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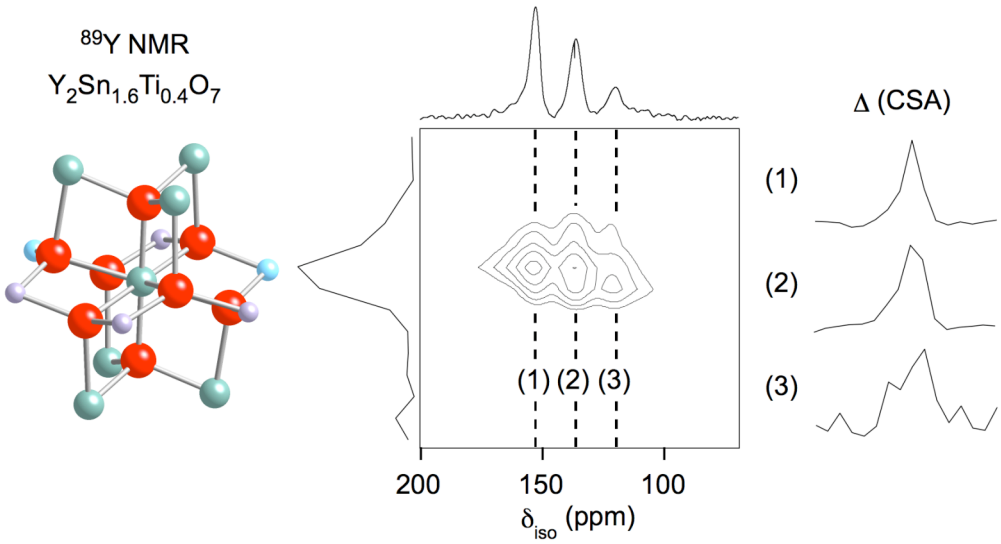
Royal Society of Chemistry

NMR Discussion Group



Postgraduate Meeting 2010

School of Chemistry
University of Leeds
24th June 2010

Dear Delegate,

Welcome to the third RSC NMR Discussion Group one-day "postgraduate" symposium. Following previous versions of this meeting at Astra Zeneca, Charnwood, and the University of Manchester, 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.

This year, we have introduced two overview lectures, given by leaders in their field, to highlight the power of magnetic resonance methods across a broad range of topcis. This year Dr Paul Hodgkinson (University of Durham) and Prof. Mike Williamson (University of Sheffield) will present overviews of solid-state NMR and applications to biological systems respectively.

The varied programme has been arranged to allow delegates to listen to the talks, 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	**Julie Fisher**	**Mike Williamson**
University of Sussex Meeting Organiser	University of Leeds Local Organiser	University of Sheffield NMRDG Chairman

Local organisation and acknowledgements

Meeting coordinated by:	Iain Day, University of Sussex
Local organisation coordinated by:	Julie Fisher, University of Leeds
Online registration coordinated by:	John Parkinson, University of Strathclyde

The organisers would like to thank Stephen Byard (Sanofi-Aventis) 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:

Jeol UK

Sanofi-Aventis

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 **Paul Hodgkinson**, University of Durham
An overview of Current Solid-State NMR and its Applications

1110 – 1130 **Jonathan Bradley**, University of Warwick
^{1}H Double-Quantum Build-Up Curves From DQ Filtered ^{1}H-^{13}C Correlation Spectra

1130 – 1150 **Jan Novak**, University of Birmingham
Magnetic Resonance Imaging of Chemistry in Flow

1150 – 1210 **Catherine Cropper**, University of Liverpool
NMR Studies of Ionic Liquids Confined in Mesoporous Silica

1210 – 1230 **Catharine Jones**, University of Bristol
Interproton Distances from Nuclear Overhauser Effect (NOE) Data: Rigid and Flexible Systems

1230 – 1250 **Alexandria Rogerson**, University of Manchester
The Determination of the Components of Monoacetin Using DOSY

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 **Mike Williamson**, University of Sheffield
If I knew then what I know now…

1510 – 1530 **Theodoros Karamanos**, University of Leeds
Structural Determination of the Dimeric Apo-ZitB Cytoplasmic Domain

1530 – 1550 **Judy Fonville**, Imperial College
The Use of J-resolved ^{1}H NMR Spectroscopy in Metabolic Profiling

1550 – 1610 **Wing Ying Chow**, University of Cambridge
Solid-state NMR as a Structural Probe for Collagen Matrices

Close

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

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Abstracts of Talks

(Talk 001)

Paul Hodgkinson, University of Durham, paul.hodgkinson@durham.ac.uk

An Overview of Current Solid-State NMR and its Applications

Paul Hodgkinson

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As a probe of local chemical environment, NMR can be applied to any type of material, from simple solutions to heterogeneous solids. NMR in the solution state has the advantage that the NMR interactions are reduced to isotropic averages, leaving just the isotropic component of the chemical shift and scalar (J) couplings. In contrast, solid-state NMR spectra are influenced by interactions such as the dipolar and quadrupolar couplings, and the anisotropy of the chemical shift. Hence the challenge in solid-state NMR is often one of too much information. Moreover, solid-state NMR is often being applied to "difficult" samples where conventional solid-state characterisation techniques, i.e. single-crystal X-ray diffraction, are unsuitable.

This tutorial lecture discusses how solid-state NMR differs from its solution-state counterpart, and describes how high-resolution spectra may be obtained, using techniques such magic-angle spinning and homonuclear decoupling. The range of problems to which solid-state NMR can be applied is illustrated, and the information on structure and dynamics that can be obtained from solid-state NMR experiments is discussed. The role of computational chemistry techniques, particularly new DFT-based methods, to connect NMR observations and structural information from diffraction-based techniques is highlighted.

(Talk and Poster 002)

Jonathan Bradley, University of Warwick, jonathan.bradley@warwick.ac.uk

^{1}H Double-Quantum Build-Up Curves From DQ Filtered ^{1}H-^{13}C Correlation Spectra

Jonathan P. Bradley[1], S.P. Velaga[2], O. Antzutkin[1,2] and Steven P. Brown[1].

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[2]Luleå University, Sweden

^{1}H double-quantum (DQ) spectroscopy is a well established method for obtaining structural information regarding proton proximities in solids, the presence of peaks typically indicating a H-H proximity of up to 3.5 Å. We have recently shown that quantitative information about H-H proximities can be obtained from the build-up of DQ peak intensity in ^{1}H DQ CRAMPS spectra recorded with increasing numbers of POST-C7 recoupling elements. These build-up curves allow the reliable determination of relative H-H distances, even in dense networks of many dipolar-coupled spins, such as are present in organic molecules.

Solid-state NMR spectra have been recorded for the gamma polymorph of the active pharmaceutical ingredient (API), indomethacin. The 1H chemical shifts were assigned on the basis of ^{1}H-^{13}C MAS-*J*-INEPT correlation spectra and first-principles GIPAW calculations of the chemical shifts using the known crystal structures.

The ^{1}H DQ CRAMPS spectrum of indomethacin-γ features many overlapping peaks, making the extraction of build-up curves impossible for the majority of the 1H nuclei in the system. A ^{1}H DQ - ^{13}C correlation experiment has been used to exploit the narrower lines and wider chemical shift range of a ^{13}C spectrum. The resulting spectrum separates the DQ peaks and consequently allows the extraction of DQ build-up curves from regions of the spectrum that are too crowded in a standard ^{1}H DQ CRAMPS spectrum.

