NMR Spectroscopy

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Multiple Parallel 2D NMR Acquisitions in a Single Scan**

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Parallel receiving technologies have recently crossed the boundary separating magnetic resonance imaging from nuclear magnetic resonance (NMR) spectroscopy. In the latter case they promise significant time-saving advantages, by enabling the detection of multiple spectra simultaneously rather than in series. Moreover the full compatibility of parallel receiving with all other advances of contemporary NMR spectroscopy promises to open even further synergies in terms of speed and analytical capabilities. The present study shows one such instance, whereby the combination of parallel receiving multinuclear technologies is made with gradient-based spatial encoding methods, to yield multiple multidimensional NMR spectra in a single scan. The potential of this combination is demonstrated by the parallel acquisition of 2D ^{1}H–^{1}H and ^{1}H–S correlation spectra involving different S nuclei (^{19}F, ^{31}P), within a single transient. Besides its potential conceptual message about what is nowadays within reach of NMR spectroscopy, the ensuing two-dimensional parallel ultrafast NMR spectroscopy (2D PUFSY) experiment carries new opportunities for high-throughput analyses, chemical kinetics, and fast experiments on metastable hyperpolarized solutions.

Parallel receiving is an integral component of modern magnetic resonance; particularly in imaging applications where it can lead to substantial accelerating factors by scanning separate regions in space.[1–3] The advent of multiple receivers is also beginning to influence NMR spectroscopy technologies; not by providing spatial multiplexing, but rather by enabling the simultaneous acquisition of two or more signals arising from different nuclei.[4–11] Following the introduction of parallel NMR spectroscopy, it was shown that one of its main advantages results from its use to collect two or more different kinds of multidimensional correlation experiments, within the time duration that would normally entail to collect a single spectrum.[4] This is the principle of parallel acquisition NMR spectroscopy (PANSY), which eventually evolved into more sophisticated pulse sequences capable of affording all the 2D correlation spectra necessary for a complete assignment of small molecules—within the timescale of the slowest experiment in the multiple set.[5–7] These "parallel acquisition NMR, all-in-one combination of experimental applications" (PANACEA) strategies, have since been extended to systems of various heteronuclei[8,9] and adapted to protein liquid-state[10,11] and solid-state NMR experiments.[12–14] While these multiple receiver techniques have demonstrated that substantial time savings are possible, they have still conformed to the classical means of indirect frequency encoding,[15,16] whereby a series of independent scans are charged with encoding in a step-wise manner the evolution of the F_1 indirect spectral domain. The incremented repetitions thus required to discretely sample the indirect time domain t_1 implies that, even if sufficient sensitivity is available, sampling considerations associated with the slowest of all experiments still dictate the execution of all remaining 2D acquisitions. It was recently shown that sparse sampling coupled to non-Fourier processing techniques can alleviate this constraint, and break the Nyquist criteria without sacrifices in resolution or spectral bandwidth.[17,18] Herein we present an alternative—and arguably ultimate—form of compressing multiple 2D experiments, involving their parallel implementation while following the spatially encoded protocol enabling the multiplexing of all the information involved in every indirect dimension,[19] in a single scan.

The spatiotemporal encoding principles underlying the acquisition of 2D NMR spectra/images in a single scan have been described elsewhere in detail,[20,21] and hence they are only briefly described and within the context of the parallelized experiments presented here. "Ultrafast" NMR spectroscopy is based on endowing different z positions within a sample, with the different degrees of chemical shift evolution that would normally be associated with differing t_1 values. If implemented in a one-to-one z–t_1 fashion, this spatiotemporal encoding leads to a linear spatial winding of the magnetizations/coherences [Eq. (1)],

$$M_{+}(z) = M_o \exp[iC\Omega_1(z - z_o)] \quad (1)$$

where Ω_1 is the indirect domain frequency being targeted and $C \approx t_1^{\max}/L$ is a constant defined by the overall duration $t_1^{\max}$ of the encoding process, and by the sample length L being encoded. During the acquisition time t_2 a gradient G_{acq} enables one to unravel these spatially encoded magnetization windings, leading to echoes positioned at acquisition wavenumbers $k = -C\Omega_1$. These echoes are equivalent, in essence, to the indirect domain F_1 spectrum. By oscillating G_{acq} one can then monitor these traces repeatedly as they evolve as

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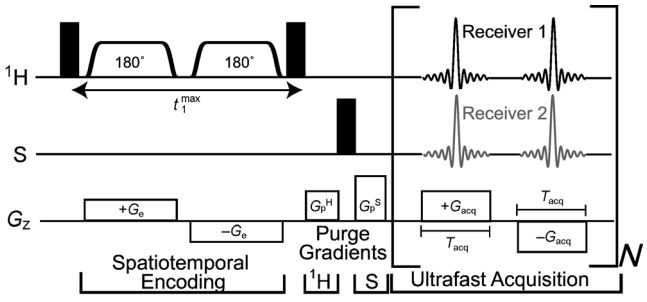

