High-Resolution Solid-State NMR Applied to Polypeptides and Membrane Proteins

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ABSTRACT

Solid-state NMR provides unique possibilities to study insoluble or noncrystalline molecules at the atomic level. High-resolution conditions can be established by employing magic-angle spinning at ultrahigh magnetic fields. We discuss NMR methods that make use of these experimental improvements and allow for the study of multiply or uniformly [^{13}C,^{15}N]-labeled polypeptides and proteins. Recent biophysical applications are reviewed.

1. Introduction

In many research fields, nuclear magnetic resonance (NMR), which probes the interaction of microscopic, nuclear magnetic moments (such as ^{1}H, ^{13}C, or ^{15}N spins) with a static magnetic field (B_0) and an external radio frequency (rf) field, has made important contributions to our present understanding of molecular structure and function. In particular, NMR has become a standard method for the characterization of three-dimensional (3D) structure and dynamics of proteins that undergo fast molecular reorientations in solution.[1] Such macroscopic sample conditions are, however, difficult to establish for protein aggregates, which are associated with a growing number of protein folding diseases.[2] Moreover, integral membrane proteins, which constitute about 30% of all proteins, are often difficult to solubilize or crystallize in functional form. Membrane receptors or channels frequently interact with ligands and are involved in a number of important physiological processes including cellular communication and selective transport. In all these cases, NMR techniques particularly designed for the study of solid-phase systems ("solid-state NMR") offer unique possibilities to elucidate structural or dynamic parameters at atomic resolution.

Unlike in solution, the spectral resolution and the overall sensitivity of solid-state NMR is influenced by the size and the orientation dependence of the nuclear spin interactions, i.e., the chemical shielding and the homo- and heteronuclear dipolar spin−spin couplings. Recent progress in NMR instrumentation, in particular the combination of ultrahigh magnetic fields (i.e., above 14 T) with rapid sample rotation about the "magic" angle (magic angle spinning, MAS[3]), where the orientation dependence of the nuclear spin interactions is minimized, have considerably improved the use of solid-state NMR in biophysical applications. Such conditions have, thus far, not permitted a widespread application of high-resolution ^{1}H NMR spectroscopy, the most sensitive detection method in solution, but they have greatly extended the possibilities to study multiply or uniformly [^{13}C,^{15}N]-isotope-labeled polypeptides in high sensitivity and adequate spectral resolution.

In this Account, we will give a summary of rf pulse schemes that make use of these *high-resolution* solid-state NMR (HR-SSNMR) conditions and permit the detection of multiple structural parameters from a single protein sample or NMR data set. Recent applications in polypeptides and (membrane) proteins are discussed and underline the growing potential of HR-SSNMR to provide exclusive insight into the microscopic details of biological functioning.

2. Methodology

The NMR spectrum of randomly oriented solid-phase molecules is extensively broadened by the anisotropic interactions. High-resolution conditions can be established by the combined use of MAS, rf decoupling schemes, and ultrahigh magnetic fields (Figure 1). Two-dimensional (2D) spectroscopy[4] permits the spectral separation of NMR signals of multiply labeled molecules and the detection of coherence transfer between the individual nuclei. Such transfer can result from isotropic J (through-bond[5]) and anisotropic dipolar (through-space) couplings. While J couplings report on the chemical bonding, dipolar couplings represent a direct measure for the internuclear distance. In polypeptides, the strongest dipolar couplings usually dominate through-bond interactions and represent the most direct instrument for structure determination in the solid state. To observe these interactions under MAS conditions, a variety of rf (dipolar recoupling) techniques have been developed.[6,7]

Depending on the structural parameter of interest, homo- or heteronuclear dipolar recoupling can be achieved for all nuclei independently of their chemical shift range

