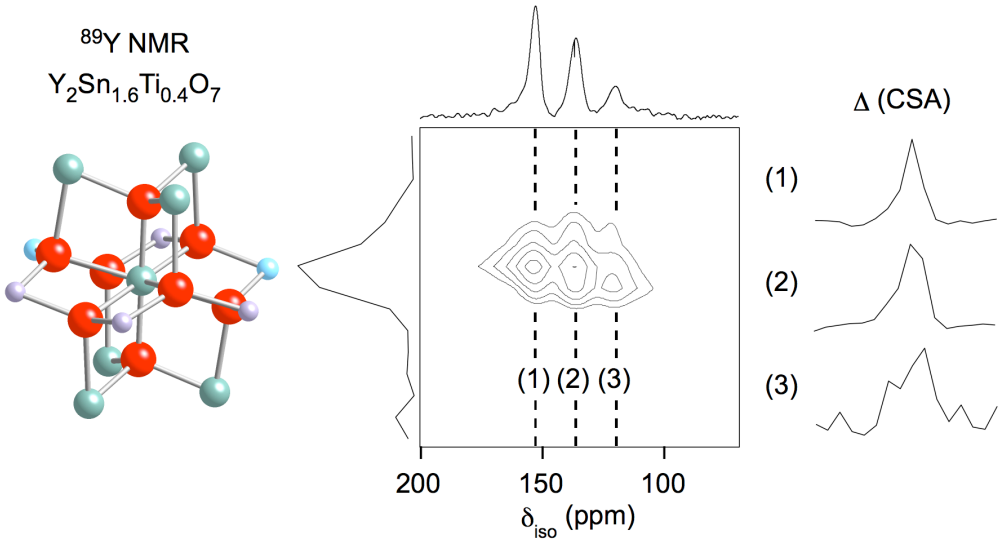
RSC | Advancing the Chemical Sciences

Royal Society of Chemistry

NMR Discussion Group



Postgraduate Meeting 2011

College of Medical and Dental Sciences
University of Birmingham
22nd June 2011

Dear Delegate,

Welcome to the fourth one-day "postgraduate" symposium of the RSC NMR Discussion Group. Following previous meetings at Astra Zeneca, Charnwood, and the Universities of Manchester and Leeds, this meeting has a broadly similar format. The meeting brings together early career researchers, broadly defined as postgraduates, early career post doctorial workers, and young industrialists, who all have a strong research interest in magnetic resonance and provides a forum to showcase their work.

With their successful introduction year, we are continuing to have two overview lectures, given by leaders in their field, to highlight the power of magnetic resonance methods across a broad range of topics. This year Prof. Ulrich Günther (University of Birmingham) and Prof. Clare Grey (University of Cambridge) will present overviews of using NMR to investigate metabolomics and applications of solid state NMR to materials chemistry.

The varied programme has been arranged to allow delegates to listen to the wide range of talks, and also present and discuss their posters with other early career researchers and more established colleagues. There is also ample opportunity for further discussions over tea/coffee and lunch.

We hope that you will make the most of this opportunity and that you enjoy the meeting.

Iain Day	**Michael Overduin** **Sara Whittaker**	**Mike Williamson**
University of Sussex	University of Birmingham	University of Sheffield
Meeting Organiser	Local Organisers	NMRDG Chairman

Local organisation and acknowledgements

Meeting coordinated by:	Iain Day, University of Sussex
Local organisation coordinated by:	Michael Overduin, University of Birmingham
	Sara Whittaker, University of Birmingham
Online registration coordinated by:	John Parkinson, University of Strathclyde

The organisers would like to thank Stephen Byard (Covance) for significant help "behind the scenes".

Thanks go to Mike Williamson (University of Sheffield), Iain Day (University of Sussex) and John Parkinson (University of Strathclyde) for acting as Judges for the prize giving.

The NMR Discussion Group gratefully acknowledges the following sponsorship for their generous support of this meeting:

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Posters

Posters should be mounted on the poster boards during the arrival period prior to the formal welcome and start of the program and should be attached to the board for which the poster number has been designated. Posters should be removed after the close of the meeting.

Programme

1000 – 1025 Arrival, Registration, Poster mounting and Coffee
1025 – 1030 Welcome, Mike Williamson, NMRDG Chairman

Oral Presentation Session 1, session chair: Iain Day

1030 – 1110 **Ulrich Günther**, University of Birmingham
Cancer Metabolomics

1110 – 1130 **Nur Adeela Yasid**, University of Sheffield
Metabolite analysis of Escherichia coli *in response to oxygen levels*

1130 – 1150 **Andrew Tatton**, University of Warwick
^{14}N-^{1}H Correlation Spectra at 850 MHz

1150 – 1210 **Ryan Mewis**, University of York
Automation of the Signal Amplification By Reversible Exchange (SABRE) process produces a viable NMR and MRI tool

1210 – 1230 **Andrew Grigg**, University of Warwick
^{71}Ga-^{31}P and ^{27}Al-^{31}P R^3-HMQC on Ga and Al substituted β-tricalcium phosphate

1230 – 1250 **Hugh Dannatt**, University of Sheffield
Dynamics in the Catalytic Cycle of a β-Phosphoglucomutase

Lunch

1250 – 1330 Buffet lunch and mixing

Poster Session

1330 – 1400 Odd numbered posters manned
1400 – 1430 Even numbered posters manned

Oral Presentation Session 2, session chair: Stephen Byard

1430 – 1510 **Clare Grey**, University of Cambridge
Solid state NMR - tbc

1510 – 1530 **Gregory Rees**, University of Warwick
A Multinuclear Solid State NMR and DFT Study of Solid Oxide Fuel Cells

1530 – 1550 **Adam Colbourne**, University of Manchester
A Powerful New Tool for Mixture Analysis: Local Covariance Order Diffusion-Ordered Spectroscopy (LOCODOSY)

1550 – 1610 **Ivanhoe Leung**, University of Oxford
Mechanistic NMR Studies on the Enzyme γ-Butyrobetaine Hydroxylase (BBOX)

Close

1610 Tea and Coffee
1620 Award of Best Oral Presentation and Best Poster Presentation

Index to Abstracts

Abstracts of Talks

(Talk 001)

Ulrich Günther, University of Birmingham, u.l.gunther@bham.ac.uk

Cancer Metabolomics

Ulrich Günther

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Metabolomics is a fast growing discipline that holds great promise for the study of complex medical processes including transitions between health and disease, disease progression and therapeutic responses. We have performed NMR based metabolomics on serum and plasma samples from patients with different cancers, including head and neck cancer, multiple myeloma, and oesophageal cancer. All of these studies show considerable potential for metabolomics as a diagnostic method. We have also used metabolomics in cancer drug discovery, assessing mechanistically relevant changes in metabolite profiles in response to drug treatments. Many of the metabolomics results obtained for cancer reemphasize changes in metabolism in many cancers.

