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Tailored low-power cross-polarization under fast magic-angle spinning

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ABSTRACT

High static magnetic fields and very fast magic-angle spinning (MAS) promise to improve resolution and sensitivity of solid-state NMR experiments. The fast MAS regime has permitted the development of low-power cross-polarization schemes, such as second-order cross-polarization (SOCP), which prevent heat deposition in the sample. Those schemes are however limited in bandwidth, as weak radio-frequency (RF) fields only cover a small chemical shift range for rare nuclei (e.g. ^{13}C). Another consideration is that the efficiency of cross-polarization is very sensitive to magnetization decay that occurs during the spin-lock pulse on the abundant nuclei (e.g. ^{1}H). Having characterized this decay in glutamine at 60 kHz MAS, we propose two complementary strategies to tailor cross-polarization to desired spectral regions at low RF power. In the case of multiple sites with small chemical shift dispersion, a larger bandwidth for SOCP is obtained by slightly increasing the RF power while avoiding recoupling conditions that lead to fast spin-lock decay. In the case of two spectral regions with large chemical shift offset, an extension of the existing low-power schemes, called MOD-CP, is introduced. It consists of a spin-lock on ^{1}H and an amplitude-modulated spin-lock on the rare nucleus. The range of excited chemical shifts is assessed by experimental excitation profiles and numerical simulation of an I_2S spin system. All SOCP-based schemes exhibit higher sensitivity than high-power CP schemes, as demonstrated on solid (glutamine) and semi-solid (hydrated, micro-crystalline ubiquitin) samples.

1. Introduction

The past decade has seen a tremendous development in the field of solid-state NMR [1,2]. Part of this development can be attributed to the widespread adoption of high static magnetic fields. High fields provide better sensitivity, since the improvement in signal-to-noise ratio (S/N) is roughly proportional to $B_0^{3/2}$. Furthermore, chemical shift dispersion directly scales with the field strength, which is beneficial for the resolution of crowded spectra. Similar to the progress provided by high B_0 fields, a recent wave of improvement in resolution and sensitivity has been brought about by the development of very fast MAS probe heads and pulse techniques. MAS frequencies of up to 67 kHz are now reached by rotors with an o.d. of 1.3 mm. As a consequence of the very fast rotation, chemical shift anisotropies are efficiently averaged and dipolar couplings are greatly reduced [3], resulting in narrow line-widths. The large decrease in sample volume reduces the sensitivity; this effect is partially compensated since the S/N per unit volume follows the inverse of the RF coil diameter [4,5]. Therefore, at equal MAS speed, the absolute S/N is roughly proportional to the rotor diameter.

At high static field, large chemical shift dispersions entail the generation of larger applied B_1 fields in order to excite the full spectral width of nuclei such as ^{13}C and ^{15}N. Strong radio-frequency (RF) irradiation can deposit a high amount of energy in the sample. The heat contributed can lead to irremediable alteration of the sample. This situation is particularly critical for the study of biological samples, which are fragile and often preserved in ionic buffers [6]. In fast MAS experiments, additional heating is caused by the friction of the MAS rotor with the surrounding gas, which can increase the temperature by about 60 K at 60 kHz MAS. To overcome the heating problem, one strategy is to reduce electric fields through modifications of the RF coil design [7–13]. A concurrent and complementary strategy is the development of pulse sequences requiring minimal amounts of irradiation power. This additionally mitigates the strain that strong RF generation imposes on the instrumentation. Substantial gains in sensitivity can further be obtained by combining low-power pulse sequences and fast MAS with shortening of the recycling delay. For instance, in the presence of paramagnetic nuclei, the ^{1}H longitudinal relaxation times are reduced, thus allowing fast acquisition [14–18].

Low-power alternatives compatible with very fast magic-angle spinning of the rotor have been recently introduced for many of the fundamental building blocks of solid-state NMR pulse

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sequences. This comprises proton hetero-nuclear decoupling (e.g. XiX [19–21], TPPM [22–24], PISSARRO [25]), mixing schemes (e.g. HORROR [26], ocHORROR [27], DREAM [21,28,29], RFDR [30–32], MIRROR [33], PARIS [34], PAR [35,36]), and cross-polarization [37,38]. Low-power cross-polarization schemes, such as band-selective ^{1}H–^{13}C cross-polarization [37], and second-order cross-polarization (SOCP) [38], are easily incorporated into multidimensional experiments. The band-selective ^{1}H–^{13}C CP was included in the low-power pulse sequence for CC-RFDR [17]. We have recently presented a set of low-power solid-state NMR experiments assembled based on SOCP, (NCA, N(CO)CX and CC), that are sufficient for protein resonance assignment under fast MAS, and which include sequential ^{15}N–^{13}C correlation [18]. SOCP is a second order recoupling experiment, part of the growing family that presently consists of PAIN-CP [39], PAR [35,36,40], MIRROR [33], RESORT [41] and SOCP.

An intrinsic limitation of the low-power CP schemes is their band-selective aspect, since a low-power irradiation cannot efficiently spin-lock the full range of chemical shifts for rare nuclei. In the current article, we present two strategies to alleviate this limitation. In case of multiple sites with small chemical shift dispersion, we employ SOCP with increased RF frequency. In case of regions with large chemical shift dispersion, low-power CP is employed with an amplitude modulation on the rare nucleus (the "S" spin) spin-lock pulse. This constitutes an extension of the present schemes which restores the high information content per spectrum that is found in broadband excitation while conserving the benefits of low-power solid-state NMR pulse sequences.

