Identify Inter-helical Distances of Full-length Influenza A M2 (M2FL) Protein from ^{13}C-DARR NMR Experiments

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Introduction

Influenza A M2 protein is an essential protein for this virus and a drug target for the treatment of this infectious disease. NMR has been widely used as a tool to study the structure and function of this homo-tetrameric proton channel. Identifying interhelical distances of M2FL is critical to obtain its accurate structure and to develop therapeutic agents like channel blockers. Although it has been attempted to obtain interhelical distances of different M2 protein constructs using different NMR techniques, ambiguity in the resonance assignments have complicated the interpretation of the spectral results for distance restraints.

Experimental

Isotopically labeled M2FL with N-terminus his tag is expressed in *E. coli* followed by purification with Ni^+ affinity chromatography. Then tetrameric samples were prepared with a mix of isotopically labeled M2FL protein monomers in detergents followed by detergent removal and reconstitution of M2FL into lipid bilayers. DARR (Dipolar Assisted Rotational Resonance) solid-state NMR experiments were performed on this isotopically mixed M2FL in lipid bilayers. Since crosspeaks closer to the diagonal of the DARR spectra are difficult to identify, difference spectroscopy was used to improve the resolution of these mixed labeled spectra.

Results and Discussion

Since mixed isotopic labeled samples were utilized in these experiments, it can be confirmed that the crosspeaks appearing in the DARR spectra arise from interactions between neighboring helices in the tetramer. Difference spectroscopy provided a significant enhancement of spectral resolution when the crosspeaks from two helices are close to the diagonal. The challenges due to scrambling of labeled residues, which occurs during reversed-labeled M2FL expression, can be prevented or minimized by forward labeling strategy.

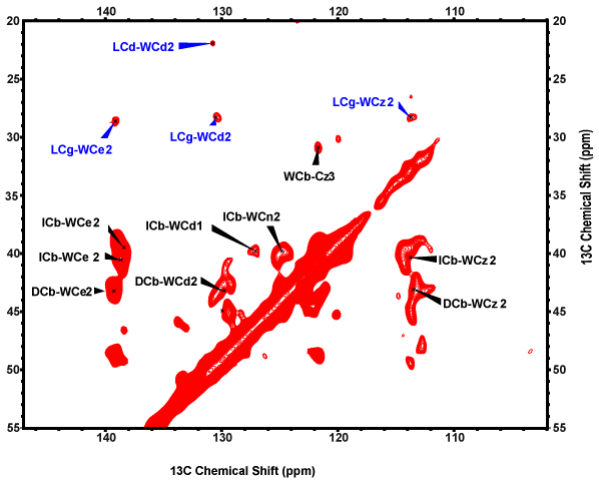

Figure 1: Alipatic-aromatic region of DARR Spectrum of ^{13}C Leu M2FL mixed with ^{13}C Ile/Trp M2FL with a 500 ms mixing time. The blue labels highlight resonances from interhelical contacts.

Conclusions

Here, the ability to identify interhelical contacts in M2FL by mixed isotopic labeled strategy is demonstrated. Enhancement of the sensitivity of these resonances is being attempted by developing sample preparation protocol and/or optimizing the mixing time in the DARR experiment.

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