CCPN Conference 2014 Poster Abstracts

Yaser Alnaam

Effects of dietary fats on heart-liver lipids compositions: analysed by nuclear magnetic resonance

Accumulation of lipids in various organs cause many diseases such as obesity, heart failure, dysfunction of liver metabolism. These diseases are vital cause of death in the developing and developed countries. A key process in the pathogenesis of lipid accumulation is the development of various physiological and metabolic processes, triggered by several independent risk factors such as myocardiac infraction, hypertension and liver sclerosis. Exposure of the surplus lipids, as occurs in obesity, may lead to long term deregulation of lipid uptake and metabolism resulting in inappropriate lipid accumulation, cell death and further cause lipid toxicity in various organs such as liver and failure of heart. The aim of this project was to investigate the lipid composition of fat diet co-related with heart disease, toxicity in liver. Pressure overload hypertrophy was induced surgically in male Sprague-Dawley rats by abdominal aortic constriction. Animals were maintained for 9-12 weeks post-surgery and fed either standard diet (7.4% kcal from fats) or western diet (45-60% kcal from fat). Subsequently, lipid contents of heart-liver tissues were investigated with 1H NMR and 31P NMR spectroscopy and data are statically analysed by PCA (principle component analysis).

Stefi Benjamin

Comparison of the use of CCPNmrAnalysis and NMRView softwares for structure determination of PfIMP1

Immune Mapped protein 1 (IMP1) is a soluble protein that is conserved across apicomplexan parasites. The structure and function of this protein is unknown. Recent studies have shown that when mice were vaccinated with a DNA vaccine of *Toxoplasma gondii* IMP1, they had a longer life span and increased immunity against *Toxoplasma gondii* infections compared to mice which were not vaccinated (Cui X et al, 2012). Similar results were found with *Eimeria tenella* homologue of IMP1. When a fragment of EtIMP1 was fused with an adjuvant and used as a vaccine in chickens, the vaccinated chickens had higher immunity against *Eimeria tenella* infections (Yin G, et al, 2013). These studies show that the IMP1 homologue improves the immunity of the host against infections in a species-specific manner. In this study, we studied the Plasmodium falciparum homologue of IMP1. *Plasmodium falciparum* is an apicomplexan parasite that is responsible for causing malaria in humans. It is the deadliest of the five *Plasmodium* species and is responsible for majority of deaths caused by cerebral malaria (Sherman IW, 1998). Structure determination of PfIMP1 was performed using NMR followed by data analysis using CCPNmr Analysis and NMRView. This poster will show a comparison of CCPNmr Analysis and NMRView and discuss the pros and cons of both softwares in structure determination.

Sandra Berndt

Activation dependent conformational changes of the beta-1 adrenergic receptor visualized by methionine NMR spectroscopy

The beta-1 adrenergic receptor is a typical member of class A G protein-coupled receptors (GPCRs). This membrane protein is involved in the regulation of peripheral blood circulation, muscle contraction, central neuronal activities and also in metabolic regulation. Improved ligands could be used for the selective treatment of asthma, hypertension and even cardiac dysfunction. Hence, increasing the mechanistic understanding by characterizing the structural and dynamical changes of this pharmacologically important receptor during the activation process is key. The functional expression of this receptor was achieved using recombinant baculovirus-infected insect cells. Receptor functionality was verified by radioligand binding. A thermostabilized functional beta-1 adrenergic receptor was used in LMNG-(Lauryl Maltose Neopentyl Glycol)-micelles. These detergent molecules form stable micelles, which are suitable for solution NMR spectroscopy studies. Methyl groups show favourable relaxation properties for NMR spectroscopy. Isotopic selective labelling of methionine residues was performed by the addition of 13C-labelled methionine during the protein expression. Activation of the receptor through different agonists (inverse, neutral, partial, biased and full) and in the presence of a G protein-mimicking nanobody results in significant chemical shift changes of the methionine residues in $^{1}H,^{13}C$-HMQC (Heteronuclear Multiple Quantum Coherence) NMR experiments. The changes are the result of major structural rearrangements taking place in the ligand bound adrenergic receptor. Our study offers insight into the dynamical properties and the conformational mobility during the receptor activation process. This work is supported by BBSRC Grant BB/K01983X/1.

Eve Blumson

The structure of the type III connecting segment (IIICS) of fibronectin

The type III connecting segment (IIICS) domain of fibronectin (Fn) is able to facilitate the adherence and spreading of leukocytes and melanoma cells. Fn is mainly composed of three repeated domains, FI, FII and FIII, with the IIICS located between the 14th and 15th FIII domains (FIII14 and FIII15). The structure of the 13kDa IIICS is unknown, with it having no sequence homology to any of the repeated domains. Interestingly, the IIICS can be divided into three subdomains, which combine via alternative splicing to give one of five variants in human Fn. The full-length IIICS contains two integrin binding sites and proteoglycan binding site, which are able to facilitate its cell binding properties. The structure of the IIICS has been investigated by NMR using a 55kDa construct containing the full length IIICS flanked by three FIII domains (FIII12-14) at the N-terminus and the FIII15 domain at the C-terminus. The structure of FIII15 is also unknown, but it is predicted to be lacking an N-terminal strand that would allow it to form a typical FIII fold of a seven-stranded beta sandwich. A triple labelled sample of this construct was expressed and used to record a standard set of triple resonance experiments.

Through these experiments almost complete backbone assignment of the IIICS and FIII15 domain was possible. Peaks from residues in the FIII12-14 domains were not visible in these experiments. The dynamics of the construct were investigated by recording ^{15}N relaxation experiments on a ^{2}H, ^{15}N labelled sample. This data showed the IIICS to have increased flexibility compared to the FIII15 domain. Analysis of the chemical shift data suggests that the FIII15 module forms an six-stranded beta sandwich fold and that the IIICS does not contribute the seventh strand to form a typical FIII fold. The chemical shift data also suggests that the IIICS contains areas of structure within it. Further experiments will involve generating structural restraints, initially by side chain assignment and NOESY experiments.

Fedir Bokhovchuk

Structural and functional aspects of TRPV5 channel regulation

The epithelial Ca^{2+} channel TRPV5 (Transient Receptor Potential Vanilloid 5) is mainly expressed in the kidney, where it plays an essential role in Ca^{2+} homeostasis. The activity of TRPV5 and, therefore, Ca^{2+} influx, is tightly regulated by various hormonal stimuli mediated by interactions with numerous intracellular binding partners. Previously, we have shown that the Ca^{2+}-binding protein Calmodulin (CaM) is directly involved in the Ca^{2+}-induced inactivation of TRPV5 [1]. *In silico* analysis of TRPV5 identified further six putative CaM binding sites, which could also be involved in channel regulation and CaM binding sites 1 and 2 were studied in detail (Fig.1) [2]. Calmodulin has 4 EF-hand motifs, each of which binds a Ca^{2+} ion, located on two approximately symmetrical globular domains (the N- and C-domain). C-domain EF-motifs have a higher affinity for Ca^{2+} compared to N-domain, which highlights the role of CaM as a sensor of intracellular Ca^{2+}. The focus of the current work is to determine how the CaM binding site 1 interacts with the CaM N- and C-domains and thus, to provide new insights into the regulation of TRPV5 by CaM under different Ca^{2+} conditions.

Louise Bolton

Structural Plasticity and Antigen Selection by MHC class I

The selection of immunogenic peptides by MHC class I molecules in the endoplasmic reticulum has major implications for immune system stimulation and may present an intervention point to treat disease. We have developed a systems biology model for antigen processing that allows us to identify critical molecular parameters from complex biological experiments [1]. Mature MHC class I on the cell surface contains high-affinity peptide but has a structure almost identical to MHC class I in complex with low-affinity peptides that are retained in the ER, indicating that cellular quality control is not based on the structure of the final complex. We hypothesis that it is the structural dynamics of MHC class I that play a central role in determining peptide selection. We are investigating the structural dynamics of the 45kDa MHC-peptide complex, H2-Db-FAPGNYPAL, using NMR and molecular dynamics simulations. The backbone atom assignment of MHC class I, using triple resonance experiments and amino acid-selective labelling strategies, allows us to study the

structure and dynamics of this complex using residual dipolar coupling and heteronuclear relaxation data. The data suggest the presence of significant local and inter-domain flexibility in both the H2-Db and β2m subunits, as well as differences in inter-domain orientations with regards to the crystal structure. Extensive molecular dynamics simulations of H-2Db suggest allosteric coupling between the peptide binding site and the membrane-proximal α3 domain. Taken together, the data suggest that MHC class I exhibits significant internal dynamics on a wide range of timescales, and that dynamic coupling of these motions has functional implications for the selection of high affinity peptides. [1] N. Dalchau et al 2011, PLOS Comput. Biol. 7, e1002144

Mark Bostock

Influence of the sampling properties on the quality of compressed sensing reconstructions

Mark Bostock and Daniel Nietlispach

Multidimensional experiments are indispensable to acquire high quality structural data for biomolecules. Conventionally, such experiments are recorded with Nyquist sampling and processed using the Fourier transform (FT), leading to lengthy experiment times and limiting sensitivity and resolution. Increasingly, undersampling approaches are favoured along with non-FT reconstruction allowing increases in sensitivity and resolution or reduced experiment time, of particular benefit to studies of high molecular weight systems.

