Thirteen Annual Symposium of the UK High-Field solid-state NMR facility

Thursday April 18th, 2024
Scarman Conference Centre, University of Warwick

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The UK High-Field Solid-State NMR Facility
Annual Symposium Programme
Thursday April 18th, 2024

10.30 – 11:00: Registration

Morning Session, Chair: Prof. Phil Williamson, University of Southampton

11:00 – 11.40: *Methods for Structure Determination of RNA and RNA-protein Complexes by Solid-State NMR*, Prof. Teresa Carlomagno, University of Birmingham

11:40 – 12:05: *High Field Solid State NMR Explorations of Protein-Lipid Interactions in Cardiovascular Disease*, Dr. Sophie Rawnsley-Lau, Lancaster University

12:05 – 12:30: *How High-Field Solid-State NMR Improves our Understanding of Zeolite Structures*, Joseph Hurd, University of Manchester

12.30 – 13:40: Lunch

First Afternoon Session, Chair: Prof. Frédéric Blanc, University of Liverpool

13:40 – 13:50: *Update on the UK High-Field Solid-State NMR National Research Facility*, Prof. Steven Brown, University of Warwick

13:50 – 14:30: *Structure and Dynamics of Glass Forming Metal Organic Frameworks: NMR study of Zeolitic Imidazolate Framework ZIF-62*, Dr. Pierre Florian, CNRS Orléans

14:30 – 14:55: *In-situ Solid-State NMR Spectroscopy for the Study of Novel Zeolites*, Dr. Nicole Kelly, St Andrews University

14:55 – 15:20: *NMR at Fast Speeds: Progress in Fast Spinning Experiments at the National Research Facility*, Dr. Trent Franks, University of Warwick

15.20 – 15:50: Coffee break

Second Afternoon Session, Chair: Prof. Sharon Ashbrook, St Andrews University

15:50 – 16:25: *Reaction Mechanisms, Kinetics, and Nanostructural Evolution of Cements and Related Materials: Insights from High-Field Solid-State NMR*, Dr. Brent Walkley, University of Sheffield

16:25 – 17:00: *Stretchy Skin and Resilient Fungi - New Applications of ssNMR to Extracellular Matrices*, Dr. Wing Ying Chow, University of Warwick

17:00: End of symposium

Methods for Structure Determination of RNA and RNA-Protein Complexes by Solid-State NMR

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Nucleic acids and ribonucleoprotein complexes (RNP) play both a regulatory and a functional role in cellular processes; the elucidation of their activity mechanism requires knowledge of their structure. Nucleic acids and RNP are difficult to crystallize due to their conformational plasticity. Moreover, the size of RNPs easily exceeds the molecular weight limit of solution-state NMR. Solid-state NMR (ssNMR) has become a critical instrument in the elucidation of structure-function relationships of large biomolecular complexes, being in principle applicable to molecules of any size. While enormous progress has been made in the structure determination of membrane proteins and amyloid fibrils by ssNMR, significantly fewer studies have been performed on RNA or RNP by ssNMR.

Our laboratory has pioneered the application of ssNMR to structural studies of RNA at atomic resolution, solving the first structures of both RNA and an RNA–protein complex by ssNMR using conventional ^{13}C- and ^{15}N-detection [1–4]. This approach is limited by the severe overlap of the RNA peaks together with the low sensitivity of multidimensional experiments. Here, I will show how we overcome the limitations in sensitivity and resolution using ^{1}H-detection at fast MAS rates. I will demonstrate that ultrafast magic angle spinning (MAS) yields narrow ^{1}H resonances for the 26mer Box C/D RNA bound to L7Ae protein from *Pyrococcus furiosus* (*Pf*) [5]. I will discuss experiments that allow complete assignment of RNA resonances using ^{1}H detection through 4D HCCH-TOCSY for the assignment of ribose resonances, 3D (H)NCH and 4D HNCH experiments for the connection of ribose and base resonances, and 3D (H)CCH, (H)CNH, (H)N(C)CH, (H)NCH, (H)N(HH)CH and (H)N(HH)NH experiments for the complete assignment of base resonances. Moreover, the last two experiments allow accurate site-specific determination of the RNA secondary structure, including Watson-Crick (WC) and non-WC base pairs. Thanks to the high sensitivity of ^{1}H detection, 2D versions of these experiments allow the identification of base pairing patterns within hours using sub-milligram quantities of uniformly labelled RNA [6]. We are currently working on the development of experiments for the collection of structural restraints based on ^{1}H detection. We believe that these results will permit structure determination of RNAs embedded in complexes of large size by ssNMR.

