

## **Mycobacterium tuberculosis: solid state NMR study of Rv2433c protein**

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Membrane protein structure determination remains a challenging task in structural biology. Predicted membrane proteins account for 30% of sequenced genomes. However, less than 1% of protein structures in Protein Data Bank represent membrane proteins. The Structural Genomics Consortium for membrane proteins of *Mycobacterium tuberculosis* (TB) concentrates on solving structures of multiple membrane proteins of the pathogenic bacteria.

Rv2433c is a 96 residue protein with hypothetical function and structure in *Mycobacterium tuberculosis* (TB) genome. Any structural and functional information of TB proteins is extremely valuable for the reason that this bacillus is responsible for nearly 3 million human deaths annually. Rv2433c was recently successfully expressed in our laboratory and HSQC, <sup>15</sup>N-edited NOESY, HNHA, and TOCSY, suggest a well folded 2° structure (1). Two transmembrane (TM) regions are predicted based on the statistical analysis for TM domains. TM helices are ordered in the lipid environment providing an advantage of using the solid state NMR of aligned samples.

In this poster we present both the preliminary solution and solid state NMR spectra towards structural determination of Rv2433c. Solid state NMR has an advantage of studying TM protein in native like environment compared to both liquid NMR and X-ray crystallography. We use <sup>15</sup>N and <sup>31</sup>P spectroscopy to assess protein orientation in POPC/POPG hydrated lipid bilayers. Three options for TM protein orientation in lipid bilayers are possible: (i) all domains are oriented, (ii) none of the domains are oriented and (iii) only TM segments are primarily oriented while the other domains are dynamically or conformationally disordered. The first and latter alternatives would result in powder pattern-like 1D <sup>15</sup>N NMR spectrum obtained from uniformly labeled <sup>15</sup>N Rv2433c by cross-polarization from abundant <sup>1</sup>H nuclei followed by <sup>1</sup>H decoupling. Combined with low signal to noise ratio (lipid to protein ratio is 100:1) the spectrum interpretation becomes ambiguous. A more reliable approach presented here employs selective amino acid labeling. Three amino acid residues primarily occur in the predicted TM domains of Rv2433c: Leu (8 out of 10), Val (8 out of 12) and Ile (3 out 4). <sup>15</sup>N spectra of those Rv2433c modifications help to establish whether or not the TM domains are oriented in lipid bilayers without any substantial signal interference from the other <sup>15</sup>N sites. However, more importantly to the analysis and peak assignments in PISEMA experiments, which determine the tilt angle of TM helices, are significantly simplified as well.

(1) R.C. Page, J. Moore, A. Korepanova and T.A. Cross, Expression, Purification, and Initial Characterization by NMR Spectroscopy of Multiple Integral Membrane Proteins from *Mycobacterium tuberculosis*, 33rd Southeastern Magnetic Resonance Conference, Tallahassee, FL, October 17-19 (2003).