(Talk and Poster 002)

Nur Adella Yasid, University of Sheffield, mbp08ny@sheffield.ac.uk

Metabolite analysis of Escherichia coli in response to oxygen levels

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E. coli is a versatile bacterium that has three metabolic modes; aerobic, anaerobic and fermentation. The major environmental factor that controls the switching between these metabolic modes is oxygen availability. *E. coli* in the intestinal tract is completely anaerobic but certain parts of the intestinal tract close to the gut are microaerobic. The change from anaerobic to aerobic or microaerobic is very rapid and leads to major metabolic change. The changes have been characterized by using NMR. We have developed a quenching technique by using ethanol/NaCl. This technique cools the cells very rapidly after removal from the chemostat (less than 30 seconds) to inactivate the metabolic activity. The measurement error from most sources is small with acceptable error from extraction of intracellular metabolites. The technique is being used to studying the transition from anaerobic to aerobic at different levels of oxygen. The change to aerobic conditions showed a decrease in pyruvate, since the pyruvate dehydrogenase complex (PDHC) is activated with the presence of oxygen. Changes in the major intracellular and extracellular metabolites have been measured and are being modelled. An unknown peak (1.53 ppm) has been detected during the switch and identification of the peak is being carried out by using HPLC.

(Talk and Poster 003)

Andrew Tatton, University of Warwick, a.s.tatton@warwick.ac.uk

^{14}N-^{1}H Correlation Spectra at 850 MHz

Andrew S. Tatton[1] Dinu Iuga[1] Tran N. Pham[2] Fred G. Vogt[2] and Steven P. Brown[1]

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[2]GlaxoSmithKline

^{14}N-^{1}H solid state NMR experiments allow for observation of N-H correlations without the requirement of isotopic labelling.[1, 2] N-H correlations are achieved using a HMQC pulse sequence employing R^3 recoupling of the ^{14}N-^{1}H dipolar couplings.[2]

Spectra are presented for a variety of organic compounds with CQ values ranging from approximately 1-4 MHz. It was found that longer recoupling times allow for longer through space N-H distances to be probed which have no through bond coupling. The improvement noted at faster spinning frequencies and higher ^{14}N nutation frequencies obtained using a 1.3 mm probe is also presented.

1. Cavadini, S., A. Abraham, and G. Bodenhausen, Chemical Physics Letters, 2007 **445** 1.
2. Gan, Z.H., J.P. Amoureux, and J. Trebosc, Chemical Physics Letters, 2007 **435** 163.

(Talk and Poster 004)

Ryan Mewis, University of York, ryan.mewis@york.ac.uk

Automation of the Signal Amplification By Reversible Exchange (SABRE) process produces a viable NMR and MRI tool

Ryan E. Mewis[1] Kevin D. Atkinson[1] Simon B. Duckett[1] Gary G. R. Green[2] Louise A. R. Highton[1] and Lyrelle S. Lloyd[1]

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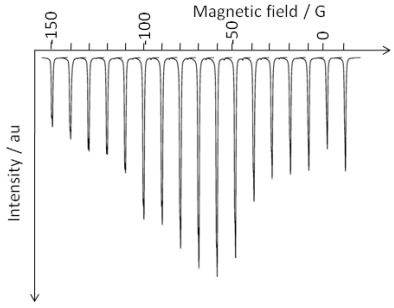
[2]York Neuroimaging Centre, The Biocentre, Innovation Way, Heslington, York, YO10 5DG

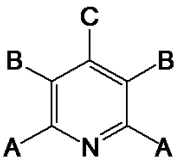
Hyperpolarization techniques utilising the spin isomer parahydrogen have been highly advantageous to both NMR and MRI by increasing detectable signal intensities by many orders of magnitude. At York, a new *para*hydrogen based hyperpolarization technique has been developed; SABRE (Signal Amplification By Reversible Exchange).[1] Unlike classical *para*hydrogen based methods in which the *para*hydrogen molecule is incorporated into an unsaturated substrate, polarization is instead transferred by the ^{1}H scalar coupling which exists between a hydride ligand derived from *para*hydrogen and that of the ^{1}H nuclei on a substrate molecule also residing at the same metal centre. Compared with classical methods, after its dissociation the substrate molecule is chemically unchanged. Furthermore, a wide range of molecules can be polarized using this technique.

An automated flow system for preparing hyperpolarized molecules via SABRE followed by transfer to the NMR spectrometer for interrogation by an appropriate read pulse has been developed. Use of this apparatus has enabled the magnetic states populated after polarization transfer to be identified using OPSY (Only *Para*hydrogen SpectroscopY) and used in the collection of 2D hyperpolarised spectra (e.g. COSY, HMQC and HMBC). In addition, it has also been used to investigate the polarization transfer efficiency by varying the strength of the magnetic field at which transfer is conducted. This presentation will highlight some of the most recent results obtained using this system for both NMR and MRI.

1. Adams, R. W. et. al., Reversible Interactions with para-Hydrogen Enhance NMR Sensitivity by Polarization Transfer. Science 2009, 323, 1708-1711



^{1}H NMR intensities of the *ortho* resonance (A) of hyperpolarised pyridine (below) prepared using SABRE after polarization transfer in a magnetic field of the range −150 to +10 G



(Talk and Poster 005)

Andrew Grigg, University of Warwick, a.t.grigg@warwick.ac.uk

^{71}Ga-^{31}P and ^{27}Al-^{31}P R^3-HMQC on Ga and Al substituted β-tricalcium phosphate

Andrew T Grigg[1] Diane Holland[1] Ray Dupree[1]

[1]Department of Physics, University of Warwick, Gibbet Hill Rd, Coventry, CV4 7AL

β-tricalcium phosphate (β-TCP) is of interest due to the ease with which active metal cations can substitute for the abundant and varied calcium sites, making it useful as a matrix for nuclear waste immobilisation. β-TCP has been shown to provide a host for a range of cations, including Ga, Al, Mg and Sm . Indirect methods have been widely used to examine the doped structure, including X-ray and neutron diffraction, and ^{31}P NMR. Direct probes of the cation sites are preferable, and ^{43}Ca NMR has been performed at the UK 850 MHz Solid-State NMR Facility. However, the low γ and low natural abundance of ^{43}Ca resulted in low signal intensity, even at high field. Fitting the ^{43}Ca spectra to five pseudo-Voigt profiles (corresponding to the 5 Ca sites) is possible; though the poor signal and broad lineshape of the spectra make any fits ambiguous. Rotary resonance recoupling heteronuclear multiple quantum coherence (R^3-HMQC) experiments have been performed on both Ga and Al doped β-TCP, providing an excellent and relatively simple method of determining which P environments are coupled to specific cations. ^{31}P-^{71}Ga R^3-HMQC has conclusively shown that Ga substitutes onto the Ca(5) site, and preliminary data from the analogous ^{31}P-^{27}Al experiment are very promising. The possibility of a similar experiment on Cd-doped β-TCP is planned, with the Cd serving as a Ca surrogate. Since Cd^{2+} is of a very similar size to Ca^{2+}, it should allow ^{31}P-^{113}Cd experiments to probe the 'undoped' β-TCP structure

(Talk and Poster 006)

