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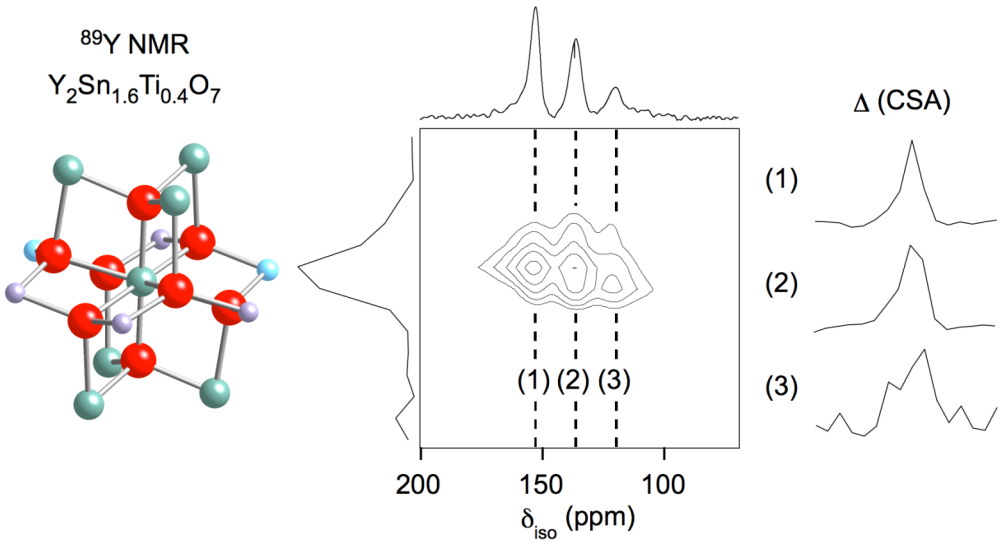
Royal Society of Chemistry

NMR Discussion Group



Postgraduate Meeting 2013

School of Chemistry
University of Edinburgh
20th June 2013

Dear Delegate,

Welcome to the sixth Postgraduate Symposium of the RSC NMR Discussion Group! This meeting has a broadly similar format to the previous meetings and 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. The idea is that it provides a forum to showcase their work and networking opportunities within the NMR community.

As in previous years, we continue to have two overview lectures, given by leaders in their field, to highlight the power of magnetic resonance methods across a broad range of topics. This year Dr Sharon Ashbrook (University of St Andrews) will discuss the combined power of solid-state NMR and first principles calculations while Prof. Paul Barlow (University of Edinburgh) will present an introduction to the use of NMR in structural biology.

The programme is designed to be varied, with a wide range of talks covering the wealth of topics in our field. Please take the time to visit the posters which allow delegates to discuss their work with other early career researchers and more established colleagues in a friendly and informal setting. 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
University of Sussex

Yaroslav Khimyak
University of East Anglia

Meeting Organisers

Dusan Uhrin
University of Edinburgh

Juraj Bella
University of Edinburgh

Local Organisers

James Keeler
University of Cambridge

NMRDG Chairman

Local organisation and acknowledgements

Meeting coordinated by:	Iain Day, University of Sussex
	Yaroslav Khimyak, University of East Anglia
Local organisation coordinated by:	Dusan Uhrin, University of Edinburgh
	Juraj Bella, University of Edinburgh
Online registration coordinated by:	John Parkinson, University of Strathclyde

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

Thanks go to Dusan Uhrin (University of Edinburgh), Yaroslav Khimyak (University of East Anglia) 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:

Goss Scientific Instruments

Dalton Transactions

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, Iain Day, NMRDG

Oral Presentation Session 1, session chair: Tim Claridge

1030 – 1110 **Sharon Ashbrook**, University of St Andrews
Structure, Disorder and Dynamics in Solids: Multinuclear NMR and First-Principles Calculations

1110 – 1130 **Alexander Forse**, University of Cambridge
NMR Studies of Ion Adsorption on Porous Supercapacitor Electrode Materials

1130 – 1150 **Matthew Renshaw**, University of Cambridge
Operando MR: Fixed-bed heterogeneous catalysis at elevated temperature and pressure

1150 – 1210 **Nicholle Bell**, University of Edinburgh
"Separation" by NMR: 3D and 4D NMR experiments for complex mixtures

1210 – 1230 **Jonathan Katz**, University of Sussex
Exploring Small Molecule Aggregation Phenomena using NMR Spectroscopy and Small Molecule Probes

1230 – 1250 **Nicole Fauré**, University of Glasgow
Solid-State NMR Studies of an Immobilised Enzyme

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: John Parkinson

1430 – 1510 **Paul Barlow**, University of Edinburgh
An Introduction to Structural Biology

1510 – 1530 **Marcin Skotnicki**, University of Durham
Characterisation of two amorphous forms of Valsartan by multi-technique solid-state NMR

1530 – 1550 **Henri Colaux**, University of St Andrews
Novel Conversion Pulse Schemes for MQMAS experiments

1550 – 1610 **Richard Hopkinson**, University of Oxford
Using NMR to Study Histone Demethylase Catalysis

Close

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

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Abstracts of Talks

(Talk 001)

Sharon Ashbrook, University of St Andrews, sema@st-andrews.ac.uk

Structure, Disorder and Dynamics in Solids: Multinuclear NMR and First-Principles Calculations

Sharon Ashbrook

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NMR spectroscopy provides an element-specific, sensitive probe of the local environment, enabling detailed information to be extracted. However, in the solid state the vast majority of this information remains unexploited, owing to the challenges associated with obtaining high-resolution spectra and the ease with which these can be interpreted. For inorganic solids this problem is amplified by the large range of nuclides studied, the lack of prior information in the literature and the practical challenges of experimental implementation for species with long relaxation times, low sensitivity and large quadrupolar broadening.

Recent advances enabling accurate and efficient calculation of NMR parameters in periodic systems have revolutionized the application of such approaches in solid-state NMR spectroscopy, particularly among experimentalists. The use of first-principles calculations aids in the interpretation and assignment of the complex spectral lineshapes observed for solids. Furthermore, for materials with poorly characterized structures calculations provide a method for evaluating potential structural models against experimental measurements. As NMR is sensitive to the atomic-scale environment, it provides a potentially useful tool for studying disordered materials, and the combination of experiment with first-principles calculations offers a particularly attractive approach. After introducing the experimental methods used in solid-state NMR, I will then discuss some of the issues associated with the practical implementation of first-principles calculations of NMR parameters in the solid state. I will then illustrate the insight into local structure and disorder in inorganic materials that can be obtained when computational and experimental approaches are combined, using a case study investigating ceramic materials proposed for the encapsulation of radioactive waste.

(Talk and Poster 002)

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NMR Studies of Ion Adsorption on Porous Supercapacitor Electrode Materials

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Supercapacitors are high power energy storage devices with essentially unlimited cycle lives.[1] Porous carbons are popular supercapacitor electrode materials as they have high surface areas for charge storage by ion adsorption. In particular, titanium carbide-derived carbon (TiC-CDC) has recently attracted great attention as it has a pore size that is tuneable on the nanoscale.[2] Control of the carbon pore size has shown that the highest capacitances are achieved at subnanometre pore sizes.[3] In spite of recent advances, the charge storage mechanism in porous carbons is not well understood.

Nuclear magnetic resonance (NMR) spectroscopy has been used to study the structure of the electrode-electrolyte interface in TiC-CDC.[4] NMR experiments on carbons soaked with different volumes of NEt_4BF_4/acetonitrile electrolyte reveal two main ion environments. In-pore ions are adsorbed to the carbon inside the pores, whilst ex-pore ions (observed at higher frequencies) are not adsorbed and are in large reservoirs of electrolyte between carbon particles. For carbons with smaller pore sizes, fewer adsorbed ions are observed. A ^{13}C - ^{1}H inverse cross polarisation experiment enables magnetisation transfer from the carbon architecture to the adsorbed species, allowing their selective observation (Fig. 1). To study working supercapacitor devices, in situ NMR methods have been developed, where NMR spectra are acquired during charge and discharge. Shifts of the in-pore resonance are observed as the applied potential is varied, along with changes in intensity of peaks from the different environments. The effect of pore size on this behavior is currently being investigated.

