Structural Characterization of FtsX by Solid-State NMR

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Introduction
FtsX is a transmembrane protein of 32 kDa with 4 transmembrane (TM) helices, which in complex with FtsE constitutes an ABC transporter. It has been demonstrated that it plays an important role during the final steps of cell division in *E. coli* and experimental evidence suggests a similar role in *M. tuberculosis*, which makes this protein an interesting target for drug development against *M. tuberculosis*. In this study, ssNMR will be used to characterize the structure of FtsX from *M. tuberculosis* because it offers the possibility to study this and other membrane proteins in a membrane-like environment.

Experimental
Recombinant His-tagged FtsX has been over expressed in *E. coli* and purified using detergent and nickel affinity chromatography. The protein can be reconstituted into a membrane-like environment using POPC and POPG (4:1 ratio) in a protein to lipid ratio of 1:150. For preliminary NMR experiments, the protein has been \(^{15}\text{N}\) uniformly labeled and specifically labeled using \(^{15}\text{N}\) isoleucine.

Results and Discussion
During the first steps of FtsX characterization, \(^{15}\text{N}\) 1D Cross Polarization of mechanically aligned samples have been used to recognize the quality of the aligned sample. The \(^{15}\text{N}\) uniformly labeled sample suggest the protein is significantly aligned, but an important amount of unaligned protein is observed with a signal centered at 82 ppm (Figure 1A). This may be explained by limited alignment of TM helices or the signal contribution from the loops, that in FtsX can be as large as 100 amino acids. However, due to the \(^{15}\text{N}\) isoleucine sample spectrum is similar to the \(^{15}\text{N}\) uniformly labeled sample (Figure 1B), it helps to conclude the most of the signal at 82 ppm is unaligned protein. Additional \(^{31}\text{P}\) experiments also show a low degree of phospholipids alignment for the sample.

![Figure 1: 1D \(^{15}\text{N}\) CP experiments of FtsX.](image)

Conclusions
From these results it is possible to conclude that optimization of sample alignment is still needed.

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References