Separating the coherence transfer from chemical shift evolution in high-resolution Pure Shift COSY NMR.

Juan A. Aguilar[1], Raquel Belda[1], Benjamin R. Gaunt[1], Alan M. Kenwright[1] and Ilya Kuprov[2]

1 University of Durham, Department of Chemistry (Durham, United Kingdom); **2** University of Southampton, Department of Chemistry (Southampton, United Kingdom)

Abstract

Recent developments in data sampling and processing techniques have made it possible to acquire two-dimensional NMR spectra of small molecules at digital resolutions in both dimensions approaching the intrinsic limitations of the equipment and sample on a realistic timescale. These developments offer the possibility of enormously increased effective resolution (peak dispersion) and the ability to effectively study samples where peak overlap was previously a limiting factor. Examples of such spectra have been produced for a number of two-dimensional techniques including TOCSY and HSQC. In this paper we investigate some of the problems in applying such techniques to COSY spectra, and suggest a modification to the classic experiment that alleviates some of these problems.

Introduction

The synergic use of Pure Shift NMR techniques[1,2] and Compressive Sensing[3-6], allows the realistic recording of two-dimensional NMR spectra of small molecules at digital resolutions approaching the intrinsic limits of the samples and equipment used.[7] The systematic use of this combination has been termed “Compressed NMR”[7], and is the subject of an companying article

in this edition. The advantages this can bring when dealing with spectra where peak overlap is a limiting factor, such as the spectra of mixtures of closely related structures, have been amply demonstrated in TOCSY and HSQC spectra. Thus far, the same approach has found limited applicability for COSY spectra for reasons discussed below. It is experimentally very challenging to produce a COSY spectrum that is intrinsically Pure Shift in both dimensions, not least because the desired correlations depend on the homonuclear J-couplings that Pure Shift techniques seek to suppress.

The commonest approach to the problem is to suppress J evolution in only one dimension. The classic way of doing this is to use a constant-time t_1 evolution period to suppress modulation due to J evolution in t_1[8–10]. This yields a COSY spectrum that is Pure Shift in f_1. If a spectrum that appears to be Pure Shift in both dimensions is desired, this can, in principle, be obtained by covariance processing the CT-COSY[11] although, in practice, this can also introduce artefacts and so should be used with caution.

One alternative approach would be to run a "normal" COSY experiment (i.e. with an incremented t_1 evolution period) and then detect the fid for each increment using a "real-time Pure Shift" technique[12,13]. In practice this is difficult to implement because real-time Pure Shift techniques tend to have limited effective bandwidth and tend to introduce artefacts into the fid that would subsequently turn up in the processed 2D spectrum as "t_1 noise", and also because antiphase multiplets that are typically present in COSY correlations would tend to cancel if decoupled. Another alternative would be to use homonuclear decoupling on a version of the COSY experiment that produces in-phase coherence multiplets, such as CLIP-COSY.[14]

Our approach decouples the f_1 dimension using a PSYCHE element and uses compressed sensing to reduce the overall experiment time, yielding COSY spectra with J coupling suppressed in the f_1 dimension at the highest possible effective resolution, while separating the J-coupling evolution

from the chemical shift evolution, thereby allowing us greater selectivity over the correlations observed.

Results and discussion

Our initial investigations involved CT-COSY experiments acquired using compressive sensing to achieve high digital resolution in both dimensions. At the same time we ran classic incremented evolution COSY experiments under similar conditions for comparison. Typically, when interpreting ^{1}H COSY spectra, we assume that the majority of the observed correlations arise from ^{2}J and ^{3}J interactions, with the occasional observation of ^{4}J interactions in specific cases (allylic couplings, "W" couplings through four planar sigma bonds, meta couplings in aromatic systems with an electron withdrawing group in the ortho position). It is this limiting of the range of COSY interactions that facilitates interpretation, particularly when the structure of the molecule is unknown or uncertain. However, as we increased the digital resolution by going to longer t_1 evolution times we found that we could detect an increasing number of long-range correlations due to couplings not typically apparent in the one-dimensional proton spectrum. While this is an interesting observation for a sample whose structure is already established, it would be a source of confusion when interpreting the spectrum of an unknown.

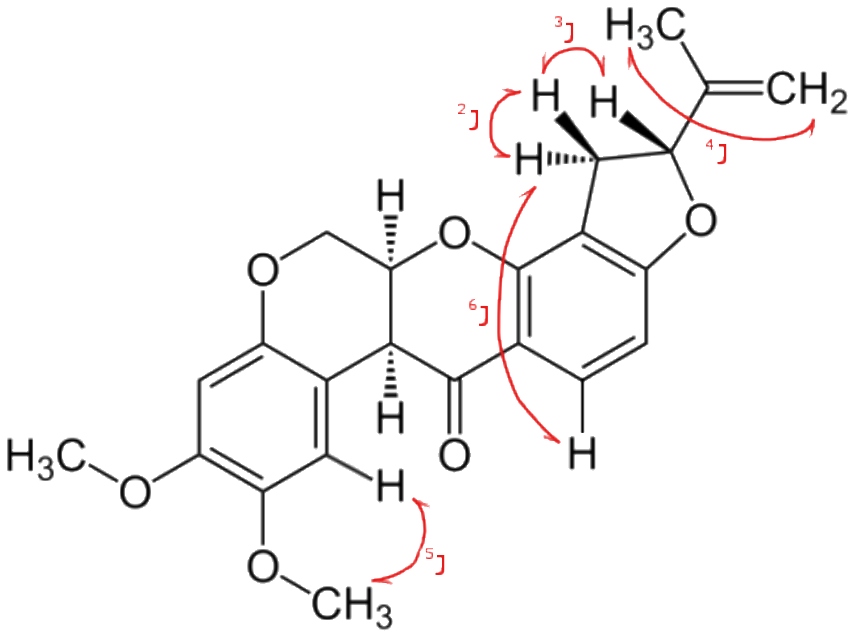


Figure 1. Molecular structure of rotenone with a selection of couplings observed in high digital resolution COSY spectra indicated.

