

## Structural Elucidation of Biomaterials by Very High Speed MAS Proton and Oxygen-17 NMR at Ultrahigh Fields

### Overview and Context of Research Programme

Structural resolution of large ( $M_{r(\text{eff})} \sim \text{MDa}$ ) biomolecules is at the forefront of structural biology to allow functional descriptions as well as in therapeutic design (drugs, etc.). Membrane proteins provide one of the major challenges in this area, due to the difficulty in their crystallisation and hydrophobic character, but the need for detailed information at the atomic level is paramount - >70% of all drugs act on them and 55 - 85% of all new drug targets for the next decade are identified as membrane proteins.

Solid state NMR is proving to be a successful way of obtaining detailed information about functional membrane proteins. In particular, exploring ligand binding sites and the interactions involved in receptor activation resulting from such binding is an important area. One aspect which has not received much attention is the determination of hydrogen bonding both within ligand sites in a protein, as well as within an environment of the membrane core (low dielectric constant of 2) where they are assumed to stabilise a protein. Although implicit in descriptions of such interactions, H-bonds have not been described (length or strength) directly in these important systems. Solid State  $^{17}\text{O}$  NMR provides a new opportunity to examine and detect H-bonding in a wide range of systems but, since it has not been routinely studied previously, proof of principle needs to be demonstrated under ideal conditions, as initiated here, for characterisation of hydrogen-bonding.

Although deuterium is a good nucleus for determination of orientation and dynamics from the quadrupolar interaction and relaxation phenomena respectively, it is also a valuable substitution to reduce dipolar contributions to spectral broadening from a range of NMR nuclei, particularly  $^1\text{H}$ . Thus, in  $^{13}\text{C}$  or  $^1\text{H}$  NMR of membrane peptides and bilayers, a deuterated lipid bilayer gives the opportunity for reduced line broadening as well as permitting the  $^1\text{H}$  spectra to be revealed without the background signal from the predominant lipid protons. Hence we have carried out a range of experiments to look for resolved detail in  $^1\text{H}$  NMR spectra from biologically significant materials.

### Changes to Research Programme

The original grant application was an interdisciplinary 2 site application at the Life Sciences interface. The proposal had the ultimate goal of applying the solid state NMR techniques developed at the start of the project to a detailed structural study of membrane proteins such as the M4 peptide of the acetyl choline receptor which has many biochemical implications. The panel thought that original application was "highly adventurous" and perceived a significant associated level of risk. Hence the panel decision was to fund a much more limited proof of concept technique development project to be largely based at one site (Warwick). The severe curtailment of resources can be gauged by the reduction of PDRA support from 6 to only 2 man-years, a similar reduction in the amount of technical support and a significant cut in equipment. The panel acknowledged that "it is not expected that with the reduced level of resource that the applicants will complete the full programme of work as set out in the original application". Hence the aims of this project became much more technique development oriented with the new goals (stated on the form) of examining the feasibility of ultrafast MAS, deuteration schemes to reduce the dipolar coupling, and to carry out initial development of  $^{17}\text{O}$  in simple biomolecules. The non award of the PDRA at Oxford meant that only limited progress on  $^2\text{H}$  and  $^{17}\text{O}$  labelling could be made. A further difficulty was that the world-leading prototype wide-bore 800 MHz spectrometer that should have been ready from mid-2000 has been plagued with technical problems, and was not available for this project. One of the approaches suggested in the original proposal was to render the proton dipolar more inhomogeneous by increasing the chemical shift between interacting species at higher fields. Hence the improvements in resolution reported here carried out at 600 MHz are expected to be even better at higher fields since it has been shown whilst this work was in progress that for  $^1\text{H}$  spectra the homogeneous linebroadening is inversely proportional to the product of the field strength and the spinning speed.<sup>17</sup> Despite all the difficulties, an ambitious modified set of objectives based on technique development but still with biomolecular impact was proposed.

