ULTRA HIGH-RESOLUTION HSQC: APPLICATION TO THE EFFICIENT AND ACCURATE MEASUREMENT OF HETERONUCLEAR COUPLING CONSTANTS

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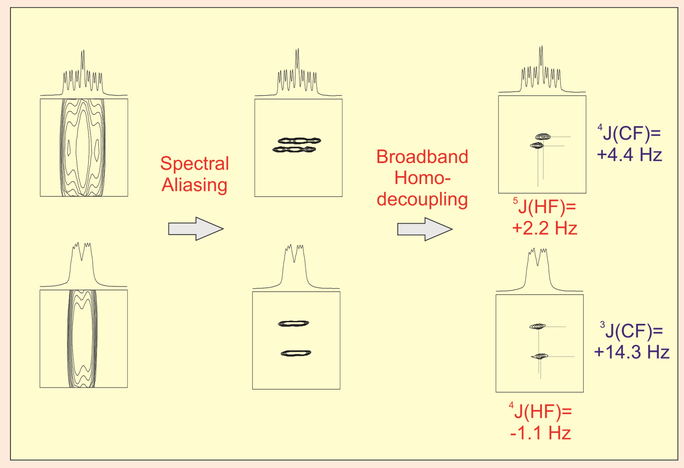
Introduction

Digital resolution and signal resolution are two important concepts in multidimensional NMR spectroscopy. A key parameter defining the total acquisition time of a 2D NMR experiment is the number of variable t_1 evolution times required to achieve a satisfactory digital resolution along its indirect F1 dimension. In this study, the success in implementing **spectral aliasing along the indirect F1 dimension of HSQC experiments**[1-3] is demonstrated by the easy and fast measurement of heteronuclear coupling constants from the indirect dimension of 2D HSQC spectra, without any significant increase of the experimental time. It is also shown that these gains can be further improved with the large spectral resolution achieved by the collapse of the J(HH) multiplet structures to singlets by **broadband ^{1}H homodecoupling along the F2 dimension**.[4-5] The resulting 2D cross-peaks exhibit ultra simplified multiplet patterns from which the efficient measurement of J coupling values is performed in a straightforward manner. Experimental data are provided for the **simultaneous determination of the magnitude and the sign of J(CX) and J(HX) coupling constants** from a single cross-peak (X = ^{19}F, ^{31}P or ^{2}H).[6]



Results and Discussion

Spectral aliasing in HSQC experiments is easily achieved by setting a small ^{13}C spectral width (SW(^{13}C)), and the practical consequence is a tremendous resolution enhancement in the F1 dimension without any other special requirements such as pulse sequence modification, particular hardware configuration, additional set-up or the need for post-processing tools. The use of complementary broadband BIRD-based homodecoupling along the F2 dimension affords ultra resolved cross-peaks (Fig. 2) .

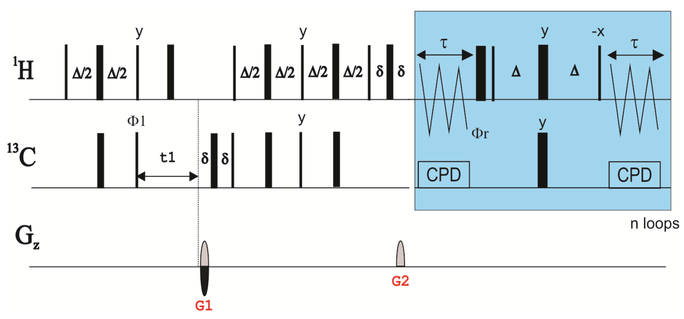


Figure 1: Sensitivity-enhanced HSQC pulse sequence incorporating BIRD-based broadband ^{1}H homodecoupling during acquisition. All NMR experiments were acquired on a Bruker AVANCE spectrometer operating at 400.13 MHz proton frequency, equipped with a 5 mm BBOF probe and a z-axis pulsed field gradient accessory (maximum strength of 53.5 G/cm). The Δ delays were optimized to 140-160 Hz (Δ=1/(2*JCH)) depending of the sample.

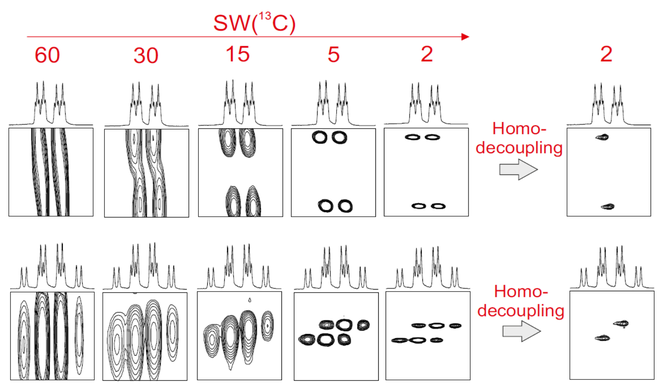


Figure 2: Experimental effects achieved on signal resolution after reducing SW(^{13}C) (from 60 ppm to 2 ppm) in HSQC spectra. In the right column, the additional benefits to add broadband ^{1}H homodecoupling along the detected F2 dimension can be appreciated.

