Slice-Selective HSQMBC Experiments

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Introduction

The determination of long-range heteronuclear coupling constants ($^nJ_{XH}$, n > 1) is an important parameter in the structural and conformational analysis of natural-abundance molecules. A ^{1}H-selective α/β–HSQMBC (selHSQMBC)[1] experiment has been proposed for the accurate measurement of small $^nJ_{CH}$ on protonated and non-protonated carbons using the IPAP technique. In principle, a separate experiment is required for each individual resonance although the method can be successfully applied for multiple signals using band-selection or multiple-frequency excitation.

In this work, we incorporate the spatial-encoding concept into the selHSQMBC pulse scheme to invert each individual resonance in a different slice along the z dimension, thereby avoiding unwanted J_{HH} coupling evolution during the INEPT transfer. In this new spatially-encoded selHSQMBC (se-selHSQMBC) experiment, all resonances can be simultaneously and selectively excited in a single NMR experiment without prior knowledge of their frequencies. In order to improve the low sensitivity levels inherent to spatially-encoded NMR experiments, we apply a multi-slice selection technique based on the use of multiple-frequency modulated pulses.

Methodology

NMR Pulse Sequences

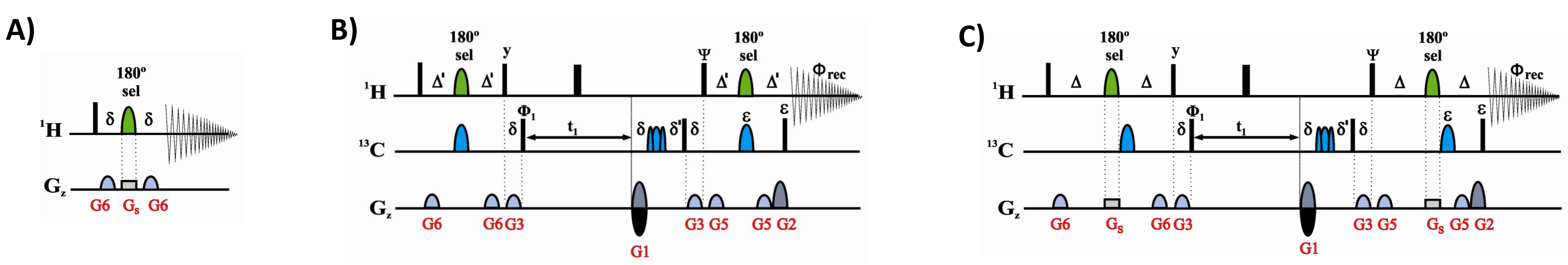

Figure 1: Pulse sequences used in this work: A) 1D spatially-encoded single pulsed-field gradient echo (se-SPFGE), B) 2D selHSQMBC[1], and C) 2D se-selHSQMBC experiments. Spatial frequency encoding is achieved by simultaneous application of a spatial-encoding gradient (G_s) and a frequency-selective 180º ^{1}H pulse. The G_s gradient is adjusted to cover a spectral width of $k*SW_{1H}$ (k≥1). G3 gradient acts as zz-purge gradient filters, G5 and G6 are used for refocused heteronuclear gradient echo and G1 and G2 are used for coherence selection. The sign of the G1 encoding gradient is alternated for echo/anti-echo coherence selection. δ is the duration of the gradients. The interpulse Δ delays (Δ'=Δ-p(180ºsel)/2) are optimized to $1/(4*^nJ_{CH})$. For the IPAP experiments, two independent in-phase (Ψ=y, ε=on) and anti-phase (Ψ=x, ε=off) data are collected and further combined as shown in Fig. 2.