(Talk and Poster 003)

Jan Novak, University of Birmingham, jxn624@bham.ac.uk

Magnetic Resonance Imaging of Chemistry in Flow

Jan Novak,[1] Annette Taylor,[2] Melanie Britton[1]

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The study of chemical waves under flow is of increasing interest as they provide alternative mechanisms for pattern formation in nature, particularly in biological systems. The manganese-catalysed Belousov-Zhabotinsky (BZ) reaction[1] has previously been studied under flow using magnetic resonance imaging (MRI)[2]. The BZ reaction is known to form travelling waves and recently it has been shown that stationary waves form when the reaction is coupled with flow in a packed bed reactor. We have extended the range of flow environments studied to include Taylor vortices in a Couette cell and Poiseuille flow.

MRI visualisation of the chemical waves is made possible by a difference in the NMR relaxation times of water solvent molecules in the presence of the two oxidative states of the metal catalyst. In this study we look at chemical waves propagating through a series of Taylor vortices[3] (Fig 1.). Taylor vortices are a hydrodynamic instability produced in Couette flow, composed of torroidal counter-rotating vortices within the annulus of the cell.

Previous work has shown that MRI is able to produce velocity images of flow within Taylor vortices[4]. We have combined the visualisation of chemical waves with of NMR velocity and diffusion maps.

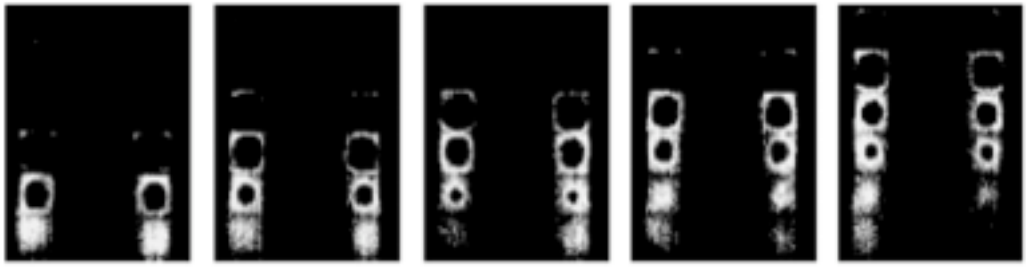

Fig 1. A time series of chemical waves propagating through Taylor vortices

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(Talk and Poster 004)

Catherine Cropper, University of Liverpool, c.cropper@liv.ac.uk

NMR Studies of Ionic liquids confined in Mesoporous Silica

C. Cropper,[1] J. A. Iggo, N. Winterton, Y. Z. Khimyak

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Room temperature ionic liquids (RT-ILs) have attracted considerable attention recently due to their physical properties such as lack of vapour pressure, high thermal stability and conductivity. The tunable properties of RT-ILs allow for a vast range of applications and are achieved by careful selection of cation and anion components[1].

Porous silicas have tunable pore dimensions and can be easily functionalised for use in various catalytic processes, such as salt supports for phase transfer catalysis[3]. Combination of RT-ILs and silica mesoporous supports might create unique materials with the advantageous properties of both components. Such properties will need to be characterised on a molecular level.

Solid-state and HR/MAS NMR spectroscopy have been used to analyse the changes in dynamics and thermodynamic properties of encapsulated RT-IL compared to bulk RT-IL. We have shown the dynamics of encapsulated RT-IL to depend on its loading level and type of porous support. Thus, we have observed changes in ^{1}H T_1 relaxation times with different loadings of RT-IL in the porous support and at various temperatures. The combination of ^{1}H and ^{19}F NMR studies suggests a difference in motional regimes for the cation and anion components.

Further understanding of the cation motions have been gained from ^{1}H- ^{13}C CP dynamics. The interaction of cation protons with the silicate units in the pore walls has been studied by ^{1}H-^{29}Si correlation experiments.

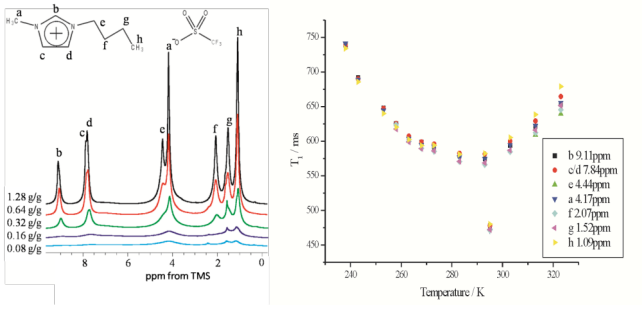

Figure (left) 1H MAS NMR spectra of [bmim]OTf encapsulated in SBA-15 showing linewidth broadening with decreasing loading. (right) Temperature dependence of ^{1}H T_1 times of [bmim]OTf encapsulated in SBA-15 at 0.64 g/g loading level.

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(Talk and Poster 005)

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

Interproton Distances from Nuclear Overhauser Effect (NOE) data: Rigid and Flexible Systems

Catharine R. Jones,[1] Craig P. Butts,[1]

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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.[1,2] 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[3] produces a mean absolute error of only 2.97% (0.09Å).

This technique is then applied to flexible molecules, where the possibility of more than one conformation contributing to the overall structure exists. 4-propylaniline is used as a model system, and we introduce results that suggest that NOE data can reliably predict average distances that compare very well with Boltzmann-averaged computational distances. Some ambiguities exist in literature over whether an r^{-6} or r^{-3} relationship should be used to describe internal motions faster than the overall tumbling of the molecule, and we demonstrate that the r^{-6} relationship holds in flexible, small molecules.

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(Talk and Poster 006)

Alexandria Rogerson, University of Manchester,
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The Determination of the Components of Monoacetin using DOSY

Alexandria K Rogerson,[1] Gareth Morris,[1] Mathias Nilsson[1]

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Diffusion-Ordered Spectroscopy (DOSY)[1] achieves a virtual separation of a mixture, using results from a series of pulsed-field gradient spin echo experiments[2]. These are analysed to determine diffusion coefficients for the individual signals in the spectrum, distinguishing between the different components of a mixture.

A recent study of commercial mixtures of the acetylglycerols used 1D NMR, HPLC and GC to analyse the mixtures and to give partial NMR assignments[3]. The present investigation aimed to explore how far NMR alone could be used to achieve a similar result, using DOSY to distinguish between components.

Technical grade "monoacetin" contains six structurally similar components in the sample, including families of structural isomers of the mon-, di- and triacetins, plus unreacted glycerol. The four distinct diffusion coefficients for these species were identified without difficulty but the different mono- and diacetin isomers had similar diffusion coefficients.

Approximately 90% of the spectrum could be assigned straightforwardly to one or other of the four types of species using DOSY data. Homo-and heteronuclear 2D experiments were carried out to assign signals to individual isomers giving an almost complete assignment of all peaks in the highly complex ^{1}H NMR spectrum.

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(Talk 007)

Mike Williamson, University of Sheffield, m.williamson@sheffield.ac.uk

If I knew then what I know now…

Mike Williamson

Dept of Molecular Biology and Biotechnology, University of Sheffield

In this talk I discuss some aspects of my research, drawing lessons that might be relevant for young researchers in NMR. I discuss the importance of luck and the value of persistence; the importance of postdocing in the right place; how to choose a good research field; how much you need to understand; and how my research areas developed. The main conclusion is that it helps to be genuinely interested in the research. In an era of reduced funding for research, it certainly pays to have wide interests, and it does no harm to be working in something of medical interest.

