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Simultaneous homonuclear and heteronuclear spin decoupling in magic-angle spinning solid-state NMR

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ABSTRACT

We show here an effective way of implementing simultaneously homonuclear and heteronuclear dipolar decoupling in magic-angle spinning (MAS) solid-state NMR. Whilst the homonuclear spin decoupling is applied on the ^{1}H channel, heteronuclear spin decoupling is applied on the ^{13}C channel. The ^{1}H spins are observed in a windowed fashion in this case. The resultant ^{1}H spectrum has higher resolution due to the attenuation of broadening arising from both homonuclear ^{1}H-^{1}H and heteronuclear ^{1}H-^{13}C interactions, with the latter normally leading to additional line broadening in ^{13}C labelled samples. The experiments are performed at MAS frequencies of ca. 60 kHz.

1. Introduction

^{1}H spectral resolution in magic-angle-spinning (MAS) solid-state NMR is vital for characterising a wide range of molecules, ranging from organic to biomolecules. The resolution is normally poor compared to that of ^{13}C or ^{15}N due to the very strong homonuclear ^{1}H-^{1}H dipolar couplings which can reach up to 20 kHz in many cases [1]. Homonuclear decoupling methods, like PMLG and DUMBO schemes, have made it possible to achieve relatively higher resolution in the ^{1}H spectra over a wide range of MAS frequencies [1–3]. Recent research has also shown remarkable ^{1}H resolution in biomolecular systems, consisting of fully protonated proteins, under MAS frequencies of 100–120 kHz [4–6] without the need of homonuclear decoupling methods. The typical linewidths obtained at these spinning frequencies are still a factor of 4–5 more than those observed in solution NMR. A systematic analysis of the dependence of ^{1}H resolution on MAS frequency by Meier and co-workers has shown that averaging of the ^{1}H-^{1}H homonuclear couplings to give a resolution comparable to that obtained in solution NMR, using MAS alone, will require spinning frequencies in excess of 250 kHz [7]. Thus, Combined Rotation and Multiple Pulse Schemes (CRAMPS) still remain an attractive option to improve upon resolution gains from MAS alone. A few studies have shown that even at high MAS frequencies of 60–110 kHz, homonuclear decoupling further helps in narrowing ^{1}H line widths or extending the inherent T_2 relaxation times, the so-called T_2' values [1]. These methods are, thus, of immense utility when working with protonated samples, even at MAS frequencies $\geq$ 60 kHz. However, a limitation of the methods shows up in the fast MAS regime when the ^{1}H nuclei in question are also connected to an NMR-active hetero-nucleus, for example, the case of isotopically ^{13}C enriched samples. The contribution of the heteronuclear dipolar interaction is such cases is significant and in some cases, heteronuclear decoupling alone can result in better resolution than homonuclear decoupling alone (*vide infra*). Simultaneous application of heteronuclear and homonuclear decoupling can in principle give the best resolution, but the interference between these time dependent processes (including MAS) makes finding of a suitable condition non-trivial.

We here report on a strategy to obtain or recover high-resolution ^{1}H spectrum in case of isotopically enriched samples as in case of non-labelled samples. It involves the application of PMLG $\frac{x\bar{x}}{mm}$ with low radio-frequency (RF) amplitude and comparatively long cycle time at a MAS frequency ~60 kHz and *r*CW^{ApA} decoupling [8] with a cycle time much longer than homonuclear decoupling and MAS cycle times. The choice of *r*CW^{ApA} is not unique; we also show the comparison with the often used WALTZ-16 decoupling [9] under these conditions. These results point towards a MAS and homonuclear decoupling regime which can be effectively targeted for the development of heteronuclear decoupling schemes that are compatible with simultaneous homonuclear