IPAP Methodology

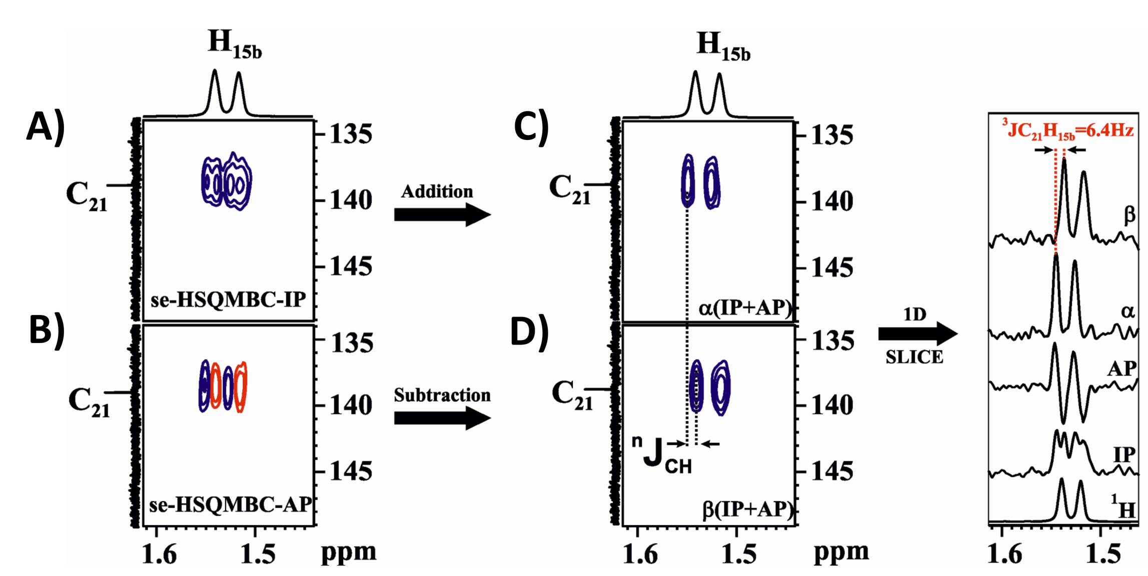

Figure 2: Our proposal uses the IPAP principle where the extraction of $^nJ_{CH}$ is realized by measuring of relative displacement of separate α- and β-cross-peak (C and D, respectively) resulting of the addition/subtraction procedure of complementary pure-phase In-Phase (IP) and Anti-Phase (AP) HSQMBC data (A and B, respectively).

Experimental Part

Multiple-frequency pulses

The se-SPFGE experiment has been used to evaluate the effectiveness of multiple-frequency pulses for improving the sensitivity of spatially-encoded experiments by simultaneous multi-slice selection.

