, Nevzorov AA, Park SH, Howell SC, Mrse AA, Opella SJ. High-resolution NMR spectroscopy of membrane proteins in aligned bicelles. *J Am Chem Soc.* 2004; 126:15340–15341. [PubMed: 15563135]
15. Durr UH, 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]
16. Traaseth NJ, Shi L, Verardi R, Mullen D, Barany G, Veglia G. Determination of Membrane Protein Structure and Topology Using a Hybrid Solution and Solid-State NMR approach. *Proc Natl Acad Sci.* 2009; 106:10165–70. [PubMed: 19509339]
17. Hu J, Asbury T, Achuthan S, Li C, Bertram R, Quine JR, Fu R, Cross TA. Backbone structure of the amantadine-blocked trans-membrane domain M2 proton channel from Influenza A virus. *Biophys J.* 2007; 92:4335–4343. [PubMed: 17384070]
18. Aisenbrey C, Bechinger B. Investigations of polypeptide rotational diffusion in aligned membranes by ²H and ¹⁵N solid-state NMR spectroscopy. *J Am Chem Soc.* 2004; 126:16676–16683. [PubMed: 15600374]
19. Naito A. Structure elucidation of membrane-associated peptides and proteins in oriented bilayers by solid-state NMR spectroscopy. *Solid State Nucl Magn Reson.* 2009; 36:67–76. [PubMed: 19647984]
20. Vosegaard T, Kamihira-Ishijima M, Watts A, Nielsen NC. Helix conformations in 7TM membrane proteins determined using oriented-sample solid-state NMR with multiple residue-specific ¹⁵N labeling. *Biophys J.* 2008; 94:241–250. [PubMed: 17827220]
21. Fu R, Gordon ED, Hibbard DJ, Cotten M. High resolution heteronuclear correlation NMR spectroscopy of an antimicrobial peptide in aligned lipid bilayers: peptide-water interactions at the water-bilayer interface. *J Am Chem Soc.* 2009; 131:10830–10831. [PubMed: 19621928]
22. Li Y, Berthold DA, Gennis RB, Rienstra CM. Chemical shift assignment of the transmembrane helices of DsbB, a 20-kDa integral membrane enzyme, by 3D magic-angle spinning NMR spectroscopy. *Protein Sci.* 2008; 17:199–204. [PubMed: 18227427]
23. Huang L, McDermott AE. Partial site-specific assignment of a uniformly (¹³C), (¹⁵N) enriched membrane protein, light-harvesting complex 1 (LH1), by solid state NMR. *Biochim Biophys Acta.* 2008; 1777:1098–1108. [PubMed: 18267103]
24. Yang J, Tasayco ML, Polenova T. Magic angle spinning NMR experiments for structural studies of differentially enriched protein interfaces and protein assemblies. *J Am Chem Soc.* 2008; 130:5798–5807. [PubMed: 18393505]