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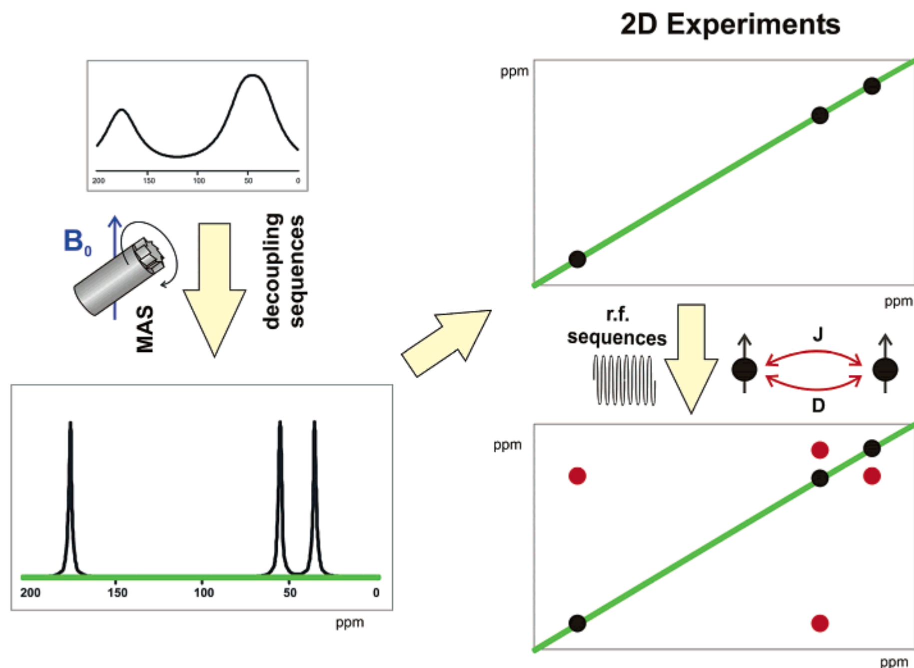

FIGURE 1. Basic tools for the study of multiply labeled biomolecules in solid-state NMR: line-narrowing techniques and 2D NMR spectroscopy.

(broad-band recoupling), for a defined spectral range (band-selective recoupling) or even specifically for spin pairs exhibiting particular chemical shifts (chemical-shift-selective). In this section, 2D experiments that make use of these pulse sequences and lead to resonance assignments or help to extract structural parameters in multiply labeled immobilized proteins are reviewed.

2.1. Spectral Assignment. Before a structural characterization in a multiply labeled sample can proceed, the observed signals must be identified and attributed to the NMR-detectable nuclei. As a spectroscopic quantity that measures local electronic environments, the isotropic chemical shift is exquisitely sensitive to amino acid type. As a result, *intraresidue* resonance assignment in polypeptides usually begins with homonuclear (^{13}C,^{13}C) correlation experiments that allow identification of different amino acid types based on their characteristic chemical shift correlation patterns. In Figure 2, results of two alternative 2D correlation experiments applied to the tripeptide Ala-Gly-Gly are shown. In a single-quantum (1Q) correlation experiment, through-space or through-bond correlations give rise to cross-peaks labeled with the isotropic chemical shifts Ω_j and Ω_k of the interacting spins. Magnetization transfer among spins can be enhanced by through-bond[5] and adiabatic through-space transfer.[8] Signal intensities on the diagonal can also be eliminated by employing double-quantum (2Q) correlation experiments. Here, the scalar or dipolar coupling can be exploited to excite a coupled spin state (2Q coherence) that evolves under the influence of both spins during t_1. The two isotropic signals appear at the sum of the respective isotropic shifts $\Omega_j + \Omega_k$ in the 2Q dimension.[4,7]

Carbon−carbon correlations can also be related to nearest neighbor ^{15}N spins, as shown in Figure 3. For example, selective polarization transfer from backbone ^{15}N to $^{13}C_\alpha$ carbon atoms[9] can, in conjunction with subsequent (^{13}C,^{13}C) transfer steps, be used to complete heteronuclear ^{15}N/^{13}C intraresidue spectral assignments. The connectivity between adjacent polypeptide residues (*interresidue* assignment) can be established by directing the ^{15}N/^{13}C transfer from the backbone ^{15}N resonance to the nearest neighbor carbonyl group (i.e., of the previous residue). Due to the small chemical shift dispersion of carbonyl NMR signals, a further homonuclear broad-band magnetization transfer to $^{13}C_{\alpha,\beta}$ is usually mandatory.[7]

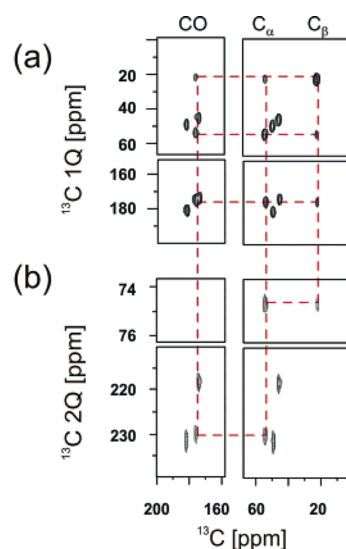

FIGURE 2. Two examples of a homonuclear (^{13}C,^{13}C) correlation experiment: (a) proton-driven spin diffusion (1Q,1Q) correlation spectrum and (b) a double quantum (2Q,1Q) correlation experiment. Both methods are applied on the uniformly [^{13}C,^{15}N]-labeled tripeptide Ala-Gly-Gly. Connecting (red) lines relate to the Ala residue.

