# Sensitivity Enhancement of $^{13}C$-$^{15}N$ HETCOR Experiment in Static Solid-State NMR

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## Introduction

Orientation of membrane proteins is studied using double resonance ($^{1}H$-$^{15}N$) static solid-state NMR experiments. These experiments take advantage of $^{15}N$ detection to resolve amide resonances.[1] For larger proteins, however, these spectra are rather crowded and triple resonance experiments with $^{13}C$ detection are often needed. However, $^{13}C$-$^{13}C$ dipolar couplings (DCs) make $^{13}C$-detected spectra rather complex. An alternative approach is to obtain $^{13}C/^{15}N$ chemical shifts correlations using $^{15}N$-detected triple resonance heteronuclear correlation (HETCOR) experiments. Here, we propose a sensitivity enhanced version of [$^{13}C$,$^{15}N$]-HETCOR experiment based on windowless-isotropic-mixing cross-polarization (WIM-CP) that can speed up the spectroscopy of oriented membrane proteins.

## Experimental

All experiments were carried out on a U-$^{13}C$,$^{5}N$ labeled NAVL single crystal using a 600 MHz wide bore spectrometer equipped with a triple resonance low-E probe developed at the NHMFL.[2]

## Results and Discussion

In both HETCOR and sensitivity-enhanced (SE) HETCOR, homonuclear $^{13}C$-$^{13}C$ DCs are suppressed during $t_1$ evolution by the Frequency-Shifted Lee-Goldberg (FSLG) sequence. Unlike the classical HETCOR, our modified experiments incorporate a WIM-CP sequence, which transfers both the x and y components of $^{13}C$ polarization to $^{15}N$. This leads to a theoretical sensitivity enhancement of 40%. We also found that WIM-CP suppresses $^{13}C$-$^{13}C$ DCs. For the regular HETCOR, spin diffusion among $^{13}C$ nuclei during $^{13}C$-$^{15}N$ cross polarization results into inefficient $^{13}C$-$^{15}N$ transfer and lower sensitivity as shown by peak 3 in **Figure 1**. The exclusive $^{13}C/^{15}N$ transfer using WIM-CP increases the efficiency of the HETCOR experiment and allows for measurement of $^{13}C/^{15}N$ dipolar coupling from the transient oscillation of the polarization transfer.

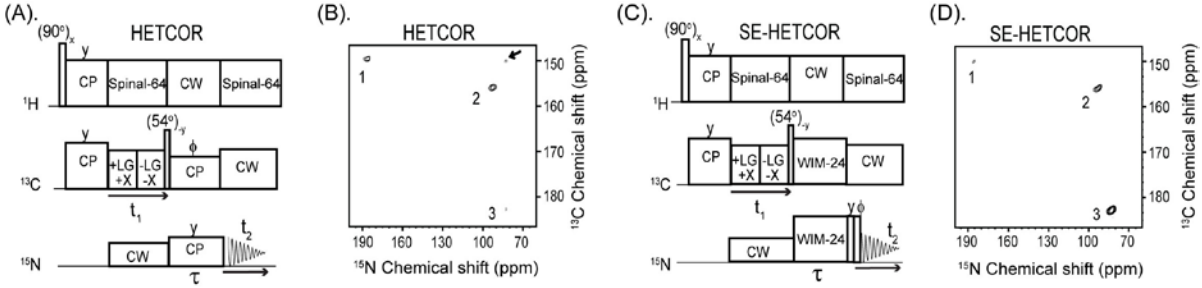


**Figure 1**:$^{13}CO$-$^{15}N$ heteronuclear correlation experiment in static solids. Pulse sequences of conventional HETCOR and SE-HETCOR are shown in (A) and (C) respectively. HETCOR and SE-HETCOR spectra drawn at same noise level are shown in (B) and (D) respectively. Sensitivity of peaks 2 and 3 is optimized by setting the $\tau$ periods to 700 and 400 $\mu$s for HETCOR and SE-HETCOR, respectively.

## Conclusions

The $^{13}C$-$^{15}N$ HETCOR is central to sequential assignment protocols and structure determination of oriented membrane proteins. We designed a new method that enhances the sensitivity of $^{13}C$-$^{15}N$ HETCOR experiments that can be applied to oriented membrane proteins.

## Acknowledgements

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## References

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