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High Field Solid State NMR Explorations of Protein-Lipid Interactions in Cardiovascular Disease

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We have utilised and developed solid-state NMR (ssNMR) methods, including at high-field, to investigate functionally-important phospholipid-protein interactions associated with cardiovascular disease. Protein-lipid interactions may be a common factor in the formation of arterial plaques and cholesterol transport. The aortic amyloid polypeptide, medin, is highly prevalent as amyloid plaques in blood vessels of individuals over the age of 50 and has possible associations with AD via its co-aggregation with the Alzheimer's amyloid-beta peptide.[1, 2]

Using the high-field ssNMR facility, we obtained the first structural details of medin fibrils[3], and more recently have used magic-angle spinning (MAS) and oriented sample NMR to characterise medin on the surface of liposomes mimicking extracellular vesicles isolated from smooth muscle cells. The results provide insight into the aortic amyloid formation pathway and the role of phosphatidylserine lipids.

Second, we have developed ssNMR methods to probe protein-lipid interactions in high-density lipoprotein (HDL), also known as "good cholesterol", because of its ability to carry cholesterol to the liver for excretion. We have developed ssNMR methods to detect the particle morphology of model HDL-C (rHDL-C) particles using oriented ^{31}P ssNMR spectroscopy, wherein the observed NMR lineshapes are highly sensitive to the surface curvature of the lipid headgroups.[4] Using the dynamically-averaged ^{13}C-^{13}C and ^{13}C-^{1}H dipolar couplings we have determined the orientational distribution of [2,3,4-$^{13}C_3$]cholesterol in discoidal rHDL-C.[5, 6]. We are now investigating differences in the structure and function of atheroprotective and dysfunctional variants of HDL-C containing mutants of the main protein, apoA-I. To obtain higher resolution spectra and site-specific structural conformations of apoA-I in rHDL-C nanoparticles we obtained 2D ^{13}C-^{13}C and ^{1}H-^{15}N-^{13}C triple resonance measurements at a higher magnetic field (1 GHz). These recent results will be presented. Our overarching goal is to use the structural knowledge to develop improved diagnostic and preventative approaches to cardiovascular disease.

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How High-Field Solid-State NMR Improves our Understanding of Zeolite Structures

Joseph Hurd, Daniel Lee

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Zeolites are microporous materials widely used for adsorption and catalysis across industry. The positions and strengths of their active sites (both Brønsted-acid and Lewis-acid type (BAS/LAS)) play a key role in their properties and selectivity. Thus, it is vital to characterise these sites to elucidate their chemical environments, locations within pores, and distances from one another so that they can be optimised for desired applications. A variety of studies will be presented highlighting the importance of advanced technologies for NMR, as provided by the UK High-Field Solid-State NMR Facility, to solve structural challenges within novel catalysts.

High-field and fast magic angle spinning (MAS) NMR spectroscopy has been used to identify changes in the structure of zeolite Y induced by post-synthesis treatments aimed at improving functional properties. This enabled elucidation of local environmental changes for both Brønsted (H) and Lewis (Al) acid sites in these porous catalysts as well as evidence for synergistic effects between them. Through the combination of the high-resolution NMR spectra and catalytic activity data, structure can be related to function.

Basing design principles on zeolite catalysts, mixed-metal-doped mesoporous silica-based materials have been developed where the larger pores accommodate bigger molecules and prevent coking. Understanding the roles of the different metal sites in catalysis is just as crucial as it is for zeolites. When one or both of these metal dopants has a large quadrupole moment, high magnetic fields are vital to obtain spectra where important structural information can be extracted. Here, it will be shown how ^{93}Nb NMR has been used to determine a biomass conversion reaction mechanism using Al-Nb-MCM-41 as an efficient and product-selective catalyst.

Adsorption of pyridine and pyridine derivatives has been widely used to characterise active sites on material surfaces through FTIR [1] and NMR [2] spectroscopies, and is a relatively standard technique. Fluoropyridine molecules are also able to coordinate to active sites, and exhibit beneficial properties compared to their non-fluorinated analogues. The foundations for using these as informative probe molecules has been established using moderate- and high-field MAS NMR, as well as with low temperature (100 K) MAS NMR.