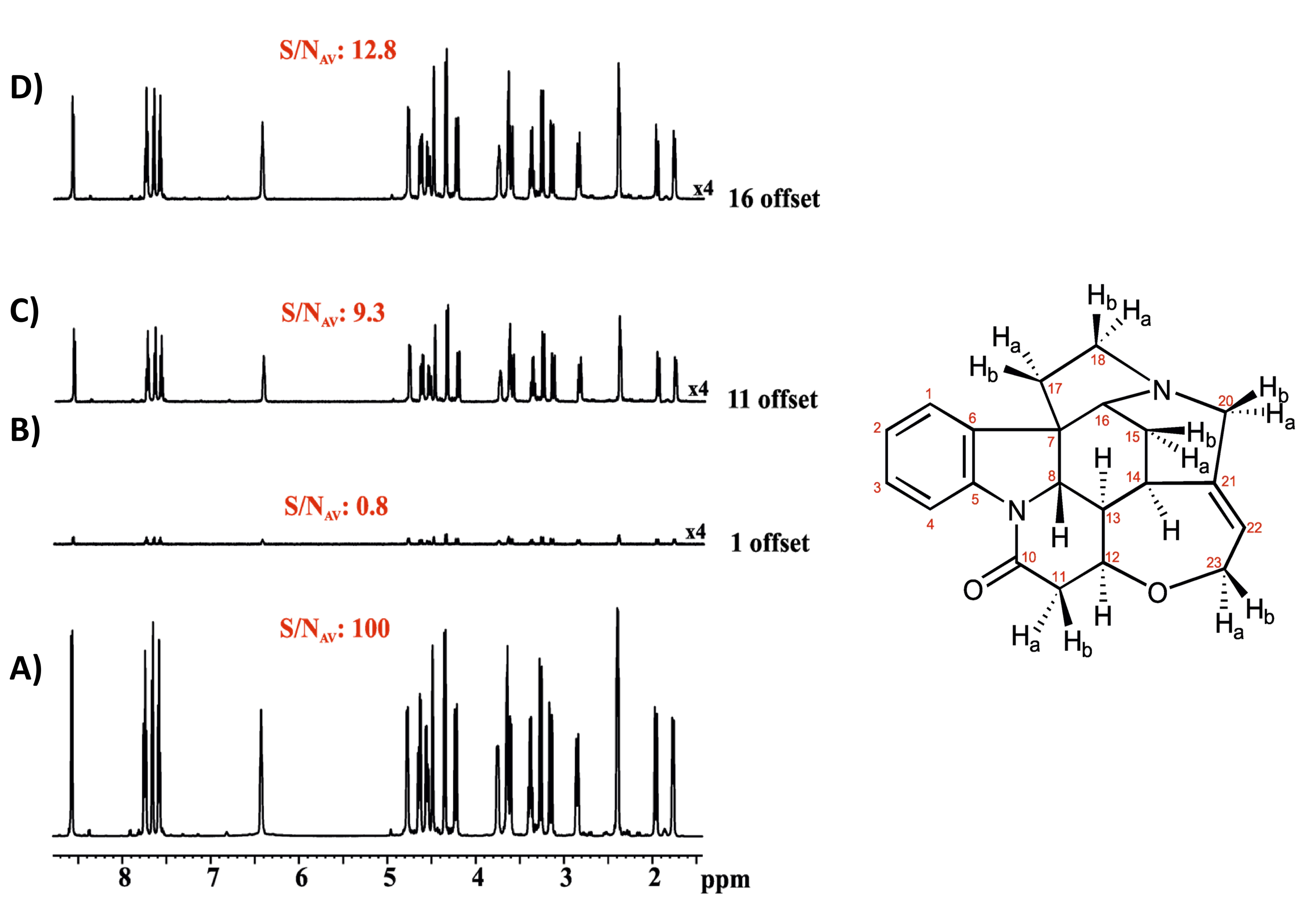

Figure 3: A) ^{1}H NMR spectrum of 0.1M strychnine in $CDCl_3$; B) Single-slice se-SPFGE spectrum using a selective Gaussian-shaped 180º ^{1}H pulse of 30ms and an encoding gradient (G_s) of 1.1 G/cm; C) Multi-slice se-SPFGE experiment acquired as B) but using a 11-site multiple-frequency pulse (restricted condition: only offsets that contain all signals into the volume coil are allowed); D) The same as C) but using a 16-site multiple-frequency pulse with offsets that include some protons out of the limits of the coil. The averaged signal-to-noise ratio percentage (S/N_{av}) is shown in each spectrum.

Spatially-Encoded *vs* conventional selHSQMBC experiments

The 16-sites multiple-frequency 180º ^{1}H pulse described in Fig. 3D has been successfully implemented into the se-selHSQMBC experiment. The results obtained are compared with some equivalent single-frequency selHSQMBC spectra, both acquired in the same conditions.

