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Communication: Ultrafast homonuclear correlation spectroscopy with diagonal suppression[a)]

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A novel ultrafast 2D NMR experiment is introduced for homonuclear correlation spectroscopy in solution state, with diagonal peak suppression in each scan of a two scan procedure. This experiment permits clear visualization of cross peaks between spins whose chemical shifts are very close, which could otherwise be masked by diagonal peaks. The present report describes the principles of its design and illustrates actual performance. © *2014 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4884385]

Nuclear magnetic resonance (NMR) has emerged as a powerful technique in physics, chemistry, materials science, biochemistry, and biology owing to its high resolution, which results in the ability to study molecular structure and dynamics in great detail. In particular, spin connectivity in molecules may be visualized handily by multi-dimensional NMR[1,2] not only in small molecules, but also in the case of biomolecules or mixtures of metabolites. 2D correlation spectroscopy (COSY), which allows visualization of coupled networks of homonuclear spin systems by driving coherent magnetization transfer among coupled spins, is among the most popular multi-dimensional (*n*D) NMR experiments.[2] *n*D NMR experiments are, however, generally time consuming when standard data acquisition strategies are employed, requiring multiple repetitions of the experiment with evolution time incrementation for the indirect dimensions. Several methods have been proposed in the last decade to speed up *n*D experiments significantly.[3–6] Frydman and co-workers proposed single scan or ultrafast (UF) *n*D NMR, which replaces parametric evolution time incrementation with spatial encoding and Echo Planar Imaging (EPI) type of acquisition.[7–10] Practical applications of the UF method have been demonstrated in several recent studies[11–13] including magnetic resonance imaging.[14] The UF implementation of 2D COSY has been employed to study dynamic processes,[15] as also for quantification of metabolites.[16]

Spin echo correlation spectroscopy (SECSY), which may be viewed as a delayed COSY experiment, generates information similar to that from COSY, but gives rise to mixed phase (or phase twisted) lineshapes. It is based on coherence transfer echo pathway selection, and acquisition of the signal from the echo top. SECSY has some advantages over COSY, and comes into its own especially in inhomogeneous media, and in the study of biological macromolecules,[17] as well as in *in vivo* applications.[18] An ultrafast version of SECSY has also been reported recently.[19,20] One of the interesting features of SECSY is that it requires a reduced spectral width in the indirect dimension compared to COSY, and hence smaller acquisition gradients[9] (G_a) may be employed in its UF version.

While mapping spin connectivity by way of coherence transfer is the focus of correlation spectroscopy, a major concern with COSY, SECSY, and their ultrafast variants however is that these experiments also give rise in general to peaks from magnetization components that have *not* been involved in any coherence transfer. Such spectral multiplets are centred at the same frequency in both dimensions in COSY, and at zero frequency in the virtual frequency dimension F_1 in SECSY. In a generalized sense we may call these peaks as "diagonal" peaks in both experiments. They are to be distinguished from peaks that could arise from longitudinal magnetization that is brought into the transverse plane by the second pulse, which are routinely eliminated however by standard procedures. These latter multiplets are centred at $F_1 = 0$ in COSY, but are centred in SECSY at an F_1 frequency that equals half the individual chemical shift, i.e., half the frequency in F_2. These peaks may in a generalized sense be called "axial" peaks in both experiments.

Here we propose and demonstrate a simple strategy that basically suppresses diagonal peaks in each scan in the ultrafast SECSY environment, and leads ultimately to a two scan procedure. This approach is in fact valid under any general conditions of limited resolution and short acquisition time in the directly detected dimension; such conditions are commonly encountered especially in volume localized Magnetic Resonance Spectroscopy (MRS), UF NMR, and Overhauser dynamic nuclear polarization (ODNP).