Hugh Dannatt, University of Sheffield, h.dannatt@shef.ac.uk

Dynamics in the Catalytic Cycle of a β−Phosphoglucomutase

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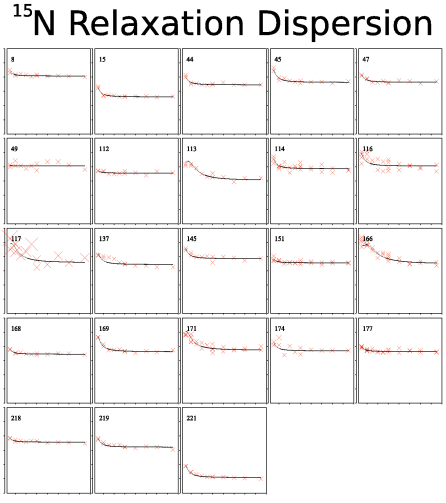
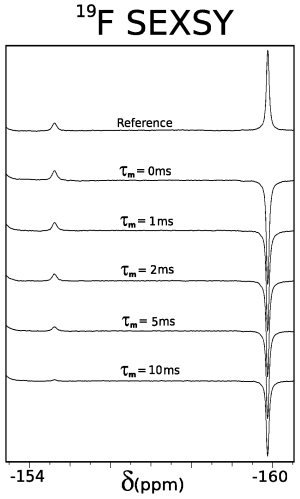
Of all the reactions that occur in biology, phosphate monoester hydrolysis is among the slowest to proceed without a catalyst. The enzymes which catalyse these reactions are able to achieve rate enhancements on the order of 10^{21}, the highest acceleration factor currently identified. This work focuses on the involvement of protein dynamics in achieving this extraordinary rate enhancement. The transition state of phosphoryl transfer reactions can be mimicked by metal fluorides – and as such these can be used to trap the enzymes in 'active' transition state analogue complexes which can then be studied. Trifluoromagnesate (MgF_3^-) ions are both isosteric and isoelectric with a transferring phosphate ion and therefore make excellent transition state analogues.

β-Phosphoglucomutase (βPGM) catalyses the interchange between β-Glucose-1-Phosphate and β-Glucose-6-Phosphate (βG6P). In the presence of βG6P and both Mg^{2+} & F^- ions, a βPGM-MgF3--βG6P transition state analogue complex spontaneously forms, allowing probing of an active conformation. Study by either ^{15}N Relaxation Dispersion or ^{19}F Selective Exchange SpectroscopY (SEXSY) reveal the presence of 2 conformations which interchange at the same rate as catalysis, suggesting the minor conformer is of catalytic importance





(Talk 007)

Clare Grey, University of Cambridge, cpg27@cam.ac.uk

Solid-state NMR - tbc

Clare Grey

Dept of Chemistry, University of Cambridge

To be annoucned

(Talk and Poster 008)

Gregory Rees, Univresity of Warwick, g.j.rees@warwick.ac.uk

A Multinuclear Solid State NMR and DFT Study of Solid Oxide Fuel Cells

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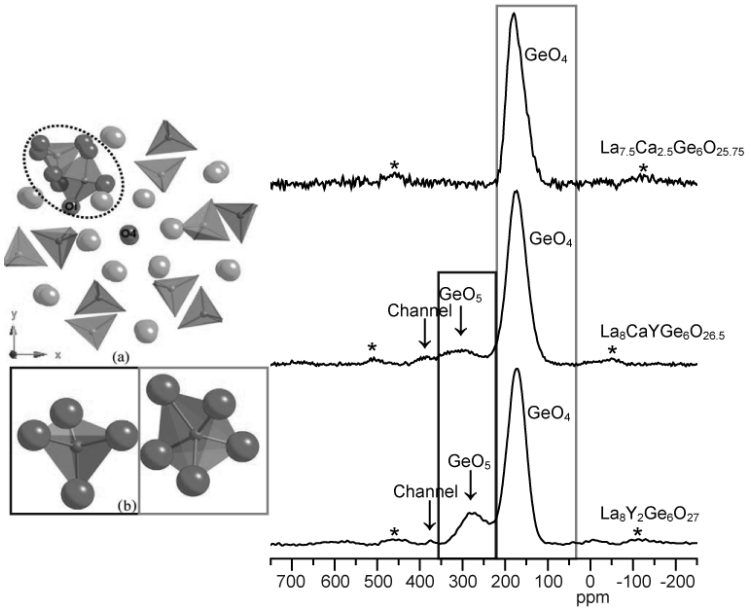
[2]Department of Chemistry, University of Birmingham, Edgbaston, Birmingham. B15 2TT

Materials displaying high oxide-ion conductivity have attracted considerable interest due to technological applications in solid oxide fuel cells (SOFC), oxygen sensors and separation membranes. ^{17}O solid state NMR data have been recorded for the apatite series $La_{8+x}M_{2-x}(GeO_4)_6O_{2+x/2}$ ($0 < x < 1.0$). For $x = 0$ a single NMR resonance is observed at a chemical shift of ~δ 175 ppm; as the La:M ratio is raised the interstitial oxygen content also increases and a second chemical shift at ~δ 300 ppm is observed. This has been attributed to the formation of a GeO_5 unit via the presence of O interstitial species.

This data has been used to predict the number of GeO_5 units and Frenkel-type disorders. The increased intensity in this low field peak is shown to correlate with enhanced conductivity. ^{17}O labelling shows bias towards the GeO_4 and interstitial oxygen speciation, and not the two channel oxygen's thus suggesting that the route of conductivity is due to the mobility of the oxygen's around the germanium centres. Hence, ^{17}O solid state NMR has given an insight into the conduction pathway and environment of the varying oxide-ion conductors.



(Talk and Poster 009)

Adam Colbourne, University of Manchester
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A Powerful New Tool for Mixture Analysis: Local Covariance Order Diffusion-Ordered Spectroscopy (LOCODOSY)

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Diffusion-ordered spectroscopy (DOSY) is a useful, widespread tool for analyzing mixtures. In general, one aims to separate out the NMR spectra of individual mixture components, gaining information on the physical and chemical properties of the analytes: namely hydrodynamic radii, diffusion coefficients and intermolecular interactions. The two most common data processing approaches are univariate (e.g. HR-DOSY[1]) and multivariate methods (e.g. SCORE[2] and DECRA[3]). The former of these breaks down where peaks from different components overlap and the latter struggles with increasing number of components (2-4 is a practical limit). A hybrid approach has been developed[4] combining the strengths of both, the principle of which is to break the spectrum into regions for independent multivariate analysis before rebuilding the dataset from the processing results. The total number of components resolvable rises dramatically, as fewer components will be present in each spectral window. LOCODOSY allows the clean resolution of significantly more chemical components than previously possible.

1. Barjat, H., Morris G. A., Smart, S., Swanson, A. G., Williams, S. C. R., J. Magn. Reson., 108, 170-172. (1995)
2. Nilsson M., Morris G. A., Anal. Chem., 80, 3777-3782. (2008)
3. Windig W., Antalek B., Chemom. Intell. Lab. Syst., 37, 241-254. (1997)
4. Colbourne A., Morris G. A., Nilsson M., J. Am. Chem. Soc., in press.

(Talk and Poster 010)

Ivanhoe Leung, University of Oxford, ivanhoe.leung@chem.ox.ac.uk

Mechanistic NMR Studies on the Enzyme γ-Butyrobetaine Hydroxylase (BBOX)

Ivanhoe K. H. Leung[1] Luc Henry[1] Grazyna T. Kochan[2] Christopher J. Schofield[1] Timothy D. W. Claridge[1]

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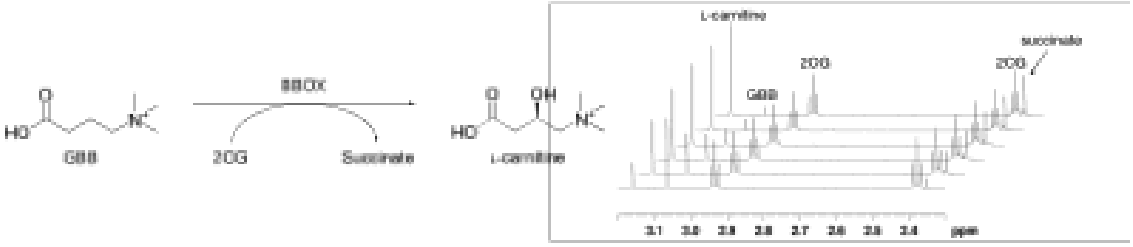
L-Carnitine is essential for energy metabolism. In human, L-carnitine is obtained from both diet and by endogenous biosynthesis. The final step of the L-carnitine biosynthesis is catalysed by γ-butyrobetaine hydroxylase (BBOX), a 2-oxoglutarate (2OG) and Fe^{2+} dependent dioxygenase. BBOX is an inhibition target of 3-(2,2,2-trimethylhydrazine)propionate (THP), a drug used clinically for the treatment of coronary heart diseases.

