

Intramolecular ^1H – ^{13}C distance measurement in uniformly ^{13}C , ^{15}N labeled peptides by solid-state NMR

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ABSTRACT

A ^1H – ^{13}C frequency-selective REDOR (FS-REDOR) experiment is developed for measuring intramolecular ^1H – ^{13}C distances in uniformly ^{13}C , ^{15}N -labeled molecules. Theory and simulations show that the experiment removes the interfering homonuclear ^1H – ^1H , ^{13}C – ^{13}C and heteronuclear ^1H – ^{15}N , ^{13}C – ^{15}N dipolar interactions while retaining the desired heteronuclear ^1H – ^{13}C dipolar interaction. Our results indicate that this technique, combined with the numerical fitting, can be used to measure a ^1H – ^{13}C distance up to 5 Å. We also demonstrate that the measured intramolecular ^1H – ^{13}C distances are useful to determine dihedral angles in proteins.

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1. Introduction

High-resolution magic-angle-spinning solid-state NMR (SSNMR) spectroscopy has become a complementary method to X-ray crystallography and solution NMR for determining three-dimensional structures of proteins. This structure determination relies on the measurement of torsion angles and distances [1] and orientational constraints using aligned samples [2,3]. Backbone ^{13}C and ^{15}N chemical shifts are well known to be sensitive to protein (ϕ , ψ) torsion angles, and can be measured straightforwardly to constrain the protein secondary structure [4–6]. In addition to chemical shifts, a number of heteronuclear dipolar-correlation techniques such as HNCH [7], NCCN [8] and HCCH [9,10] have been developed to quantitatively measure the backbone and sidechain dihedral angles.

Solid state NMR has been proven to be a reliable technique to obtain the quantitative distance information in various systems [11–25]. Distances between non-proton atoms in proteins can be measured using 2D and 3D correlation experiments such as DARR [26], RFDR [20], CHHC [27], PAR [28], R^2W [29], FS-REDOR [30] and ZF-TEDOR [31]. Homonuclear correlation experiments such as DARR and CHHC yield qualitative ^{13}C – ^{13}C or ^{15}N – ^{15}N distance

constraints based on the growth of cross peak intensities as a function of mixing time; short distances manifest as fast build-up curves whereas long-range distances exhibit slow build-up curves. Alternatively, the rotational resonance width (R^2W) experiment [29,32,33] yields more quantitative ^{13}C – ^{13}C distance constraints by monitoring cross peak intensities as a function of MAS frequency while the spin diffusion mixing time is held constant. To determine heteronuclear distances, one can use the frequency-selective (FS) REDOR experiment [30] or the 3D ZF (zero-filter) TEDOR experiment [31]. These methods have so far been applied mostly to ^{13}C – ^{15}N distance determination in proteins [34,35].

While heavy-atom ^{13}C – ^{13}C , ^{13}C – ^{15}N and ^{15}N – ^{15}N distances are important for protein structure determination, distances involving protons, such as ^1H – ^{13}C and ^1H – ^{15}N distances, also provide valuable constraints to the backbone and sidechain conformation of proteins. The difficulty presented by the dense multi-spin network of protons in proteins was recently shown to be possibly overcome using a Y-nucleus detected ^1H –X REDOR [11] technique [36,37,38], where X and Y represent two different types of nuclei. For example, a ^{15}N -detected ^1H – ^{13}C REDOR experiment allows the measurement of the distance of a carbonyl carbon to an amide proton involved in N–H...O=C hydrogen bond. However, the Y-detected ^1H –X REDOR experiment has the limitation that it requires the samples to be site-specifically labeled in the X nucleus in order to avoid the complication of multiple X nuclei simultaneously coupled to each ^1H spin. It is thus desirable to extend the technique to uniformly ^{13}C , ^{15}N labeled proteins to measure multiple ^1H – ^{13}C distances.

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In this work, we describe the theory, simulation and application of a ^{13}C -detected FS-REDOR experiment to extract ^1H - ^{13}C distances. By using frequency-selective REDOR, we not only extend the ^1H -X REDOR principle to uniformly ^{13}C , ^{15}N -labeled samples but also show that the allowance of ^{13}C detection rather than ^{15}N detection in the previous study[36–38] significantly increases the sensitivity of this approach. The feasibility of our ^1H - ^{13}C FS-REDOR experiment has been evaluated on two ^{13}C , ^{15}N -labeled model compounds, formyl-Met-Leu-Phe-OH (f-MLF) and histidine.

2. Experimental

2.1. Materials

Uniformly ^{13}C , ^{15}N -labeled f-MLF was purchased from Cambridge Isotope Laboratories and used without further purification. 98% ^{13}C , ^{15}N -labeled Histidine·HCl·H₂O was purchased from Sigma-Aldrich and was diluted to 20% by codissolving with 80% unlabeled histidine to remove the interfering intermolecular dipolar couplings. The mixture was recrystallized at pH 8.0 as described before.[39] The solid-state NMR structure of f-MLF (PDB code: 1Q70) [40] was referenced to compare with the measured distances. For histidine, the measured intramolecular ^1H - ^{13}C distances and deduced sidechain dihedral angles were compared with the values in the crystal structure of L-histidine (CSD code: LHISTD02).

2.2. Instrumentation

Solid-state NMR experiments were carried out on a wide-bore Bruker AVANCE-600 spectrometer using a double-resonance 4-mm MAS probe. Samples were spun at 5.0 kHz at room temperature for all experiments. Typical radiofrequency field strengths were 50–63 kHz for ^{13}C and 62–83 kHz for ^1H . ^{13}C chemical shifts was referenced to the α -Gly C' signal at 176.49 ppm on the TMS scale.

