Journal of Magnetic Resonance 214 (2012) 324–328

Contents lists available at SciVerse ScienceDirect

Journal of Magnetic Resonance

journal homepage: www.elsevier.com/locate/jmr

Communication

Rapid solid-state NMR of deuterated proteins by interleaved cross-polarization from ^{1}H and ^{2}H nuclei

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ARTICLE INFO

Article history:
Received 9 August 2011
Revised 27 October 2011
Available online 9 November 2011

Keywords:
Solid-state NMR
Deuterated proteins
Multiple acquisition
^{2}H NMR

ABSTRACT

We present a novel sampling strategy, interleaving acquisition of multiple NMR spectra by exploiting initial polarization subsequently from ^{1}H and ^{2}H spins, taking advantage of their different T_1 relaxation times. Different ^{1}H- and ^{2}H-polarization based spectra are in this way simultaneously recorded improving either information content or sensitivity by adding spectra. The so-called Relaxation-optimized Acquisition of Proton Interleaved with Deuterium (RAPID) $^{1}H \rightarrow {}^{13}C/{}^{2}H \rightarrow {}^{13}C$ CP/MAS multiple-acquisition method is demonstrated by 1D and 2D experiments using a uniformly ^{2}H, ^{15}N,^{13}C-labeled α-spectrin SH3 domain sample with all or 30% back-exchanged labile ^{2}H to ^{1}H. It is demonstrated how 1D ^{13}C CP/MAS or 2D ^{13}C–^{13}C correlation spectra initialized with polarization from either ^{1}H or ^{2}H may be recorded simultaneously with flexibility to be added or used individually for spectral editing. It is also shown how 2D ^{13}C–^{13}C correlation spectra may be recorded interleaved with ^{2}H–^{13}C correlation spectra to obtain ^{13}C–^{13}C correlations along with information about dynamics from ^{2}H sideband patterns.

1. Introduction

Solid-state NMR spectroscopy is rapidly being established as a major tool in structural biology to provide atomic resolution information about structure and dynamics, for example, of insoluble proteins residing in membranes [1], heterogeneous aggregates/assemblies [2,3], or fibrillar structures [4,5]. This development is mediated by fast development of improved instrumentation, novel isotope labeling protocols, advanced pulse techniques and sampling procedures, as well as efficient data interpretation methods. We here address attention to the increasing use of ^{2}H labeling and multiple-sampling protocols to device new ways to accelerate acquisition of multiple-dimensional spectra of deuterated proteins on standard instrumentation. The aim is to obtain high sensitivity through efficient exploitation of available ^{1}H and ^{2}H polarization and facilitate isotope-mediated spectral editing.

Lately there has been considerable interest in extensive deuteration of ^{13}C, ^{15}N-labeled proteins to dilute proton spins and thereby facilitate ^{1}H detection and the use of ^{1}H chemical shifts and dipole–dipole couplings as sources to resolution and information [6–11]. This typically involves expression of perdeuterated proteins and subsequent (e.g., 30–100%) back-exchange of labile (primarily amide) ^{2}H with ^{1}H. This may be combined with low (e.g., 5%) protonation of non-exchangeable sites [9] or full protonation of CH_3 groups in alanine, valine, isoleucine, and leucine at the stage of protein expression [12]. While meeting the wish of diluting protons spins, it is also clear that such procedures lead to loss of most of the polarization originally available through proton spins. This has motivated interest in alternatively using the deuterons as a direct source to high-resolution spectra, e.g. via monitoring ^{2}H double-quantum evolution [13], and in the establishment of excitation methods which facilitate efficient polarization transfer from ^{2}H to ^{13}C [14], potentially in combination with simultaneous transfer from ^{1}H to ^{13}C [15]. Furthermore, ^{2}H quadrupolar couplings are important probes of local dynamics [16–18] and applied often in investigations of polymers [19,20] and proteins [21,22].

In this Communication, we present a novel simple sampling strategy, which through multiple-acquisition efficiently takes advantage of the polarization available from both ^{1}H and ^{2}H spins in extensively deuterated proteins.