As an example, and model system for subsequent investigations, we looked at rotenone (Figure 1). In both classic COSY experiments and CT-COSY experiments run at a digital resolution in f_1, prior to zero filling, of 1.25 Hz/point, correlations over 5 and 6 bonds, as indicated in Figure 1, were readily detected and are highlighted in Figure 2.

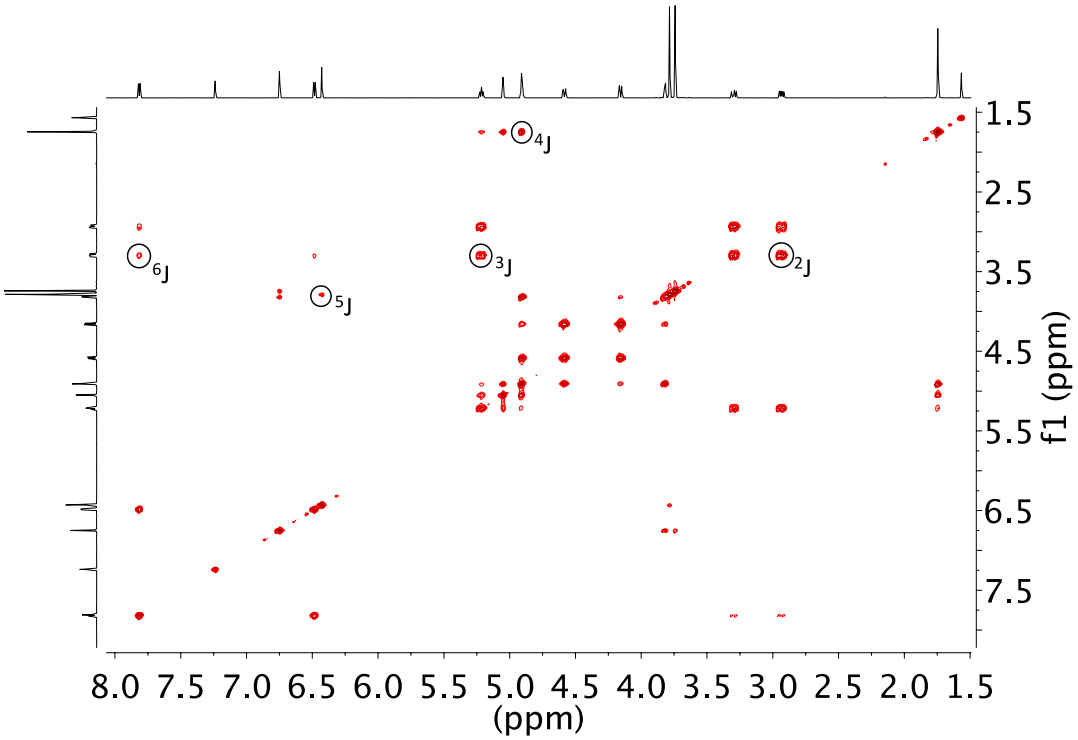


Figure 2. COSY spectrum of rotenone at high digital resolution. The spectrum was acquired on a Varian VNMRS-600 using compressed sensing. The spectral window in both dimensions was 5kHz. 4096 complex data points were acquired in t_2 (acquisition time = 819.2 ms). 512 non-uniformly (incoherently) spaced increments were acquired in t_1, using a sampling schedule suitable for reconstruction to 4096 uniformly spaced increments. Reconstruction was carried out using the Iterative Soft Thresholding (IST) algorithm implemented in the Mnova software (MestreLab Research). The final Fourier transform size was 8k x 8k. The total experiment time was 54 minutes

The reason for this is readily apparent. In the classic incremented evolution COSY (Figure 3 top), the evolution of J coupling takes place over the same time period as the evolution of the chemical shift. Thus, by going to longer t_1 evolution periods we are not only increasing the time for chemical shift evolution and thereby generating higher effective resolution, but also increasing the limiting value for J evolution, and thereby making it possible to detect smaller couplings. Indeed, the limitation on the size of coupling that can be detected is set by the T_2 of the sample. For small

molecules in non-viscous solvents this can often be several seconds, meaning that J couplings substantially less than 1 Hz can be detected.

