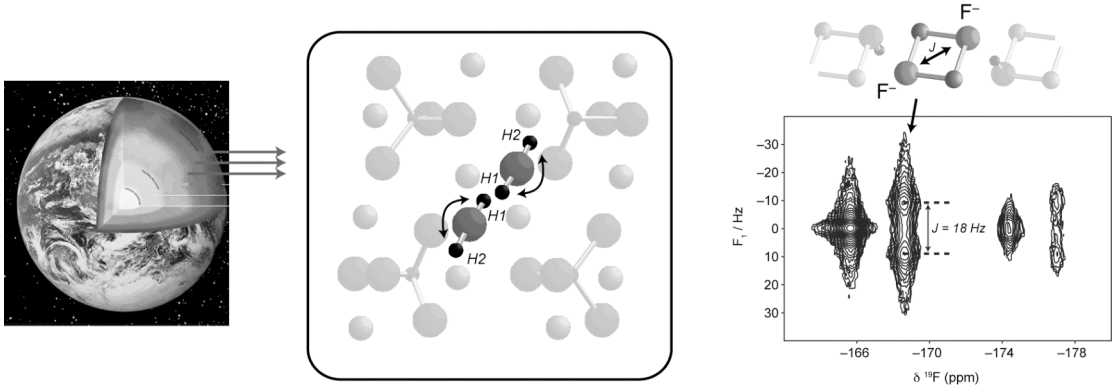
RSC | Advancing the Chemical Sciences

Royal Society of Chemistry

NMR Discussion Group

Postgraduate Meeting 2009

School of Mathematics
University of Manchester
25th June 2009



Dear Delegate,

Welcome to the second RSC NMR Discussion Group one-day "postgraduate" symposium. Following the successful meeting at Astra Zenica, Charnwood, last June this meeting follows 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.

The varied programme has been arranged to allow all 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	**Gareth Morris** **Mathias Nilsson**	**Tim Claridge**
University of Sussex Meeting Organiser	University of Manchester Local Organisers	University of Oxford NMRDG Chairman

Local organisation and acknowledgements

Meeting coordinated by:	Iain Day, University of Sussex
Local Organisation coordinated by:	Gareth Morris, University of Manchester
	Mathias Nilsson, University of Manchester

Thanks go to Tim Claridge (University of Oxford), 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:

Oxford Instruments

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, Tim Claridge, NMRDG Chairman

Oral Presentation Session 1, session chair: Iain Day

1030 – 1050 **Rob Evans**, University of Manchester
Isomer Resolution by Matrix-Assisted DOSY

1050 – 1110 **Sarah Bohndiek**, University of Cambridge
Detecting tumour responses to anti-vascular therapy using hyperpolarized [1-^{13}C]pyruvate and [1,4-^{13}C]fumarate magnetic resonance spectroscopy

1110 – 1130 **Tony Cheung**, University of Manchester
NMR investigation of mismatched DNA and its interactions with Escherichia coli MutS

1130 – 1150 **Thomas Garner**, University of Nottingham
Effects on ubiquitin binding of Paget's disease mutations in the UBA domain of P62(SQSTM1)

1150 – 1210 **John Griffin**, University of St. Andrews
Understanding Water Storage Inside the Earth: Disorder and Dynamics in Hydrous Magnesium Silicates Probed by DFT Calculations and Solid-State NMR

1210 – 1230 **Tim Knowles**, University of Birmingham
Structural studies of the β-barrel assembly machinery, the key complex in outer membrane protein assembly

Lunch

1230 – 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: Mathias Nilsson

1430 – 1490 **Nicky Farrer**, University of Warwick
Photoactivated PtIV-azido anticancer complexes

1490 – 1510 **Refaat Hamed**, University of Oxford
Monitoring the activities of the crotonase superfamily enzymes by ^{1}H-NMR in real time

1510 – 1530 **Adolfo Botana**, University of Manchester
Suppression of J-modulation in DOSY experiments

1530 – 1550 **John King**, University of Manchester
NMR studies of the binding of peptidyl transferase inhibitor antibiotics to conserved secondary structural motifs of 23S ribosomal RNAs.

Close

1550 Tea and Coffee
1600 Award of Best Oral Presentation and Best Poster Presentation

Index to Posters

Abstracts of Talks

(Talk and Poster 001)