References

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Structure and Dynamics of Glass Forming Metal Organic Frameworks: NMR study of Zeolitic Imidazolate Framework ZIF-62

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Zeolitic Imidazolate Framework (ZIFs) are microporous Metal Organic Frameworks built from inorganic metal-ion nodes, such as Zn^{2+}, interconnected by organic linkers, such as imidazole (Im, $C_3H_3N_2^-$) and/or benzimidazole (bIm, $C_7H_5N_2^-$)in the case of ZIF-62. Those compounds have an intrinsically disordered structure in their crystalline form since bIm is statistical distributed around the zinc cations and are able to form glasses upon cooling from their melts. The ability to form glasses is viewed as a highly desirable property, given the potential to form moldable, porous, macroscale structures and grain boundary free membranes. They have found applications as membranes for gas separation owing to their higher porosity than their inorganic cousins and their molding ability. Due to their intrinsic disorder, the structure and dynamics of both crystalline and glassy forms are complex, and ssNMR is a unique tool to address those properties vital for the -still unknown- glass formability.

We have followed ex-situ, the structural transformation(s) of ZIF-62 occurring as a function of composition using magic-angle spinning NMR, applying a variety of experiments involving several nuclei. We address the efficiency of CPMAS (^{13}C or ^{1}H detected) and INEPT as a function of spinning speeds up to above 100 kHz and magnetic fields up to 1.2 GHz to extract the most out of those broadened lines. ^{1}H DQ/SQ experiments evidence linker proximities which suggest possible steric hindrance between linkers and high field ^{67}Zn allows discussing directly the type of structural disorder.

Although technically challenging, we also performed in-situ high-temperature deuterium MAS NMR experiments, from -173°C to the molten state (450°C). Those data, associated with EXPRESS lineshape simulations, highlight two types of movements of the Im organic linker at frequencies (in the range 10 MHz to 160 MHz) and angular amplitudes (from 5° to 25°) increasing with temperature and stabilizing in the melt. Frequency and angle composition and temperature dependence show that it is steric interactions which underpin the origin of the glass transition itself.

This study shed some light, for the first time, on the dynamics taking place in those MOFs. Unlike the inorganic-type melting behavior identified previously in the literature, their behavior upon heating through the glass transition is demonstrated to be reminiscent of organic polymers, thus significantly impacting the design of this new family of glasses in the context of processing and optimizing their applications in several fields ranging from separation to catalysis and optics.

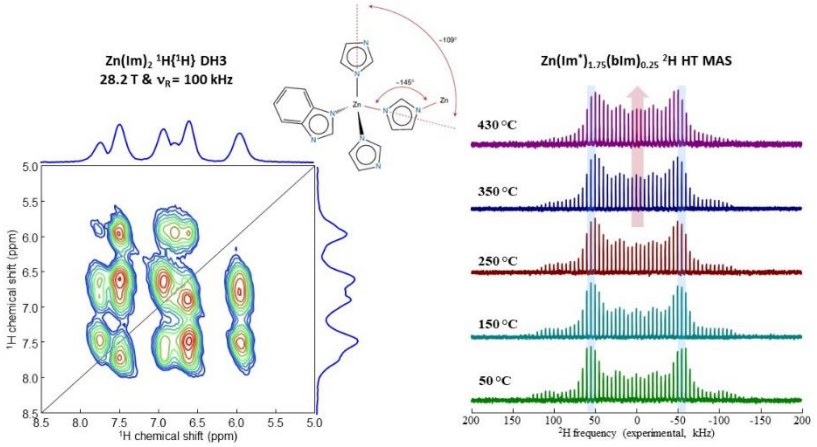

Figure: High-field/high speed DQ/1Q ^{1}H spectroscopy on crystalline ZIF-62 (left) and ^{2}H MAS NMR at several temperatures on glassy ZIF-62 (right)