2. Experimental

The NMR experiments were carried out on a Bruker Avance 600 MHz (3.2 mm triple resonance probe) (Figs. 1 and 2), and a Bruker Avance 400 MHz (2.5 mm triple resonance probe) (Figs. 1 and 3) using 10 and 20 kHz spinning. Spectra of uniformly ^{2}H, ^{13}C, ^{15}N-labeled α-spectrin SH3 domain with 30% (Figs. 1 and 2) or all (Figs. 1 and 3) labile deuterons replaced by protons (henceforth referred to as [30%–U–^{2}H/^{1}H, ^{13}C, ^{15}N]SH3) and

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doi:10.1016/j.jmr.2011.10.020

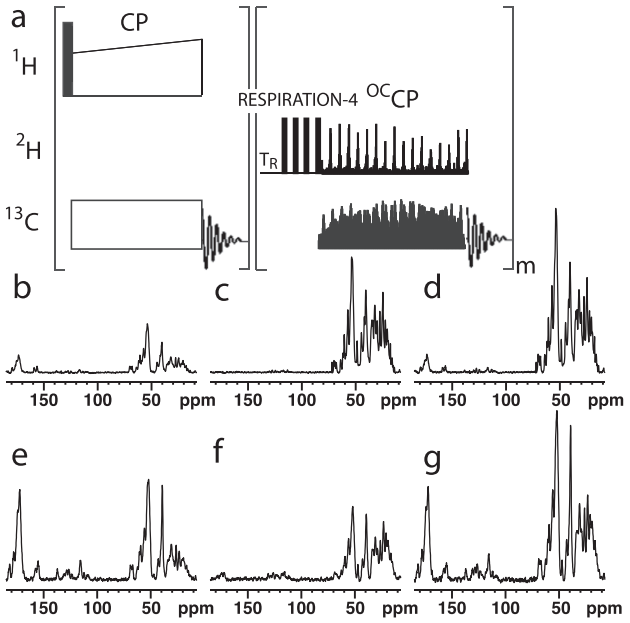

Fig. 1. (a) Schematic representation of the RAPID $^1H \rightarrow {}^{13}C/{}^2H \rightarrow {}^{13}C$ CP/MAS experiment with the left part representing the ^{1}H-based experiment, interleaved with m ^{2}H RESPIRATION-4 ^{OC}CP experiments [14] (right) (see text). (b and e) $^1H \rightarrow {}^{13}C$ CP/MAS and (c and f) $^2H \rightarrow {}^{13}C$ RESPIRATION-4 ^{OC}CP/MAS spectra extracted from a RAPID $^1H \rightarrow {}^{13}C/{}^2H \rightarrow {}^{13}C$ CP/MAS dataset recorded on (b–d) [30%–U–^{2}H/^{1}H, ^{13}C, ^{15}N]SH3 and (e–g) [100%–U–^{2}H/^{1}H, ^{13}C, ^{15}N]SH3. Both ^{1}H-based (b,e) and ^{2}H-based spectra (c,f) (scaled to the same noise level in the figure) are identical to individual experiments (each taking the same amount of time), however, here recorded in parallel within the time of the $^1H \rightarrow {}^{13}C$ CP/MAS experiment. (d and g) Sum of the two spectra in (b and c) and (e and f). The spectra in (b and c) are acquired using 256 scans, $t^H_{acq} = t^D_{acq} = 0.02$ s, $m = 16$, $T_R = 0.2$ s, corresponding to a repetition rate of $(0.2 + 0.02) * 16$ s = 3.52 s for the ^{1}H-polarization based experiment, while the spectra in (e and f) are aquired using 2048 scans, $t^H_{acq} = t^D_{acq} = 0.03$ s, $m = 8$, $T_R = 0.2$ s, corresponding to a repetition rate of $(0.2 + 0.03) * 8$ s = 1.84 s for the ^{1}H-polarization based experiment.