We and others have pioneered application of the compressed sensing reconstruction approach, using l1- norm minimisation, to NMR spectra, demonstrating high quality reconstructions which faithfully reproduce spectral frequency, linewidth and intensity information.[1–4] However, it is known that the sampling schedule can have a strong influence on the quality of the reconstruction. Exponential sampling[5] and weighted Poisson Gap sampling[6] have particularly favourable properties for NMR. Nevertheless, within a particular family of sampling schedules, variability in the schedule quality due to the seed for the random number generator has also been reported.[6] Consequently criteria to distinguish a high quality schedule from a poor schedule are essential to minimise spectral artefacts. Data is presented for exponential and Poisson schedules demonstrating a variety of possible scoring methods for sampling schedules, building on work by Aoto et al.[7], as well as the differing dependence of reconstruction quality on the underlying sampling schedule.

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Eva Caamano-Gutierrez

Unraveling the mysteries of the metabolism of Plasmodium falciparum

The most deadly malarial parasite *Plasmodium falciparum* has a complex life cycle with different stages and hosts. Furthermore, the ability of *P. falciparum* to adapt in response to the different environmental conditions results in a huge plasticity in its metabolic make-up. Similarly than other rapidly proliferating cells such as cancer cells, *P. falciparum* in its intra-erythrocytic stage has a highly effective glycolysis with an uptake to up to 100x more glucose than uninfected erythrocytes. However, it does not present a proportional flux to TCA cycle. We hypothesize that this high glucose uptake yet low TCA activity is due to *P. falciparum* redirection of glycolytic intermediates into anabolic reactions to provide the 'building blocks' for biomass generation (nucleic acids, fatty acids, isoprenoids, etc.) together with an effective reduction in the production of ROS (Reactive Oxygen Species) as a consequence of a less active TCA. By inducing changes in the environment/culture medium we hope to test the commitment to schizogony and thus the amount of biomass/daughter cells produced. We are monitoring by ^{1}H 1D NOE and ^{1}H ^{13}C 2D HSQC the quantities of various metabolic components during the 48h intra-erythrocytic life cycle of the parasite after enrichment with U-^{13}C glucose. Identification and quantification of the most abundant metabolites has been achieved by combination of the standard CCPNmr metabolomics project, the Madison Metabolomics Consortium database and in-house acquisition of calibrated standards. Here we discuss our preliminary findings and the limitations associated with this process.

Hugh Dannatt

Ultra-fast magic angle spinning solid-state NMR of 7-helix trans-membrane proteins

There is great interest in obtaining high-resolution structural data on 7-helix trans-membrane (7TM) proteins due to their importance as drug targets. However, crystallisation of these proteins is extremely challenging, and relies on the use of conditions that differ greatly from those found in native membranes. Solution NMR presents similar difficulties since non-native detergent environments are used in order to solubilise the molecules. Solid-state NMR has the potential to provide high-resolution structural information within a native or native-like membrane environments, preserving the native structure and function of the molecules in question. A key problem with all methods for protein structure determination is the large amount of sample required, which contrasts with the typically very low expression levels exhibited by membrane proteins. Recent solid-state magic-angle spinning (MAS) solid-state NMR studies of fibrillar and microcrystalline proteins have shown that high-quality spectra can be obtained using ultra-fast spinning of samples which contain very small amounts (<4 mg) of protein. In addition to allowing the use of low-power carbon-detected pulse sequences, the ultra-fast MAS regime also enables the use of more sensitive proton-detected solid-state NMR methods. Here we use MAS frequencies of 60 kHz in combination with high magnetic fields to acquire low-power ^{13}C- and ^{1}H-detected experiments of the well-characterised bacteriorhodopsin 7TM protein in its native purple membranes. We

demonstrate that ^{13}C-detected experiments under these conditions yield sensitive spectra with comparable resolution to those recorded under moderate spinning speeds. Furthermore, bacteriorhodopsin yields excellent 1H-detected spectra at 60 kHz. These results show that ultra-fast MAS frequencies may open the door to the study of more challenging and previously inaccessible membrane protein systems.

Oliver Deacon and Andreas Ioannis Karsisiotis

Fat(al) attraction of cytochrome c: A new approach to study protein-lipid interactions

Proteins are the fundamental biological molecules that control cell function. Phospholipids, a type of fat, can modulate protein function and signal major biological events. An example is the phospholipid, cardiolipin, which binds to the protein cytochrome c and triggers programmed cell death. To understand how the interaction retunes the protein's function, it is necessary to understand the details of how the protein binds the phospholipid. This is very difficult to determine by conventional means. Here, we propose a new NMR structure-based method using an interrupted hydrogen deuterium exchange approach, that will both inform our cytochrome c research, and have applications in a wide variety of other biological systems.

Claudio Dos Santos

An NMR-based Kinetic Isotope Assay of Enzyme-Catalysed Methyl Transfer Reactions

Catechol-O-methyltransferase (COMT) performs an important role in the metabolism of catecholamine neurotransmitters, by transferring a methyl group from S-adenosyl-Methionine [(-) SAM] to catechol substrates such as dopamine. In humans, COMT is used as a drug target for the treatment of Parkinson's diseases in combination with the decarboxylation inhibitor catecolamine. However, this approach can be improved but new drug design requires a better understanding of this methyl transfer reaction mechanism. An invaluable physical organic methodology that provides insight into chemical and biological reaction mechanisms is that of kinetic isotope effect (KIE) measurement and analysis. Different reaction features such as geometry, extent of bond cleavage and formation can be provided by measuring reaction rate changes by isotopic substitution of heavy atoms involved in the rate-limiting step of the reaction. The experimental challenge has been to design a sufficiently sensitive method using nuclear magnetic resonance (NMR) ^{1}H spectroscopy that can measure relatively small ^{13}C isotope effects accurately, using a mixture of ^{12}C and ^{13}C labeled SAM (synthesised using SAM synthetase) and different catechol substrates. The methyl groups of ^{12}C and ^{13}C [(-) SAM] produce 1H NMR resonances, which are resolved and their intensity can therefore be determined. The rate of consumption of ^{13}C-SAM and ^{12}C-SAM during the COMT-catalysed reaction was determined and remaining fractions of the two isotopes species allows the calculation of the kinetic isotope effect using competitive KIE analysis. We will discuss the measured KIEs in the context of the COMT reaction mechanism.

Aleksandra Dziewulska

Three types of intra-domain crosslinks in an S. pneumoniae TIEd protein

Streptococcus pneumoniae (pneumococcus) is the main agent causing community-acquired pneumonia, and the most common respiratory pathogen in the United Kingdom and worldwide. Like other pathogenic bacteria, pneumococci rely on surface proteins to facilitate adhesion to and invasion of host cells. The TIE protein from *S. pneumoniae* is the first representative of a new class of Gram-positive surface proteins that comprise domains containing three different self-generating crosslinks between amino acid side chains, namely thioester, isopeptide and ester bonds. NMR is used among other techniques for the characterisation of TIE domains.

John Edwards

Covalent Binding of Gram-Positive Thioester Domains to a Host Protein.

Authors: John Edwards, Aleksandra Dziewulska, Su-Yin Kan, Miriam Walden, Mark Banfield, Uli Schwarz-Linek. Gram-positive pathogens are responsible for a wide range of diseases including lethal invasive infections, and represent a major health concern due to growing resistance to antimicrobial agents and lack of effective vaccines. A critical step in infection by these pathogens is the adhesion of the bacteria to host cells, which is generally thought to be mediated by non-covalent molecular recognition. Bacterial adhesins are often presented at the tip of pili or surface-associated proteins, projecting out from the cell surface. Structural studies of pilus proteins in *Streptococcus pyogenes* have discovered unusual internal thioester bonds formed from the side chains of cysteine and glutamine residues. These bonds have only been observed previously in complement proteins, where they react to form covalent links to the surface of pathogens. Their presence in surface associated proteins of pathogens suggests a similar mechanism for covalent attachment of bacteria to host tissue. We have predicted and confirmed the presence of thioester domains (TEDs) in cell wall anchored proteins from a wide variety of Gram-positive pathogens, including *S. pyogenes*, *Streptococcus pneumoniae* and *Clostridium difficile*. NMR was used to assess the role of the thioester bond in TEDs for protein structure and stability. Conformational changes of TEDs upon thioester opening by small nucleophiles were studied. Using a doubly-covalent pull-down assay a first target for a bacterial TED was identified in a complex biological sample. Lysine side chains have been identified as the most probable reaction partners on the host protein. Covalent bond formation between the binding partners was strictly dependent on the thioester, and removal by point mutation of the cysteine residue greatly reduced the ability of the bacterial protein to bind to its target.