### Achievements of Scientific Programme: Summary of Outcomes

1. Optimisation of biosynthetic ways of labeling lipids with  $^2\text{H}$  and amino acids with  $^{17}\text{O}$ .
2. Installation of a new ultrafast (> 45 kHz) non commercial dual channel MAS probe with high power  $^1\text{H}$  decoupling
3. Recording fast  $^1\text{H}$  MAS spectra from a range of biologically relevant molecules to show the effect of spinning speed and the effect of strong hydrogen bonding.
4.  $^1\text{H}$  Fast MAS and MAOSS (magic angle oriented sample spinning) of deuterated samples including the first ever reported  $^1\text{H}$  spectrum from a membrane embedded protein and NOESY spectra.
5. Showing that in simple amino acids with even modest  $^{17}\text{O}$  enrichment, good quality NMR spectra could be obtained that distinguished different oxygen environments and the solid state NMR spectra are much more informative than the solution spectra.

6. Developed schemes for significant ( $> 2$ ) enhancement of the signal for  $I = 5/2$  spins (e.g.  $^{17}\text{O}$ ).<sup>3</sup>
7. Recording  $^{17}\text{O}$  data from a wide range of model compounds showing that the NMR interaction parameters are very sensitive to the hydrogen bonding arrangements
8. Shown that computer simulations of the NMR parameters can aid structural interpretation of the  $^{17}\text{O}$  NMR data.
9. Shown that  $^{17}\text{O}$  spectra of the D and the L forms, and the D,L- (racemic crystal) form of glutamic acid are very different and are also very different for monosodium glutamate (MSG).

## Detailed Description of Work Carried Out

### Isotopic Labeling Schemes and Sensitivity of $^{17}\text{O}$ NMR

Some work was carried out on optimising biosynthetic ways of labeling lipids to make bilayer forming lipids for use in novel solid state NMR studies. The way in which lipids are made is to acylate deuterated acyl chains and condense them to benzyl-protected glycerol. Usually this part of the molecule is not deuterated, but we have now extended the method to include this moiety, not least because the  $^1\text{H}$  resonances can overlap resonances from peptides and proteins in membranes. This is a useful step forward and some small stocks of lipids have been made and used.

Various procedures were investigated for efficiently  $^{17}\text{O}$  enriching amino acids. One optimised procedure involved suspending the amino acid in a mixture of  $^{17}\text{O}$  enriched water and dioxane (1:3,v/v). Water was used in 25 fold molar excess over the amino acid. The mixture was treated with a continuous stream of HCl gas obtained from  $\text{MgCl}_2/\text{H}_2\text{SO}_4$  for two hours while kept at  $90^\circ\text{C}$ . The sample was lyophilised and the  $\text{H}_2^{17}\text{O}$ /dioxane recovered. These amino acids would be the source for the manufacture of more complex molecules. Below it is shown that the electric field gradients (efgs) are typically quite large such that high fields and fast spinning are needed to obtain good spectra. It is worth noting that the typical separation of the carbonyl and hydroxyl lines in the spectrum combined with their large width is such that on a 600 MHz spectrometer one needs to spin at  $> \sim 17$  kHz to ensure no overlap of the lines. This necessitates using a small volume rotor and much of our work was done in a 3.2 mm rotor with a volume of  $\sim 11$   $\mu\text{l}$ . Nevertheless enrichment using 10%  $^{17}\text{O}$   $\text{H}_2\text{O}$  was found to be sufficient to give an adequate signal-to-noise ratio. This means that for more complex molecules and for peptides embedded in membranes much higher enrichment would offer sufficient sensitivity to examine oxygen in these cases.

### NMR Spectroscopy

Before extensive studies were carried out on biomolecularly relevant materials it was important to establish the variations in the  $^1\text{H}$  and  $^{17}\text{O}$  NMR interaction parameters with hydrogen-bonding. Hence surveying the literature a range of carboxylic acids and their salts were identified where there were accurate structural details and large variations in hydrogen-bonding. Phthalates, maleates and chloromaleates contain examples where there is a wide range of hydrogen-bonding from "typical" medium strength hydrogen-bonding to some of the strongest hydrogen-bonding known in such organic materials. The large variation in hydrogen bond strength of these materials allowed the likely range of  $^1\text{H}$  and  $^{17}\text{O}$  NMR parameters in biomolecular materials to be investigated. This was extended to amino acids and membrane embedded proteins.