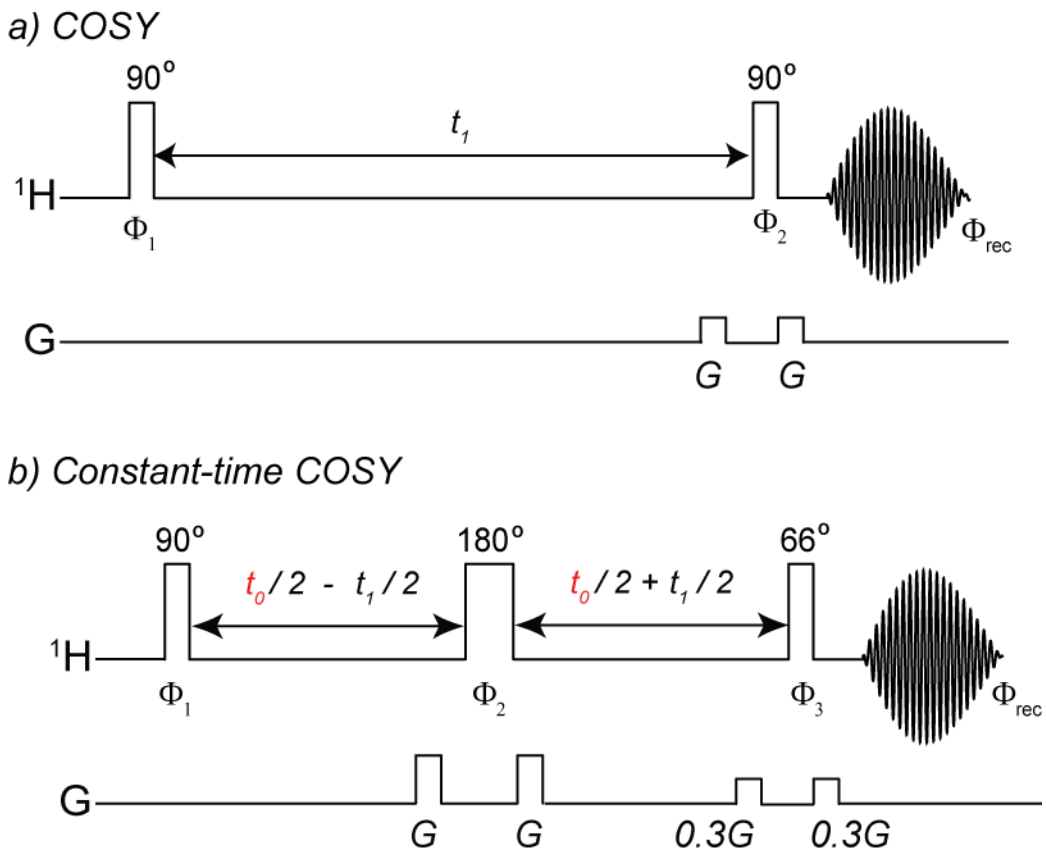


Figure 3. Pulse sequences for the classic COSY experiment (top) and the CT-COSY experiment (bottom) used in this work. For the CT-COSY experiment, t_0 is the total evolution time and represents the time for the evolution of J coupling in the first dimension.

A similar situation applies in the CT-COSY experiment (Figure 3, bottom), except that in this case the time for evolution of J coupling (t_e) is fixed. Its duration is determined by the combination of spectral window and the number of data points in t_1 (effectively the digital resolution in f_1), but the J evolution time is at least twice as long as the final J evolution time in the classic COSY experiment run at the same digital resolution in f_1. This still means that as the digital resolution in f_1 is increased the time for evolution of J coupling is also increased, so smaller couplings can be detected, and the user has no control over this.

To address this problem we designed a COSY sequence in which chemical shift evolution and coherence transfer evolution occur in separate periods so that the f_1 digital resolution can be controlled separately from the coherence transfer. The sequence we propose (Figure 4) consists of a chemical shift (only) evolution period with a Pure Shift refocusing element in the centre of it, followed by a fixed period during which evolution of both chemical shift and J-coupling occurs. The idea of using the PSYCHE refocusing element in the t1 evolution period of two-dimensional NMR experiments to generate homonuclear correlation spectra that are Pure Shift in f_1 is not new. The element has already been successfully incorporated into a number of experiments, notably PSYCHE-TOCSY[15,16]. We chose the pair of low flip angle Saltire CHIRP pulses used in the PSYCHE sequence[17] as the pure shift refocusing element because they are effective over a wide bandwidth and yield relatively high sensitivity, but a number of other possibilities also exists including the method of Zangger and Sterk[18] and the use of BIRD pulses.[19] We refer to this experiment as PSYCOSY (PSYCHE-COSY).

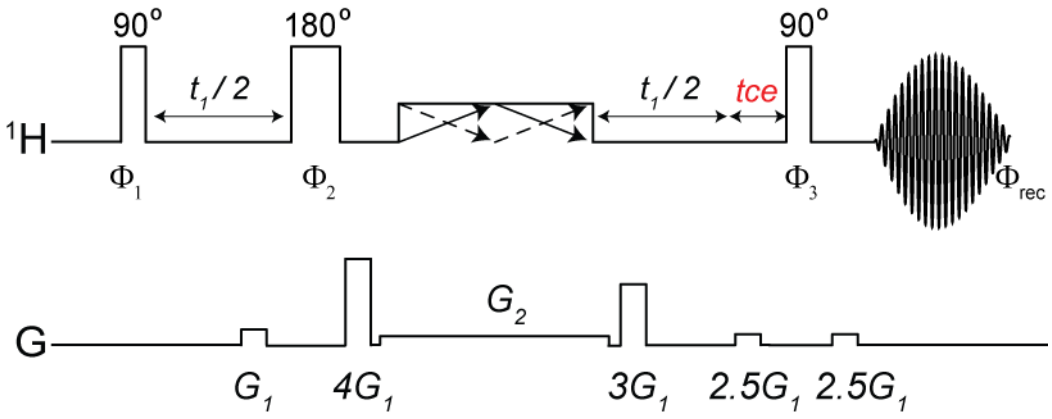


Figure 4. PSYCOSY pulse sequence. Evolution of J coupling is refocused during t_1 and is therefore only effective during the delay added to the end of t_1 to facilitate coherence evolution (t_{ce}). The duration of this delay is under the control of the experimenter, and is independent of the digital resolution in f_1. In the examples presented below, the pair of Saltire CHIRP pulses had a bandwidth of 7kHz, a flip angle of 10 degrees and a duration of 60ms (2x30ms). The amplitudes of the six gradients shown in the sequence were 4, 16, 0.7, 12, 10, and 10 Gauss cm^{-1}, respectively.

The duration of the 0.7 Gauss cm^{-1} gradient was 60ms (to match the CHIRP pulses). The duration of the other gradients was 2.5ms each.

We then investigated the behaviour of the PSYCOSY experiment as a function of the coherence evolution time (t_{ce}). We measured the intensities of observed correlations relative to a signal with no couplings (residual solvent signal) which was set in all cases to have a constant arbitrary value of 100. The expectation for an isolated 2-spin system would be that the coherence transfer would take the form of a sin curve as a function of the coherence evolution time, with a slower build-up of coherence for smaller values of J. Initial investigations indicated that this behaviour was indeed observed for an isolated two-spin system, but that the behaviour of spin systems containing more than two spins was more complicated. Figure 5 shows the intensity of the observed correlation as a function of the coherence evolution time for a 6-bond coupling in rotenone, as highlighted in Figure 1. Corresponding plots for the other couplings highlighted in Figure 1 are available in the supplementary information (Figures S1 to S4).