To put this in perspective, we recall that while axial peaks may be easily suppressed by suitable phase cycling (for example, by phase alternation of the first pulse together with the receiver phase), suppression of diagonal peaks, which could arise both from coupled spins, as well as from "isolated" spins (i.e., spins that are not coupled to others), has thus far required more elaborate strategies. Diagonal peaks could often obscure the more informative cross peaks, and indeed overlap between cross and diagonal peaks could be especially

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severe in UF experiments due to their typical linewidths, which are larger in both dimensions – and especially so in the indirect dimension – than in standard COSY or SECSY. Several approaches have been reported in the literature to suppress diagonal peaks in COSY spectra. Double quantum filtered COSY[21] is a popular approach to get a correlation spectrum with reduced diagonal peaks. This method, which has half the sensitivity of COSY, effectively suppresses diagonal peaks arising from isolated spins, but not those from coupled spins. Some other methods have been proposed for diagonal suppression, based on the subtraction of two spectra.[22,23] Typically, one spectrum is acquired with both cross and diagonal peaks, while a second is the spectrum with only diagonal peaks, acquired with a modified pulse sequence. Neglecting relaxation losses during the mixing time, the efficiency of diagonal suppression in such experiments depends on the reproducibility of the two different experiments, as well as the efficiency of the relevant additional modules, e.g., refocusing pulses[22] or the z-filter.[23] Diagonal peak suppression was also recently investigated with the help of spatially selective and frequency selective pulses:[24] the sequence uses a selective pulse combined with a weak field gradient to excite the sample. The magnetization that does not get transferred during the mixing time (and thus generates diagonal peaks) is suppressed with an excitation sculpting block before signal acquisition. The disadvantages of such an approach include the considerable loss of sensitivity owing to slice selective excitation, the dependence of the efficiency of diagonal suppression on the selectivity of the 180° pulse in the excitation sculpting block, and the suppression of cross peaks in the close vicinity of diagonal peaks as well.

In contrast, our present approach is a simple single scan strategy for diagonal suppression in the ultrafast SECSY environment, that leads ultimately to a two step phase cycle. We term our experiment UF-DISSECT (UltraFast DIagonal Suppressed Spin-Echo Correlation specTroscopy). The basic sequence is very similar to UF-SECSY except for an additional 90° pulse with specified phase just before the start of data acquisition (the "DISSECT pulse"). In UF-DISSECT, as shown in Fig. 1, this additional 90° pulse is used at the top of the coherence transfer echo.

We give below an expression for the observable part of the spin density matrix of a spin-1/2 AX system after the final DISSECT pulse, starting with the longitudinal magnetization of spin 1 (which stands for spin A). [Of course, terms originating from the longitudinal magnetization of spin 2, i.e., spin X, also contribute to the final density matrix, but these may be written down simply by substituting 1 with 2 and vice versa in the spin indices of Eq. (1).] The expression recognizes that the pulse sequence corresponds to a constant time evolution experiment, and incorporates the results of standard echo pathway selection, supplemented by phase alternation of the final DISSECT pulse with co-addition of signals:

$$I_{1z} \rightarrow \tfrac{1}{2}\left[\sin\left(\frac{(\omega_1-\omega_2)t_1}{2}\right)\sin^2\left(\frac{\pi J\Delta}{2}\right)I_{2y} - \sin\left(\frac{(\omega_1-\omega_2)t_1}{2}\right)\sin(\pi J\Delta)I_{1x}I_{2z}\right]. \quad (1)$$

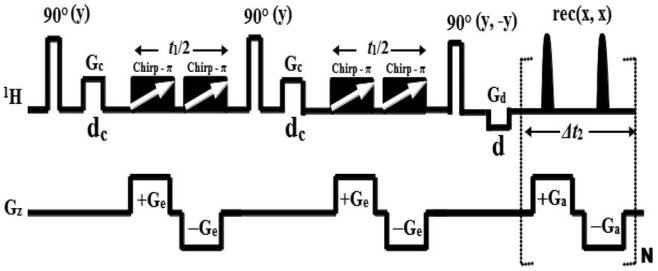

FIG. 1. Pulse sequence proposed and implemented for the acquisition of 2D UF-DISSECT spectra. The three narrow open rectangles on the ^{1}H channel are hard 90° pulses. Spatial encoding in the indirect dimension is achieved with four chirp pulses (i.e., pulses that are linearly frequency modulated or swept; marked with arrows), issued in the presence of gradients G_e in the z-direction. Two gradients (G_c) of equal amplitude and duration are used for coherence pathway selection. G_d is the purging gradient used just before the data acquisition to remove undesired residual magnetization and is adjusted to shift the centre of the chemical shift range to the centre of the sampling window of the indirect dimension.[25] Signal acquisition is performed under the oscillating gradient (G_a), which is repeated N times. Phase cycling is shown in the figure.

