Communication

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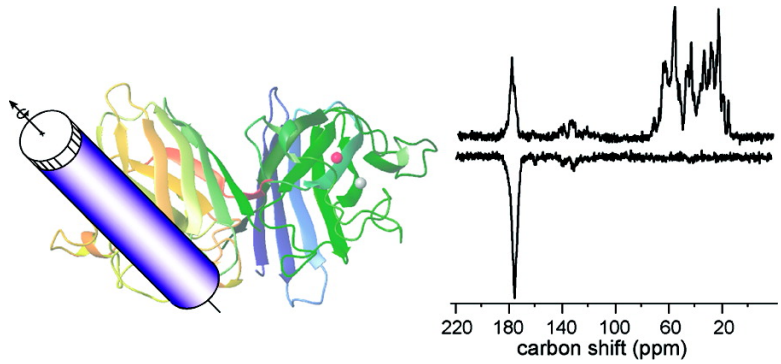

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Band-Selective $^{1}H-^{13}C$ Cross-Polarization in Fast Magic Angle Spinning Solid-State NMR Spectroscopy

Ségolène Laage,[†] Alessandro Marchetti,[†] Julien Sein,[†] Roberta Pierattelli,[‡] Hans Juergen Sass,[§] Stephan Grzesiek,[§] Anne Lesage,[†] Guido Pintacuda,*[,†] and Lyndon Emsley*[,†]

Université de Lyon, CNRS/ ENS Lyon/ UCB-Lyon 1, Centre RMN à Très Hauts Champs, 5 rue de la Doua, 69100 Villeurbanne, France, Department of Chemistry and Magnetic Resonance Center (CERM), University of Florence, Via Luigi Sacconi 6, 50019 Sesto Fiorentino (Firenze), Italy, and Biozentrum, University Basel, Klingelbergstrasse 70, 4056 Basel, Switzerland

Received July 29, 2008; E-mail: Guido.Pintacuda@ens-lyon.fr; Lyndon.Emsley@ens-lyon.fr

Solid-state NMR is rapidly growing as a tool for structural and dynamical characterization of a range of highly pertinent biomolecular systems.[1] Recently, striking examples have been provided for microcrystalline globular proteins,[2] fibrils,[3] and membrane complexes.[4] All of these studies rely on 2D or 3D homonuclear and heteronuclear experiments correlating ^{13}C, ^{15}N resonances of uniformly ^{13}C- and ^{15}N-labeled proteins. These experiments provide both the assignment of the resonances, and the constraints for the structural or dynamical determination.[5]

Precise control of coherence transfer between groups of spins is essential in these studies to obtain unambiguous correlations. For example, in the quest for controlled transfers, a series of one-bond correlation experiments have been developed using *J*-coupling between neighboring nuclei.[6] Another key method is the band-selective SPECIFIC-CP (spectrally induced filtering in combination with cross-polarization) experiment, which yields sequential assignments by providing distinct N-CO and N-CA transfers along the protein backbone.[7] This experiment depends on the possibility of directing dipolar coherence transfer between N and C spins, based on the difference in the chemical shifts of the carbon resonances.

Analogs of the SPECIFIC-CP experiments for homonuclear ^{13}C correlations have so far only been proposed using selective pulses combined with phase-cycled *z*-filters after a broadband cross-polarization (CP) to remove undesired polarization from portions of the ^{13}C spectrum.[8] In the following, we demonstrate an approach that allows simple, band-selective $^{1}H-^{13}C$ CP. In turn, this enables the rapid acquisition of directed homonuclear $^{13}C-^{13}C$ 2D correlations with high sensitivity. This is demonstrated with the acquisition of a high-resolution aliphatic correlation of a microcrystalline sample of the paramagnetic, oxidized form of human superoxide dismutase (SOD),[9] and with the acquisition of a high-resolution $CO-C^{\alpha}$ correlation for the protein domain GB1.[10]

At conventional magic angle spinning (MAS) frequencies, $^{1}H-^{13}C$ CP is intrinsically broadband, with the whole proton bath being in contact with all the carbons.[11] Selective transfer would require low-power irradiation on ^{13}C resonances. Low power CP has indeed been considered with success in solution NMR.[12] In solids, because of the overlap of several broad zero-quantum (ZQ) and double-quantum (DQ) Hartmann−Hahn matching conditions,[13−15] low-power irradiation entails a substantial price in the transfer efficiency.

