Sample Preparation for MAS and PISEMA Spectroscopy of M2 Protein

S. Luca (NHMFL, FSU), D.T. Murray (NHMFL, FSU), H. Qin (NHMFL, FSU), T.A. Cross (NHMFL, FSU)

Introduction
The M2 protein of influenza virus A is a proton-selective ion channel activated by acidic pH. Structure determination by various techniques has so far focused almost exclusively on truncated versions of the M2 protein. This is the result of both limitations on sample preparation imposed by these methods and difficulties associated with handling of the full-length protein. Importantly, some big structural differences have been observed between these constructs. This suggests that the properties of the native M2 channel may not be correctly reflected in these studies. Our goal was to prepare pure full-length M2 protein and reconstitute it in model phospholipid bilayers. Solid-state NMR studies on both liposome preparation by magic angle spinning (MAS) NMR and on oriented bilayers by PISEMA experiments are currently ongoing.

Experimental
A robust protocol for fast and efficient purification of the M2 protein recombinantly expressed in E. coli has been developed. About 25mg of highly pure protein can be obtained per liter of bacteria culture under conditions that allow isotope labeling for NMR studies. Sample purity and homogeneity was verified by a number of techniques including SDS-PAGE electrophoresis and size exclusion chromatography. The protein was reconstituted in lipid bilayers composed of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DMPG) with a molar ratio of 4:1.

Results and Discussion
Although the reconstitution yields of M2 into DMPC/DMPG bilayers are currently not very high (only about 1:400 protein to lipid molar ratio), solid-state NMR studies can still be performed. We report here initial one-dimensional $^{13}$C MAS NMR spectra acquired on a 900MHz spectrometer using a uniformly $[^{13}$C,$^{15}$N]-labeled M2 protein sample. Some $^{13}$C resonances with a line width of about 1.0 to 1.5 ppm are observed and indicate that a homogeneous preparation of M2 channel in lipid bilayers has been achieved. These preliminary MAS NMR data demonstrate that further solid-state NMR studies are feasible. Samples with different $^{13}$C and $^{15}$N labeling schemes will be prepared and the structure and dynamics of the M2 channel will be investigated to further elucidate its biological functioning.

Acknowledgements
This work was supported by the NIH/NIAID grant number R01 AI-023007 and the National High Magnetic Field Laboratory, a cooperative agreement between the NSF (DMR-0084173) and the State of Florida.