Open Journal of Biophysics, **2012, 2, 109-116**
http://dx.doi.org/10.4236/ojbiphy.2012.24014 Published Online October 2012 (http://www.SciRP.org/journal/ojbiphy)

Solid-State NMR Spectroscopic Approaches to Investigate Dynamics, Secondary Structure and Topology of Membrane Proteins

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Received June 15, 2012; revised July 29, 2012; accepted August 20, 2012

ABSTRACT

Solid-state NMR spectroscopy is routinely used to determine the structural and dynamic properties of both membrane proteins and peptides in phospholipid bilayers [1-26]. From the perspective of the perpetuated lipids, ^{2}H solid-state NMR spectroscopy can be used to probe the effect of embedded proteins on the order and dynamics of the acyl chains of phospholipid bilayers [8-13]. Moreover, ^{31}P solid-state NMR spectroscopy can be used to investigate the interaction of peptides, proteins and drugs with phospholipid head groups [11-14]. The secondary structure of ^{13}C=O site-specific isotopically labeled peptides or proteins inserted into lipid bilayers can be probed utilizing ^{13}C CPMAS solid-state NMR spectroscopy [15-18]. Also, solid-state NMR spectroscopic studies can be utilized to ascertain pertinent information on the backbone and side-chain dynamics of ^{2}H- and ^{15}N-labeled proteins, respectively, in phospholipid bilayers [19-26]. Finally, specific ^{15}N-labeled amide sites on a protein embedded inside oriented bilayers can be used to probe the alignment of the helices with respect to the bilayer normal [2]. A brief summary of all these solid-state NMR approaches are provided in this minireview.

Keywords: Solid-State NMR; Structure and Dynamics; Membrane Proteins

1. Introduction

Membrane proteins make up approximately one-third of the total number of known proteins [27]. They play several significant roles in biological systems that includes transporting ions, acting as receptors, participating in membrane fusion and destabilization, and many others. Despite the abundance and clear importance of membrane-associated proteins, limited information about these systems exists. Structural studies of these membrane proteins are key to understand their biological functions. X-ray crystallography has been used to elucidate structural information of biologically significant protein systems [28-35]. However, the hydrophobic surfaces associated with membrane-bound protein systems make the crystallization process very challenging. Although investigators are making progress with X-ray techniques, still only a limited list of membrane protein structures have been obtain via X-ray crystallography [36]. Alternatively, solid-state NMR spectroscopy is a powerful technique that can be used to provide structural, orientational, and dynamic information about membrane protein systems in model membrane systems [2,37,38].

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2. Solid-State NMR Approaches

2.1. ^{2}H Solid-State NMR Spectroscopy of Lipids with ^{2}H-Lableled Acyl Chains

Multilamellar vesicles (MLVs) can be prepared from phospholipids such as 1-palmitoyl-2-oleoyl-sn-glycero-phosphocholine (POPC) and the details of sample preparation and type of lipid was reported previously [3-7]. Approximately 10% of deuterated POPC (*sn*-1 chain, POPC-d_{31}, see **Figure 1**) or any other deuterated lipid of interest can be added to each sample. Other lipids can be used such as the negatively charged lipids such as 1-palmitoyl(d_{31})-2-oleoyl-sn-glycero-3-[phospho-L-serine] phospholipids (POPS-d_{31}) used in Sap C studies (see **Figure 2**) and Distearoyl-d_{70}-phosphatidylglycerol (DSPG-d_{70}) (see **Figure 3**).

The effect of embedded proteins on the order and dynamics of the acyl chains of POPC-d_{31} bilayers can be investigated utilizing ^{2}H solid-state NMR spectroscopy in the absence as well as in the presence of (X) mol% of the protein with respect to the lipids at different temperatures [40]. For example, in **Figure 4(a)**, the central resonance doublet corresponds to the terminal CD_3 groups (Carbon # 1 in **Figure 1**) and the remaining over-

lapped doublets result from the different CD_2 segments of the acyl chain of POPC-d_{31}. The addition of (X) mol% of specific protein with respect to the lipids to POPC MLVs could alter (depending on the protein of interest) the lineshape and the spectral resolution of the ^2H NMR spectra (**Figure 4(b)**) when compared to the control (**Figure 4(a)**) sample.