[100%–U–^{2}H/^{1}H, ^{13}C, ^{15}N]SH3) were recorded with the sample temperature adjusted to 5 °C. For the experiments at 20 kHz spinning, standard ramped CP/MAS experiments were used for $^1H \rightarrow {}^{13}C$ transfer (3.5/2.5 ms mixing time, constant amplitude rf of 55 kHz on ^{13}C, and rf with a 80–100% linear ramp on ^{1}H with average amplitude 35 kHz). RESPIRATION-4 [14] (rf amplitude of 45 kHz) and ^{OC}CP [14] (800 μs mixing time, peak amplitude of 50/50 kHz and average amplitude (RMS power) 11.6/28.7 kHz were used for ^{2}H/^{13}C). The experiments at 10 kHz spinning were carried out with 80–100% ramped $^1H \rightarrow {}^{13}C$ (average rf amplitudes of 35 kHz and 55 kHz) and $^2H \rightarrow {}^{13}C$ (average rf amplitudes of 55 kHz and 45 kHz, ^{2}H excitation using RESPIRATION-4) CP with 2.5 ms mixing times. For ^{13}C–^{13}C mixing in the experiments for Fig. 2, finite pulse RFDR (fp-RFDR) [23] was used with 5 ms mixing times. For the DONER mixing [24] in Fig. 3a, a mixing time of 50 ms was used with simultaneous irradiation on the ^{1}H and ^{2}H rf channels with amplitude 10 kHz. The interleaved 1D experiments (Fig. 1) were carried out in a 2D set up with two acquisition buffers (pseudo 2D experiment), and the two 1D spectra were processed individually. Interleaved 2D spectra (Figs. 2 and 3) were acquired in a pseudo 3D mode with two acquisition buffers. The [100%–U–^{2}H/^{1}H, ^{13}C, ^{15}N]SH3 and [30%–U–^{2}H/^{1}H, ^{13}C, ^{15}N]SH3 samples were produced as described in Ref. [8]. We note the samples were not doped by paramagnetic ions.

3. Results and discussion

Aimed at exploiting the rich source of polarization and information intrinsic in the fast relaxing ^{2}H spin bath, we propose here an experimental protocol resembling recent NMR multiple-acquisition methods. Here, however, we can confine ourselves to single-receiver detection and operation without gradients. Multiple-acquisition methods have previously been implemented using pulsed field gradients to facilitate acquisition of multiple-dimensional spectra slice-wise in one scan [25,26] or using multiple-receivers to simultaneously acquire multiple spectra in parallel [27,28]. Addressing studies of extensively deuterated proteins, we here exploit the widely different longitudinal relaxation of ^{1}H and ^{2}H spins in proteins to establish multiple-acquisition schemes. In these $^1H \rightarrow {}^{13}C$ and $^2H \rightarrow {}^{13}C$ cross-polarization (CP) magic-angle-spinning (MAS) based experiments are interleaved to harvest polarization from both ^{1}H and ^{2}H spins within the same overall time as a standard ^{1}H or $^2H \rightarrow {}^{13}C$ based experiment. While typical ^{1}H T_1's may be in the order of seconds, ^{2}H T_1's are often 2–3 orders of magnitude smaller with their absolute values depending highly on the level of deuteration as recently reported for ^{1}H in α-spectrin SH3 by Akbey et al. [8] We note that the long T_1's for protons have motivated the use of paramagnetic doping [29–31] to facilitate faster repetition rates, which is also enabled by extensive deuteration, since it removes the need for high-power ^{1}H decoupling. We should note, that use of faster repetition rates has also been explored in the RELOAD experiment, where different ^{13}C T_1 relaxation rates were exploited through selective rf irradiation [32].

