

# Enhancing resolution and sensitivity of $^{17}\text{O}$ solid-state NMR through combining double rotation, $^1\text{H}$ decoupling and satellite modulation for biomolecular applications

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## Abstract

$^{17}\text{O}$  solid-state NMR is a highly sensitive probe of structural detail of organic solids but improvements in sensitivity and resolution are crucial for it to be applied to larger biological molecules. Here it is shown that high resolution ( $\sim 1$  ppm) and significant signal enhancement can be achieved by combining  $^1\text{H}$  decoupled double rotation (DOR), which narrows the lines by a factor of  $\sim 100$  compared to conventional magic angle spinning, and manipulation of the satellite transition populations to transfer magnetisation to the central transition, which produces a signal enhancement of  $\sim 2$ .

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## 1. Introduction

$^{17}\text{O}$  solid-state NMR offers a novel, practical approach to tackle one of the key experimental challenges for biomolecular chemistry to provide high quality, detailed and unambiguous atomic scale information about the molecular bonding arrangement and changes that occur upon ligand-receptor interaction. The ubiquity of oxygen throughout living systems should imply that  $^{17}\text{O}$  plays a central role in such studies. Oxygen plays a key role in intra- and intermolecular interactions with hydrogen-bonding important in biological processes so that  $^{17}\text{O}$  could provide detailed information about the dynamics and structure of biomolecules. Since amino acids are key building blocks of many biological molecules they are genuine models for the development of methodology for larger biomolecules.

In recent years there has been a significant increase in the reports of  $^{17}\text{O}$  NMR from biomolecular solids, all of which rely on conventional magic angle spinning (MAS) NMR [1–8]. It has been clearly demonstrated that the NMR interaction parameters (i.e. the isotropic chemical shift and the quadrupole interaction which is characterised by the quadrupole coupling constant  $\chi_Q$  and the asymmetry parameter  $\eta$  [9] ( $\chi_Q = e^2 Qq / (2I(2I - 1))\hbar$ ,  $eQ$  is the quadrupole moment and  $eq$  is the maximum component of the electric field)) depend very sensitively on local environment. Comparison of alanine in a range of materials show significant variation of its  $^{17}\text{O}$  NMR interaction parameters, including direct observation when inserted into position 12 of the 23 amino acid peptide WALP embedded within a hydrated membrane [4]. The work on WALP used 70 at.% enriched water and specifically labelled a single site. However to be a widely useful tool several key challenges still need to be addressed: (i) narrowing lines to overcome the significant overlap that will occur when multiple sites are present

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due to residual second-order quadrupole effects under MAS and (ii) signal enhancement to improve sensitivity. Addressing these will allow new  $^{17}\text{O}$ -based solid-state NMR approaches for structure determination and application to larger biomolecules to be contemplated.

Magic angle spinning is the most commonly used approach in solid-state NMR since it is a straightforward one-dimensional experiment. For  $^{17}\text{O}$  in organic solids a limitation is that  $\chi_Q$  is typically in the range 6–9.5 MHz such that residual width due to second-order quadrupole effects can be substantial ( $>10$  kHz even at 14.1 T, with the effect scaling inversely with the applied magnetic field). This has three direct consequences: (i) fast MAS is required even at high magnetic field, (ii) the sensitivity is not as high as if the line was more completely narrowed and (iii) there is likely to be significant spectral overlap if multiple sites are present. Hence higher resolution techniques that potentially offer significant advantage are needed. Multiple-quantum (MQ) MAS that removes anisotropic quadrupolar broadening through the correlation of symmetric ( $+m \leftrightarrow -m$ ) MQ transitions with the single-quantum central-transition under MAS is one that has proved popular since it requires only a conventional MAS probehead [10]. However this approach presents challenges to overall sensitivity and the quantitative integrity of the spectrum since it demands excitation and conversion of multiple-quantum coherences [11] which becomes less and less efficient as the electric field gradient increases, and is a 2D experiment. An alternate method is double rotation (DOR) where the sample is spun about two angles (usually  $54.736^\circ$  and  $30.12^\circ$ ) simultaneously through the use of a sophisticated double rotor, which removes both second- and fourth-rank broadenings to produce a truly high resolution spectrum [12,13]. DOR offers the conceptually simplest approach to producing an isotropic spectrum, with reasonable sensitivity in a one-dimensional experiment.