The correlation experiments discussed so far involve transfer among ^{13}C and ^{15}N spins that provide the highest spectral resolution under HR-SSNMR conditions. Additional information about intra- and interresidue con-

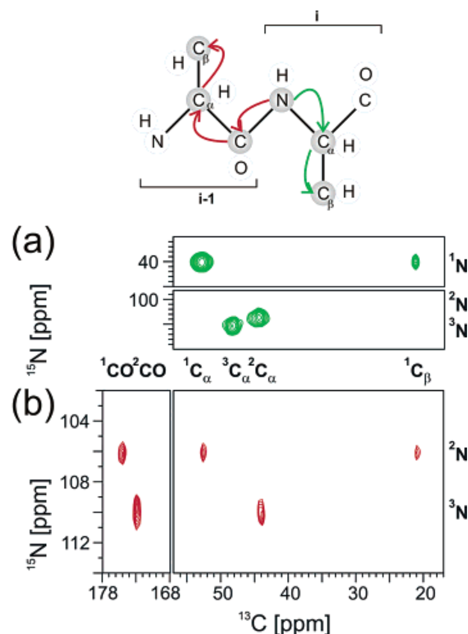

FIGURE 3. Heteronuclear experiments that permit intraresidue (a) or interresidue (b) assignments in the solid state. In (a), magnetization is selectively transferred from the amide backbone ^{15}N to the side chain of the same residue (via $^{i}C_{\alpha}$, green). In (b), backbone ^{15}N spins are correlated with the previous residue (via $^{i-1}$CO and $^{i-1}C_{\alpha}$, red). These correlation experiments are in general referred to as NCACB (a) and NCOCACB (b) methods and are here applied on the tripeptide Ala-Gly-Gly.

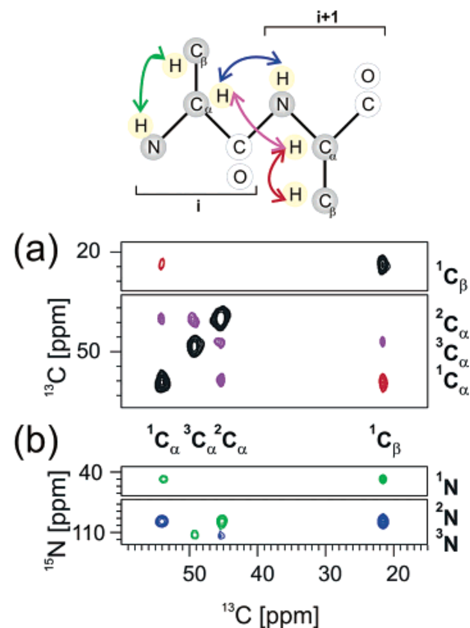

FIGURE 4. Proton−proton connectivities encoded via directly bonded $^{15}N^{13}C$ (green) or $^{13}C^{13}C$ nuclei (red). Some of these contacts may be interresidual (blue and purple). Results of CHHC (a) and NHHC (b) experiments on the uniformly labeled tripeptide Ala-Gly-Gly are shown.

nectivities can be obtained from 2D proton-mediated correlation data.[10] Although the inherent line width and chemical shift dispersion limits the use of ^{1}H detection periods, proton−proton interactions can serve as valuable probes for internuclear distances in biomolecules. Cross-peaks in the resulting CHHC or NHHC spectra indicate close proximity between the protons directly bonded to the correlated (^{13}C,^{13}C) or (^{15}N,^{13}C) nuclei, respectively (Figure 4). These correlations may not only help to validate spectral assignments but, as discussed below, contain valuable structural information about the polypeptide of interest.

2.2. Secondary Structure. The chemical shift detected in NMR not only provides the basis for the spectral discrimination of NMR signals but also represents a sensitive probe of local molecular conformation. In polypeptides, the spectral assignment methods discussed above directly allow for structural investigation of the backbone conformation. For this purpose, one can rely on empirical[11,12] and theoretical[13] results that define for each amino acid residue type an average ("random coil") chemical shift. For example, empirical studies conducted in solution[12] and in the solid state[11,14,15] reveal that deviations from these reference $^{13}C_{\alpha}$ and $^{13}C_{\beta}$ chemical shift values can be interpreted in terms of the local backbone conformation. In fact, a statistical analysis and DFT calculations[16] can be combined to predict how the *conformation-dependent chemical shift* changes as a function of the dihedral angles (ψ,ϕ). For illustration, Figure 5 shows the functional dependence of C_{α} and C_{β} chemical shifts ($\Omega_{C_{\alpha}}$ and $\Omega_{C_{\beta}}$, respectively) for the central Tyr residue

