CCPN Conference 2015

20-22nd July 2015

Monday 20th July

12:00 Registration (Dome) / Check-in (Halls of Residence)

12:30 Lunch

13:30 Introduction (Main Lecture Theatre)

Session 1: Enhanced Sensitivity and Fast Methods for the Detection of NMR Signals.
Session Chair : Ulrich Günther

13:40 **Goran Karlsson** (University of Gothenburg)
Optimized sensitivity for protein structure determination - probe performance, NMR data acquisition and processing

14:20 **Marcel Utz** (University of Southampton)
Integration of High-resolution Nuclear Magnetic Resonance Spectroscopy with Lab-on-a-Chip Devices

14:50 Break (Tea/Coffee)

15:20 **Ericks Kupce** (Bruker BioSpin, Coventry)
New Ways to Record Multi-Dimensional NMR Spectra

15:50 **Hans Koss** (NIMR)
Merging NMR Data Dets in the Context of Structural and Functional Studies on Phospholipase Cγ and its Mutants

16:30 **Discussion**

17:00 **Geerten Vuister** (University of Leicester)
"CCPN : New Developments"

17:45 **Rasmus Fogh**
CCPN Assembly Meeting

18:15 Poster Session (Main Hall)

19:00 Conference BBQ (Outside weather permitting/Main Hall)

Tuesday 21st July

8:00 Breakfast in Bistro

8:55 Meeting Starts

Session 2 : Extracting structural and dynamics data from NMR spectra
Session Chair : Joern Werner

9:00 **Jonathan Yates** (University of Oxford)
CCP NMR Crystallography: Prediction and Interpretation of NMR parameters in Solids.

9:40 **Ilya Kuprov** (University of Southampton)
Fokker-Planck Equation as a Unifying Simulation Formalism for all Types of Magnetic Resonance that Involve Spatial Degrees of Freedom

10:10 **Alfonso de Simone** (Imperial College London)
Probing the Structure and Dynamics of Proteins using Residual Dipolar Couplings

10:40 Break (Tea /Coffee)

11:10 **Mike Plevin** (University of York)
Stereo-specific Methyl Labelling as a Tool for Assignment and NOE Measurement

11:40 **Discussion**

12:00 Lunch (Bistro)

13:30 Walk (Meet in main hall – to leave by coach at 13:30)

18:00 Poster Session

19:15 Conference Dinner and Poster Prize Presentation (Main Hall)

Wednesday 22nd July

8:00 Breakfast in Bistro

8:55 Meeting Starts

Session 3 : New tools for studying molecular structure by NMR
Session Chair : Gary Thompson

9:00 **Emeline Barbet-Massin** (Technische Universität München)
Study of Bio-macromolecular Complexes by Solid-state NMR: New Approaches and Examples

9:40 **Steven Brown** (University of Warwick)
NMR Crystallography of Organic Solids

10:10 **David Middleton** (University of Lancaster)
Paramagnetic Relaxation Enhancement as a Tool for Investigating Biomolecular Orientations by Solid-state NMR

10:40 Break (Tea /Coffee)

11:10 Theo Karamanos (University of Leeds)
Invisible States in Amyloid Assembly Visualized by Paramagnetic NMR

11:40 Discussion

12:10 Discussion Overall Meeting

12:30 Closing Remarks

12:45 Conference Ends

13:00 Lunch (if required).

Session 1

Optimized sensitivity for protein structure determination- probe performance, NMR data acquisition and processing

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In this talk I will describe the mode of operation for protein structure determination at the Swedish NMR Centre. The sample and probe geometry will be discussed as well as strategies employed in performing, processing and analyzing multidimensional NMR experiments. Pros and cons of non-uniform sampling and targeted acquisition for back-bone assignment will be discussed and exemplified, as well as strategies, pulse program modification and algorithms used for processing of 3D- and 4D-NOESY experiments.