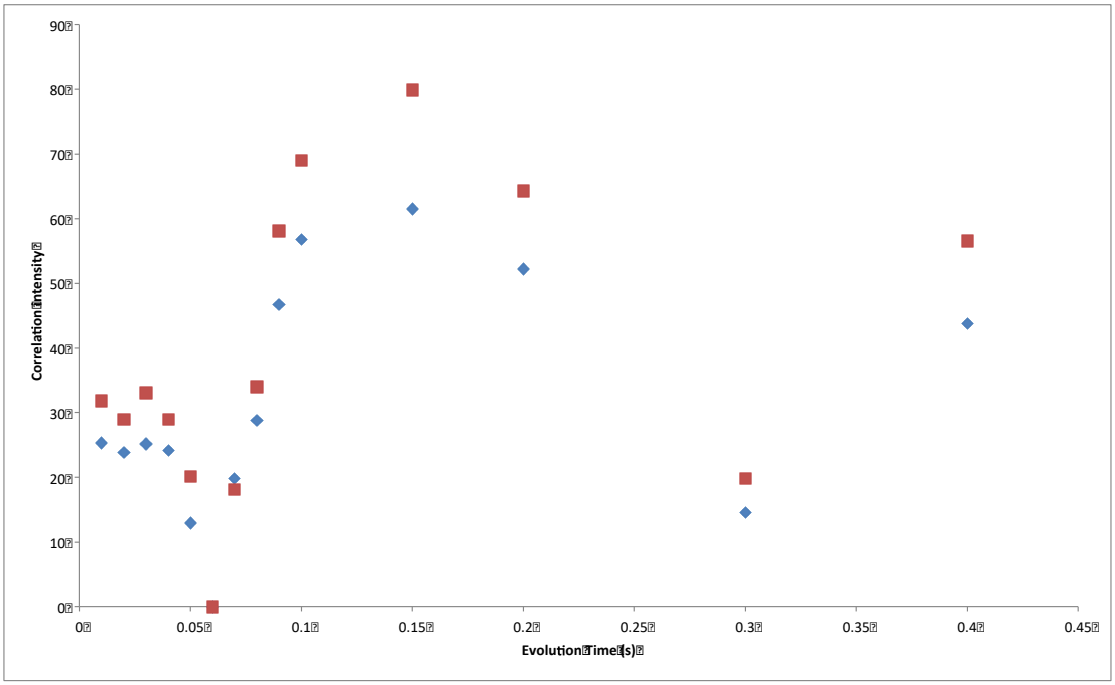


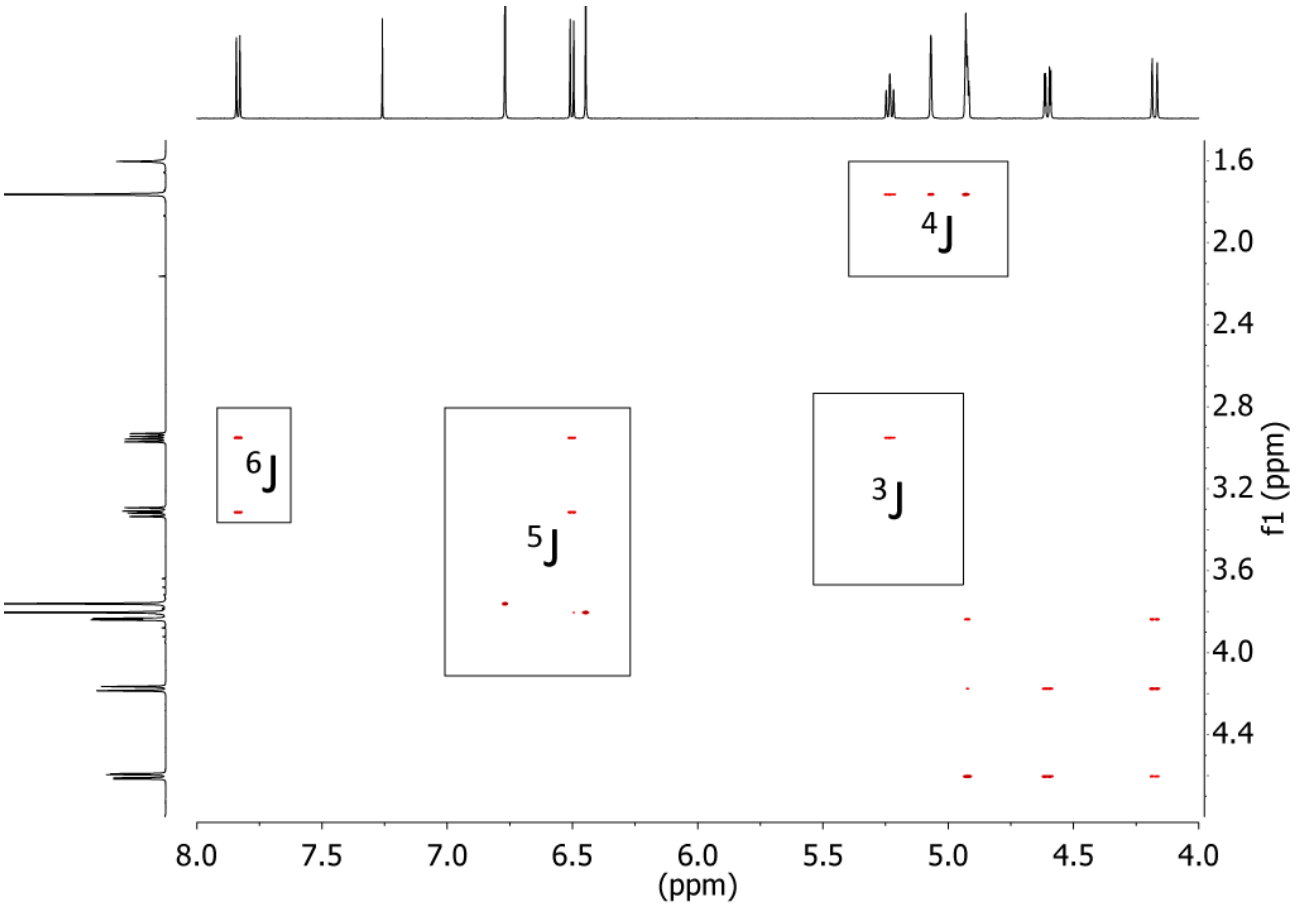
Figure 5. Intensity of the observed ^{6}J correlations as a function of the coherence evolution time in PSYCOSY

