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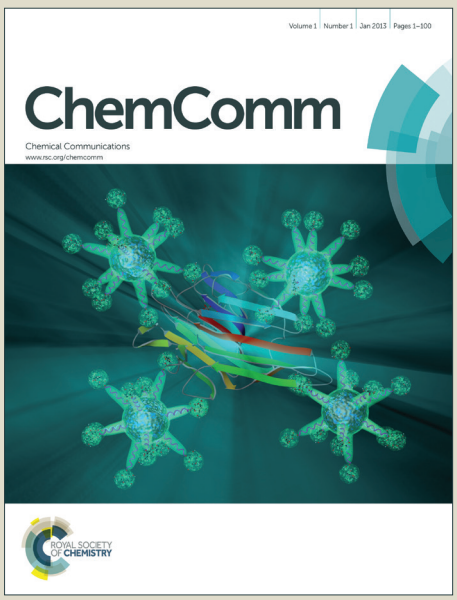

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ARTICLE TYPE

Ultra high-resolution HSQC: Application to the efficient and accurate measurement of heteronuclear coupling constants

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A rapid NMR data acquisition strategy in terms of enhanced resolution per time unit for the simple and efficient determination of multiple coupling constants is described. The use of ^{13}C spectral aliasing combined by broadband ^{1}H homodecoupling allows accurate measurements from ultra high resolved 2D HSQC cross-peaks.

Digital resolution and signal resolution are two important concepts in NMR spectroscopy. One of the more critical parameters defining the total acquisition time of a 2D NMR experiment is the number of t_1 evolution times required to achieve a satisfactory digital resolution in its indirect F1 dimension. This is particularly important when analyzing highly congested areas where signal overlap can occur due to the lack of resolution. Many different solutions to improve this limitation have been proposed over the years such as the use of region-selective pulses,[1] spectral-aliasing,[2–8] non-uniform sampling or maximum-entropy reconstruction algorithms,[9] among others. Of these, the use of spectral aliasing plays a particular role for its great simplicity, general application and high efficiency, as demonstrated by the interesting applications reported for kinetic, diffusion and titration NMR studies, in addition to structural characterization of similar compounds or the analysis of highly overlapped spectra and complex mixtures.

In this study, the success to implement spectral aliasing into routine NMR experiments is expanded by demonstrating its high relevance in the easy measurement of coupling constants from the indirect dimension of 2D HSQC spectra. It is also shown its full compatibility with modern pure shift NMR techniques,[10–13] enhancing even more signal dispersion, as recently reported for the determination of small chemical shift differences in enantiodifferenation studies.[14] The joint effects resulting to combine ^{13}C spectral aliasing in the F1 dimension and broadband ^{1}H homonuclear decoupling in the detected F2 dimension of a 2D HSQC experiment affords ultra high resolved cross-peaks from which the analysis and the extraction of accurate J values becomes more efficient.

For proof of principle, we illustrate our proposal by measuring the sign and the magnitude of both J(CF) and J(HF) coupling constants in fluorinated compounds from the clean E.COSY pattern obtained in high-resolved HSQC spectra.[15–21] Attempts to measure these couplings from a regular 2D HSQC spectrum frequently meets with the lack of spectral resolution along the F1 dimension. Spectral aliasing is easily achieved by setting a very small ^{13}C spectral width (SW(^{13}C)), and the practical consequence is a tremendous resolution enhancement without any other special requirements for pulse sequence modification, particular hardware configuration, additional set-up or the need for post-processing tools. For instance, using a conventional SW(^{13}C) of 160 ppm and 128 t_1 increments, a poor digital resolution of 251.5 Hz/Pt is achieved before data processing. Reducing SW(^{13}C) to 2 ppm, an improved digital resolution of 3.1 Hz is automatically achieved which should be equivalent to acquire 10200 t_1 increments, representing an increased factor in terms of resolution or acquisition time of about 80. As an example, Figure 1A shows the spectral-aliased HSQC spectrum of 2-fluoropyridine recorded in a 400 MHz spectrometer equipped with a standard broadband probehead. Excellent resolution levels are achieved using SW(^{13}C) of 2 ppm and 128 t_1 increments, within a short experimental time of 7 minutes and without need of any additional prior calibration or set-up. After data processing, resolution in the F2 and F1 dimensions is 0.5 and 0.2 Hz/Pt, respectively.