In **Figure 4(b)**, the loss in spectral resolution is manifested by the disappearance of sharp edges of the ^2H NMR peaks. The changes in spectral resolution of the ^2H NMR spectra indicate that the protein interacts with the POPC-d_{31}-containing MLVs when compared to the control. If the spectral resolution and the line shapes did not change when compared to the control, this indicates that the protein does not interact significantly with the POPC MLVs as indicated in a previous study from the Lorigan Lab using the transmembrane domain segment of WT-PLB [12].

All the powder-type ^2H NMR spectra of multilamellar dispersions of POPC-d_{31} can be numerically deconvoluted (dePaked) using the algorithm of McCabe and Wassal [41,42]. These spectra can be dePaked such that the bilayer normal was perpendicular with respect to the direction of the static magnetic field. Then, the quadrupolar splittings can be directly measured from the dePaked spectra and converted into order parameters as described before [11,12]. The quadrupolar splittings of the CD_3 methyl groups (see **Figure 1**) at the end of the acyl chains are the smallest and close to 0 kHz because they rotate at the fastest frequency. The second smallest splitting was assigned to the ^2H attached to C-14 and so forth along the acyl chain. The quadrupolar splittings for the deuterons in the plateau region were estimated by integration of the last broad peak.

The quadrupolar splittings can be directly measured from the dePaked spectra and converted into the S_{CD} order parameter using the following expression; $\Delta\nu_Q^i = 3/4(e^2qQ/h)\, S_{CD}^i$ [43-45]. Where $\Delta\nu_Q^i$ is the quadrupolar splitting for a deuteron attached to the ith carbon, e^2qQ/h is the quadrupolar splitting constant (168 kHz for deuterons in C-^{2}H bonds), and S_{CD}^i is the chain order parameter for a deuteron attached to the *i*th carbon of the acyl chain of POPC-d_{31}. The order parameters calculated for the CD_3 quadrupolar splitting should be multiplied by three according to procedures in the literature [46,47].

Figure 5 displays the smoothed segmental C-D bond order parameters (S_{CD}) of the POPC-d_{31} acyl chains calculated by dePakeing the corresponding ^2H NMR powder spectra shown in **Figure 4**. **Figure 5** reveals a characteristic profile of decreasing order (S_{CD}) with increasing distance from the glycerol backbone of POPC-d_{31} MLVs. Any decrease (if any) in the order parameter profile of POPC-d_{31}-containing bilayers in the presence of (X) mol% of specific protein indicates more disorder and motion in the acyl chain region of the lipids when compared to the control sample (without protein). If the order parameter profile of POPC-d_{31}-containing bilayers in the presence of (X) mol% protein is similar to the control sample, this indicates that this particular protein is not altering the order and motion in the acyl chain region of the lipids.

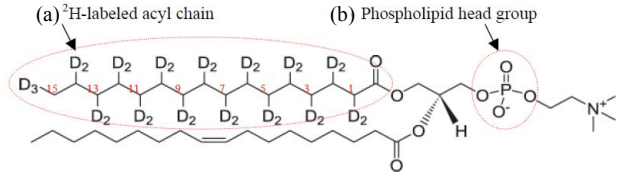

Figure 1. POPC-d_{31} structure [39]. The ^2H-labeled acyl chain and phospholipids head groups are labeled with dashed red circles. The numbering of the ^2H-labeled carbon skeleton of the acyl chain is shown is red.

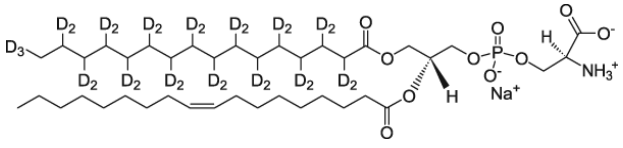

Figure 2. POPS-d_{31} structure [39]. Only one acyl chain is ^2H-labeled.

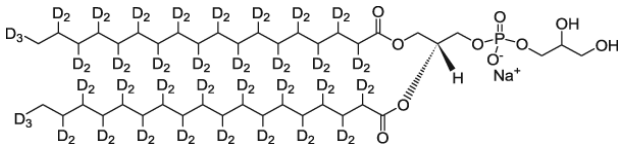

Figure 3. Distearoyl-d_{70}-phosphatidylglycerol (DSPG-d_{70}) structure [39].

