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Sensitivity and resolution enhancement of oriented solid-state NMR: Application to membrane proteins

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ABSTRACT

Oriented solid-state NMR (O-ssNMR) spectroscopy is a major technique for the high-resolution analysis of the structure and topology of transmembrane proteins in native-like environments. Unlike magic angle spinning (MAS) techniques, O-ssNMR spectroscopy requires membrane protein preparations that are uniformly oriented (mechanically or magnetically) so that anisotropic NMR parameters, such as dipolar and chemical shift interactions, can be measured to determine structure and orientation of membrane proteins in lipid bilayers. Traditional sample preparations involving mechanically aligned lipids often result in short relaxation times which broaden the ^{15}N resonances and encumber the manipulation of nuclear spin coherences. The introduction of lipid bicelles as membrane mimicking systems has changed this scenario, and the more favorable relaxation properties of membrane protein ^{15}N and ^{13}C resonances make it possible to develop new, more elaborate pulse sequences for higher spectral resolution and sensitivity. Here, we describe our recent progress in the optimization of O-ssNMR pulse sequences. We explain the theory behind these experiments, demonstrate their application to small and medium size proteins, and describe the technical details for setting up these new experiments on the new generation of NMR spectrometers.

Contents

Abbreviations: 5CB, 4-pentyl-4′-cyanobiphenyl; BLEW, Burum Linser Ernst Windowless; CP, cross polarization; CSA, chemical shift anisotropy; DC, dipolar coupling; DTRF, doubly tilted rotating frame; FSLG, Frequency-Switched Lee-Goldberg; HETCOR, heteronuclear correlation; HIMSELF, Heteronuclear Isotropic Mixing Separated Local Field; LG, Lee-Goldberg; MAS, magic angle spinning; MLEV, Malcolm Levitt Decoupling Sequence; MSHOT, Magic Sandwich Higher Order Truncation; NAL, N-acetyl-leucine; NAVL, N-acetyl-valine-leucine; O-ssNMR, oriented solid-state NMR; PDSD, proton driven spin diffusion; PELF, Proton Evolved Local Field; PI, polarization inversion; PISEMA, polarization inversion spin exchange at the magic angle; PLN, phospholamban; RMS, root mean square; SAMPI4, Selective Averaging Magic Sandwich with Polarization Inversion; SE, sensitivity enhancement; SLN, Sarcolipin; SNR, signal to noise ratio; WIM24, Windowless Isotropic Mixing 24.

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1. Introduction

High-resolution structures of membrane proteins are being solved with an exponential progression similar to that of soluble membrane proteins. To date, however, the total number of membrane proteins determined is dramatically lower, with only 300 unique folds identified compared to ~70,000 for the soluble counterpart. Without any doubt, X-ray spectroscopy plays a major role in the structure determination of membrane proteins. Nonetheless, crystallized membrane proteins do not contain physiologically relevant concentrations of the lipids that enable biological function. In contrast, solid-state NMR is the only spectroscopic technique that provides atomic-resolution structural information of membrane proteins in lipid preparations and under functional conditions [1–3]. Unfortunately, the throughput of solid-state NMR structures has been very low. In fact, solving membrane protein structure at high resolution by solid-state NMR methods requires not only great skill in protein expression and sample preparation [1,4] but also a great deal of knowledge of spectroscopic methods that need to be adapted to the sample behavior.

