Solid-State NMR Spectroscopy

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Long-Range Correlations between Aliphatic ^{13}C Nuclei in Protein MAS NMR Spectroscopy**

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Solid-state NMR spectroscopy is a powerful technique for the investigation of complex biological systems such as membrane proteins and amyloid fibrils. In magic-angle spinning (MAS) NMR spectroscopy, structural information is obtained by the reintroduction of anisotropic interactions.[1] In particular, a large number of homonuclear dipolar recoupling schemes have been developed and applied to record correlation spectra and measure internuclear distances in peptides and proteins.[1] While many recoupling techniques efficiently transfer polarization between directly bonded ^{13}C nuclei, their effectiveness can be reduced significantly when distant ^{13}C spins with weak dipolar couplings are involved. This limitation is generally imposed both by the experimental constraints that must be maintained during long mixing periods and by the inherent complexities of multiple-spin systems such as dipolar truncation,[2,3] that is, the attenuation of weak dipolar couplings by stronger dipolar couplings in the recoupled dipolar Hamiltonian. As a consequence, spin diffusion techniques,[4–8] which circumvent some of these limitations,[9] have been widely utilized to estimate long-range homonuclear structural constraints in protein solid-state NMR studies.[10,11] We present herein an experimental approach that provides highly sensitive long-range correlations between aliphatic ^{13}C nuclei through the combination of isotope dilution and efficient polarization transfer by a band-selective radio-frequency dipolar recoupling (BASE RFDR) scheme. We demonstrate this method with a sample of PI3-SH3 (the SH3 domain of the p85α subunit of phosphatidylinositol 3 kinase) in amyloid fibril form.

The ^{13}C alternating labeling scheme devised by LeMaster and Kushlan,[12] and introduced to solid-state NMR studies by Hong,[13] which employs [2-$^{13}C_1$]- or [1,3-$^{13}C_2$]glycerol as the carbon source, results in amino acids labeled with ^{13}C at approximately every other carbon position (“odd/even” labeling). The approach simplifies ^{13}C–^{13}C correlation spectra by reducing the number of spectral lines and narrows the linewidths because *J* couplings are eliminated. It thus offers significant advantages over uniform ^{13}C labeling.[10] From the perspective of the dipolar recoupling dynamics of the spin system, this type of ^{13}C spin dilution provides two additional benefits. First, relayed polarization transfer, which is dominated by rapid diffusion between directly bonded nuclei in uniformly ^{13}C-labeled samples, is partially eliminated in odd/even ^{13}C-labeled spin systems; magnetization originating from a given site therefore propagates more directly to fewer spins, resulting in correlation spectra that present improved long-range transfer efficiency between structurally interesting pairs of nuclei. Secondly, the elimination of directly bonded ^{13}C nuclei in most amino acid spin systems results in the partial attenuation of dipolar truncation effects, thus enabling the observation of weak dipolar couplings corresponding to long internuclear distances. However, dipolar truncation is most severe in pulse sequences that generate a first-order recoupled dipolar Hamiltonian and is considerably less pronounced in schemes that utilize second-order effects to achieve polarization transfer.[9,14,15] Therefore, the enhanced observation of long-range correlations in spin diffusion spectra of odd/even-labeled proteins (compared to uniformly labeled samples) is likely a result of the reduction of relayed polarization transfer and not of the attenuation of dipolar truncation in these dilute spin systems. In contrast, as examined recently,[3] certain zero-quantum (ZQ) recoupling schemes may benefit directly from the attenuation of dipolar truncation in such spin systems.

In order to explore the advantages of alternating labeling for techniques other than spin diffusion, we investigated the application of efficient recoupling methods to a fibril sample of PI3-SH3 (86 residues) prepared with [2-^{13}C]glycerol as the carbon source (2-PI3-SH3). As may be expected, the elimination of directly bonded ^{13}C nuclei interrupts relayed polarization transfer and attenuates dipolar truncation in broadband radio frequency-driven recoupling[16–20] (RFDR) experiments. The polarization transfer dynamics in this ZQ recoupling approach are heavily dominated by the strongest couplings present, which in the case of [2-^{13}C]glycerol labeling are typically the two-bond couplings between sequential $^{13}C_\alpha(i)$ and $^{13}C'(i-1)$ resonances. As a consequence, the most prominent cross-peaks in broadband RFDR spectra of 2-PI3-SH3 are medium- and long-range correlations between C′ and aliphatic nuclei, while correlations between distant aliphatic nuclei, rich in structural information, are generally too weak

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to be observed. A representative broadband RFDR spectrum is shown in the Supporting Information.