Luke Evans

Using Synchrotron Radiation Circular Dichroism (SRCD) to Probe the Structure and Interactions of Membrane Proteins

Membrane proteins form a large percentage of total cellular proteins and also account for a majority of drug targets. Despite this, difficulties in their expression, purification and characterisation, mostly as a result of their inherent hydrophobicity, means relatively few membrane proteins structures have been solved compared to soluble proteins. Available structures solved by crystallography and solution state NMR provide valuable information but typically do not examine the protein in a native lipid environment, and instead rely on detergent. We aim to combine solid-state NMR with SRCD to characterise both the structure of membrane proteins and their interactions within a physiologically relevant lipid environment.

Stuart Findlow

Role of NS4B in remodelling the ER membrane and implications for the viral lifecycle

Approximately 170 million people are chronically infected with Hepatitis C virus worldwide. Mortality rates are high, no prophylactic vaccines exist and standard treatment is only effective in 50% of patients. In this study we apply deuterium and phosphorous solid state NMR to investigate the viral protein NS4B which is involved in membranous web (MW) formation. MWs are altered intracellular membranes that form multi-vesiculated structures that are the site of viral RNA replication. The MWs are thought to provide a scaffold for the replication complex and protect the viral RNA from host defence mechanisms. NS4B remodelling of membranes is thought to occur by NS4B oligomerisation with the amphipathic alpha helix AH2 being a major determinant. Here we present our findings on the AH2 component of NS4B; its interaction with and effects on variably charged membranes, its oligomeric state and we demonstrate our results are consistent with AH2's importance within NS4B for remodelling ER membranes and coordinating NS4B's oligomeric state and thus its structure.

Jaelle Foot

Structural investigation of the RNA binding protein Sam68

The STAR (Signal Transduction and Activation of RNA) family of proteins provide a clear link between alternative splicing and signal transduction [1]. In particular, Sam68, the best-characterized member of this family, is subject to various post-translational modifications and affects a diverse range of cellular processes such as cell cycle regulation, apoptosis and differentiation, through its direct involvement in RNA export, signal transduction and alternative splicing. It has been shown that Sam68 has enhanced splicing activity following Serine/Threonine phosphorylation by kinases such as ERK, Cdc2 and Nek2. It is unclear, however, whether this affect is due to changes in RNA binding or in interactions with other members of the spliceosome. We have shown by radioactive in vitro kinase assay that residues within the STAR (RNA binding) domain of Sam68 are phosphorylated by these

kinases. Following assignment of a portion of the STAR domain, we are using NMR as a tool to determine specific sites of phosphorylation. In addition, we are investigating the mechanism of RNA binding to Sam68 using NMR and other techniques.

Alexander Geddes

High pressure NMR as a probe of conformational dynamics relevant to catalysis in pentaerythritol tetranitrate reductase

Pentaerythritol tetranitrate reductase (PETNR) is a flavin mononucleotide (FMN) containing oxidoreductase enzyme that utilizes a reduced nicotinamide adenine dinucleotide phosphate (NADPH) coenzyme for hydride donation, and catalyses the reduction of a chemically diverse range of unsaturated alkene moieties. H-transfers in the catalytic cycle of PETNR are thought to be initiated through transient modulation of the hydrogen transfer distance, through high frequency bond-stretch motions, which permit hydride transfer to occur via a tunnelling mechanism. To investigate high frequency motions involved in promoting H-transfer via tunnelling, the structure of PETNR in the presence of oxidative substrate, 2-cyclohexen-1-one, was examined via heteronuclear TROSY-NMR. Following the identification of dynamic side-chains located proximally to the site of catalysis, mutagenesis and a subsequent stopped flow characterisation of reductive kinetics observed with PETNR-variants was undertaken. Modification of residues at certain loci appears to have resulted in a modulation of the temperature dependence of the kinetic isotope affect observed for H-transfer. This indicates the importance of such side chains for aligning donor and acceptor groups in the reactive complex prior to tunnelling and may highlight their involvement in catalytically pertinent high-frequency motions. Further investigation of high-frequency motions in PETNR:substrate complexes will involve tandem high pressure NMR studies. This approach will be used to interrogate how internal bond compressibility is modulated in enzyme:ligand complexes and thus, could provide a framework for describing fast timescale dynamic events that occur along the reaction coordinate at an atomic level of detail.

Alice Goode

FUNCTIONAL AND STRUCTURAL STUDIES OF TWO UBA DOMAIN MISSENSE MUTATIONS OF SEQUESTOSOME 1 (SQSTM1) IN PAGET'S DISEASE OF BONE (PDB)

Mutations within the SQSTM1 gene occur in 25-30% of patients with familial Paget's disease of bone (PDB). Over 30 mutations have been described commonly affecting the C-terminal ubiquitin-associated (UBA) domain of the SQSTM1 protein. Two PDB patients were recently identified in an Italian cohort with a C1320A base mutation within the UBA domain (A427D), associated with a particularly severe phenotype involving 7.00 ± 2.8 affected pagetic sites. Another study identified five PDB patients from the UK (from a total of 80 patients with SQSTM1 mutations) with the base mutation T1311G, resulting in an I424S substitution in the UBA domain. Here we describe the functional and structural characterisation of the I424S and A427D mutants. In vitro pull-down assays showed both mutations abolished SQSTM1ís

ability to bind to ubiquitin. NF-kB reporter assays using HEK293T cells indicated both mutants were associated with an activation of signalling compared to wt-SQSTM1, with the A427D mutant giving a higher NF-kB activation level than all other PDB-associated SQSTM1 mutants tested to date. Both mutations occur in the third helix of the UBA domain and are predicted to disrupt the helical structure. Although the A247D-UBA domain polypeptide was not amenable to biophysical analysis, probably reflecting its instability, initial NMR chemical shift analysis of the I424S-UBA suggests significant structural differences not only around the mutation site in helix 3 but across the whole domain. These observations support the hypothesis that changes in SQSTM1's ability to regulate NF-kB signalling may be related to disease severity in PDB patients, and represent the first description of a SQSTM1 mutation in helix 3 associated with significant effects on the UBA domain structure. This work was supported by funding from Arthritis Research UK

Matt Goodwin

Fragment Screening by Microflow NMR

Fragment screening by tube based NMR typically requires high sample consumption. By using a microflow NMR system 50 µL injections are routinely carried out overcoming this problem. The flow cell consists of a glass cell of 3 mm width that can be lowered into the NMR. A liquid handler can inject samples from 96 or 384 well plates, and the sample flows into the cell as a plug of liquid inside of an immiscible magnetically matched fluorinated solvent. This allows ^{1}H-^{15}N HSQC spectra and STD spectra to be routinely acquired on 87 µg and 2.6 µg of protein respectively.

Aleksandras Gutmanas

The wwPDB NMR validation pipeline

The Protein Data Bank in Europe (PDBe; pdbe.org) is a founding member of the Worldwide PDB (wwPDB; wwpdb.org) consortium, which manages the Protein Data Bank (PDB), the single global archive of three-dimensional structures of biological macromolecules and their complexes. PDBe has been tasked by the wwPDB partners to develop software pipelines implementing the recommendations of the wwPDB and EMDataBank validation task forces (VTFs) for X-ray crystallography, Nuclear Magnetic Resonance and 3D Electron Microscopy. These pipelines are developed for use in structure deposition and annotation by depositors and wwPDB curators and are (being) integrated into the new wwPDB Deposition and Annotation tool that was launched in early 2014. The pipelines produce concise validation and annotation reports, which can be submitted along with manuscripts describing the structures and will become publicly available with the release of PDB entries. In addition, the validation pipelines are made available as stand-alone anonymous servers. As recommended by the wwPDB NMR VTF, the NMR pipeline applies the same knowledge-based validation criteria for structure quality assessment as for crystal structures. However, global parameters are reported only for well-defined structural regions of proteins. The validation of chemical shifts is presently limited

to completeness of assignments, referencing corrections and identification of statistical outliers. The developers of the most commonly used software packages for structure determination by NMR have agreed to specify and implement a new mmCIF/STAR-like format to capture experimental NMR restraints and peaks to enable automatic validation of these types of data. PDBe has previously developed a prototype resource for visualisation of NMR validation resource (Vivaldi; pdbe.org/vivaldi). This resource along with the PDB entry pages will be updated to reflect the recommendations of the wwPDB NMR VTF. It will also merge into a single validation portal for presentation of X-ray, NMR and EM validation information.