DOR was first proposed in the late 1980s but early probes suffered from a number of drawbacks in that they could only manage outer rotor speeds of  $\sim 1$  kHz and long term spinning was difficult. Hence although there were some reports of  $^{17}\text{O}$  DOR from inorganic solids (see Ref. [14] and references therein) almost all DOR experiments have involved sensitive nuclei (e.g.  $^{23}\text{Na}$ ,  $^{27}\text{Al}$ ). In addition a few early DOR studies used a double channel (i.e. decoupling and cross-polarisation) probe,  $^1\text{H}$ – $^{27}\text{Al}$ , to allow identification of those aluminium sites that are directly connected to protons in microporous aluminophosphates (AlPOs) [e.g. [15]]. This paper reports the use of a new optimised  $^1\text{H}$ – $^{17}\text{O}$  DOR probehead with computer-assisted control of the probehead air pressures. The outer rotor of this new probehead will spin at up to 2 kHz for effectively an indefinite period allowing weak signals to be investigated and more complex experiments contemplated. The high power  $^1\text{H}$  decoupling (up to  $\sim 50$  kHz) allows dipolar coupled proton-rich solids to be studied.

This Letter compares  $^{17}\text{O}$  DOR on two amino acids L-alanine and glycine  $\cdot$  HCl since they offer highly contrasting

oxygen sites that are typical in such materials. Glycine  $\cdot$  HCl has two very distinct oxygens C=O and O–H, whereas for alanine the two oxygen signals arise from O(1), the conventional hydrogen-bond with  $\text{O} \cdots \text{N}$ , and the O(2) site, a bifurcated oxygen as the attached proton is also bonded to two other nitrogen atoms. For L-alanine a two field dynamic angle spinning (DAS) study of a uniformly  $^{17}\text{O}$ -labelled sample showed two completely resolved  $^{17}\text{O}$  signals with their shift at 11.7 T differing by 29 ppm. High power  $^1\text{H}$  decoupling substantially narrowed the DAS lines [16]. The  $^{17}\text{O}$  3Q MAS NMR spectrum of L-alanine also shows that the two distinct resonances could be completely resolved although the residual width in the isotropic dimension was 6–7 ppm [2]. A key feature of the present paper is that in addition to the sensitivity enhancement produced by the line narrowing of DOR, it shows that further enhancement can be achieved using manipulation of the satellite populations to enhance the intensity of the central transition. Recently there has been much development of pulse sequences to manipulate the satellite populations so as to increase the population difference across the central transition prior to the observed pulse. Sequences that have been applied to MAS of  $I = 5/2$  nuclei include rotor assisted population transfer (RAPT) using  $(X-\tau-\bar{X})_n$  [17,18] or frequency switched Gaussian pulses (FSG) [19], and double frequency sweeps (DFS) [20]. For  $^{17}\text{O}$  MAS an enhancement factor of  $\sim 2.1$  has been reported using a variant of RAPT [18]. Here we report RAPT under DOR for the first time.

## 2. Experimental details

### 2.1. Sample preparation

The  $^{17}\text{O}$  enriched samples were prepared by acid-catalysed exchange as described previously [2,21] from 20 at.%  $\text{H}_2^{17}\text{O}$ .

### 2.2. Solid-state $^{17}\text{O}$ NMR

$^{17}\text{O}$  NMR spectra were recorded on a chemagnetics infinity 600 spectrometer at an applied magnetic field of 14.1 T operating at a frequency of 81.345 MHz. A 4 mm MAS probe was used for alanine  $^{17}\text{O}$  MAS experiments with the sample spinning frequency 15.3 kHz and a 3.2 mm MAS probe spinning at 21 kHz was used for glycine. A spin-echo experiment was used to record all  $^{17}\text{O}$  MAS spectra with the echo delay set to an integer number of rotor periods. Approximately 18000 transients were recorded with a recycle delay of 5 s for alanine and 3 s for glycine. All spectra were referenced to water at 0.0 ppm.

$^{17}\text{O}$  DOR NMR experiments were carried out using odd-order sideband suppression [13] and the outer rotor speed was varied between 1300 and 1800 Hz to determine the centreband. Typical CW  $^1\text{H}$  decoupling was 34 kHz. The  $^{17}\text{O}$  pulse length was 3  $\mu\text{s}$ . Two RAPT methods FSG and  $(X-\tau-\bar{X})_n$  were used to enhance the  $^{17}\text{O}$  NMR signal.

