J|A|C|S COMMUNICATIONS

Published on Web 08/26/2010

True Chemical Shift Correlation Maps: A TOCSY Experiment with Pure Shifts in Both Dimensions

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Abstract: Signal resolution in ^{1}H NMR is limited primarily by multiplet structure. Recent advances in pure shift NMR, in which the effects of homonuclear couplings are suppressed, have allowed this limitation to be circumvented in 1D NMR, gaining almost an order of magnitude in spectral resolution. Here for the first time an experiment is demonstrated that suppresses multiplet structure in both domains of a homonuclear two-dimensional spectrum. The principle is demonstrated for the TOCSY experiment, generating a chemical shift correlation map in which a single peak is seen for each coupled relationship, but the principle is general and readily extensible to other homonuclear correlation experiments. Such spectra greatly simplify manual spectral analysis and should be well-suited to automated methods for structure elucidation.

Signal resolution in ^{1}H NMR is limited primarily by multiplet structure. Recent advances in "pure shift"[1−6] (or "chemical-shift"[7] or "δ-resolved"[8]) NMR, in which the effects of homonuclear couplings are suppressed, can circumvent this limitation, gaining almost a factor of 10 in resolution in 1D NMR. A key concept in early 2D NMR was that of a chemical shift correlation map.[9] Homonuclear maps have full multiplet structure in at least one[10] dimension and normally in both. We show here a method for obtaining experimental homonuclear shift correlation maps that are fully decoupled in both dimensions, giving an increase in resolving power that can approach 2 orders of magnitude.

Methods for measuring heteronuclear shift correlation maps without multiplet structure, for example ^{13}C-observed HETCOR experiments with ^{1}H homonuclear decoupling, have been known and used for many years,[11] and recently a pure shift HSQC experiment has been described.[4] Decoupled *homonuclear* correlation spectra have to date only been produced by postprocessing fully coupled 2D spectra using methods such as pattern recognition, with or without prior knowledge.[12−14] The new method produces for the first time an experimental fully decoupled homonuclear correlation spectrum, using a conventional 2D pulse sequence adapted to suppress homonuclear J evolution in t_2, followed by covariance, as opposed to double Fourier transform (FT), data processing.[15−17] The principle is demonstrated for TOCSY,[18] generating a map in which a single peak is seen for each coupling relationship, but is very general and may be applied to a variety of homonuclear correlation experiments.

Heteronuclear correlation experiments with homonuclear decoupling exploit couplings to isotopically dilute spins (e.g., ^{13}C), using the BIRD pulse sequence element.[19] Here a similar selection of a subset of molecules is made using a 180° pulse that is simultaneously chemical shift and spatially selective. This elegant device, originated by Zangger and Sterk (ZS),[20] has been used to produce pure shift 1D and DOSY ^{1}H spectra,[1,5,6] absorption-mode ^{1}H 2D J spectra,[3] and δ-resolved spectra of enantiomers.[8]