In order to select optimal conditions, we first characterized the decay of ^{1}H spin-locked magnetization as a function of RF-field strength and MAS frequency. The effect of amplitude modulation on cross-polarization is then analyzed through experimental and simulated excitation profiles. The efficiency of the cross-polarization schemes is demonstrated on solid (glutamine as a dry powder) and semi-solid (hydrated, micro-crystalline ubiquitin [42]) samples. Finally, SOCP with increased bandwidth is used to record an entirely low-power 2D ^{13}C–^{13}C correlation experiment on ubiquitin. The obtained spectrum contains correlations for complete amino acid spin systems, including carbonyl, aromatic and methyl carbons.

2. Materials and methods

2.1. Sample preparation

Uniformly [^{13}C, ^{15}N]-labeled ubiquitin was recombinantly expressed in *E. coli* and purified as previously described [42,43]. Micro-crystals were obtained by precipitating the sample with polyethylene glycol [44]. Approximately 1 mg of micro-crystalline protein was filled into a rotor of o.d. 1.3 mm. A 1.3-mm rotor was packed with 2.91 mg of uniformly [^{13}C, ^{15}N]-labeled L-glutamine purchased from Cambridge Isotope Laboratories (Cambridge, MA).

2.2. Solid-state NMR spectroscopy

Spectra were recorded at 18.8 T (800 MHz ^{1}H Larmor frequency) on a Bruker Avance III standard-bore spectrometer equipped with a 1.3-mm triple-resonance probe head (Bruker). Three MAS frequencies were used for the spin-lock experiments: 40.2, 49.9, and 60.0 kHz. All other experiments were performed at 60.0 kHz. The temperature of the ubiquitin sample was estimated to be +6.7 °C at a MAS frequency of 40 kHz, +17.0 °C at 50 kHz and +31.0 °C at 60 kHz. This estimate was obtained by comparison of the isotropic ^{1}H chemical shift of water at high-speed MAS to published chemical shifts [45]. For all experiments, the ^{1}H–^{13}C and ^{1}H–^{15}N dipolar couplings were decoupled during acquisition using 12 kHz of XiX decoupling on ^{1}H [19,20]. A recycling delay of 2 s was employed. Chemical shifts are reported in ppm from DSS, calibrated using adamantane as external reference [46]. CP-MAS spectra were acquired with 128 scans for ubiquitin (Fig. 4a,b and d) and 32 scans for glutamine (Figs. 4 and 6). The 2D ^{13}C–^{13}C DREAM spectrum was recorded with 250 t_1 points and 288 scans, for a total experimental time of 40 h. The maximum acquisition time was 6.2 ms in the t_1 and 14.4 ms in the t_2 dimension. Double-quantum ^{13}C–^{13}C mixing was accomplished by a tangential amplitude sweep [28,29] from ~25 to ~35 kHz during 5 ms.

2.3. Measurement of excitation profiles

Signal intensity was detected after a cross-polarization of 5 ms through a series of ^{1}H–^{15}N CP-MAS spectra of glutamine (Fig. 2). To record a complete profile, the ^{15}N carrier was swept from 102 kHz upfield to 102 kHz downfield of the resonances in steps of 750 Hz. The glutamine ^{15}N spectrum has two resonances corresponding to the side-chain amide and to the backbone amine at 111.1 ppm and 38.16 ppm, respectively. The homonuclear dipolar couplings between ^{15}N are considered to be insignificant, based on the large distance separation between two nitrogen atoms in the crystal structure (Refs. [47,48]). Hence, signals from both side-chain and backbone were combined in the analysis to increase *S*/*N*.

2.4. Quantum mechanical simulations

The simulated excitation profiles of ^{1}H–^{15}N CP in glutamine were obtained from a step-wise integration procedure of the Liouville–von Neumann equation within the numerical simulation routine GAMMA [49]. Neglecting scalar through-bond couplings, the relevant Hamiltonian in the Zeeman interaction frame contains ^{15}N isotropic chemical shift, dipolar couplings (^{1}H–^{1}H and ^{1}H–^{15}N) and RF irradiation on ^{1}H and ^{15}N (time-dependent in case of MOD-CP). Mimicking the situation in an NH_2 group, a proton-proton distance of 1.74 Å (corresponding dipolar coupling: 23.2 kHz) and proton-nitrogen distances of 1.05 Å (dipolar coupling: 10.5 kHz) were used. To obtain a complete excitation profile, simulations were carried out in steps of 375 Hz such that the ^{15}N isotropic chemical shift covered a range from –102 kHz to +102 kHz. The expectation value of spin-locked ^{15}N magnetization was averaged for all time points between 6 ms and 8 ms. Powder averaging involved 120 orientations. All simulated profiles were adjusted with a single, global scaling factor in order to be compared with experimental profiles. This global scaling factor was found by least-squares fitting of simulated to observed signal intensities.

3. Results and discussion

3.1. Characteristics of spin-locked magnetization

A ubiquitous building block in solid-state NMR pulse sequences is the spin-lock RF pulse. Magnetization is preserved along a given axis in the rotating frame by the application of an external field $\vec{B}_1$ parallel to this axis. If the RF field is sufficiently strong, then dephasing due to dipolar couplings and chemical shifts is small or negligible. Magnetization can thus be stored for relatively long periods, limited only by the spin–lattice relaxation in the rotating frame with a time constant $T_{1\rho}$. However, at specific amplitudes of the RF field, some spin interactions are reintroduced because of the interference between RF irradiation and MAS. These