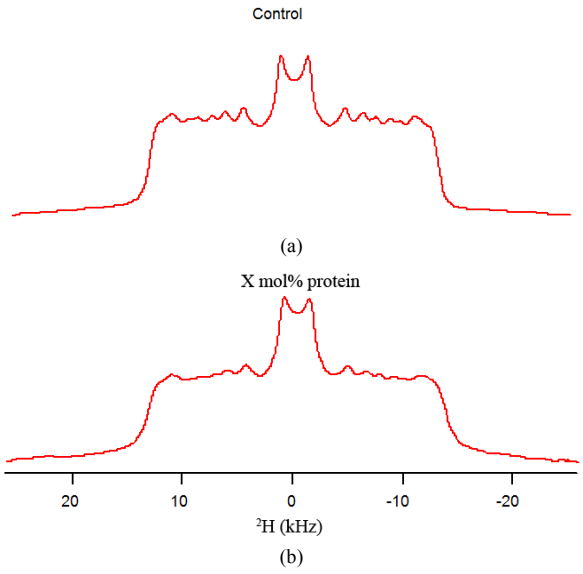

Figure 4. ^2H NMR powder-pattern spectra of POPC-d_{31} bilayers in the absence (a) and in the presence of (X) mol% protein with respect to the lipids.

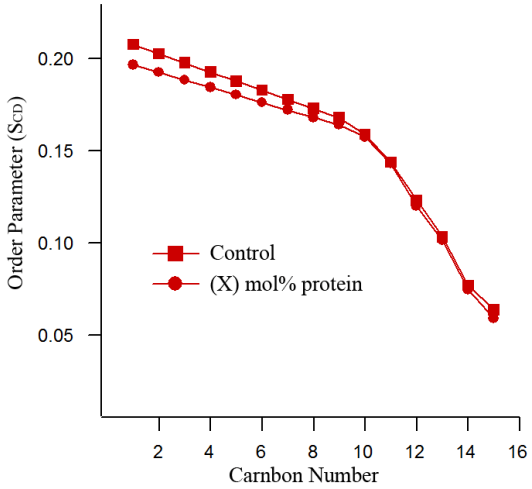

Figure 5. The smoothed acyl chains orientational order parameter (S_{CD}) profiles calculated from the dePaked ^{2}H NMR spectra of POPC-d_{31} (Figure 4). The closed squares and circles represent POPC bilayers with 0 mol% and (X) mol% protein, respectively.

2.2. ^{31}P Solid-State NMR Spectroscopy of Phospholipid Head Groups

The motionally averaged powder pattern spectra are characteristic of MLVs in the liquid crystalline phase (L_α) and are expected for phospholipids bilayers at a temperature well above the chain melting point transition temperature (T_m) [48]. **Figure 6(a)** shows the structure of the neutral phospholipid POPC (T_m = –2˚C) [39]. In addition, other phospholipids that includes 1-palmitoyl-2-oleoyl-sn-glycero-3-[phospho-L-serine] (POPS, T_m = 14 ˚C, **Figure 6(b)**), dioleoylphosphatidyl-glycerol (DOPG, T_m = –18˚C, **Figure 6(c)**) and dioleoylphosphatidylserine (DOPS, T_m = –11˚C, **Figure 6(d)**) structures are shown [39]. The static ^{31}P NMR spectrum of each phospholipid head group shown in **Figures 6(a)-(d)** have a specific ^{31}P chemical shift anisotropy (CSA; is equal σ_{33}-σ_{11}) width and lineshape. In several cases, the lineshapes of the static ^{31}P NMR spectra and its corresponding CSA values have been successfully used to study the perturbation effect induced by drugs and proteins on phospholipids [11,14,49,50]. The addition of (X) mol% of specific protein to specific MLVs could alter the ^{31}P CSA and line shape (**Figure 7(b)**) when compared to the control (**Figure 7(a)**) sample. This indicates that the protein of interest is interacting with the head group region of the MLVs.