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Lisa Hale

Crystal Structure & NMR Studies of sNT-Als1 - the N-terminal Adhesin Domain of a Host-Cell Adhesion and Biofilm Virulence Factor of Pathogenic Fungus Candida albicans

The dimorphic fungus *C. albicans*, a harmless commensal in ~60% of healthy adults, is also an opportunistic pathogen in humans. Immunocompromised individuals are mainly at risk, for example those under immunosuppressive therapies, HIV/AIDS patients, the elderly and early neonates. Diseases caused include invasive candidiasis which has a 40-60% mortality rate. The majority of invasive candidiasis is caused by *C. albicans* forming biofilms on medical devices (e.g. catheters, heart valves) from which yeast cells disperse and enter the blood stream. The adherence properties of *C. albicans* are crucial for colonisation and biofilm formation. An important adhesive factor in the early stages of colonisation is Als1 (Agglutinin-like Sequence protein 1). This protein belongs to the Als family of glycoproteins (Als1-7 and Als9), all of which share similar domain organisation: an N-terminal adhesion region comprised of two immunoglobulin-like domains, plus central and C-terminal regions, which are highly glycosylated. Previously, the N-terminus of Als9 had been shown to bind the flexible C-termini of host cell proteins as ligands. Here I describe the X-ray crystallographic structure of SNT-Als1 with a peptide ligand, the backbone resonance assignments by Nuclear Magnetic Resonance (NMR) of the ligand-free protein and ligand-binding experiments using Differential Scanning Fluorimetry (DSF) and Chemical Shift Perturbation (CSP) by NMR. Comparison of the structure and ligand binding behavior of SNT-Als1 with other Als proteins will guide the design of therapeutic molecules that block the adhesive properties of these adhesins, and thus the formation of disease-causing biofilms by *C. albicans*.

Kieran Hand

Developing a method to observe the phosphorylation and aggregation of amyloid proteins using NMR spectroscopy

Institute of Integrative Biology, University Of Liverpool. Kieran Hand, Dr Jill Madine, Dr Hannah Davies, and Dr Marie Phelan. Protein folding, and misfolding in relation to amyloidosis (a disorder characterised by the deposition of insoluble protein) has been studied extensively. Amyloidogenic proteins are implicated in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Thus it is of great biological interest to characterise the mechanisms of folding, and the pathways that lead to amyloidosis in order to identify putative targets for therapeutics. One particular protein that has been the basis of many biochemical and biophysical studies is alpha-synuclein, an intrinsically disordered soluble presynaptic protein that displays the ability to self-assemble, and deposit as insoluble amyloid fibrils. To date, the exact mechanisms of misfolding that lead to a protein adopting a toxic conformation are still yet to be fully elucidated. One factor that could influence the aggregation of alpha-synuclein is phosphorylation. Initial studies employed 1D NMR spectroscopy to assess the activity of a recombinantly produced kinase, Casein Kinase 2, and observe the phosphorylation of a control peptide in a time-resolved manner. ^{15}N-^{1}H HSQC NMR was then employed to monitor the phosphorylation, and subsequent aggregation of alpha synuclein both in buffer, and a cell lysate, a more physiologically relevant environment. Through the analysis of site specific changes upon phosphorylation this investigation demonstrates that phosphorylation alters the aggregation properties of alpha-synuclein.

William Hawthorne

Structural Studies of the Functional Amyloid Regulatory protein CsgH from E. coli

Although amyloids are usually associated with human neurodegenerative diseases such as Alzheimer's and Parkinson's, functional amyloidogenic systems exist such as the Curli system in E. coli. Curli involves the secretion of a polymerising unit, CsgA on the cell surface of E. coli. CsgC has been determined to have a role in keeping CsgA in an unpolymerised form in the periplasm. CsgC inhibits CsgA fibrillation at substoiochiometric molar ratios, however its anti-amyloid mechanism of action remains unclear. A structural homologue of CsgC, termed CsgH was recently identified in several alphaproteobacterial species and has recently been demonstrated to exhibit similar levels of CsgA amyloid attenuation. A structure of CsgH would enable other insights in the anti-amyloid mechanism of this class of chaperones and their evolution. Unlike CsgC, CsgH has proven to be difficult to crystallise, here we show our NMR data, including backbone and side chain assignments with a view towards solving an NMR structure of CsgH.

Luke Johnson

Characterisation of β-phosphoglucomutase ground states through metal fluoride analogues and site directed mutagenesis.

Phosphoryl transfer enzymes are highly proficient, and have some of the largest rate enhancements identified. This enables rapid manipulation of phosphate mono- & di-esters essential for energy production and cell signalling, but equally means that phosphate esters are stable enough to provide the durability necessary for genetic material and phospholipids. It is of importance to identify the mechanisms employed by phosphoryl transfer enzymes which facilitate this catalytic efficiency. β-phosphoglucomutase (PGM) from *Lactococcus lactis* catalyses the inter-conversion of β-glucose-1-phosphate (βG1P) and glucose-6-phosphate (G6P) through a β-glucose-1,6-bisphosphate intermediate. By using fluoroberyllate as a phosphate analogue we successfully trap both βG1P and G6P ground-states of PGM. These have been investigated by a combination of ^{19}F, ^{31}P and protein NMR techniques together with crystallography. The trifluoroberyllate G6P complex of PGM forms as two near attack conformers. These states demonstrate how PGM rotates its general base to prevent unproductive hydrolysis of the phosphoenzyme. Additionally, by increasing the pH we show that one of the fluoride ligands of the trifluoroberyllate moiety can be replaced by a hydroxyl which results in βG1P preferentially binding. To complete the reaction co-ordinate and form a β-glucose-1,6-bisphosphate ground-state complex we mutated the general base Asp10 to Asn. BEST-TROSY experiments show that upon exposure to substrates a slow transition occurs resulting in trapping of the β-glucose-1,6-bisphosphate intermediate. β-glucose-1,6-bisphosphate orientates with the C1' phosphate in the active site and with Asp-8 positioned for 'in-line' nucleophilic attack. This work together with previous work using transition state analogues allows a detailed description of how PGM progresses through catalysis to be developed.

Hannah Johnston

Using NMR and PCA to Correlate Metabolite Profile of Lung Cancer Cells with Intracellular Redox Potential

The Warburg effect describes the survival advantage of cancer cells in that they can proliferate under low oxygen conditions via a less efficient pathway known as glycolysis. This process provides the key carbon precursors needed for cell proliferation, nucleotide and amino acid biosynthesis. The complete picture for this redirection of metabolism and increased survivability of cancer cells remains elusive. The tumour microenvironment can have a profound effect on biological functions such as transcription regulation, epigenetic alterations and post-translational modifications, all of which play an important role in the shift of cellular metabolism toward glycolysis. Tumour progression, metastasis and therapeutic resistance are associated with hypoxia, therefore comparing the metabolic fingerprint of a normal cell to a cancerous cell can help provide biomarkers for anticancer therapy. Our newly developed novel-class of SERS nanoparticles can quantitatively and non-invasively measure the redox potential of cancer cells in

vitro. An analytical approach using ^{1}H NMR spectroscopy and multivariate analysis can provide a comprehensive snapshot of the metabolic profile alterations caused by cancer. These techniques combined can help us to better understand the metabolomics and thermodynamic factors underpinning redox signalling at different redox potentials.

Michael Jolly

Paramagnetic Relaxation Agents in Biomolecular NMR

Paramagnetic Relaxation Agents in Biomolecular NMR. Michael M. Jolly[1], Malcolm H. Levitt[1], Philip T.F. Williamson[2]. [1]School of Chemistry, University of Southampton, Highfield Campus, Southampton, SO17 1BJ, United Kingdom. [2]Centre for Biological Sciences/Institute for Life Sciences, University of Southampton, Highfield Campus, Southampton, SO17 1BJ, United Kingdom.

