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Ultrahigh-Resolution Total Correlation NMR Spectroscopy

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Supporting Information

ABSTRACT: Resolution and sensitivity are paramount for extracting detailed structural information using NMR spectroscopy. Recently developed "pure shift" techniques have greatly improved the resolution attainable in one- and two-dimensional NMR, but at a considerable cost in sensitivity. A newly introduced method, PSYCHE, greatly reduces this loss. It produces pure shift spectra with significantly improved sensitivity, spectral purity, and tolerance of strong coupling compared to previous methods. Here PSYCHE is applied to the TOCSY experiment. In combination with covariance processing, the result is a high-quality, high-resolution TOCSY spectrum with singlets in both dimensions: a pure chemical shift correlation map. Such spectra should greatly simplify both manual spectral analysis and automated structure elucidation.

Since the advent of 2D NMR spectroscopy,[1−3] new methods have continually been sought to improve the resolution and sensitivity of homonuclear correlation experiments. In such experiments, coupling interactions between nuclei split signals into multiplets, degrading both resolution and sensitivity. The additional dimension introduced in 2D NMR experiments like TOCSY and NOESY can reduce the impact of signal overlap, but in many applications it remains a significant problem, impeding reliable assignment of chemical sites. Although in principle the multiplet structure contains valuable information, in most 2D correlation experiments it is an unnecessary and unhelpful complication. Here an improved method for removing this complication is presented.

Multiplet structure can arise from both homonuclear and heteronuclear couplings. The latter are relatively rare in homonuclear correlation, and if necessary they can easily be suppressed by broadband irradiation during acquisition and/or evolution. For homonuclear couplings, however, the situation is quite different, and it is only recently that practical general methods for broadband decoupling have emerged.[4−7] Such "pure shift" methods yield spectra containing only a single signal for each chemically distinct site, but at a significant cost in sensitivity. The most common methods use the Zangger and Sterk (ZS)[4] and BIRD[7,8] pulse sequence elements, which are both based on dividing the ^{1}H spins into active and passive subgroups that are manipulated differentially. The magnetization from the active spins gives rise to the detected signal, while the passive spins are manipulated to refocus the effect of homonuclear couplings, and their signals are suppressed. In the ZS and BIRD methods the active subgroup is typically much smaller than the passive subgroup, exacting a high cost in sensitivity.

The ZS method relies on spatially and frequency selective 180° pulses; it has been enhanced and adapted for 1D NMR,[6,9,10] DOSY,[11] and 2D experiments like TOCSY[10,12,13] and NOESY.[14] Although the ZS method can be very effective, when the chemical shift difference between resonances to be decoupled is small, highly selective 180° pulses are needed, and the sensitivity penalty is very great. The BIRD[7,8,15] method relies on isotopically sparse heteronuclei. It typically selects as active spins those protons directly bonded to ^{13}C (or ^{15}N) at natural abundance, so it has a minimum sensitivity penalty of 2 orders of magnitude, and it does not decouple geminal interactions. It is particularly helpful where ^{12}C-attached protons are strongly coupled, but it may suffer similarly from strong coupling when a ^{12}C-attached proton is strongly coupled to a ^{13}C satellite of another proton. In experiments such as natural abundance HSQC, that already rely on the presence of ^{13}C, there is no additional sensitivity penalty, and BIRD pure shift methods can be highly effective.[16]

Both ZS and BIRD data are typically acquired as interferograms: a pure shift FID is constructed from a series of short chunks of data acquisition of duration $1/SW_1$.[6] Real-time windowed acquisition can sometimes be used to speed up experiments, at some cost in spectral quality and resolution.[10,17,18] In the special case of heteronuclear shift correlation, spectra without (homonuclear) multiplet structure have been available for many years. For example, in ^{13}C-observed HETCOR experiments[19] BIRD can be used to suppress ^{1}H−^{1}H couplings in F_1, although HETCOR has now been almost completely supplanted by the more sensitive HSQC and HMQC experiments. Pure shift HSQC experiments have recently been introduced,[16,20,21] as well as specialized experiments for fully ^{13}C-labeled samples.[22,23]

The biggest challenge in general with pure shift methods is the cost in sensitivity; other significant issues include spectral artifacts and failure to decouple some interactions (e.g., in the case of strong coupling). Very recently, a new and very general pure shift method, PSYCHE (Pure Shift Yielded by Chirp Excitation), has been introduced, which addresses all these problems, offering almost an order of magnitude improvement in performance over most previous methods.[24]

The PSYCHE method is related to the anti z-COSY[5,25] experiment, in which low flip angle pulses (β) are used to quench