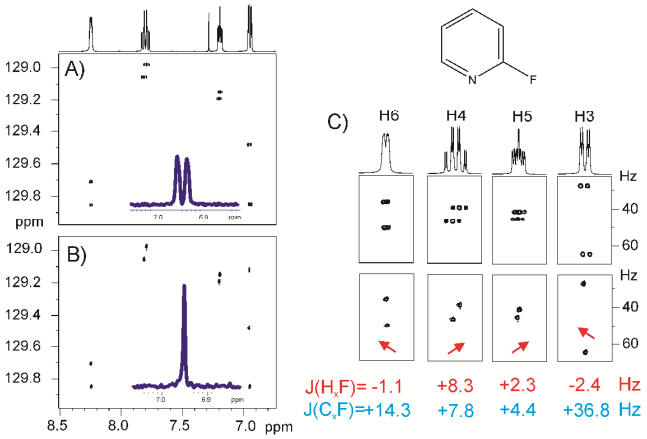

Figure 1: 2D spectral-aliased ^{1}H-^{13}C HSQC spectrum of 2-fluoropyridine acquired with a reduced SW(^{13}C) of 2 ppm: A) without and B) with broadband ^{1}H homodecoupling in the F2 dimension. C) Expanded cross-peaks showing the high levels of digitization and signal dispersion achieved for each experiment.

Spectral aliasing depends of the quadrature detection mode used in the F1 dimension and, in contrast to the effects associated to spectral folding, the phase properties and the appearance of the E.COSY multiplet structure in the reported HSQC are retained as in the original experiment. Hence, the magnitude and the relative

sign between CF and HF couplings can be extracted by a direct and simple analysis of each individual signal. For instance, note the clear splitting and the relative positive/negative slope for all cross-peaks, even for the small couplings of J(C3-F)=+4.38 Hz and J(H3-F)=+2.25 Hz displayed for the C3-H3 correlation or the small J(H6-F)=-1.10 Hz. All data agree with previously reported results[22] and simple modifications of the basic pulse sequence can offer additional measurements, such as the simultaneous determination of a complete set of the magnitude and the sign of $^{1}J(CH)$, J(FH) and J(FC) coupling constants from a F2-^{13}C-coupled spectral-aliased HSQC spectrum (Figure S1). In these spectra, the observed chemical shift value from signals outside of the active window deviates from its true value due to the extensive signal aliasing. In practice, this is not a problem because the determination of coupling constants is usually performed after a chemical shift assignment process and, therefore the real chemical shift can be reestablished, if needed, comparing aliased data from a reference HSQC or 1D ^{13}C spectrum,[2] by recording two differently SW(^{13}C)-optimized datasets[2,3,8] or using computer-optimized methods.[4] Anyway, the ambiguity about the incorrect δ(^{13}C) assignment in HSQC spectra is easily resolved because each individual proton only yields a single cross-peak

Figure 2 compares the different 2D cross-peak resolution exclusively as a function of SW(^{13}C), whereas all other experimental parameters remain exactly the same. Clearly, the use of SW(^{13}C) between 2-5 ppm resolves most of the coupling patterns. It is also shown how signal dispersion is further enhanced from the spectral-aliased pure shift HSQC experiment which uses a BIRD-based element for homonuclear decoupling during acquisition,[13,14] (see Figures 1B and right column in 2B). In addition to the evident simplification of the multiplet structure, a relative sensitivity gain is also achieved by signal collapsing as shown in 1D traces of Fig. 1.

