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Higher Sensitivity through Selective ^{13}C Excitation in Solid-State NMR Spectroscopy

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In solid-state NMR (ssNMR) spectroscopy, it has become feasible to determine the structures of small crystalline proteins (<100 residues) at atomic resolution. This is an important development for structural biology, as it potentially makes accessible many macromolecular systems that have proven to be unsuited to diffraction methods or solution-state NMR analysis. However, while the first successful ssNMR studies of membrane proteins and amyloid samples have indeed recently been reported,[1] many protein systems of interest are still difficult or impossible to tackle because of the low intrinsic sensitivity of ssNMR.[2] Long recycle delays (~2−4 s) are required for the spin systems to return to equilibrium after each scan, and in current multidimensional ssNMR schemes designed for biomolecules, over 95% of the spectrometer time consists only of idling delays. Methods for making ssNMR faster, i.e. for improving the signal-to-noise ratio per unit time, are clearly a priority.[2] Considerable research effort is being invested in strategies to maximize the experimental efficiency, e.g. by using nonlinear sampling schemes[3] or band-selective excitation[4] to reduce sampling rates of multidimensional experiments or by using alternative signal-processing options.[5−7] Other attempts involve enhancing either the intrinsic longitudinal relaxation of the sample with paramagnetic compounds[8−12] or the signal intensity using dynamic nuclear polarization.[13]

Here, we introduce a time-saving ssNMR experiment using band-selective excitation of ^{13}C nuclei in a spin network. The experimental idea is based on the spin-temperature difference between a large pool of nonexcited spins and a number of excited spins. For these pools of ^{13}C nuclei, proton-driven spin diffusion (PDSD) offers a conduit for magnetization exchange.[14] The result is an accelerated return to I_Z magnetization for the excited ^{13}C nuclei, with a rate constant that we will denote as T_1*, in order to distinguish it from T_1, which is reserved for longitudinal spin−lattice relaxation. In such an experiment, the recycle delay time between successive scans may be reduced, leading to a higher rate of data acquisition. In the following, we will use the acronym RELOAD to denote the effect of *r*elaxation *e*nhancement by a *lo*wer temperature of *ad*jacent spins. This strategy is reminiscent of rapid-acquisition experiments found in solution-state NMR spectroscopy, such as SOFAST NMR[15,16] and COST-HSQC,[17] but is aimed at ^{13}C instead of ^{1}H nuclei.

In order to utilize RELOAD in the Hartmann−Hahn cross-polarization (CP) experiment depicted in Figure 1a, changes are suggested as shown in Figure 1b: CP is used to enhance ^{13}C magnetization in a uniformly labeled sample. A ^{13}C 90° pulse then stores ^{13}C transverse magnetization in the longitudinal state, and after a selective ^{13}C 90° readout pulse is applied, the first free-induction decay (FID) is recorded. As explained above, selectively excited nuclei return to I_Z magnetization much more quickly via PDSD, which is now initiated simply by switching off ^{1}H decoupling for a period d_{REL}, in order to prepare the spins for another round of selective excitation/measurement.

For ^{13}C CP ssNMR experiments (Figure 1a), the limiting factor preventing fast data acquisition is the ^{1}H spin−lattice relaxation time. Whether a RELOAD experiment is associated with an improvement