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

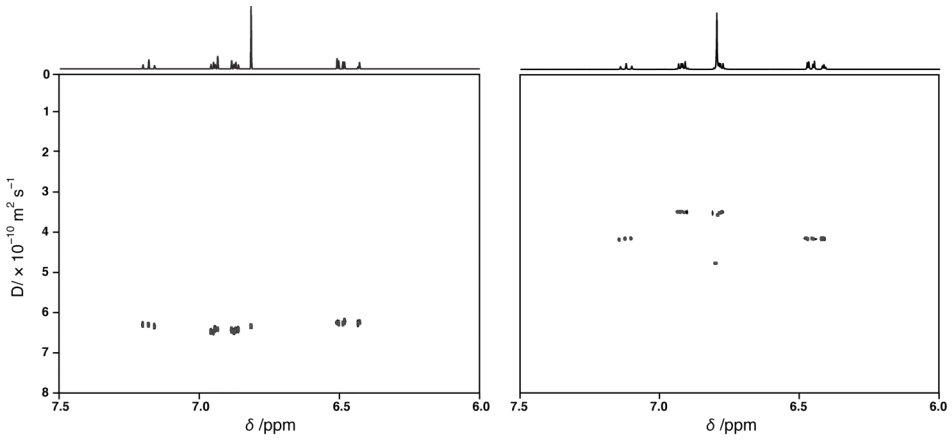
Isomer Resolution by Matrix-Assisted DOSY

Diffusion-ordered NMR spectroscopy (DOSY) is a very useful tool in the analysis of mixtures, separating the NMR signals of different components according to their diffusion coefficients, but relies on the different species having sufficiently different hydrodynamic radii. The great majority of DOSY experiments have used solutions of mixtures in simple solvents but there is considerable scope for the manipulation of relative diffusion coefficients by the use of modified solvents. One simple and effective manipulation of the solvent is to a use a surfactant co-solute above its critical micelle concentration. This allows species of a similar size, such as isomers, to be distinguished from one another by virtue of their differing degrees of interaction with the micellar structures formed.

Using a common surfactant such as SDS in aqueous solution, excellent separation and resolution of the proton spectra of isomers can be obtained. The same principle can be exploited in non-aqueous solvents by using reversed micelles, such as AOT in chloroform; good separation and resolution are again readily available, and there is added flexibility afforded by the ability to control the water content of the reversed micelles. There is a close analogy here between DOSY and chromatography. Much of the richness of chromatography as an analytical tool stems from the control allowed by varying the nature of the stationary phase. The wide variety of potential surfactants, co-solutes and micellar structures formed gives matrix-assisted DOSY a comparable flexibility.

References:

Morris, K. F., Stilbs, P. Johnson, C. S. *Anal. Chem.* **1994**, *66*, 211-215
Zielinski, M. E., Morris, K. F. *Magn. Reson. Chem.* **2009**, *47*, 53-56
Kavakka, J. S., Kilpelainen, I., Heikkinen, S. *Org. Lett.* **2009**, *11*, 1349-1352
Evans, R., Haiber, S. Nilsson, R., Morris, G. A. *Anal. Chem.* in press, DOI: 10.1021/ac9005777



(Talk and Poster 002)

Sarah Bohndiek, University of Cambridge, seb53@cam.ac.uk

Detecting tumour responses to anti-vascular therapy using hyperpolarized [1-^{13}C]pyruvate and [1,4-^{13}C]fumarate magnetic resonance spectroscopy

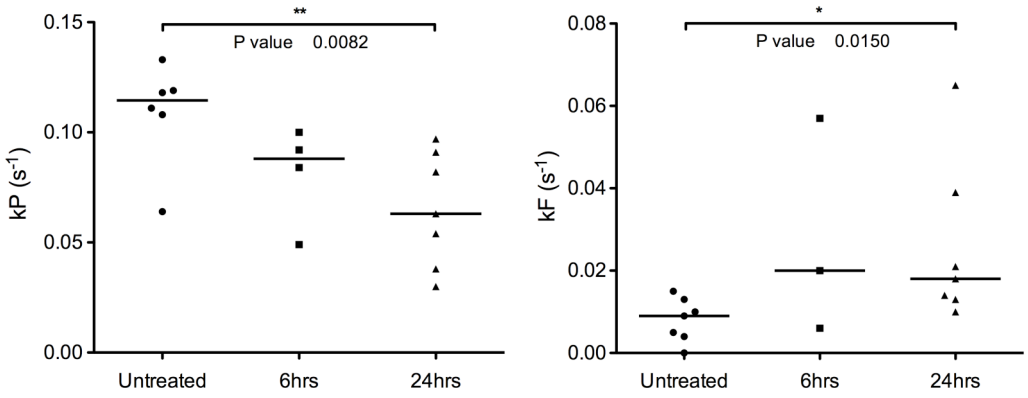
Dynamic nuclear polarization of ^{13}C labelled metabolic substrates substantially increases the sensitivity of the ^{13}C experiment by 10,000-fold or more [1], enabling in vivo magnetic resonance spectroscopic imaging of the labelled molecule and its metabolites. Hyperpolarized [1-^{13}C]pyruvate can detect early chemotherapy response in a number of murine models of cancer, including lymphoma and prostate cancer [2,3]. Recent work has also shown that [1,4-^{13}C]fumarate, a tricarboxylic acid cycle intermediate, is a positive contrast marker of tumour necrosis [4].