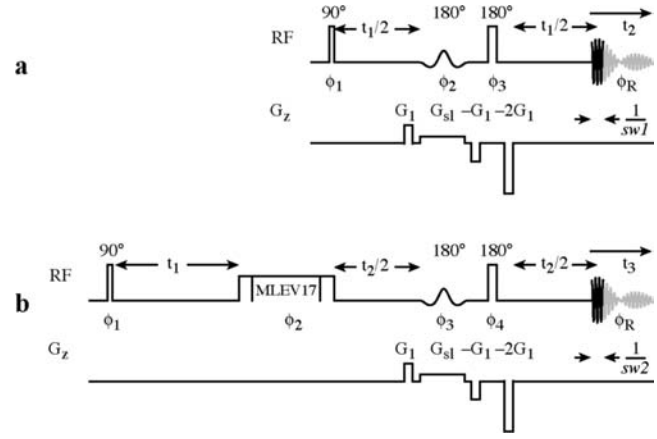

Figure 1. Pulse sequences for (a) pure shift 1D NMR and (b) pure shift TOCSY. The slice select gradient G_{sl} of 0.6 G cm^{-1} was chosen such that $|\gamma G_{sl} L| > 2\pi sw$, where L is the length of the active volume of the RF coil. 1 ms gradient pulses were used with $G_1 = 15$ G cm^{-1}. Phase cycling in multiples of 90°: (a) $\phi_1 = 0123$; $\phi_2 = 0_4 1_4 2_4 3_4$; $\phi_3 = 0_{16} 1_{16} 2_{16} 3_{16}$; $\phi_R = \phi_1 + 2(\phi_2 + \phi_3)$; (b) $\phi_1 = 0123$ + hypercomplex; $\phi_2 = 01$; $\phi_3 = 0_2 1_2 2_2 3_2$; $\phi_4 = 0_8 1_8 2_8 3_8$; $\phi_R = \phi_1 + 2(\phi_3 + \phi_4)$.

The pulse sequences used here for acquiring 1D and 2D pure shift spectra are shown in Figure 1a and 1b respectively. Figure 2 shows a comparison between the normal 400 MHz ^{1}H TOCSY spectrum of the macrolide antibiotic clarithromycin and the pure shift version obtained with the pulse sequence of Figure 1b; MLEV-17 is used here for simplicity, but a wide range of isotropic mixing sequences may be used. The conventional 1D spectrum is shown in Figure 2a top and left, and the pure shift 1D spectrum in Figure 2b top and left. Comparison between the normal (a) and pure shift (b) spectra shows essentially complete decoupling in this system, where there is little strong coupling.

In the general case the limiting factors are 2-fold: the bandwidth bw of the soft 180° pulse in Figure 1a and 1b, which determines the minimum shift difference $\Delta\delta$ between coupled spins for which decoupling is obtained; and second order effects, which can complicate the spin responses. For the most part the method fails gracefully. If coupled multiplets are less than bw Hz apart but not very strongly coupled ($bw > \Delta\delta > J$), couplings within bw become active but the effects of couplings to other spins remain suppressed, retaining much of the resolution advantage. Where spins are fairly strongly coupled ($bw > \Delta\delta \approx J$), weak extra signals appear at intermediate frequencies, and very strong coupling ($bw > J > \Delta\delta$) typically yields broad signals.