Figure 1. Pulse sequence for the simultaneous acquisition of ^{1}H–^{1}H and ^{1}H–S correlations; S denotes a generic heteronuclear spin. Solid rectangles represent hard 90° pulses, rounded trapezoidal shapes in the ^{1}H channel are 180° frequency-swept adiabatic inversion pulses. The ultrafast acquisition involves *N* cycles of alternating gradients that monitor the evolving F_1 traces in t_2.

a function of t_2, leading after 1D Fourier transformation (FT) against this variable to the full I(F_1,F_2) correlation being sought.

Figure 1 shows one of the manners by which these concepts were adapted to the simultaneous recording of two single-scan 2D NMR spectra using parallel receiving technologies. This version of parallel ultrafast 2D spectroscopy (PUFSY) aims to provide simultaneous homo- and heteronuclear *J*-based COSY correlations between neighboring sites[22–24]—in a single shot. As the homonuclear portion of this correlation requires preserving both the in- and the antiphase terms evolving during t_1, spin coherences such as $2H_{1z}H_{2\pm}$, $2H_{1\pm}H_{2z}$ and $2H_{\pm}S_z$, this calls for the use of a phase-modulated encoding. In the present case this was executed by using an initial hard 90° excitation ^{1}H pulse followed by a constant-time spatiotemporal encoding period involving adiabatic 180° sweeps imparted under the action of a bipolar $\pm G_e$ encoding gradient.[25] If followed by another pair of hard 90° read pulses acting on both the ^{1}H and the S heteronuclei, the longitudinal and transverse nature of the encoded spin coherences will be exchanged. This leads to the possibility of decoding both homo- and heteronuclear correlations, with the aid of an oscillating acquisition gradient G_{acq} and of signal acquisitions on both the I and S channels. A data set acquired on the proton receiver (e.g., #1) thus yields a classical ^{1}H–^{1}H COSY spectrum, whereas data acquired on receiver #2 operating at the S spin Larmor frequency, gives a ^{1}H–S heteronuclear correlation spectrum. As usual in this kind of experiments, pre- and post-mixing "purging" gradients flanking the read 90° pulses can be used to select a desired coherence transfer pathway and to position the indirect domain observation in the middle (i.e., $T_{acq}/2$) of each bipolar $\pm G_{acq}$ gradient.

Three evident limitations arise from this mode of operation. One concerns the identical encoding procedure that both correlation spectra will share; certain parameters such as the central offset of the indirect domain axes and/or the indirect domain spectral resolutions, will consequently also have to be common (this feature is shared by parallel acquisition experiments even when relying on conventional 2D encoding modes). A second constraint arises from the common train of oscillating acquisition gradients used to decode the indirect domain information. These amplitudes and durations define the spectral bandwidths that will characterize the F_1 and F_2 domains in both single-scan experiments collected; for the strategy illustrated in Figure 1 the latter will be $(2T_a)^{-1}$ regardless of the nucleus observed in the F_2 domain, whereas the former will be $SW1_{H,S} = |\gamma_{H,S} G_{acq} T_{acq}/(2\pi C)|$ for the homo/heteronuclear F_1 traces being correlated. The dual sharing of both the central offset and the bandwidth characteristics along F_1 proved too constraining for the acquisition of the desired spectra; to alleviate this, both nuclear species were allowed to experience different purging gradient pulses so as to shift their respective F_1 echo peaks to the center of the common acquisition gradient windows. A final constraint associated to the PUFSY acquisition mode (shared in general with PANSY experiments) is a lack of F_2 decoupling: irradiating either nucleus during the course of the acquisition, would null one of the F_2 shift dimensions being sought by the 2D experiments. All resulting spectra will therefore show both homo- and heteronuclear ^{1}H–S couplings.

With these considerations as background, Figure 2 shows the spectra acquired for two different fluorine-containing samples. The pulse sequence shown in Figure 1 was used, with ^{19}F taken as the S nucleus and additional experimental parameters as given in the figure caption. The ^{1}H–^{1}H COSY spectra of 2,3,4-trifluoro cinnamic acid show two independent AX spin systems, although the downfield peaks of both systems overlap somewhat partially. The ^{1}H–^{19}F spectrum shows heteronuclear correlations at two different proton sites. The spectra shown in the bottom row of Figure 2 were acquired from a mixture of three different compounds. The assignments are provided on the spectra; it should be noted, however, that owing to the smaller spectral window used for the ^{19}F directly detected dimension, some of the peaks have experienced aliasing. Still, all peaks are very well resolved in the two dimensions, thus illustrating the suitability of PUFSY for analyzing mixtures at sub-second timescales for samples whose 1D spectra are too crowded to allow a meaningful analysis.