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 (Fig. 3-4).

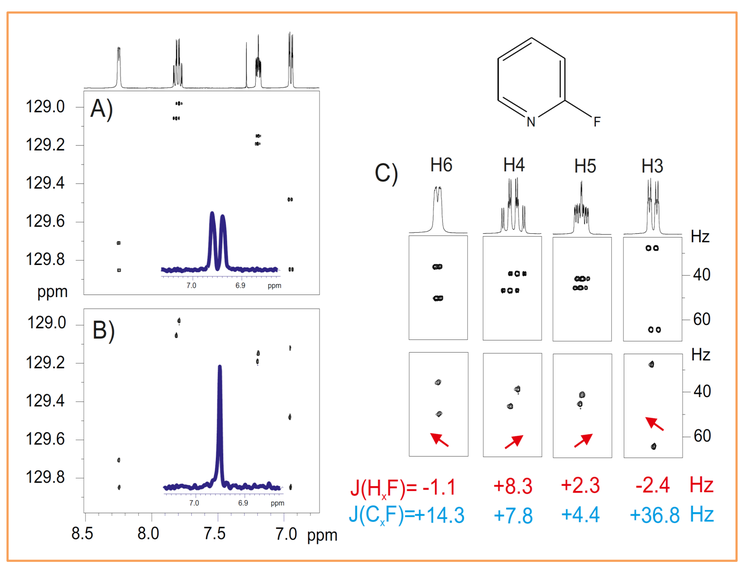


Figure 3: 2D spectral-aliased ^{1}H–^{13}C HSQC spectrum of 2-fluoropyridine (20 mg in 0.6 ml of $CDCl_3$) acquired with a reduced SW(^{13}C) of 2 ppm: (A) without and (B) with broadband ^{1}H homodecoupling in the F2 dimension. (C) Expanded cross-peaks showing the high levels of digitization and signal dispersion achieved for each experiment. Two scans were collected for each of the 128 t_1 values with 2048 complex points in the corresponding FID. The total experimental time was about 7 minutes for each 2D spectrum. Broadband homodecoupling during acquisition was achieved applying 130 loops (n) with τ =8-10 ms. After standard data processing, the resolution in the F2 and F1 dimensions is 0.5 was 0.2 Hz/pt, respectively.

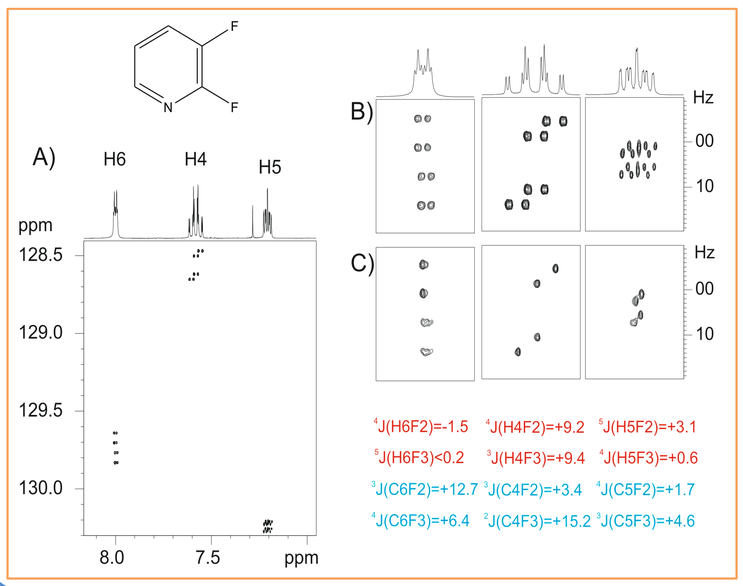
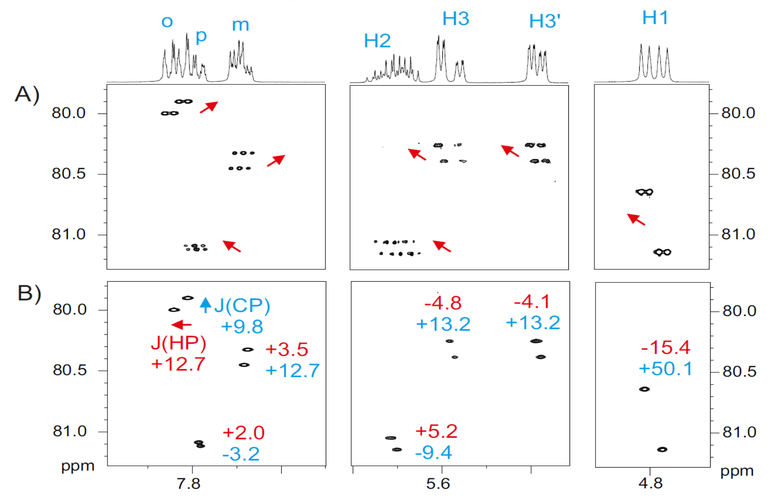