Significant enhancements in sensitivity can be obtained through the acquisition of NMR data at cryogenic temperatures with the potential to revolutionise the study of biomolecular systems. Sensitivity enhancements will greatly facilitate the analysis of biomolecular systems such as fibrillar or membrane proteins, proteins that express poorly, and of protein states that are not highly populated. The existence of a paramagnetic center in a biological macromolecule has the effect of enhancing longitudinal (R_1) and transverse (R_2, $R_{1\rho}$) nuclear spin relaxation rates due to the formation of fluctuating magnetic fields at the sites of the nuclei. These relaxation enhancements are in general caused by a combination of electron spin relaxation and molecular dynamics. For biological macromolecules in the solid phase, Solomon dipolar relaxation, which originates from the modulation of the electron-nucleus hyperfine coupling, is the main mechanism responsible for generating nuclear paramagnetic relaxation enhancements (PRE). The precise effects on NMR spectra induced by the presence of a given paramagnetic center will generally depend on multiple factors, metal ions with highly anisotropic magnetic susceptibility tensors tend to relax very rapidly (on the picosecond or sub-picosecond time scale) and thus generate negligible transverse PREs along with significant pseudocontact shifts (PCS). On the other hand, the unpaired electron spins of nitroxide radicals such as TEMPO and metal ions such as Cu^{2+}, Mn^{2+} and Gd^{3+} relax considerably slower (typically on the order of a few nanoseconds to tens or hundreds of nanoseconds) and are associated with more significant transverse nuclear PREs as well as negligible PCSs1. Cryogenic MAS offers the potential for significant gains in sensitivity for biomolecular NMR as the thermal nuclear magnetization depends on T-1 (Curie Law). Additional enhancements with a T-1/2 dependence are seen as the receiver/transmitter coils are cooled due to a reduction in Johnson-Nyquist noise2. To assess the utility of this method on biomolecular systems ^{13}C NMR spectra have previously been recorded over a range of temperatures from 293K to 22K. At 40K enhancements of a factor of 10 have been realized on fibrillar proteins. Gains in sensitivity are offset in part however by increases in longitudinal relaxation at lower temperatures. Efforts are underway to investigate the use of paramagnetic ions to enhance the longitudinal relaxation without further affecting the resolution in the spectra. To reduce the T_1 at cryogenic temperatures we are investigating the use of

paramagetic relaxation agents in biomolecular samples. Samples of microcrystalline $^{13}C/^{15}N$ labelled GB3 have been prepared in the presence of relaxation agents including Gd^{3+}, Ho^{3+} and TEMPO. Preliminary studies at room temperature have revealed that in the presence of 1mM Gd-DTPA, a 11-fold reduction in T_1, and inspection of the ^{15}N CP-MAS spectra revealed only a small increase in linewidth. If these results are mirrored at cryogenic temperatures, this would enable the full sensitivity gains made by cryogenic MAS to be realised. Work is currently underway to conduct detailed T_1 and T_2 measurements over a range of cryogenic temperatures.

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Tokuwa Kanno

Can ROCK Regulate Metabolism? HR-MAS NMR Studies of Metastatic Melanoma Cells

Tokuwa Kanno, Pahini Pandya, Justyna Kozlowska, Francesca Di Giuseppe, Geoff Charles-Edwards, Gilbert O. Fruhwirth, Victoria Sanz-Moreno & A. James Mason

Metabolic reprogramming of cancer cells is an established hallmark of cancer. 90% of cancer-related deaths are caused by metastasis, which is defined as the spread of cancer cells throughout the body. Metastasis relies heavily upon altered cell migration and invasion, both of which are regulated by actomyosin contractility. High actomyosin contractility is driven by increased ROCK (Rho Kinase) activity: ROCK inhibitors are currently being clinically investigated for their therapeutic value. In this study we use metabolomic techniques to investigate what changes occur after manipulating actomyosin contractility regulated by ROCK. Changes in metabolic signatures can be used as biomarkers of cancer progression and unveil novel therapeutic targets.

A375 M2 human melanoma cells were treated for 24 hours with ROCK inhibitors H1152 (5 μM), Y27632 (10 μM, 20 μM) and GSK269 (0.1 μM), pelleted and lyophilized before re-hydration with D2O. Samples were analysed with whole cell High Resolution Magic Angle Spinning (HR-MAS) NMR. Multivariate analysis, in particular orthogonal partial least squares discriminant analysis (OPLS-DA) was used to characterise the effects of the ROCK inhibitors and identify relevant metabolites.

Treatment of A375 M2 human melanoma cells with the ROCK inhibitors caused significant changes (H1152 Q2 = 0.27 ; Y27632 Q2 = 0.46 ; GSK269 Q2 = 0.31) in several intracellular metabolite concentrations, in particular an increase in fatty acids and a decrease in myo-inositol, glycerophosphocholine, glutamine, and glutamate.

Altering ROCK activity in melanoma cells significantly alters intra-cellular metabolites. Many of the metabolites identified in our studies have been previously implicated in cancer cell proliferation: Our ongoing work will identify which associated pathways may be related to metastasic dissemination. This study enables future comparison of the metabolic phenotype obtained in vitro with

patient-derived samples, which will determine whether a metabolite fingerprint associated with altered contractility and invasion has diagnostic value.
These metabolomic signatures may enable identification of new therapeutic targets when integrated with ongoing transcriptomic/proteomic studies.

Hans Koss

Multidisciplinary investigation of the solution structure properties and regulatory mechanism of the γ-specific array components of phosphatidylinositol phospholipase Cγ 1

Koss H, Bunney TD, Esposito D, Coveney PV, Katan M, Driscoll PC.
Phosphatidylinositol phospholipase Cγ 1 (PI-PLCγ1) is an intracellular membrane-associated second messenger signalling protein, which is activated by receptor tyrosine kinases, such as fibroblast growth factor receptor (FGFR) family members. PLCγ contains the pseudocyclic γ-specific array (γSA), in which a tandem SH2 domain pair, a SH3 domain and a split-PH domain are connected by linker segments, effectively within a loop that is an insert in the lipase catalytic domain. Activated FGFR binding to the nSH2 domain leads to phosphorylation of Y783, a residue in the cSH2-SH3 linker. Reports suggest that this modification reduces nSH2 affinity to FGFR as well as termination of PLCγ autoinhibition by the cSH2 domain (1). The communication of the phosphorylation event through the cSH2 domain and between the both SH2 domains is currently being studied to understand FGFR dissociation, cSH2 release as well as recently discovered activating mutations in PI-PLCγ which are relevant for human disease (2), (3). Crystallographic studies did not reveal substantial differences between the non-phosphorylated and the phosphorylated form of the tandem nSH2-cSH2 construct. We have performed atomistic molecular dynamics simulations to generate hypotheses about both segmental dynamics and ligand-dependent allosteric interdomain communication within tandem-SH2 protein. We are experimentally testing these hypotheses and investigating the structural, functional, and dynamic properties of the nSH2-cSH2 subunit and its mutants in non-phosphorylated and phosphorylated states. Experimental approaches including relaxation data analysis, chemical shift perturbation analysis, residual dipolar coupling experiments and SAXS have been established and used to generate preliminary data, at the time of writing mostly for non-phosphorylated tandem-SH2, for which the backbone resonance assignment has been completed. (1) Bunney TD, Esposito D, Ö, Driscoll PC, Katan M. Structural and functional integration of the PLCg interaction domains critical for regulatory mechanisms and signalling deregulation. Structure 2012. 20(12), 2062. (2) Behjati S, Ö, Koss H, Ö, Katan M, et al. Recurrent PTRB and PLCg1 mutations in angiosarcoma. Nat Genet 2014. 46(4), 376. (3) Zhou Q, Ö, Katan M, Ö Bunney TD, et al. A hypermorphic missense mutation in PLCG2, encoding phospholipase Cγ2, causes a dominantly inherited autoinflammatory disease with immunodeficiency. Am J Hum Genet 2012. 91(4), 713.

Ewelina Krysztofinska

Biophysical studies on centrosome-associated proteins

Centrosomes are found in most animal cells, where they serve as the main microtubule-organising centre and thus influence many cell processes, including cell shape, polarity and division. The centrosome comprises of two centrioles surrounded by pericentriolar material (PCM). Hundreds of proteins are localized within the PCM whose structures and functions are poorly understood, such as CDK5RAP2 and PCNT. Several studies indicate that their interaction is necessary for centrosome maturation and mutations in genes encoding these proteins are found to be disease related. The aim of this project is to characterise the structure and function of centrosome proteins within PCM to expand the current knowledge and better understand how disruption leads to disease. Domains from CDK5RAP2 and PCNT have been designed and recombinantly expressed to study their structures and interactions using a number of biophysical techniques. The PCNT domain appears to be monomeric based on SEC-MALLS experiments, and unfolded when analysed by solution NMR. The N-terminal domain of CDK5RAP2 can form a dimer as judged by SEC-MALLS. However, NMR analysis of both proteins indicates that they are unstructured, though there was some evidence of secondary structure from circular dichroism spectroscopy. The C-terminal domain of CDK5RAP2 was insoluble and therefore had to be purified under denaturing conditions, however refolded protein precipitated rapidly. This precipitation could be prevented upon addition of its binding partner, PCNT, which indicates a possibility that PCNT is interacting with the C-terminal domain of CDK5RAP2. Protein-protein interactions were also analysed by SEC-MALLS and by chemical cross-linking, both of which confirmed the formation of a complex. Future work will involve the further optimisation of current targets and exploration of other centrosomal proteins.