Combretastatin A-4 phosphate (CA-4-P) is a vascular disrupting agent (VDA) currently in Phase II/III clinical trials in the UK. While treatment response is usually measured by changes in tumour size, VDAs do not significantly affect tumour growth, instead causing collapse of tumour blood vessels. Previous work in our laboratory has shown changes in tumour metabolism detected by ^{31}P MRS can more sensitively assess response to CA-4-P treatment than DCE-MRI of the vasculature [5]. The advent of hyperpolarized MRS may make this approach feasible in the clinic.

Here, we show that hyperpolarized [1-^{13}C]pyruvate and [1,4-^{13}C]fumarate can sensitively detect response to CA-4-P treatment in a murine lymphoma model. The apparent rate constant, kP, of ^{13}C label flux between pyruvate and lactate (Figure 1) is significantly decreased 24 hours after treatment (kP=0.109±0.024s-1 to 0.065±0.026s-1, mean±s.d., $p<0.01$). Concurrently, the production of [1,4-^{13}C]malate from injected fumarate increases 3-fold ($p<0.02$) due to massive haemorrhagic necrosis, as shown by histological analysis. These results indicate that hyperpolarized ^{13}C MRS could be used as an imaging biomarker in future VDA trials.

[1] J-H Ardenkjaer-Larsen et al (2003) PNAS **100** 10158-10163
[2] S E Day, M I Kettunen et al (2007) Nat Med **13** 1382-1387
[3] M J Albers et al (2008) Cancer Res **68** 8607-8615
[4] R in ‘t Zandt et al (2009) Proc Int Soc Mag Res Med
[5] D A Beauregard et al (2001) Cancer Res **61** 6811-6815

Figure 1: Vascular disruption due to CA-4-P causes significant changes in tumour metabolic status, as detected via the apparent rate constants of both pyruvate-lactate (kP) and fumarate-malate (kF) flux *in vivo*.



(Talk and Poster 003)

Tony Cheung, University of Manchester, tony.cheung@postgrad.mancheseter.ac.uk

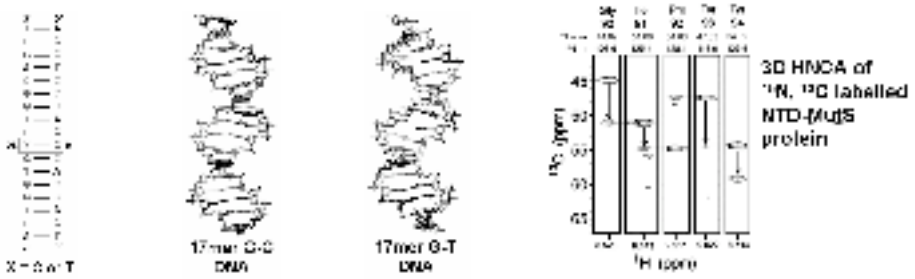
NMR investigation of mismatched DNA and its interactions with *Escherichia coli* MutS

Tony Cheung and Vasudevan Ramesh, School of Chemistry, Manchester Interdisciplinary Biocentre, The University of Manchester, 131 Princess Street, Manchester, M1 7DN, U.K.

E. coli MutS is a dimeric DNA repair protein (97 kDa, monomer) that recognises mismatches due to errors which occur during DNA replication.

The NMR structures of 17mer canonical G-C and mismatched G-T DNAs have been successfully determined and both adopt B-form type DNA conformation. Selective changes to the helical parameters at the mismatch site are induced by the G-T mispair. The NMR results highlight that mismatch stability is independent of the length, orientation and sequence context of the DNA. Furthermore, the NMR structure of a modified 6-thioguanine (6-TG) DNA is being determined to investigate its role as a potential anticancer agent.

The key N-terminal domain (NTD, 15 kDa) of E. coli MutS has been successfully expressed and isolated for the very first time. The ^{15}N–^{1}H correlated HSQC spectrum shows a stable and well-folded tertiary structure, which is not observed in the X-ray crystal structures. Structure determination of unbound NTD-MutS protein using isotope aided 3D NMR techniques, computational and biophysical methods is underway. The unbound structure is important as the conformational change upon mismatch recognition within this domain is still not known. Titration of mismatched G-T DNA with E. coli MutS protein has shown specific interaction by means of changes to chemical shifts and linewidths of mismatch base pair imino proton resonances.



(Talk and Poster 004)

Thomas Garner, University of Nottingham, pcxtpg@nottingham.ac.uk

Effects on ubiquitin binding of Paget's disease mutations in the UBA domain of P62(SQSTM1).

T. Garner, J. Long, J. Cavey, R. Layfield and M. Searle*

School of Chemistry, Centre for Biomolecular Sciences, University Park, Nottingham, NG7 2RD, UK.