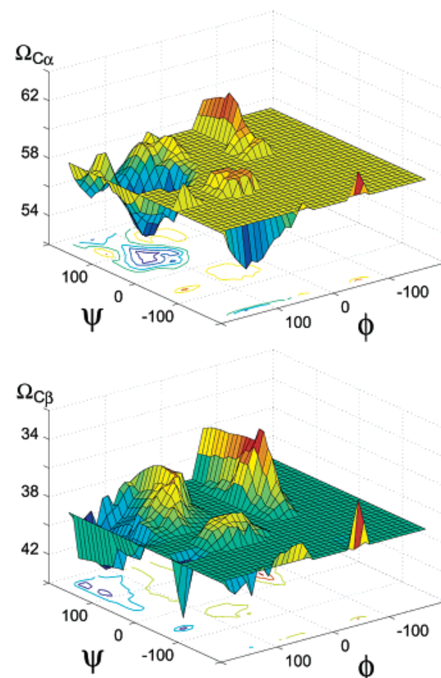

FIGURE 5. Ramachandran plot of the isotropic chemical shifts of C_{α} (top) and C_{β} (bottom) spins for a Tyr residue within the tripeptide Pro-Tyr-Ile. PDB structures spanning all possible conformations (ψ,ϕ) of the tripeptide Pro-Tyr-Ile (in steps of 10°) were generated using Insight II (Biosym Technologies, CA) in an automated manner. These structures were subsequently used as entry parameters for the SHIFTS program[16] to predict the indicated chemical shifts.

within the tripeptide Pro-Tyr-Ile on the torsional angles ψ and ϕ.

The size of the conformation-dependent deviations can significantly exceed the residual ^{13}C line width (0.5−1

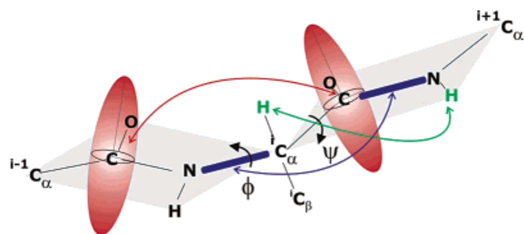

FIGURE 6. Alternative approaches to directly determine secondary structure elements: correlating dipolar tensors such as (C_α,N)−(CO,N) (blue) or carbonyl CSA tensors (red) and measuring $H_\alpha(i)$−$H_N(i+1)$ distances (green).

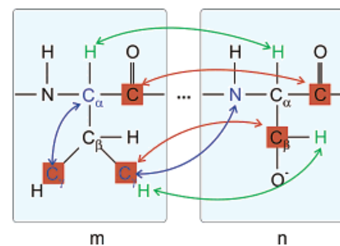

FIGURE 7. Different means to obtain distance constraints between two, not necessarily sequential, residues **m** and **n**. Proton−proton contacts may be encoded via neighboring rare spins (green). Selective recoupling of spectrally resolved pairs of spins results in precise internuclear distances (blue). Dilution of rare spins by block labeling allows for the detection of nontrivial (^{13}C,^{13}C) distance restraints (orange).

ppm) observed in well-ordered polypeptides. Note that there exists a remarkable correlation between the observed variations of Ω_{C_α} and Ω_{C_β}. This dependence can be used to enhance or suppress conformation-dependent chemical shift variations during a 2D correlation experiment leading to a better spectral dispersion among different amino acid residue types or within residues of variable secondary structure.[17]

In addition to the conformation-dependent chemical shift, *distances* (encoded as dipolar couplings) can be used to refine the local polypeptide conformation in the solid state. For example, the backbone (H_α,H_N) distance between sequential residues strongly correlates with the backbone dihedral angle ψ and can easily be detected during a NHHC correlation experiment.[18] In addition, a variety of distances sensitive to backbone conformation have been measured in doubly labeled samples and may be used to refine the secondary structure (reviewed elsewhere[7]).