Here, $\Delta/2$ is essentially the fixed time interval between the first two, or the last two 90° pulses of the sequence; J evolution occurs essentially during the entire period Δ, while chemical shift evolution occurs for a variable duration (that depends on the spatial co-ordinate of each portion of the sample in the G_e gradient direction). In the following, we summarize the manner in which the UF-DISSECT sequence functions. The last 90° pulse in the DISSECT sequence converts in-phase as well as anti-phase magnetization components that are modulated by only the coupling ("diagonal peak" components) into longitudinal and multiple quantum terms, respectively, thereby suppressing the "diagonal" peaks in a single scan procedure. This last pulse also similarly renders unobservable other magnetization components that oscillate at higher frequencies related to shifts, if they have a single operator in phase quadrature to the pulse phase, while it leaves unmodified in-phase cross peak components that have the same phase as this pulse; finally, it effects further coherence transfer on anti-phase magnetization terms with both operator components orthogonal to the pulse phase. Longitudinal and multiple quantum terms present at the end of the t_1 period, which are converted to observable terms by the DISSECT pulse, are all easily suppressed with a two-step phase cycle without affecting the desired observable terms, simply by phase alternating the DISSECT pulse while keeping the receiver phase constant. The first observable term on the right hand side of Eq. (1) represents a cross-peak multiplet that is anti-phase in F_1, centred at half the chemical shift difference between spins 1 and 2 (i.e., spins A and X), while it is in-phase in F_2, centred at the shift of the "destination" spin 2. The second term on the other hand represents a cross-peak that is anti-phase in both dimensions, as well as being in phase quadrature to the first term in both F_1 and F_2; while it too is centred at half the chemical shift difference in F_1, it is centred in F_2 at the shift of the parent (or "source") spin 1: this second term results from the additional coherence transfer effected by the final DISSECT pulse. In larger clusters of coupled spins, combination single quantum coherences may also