Integration of High-resolution Nuclear Magnetic Resonance Spectroscopy with Lab-on-a-Chip Devices

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Nuclear magnetic resonance spectroscopy is one of the most versatile tools for the study of molecular structure and dynamics and the analysis of complex mixtures. It is generic (any molecule containing protons or other NMR active nuclei will yield a distinctive spectrum), and non-invasive. NMR would therefore be an ideal readout technique for microfluidic lab-on-a-chip devices, particularly ones that are designed to support live systems such as cells or tissue slices. However, the integration of NMR spectroscopy with microfluidics is challenging due to the inherently limited sensitivity of magnetic resonance experiments, and due to the requirement for extremely homogeneous magnetic fields. In this talk, I will briefly review the state of the art in this field, and present some of our recent contributions to the design of high-resolution and high-sensitivity NMR detectors and NMR-compatible lab-on-a-chip devices. We have recently succeeded in directly observing the metabolism of a population of human adenocarcinoma cells supported on a chip in a volume of only 4 µL.

New Ways to Record Multi-Dimensional NMR Spectra

Ēriks Kupče,

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The recent developments in cryoprobe technology offer significant increases in sensitivity. On the other hand, the introduction of NMR spectrometers with multiple receivers permits spectra from several different nuclear species to be recorded in parallel, and several standard pulse sequences to be combined into a single entity. These developments offer an exciting possibility of changing the conventional paradigm of NMR data mining to better optimize the efficiency of spectrometer use. It is shown how these improvements in the flow and quality of spectral information can be significantly augmented by compressive sensing techniques – controlled aliasing, Hadamard spectroscopy, random sampling, projection-reconstruction, and ultra fast NMR Spectroscopy. Future developments of these techniques are confidently expected to mitigate one of the most serious limitations in multidimensional NMR – the excessive duration of the measurements.

We also propose a new general form of two dimensional spectroscopy where the indirect "evolution" dimension is derived using the Radon transform. This idea is applicable to several types of spectroscopy but is illustrated here for the case of NMR spectroscopy. This "projection spectroscopy" displays characteristic correlation peaks that highlight perturbations of chemical shifts caused by temperature, pressure, solvent, molecular binding, chemical exchange, hydrogen bonding, pH variations, conformational changes, or paramagnetic agents. The results are displayed in a convenient format that allows the chemist to see all of the chemical shift perturbations at a glance and assess their rates of change and directions. As a proof of principle, we present several simple, practical examples that display two-dimensional representations of the effects of temperature and solvent on NMR spectra.

Merging NMR data sets in the context of structural and functional studies on phospholipase Cγ and its mutants.

Hans Koss,

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Phosphatidylinositol phospholipase Cγ (PLCγ) is an intracellular membrane-associated second messenger signalling protein, which is activated by tyrosine kinases, such as fibroblast growth factor receptor 1 (FGFR1). PLCγ contains the pseudocyclic γ-specific array (γSA), in which a SH2 domain pair (tandem-SH2), a SH3 domain and a split-PH domain are connected by linker segments, effectively within a loop that is an insert in the catalytic domain. Activated FGFR1 binding to the nSH2 domain phosphorylates Y783, a residue in the cSH2-SH3 linker. This modification terminates PLCγ autoinhibition by the cSH2 domain and reduces nSH2 affinity to FGFR1 (1). Crystallographic studies did not reveal static structural features explaining how this release of FGFR1 from nSH2, triggered by Y783 phosphorylation in the cSH2-SH3 linker, is brought about. The communication of Y783 phosphorylation through the cSH2 domain and between the both SH2 domains is being studied to

understand FGFR dissociation, cSH2 release as well as activating mutations in PLCγ which are relevant for human disease including cancer and autoimmune disease (2), (3).
Molecular dynamics simulations yielded hypotheses about ligand-dependent allosteric interdomain communication within tandem-SH2. We have tested these hypotheses experimentally and we are investigating the structural, functional, and dynamic properties of tandem-SH2 and its mutants in non-phosphorylated and phosphorylated states. We collected evidence for a structural change of tandem-SH2 in solution upon phosphorylation, which might lead to FGFR kinase release. We also investigated the role of the cSH2-Y783 linker in this context. Experimental approaches include residual dipolar coupling experiments, chemical shift perturbation analysis, paramagnetic relaxation enhancement (spin labelling and solvent PRE), relaxation data analysis, in-tube (de)phosphorylation assays, ITC and SAXS. We are now also classifying disease-relevant mutants based on their structural and functional effects in context of both the tandem-SH2 construct and the gamma-specific array.

