**Toll Like Receptor-2 and 1TM-TM Interaction Studies by Solid State NMR Spectroscopy**

Das, N., de Jesus, A.J. and Yin, H.H (Colorado U., Boulder, Chemistry and Biochemistry, Biofrontier)

**Introduction**

Toll-like receptors (TLRs) are important components of the innate immune system that regulate detection of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs; a.k.a. “alarmins”). Upon PAMP recognition, TLRs recruit a series of adaptor proteins, which trigger the proinflammatory signaling cascades that result in activation of nuclear factor (NF)-κB and upregulation of inflammatory cytokines and chemokines. This TLR response is crucial for helping to eliminate the pathogen and establishing long lasting adaptive responses, but also can cause various autoimmune diseases and inflammatory disorders. Here we present the structural studies of human TLR2/1 TM-TM interaction studies in lipid bilayer by ssNMR spectroscopy. We applied Oriented State solid state NMR (OS ssNMR) experiments to obtain uniaxial orientations of TLR2 TMD in POPC:POPG lipid bilayers and MAS ssNMR to obtain unique inter-helical distances.

**Experimental**

15N and 13C amino acid specific labeled TLR2 and 1 TMDs were expressed, purified and ~5-6 mg of proteins were reconstituted together (1:1 mol/mol) or separate in to POPC-POPG liposomes (1:80 protein to lipid molar ratio). 2D SAMPI4/PISEMA experiments (Figure 1A) were performed using the 720 MHz spectrometer at the NHMFL at 286K for TLR2 TMD only and for TLR2/TLR1. Complexes were prepared with 1:1 mol/mol with different amino acids labels for MAS mediated distance determination.

**Results and Conclusions**

C:\Users\biswas\Downloads\tlr2-tlr1.TIF TLR2/TLR1 TM-TM complex structure (Figure 1B) was determined by Xplor-NIH using ssNMR restraints. The Xplor structure also shows a tilt angle of 25° for TLR2 TMD, while TLR1 TMD was constructed as a uniform α helix aligned with respect to TLR2 TMD by four unique MAS distances. Restrained MD simulation work is currently on going to determine the high resolution atomic structure of complex TM-TM in an explicit lipid bilayer environment.

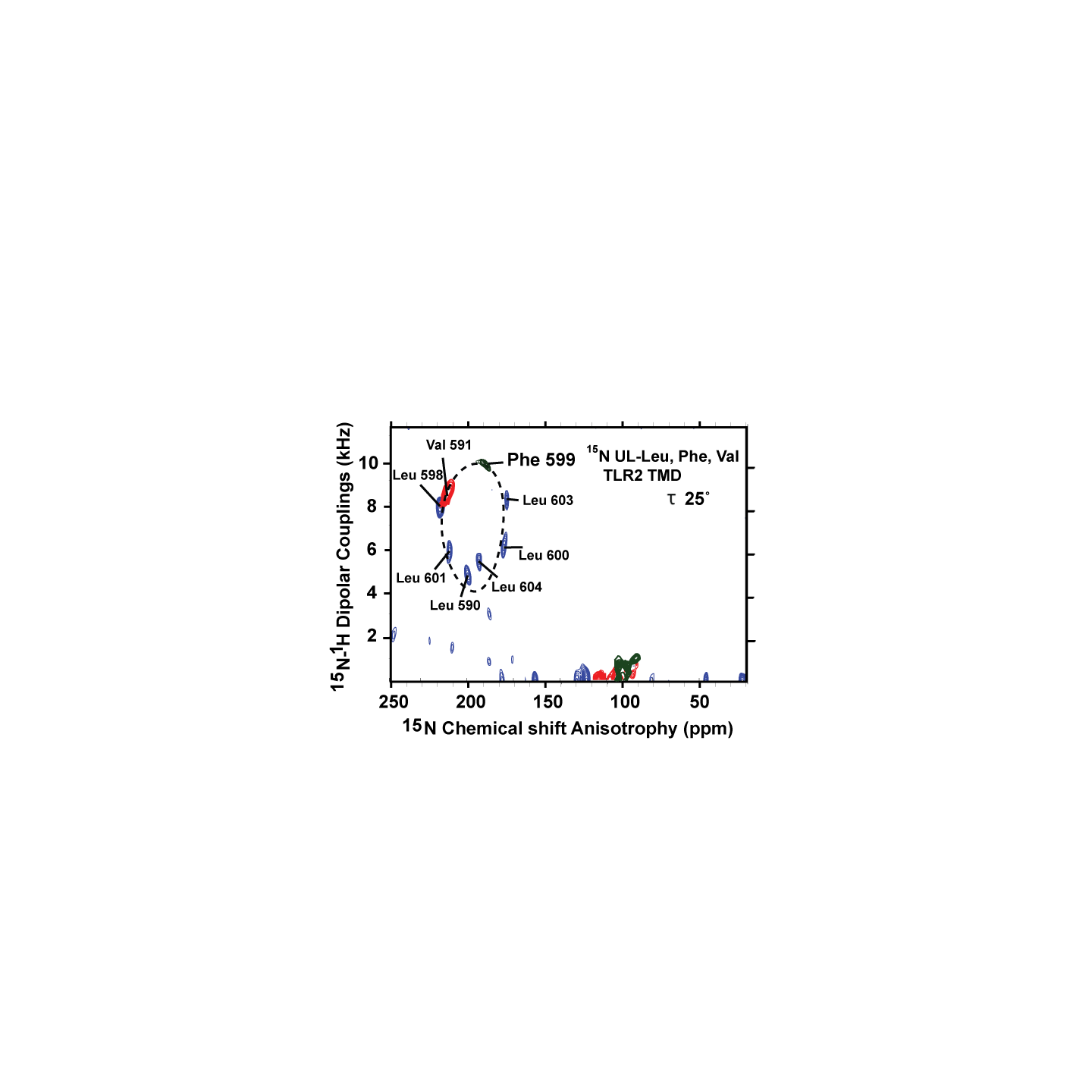


Figure 1: 2D SAMPI4 Spectra of 15N amino acid specific labeled TLR2 TMD (1A) and Xplor-NIH determined structure of TLR2/1 TM-TM interactions in lipid bilayer with three interhelical distance restraints obtained by 2D 500ms DARR MAS experiments of TLR2/1 single amino acid labeled TMD complexes in POPC/POPG lipid environment (1B).

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