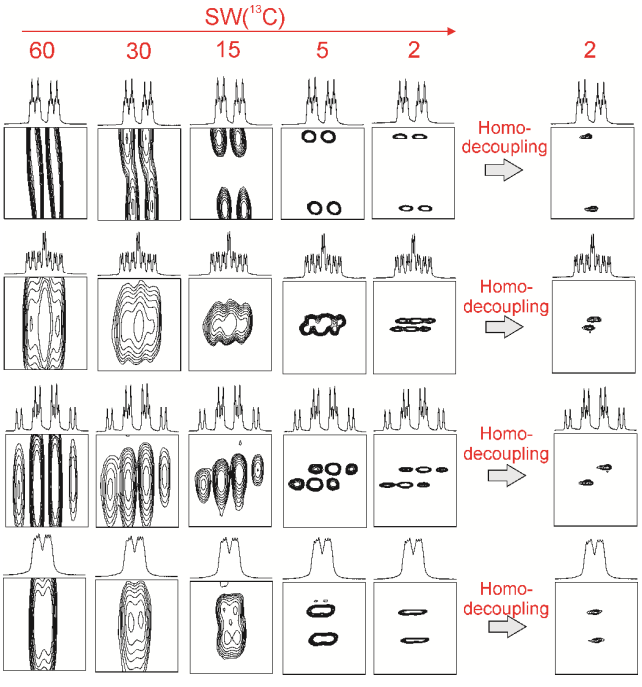

Figure 2: Experimental effects on signal resolution after reducing SW(^{13}C) in HSQC experiments. In the right column, the additional benefits to add broadband ^{1}H homodeocupling along the detected F2 dimension can be appreciated.

The performance of the experiment has also been verified with albaconazole, a triazole derivative with potent and broad spectrum antifungal activity containing two fluorine atoms in its structure. The advantages of 2D multiplet simplification are visible from the results obtained from the double E.COSY nature of some cross-peaks (Figure 3). The relative sign and the magnitude of four- and five bonds J(FH) and J(CF) couplings are readily and simultaneously measured. It can be seen how the highly overlapped H-21 and H23 can be clearly distinguished, allowing the easy measurement of their couplings. In the case of H-23, note the different positive/negative skew observed for their $^{3}J(HF)$ and $^{5}J(HF)$ correlations. Note that in the case of the diastereotopic H-13 and H-13' protons, the geminal $^{2}J(HH)$ is still observed because BIRD cannot homodecouple these interactions. In these protons, small and positive five-bond $^{5}J(H13\text{-}F20)$ couplings smaller than the linewidth can be determined, even without being resolved in the conventional ^{1}H multiplet.

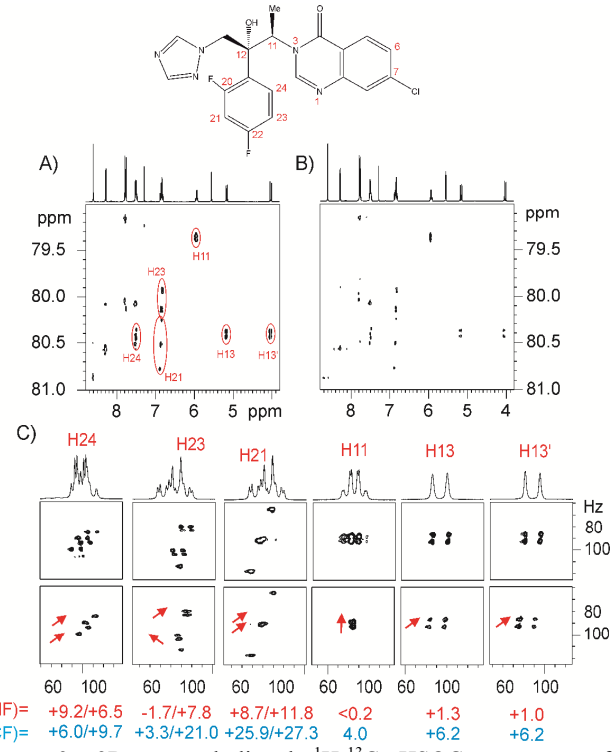

Figure 3: 2D spectral-aliased ^{1}H-^{13}C HSQC spectrum of albaconazole acquired with a reduced SW(^{13}C) of 2 ppm: A) without and B) with broadband ^{1}H homodecoupling in the detected dimension. C) Expanded 2D cross-peaks showing the high levels of digitization and signal dispersion for each experiment.