### Proton NMR

An ultrafast MAS probe was installed which was capable of MAS rates of up to 45 kHz. It can be seen in Fig. 1 that in alanine the  $^1\text{H}$  spectra continue to narrow up to 45 kHz. Fast  $^1\text{H}$  was carried out on a wide range of compounds and some examples of these are shown in Fig. 1. Three of the organic salts shown in Fig. 1 have very strong hydrogen bonds and show the strongly hydrogen bonded proton well separated at 20 ppm. Such shifts are extremely large for protons and fast MAS alone would be able to identify such sites even in complex biomolecules with many protons. Ultrafast MAS also allows new possibilities for efficient decoupling schemes for  $^1\text{H}$ - $^{13}\text{C}$ .<sup>18</sup>

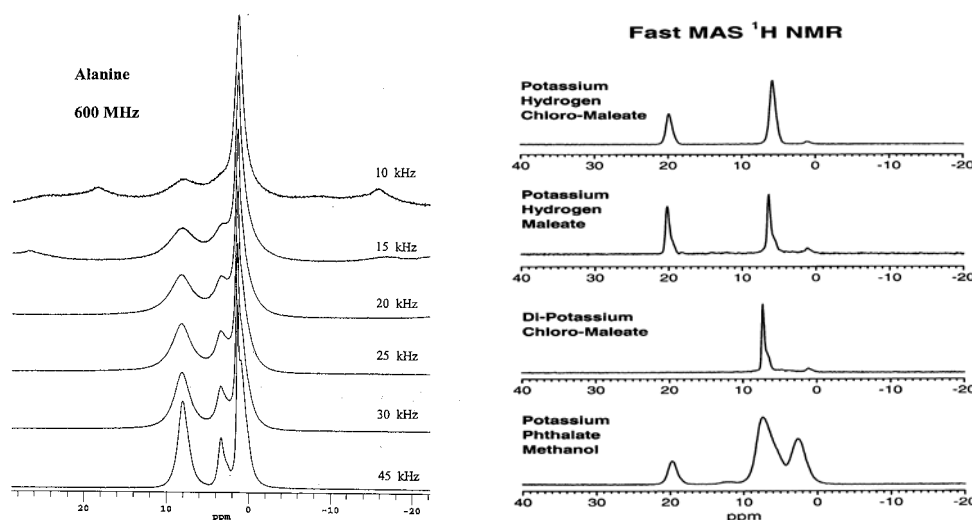


Figure 1:  $^1\text{H}$  NMR showing the effect of (a) spinning speed on the spectrum from alanine and (b) strong hydrogen bonding in some maleate salts.

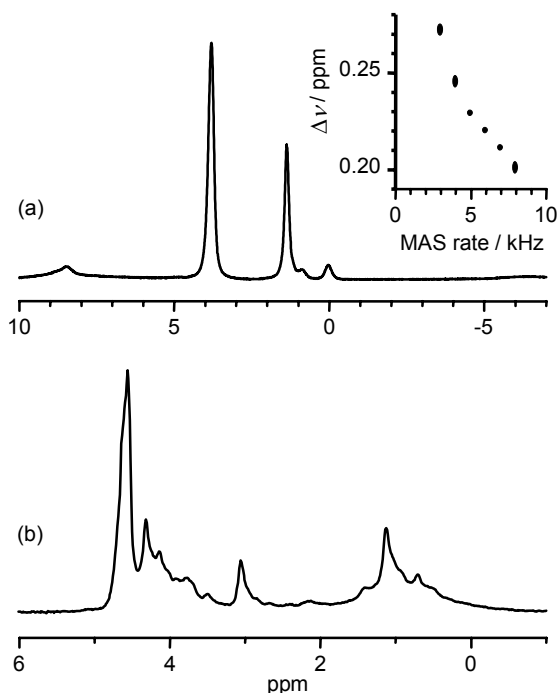


Figure 2:  $^1\text{H}$  MAS of (a) 98% deuterated alanine with the inset showing the effect of spinning speed on linewidth, and (b) deuterated lipid  $\text{d}_{54}$ -DMPC

