Stability and Application of $^1$H Amide Chemical Shift Tensors for Structural Restraints

M. L. Truong (NHMFL, FSU), R. Fu (NHMFL), M. Cotten (Hamilton), T. A. Cross (NHMFL, FSU)

Introduction
Chemical shift anisotropy (CSA) tensors are important to interpret orientation-dependent interactions in solid state NMR. The CSA tensors of the amide proton ($^1$H$^N$) in proteins in conjunction with existing $^{15}$N CSA tensors can provide orientational information for protein structure determination. The aim of this research is to show that recently obtained amide proton CSA tensors shows limited variability across different peptides and could greatly aid in elucidating the structures of transmembrane and membrane associated proteins.

Experimental
Two-dimensional heteronuclear correlation (HETCOR) spectroscopy was used on a static powder sample of the dipeptide, alanyl-$^{15}$N-leucine, to obtain proton CSA measurements. The HETCOR experiment was performed on the 600 MHz High Resolution Wide Bore NMR magnet, using a low-e double-tuned (nitrogen, proton) probe built at NHMFL. The principal magnitudes of the CSA tensors were obtained along with the orientation. Additionally, HETCOR was performed on samples of gramicidin A (gA), a small transmembrane peptide, with different residues $^{15}$N isotopically labeled along the peptide backbone and side-chains to determine the variability of the proton CSA tensors.

Results and Discussion
There is very little variability in the amide proton CSA, as shown in Fig. 1. The two samples of gA displayed nearly identical powder patterns, with the discontinuities used to measure the principal magnitudes of the CSA, occurring in similar positions. Spectra of the indole side-chain of two different sites of gA (not shown) also showed a similar correlation. The $^1$H$^N$ CSA parameters were also applied to piscidin I, a helical antimicrobial membrane associated peptide. A proton chemical shift wave analysis was performed on experimental $^1$H chemical shifts for different residues of piscidin I, obtained via HETCOR. As seen in Fig. 2, the experimental $^1$H chemical shift data fits almost perfectly to the theoretical $^1$H chemical shift wave of two different tilt angles. The analysis clearly shows a small kink in the peptide, and that the peptide lies nearly parallel to the lipid bilayer. With the data collected from experiments conducted on gramicidin A and piscidin I, there is now evidence that little variability exist in the principal magnitudes and orientation of the $^1$H$^N$ CSA tensors, and can be applied as additional structural restraints for membrane protein structure determination.

Acknowledgements
This work was supported in part by the NIH R01 AJ23007. All experiments were performed at the National High Magnetic Field Laboratory, supported by the NSF Cooperative Agreement DMR-0654118 and the State of Florida.