25. Han Y, Ahn J, Concel J, Byeon JJ, Gronenborn AM, Yang J, Polenova T. Solid-state NMR studies of HIV-1 capsid protein assemblies. *J Am Chem Soc.* 2010; 132:1976–1987. [PubMed: 20092249]
26. Andronesi OC, Becker S, Seidel K, Heise H, Young HS, Baldus M. Determination of membrane protein structure and dynamics by magic-angle-spinning solid-state NMR spectroscopy. *J Am Chem Soc.* 2005; 127:12965–12974. [PubMed: 16159291]
27. Ulmschneider MB, Sansom MS. Amino acid distributions in integral membrane protein structures. *Biochim Biophys Acta.* 2001; 1512:1–14. [PubMed: 11334619]
28. Shi L, Ahmed MA, Zhang W, Whited G, Brown LS, Ladizhansky V. Three-dimensional solid-state NMR study of a seven-helical integral membrane proton pump--structural insights. *J Mol Biol.* 2009; 386:1078–1093. [PubMed: 19244620]
29. Etzkorn M, Martell S, Andronesi OC, Seidel K, Engelhard M, Baldus M. Secondary structure, dynamics, and topology of a seven-helix receptor in native membranes, studied by solid-state NMR spectroscopy. *Angew Chem Int Ed Engl.* 2007; 46:459–462. [PubMed: 17001715]
30. Ader C, Schneider R, Hornig S, Velisetty P, Wilson EM, Lange A, Giller K, Ohmert I, Martin-Eaucclair MF, Trauner D, Becker S, Pongs O, Baldus M. A structural link between inactivation and block of a K⁺ channel. *Nat Struct Mol Biol.* 2008; 15:605–612. [PubMed: 18488040]
31. Bhate MP, Wylie BJ, Tian L, McDermott AE. Conformational dynamics in the selectivity filter of KcsA in response to potassium ion concentration. *J Mol Biol.* 2010; 401:155–166. [PubMed: 20600123]
32. Englander SW, Wand AJ. Main-chain-directed strategy for the assignment of ¹H NMR spectra of proteins. *Biochemistry.* 1987; 26:5953–5958. [PubMed: 3689754]
33. Seidel K, Andronesi OC, Krebs J, Griesinger C, Young HS, Becker S, Baldus M. Structural characterization of Ca(2+)-ATPase-bound phospholamban in lipid bilayers by solid-state nuclear magnetic resonance (NMR) spectroscopy. *Biochemistry.* 2008; 47:4369–4376. [PubMed: 18355039]
34. Jaroniec CP, MacPhee CE, Bajaj VS, McMahon MT, Dobson CM, Griffin RG. High-resolution molecular structure of a peptide in an amyloid fibril determined by magic angle spinning NMR spectroscopy. *Proc Natl Acad Sci U S A.* 2004; 101:711–716. [PubMed: 14715898]
35. Petkova AT, Ishii Y, Balbach JJ, Antzutkin ON, Leapman RD, Delaglio F, Tycko R. A structural model for Alzheimer's beta -amyloid fibrils based on experimental constraints from solid state NMR. *Proc Natl Acad Sci U S A.* 2002; 99:16742–16747. [PubMed: 12481027]
36. Smith SO, Kawakami T, Liu W, Ziliox M, Aimoto S. Helical structure of phospholamban in membrane bilayers. *J Mol Biol.* 2001; 313:1139–48. [PubMed: 11700069]
37. Hughes E, Middleton DA. Solid-state NMR reveals structural changes in phospholamban accompanying the functional regulation of Ca²⁺-ATPase. *J Biol Chem.* 2003; 278:20835–42. [PubMed: 12556441]
38. Cady SD, Schmidt-Rohr K, Wang J, Soto CS, Degradó WF, Hong M. Structure of the amantadine binding site of influenza M2 proton channels in lipid bilayers. *Nature.* 2010; 463:689–692. [PubMed: 20130653]
39. Ahuja S, Hornak V, Yan EC, Syrett N, Goncalves JA, Hirshfeld A, Ziliox M, Sakmar TP, Sheves M, Reeves PJ, Smith SO, Eilers M. Helix movement is coupled to displacement of the second extracellular loop in rhodopsin activation. *Nat Struct Mol Biol.* 2009; 16:168–175. [PubMed: 19182802]
40. Vuister GW, Kim S, Wu C, Bax A. 2D and 3D NMR Study of Phenylalanine Residues in Proteins by Reverse Isotopic Labeling. *J Am Chem Soc.* 1994; 116:9206–9210.
41. Hong M, Jakes K. Selective and extensive ¹³C labeling of a membrane protein for solid-state NMR investigations. *J Biomol NMR.* 1999; 14:71–74. [PubMed: 10382307]
42. Heise H, Hoyer W, Becker S, Andronesi OC, Riedel D, Baldus M. Molecular-level secondary structure, polymorphism, and dynamics of full-length alpha-synuclein fibrils studied by solid-state NMR. *Proc Natl Acad Sci U S A.* 2005; 102:15871–15876. [PubMed: 16247008]
43. Castellani F, van Rossum B, Diehl A, Schubert M, Rehbein K, Oschkinat H. Structure of a protein determined by solid-state magic-angle-spinning NMR spectroscopy. *Nature.* 2002; 420:98–102. [PubMed: 12422222]