Figure 4: A) 2D spectral-aliased ^{1}H–^{13}C HSQC spectrum of 2,3-difluoropyridine (20 mg in 0.6 ml of $CDCl_3$) acquired with a reduced SW(^{13}C) of 2 ppm: (B,C) Expanded cross-peaks showing the high levels of digitization and signal dispersion achieved for each experiment B) without and C) with broadband ^{1}H homodecoupling in the F2 dimension. Experimental conditions as described in Fig. 3.

The phase properties and the appearance of the E.COSY multiplet structure in the reported spectral-aliased HSQC are retained as in the original experiment. A further example involves the measurement of the magnitude and sign of J(CP) and J(HP) in phosphorus-containing molecules (Figure 5). The analysis of the positive/negative slope of a single cross-peak only provides information about if the involved couplings have the same or opposite sign. Alternatively, the analysis of a reference cross-peak or the positive/negative slope for a set of cross-peaks into the same column (they have the same J(HP) coupling) or the same row (they have the same J(CP)) in a complementary spectral-aliased HSQC-TOCSY experiment helps in the absolute determination of the positive/negative sign of the involved couplings.



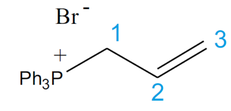


Figure 5: 2D spectral-aliased ^{1}H–^{13}C HSQC spectrum of allyltriphenylphosphonium bromide (20 mg in 0.6 ml of $CDCl_3$) acquired with a reduced SW(^{13}C) of 2 ppm: (A) without and (B) with broadband ^{1}H homodecoupling in the F2 dimension. All data were collected under the same experimental conditions as described in Fig. 3.

The method has been also applied to a mixture of common deuterated solvents for the fast and efficient measurement of J(HD) and J(CD) in residual mono-deuterated isotopomeric derivatives (Figure 6). 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 can make of these experiments an interesting tool to obtain H/D and ^{12}C/^{13}C isotope effects on both ^{1}H and ^{13}C chemical shifts.

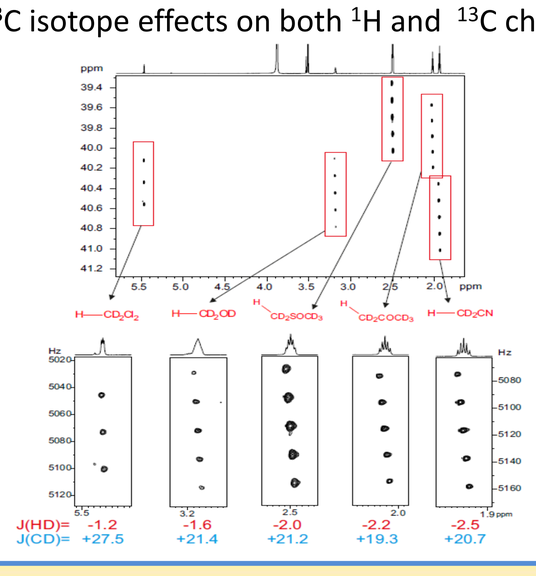


Figure 6: 2D spectral-aliased ^{1}H–^{13}C HSQC spectrum of a mixture of deuterium solvents, consisting of acetonitrile-d_3 (99,8%), acetone-d_6 (99,8%), DMSOd_6 (99,8%), metanol-d_4 (99,8) and CD_2Cl_2 (99.9%). Data were acquired and processed as described in Fig. 3.

- ✓ Ultra high-resolution HSQC spectra are quickly obtained combining spectral aliasing along the indirect F1 dimension and broadband homodecoupling along the F2 dimension.
- ✓ Easy and simultaneous determination of the magnitude and the sign of J(CX) and J(HX) coupling constants are performed in short acquisition times.
- ✓ Precise measurement using standard data acquisition and processing.
- ✓ This approach is fully compatible with non-uniform sampling (NUS), improving even more spectral resolution and shortening experimental times

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