We have analysed the expected behaviour for a multispin system using the product operator formalism (supplementary information). The key result from the analysis is that, to a first approximation, in a multi-spin system containing large and small couplings, the transfer function for the small coupling is minimised close to the point where the transfer for the large coupling is a maximum. This behaviour has been observed previously in CT-COSY spectra, and has even been suggested as a way of determining coupling constants.[20] The detailed evolution of the coherence intensity as a function of the evolution time depends on all of the possible sums and differences of the frequencies corresponding to the coupling constants in the coupled system. The frequencies are fixed, but the amplitude coefficients of the different frequencies vary for the various correlations. To check that the observed behaviour corresponds to our analysis, the behaviour of the 4-spin system containing the ^{6}J, ^{3}J, and ^{2}J couplings highlighted in Figure 1 were modeled. Measured

coupling constants of 15.8 Hz (^{2}J), 10.0 and 8.2 Hz (^{3}J) were used. The ^{6}J coupling constants were too small to be accurately measured from the 1-dimensional proton spectrum, so values of 0.5 Hz were assumed in both cases. The amplitudes of the combination frequencies were adjusted by least squares fitting. The results show excellent agreement – examples for ^{2}J, ^{3}J, and ^{6}J are shown in Figures S11 to S13, and the Matlab code used to generate the simulations is also provided in the supplementary information.

By choosing a coherence evolution delay of 0.5/J (where J is a coupling constant typical of ^{3}J interactions) we are able to maximise coherence transfer for those interactions while, at the same time, minimising coherence transfer for smaller couplings in the same multi-spin system. We chose a coherence evolution delay of 63ms, corresponding to a J value of 8Hz. This is a slightly larger J value than the average ^{3}J couplings observed in alkyl chains and aromatic rings, and is a compromise to ensure that correlations due to larger ^{2}J couplings are still observed at reasonable intensity. It is also a reasonable approximation to the sort of limiting evolution delay that would typically be encountered in a lower resolution classic COSY experiment with which most chemists are familiar. The effect of this can be seen by comparing the results obtained from a PSYCOSY experiment run using a coherence evolution delay of 63 ms and a digital resolution in f_1, prior to zero filling, of 1.25 Hz/point, with a CT-COSY experiment run at the same digital resolution. Figure 6 shows an expansion of part of the relevant spectra rich in long-range couplings. The corresponding full spectra are available in the supplementary information (Figures S5 to S7). The PSYCOSY experiment shows the correlations that would be expected in a lower-resolution classic COSY, while the CT-COSY experiment shows additional correlations over 5 and 6 bonds. The 6-bond correlations are not apparent in the PSYCOSY experiment. Some long range couplings are still visible in the PSYCOSY experiment, notably allylic 4-bond couplings and some 5-bond couplings between isolated spin systems that appear as singlets in the 1-dimensional proton spectrum but, interestingly, a non-allylic 4-bond coupling that was visible in the CT-COSY

spectrum has been suppressed. It is also noteworthy that some 3-bond couplings that did not appear in the CT-COSY spectrum are visible in the PSYCOSY.


4J
6J
5J
3J
1.6
2.0
2.4
2.8
3.2
3.6
4.0
4.4
f1 (ppm)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
(ppm)