Recently, new hardware developments have opened the way to ultrafast (>60 kHz) MAS with small diameter rotors (e.g. 1.3 mm). These spinning rates enter a regime where homonuclear $^{1}H-^{1}H$ couplings are efficiently averaged,[16] and DQ and ZQ matching conditions become well spaced. We demonstrated that, in this regime, broadband CP could be achieved using low rf amplitudes.[15]

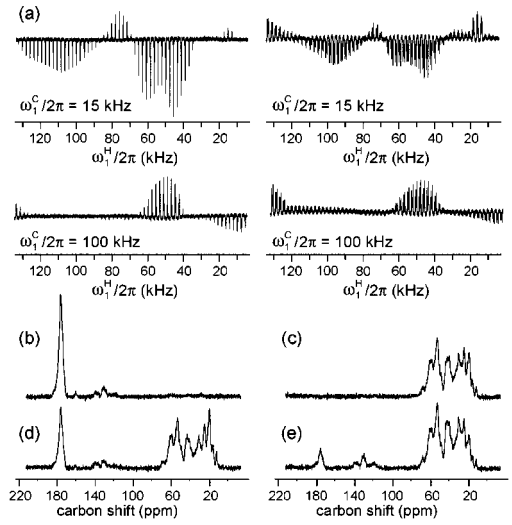

Figure 1. ^{13}C spectra of (^{15}N,^{13}C)-labeled SOD recorded at 60 kHz MAS. (a) Matching profiles for specific (upper panels) and broadband (lower panels) CP. Intensity of the CO carbon signal (left panels) and of the aliphatic carbon signals (right panels) at variable proton rf-field strength. In the two upper panels, the carbon rf-field strength $\omega_1^C/2\pi$ is 15 kHz and the ^{13}C carrier Ω_C is 176 (right) and 40 ppm (left), respectively. In the two lower panels, the ^{13}C rf-field strength $\omega_1^C/2\pi$ is 100 kHz, and the ^{13}C carrier is placed at 100 ppm. (b−e) Comparison between a specific CP transfer to CO carbons (b, $\Omega_C = 176$ ppm, $\omega_C/2\pi = 14$ kHz $\omega_H/2\pi = 46$ kHz, 1.5 ms contact time), a specific CP to aliphatic carbons (c, $\Omega_C = 35$ ppm, $\omega_C/2\pi = 14$ kHz $\omega_H/2\pi = 46$ kHz, 1.35 ms contact time), and two conventional nonselective, high-power CPs (d,e, $\Omega_C = 100$ ppm, $\omega_C/2\pi = 100$ kHz, $\omega_H/2\pi = 40$ kHz, 4.9 ms and 750 μs contact times to yield a maximum CO and aliphatic ^{13}C signal, respectively). All experiments were performed on a Bruker Avance III spectrometer operating at a proton frequency of 900 MHz and equipped with a double-resonance 1.3 mm CP-MAS probe.

Here we show that band-selective $^{1}H-^{13}C$ CP transfer can be achieved using either ZQ or DQ matching conditions if careful attention is paid to the choice of rf field amplitudes and ^{13}C carrier. In particular, if the carrier is placed on the CO or the aliphatic ^{13}C regions, and the matching condition is chosen so that the ^{13}C amplitude is smaller than the frequency differences, then perfectly selective CP is achieved with no loss in sensitivity.

Figure 1 shows an investigation of optimal conditions for band-selective $^{1}H-^{13}C$ CP to the carbonyl and aliphatic carbons of fully-(^{15}N,^{13}C)-labeled, oxidized human SOD. Figure 1a shows the Hartmann−Hahn profiles obtained by varying the proton rf-field

[†] CNRS/ ENS Lyon/ UCB-Lyon 1.
[‡] University of Florence.
[§] University Basel.

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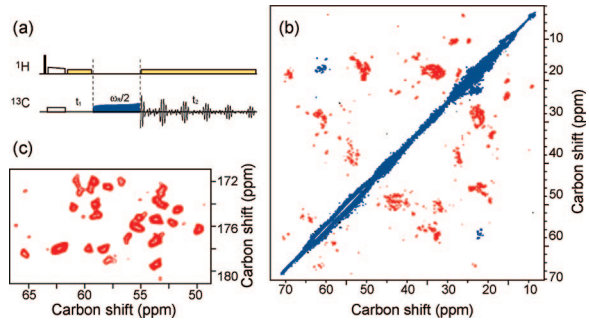