(Talk and Poster 008)

Theodoros Karamanos, Univresity of Leeds, bstk@leeds.ac.uk

Structural Determination Of The Dimeric Apo-ZitB Cytoplasmic Domain

Theodoros Karamanos,[1] Gary Thompson,[1] Steve Homans[1]

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Heavy metal ions are known to be of crucial importance for many cellular processes that occur naturally and physiologically but in high concentrations they are toxic. In order to manipulate and control heavy metal ion homeostasis, cells have developed import and export mechanisms. Apo-ZitB is a transmembrane protein that belongs to the cation diffusion facilitator family of zinc transporters. Crystallographic studies on other members of the CDF family, propose a mechanism for zinc export/import, which involves rearrangements of the cytoplasmic domain relative to the transmembrane part of the molecule. To examine this model, we used an NMR approach to determine the dimeric structure of Apo-ZitB cytoplasmic domain. Previous work on the same project, failed to successfully determine the dimeric form of the protein using an nOe based NMR calculation approach, but resulted in the calculation of Apo-ZitB monomer by CS-ROSETTA. In this study we use a different approach that relies on residual dipolar couplings (RDCs) to try and resolve the structure of the dimer. Using this methodology the symmetry axis between the monomers was calculated without using any nOe assignments, reducing the time required from months to weeks. Using RDC derived information symmetry related dimers were built and evaluated. Here we report the first preliminary structure of Apo-ZitB dimer based on RDCs. This structure has many common features to other structures of proteins belonging to the same family of transporters and opens the way to efficiently calculating the full high resolution structure.

(Talk and Poster 009)

Judy Fonville, Imperial College, j.fonville07@imperial.ac.uk

The use of *J*-resolved ^{1}H NMR spectroscopy in metabolic profiling

J.M. Fonville, A.D. Maher, M. Coen, E. Holmes, J.C. Lindon and J.K. Nicholson.

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Metabonomics is a key area in systems biology which exploits nuclear magnetic resonance (NMR) as an analytical technique to obtain metabolic profiles.[1] These generated metabolic profiles are subsequently analysed using chemometric tools to elucidate, for example, class differences between 'healthy' versus 'diseased', and build models that uncover the underlying biomolecular pathways.
Routinely used experiments in NMR spectroscopic profiling of biofluids (such as the first increment of a NOESY experiment, and the Carr-Purcell-Meiboom-Gill experiment) suffer from peak overlap, which hinders biomarker identification, both from inspection of the raw spectral data and the multivariate models. Here, we evaluate the novel use of *J*-resolved ^{1}H NMR spectroscopy and the high-resolution one-dimensional spectral projections (which are effectively ^{1}H-decoupled proton spectra) to overcome the peak overlap problem in metabonomics.[2] The advantages and drawbacks of *J*-resolved NMR spectroscopy are investigated with a particular focus on information recovery in metabonomics. This is illustrated with a systematic evaluation of spectra from plasma and urine from a galactosamine toxicity study.[3]

This study used, for the first time, full-resolution *J*-resolved projections in metabonomics, and established that peak alignment is required to obtain interpretable statistical correlations and multivariate models. Previously, binned data have been used but the limitations on data interpretation and information recovery are pronounced. Aligned full-resolution *J*-resolved spectra resulted in improved pattern recognition models compared to conventional one-dimensional spectra. The two types of biofluid samples, with numerous highly-coupled peaks from the toxin and its metabolites, highlight nicely the increased peak dispersion, improved interpretation, and also demonstrate the relaxation editing and lack of quantification. We evaluated the use of *J*-resolved projections for regression analysis to aid biomarker identification, but found that the level of quantification is currently not sufficient for implementation of this regression spectroscopy.

The combination of projections and the original two-dimensional spectra increase the information recovery from NMR-based metabonomic studies, resulting in enhanced biomarker identification. Summarising, the results indicate the overall usefulness of addition of the *J*-resolved experiment and its projection in the 'metabonomics toolbox'.

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(Talk and Poster 010)

Wing Ying Chow, University of Cambridge, wyc25@cam.ac.uk

Solid-state NMR as a Structural Probe for Collagen Matrices

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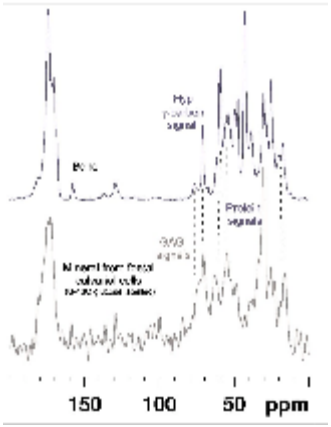
Collagen is the most abundant structural protein in the human body, and a major component of the extracellular matrix. However, our understanding of collagen structure is derived mainly from model peptides that can be synthesised, crystallised and analysed by X-ray diffraction. Much is still not understood on the fibrillar level structure, which exhibits disorder and complex cross links.

Previous attempts to grow collagen matrix in cell cultures have been followed with solid-state NMR. While upstream biochemical markers indicate that the cell had attempted to synthesise collagen, and binding stains show the presence of long molecules being produced, solid-state NMR indicates that the necessary post-translational modification of proline have not always been carried out. An NMR toolkit for collagen structure that can directly pick out key motifs of collagen, such as triple helical units and fibrillar packing, will allow us to characterise such synthetic attempts with even greater precision.

To increase the information on native collagen structure available from NMR, we are developing in vitro and in vivo techniques for isotopic labelling. To understand the data from these complex systems, we are undertaking detailed structural studies on model, isotope labelled, synthetic collagen fragments, so that we can identify spectroscopic fingerprints of collagen structural motifs using a toolkit of key NMR experiments. To this end, we have synthesised ^{15}N labelled model collagen peptides and have initiated ^{13}C CSA, ^{15}N CSA, ^{13}C{^{15}N} REDOR and ^{15}N-^{15}N DQ experiments. These are being analysed to provide constraints on the triple helical geometry.



Abstracts of Posters

Poster 011

Richard Hopton, University of Leeds, chm2r2ph@leeds.ac.uk

An NMR-based metabonomics approach to determine the effects of a probiotic intervention on aflatoxins B1 exposed turkeys

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[2]Molecular Epidemiology Unit, Centre for Epidemiology and Biostatistics, The LIGHT laboratories, University of Leeds, Leeds, United Kingdom,
[3]Department of Veterinary Science, and Graduate Toxicology Program, Utah State University, Logan, UT USA.
[4]School of Biological Sciences, University of Hong Kong, China

Aflatoxin B_1 (AFB_1) is a potent liver carcinogen, and human exposure in parts of Africa and Asia is frequent and occurs at high levels. Targeted intervention strategies are important to alleviate the burden of aflatoxin exposure. One potential approach is the use of lactobacillus rhamnosus (LGG), a probiotic that restricts AFB_1 bioavailability. In this study the effectiveness of LGG to alter the serum metabonome of AFB_1 treated turkeys was examined using ^{1}H-NMR based metabonomics with principle components analysis (PCA). Turkeys received LGG or PBS control (n=20 per group) on days 11-30, and 10 animals in each group received an additional dose of AFB_1 (1ppm) or PBS control on days 21-30, after which serum was isolated. No significant differences were observed in the PCA between control and LGG (p=0.087), whilst AFB_1 treatment was significantly different from the control (p=0.012). Regions identified as varying in PCA plots are being further examined using 2D-spectroscopy. There were no significant difference in the AFB_1 and LGG + AFB1 (p = 0.259) suggesting that LGG intervention was not successful in sufficiently restricting AFB_1 uptake. An observation reflected by the modest reduction in aflatoxin-albumin (AF-alb) adduct level (an exposure biomarker for AFB_1 intake) between these two groups; AFB_1 only mean AF-alb adduct level 2.17ng/mg SD 0.65ng/mg and LGG + AFB_1 mean 1.77 ng/mg; SD 0.65ng/mg (p=0.19). This is the first study using ^{1}H-NMR to show a difference in the serum metabonome following exposure to AFB_1.