In contrast, the application of RFDR using low-power, band-selective π pulses (Figure 1 a) with the minimal bandwidth necessary to cover the aliphatic ^{13}C spectral region yields intense correlation peaks between distant aliphatic nuclei (Figure 1 b). This spectrum was recorded at a spinning frequency ($\omega_r/2\pi$) of 12.5 kHz and employed ^{13}C recoupling π pulses with a 12.5 kHz radio-frequency (rf) field ($\omega_1/2\pi$) in a manner similar in appearance to conventional RFDR. During the mixing period ($\tau_{\text{mix}} = 17.92$ ms), a 80 kHz ^{1}H continuous-wave (cw) decoupling field was applied and the ^{13}C recoupling π pulses followed a 32-step phase sequence ($XY-16$, $Y\bar{X}-16$) optimal for compensation of chemical shift offsets and rf inhomogeneity for weak π pulses that is based on the $XY-16$ scheme.[21]

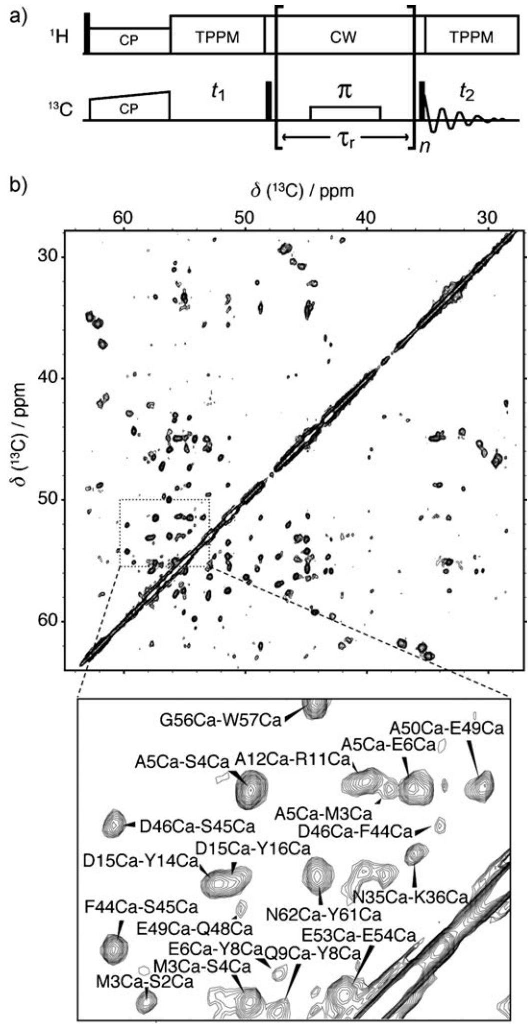

Figure 1. a) Band-selective RFDR pulse sequence for two-dimensional homonuclear correlation spectroscopy. The recoupling π pulses occupy half or more of the rotor period, τ_r, and follow a 32-step phase scheme. b) Long-range aliphatic correlation spectrum of 2-PI3-SH3 amyloid fibrils obtained with 17.92 ms of longitudinal mixing by BASE RFDR. The mixing time was optimized for 3.8 Å, the typical internuclear distance of sequential C_α–C_α contacts. The spectrum was recorded in 27 h at a magnetic field of 16.4 T and MAS frequency of 12.5 kHz, with ^{13}C recoupling π pulses of 12.5 kHz centered at 48 ppm. CP = cross-polarization, TPPM = two-pulse phase modulation.

The long-range polarization-transfer efficiency of this BASE RFDR approach is a product of the interplay between several factors in addition to the attenuation of dipolar truncation afforded by alternating labeling. First, the selective bandwidth of the recoupling π pulses centered on the aliphatic region of the ^{13}C NMR spectrum exclude downfield resonances from the aliphatic–aliphatic recoupling dynamics, resulting in the elimination of relayed polarization transfer through neighboring C′ spins, which enhances the direct polarization transfer between distant aliphatic ^{13}C spins. Concurrently, the application of long ^{13}C π pulses occupying a large fraction of the rotor period entails a significant finite-pulse effect, even for moderate spinning frequencies, which facilitates dipolar recoupling between nuclei with small chemical shift differences such as aliphatic spins.[18,19] Furthermore, heteronuclear interference conditions[20] are readily avoided by the application of moderate to strong ^{1}H decoupling fields owing to the weak effective ^{13}C rf field employed during mixing, leading to minimal polarization losses during the recoupling period. Finally, numerical simulations indicate that while polarization transfer in BASE RFDR is primarily mediated by direct ^{13}C–^{13}C dipolar interactions, third-spin-assisted recoupling[14,15] (TSAR), which relies on higher-order heteronuclear interactions, is active during these (^{13}C/^{1}H) rf field conditions and is compatible with the longitudinal mixing mechanism of BASE RFDR, potentially enhancing the attainable polarization-transfer efficiency. Numerical simulations illustrating finite-pulse and TSAR effects are provided in the Supporting Information.