NMR spectroscopy is applied to characterise recombinant human BBOX and to conduct mechanistic and inhibition studies. Using a combination of 1D and 2D NMR techniques, the results show that BBOX can accept a wide range of substrates. Further investigations using ^{13}C labelled THP led to the identification of an unexpected product with an additional carbon-carbon bond resulting from a novel enzyme-catalysed rearrangement reaction.

Overall, the application of NMR in this study has allowed the identification and characterisation of unexpected substrates and a new reaction mechanism, which would be difficult to detect using conventional biochemical assays. NMR is particularly useful to study systems involving multiple substrates and products, and can be applied routinely for targeted or non-targeted analyses. These results also provide a basis for future development of improved BBOX inhibitors.

Reference:
Leung, I. K. H.; Krojer, T. J.; Kochan, G. T.; Henry, L.; von Delft, F.; Claridge, T. D. W.; Oppermann, U.; McDonough, M. A.; Schofield, C. J. Structural and mechanistic studies on γ-butyrobetaine hydroxylase. Chem. Biol. 2010, 17, 1316–1324.



Abstracts of Posters

Poster 011

Catharine Jones, University of Bristol, chcrj@bris.ac.uk

Interproton Distances from Nuclear Overhauser Effect (NOE) Data

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The determination of accurate interproton distances in solution using NOE data is an area of significant interest and complexity – the large majority of approaches rely on full relaxation matrix analysis of these data. We present a much simpler method that can be used to derive accurate interproton distances from within rigid systems using 1D or 2D NOESY data. Strychnine is used as a model system to test the validity of this method. A comparison between the 1D NOE-derived distances and the best solvent-corrected gas-phase structure of strychnine[1] (Figure 1) produces a mean absolute error of only 2.97% (0.09Å).[2]

A second low-level conformer of strychnine is subsequently identified experimentally by the NOE data and confirmed by computation, thereby demonstrating the potential of accurate NOE measurements to determine minute contributions to structure ensembles in solution.[3]

NOE data is further applied to the small flexible molecule, 4-propylaniline, in the confirmation and prediction of the relative populations of the multiple possible conformers.[4] It is suggested that with the highly accurate interproton distances determined using this method, there is less need for reliance on large numbers of loose restraints, such as scalar couplings, which are typically used in the dynamical analysis of flexible molecules.

[1] A. Bagno, F. Rastrelli and G. Saielli, Chem. Eur. J., 12, 5514 – 5525 (2006)
[2] C.P. Butts et al., Org. Biomol. Chem., 9 (1), 177-184 (2011)
[3] C.P. Butts, C.R. Jones and J.N. Harvey, Chem. Comm., 47 (4), 1193-1195 (2011)
[4] C.R. Jones, C.P. Butts and J.N. Harvey, Belstein J. Org. Chem., 7, 145-150 (2011).

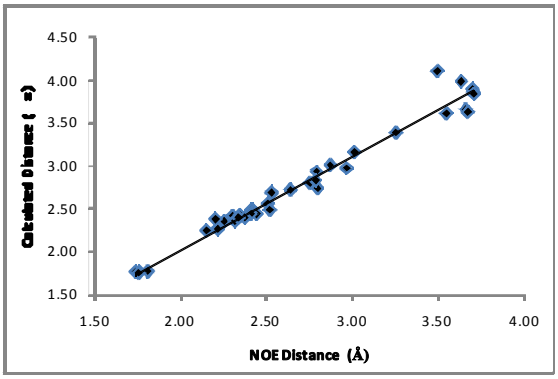

Figure 1: Comparison of calculated distances[3] and NOE-derived distances in strychnine.

Poster 012

Sabah Abu-Khumra, University of Nottingham, ppxsmma@nottingham.ac.uk

Dynamic Tunnelling Polarization: a quantum rotor analogue of DNP and the solid effect

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Nuclear spin-isomers are identified in symmetric molecules that possess identical nuclei with finite spin. They arise as a result of the antisymmetry principle which is the most general form of the Pauli Exclusion Principle. Due to the entanglement of space and spin degrees of freedom, spin-isomers are distinct species that generally possess little capacity to interconvert. Experiments will be described that facilitate a bespoke manipulation of the populations of the spin-isomers, or spin-symmetry species, of methyl (CH_3) rotors by r.f. irradiation of weakly allowed sideband transitions within the manifold of tunnelling-magnetic levels. By suitable design of the experiment this results in a dynamic polarization of the CH_3 spin-symmetry states, analogous to dynamic nuclear polarization in NMR. Substantial positive and negative CH_3 tunnelling polarizations are observed, providing a quantum rotor analogue of dynamic nuclear polarization and the solid effect in NMR. The experiments employ field-cycling NMR and level-crossings between tunnelling and Zeeman systems are employed to report on the tunnelling polarization. The tunnelling lifetimes are measured and the field dependence investigated..

Poster 013

Alexandria Rogerson, University of Manchester,
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Simultaneous Enhancement Of Chemical Shift Dispersion And Diffusion Resolution In Mixture Analysis By Diffusion-Ordered NMR Spectroscopy

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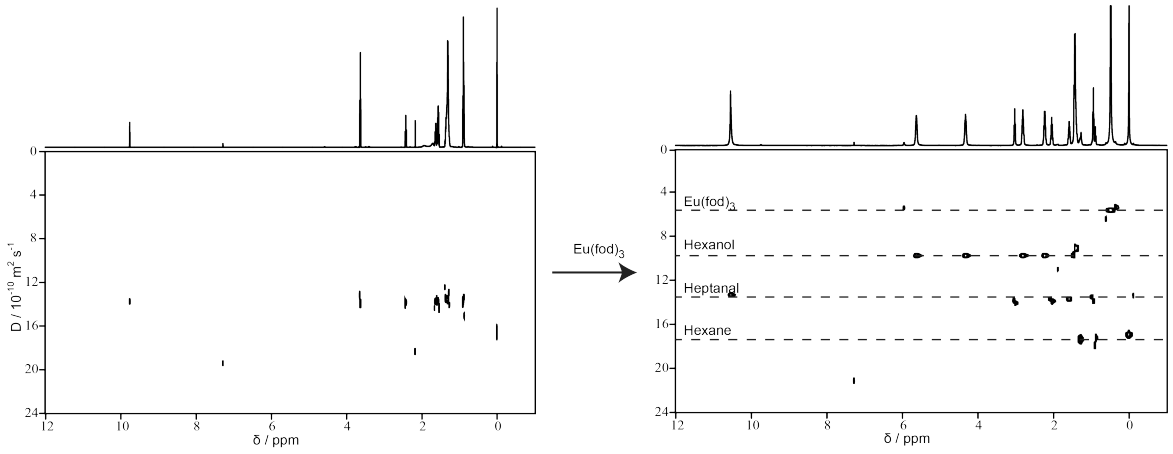
NMR spectroscopy is a powerful tool for the elucidation of molecular structure, but is relatively little used for mixture analysis. High resolution diffusion-ordered spectroscopy (HR-DOSY)[1] allows the separation of signals from different species, but for the best results requires both that the mixture components have different diffusion coefficients, and that their signals do not overlap.

