Structure characterization of *Mtb* ChiZ-TM by Oriented Solid-State NMR Spectroscopy

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Introduction

Chiz (Rv2719c) is a membrane protein that has 165 amino acids with one transmembrane helix from *Mycobacterium tuberculosis*. ChiZ interacts with the PG synthase, FtsI, to regulate cell wall synthesis at septal and polar sites, and interacts with FtsQ, a protein essential for cell division and an interaction partner for multiple cell division proteins. ChiZ also helps focus the FtsZ assembly to midcell sites [1].

Experimental

ChiZ gene was truncated to T57-G90, including the transmembrane helix (TM), and was fused to MBP (maltose binding protein). The recombinant protein was overexpressed in *E. coli* and purified by Ni^{2+} affinity column. The MBP was removed by TEV cleavage. The purified protein was reconstituted in POPC-POPG liposomes. The ChiZ-TM oriented sample was prepared with a protein:lipid ratio of 1:80 with the lipid ratio for POPC:POPG being 4:1. The Low-E static ^{15}N-^{1}H 2D SAMPI4 spectra were obtained on a 600 MHz magnet with a Bruker console.

Results and Discussion

The protein sequence after cleavage is SNA $_{57}$TGHGSRP VPPATTVGLA LLAAAITLWL GLVAQFG$_{90}$ and the transmembrane helix sequence is $_{64}$VPPATTVGLA LLAAAITLWL GLV$_{86}$. The 1D and SAMPI4 spectra of uniform ^{15}N labeled ChiZ-TM showed aligned content around 210ppm chemical shift (**Fig. 1 & Fig. 2**).

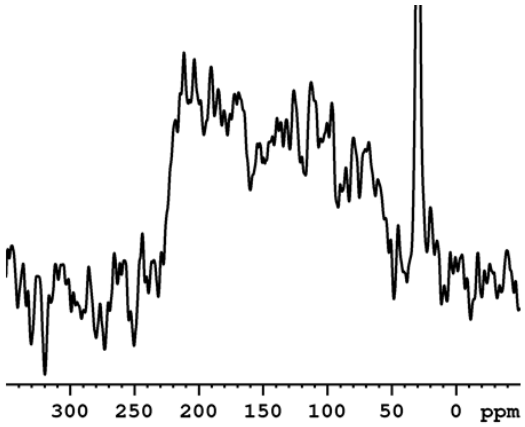

Fig. 1 1D spectrum of ChiZ-TM-^{15}N uniform labeled. The spectrum was collected at 15° C.

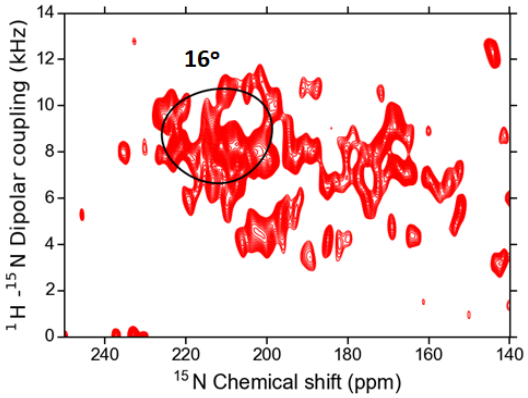

Fig. 2 2D SAMPI4 spectrum of ChiZ-TM oriented sample with uniform ^{15}N labeled was collected at 15° C.

Conclusions

Based on the SAMPI4 spectrum, the aligned portion fit a 16° pisa wheel suggesting a tilt angle for Chiz TM helix of 16°.

Acknowledgements

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References

[1] Vadrevu, I.S., *et al*., Tuberculosis (Edinb), **91(1)**, S128-35 (2011).