2.3. ^{13}C Solid-State NMR Spectroscopy of Site-Specific ^{13}C-Labeled Peptides in MLVs

The secondary structure of ^{13}C=O site-specific isotopically labeled peptides or proteins inserted into lipid bilayers can be probed utilizing ^{13}C CPMAS solid-state NMR spectroscopy [15-18,50]. The local conformations of peptides and proteins can be characterized by examining the ^{13}C=O chemical shifts of the ^{13}C-labeled carbonyl of Ala, Leu, and Val. In general, the α-helical structure chemical shifts may vary within the data range (174 - 177 ppm) for different conformational-dependent changes (see **Figure 8(a)**) [17]. Also, a single ^{13}C NMR peak at approximately 172.4 ppm can be attributed to either a β-sheet or an unstructured structural conformation of the peptide (see **Figure 8(b)**); whereas a single peak at approximately 176 ppm can be attributed to one α-helical structure conformation (see **Figure 8(c)**) [15].

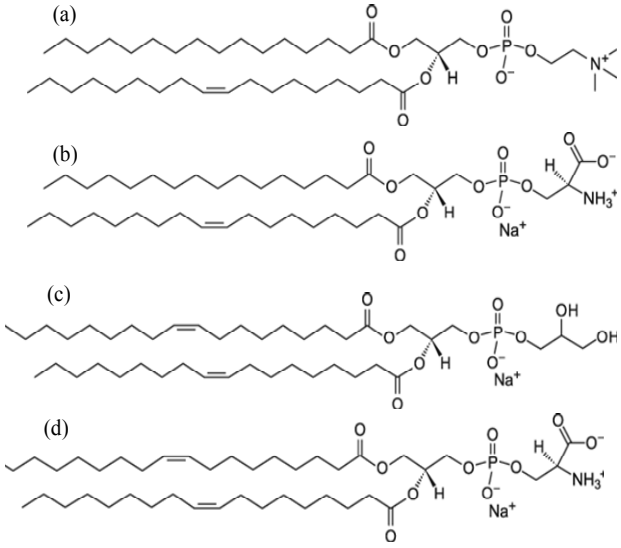

Figure 6. The structure of (a) POPC, (b) POPS, (c) DOPG and (d) DOPS phospholipids [39].

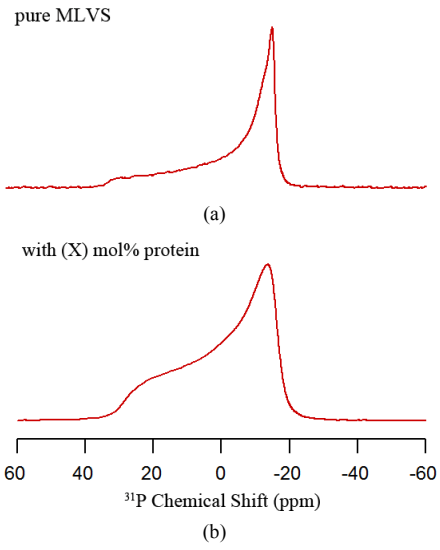

Figure 7. Static ^{31}P solid-state NMR spectra of phospholipid MLVs in the absence (a) and in the presence (b) of X mol% protein.

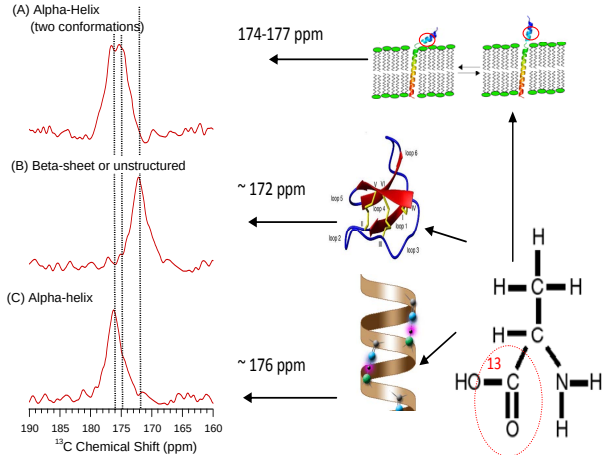

Figure 8. ^{13}C=O chemical shifts of ^{13}C-labeled carbonyl of Ala located at (A) α-helix with two structural conformations, (B) Unstructured or β-sheet structural conformation and (C) α-helical with one structure conformation.