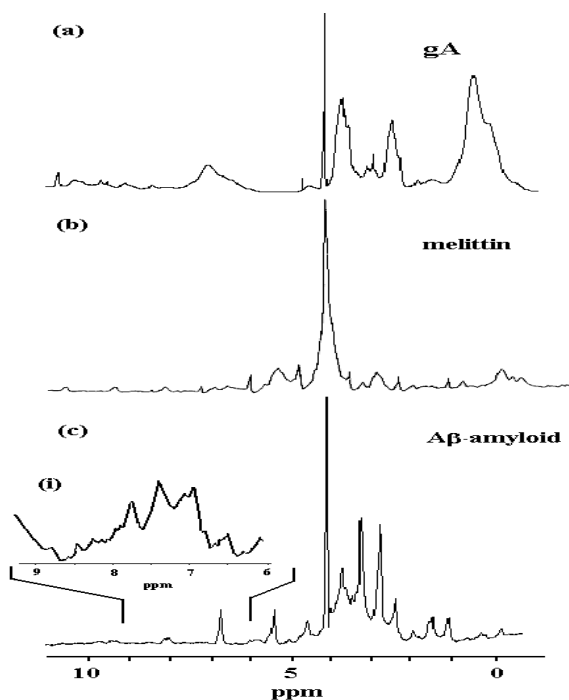


Figure 3:  $^1\text{H}$  MAOSS NMR of the peptides gramicidin A (a), melittin (b) and  $\text{A}\beta_{(1-40)}$ -amyloid (c) incorporated into  $\text{d}_{67}$ -DMPC membranes in 1:15, peptide: lipid molar ratio, hydrated with  $\text{D}_2\text{O}$  of 50% w/v.

More routinely accessible MAS speeds were used to study some deuterated amino acids and lipids. It can be seen that in the deuterated samples narrow lines are observed giving high resolution spectra (Figure 2). The  $^1\text{H}$  lines are significantly narrower than for protonated samples with ultrafast MAS and narrower than those produced from such samples under any line narrowing scheme in the literature. These studies were extended by the incorporation of protonated peptides in deuterated bilayers. For molecules incorporated in lipid bilayers the intensity of the usually dominant proton NMR resonances from the bilayer lipids is significantly reduced, as an added benefit to the decrease in the line-broadening due to the dipolar couplings from protons to the peptides ( $d_{(\text{protons})} \gg d_{(\text{deuterons})}$ ). We have now coupled this deuteration with a new methodology (Magic Angle Spinning of Oriented Samples, MAOSS) where the fast ( $\tau_r \sim 10^{-9}\text{s}$ ) long axis rotation of the components can average out  $^1\text{H}$  dipolar coupling to give narrow, high resolution-like spectra. This initial proton spectrum (Figure 3 and *first ever reported*) for three membrane embedded peptides show this much enhanced resolution and even assignments can be made for some obvious residues.

This shows that structural studies using conventional solution state NMR methods, may indeed be possible with unlabelled peptides. This is illustrated by the first NOESY spectra being generated for three different, but similar sized peptides, gramicidin A (an ion channel), Alzheimer's  $\beta_{(1-40)}$  amyloid peptide and melittin (a fusogenic peptide from bee venom). Only gramicidin has been described structurally. We are pursuing this approach to get full structures as a general method.<sup>7</sup> This natural abundance work will augment  $^{15}\text{N}$  and  $^{13}\text{C}$  labelling as well as extension to  $^{17}\text{O}$  labelling for full structures.