References:
1. P. Simon and Y. Gogotsi, Nat. Mater., 2008, 7, 845–854.
2. V. Presser, M. Heon, and Y. Gogotsi, Adv. Funct. Mater., 2011, 21, 810–833.
3. J. Chmiola, G. Yushin, Y. Gogotsi, C. Portet, P. Simon, and P. L. Taberna, Science, 2006, 313, 1760–1763.

4. A. C. Forse, J. M. Griffin, H. Wang, N. M. Trease, V. Presser, Y. Gogotsi, P. Simon, and C. P. Grey, Phys. Chem. Chem. Phys., 2013, 10.1039/c3cp51210j.

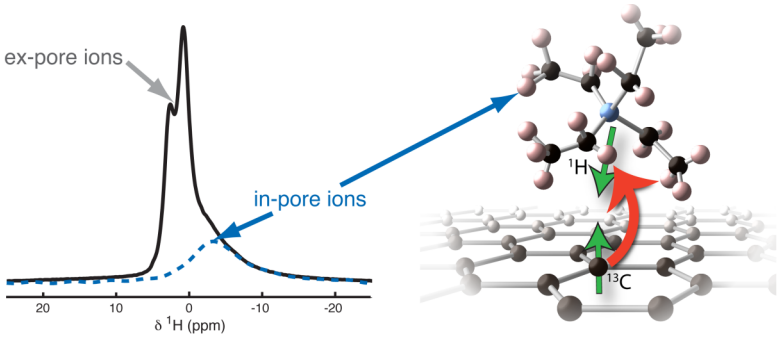

Fig. 1
Left panel; direct (black) and ^{13}C - ^{1}H inverse cross polarisation (blue, dashed) spectra of a porous carbon soaked with electrolyte. Right panel: Schematic illustrating transfer in the inverse cross polarisation experiment.

(Talk and Poster 003)

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Operando MR: Fixed-bed heterogeneous catalysis at elevated temperature and pressure

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The ability to perform operando studies of fixed-bed heterogeneous catalytic systems has long been a goal of catalysis research; Magnetic Resonance (MR) methods, being non-invasive and non-destructive, are ideal for this purpose [1]. However, until recently, the processes studied using MR have been performed under mild conditions (typically < 5 atm and < 200 °C). We have recently commissioned a fixed-bed reactor, compatible with operation inside a superconducting magnet, which can be operated up to a temperature of 350 °C and a pressure of 31 atm while simultaneously performing MR experiments. To demonstrate its capability, two different systems have been studied.

First, the effect of confinement in mesopores on the vapour-liquid phase change of cyclohexane to elucidate pore filling and emptying mechanisms. The understanding of confinement effects, particularly at realistic reaction conditions, is an important consideration in heterogeneous catalysis. Through ^{1}H spin density images, the isothermal (188 °C) vapour-liquid phase changes of cyclohexane in a bed of titania pellets was studied across the phase boundary revealing pores with liquid remaining after bulk liquid has vaporised. The data are analogous to N_2 adsorption isotherms that characterise pore diameter and surface area, but are conducted for a relevant species at realistic conditions.

Second, the multi-phase ethene oligomerisation reaction, conducted at 110 °C and 200 °C and at a pressure of 29 atm, was investigated. Ethene oligomerisation produces predominantly primary alkenes in the C_4-C_{20+} range which can then be used in the production of detergents, plasticizers and low-density polyethene [2] and has been proposed as a step in the formation of liquid fuels from natural gas via the oxidative coupling of methane [3]. The relationships between operating temperature, phase distribution and catalyst performance have been explored using MR techniques.

[1] L. F. Gladden et al., Adv. Catal. 50 (2006) 1.
[2] D. Vogt, Applied Homogeneous Catalysis with Organometallic Compounds, B. Cornils, W. A. Herrmann (Eds) VCH, New York (1996) 245.
[3] C. Marcilly, J. Catal. 216 (2003) 47.

(Talk and Poster 004)

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"Separation" by NMR: 3D and 4D NMR experiments for complex mixtures

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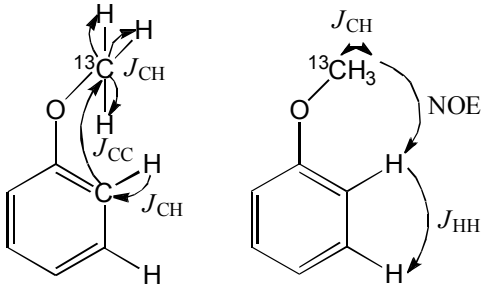
One of the frontiers of NMR spectroscopy is the structure determination of small molecules contained within complex mixtures. Examples of such mixtures include plant extracts, raw and processed food, environmental mixtures such as humic substances, or chemically/enzymatically degraded biomatter such as lignin.

These mixtures are composed of carbon- and oxygen-rich compounds containing numerous OH groups (aliphatic, phenolic, and carboxylic). Of particular interest are aromatic compounds classified as phenolics or polyphenols. By methylating their OH moieties with ^{13}C labelled methyl groups, we introduce an NMR active nucleus, which allows us to filter out a vast majority of resonances and to detect signals only from the immediate neighbourhood of the $^{13}CH_3O$- groups. By comparing the obtained ^{1}H and ^{13}C chemical shifts with database information we can suggest structural fragments present in these compounds.

Towards this end we have developed novel 3D and 4D NMR experiments that use proton-carbon, carbon-carbon and proton-proton couplings or NOEs to transfer the magnetisation between aromatic protons and $^{13}CH_3O$- groups as illustrated below.



We have utilised these polarisation transfer pathways to propose several novel NMR experiments such as:

3D $HcCH_3$, 3D $HCcH_3$ and 4D $HCCH_3$
3D INADEQUATE-HSQC
3D HMQC-HMBC
4D HMQC-NOESY-TOCSY

We illustrate the use of these experiments on a complex model mixture of phenolics.

(Talk and Poster 005)

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Exploring Small Molecule Aggregation Phenomena using NMR Spectroscopy and Small Molecule Probes

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Driven by non-covalent π -π interactions, planar aromatic molecules such as dyes,[1] pigments[2] and drug compounds[3] are known to spontaneously self-aggregate. Moreover, as these associations underpin phenomena such as DNA base-pair stacking,[4] protein folding that leads to various neurodegenerative disorders,[5] and metal-organic frameworks responsible for hydrogen storage,[6] it is crucial to understand their interaction with other small molecules.

The aggregation process has been shown to play a critical role in various solute and solvent properties. Not only will increasingly larger assemblages result in lower diffusion coefficients[7] and increased viscosity,[1] but the unique magnetic environments within each layer will be altered by an enhancement of the ring shielding effect in neighbouring molecules.[8]

Nuclear Magnetic Resonance can be employed as an effective tool in such an investigation. Using a pulsed field gradient echo experiment to monitor diffusion coefficients or tracking concentration dependent changes in chemical shifts, it becomes possible to quantify the size and degree of aggregation. Here we further aim to apply the already accepted NMR toolbox in a new way. By introducing a small reporter probe molecule, Fluorophenol (1 mol%), into a range of samples with varying amounts of the self-aggregating dye Sunset Yellow FCF, we will show that it becomes possible to interpret their association in terms of simple diffusion and thermodynamic models.

References

[1] M. P. Renshaw and I. J. Day, The Journal of Physical Chemistry B, 2010, 114, 10032–10038.
[2] D. Hazafy, M.-V. Salvia, A. Mills, M. G. Hutchings, M. P. Evstigneev and J. A. Parkinson, Dyes and Pigments, 2011, 88, 315–325.
[3] D. B. Davies, L. N. Djimant and A. N. Veselkovb, Journal of Chemical Society, Faraday Transactions, 1996, 92, 383–390.
[4] F. Crick and J. Watson, Nature, 1953, 171, 737—-738.
[5] F. Chiti and C. M. Dobson, Annual Review of Biochemistry, 2006, 75, 333–66.
[6] T. Cook, Y. Zheng and S. P, Chemical Reviews, 2013, 113, 734–777.
[7] C. J. Johnson, Progress in Nuclear Magnetic Resonance Spectroscopy, 1999, 34, 203–256.
[8] A. Veselkov, A. Lantushenko, D. Veselkov and D. Davies, Journal of Structural Chemistry, 2002, 43, 234–241.