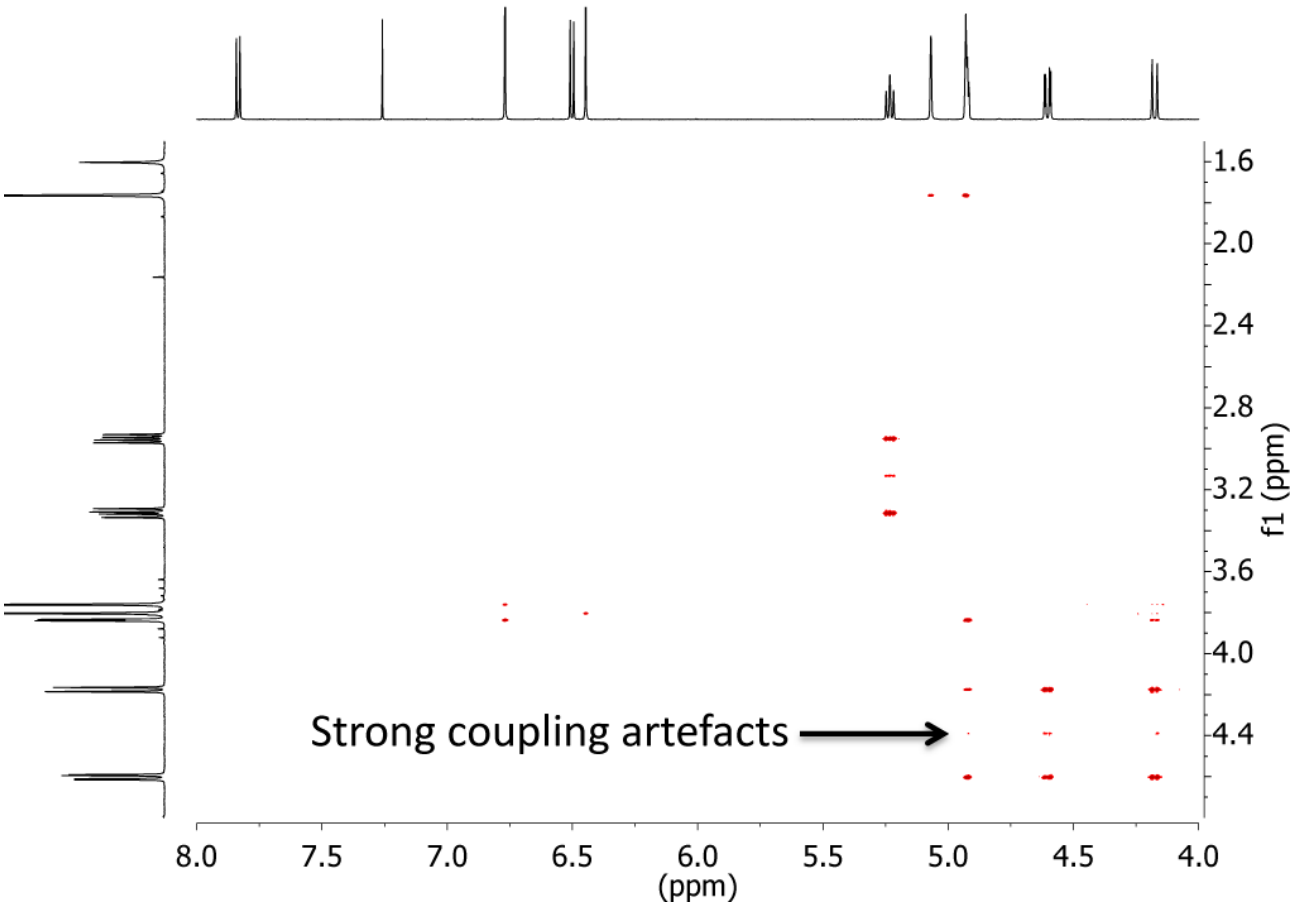
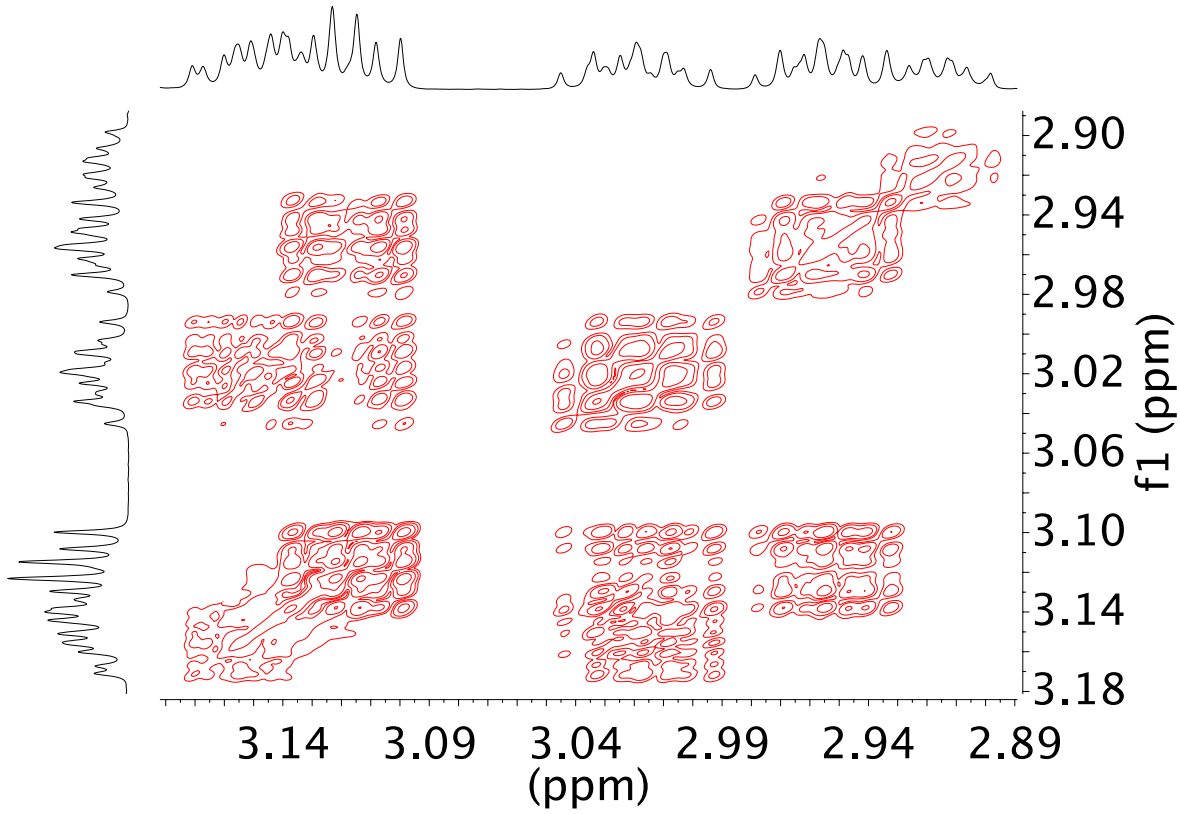

Strong coupling artefacts
1.6
2.0
2.4
2.8
3.2
3.6
4.0
4.4
f1 (ppm)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
(ppm)

Figure 6. PSYCOSY (bottom) vs CT-COSY (top) at high digital resolution. Both spectra were acquired on a Varian VNMRS-600 using compressed sensing. The spectral window in both dimensions was 5kHz. In each case 2048 complex data points were acquired in t_2 (acquisition time = 409.6 ms). 512 non-uniformly (incoherently) spaced increments were acquired in t_1, using a sampling schedule suitable for reconstruction to 4096 uniformly spaced increments. Reconstruction was carried out using the Iterative Soft Thresholding (IST) algorithm implemented in the Mnova software (MestreLab Research). The final Fourier transform size was 8k x 8k. For the CT-COSY, the limiting t_1 evolution time (t_e) was 819.2 ms. The coherence evolution time (t_{ce}) in the PSYCOSY was 63ms, corresponding to a J value of 8 Hz. The total experiment time (55 minutes) was the same for both spectra

