TACKLING LOW NATURAL ABUNDANCE

^{17}O Solid-State NMR with CPMAS™ CryoProbe

^{17}O, an important but elusive nucleus

^{17}O has tremendous potential for analyzing biomolecular structures. It represents an ideal and robust candidate to study hydrogen bonding within secondary and tertiary protein structures, which can range from glucose binding proteins to glucose metabolism of live cells.

Applications of ^{17}O NMR spectroscopy so far have been quite limited due to low natural abundance, 0.04%, spin 5/2 with high quadrupolar coupling constants, and very long relaxation times.

Tackling ^{17}O

Advancements of ^{17}O enrichment and solid-state NMR technology began opening the door for studying many biological molecules that are usually considered too difficult for ^{17}O NMR spectroscopy. Combination of improved labeling procedures, paramagnetic doping, high field instruments and high sensitivity MAS CryoProbes, have dramatically revolutionized the field.

Here, we report three examples where such combined approach led to results of unprecedented sensitivity, resolution and analytical insight.

Carbohydrates

Carbohydrates are among the most difficult classes of organic compounds to study by solid-state ^{17}O NMR: ^{17}O-labeled carbohydrates are difficult to synthesize, have ^{17}O Cq's in the range of 9-11 MHz, and often exhibit very long T_1's for both ^{1}H and ^{17}O.