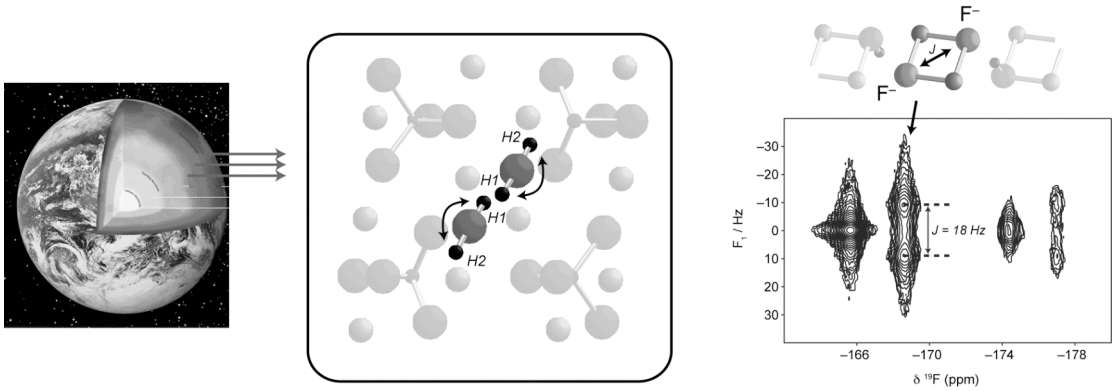
The human scaffold protein p62 is a multi-domain protein primarily associated with RANK ligand induced Nuclear Factor Kappa B (NF-κB) activation. The p62 protein plays a major role in cellular proliferation and has been implicated in bone, muscle and T-cell differentiation.
Mutations in the C-terminal 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. However, studies on the isolated domain reveal that several mutations have no clear effect on ubiquitin recognition. This has led to a detailed investigation of ubiquitin recognition and how mutations in the UBA domain can affect this process.
Previous studies have shown the UBA domain undergoes a conformational change prior to ubiquitin binding. It is therefore possible that PDB mutations could effect not only the direct interaction with ubiquitin but also the equilibrium between the ground state and its ubiquitin binding state. We present here preliminary studies of ubiquitin binding by PDB mutants of the isolated UBA domain. We have focused on four PDB mutants: the most common disease mutant (P392L); a stabilising, very low affinity mutant (G425R); and two destabilising mutants with little clear effect on ubiquitin binding (S399P and G411S).

(Talk and Poster 005)

John Griffin, University of St. Andrews, jmg21@st-andrews.ac.uk

Understanding Water Storage Inside the Earth: Disorder and Dynamics in Hydrous Magnesium Silicates Probed by DFT Calculations and Solid-State NMR

For a number of years it has been hypothesised that the Earth's mantle may contain a vast amount of water. Indeed, it is thought the total amount may be equivalent to or greater than that present on the Earth's surface in the oceans and the atmosphere. However, the exact mechanism by which water is stored is not understood. It is currently thought that defect sites within the nominally anhydrous silicates that make up the Earth's mantle may hold sufficient amounts of structurally-bound hydrogen to account for the inner-Earth water budget. We present a combined experimental and computational solid-state NMR study of a group of hydrous magnesium silicates that provide models for water incorporation within forsterite (α-Mg_2SiO_4) which exists in the Earth's mantle to a depth of 410 km. DFT calculations using CASTEP show that experimental ^{17}O high-resolution two-dimensional NMR spectra can be explained by a structural model that involves dynamic exchange of hydroxyl protons between two sites. This contrasts previous diffraction studies on the same materials, which only identified disordered static occupancy of the two sites. The effect of fluorine substitution into the crystal structure is also investigated. ^{2}H magic-angle spinning (MAS) NMR reveals that this restricts hydroxyl-group dynamics, resulting in only a single hydroxyl proton site. ^{19}F MAS NMR indicates the existence of rarely observed 'through-space' J-couplings between F– sites, which allow the observation of two-dimensional correlation spectra



(Talk and Poster 006)

Tim Knowles, University of Birmingham, t.j.knowles@bham.ac.uk

Structural studies of the β-barrel assembly machinery, the key complex in outer membrane protein assembly

The folding of transmembrane proteins into the outer membrane presents formidable challenges to bacteria, chloroplasts and mitochondria. The long, unfolded polypeptides of outer membrane proteins must navigate the cytoplasm, inner membrane and periplasmic space. The Omp85 complex must then recognize them and initiate the delicate task of sewing up their β-barrel folds in to the membrane. Five proteins are currently known to form this complex, two of which are critical for the survival of all Gram-negative bacteria. Using NMR we have determined the structures of two of these components. Here we discuss the implications of these structures with regards to the mechanisms that coordinate membrane protein trafficking in Gram-negative bacteria.