Finally, information about the backbone conformation in the solid state can be obtained by correlating two *anisotropic interactions* such as the chemical shift anisotropy (CSA) or the dipolar coupling in a two-dimensional experiment. Here, one exploits the defined orientation of dipolar tensors along the internuclear vector and empirical knowledge regarding the orientation of CSA tensors (Figure 6). These anisotropic interactions can be recoupled during the evolution and/or detection period of a homonuclear or heteronuclear 2D correlation experiment by appropriate rf schemes. The resulting cross-peak pattern is characteristic for the relative orientation of the two anisotropic interactions. In uniformly labeled peptides, correlations between NH/NH[19] and NH/CH[20] dipolar tensors have been used for backbone dihedral angle determinations. Alternatively, relative tensor orientation may be encoded in the evolution of a 2Q two-spin state under the effect of two anisotropic interactions. Applications correlating CH/NH[21] and CN/CN[22,23] dipolar couplings or sequential carbonyl CSAs[24] have been demonstrated. In a 2Q correlation experiment under CN dipolar dephasing, the signal amplitude can directly report on the backbone torsion angle and can hence conveniently be applied in multiply labeled polypeptides.[7]

2.3. Determining Tertiary Structure. Internuclear distances and torsion angles define the three-dimensional conformation of a protein. In particular, medium and long-range distance parameters that connect different residues **m** and **n** are invaluable tools to restrain the overall fold (Figure 7). The strongest dipolar interactions dominate the spin system dynamics in solid-state NMR, and in a uniformly labeled polypeptide, these interactions reflect one-bond (^{13}C,^{13}C) or (^{13}C,^{15}N) distances. As demonstrated before, they are of great help for spectral assignments and for defining backbone or side chain conformation. Unfortunately, these dipolar couplings are of limited use for 3D structure determination. Knowledge of available solution-state NMR methods has helped to propose alternative experimental approaches that circumvent these problems. The corresponding techniques provide routes complementary to the 3D structure determination of a polypeptide in the solid state and will be discussed below. Similar to that in the solution state,[1] NMR structure determination in the solid state subsequently involves the calculation of families of molecular conformations that are consistent with the experimentally derived distance or angle constraints. The number and precision of these parameters determine the accuracy of the resulting 3D structure.

Once resonance assignments have been obtained, the chemical shift provides a spectroscopic tag for each spin pair. Certain (^{13}C,^{15}N) or (^{13}C,^{13}C) dipolar couplings can be measured in uniformly [^{13}C,^{15}N]-labeled samples by *chemical-shift-selective* rf pulse sequences. The weak coupling of interest is actively reintroduced based on the chemical shift separation and can be determined in the presence of all other couplings. Chemical-shift-selective transfer in (^{13}C,^{13}C) and (^{15}N,^{13}C) spin pairs is possible under a variety of selective recoupling conditions, where MAS rate, chemical shift separation, and (if necessary) an rf field fulfill particular selection rules.[9,25,26] In addition, selective pulses can render broad-band recoupling techniques frequency selective.[27] Both types of methods have been successfully employed for the measurement of a variety of internuclear distances in di- and tripeptides. Recent studies in our group[28] indicate that chemical-shift-selective recoupling is also possible in larger polypeptides and may assist the 3D structure determination process.

Unlike (^{13}C,^{13}C) and (^{15}N,^{13}C) dipolar couplings in a uniformly labeled protein, the strongest *(^{1}H,^{1}H) interactions* can provide valuable information about the 3D structure of a protein.[1] Not surprisingly, these constraints

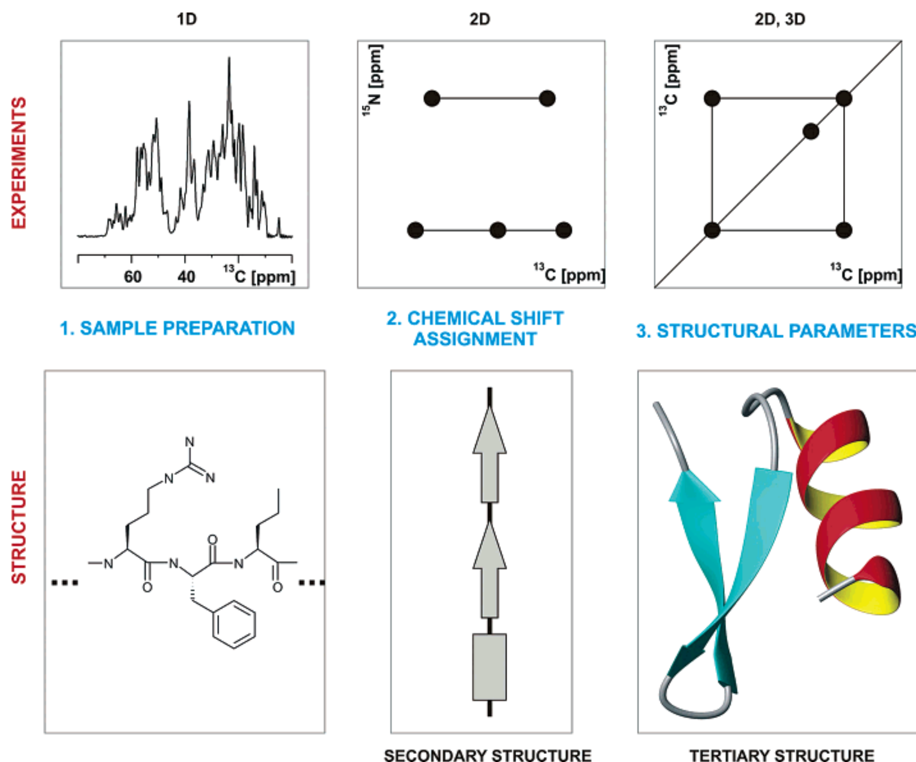

FIGURE 8. General approach for determining the structure of multiply labeled proteins in solid-state NMR under MAS conditions.