In-situ Solid-State NMR Spectroscopy for the Study of Novel Zeolites

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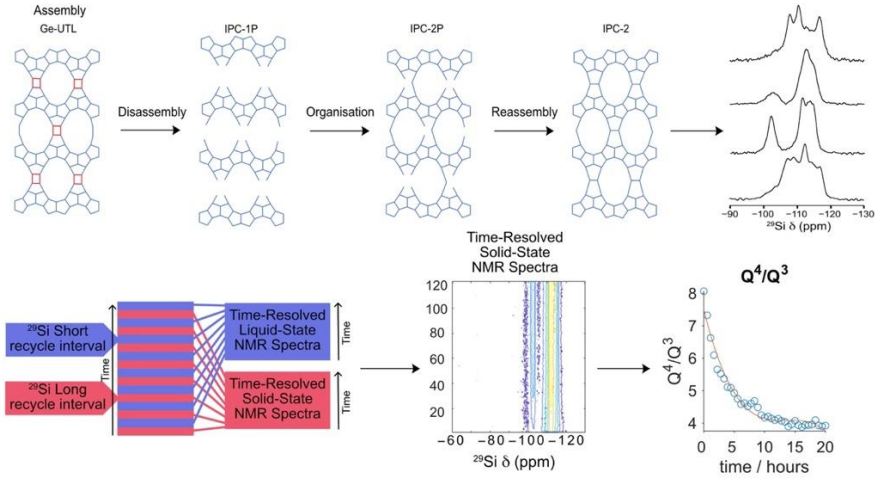
The ADOR process is an effective way of producing zeolites that would not be feasible through traditional routes.[1] The ADOR process consists of four stages, assembly-disassembly- organization-reassembly. The structure and chemistry of the parent zeolite are an important consideration, with the current focus on zeolites with silica-rich layers linked by germanium- rich cubic units. Germanosilicate zeolites are ideal for ADOR as they have hydrolytically sensitive Ge–O bonds that are preferentially hydrolysed over more stable Si–O bonds. ^{29}Si solid-state MAS NMR spectroscopy has been utilised in previous studies to investigate the ratio of Q^4/Q^3 species (which would be 2.5 and 7 for idealized IPC-1P and IPC-2P, respectively). The Q^4/Q^3 ratio can be used to track the ADOR process both ex-situ and in-situ.[2]

CLASSIC NMR (Combined Liquid- and Solid-State In-situ Crystallisation NMR) is an experimental approach that utilises the different response of solids and liquids in NMR experiments to study *in-situ* reactions.[3] CLASSIC NMR is achieved by alternating two different pulse sequences that alternate between collecting solid-state NMR and liquid-state NMR spectra. CLASSIC NMR has previously been used to study crystallisation processes and for the identification of polymorphs.

Here we implement CLASSIC NMR to study the ADOR process under different conditions to understand the effect temperature and pH have on the reaction rate and completion. In order to confirm the products of the reaction they will be compared to a model set of 4 ADOR intermediates and products. The model set has used a combination of experimental MAS NMR and powder XRD, along with periodic DFT calculations to understand the structure of the ADOR intermediates and products.



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Progress in Fast Spinning Experiments at the National Research Facility

W. Trent Franks

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I will present a brief overview of the fast-spinning probes available at the NRF and how they are used. In particular, the methods we have available to pack the tiny rotors. I will present several applications from NRF projects which include natural products, traditional biological NMR, pharmaceuticals, and materials where fast spinning was used. Finally, I will describe some new experiments that have been developed in collaborations at the NRF.

Stretchy Skin and Resilient Fungi - New Applications of ssNMR to Extracellular Matrices

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The extracellular matrix (ECM) is a complex and heterogeneous material that supports and enables life. Building on previous work on using solid-state NMR to probe collagen proteins in the ECM in bone and cartilage, we are using a similar approach to investigate Ehlers-Danlos syndrome (EDS). In patients suffering from EDS, a common symptom is overly mobile joints and stretchy skin. Using ^{13}C-labelling of EDS fibroblast culture, we aim to uncover the molecular basis underlying the key symptoms of this disease, with the eventual aim of improving diagnosis and treatment. A second novel area of application involves the fission yeast *Schizosaccharomyces pombe* which, like all fungi, has a glycan-based cell wall. *S. pombe* is not a pathogenic fungus; however, as a model organism, it is open to genetic manipulation and enables a mechanistic understanding of fungal cell wall formation that can drive the development of new antifungal drugs.

Reaction Mechanisms, Kinetics, and Nanostructural Evolution of Cements and Related Materials: Insights from High-Field Solid-State NMR

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Cement, the 'glue' in concrete, is the ubiquitous material upon which modern civilisation is built, providing long-term strength, impermeability and durability for housing and infrastructure, as well as a binding agent for immobilisation and safe disposal of radioactive and toxic wastes. The fundamental chemical interactions which control the structure and performance of cements have been the subject of intense research for decades, but the complex, crystallographically disordered nature of the key phases which form in hardened cements has caused difficulty in obtaining detailed information about local structure, reaction mechanisms, and kinetics which ultimately underpin their performance during use. Solid-state NMR spectroscopy can resolve key atomic structural details within these materials and has emerged as a crucial tool in characterising cement structure and properties. Here I will discuss recent work performed in our group at The University of Sheffield, UK, where we use high-field solid-state NMR experiments to resolve reaction mechanisms, kinetics, and nanostructural evolution of cements and related materials for applications in construction and nuclear sectors.