# Identifying Inter-Residue Resonances in Crowded 2D $^{13}C$-$^{13}C$ Chemical Shift Correlation Spectra of Membrane Proteins by Solid-State MAS NMR Difference Spectroscopy

Y. Miao (FSU, Chemistry); T.A. Cross (FSU, Chemistry and Biochemistry, NHMFL); R. Fu (NHMFL)

## Introduction

With the development of dipolar recoupling techniques in solid-state MAS NMR, spin diffusion has become one of the most powerful tools to obtain carbon-carbon distance restraints in protein structural elucidation. In the spin-diffusion based $^{13}C$-$^{13}C$ correlation experiments, a short mixing time (typically a few milliseconds) can generate cross peaks between directly bonded carbons to establish the intra-residual connectivity. However, a much longer mixing time is needed in order to obtain the correlations between those sterically close carbons from different residues. Since spin diffusion is based on through-spaced dipolar couplings, both intra- and inter-residue resonances coexist in the same spectrum, with the former giving rise to much stronger correlation signals than the latter, making it extremely difficult to identify those inter-residue resonances from this crowded spectrum. Here, we demonstrate the feasibility of using difference spectroscopy to identify some inter-residue correlations for resonance assignments in the crowded 2D $^{13}C$-$^{13}C$ chemical shift correlation spectra of uniformly $^{13}C$, $^{15}N$-labeled full length M2 protein [1].

## Experimental

$^{13}C$-$^{13}C$ chemical shift correlation experiments were performed at 243K on a Bruker Avance 600 NMR spectrometer using a home-made 3.2 mm low-E triple-resonance MAS NMR probe. The sample was spinning at 10 kHz.

## Results and Discussion

Fig. 1A shows the regular $^{13}C$-$^{13}C$ DARR spectrum with a mixing time of 50 ms. Strong cross peaks from intra-residues make it difficult to identify those inter-residue resonances from this severely overlapped spectrum. Clearly the difference spectrum (Fig. 1B) gives a much better resolution in terms of identifying the inter-residue resonances [1].

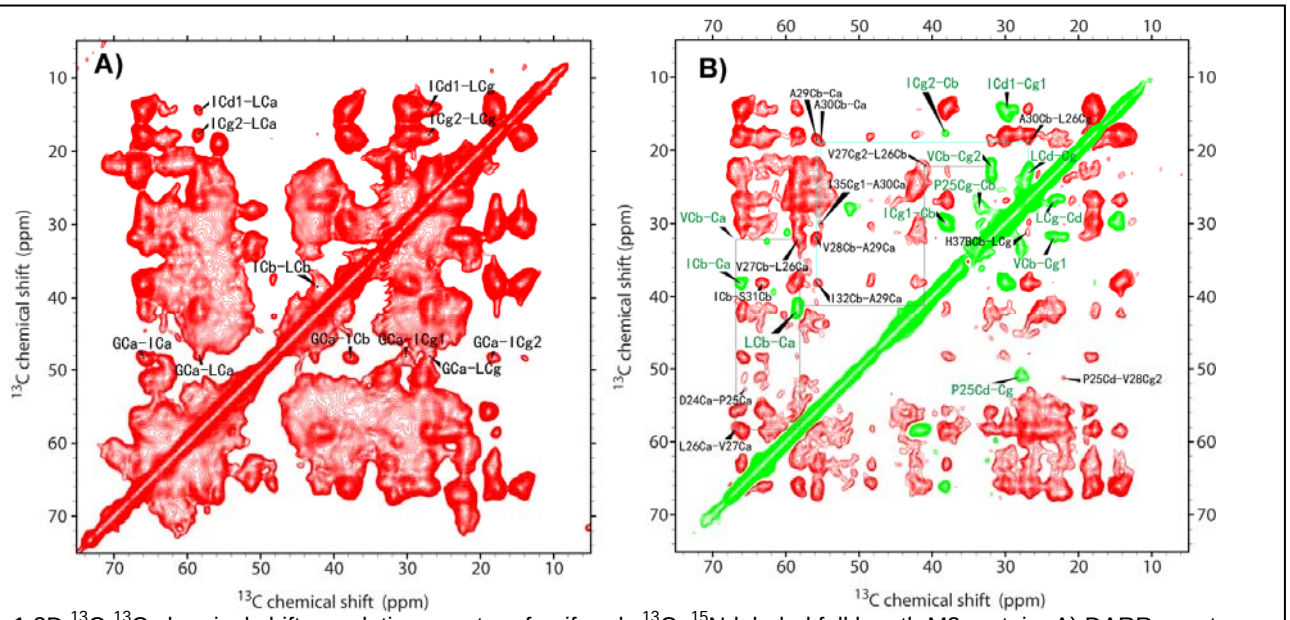


Fig. 1 2D $^{13}C$-$^{13}C$ chemical shift correlation spectra of uniformly $^{13}C$, $^{15}N$-labeled full length M2 protein. A) DARR spectrum with 50 ms mixing time. B) Difference spectrum between the DARR spectra with 50 and 20 ms mixing times.

## Acknowledgement

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## References

[1] Miao, Y., *et al.,* J. Biomol. NMR, **56**, 265-273 (2013).