This work was supported by the White Rose Doctoral Training Centre, UK, and by a grant from the Community University Research Initiative, Utah State University.

Poster 012

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

NMR-Based Profiling of Ovarian Follicular Fluid and Plasma

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[2]The Leeds Centre for Reproductive Medicine, Leeds Teaching Hospitals NHS Trust, Seacroft Hospital, Leeds, UK

The success rates of infertility treatments such as in vitro fertilisation (IVF) and inter-cytoplasmic sperm injection (ICSI) are unsatisfactory, and there is currently no reliable method of assessing oocyte quality and potential. It has been shown that follicular fluid (FF), the fluid surrounding an oocyte as it develops within its follicle in the ovary, contains substances essential to follicle growth and 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 infertility within human FF and plasma.

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

Among this small cohort some interesting and potentially valuable trends are already arising. Most strikingly, is a difference in composition of FF taken at the pre-ovulatory and ovulatory stages, particularly at 1.33 and 4.13 ppm; the lactate signals. A difference in the corresponding plasma samples was also observed.

To our knowledge this is the first study to use NMR spectroscopy and metabolomics techniques together to identify chemical markers of oocyte quality within human FF. The findings of the study indicate that it may be worthwhile to investigate FF composition among women undergoing infertility treatment more extensively.

Poster 013

Robert Evans, University of Manchester, robert.evans@manchester.ac.uk

Pure Shift DOSY Techniques

R. Evans[1], J. Aguilar[1], S. Haiber[2], M. Nilsson[1] and G. A. Morris[1]

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[2].Givaudan, Dept. for Analytical Research, Huizerstr. 28, NL – 1411, Naarden, Netherlands

Diffusion-ordered NMR spectroscopy (DOSY) allows the spectrum of a mixture to be resolved into individual components on the basis of their diffusion coefficients. Good results require well-resolved spectra; peak overlap in the frequency dimension, almost unavoidable in ^{1}H NMR, leads to artefacts such as peaks appearing in compromise positions in the diffusion dimension. Signal overlap, and its attendant problems, can be greatly reduced by simplifying the proton spectrum to give a homodecoupled or 'pure shift' spectrum. Pure shift techniques based on homonuclear 2D J spectroscopy [1] have been long available but are all more or less unsatisfactory. The properties of the phasetwist lineshape that is inherent to the technique [2] necessitate the use of severe weighting functions and absolute value display, so that 45° projection of absolute value 2D J spectra yields pure shift spectra with broad lines and distorted intensities.

The introduction of the Zangger-Sterk pulse sequence element [3] has led to significant improvements. This combination of selective pulse and magnetic field gradient, simultaneously slice- and shift-selective, allows a subset of the spins to be treated as heteronuclei which can then be manipulated independently of the rest of the sample. A number of pure shift experiments [4, 5] have been developed that show resolution of complicated spectra and pure absorption mode lineshapes. The extension of such sequences to produce 2D [4] and 3D pure shift DOSY experiments will be demonstrated, alongside a number of illustrative examples and applications.

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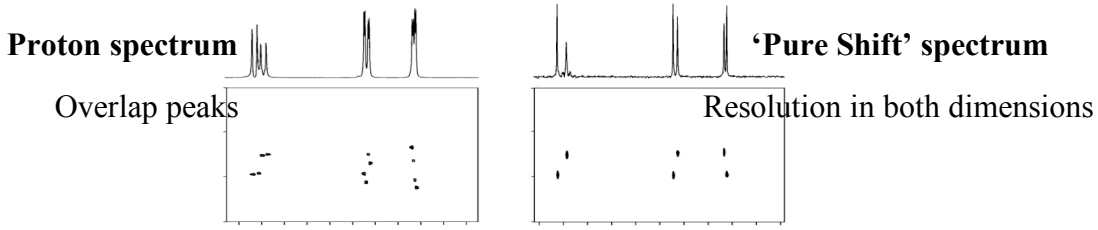
Mixture of 100 mM of quercetin and rutin in DMSO-d_6



Poster 014

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Structural studies of the unfolded state of immunity protein 7

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Immunity protein 7 (Im7) is a four-helix, 9.5 kDa protein that inhibits bacterial colicin DNase E7, conferring colicin E7 resistance to *Escherichia coli* cells that produce Im7. The folding mechanism of Im7 has been intensively studied and it is now known that folding proceeds via a transiently populated intermediate. The unfolded state of Im7 is of great significance towards our goal of understanding folding at an atomic level, since local conformational preferences could initiate or inhibit folding events. Characterisation of the unfolded state of Im7 remains elusive since it is not populated under physiological conditions. Structural studies of urea-denatured Im7 showed hydrophobic clustering in regions corresponding to helices in the native structure, indicating local structural preferences even in the presence of high denaturant concentration. Since urea disrupts structure formation, unfolded Im7 could have a very different conformation in the absence of urea. Mutations have been introduced into Im7 to significantly destabilise the folded and intermediate states, hence populating the unfolded state in aqueous solution, providing an opportunity to structurally and energetically characterise the unfolded state. Results show the unfolded state has been successfully populated under non-denaturing conditions, and preliminary structural characterisation of the unfolded ensemble using NMR will be presented

Poster 015

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Practical Aspects Of Timecourse Dosy

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Analysis of chemical reactions is a daunting task which has been tackled by a variety of methods with different degrees of success and complexity. One of the latest methods is Timecourse DOSY, [1-2] which aims to extract NMR spectra, diffusivities and kinetic information through PARAFAC[3] (Parallel Factor analysis). In order to perform this multiway decomposition it is necessary to obtain high quality data. Critical requirements such as temperature stability and sufficient signal-to-noise ratio are discussed, along with illustrative examples at the limits of successful decomposition. A Matlab-based toolbox has been created to facilitate the task of pre-processing data to optimise data quality and to post-process PARAFAC results, providing publication quality plots with minimal intervention.