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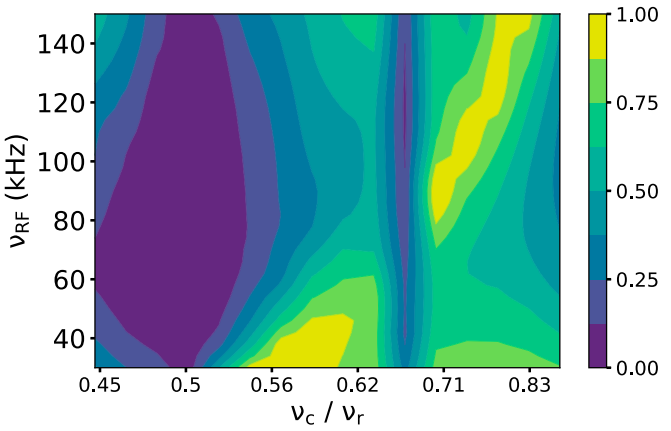

Fig. 1. Simulated intensity (normalized to the maximum of the data) of one of the Glycine CH_2 protons in a spin system containing 3 protons as a function of the cycle time of the homonuclear decoupling sequence (PMLG $\frac{x\bar{x}}{mm}$) and the RF amplitude. Other than the good conditions at high RF amplitude and short PMLG $\frac{x\bar{x}}{mm}$ cycle times, we also see good conditions at ν_{RF} = 35–45 kHz and a ν_r/ν_c = 0.55–0.60.

decoupling as well. The significantly improved resolution at low RF amplitudes for both homonuclear and heteronuclear decoupling scheme makes this strategy particularly suitable to study fully protonated biomolecules at MAS frequencies ≥60 kHz.

2. Experimental

Experiments were performed on natural abundance sample of glycine (NA-Gly), uniformly ^{13}C and ^{15}N labelled glycine (CN-Gly) and uniformly ^{13}C and ^{15}N labelled tripeptide N-formyl-methionyl-leucyl-phenylalanine (MLF) at MAS frequencies of 60.0, 60.1 and 59.6 kHz, respectively, on an AV-III Bruker 700 MHz spectrometer (~16.5 T) equipped with a two-channel 1.3 mm probe and TopSpin 2.1. All samples were fully packed in 1.3 mm rotors. Supercyled PMLG $\frac{x\bar{x}}{mm}$ was used for homonuclear dipolar decoupling [1,10,11]. Windowed ^1H detection using PMLG $\frac{x\bar{x}}{mm}$ was optimised independently on all samples in the low RF amplitude regime following strategies described previously [1,12,13]. Briefly, we used a PMLG^x block with 6 pulses with the following phases: 325.36°, 256.08°, 186.79°, 6.8°, 76.08°, 145.36° and varied the length of the single PMLG^x block from 6 μs to 15 μs in steps of 0.3 μs and the RF amplitude from 30 kHz to 100 kHz. The window duration was kept to the minimal value that allowed us to obtain a 16-point oversampling for each point collected (requiring 1.6 μs on the available digital receiver) and minimum noise artefacts (2.8–3.0 μs). ^1H offset was optimised at the end to achieve the best possible resolution. Optimal decoupling was judged based on the resolution of the CH_2 protons for the glycine samples and the aliphatic protons in the MLF sample. All spectra were scaled by the PMLG scaling factor that gave the correct chemical shift values for the CH_2 and the NH_3 protons in glycine and the H_α protons in MLF (0.88–0.90). Windowed acquisition leads to a loss in sensitivity compared to non-windowed detection, and as expected, based on our previous findings [12], we saw that the ^1H spectra acquired using windowed acquisition and without homonuclear decoupling had a signal-to-noise ratio ~20–25% of the spectra obtained in a non-windowed fashion. Consequently, 2D ^{13}C-^1H correlation spectra were recorded on MLF with 4 (non-windowed detection) or 32 scans (windowed acquisition), which gave signal-to-noise ratios ~58 (non--windowed detection) and ~42 (windowed detection) for the first indirect point. We used a direct acquisition time of 6 ms and an indirect evolution time of 12.8 ms with the States-TPPI method for quadrature detection. Heteronuclear decoupling schemes were applied on the ^{13}C channel with an RF amplitude of 12.5 kHz. In the *r*CW^{ApA} scheme [14], the condition for optimal decoupling was obtained by varying the length of the CW-block while keeping the length of the π-block as 40 μs. For WALTZ-16, the base pulse length (which is theoretically equal to the $\pi/2$ pulse) was varied to find the optimal value.