It is noticeable in the PSYCOSY spectra that there are strong coupling artefacts in f1, equidistant between pairs of peaks that are coupled. These correspond to the strong coupling artefacts observed in the 1-dimensional PSYCHE experiment, but seem to be more apparent in the two-dimensional experiment. These effects were further investigated by simulation using the Spinach software package and, in particular, the recently introduced modules to deal with spatial encoding.[21, 22] To keep the simulation time to a minimum we simulated the isolated proton spin system on the right hand side of the molecule, which contains observable couplings in the COSY spectrum arising from ^{2}J, ^{3}J, ^{4}J, and ^{6}J couplings (see Figure 1). Where values for the relevant coupling constants could be easily measured from the 1-dimensional proton spectrum these were used in the simulations. Where the couplings in question were too small to be readily determined from the 1-dimensional proton spectrum a value of 0.5 Hz was used in the simulation. Even so, the ability to practically simulate what is effectively a spatially encoded experiment for an 11-spin system at a resolution of 4096 x 4096 data points is only possible thanks to the development of new features in the Spinach kernel, and represents a significant development in spin dynamics simulation. The results of the simulations are shown in Figures S8 and S9 in the supplementary information. The simulations confirm that the observed strong coupling artefacts arise from the fundamental form of the

experiment, rather than as the result of imperfect coherence pathway selection. The artefacts are the subject of ongoing investigations. It is possible that they could be reduced by using the triple spin echo (TSE) technique suggested by Foroozandeh et al [23], but this has yet to be verified experimentally. The simulations also confirm that the relative intensities of the correlations observed are independent of the digital resolution (where a single value of T_2* is common to all the signals).

Having established the utility of the experiment, we have been able to use it profitably in a number of cases where the achievable effective resolution in classic COSY experiments was limited by multiplet overlap. An example is shown in Figure 7.


2.90
2.94
2.98
3.02
3.06
3.10
3.14
3.18
f1 (ppm)
3.14
3.09
3.04
2.99
2.94
2.89
(ppm)

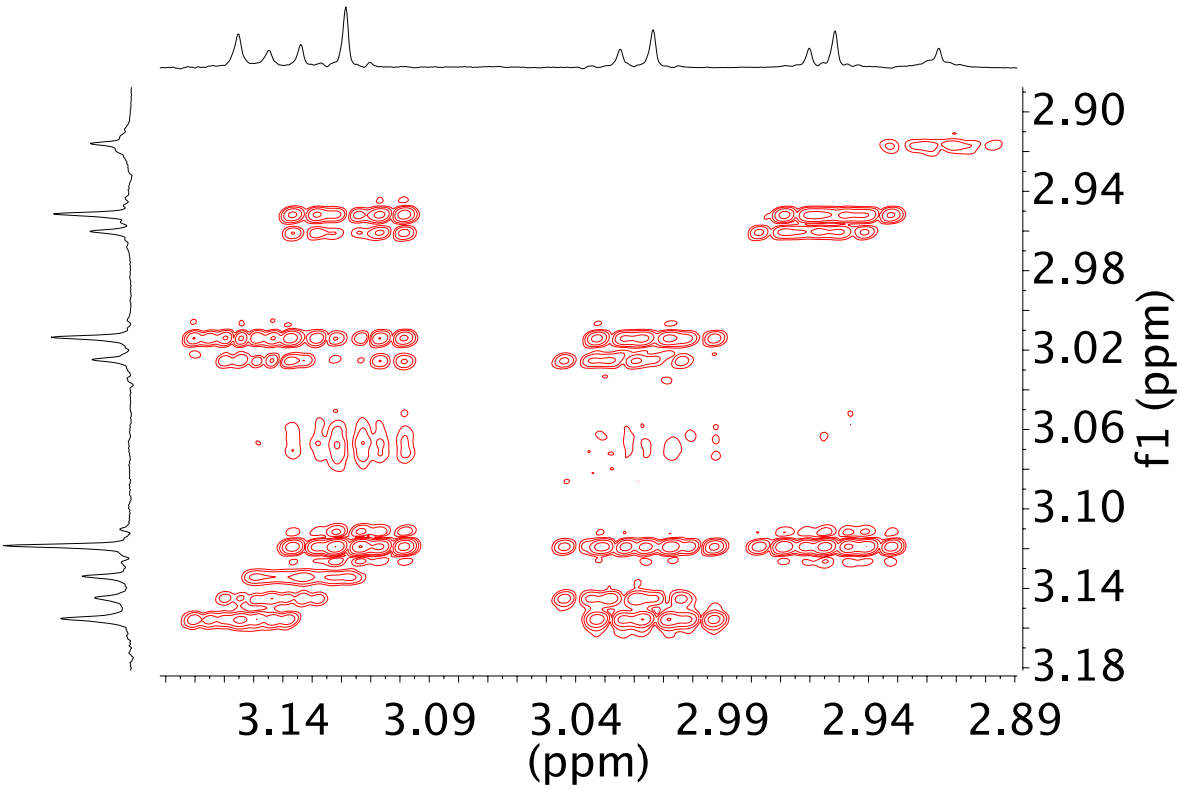

2.90
2.94
2.98
3.02
3.06
3.10
3.14
3.18
f1 (ppm)
3.14
3.09
3.04
2.99
2.94
2.89
(ppm)

Figure 7. Top: Expansion of part of the gCOSY spectrum of cellobiose (a 1,4-dimer of glucose) in DMSO-d_6 at 600 MHz. Bottom: Expansion of part of the PSYCOSY spectrum of cellobiose (a 1,4-dimer of glucose) in DMSO-d_6 at 600 MHz. Each cellobiose molecule contains two glucose rings and one of the rings can exist as one of two anomers. The ability of the PSYCOSY spectrum to resolve separate correlations in heavily overlapped multiplets is clearly demonstrated. In both spectra the spectral window in each dimension was 6kHz. For each FID 4096 complex data points were acquired in t_2 (acquisition time = 681.6 ms). 512 non-uniformly (incoherently) spaced increments were acquired in t_1, using a sampling schedule suitable for reconstruction to 4096 uniformly spaced increments. Reconstruction was carried out using the Iterative Soft Thresholding (IST) algorithm implemented in the Mnova software (MestreLab Research). The final Fourier transform size was 8k x 8k. The coherence evolution time (t_{ce}) in the PSYCOSY was 71ms, corresponding to a J value of 7 Hz. The total experiment time was 55 minutes in both cases. The full PSYCOSY spectrum of cellobiose is shown in the supplementary information (Figure S10), together with the complete assignment of the proton chemical shifts of both anomers (Table S1).