Figure 2. (a) Band-selective DREAM pulse sequence. (b-c) DREAM[17] correlation spectra at 60 kHz MAS: (b) $C^{aliph}-C^{aliph}$ spectrum of paramagnetic SOD (the CO region is empty) (CP on aliphatic carbons, $\Omega_C = 35$ ppm, $\omega_1^C/2\pi = 14$ kHz, $\omega_1^H/2\pi = 46$ kHz, contact time 1500 μs, mixing time 5 ms, 384 scans, 0.5 s interscan delay, 300 t_1 increments, $t_1^{MAX} = 10$ ms, $t_2^{MAX} = 20$ ms, total time = 17 h) and (c) $CO-C^{\alpha}$ spectrum of GB1 (CP on carbonyls, $\Omega_C = 176$ ppm during CP and 105 ppm during the rest of the experiment, $\omega_1^C/2\pi = 14$ kHz, $\omega_1^H/2\pi = 46$ kHz, contact time 350 μs, mixing time 5 ms, 192 scans, 2 s interscan delay, 80 t_1 increments, $t_1^{MAX} = 10$ ms, $t_2^{MAX} = 20$ ms, total time = 8.5 h). XiX proton-decoupling[18] ($\omega_1^H = 13$ kHz, $\tau_p = 70$ μs) was used in direct and indirect acquisition. Under these conditions we predominantly see one-bond tranfers. The two microcrystalline samples each containing about 1 mg of the ^{13}C, ^{15}N-labeled proteins were prepared as previously described[9,10] and directly centrifuged into the NMR rotor.

strength $\omega_H/2\pi$ from 130 to 5 kHz while keeping the carbon field amplitude fixed either at low ($\omega_C/2\pi = 14$ kHz) or at relatively high values ($\omega_C/2\pi = 100$ kHz). In each profile, ZQ and DQ Hartmann−Hahn conditions show positive and negative intensity, respectively, as previously shown.[13] Figure 1b−e compares the whole ^{13}C spectra of SOD recorded for the low carbon field DQ condition at 60 kHz MAS with ordinary broadband, ZQ CP spectra. Highly specific $^1H-^{13}C$ CP transfers to the CO or to the ^{13}C aliphatic could be recorded with carbon and proton fields of 15 and 45 kHz, and a carbon carrier placed at 176 and 40 ppm, respectively. We note that the ^{13}C bandwidth will be roughly inversely proportional to the amplitude of the ^{13}C rf field and subject to broadening by homonuclear couplings, as illustrated by the offset profile in the SI. Finally, the selectivity does not involve a compromise in sensitivity. On the contrary, in the broadband scheme, spin diffusion and dipolar truncation effects yield less efficient CP for carbonyl, which experience a relatively weak dipolar coupling to protons. For these nuclei, the low power conditions bring a substantial enhancement of intensity (in this case, there is a factor of about 2 in the integral of the CO region between Figure 1 panels d and b), as the polarization transfer does not suffer from the simultaneous CP process occurring to the aliphatic carbons.

Capitalizing on these effects, Figure 2 shows the application of the band-selective $^1H-^{13}C$ CP scheme implemented as part of 2D homonuclear $^{13}C-^{13}C$ correlation experiments on microcrystalline protein samples. Here, long experimental times are routinely required for sampling the large spectral widths that include all the carbon resonances, while much of the information is often contained in small regions of the spectrum ($CO-C^{\alpha}$, or $C^{aliph}-C^{aliph}$). The selectivity provided by the low-power CP scheme allows reducing the spectral width in the indirect dimension without the drawbacks connected to folding.

Figure 2b shows a $C^{aliph}-C^{aliph}$-selective DREAM experiment performed on SOD. The DREAM scheme described previously[17] was preceded by an aliphatic-selective $^1H-^{13}C$ CP. Notably, in addition to the selectivity of the transfer, the low powers used for both CP and ^1H decoupling allow a reduction in the probe duty cycle and limits sample heating. This in turn enables the use of fast recycle delays if the ^1H T_1s are suitably short.[15,19] Figure 2 c shows the $CO-C^{\alpha}$ selective DREAM experiment performed on fully-(^{15}N,^{13}C)-labeled GB1. Here, the narrow CO indirect dimension can be sampled with only a few t_1 increments in a particularly short time. This is, for example, extremely useful in experiments to quickly screen protein preparations in solids in the same way that ^1H,^{15}N-HSQC experiments are used in liquids.

In conclusion, we have introduced a method to selectively orient polarization from the whole proton bath to a specific part of the carbon spectra, without any loss of sensitivity for the aliphatic signals and with a gain for carbonyls, which are more difficult to polarize with conventional CP. This $^1H-^{13}C$ SPECIFIC-CP technique may advantageously be combined with 2D correlation experiments to reduce experimental times by sampling selectively the bandwidths which contain information. The fact that it may result in totally low rf-field experiments makes it an ideal choice for the study of biological molecules, and we thus expect this to become a building block in many correlation methods used for either assignment or structure determination in solid proteins.

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Supporting Information Available: Analysis of selective CP offset and MAS rate dependence. This material is available free of chare via the Internet at http://pubs.acs.org.

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