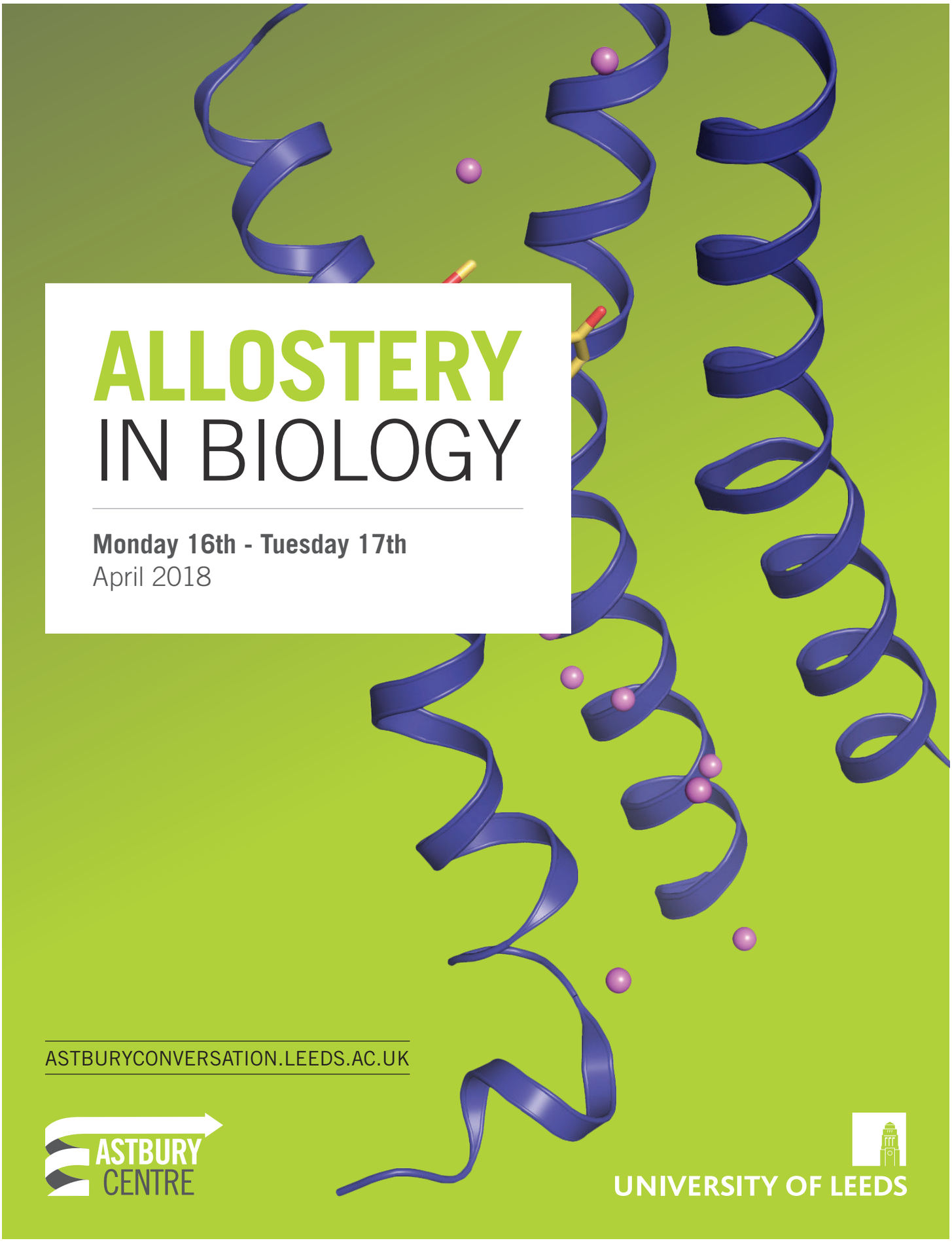
JOIN
THE ASTBURY CONVERSATION



ALLOSTERY IN BIOLOGY

Monday 16th - Tuesday 17th
April 2018

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ASTBURY CENTRE

UNIVERSITY OF LEEDS

PROGRAMME

Monday 16th April

10:00	**Arrival and registration: Parkinson Court**
SESSION 1: The Great Hall **Chair:** Tom Edwards, University of Leeds	
10:35	Welcome to the Astbury Conversation (Sheena Radford, Director of the Astbury Centre)
10:40	Introduction to the Astbury Conversation Symposium (Tom Edwards, Deputy Director of the Astbury Centre)
10:45	Richard Bayliss (University of Leeds): Dynamics and disorder in kinases and their binding partners
11:15	Elizabeth Morris (The Francis Crick Institute, London): Allostery and dynamics in cellular dNTP regulation by HIV-1 restriction factor SAMHD1
11:30	Lotte van Beek (University of York): Tandem domains go a long way: Repetitive domains form an elongated stalk in a biofilm-forming protein
11:45	Carol Robinson (University of Oxford): From peripheral proteins to membrane motors - mass spectrometry comes of age
12:15	Flash presentations
12:30	**Lunch: The Refectory**
SESSION 2: The Great Hall **Chair:** Jenn Potts, University of York	
13:45	David Agard (University of California, San Francisco): Protein Folding as an Allosteric Strategy: The Yin and Yang of Hsp90-mediated Client Activation
14:15	Anastasia Zhuruvleva (University of Leeds): Conformational and functional flexibility of the molecular chaperone BiP
14:30	Lisa Jones (University of Maryland): Development of In-Cell and In Vivo Footprinting Coupled with Mass Spectrometry for the Structural Analysis of Proteins in their Native Environment
14:45	Jim Naismith (University of Oxford): Enzymatic manipulation of peptides: making what is hard look easy
15:15	Flash presentations
15:30	**Coffee Break: (Parkinson)**
SESSION 3: The Great Hall **Chair:** Christina Redfield, University of Oxford	
16:00	Babis Kalodimos (University of Minnesota): Allosteric interactions in protein kinases
16:30	Anna Higgins (University of Leeds): Determining the mechanism of BAM-assisted OMP folding
16:45	Jeremy Tame (Yokohama City University): Photoactivation of a light-regulated adenylate cyclase
17:00	Katrin Rittinger (The Francis Crick Institute, London): Protein ubiquitination: there's more than one way to get modified
17:30	**Photo Session: Outside The Great Hall**
18:00	**Meet the editors panel discussion**
18:30	**Brian Kobilka (Stanford University):** Structural insights into the dynamic process of G-Protein-Coupled receptor activation
19:00	**Posters with pizza & beer: The Refectory** Poster session A 19:00 – 20:00, session B 20:00 – 21:00

Tuesday 17th April

8:30	**Arrival: Coffee Room UG09**
9:00	Paul Varley (Medimmune): Structural Biology and BioPharmaceutical Development
9:15	Zara Sands (UCB): The discovery of a novel dual acting A2A/NR2B antagonist and how it has helped to further chart A2A conformational space