2.4. ^{2}H Solid-State NMR Spectroscopy of Site-Specific ^{2}H-Labeled Peptides in MLVs

As mentioned previously, probing of how both segments of phosphorylated and unphosphorylated membrane proteins are moving within the phospholipid bilayers is crucial to describe the physiological functions. ^{2}H solid-state NMR spectroscopy is a powerful well developed technique to study the structural and side-chain dynamic properties of membrane proteins in phospholipid bilayers [8,51-53]. The corresponding quadrupolar splitting and lineshapes of the ^{2}H solid-state NMR spectra can be used to probe the molecular dynamics of the sidechain of selectively labeled residues in site-specific ^{2}H-labeled integral membrane proteins [19-24].

In previous studies, methyl group motions have been well-characterized utilizing ^{2}H NMR studies of CD_3-labeled sites of alanines, valines and leucines [19,24,25, 54-57]. For the isotopically labeled alanines (short aliphatic side-chains), the deuterated methyl group (CD_3) rotates along the C_α-C_β bond and allows the deuterons to make jumps between three-sites described by a tertrahedral geometry (see **Figure 9(a)**) [58,59]. However, for Leu, the long aliphatic side-chain can be isotopically labeled at the δ- and/or ε-CD_3 sites (see **Figure 9 (b)**) and the deuterium NMR powder pattern lineshapes will be strongly influenced by the motions about the C_γ-C_δ bond axis as well as by additional librational motion about the C_α-C_β and C_β-C_γ bond axes at various temperatures [24,60].

It has been reported that if the CD_3-methyl probe of a protein undergoes no motion other than those associated with the axial rotation about the C-CD_3 bond in a randomly dispersed sample, the resultant ^{2}H NMR spectra will consist of a Pake pattern with a 40 kHz quadrupolar splitting (see **Figure 10(a)**) [61]. However, residues located outside the membrane are expected to be more motionally averaged and yield an isotropic peak (see **Figure 10(b)**) [62].

2.5. ^{15}N Solid-State NMR Spectroscopy of Site Specific ^{15}N-Labeled Peptides in MLVs

^{15}N solid-state NMR spectroscopic studies are utilized to ascertain pertinent information on the backbone structure and dynamics of membrane proteins in phospholipid bilayers [25,26]. It provides important physiological and mechanistic information regarding the regulatory role of membrane proteins and it phosphorylated form in biological systems [64-66]. Generally, the immobile (without large amplitude motions) amide sites of specific ^{15}N-labeled proteins yield a broad static ^{15}N powder pattern (see **Figure 11(a)**); whereas, motionally averaged amide sites reveal isotropic peaks (see **Figure 11(b)**) [26].

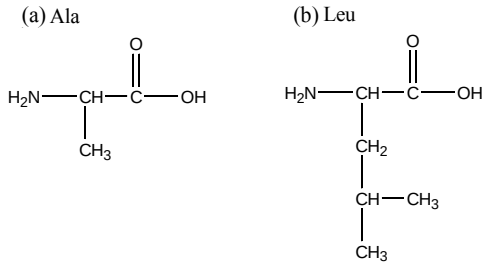

Figure 9. The structure of (a) alanine and (b) leucine residues.

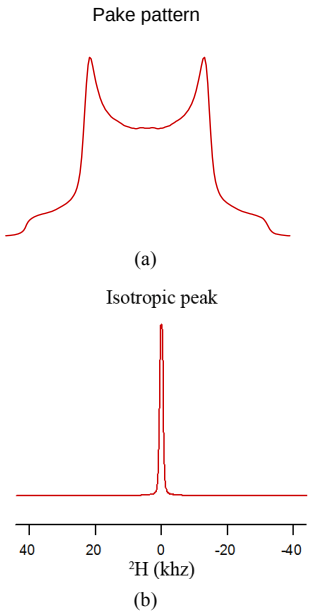

Figure 10. ^{2}H NMR simulations of CD_3-Ala using Multiple Axis Quadrupolar Echo Tailing (MXQET) program [63]. The model simulations show (a) Pake pattern and (b) isotropic peak spectra.

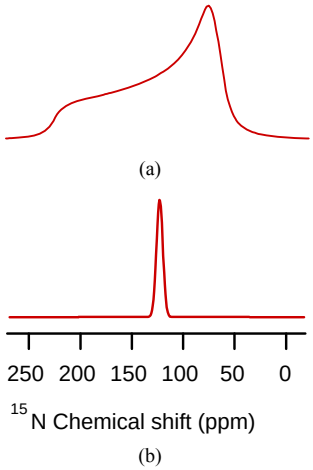

Figure 11. Simulations of the ^{15}N NMR spectra using the Dominique Massiot's Fit (DMFIT) software program [67]. The model simulations show (a) a powder pattern and (b) an isotropic peak.