(Talk and Poster 007)

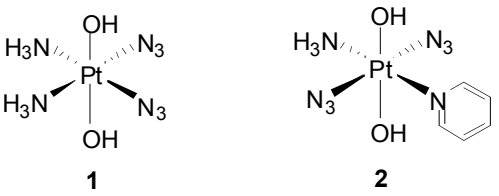
Nicky Farrer, University of Warwick, n.farrer@warwick.ac.uk

Photoactivated PtIV-azido anticancer complexes

Low-spin octahedral Pt^{IV} complexes are well-known for their kinetic inertness. Therefore Pt^{IV}-based complexes exhibit several advantages over their Pt^{II} analogues (such as the mainstream treatment cisplatin) as anticancer drugs. Pt^{IV} complexes are prodrugs; ultimately reduction is required for reactivity in vivo. Several non-toxic, photolabile Pt^{IV}-azido complexes have demonstrated potent cytotoxicity following light-induced reduction to Pt^{II} species, with little or no toxicity in the absence of irradiation.

The photodecomposition of cis-trans-cis-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ has been investigated previously in some detail and in addition to the generation of Pt^{II} species, under aqueous conditions generates N_2[1a] whereas in buffer the formation of O_2 is detected[1b] Here we present investigations into the photoactivity of the more recently reported trans-trans-trans-$[Pt(N_3)_2(OH)_2(py)(NH_3)]$[2]. We have used multinuclear NMR methods, including ^{14}N NMR spectroscopy, to track photoreaction pathways involving the azido ligands. ^{15}N labelling has been used to aid d signal assignment.

1) a) L. Ronconi and P. J. Sadler, Chem. Commun., 2008, 2, 235, b) H. I. A. Phillips, L. Ronconi, and P. J. Sadler, Chem. Eur. J., 2009, 15, 1588.
2) F. S. Mackay, J. A. Woods, P. Heringová, J. Kašparková, A. M. Pizzaro, S. A. Moggach, S. Parsons, V. Brabec and P. J. Sadler, Proc. Natl. Acad. Sci., 2007, 104, 20748



Structures of *cis-trans-cis*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ (**1**) and *trans-trans-trans*-$[Pt(N_3)_2(OH)_2(py)(NH_3)]$ (**2**)

(Talk and Poster 008)

Refaat Hamed, University of Oxford, refaat.hamed@chem.ox.ac.uk

Monitoring the activities of the crotonase superfamily enzymes by ^{1}H-NMR in real time

Refaat Hamed, Edward T. Batchelar, Timothy D. W. Claridge and Christopher J. Schofield
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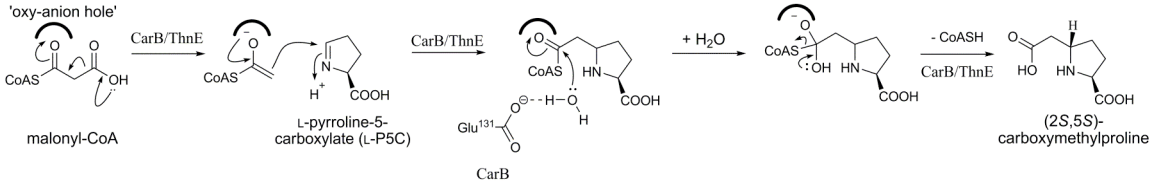
Carboxymethylproline synthases (CarB and ThnE) are unique members of the crotonase enzyme superfamily. CarB and ThnE are involved in the early stages of carbapenem antibiotic biosynthesis, compounds of significant biomedical interest. CarB/ThnE catalyse the formation of *t*-carboxymethylproline (*t*-CMP) from malonyl-coenzymeA and glutamate semialdehyde. The mechanism of catalysis is understood to proceed via decarboxylation of malonyl-coenzyme A, C-C bond formation between the formed enolate and pyroline-5-carboxylate, and hydrolysis of the resultant thioester into *t*-CMP and CoASH. Real time *in-situ* ^{1}H-NMR monitoring of the CarB/ThnE catalysed reactions is an efficient method to follow the kinetics of various transformations and allows one to calculate specific enzyme activities. Significantly, it provides direct simultaneous information on the processing of substrate, co-factors and products, providing considerable advantages over other biophysical methods that are often able to monitor only a subset of the total reaction components (either directly or indirectly). The same method has been used to follow the reactions catalysed by other crotonases in real time.



(Talk and Poster 009)

Adolfo Botana, University of Manchester,
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Suppression of J-modulation in DOSY experiments

