PROTEIN-PROTEIN INTERACTIONS BY SOLID-STATE NMR SPECTROSCOPY

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Introduction

We are investigating a protein-protein interaction between two integral membrane proteins involved in cardiac muscle contractility, sarcolipin (SLN) or phospholamban (PLB) and sarco(endo)plasmic reticulum calcium pump (SERCA). The solution NMR structure of PLB has been solved in detergent micelles to be an *L-shape* structure: a cytoplasmic helix (residues 1-17) connected to a transmembrane helix (residues 22-52) via a flexible hinge region (residues 17-21)[1]. Likewise, the solution structure of SLN, in detergent micelles, has been determined to be similar to the transmembrane domain of PLB[2].

Experimental

PISEMA spectra were acquired on a ^{1}H-^{15}N Low-E PISEMA probe (custom built at the NHMFL) using a Bruker DRX 600 NMR, operating at a frequency of 600.1 MHz for ^{1}H and 60.8 for ^{15}N and a Bruker Avance II 900 UWB spectrometer, operating at a frequency of ~894 MHz for ^{1}H and 89.4 MHz for ^{15}N using uniformly and selectively labeled ^{15}N SLN or PLB reconstituted in mechanically oriented lipid bilayers.

Results and Discussion

Figure 1 illustrates the benefits obtained at higher fields, as well as the benefits offered by the Low-E probe technology developed by Peter Gor'kov and Bill Brey at the NHMFL. The quality of spectra obtained at 900 MHz will allow residue-by-residue resolution of the interaction of these regulatory proteins with the enzyme SERCA. The PISEMA spectra represent a 19° orientation of the helix axis with respect to the bilayer normal.

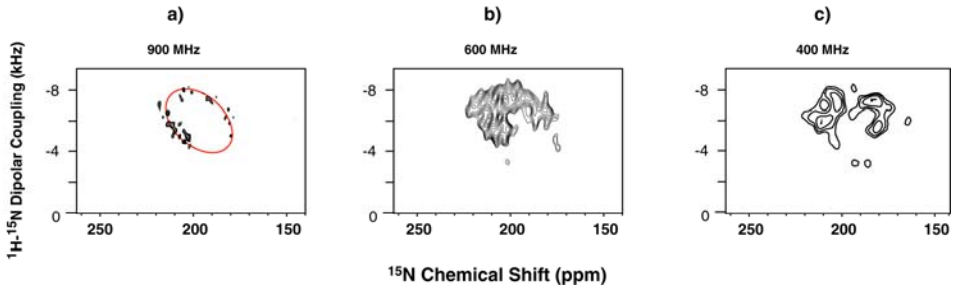

Figure 1. PISEMA of uniformly ^{15}N-labeled protein in oriented lipid bilayers at (a) 900 MHz, (b) 600 MHz, (c) 400 MHz. The "PISA Wheel" in (a) represents an angle of 19° with respect to the bilayer normal.

Additionally, we have collected PISEMA on ^{15}N-Leu SLN in oriented lipid bilayers at 600 MHZ, Figure 2. The acquisition of PISEMA on selectively ^{15}N-labeled samples will facilitate the assignment of the full protein.

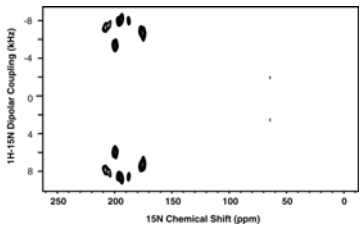

Figure 2. PISEMA of ^{15}N-Leu protein in oriented lipid bilayers at 600 MHz.

References

1. Zamoon, J., *et al*., Biophys. J., **85**, 4, 2589-98 (2003).
2. Mascioni, A., *et al*., Biochemistry, **41**, 2, 475-82 (2002).