Structural Data for *Mtb* LspA by Oriented Solid State NMR Spectroscopy

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Introduction

LspA, a lipoprotein signal peptidase II, is a membrane protein In *Mycobacterium tuberculosis.* It consists of 202 amino acids with four TMs [1]. LspA and another two enzymes, Lgt and Lnt, are involved in the posttranslational processing of lipoproteins. Lipoproteins are abundant in *Mycobacterium tuberculosis* and often involved in virulence and immunoregulatory processes [2]. To investigate the structure of LspA, Oriented Sample ssNMR was used for this study.

Experimental

LspA protein was overexpressed in *E. coli* and purified by Ni^{2+} affinity column chromatography using FPLC. The purified protein was reconstituted in POPC-POPG liposomes. The LspA oriented sample was prepared with a protein: lipid ratio of 1:120 and a lipid ratio are 4:1 of POPC: POPG. The Low-E static ^{15}N-^{1}H 2D PISEMA (Polarization Inversion Spin Exchange at the Magic Angle) spectra were performed on a 600 MHz magnet with a Bruker console.

Results and Discussion

The PISEMA results showed that samples of ^{15}N labeled Tryptophan, Methionine, Phenylalanine and Threonine of LspA in lipid bilayers are aligned (**Fig.1 and Fig. 2)**. The sequence of LspA TM2 helix is 89**YT WVLTLIATGV VVGIFWMGR**109 and TM3 helix is 113**SPWWALGLGMILGGAMGN LVDRF**135. Typically, the residues in the transmembrane helix will show in PISEMA spectral regions shown in Figs. 1 and 2 and fit with a pisa wheel. The residues in first turn and last turn of TM may not show in the spectra or not fit the pisa wheel.

Conclusions

Base on the PISEMA spectra, the tilt angle of LspA TM2 is 23° (**Fig. 1**) and TM3 is 25° (**Fig. 2**). When more spectra of specific amino acids ^{15}N labeled samples will be done, the tilt angle of TM1 and TM4 of LspA will be characterized.

Acknowledgements

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References

[1] Senders, P., *et al.,* Molecular Microbiology, **52(6)**, 1543–1552 (2004).
[2] Becker, K., *et al.,* FEBS Letters, **590**, 3800–3819 (2016).

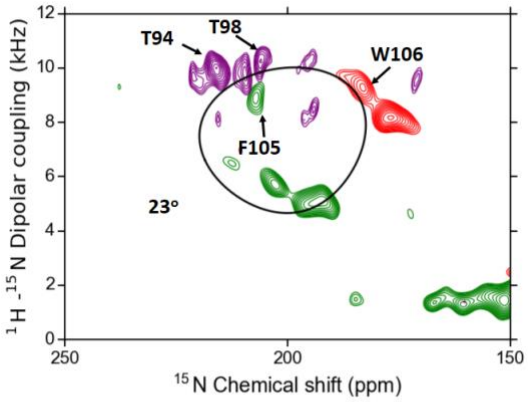

Fig. 1 PISEMA spectra of LspA oriented samples ^{15}N-Trp(Red), ^{15}N-Phe(Green) and ^{15}N-Thr(Purple) were collected at 15°C.

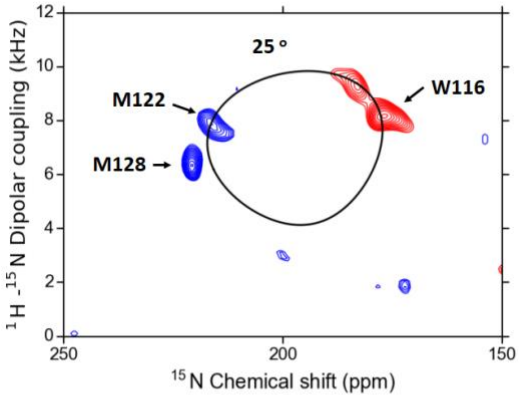

Fig. 2 PISEMA spectra of LspA oriented samples ^{15}N-Trp(Red) and ^{15}N-Met(Blue) were collected at 15°C.