### $^{17}\text{O}$ NMR (a) Model carboxylic acids and salts

The variation in the oxygen spectra is very marked in these compounds. Some show "typical"  $^{17}\text{O}$  NMR spectra from carboxylic acids<sup>1</sup> with medium strength hydrogen-bonding where the 2 distinct oxygen environments are expected with well separated resonances which have distinct well defined quadrupole lineshapes (typical spectra of this type can be seen below from some of the amino acids) from the carbonyl and the hydroxyl oxygen. Subtle variations in hydrogen bonding produce readily observed spectral changes. To understand better these variations with structure, the NMR parameters were computed using the Gaussian software. In phthalic acid preliminary calculations showed the  $^{17}\text{O}$  parameters are very sensitive to the position of the intermediate proton and could provide very detailed information about the local hydrogen-bonding. As the proton becomes more centrally located between the oxygens the hydrogen-bonding strength increases and the difference between the oxygen sites is reduced. The quadrupole interaction was seen to decrease by a factor of 1.4 as the hydrogen-bonding increases, and in some cases there is an additional a large change in the chemical shift of  $> 250$  ppm.<sup>1,5</sup> A surprising result was that even in materials with only moderate hydrogen-bonding, the spin-lattice relaxation time ( $T_1$ ) varied by more than 3 orders of magnitude. Based on this data there is much encouragement that large variations in the  $^{17}\text{O}$  NMR interaction parameters reflect molecular conditions in a sensitive way.

## (b) Amino acids

A number of amino acids were enriched with  $^{17}\text{O}$  and spectra of two of these, asparagine and tyrosine, are shown in Fig. 4. In contrast to solution state NMR, where tyrosine gives a single resonance at 256 ppm, the two oxygen sites are clearly resolved. Furthermore, there are clear differences between the NMR parameters of the different amino acids. In general the carbonyl oxygen shift is typically 350 - 315 ppm with a near axial electric field gradient of 8.15 - 8.55 MHz.<sup>6</sup> The hydroxyl oxygen has a smaller shift range and efg, of typically 187 - 172 ppm and 7.60 - 7.35 MHz respectively. In this case the electric field gradient is distinctly non-axial with an asymmetry parameter of  $\sim 0.20 - 0.30$ : the typical measurement precision is  $\pm 1$  ppm,  $\pm 0.03$  MHz and  $\pm 0.03$  respectively so that different amino acids can be readily distinguished.

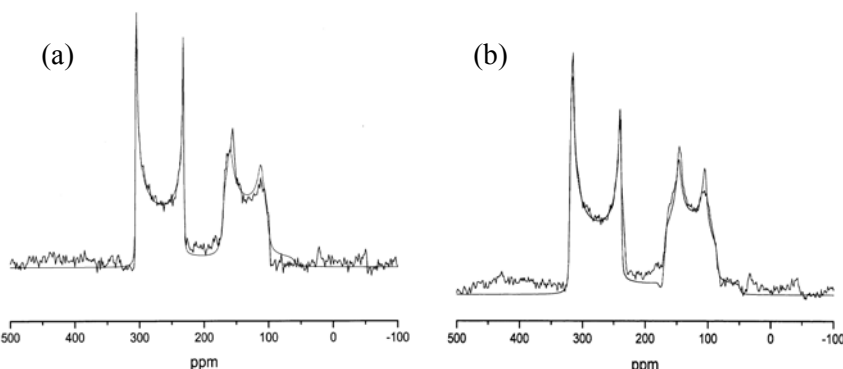


Figure 4:  $^{17}\text{O}$  MAS NMR of the amino acids (a) tyrosine.HCl and (b) asparagine.

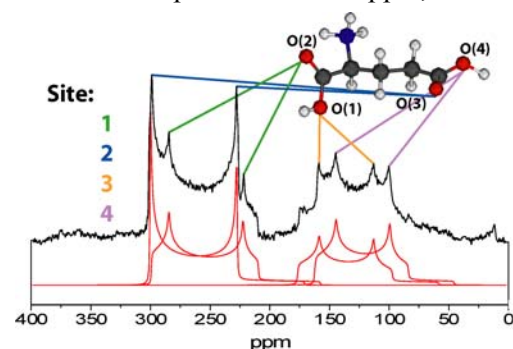


Figure 5:  $^{17}\text{O}$  MAS NMR of L-glutamic acid with the assignments of the oxygen peaks to the molecular sites.

introduced XiX decoupling pulse sequence was the most effective scheme for resolving these features.<sup>19</sup>