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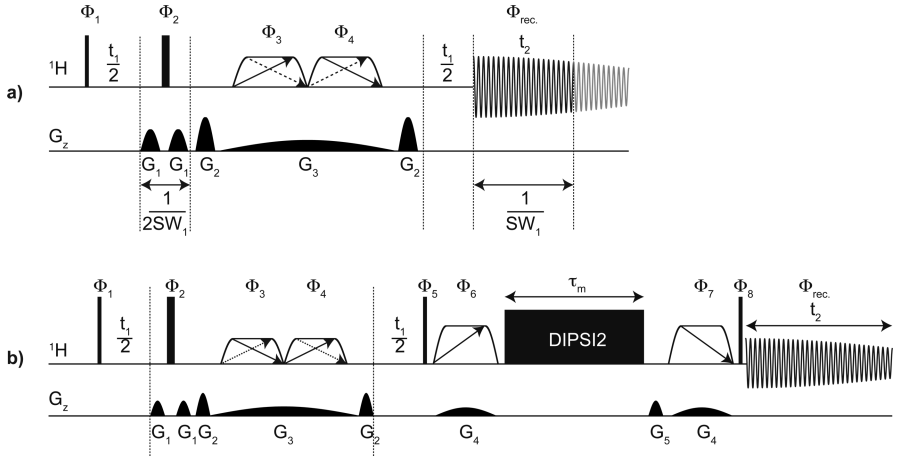

Figure 1. Pulse sequences for 1D PSYCHE (a) and 2D F_1-PSYCHE-TOCSY (b). Narrow rectangles are 90° RF pulses, wide 180°, and trapezoids with cross-diagonal arrows are low-power chirp pulses of small flip angle β (here 20°), which sweep frequency in opposite directions simultaneously with duration 15 ms and RF amplitude 46 Hz. Trapezoids on either side of the mixing element (DIPSI2) in (b) are 180° chirp pulses of 30 ms duration and 894 Hz amplitude, to suppress zero quantum coherences. Opposite directions of frequency sweep are used to avoid refocusing of undesired coherences.

the effects of scalar couplings among spins. In PSYCHE, a proportion $\sin^2\beta$ of magnetization (active spins) is refocused in a stimulated echo by a pair of low flip angle, swept-frequency chirp pulses, while the remaining (passive) spins are left unaffected; this division of spin populations is purely statistical. In combination with a hard 180° pulse, the net effect is to refocus the effects of J but not those of chemical shift. The simultaneous field gradient dephases unwanted coherence transfer pathways including cross-peak,[5] zero quantum coherence (ZQC),[26] and strong coupling pathways.[27] Currently, the only class of pure shift experiments that can compete in sensitivity with PSYCHE is that of band-selective (BASH) methods,[17,18,28] but these are not broadband, only decoupling part of a spectrum.

Here we combine PSYCHE with 2D TOCSY[29] to suppress homonuclear J evolution in t_1, with the option of using indirect covariance data processing[30] to give a 2D correlation spectrum with full homonuclear decoupling in both dimensions. Figure 1 shows the pulse sequences used for acquiring 1D and 2D pure shift spectra, respectively. In the F_1-PSYCHE-TOCSY sequence (Figure 1b), the pure shift element, consisting of a hard 180° pulse and two low flip angle chirp pulses applied during a weak pulsed field gradient, is implemented in the middle of the evolution period (t_1) of a 2D TOCSY pulse sequence. For the active spins, chemical shifts continue to evolve, while homonuclear couplings are refocused. The net effect is a 2D spectrum in which all multiplets in the indirect dimension (F_1) are collapsed to singlets. The F_1-PSYCHE-TOCSY experiment is an order of magnitude more sensitive than the previously published F_2-ZS-TOCSY experiment[12] and gives significantly cleaner results (see Figures S4 and S7 in the Supporting Information).

Using decoupling in the F_1 rather than the F_2 dimension has the advantages that standard processing can be used and that the small artifacts caused by F_2 chunked acquisition are avoided. In addition, using conventional acquisition in t_2 allows high resolution (albeit with multiplet structure) in F_2 at no extra cost. In general this produces higher quality and more easily interpretable spectra (Figure S6) that lend themselves more readily to covariance processing (Figure S7). F_1-decoupled spectra require a large number of t_1 increments to achieve the digitization necessary to take advantage of the increased resolution, but this potential time disadvantage is normally more than offset by the need to acquire multiple chunks of data where pure shift acquisition is used in the t_2 domain.