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10.1021/ja1039715

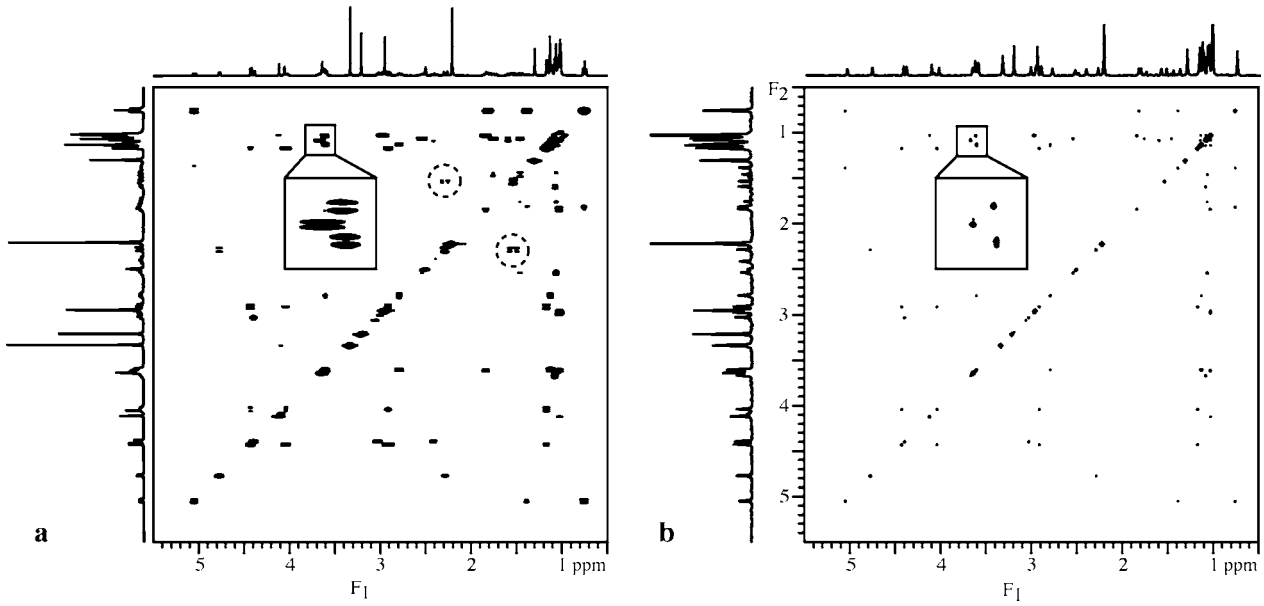

Figure 2. (a) Conventional TOCSY spectrum and 1D spectra of a 1.6% w/w solution of clarithromycin in dimethylsulfoxide-d_6, recorded using 128 t_1 increments in 19 min; antiphase cross-peaks circled. A 60 ms MLEV-17 mixing period flanked by 10 ms spin-lock periods was used. (b) Double pure shift TOCSY spectrum, with (top and far left) pure shift 1D spectra (4 transients) measured with the sequence of Figure 1a; 2D data were acquired using the sequence of Figure 1b with the same first mixing period as that for Figure 2a and an 18.5 ms rsnob[21] selective 180° pulse. 128 t_1 increments and 24 t_2 increments of 2 transients were acquired in 7.5 h. Spectral widths were 2400, 40, and 2400 Hz in F_1, F_2, and F_3 respectively. After FT with respect to t_2 the data were subjected to covariance processing[15−17] (<1 min on a standard PC) using a Mathematica[22] module called from the VnmrJ software of the Varian INOVA 400 spectrometer used. Both TOCSY experiments used hypercomplex F_1 quadrature detection and phase sensitive display.

The sensitivity penalty paid for the pure shift gain in resolution is of the order of *sw*/*bw*, typically 1 to 2 orders of magnitude, where *sw* is the spectral width; the trade-off between sensitivity and resolution is under the control of the experimenter through the choice of *bw*. Antiphase, COSY/ZQ-type, cross-peaks in Figure 2a (e.g., those circled) are absent from the pure shift spectrum; collapsing the multiplet structure cancels out such spurious responses (though a more fortunate choice of mixing time would give true TOCSY cross-peaks).

There are two distinct steps in the production of a doubly pure shift homonuclear correlation spectrum such as that of Figure 2b. The first is to generate an experimental data set with one dimension decoupled, and the second to use covariance processing to produce a doubly pure shift 2D spectrum. The starting point is a pulse sequence such as that of Figure 1a, an improved version of the original ZS sequence. Consider first the behavior of a coupled spin system if the slice-select gradient G_{sl} in Figure 1a were zero. The remaining gradient pulses would restrict the signal observed to that from spins affected by the selective pulse, while the net evolution for those spins at the end of the evolution period t_1 would be determined by the chemical shift alone, the hard and soft 180° pulses together refocusing the effects of scalar couplings. The effect of reintroducing the slice-select gradient G_{sl} is then to place each different chemical shift on resonance for the selective 180° pulse at a different position in the sample. Setting $G_{sl} \approx 2\pi\, sw/\gamma L$, where γ is the magnetogyric ratio and L the length of the active volume of the probe, places one end of the spectrum on resonance at the top of the sample and the other at the bottom. The result is a dual selection of chemical shift and of spatial position, so that signals with different chemical shifts are excited at different positions in the sample. All signals in the spectrum are recorded at the same time, but for each signal the active spins come from a different thin slice; field gradient pulses and/or phase cycling ensure that signals from spins outside the selected slices are suppressed.