A representative 1D variant of our so-called RAPID (Relaxation-optimized Acquisition of Proton Interleaved with Deuterium) $^1H \rightarrow {}^{13}C/{}^2H \rightarrow {}^{13}C$ CP/MAS experiment is shown in Fig. 1a. Using standard protocols, the $^1H \rightarrow {}^{13}C$ CP/MAS experiment (left part) is conducted with the waiting time adjusted to allow for reasonable repolarization of the ^{1}H spins and recorded with a sufficient number of scans to obtain a decent signal-to-noise-ratio. In the RAPID approach this waiting time is filled with a number (m) of $^2H \rightarrow {}^{13}C$ CP/MAS experiments, here implemented using a RESPIRATION-4 pulse for the initial ^{2}H excitation and an optimal control ^{OC}CP/MAS element for $^2H \rightarrow {}^{13}C$ cross-polarization [14]. The ^{13}C spectra of [30%–U–^{2}H/^{1}H, ^{13}C, ^{15}N]SH3 shown in Fig. 1b and c, reflecting coherence transfer from ^{1}H and ^{2}H, respectively, were acquired using the RAPID $^1H \rightarrow {}^{13}C/{}^2H \rightarrow {}^{13}C$ CP/MAS pulse sequence in Fig. 1a with $T_R = 200$ ms, $t^H_{acq} = t^D_{acq} = 20$ ms, and $m = 16$ corresponding to approximately 3.5 s repetition delay for the ^{1}H experiments. Similar spectra of [100%–U–^{2}H/^{1}H, ^{13}C, ^{15}N]SH3 are shown in Fig. 1e and f with $T_R = 200$ ms, $t^H_{acq} = t^D_{acq} = 30$ ms, and $m = 8$ corresponding to approximately 2 s waiting time between every ^{1}H based scan in accordance with the faster ^{1}H relaxation time of [100%–U–^{2}H/^{1}H, ^{13}C, ^{15}N]SH3 compared to [30%–U–^{2}H/^{1}H, ^{13}C, ^{15}N]SH3. The spectra in Fig. 1b and e are identical to the standard $^1H \rightarrow {}^{13}C$ CP/MAS spectra, while the ones in Fig. 1c and f are identical to the standard $^2H \rightarrow {}^{13}C$ RESPIRATION-4 ^{OC}CP/MAS spectra (note this experiment in itself is up to an order of magnitude more sensitive than the standard $^2H \rightarrow {}^{13}C$ CP/MAS experiment as demonstrated recently [14]), here with the ^{1}H- and ^{2}H-based spectra recorded in parallel using the time of one of them. This implies that the spectrum in Fig. 1c (and 1f) may be considered a gain in sensitivity or a source to complementary information exploiting an alternative mixing scheme (long-range vs. short-range) or selecting potentially different locations in the protein by exciting via ^{2}H instead of ^{1}H. In the former case the two spectra should be added upon mutual scaling to have the same noise figure (i.e., factors of 1 and $m^{-1/2}$ for ^{1}H- and ^{2}H-polarization based experiments with similar signal intensities, respectively) leading to the spectra in Fig. 1d and g. For the [30%–U–^{2}H/^{1}H, ^{13}C, ^{15}N]SH3 sample the $^2H \rightarrow {}^{13}C$ CP/MAS spectrum (Fig. 1c) shows superior sensitivity for the aliphatic carbons compared to the $^1H \rightarrow {}^{13}C$ CP/MAS spectrum (Fig. 1b), with the most pronounced gains for signals from carbons furthest away from the back-exchanged protons. Naturally, the relative gain in sensitivity for the ^{2}H-polarization-based spectrum is less pronounced in a