A further example involves the measurement of the magnitude and sign of J(CP) and J(HP) in phosphorus-containing molecules (Figure 4). Previous studies performed these measurements from conventional experiments applying numerous t_1 increments, using scaling J factors along the F1 dimension or triple resonance $^{1}H/^{13}C/^{31}P$ NMR experiments.[23,24] Note, for instance, the advantageous resolution conditions resolution for the wide and highly complex ^{1}H resonance corresponding to the olefinic H2 proton in allyltriphenylphosphonium bromide, which present an overall multiplet width of 45.9 Hz. The H2-C2 HSQC cross-peak is reduced to an ultra simplified and well resolved two-component E.COSY multiplet pattern with line widths of only 3.5

Hz (Figure S4). It must also be highlighted that $^{4}J(CP)$ and $^{5}J(PH)$ are precisely measured. The absolute signs of the involved couplings can be obtained taken a known coupling as a reference cross-peak. In absence of this reference, a spectral-aliased HSQC-TOCSY experiment can be very helpful because provides different cross-peaks for the same ^{1}H or ^{13}C peak (Figure S5). Thus, comparison the skew pattern of all cross-peaks for a determined proton (selected column) or a specific carbon (selected row) can facilitate this determination.

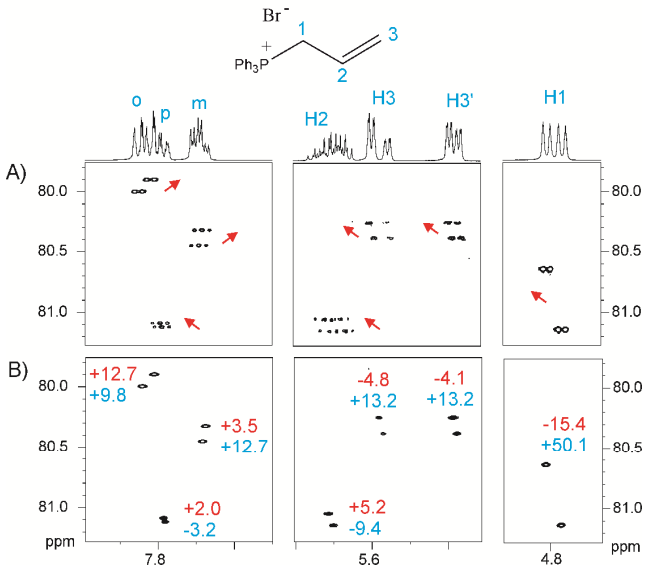

Figure 4: A) spectral-aliased 2D ^{1}H-^{13}C HSQC spectrum of allyltriphenylphosphonium bromide acquired with a SW(^{13}C) of 2 ppm. B) Pure shift version showing ultra simplified cross-peaks. The sign and the magnitude of (top) $J(H_xP)$ and (bottom) $J(C_xP)$ couplings are shown for each cross-peak.

Finally, the method has been applied to a mixture of common deuterated solvents (acetonitrile, acetone, dimethyl sulfoxide, methanol and methylene chloride) for the fast and efficient measurement of J(HD) and J(CD) in residual mono-deuterated isotopomeric derivaatives (Figure 5). The negative slope for all observed cross-peaks confirms the negative sign of the small $^{2}J(HD)$ couplings, assuming that $^{1}J(CD)$ is positive. The high precision achieved in the indirect dimension makes of these experiments very interesting to obtain H/D and $^{12}C/^{13}C$ isotope effects on both ^{1}H and ^{13}C chemical shifts.

It can be anticipated that spectral aliasing can be extended in a variety of NMR experiments involving J measurement from the F1 dimension of a 2D spectrum. The most obvious applications should be the measurement of the reported J(XH) and J(CX) couplings on non-protonated carbons from spectral aliased HMBC or HSQMBC experiments or the measurement of $^{1}J(CH)$ along the F1 dimension of F1-coupled HSQC spectra, with a particular interest in the measurement of residual dipolar couplings (RDCs) in small molecules dissolved in weakly aligned anisotropic media.[25] Also of interest should be the measurement of long-range proton-carbon coupling constants, as reported for the SIS-HSQC experiment which provide the sign and the magnitude of J(HH) and $^{n}J(CH)$[26] (see Figure S6). The feasibility of simplifying multiplet patterns by broadband homodecoupling in this type of experiments is under investigation and will be published elsewhere.

