

Towards ^{17}O Solid State NMR Spectroscopy of Ion-selective Channels at Ultra-high Magnetic Fields

Eduard Y. Chekmenev^{1,2}; Lee N. Miller¹; Jun Hu¹; Yiming Mo¹; Peter L. Gor'kov¹; William W. Brey¹; Kevin W. Waddell^{3,4}; Richard J. Wittebort⁴; Timothy A. Cross¹

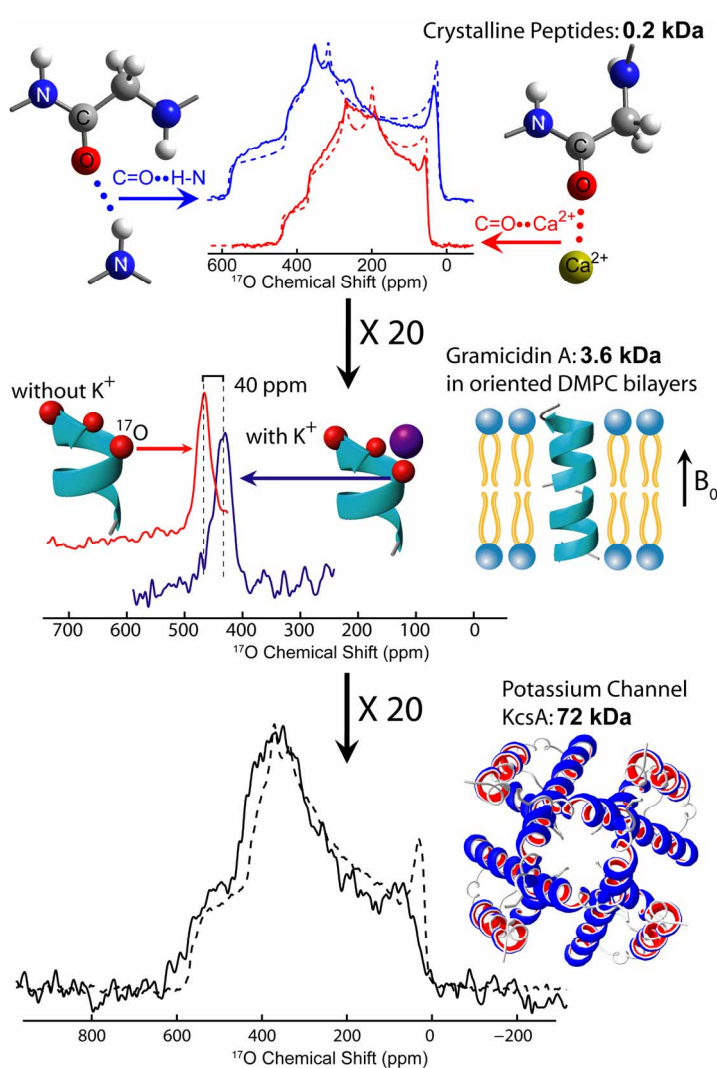
¹National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL 32310

²MR Spectroscopy Unit, Huntington Medical Research Institutes, Pasadena, CA 91105

³Vanderbilt University, Institute of Imaging Science, Nashville, TN 37232

⁴University of Louisville, Louisville, KY 40292

^{17}O , spin 5/2 quadrupole nuclei have recently been extensively employed to study ion-binding in proteins because of the high sensitivity of its quadrupolar coupling (QC) and chemical shift (CS) to the intermolecular interactions. While ^{17}O spectroscopy is considered difficult, the advent of high magnetic fields potentially allows for functional studies of large ion channels, the Holy Grail for many biochemists today. Here, we provide the progress on ^{17}O spectroscopy in the ultra-wide bore **21.2T** NMR at the NHMFL.



Much of the ^{17}O results to date were largely obtained from relatively small molecules such as individual amino acids. Recently we were able to demonstrate that ion binding significantly affects both the CS and QC of carbonyl oxygens in polycrystalline **Gly-Gly-Gly** (Figure 1-top spectra). Moreover, it was found that ^{17}O is a significantly more sensitive probe for ion binding than the more typically used ^{15}N nuclei of the peptide backbone. We also studied ion binding by ^{17}O anisotropic CS in the cation conductive pore of **gramicidin A**, the binding site of which has similar intermolecular interactions that contribute to the biologically important function of high selectivity and high conductance rate in ion selective channels. While the sensitivity is always a challenge for NMR spectroscopy, we take advantage of high fields to aid the sensitivity in addition to high ^{17}O isotopic enrichment ($\sim 60\%$), favorable relaxation ($T_1 \sim 0.6$ ms, $T_2 \sim 0.25$ ms) and orienting the channels, which resulted in reducing the line width from >500 ppm to ~ 25 ppm, a 20 fold reduction. The insights gained from ion binding effects on CS in the relatively small gramicidin A pore (Figure 1-middle) helps potentially to approach the **KcsA potassium channel**. The preliminary results (Figure 1-bottom, solid line) suggest that librational motions have negligible effects, CS tensor span and CS distribution at various amino acid positions are similar to those observed in crystalline solids (Figure 1-bottom, dashed line, polycrystalline Gly-Gly-Gly).

Solid-state ^{17}O NMR is a sensitive and accurate probe for ion binding in proteins. Our preliminary results obtained in a full length 72 kDa bundle of KcsA suggest that ^{17}O can be successfully applied to mechanistic studies of ion solvation in ion-selective channels including KcsA in the native membrane environment.

Figure 1. High Field ion channel studies by ^{17}O solid-state NMR spectroscopy: (top) ^1H decoupled spectra and simulations of Gly- ^{17}O -Gly-Gly with hydrogen bonding and ion interactions at 19.6T;(middle) spectra of oriented [^{17}O -Leu10] gramicidin A in DMPC bilayers in the absence and presence of 2.4M KCl at 21.2T;(bottom) ^1H decoupled spectra of [^{17}O -Gly] KcsA potassium channel and Gly- ^{17}O -Gly-Gly (dashed) obtained by cross-polarization from protons at 21.2T.

(1) Hu, J.; Chekmenev, E. Y.; Gan, Z. H.; Gor'kov, P. L.; Saha, S.; Brey, W. W.; Cross, T. A. *J. Am. Chem. Soc.* **2005**, *127*, 11922-11923.

(2) Fu, R.; Brey, W. W.; Shetty, K.; Gor'kov, P.; Saha, S.; Long, J. R.; Grant, S. C.; Chekmenev, E. Y.; Hu, J.; Gan, Z.; Sharma, M.; Zhang, F.; Logan, T. M.; Bruschweller, R.; Edison, A.; Blue, A.; Dixon, I. R.; Markiewicz, W. D. and Cross, T. A. *J. Magn. Reson.* **2005**, *177*, 1-8.