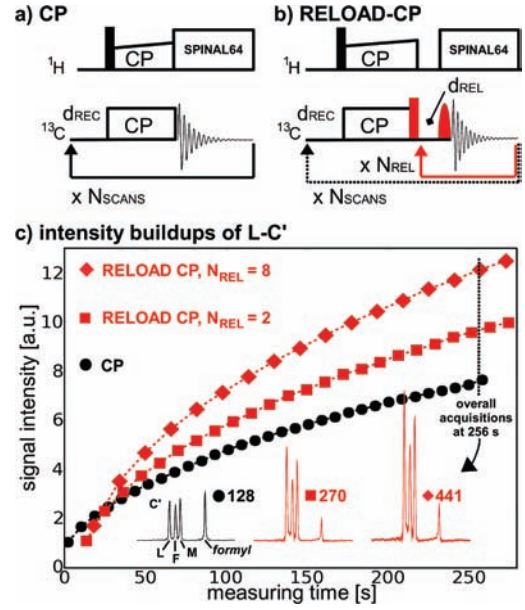

Figure 1. To take advantage of RELOAD, the CP pulse sequence in (a) is modified as indicated in red in (b). A higher acquisition rate of RELOAD−CP (N_{REL} indicated in the figure) leads to stronger signals in the same amount of time, as shown in (c) for L-C′ in [U-^{13}C]MLF, acquired with d_{REC} = 2 s and d_{REL} = 0.4 s. For comparison, the C′ peaks of [U-^{13}C]MLF after an acquisition time of 256 s are also depicted. The different intensities of the formyl signal are caused by the selective pulse excitation profile (for further experimental details, see the Supporting Information).

in signal-to-noise ratio per unit time depends on the ratio of the ^{1}H spin−lattice relaxation rate, which determines d_{REC}, and the ^{13}C magnetization recovery rate T_1*, which determines d_{REL}. While d_{REC} is usually on the order of seconds, inversion recovery experiments show that selectively excited ^{13}C spins return to I_Z magnetization ~100 times faster. For example, the C′ nuclei of [U-^{13}C]MLF (MLF = Met-Leu-Phe-OH) exhibit longitudinal relaxation rates (in s) of 1.34(5) (M), 1.78(2) (L), and 1.62(7) (F), whereas selective excitation leads to respective values of only 0.0117(4), 0.0106(4), and 0.0114(5) (for details, see the Supporting Information). Consequently, a single CP scan associated with a recycle delay time d_{REC} of 1.0−3.0 s may be replaced by a higher number of N_{REL} acquisitions, typically using delays d_{REL} of 0.1−0.3 s.

For RELOAD−CP, the higher acquisition rates lead to an enhanced signal intensity buildup, as depicted in Figure 1c for L-C′ in [U-^{13}C]MLF. N_{REL} transients are acquired before insertion of a recycle delay d_{REC}, and the overall number of acquisitions of a RELOAD experiment is then given by the number of scans N_{SCANS} multiplied by the number of RELOAD cycles N_{REL}. In Figure 1c, after 256 s, the RELOAD-CP experiments reached acquisition numbers of 270 (N_{REL} = 2, d_{REL} = 400 ms) and 441 (N_{REL} = 8, d_{REL} = 400 ms), while the CP experiment achieved a lower number of 128. For comparison, the C′ peaks for the CP and RELOAD−CP experiments

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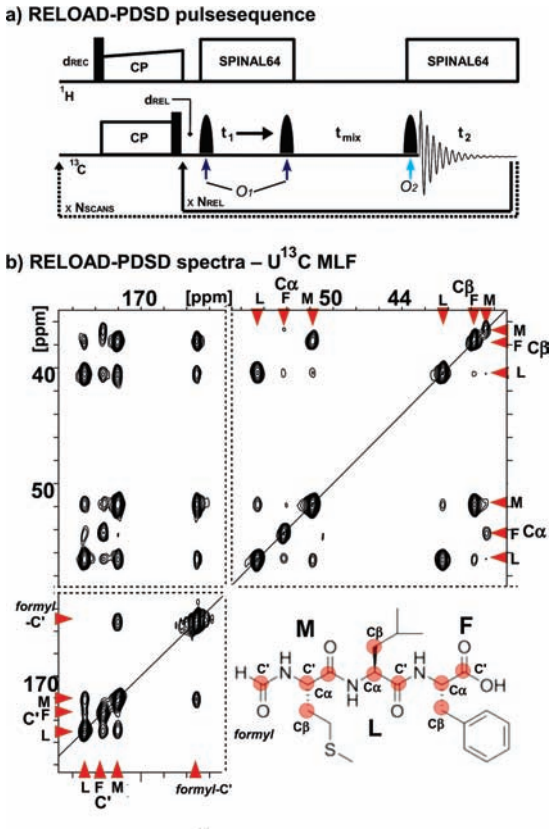