The pulse train for the RAPT modulation  $(X-\tau-\bar{X})_n$  sequence consisted of 32 sets ( $=n$ ) of 1  $\mu$ s pulses separated by 0.6  $\mu$ s with an rf field strength of 27 kHz. For the FSG sequence 10 Gaussian pulses, 21  $\mu$ s long, spacing 2  $\mu$ s, offset from the central spectral alternately by  $\pm 350$  kHz were used.

### 3. Results and discussion

Fig. 1a shows the  $^{17}\text{O}$  MAS (upper) and DOR (lower) spectra of L-alanine. The MAS spectrum clearly shows the presence of two overlapping oxygen lines whereas two sharp resonances separated by  $\sim 10$  ppm, along with their spinning sidebands, can be clearly identified in the DOR spectrum. The narrowing under DOR compared to MAS is very marked, a factor of  $\sim 100$ . Fig. 1b shows the MAS and DOR spectra for glycine  $\cdot$  HCl. An MAS experiment is all that is needed to determine the NMR parameters as two completely separated oxygen resonances are observed because two structurally highly distinct sites, a definite C=O and an OH site with an OH distance  $< 1.0$  Å are present. The DOR spectrum again shows very significant narrowing by a factor of  $> 100$  compared to MAS, but in this case however, only a single narrow peak with extensive spinning sidebands centred under the MAS line from the C=O oxygen at 336 ppm is observed. The large sideband manifold is because the chemical shift anisotropy of the

C=O site in glycine  $\cdot$  HCl is about 600 ppm [7]. There is very little intensity (if any) from the directly bonded OH site where the O–H distance is  $\lesssim 1.0$  Å. This is probably due to the relatively low decoupling power and spinning speed used for this experiment being insufficient to narrow the dipolar coupling, whereas for alanine, where the O–H distances are  $\sim 1.7$ – $1.8$  Å, these experimental conditions are more than ample to fully narrow the resonance. It should be noted that although DOR is the major cause of the narrowing compared to the MAS, the  $^1\text{H}$  decoupling also plays a significant role since the resonances narrow by a factor of  $\sim 2$  compared to the uncoupled spectra.

Fig. 2 shows the enhancement obtained for the DOR centrebands of: (a) alanine and (b) glycine. For alanine both the RAPT sequences give very similar performance with an enhancement of  $\sim 1.6$ , while for glycine only the FSG sequence was used and an enhancement of  $\sim 2.0$  was obtained, not far off that achieved under simple MAS for  $^{17}\text{O}$  in silica [18]. Both these sequences apply rf irradiation which is modulated in such a way that it has a frequency profile designed to affect the satellite transitions but not the central transition. The  $(X-\tau-\bar{X})_n$  sequence was optimised empirically. For the FSG sequence the enhancement was not very sensitive to the offset once this was greater than  $\sim 250$  kHz. For offsets much above 350 kHz the bandwidth of the probe means that most of the power is reflected back to the transmitter reducing that