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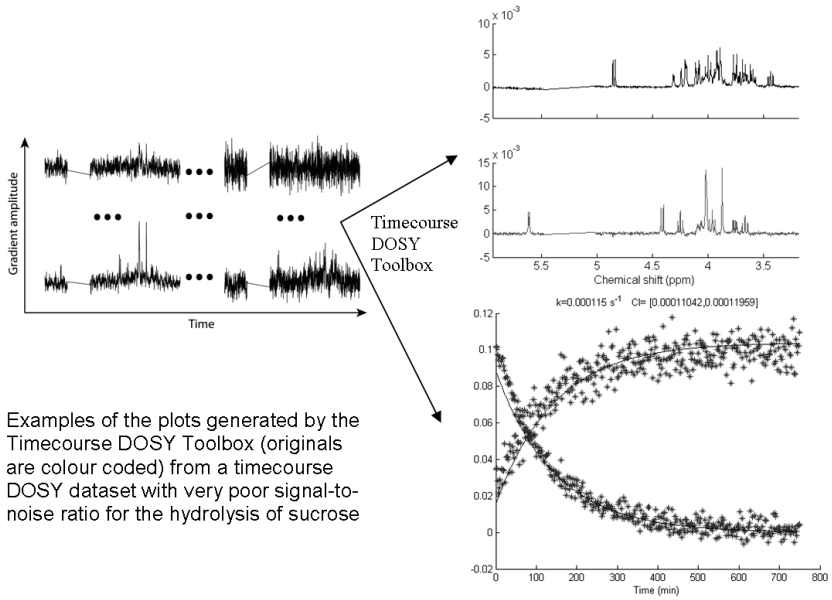

Examples of the plots generated by the Timecourse DOSY Toolbox (originals are colour coded) from a timecourse DOSY dataset with very poor signal-to-noise ratio for the hydrolysis of sucrose

Poster 016

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NMR Based Metabolomic Studies of Arterial Disease

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Atherosclerosis is a disease of large and medium-sized arteries and contributes to the increasing worldwide burden of myocardial infarction (heart attack) and stroke. Plasma has been studied from three groups of patients, two suffering from arterial disease, either coronary or peripheral, and one without arterial disease. Peripheral arterial disease can cause intermittent claudication, which is aching or pain in the legs reproducibly brought on by walking and relieved by rest.

Metabolomics provides a snapshot of the metabolome. Changes to the metabolome can provide evidence of endpoint metabolite markers for disease, drug treatment, diet, lifestyle and genetic composition. These can cause alterations to body conditions that disrupt the normal ratio of endogenous biochemicals in cellular metabolic pathways as the body tries to retain homeostasis. When analysing the complex information from biological samples, multivariate statistical analysis is necessary for reduction of the data and extraction of the relevant biomarker information.

For this preliminary study, sample data from 80 patients, with similar numbers in each group, has been acquired using ^{1}H-NMR spectroscopy and various bin widths investigated, initially using principal component analysis (PCA) then partial least squares discriminant analysis (PLS-DA). Firstly, PCA showed diet was strongly influencing results, principally glucose and lactate, but other interesting trends have been shown. These will be investigated through further analysis and the acquisition of more samples from new patients in all groups.

Poster 017

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Structure, Disorder and Phase Transitions in AlPO-53

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Aluminophosphates $(AlPOs)_1$ are an important class of crystalline microporous framework materials with applications in catalysis, gas storage and materials science. Their structure consists of a neutral framework of alternating corner-sharing and tetrahedra, with micropores capable of accommodating small molecule guest species. AlPO-53 displays an unusual number of phase changes related to hydration, dehydration and calcination.[2,3] Here, multinuclear solid-state NMR is used to provide structural and kinetic information on these phases and transitions. Each phase can be characterised by a distinctive ^{31}P spectrum, while more detailed structural insight is obtained from high-resolution ^{27}Al multiple-quantum MAS and $^{31}P/^{27}Al$ INEPT/MQ-INEPT experiments (where magnetization is transferred between nuclei using the *J* coupling). While the sensitivity of the MQMAS experiments is lower than conventional experiments, the resolution gain in many cases is significant. Disorder of the methylamine template is investigated by ^{1}H ultrafast MAS NMR and double-quantum spectra, natural abundance ^{13}C and ^{15}N cross-polarisation and $^{1}H/^{13}C$ INEPT experiments.

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Poster 018

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Investigating Disorder in Pyrochlore Materials by MAS NMR and First Principles Calculations

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NMR spectroscopy provides an element-specific probe of local structure and dynamics in solids, without the requirement for long-range order. Magic-angle spinning (MAS) removes anisotropic interactions which broaden solid-state NMR spectra, and achieves high-resolution spectra. For disordered systems, we typically see a distribution of NMR parameters and corresponding broadening or splittings in the spectrum, hindering analysis. Here, we combine high-resolution NMR experiments with DFT calculations (CASTEP code[1]) to investigate disorder in pyrochlore ($A_2B_2O_7$) ceramics. These materials are of particular interest for their application in the long-term storage of radioactive waste. The chemical shift anisotropy (CSA) can also be used alongside isotropic chemical shifts, and similarly compared to calculations to provide an additional aid to spectral assignment. However, in complex materials it is often difficult to measure the CSA using slow MAS or static experiments. Alternatively, 2D CSA-amplified PASS,[2] a 2D experiment where the CSA is reintroduced and measured in the indirect dimension, whilst retaining the practical advantages of faster MAS can be implemented. Here, disorder in the pyrochlore solid solution $Y_2Ti_{2-x}Sn_xO_7$ is investigated using ^{89}Y and ^{119}Sn NMR and calculations. Using ^{89}Y NMR, it is possible to resolve sites with different numbers of Sn next nearest neighbours. Through the assignment and integration of these peaks, the distribution of Sn and Ti on the pyrochlore B-sites surrounding the ^{89}Y can be compared to theoretical population distributions.[3-4] In contrast, the ^{119}Sn NMR spectra appear less resolved and calculations are required in order to interpret the complex lineshapes which result.

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2. R. M. Orr, M. J. Duer, S. E. Ashbrook, J. Magn. Reson. 2005, 174, 301-309
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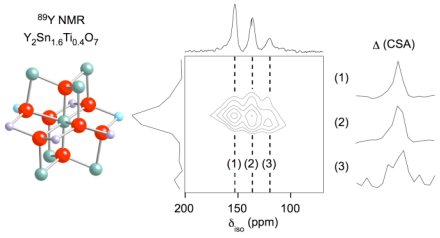

Poster 019

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Solid State NMR investigation into local structure and order in aluminophosphates

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Aluminophosphates (AlPOs) are porous framework materials with industrial applications, including catalysis and gas storage. First synthesised by Wilson et al. [1] in the 1980s, AlPOs consist of alternating corner sharing AlO_4 and PO_4 tetrahedra. Solid-state NMR is an excellent probe of local structure and order, particularly suited to the study of AlPOs, as the basic components of the framework are NMR active (^{27}Al, ^{31}P, ^{17}O), as are the templates molecules. ^{27}Al is quadrupolar (I=5/2) and two-dimensional multiple-quantum MAS experiments are needed to remove the quadrupolar broadening and achieve high-resolution.

In this work, we investigate the structure of two new aluminophosphates, STA-2 and STA-15. Typically, AlPOs are synthesized using template molecules, which are often charged, requiring the incorporation into the structure of charge-balancing anions, such as hydroxide or fluoride. In these two materials, the number and location of the OH– anions is difficult to extract from x-ray diffraction and solid-state NMR is used to examine the nature and role of these species in more detail. We employ high-resolution NMR experiments to compare the framework structures in templated and calcined materials and to provide insight into the location and order of the charge-balancing OH– species. In addition, heteronuclear correlation experiments provide information on through-bond connectivity and probe the framework structure.