(Talk and Poster 006)

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Solid-State NMR Studies of an Immobilised Enzyme

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Enzymes, when used as industrial biocatalysts, possess the astonishing virtue of leading to product formation at mild and environmentally friendly conditions with a high specificity. A core technology is the immobilisation of enzymes – the conversion of the soluble protein molecules into a solid particle form that can be easily separated from the reaction mixture. Since the advent of immobilisation of single enzymes in the 1940s, numerous methods have been developed. Despite extensive study on different systems, there is no clear approach for a given process and enzyme. One reason for this is that little is known about the state of the protein molecules in the preparation except what is deduced from the catalytic activity.

With this in mind and aiming towards a better understanding of immobilised enzymes, this contribution describes a comprehensive study of the covalent immobilisation of α-chymotrypsin on functionalised silica particles (glycidoxypropyltrimethoxysilane, GOPS, grafted onto the surface of silica gel). Using one- and two-dimensional ^{13}C, ^{29}Si and ^{1}H NMR techniques, we have been able to characterise this bio-functionalised heterogeneous enzymatic and inorganic support system, demonstrating the power of multinuclear solid-state NMR to provide a better understanding of immobilised enzymes at the molecular level.

(Talk 007)

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An Introduction to Structural Biology

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(Talk and Poster 008)

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Characterisation of two amorphous forms of Valsartan by multi-technique solid-state NMR

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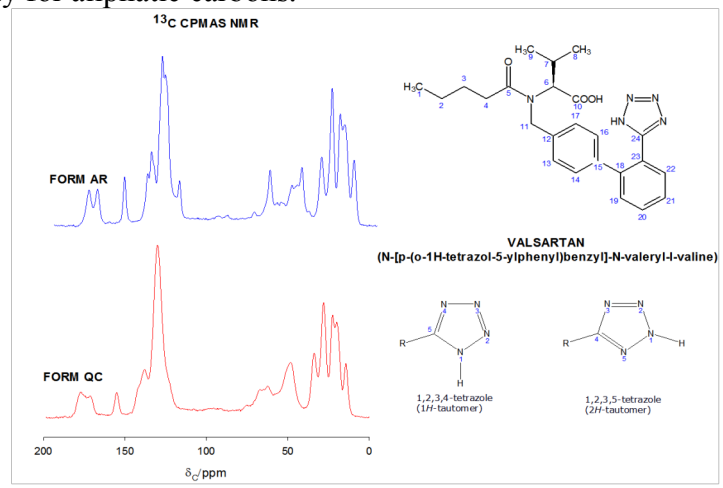
Amorphous active pharmaceutical ingredients (APIs) have been used in the development of pharmaceutical solid formulations due to their advantages over crystalline forms such as increased solubility and dissolution rate. Differences in amorphous forms regarding stability and physicochemical properties have been reported in the literature (1). Valsartan, a tetrazole-containing molecule, angiotensin II type 1 (AT_1) receptor antagonist used in the management of hypertension, has been found to exist in two different amorphous forms: Form AR (as-received sample) and QC (quench-cooled sample). Both forms are stable and do not recrystalise in the analysed range of temperatures. Samples were characterised by differential scanning calorimetry (DSC) and variable-temperature ^{1}H, ^{13}C and ^{15}N solid-state NMR. Different spectral features between two forms were observed, indicating high sensitivity of solid-state NMR to the variation in local environments and short range orders. Form AR has been found to exist in higher order of structure arrangement than Form QC. ^{15}N inversion-recovery CPMAS experiments suggest that Form AR and QC exist as a mixture of two tautomeric forms, with the 1,2,3,4-tautomer predominating. There is more 1,2,3,5-tetrazole tautomer in the quench-cooled sample. The physicochemical properties of solid API are strongly related not only to its structural features but also to its molecular dynamics. Proton and carbon NMR relaxation times (T_1 and $T_{1\rho}$) measurements have provided valuable information about the molecular motion in the two forms, suggesting a slightly higher overall molecular mobility of Form QC especially of phenyl and tetrazole rings than for Form AR and similar mobility for aliphatic carbons.



1. M. Savolainen, A. Heinz, C. Strachan, K.C. Gordon, J. Yliruusi, T. Rades, and N. Sandler. Screening for differences in the amorphous state of indomethacin using multivariate visualization. Eur. J. Pharm. Sci. 30:113-123 (2007).

(Talk and Poster 009)

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Novel Conversion Pulse Schemes for MQMAS experiments

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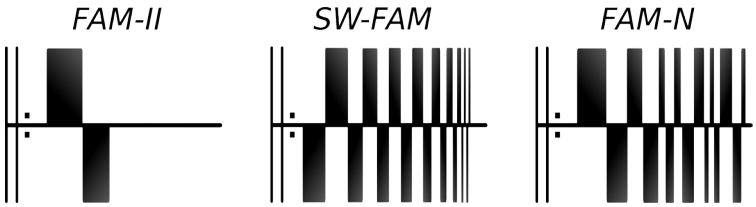

Figure: Comparison between FAM-II, SW-FAM [1] and FAM-N pulse schemes

As MAS cannot remove completely the second-order quadrupolar broadening for nuclei with $I > 1/2$, there has been widespread application of the multiple-quantum MAS experiment for achieving high-resolution or isotropic spectra. However, this experiment suffers from poor sensitivity, particularly during the conversion of triple- to single-quantum coherences, and a number of approaches (e.g., FAM [1], DFS [2], etc.) have been used to improve this technique, but can be time consuming to optimize. Here we introduce a new conversion pulse, based on the FAM approach, consisting of N consecutive pulses with opposite phase, easily and quickly optimized. We demonstrate that this can bring about significant improvements in sensitivity (typically 2-5 times higher compared to a single pulse), improves on the sensitivity achieved by FAM and is of similar efficiency to DFS. This approach, demonstrated using ^{87}Rb, ^{23}Na (I=3/2), and ^{27}Al and ^{17}O (I=5/2) NMR at 14.1 T and 12.5 kHz MAS, provides similar efficiency at both moderate and high RF field strengths. The computational optimization is efficient and easy and can be implemented directly within the experiment with minimum re-optimization. The initial requirements are approximate values for the inherent RF nutation rate and, if available, the quadrupolar parameters. We also investigate the robustness of this new approach to variation in these parameters, and consider the effects of faster MAS rates.

[1] Bräuniger, T. and Madhu, P., J. Magn. Reson., 2008, 193, 102.
[2] Kentgens, A. & Verhagen, R., Chem. Phys. Let., 300, 435

(Talk and Poster 010)

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Using NMR to Study Histone Demethylase Catalysis

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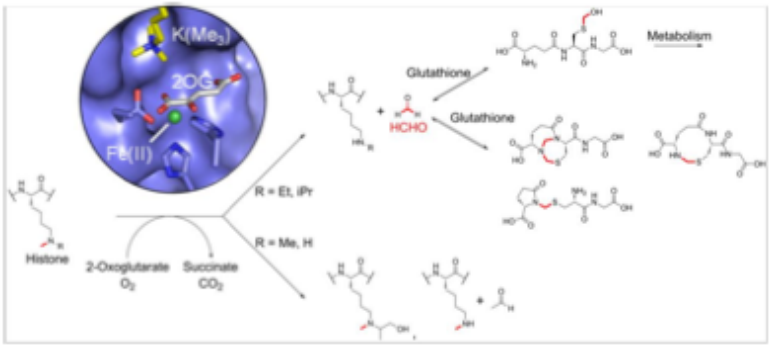
Chemical modifications to histone amino-termini regulate transcription by modulating chromatin structure and dynamics.[1] Histone modifications, which include methylation, acetylation and phosphorylation, are often dynamic and are catalysed by various histone modifying enzymes. Jumonji histone lysyl demethylases (KDMs), a subfamily of iron(II) and 2-oxoglutarate dependent dioxygenases, remove methyl groups from methylated lysine residues on histones and are linked to diseases including cancers.[2] Detailed analyses by NMR using a Bruker 700 MHz spectrometer equipped with a TCI cryoprobe have allowed the first direct observation of multiple substrates and products during KDM catalysis.[3] Further, ^{13}C-labelling of the lysyl methyl groups, coupled to HSQC experiments, have allowed identification of the toxin and carcinogen formaldehyde (HCHO) as a side product during demethylation.[3] In a separate study using synthetic histone fragments, NMR techniques have been used to identify and characterise novel oxidative reactions catalysed by KDMs.[4]
The discovery of HCHO release during histone demethylation suggests the presence of efficient HCHO metabolism pathways. In humans, a predominant pathway of HCHO metabolism involves the ubiquitous tripeptide glutathione, which is proposed to sequester HCHO in cells via non-enzymatic reaction through a cysteinyl thiol (forming a hemithioacetal).[5,6] Detailed NMR analyses on the reactions of HCHO with glutathione have identified multiple glutathione-HCHO adducts, including macrocycles stable under physiologically relevant conditions.[7] These findings imply that the cellular reactions of HCHO, at least with glutathione, are more complex than presently perceived, and may suggest that HCHO production is important for cellular function.