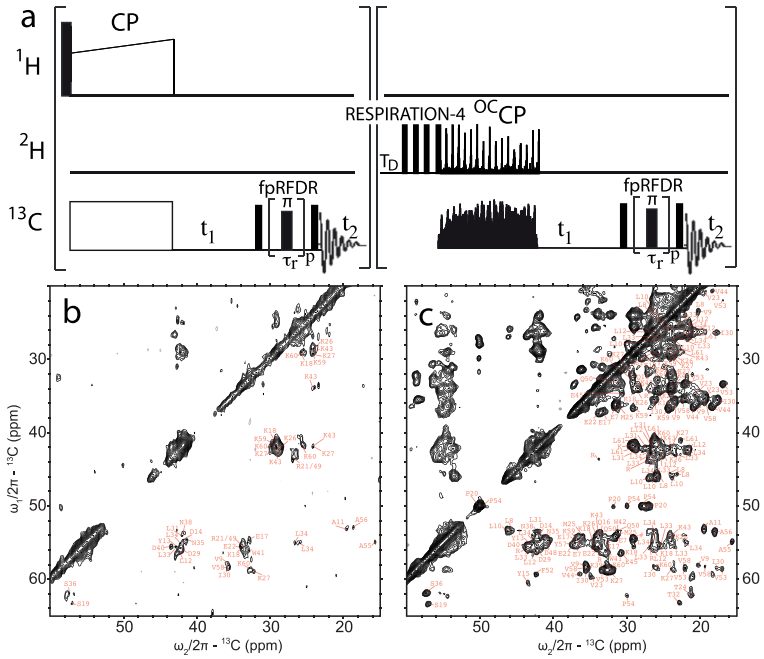

Fig. 2. (a) RAPID ^{1}H → ^{13}C/^{2}H → ^{13}C CP/MAS 2D ^{13}C–^{13}C correlation experiments interleaving two 2D pulse sequences with polarization originating from ^{1}H (left part; based on standard ramped CP) and ^{2}H (right part; based on RESPIRATION-4 ^{OC}CP [14]). Finite pulse RFDR (fpRFDR) [23] is used for ^{13}C–^{13}C mixing. (b and c) Representative spectra obtained for [30%–U–^{2}H/^{1}H, ^{13}C, ^{15}N]SH3 with 30% back-exchange of ^{1}H on labile sites initialized with ^{1}H → ^{13}C (b) and ^{2}H → ^{13}C (c) ^{OC}CP. Assignments are taken from Ref. [35], # indicates L61, K59, Q16, L31. Note the ^{1}H polarization based spectrum (b) is seen to exhibit signals mainly below the diagonal due to excitation of carbons closest to functional groups with labile protons that display higher chemical shift than the ^{13}C being transferred to, with exception of the ^{13}CO. The spectra are acquired using 16 scans, $t_2^{max.H} = t_2^{max.D} = 0.03$ s, $m = 16$, $T_R = 0.2$ s, 512 increments in the indirect dimension, $t_1^{max.H} = t_1^{max.D} = 15$ ms.