Peak broadening due to intermediate exchange, partial dimerization of some constructs and sample instability hampered resonance assignment and NMR data analysis. For that reason, the possibility of merging NMR data sets was exploited. Specifically, non-equivalent triple resonance data were combined where feasible, and time resolution of in-tube (de)phosphorylation assay datasets was adjusted during or after data acquisition.

(1) Bunney TD, ..., Driscoll PC, Katan M. Structure 2012. 20(12), 2062.
(2) Behjati S, …, **Koss H**, ..., Katan M, …, Campbell PJ. Recurrent PTRB and PLCG1 mutations in angiosarcoma. Nature Genetics 2014, 46(4).
(3) **Koss H**, Bunney TD, Behjati S, Katan M. Dysfunction of phospholipase Cγ in immune disorders and cancer.

Session 2

CCP NMR Crystallography: Prediction and Interpretation of NMR parameters in Solids

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In recent years in has become possible to calculate NMR parameters in solid materials using first-principles quantum mechanical simulations[1]. Calculations provide full tensorial information on the chemical shielding, electric field gradient, direct and indirect (J) spin-spin coupling. Work is on-going to improve the accuracy of such simulations - for example, incorporating the effects of dynamics and disorder - as well as increasing the size of the systems which can be addressed. In support of this work CCP-NC is also developing tools to aid the visualisation and processing of the data from simulations.

[1] See e.g. Chemical Reviews 112 (11), 5733 (2012)

Fokker-Planck equation as a unifying simulation formalism for all types of magnetic resonance that involve spatial degrees of freedom

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Fokker-Planck equations that describe the time evolution of probability distributions [1,2] were first used in a magnetic resonance context in the 1970s as part of the formalism that became known as the Stochastic Liouville Equation (SLE) [3-5]. To this day SLE remains the most general relaxation theory in magnetic resonance – it is non-perturbative and works at all magnetic fields and correlation times, from extreme narrowing to the solid limit.

Initial adoption of Fokker-Planck equations was complicated by large matrix dimensions and subsequent work had to rely heavily on sparse matrix libraries and Lanczos techniques [3], but the exponential rise in computing power in the following 40 years has removed the problem – the same calculations take just a few seconds on a contemporary workstation. The advances in computing power make the Fokker-Planck formalism worth re-visiting. One particular feature that to our knowledge remains unexplored in magnetic resonance is the possibility of treating *any* spatial dynamics that can be generated by a linear operator – the formalism itself is not restricted to diffusion.

Although matrix dimensions do increase, the reduction in *formulaic* complexity is very remarkable: MAS, DOR, diffusion, PFGs, hydrodynamics, polychromatic irradiation and other methods that normally introduce inconvenient time dependence into the Hamiltonian become, within Fokker-Planck formalism, *time-independent operators* that are simply added to the background evolution Liouvillian. Even spherical grids are no longer needed – the Fokker-Planck formalism can solve directly for powder averages [6].

[1] A.D. Fokker, *Die mittlere Energie rotierender elektrischer Dipole im Strahlungsfeld*, Annalen der Physik 348 (**1914**) 810-820.

[2] M. Planck, *Über einen Satz der statistischen Dynamik und seine Erweiterung in der Quantentheorie*, Sitzungsber. Kön. Preuss. Akad. Wiss. (**1917**) 324-341.
[3] G. Moro, J.H. Freed, *Calculation of ESR Spectra and Related Fokker-Planck Forms by the Use of the Lanczos Algorithm*, Journal of Chemical Physics 74 (**1981**) 3757-3773.
[4] R.P. Mason, J.H. Freed, *Estimating Microsecond Rotational Correlation Times from Lifetime Broadening of Nitroxide Electron-Spin Resonance-Spectra near Rigid Limit*, Journal of Physical Chemistry 78 (**1974**) 1321-1323.
[5] J.H. Freed, G.V. Bruno, C.F. Polnaszek, *Electron Spin Resonance Line Shapes and Saturation in Slow Motional Region*, Journal of Physical Chemistry 75 (**1971**) 3385.
[6] L.J. Edwards, D.V. Savostyanov, A.A. Nevzorov, M. Concistre, G. Pileio, I. Kuprov, *Grid-free powder averages: on the applications of the Fokker-Planck equation to solid state NMR*, Journal of Magnetic Resonance 235 (**2013**) 121-129.