Figure 2 demonstrates that BASE RFDR leads to improved sensitivity in aliphatic–aliphatic correlation spectra of 2-PI3-SH3 compared to that obtained with proton-driven spin diffusion[5,6] or DARR (dipolar-assisted rotational resonance)[7,8] for cross-peaks corresponding to internuclear distances of roughly 3.8 Å and above, arising from sequential interresidue ^{13}C–^{13}C couplings and longer contacts. However, a few specific exceptions, where cross-peak intensities are attenuated, can be observed most likely as a consequence of strong dipolar truncation effects remaining in certain spin systems in 2-PI3-SH3. We must note that while mixing times in Figure 2 were optimized for C_α–C_α contacts (≈ 3.8 Å) for direct comparison, in the case of DARR and spin diffusion experiments these cross-peaks build up slowly at such long mixing times (300–500 ms) that longer contacts could potentially be observed.

Optimal experimental conditions for efficient low-power recoupling are determined by the spectral bandwidth required. In the case of aliphatic carbons at 16.4 T (700 MHz ^{1}H Larmor frequency), with a spectral dispersion of ≈ 10 kHz, ^{13}C rf fields of similar magnitude are required for efficient recoupling within the aliphatic region. At the same time, the rotor-synchronized ^{13}C-recoupling π pulses must occupy a significant fraction of the rotor period (half or more) in order to produce the most favorable finite-pulse effects. Thus, for ^{13}C π pulses of 12 kHz, BASE RFDR can be performed at MAS frequencies ranging from approximately

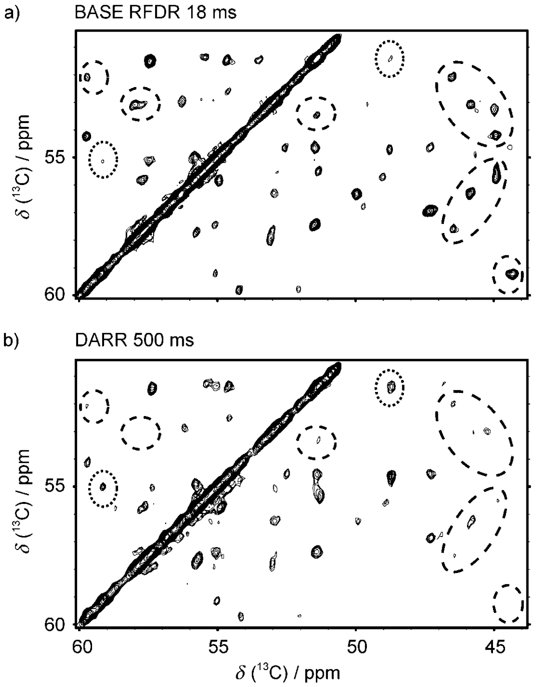

Figure 2. Long-range aliphatic correlations in 2-PI3-SH3 obtained with 17.92 ms of BASE RFDR (a) and 500 ms of DARR (b). Both spectra were recorded in 6.8 h under experimental conditions similar to those described for Figure 1 b. BASE RFDR shows improved overall polarization-transfer efficiency, highlighted by dashed ellipses. Dotted ellipses mark a few exceptions, likely the result of remaining dipolar truncation for certain spin systems. See the Supporting Information for full spectral views.

12 kHz to 24 kHz, the latter being the windowless recoupling irradiation limit in which the recoupling π pulse occupies the entire rotor period. Conversely, at a spinning frequency of 12 kHz, BASE RFDR can be applied with ^{13}C rf fields ranging from 6 to 12 kHz.

The aliphatic–aliphatic polarization-transfer we have demonstrated here in studies of PI3-SH3 fibrils has potential applications to a variety of aspects of protein MAS NMR analysis, including sequential resonance assignment, long-range constraints generation, and distance measurements. The BASE RFDR pulse scheme is an extension of RFDR[16–20] into a regime where the majority of the rotor period during the mixing time is occupied by weak ^{13}C irradiation, at moderate spinning frequencies. While the efficiency of BASE RFDR has been delineated here and exploited to procure highly sensitive long-range aliphatic correlations, further methodological investigation of this dipolar recoupling scheme is currently in progress.

Experimental Section

The sample of 2-PI3-SH3 fibrils was prepared as described previously[22] but using [2-^{13}C]glycerol and NaH^{13}CO$_3$ as the sole sources of carbon. The total amount of protein in each packed sample was approximately 8 mg. All experiments were performed using a custom-designed spectrometer operating at 700 MHz ^{1}H Larmor frequency (courtesy of Dr. David J. Ruben at the Francis Bitter Magnet Laboratory, Massachusetts Institute of Technology) equipped with a 3.2 mm Varian/Chemagnetics probe (Palo Alto, CA). Correlation experiments consisted of ^{1}H–^{13}C cross-polarization, chemical shift evolution, longitudinal homonuclear mixing, and detection periods. TPPM[23] decoupling (100 kHz ^{1}H rf field) was applied during the evolution and detection periods. Such high-power decoupling was desirable for improved resolution but not strictly necessary for experimental performance. Further details on data acquisition are provided in the Supporting Information.

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