Integration of ^{17}O for Solid-State NMR Studies of Peptides and Proteins

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Nuclear magnetic resonance (NMR) spectroscopy is a powerful technique for obtaining molecular-level information on structure and dynamics in biomolecules like peptides and proteins. For the most important elements in biomolecules (*i.e.*, H, C, N, and O), most NMR observations are carried out with "NMR friendly" nuclei like ^{1}H, ^{13}C, and ^{15}N, which all have a nuclear spin of I = 1/2. *By comparison, the remaining key element, oxygen, is rarely studied with NMR because its only NMR-active isotope, ^{17}O, has very low natural abundance (0.037%) and a nuclear spin I = 5/2*. Any nucleus with I > 1/2 belongs to a special category known as quadrupolar nuclei, which often have large quadrupolar interactions that broaden peaks and make it difficult to obtain NMR spectra with high signal-to-noise and high resolution.

We have developed a suite of NMR methods and new NMR instrumentation to overcome issues of sensitivity and resolution in ^{17}O NMR, with a focus on detecting the ^{17}O signal indirectly via protons (^{1}H). The strong ^{1}H NMR signal at a high magnetic field, in combination with ultrafast magic-angle spinning (MAS) and high-resolution, multiple-quantum MAS (MQMAS) NMR methods for quadrupolar nuclei, allow for two- and three-dimensional experiments that enable the measurement of ^{17}O chemical shifts and quadrupolar interactions; determination of internuclear connections between ^{17}O and ^{1}H, ^{13}C, and ^{15}N; and characterization of hydrogen bonds in biomolecules, as demonstrated with a model peptide (**Figure**).[1,2]

This work expands the NMR toolkit for biomolecules to ^{17}O, opening new opportunities to study oxygen environments in proteins, and expanding our understanding of the roles of hydrogen bonds in 3D protein structure, including protein folding, conformational changes, bonding to water and other molecules, and protein-protein intermolecular interactions.

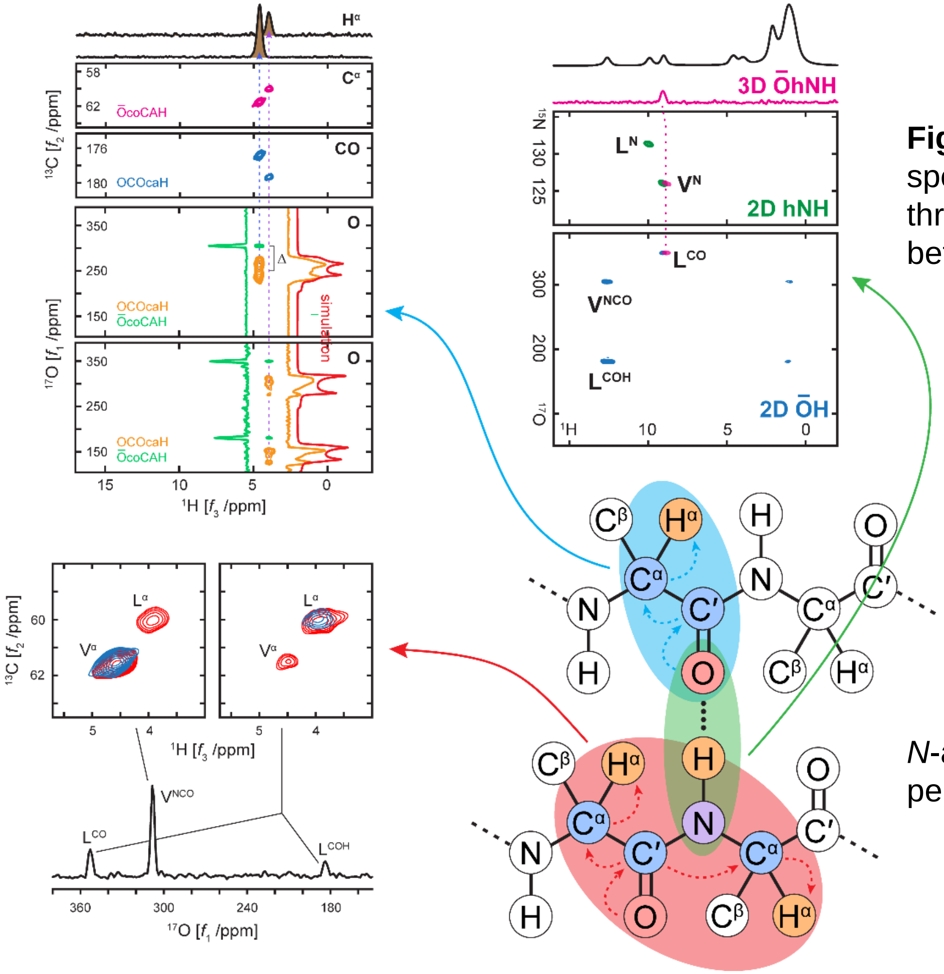

Figure: 2D and 3D NMR spectra, demonstrating through-bond correlations between ^{1}H, ^{13}C, and ^{17}O (**blue**/**top left**); through-space intra- and inter-residue O-C$^{\alpha}$ correlations (**red**/**bottom left**); ^{1}H, ^{15}N, and ^{17}O correlations highlighting hydrogen bonds (**green**/**top right**) within a [U-^{13}C, ^{15}N, 70%-^{17}O] enriched *N*-acetyl-*L*-valyl-*L*-leucine peptide.

Facilities and instrumentation used: NMR/MRI Facility: NHMFL 18.8 T/800 MHz and a 0.75 mm ultra-fast magic-angle spinning probe built in-house at MagLab.

Citations: [1] Hung, I.; Mao, W.; Keeler, E.G.; Griffin, R.G.; Gor'kov, P.L.; Gan, Z., *Characterization of peptide O······N hydrogen bonds via ^{1}H-detected $^{15}N/^{17}O$ solid-state NMR spectroscopy,* **Chemical Communications, 59** (21), 3111-3113 (2023) **doi.org/10.1039/d2cc07004a**;

[2] Hung, I.; Keeler, E.G.; Mao, W.; Gor'kov, P.L.; Griffin, R.G.; Gan, Z., *Residue-Specific High-Resolution ^{17}O Solid-State Nuclear Magnetic Resonance of Peptides: Multidimensional Indirect ^{1}H Detection and Magic-Angle Spinning,* **Journal of Physical Chemistry Letters, 13** (28), 6549-6558 (2022) **doi.org/10.1021/acs.jpclett.2c01777**