# Structural Characterization of ChiZ by Solution and Solid-state NMR

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## Introduction

ChiZ is a 17.3 kDa membrane protein from *Mycobacterium tuberculosis,* which has been shown to be involved in cell division [1]. This protein has one C-terminal domain (residues 91 to 165) that contains a LysM domain for peptidoglycan binding in the periplasm region. The N-terminal cytoplasmatic domain (residues 1 to 64) has been shown to have petidoglycan hydrolysis activity, and it is predicted to be intrinsically disordered [1]. Both domains are separated by a transmembrane helix. In this study, solid state NMR will be used to characterize the structure of transmembrane region of ChiZ in a membrane-like environment, while solution NMR will be used to characterize the soluble domains independently.

## Experimental

Recombinant His-tagged ChiZ soluble domains have been over expressed in *E. coli* and purified using nickel affinity chromatography. For preliminary NMR experiments, the protein has been $^{15}$N and $^{13}$C uniformly labeled. 2D HSQC has been used during the initial characterization of soluble domains under different conditions.

## Results and Discussion

2D $^{1}$H-$^{15}$N HSQC of ChiZ N-terminal domain shows a narrow dispersion of signal in the proton dimension of just 0.75 ppm (Fig. 1A). This small distribution of signal is typical of unordered proteins and it correlates well with previous circular dichroism spectra that shows the unordered nature of the domain. Although the domain is mostly unfolded, it is possible to observe that the protein can be found in at least two different conformation depending the source used during purification. In the case of the C-terminal domain, the 2D $^{1}$H-$^{15}$N HSQC also shows a small dispersion of the signal in the proton dimension (Fig. 1B). In this case, it is expected the C-terminal to be folded [2].

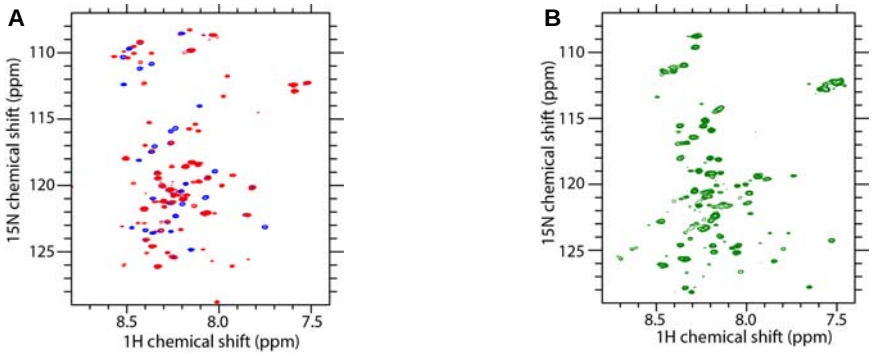


Fig.1. 2D $^{1}$H-$^{15}$N HSQC spectra of ChiZ soluble domains. (**A**) Spectra of ChiZ N-terminal domain purified from soluble lysate (Red) and inclusion bodies using urea (Blue). (**B**) Spectrum of C-terminal domain of ChiZ. spectra were collected at 37°C in a 800 MHz magnet at NHMFL.

## Conclusions

It is clear that dramatic structural differences are present in the samples from soluble fraction of the cells and from the precipitated fraction from inclusion bodies. More studies need to be carried out to fully characterize the different structures of the N-terminal domains. In addition, more work needs to be done in order to find the best condition for the C-terminal domain samples.

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## References

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[2] Mesnage, S., *et al*., Nature Communications, **5**, Article number: 4269.