The two requirements can be addressed simultaneously using lanthanide shift reagents[2] (LSRs): the chemically-selective binding of solutes to an LSR both increases chemical shift dispersion, reducing signal overlap, and changes the diffusion coefficients seen for the different species. The latter effect is an example of matrix-assisted DOSY[3,4], in which the relative diffusion coefficients of different mixture components are manipulated by changing the matrix in which they diffuse.

1. C. S. Johnson, Prog. Nucl. Mag. Res. Sp., 1999, **34**, 203-256.
2. J. K. M. Sanders and D. H. Williams, Nature, 1972, **240**, 385-390.
3. R. Evans, S. Haiber, M. Nilsson and G. A. Morris, Anal. Chem., 2009, **81**, 4548-4550.
4. C. F. Tormena, R. Evans, S. Haiber, M. Nilsson and G. A. Morris, Magn. Reson. Chem., 2010, **48**, 550-553.



Poster 014

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Multinuclear Solid State NMR Characterisation of Zinc and Bismuth Incorporation in Borosilicate Glass Systems

J.R Higgs[1] M.E. Smith[1] J.V. Hanna[1] P.T. Bishop[2] J.C.S. Booth[2] H. Zheng[2]

[1]Department of Physics, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL.
[2]Johnson Matthey Technology Centre, Blounts Court, Sonning Common, Reading, Berkshire, RG4 9NH.

The structures of borosilicate glasses, which are basic components of automotive glass enamels, are being investigated using multinuclear solid state NMR. Some of these glass materials are already commercially used, although there is a desire in the automotive industry to further improve their acid resistance and lower the corresponding melting temperature.

Series of model glass systems are being studied in order to investigate the basic influences that metals such as bismuth and zinc exert on glass networks close to the commercial compositions. Solid state NMR is used to examine the structural features of these technologically important glasses on the atomic scale in order to learn how the local structure affects the properties of interest. In addition to conventional magic angle spinning (MAS) NMR techniques such as multiple quantum magic angle spinning (MQMAS) and double rotation (DOR) have been employed to elucidate additional information about glass structure and structural distributions that characterise the base borosilicate glass and its metal-incorporated counterparts.

Poster 015

Cassey McRae, University of Leeds, chm4cm@leeds.ac.uk

NMR-Based Profiling of Ovarian Follicular Fluid and Plasma

McRae, C.[1] Baskind, N.E.[2] Sharma, V.[2] and Fisher, J.[1]

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The success rates of in vitro fertilisation (IVF) cycles are unsatisfactory, and there is no reliable method of assessing oocyte potential. Follicular fluid (FF), which surrounds the oocyte in the ovary, contains substances essential to oocyte fertilisation. Therefore it may be possible to identify chemical markers of oocyte quality within this fluid. The aim of this study was to use high resolution nuclear magnetic resonance (NMR) spectroscopy and metabolomics techniques to identify biomarkers of oocyte quality within human FF and plasma.

FF and plasma were collected from 10 IVF patients at the ovulatory and a pre-ovulatory stage. The Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence was used to obtain metabolic profiles of the fluids before principal components analysis (PCA).

PCA identified a difference in composition of FF taken at the pre-ovulatory and ovulatory stages, due to the signals of metabolites associated with anaerobic respiration. A patient who had a live birth appeared different due to signals at 1.47, 2.14 and 2.46 ppm, which correspond to alanine and glutamine, respectively. A patient who had a miscarriage appeared to have high intensities of signals at 1.33 and 4.13 ppm; the lactate signals, and at 1.21 and 1.28 ppm; ketone signals. Similarly, the plasma compositions were different between the two menstrual stages, and the miscarriage patient's plasma contained high levels of ketones.

To our knowledge this is the first study to use NMR spectroscopy and metabolomics to identify biomarkers of oocyte quality within human FF. The findings indicate that it may be worthwhile to investigate FF composition among IVF patients more extensively.

Poster 016

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SEC-DOSY: Diffusion Ordered Spectroscopy in the presence of a size exclusion chromatographic stationary phase

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Diffusion-ordered spectroscopy (DOSY) uses pulsed field gradients to measure translational diffusion coefficients and is a useful tool for the analysis of mixtures. Recent developments of the experiment include the addition of chromatographic stationary phases, such as silica gels[1], as a method of altering the diffusion behaviour of a molecule.

Here we present the application of size-exclusion chromatographic stationary phases to diffusion-ordered spectroscopy. Size-exclusion chromatography (SEC), also known as gel filtration or gel permeation chromatography is a liquid chromatography technique which uses porous stationary phases to separate molecules by molecular size. Small molecules can access more of the pores than the larger molecules, in a chromatographic separation they spend more time on the column and therefore elute later than larger molecules.

The effect of the addition of three SEC stationary phases on the observed diffusion coefficient has been characterised using poly(styrene-4-sulfonate) molecular weight reference standards with a range of molecular weights. For each stationary phase there is a characteristic decrease in observed diffusion coefficient related to the molecular weight of each standard.

The addition of these stationary phases produces a semi-solid slurry which generates inhomogeneous line broadening caused by increased dipole-dipole interactions and susceptibility broadening. This can lead to decreased spectral resolution which makes the analysis of the DOSY data challenging. A proposed solution to this is the use of an HR-MAS probe[2]; spinning the semi-solid samples at the magic angle should reduce the overlapping of peaks and yield more liquid-like line widths and line shapes. Preliminary HR-MAS results for these systems will be presented.

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Poster 017

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Magnetic Resonance Velocimetry In A Vortex Flow Reactor

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Taylor Vortex Flow (TVF) occurs in the annulus of two concentric cylinders, when the inner one is rotated above a critical rotation rate. TVF is characterised by counter-rotating axi-symmetric vortices along the length of the tube. Kose[1] (1994) and Seymour *et al*[2] (1999) produced NMR velocity maps of this flow. By adding axial flow, a Vortex Flow Reactor (VFR) is produced, causing a translational movement of the vortices (fig. 1a). This type of reactor is used for numerous applications (such as catalytic, electrochemical and enzymatic reactions) due to its plug-like flow and mixing properties. Despite widespread application, many questions concerning this flow remain, including plug-flow properties and inter/intra vortex mixing. It is expected that MR velocity and diffusion maps will provide answers to these questions.

A challenge for imaging flow in this system lies in the periodic motion caused by the moving vortices. This causes imaging artifacts and errors in velocity measurements. However, by adapting the pulse sequence timing to the flow period[3], it is possible to simulate steady state and obtain velocity maps. We used PGSE imaging sequence and timed data acquisition to the Taylor vortices translation period to produce the first high-resolution NMR velocity maps of the VFR flow (fig. 1b).