Sebastian Lambert

NMR Indicates an Interaction of Dimeric HCV Inhibitors with NS5A

Hepatitis C Virus (HCV) is expected to be an increasing global healthcare burden. HCV Infection can progress to major chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC). No protective vaccine currently exists against hepatitis C and current therapies are ineffective. NS5A is an essential component of the HCV with roles in RNA replication, modulation of the host cell and assembly of viral particles, however no precise function of NS5A has been established. New direct acting antivirals (DAA) such as daclatasvir (DCV), a dimeric compound, which target NS5A with picomolar potency, are showing promise in clinical trials. The exact nature of how these compounds have an inhibitory effect on HCV is unknown; however resistance mutations appear in the NS5A domain 1. Genotype 1a HCV is less affected by these compounds and resistance mutations have a greater effect than in the 1b genotypes. Using nuclear magnetic resonance (NMR) spectroscopy, we investigated the binding of DCV to NS5A constructs, which included the key resistance mutation sites. These extended constructs appeared to have slightly altered conformations, which allow a weak interaction with DCV. This data and

subsequent studies will give new insights into the roles of NS5A in the HCV replication complex and the mechanism of action of this class of DAA.

Blanca López-Méndez

Fragment Based Ligand Discovery againts FAK using 19F-NMR/SPR

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Fragment-based drug discovery is an alternative approach to target-focused drug discovery based on high throughput cell or biochemical assays, which has gained widespread interest and application. It is based on the idea of building drugs piece by piece, detecting first the weak binding of low molecular weight (typically MW < 150-300 Da) and low complexity compounds (fragments), that could subsequently be chemically developed into compounds with lead-like properties. The approach is particularly promising to target protein-protein interactions, and to unveil protein allosteric sites that may or may not be physiologically exploited. In our Unit we have developed tools to conduct NMR-based screening to identify and characterize the binding of small molecules to protein targets, potentially providing hits for drug discovery research and chemical tools for biophysical and functional applications. In particular, we put together a collection of ~370 fluorinated small molecule compounds with good solubility in aqueous buffer and assembled them into mixtures of eight each. Using 1D 19F NMR spectroscopy we were able to monitor the possible binding of the compounds present in each cocktail to different protein targets by monitoring the increase of 19F NMR signal linewidth upon protein addition. Typically, 20 μM concentration in each CF3-containing fragment, or 50 μM for those with CF groups, are sufficient to obtain high quality spectra, and as low as 1:50 of protein equivalents produce a detectable effect on fragment binders, therefore requiring very small amounts of protein. With a sample changer and a dual H-F probe in our 700 MHz instrument, screening of the complete fluorinated fragment library can be performed in less than 2 days. Results obtained in the initial screening and the follow-up by both 1H NMR and SPR of hits discovered against the kinase and auto-inhibitory domains of the focal adhesion kinase (FAK) a non-receptor tyrosine kinase that localizes to focal adhesions in adherent cells, will be presented in this poster.

Matthew Lougher

Investigating MmyJ: An ArsR-Like Transcriptional Repressor Protein

Methylenomycin A is an antibiotic effective against both Gram-positive and Gram-negative bacteria, and was the first identified antibiotic whose synthesis and resistance is entirely plasmid determined [1]. However, as with all antibiotics, the organism that produces it, in this case *Streptomyces coelicolor* A3(2), must itself be able to resist the effects of the compound. It is known that *S. coelicolor* resists

Methylenomycin A through an efflux pump protein, Mmr [2], but this protein is not constitutively expressed. It is theorised that the antisense gene preceding mmr encodes a protein that not only regulates its own transcription, but also that of the mmr gene until it senses Methylenomycin production; this protein has been termed MmyJ. Homology modelling indicates that MmyJ belongs to the ArsR family of transcriptional regulators, which bind DNA through the formation of hydrogen bonded dimers, and release the DNA upon receiving a binding ligand [3]. The aim of this project is to prove that this is the function of MmyJ, as well as work towards structural and dynamics information about the protein, plus its binding properties to both DNA and the as yet unidentified ligand, which is suspected to be an intermediate in the Methylenomycin synthetic pathway.

Santiago Martínez Lumbreras

Structural and biophysical studies of SGTA

The small glutamine-rich tetratricopeptide repeat protein A (SGTA) is a co-chaperone involved in diverse cellular processes such as protein quality control, tail anchored membrane protein biogenesis, and steroid receptor signalling. Unsurprisingly, SGTA has been implicated in many human diseases such as in prostate cancer, polycystic ovary syndrome and in viral infections. SGTA exerts its protective effects through the identification of exposed hydrophobicity present in client proteins, protects their exposure to the cytosol, and facilitates either targeted degradation, or rescue, of secretory and membrane proteins mislocalised to the cytoplasm. This is achieved in a quality control cycle together with the heterotrimeric BAG6 complex. In addition, SGTA participates in an early stage of the GET (Guided Entry for Tail anchored proteins) pathway through this ability to recognise exposed hydrophobic regions that are present in transmembrane domain (TMD) helices of tail-anchored proteins. Recently, SGTA has been shown to be involved in modulating androgen receptor sensitivity to its different steroid ligands, together with its cellular compartmentalisation. It is known that the C-terminal domain of SGTA is crucial in the recognition of exposed hydrophobicity, yet both structural and mechanistic details of this key process remain unclear. We thus aim to structurally characterise this C-terminal substrate-binding domain of SGTA in complex with TMD helices from tail-anchored proteins, and in complex with mislocalised membrane protein substrates, by solution NMR spectroscopy. Furthermore, we aim to investigate the different interactions occurring between regions of SGTA and its binding partners in various cellular contexts.

Karen McClymont

Characterisation of an endotoxin-sensing molecular switch

Bacterial endotoxin is the lipopolysaccharide (LPS) found in the outer cell membrane of gram-negative bacteria. It is the first indicator of a gram-negative bacterial infection and is thought to be the primary cause of sepsis - a severe reaction to a systemic infection which can result in multiple organ failure and often death. The Limulus Amebocyte Lysate (LAL) test developed by Levin and Bang in

1968 is used for the detection of endotoxin. It is extremely sensitive to LPS, simple and reliable and is in high demand due to the lack of a more sensitive test. Characterisation of the key proteins involved in binding endotoxin and a better understanding of the molecular details surrounding LPS is necessary to advance knowledge and develop new, highly sensitive biosensors to benefit pharmaceutical and medical device screening as the increase in demand for horseshoe crab blood has led to an unsustainable burden on the wild population of horseshoe crabs. My project will involve characterising factor C to understand and exploit its recognition of LPS and to identify the conformational changes that activate the enzyme as a consequence of this. Factor C is a serine protease zymogen that works as a 'biosensor' in response to LPS. The protein binds LPS and becomes enzymatically active as a consequence. Such activation events must involve a conformational change in the enzyme in response to LPS binding. The ultimate goal of this work will be to design a highly sensitive and specific novel endotoxin sensor to replicate the sensitivity of Factor C without the use of the enzymatic activity as a read out. This may help to decrease the chance of false negative or positive results that have prevented current synthetic factor C based LPS testing products from gaining regulatory approval along with their significantly lower differences in sensitivity (up to 300-fold) compared to LAL-based tests. In order to achieve this I aim both to purify full length factor C to characterize how its conformation changes on binding LPS using low resolution techniques, and to express and purify smaller LPS-binding fragments to derive a detailed picture of how the protein recognizes LPS specifically. For both strands of the project I will have to develop methods that allow me to produce multi-milligram quantities of purified protein. Full length factor C will be purified in its native form from the amebocytes themselves as well as from recombinant sources, and biophysical techniques to determine its size and shape applied. LPS binding fragments will be purified exclusively from recombinant sources, and since the aim here is to determine three dimensional structures of the protein fragments in complex with LPS, expression systems that are the most convenient for the production of NMR and X-ray crystallography samples are being explored first.

Luisa Moretto

Interdomain communication in modular type I polyketide synthases

To improve yields from engineered polyketide synthase complexes, it is important to understand how component protein domains communicate. Within a minimal substrate extension module, the enzymatic domains recognize acyl carrier protein (ACP) domains via specific protein-protein interfaces. By contrast, preliminary results suggest that the interaction between ACP and an optional reductive domain is governed by substrate recognition.