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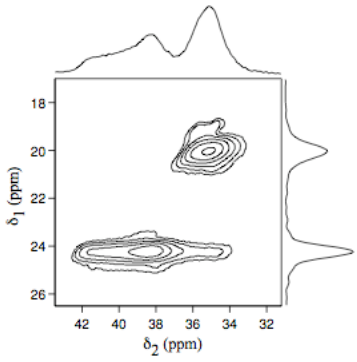

Poster 020

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Solid-State NMR Studies Of A Cardiac Glycoside Bound To The Digitalis Receptor

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Solid-state NMR is a valuable technique for the structural analysis of small molecule and peptide ligands bound to protein receptors embedded in lipid membranes. In this work SSNMR is used to examine the structure and orientation of a ^{13}C-labelled cardiac glycoside in the high affinity site of the Na+/K+, ATPase (NKA), also known as the digitalis receptor. NKA is an ion pump which maintains the ionic gradient in eukarotic cells vital for several processes including maintaining cell volume and transporting secondary solutes. Although it is well understood that cardiac glycosides such as ouabain are highly selective and potent inhibitors of NKA (and centuries-old drugs for heart failure), their orientation within the high-affinity inhibitory site of the membrane embedded protein is unclear. There is evidence that ouabain analogues lie across the NKA surface with the mobile sugar group facing away from the protein. However, more recently, X-ray crystallography of ouabain soaked into lipid-free NKA crystals suggest that the inhibitor is inserted deeply into the transmembrane domain. Cross-polarisation magic-angle spinning (CPMAS) SSNMR in conjunction with paramagnetic broadening agents is used here to provide a clearer picture of the cardiac glycoside orientation within NKA embedded in its native membrane, by exploiting a double ^{13}C-labelled diacetylated derivative of ouabain.

Poster 021

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Solid-State NMR Analysis Of Fibrils Derived From The Aortic Amyloid Polypeptide Medin

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The amyloidoses are a collection of diseases that are characterised by the presence of highly ordered protein fibrils. Solid-state NMR (SSNMR) is providing insights into the structure of these fibrils that could lead to the design of aggregation inhibitors for potential drug therapies. In this work SSNMR is used to study the fibril architecture of medin, the principal component of the amyloid plaques found in patients with aortic medial amyloid, a condition found in over 97% of the Caucasian population over the age of 50. We examine the structural properties of fibrils formed by full-length medin and by several shorter medin-derived peptides, each containing the amyloidogenic C-terminal residues 42-49. Two-dimensional ^{13}C-^{13}C dipolar correlation spectra detect intermolecular couplings which report on the arrangement of β-sheet layers in the fibrils. An octameric medin-derived peptide with the sequence N42FGSVQFV adopts an anti-parallel orientation of β-sheet layers with close contacts between F43 and V46 and between S45 and V46. By contrast, the full length 50 amino acid medin sequence appears to lack these close contacts suggesting a parallel orientation of β-sheet layers. Structural comparison with fibrils from intermediate-size medin peptides (residues 37-50 and residues 29-50) reveals which residues may cause the transition between these two β-sheet arrangements, and highlights sites which could be targeted by small molecules or peptides to inhibit aggregation into toxic amyloid species.

Poster 022

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The Elusive Minor Form of β-phosphoglucomutase

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Enzymes have evolved to catalyse reactions that would otherwise not occur on the biological timescale. Protein and inositol phosphates accelerate the reaction 10^{21} fold, among the highest rate accelerations amongst enzymes. Phosphate transfer is fundamental throughout the cell, including energy storage and usage through ATP, signalling, giving stability to DNA/RNA and the maintenance of lipid bilayer asymmetry.

Metal fluorides have been used as analogues of the phosphate group. There are two types of analogue – the ground state analogue (GSA), mimicking the phosphate group bound to the enzyme or substrate in a tetrahedral geometry and the transition state analogue (TSA), mimicking the transferring phosphate group in the associative transfer mechanism.

B-phosphogulcomutase (β-PGM) from Lactococcus Lactis has been well established as a model for phosphate transfer. The enzyme converts β-Glucose-1-Phosphate to β-Glucose-6-Phosphate via the bis-phosphate intermediate. The G6P, once it is produced, enters glycolysis, making β-PGM a fundamental enzyme in energy storage.

The ^{19}F NMR of the β-PGM:MgF3:G6P TSA complex shows a minor form at 10% of the population at 298 K, which increases to 22 % of the population at 278 K. NMR has been used extensively to study the nature of this minor form; ^{19}F NMR, backbone assignment and deuterium isotope shift analysis using 60 % D_2O buffer. Two of the minor form peaks overlap in the ^{19}F NMR, so a saturation transfer experiment has been developed that removes one of the overlapping signals, allowing visualisation of the minor form deuterium isotope shifts.

Poster 023

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Astringency Reduction In Toothpaste

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Astringency is not a taste, but rather a sensation created by the de-wetting of the oral membranes leaving them dry and feeling constricted. This de-wetting has been shown to be a result of the aggregation and precipitation of salivary proline rich proteins when bound to dietary tannins found in tea and red wine. It is thought that zinc, an antimicrobial agent in many toothpaste bases, also causes the astringent sensation and it is of commercial interest to reduce this undesirable effect.

As a result of studying zinc retention through human trials and ICP-AES, aggregation reduction through turbidity and a human tasting panel on "active" ingredients and zinc binding through 1D ^{1}H NMR, it has been possible to determine three "active" ingredients that reduce perceived astringency without affecting the antimicrobial effects.

After the first rinse post-brushing, present in the oral cavity is zinc at 3 μM; maltol/ethyl maltol/cyclotene at 0.4 mM; PRPs at 30 μM; and K_ds of zinc for maltol, ethyl maltol and PRP respectively of 19 mM, 21 mM, and 500 μM. Resulting calculations suggest that the bound complex would use only approximately 2% of the zinc present. However, the amount that does bind is thought to be sufficient in disrupting the equilibrium between the zinc and the PRPs, resulting in the reduction of turbidity and perceived astringency, and is not thought to affect the antimicrobial properties.

Poster 024

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Investigating the RNA Binding Properties of EDEN-BP

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EDEN-BP is a 53kDa, 489 residue RNA binding protein, which targets maternal mRNAs for deadenylation by binding to EDEN motifs in the 3' untranslated region. The protein contains three RNA recognition motifs, two in the N-terminal region and one in the C-terminal region. This third motif is believed to be unnecessary for specific binding. EDEN-BP shares an 88.4% sequence identity with the human protein CUG-BP, for which an NMR solution structure of the two N-terminal RNA recognition motifs is available. This structure shows two compact domains, joined by a flexible linker.

This study focuses on the corresponding 187 residue region of EDEN-BP, which is believed to have a similar structure. In addition to this 187 residue protein, the two domains it is composed of have been produced separately with the aim of determining their individual RNA binding properties. Comparison of chemical shifts from the separate domains with those seen in the larger protein suggests these domains are largely independent.

Titrations of these separate domains with short RNA sequences based on the EDEN motif indicate that they retain the ability to bind RNA. With ^{15}N TROSY experiments the changes in chemical shift of the various NH protons in the protein on binding to RNA can be tracked, providing information on the bound conformation of the individual domains, and hence EDEN-BP as a whole.

Poster 025

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An inserted alpha-helix redirects the polypeptide backbone in a novel homologue of the protein RsmA from *Pseudomonas aeruginosa*

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University of Nottingham

We have determined a 2.0 angstrom X-ray crystal structure of a novel homologue of the protein RsmA from *Pseudomonas aeruginosa*, whose infection of the Cystic Fibrosis lung leads to early mortality in 80-95% of these patients. The excretion of toxic factors makes this bacterium highly pathogenic, whilst its adaptability to its environment makes *P. aeruginosa* infection difficult to treat. RsmA is a small (<8 kDa) protein that post-transcriptionally regulates these processes. A symmetrical homodimer with two identical sites for binding to the ribosome binding sites of certain mRNA molecules, RsmA functions by preventing translation initiation and thereby modulates the expression of a wide range of bacterial genes.