have been extensively used in solution-state NMR to determine the structure of proteins. In the absence of high-resolution ^{1}H evolution and detection periods, CHHC and NHHC 2D experiments[10] may be used to indirectly probe proton–proton contacts (Figure 7). For example, one NHHC correlation experiment has been sufficient to determine the 3D structure of the tripeptide Ala-Gly-Gly to a backbone root-mean-square deviation of 0.37 Å.[18] CHHC correlations have been invaluable to detect medium- and long-range restraints in the 76-residue protein ubiquitin[10] and are currently utilized to define its structure in the solid state.

The problem of detecting structurally relevant distance constraints in solid phase polypeptides can alternatively be solved by biochemistry, i.e., *advanced isotope-labeling* schemes. Techniques such as proton dilution by deuteration[29] or ^{13}C block labeling[30] can simplify the requirements regarding NMR methodology but generally involve additional efforts regarding sample preparation. If possible, these labeling patterns are most economically generated during bacterial growth in minimal media containing (^{2}H)glucose and ammonium chloride (complete deuteration) or by using [1,3-^{13}C]- or [2-^{13}C]glycerol as carbon sources[31] (^{13}C block labeling). As recently demonstrated in the α-spectrin SH3 domain, the biochemical removal of nearest neighbor (^{1}H,^{1}H)[32] and (^{13}C,^{13}C)[33] interactions simplifies the problem of detecting nontrivial structural constraints in polypeptides. On the other hand, spin dilution can complicate spectral assignment procedures, in particular in the presence of a large natural abundance background signal and overall low signal-to-noise ratios. We conclude this section by summarizing the different aspects of a structural study by HR-SSNMR methods in Figure 8.

3. Applications

Solid-state NMR has long been utilized to address important biophysical problems. A detailed discussion of these studies is beyond the scope of this Account, and the interested reader is referred to recent reviews.[6,34]

In the following, we will concentrate on NMR studies in uniformly or multiply labeled samples that have made use of or are amenable to the correlation experiments discussed above. Since these methods have been developed relatively recently, NMR studies in well-characterized proteins continue to serve as valuable templates to validate and optimize the presented methodology.

3.1. Immobilized Polypeptides and Proteins. Small well-ordered (often microcrystalline) proteins with known 3D structures and chemical shifts available from solution-state NMR have been ideal to test solid-state NMR methodology and to assess the influence and requirements of NMR instrumentation. In a first stage, results of 2D correlation experiments aiming at the spectral assignment of protein resonances in the solid state were reported. The first protein for which nearly complete ^{13}C and ^{15}N sequential resonance assignments were obtained is the SH3 domain of α-spectrin (62 residues).[35] Results of an intraresidue NCACB correlation experiment obtained on a wide-bore 750 MHz instrument are shown in Figure 9. The resulting resonance assignments provided the basis for the interpretation of nontrivial (^{15}N,^{15}N) and (^{13}C,^{13}C) contacts of a ^{13}C block labeled variant of the α-spectrin SH3 domain and a subsequent 3D structure calculation.[33]

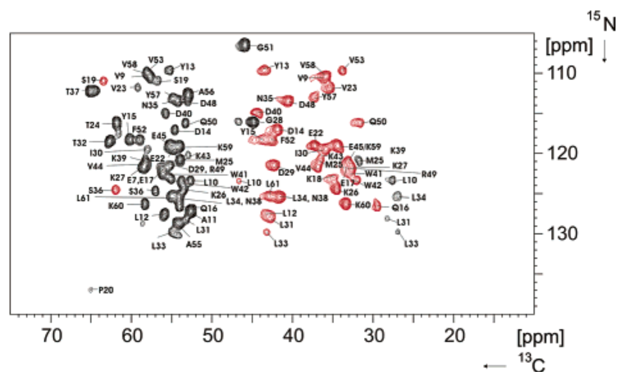

FIGURE 9. Aliphatic region of the NCACB spectrum of the α-spectrin SH3 domain.[35] The double-quantum (2Q) CC transfer unit used in this application results in C_β signals with negative peak intensities (indicated in red).