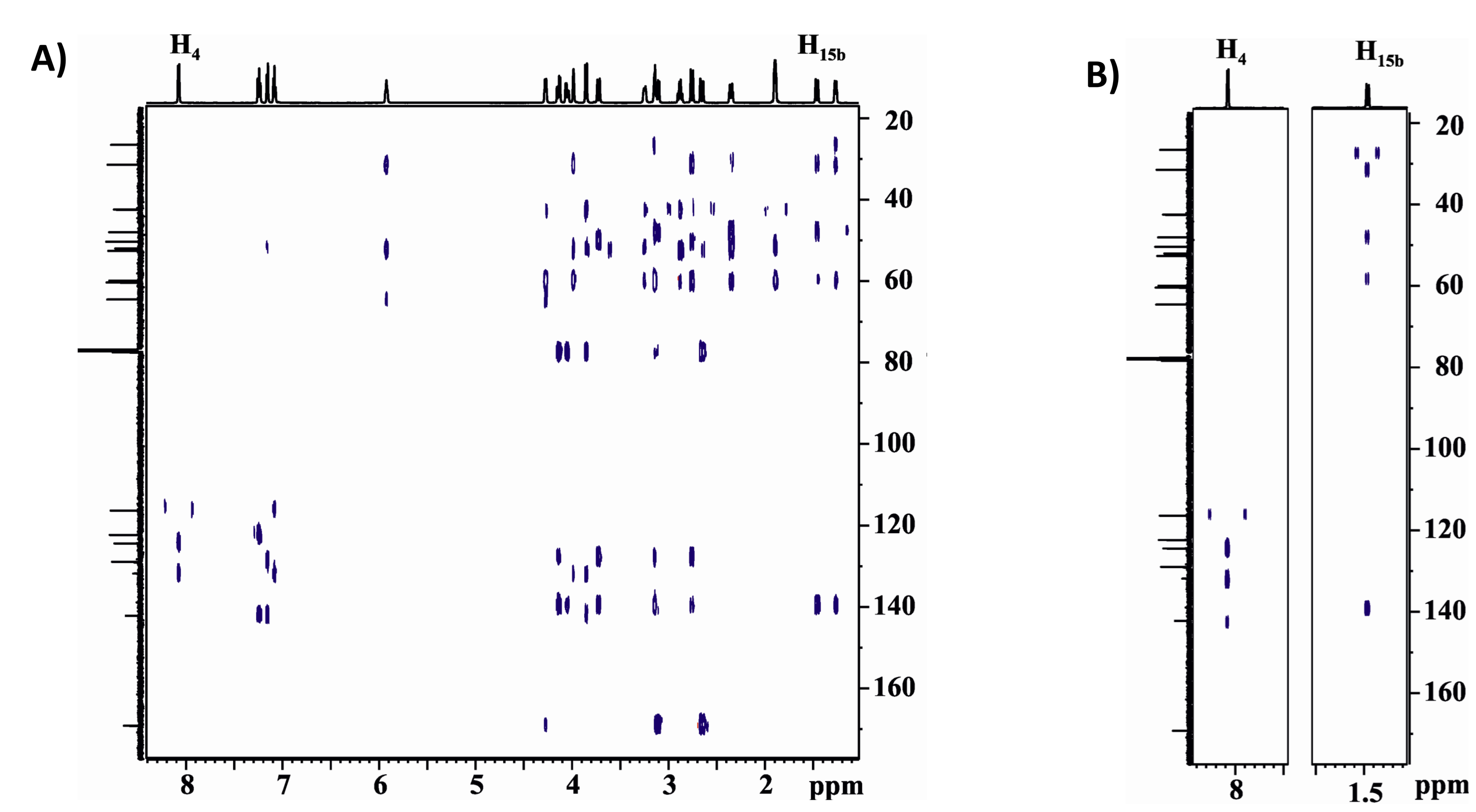

Figure 4: A) 2D se-selHSQMBC-IP spectrum using a 16-site multiple-frequency selective Gaussian-shaped 180º ^{1}H pulse of 30ms and an encoding gradient (G_s) of 1.1 G/cm; B) 2D selHSQMBC-IP spectra of the H_4 (left) and H_{15b} (right) protons using a selective Gaussian-shaped 180º ^{1}H pulse of 30ms. All the experiments were acquired with 64 scans with 64 t_1 increments for each one of them, the number of data points in t_2 was set to 4096 and the interpulse Δ delays were optimized to 8Hz (t_{exp}= 1h 40min). Prior to Fourier-transformation of each data, zero filling to 1024 in F1, 8192 points in F2 and a sine squared function in both dimensions were applied.