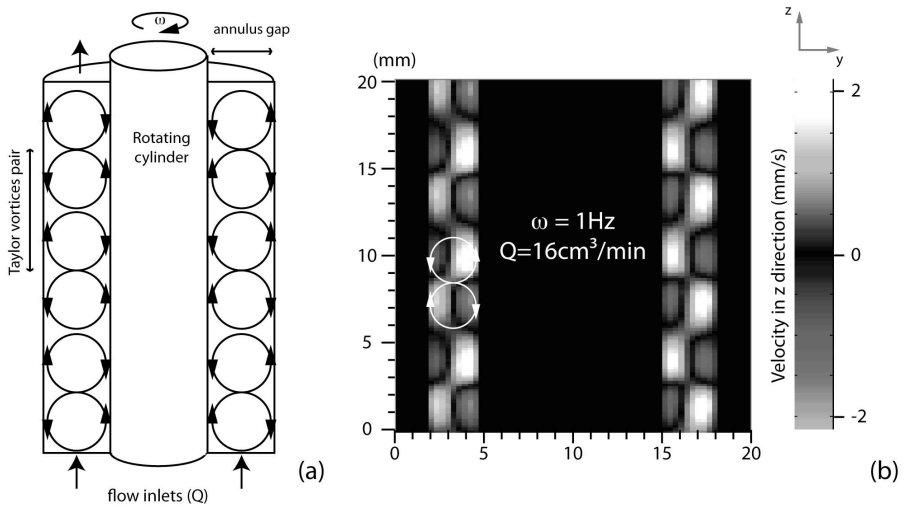

Fig.1. (a) Vortex Flow Reactor (VFR) schematic, (b) Magnetic Resonance velocity map of the VFR flow in the z direction at ω=1Hz and Q=16cm^3/min

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Poster 018

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Using NMR for mechanistic studies on a 2-oxoglutarate dependent oxygenase

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Deacetoxycephalosporin C synthase (DAOCS) is a Fe(II) and 2-oxoglutarate (2OG) dependent oxygenase that catalyses the ring expansion of penicillins to cephalosporins.1 The mechanism proposed for DAOCS2 is fundamentally different from the consensus mechanism proposed for the 2OG oxygenase family of enzymes (Figure 1). NMR spectroscopy is employed to test the validity of the proposed mechanisms.

Solvent-water longitudinal relaxation (T_1) measurements can provide quantitative information regarding of ligand binding close to a paramagnetic metal ion in the active site.3,4 Binding constants obtained suggest that having one substrate bound in the active site would not prevent the other substrate from binding. Thus the formation of a ternary complex would be possible.

The proposed ping-pong mechanism suggests that the two substrates bind at overlapping sites. Displacement experiments using 1D HSQC with ^{13}C labelled substrates show that the binding of substrate and co-substrate do not interfere with each other, thus indicating that the binding sites for 2OG and the penicillin substrate do not overlap.

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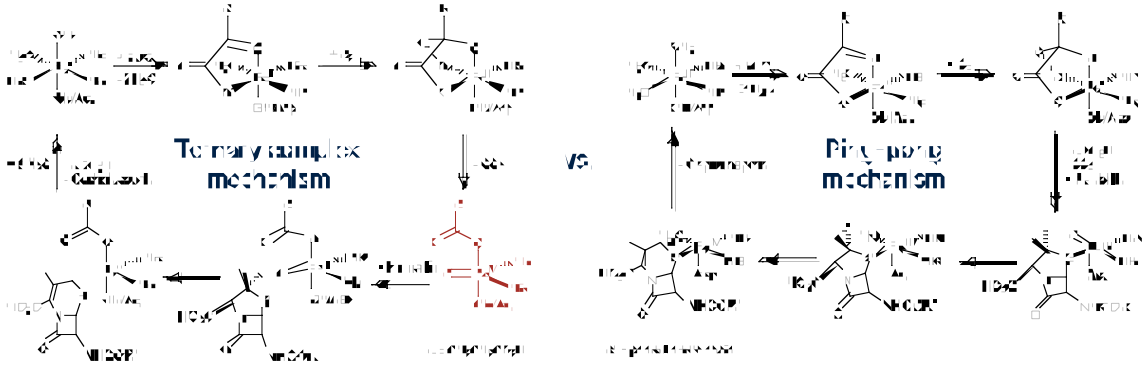

Poster 019

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Using solid-state NMR to investigate framework materials

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Framework materials with the formula AM_2O_7 and AM_2O_8 are of great interest and have been shown to exhibit negative thermal expansion (NTE) and interesting oxygen dynamics. Solid-state NMR was used to probe the local environment of the nuclei, which often complements information obtained from diffraction based techniques. Ab initio calculations were also used and compared to experimental spectra.

RbNbOP2O7 exhibits NTE and from powder X-ray diffraction, 2 reversible phase transitions occur at 346 K and 276 K. The data suggests the first phase transition on warming changes from low to high symmetry, followed by a second phase transition from high to low symmetry to a structure similar to the low temperature structure. However, the intermediate phase has not yet been characterised and NMR has been employed to help complement other diffraction methods. ^{31}P 1D NMR and ^{97}Nb echo experiments showed little difference in the NMR spectra obtained at different temperatures. Originally Rb ion was thought to be only acting as the counter-ion. However, much larger changes have been observed in ^{87}Rb echo NMR performed on a 400 MHz spectrometer and the 850 MHz National Facility spectrometer. The data shows the intermediate phase structure is related to the low temperature phase.

$SnMo_2O_8$ differs in its behaviour compared to other members of AM_2O_8 family, in that it exhibits positive thermal expansion with a phase transition at approximately 300 K. In this case, broad features that do not vary significantly with temperature, suggesting static disorder.

Poster 020

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Predicting Diffusion Coefficients for Small Molecules

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Diffusion-ordered NMR spectroscopy (DOSY) has found increasing use as an analytical tool, capable of identifying components in a mixture[1]. However, the diffusion information obtained is of limited use because it is difficult to interpret.

Diffusion coefficients are often predicted using the most basic relationship, the Stokes-Einstein equation[2], which balances the kinetic energy of the system against the friction acting on the molecules. Unfortunately this is a very poor approximation for small molecules, because of the finite size of solvent molecules, and of the flexibility, solvation and non-spherical shapes of solute molecules. Various refinements of the original equation have been suggested[3, 4, 5], with that proposed by Chen finding some use in a range of applications[6]. Diffusion coefficients have also been parameterised using fractal models[7].

Here a new model is proposed for predicting the diffusion coefficients of small molecules. It has a simple physical basis, is independent of solvent, uses only one adjustable parameter, and is shown to allow diffusion coefficients to be predicted to an accuracy of around 10% using only the solute molecular weight and solvent viscosity.