Partial or complete resonance assignments have also been published for the 76-residue protein ubiquitin,[36,37] the 58-residue peptide basic pancreatic trypsin inhibitor BPTI,[38] and the cyclic decapeptide antamanide.[39]

Resonance assignments were also recently reported[40] for the 85-residue catabolite repression histidine containing protein Crh that predominantly occurs in a monomeric form in solution. Application of the 2D correlation techniques discussed above has led to a first structural characterization of a microcrystalline form of Crh in the solid state. Here, the NMR data are consistent with a dimeric, domain-swapped form of Crh that is also observed in single crystals. These results provide a valuable reference for further solid-state NMR studies to elucidate the folding pathways from the monomeric form in solution to the dimeric fold in the solid phase. Furthermore, three-dimensional structures purely based on solid-state NMR data have been determined for the uniformly labeled di- and tripeptides Gly-Ile,[41] Met-Leu-Phe,[42] and Ala-Gly-Gly.[18]

Another class of polypeptides, which have been extensively studied by solid-state NMR, are amyloid fibrils formed by a large variety of peptides and proteins.[2] Such protein fibrils are noncrystalline and insoluble, making them ideal targets for solid-state NMR spectroscopy. A nearly complete assignment of ^{13}C NMR signals was obtained for amyloid fibrils formed from a uniformly labeled seven-residue fragment of the 40 residue Alzheimer β (Aβ) peptide.[14] Recently, a structural model for the full-length Aβ amyloid fibrils could be proposed based on solid-state NMR studies on samples containing selected five to seven uniformly labeled amino acids.[43] Full sequential assignment of ^{13}C and ^{15}N resonances was also reported for a 10-residue peptide fragment of transthyretin in an amyloid fibril based on a spectral analysis of samples containing different stretches of four consecutive uniformly labeled amino acids. Conformation-dependent chemical shifts provided the basis to predict the backbone conformation of fibrillized transthyretin.[44]

3.2. Membrane Proteins. Intrinsic membrane or membrane-associated proteins comprise a large fraction of most genomes and represent key mediators of cellular function. Despite recent advancements in X-ray crystallography and solution-state NMR, the structural characterization of these protein classes at atomic resolution still represents a considerable biophysical challenge. Likewise, membrane protein expression and purification stand for an active field of biomolecular research.

If significant structural information is available from other resources, solid-state NMR on partially or uniformly labeled protein samples can help to address a particular biophysical problem at atomic resolution. For example, this approach was taken to elucidate interatomic distances in a uniformly [^{13}C,^{15}N]-labeled version of the light-driven proton pump bacteriorhodopsin (bR). Frequency-selective methods allowed for distance measurements between the spectrally isolated N_ζ of Lys216 involved in the covalent binding of the retinal and C_γ of neighboring aspartic acid residues.[45] Chemical-shift-selective transfer methods were also employed to monitor conformational changes during the photocycle of bR.[46]

Membrane protein (i.e., receptor) function often involves interactions with ligand molecules and represents an area of great pharmacological relevance. In these cases, working with uniformly labeled ligands permits spectroscopic investigations with greater flexibility. For example, ^{1}H and ^{13}C resonance assignments obtained on a uniformly ^{13}C-labeled retinal chromophore, the covalently bound natural ligand of rhodopsin, were obtained in the dark-adapted state of the photoactive protein. Comparison to resonance assignments obtained on retinal in free form were qualitatively interpreted in terms of conformational rearrangements due to retinal−receptor interactions.[47]

To date, no structural information of a high-affinity ligand bound to a G protein coupled receptor (GPCR) is available. Unlike rhodopsin, the recombinant expression of GPCR in large quantities is usually difficult and must involve carefully optimized biochemical procedures. Restrictions regarding the availability of functional receptors also affect ligand quantities that can be studied. Moreover, the chemical environment including lipids and receptor protein can hamper the unambiguous spectral identification of a bound ligand in a solid-state NMR experiment. We have recently shown how 2D double-quantum correlation experiments (such as depicted in Figure 2b) can be utilized to detect microgram quantities of bound neurotensin, a 13-residue neuropeptide that binds in high affinity to the NTS-1 (101 kDa) receptor. For the 6-residue, biologically active, C-terminal sequence of neurotensin, a homonuclear (2Q,1Q) correlation spectrum is sufficient to assign all C_α and C_β resonances of the uniformly [^{13}C,^{15}N]-labeled ligand. As discussed in section 2.2, these chemical shift assignments can be used to construct the backbone model of the ligand in complex with the receptor (Figure 10). Our solid-state NMR data[48] indicate that the resulting beta-strand conformation is only adopted in the presence of the receptor and may serve as a structural template for future pharmacological studies.