44. Gullion T, Schaefer J. Rotational-Echo Double-Resonance NMR. *J Magn Reson.* 1989; 81:196–200.
45. Yang J, Parkanzky PD, Bodner ML, Duskin CA, Weliky DP. Application of REDOR subtraction for filtered MAS observation of labeled backbone carbons of membrane-bound fusion peptides. *J Magn Reson.* 2002; 159:101–110. [PubMed: 12482688]
46. Matsuoka S, Schaefer J. Double-quantum filtered rotational-echo double resonance. *J Magn Reson.* 2006; 183:252–258. [PubMed: 17008112]
47. Vuister GW, Wang AC, Bax A. Measurement of Three-Bond Nitrogen-Carbon J Couplings in Proteins Uniformly Enriched in ^{15}N and ^{13}C . *J Am Chem Soc.* 1993; 115:5334–5.
48. Masterson LR, Tonelli M, Markley JL, Veglia G. Simultaneous detection and deconvolution of congested NMR spectra containing three isotopically labeled species. *J Am Chem Soc.* 2008; 130:7818–7819. [PubMed: 18512910]
49. Tonelli M, Masterson LR, Hallenga K, Veglia G, Markley JL. Carbonyl carbon label selective (CCLS) ^1H - ^{15}N HSQC experiment for improved detection of backbone ^{13}C - ^{15}N cross peaks in larger proteins. *J Biomol NMR.* 2007; 39:177–185. [PubMed: 17828465]
50. Hing AW, Vega S, Schaefer J. Transferred-echo double-resonance NMR. *J Magn Reson.* 1992; 96:205–209.
51. Jaroniec CP, Tounge BA, Herzfeld J, Griffin RG. Frequency selective heteronuclear dipolar recoupling in rotating solids: accurate (^{13}C) - (^{15}N) distance measurements in uniformly (^{13}C) , (^{15}N) -labeled peptides. *J Am Chem Soc.* 2001; 123:3507–3519. [PubMed: 11472123]
52. Li S, Su Y, Luo W, Hong M. Water-protein interactions of an arginine-rich membrane peptide in lipid bilayers investigated by solid-state nuclear magnetic resonance spectroscopy. *J Phys Chem B.* 2010; 114:4063–4069. [PubMed: 20199036]
53. Hong M, Griffin RG. Resonance Assignments for Solid Peptides by Dipolar-Mediated $^{13}\text{C}/^{15}\text{N}$ Correlation Solid-State NMR. *J Am Chem Soc.* 1998; 120:7113–7114.
54. Chevelkov V, Faelber K, Diehl A, Heinemann U, Oschkinat H, Reif B. Detection of dynamic water molecules in a microcrystalline sample of the SH3 domain of alpha-spectrin by MAS solid-state NMR. *J Biomol NMR.* 2005; 31:295–310. [PubMed: 15928996]
55. Kaustov L, Kababya S, Belakhov V, Baasov T, Shoham Y, Schmidt A. Inhibition mode of a bisubstrate inhibitor of KDO8P synthase: a frequency-selective REDOR solid-state and solution NMR characterization. *J Am Chem Soc.* 2003; 125:4662–4669. [PubMed: 12683839]
56. Klug CA, Zhu WL, Tasaki K, Schaefer J. Orientational order of locally parallel chain segments in glassy polycarbonate from C- 13 -C- 13 dipolar couplings. *Macromolecules.* 1997; 30:1734–1740.
57. Bennett AE, Rienstra CM, Lansbury PT, Griffin RG. Frequency - selective heteronuclear dephasing by dipole couplings in spinning and static solids. *J Chem Phys.* 1996; 105:10289–10299.
58. Bennett AE, Becerra LR, Griffin RG. Frequency - selective heteronuclear recoupling in rotating solids. *J Chem Phys.* 1994; 100:812–814.
59. Schnell I, Langer B, Sontjens SH, van Genderen MH, Sijbesma RP, Spiess HW. Inverse detection and heteronuclear editing in ^1H - ^{15}N correlation and ^1H - ^1H double-quantum NMR spectroscopy in the solid state under fast MAS. *J Magn Reson.* 2001; 150:57–70. [PubMed: 11330984]
60. Yao XL, Schmidt-Rohr K, Hong M. Medium- and Long-Distance ^1H - ^{13}C Heteronuclear Correlation NMR in Solids. *J Magn Reson.* 2001; 149:139–143.
61. Levitt M, Freeman R, Frenkel T. Broadband heteronuclear decoupling. *J Magn Reson.* 1982; 47:328–30.
62. Gullion T, Baker DB, Conradi MS. New, compensated Carr-Purcell sequences. *J Magn Reson.* 1990; 89:479–484.
63. Mueller KT. Analytic solutions for the time evolution of dipolar-dephasing NMR signals. *J Magn Reson A.* 1995; 113:81–93.
64. Bennett AE, Rienstra CM, Auger M, Lakshmi KV, Griffin RG. Heteronuclear Decoupling in Rotating Solids. *J Chem Phys.* 1995; 103:6951–8.
65. Baldus M, Petkova AT, Herzfeld JH, Griffin RG. Cross polarization in the tilted frame: assignment and spectral simplification in heteronuclear spin systems. *Mol Phys.* 1998; 95:1197–1207.