We recently identified a novel RsmA homologue (RsmN) in *P. aeruginosa* that shares >50% homology with RsmA. However, our X-ray crystal structure of RsmN differed from prediction. An inserted 16-residue alpha-helical sequence in a loop of RsmN directs an alternative arrangement of structural elements within a similar overall fold. Although RsmN presents a similar RNA-binding surface, our in vivo studies demonstrated that RsmA and RsmN are not functionally interchangeable. We are currently exploring the affinities of RsmA and RsmN for various mRNA target sequences using NMR spectroscopy to elucidate reasons for the observed differences in the regulatory mechanisms of these proteins.

Poster 026

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Insights into the enzymatic mechanism of phosphoryl transfer

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-Phosphoglucomutase (PGM) catalyses the interchange between Glucose-1-Phosphate and Glucose-6-Phosphate; it is a phosphoryl transfer enzyme. The planar geometry and charge of the transferring phosphate in the transition state of the reaction is mimicked by metal fluorides, which therefore form transition state analogue complexes with a variety of phosphoryl transfer enzymes. Enzyme-catalysed phosphoryl transfer displays the largest rate acceleration by enzymes found so far when compared to the uncatalysed reaction in solution, and as such it is an excellent candidate to study the basis of the catalytic proficiency of enzymes. The use of various NMR techniques to analyse transition state analogue complexes of PGM allows for evaluation of the various theories of enzyme catalytic proficiency. Techniques such as chemical shift analysis, J-coupling analysis, exchange spectroscopy, relaxation dispersion, and deuterium isotope shift analysis shed light on ideas such as electrostatic pre-organisation, substrate destabilisation, entropy, and protein dynamics.

Poster 027

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The effect of acute rejection on erythrocyte metabolic profiles: A ^{1}H-NMR study of renal transplantation

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Background: Acute rejection is associated with an increase in the risk of graft loss in kidney transplantation. Current clinical tests are non-specific and often the only way to accurately diagnose acute rejection is via an invasive biopsy, posing risks to both patient and graft, and potentially leading to delayed detection of acute rejection episodes. As a result the condition may be allowed to progress, with a greater degree of damage inflicted to the graft before intervention is carried out. Hence an early onset biomarker is sought.

Method: A Nuclear Magnetic Resonance (NMR) based approach using lipidic and aqueous extracts from erythrocyte samples from eighteen patients, taken at regular intervals up to 7 days post-transplant has been performed. Integration of characteristic signals was carried out in order to observe differences in the lipid profiles between rejecting and non-rejecting patients. A chemometric approach involving pattern recognition methods and multivariate statistical analysis (primarily Principal Component Analysis) will also be used to analyse the NMR spectra in search of a biomarker indicating acute rejection.

Results: Differences were observed in the relative concentrations of particular lipid classes between rejecting and non-rejecting patients.

Conclusion: These early results suggest that NMR-based metabolic profiling may provide some insight to a biomarker of acute rejection.

Poster 028

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Investigating the dimerisation and ubiquitin binding by the human scaffold protein p62.

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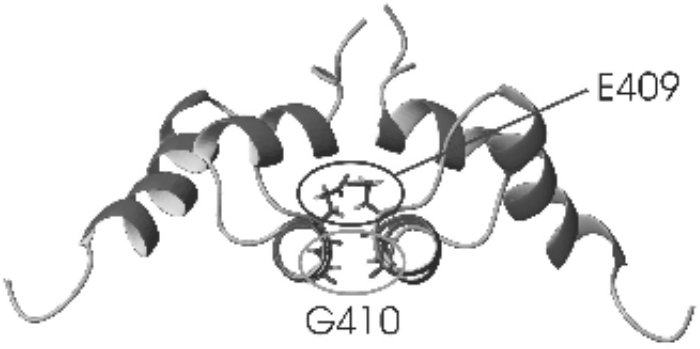
The human scaffold protein p62 is a multi-domain protein primarily associated with RANK ligand induced Nuclear Factor Kappa B (NF-κB) activation. The p62 protein plays a major role in cellular proliferation and has been implicated in bone, muscle and T-cell differentiation.

Mutations in the C-terminal Ubiquitin Associated (UBA) domain have been shown to cause a predisposition to Paget's disease of bone (PDB), a debilitating bone disease characterised by excessive bone turnover. All current pagetic p62 mutations result in the loss of ubiquitin binding in the full length protein.

Recent Studies have suggested that the p62-UBA exists as a dimer in solution, and that dimerisation competes with ubiquitin binding. This raises the prospect that PDB mutations instead of directly interfering with ubiquitin recognition may modulate dimerisation to influence binding. We present here an investigation into this hypothesis using non PDB mutations at key dimer interface residues (Fig) to disrupt dimerisation in order to investigate the functional consequences of dimerisation



Poster 029

Claire Merrifield, Imperial College, c.merrifield07@imperial.ac.uk

NMR-Based Urinary Metabolic Profiling of the Pig Reveals a Sustainable Metabolic Reprogramming Event Related to Weaning Diet

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A combination of one and two dimensional ^{1}H and ^{13}C Nuclear Magnetic Resonance Spectroscopy (NMR) and High Resolution Magic Angle Spinning (HR-MAS) NMR was used to characterise the porcine urine, serum, liver and kidney metabolome. Urine is found to be the most metabolite-dense matrix investigated and thus was interrogated further in a probiotic intervention study where pigs were weaned on to different starter diets and divided into a subset which received probiotics and a control group. The diets were subsequently standardised four weeks prior to urine collection.

Established chemometric techniques such as orthogonal projection to latent structures discriminant analysis (O-PLS-DA) were used to identify the metabolites involved in discriminating the groups. These data indicate that the initial weaning diet is found to induce a sustainable metabolic reprogramming event in the young mammal even after a four-week dietary washout (Q2-0.86). This persistent metabolic signature differs mainly due to end products of dissimilatory amino acid metabolism by the gut microbiota such as p-cresol glucuronide, indoleacetylglycine and phenylacetylglycine as well as endogenous metabolites involved in energy metabolism. The initial weaning diet is also shown to affect the metabolic consequences of probiotic intervention around weaning mainly with respect to gut microbial co-metabolites.

This work indicates that despite the four week dietary washout period, weaning diet appears to have a sustainable reprogramming effect on the metabolic profile of these animals. Importantly, this effect leads to a differential action of the probiotic in terms of gut-microbial co-metabolism and validates the use of metabolic profiling of biofluids in this important test species to ascertain the metabolic impact of dietary interventions.

Poster 030

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Binding Interactions Of Obp3 Using NMR And ITC

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Odour Binding Proteins (OBPs) are small proteins (18-20kDa), found in the nasal mucus of vertebrate mammals. The exact function of these proteins is undetermined; they are known to bind a large number of volatile, hydrophobic odorant molecules, as part of their role within the olfactory system.

In rat there are three known subtypes of OBPs. Designated OBP1, OBP2 and OBP3, they share less than 30% sequence identity. Work has been carried out showing that each of the three subtypes interact with distinct chemical classes (Lobel 2002). Such specificity may suggest a more active role, rather than idea that OBPs, like other proteins in the lipocalin family of which they are part, are merely passive transporters, helping odorants from the gas phase across the mucus layer to Olfactory Receptor Neurons.