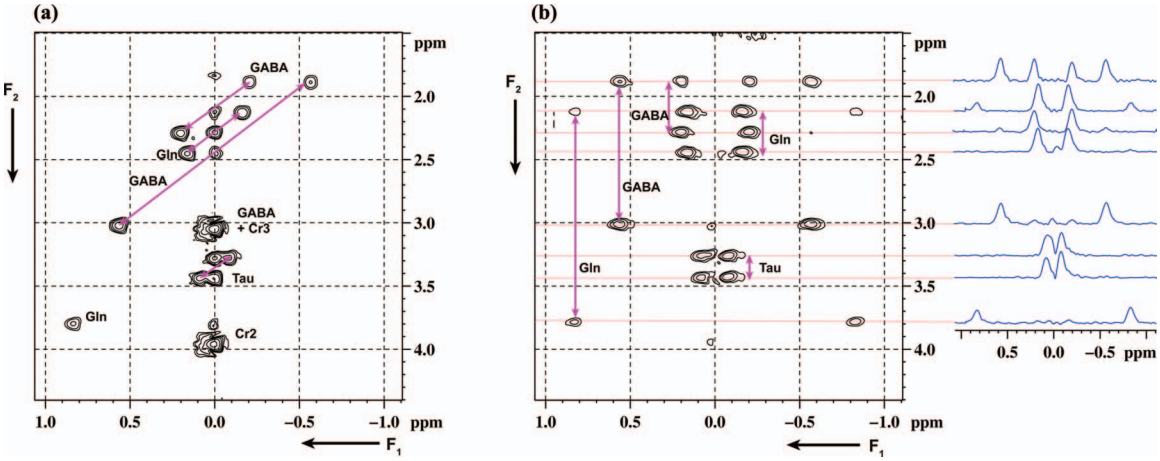

FIG. 2. 2D NMR spectrum of a metabolite mixture in D_2O (creatine, taurine, glutamine, and γ-amino butyric acid, 50 mM each) obtained with: (a) UF-SECSY and (b) UF-DISSECT. Both spectra were acquired with 16 scans in 39 s, other parameters: recycle delay = 2 s; N = 128; 7.5 ms chirp pulses (i.e., pulses that are linearly frequency modulated or swept), bandwidth BW = 40 kHz, $G_e = \pm 6.5$ G/cm, $G_c = 7.5$ G/cm, $d_c = 2$ ms, $G_d = 17.5$ G/cm, d = 270 μs, $G_a = \pm 36.56$ G/cm, $\Delta t_2 = 558.2$ μs. Metabolite cross-peaks are shown connected with arrows in both the spectra. Additional F_1 traces (in blue) are shown in the UF-DISSECT spectrum to highlight the efficiency of diagonal suppression. The cross-peak intensities in UF-SECSY are 1.9–2.2 times higher than in UF-DISSECT.

give rise to anti-phase transverse magnetization components (*vide* the supplementary material,[26] which includes an analytical treatment of the density matrix for spin-1/2 AX and AX_2 systems). It may be noted however that such terms which are anti-phase in F_2 are rendered essentially unobservable in experiments that involve large spectral linewidths and have correspondingly short acquisition times, as in ultrafast NMR, in solution state ODNP, and typically also in MRS even with standard acquisition mode; the typical linewidths in ultrafast NMR, for example, are in the range of 30–45 Hz and possibly even more in inhomogeneous media. From Eq. (1) it is clear that pure phase spectra may be obtained because of amplitude modulation of the signal in t_1 under UF, ODNP, and/or *in vivo* conditions; however, the cross-peak intensity would be reduced compared to that in SECSY. Simulations of DISSECT for larger spin systems at a number of field strengths show its general validity for diagonal suppression.[27]

Fig. 2(a) shows the magnitude mode 2D UF-SECSY spectrum of a mixture composed of biologically important metabolites like creatine (Cr), taurine (Tau), glutamine (Glu), and γ-amino butyric acid (GABA), 50 mM each in D_2O. Cross-peaks of all the metabolites are marked with arrows. The appearance of the UF-SECSY spectrum is as expected: cross peaks, which correlate scalar coupled spins, have a multiplet centred in F_1 at half the difference of their chemical shifts, while in F_2 they are centred at the respective shifts of the two coupled spins. On the other hand, "diagonal" peaks are centred at $F_1 = 0$, and at the respective shifts in F_2. Cr, with methyl protons at 3.02 ppm and methylene protons at 3.91 ppm, does not show any cross-peaks because there are no couplings in the spin system.

The 2D UF-DISSECT spectrum of the same sample is shown in Fig. 2(b), also in magnitude mode. Here, diagonal peaks are well suppressed, while cross peaks, which result from amplitude modulation in t_1, occur symmetrically around $F_1 = 0$ at half the difference in chemical shifts of the spins in question. It is noticeable that diagonal suppression in UF-DISSECT is excellent for both coupled and uncoupled spin systems, only a minor residual peak of Cr ($-CH_3$) being found near 3.02 ppm. Particular attention may be drawn to taurine, which shows cross peaks at F_2 frequencies of 3.24 ppm and 3.42 ppm, the F_1 frequency being ± 0.09 ppm. In UF-SECSY the two cross peaks of Tau overlap with the strong "diagonal" peaks due to the small chemical shift difference between the resonances, even at 500 MHz. UF-COSY shows similar behavior (see the spectrum included in the supplementary material[26]). In the UF-DISSECT spectrum on the other hand the suppression of diagonal peaks is entirely satisfactory and all four Tau cross-peaks are well resolved. The effect of amplitude modulation is also seen from the linewidth of the cross-peaks in the spectrum which, even in magnitude mode, is typically about 10%–15% less in UF-DISSECT compared to UF-SECSY.