Figure 2. RELOAD in 2D ^{13}C-PDSD ssNMR experiments. (a) RELOAD−PDSD pulse sequence. (b) Homonuclear ^{13}C backbone correlations and a cartoon of [U-^{13}C]MLF. Three RELOAD−PDSD spectra were acquired separately, with $d_{REC} = 2$ s, $d_{REL} = 0.2$ s, $t_{mix} = 0.12$ s, $N_{REC} = 8$, $N_{SCANS} = 16$ (for further experimental details, see the text and the Supporting Information). Assignments are indicated in the spectra (labels and red arrows) and on the cartoon structure (labels and red circles).

after an acquisition time of 256 s are depicted at the bottom of Figure 1c. For a RELOAD cycle number $N_{REL} = 8$, the signal has about twice the intensity of the conventional CP experiment, which translates into a 4-fold reduction of measuring time.

The maximum consecutive number N_{REL} of such "RELOAD cycles" is ultimately limited by sample heating effects from ^{1}H decoupling during acquisition. It is worth noting that new "E-free" probe technology is commercially available, and heating has proven to be negligible in preliminary RELOAD experiments with an "E-free" NMR probehead. Furthermore, high-speed magic-angle spinning also allows lower proton decoupling.[18]

As an example of RELOAD in 2D ssNMR, Figure 2a depicts the pulse sequence of a 2D RELOAD−PDSD experiment: RELOAD−CP is followed by selective initiation of chemical-shift evolution and sampling of the FID in the indirect dimension (t_1, offset O_1). A selective readout pulse samples the FID in the direct dimension (t_2, offset O_2). As indicated in Figure 2a, N_{REL} rapid acquisitions are carried out, for which proton decoupling is switched off for the duration of the delay d_{REL}. The homonuclear correlations of the backbone C′, C$^\alpha$, and C$^\beta$ nuclei of [U-^{13}C]MLF are depicted in Figure 2b as a mosaic of three RELOAD−PDSD spectra, together with peak assignments and a cartoon of [U-^{13}C]MLF (backbone nuclei are indicated). For all three experiments, all of the parameters except the offsets O_1 and O_2 were identical: the overall number of scans per increment was 128 ($N_{SCANS} = 16$, $N_{REL} = 8$), and the spectral width and number of increments in t_1 were 35 ppm and 64, respectively, yielding an FID resolution of 118 Hz. With a recycle delay of $d_{REC} = 2$ s and a RELOAD delay of $d_{REL} = 0.2$ s, the measuring time for each spectrum was 0.97 h, leading to an overall measuring time of ∼3 h for the complete mosaic (for more details, see the Supporting Information). In comparison, a full PDSD spectrum with the same number of acquisitions and an FID resolution of 125 Hz (sweep width 300 ppm, 512 increments) would take 36.4 h to complete. This dramatic reduction in measuring time may be explained by two factors. First, time is saved by using a smaller number of increments in t_1, as has already been demonstrated elsewhere;[4] this reduces the measuring time to 4.55 h. Second, RELOAD reduces this time to 0.97 h by acquiring $N_{REL} = 8$ RELOAD cycles with every one of the $N_{REC} = 16$ scans, in which the delay $d_{REC} = 2$ s is replaced by $d_{REL} = 0.2$ s.

In summary, we have introduced a strategy for increasing the sensitivity of ssNMR experiments. The maximum possible RELOAD enhancement depends on a variety of parameters, such as the number of RELOAD cycles N_{REL} per recycle delay d_{REC} and the length of the RELOAD delay d_{REL}. In addition, the type of selective-excitation scheme, the temperature dependence, the sample size, and the ^{13}C labeling scheme play a role and will be discussed in detail elsewhere. We have demonstrated the first examples of 2D RELOAD ssNMR experiments with ^{13}C homonuclear correlation spectra, in which the measuring time was reduced from 36 to 3 h. This approach does not depend on sample or hardware modifications and is applicable to several ssNMR experiments, specifically for backbone ^{13}C nuclei in magnetization transfer experiments designed for sequential peak assignments.[19] We believe that the ssNMR RELOAD approach should be of major interest to structural biologists involved in the study of macromolecular complexes.

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Supporting Information Available: Details of inversion recovery and of RELOAD−PDSD experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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