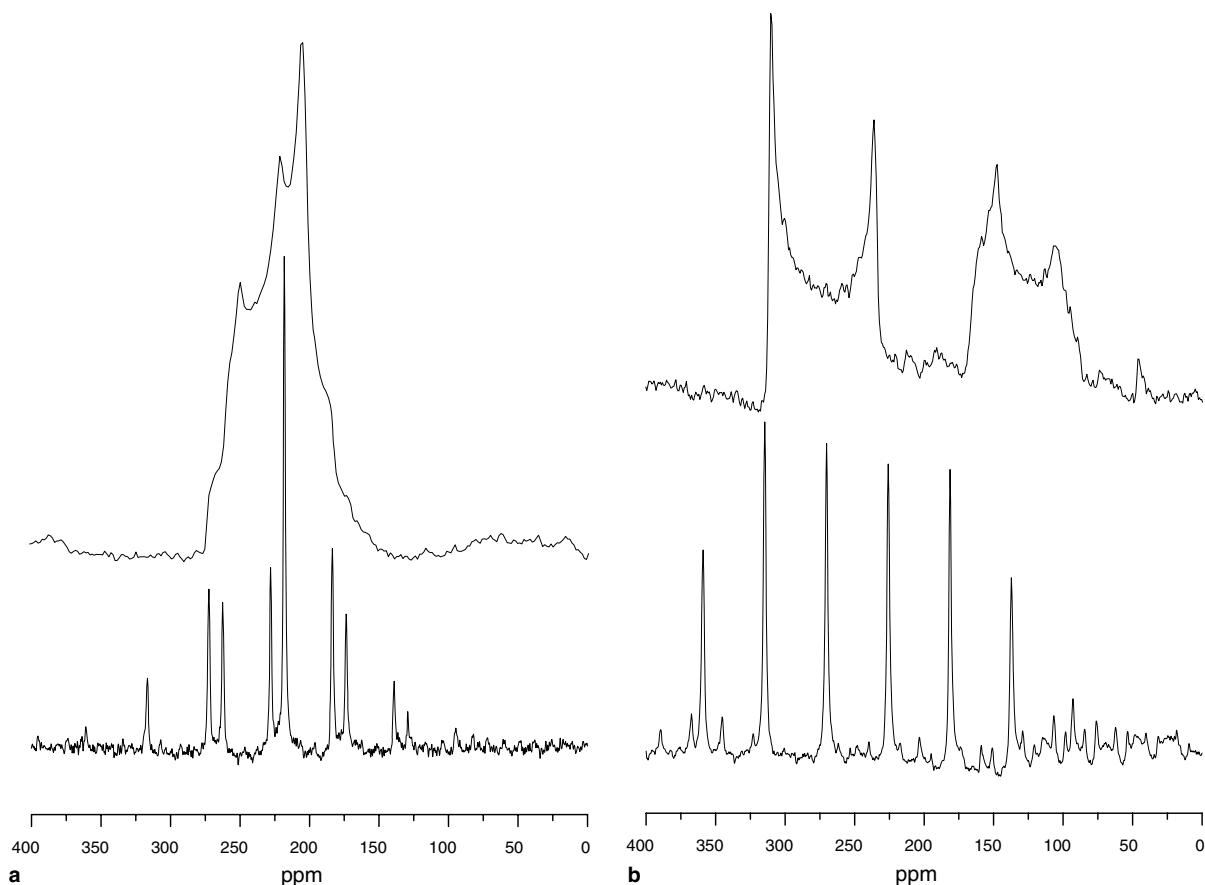


Fig. 1. Comparison of  $^{17}\text{O}$  MAS and DOR spectra (1800 Hz outer rotor) at 14.1 T of (a) L-alanine and (b) glycine  $\cdot$  HCl.

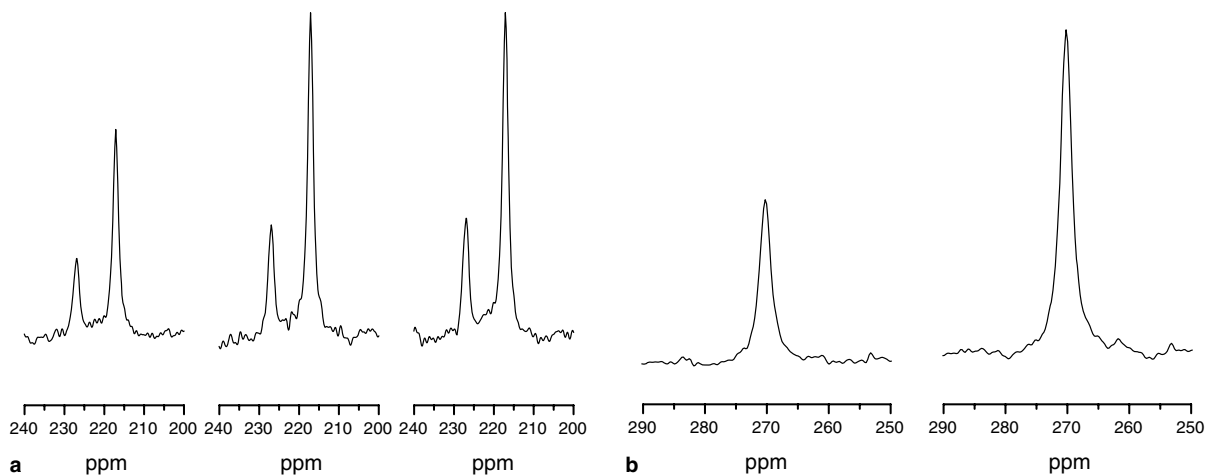


Fig. 2.  $^{17}\text{O}$  signal NMR signal enhancement under DOR for (a) L-alanine using RAPT  $(X-\tau-\bar{X})_n$  and FSG, no RAPT (left),  $(X-\tau-\bar{X})_n$  (middle), FSG (right); (b) glycine · HCl no RAPT (left), FSG RAPT (right). All spectra outer rotor 1800 Hz, 3200 transients with 1 s pulse delay