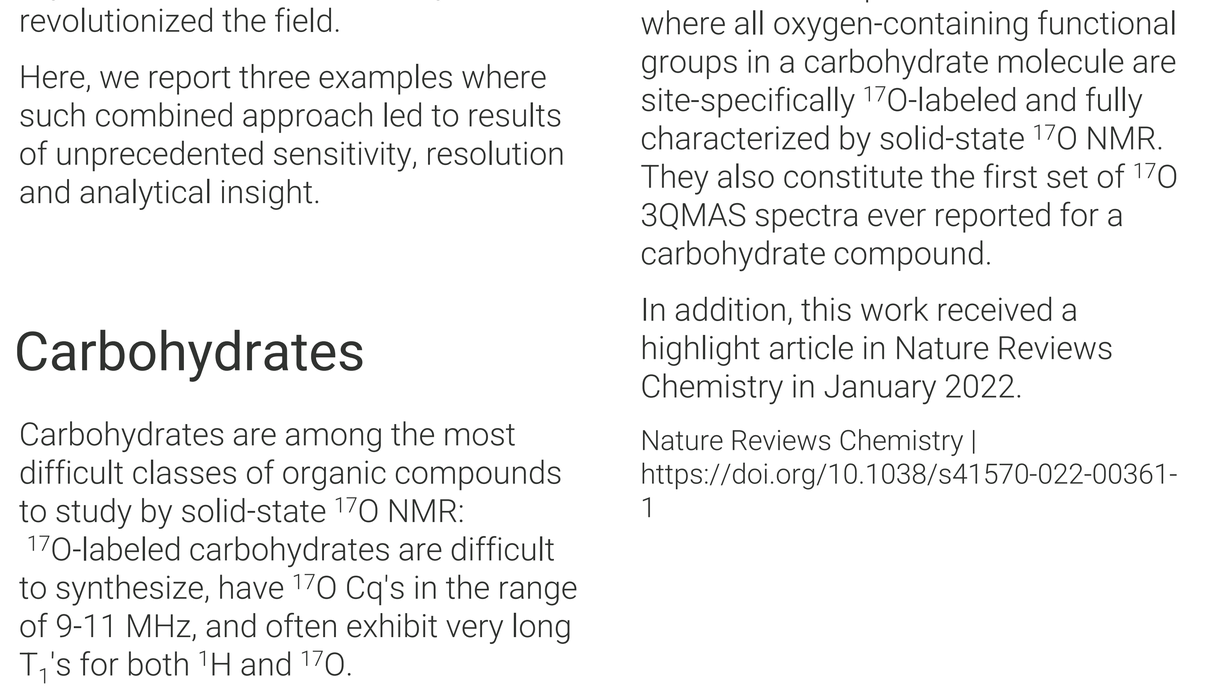

Fig. 2: 2D ^{17}O 3QMAS (left) and 2D ^{13}C refocused-INADEQUATE (right) spectra of 100 mg 10% ^{17}O [3/5/6-^{17}O]-α-D-glucose, 10% (w/w) Cu-EDTA). Total experimental time 6 days and 30 hours, with apodization weighted sampling. See J. Shen et al., Chem. Sci., 2022, 13, 2591 for further details.

Fig. 1 Showcasing O-17 research from Professor Gang Wu's laboratory, Department of Chemistry, Queen's University, Ontario, Canada. Reproduced from Chemical Science rsc.li/chemical-science.

By combining paramagnetic Cu(II) doping with the new CPMAS CryoProbe technology, and apodization weighted sampling we achieved a sensitivity boost for solid-state ^{17}O NMR by a factor of 6-8, enabling acquisition of high-quality ^{17}O multiple-quantum (MQ) MAS 2D spectra for carbohydrate compounds. The unprecedented spectral resolution found in these ^{17}O MQMAS spectra permitted detection of a key structural difference for a single hydrogen bond between two types of crystallographically distinct α-D-glucose molecules.
These results represent the first case where all oxygen-containing functional groups in a carbohydrate molecule are site-specifically ^{17}O-labeled and fully characterized by solid-state ^{17}O NMR. They also constitute the first set of ^{17}O 3QMAS spectra ever reported for a carbohydrate compound.

In addition, this work received a highlight article in Nature Reviews Chemistry in January 2022.

