Journal of Magnetic Resonance 287 (2018) 140–152

Contents lists available at ScienceDirect

Journal of Magnetic Resonance

journal homepage: www.elsevier.com/locate/jmr

Perspectives in Magnetic Resonance

^{1}H magic-angle spinning NMR evolves as a powerful new tool for membrane proteins

Tobias Schubeis [a], Tanguy Le Marchand [a], Loren B. Andreas [b], Guido Pintacuda [a,*]

[a] Centre de RMN à Très Hauts Champs, Institut des Sciences Analytiques (UMR 5280 – CNRS, ENS Lyon, UCB Lyon 1), Université de Lyon, 5 rue de la Doua, 69100 Villeurbanne, France
[b] Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, D-37077 Göttingen, Germany

ARTICLE INFO

Article history:
Received 25 September 2017
Revised 20 November 2017
Accepted 22 November 2017

Keywords:
Fast MAS
^{1}H detection
Resolution
Sensitivity
Deuteration
Membrane proteins

ABSTRACT

Building on a decade of continuous advances of the community, the recent development of very fast (60 kHz and above) magic-angle spinning (MAS) probes has revolutionised the field of solid-state NMR. This new spinning regime reduces the ^{1}H-^{1}H dipolar couplings, so that direct detection of the larger magnetic moment available from ^{1}H is now possible at high resolution, not only in deuterated molecules but also in fully-protonated substrates. Such capabilities allow rapid "fingerprinting" of samples with a ten-fold reduction of the required sample amounts with respect to conventional approaches, and permit extensive, robust and expeditious assignment of small-to-medium sized proteins (up to ca. 300 residues), and the determination of inter-nuclear proximities, relative orientations of secondary structural elements, protein-cofactor interactions, local and global dynamics.

Fast MAS and ^{1}H detection techniques have nowadays been shown to be applicable to membrane-bound systems. This paper reviews the strategies underlying this recent leap forward in sensitivity and resolution, describing its potential for the detailed characterization of membrane proteins.

1. Introduction

Membrane proteins are an important class of biomacromolecules, comprising roughly 30% of the proteome. They are responsible for many critical cellular processes, including signaling across cell membranes and both passive and active ion and small molecule transportation. It is no surprise that so many drugs and drug candidates target membrane proteins.

Unfortunately, membrane proteins pose a particular challenge in structural biology. They are often difficult to express, and even when they can be produced in large amounts, they are often refractory to standard techniques. Obtaining high quality crystals in detergent or lipid cubic phases for X-ray diffraction studies remains a difficult undertaking, and solution NMR investigations are easily jeopardized by the slowly tumbling rates of the micellar preparations. Recently, developments in cryo-electron microscopy have led to an explosion in structures for larger proteins, above about 100 kDa in size, however this technique has difficulties below about 50–100 kDa.

In the last two decades, Magic Angle Spinning (MAS) NMR has developed as a generally applicable structural biology technique, capable of complementing liquid-state NMR, X-ray crystallography and electron microscopy. Following seminal work on microcrystalline proteins [1], a growing number of studies demonstrates the utility of MAS to calculate three-dimensional structures and to determine dynamics of complex assemblies such as amyloid fibrils [2–5] bacterial virulence factors [6] and intact viral capsids [7,8]. In this context, a variety of reports have been published for membrane channels [9–16] and membrane-bound drug targets [17,18] at an atomic level in reconstituted lipid bilayers or even directly in native cell membranes [19–21]. However, while targeted protocols have been designed for sample preparation, sequence-specific resonance assignment, and collection of conformational restraints, these procedures are still far from routine [22], and for example only for very few transmembrane proteins a complete structure determination by MAS NMR has been reported in lipid bilayers [18,23–27].

Although the topic of this review concerns MAS, it is also important to remember that static NMR using carefully oriented sample was developed even earlier, and successfully applied to determine small alpha helical structures [28,29]. Such studies were the first of any kind to accurately determine helical tilt angles in fluid bilayers, and also could detect the influence of protein dynamics. Nevertheless, it is our perspective that the relative ease of sample preparation and resonance assignment for MAS conditions will propel the

* Corresponding author.
E-mail address: guido.pintacuda@ens-lyon.fr (G. Pintacuda).

https://doi.org/10.1016/j.jmr.2017.11.014
1090-7807/

technique to widespread use, despite missing the benefit of long-range correlation possible with oriented samples.

We have divided our discussion into the following 9 sections (2–10). In Section 2 we discuss physical considerations having to do with the MAS rate, and deuteration strategies that work synergistically to narrow proton resonances. In Section 3, we discuss the problem of proton exchange in deuterated proteins, and how this can be overcome with the latest technology and labelling strategies. In Section 4, we review the various methods to obtain information from protein side-chains. In Section 5, we discuss the resolution and sensitivity of membrane samples in relation to current and future instrumentation. Section 6 details the workflow for sample preparation, and the advantages of sample optimization using fast MAS conditions. Sections 7 and 8 review resonance assignment and structure determination, respectively. Finally, Section 9 concerns measurement of dynamic processes that can be efficiently measured using 1H detection, even for bilayer embedded membrane proteins, as highlighted by several recent reports.

2. Proton detection, deuteration, faster MAS

Most of the biomolecular studies by MAS-NMR performed so far are primarily based on double and triple resonance spectra, making use of ^{13}C-detected correlations between ^{13}C and ^{15}N signals under relatively slow MAS [30,31]. Most transporters and membrane-bound enzymes are predicted to contain more than 6 transmembrane α-helices or 8 β-strands (>20 kDa). The resonance assignment of a protein in this size range requires the acquisition of a large set of multidimensional spectra. The long acquisition times required for ^{13}C-detected strategies are thus a severe obstacle to the study of such proteins by MAS NMR. This is mainly due to the fact that ^{13}C-detected experiments are relatively insensitive, in contrast to the standard solution NMR acquisition and analysis protocols, where ^{1}H-detected triple-resonance pulse schemes provide rapid sequential assignment and structural determination [32,33].