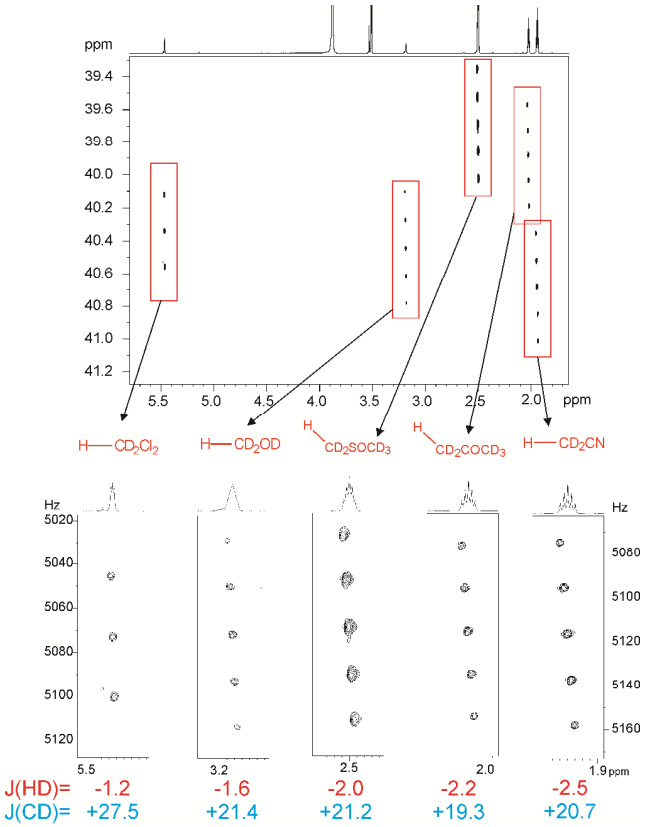

Figure 5: 2D spectral aliased ^{1}H-^{13}C HSQC spectrum of a mixture of deuterated solvents acquired with a SW(^{13}C) of 2 ppm. The residual mono-protonated isotopomers are quickly observed in the HSQC spectrum, allowing the fast measurement of $^{2}J(HD)$ and $^{1}J(CD)$.

The proposed strategy is far superior to other NMR methods which have been recently introduced to measure these same heteronuclear couplings, some of them requiring sophisticated pulse sequences with specialized set-up, requiring special hardware configuration such as the need for requiring triple-resonance hardware capabilities to perform fluorine detection,[15,16] others only provide the magnitude of J(HF) couplings[17–20] or/and do not determine the positive/negative sign of the coupling.[19–21] In addition, despite the relative sensitivity losses and the minimum increment of total acquisition time associated with the very narrow SW(^{13}C) and consequent long evolution times required for the multiple aliasing method (Figure S2), our method offers optimum sensitivity without the important losses associated to other related pure-shift methods. Also, its 2D nature allows it to be used for assignment purposes, avoiding the limitation of signal overlap signals of 1D NMR methods.

Conclusions

In summary, it has been shown that the superb digital resolution achieved in spectral aliased HSQC experiments allows the easy and simultaneous determination of the magnitude and the sign of J(CX) and J(HX) coupling constants (X=^{19}F, ^{31}P or ^{2}H). A common feature of spectral aliasing is its general implementation in many routine experiments, even in low field magnets, improving the attainable resolution along the F1 dimension up to two orders of magnitude by a simple change of the ^{13}C spectral width. It has been shown that the gains of introducing aliasing are further improved with the large signal

resolution achieved by the collapsing of J(HH) multiplet structure by broadband ^{1}H homodecoupling in the F2 dimension. The resulting 2D cross-peaks exhibit ultra simplified multiplet patterns from which the measurement of the active J values is determined in a straightforward manner. As pointed out already, this general approach introduced in this study can be applicable in many experiments aimed at determining coupling constants with high accuracy. Finally, it should be added that the presented approach is fully compatible with other enhancing methods, such as non-uniform sampling, improving even more the signal resolution obtained per time unit.

Notes and references

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