available to be delivered to the sample and calculations (under MAS) have shown the enhancement is fairly insensitive to offset once it is greater than  $\sim 250$  kHz for a wide range of  $\chi_Q$  [19]. For all these modulation sequences the initial irradiation combined with sample rotation causes the population of the satellite transitions to become saturated thereby enhancing the central transition signal. This is the first time that it has been demonstrated that under the more complex reorientation of DOR these enhancement schemes are as effective as under simple MAS. For  $^{17}\text{O}$  any, even small, increase in the signal intensity has significant consequences in opening up new possibilities for the use of  $^{17}\text{O}$  solid-state NMR. These data also confirm that a previous numerical calculation of the dependence of the signal enhancement on the magnitude of  $\chi_Q$  for a spin 5/2 system under MAS [18] which showed that the enhancement was fairly independent of  $\chi_Q$  at a factor of 2.0–2.2. The enhancements under DOR obtained here are similar,  $\sim 2$ , and raise the maximum  $\chi_Q$  values for which enhancement has been observed for  $^{17}\text{O}$  to 8.3 MHz.

The very great narrowing observed under DOR opens up many real and exciting new NMR possibilities for  $^{17}\text{O}$ . Linewidths for alanine and glycine are  $\sim 1$  ppm (and our observations include some  $^{17}\text{O}$  DOR lines with significantly smaller linewidths [22,23]) are now comparable with those for spin-1/2 nuclei (e.g.  $^{13}\text{C}$ ,  $^{15}\text{N}$ ) from comparable samples. A whole spin-1/2 methodology has developed that allows connectivity of molecules to be considered via for example correlation and recoupling sequences. With the linewidths observed here much of the methodology can, in principle, be transferred to  $^{17}\text{O}$  (although detailed complications such as the more complex motion under DOR compared to MAS need to be resolved). These new possibilities are further encouraged by the gains in sensitivity compared to MAS as a result of the significant DOR line narrowing combined with RAPT. Less than an hour is required for good S/N DOR spectra for samples with relatively modest isotopic enrichment (20 at.% enriched water) compared with overnight for an MAS spectrum.

Considerably higher enrichments are possible so that  $^{17}\text{O}$  DOR observation of much larger biomolecules is feasible and, with the much high spectral resolution achieved, multiply labelled molecules can be studied [23,24]. The improvements in DOR probe technology, particularly the high rotor speed combined with the stability of the system, means that two-dimensional experiments lasting several days can be contemplated. Although two fields are required to extract the NMR interaction parameters [24], this information is not needed for correlation/recoupling experiments. Comparison should also be made to the more common techniques for producing high resolution spectra from half-integer spin quadrupole nuclei, MQ-MAS and ST-MAS. To date using  $^{17}\text{O}$  MQ-MAS on such samples much inferior resolution has been achieved. The resolution from such experiments is determined by the acquisition time in the second dimension. However the two-dimensional nature of these experiments combined with the loss of sensitivity in excitation and conversion of multiple-quantum coherences means that they become very time consuming, especially if comparable resolution to the DOR is required.

#### 4. Conclusion

Under DOR line narrowing of  $^{17}\text{O}$  in proton-rich samples by a factor of  $>100$  compared to conventional MAS offers both genuinely high resolution spectra and yields a large enhancement of the signal sensitivity. CW  $^1\text{H}$  decoupling is shown to be effective at producing further narrowing provided that the proton coupling is not too strong. It has also been shown that further signal enhancement under DOR is possible using both RAPT and FSG sequences <sup>1</sup>.

<sup>1</sup> During preparation of this manuscript it was brought to our attention that work using a similar DOR probe reported enhancement of  $^{27}\text{Al}$  using DFS [25]. This is distinct from RAPT sequences needing, in general, specialised hardware. No decoupling, which is essential for  $^{17}\text{O}$  in biomolecules, was reported.

These observations combine to suggest that DOR NMR of  $^{17}\text{O}$  has the potential to offer exciting new applications as a probe of biomolecular structure.

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