To extend the applicability of MAS-NMR to the detailed characterization of membrane proteins requires a leap forward in sensitivity and resolution, and many strategies have been employed over the last two decades to achieve this.

The direct detection of proton coherences, exploiting the high gyromagnetic ratio and abundance of proton nuclei, is the most natural way to enhance the spectral sensitivity [34,35]. The very same high gyromagnetic ratio and abundance, coupled to the small spectral dispersion of ^{1}H resonances, however generates a network of strong dipolar couplings which results in severe line-broadening at moderate (10–20 kHz) MAS rates, preventing the straightforward application of ^{1}H detection to proteins in the solid state.

Fig. 1 provides an overview of the efforts of numerous groups to overcome these challenges. Despite encouraging proof-of-principles on fully-protonated model systems [36], the most commonly used strategy to retrieve resolution has been proton dilution [37,38]. This is typically achieved by perdeuteration and back-protonation at the exchangeable sites in a suitable H_2O/D_2O mixture, so that the network of strong ^{1}H-^{1}H dipolar couplings that broaden the NMR signals is broken [39,40]. Following up on pioneering studies on microcrystalline proteins, Linser et al. presented the first high-resolution ^{1}H-^{15}N correlation spectra of two membrane proteins in lipid bilayers, the outer membrane protein G (OmpG) and bacteriorhodopsin (bR) [41]. For these systems, the highest resolution was obtained combining 10–20 kHz MAS with extreme dilution of the proton content in the sample by chemical exchange in 10–40% H_2O/D_2O. With such an approach, however, the optimal deuteration level is a trade-off between high resolution and detection sensitivity, as increased dilution results in a loss of signal of the observed nuclei, in particular for ^{1}H-^{1}H correlations [42].

In parallel, the first encouraging studies on the effects of rapid sample rotation on ^{1}H resonances have driven the development of probes capable of faster MAS, which have been increasingly more effective in averaging ^{1}H dipolar interactions [34,35]. At faster MAS rates and high magnetic fields, the proton dilution requirements become less stringent, and ^{1}H detection techniques were shown to be applicable to a seven-helical integral membrane protonpump, proteorhodopsin (PR) [43], and the four-helical transmembrane domain DsbB [44], with high reprotonation levels (70–100%) in the 30–40 kHz MAS range.

At 60 kHz MAS, sensitive cross-polarization (CP) HSQC spectra with $^{1}H^{N}$ linewidths of about 100 Hz were obtained for uniformly [^{2}H,^{13}C,^{15}N]-labelled microcrystalline protein samples fully back-exchanged at amide sites [45,46]. Despite substantial reduction of the sample amount (to 2–3 mg for a 1.3 mm rotor), this enables the acquisition of ^{1}H resonances with extremely high spectral resolution and high signal sensitivity, as reviewed recently [47].

An increase in the spinning rate requires reduced sample dimensions, which in principle entails a major loss in sensitivity. Interestingly, the loss in sample volume is partially offset by the increased detection sensitivity associated to improved inductive coupling of smaller coils, and most importantly is compensated by the significantly longer coherence lifetimes which can be obtained with fast spinning. Longer coherence lifetimes translate into narrower proton lines and improved coherence transfer efficiencies, with improved sensitivity and resolution in multidimensional correlation experiments [48], in particular those involving many coherence transfer steps (see Section 7).

Such capabilities not only allow rapid “fingerprinting” of samples but also permit extensive, robust and expeditious assignment [49], and the determination of structurally important parameters such as inter-nuclear proximities and local dynamics [50]. Notably, this approach was shown to be applicable to small-to-medium sized transmembrane systems, and for example highly resolved spectra from the influenza A M2 channel in reconstituted lipid bilayers were obtained with complete backbone assignments determined from a single sample of 1.5 mg and only two weeks of experimental time [49]. These developments speed up by almost two orders of magnitude the analysis of membrane proteins in lipid bilayers, and more complex targets of higher molecular weight, such as OmpG [49,51], the β-barrel Outer Membrane Protein from *Klebsiella pneumoniae* (KpOmpA) [52] or the human voltage dependent anion channel (VDAC) [53], have already been reported.

The advent of even faster MAS rates in smaller rotors has reduced further the amount of sample required, and larger sensitivity gains per sample volume have been reported (0.5 mg or less is required for 0.8 mm and 0.7 mm rotors) [54–59].

3. Solvent accessibility and H/D exchange issues

Combined with MAS, deuteration is still the most effective way to improve resolution [60] without compromising the sensitivity of the remaining protons. Protein deuteration requires expression in D_2O, which reduces the protein yield in bacteria and is incompatible with certain expression systems, *e.g.* insect or mammalian cells. If feasible, NMR spectra of folded proteins might still suffer from incomplete re-protonation at the exchangeable sites during purification. This problem is particularly relevant for membrane proteins, which often lack refolding protocols, so that the reintroduction of protons is limited to exchangeable and solvent accessible sites, with a drastic loss of information for the extensive hydrophobic transmembrane regions shielded by the lipids or detergents.

This effect may be used in a constructive way. For example, Ladizhansky and coworkers have exploited this phenomenon to study the solvent-exposed regions of Proteorhodopsin (PR) [43].