Currently, there are two major methodologies that enable the structure determination of membrane proteins: magic angle spinning (MAS), and oriented-solid-state (O-ssNMR). In MAS-NMR, membrane protein samples reconstituted in lipid membranes are spun at the magic angle (54.7°) to attenuate dipolar and chemical shift anisotropic interactions with resonances that are dispersed according to their local chemical environment. However, dipolar couplings (DC) and chemical shift anisotropy (CSA) can be recovered using recoupling techniques and used for structure determination [5–8]. In contrast, O-ssNMR experiments that directly measure DCs and CSAs require oriented samples, whose preparation is more laborious. Traditionally, uniaxially oriented membrane protein preparations involved the reconstitution of membrane proteins in aligned membrane bilayers immobilized onto glass plates [5–16]. Excellent references regarding these preparations can be found in many reviews [17–22]. These preparations suffer from lack of control over sample hydration and pH, and are also not optimal with regards to coil-filling and compatibility with all proteins. More recently, oriented samples of membrane proteins have been prepared in lipid bicelles [23,24], which are preparations of mixtures of short-chain detergent-like lipids (e.g., DHPC. CHAPSO, TritonX-100, DPC) and long-chain lipids (typically DMPC or DMPC doped with 20% unsaturated lipids such as POPC) [24,25]. Bicelles with a sufficiently large '*q*-ratio', i.e., the molar ratio of long to short chain lipids, are capable of spontaneously aligning in the high field of the NMR spectrometers and are thus an alternative to mechanically aligned bilayers for oriented solid state NMR experiments [24]. Fully hydrated bicelles undergo fast rotational diffusion, averaging out part of the anisotropy and increasing the $T_{1\rho}$. The latter renders line-shapes for amide resonances of membrane proteins that are similar to those of globular proteins [26,27]. In addition, bicelles offer complete control over sample hydration and pH, and the preparations, although limited to a small subset of lipids, are generally compatible with single TM and polytopic membrane proteins [28]. The preparations tend to minimize the conformational heterogeneity of membrane proteins as compared to the mechanically aligned bilayers, giving spectra with higher resolution [29].

A significant advantage of O-ssNMR over MAS is that the resonances are more dispersed because there is an anisotropic contribution to nuclear shielding, which produces a larger breadth of chemical shifts. More importantly, the anisotropic interactions with the external magnetic field obtained from these experiments allow the calculation of the entire membrane protein topology as parameters such as tilt and rotation angle of transmembrane segments are encoded directly in these spectra [30,31]. In order to take advantage of these spectral features, we recently developed new methods to obtain higher resolution and sensitivity. We recrafted the classical separated local field (SLF) experiments into sensitivity enhanced (SE) versions [32–35] as well as constant time (CT) variants [36] that enabled us to carry out 2D and 3D NMR experiments on membrane proteins. In this review, we describe the theory, the technical details as well as our recent applications of these new techniques for the structure determination of membrane proteins.

2. Sensitivity enhancement (SE) of separated local field (SLF) experiments

2.1. Theory of SE-SLF

Transient heteronuclear dipolar oscillations during Hartmann–Hahn cross polarization (CP) were first observed by Muller et al. on ferrocene single crystal [37]. In the case of a weak ^{1}H–^{1}H dipolar-coupled network such as in liquid crystals and ferrocene single crystal, the CP evolution of the *I*–*S* (^{1}H–^{13}C or ^{1}H–^{15}N) spin system gives rise to oscillating dipolar coherences that can be Fourier transformed to obtain the *I*–*S* DC [38]. However, for most solids including membrane proteins, the spin-diffusion among protons mediated by the strong homonuclear dipolar couplings (~50–150 kHz) attenuate heteronuclear oscillations [39]. A solution to this problem was introduced by Waugh, who pioneered the SLF spectroscopy that suppresses the ^{1}H–^{1}H DCs during heteronuclear DC evolution in the indirect dimension [40]. This experiment was the first example of two-dimensional separated local field (SLF) spectroscopy and enabled one to separate the chemical shifts of the low-abundant nucleus (*S*) from the *I*–*S* heteronuclear DC. Note that the spin–spin J_{IS} coupling constants contribute to the splitting in the indirect dimension of SLF spectra (J_{IS} values are few tens of Hz). However, the contribution of J_{IS} is neglected in the SLF spectra of membrane proteins due to relatively larger line widths.

A typical SLF experiment consists of a CP from the abundant *I* spin bath to the less abundant *S* spins (typically ^{15}N or ^{13}C) to generate transverse *S* spin magnetization that evolves for a t_1 period under *I*–*S* DC and is followed by the *S*-spin chemical shift evolution.