It may be noted that for a two-spin-1/2 system precisely four peaks result in UF-COSY, UF-SECSY, as well as in UF-DISSECT. While two of the four are diagonal peaks in the first two cases, all four are cross-peaks in the latter case. As a measure of "spectral crowding" it may be noted that both for UF-COSY and UF-DISSECT, the area in the frequency plane enclosed by the four-peak pattern is the square of the chemical shift difference between the two spins, while for UF-SECSY it has only one half this value. The characteristic rectangular pattern of the four cross-peaks between every pair of coupled, chemically shifted spins in UF-DISSECT may be deemed an aid in identification in terms of pattern recognition; it may be noted however that suitable post-processing may in principle be applied to recover the standard SECSY cross-peak pattern if desired.

To further validate the diagonal suppression efficiency of our sequence, we also demonstrate the method on strychnine in $CDCl_3$. Fig. 3 shows the 2D UF-DISSECT spectrum of strychnine. In our study we have focused on the region of its spectrum from 1 ppm to 4.5 ppm. To avoid any aliasing or fold-over in F_2 we have replaced all hard 90° pulses

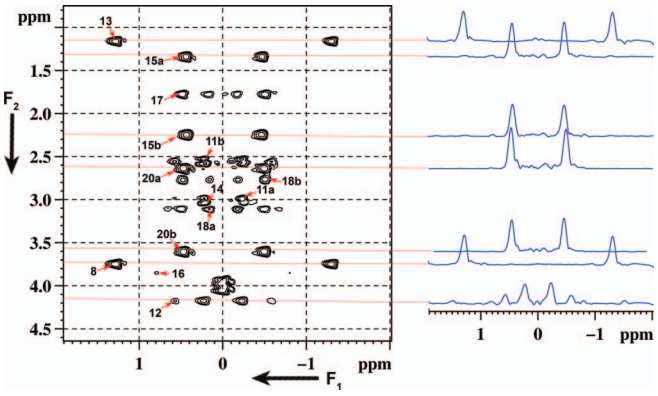

FIG. 3. 2D UF-DISSECT magnitude mode spectrum of strychnine (0.5 M) in $CDCl_3$ (reference 2D COSY with structure in the supplementary material[26]). All peaks are labeled according to the above structure. F_1 traces of few peaks are shown in blue. The spectrum is acquired with 8 scans in 22 s with a recycle delay of 2 s; N = 128, 7.5 ms chirp pulse with BW of 60 kHz, $G_e = \pm 5.5$ G/cm, $G_c = 7.5$ G/cm, $d_c = 2$ ms, $G_d = 17.5$ G/cm, d = 270 μs, $G_a = \pm 41.775$ G/cm, $\Delta t_2 = 477$ μs, three hard pulse are replaced by three selective Shinnar-Le Roux SLR pulses (5.314 ms and BW = 2 kHz).

in our sequence with selective 90° Shinnar-Le Roux (SLR) pulses (bandwidth = 2 kHz). The 2D UF-DISSECT spectrum shows complete diagonal suppression as confirmed from the F_1 traces, which are also shown in the figure. It may be noted that cross-peaks in the region 3.88–4.12 ppm (from ^{1}H 23a, b) lie very close together indeed on this extended F_1 range, and give the semblance of a diagonal peak.

In summary, we have presented a new experiment that generates ultrafast 2D spin echo correlation spectra with excellent suppression of diagonal peaks, thus making available cross-peak information of close lying chemically shifted peaks. Our experiments were implemented on a standard high resolution NMR spectrometer employing a standard 5 mm probe with z gradient. We have documented in this report the efficiency of suppression of diagonal peaks with two different samples, one of them a mixture of metabolites, and the other an alkaloid. We expect that the experiment will have wide general validity. We are currently exploring in our laboratory the adaptation of DISSECT to exchange spectroscopy,[28] both in standard and ultrafast modes; the results will be reported elsewhere.

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