Structure and Disorder in the CrgA Cell Division Protein from *M. tuberculosis*

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Introduction

Understanding the structure and function of the cell division apparatus of *Mycobacterium tuberculosis* is crucial for advancing drug development against tuberculosis. Here, we report the solid-state NMR structure of a transmembrane protein, CrgA, that is a central component of the *tuberculosis* divisome. Small helical membrane protein structures are particularly sensitive to their environment and consequently we characterized CrgA in an environment that models well the biophysical properties of the native membrane. To determine the structure, both oriented sample and magic angle spinning NMR data from liquid-crystalline lipid bilayer preparations were used along with refinement by restrained molecular dynamics simulations in the same lipid environment. The structure suggests how CrgA serves as a platform for other proteins of the divisome. Intrinsically disordered regions (IDRs) have recently been recognized to serve important roles in protein –protein interactions, yet little is known about membrane protein IDRs. Here, we demonstrate that CrgA has an IDR.

Results & Discussion

Both Oriented Sample (OS) and Magic Angle Spinning (MAS) solid state NMR have been used to characterize the structure and disorder of CrgA, a 93-residue protein that is known to recruit 5 other proteins to the cell division apparatus. Here, the structure of CrgA was determined in a liquid-crystalline lipid bilayer environment. The ssNMR data define a complete structure for the transmembrane domain that was refined using restrained molecular dynamics simulations in an all-atom representation of the same lipid bilayer environment as in the NMR samples [1]. Uniquely, in this study the residual anisotropic restraints were used to evaluate the nascent structure in the 18 residues of the N-terminus.

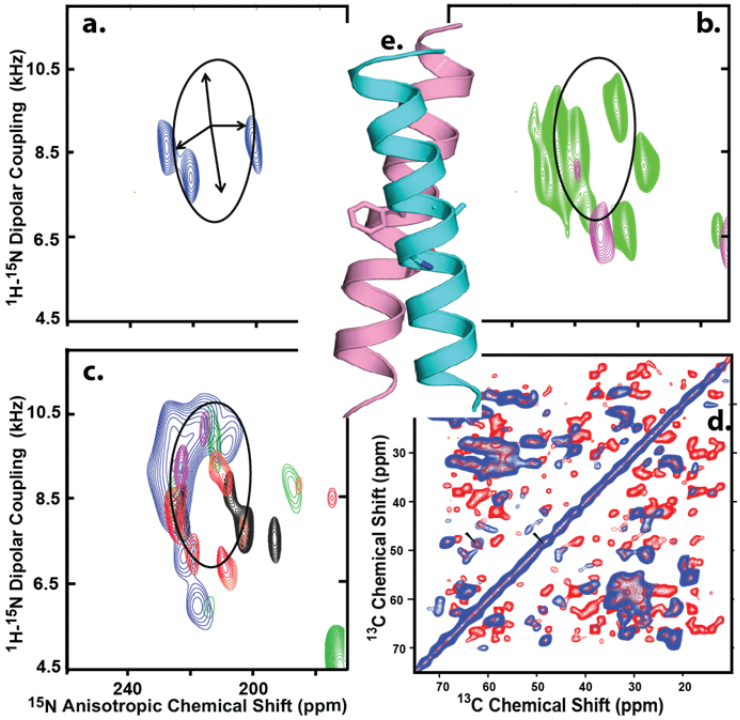

Figure 1: All spectra obtained in a NHMFL WB600 spectrometer with a NHMFL Low-E probe.
a,b,c) OS ssNMR spectroscopy of full length CrgA. Different amino acid specific labeled samples are distinguished by color from which orientational restraints are obtained.
d) MAS ssNMR of full length CrgA from which interhelical restraints were obtained.
e) The combination of orientational and distance restraints led to the structure of the transmembrane domain of CrgA.

Conclusions

This is the first multi-helix full length membrane protein to be characterized in liquid crystalline lipid bilayers. The structure provides insights into how other proteins bind. At least two binding sites in the transmembrane region of the protein were identified by the presence of conserved small residues (glycine & alanine). These sites maybe for binding two other transmembrane proteins, FtsQ or CwsA. Furthermore the characterization of the intrinsically disordered domain at the cytoplasmic N-terminus suggests that either FtsZ or FtsI bind to this site. The fifth protein to bind to CrgA is PbpA that is likely to bind to the interhelical loop.

Acknowledgements

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Reference

[1] Das, N., *et al.,* PNAS, early edition doi/10.1073/pnas/1415908112 (2014).