Refining Powder Pattern HETCOR: Correlating $^1$H and $^{15}$N Chemical Shift Tensors

M. L. Truong (NHMFL, FSU, Chemistry and Biochemistry); R. Fu (NHMFL)

Introduction
Accurate chemical shift anisotropy (CSA) values are important not only for orientation-dependent interactions in solid state NMR of aligned membrane-bound peptides and proteins, but also for both the structural and dynamic interpretation of NMR spectra in general. In PISEMA (Polarization Inversion Spin Exchange at the Magic Angle) experiments, spectra of membrane proteins embedded in aligned lipid bilayers largely depend on the orientation of amide $^{15}$N CSA tensors with respect to the amide $^{15}$N-$^1$H bonds. The CSA tensors of the amide proton in proteins also provide similar orientational information. However, information on the CSA tensors of amide protons in peptides and proteins is limited. Only a solid-state study [1] and two solution NMR studies [2,3] based on the transverse CSA/DD cross-correlation rate have been published. The aim of this work is to refine powder pattern heteronuclear correlation (HETCOR) experiments in solids in order to obtain accurate amide proton CSA tensors and a framework for amide proton chemical shifts as structural restraints for solid state NMR of membrane-bound peptides and proteins.

Experimental
The NMR experiments were performed on a Bruker Avance WB600 spectrometer using a low electrical field PISEMA probe with a rectangular coil dimension of 7.6 x 5.6 x 11 mm.

Results and Discussion
In polycrystalline solids, all spin interactions are anisotropic. In order to obtain ideal HETCOR spectra, the following assumptions must be fulfilled: 1) the $^1$H homonuclear interactions are suppressed uniformly over the entire $^1$H chemical shift range (~30 ppm); 2) $^{15}$N spin needs to be decoupled over its entire chemical shift range (~200 ppm); 3) The magnetization transfer can only take place from the amide $^1$H to its bonded $^{15}$N. Fig. 1 shows such a sequence that fulfills all these conditions. In the $t_1$ dimension, the $^1$H homonuclear dipolar interactions are suppressed by a decoupling sequence based on magic sandwiches, which is efficient over a large chemical shift range [4], while the $^1$H-$^{15}$N dipolar couplings are removed by a $^{15}$N $\pi$ pulse applied in the middle of the $t_1$ period. During the mixing time, a windowless isotropic mixing (WIM) sequence [5] is used. Since the proton homonuclear interactions are suppressed during the mixing time, only the amide $^1$H magnetization is transferred to its bonded $^{15}$N. Fig. 2 shows the HETCOR spectra of a polycrystalline sample of Ala-$^{15}$N-Leu peptide at different mixing times. Through the computer simulations, the $^1$H CSA values were obtained, which are in agreement with the solution NMR data [2,3].

Acknowledgements
This work was supported by NIH R01 AI-023007 and the experiments were performed at the NHMFL supported by the NSF Cooperative Agreement DMR-0084173 and the State of Florida.

References