

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

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^{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. 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**. 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 (19.6T and 21.1T) to aid the sensitivity in addition to high ^{17}O isotopic enrichment (~60%), favorable relaxation ($T_1 \sim 0.6$ ms, $T_2 \sim 0.25$ ms) and orienting the channels with respect to B_0 , 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 helps potentially to approach the **KcsA potassium channel**. The preliminary results 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.