The PSYCHE method in its present form has the disadvantage compared to ZS- and BIRD-based techniques that it is not suitable for real-time acquisition of pure shift spectra. Despite this, it is more than competitive in sensitivity; only band-selective homodecoupling, which is not broadband, routinely offers better signal-to-noise ratio for homonuclear experiments.[17,28] While the well-known constant-time F_1 decoupling method[31] can offer high sensitivity at reasonably good resolution in favorable cases, it is not a general method, with signal signs and amplitudes depending on the details of the spin system and sequence timing. For complex spin systems and/or a wide range of coupling constants, PSYCHE is again highly competitive.

Figure 2 illustrates the application of F_1-PSYCHE-TOCSY, combined with indirect covariance processing,[30] to the challenging case of estradiol, which has a very crowded spectrum with significant strong coupling (Figure 2a). Figure 2b shows the result of F_1-PSYCHE-TOCSY, with the decoupling giving a single peak in F_1 for each distinct chemical shift. This spectrum contains the same information as the conventional TOCSY, but with multiplet structure in only one dimension (F_2). Application of indirect covariance processing[30] to the data of Figure 2b yields Figure 2c, in which all homonuclear multiplet structure has been removed to yield singlet signals in both dimensions. The result is a fully resolved map of the interproton coupling relationships in this complex spin system, greatly facilitating both manual and automatic analysis compared to the thicket of cross-peaks in Figure 2a.

The assignment of ^{1}H spectra of complex samples, including mixtures, can be a significant challenge even at the highest magnetic fields currently available. The resolution gain in homonuclear correlation afforded by PSYCHE is considerably greater than any gains anticipated from increases in magnetic field in the foreseeable future.

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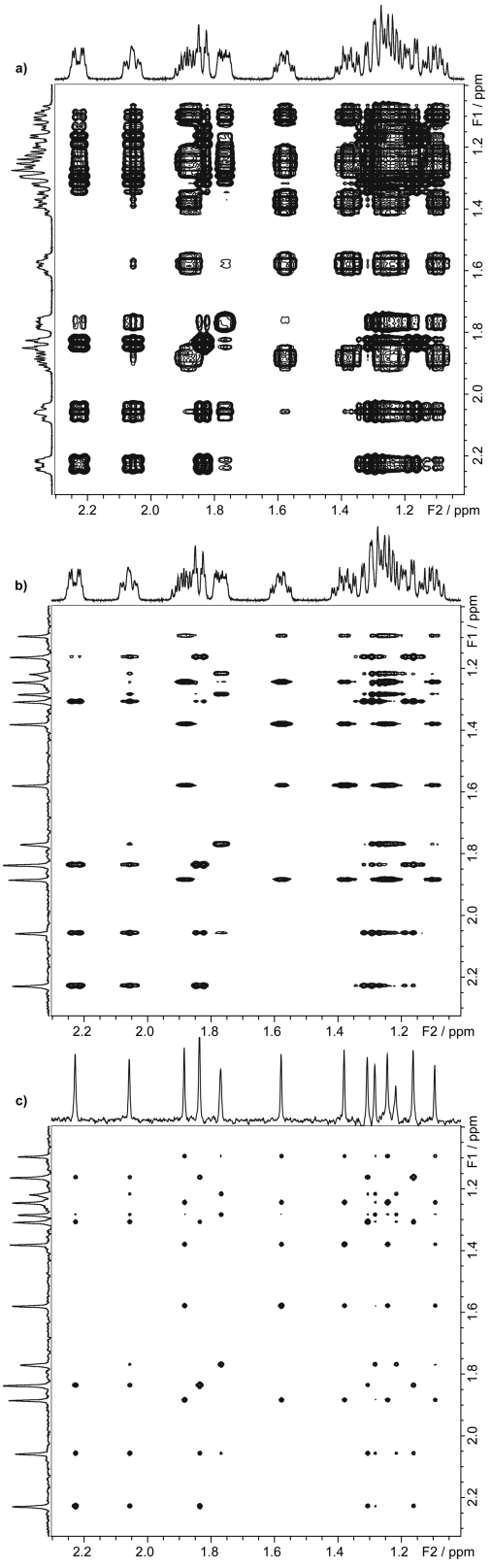

Figure 2. Spectra of normal TOCSY (a), F_1-PSYCHE-TOCSY (b), and double pure shift TOCSY using PSYCHE in F_1 and covariance processing in F_2 (c) of a sample of estradiol in DMSO-d_6.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Instructions for generation of chirp pulses for PSYCHE element, pulse sequence code, assignment of estradiol in 1D and 2D pure shift spectra, comparison of F_1-PSYCHE-TOCSY with F_2-ZS-TOCSY, and some simulation results. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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