This data from the L- and D-glutamic acids individually makes interesting comparison with that reported during the course of this project for D,L-glutamic acid<sup>20</sup> where the spectrum showed two resonances in approximately the same position as each of the pairs of lines that are observed here for both the L- and D-glutamic acids. Even under 3Q conditions there was no resolution into separate lines. There was, in addition, an extra resonance of twice the intensity centred around  $\sim 200$  ppm between the two peaks observed here in L-glutamic acid. The assignment of the resonances do not correspond to ours. The outer two resonances are from carbonyls and hydroxyls that are closely similar to those present in the L structure since their parameters are in close agreement with the mean parameters observed for the two pairs of sites observed here. Then the intermediate resonance arises from oxygens (appearing to have similar NMR parameters) that are very different to those in the chiral forms and result from the differing bonding in the D,L form. The D,L form of the glutamate could be either be a racemic conglomerate of chiral crystals or a racemic crystal. However the presence of the additional resonance in the spectrum with  $\sim 50\%$  of the total intensity strongly implies the latter (see ref. 4 for more details). Hence differences in the packing conformation between the chiral forms and the D,L racemate leads to changes in the oxygen bonding of the network so that in the racemate half the hydroxyls and carbonyls must be in very different (hydrogen) bonding environments. The sensitivity of oxygen to changes in bonding is emphasised by comparison with MSG where there are two distinct but very similar glutamate anions doubling, in principle, the number of oxygen sites. In MSG a single, almost featureless line with some minor structure in the MAS spectrum at an intermediate shift to the signals from L-glutamic acid.HCl is seen (Fig. 6(a)). A 3Q MAS spectrum shows 5 lines in the centreband in Fig. 6(b). These changes must be due to the changed bonding in MSG. The ability to resolve

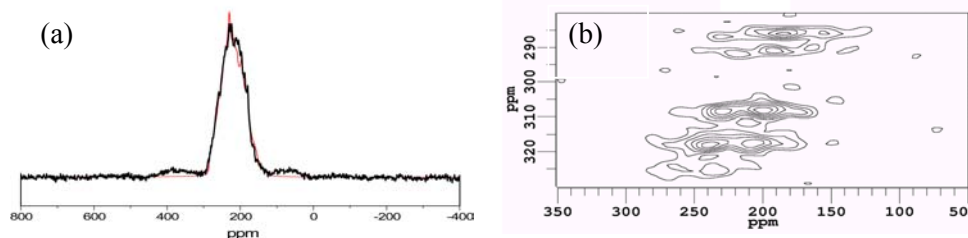


Figure 6:  $^{17}\text{O}$  of MSG showing (a) MAS and (b) 3Q centrebands.

the different sites and to detect changes from  $^{17}\text{O}$  NMR spectra suggest that this will become a fruitful experimental approach to elucidate molecular pathways of biochemical recognition and could find widespread application.

## Impact and Beneficiaries of Research

It is too early to gauge directly the impact of the research carried out in this 2 year project but much of the work has been laying the foundations to enable further structural elucidation of a wide range of organic materials by solid state NMR. So far both deuteration and  $^{17}\text{O}$ -enrichment procedures of various simple biomolecules have been shown to be effective. These will be necessary for all such studies as the first step of using this approach to investigate more complex biomolecules. Techniques like  $^1\text{H}$  MAOSS of deuterated peptides in lipid membranes provided high resolution  $^1\text{H}$  spectra that allowed NOESY experiments to be carried out, an approach that could be widely applicable. The  $^{17}\text{O}$  parameters collected here show that oxygen has a very high sensitivity to even small structural variations such that  $^{17}\text{O}$  could be a very useful addition to the multinuclear study of biomolecules. It is predicted that the next few years will see a dramatic expansion of the use of  $^{17}\text{O}$  to the study of such molecules as higher fields and improved probes become available.

The technique development could find beneficiaries from a wide range of scientific disciplines. This work could be applied to the study of many biochemically important problems. These include of glutamate that plays a significant role in many biochemical processes acting as one of the most important neurotransmitters activating several families of brain-receptors with implications in for example the food industry. The resolution of membrane structure has implications for drug design used for the treatment of diseases. The possibility of determining structures from solid state NMR spectra of membrane proteins, and characteristics of ligand binding, could well open up new avenues of pharmaceutical research.