Not only can solid-state NMR report on the complete structure of a membrane protein, but it can also probe dynamics in the protein of interest. Both aspects have recently been investigated[15,49] in the context of a uniformly

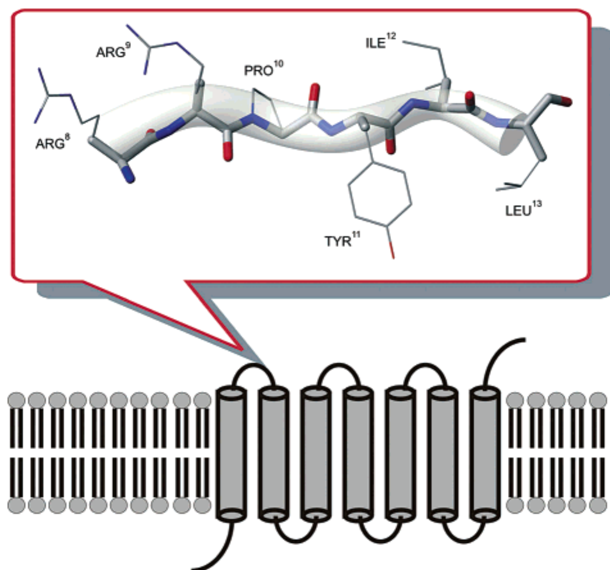

FIGURE 10. Representative backbone conformation of neurotensin-(8–13) bound to its G-protein coupled receptor NTS-1 (of currently unknown structure) as determined by solid-state NMR.[48]

[^{13}C,^{15}N]-labeled version of the LH2 light-harvesting complex, one of the largest systems studied by solid-state NMR (approximately 150 kDa) to date. Here, the application of ultrahigh magnetic fields (up to 750 MHz) was crucial to establish high-resolution conditions in the solid state. Two-dimensional (^{15}N,^{13}C) correlation experiments (such as discussed in section 2.1) were conducted to assign the backbone and side chain resonances for different temperatures. At −10 °C, a variety of spectral assignments within the transmembrane sections of the protein were possible.[49] Additional correlations occur at lower temperatures, consistent with the immobilization of flexible loop regions of the protein. A more detailed structural study in membrane proteins could include (C,C) or N/CHHC correlation experiments, possibly in conjunction with advanced isotope labeling approaches to determine the complete 3D structure of a membrane protein in the solid state.

4. Conclusions and Future Prospects

Similar to other spectroscopic methods, the applicability of solid-state NMR in investigating structural problems is often determined by the spectral resolution and the signal-to-noise ratio. For a long time, solid-state NMR has successfully been utilized to determine *individual* structural and dynamical parameters in insoluble and noncrystalline systems at atomic resolution. Here, site-specific single or pairwise isotope labeling ensured spectral resolution and sensitivity. Recent studies have shown that both parameters can be significantly improved by increasing the strength of the static magnetic field in which the sample of interest is placed and by employing state-of-the-art NMR instrumentation. We have discussed a series of NMR pulse schemes that make use of these technical advancements and are tailored to retrieving *multiple* structural parameters or even the complete three-dimensional protein structure from a single, isotope-labeled sample.

The solid-state NMR spectroscopist can hence address structural problems in chemistry and biophysics with greater flexibility. Recent results have established solid-state NMR as an invaluable tool to characterize fibrous proteins on the molecular level. In addition, solid-state NMR could play a key role in investigating high-affinity ligand binding in membrane proteins, an area of great pharmacological relevance. Many of the 2D correlation methods discussed here are readily applicable in non-peptidic ligands, or they can be adapted for the study of ion topologies in biomolecules.

Advances regarding sample preparation (for example, including modular labeling, in vitro expression, and intein technology[50]) and improvements in NMR hardware instrumentation could open up new areas of solid-state NMR research such as the investigation of large protein–protein complexes or the complete 3D characterization of larger membrane proteins. Solid-state NMR studies of multiply labeled biomolecules will furthermore profit from improved procedures for calculating 3D structures, in particular in the presence of ambiguous or a limited number of structural constraints.

Unlike X-ray crystallography, protein motion does not hinder solid-state NMR methods. In fact, complementary to solution-state NMR, it may provide a very efficient means to study protein folding, flexibility, and function under biologically relevant conditions. Hand in hand with solution-state techniques and crystallographic methods, solid-state NMR could give insight into protein function and the chemistry of life with unprecedented accuracy and flexibility.

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