Juliet Morgan

Structural Studies of Integrin Mac-1 I Domain by NMR Spectroscopy using Unlabeling Methods and Paramagnetic Relaxation Enhancement

Juliet Morgan[1], Alice Fickling[1], Adam Tibbles[1], Huw Williams[1], Jonas Emsley[2] & Mark Searle[1]. [1]School of Chemistry, Centre for Biomolecular Sciences, The University of Nottingham. [2]School of Pharmacy, Centre for Biomolecular Sciences, The University of Nottingham. In response to vascular injury, leukocyte integrin macrophage-1 antigen (Mac-1) is activated, enabling the firm adhesion of leukocytes to the newly formed thrombus. The arrest of the leukocytes on the thrombus is governed by the interaction between Mac-1 and the platelet counter-receptor glycoprotein Ibα (GPIbα). This interaction facilitates leukocyte migration into the peripheral tissue resulting in inflammation and therefore provides a novel molecular target for the treatment of vascular inflammatory diseases. Within the Mac-1 heterodimer complex the major ligand-binding region is the I domain situated at the extracellular N-terminus of the α-chain. Integral to binding interactions is the Mg^{2+} bound at the metal ion adhesion site (MIDAS) located on the top face of the I domain. For the purposes of assigning the I domain backbone 3D NMR experiments were acquired using a ^{15}N, ^{13}C isotopically labeled *Mus musculus* construct. This data has been further supplemented through the acquisition of 2D ^{15}N-TROSY NMR experiments using amino-acid selective unlabeling techniques, providing the simple spectral identification of residue type. In addition to this the cation binding property of the I domain was investigated by substituting paramagnetic Mn^{2+} for Mg^{2+}, enabling the visualization of the MIDAS through paramagnetic relaxation enhancement (PRE).

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Ryan Nicholls

Generating a high affinity Insulin-like growth factor 2 trap

Generating a high affinity IGF2 receptor. Ryan Nicholls[1], Christopher Williams[1], Susana Frago[2], Bassim Hassan[2] and Matthew Crump[1]. [1]Organic and Biological Section Chemistry, School of Chemistry, University of Bristol, Cantock's Close, Bristol. BS8 1TS . [2]Cancer Research UK Tumour Growth Control Group, Oxford Molecular Pathology Institute, Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE. EMAIL: rn12576@bristol.ac.uk

The misregulation of the embryonic growth factor Insulin-like growth factor 2 (IGF2) has been linked to the growth and proliferation of many cancer cell types [1]. Here we seek a novel IGF2 targeting therapeutic through directed evolution of an existing IGF2 binder. The Insulin-like growth factor 2 receptor (IGF2R), a 300kDa trans-membrane receptor composed of 15 homologous extracellular repeats, is involved in the transport and bio-availability of IGF2; as well as many other ligands,

including mannose-6-phosphate tagged proteins. Domain 11 (D11) of IGF2R is responsible for IGF2 binding with assistance from domain 13, which increases D11 affinity for IGF2 [2]. D11 is composed of a β-barrel core with four loops (AB, CD, FG and HI) that form the IGF2 binding site. The assistive fibronectin insert of domain 13 interacts with the AB loop, changing its structure to generate higher affinity binding compared with lone D11. Previous work uncovered an AB loop mutant (E4) that had a 10-fold increase in IGF2 affinity, which was stable enough to generate a NMR structure of the IGF2-D11 complex [3]. Herein we have continued to engineer a recombinant high binding affinity domain 11 for IGF2 by further mutating the binding loops of D11. This has been achieved by systematically mutating residues in the loops to generate increased electrostatic and hydrophobic interactions with the IGF2-domain 11 complex. This work has led to a >100 fold increase in affinity of D11 for IGF2 with the potential therapeutic applications in treating IGF2 related tumours. My current work is to fully understand the structural and dynamic basis for the increased IGF2 affinity.

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Carl Oster

Solvent PREs for studying protein complexes in the solid state

Solvent PREs (Paramagnetic relaxation enhancements) are becoming increasingly popular for studying protein complexes in solution. Here we apply solvent PREs in the solid state to study a protein-antibody complex; the B1 domain of protein G (GB1) in a > 300 kDa precipitated complex with full length human immunoglobulin (IgG). By adding 10 mM Gadodiamide (Omniscan) to the protein complex we could use a much shorter recycle delay compared to a sample without. We could then measure 15N R1 relaxation rates of the protein. From these measurements we could get information on which residues of GB1 are involved in the binding to IgG.

Stanislava Panova

Characterization of reversible protein self-association by NMR spectroscopy

Protein self-association is a process intrinsic for all proteins at high concentrations. It leads to increased viscosity, gelation and possible precipitation, which cause problems in protein manufacturing, stability and delivery. If protein drugs require high dosing, special approaches are needed. At high concentrations proteins experience conditions close to crystal state, therefore interactions in solution could potentially coincide with crystal lattice contacts. A range of diverse methods are

used to study this process, but the complexity of the mechanism makes it hard to build a reliable model. Here, the self-association of streptococcal Protein G (PrtG) was studied using Nuclear Magnetic Resonance (NMR) spectroscopy in solution. The properties of protein-protein interactions at high concentration, up to ~ 200 mg/ml, were studied on residue-level resolution. Residue specific information on protein dynamics was obtained using ^{15}N relaxation measurements. The experiments were carried out at multiple concentrations. Variation in the rotational correlation time over these concentrations showed changes in the protein dynamics, which reflected oligomerisation processes occurring in solution. Pulsed-field gradient NMR spectroscopy was used to monitor translational diffusion coefficients in order to estimate the degree of protein self-association. Oligomer formation was also monitored by looking at variations in ^{1}H and ^{15}N amide chemical shifts. Future thorough analysis of the relaxation data and calculation of chemical exchange contributions will help to allocate specific sites involved in oligomer formation. Better understanding of protein self-association mechanisms under different conditions could assist in developing methods to reduce the level of reversible protein self-association in solution at high protein concentrations.

Marie Phelan

Metabolomics Studies of Pseudomonas

Pseudomonads are highly versatile bacteria with the ability to adapt to a wide range of environments. Through varying the growth media of the bacteria, the metabolic processes of *Pseudomonas fluorescens* have been investigated. The metabolic variation of these cultures has been monitored via NMR with metabonomic analysis undertaken on both intracellular extracts and extracellular washes to build up metabolite profiles for different nutritional states.

Sophie Powell

Using NMR spectroscopy to investigate the mechanism of TSG-6 aggregation

TSG-6 is a multi-functional protein, which is expressed in response to inflammatory mediators and it is believed to protect tissues from the damaging effects of acute inflammation. The structure of the Link module from human TSG-6 (a 98 amino acid construct termed Link_TSG6), which mediates binding to a large number of ligands (including the glycosaminoglycan hyaluronan (see Higman et al. (2014) J. Biol. Chem. 289, 5619-5634) and CXCL8 (Dyer et al. (2014) J. Immunol. 192, 2177-2185)), has been previously characterized by both NMR spectroscopy in solution and X-ray crystallography. We have recently observed differences in post-translational modifications on recombinant Link_TSG6 protein produced in two different strains of E. coli. These protein preparations were found to have differing colloidal stabilities and NMR has revealed subtle structural differences between the two. Further NMR analysis is being used to examine more closely these differences as well as the effects of post-translational modification on the mechanism of Link_TSG6 aggregation. Due to the fact that the Link_TSG6 structure has been so well characterized and because preparations can be produced with differing

propensities for aggregation, Link_TSG6 makes an ideal model protein for the development of NMR and computational methodologies to examine the weak protein-protein interactions that drive the aggregation process.