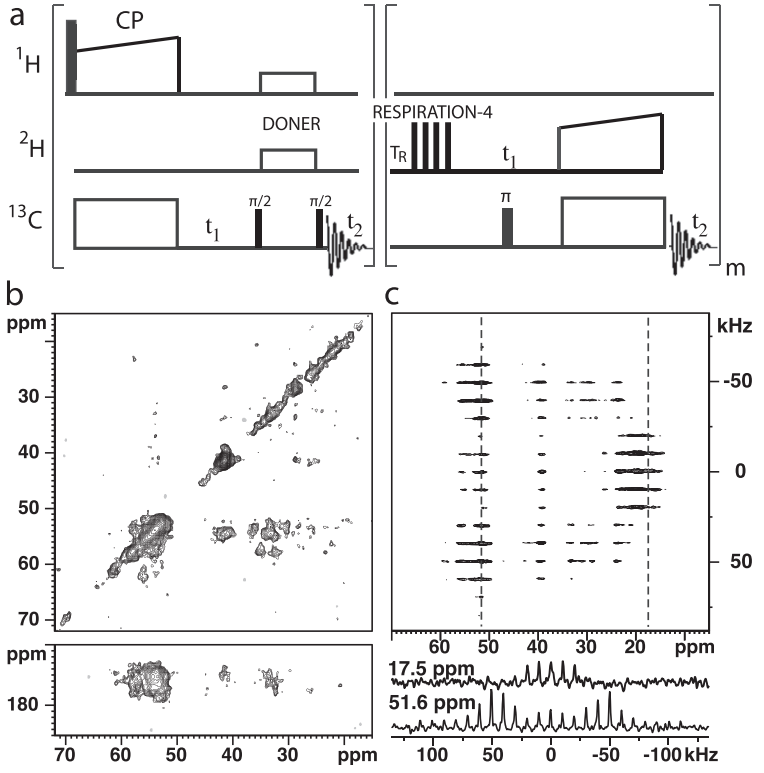

Fig. 3. (a) 2D RAPID experiment with interleaved sampling of a ^{1}H → ^{13}C CP/MAS ^{13}C–^{13}C DONER correlation spectrum and a ^{2}H → ^{13}C RESPIRATION-4 CP/MAS ^{2}H single-quantum spinning sideband vs ^{13}C chemical shift correlated spectrum. (b and c) Experimental 2D ^{13}C–^{13}C (b) and ^{2}H–^{13}C (c) subspectra recorded using the pulse sequence in (a) using 10 kHz sample spinning for [100%–U–^{2}H/^{1}H, ^{13}C, ^{15}N]SH3. The spectra are acquired using 440 scans, $t_2^{max.H} = t_2^{max.D} = 0.03$ s, $m = 8$, $T_R = 0.2$ s, 500 increments in the indirect dimension, $t_1^{max.H} = 12.4$ ms, $t_1^{max.D} = 0.744$ ms.

100% ^{1}H back exchanged sample where the signal-to-noise ratios for ^{1}H- and ^{2}H-polarization based spectra are comparable (noting that the spectra are recorded with different number of scans but using the same time) as seen in Fig. 1e and f. No ^{1}H decoupling

has been used. For these extensively deuterated samples this would only give a very modest improvement in resolution [24], while significantly increasing the rf duty cycle. Likewise, no ^{2}H decoupling was applied with the argument that dipole–dipole couplings are efficiently averaged out by MAS and decoupling of the ^{2}H–^{13}C scalar decoupling will only have an effect in very high resolution spectra [24,33]. We should note that depending on the applied polarization transfer method deuterium isotope effects may influence the ^{13}C resonance positions (and lead to multiple patterns due to presence of different $^{13}C(^1H)_n(^2H)_m$ isotopomers) in ^{1}H- and ^{2}H-polarization based experiments differently [9,15,33], in which case one may consider shifting the spectra mutually prior to addition. In the present case we did not shift the spectra as the use of long-range ^{1}H → ^{13}C CP polarized all ^{13}C spins with high efficiency.

Fig. 2 illustrates the application of RAPID to obtain 2D ^{13}C–^{13}C correlated MAS fpRFDR NMR spectra of [30%–U–^{2}H/^{1}H, ^{13}C, ^{15}N]SH3 exploiting original polarization from ^{1}H as well as ^{2}H. The two polarization sources were exploited simultaneously using the pulse sequence in Fig. 2a, interleaving experiments initialized with ^{1}H → ^{13}C CP (left) and ^{2}H → ^{13}C RESPIRATION-4 ^{OC}CP (right) to obtain two individual 2D spectra, where the polarization also can be added to obtain a sensitivity-enhanced 2D spectrum taking polarization from both ^{1}H and ^{2}H (not shown). The spectra were sampled using $m = 16$ interleaved ^{2}H → ^{13}C CP recordings ($T_R = 200$ ms, $t^H_{acq} = t^D_{acq} = 20$ ms) for each ^{1}H → ^{13}C CP recording hereby filling up the 3.5 s relaxation delay of the latter experiments.

Comparing the two 2D spectra in Fig. 2 reveals the much better sensitivity in the experiment based on ^{2}H polarization for the [30%–U–^{2}H/^{1}H, ^{13}C, ^{15}N]SH3 sample, underlining the enormous potential in the exploitation of the ^{2}H polarization, which has become possible through the introduction of the RESPIRATION and ^{OC}CP techniques. Using the [100%–U–^{2}H/^{1}H, ^{13}C, ^{15}N]SH3 sample, the signal-to-noise are in the two 2D spectra ratios comparable (data not shown) as implied from the 1D spectra in Fig. 1e and f. Simultaneous recording of these two types of spectra open the possibility to exploit the differences in the intensities/occurrence of ^{1}H-polarization based ^{13}C–^{13}C cross-peaks relative to the ^{2}H-polarization based ones for assignment purposes. For instance signals from Pro, lacking a backbone amide proton, will only arise in the ^{2}H-polarization based experiment, as seen by the lack of peaks around 50 ppm in the ^{1}H-polarization based experiment (Fig. 2b). Finally, one may envisage that different degrees of hydrophobic packing may affect ^{2}H–^{1}H exchange and thereby be discriminated through comparison of ^{1}H- and ^{2}H-polarization based spectra. Such discrimination comes in addition to the possibility to add the spectra (potentially after correction for deuterium isotope shift effects) to obtain a significant sensitivity gain as discussed already in relation to the spectra in Fig. 1d and g. If this is the primary aim, and deuterium isotope shift effects is not a an issue, we note that the left-hand side experiment in Fig. 2a may be replaced by the recent triple-resonance cross-polarization experiment by Akbey et al. [15]. We should also note that it is not possible to add ^{1}H → ^{13}C CP in the fast repeated experiment (Figs. 1a and 2a, right-hand side) as this will interfere with the build up of ^{1}H polarization during the train of ^{2}H → ^{13}C CP polarization transfer experiments.