In principle a pure shift spectrum of width *sw* Hz could then be obtained by using the sequence of Figure 1a to map out the shift evolution one point at a time, incrementing t_1 in steps of 1/*sw* s, but this would be extremely slow. A second key recognition by ZS[20] was that the different time scales of chemical shifts and scalar couplings make it possible to acquire a relatively large number $N = sw/sw1$ of complex t_2 data points in a time 1/*sw1* s, rather than a single point, per transient. Provided $J \ll sw1$, the effect of J evolution on the signal remains small. A complete interferogram mapping out the chemical shift evolution can then be obtained by incrementing t_1 in only *ni* steps (typically a few dozen) of 1/*sw1* s and stitching together the first 1/*sw1* s of each successive free induction decay (FID) $s(t_2)$; the incrementation of t_1 in steps of 1/*sw1* ensures that the chemical shift evolves smoothly from one chunk of FID to the next.[6] Data acquisition follows the same logic as in 2D NMR, with *ni* as the number of increments of the evolution time t_1 and *sw1* as the spectral width in the indirect dimension. The result is that pure shift data can be acquired far more rapidly than would be the case for point-by-point acquisition. The traces at the top and left of Figure 2b were obtained in this way, with $sw = 2400$ and $sw1 = 40$ Hz.

The sequence of Figure 1a can be incorporated into many existing NMR techniques, both 1D and 2D. Replacing the first part of the sequence with the preparation, evolution, and mixing periods of a simple TOCSY sequence gives the sequence of Figure 1b. t_1 is now the evolution period and *ni1* is the number of increments for the homonuclear correlation, t_2 and *ni2* are those for the pure shift evolution, and t_3 is the real time for data acquisition. FIDs $s(t_3)$ are acquired with spectral width *sw* in t_1 and in t_3, but $sw2 = sw/N$ in t_2. Provided $sw2 \gg J$, the effects of J modulation over the first 1/*sw2* of t_3 are small, as in the 1D case of Figure 1a. A t'_2 interferogram can then be constructed by stitching together the first 1/*sw2* seconds of successive FIDs $s(t_1, t_2, t_3)$. The result is a data matrix $s(t_1, t'_2)$ in which signals evolve as normal as a function of t_1, but with chemical shift modulation only as a function of t'_2. Double FT then gives the spectrum of Figure 3, in which

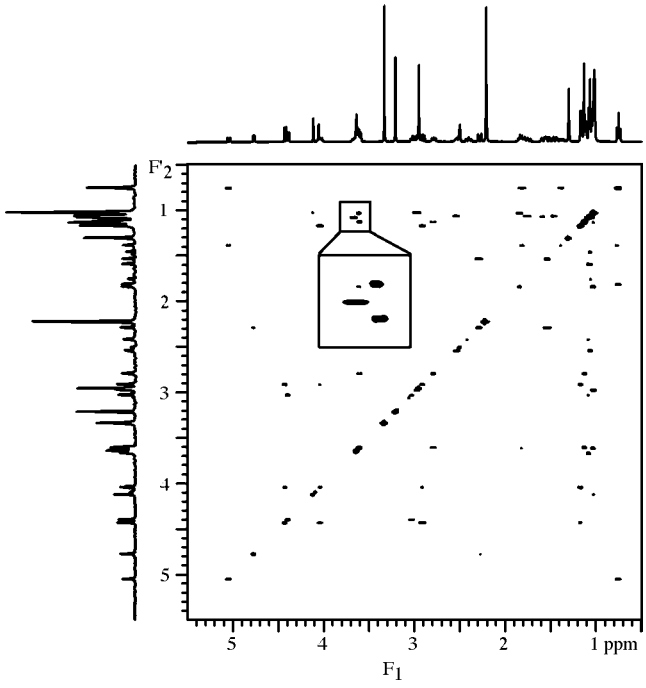

Figure 3. Single pure shift TOCSY spectrum of clarithromycin obtained by direct double Fourier transformation of the data used to produce Figure 2b.

homonuclear multiplet structure appears in F_1 but not in F'_2: a singly pure shift homonuclear correlation spectrum.