[1] M. Berdasco, M. Esteller, Dev. Cell. 2010, 19, 698-711.
[2] L. J. Walport, et al., Curr. Opin. Chem. Biol. 2012, 16, 525-534.
[3] R. J. Hopkinson, et al., ChemBioChem 2010, 11, 506-510.
[4] R. J. Hopkinson, et al., Angew. Chem. Int. Ed. 2013, in press.
[5] B. K. Hunter, et al., Biochemistry 1984, 23, 508-514.
[6] H. Kalasz, Mini Rev. Med. Chem. 2003, 3, 175-192.
[7] R. J. Hopkinson, Org. Biomol. Chem. 2010, 8, 4915-4920.

Abstracts of Posters

Poster 011

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Solid-State NMR Investigation of GaPO-34

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Gallophosphates (GaPOs) are a family of microporous metallophosphates closely related to the better-known aluminophosphate zeolites (AlPOs). However, the different chemical behaviour of Ga means that GaPOs have the potential to catalyse different reactions or selectively absorb different species, extending the range of applications of zeolites. GaPOs are synthesised in the presence of fluoride and an organic structure-directing agent (SDA), both of which are typically incorporated into the "as-prepared" material. To "activate" GaPOs for potential applications, the SDA and fluoride must be removed by calcination - heating the material until the SDA and fluoride are released. However, calcined GaPOs are believed to be less stable (particularly to water) than the analogous AlPOs.

Here, we focus on some recent work on GaPO-34.[1,2] GaPO-34 can be prepared with two SDAs; pyridinium or 1-methylimidazolium, which display distinctly different behaviour on calcination. Calcination of the pyridinium variant leads directly to the fully-calcined material, whereas calcination of the 1-methylimidazolium variant proceeds via a dehydrofluorinated intermediate. The calcined framework is stable indefinitely, even when hydrated. However, attempts to dry the material lead to an amorphous phase, rather than the calcined $GaPO_4$ framework. This work investigates the many phases of GaPO-34 through a combination of solid-state NMR spectroscopy, in situ variable-temperature X-ray diffraction and first-principles DFT calculations, giving a deeper understanding of the structural and chemical transformations occurring within GaPO-34.

References
[1]. C. Schott-Darie et al., Stud. Surf. Sci. Catal. 1994, 84, 101.
[2]. M. Amri et al., J. Phys. Chem. C, 2012, 116, 15048.

Poster 012

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Combined Solid-State NMR and DFT Study of Hydrated AlPO-34

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Aluminophosphates (AlPOs) are an important class of microporous materials. They consist of corner sharing aluminate and phoshate tetrahedra, which form pores of molecular size. AlPO-34 (CHA framework topology) can be synthesised using many different structure-directing agents. SIZ-4 (St Andrews Ionothermal Zeolite 4) is synthesised ionothermally[1,2] facilitating the enrichment of the framework with ^{17}O, which enables ^{17}O spectra to be obtained more easily (the natural abundance of ^{17}O is 0.037%).[3] Multinuclear solid-state NMR (^{27}Al, ^{31}P, ^{1}H and ^{17}O) was used to study the calcined-hydrated framework to examine host-guest interactions (CHA framework-H_2O). There are two phases of the hydrated material (partial and full), with the nature and relative proportions obtained dependant on sample preparation conditions. First-principles DFT calculations were also carried out on model structures to guide experiments and aid spectral assignment.

1. E. R. Cooper et al., Nature, 2004, 430, 1012
2. E. R. Parnham and R. E. Morris, Chem. Mater., 2006, 18, 4882
3. J. M. Griffin et al., Chem Sci., 2012, 3, 2293

Poster 013

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Exploiting the ^{31}P Chemical Shift Anisotropy of Aluminophosphates

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Aluminophosphates (AlPOs) are an important class of microporous materials that consist of alternating corner-sharing AlO_4 and PO_4 tetrahedra. Solid-state NMR spectroscopy can be used to probe the local structure and order, since the basic components of the framework and the structure-directing agents are NMR active (^{27}Al, ^{31}P, ^{1}H, ^{13}C). The ability to quantify the isotropic and anisotropic chemical shift parameters, both experimentally and using first-principles density functional theory (DFT) calculations would greatly ease the challenge of spectral assignment. To calculate NMR parameters an initial structural model is required, the accuracy of which typically depends on the type of diffraction used. In many cases, optimisation of the geometry is required to minimise the forces upon the atoms, prior to the calculation of NMR parameters. Many traditional DFT calculations are not able to adequately describe the long-range forces, owing to an underestimation of dispersion interactions in many exchange-correlation functionals.

This work evaluates two dispersion correction schemes implemented within the CASTEP code,[1] on the ability to optimise the geometry of the structure of AlPOs. We also consider the experimental measurement of the 31P chemical shift anisotropy of as-made and calcined AlPOs, using CSA-amplified PASS experiments. As these can be very small for more symmetrical AlPO frameworks, experiments are performed at both 14.1 T and 20.0 T to enable accurate extraction of this parameter. These values are compared to calculated values and their dependence upon the local geometry explored. It is hoped that a deeper understanding of the local structure and interactions will afford greater insight into these widely-relevant materials.

[1] E. R. McNellis, et al., Phys. Rev. B, 2009, 80, 205414
[2] R. M. Orr, et al., J. Magn. Reson., 2005, 174, 301

Poster 014

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Synthesis, Structure and Spectroscopic Properties of Selenium, Sulfur and Phosphorus Heterocycles

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P-Se and P-S heterocycles have been previously studied and several heterocycles are already reported in the literature. The Woollins group has undertaken extensive studies in this field.[1] Two important reagents, Lawesson's reagent and the Se analogue, Woollins' reagent, have enabled the chemistry of organophosphorus-sulfur/selenium heterocycles to be extended in recent years. Moreover, the commercialization of both reagents opens the door for applications in industry and the laboratory, especially in the fields of organic and organometallic chemistry.[2]

We are developing routes to new peri-backbone molecules which have unusual bonding and the potential to form radicals for use in spintronics. In this work, we present new sulfur/selenium and phosphorus heterocycles using an adapted synthetic route.[3] Figure 1 shows the structures of these new materials. This new route uses chalcogen- containing peri-substituted naphthalenes as starting materials and phosphine chlorides for the introduction of the phosphorus atom.

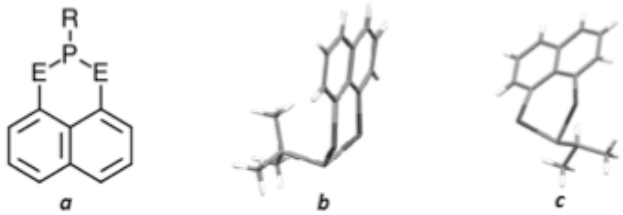

Figure 1: (a)Model of sulphur/selenium and phosphorus heterocycles, where E= S, Se and R =isopropyl or tert-butyl (b) View of crystal structure of tert-butyl-phosphorus diselenolenapthalene along the a axis (c) View of crystal structure of isopropyl-phosphorus diselenolenapthalene along the c axis.

In this work, we present crystallographic studies combined with multinuclear solid-state NMR Spectroscopy, for the full characterization of these compounds. ^{77}Se and ^{13}C CP MAS NMR spectra of these organo-heterocycles are shown, as well as ^{31}P solid-state MAS NMR spectra in order to gain insight into the bonding and crystal packing in these unusual molecules.