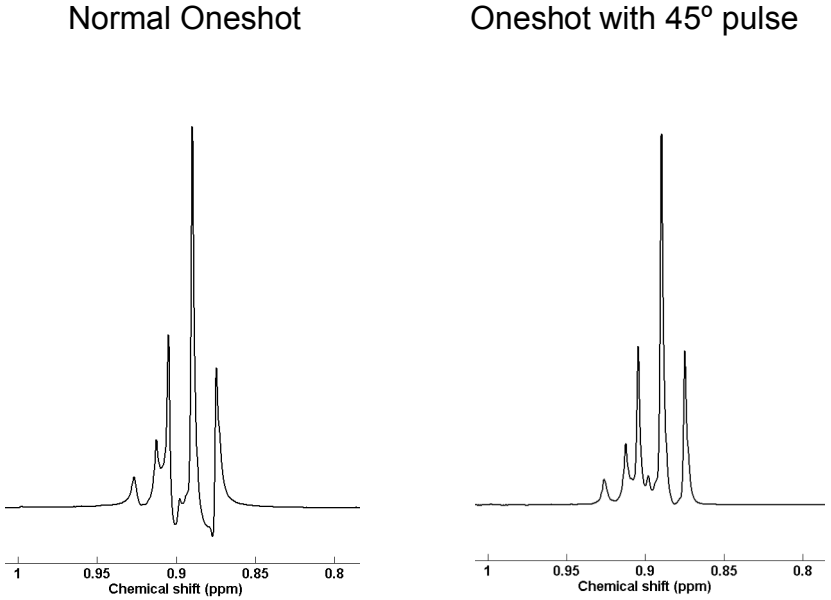
DOSY experiments are very demanding of spectral quality, even very small phase errors in signals causing visible distortions in the diffusion domain. All the basic echo experiments used for diffusion measurement show multiplet phase distortions caused by J-modulation, requiring a trade-off between phase errors and gradient pulse width. Two methods are commonly used to suppress its effects, a purging spin-lock pulse[1,2] and a z-filter[3] (also known as a Longitudinal Echo Delay). Neither is entirely satisfactory: spin locking causes sample heating and LED requires extensive phase cycling, and both lose sensitivity and mask warning signs of instrumental problems such as convection. We propose a simple and effective solution to remove the unwanted anti-phase terms that avoids all these disadvantages, adding a 45º purging pulse immediately before the onset of acquisition. The new method is illustrated for a widely-used general purpose DOSY technique, the Oneshot sequence[4].

1. Pelta, M. D., Barjat, H., Morris, G. A., Davis, A. L., Hammond, S. J. Pulse sequences for high-resolution diffusion-ordered spectroscopy (HR-DOSY). *Magn. Reson. Chem.* **1998**, 36 (10), 706-714.

2. Torres, A. M., Cruz, R. D., Price, W. S. Removal of J-coupling peak distortion in PGSE experiments. *J. Magn. Reson.* **2008**, 193 (2), 311-316.

3. Gibbs, S. J., Johnson, C. S. A PFG NMR experiment for accurate diffusion and flow studies in the presence of eddy currents. *J. Magn. Reson.* **1991**, 93 (2), 395-402.

4. Pelta, M. D., Morris, G. A., Stchedroff, M. J., Hammond, S. J. A one-shot sequence for high-resolution diffusion-ordered spectroscopy. *Magn. Reson. Chem.* **2002**, S147-S152.



Echo experiments used for diffusion measurement show multiplet phase distortions caused by J-modulation. The addition of a 45º purging pulse immediately before the onset of acquisition removes the anti-phase terms, producing spectra without these distortions.

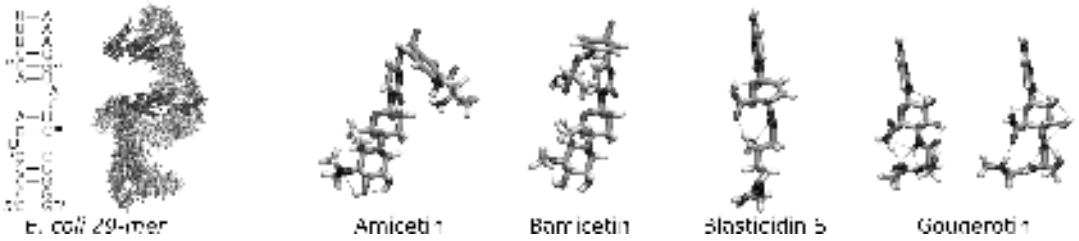
(Talk and Poster 010)

John King, University of Manchester, john.king.jpk@googlemail.com

NMR studies of the binding of peptidyl transferase inhibitor antibiotics to conserved secondary structural motifs of 23S ribosomal RNAs.

John King, Misbah Nareen and Vasudevan Ramesh, School of Chemistry, Manchester Interdisciplinary Biocentre, The University of Manchester, 131 Princess Street, Manchester, M1 7DN, U.K.

RNA binding peptidyl transferase (PT) antibiotics play a fundamental role in the inhibition of protein synthesis and their mode of action is not well understood. We have successfully determined the NMR structures of the PT inhibitor antibiotics Amicetin, Bamicetin, Blasticidin S and Gougerotin in native solution state. The structures all exhibit a stable conformation, stabilised by a tight network of intramolecular hydrogen bonds. Also, all the structures show a similar conformation in the analogous regions of their chemical structure. Three highly conserved and homologous ribosomal RNA motifs (27-37mers) of H. halobium, E. coli and B. subtilis 23S RNA have been chosen to investigate their interaction with Amicetin. The NMR structure of the E. coli 29-mer motif has been determined and it adopts a well folded A-RNA conformation. It however differs from the X-ray structure as the highly conserved adenine residue, which resides in a bulge strongly implicated with Amicetin binding, folds into the helix as opposed to being flipped out. Furthermore, the NMR structure calculation of a uniformly ^{13}C, ^{15}N labelled H. halobium 37-mer RNA sample is nearing completion. This will be followed by the structure determination of its complex with Amicetin.