## Explanation of Expenditure

Equipment expenditure was broadly in line with the planned breakdown of costs except that it was not possible to obtain the  $> 45$  kHz MAS probe and a network analyser for the amount available under this heading. (The network analyser has proved invaluable in optimally setting up this, and other, probes). The additional costs were found from the travel, which is underspent since the lack of a working 800 MHz spectrometer meant that there were far fewer trips from Warwick to the Rutherford Lab., and from the consumable budget.

## Further Research in this Area and Infrastructural Improvements

This project marked the first major funding for the Warwick group for work on organic/bioorganic materials and will now (funding permitting) form an important component in the research programme of the group. To carry out more background  $^{17}\text{O}$  NMR work on hydrogen-bonding MES obtained a Royal Society Leverhulme Trust Senior Research Fellowship (2001-2002). The foundations of the work laid here will form the basis of a new grant application concentrating on further developing  $^{17}\text{O}$  NMR for structural elucidation of biomolecules as envisaged in the original grant application. We will aim to investigate the possibility of using  $^{17}\text{O}$  in biological NMR of membrane peptides both alone and in bilayers. For example we have synthesised a membrane peptide (WAL hydrophobic peptide with one  $^{17}\text{O}$ -glutamate residue) to be used as to study hydrogen-bonding of amino acid ligands (such as glutamate at its receptor binder site) or in proteins (some hydrogen-bond formers are rare but vital for the structural stability within membranes). This then targets the use of  $^{17}\text{O}$  NMR to study pharmacologically important biomolecules. The data generated in this study indicates that there is a real possibility of investigating the detailed hydrogen-bonding environment in real biological environments by using high enrichment and high applied magnetic fields. We will seek to develop further and exploit  $^1\text{H}$  MAOSS on deuterated materials and/or ultrafast MAS for the characterisation of such biosystems at the very highest applied magnetic fields. The NMR work on deuterated membrane samples is being complemented by funding for neutron beam time and sample preparation.

Recently a new EPSRC Advanced Fellow (Dr S.P. Brown) joined the group at Warwick. He will apply very high resolution  $^1\text{H}$  techniques to probe fundamental non-bonding interactions in solids. The applications will include examination of the structure and dynamics of weak hydrogen bonding and for probing hydrogen bond strength through heteronuclear dipolar and J couplings. This complements and extends this work and uses the infrastructure (e.g. ultrafast MAS) put in place by this grant.

## Training

The PDRA (Dr Pike) came with significant solid state NMR experience but all his previous work had been on inorganic materials physics-based problems. This project gave him exposure to a whole new set of biomolecular problems increasing his range of experience. The probe development, optimising the rf and ultrafast spinning, was also valuable in expanding his technical background expertise. For  $^{17}\text{O}$  it is anticipated that especially when diluted in larger biomolecules obtaining as much sensitivity as possible will be vital. Hence the PDRA spent some time looking at modulation sequences to improve sensitivity by manipulating the satellite transition magnetisation<sup>2</sup> (A collaboration with Prof. Levitt's Group in Southampton). To help interpret the  $^{17}\text{O}$  data some computational work calculating the NMR parameters was carried out which developed some new skills for the PDRA. A D.Phil. student (Lemaitre) and a PDRA (Lamm) at Oxford were not employed on the grant but carried out related work directly benefiting from the funding of consumables and the infrastructure put in place by this grant. Staff costs at Oxford were used to employ Dr Fischer on a PT basis (4.75 months over 2 years) to synthesise the biomolecules used here.

## Dissemination and Collaboration

With only a 2 year project requiring the development and installation of new infrastructure, although some papers were produced, it is only now that the production of some more substantial publishable work is commencing. This will be/has been published in leading international journals, both specialist chemical physics journals and those that make directly contact with the intended beneficiaries. The group has undertaken a significant programme of dissemination through invited and contributed presentations at a range of conferences etc. (detailed at the end).