66. Schaefer J, McKay RA, Stejskal EO. Double-Cross-Polarization NMR of Solids. *J Magn Reson.* 1979; 34:443–447.
67. Li Y, Berthold DA, Frericks HL, Gennis RB, Rienstra CM. Partial (^{13}C) and (^{15}N) chemical-shift assignments of the disulfide-bond-forming enzyme DsbB by 3D magic-angle spinning NMR spectroscopy. *Chembiochem.* 2007; 8:434–442. [PubMed: 17285659]
68. Schuetz A, Wasmer C, Habenstein B, Verel R, Greenwald J, Riek R, Bockmann A, Meier BH. Protocols for the sequential solid-state NMR spectroscopic assignment of a uniformly labeled 25 kDa protein: HET-s(1-227). *Chembiochem.* 2010; 11:1543–1551. [PubMed: 20572250]
69. Traaseth NJ, Buffy JJ, Zamoon J, Veglia G. Structural dynamics and topology of phospholamban in oriented lipid bilayers using multidimensional solid-state NMR. *Biochemistry.* 2006; 45:13827–13834. [PubMed: 17105201]
70. Traaseth NJ, Ha KN, Verardi R, Shi L, Buffy JJ, Masterson LR, Veglia G. Structural and dynamic basis of phospholamban and sarcolipin inhibition of Ca^{2+} -ATPase. *Biochemistry.* 2008; 47:3–13. [PubMed: 18081313]
71. Lian LY, Middleton DA. Labelling Approaches for Protein Structural Studies by Solution-State and Solid-State NMR. *Prog Nucl Magn Reson Spectrosc.* 2001; 39:171–190.
72. LeMaster DM, Kushlan DM. Dynamical Mapping of *E. coli* Thioredoxin via C-13 NMR Relaxation Analysis. *J Am Chem Soc.* 1996; 118:9255–9264.
73. Lundstrom P, Teilum K, Carstensen T, Bezsonova I, Wiesner S, Hansen DF, Religa TL, Akke M, Kay LE. Fractional ^{13}C enrichment of isolated carbons using $[1-^{13}\text{C}]$ - or $[2-^{13}\text{C}]$ -glucose facilitates the accurate measurement of dynamics at backbone α and side-chain methyl positions in proteins. *J Biomol NMR.* 2007; 38:199–212. [PubMed: 17554498]
74. Shi J, Pelton JG, Cho HS, Wemmer DE. Protein signal assignments using specific labeling and cell-free synthesis. *J Biomol NMR.* 2004; 28:235–247. [PubMed: 14752257]
75. Wu PS, Ozawa K, Jergic S, Su XC, Dixon NE, Otting G. Amino-acid type identification in ^{15}N -HSQC spectra by combinatorial selective ^{15}N -labelling. *J Biomol NMR.* 2006; 34:13–21. [PubMed: 16505960]
76. Weigelt J, van Dongen M, Uppenberg J, Schultz J, Wikstrom M. Site-selective screening by NMR spectroscopy with labeled amino acid pairs. *J Am Chem Soc.* 2002; 124:2446–2447. [PubMed: 11890787]
77. Trbovic N, Klammt C, Koglin A, Lohr F, Bernhard F, Dotsch V. Efficient strategy for the rapid backbone assignment of membrane proteins. *J Am Chem Soc.* 2005; 127:13504–13505. [PubMed: 16190707]
78. Sweredoski MJ, Donovan KJ, Nguyen BD, Shaka AJ, Baldi P. Minimizing the overlap problem in protein NMR: a computational framework for precision amino acid labeling. *Bioinformatics.* 2007; 23:2829–2835. [PubMed: 17895278]
79. Reckel S, Sobhanifar S, Schneider B, Junge F, Schwarz D, Durst F, Lohr F, Guntert P, Bernhard F, Dotsch V. Transmembrane segment enhanced labeling as a tool for the backbone assignment of α -helical membrane proteins. *Proc Natl Acad Sci U S A.* 2008; 105:8262–8267. [PubMed: 18550820]
80. Sperling LJ, Berthold DA, Sasser TL, Jeisy-Scott V, Rienstra CM. Assignment strategies for large proteins by magic-angle spinning NMR: the 21-kDa disulfide-bond-forming enzyme DsbA. *J Mol Biol.* 2010; 399:268–282. [PubMed: 20394752]
81. Higman VA, Flinders J, Hiller M, Jehle S, Markovic S, Fiedler S, van Rossum BJ, Oschkinat H. Assigning large proteins in the solid state: a MAS NMR resonance assignment strategy using selectively and extensively ^{13}C -labelled proteins. *J Biomol NMR.* 2009; 44:245–260. [PubMed: 19609683]
82. Jehle S, Rehbein K, Diehl A, van Rossum BJ. Amino-acid selective experiments on uniformly ^{13}C and ^{15}N labeled proteins by MAS NMR: Filtering of lysines and arginines. *J Magn Reson.* 2006; 183:324–328. [PubMed: 16990042]
83. Bodner ML, Gabrys CM, Parkanzky PD, Yang J, Duskin CA, Weliky DP. Temperature dependence and resonance assignment of ^{13}C NMR spectra of selectively and uniformly labeled fusion peptides associated with membranes. *Magn Reson Chem.* 2004; 42:187–194. [PubMed: 14745799]