Figure 3 shows an additional example of this kind of acquisitions, with dual spectra acquired with the sequence in Figure 1 for adenosine triphosphate (ATP), taking ^{31}P as the S heteronucleus. Given the constraints imposed by the common processes mentioned earlier, these acquisitions focused solely on targeting the aliphatic region of the F1 domain; including as well the aromatic region would have been too onerous for the sensitivity achievable with this system. The ^{1}H–^{1}H COSY spectrum shows all of the homonuclear ^{1}H correlations in the aliphatic region arising from the five-member ring, whereas the ^{1}H–^{31}P spectrum shows two heteronuclear correlations between the alpha ^{31}P site on the triphosphate chain, and the two closest ^{1}H sites (5′ and 5″).

The present work demonstrates the kind of flexibility that has been reached by contemporary NMR hardware: not only can 2D NMR data be collected in a single scan, but multiple such correlations can be collected in either hetero- and/or homonuclear modes simultaneously. While this initial demonstration is only intended to convey feasibility principles, it is also clear that many additional, more elaborate experiments

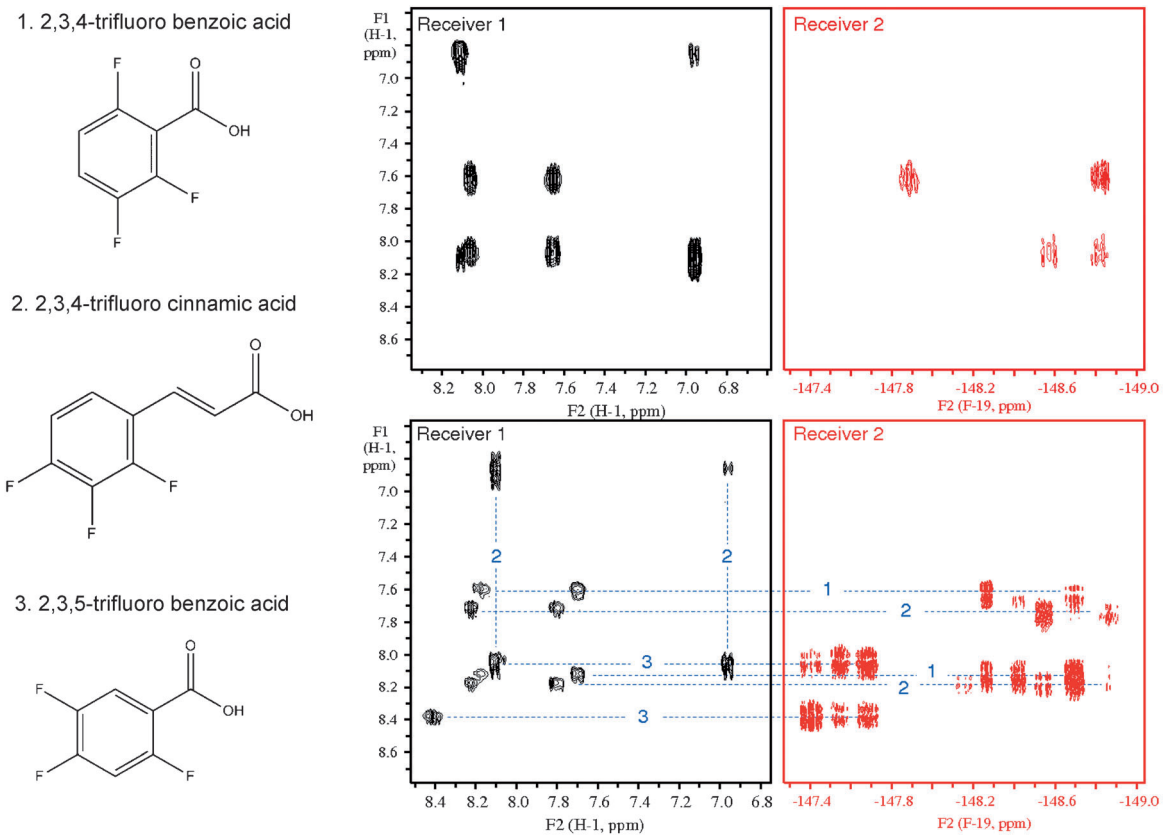

Figure 2. PUFSY COSY spectra for 2,3,4-trifluoro cinnamic acid (top), and for a mixture of 1) 2,3,6-trifluoro benzoic acid, 2) 2,3,4-trifluoro cinnamic acid, and 3) 2,4,5-trifluoro benzoic acid (bottom). ^{1}H–^{1}H correlation spectra are depicted in black and ^{1}H–^{19}F spectra are depicted in red, and they were both acquired in a single common transient. In these experiments the excitation gradient was $G_e = \pm 10\ G\,cm^{-1}$; a pair of 15 ms long chirp pulses calibrated to act as 180° inversion sweeps were used for encoding the indirect dimension, acquisition parameters included $G_{acq} = 14.9\ G\,cm^{-1}$, $T_{acq} = 488\ \mu s$ (top), and $T_{acq} = 490\ \mu s$ (bottom, mixture), and 200 *N* acquisition cycles (top) and 150 *N* acquisition cycles (bottom, mixture).

