

# Enhancing Resolution of HETCOR Spectra at 900 MHz

2009 NHMFL Science Highlight for NSF

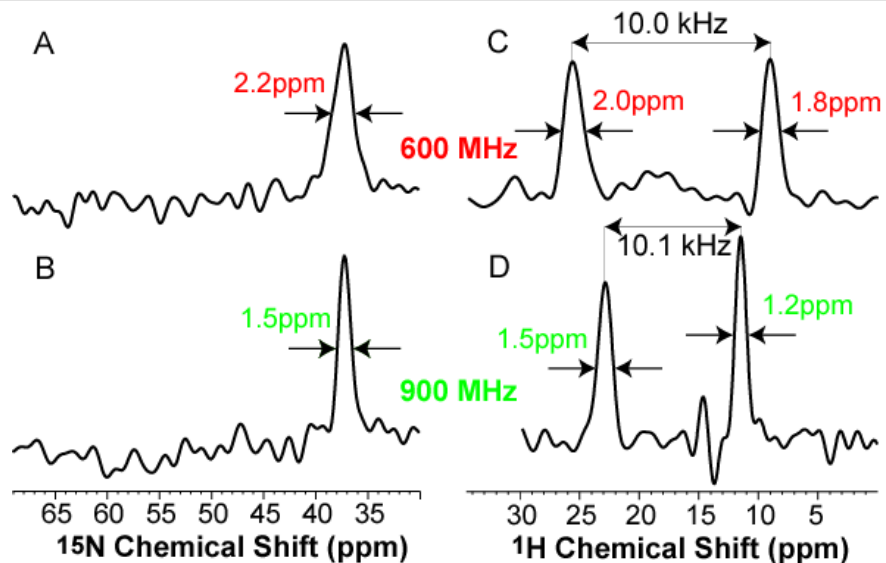
DMR-Award 0654118

NMR Spectroscopy and Imaging User Program, Florida State University

Nuclear magnetic resonance (NMR) heteronuclear correlation (HETCOR) spectroscopy permits the observation of structural data in the form of orientation restraints (i.e. anisotropic  $^1\text{H}$  and  $^{15}\text{N}$  chemical shifts as well as  $^1\text{H}$ - $^{15}\text{N}$  dipolar couplings) from membrane bound proteins and peptides aligned in hydrated lipid environments. Here, we demonstrate that the spectral resolution in both the  $^1\text{H}$  and  $^{15}\text{N}$  dimensions can be enhanced at high field (e.g. 900 MHz). This improvement results in more than a factor of 2 reduction in area occupied by each resonance in a 2-dimensional spectrum. HETCOR is emerging as an advantageous technique for structural studies of membrane bound samples at high field.

Fu, R.; Gordon, E.D.; Hibbard, D.J.; and Cotten, M., *J. Am. Chem. Soc.* **131** (31), 10830-10831 (2009)

In addition to support from the NHMFL, this research is supported by the NIH R01 AI23007, NSF CHE-0748916, Research Corporation, the Dreyfus Foundation, and the Undergraduate Research Summer program at Pacific Lutheran University.



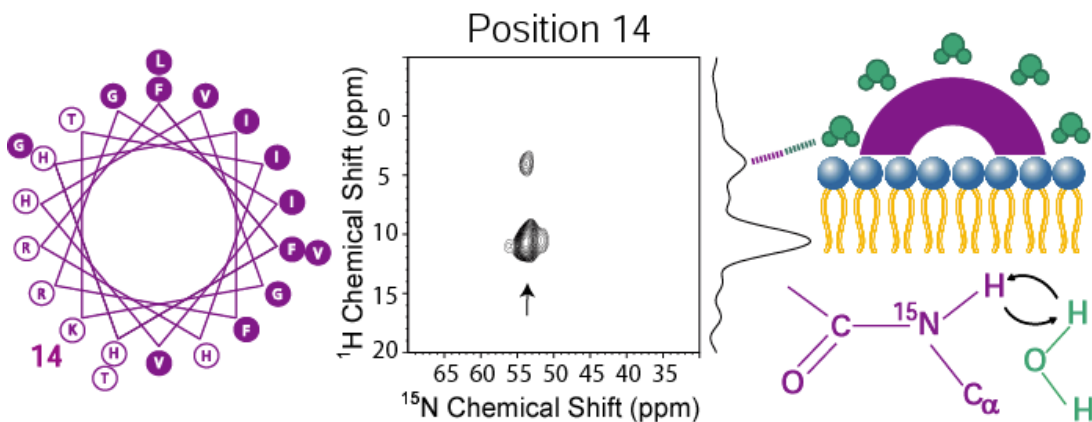
$^{15}\text{N}$  and  $^1\text{H}$  slices taken from the dipolar-encoded HETCOR spectra of a 10-site  $^{15}\text{N}$  labeled piscidin 1, an amphipathic antimicrobial peptide, oriented in lipid bilayers recorded at different fields for the resonance at the  $^{15}\text{N}$  chemical shift of 37.8 ppm. A and C) the  $^{15}\text{N}$  and  $^1\text{H}$  slices from a Bruker Avance 600 MHz NMR spectrometer; B and D) the  $^{15}\text{N}$  and  $^1\text{H}$  slices from the ultra-wide bore 900 MHz NMR spectrometer.

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(Left) Helical wheel diagram of Piscidin 1 an antibiotic from fish. The filled and open circles refer to hydrophobic and hydrophilic amino acids, respectively.

(Center) HETCOR spectrum of <sup>15</sup>N-K<sub>14</sub> Piscidin 1 oriented in bilayers recorded at 600 MHz and the slice taken at 53.8 ppm along the <sup>1</sup>H chemical shift dimension, as indicated by an arrow.

(Right) Diagram showing Piscidin 1 in the presence of lipids and the exchange between the K<sub>14</sub> amide proton and protons from water. To the best of our knowledge, this is the first direct evidence that Piscidin bound to lipid bilayers interacts with the aqueous environment.

Enhanced resolution permits the structural characterization of larger complexes as we are able to observe more signals. This spectroscopy is becoming a critical tool for studying membrane-active proteins and peptides in the presence of the membrane environment. It is only in this environment that these molecules become biological active and have their native structure, since the structures of the membrane-active proteins and peptides are strongly influenced by their environment. In addition, 2-dimensional <sup>1</sup>H-<sup>15</sup>N HETCOR spectra provide novel <sup>1</sup>H structural restraints, generating new insights about the interactions of the peptide helix with the bilayer and aqueous environments.