SOLID STATE NMR INVESTIGATION FOR MEMBRANE PROTEIN NANOCRYSTALS

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Introduction

Membrane proteins represent a wide range of challenges for structural biologists attempting to characterize three-dimensional structures. Although most of the known protein structures are from x-ray diffraction, it is extremely difficult to generate large membrane protein crystals required in X-ray crystallography. While long range order is required for x-ray diffraction quality crystals, only short range order is required for high resolution solid state NMR. In principle, high resolution structure of proteins can be determined by solid state NMR with micro and even nano-crystalline preparations. The main goal of this research is to utilize the NHMFL facility to establish methods to characterize membrane protein nanocrystals.

Experimental

Uniformly ^{13}C, ^{15}N labeled ubiquitin was prepared by overexpression in *E. coli* and was crystallized by batch methods in 60% 2-methyl-2,4-pentadiol, 20 mM citrate duffer, pH 4.0. Uniformly ^{13}C, ^{15}N labeled M2 protein was prepared by overexpression in *E. coli* and crystallized by a sitting drop method. Small crystals were observed at 25% PEG 4000, 0.1M Tris pH 8.5, 0.142M Lithium Sulfate, 2% of Octyl-glucoside. The NMR experiment was performed with a 600WB magnet and Bruker DRX console equipped with a 4mm triple-resonance MAS probe at the NHMFL.

Results and Discussion

^{13}C NMR spectra of the Ubiquitin nanocrystals taken with a 600 MHz NMR spectrometer have shown excellent improvement in spectral resolution over lyophilized protein powder. Figure 1 shows that more than half of the resonances are resolved in the 2D spectrum with ~1 ppm ^{13}C line width. In addition, extensive crystallization screening has yielded several conditions to produce microcrystals of the functional isotope labeled influenza M2 proton channel.

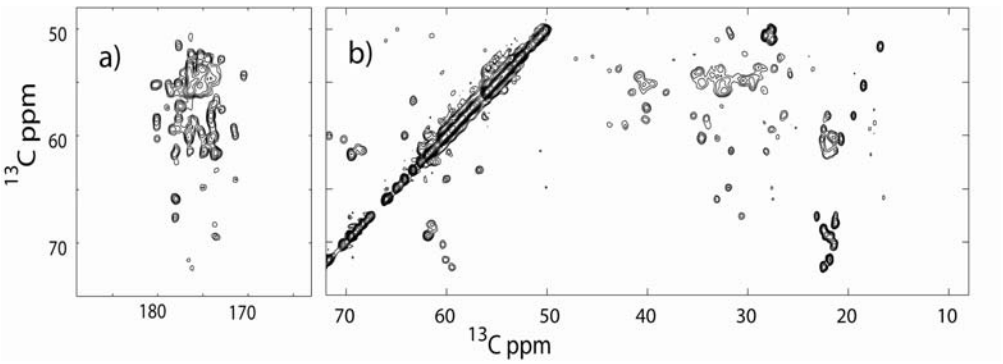

Figure 1. 2D ^{13}C-^{13}C proton driven spin-diffusion MAS spectrum of ^{13}C, ^{15}N uniformly labeled ubiquitin nanocrystals in the (a) Cα / CO correlation region and (b) in the Cα / side-chain region.

Conclusions

Smaller crystals of membrane proteins are relatively easy to prepare and they can be used to generate homogeneous preparations critical for narrow resonance line widths in magic angle spinning (MAS) NMR spectroscopy. We have used solid state NMR spectroscopy to investigate nanocrystals of ubiquitin and obtained high resolution 2D solid state NMR spectra. Progress has been made in the preparation of nanocrystals of isotope labeled membrane protein. Methods developed here will provide a powerful alternative to x-ray crystallography and significantly enhance the structural and dynamic characterization of the membrane proteins.

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