Probing the Structure and Dynamics of Proteins using Residual Dipolar Couplings

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The importance of protein dynamics as fundamental modulators of many biological processes is largely acknowledged. In this context, RDCs are emerging as powerful NMR measurements to accurately characterise protein structure and dynamics, however, their employment remains challenging because of the complex relationship between molecular structure and coupling values. A promising approach to decode this relationship involves structure-based calculations of the alignment tensors of protein conformations. By implementing this strategy to generate structural restraints in ensemble-averaged molecular dynamics simulations, it is possible to employ RDCs to characterise structural fluctuations in the native states of proteins. The talk will introduce pros and cons of this approach and show recent applications elucidating the role of structural dynamics in protein-protein interactions, enzymatic catalysis and membrane proteins. In the latter case, by extending the approach to oriented solid-state NMR, it is possible to achieve a remarkable step forward in studying membrane proteins under conditions that closely mimic the physiological environments.

Stereo-specific methyl labelling as a tool for assignment and NOE measurement

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Nuclear magnetic resonance (NMR) spectroscopy is a uniquely powerful tool for studying the structure, dynamics and interactions of biomolecules at atomic resolution. The previous 20 years have seen the development of a number of specialist isotopic labeling strategies that continue to extend the upper molecular weight limit of solution NMR spectroscopy of proteins. At the core of these labeling developments is the specific introduction of [^{1}H,^{13}C]-

labeled methyl probes into perdeuterated proteins. Typically, *E. coli* are grown in a minimal media supplemented with precursors of methyl group-containing amino acids. An array of amino acid precursors with different isotopic labelling patterns is now commercially available. As well as enabling NMR analysis of very large systems, methyl-labelled metabolic precursors can also enrich NMR studies of smaller proteins. In this talk I will provide examples of how methyl-labelling approaches can aid a wide-range of protein NMR studies, including stereo-specific assignment of prochiral methyl resonances, simplification of crowded NMR spectra, precise measurement of inter-proton distances, and the detection of very long range NOEs.

Session 3

Study of bio-macromolecular complexes by solid-state NMR:

New approaches and examples

Dr. Emeline Barbet-Massin

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Solid-state NMR has recently emerged as a key technique in modern structural biology, by providing information at atomic level for the characterization of a wide range of systems that cannot be investigated by other atomic-scale methods. There are now well established protocols for sample preparation, resonance assignment and collection of structural restraints, that have paved the way to the first three-dimensional structure determinations at atomic resolution of biomolecules in the solid state, from microcrystalline samples to fibrils and membrane-associated systems. These determinations are however still far from being routine, and larger breakthroughs are expected with further methodological and hardware developments. Accordingly, I will present new, sophisticated NMR experiments that improve the sensitivity and resolution of the standard existing schemes for resonance assignment. These schemes notably rely on the use of small rotors capable of spinning at ultra-fast magic-angle spinning rates (MAS at 60 kHz). I will show the great potential of this particular regime, which enables the use of low-power experiments and the acquisition of selective cross-polarization transfers, through-bond correlations and ^{1}H-detected correlations. Altogether, the resulting gains in sensitivity and resolution extend the capabilities of protein solid-state NMR for the analysis of large substrates. I will show applications on a range of large non-crystalline biological systems, such as viral nucleocapsids, protein fibrils or ribosome complexes.

NMR crystallography of organic solids

Prof. Steven Brown

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Working at high magnetic field results in increased sensitivity and resolution in solid-state NMR spectra. The UK 850 MHz Solid-State NMR Facility (http://go.warwick.ac.uk/850mhz) has been operational since 2010 and has enabled applications across chemistry, materials science, life sciences and environmental sciences.

