**Toll Like Receptor-2 TM Domain Structural Studies by Solid State NMR Spectroscopy**

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**Introduction**

 Past one decade solid state NMR spectroscopy has become a very powerful tool to study small helical membrane proteins in membrane environment. Here we present the structural studies of human Toll like receptor-2 (TLR2) transmembrane (TMD) in lipid bilayer by ssNMR spectroscopy. We applied Oriented Sample solid state NMR (OS ssNMR) experiments to obtain uniaxial orientations of TLR2 TMD in POPC:POPG lipid bilayers.

**Experimental**

 15N uniform labeled and amino acid specific labeled TLR2 TMD was grown in M9 media and purified by Ni2+ affinity column chromatography using a semi-automated FPLC. The purified protein was reconstituted in to POPC-POPG liposomes (1:80 protein to Lipid molar ratio). Methyl Beta Cyclodextrin (MβCD) was used for detergent removal in all sample preparation. The OS samples were prepared as mentioned in reference 1. Series of 2D SAMPI4 experiments were performed by 720 MHz spectrometer in NHMFL at 286K.

A.

B.





**Figure 1:** 2D SAMPI4 Spectra of 15N uniformly labeled (1A) and 15N Leucine labeled TLR2 TMD (1B) in POPC: POPG lipid bilayer (4:1 mole/mole) at 286K.

**Results and Conclusions**

 15N uniform labeled and 15N Leu labeled SAMPI-4 spectra (Figure 1) shows the TLR2 TMD is uniaxially oriented in lipid bilayer with a tilt angle of 25° w.r.t the bilayer normal. 15N backbone sequence specific assignments are initiated (not shown) by the simulated PISA wheel analysis superimposed on the helical wheel portion of the spectra.

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**References**

 [1] Das, N., *et al.*, Nat Protoc., **8(11)**, 2256-70 (2013).