Solid State NMR Structural Studies of Proteorhodopsin

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Introduction

Green Proteorhodopsin (GPR) is a typical retinal-binding, light-driven, transmembrane proton pump of seven-helical architecture, widely distributed in marine and freshwater bacteria. The structure of GPR is not known as it does not produce well diffracting crystals, and we are using solid state NMR (SSNMR) to determine its conformation in lipids. Three-dimensional chemical shift correlation spectroscopy has been used to assign ^{13}C and ^{15}N backbone and side chain chemical shifts for 168 residues[1,2], with a particularly high density in the transmembrane regions (~80% of residues). In contrast, many residues located at the protein-solvent interface and in the loops and termini experience vigorous motions, and could not be assigned due to the low sensitivity of their signals. Preliminary investigations have shown that the 900 MHz low-E field triple resonance HCN probe, designed and built at NHMFL, displays ~1.3-1.5x better sensitivity than the commercially available equipment of similar functionality. Additionally, the 900 MHz low-E HCN probe can recycle experiments more rapidly (1 s vs 1.8s) and can hold ~35% more sample. Our objective is to take advantage of this sensitivity improvement to extend the currently available assignments, and to obtain additional information on the dihedral angles for the exposed regions.

Experimental

The 900 MHz solid-state NMR spectrometer equipped with a low E-field triple resonance magic angle spinning probe at the NHMFL was used to perform 3D NCACX and NCOCX experiments on a uniformly ^{13}C,^{15}N (UCN) labeled sample of GPR. Spectra have been processed in NMRPipe.

Results and Discussion

Initial 3D experiments have been run in fall 2013 on UCN GPR. To demonstrate the superior sensitivity of the 900 MHz spectrometer, we compare in Fig 1 two-dimensional planes from the 3D NCOCX chemical shift correlation experiments recorded on a commercial 800 MHz Bruker Avance III spectrometer, and on the 900 MHz instrument. The spectra collected at NHMFL show better sensitivity, and allow for the identification of several additional correlations. We are currently analyzing this data to determine if it is sufficient to extend the current assignments.

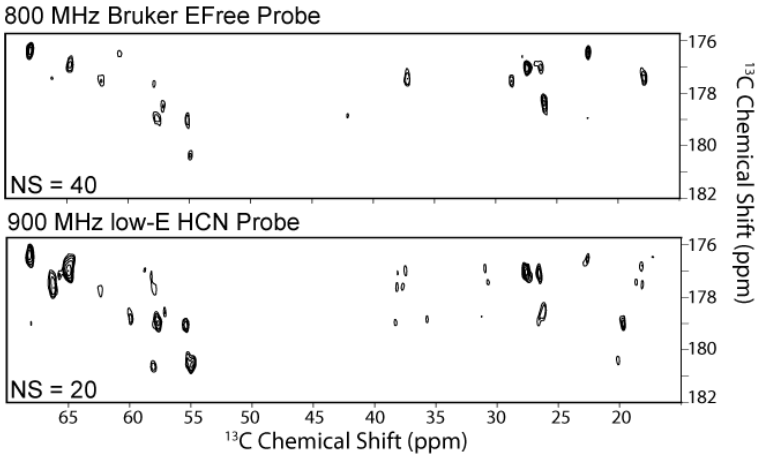

Fig. 1: 2D planes of 3D NCOCX experiments collected on the 900 MHz spectrometer at NHMFL and on the 800 MHz Bruker Avance III spectrometer at the University of Guelph. The ^{15}N dimension is cut at 120.0 ppm in both spectra. The first contour of each peak is cut at 5σ, with each additional level multiplied by 1.2.

Conclusions and Outlook

Our goal in this project is to determine the three-dimensional conformation of GPR in the native-like lipid environment. Obtaining additional structural information on the exposed regions of the protein is important to understanding the structural organization of GPR. Additional experiments will be recorded in the future to obtain the full 3D structure of GPR.

Acknowledgements

This work is supported by the Natural Sciences and Engineering Research Council of Canada.

References

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[2] Shi, L., *et al*., J Mol Biol., **386**, 1078–1093 (2009).