This presentation will focus on applications of advanced solid-state NMR methods for probing intermolecular interactions, notably hydrogen bonding, with two-dimensional high-resolution ^{1}H experiments [1] being shown to be particularly powerful. Homonuclear ^{1}H-^{1}H double-quantum (DQ) experiments reveal proximities (typically under 3.5 Angstroms) among pairs of hydrogen atoms, for example identifying an anhydrous or hydrate form of an active pharmaceutical ingredient in a tablet formulation [2] or distinguishing between ribbon-like or quartet-like self assembly in guanosine supramolecular structures [3,4]. ^{14}N-^{1}H spectra show one-bond NH connectivities or additionally longer-range NH proximities depending on the recoupling time employed. Applications include to guanosine self assembly [3,4] and proving molecular level mixing in pharmaceutical co-crystals [5,6] and a

pharmaceutical amorphous dispersion [6]. In the emerging novel NMR crystallography concept (in 2014 recognised as a new sub area by the International Union for Crystallography and by 5 years renewal funding from EPSRC for the national collaborative computational program for NMR crystallography, www.ccpnc.ac.uk), experimental solid-state NMR is complemented by first principles calculations of NMR parameters using the GIPAW (gauge-including projector augmented wave) DFT density-functional theory (DFT) planewave approach that is particularly suited to periodic solids. Recent results applying ^{13}C refocused INADEQUATE spectra to characterize biopolymer interactions in plant cell walls will also be presented [7].

References

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Paramagnetic relaxation enhancement as a tool for investigating biomolecular orientations by solid-state NMR

David A. Middleton

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NMR relaxation enhancement by paramagnetic metals has been used for many years to provide restraints on the three-dimensional structures of proteins in solution, and more recently has been exploited for a range of applications in solid-state NMR. This talk will present examples of our work utilising paramagnetic relaxation enhancement (PRE) by Mn^{2+} in combination with cross-polarisation magic-angle spinning solid-state NMR to determine molecular orientations within biological membranes and in self-assembled peptide nanostructures. PRE measurements supplemented with observations of dynamically-scaled ^{1}H-^{13}C dipolar couplings have been used to determine the orientation and anisotropic motions of the lipophilic antipsychotic drug trifluoperazine in phospholipid bilayers. We have also used PRE to investigate the interaction of a ^{13}C labelled cardiotonic steroid with the inhibitor site of the Na,K-ATPase ion-pumping enzyme embedded in its native membrane. The inhibitor, a diacetonide derivate of the cardiac glycoside ouabain is 1000-fold less potent than ouabain and PRE reveals that the acetonide modifications cause the inhibitor to adopt an inverted orientation in the binding pocket. Finally it will be shown how PRE is used to probe the accessibility of Mn^{2+} to peptide nanotube assemblies, revealing a bilayer arrangement in the nanotube walls. In each example, PRE measurements have provided valuable restraints on molecular orientation that cannot easily be obtained by other means.

Invisible states in amyloid assembly visualized by paramagnetic NMR

Dr Theodore Karamanos

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Amyloid diseases are devastating disorders caused by the polymerization of initially innocuous proteins into amyloid fibrils. Recent advances in electron microscopy and solid state NMR have allowed the characterization of amyloid fibril structures to different extents of refinement. However, structural details about the mechanism of fibril formation remain relatively poorly-defined. This is mainly due to the complex, heterogeneous and transient nature of the species responsible for assembly; properties that make them difficult to detect and characterize in structural detail using biophysical techniques. The ability of paramagnetic NMR (paramagnetic relaxation enhancement-PRE) to investigate species that are transiently and lowly populated in atomic detail renders PRE as an invaluable technique for studies of amyloid assembly. Here, we have used intermolecular PRE to study the rare biomolecular interactions that lead to enhancement or inhibition of the aggregation of human β2-microglobulin (β2m) by transient interaction with structurally similar β2m variants. Semi-quantitative analysis of the PRE data revealed key structural differences in the interfaces of different complexes that dictate the fate of assembly, providing crucial insights into the early events during the amyloid cascade.