References:
[1] G. Hua and J. D. Woollins; Angew. Chem. Int 2009, 48, 1368.
[2](a)M. Jesberger, T. P. Davis and L. Barner; Synthesis 2003, 1929.(b)J. Kaschel, et al.; Chem. Commun 2013, 49, 4403.
[3]M. Baudler, A. Moog, K. Glinka and U. Kelsch; Z. Naturforsch 1973, 28, 363.

Poster 015

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Characterization of reversible protein self-association by NMR spectroscopy

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Protein self-association is a process intrinsic for all proteins at high concentrations. It leads to the problems in protein manufacturing, stability and delivery. At high concentration protein undergoes conditions close to crystal state and lattice contacts can coincide with biological interactions in solution. The mechanism of protein self-association is studied using Nuclear Magnetic Resonance (NMR) spectroscopy in solution. The properties of protein-protein interactions at high concentration ~ 200 mg/ml are studied on residual resolution. Residue specific information on protein dynamics is obtained using ^{15}N relaxation measurements. The experiments are carried out at multiple concentrations under different conditions. Calculation of chemical exchange contribution to the transverse relaxation can help to allocate binding sites of the process. Rotational correlation time variation shows changes to the protein dynamics, which reflects oligomerisation process occurring in solution. Pulsed-field gradient NMR spectroscopy is used to monitor translational diffusion coefficient in order to estimate the degree of protein self-association. Oligomer formation is also monitored by variation of ^{1}H and ^{15}N amide chemical shifts. Better understanding of the protein self-association mechanism under different conditions will provide opportunity to develop methods to reduce the level of reversible protein self-association in solution at high protein concentration.

Poster 016

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Probing Ras:Sos:nucleotide interactions using NMR spectroscopy

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Ras proteins are mutated in 30% of all human tumours contributing to several malignant phenotypes including abnormal cell growth. The activity of Ras is partly regulated by the binding of guanine nucleotide exchange factors, such as Sos. The mechanism of Ras activation via its interactions with Sos remains unclear making it a challenging system for effective drug design. The aim of this work is to understand the molecular interactions of the Ras: Sos complex supported mainly by NMR spectroscopy. Comprehensive signal assignments in the NMR spectra of Ras, which we have completed, allows observations of the changes to specific residues in the spectra upon Ras binding to its binding partner e,g, Sos. The sequence-specific signal assignments of K-Ras by NMR have provided details on important binding site regions of K-Ras that were missing in previous literature. This has allowed us to identify binding site hotspots in the NMR spectra of Ras upon interactions with Sos. To gain a further understanding into the binding events of the Ras: Sos complex, we carried out a series of NMR-titration experiments, whereby increasing concentrations of Ras were added to Sos and analysed by NMR. Analysis of these NMR spectra enables us to monitor signals at the Ras: Sos binding sites under physiological conditions.

Poster 017

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Twisting One Polymorph into Another: The $Li_xNa_{1-x}NbO_3$ Perovskite System

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Sodium niobate, $NaNbO_3$, is one of the most structurally complex ferroelectric perovskites known, and has enjoyed renewed interest as a potential replacement for PZT, owing to its high piezoelectric responses. However, much work has recently focused on modifying this functional material to improve its piezoelectric properties, mainly by A-site substitution with Li^+.

The centro-symmetric, ambient temperature phase of $NaNbO_3$ undergoes a transition to a polar $P2_1ma$ orthorhombic phase when $x = 0.02 – 0.08$, well-characterised by ^{23}Na MQMAS NMR. Beyond this region, a Na-rich trigonal pseudo-perovskite phase is formed (R3c) and the two phases can co-exist until $x = 0.2$. By varying the calcination temperature and cooling rate, the phase fractions in this region can be controlled and the orthorhombic phase removed completely. Modification of the synthetic conditions, including annealing temperature and cooling rate, play an important role in determining the relative phase fractions formed, and consequently, the electrical properties. The relative phase fractions and subtle changes in the rhombohedral structure are well determined by neutron powder diffraction, yet ^{23}Na MQMAS spectra suggest that an additional phase change occurs around $x = 0.7$. However, the polar orthorhombic phase is now believed to undergo a non-diffuse phase transition to the Na-rich trigonal phase, which can happen over several weeks. High-resolution ^{23}Na MQMAS NMR and complementary XRD experiments are used to confirm the gradual reduction of the orthorhombic phase fraction.

Poster 018

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The effects of magic angle spinning on composite pulses in solid-state NMR

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A vast number of composite pulses have been designed for use in NMR experiments to compensate for imperfections in radiofrequency pulses, alleviating the common problems of B_1 inhomogeneity and resonance offset. Recently, we have shown that phase-antisymmetric refocusing pulses are the correct type to use when forming a spin echo [1]. In solid-state NMR of spin $I = 1/2$ nuclei such as ^{13}C or ^{31}P, there is the added problem that magic angle spinning (MAS) introduces a time dependence to the chemical shift of each crystallite over each rotor period. This variation of the resonance offset of each crystallite during the application of the composite pulse may be neglected if the pulse is of short duration. We find that as the duration of the composite pulse becomes comparable to the duration of the rotor period, the performance of the composite pulse is liable to deteriorate. Here, we show the effect of MAS on various composite refocusing pulses, including simulations demonstrating the performance of dual-compensated refocusing pulses from the ASBO family of antisymmetric composite pulse sequences [2]. The results of ^{31}P spin-echo experiments incorporating composite 180° pulses are also presented.

1. S. Odedra and S. Wimperis, J. Magn. Reson. 214, 68 (2012).
2. S. Odedra, M. J. Thrippleton and S. Wimperis, J. Magn. Reson. 225, 81 (2012).

Poster 019

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Structural characterisation of marine glycosaminoglycans

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Glycosaminoglycans (GAGs) are a group of structurally related polysaccharides found as the carbohydrate moieties of proteoglycans and sometimes as free polysaccharides. They are widely distributed throughout the animal kingdom1. GAGs are usually isolated from animal tissues, e.g. pharmaceutical grade heparin is derived from mucosal tissues of slaughtered meat animals, porcine intestines or bovine lungs2. During the past decade there has been an increased interest in analysing sulfated polysaccharides from marine organisms, such as fucosylated chondroitin sulphates from Echinoderms 3, due to their unique structures and properties.

Here we report a structure of an oversulfated dermatan sulfate, I, isolated from a sea squirt, as determined by a combination of 2D HSQC, 2D HSQC-TOCSY, 2D HMBC and 2D HSQC-NOESY. This polysaccharide has significant anti-inflammatory, but not anticoagulant properties and is resistant to enzymatic depolymerisation. In order to prepare low molecular fragments of I, free radical depolymerisation was used. Such fragments are of interest as potential active compounds but can also help during the structure elucidation process. However, radical depolymerisation generates heterogeneous samples. In order to better understand this procedure we have analyzed, using NMR, the products of the free radical depolymerisation of porcine dermatan sulfate.

References

1. Cássaro CM, Dietrich CP. Distribution of sulfated mucopolysaccharides in invertebrates. J Biol Chem. Apr 1977;252(7):2254-2261.
2. Linhardt RJ, Gunay NS. Production and chemical processing of low molecular weight heparins. Semin Thromb Hemost. 1999;25 Suppl 3:5-16.
3. Mourão PA, Pereira MS, Pavão MS, et al. Structure and anticoagulant activity of a fucosylated chondroitin sulfate from echinoderm. Sulfated fucose branches on the polysaccharide account for its high anticoagulant action. J Biol Chem. Sep 1996;271(39):23973-23984.