3. Results and discussion

3.1. ^1H - ^{13}C FS-REDOR experiment

Fig. 1 illustrates the pulse sequence for the ^1H - ^{13}C FS-REDOR experiment. After an initial 90° pulse and a magic-angle pulse of 54.7° (θ_m), the ^1H magnetization is tilted to the $-x$ direction, perpendicular to the effective field direction of the subsequent Frequency Switched Lee-Goldburg (FSLG) pulses, which remove the ^1H - ^1H homonuclear coupling [43–45]. ^1H magnetization evolves under the interactions of the ^1H chemical shift and ^1H - ^{13}C dipolar coupling for $2N$ rotor periods with a total duration of τ_m . The ^1H - ^{13}C dipolar interaction is recoupled under MAS by two

symmetric π -pulse trains, which contain two ^{13}C pulses per rotor period. A rectangular selective ^{13}C π pulse is applied in the middle of ^1H - ^{13}C dipolar recoupling to selectively refocus the ^{13}C spins of interest. Synchronously on ^1H channel, a WIM-24 pulse train [46] is applied to remove the residual homonuclear ^1H - ^1H and heteronuclear ^1H - ^{13}C dipolar couplings under MAS. In the middle of WIM-24 decoupling, a π pulse of $6.1\ \mu\text{s}$ was applied to recouple the ^1H - ^{13}C dipolar interaction. The rest ^1H - ^1H decoupling during the middle two rotor periods is achieved by using FSLG pulses. We used a ^{13}C selective π pulse of $320\ \mu\text{s}$, which is supposed to selectively invert a ^{13}C frequency range of around 6 ppm, considering the ^{13}C Larmor frequency of 150 MHz in our study.

In order to detect the dipolar modulation of the ^1H spins by observing the resolved ^{13}C intensity, the ^1H magnetization is transferred to the coupled ^{13}C spin through a short, $40\ \mu\text{s}$, Lee-Goldberg cross-polarization (LG-CP) period, which eliminates the possible ^1H spin diffusion. The effective spin-lock field strength of LG-CP is 62.5 kHz. In the REDOR mixing period, the effective field strength of the FSLG decoupling and the transverse field of the WIM-24 sequence is set to be 102.1 kHz and 81.7 kHz, respectively.

3.2. Theoretical background

We consider a multi-spin system consisting of m ^{13}C spins and n protons. In the ^1H - ^{13}C FS-REDOR experiment, ^{13}C - ^{13}C , ^1H - ^{15}N and ^{13}C - ^{15}N dipolar interactions commute with the ^1H chemical shift interaction and ^1H - ^{13}C dipolar coupling, thus do not interfere the measurement of ^1H - ^{13}C dipolar couplings. For directly bonded ^1H - ^{13}C spin pairs, the ^1H - ^{13}C J coupling is present, while the multi-bond ^1H - ^{13}C J coupling is neglectable. Therefore, we only consider ^1H - ^{13}C dipolar coupling and ^{13}C - ^{13}C J coupling as the effective Hamiltonian in our FS-REDOR experiment:

$$H = \sum_{i=1}^n \sum_{j=1}^m \kappa \omega_{ij} 2I_{iz} S_{jz} + \sum_{k \neq j} \pi J_{jk} 2S_{jz} S_{kz} \quad (1)$$

Here J_{jk} is the ^{13}C - ^{13}C scalar coupling between directly bonded j and k spins, and κ is the FSLG scaling factor for the ^1H - ^{13}C dipolar coupling. ω_{ij} represents the ^1H - ^{13}C dipolar coupling between the ^1H spin i and the ^{13}C spin j , and its value depends on the internuclear distance r_{ij} and orientation angles (θ , ϕ) of the dipolar vector relative to the magnetic field according to the following equation:

$$\omega_{ij} = \frac{\sqrt{2}}{\pi} \frac{\mu_0 \gamma_i \gamma_j}{4\pi r_{ij}^3} \sin 2\theta \sin \phi, \quad (2)$$

In the tilted frame of the FSLG sequence, the initial ^1H magnetization after the excitation pulses is $\rho(0)$:

$$\rho(0) = \sum_i p_i I_{ix}, \quad (3)$$

where p_i represents the initial polarization of the i th ^1H spin. The evolution of the ^1H magnetization under the effective

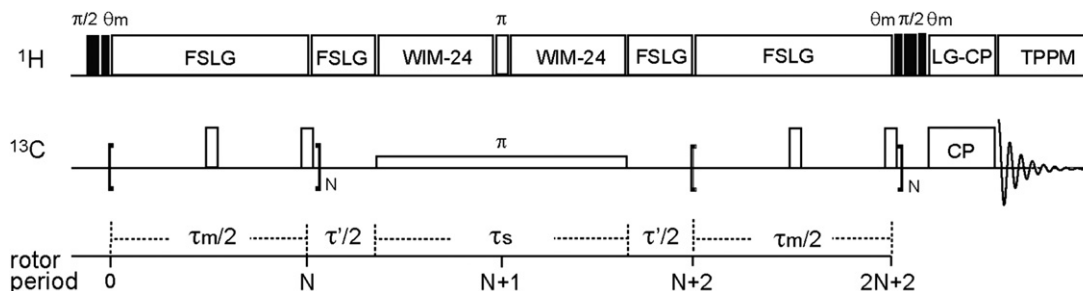


Fig. 1. Pulse sequence for ^1H - ^{13}C frequency selective (FS) REDOR experiment.

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