Relative Sensitivity

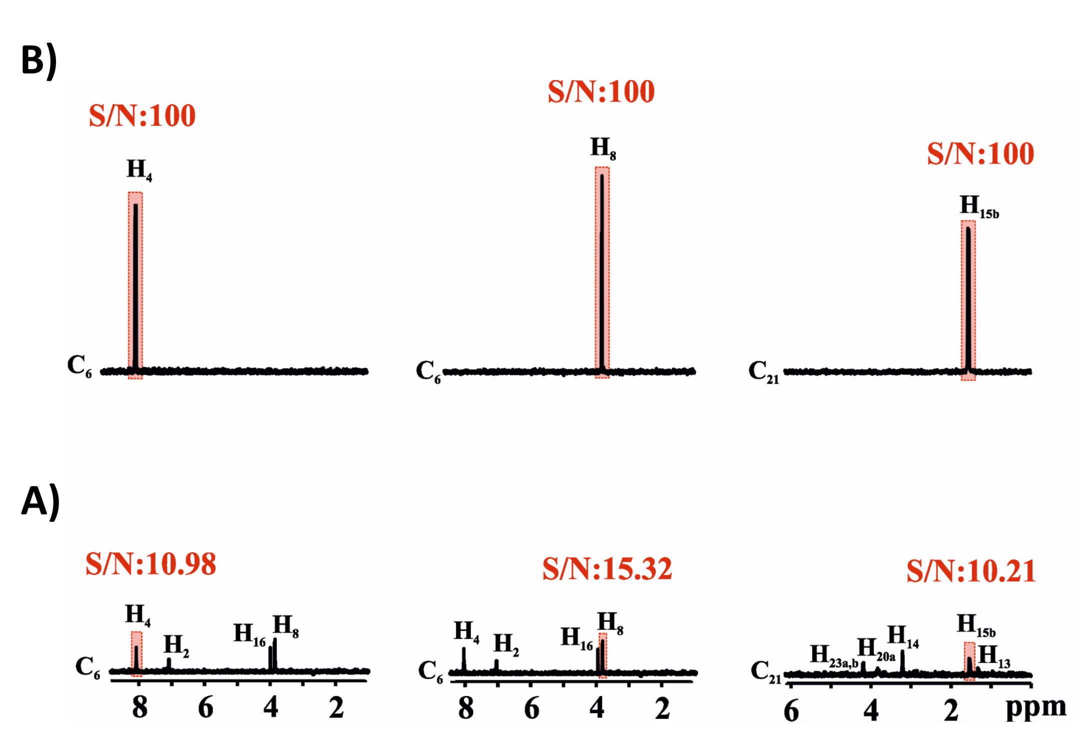

Figure 5: 1D slices extracted at the C_6 and C_{21} carbon frequencies of the A) 2D se-selHSQMBC-IP spectrum of Fig. 4A and B) of individual 2D selHSQMBC spectra of Fig. 4B. The signal-to-noise ratio percentage is shown in each case.

Experimental Determination of $^nJ_{CH}$

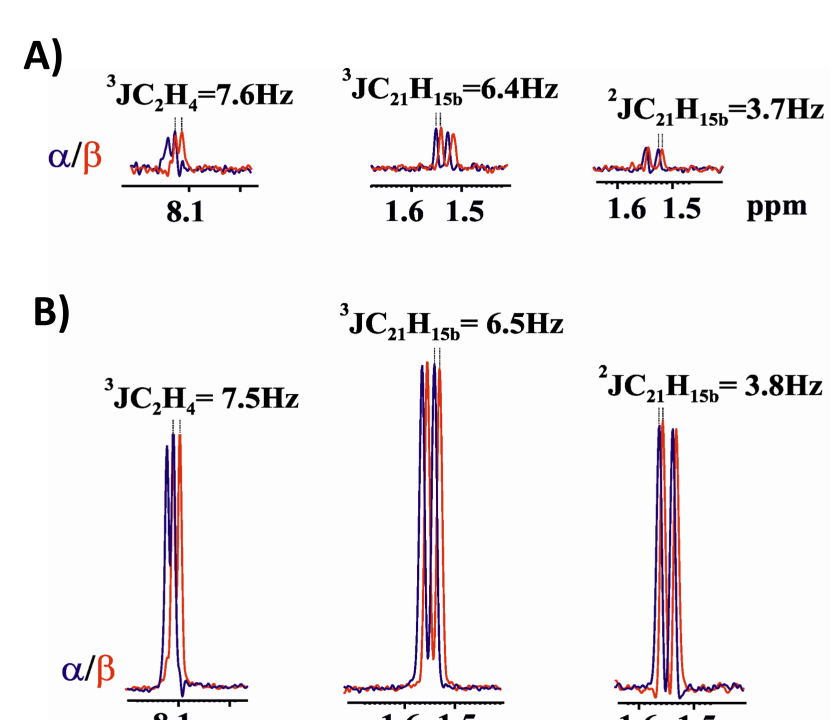

Figure 6: Comparison of the measured J values obtained from the 2D A) se-selHSQMBC vs B) individual selHSQMBC spectra.

Conclusion

In summary, we have developed a new 2D se-selHSQMBC experiment for the one-shot acquisition of multiple selective NMR experiments. In this experiment all resonances can be monitored in a single NMR experiment without prior knowledge of existing frequencies thanks to the spatial frequency encoding feature. In addition, it has been shown that the inherent low sensitivity commonly related to spatially-encoded NMR experiments can be substantially improved by applying a selective pulse modulated to multiple-frequencies.

Acknowledgements

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[1] S. Gil, J.F. Espinosa, T. Parella, *J. Magn. Reson.* **2011**, 213, 145.