Poster 020

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Signal Enhancement of Quadrupolar Satellite Transitions: Application to Satellite-Transition Magic Angle Spinning (STMAS) NMR

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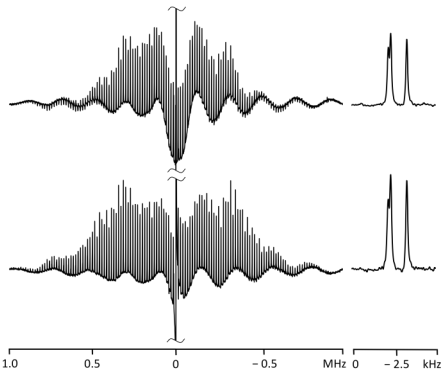
Satellite-transition magic angle spinning (STMAS) is a well-established technique for obtaining high-resolution NMR spectra of half-integer quadrupolar nuclei [1-3]. In comparison with the multiple-quantum (MQ) MAS method, which also yields isotropic spectral information, STMAS experiments have been regarded as more difficult to implement owing to technical requirements [3]. However, STMAS has a sensitivity advantage over MQMAS, arising from its single-quantum nature, making it particularly suitable for investigation of (i) low-γ nuclides, (ii) nuclides with low natural abundance, and (iii) nuclides with large quadrupolar coupling constants. Each of these situations is inevitably accompanied by inherent difficulty in obtaining good signal-to-ratio and hence necessitates investigations for possible signal enhancement.

Here we study the efficient excitation and enhancement of quadrupolar satellite transitions, with the aim of designing methods for further enhancing the sensitivity of STMAS experiments. We start by exploring the theoretical maximum population transfer efficiency into ST coherences using the Universal Bound approach [4]. Pulse sequences that lead to the desired signal enhancement for $I = 3/2$ are presented, along with the experimental results that demonstrate the validity of our signal excitation and enhancement schemes.



^{87}Rb MAS and DQF-STMAS spectra of RbNO3: conventional excitation (top) and with irradiation of the central transition followed by DANTE excitation (bottom)

[1] Z. Gan, J. Am. Chem. Soc. 122, 3242 (2000).
[2] H. T. Kwak and Z. Gan, J. Magn. Reson. 164, 369 (2003).
[3] S. E. Ashbrook and S. Wimperis, Prog. NMR. Spectrosc. 45, 53 (2004).
[4] O. W. Sørensen, J. Magn. Reson. 86, 435 (1990).

Poster 021

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Exploring the Structure of Alzheimer's Amyloid Aggregates with Copper Using Solid-State NMR

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Aggregation of amyloid-beta1-40 ($A\beta_{1-40}$) is linked to the pathology of Alzheimer's disease with current solid-state nuclear magnetic resonance (ssNMR) research focusing on the structure of stable β-sheet rich oligomers of cysteine cross-linked $A\beta_{1-42}CC$.[1] There have been reports on elevated toxicity of Aβ aggregates formed in the presence of metal ions, such as Cu^{2+}, Zn^{2+}, Fe^{2+} and Al^{3+} which may stabilise certain types of oligomers, protofibrils and amyloid fibrils.[2-4] In this work, 2D and 3D solid-state NMR (ssNMR) spectra, chemical shift values and electron microscopy images of $A\beta_{1-40}$ aggregates, formed in the presence of Cu^{2+}, are presented. Short (20 ms) and long (200 ms) mixing time ^{13}C-^{13}C MAS DARR experiments were performed at 600 MHz and 850 MHz with 10 kHz MAS. Experiments performed at 100 kHz MAS are also shown, including ^{1}H-^{13}C 2D and 3D experiments which exhibit increased resolution due to fast spinning. Fast spinning renders the employment of proton detection (PD) viable; PD and rapid relaxation, due to the paramagnetic centre, provide increased sensitivity. ^{13}C chemical shift values are compared to those obtained for different polymorphs of $A\beta_{1-40}$ fibrils incubated without metal ions [5-7] and with Cu2+ ions introduced after fibril formation.[3] Data suggest the sample is a previously unseen polymorph of $A\beta_{1-40}$ which is likely to have greater physiological relevance than amyloid fibrils formed without metals.

1. Sandberg, A. et al, Proc. Natl. Acad. Sci. USA. 107, 15595-15600 (2010)
2. Mithu, V. S. et al, Biophys. J. 101, 2825-2832 (2011)
3. Parthasarathy, S. et al, J. Am. Chem. Soc. 133, 3390-3400 (2011)
4. Mantyh, P. W. et al, J. Neurochem. 61, 1171-1174 (1993)
5. Petkova, A., Yau, W. M. and Tycko, R., Biochemistry 45, 498-512 (2006)
6. Paravastu, A. K. et al, Proc. Natl. Acad. Sci. USA. 105, 18349-18354 (2008)
7. Bertini, I. et al, J. Am. Chem. Soc. 133, 16013-16022 (2011)

Poster 022

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Characterising Molecular Self-Assembly in Supramolecular Chemistry by Solid-State NMR.

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Synthetic derivatives based on DNA/RNA bases have been shown to exhibit expansive self-assembly in the solid state. Building on recent ^{14}N-^{1}H HMQC work by Webber et al[1] and applying ^{1}H solid-state NMR methods it is possible to probe the local structure and dynamics of these systems.

Two-dimensional ^{1}H experiments are becoming increasingly important for probing structure, dynamics and interactions between organic molecules in the solid state. In particular fast sample spinning ^{1}H DQ MAS and the ^{1}H CRAMPS approach are now the preferred method for directly probing proton-proton proximities,[2] with the latter technique enjoying enhanced resolution over the former, owing to the inclusion of carefully synchronised homonuclear decoupling in the pulse sequence.

The emerging method of ^{14}N-^{1}H HMQC spectroscopy provides valuable information and is well suited for the assignment of ^{1}H chemical shifts, benefitting from the larger chemical shift range in the heteronuclear dimension.[3] This experiment has recently been shown to identify hydrogen bonding motifs in pharmaceutical systems.[4,5] Spectral assignment is further enhanced by the application of ^{13}C-^{1}H INEPT experiment for both labelled and unlabelled sample.

1. A. L. Webber, S. Masiero, S. Pieraccini, J. C. Burley, A. S. Tatton, D. Iuga, T. N. Pham, G. P. Spada and S. P. Brown, J. Am. Chem. Soc. 2011, 133, 19777.
2. S. P. Brown, Solid State Nucl. Magn. Reson. 2012, 41,1.
3. A. S. Tatton, J. P. Bradley, D. Iuga and S. P. Brown, Z. Phys. Chem. 2012, 226, 1187.
4. A. S. Tatton, T. N. Pham, F. G. Vogt, D. Iuga, A. J. Edwards and S. P. Brown, Crystengcomm. 2012, 14, 2654.
5. A. S. Tatton, T. N. Pham, F. G. Vogt, D. Iuga, A.J. Edwards and S. P. Brown, Mol. Pharmaceutics. 2013, 10, 999.

Poster 023

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Constant time gradient CRISIS HMBC (CTgc2HMBC)- Method for suppressing ^{1}H-^{1}H modulation to improve SNR, peak intensity and narrowing of ^{1}H-^{13}C cross peaks

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Since its introduction, the two-dimensional heteronuclear multiple bond correlation (HMBC) experiment has rapidly become the standard way of detecting the presence of long-range (two- and three bond), proton-carbon couplings in small- to medium-size molecules. Though widely used for detection of long -range couplings, HMBC is found to have limitations of *J*-modulation due to ^{1}H-^{1}H coupling during t_1 period causing line broadening of the ^{13}C signals in the F_1 dimension which leads to difficulty in analysing some of the ^{13}C-^{1}H cross peaks of complicated molecules with poorly separated ^{13}C signals. In order to quantify improvements to the HMBC experiment we designed a constant time gradient HMBC experiment with adiabatic pulses to remove ^{1}H-^{1}H modulation. We compare its performance against comparable non-constant time experiments. This experiment termed CTgc2HMBC gave better peak separation, signal-to-noise ratio and resolution than normal HMBC experiment at both low and high resolution.

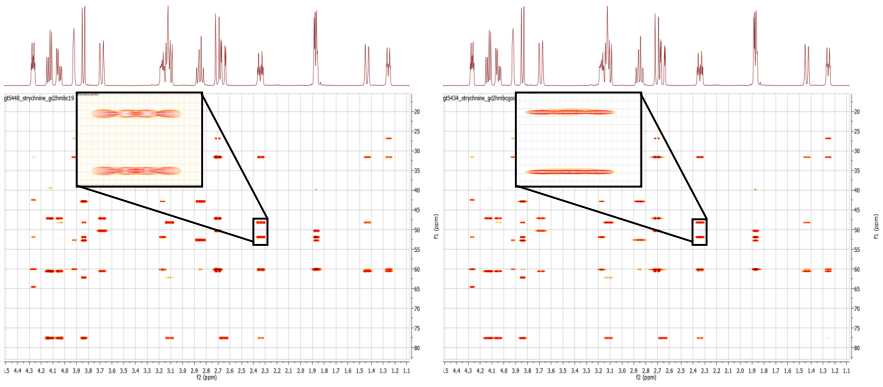

Figure 1: Part of (a) gc2HMBC spectrum (b) CTgc2HMBC of 30 mg of strychnine in 700 µl of chloroform. Both spectra were obtained using 220 ppm ^{13}C spectral width F_1 digital resolution of 150 Hz, 4 scans/increment and constant time delay was set to t1max/2.