The aim of this work is to use biophysical techniques, including Nuclear Magnetic Resonance Spectroscopy and Isothermal Titration Calorimetry to gain structural and dynamic informa¬tion and an understanding of the binding properties of OBPs, the nature of specificity between the three subtypes and the relevance this may have. A large percentage of OBP3 has been assigned, including side chains, with the aid of direct carbon observation experiments. A number of mutants have also been produced to further probe binding specificity.

Poster 031

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Solid State Nuclear Magnetic Resonance Study of Apatite Oxide Ion Conductors

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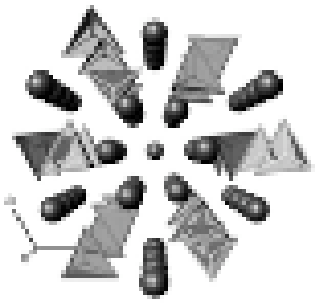
Materials displaying high oxide-ion conductivity have attracted considerable interest due to technological applications in solid oxide fuel cells (SOFC), oxygen sensors and separation membranes[1]. ^{17}O solid state NMR data have been recorded for the apatite series $La_{8+x}M_{2-x}(GeO_4)_6O_{2+x/2}$ ($0 < x < 1.0$). 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.

An increase in intensity of the low field resonance is observed with increasing x, which is thus consistent with an increase in oxide ion content. These data have been used to predict the number of GeO_5 units and Frenkel-type disorders[2]. 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.



Poster 032

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

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The structures of borosilicate glasses, used as components of automobile windscreens, are being studied using multinuclear solid state NMR. Some of these materials are already commercially used, although their properties need to be improved. The aim is to produce a new glass with much higher acid resistance which can pass a new industry test, while keeping the firing temperature the same as that for conventional windscreen glasses.

Two series of model samples have been made in order to understand the roles of Bi and Zn in the glass networks of systems close to the commercial compositions. The samples are sodium borosilicates; in the compositions Bi or Zn are substituted for boron and NMR has been used to determine whether they also substitute the role boron in the glass network, forming bonds, or whether they form fewer bonds and make the network less connected. Bloch decay ^{11}B, ^{23}Na and ^{29}Si experiments and two dimensional ^{11}B MQMAS studies have been undertaken to understand the evolving structural changes with increasing metal content. It has been found that as the metal content increases the glass networks become less connected and so Bi and Zn are not taking the place of boron in the glass network.

Poster 033

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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 (fig. 1a). This flow has been studied intensively since Taylor's original work in 1923, and in 1999 Seymour et al[1] provided the first NMR velocity maps using a pulsed gradient spin echo (PGSE) experiment. By adding axial flow, a Vortex Flow Reactor (VFR) is produced. 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, involving plug-flow properties and inter/intra vortex mixing. It is expected that velocity and diffusion maps of this flow will provide answers for these questions.

A challenge for imaging flow in this system lies in the periodic motion caused by the moving vortices. This causes imaging artefacts and errors in velocity measurements. However, by adapting the pulse sequence timing to the flow period, it is possible to simulate steady state and obtain good quality velocity maps. We used PGSE imaging sequence and timed data acquisition to the Taylor vortices translation period to produce velocity maps in a VFR (fig. 1b).

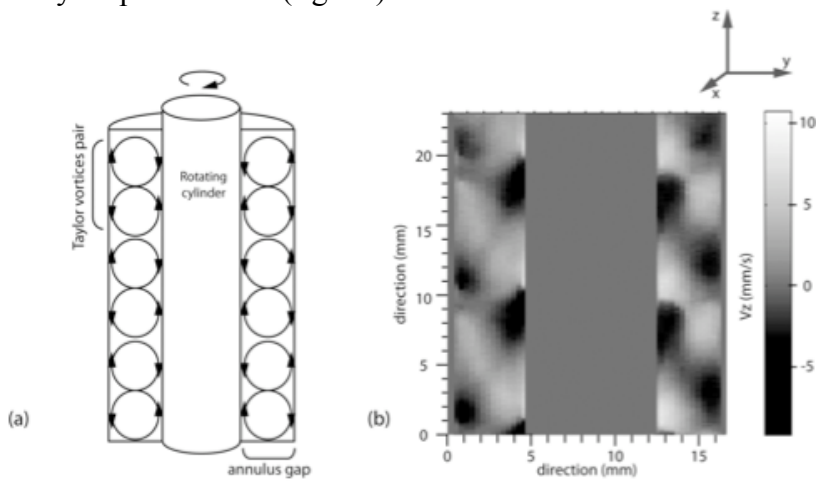

Fig.1. (a) Taylor vortices flow, (b) NMR velocity map of the VFR with flow in the z direction

(1) Seymour et al., Phys. Fluid., 1999, 11, 1104.

Poster 034

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NMR Study of Green Solvent Cellulose Regeneration

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Ionic liquids (ILS) are effective solvents for the dissolution of cellulose. When cellulose is dissolved in ionic liquids, it results in a variety of structural forms without requiring the use of toxic and volatile organic solvents. This project will investigate the relationship between micro and macroscopic properties of regenerated cellulose. Nuclear Magnetic Resonance (NMR) will be used to study how the properties of cellulose depend on the kinetics of its regeneration process. In this work the mechanism of solvation of cellulose in ionic liquids at a microscopic level has been investigated.^{1}H NMR techniques have been performed to measure longitudinal and transverse (T_1 and T_2) relaxation times and the self-diffusion coefficients of the functional groups in IL/cellulose solutions as a function of temperature and concentration. Variations in relaxation times can yield information on the dynamics of both cation and anion that the solvent consists of, providing quantitative data regarding their interaction with solutes. Changes in chemical shift of all hydrogen atoms in NMR spectra also give information on formation of hydrogen bonding between ionic liquid and cellulose.

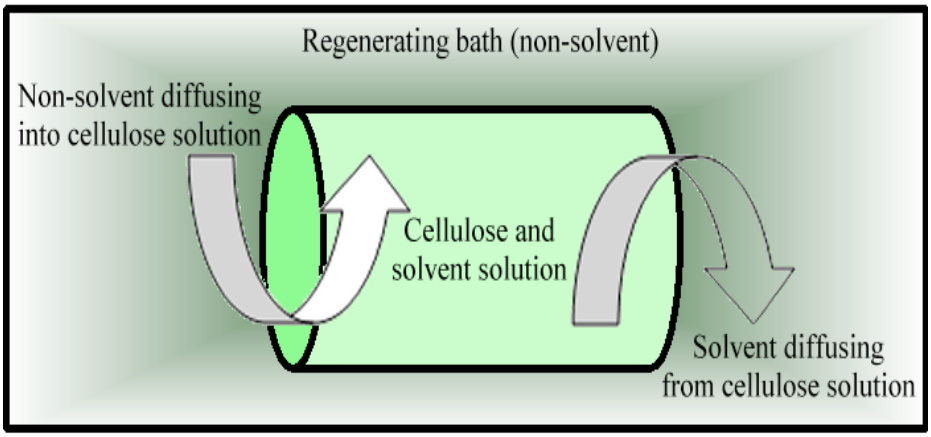

Illustration of cellulose regeneration: cellulose solution is placed in a non-solvent bath (for example, water); the non-solvent is shown diffusing into the cellulose solution and the solvent diffusing out.

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