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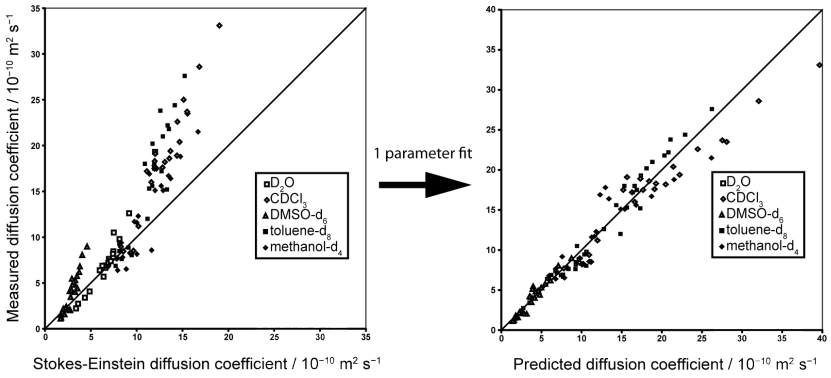

Poster 021

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Double rotation B-11 NMR applied to crystalline borates

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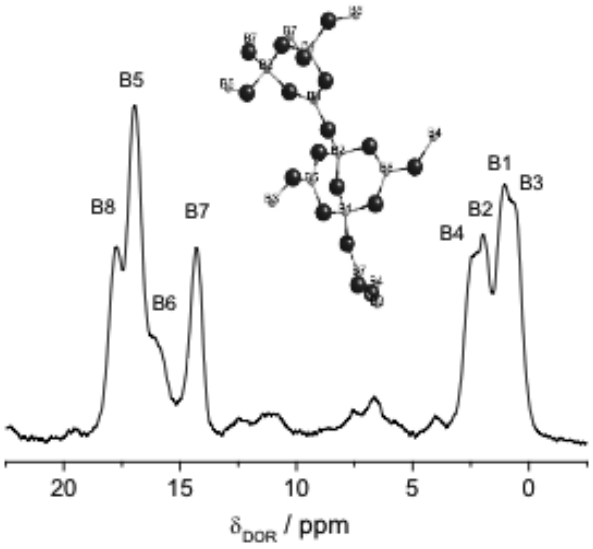
Determination of the local micro-structure is seen as fundamental to the understanding of borate chemistry in all states: vitreous, crystalline, and liquid. Despite this, in the binary borate systems alone there exist a large number of polymorphs with unknown structure. Double rotation (DOR) NMR gives high resolution B-11 spectra, via averaging of the second-order quadrupolar broadening that dominates MAS line shapes for three-coordinated boron species, allowing resolution of all crystallographically distinct sites in many systems. 2D B-11 spin diffusion DOR experiments probe site connectivity through homonuclear polarisation transfer via dipolar coupling which assists in assignment of the spectra where the structure is known and gives additional information on the structure when it is not known a priori. The barium diborate system, BaB4O7, has been taken as an interesting example of a binary borate composition for which at least four crystalline polymorphs exist, α-BaB_4O_7 of known structure, with the rest unknown. DOR reveals the existence of eight sites (4 B(III) and 4 B(IV)) in the α-phase, and indicates the number of three- and four-coordinated sites present in the other polymorphs, e.g. 2 B(III) and 2 B(IV) for γ-BaB_4O_7. Preliminary data (on this and other systems) indicate the shifts of individual B(III) and B(IV) peaks may be characteristic of specific superstructural units, further indicating the potential of DOR NMR in the study of borates with unknown structure.



Poster 022

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Exploiting the Rich Information Content in Solid-State NMR Spectra to Provide New Insight into the Structure of Disordered Materials

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Recent advances in Solid State NMR has led to new and exciting pulse sequences being developed for use in extracting specific structural information from materials. In particular the interest in extracting through bond interactions (J coupling) is a recent development previously overshadowed by the large dipolar couplings that are present in the solid state. A modified version of the INADEQUATE pulse sequence, used for many years in solution state NMR, the refocused INADEQUATE experiment has been shown to be valuable method for probing through bond connectivities. Further improvement to the refocused INADEQUATE with the appending of an extra spin echo to the end has led to individual variations in J couplings being able to be extracted from a disordered glass structure [Guerry et al., J. Am. Chem. Soc. 131, 11861, 2009] . This so called REINE sequence (Refocused INADEQUATE spin-Echo) has started to be implemented using ^{31}P NMR on a range of glasses, enabling distributions of J couplings throughout the glass structure to be extracted. From these results details on chain lengths present can be deduced, with the ultimate aim to build up an overall structural model of glass structure.

Poster 023

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High-Field Solid-State NMR of the Transmembrane Domain of the Epidermal Growth Factor Receptor Neu*/ErbB-2

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Solid state NMR is used to study the structure of the transmembrane protein Neu in a native-like bilayer environment. Neu is an epidermal growth factor receptor in rats and is similar to the human version ErbB-2. Neu is responsible for triggering cell division but a mutation in the transmembrane (TM) domain causes continuous activation resulting in oncogenic activity.

Neu is known to be a homo-dimer consisting of two α-helices and the 3D structure of the extracellular and intracellular regions are well studied. However, the molecular structure of the important TM domain is still a matter of dispute, with two opposing models suggested. The TM domain is known to be the site of key protein-protein interactions so a structural study of this region is of great importance in understanding the biological function of Neu and ErbB-2 and their role in disease. This research aims to obtain structural constraints to refine the structural models of the mutated Neu in a native-like bilayer environment using high-field solid-state NMR.

Two samples have been prepared, one based on each of the existing models by ^{13}C and ^{15}N labelling two amino acids, one on each helix, which are thought to be on the interface. 2D ^{13}C - ^{13}C DARR spectra are presented for both samples to show dipolar interactions between nearby ^{13}C atoms. All intra-residue cross peaks have been assigned, however no inter-helical correlations have been observed so far. ^{13}C chemical shift analysis of both samples is presented and confirms α-helical secondary structure for both samples.

Poster 024

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Polarisation transfer from parahydrogen to Spin-1 Nuclei

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This work investigates the transfer of hyperpolarisation, in the NMR experiment under ALTADENA conditions, from the former parahydrogen nuclei to deuterium nuclei in the product molecule resulting from the catalytic hydrogenation of acetylene-d_2 to ethylene-d_2 using parahydrogen. Current thinking is that such transfer occurs via and is mediated by the ^{13}C nuclei present in natural abundance in the substrate.[1] More recently, however, the discovery at York of the spontaneous transfer of polarisation from parahydrogen to a target molecule at low field in non-hydrogenative SABRE experiments[2] and our subsequent theoretical work[3] so far indicates that transfer of hyperpolarisation via an organo-metallic complex to ^1H and heteronuclei on a target substrate is propagated predominantly by the ^1H scalar coupled spin system.

We revisit the question of polarisation transfer to spin-1 heteronuclei in the hydrogenation reaction and investigate further the theory of the potential route by which this may occur. All couplings in the spin system of the product molecule are taken into account using literature values. Deuterium nuclei are properly treated using a full basis set of spin-1 operators. These are coupled, as appropriate, with members of the spin-½ basis set in forming Hamiltonians and density operators. A full nuclear spin density operator treatment is applied in solving the equations of motion within the approximations of average Hamiltonian theory and the absence of relaxation. Simulated spectra resulting from these calculations will be shown and a potential alternative theory of the hyperpolarisation transfer presented.

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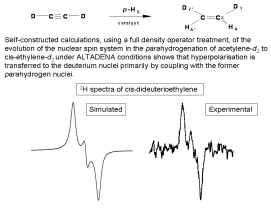

Poster 025

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β-strand alignment and seeding properties of nanotubes assembled from a modified amyloid-β peptide fragment.

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The peptide AAKLVFF, corresponding to residues 16-20 of the Alzheimer's beta-amyloid (Aβ) polypeptide extended at the N-terminus by two alanine residues), spontaneously self-assembles into fibrils in water and into nanotubular structures in methanol. Both morphologies have the amyloid-like cross-β motif. Here, high-resolution solid-state nuclear magnetic resonance (SSNMR) measurements of intermolecular 13C-13C dipolar couplings between Ala2 and Val5 confirm that the peptide β-strands have an antiparallel configuration in both morphologies, and suggest that Ala2 of each strand is in register with Val5 of neighbouring hydrogen bonded strands. The SSNMR measurements indicate that the nanotubes have greater long-range order than the fibrils, with a possible shorter repeating distance along the hydrogen bonding axis. Interestingly, the nanotubes produced in methanol are able to seed the assembly of AAKLVFF nanotubes in aqueous solution.