References

Bax, A. Summers, M. Journal of the American Chemical Society 1986, 108, 2093–2094.

Furihata, K.; Seto, H. Tetrahedron Letters 1998, 39, 7337–7340.

Poster 024

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Predicting the stability of polymer stabilised amorphous dispersions for oral drug delivery: probing the mobility of Indomethacin/PVP-VA dispersions using solid-state NMR

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The aim of this study was to focus on the potential of solid-state NMR as a tool to provide molecular level information on structure and dynamics of polymer-drug amorphous dispersions using Indomethacin and PVP-VA as a model system. This could pave the way to enable the prediction of potential storage issues, since molecular mobility of polymer stabilised amorphous systems is a key factor in their physical stability.

γ-Indomethacin (Sigma-Aldrich, UK) was formulated with PVP-VA (Kollidon 64, BASF, Germany) into amorphous dispersions of varying drug: polymer ratios by solvent evaporation from methanol, ethanol and acetonitrile. Amorphous indomethacin was prepared via melt cooling with liquid Nitrogen. Variable temperature (VT) ^{1}H-^{13}C CP MAS, ^{13}C $\{^{1}H\}$ MAS and spin lock $T_{1\rho}^{H}$ relaxation solid state NMR were used to probe differences in local mobility between the drug and polymer components of the amorphous dispersions on the kHz scale using a Bruker Avance 400 MHz spectrometer between 298 and 373 K.

Different motional regimes for the drug and polymer were identified depending on the drug loading levels (figure 1). A transition to the α-polymorph of Indomethacin was observed above 333 K for amorphous Indomethacin and 80:20 dispersion prepared from methanol and acetonitrile. Such polymorphic transitions of Indomethacin are solvent-dependent, with ethanol evaporation yielding the γ-polymorph.

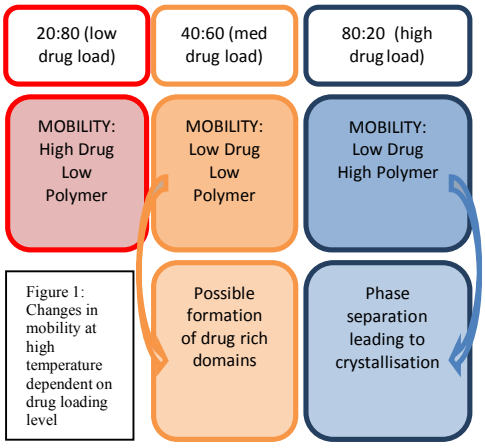

Figure 1: Changes in mobility at high temperature dependent on drug loading level

In conclusion, differences in the mobility of Indomethacin and PVP-VA dependent on drug loading levels were highlighted, and have been ascribed to domain formation and phase separation within the dispersions. Solvent dependent polymorphic transitions of the drug were also identified. VT solid-state NMR has proved a valuable tool for probing molecular mobility in solid dispersions, and therefore aiding the prediction of potential long term stability issues with amorphous dispersions.

Poster 025

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Exceptionally strong aggregation of DNA minor groove binders (MGBs) assessed by NMR Spectroscopy

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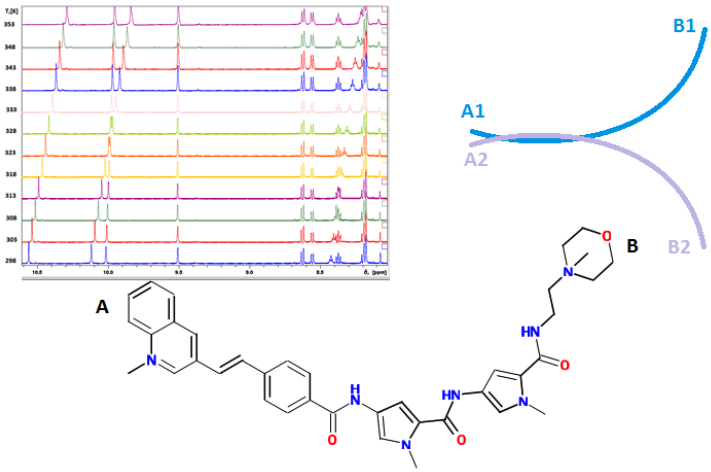
The self-assembly characteristics of a new class of antibacterial DNA minor groove binding ligand (MGB), the lead compound of which is currently entering Phase I clinical trials, are studied here by NMR spectroscopy and numerical analysis to allow the strength of assembly to be determined. NMR data in the form of diffusion coefficient and 1D ^{1}H NMR spectra were acquired as a function of temperature and concentration. Signal assignments based on TOCSY and NOESY data enabled information to be determined at the molecule-on-molecule scale to establish the nature of the aggregate building-block. These data were combined with information derived from diffusion ordered spectra, allowing parameters to be determined which described the aggregation processes, namely K_{agg}, the association constant and hydrodynamic volume (V_H). Some molecules in the class display very strong aggregation as shown by negligible changes in chemical shift as a function of concentration, a strong dependence on temperature, wide solvent peak and low signal intensity, broad resonance linewidth of MGB peaks and with properties tending toward gel formation. To check the accuracy of the NMR approach, the results were compared with similar parameters obtained using computer modelling.



Temperature dependence and intermolecular interaction of Dimethylated MGB-BP3

Poster 026

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Formation of dopamine-mediated α-synuclein soluble oligomers is require methionine oxidation

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α-synuclein is a natively unstructured presynaptic protein, and is the major component of intracellular inclusions termed Lewy bodies that are the pathological hallmark of Parkinson's diseased (PD) brains. α-synuclein consists of 140 amino acids, and its function in neurons has yet to be elucidated. Another hallmark of PD is the loss of dopaminergic neurons in the substantia nigra, and the subsequent depletion of dopamine (DA) in the striatum of the brain. It was shown that DA mediates α-synuclein aggregation into SDS-resistant soluble oligomers (Cappai et al, FASEB J (2005)). Further studies identified the ^{125}YEMPS^{129} motif on the C-terminus of α-synuclein to be important in the interaction of α-synuclein with DA (Norris et al, JBC (2005)). We have studied this interaction using NMR spectroscopy and a variety of other biophysical techniques. ^{1}H-NMR studies showed a resonance shift characteristic of methionine oxidation when α-synuclein WT is incubated with DA. Further ^{15}N-HSQC and ^{13}C-HSQC spectra identified all four methionine residues, M1, M5, M116, M127, in α-synuclein were oxidized upon incubation with DA. Mass spectrometry analysis confirmed that the predominant species of these α-synuclein:DA oligomers have a mass of 14524 Da, an addition of 64 Da to the mass of WT α-synuclein, indicating four oxidation events. The substitution of all four methionine residues with alanine significantly reduced the propensity of α-synuclein to form SDS-stable soluble oligomers in the presence of DA. The incubation of a synthetic YEMPS peptide with wild type α-synuclein and DA resulted in inhibition of SDS-stable soluble oligomerization and formation of YEM(O)PS, suggesting that the ^{125}YEMPS^{129} peptide is oxidized in preference to the methionine residues in α-synuclein and is not the interacting site. All ^{1}H-TOCSY and ^{1}H-NOESY spectra show no evidence of adduct formation. This study emphasizes the role of oxidative stress in the pathogenesis of PD.