The spectrum of Figure 3 contains all of the information needed to construct a fully decoupled, doubly pure shift, 2D spectrum. A simple route to this is to quantify the degree of similarity in the t_1 evolutions of signals at different chemical shifts in the F'_2 dimension. The covariance matrix, or correlation spectrum, **C** for the F'_2 signals can be defined as

$$\mathbf{C} = (\mathbf{X}\mathbf{X}^{\mathrm{T}})^{1/2}$$

where **X** is the *ni1* by $N \times$ *ni2* matrix $s(t_1, F'_2)$, and the symbols T and 1/2 denote the matrix transpose and the matrix square root respectively. Signals in F'_2 that have the same modulation as a function of t_1 will show strong cross-peaks in the correlation spectrum; signals that have different modulations will give little or no cross-peak intensity. In contrast to conventional 2D NMR, the number *ni1* of t_1 increments required is determined not by the target resolution in the F_1 domain of the correlation spectrum, which in covariance processing is equal to that in F'_2, but only by the need to acquire sufficient increments to avoid spurious responses. Thus in the case of Figure 2b the number of t_1 increments *ni1* used was 128 and the number of data points $N \times$ *ni2* in t'_2 was 1440, giving an F_1 digitization advantage over FT processing of more than a factor of 10 (in addition to the resolution gain from pure shift acquisition). A further reduction in experiment duration could be achieved by using nonuniform sampling in t_1, there being no requirement with covariance processing to use constant increments in t_1.

The experiment of Figure 1b uses the ZS element in t_2. It is also possible to use it in t_1; Zangger and Sterk showed such a TOCSY sequence in their original paper,[20] although the results were a little disappointing. If sufficient digitization were used in t_1, a double pure shift spectrum could be obtained by covariance processing. One advantage of using the ZS element in t_2 is that the multiplet structure associated with the pure shift dimension F'_2 can be recovered using a third FT. Double pure shift 2D spectra can also be obtained by covariance processing of constant-time 2D experiments, though the latter suffer from erratic signal amplitudes and signs.

Extension to other homonuclear correlation methods such as NOESY and ROESY should be straightforward; here the suppression of zero quantum responses would be particularly useful. COSY is a little problematic because of the antiphase cross-peaks, but *J*-selective analogues are possible, in which a rephasing delay is used to select which couplings give a net pure phase signal. Full decoupling in both dimensions can be achieved by both covariance and constant time routes. The ZS pulse sequence element can be used in t_2, as in Figure 1, or in t_1; unfortunately it cannot easily be used in both dimensions.

Clarithromycin decomposes under acidic conditions such as those encountered in the stomach to a mixture of products.[23] Assignment of the ^{1}H spectrum of such a mixture is a significant challenge at any magnetic field currently available, but pure shift 2D correlation spectra should make this and many other assignments straightforward. The resolution gain from double pure shift methods exceeds the results of any realistic current expectation of improvements in magnetic field, albeit at significant cost in sensitivity. Figure 2 represents an experimental realization of the synthetic "reduced correlation spectrum" of Sengstschmidt et al.;[14] pure shift spectra should be of great value in automated structure elucidation as well as in simplifying manual analysis.

Acknowledgment. This work was supported by the Engineering and Physical Sciences Research Council (Grant Numbers EP/E057888/1 and EP/E05899X) and the Givaudan Strategic Research Fund.

Supporting Information Available: Pulse sequence, parameter file, Mathematica notebook used for covariance processing, and selective pulse shape file. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA1039715