Abstracts of Posters

Poster 011

Ross Lennen, Schering Plough Research, ross.lennen@spcorp.com

Applications of a 5mm DCH 400 MHz NMR CryoProbe™ at Schering-Plough Research Institute, Newhouse, UK

R. J. Lennen and L. Fielding

Schering-Plough Research Institute, Newhouse, Lanarkshire, Scotland, UK, ML1 5SH.

Cryogenically cooled NMR probes for high resolution NMR spectroscopy, where electrical thermal noise is reduced in the signal detection pathway by lowering the coil and preamplifier temperature in the probe circuitry, have been available commercially since 1999 for higher field NMR spectrometers. In 2004 Bruker BioSpin launched their first ever DCH CryoProbe™ for 400 MHz NMR spectrometers. In 2007 this accessory was purchased and coupled to our existing Bruker DRX 400 MHz NMR spectrometer at Schering-Plough Research Institute, Newhouse. The substantial sensitivity gains this investment has afforded will be presented together with the benefits this has brought to our small molecule structural analysis.

Poster 012

Moustafa Ibrahim, Sheffield University, mostafa.omary@sheffield.ac.uk

Study the Effect of Substrate Binding on the Backbone Dynamics of PGK using NMR Relaxation Methods

The backbone dynamics of 15N labelled of W290Y mutant of Geobacillus of PGK samples has been studied using NMR relaxation methods. The relaxation rate constants, R1 and R2, were obtained by performing a series of different experiments at different delay times. All experiments have been recorded at frequencies of 500 MHz and 600 MHz at a temperature of 310 K. The changes in the backbone dynamics of the apo and upon binding 3-PGA to the N-domain and in the presence of the two substrates, ADP and 3-PGA, in the transition state analogue (TSA) have been monitored. R2 data provide evidence of significant changes in the backbone dynamics of the protein around residue E-174 upon binding the 3-PGA substrate. Residue E-174 is part of the hinge region, an alpha helix which connects the two domains together, also behaves significantly differently from the rest of the protein residues. This indicates that something happens in the hinge as the protein switches between its open and closed conformations to make the catalytic site. The experimental R2 data provide motion in the millisecond time scale which is of similar magnitude to the enzyme catalytic rate constant(~50msec). This result will help in understanding the contributions backbone dynamics might have to the enzyme catalytic processes.

Poster 013

John Edwards, University of Nottingham, pcxtpg@nottingham.ac.uk

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 15N 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 014

Marie-Virginie Salvia, University of Strathclyde, salvia@cpe.fr

A Sticky Problem: Harnessing self-assembly for DNA molecular recognition

Marie-Virginie SALVIA[1], Daria D. ANDREJUK[2], Hasan ALNIS[3], Simon P. MACKAY[3], Colin J. SUCKLING[1], Abedawn I. KHALAF[1], Maxim P. EVSTIGNEEV[2] & John A. PARKINSON[1*]

[1]WestCHEM Department of Pure and Applied Chemistry, University of Strathclyde, 295 Cathedral Street Glasgow G1 1XL, U.K.; [2]Department of Physics, Sevastopol National Technical University, Sevastopol 99053, Crimea, Ukraine; [3]Strathclyde Institute for Pharmaceutical and Biomedical Sciences, 27 Taylor Street, Glasgow, G4 0NR, U.K.

More than two decades of work has substantially progressed an understanding of factors responsible for DNA sequence recognition by minor groove binding small molecules (MGBs). Nevertheless, many facets of this process still remain a mystery, including those features that control MGB self-assembly and the role this plays in sequence reading, binding energetics and complex stability. Head-to-tail, side-by-side binding of MGBs to self-complementary DNA sequences is a headline mode of recognition for these molecules. By harnessing and tailoring the self-assembly process, can sequence recognition be directed and is there scope for developing exclusively hetero-assembled systems that would enable non self-complementary sequence recognition to occur? Could this lead to new molecular diagnostic or therapeutic applications? The starting point for such a study lies in understanding the self-assembly character of the MGBs themselves. Here we report association and thermodynamic results from NMR and ITC studies for an MGB molecule selected from among a class of DNA minor groove binders developed and studied at Strathclyde.