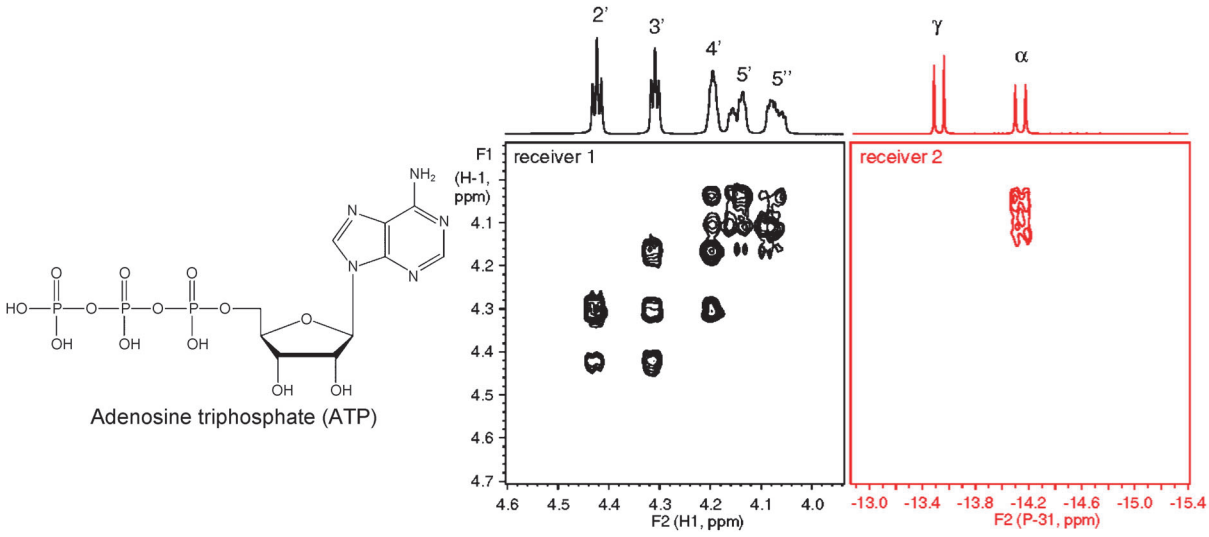

Figure 3. PUFSY spectra of ATP (10% in D_2O) acquired using the pulse sequence shown in Figure 1. The ^{1}H–^{1}H correlations are depicted in black and the ^{1}H–^{31}P spectrum is depicted in red. In addition, independently acquired 1D spectra are shown above the 2D spectra along the corresponding dimensions. These experiments used $G_{exc} = \pm 10\ G\,cm^{-1}$ excitation gradients; a pair of 30 ms long chirp pulses calibrated to act as 180° inversion sweeps, and acquisition parameters $G_{acq} = \pm 9.6\ G\,cm^{-1}$; $T_{acq} = 90\ \mu s$ and $N = 125$. 16 phase-cycled transients were averaged.

can be readily realized in this manner. A main limitation still afflicting the unprecedented speed that was presented here, stems from the fundamental sensitivity limitations that NMR spectroscopy faces vis-à-vis alternative analytical techniques. In this respect it is clear that the applicability of these sophisticated multiple-receiver parallelized-encoding techniques could improve with a number of upgrades to the hardware hereby employed; for example, by having done experiments at higher magnetic fields, or by using enhanced-sensitivity cryo-cooled probes and preamplifiers. Even more dramatic gains could result from the application of hyperpolarization procedures. In particular, the sudden-dissolution dynamic nuclear polarization experiment of Ardajkær-Larsen et al.[26] appears as a particularly good fit to highly parallelized, single-shot acquisition techniques like PUFSY.[27–33] Further investigations into expanding the flexibility of PUFSY and exploring its potential in thermally and hyperpolarized parallel acquisitions are in progress.

Experimental Section

All experiments were performed on a 600 MHz spectrometer with a direct drive console (Agilent Technologies, Santa Clara, CA) equipped with multiple receivers. The 2,3,4-trifluoro cinnamic acid sample reported in Figure 2 (top) had 5% concentration in $[D_6]$DMSO. The mixture sample reported in Figure 2 (bottom) had 5% concentration of each component in $[D_6]$DMSO. The ATP sample reported in Figure 3 had 10% concentration in D_2O. Additional information can be found in the Supporting Information.

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