A further example is given in the supplementary information (Figure S14). The use of the PSYCHE refocussing element does entail a significant reduction in sensitivity compared to the classic COSY experiment, but we have not found this to be problematic working on modern spectrometers at concentrations typically encountered in synthetic chemistry.

Experimental

Rotenone (analytical standard) was purchased from Sigma Aldrich and used without further purification. D-(+)-cellobiose was purchased from Fluka and used without further purification. Spectra of rotenone were acquired in $CDCl_3$ solution at 600 MHz on a Varian VNMRS-600. The COSY spectra (Figure 2 and Figure 7(top)) were acquired using the standard Varian implementation of the gCOSY sequence (VNMRJ4.2). The CT-COSY spectrum (Figure 6-top) was acquired using a previously described pulse sequence [15]. Theoretical simulations were

carried out for the disconnected 11-spin system on the 'right hand side' of rotenone molecule (Figure 1) using the Fokker-Planck theory [24] module of Spinach library [25]. IK-2 basis set was used [26] with 100 spatial discretisation points. All other parameters were matched to the corresponding instrumental settings. The simulation source code, with a link to this paper, will be available in the example set of Spinach library versions 2.1 and later. The simulations also include a reference signal (at 5.8 ppm) that is not coupled to anything else and whose intensity should therefore be invariant as a function of the coherence evolution time (neglecting relaxation effects).

Conclusions

By separating the coherence evolution from the chemical shift evolution and employing the compressed sensing, we are able to acquire Pure Shift ^{1}H COSY spectra at digital resolutions approaching 1 Hz/point while retaining discrimination in favour of correlations due to the larger couplings that typify short range couplings (^{n}J, where n <=4). Thus, we can greatly increase the effective resolution available in COSY experiments without introducing significant numbers of additional correlations that would make interpretation difficult. The penalty in terms of sensitivity is generally acceptable and acquisition times are not excessive.

Acknowledgements

The authors thank the University of Durham for support and gratefully acknowledge the assistance of Dr Vadim Zorin (Mestrelab Research S.L.) for assistance in importing the results of Spinach simulations into MestreNova for subsequent data processing.

Data Accessability

The raw NMR data used to generate the figures in this paper can be downloaded from DOI: (to be provided at proofs)

References

1. R. W. Adams; *eMagRes*, 2014, **3**, 295. DOI 10.1002/9780470034590.emrstm1362

2. K. Zangger; *Progress in Nuclear Magnetic Resonance Spectroscopy* 2015, **86-87**, 1.

3. E. J. Candes, M. B. Wakin; *IEEE Signal Process*. Mag. 2008, **25**, 21.

4. K. Kazimierczuk, V. Y. Orekhov; *Angewandte Chemie International Edition* 2011, **50**, 5556.

5. D. J. Holland, M. J. Bostock, L. F. Gladden, D. Nietlispach; *Angewandte Chemie International Edition* 2011, **50**, 6548.

6. J. A. Aguilar, A. M. Kenwright; *Magnetic Resonance in Chemistry*, In Press; DOI:10.1002/mrc.4705

7. J. A.Aguilar, R. Belda, A. Botana, A. M. Kenwright; *RSC Advances* 2016, **6**, 83380.

8. L. R. Brown; *Journal of Magnetic Resonance* 1984, **57**, 513.

9. M. E. Girvin; *Journal Of Magnetic Resonance Series A* 1994, **108**, 99.

10. A. Bax, R. Freeman; *Journal of Magnetic Resonance* 1981, **44**, 542.

11. J. A. Aguilar, P. Király, R. W. Adams, M. Bonneau, E. J. Grayson, M. Nilsson, A. M. Kenwright, G. A. Morris; *RSC Advances* 2015, **5**, 52902.

12. A. Lupulescu, G. L. Olsen, L. Frydman; *Journal of Magnetic Resonance* 2012, **218**, 141.

13. J. Mauhart, S. Glanzer, P. Sakhaii, W. Bermel, K. Zangger; *Journal of Magnetic Resonance* 2015, **259(C)**, 207.

14. M. R. M. Koos, G. Kummerlöwe, L. Kaltschnee, C. M. Thiele, B. Luy; *Angewandte Chemie International Edition* 2016, **55**, 7655.

15. J. A. Aguilar, J. Cassani, M. Delbianco, R. W. Adams, M. Nilsson, G. A. Morris; *Chem. Eur. J.* 2015, **21**, 6623.

16. V. M. R. Kakita, R. V. Hosur; *ChemPhysChem* 2016, **17**, 2304.

17. M. Foroozandeh, R. W. Adams, N. J. Meharry, D. Jeannerat, M. Nilsson, G. A. Morris; *Angewandte Chemie International Edition* 2014, **53**, 6990.

18. K. Zangger, H. Sterk; *Journal of Magnetic Resonance* 1997, **124**, 486.

19. J. A. Aguilar, M. Nilsson, G. A. Morris; *Angewandte Chemie International Edition* 2011, **50**, 9716.

20. Z. Wu, A. Bax; *Journal of Magnetic Resonance* 2001, **151**, 242.

21. L. Guduff, I. Kuprov, C. van Heijenoort, J-N. Dumez J-N; *Chemical Communications* 2016, **53**, 701.

22. L. Guduff, A. J. Allami, C. van Heijenoort, J-N. Dumez, I, Kuprov; *Physical Chemistry Chemical Physics* 2017, **19**, 17577.

23. M. Foroozandeh, R. W. Adams, P. Kiraly, M. Nilsson, G. A. Morris; *Chem. Commun.* 2015, **51**, 15410.

24. I. Kuprov; *Journal of Magnetic Resonance* 2016, **270**, 124.

25. H. J. Hogben, M. Krzystyniak, G. T. P. Charnock, P. J. Hore, I. Kuprov; *Journal of Magnetic Resonance* 2011, **208**, 179.

26. L. J. Edwards, D. V. Savostyanov, Z. T. Welderufael, D. Lee, I. Kuprov; *Journal of Magnetic Resonance* 2014, **243**, 107.