Poster 026

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Solid-state ^{2}H and ^{31}P NMR studies on the membrane interactions of phospholemman, a regulator of Na^{+}, K^{+}-ATPase.

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The Na^{+}, K^{+}-ATPase is a membrane protein complex responsible for maintaining an ionic gradient in all animal cells. The protein is made up of an α-subunit and a β-subunit; and in heart, liver and skeletal muscle it is associated with a third regulatory protein called phospholemman (PLM). The highly basic cytoplasmic domain of PLM was shown to inhibit Na^{+}, K^{+}-ATPase activity and, in separate NMR experiments, was found to associate with the surface of anionic lipid bilayers (1). Our recent biochemical measurements imply that PLM is capable of interacting with lipid membrane surfaces in the presence of the Na^{+}, K^{+}-ATPase, but direct evidence has been missing. Here ^{2}H static wide-line and ^{31}P magic-angle spinning NMR experiments have been conducted on Na^{+}, K^{+}-ATPase reconstituted into membranes of 2:1 dimyristoylphosphatidylcholine (DMPC)/ dioleoylphosphatidylglycerol (DOPG), with DMPC deuterated in the headgroup (DMPC-d4). Addition of the 36-residue PLM cytoplasmic domain to the membranes invokes changes in ^{2}H quadrupolar splittings and ^{31}P chemical shifts and line widths for DMPC-d_4 in the presence of Na^{+},K^{+}-ATPase, consistent with an interaction of the peptide with the membrane surface. From this information we propose a model in which the PLM cytoplasmic domain occupies Na^{+},K^{+}-ATPase associated and membrane associated states in dynamic equilibrium.

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Poster 027

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Studies of the Autophagy Marker LC3 and its Binding Partners

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Autophagy is an evolutionary conserved process that allows organisms to survive nutrient deprivation, inflammation, and hypoxia. It does this by delivering protein aggregates, intracellular microbes, and defective organelles to lysosomes to be recycled and reused. While there is a growing amount of research into this area, the mechanisms used to target cellular material for their destruction are still being elucidated. Our work focuses on the well known autophagic marker LC3, and aims to better understand its functions and role in target recognition through a combination of structural, biochemical, and in-vitro techniques. We hope to investigate the affinity of various binding partners for LC3, and evaluate the effects of phosphorylation on this binding. Initial NMR studies of our LC3 construct suggests it is purified to a level suitable for further studies, and the 15N HSQC matches well with published data of similar constructs.

Poster 028

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NMR Studies on Histone Demethylation: Detection of Enzymatically Produced Formaldehyde and Investigations into its Metabolism

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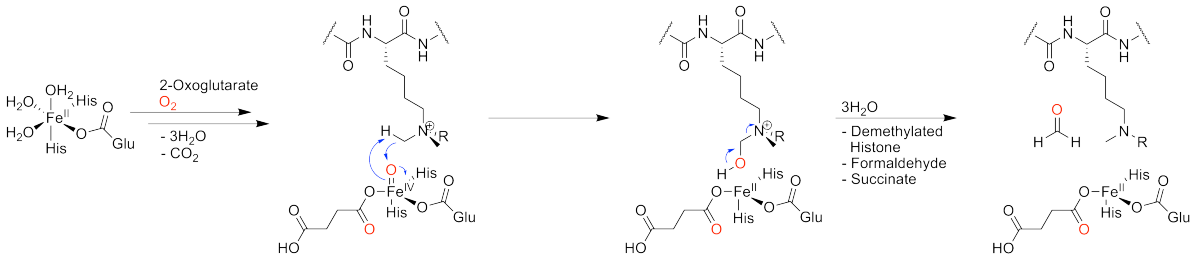
Iron(II) and 2-oxoglutarate dependent histone demethylases catalyse the removal of lysyl methyl marks from histone tails and are proposed to play vital roles in gene silencing and activation. The demethylation mechanism is thought to proceed via oxidative hydroxylation on the methyl group of the methylated lysine, resulting in fragmentation of the hemiaminal intermediate to form the demethylated histone and formaldehyde as products. Work in our laboratory has focused upon identifying formaldehyde, a known carcinogen able to react with a variety of biomolecules, as a product of demethylation and also upon investigating the reaction of formaldehyde with glutathione, which is thought to facilitate its cellular detoxification.



Using a synthesised substrate peptide with ^{13}C-labelled methyl groups on the lysine, ^{13}C-formaldehyde produced during demethylation was detected unambiguously using 1D-HSQC NMR. Kinetic parameters for demethylation were also attained using NMR, which compared favourably to data from other techniques. In addition, NMR experiments on the non-enzymatic reaction between formaldehyde and glutathione have identified two novel formaldehyde-glutathione adducts. Detailed time course and variable pH data has shown that the new adducts can be formed under neutral conditions, suggesting they may have biological roles.

Poster 029

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^{13}C-Selective EXSIDE – A method for the measurement of long-range ^{13}C-^{1}H coupling constants

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The application of 3-bond ^{13}C-^{1}H scalar coupling constants has seen a tremendous growth in elucidation of 3-dimensional structures of organic molecules. Unfortunately, in practical terms $^{3}J_{CH}$ values are difficult to extract – they are relatively small and of same magnitude as $^{3}J_{HH}$ coupling constants but also made more complicated by the low sensitivity of the ^{13}C nucleus. Many new experiments for simplifying the measurement of $^{3}J_{CH}$ have been reported in literature(1) and their main setbacks are that the interpretation of the resulting spectra is not straightforward, long selective pulse sequences lead to the loss of signal due to t2 relaxation and high-resolution 2-dimensional methods typically require extended experiment times. Of these methods, the most straightforward in our experience is the EXSIDE,(3) which gives rise to simple doublets in the F1 (^{13}C) dimension from which the $^{n}J_{CH}$ value can be read directly from the spectrum, however the extremely high F1 resolution required, means typical experiments take around 6 hours.

A new approach is reported for determination of long-range ^{13}C-^{1}H coupling constant based on ^{13}C band-selected EXSIDE, which is rapid (<10 minutes per coupling constant) and easily interpreted (Figure 1).

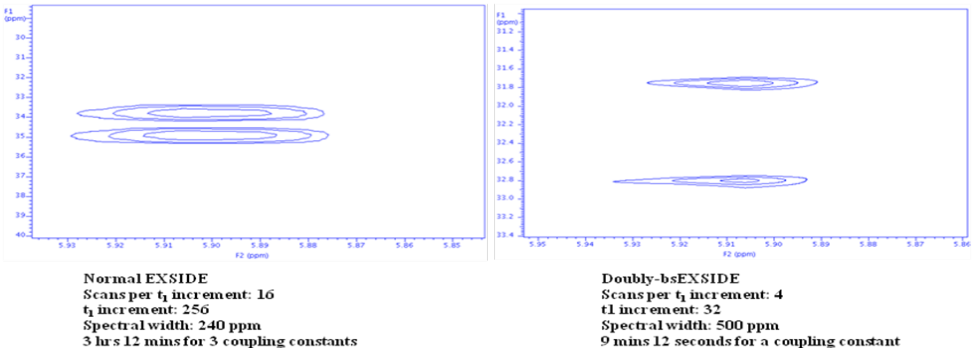

Figure 1: Comparison between normal EXSIDE and the doubly-band selected EXSIDE (strychnine sample, H22-C14)

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Delegates

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