Poster 027

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Thermodynamic parameters of small molecule assemblies determined by NMR spectroscopy

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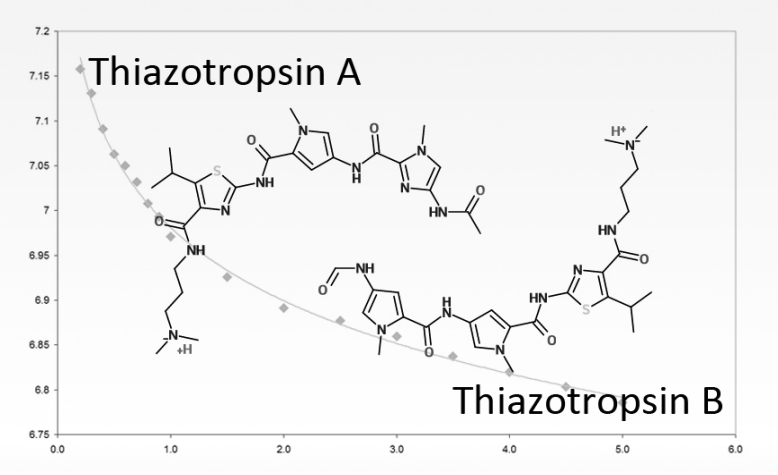
The study of molecular assembly can be of vital importance when dealing with small molecules that have the potential to be of use as medicines. DNA recognition mechanisms for instance may be influenced by the sizes and structures of ligand molecule aggregates. This study describes an additional method for determining the thermodynamic and binding affinity parameters of assembly and provides direct comparison to ITC (isothermal titration calorimetry) data. NMR spectra in the form of 1D ^{1}H and diffusion ordered data are acquired as a function of solute concentration and temperature. The results may then be fitted to a suitable mathematical model. In this study three sets of data were acquired and modeled namely for Thiazotropsin A self-assembly, Thiazotropsin B self-assembly and Thiazotropsin A – Thiazotropsin B hetero-assembly. All experimental results were fitted to the corresponding theoretical models with thermodynamic characteristics and binding affinity as the output in mind. Values obtained were compared with information derived separately by ITC and proved to be accurate. This technique can potentially be used for characterizing the binding affinity of ligands for proteins, studying DNA recognition and for similar instances of self- or hetero-assembly. Thermodynamic characterization may ultimately allow for further optimization of compounds to tailor assembly to the required specificiations.

Salvia M-V, Addison F, Alniss HY et al. Biophys. Chem. 2013, 179, 1-11



Poster 028

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NMR reporter screening method for 2OG oxygenases

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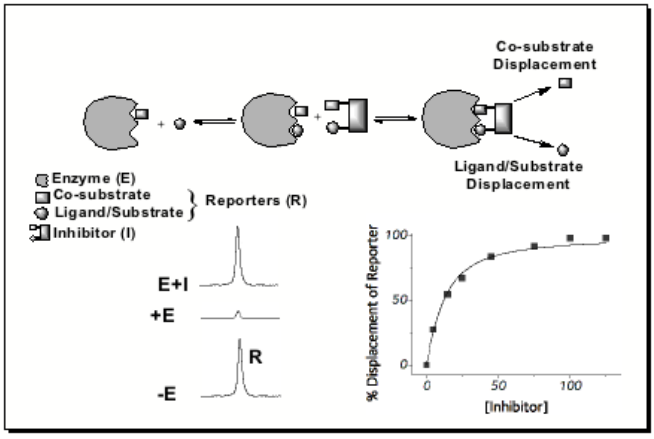
2-Oxoglutarate (2OG) dependent oxygenases are ubiquitous in plants, micro-organisms and animals. In humans, they are involved in a diverse range of important biological roles, including oxygen sensing, fatty acid metabolism and epigenetic regulation. Many 2OG oxygenases are current inhibition targets for diseases including cancer, ischemia and anaemia. There are three main strategies for 2OG oxygenase inhibitions. One class of inhibitors are designed to bind to the iron(II) in the enzyme active site and act as competitors for the enzyme co-substrate 2OG. Another class of inhibitors are designed to be substrate mimics and compete against the enzyme substrate. A third class of inhibitors are designed to compete against both the enzyme substrate and the co-substrate 2OG in order to maximise their potency and selectivity.

The NMR reporter screening method is a useful technique for the site-specific detection of both high- and low-affinity ligands. Using Hypoxia Inducible Factor (HIF) Prolyl Hydroxylase Domain 2 (PHD2), a 2OG oxygenase that is involved in our responses to hypoxia (lack of oxygen) and γ-Butyrobetaine Hydroxylase (BBOX), a 2OG oxygenase involved in carnitine biosynthesis, as model systems, we describe our progress in optimising and applying the NMR reporter method for inhibitor screening and quantification (e.g. K_D measurement).

Reference: Leung, I. K. H.; Demetriades, M.; Hardy, A. P.; Lejeune, C.; Smart, T. J.; Szöllössi, A.; Kawamura, A.; Schofield, C. J.; Claridge, T. D. W. NMR reporter ligand screening for inhibitors of 2OG oxygenases. J. Med. Chem. 2013, 56, 547–555.



Poster 029

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Monoterpene and Flavonoid Mixture Analysis by Matrix-Assisted Diffusion-Ordered Spectroscopy

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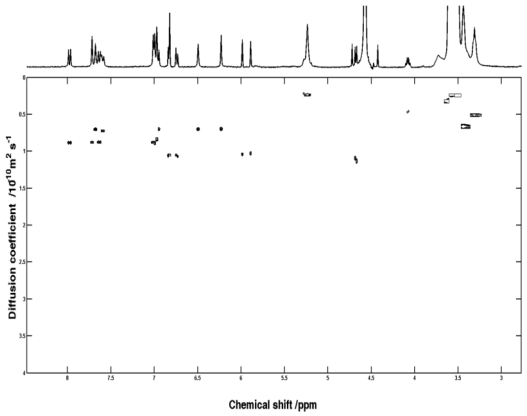
The assignment of signals of components in mixtures by NMR is difficult due to signal overlap. Diffusion-Ordered Spectroscopy has provided important progress in mixture analysis, allowing the signals originating from individual components of different molecular sizes to be distinguished. Matrix-assisted DOSY (MAD) has generated promising results in the resolution of organic mixtures, including mixtures of isomers, exploiting selective binding to a slowly-diffusing matrix. The challenge is to apply MAD to more complex molecules, for example natural products. Various surfactants, including SDS, AOT and CTAB already have been used in MAD analysis, but not the Brij family of nonionic surfactants. These consist of a hydrophilic head containing a variable number of polyoxyethylene groups, and a hydrophobic polymethylene tail. In this work, MAD was performed using Brij 78 and 98 matrices for two different mixtures of natural products. In DOSY spectra of a monoterpene mixture in 20% DMSO-d_6-D_2O using Brij 78, it was observed that thymol and carvacrol signals could be separated, which was not the case with SDS. For a flavonoid mixture in 50% DMSOd_6-D_2O using Brij 98, good resolution was obtained between fisetin, catechin and quercetin signals, allowing adequate characterization, improving on earlier results obtained with SDS. These results illustrate how MAD can help in mixture analysis, and offer Brij 78 and 98 as new matrices.



Poster 030

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Screening of matrices for mixture resolution of dihydroxybenzenes by matrix-assisted diffusion ordered spectroscopy

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Diffusion ordered spectroscopy (DOSY) produces a two-dimensional spectrum in which the signals of different compounds are separated according to their respective diffusion coefficients, which depend inter alia on hydrodynamic radius (size) and intermolecular interactions. Signals of isomeric compounds with very similar diffusion coefficients can be separated in intact mixtures by exploiting differential interactions with the matrix in which they diffuse, in a matrix-assisted DOSY (MAD) experiment. In this work, the diffusion coefficients of dihydroxybenzene isomers catechol (C), resorcinol (R) and hydroquinone (H) were studied in different matrices, including solutions of α- and β- cyclodextrins and ionic and non-ionic surfactants. The results show different interactions with each matrix studied. MAD of C, R and H using the non-ionic surfactant polyoxyethylene-(20)-stearyl ether (brij 78) in aqueous solution gave good resolution, with separations of 30 % (ΔHR), 23 % (ΔHC) and 8 % (ΔCR). The anionic surfactant sodium bis(2-ethylhexyl) sulfosuccinate (AOT) in CDCl3 showed separations of 37 % (ΔHR), 21 % (ΔHC) and 21 % (ΔCR), compared to 13 % (ΔHR), 22 % (ΔHC) and 10 % (ΔCR) for aqueous SDS.

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