84. Stringer JA, Bronnimann CE, Mullen CG, Zhou DH, Stellfox SA, Li Y, Williams EH, Rienstra CM. Reduction of RF-induced sample heating with a scroll coil resonator structure for solid-state NMR probes. *J Magn Reson.* 2005; 173:40–48. [PubMed: 15705511]
85. Takegoshi K, Nakamura S, Terao T. ^{13}C – ^1H dipolar-assisted rotational resonance in magic-angle spinning. *NMR Chem Phys Lett.* 2001; 344:631–637.
86. Morcombe CR, Zilm KW. Chemical shift referencing in MAS solid state NMR. *J Magn Reson.* 2003; 162:479–486. [PubMed: 12810033]
87. Wishart DS, Bigam CG, Yao J, Abildgaard F, Dyson HJ, Oldfield E, Markley JL, Sykes BD. ^1H , ^{13}C and ^{15}N chemical shift referencing in biomolecular NMR. *J Biomol NMR.* 1995; 6:135–40. [PubMed: 8589602]
88. Franks WT, Kloepper KD, Wylie BJ, Rienstra CM. Four-dimensional heteronuclear correlation experiments for chemical shift assignment of solid proteins. *J Biomol NMR.* 2007; 39:107–131. [PubMed: 17687624]
89. Hediger S, Meier BH, Ernst RR. Adiabatic passage Hartmann-Hahn cross-polarization in NMR under magic-angle sample-spinning. *Chem Phys Lett.* 1995; 240:449–456.

Figure 1.

Pulse sequences used to obtain (A) 2D FDR- ^{15}N - $^{13}\text{C}^\alpha$ and (B) 3D FDR- ^{15}N - $^{13}\text{C}^\alpha$ - $^{13}\text{C}^{\text{X}}$ DARR spectra. The 90° pulses on ^{13}C are phase cycled with XY-4 [62] and applied at the end of each rotor cycle, while those 180° pulses applied to ^{15}N are applied in the middle of the rotor cycle with MLEV-4 phase cycling [61]. Phase cycling is as follows: $_1 \{x, -x\}$, $_2 \{y\}$, $_3 \{x, x, y, y, -x, -x, -y, -y\}$, $_4 \{y, y, -x, -x, -y, -y, x, x\}$, $_5 \{-y, -y, x, x, y, y, -x, -x\}$, $_{\text{rec}} \{x, -x, y, -y, -x, x, -y, y\}$. Phases $_2$ and $_3$ are adjusted by 90° to generate phase-sensitive data in t_1 and t_2 , respectively.