This project initiated collaboration between the Warwick and Oxford groups and will now form a strong and on-going programme of research at the Life Sciences interface. It has also encouraged the development of projects on biomolecular materials with both the Chemistry and Biology Departments at Warwick. The planned follow-on project on the  $^{17}\text{O}$  work commenced here will involve Warwick Biology. To further exploit the  $^{17}\text{O}$  work a new international collaboration has begun with Dr Antzugin (Umea, Sweden) to aid structural work on Alzheimer's  $\beta_{(1-40)}$  amyloid fibrils. It is also clear that in these materials  $^{17}\text{O}$  double angle rotation will be highly beneficial. We have carried out some preliminary work using the world-leading probe of Dr Samoson (Tallinn, Estonia) and observed some spectacular gains in resolution – it will be important to add this technique to our methodology.

## Appendix – Details of Publications and Presentations to which GR/N29549/01 contributed

Publications published, accepted or submitted are numbered (1-4) on the form, papers in preparation and other forms of dissemination are presented here.

### (a) Papers in preparation

5. *The effect of strong hydrogen-bonding in carboxylic acids and their salts of  $^1\text{H}$  and  $^{17}\text{O}$  solid state NMR parameters*  
K.J. Pike, A.P. Howes, R. Jenkins, D.H.G. Crout, M.E. Smith and R. Dupree.
6. *Solid State  $^{17}\text{O}$  NMR of amino acids*  
K.J. Pike, A.P. Howes, A. Kukol, V Lemaitre, A. Watts, M.E. Smith and R. Dupree J. Phys. Chem.
7. *Direct observation of protons in peptides in bilayer membranes*  
S. Lamm and A. Watts, FEBS Lett.

### (b) Invited Conference Presentations (Warwick)

8. *Recent developments in solid state NMR of quadrupolar nuclei*  
M.E. Smith, BRSG meeting overview of recent developments in NMR, London, November 2001.
9. *Recent advances  $^{17}\text{O}$  and  $^{47,49}\text{Ti}$  solid state NMR*  
M.E. Smith, ANZMAG meeting, Lake Taupo, New Zealand, February 2002.
10. *Solid State NMR of quadrupolar nuclei: - from electroceramics to hydrogen bonding of biomolecules*  
R. Dupree, Wilhelm-Ostwald Institute of Physical and Theoretical Chemistry, Universität Leipzig, Germany, April 2002.

### (c) Invited Colloquia (Warwick)

11. *Solid State NMR: where are we now?*  
R. Dupree, Tag der Magnetischen Resonanz, University of Stuttgart, Germany, April 2001
12. *Solid State NMR: where are we now?*  
R. Dupree, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia May 2001
13. *Some NMR studies of (mostly) quadrupolar nuclei: from electroceramics to simple biomolecules*  
R. Dupree, Univ. Pierre et Marie Curie, Paris, France December 2002

### (d) Contributed Conference Talks (Warwick)

14. *Solid state  $^{17}\text{O}$  NMR of organic materials – characterisation of hydrogen-bonding*  
M.E. Smith, R. Dupree, K.J. Pike, A.P., Howes, A. Kukol, A. Watts, V. Lemaitre, A. Samoson, Rocky Mountain Symposium on Solid State NMR, Denver, USA, July 2002

### (e) Contributed Conference Posters (Warwick/Oxford)

15. *New Insights into the Bonding Arrangements of L-Glutamic Acid and L-Monosodium Glutamate from Solid State  $^{17}\text{O}$  NMR*  
V. Lemaitre, K.J. Pike, A. Watts, T. Anupold, A. Samoson, M.E. Smith and R. Dupree Recent Innovations in Biological Solid State NMR, a joint British Biophysical Society and IOP meeting, Oxford, 2-3 September 2002
16. *New Insights into the Bonding Arrangements of L-Glutamic Acid and L-Monosodium Glutamate from Solid State  $^{17}\text{O}$  NMR*  
.V Lemaitre; K.J. Pike; A. Watts; T. Anupold; A. Samoson; M.E. Smith; R. Dupree. ENC, Asilomar, California, April 2003

### Other References

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