Nature Reviews Chemistry | https://doi.org/10.1038/s41570-022-00361-1

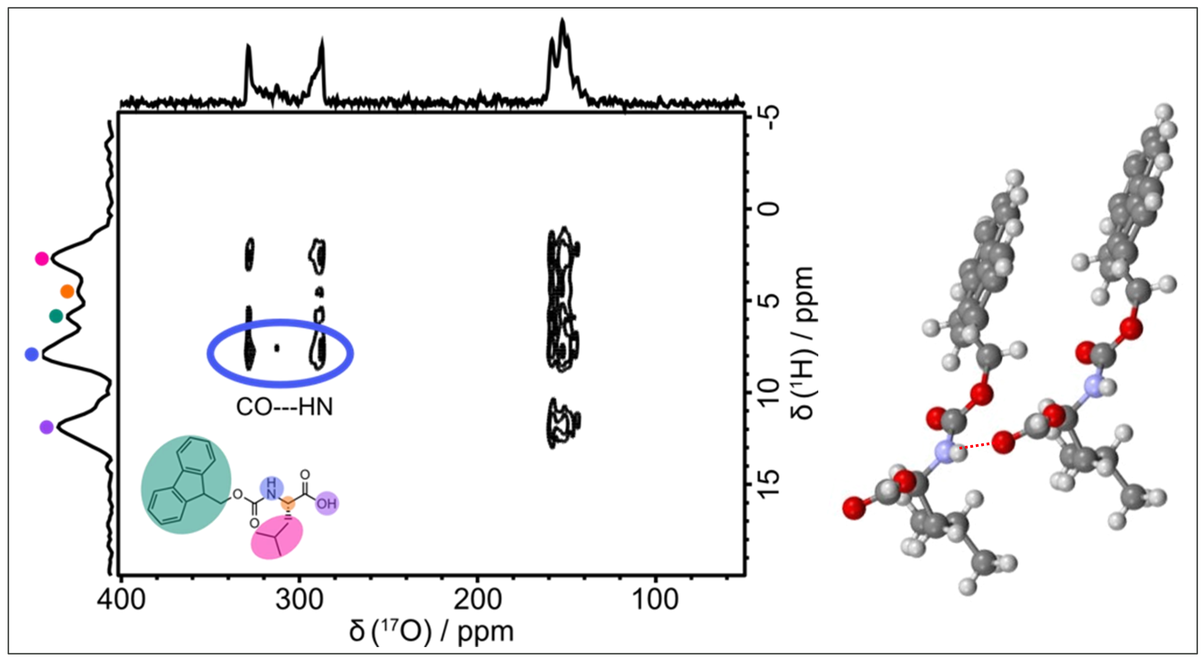

Fig. 3 Left 2D ^{1}H-^{17}O heteronuclear correlation (HETCOR) experiment – 19 mg of material; 16 hrs acquisition. Right: hydrogen bond between two amino-acids. See J. Phys. Chem. B 2021, 125, 43, 11916–11926 for more details.

Aminoacids

V. K. Michaelis and coworkers at the Department of Chemistry, of the University of Alberta, Canada, have developed an efficient (40% ^{17}O in → 40% ^{17}O label) labelling procedure for FMOC-protected amino acid building blocks. This approach permits inexpensive insertion of ^{17}O labels, an important experimental technology for biomolecular studies based on ^{17}O detection.

Employing new hardware, high field instruments and the CPMAS CryoProbe to boost unreceptive nuclei will be of fundamental importance for recording multidimensional experiments on even more complex systems in the future.

Figure 3 demonstrates the analytical potential of ^{17}O based solid-state NMR using the cryoprobe technology by revealing an intermolecular hydrogen bond between amino acids using a 2D ^{1}H-^{17}O heteronuclear correlation experiment.

The table below summarizes sensitivity gains reported by V. K. Michaelis using a conventional room temperature MAS probe (14.1 T) and a CPMAS CryoProbe (18.8 T) for 20 ± 1 mg of material and 1h acquisition (see J. Phys. Chem. B 2021, 125, 43, 11916–11926 for more details).

Method	Field Strength (B_0, MHz)	Sample Amount (mg)	S/N / mg /√scans / B_0
RT Probe Hahn-echo	600	21	1.0
Cryoprobe Hahn-echo	800	18.8	4.1

Metal carboxylate salts

I. Goldberga & D. Laurencin, (Institut Charles Gerhardt Montpellier, France), are working on NMR-crystallography, employing ^{17}O NMR spectroscopy on biologically-relevant metal carboxylate salts. A high-sensitivity and highly resolved ^{17}O 2D MQMAS (Fig.4) was obtained in an overnight experiment with the CPMAS CryoProbe despite a long relaxation delay.

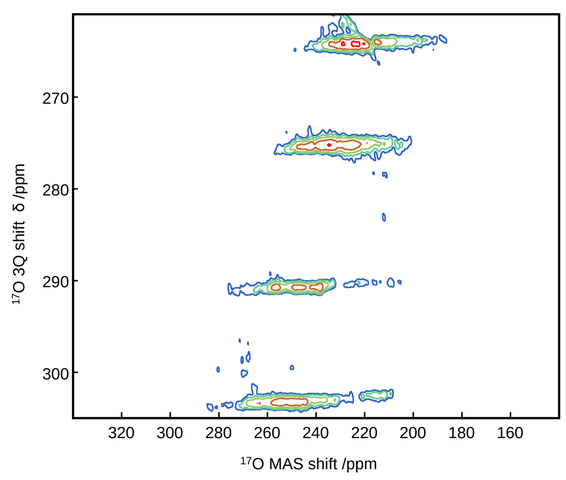

Fig. 4 ^{17}O MQMAS recorded overnight on ~60 mg of ^{17}O-enriched sample. Unpublished results.

Highlights

- Recently improved, more affordable, labeling procedure combined with high fields and highly sensitive NMR technologies promote ^{17}O NMR as a new spectroscopic tool to study many biological molecules.
- The CPMAS CryoProbe has proven to be the key technology in this field.

Technology