2.6. ^{15}N Solid-State NMR Spectroscopy of Site-Specific ^{15}N-Labeled Peptides in Mechanically Oriented Bilayers

Determining the structural topology of membrane proteins and its interaction with the lipids is critical to understand its physiological regulatory function. ^{15}N solid-state NMR spectroscopy is a powerful tool to ascertain direct information regarding the structural topology of membrane proteins in oriented phospholipid bilayers [2].

In this approach, specific ^{15}N labeled amide sites can be used to probe the alignment of the helix with respect to the bilayer normal. For example, this technique was used to probe the orientation of both the transmembrane and cytoplasmic domains of WT-PLB embedded inside mechanically oriented phospholipids [68]. Site-specific ^{15}N-labeled WT-PLB were chosen at Ala11 (in the cytoplasmic domain) and Leu 42 and Leu 51 (both in the transmembrane helix) (see **Figure 12**).

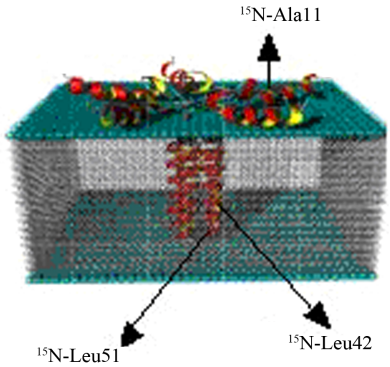

Figure 12. Side-view of a proposed structural model of site-specific ^{15}N-labeled WT-PLB at Ala11 (in the cytoplasmic domain) and Leu 42 and Leu 51 (both in the transmembrane helix) in oriented bilayers.

A resonance peak at approximately 70 ppm (close to the $\sigma_{\perp}$ component of the chemical shift tensor of the corresponding powder spectrum, see **Figure 13**), indicates that this residue is oriented approximately perpendicular to the bilayer normal (transmembrane helix as shown in **Figure 12**) [2]. Also, a ^{15}N resonance peak at approximately 210 ppm (close to the $\sigma_{||}$ component of the chemical shift tensor of the corresponding powder spectra, see **Figure 13**) indicates that the amide backbone vector of this residue is nearly parallel to the bilayer normal (the helix lies on the surface of the phospholipids bilayers as shown for cytoplasmic domain of the proposed structural model of WT-PLB presented in **Figure 12** [2].

3. Conclusion

Solid-state NMR spectroscopy is routinely used to determine the structural and dynamic properties of both membrane proteins and peptides in phospholipid bilayers. Together, ^{2}H, ^{31}P, ^{13}C and ^{15}N solid-state NMR spectroscopy can be used to probe the effect of embedded proteins on the order and dynamics of the acyl chains, phospholipid head group as well as the secondary structure of site-specific isotopically labeled amino acid and helix orientation with respect to the membrane. A summary of all these solid-state NMR approaches are provided in this mini-review.

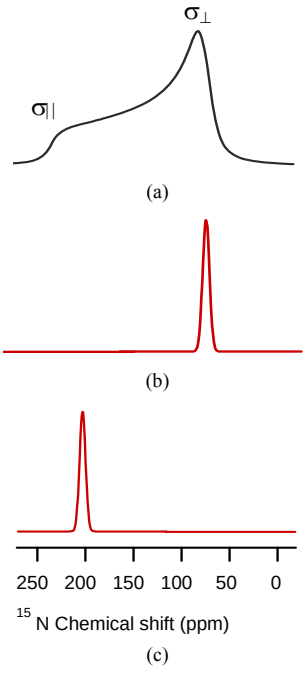

Figure 13. Simulations of the ^{15}N NMR spectra using the DMFIT software program [67]. The model simulations show (a) powder pattern and (b) ^{15}N resonance peak at approximately 70 ppm (close to the $\sigma_{\perp}$ component of the chemical shift tensor of the corresponding powder spectrum) and (c) ^{15}N resonance peak at approximately 210 ppm (close to the $\sigma_{||}$ component).

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