



## Ultra-high Field $^{17}\text{O}$ Magic-Angle Spinning NMR Using Series-Connected-Hybrid Magnet

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### Introduction

The essential role of oxygen in hydrogen bonding and in determining the chemistry, structure, and function of peptides and proteins is well known. The use of  $^{17}\text{O}$  NMR for the characterization has been challenging due to its low natural isotopic abundance (0.037%), low gyromagnetic ratio ( $-5.774 \text{ MHz T}^{-1}$ ), and spin  $I = 5/2$  quadrupolar nucleus. In this report, we present the first use of the 36 T Series-Connected-Hybrid (SCH) magnet at the NHMFL to address the resolution and sensitivity issues of  $^{17}\text{O}$  NMR.<sup>1</sup>

### Experimental

$^{17}\text{O}$  NMR were performed using the SCH, a 1.5 GHz Bruker Avance NEO NMR console and a 3.2 mm magic-angle spinning probe developed at the NHMFL. The magnetic field of the powered magnet is regulated using a customized Electronic Lock Control Board (ELCB) in the Bruker NMR console and an external LiCl solution lock sample doped with  $\text{MnCl}_2$ .<sup>2</sup> Combined active and passive shims were used to enhance the field homogeneity to less than 1 ppm over  $1 \text{ cm}^3$  DSV.

### Results and Discussion

Fig. 1 shows 1D  $^{17}\text{O}$  MAS and 2D 3-Quantum Magic-Angle Spinning (3QMAS) spectra of  $[\text{U-}^{13}\text{C}, ^{15}\text{N}, 70\% ^{17}\text{O}]$ -N-Ac-VL acquired using the SCH magnet. At the high field of 35.2 T, the 2<sup>nd</sup>-order quadrupolar broadening of  $^{17}\text{O}$  is reduced to about 10 ppm while the chemical shift spans over 100 ppm. The line narrowing makes the three  $^{17}\text{O}$  labelled sites of the model peptide completely resolved in the 1D MAS spectrum. The quadrupolar broadening can be further removed by the 3QMAS experiment as shown in Fig.1(d) and 1(e). In addition to the large span of isotropic chemical shifts, oxygen also has a large chemical shift anisotropy (CSA) amplified by the high magnetic field. Both the 1D MAS and 2D 3QMAS spectra show many spinning sidebands under the 19 kHz MAS frequency being used. The intensity profiles of the spinning sidebands and characteristic line shape from the 2<sup>nd</sup>-order quadrupolar broadening allows for a complete determination of the quadrupolar coupling and CSA parameters.<sup>1</sup> In order to resolve overlap among the spinning sidebands, a 2D sideband separation experiment was also performed to spread spinning sidebands by their order along the second-dimension (result not shown, see supporting information of ref. 1).

A stable and homogeneous magnetic field is essential for signal averaging and carrying out multidimensional NMR experiments. The combined active/passive shim and the success of field regulation using the field/frequency lock enable high-resolution NMR at a field 50% more than the highest superconducting NMR magnet available today. For quadrupolar nuclei like  $^{17}\text{O}$ , the high field brings dramatic gains in spectral sensitivity and resolution for using  $^{17}\text{O}$  NMR to probe the important oxygen element in chemistry and biology.

### Conclusion

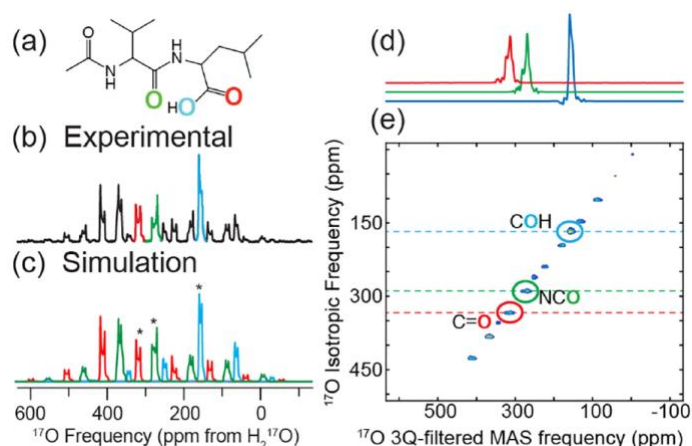
We have successfully performed 1D and 2D  $^{17}\text{O}$  NMR experiments using the newly commissioned 36 T SCH magnet. The results show great promises for  $^{17}\text{O}$  NMR of biological samples with enhanced resolution and sensitivity.

### Acknowledgements

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### References

[1] Keeler, E., *et al.*, *J. Am. Chem. Soc.*, **139**(49), 17953 (2017).



**Fig. 1.** 1D and 2D  $^{17}\text{O}$  MAS NMR spectra of  $[\text{U-}^{13}\text{C}, ^{15}\text{N}, 70\% ^{17}\text{O}]$ -N-Ac-VL recorded at 35.2 T. (a) color-coded  $^{17}\text{O}$  labelled sites (NCO, CO, and COH), (b) experimental, (c) simulated spectra with centerbands indicated with asterisks (\*), slices (d) of the anisotropic dimension and (e) 2D 3QMAS spectrum with the centerbands indicated with dashed horizontal lines and circles, the remaining peaks in the spectrum are due to spinning sidebands. The MAS frequency was 19 kHz.