Poster 015

Maryam Khajeh, University of Manchester,
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Diffusion NMR and trilinear analysis in the study of reaction kinetics

Time-resolved NMR spectroscopy can in principle allow every species involved in a chemical reaction to be monitored simultaneously, providing both real-time quantitation and information on chemical structure. However, it is not always simple to interpret the data obtained, because where signals overlap it is difficult to distinguish between the signals of different components. In principle, statistical methods such as PCA can be applied to separate the components. Unfortunately, this typically results in rotational ambiguity, where a wide range of candidate component spectra fit the experimental data equally well. Multi-linear analysis (where the data vary independently in more than 2 dimensions) offers a way around this problem, allowing experimental data to be decomposed into physically realistic component spectra where such data can be obtained experimentally.

One way to obtain trilinear NMR data for the course of a chemical reaction is to acquire successive DOSY [1] (Diffusion-Ordered SpectroscopY) datasets, in which pulsed field gradients are used to attenuate the signals of different species according to their diffusion coefficients, during the reaction. After Fourier transformation, each individual DOSY dataset records how the NMR spectrum varies with pulsed field gradient strength at a given time. Provided that each species has a different diffusion coefficient and a different timecourse, the dataset is trilinear and can be decomposed using the PARAFAC[2] (PARAllel FACtor analysis) algorithm to yield the spectrum, concentration time course, and diffusional attenuation for each component of the reaction separately[3].

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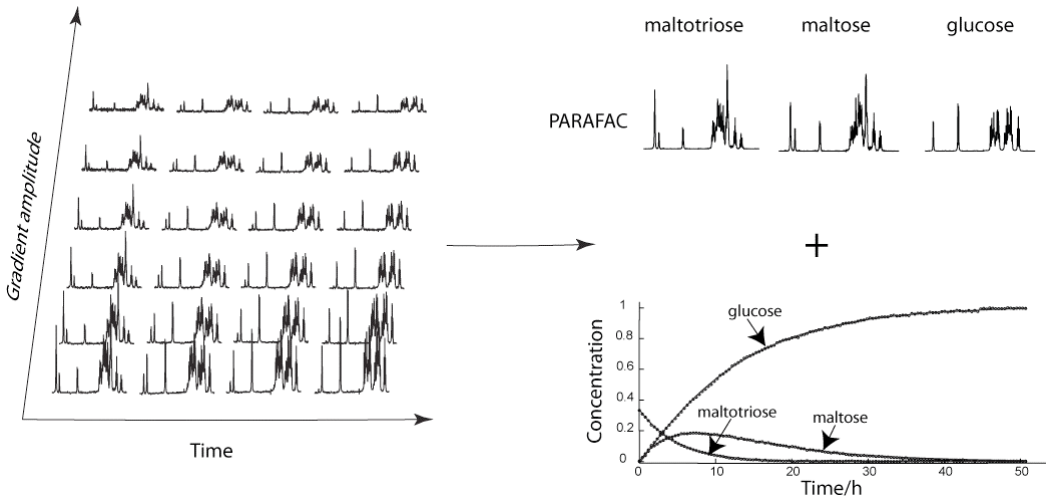

Poster 016

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Solvent relaxation as a tool to screen small molecule-metalloprotein interactions

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NMR is a valuable tool for the study of protein-ligand interactions. Most methods have limited applicability as primary screening tools but are more useful when, for example, structural information is needed. For instance STD-NMR requires a large excess of ligand molecules.[1] Transferred NOE requires a delicate ligand-protein ratio, and is inapplicable in systems such as peptide-protein interactions.[2] On the other hand, chemical shift perturbation using ^{1}H-^{15}N HSQC requires access to ^{15}N labelled protein, and is fairly difficult to apply in systems larger than 25 kDa.[3]

Water molecules are always present inside and outside the active sites of proteins. In the presence of a paramagnetic metalloprotein, the relaxation rates of water molecules are enhanced and such effects are measurable by NMR.[4] Changes in relaxation rates upon ligand binding at the paramagnetic centre are therefore indicative of these binding events. Using the catalytic domain of human Prolyl Hydroxylase Domain 2 (PHD2181-426), which is a Fe^{2+}/2-oxoglutarate (2OG) dependent enzyme involved in body's response to oxygen depletion, we describe the feasibility to screen binding activities that would be otherwise challenging to detect using other NMR techniques. PHD2 forms a ternary complex with 2OG and the 19-mer substrate peptides CODD and NODD (these peptides are fragments from its natural substrate, the α-subunit of hypoxia inducible transcription factor; HIF-α). A set of inhibitors and PHD2 mutants have also been studied. Our results are consistent with previous biophysical and inhibition studies proving the applicability of this technique for ligand screening at relatively high magnetic fields.[5-7]

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Poster 017

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Initial Characterisation of OBP3 by 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 (Figure1). Work has been carried out showing that each of the three subtypes interact with distinct chemical1 classesSuch specificity may suggest a more active role, rather than the 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 musus 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 information and an understanding of the binding properties of OBPs, the nature of specificity between the three subtypes and the relevance this may have.

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