Figure 2. FDR dephasing $^{15}\text{N}\{^{13}\text{C}\}$ curves for one-bond (1.3 Å) and two-bond (2.5 Å) $^{13}\text{C}'\text{-}^{15}\text{N}$ distances. The dotted line indicates the maximum dephasing for one-bond transfer, showing less than 0.1 dephasing for the two-bond curve. The FDR dephasing data is scaled by $1/\sqrt{2}$ with respect to the REDOR experiment [44, 58].

Figure 3. FDR- ^{15}N - $^{13}\text{C}^\alpha$ spectra for NAVL. Spectra were acquired with (FDR, panel A) and without ^{13}C 90° pulses (reference, panel B). A total dephasing time of 2 msec was used with an MAS rate of 8 kHz. (C) Subtraction of the FDR spectrum from that of the reference. Note that in the subtracted spectrum, the noise increases by a factor of $\sqrt{2}$.

Figure 4. Comparison of ^1H - ^{13}C cross-polarization, ^{15}N - $^{13}\text{C}\alpha$ SPECIFIC-CP, $^{13}\text{C}'$ - ^{15}N - $^{13}\text{C}\alpha$ double SPECIFIC-CP, and FDR- ^{15}N - $^{13}\text{C}\alpha$ (2.0 msec dephasing time) experiments. The integrated intensities from these spectra are shown in Table 1.

Figure 5. FDR- ^{15}N - ^{13}C α spectra for AFA-PLN labeled $[\text{U-}^{13}\text{C}, ^{15}\text{N}]$ at residues 30–33. 1D spectra were acquired with (FDR, panel A) and without ^{13}C 90° pulses (reference, panel B). A total dephasing time of 2 msec was used with an MAS rate of 8 kHz. (C) Subtraction of the FDR spectrum from the reference.

Figure 6. 2D FDR- ^{15}N - $^{13}\text{C}\alpha$ spectra for AFA-PLN labeled [$\text{U-}^{13}\text{C}$, ^{15}N] at residues 30–33. (A) Difference spectrum for AFA-PLN $^{30-33}$ using similar parameters as that in Figure 5. (B) ^{15}N - $^{13}\text{C}\alpha$ spectrum of AFA-PLN $^{30-33}$.

Figure 7. FDR- $^{13}\text{C}^{\alpha}$ - $^{13}\text{C}^{\text{X}}$ -DARR spectra for NAVL. (A) Spectra were acquired with (FDR, panel i) and without ^{13}C 90° pulses (reference, panel ii) using the pulse sequence in Figure 1B. A total dephasing time of 2 msec was used with an MAS rate of 8 kHz. (iii) Subtraction of the FDR spectrum from that of the reference. A 100 msec DARR mixing time was used. Panels B and C show the 1D traces from the 2D spectra in panel A.

Table 1

Transfer efficiencies calculated from integrated 1D NAVL spectra for the ^{15}N - $^{13}\text{C}^\alpha$, $^{13}\text{C}'$ - ^{15}N - $^{13}\text{C}^\alpha$, and FDR- ^{15}N - $^{13}\text{C}^\alpha$ experiments in Figure 4 expressed as a percentage from that of the cross-polarization (^1H - ^{13}C) experiment. Data are normalized with respect to each peak in NAVL. The FDR experiment was conducted using total dephasing times of 1.5 and 2.0 msec. Errors for all values are less than 0.1%.

Experiment	Peak 1 ($\text{C}^\alpha = 61.0\text{ppm}$)	Peak 2 ($\text{C}^\alpha = 56.3\text{ppm}$)
Cross-Polarization	100%	100%
N- C^α	53.3%	44.5%
C' -N- C^α	5.6%	22.0%
FDR-N- C^α (1.5 msec)	40.7%	9.6%
FDR-N- C^α (2.0 msec)	36.3%	6.3%