Masooma Rasheed

Ligand-Dependent Dynamics of the Active-Site Lid in Bacterial Dimethylarginine Dimethylaminohydrolase

The dimethylarginine dimethylaminohydrolase (DDAH) enzyme family has been the subject of substantial investigation as a potential therapeutic target for the regulation of vascular tension. DDAH enzymes catalyze the conversion of asymmetric Nη,Nη-dimethylarginine (ADMA) to L-citrulline. Here the influence of substrate and product binding on the dynamic flexibility of DDAH from *Pseudomonas aeruginosa* (PaDDAH) has been assessed. A combination of heteronuclear NMR spectroscopy, static and time-resolved fluorescence measurements, and atomistic molecular dynamics simulations was employed. A monodisperse monomeric variant of the wild-type enzyme binds the reaction product l-citrulline with a low millimolar dissociation constant. A second variant, engineered to be catalytically inactive by substitution of the nucleophilic Cys249 residue with serine, can still convert the substrate ADMA to products very slowly. This PaDDAH variant also binds L-citrulline, but with a low micromolar dissociation constant. NMR and molecular dynamics simulations indicate that the active site ìlidî, formed by residues Gly17-Asp27, exhibits a high degree of internal motion on the picosecond-to-nanosecond time scale. This suggests that the lid is open in the apo state and allows substrate access to the active site that is otherwise buried. l-Citrulline binding to both protein variants is accompanied by an ordering of the lid. Modification of PaDDAH with a coumarin fluorescence reporter allowed measurement of the kinetic mechanism of the PaDDAH reaction. A combination of NMR and kinetic data shows that the catalytic turnover of the enzyme is not limited by release of the L-citrulline product. The potential to develop the coumarin-PaDDAH adduct as an L-citrulline sensor is discussed.

Christina C. Roggatz

Influence of ocean acidification on signalling molecules of marine crustaceans

The world ocean's pH is predicted to decrease from its current level of 8.1 to a pH of 7.7 by the year 2100. This rapid acidification is unprecedented in evolutionary terms and numerous studies already reported possible severe effects on physiology and fitness of marine organisms and communities. In most marine animals chemical cues mediate a large variety of behavioural traits such as homing, habitat choice, mate selection or kin recognition, and are also important in food location, dominance interactions and predator avoidance. Reduced responsiveness of marine animals to chemical cues under reduced pH conditions has been shown in marine fish. Suggested hypotheses of the mechanisms of this infodisruption included signal affection during transmission and reduced signal detection and reception. As many of the acid dissociation constants (pK_a) of signalling molecules fall within a pH range

of 7.7 to 8.1, which is affected by ocean acidification, changes in charge and structure of these molecules are likely to occur. The present work focuses on the effect of reduced pH conditions on the chemical properties of different signalling peptides from crustaceans using ^{1}H NMR spectroscopy.

Sarah Rouse

Charactersing SUMO-SIM interactions

The small ubiquitin-like modifier (SUMO) forms polymeric chains that are key signals in a range of cellular processes such as meiosis, genome maintenance and stress response. The SUMO-targeted ubiquitin ligase RNF4 engages with SUMO chains on linked substrates and catalyses their ubiquitination, targeting them for proteasomal degradation. We used NMR spectroscopy combined with a segmental labelling approach to reveal the influence of RNF4 on the conformation of the SUMO chain. We employed molecular modelling and simulations in order to further investigate the nature of this SUMO-SIM interaction. The effect of linker length and mutations of charged motif-lining residues were also analysed *in silico.*

Arooj Shafiq

Investigating the structural basis of RalA and RalB functional diversity

RalA and RalB are highly similar members of the Ras superfamily small G-proteins with approximately 85% sequence identity. They bind to the same downstream effector proteins, but they perform highly distinct roles both in normal and cancerous cells. Differences in the dynamic properties of the G domain may be crucial for some of the functional diversity of Rals. The solution structure of C-terminal truncated RalB was published in 2009, but no NMR structure of RalA is available for comparison. In this study, we are solving the first solution structure of RalA and comparing the dynamics of RalA and RalB G domains using ^{15}N relaxation data based on T_1, T_2 and NOE experiments. Initial results showed some interesting differences between two proteins and further analysis is still in process. In addition, RalA and RalB have a hypervariable C-terminal region with less than 50% sequence identity that is rich in basic amino acids. However, no structural information is available about the C-terminus of Ral proteins. Our initial structural analysis of full length RalA reveals that the C-terminus of activate form (GMPPNP bound) undergoes exchange broadening in contrast to inactive form (GDP bound) which seems to have fast motion at C-terminus. Further analysis is still in progress. It has also been shown that calmodulin (CaM) differentially binds to the C-terminus of RalA and RalB in a Ca^{2+} dependent manner and promotes their activation. To date, no in vitro study has been done to explore the differences in the interactions of RalA and RalB with CaM. Our study shows that the C-terminus of activated RalA binds to Calmodulin (CaM) in a Ca^{2+} dependent manner with a K_d of 1.39μM. Furthermore, C-terminal phosphorylation of RalA reduces its binding affinity with CaM. Together, this study can provide us valuable information about the unique properties of Rals to understand their structural and functional differences.

Gary Thompson

XCamshift: a Fast Python Implementation of the Camshift Chemical Shift Forcefield

XCamshift: a Fast Python Implementation of the Camshift Chemical Shift Forcefield. G. Thompson. Astbury Centre for Structural Molecular Biology, University of Leeds, UK . The use of chemical shifts for the calculation of structures has provided both a simpler way to calculate folds of proteins and also provides a tool for calculating structures of transient states, which are not accessible from traditional restraints such as nOes. The camshift algorithm is the only currently available implementation of a forcefield which uses chemical shifts to efficiently restrain the calculation of structures during molecular dynamics simulations. Xcamshift and the accompanying chemical shift forcefield framework Locsmith provide a complete implementation of the Camshift forcefield in the popular and frequently used xplor nih package. Xcamshift uses the cython dialect of python to provide a forcefield written in the python scripting language that is familiar to the users of xplor-NIH, but at the same time works at the same speed as native C code. Highlights from the latest version of xcamshift and its use include: 1. Investigation of the speed of xcamshift energy and force calculations by comparing them with other forcefield components such as standard non bonded interactions. This demonstrates the efficiency of using chemical shifts in structure calculation protocols. A large increase in speed through the use of an improved non bonded list calculator resulting from this work is described. 2. Demonstration of the use of the xcamshift in restraining nOe poor structure calculations including the effects of complimentary restraints such as RDCs and hydrogen bonds is included the re-refinement of the membrane CS Rosetta structure of the P7 protomer from Hepatitis C without resorting to the use of rigid bodies in secondary structure elements during molecular dynamics calculations. 3. An initial implementation of an ensemble-averaged version of the forcefield. This implementation is suitable for the analysis and restraint of chemical shifts in regions of proteins which are dynamic in nature and whose measured shifts are an average from a number of states. 4. Implementation of scaling of sub terms within the forcefield (e.g. aromatic ring currents) during molecular dynamics based structure calculations and their use to avoid local minima 5. Initial implementation of a force calculator for hydrogen bond terms

Uybach Vo

Probing Ras:Sos interactions involved in cancer using Nuclear Magnetic Resonance spectroscopy

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 targets. The aim of this work is to understand the molecular interactions of the Ras: Sos complex supported mainly by Nuclear Magnetic Resonance spectroscopy (NMR). 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 ^{13}C-labelled the methyl-methionine residues of Sos and 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 surface of the Ras: Sos binding sites.

Felix Williams

Structural study of plasmodium falciparum IMP1

Immune Mapped Protein 1 (IMP1) is a protein of unknown function recently discovered in *Eimeria maxima*, a pathogen that causes significant economic losses to the poultry industry. Homologues of this protein are also found in the human pathogens *Toxoplasma Gondii* and *Plasmodium falciparum*. Recent studies have shown that IMP1 when injected with an adjuvant can produce a protective immune response against *Eimeria tenella*. A better understanding of this protein's function and structure could therefore lead to clinically useful information. This poster will present our preliminary data concerning the structure of PfIMP1 obtained by NMR spectroscopy.

Joanna Woods

NMR studies of the interaction between Cdc42 and Toca1

Transducer of Cdc42-dependent actin polymerisation (Toca1) is a multi-domain adaptor protein implicated in filopodia formation and endocytosis. It includes an N-terminal F-BAR domain, which binds to membranes and induces curvature, a Cdc42-binding domain known as the homology region 1 (HR1) and a C-terminal SH3 domain, which interacts with a range of other adaptor proteins including the Wiskott-Aldrich Syndrome-Like Protein (WASL). Through its interactions with membranes, Cdc42 and WASL, Toca1 is thought to regulate PIP2-stimulated actin polymerisation leading to filopodia formation and thus cell migration. Up until now there has been little structural insight into the roles and regulation of Toca1 in the pathway of filopodia formation, and no structural studies of Cdc42 in complex with a coiled-coil such as an HR1 domain. Here, a range of biophysical techniques along with NMR spectroscopy have been used to investigate the interaction between Toca1 and Cdc42, and potential means of autoregulation by Toca1. The binding interface of the Cdc42-HR1 domain complex has been mapped using NMR. The HR1 domain binds to Cdc42 via one end of the coiled-coil, with residues from both helices being involved. The binding region on Cdc42 appears to resemble that of other known Cdc42 effector proteins and involve the two switch regions. A model of the complex, based on NMR structures of the individual components and data from chemical shift mapping, is displayed here.