Fig. 3 illustrates a RAPID experiment for [100%–U–^{2}H/^{1}H, ^{13}C, ^{15}N]SH3 using the ^{1}H-polarization to generate a ^{13}C–^{13}C correlated 2D spectrum while using the ^{2}H-polarization to generate a spectrum showing ^{2}H single-quantum quadrupolar coupling induced spinning sidebands correlated with ^{13}C chemical shifts. In the former spectrum, the ^{13}C–^{13}C mixing is accomplished by the rf-mediated spin-diffusion DONER experiment involving irradiation on both the ^{1}H and ^{2}H rf channels [24,34]. The combined sampling of these two 2D spectra ($m = 8$, $T_R = 200$ ms, $t_2^{\max,H} = t_2^{\max,D} = 30$ ms) in a single experiment may provide important information about ^{13}C and ^{2}H chemical shifts and long-range ^{13}C–^{13}C correlations (as opposed to the "one- and two-bond" transfers in Fig. 2) for assignment purposes as well as dynamics measurements through the ^{2}H spinning sidebands [16,17,22]. The ^{13}C–^{13}C spectrum in Fig. 3a is the same type as those in Fig. 2, however with potential additional long-range cross-peaks. The well-resolved spinning sideband patterns in Fig. 3b, here acquired using a spinning frequency of 10 kHz to maintain sufficient anisotropic information, may be fitted to obtain information about the quadrupolar coupling and dynamics at the local site of the involved deuterons. Also in this case, one could envisage use of different pulse sequence components, as for example replacement of the right-hand pulse sequence with a ^{2}H double-quantum (2Q) excitation scheme, e.g. implemented using RESPIRATION pulses [14]. This would provide high-resolution information about ^{2}H chemical shifts through 2Q frequencies [13] rather than the less resolved information from single-quantum frequencies.

In conclusion, we have demonstrated that multiple-acquisition spectra recorded on standard solid-state NMR instrumentation without pulsed-field gradients or multiple-receivers may provide substantial sensitivity gains or increased spectral information. The so-called RAPID experiments rely on polarization from different spin reservoirs characterized by different T_1 relaxation times. A prominent example is solid-state NMR of extensively deuterated proteins with partial back-exchange of labile deuterons to protons or proteins expressed with small amounts of protons. In this case recording of ^{1}H-polarization based spectra supplemented with efficient exploitation of polarization from the highly abundant ^{2}H spins may represent a rich source of information which can be gained without any cost just through appropriate interleaving of experiments. We have demonstrated that identical experiments just exploiting polarization from different sources may be added to improve sensitivity or may be used separately exploiting the different position of ^{1}H and ^{2}H spins to provide additional structural information. Alternatively the experiments may be of different nature, to complement chemical shift/assignment information with information about, e.g., protein dynamics. We note that we restricted ourselves to interleaved acquisition of two different spectra with the time of one experiment, but obviously one could easily interleave more experiments, e.g., having one ^{1}H-polarization based experiment (long T_1) interleaved with two or more different ^{2}H-polarization based experiments (short T_1). We envisage that such combined experiments will find immediate application in the heavily expanding studies of deuterated samples in solid-state NMR of proteins and polymeric systems.

Acknowledgments

We acknowledge support from the Danish National Research Foundation, The Danish Center for Scientific Computing, and the BIONMR FP7 Project.

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