Numerical simulations were done with SIMPSON 4.2.1 [15] using the glycine spin system with 3 protons (two methylene and the closest amine proton). A crystal file using the REPULSION scheme 'rep20' [16] and 12 γ-angles was used for powder averaging. Spectra were processed and analysed using Nmrglue (0.7-dev) [17] and TopSpin (3.5-pl7) and plotted using Matplotlib (2.0.2) in Python (3.6.2). Glycine resonances were fit to Lorentzian peaks using the 'curve_fit' function in Scipy (1.0.0) and the MLF resonances were fit to 2D Lorentzian peaks in

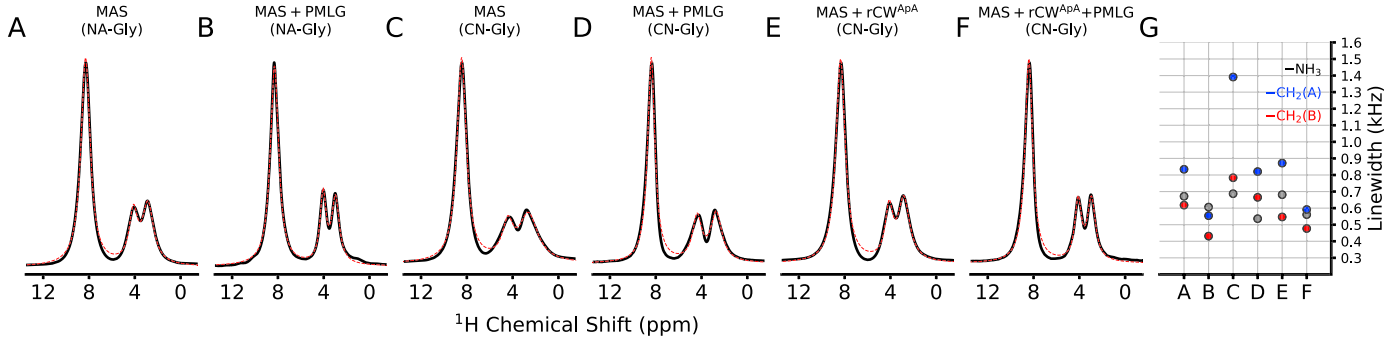

Fig. 2. Successive improvement in resolution for CN-Gly sample upon the application of various decoupling schemes and comparison with the resolution obtained in the case of NA-Gly. (A–F) 1D spectra of NA-Gly or CN-Gly under different decoupling schemes. (A) MAS alone on NA-Gly, (B) MAS + homonuclear decoupling (PMLG $\frac{x\bar{x}}{mm}$) on NA-Gly, (C) MAS alone of CN-Gly, (D) MAS + homonuclear decoupling (PMLG $\frac{x\bar{x}}{mm}$) on CN-Gly, (E) MAS + heteronuclear decoupling (*r*CW^{ApA}) on CN-Gly and (F) MAS + heteronuclear decoupling + homonuclear decoupling (PMLG $\frac{x\bar{x}}{mm}$ and *r*CW^{ApA}) on CN-Gly. The parameters for windowed detection and MAS + homonuclear decoupling were as follows: ν_{RF} = 49 kHz, ν_c = 35 kHz, τ_{window} = 3.0 μs. *r*CW^{ApA} heteronuclear decoupling was achieved with an RF amplitude of 12.5 kHz and τ_{cw} = 45 μs and τ_y = 40 μs. Each spectrum was obtained with 8 scans, an acquisition time of 15 ms, a recycle delay of 2 s and processed without any window function. Spectra are plotted normalized to the intensity of the NH_3 protons. Overlaid on each of the 1D spectra (A–F) are the fits assuming 3 Lorentzian resonances (red, dashed lines) and the line-widths obtained from these fits for all the resonances (amine protons in grey, methylene proton at 2.6 ppm in blue and methylene proton at 4.4 ppm in red) are reported in (G). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)