9:30	Adam Nelson (University of Leeds): Nature-inspired approaches for bioactive small molecule discovery
10:00	Meni Wanunu (Northeastern University): Studying Biomolecules using Force and Temperature Control in Nanopores
10:15	Jeroen van Dyk (University of Antwerp): The activation and oligomerization of BAX after treatment with different activating compounds
10:30	**Coffee Break (with exhibitors & CCPs): The Refectory**
11:15	Riki Eggert (King's College, London): A chemical approach to understanding cell division
11:45	Matt Iadanza (University of Leeds): All the same but completely different: A new cryoEM fibril structure illustrating the diverse structural underpinnings of a common amyloid architecture
12:00	Claire Friel (University of Nottingham): How sequence specifies function across the kinesin superfamily: one engine, many machines.
12:15	Matthias Rief (Technical University of Munich): Single molecule mechanics of proteins
12:45	**Lunch and posters (with exhibitors & CCPs): The Refectory**
14:30	Hagan Bayley (University of Oxford): Translocation of biopolymers through pores
15:00	Yuji Goto (IPR, Osaka): Revisiting supersaturation as a factor determining amyloid fibrillation
15:15	John Briggs (LMB, Cambridge): Understanding the structures of viruses and vesicles using cryo-electron tomography
15:45	Closing remarks & prizes
15:55	**Refreshments (exhibition will be open): Parkinson Court**
16:30	**Brian Kobilka; Plenary Lecture: The Great Hall** G-Protein-Coupled Receptors: Challenges for Drug Discovery
17:30	**Public engagement and wine reception (exhibition reopens): Parkinson Court**
19:00	**Close**

SESSION 4: The Great Hall

Chair:
Madhulika Nambiar, Heptares

SESSION 5: The Great Hall

Chair:
Travis Beddoe, La Trobe University

SESSION 6: The Great Hall

Chair:
Helen Walden, University of Glasgow

WELCOME TO THE **2018 ASTBURY CONVERSATION**

I am delighted to welcome you to the 2018 Astbury Conversation.

Two years ago we set out with a simple, but ambitious aim – to bring some of the world's leading figures in structural molecular biology together in one place to discuss recent innovations and discoveries, new techniques and technologies.

We were delighted with the response to our inaugural event in 2016 and we are excited at the prospect of another inspiring two days of cutting edge science this year. If you were with us two years ago, then welcome back – you already know what to expect. If not, I hope you enjoy the two days with us and I look forward to meeting you all.

Your programme for the next two days is set out on the previous pages and we are delighted to welcome as our Conversationalist Professor Brian Kobilka MD, who will talk about his work on G-Protein-Coupled receptors on both days – as part of our symposium and at Tuesday's public lecture.

You will have opportunities during the Conversation to network with researchers in academia and in industry, as well as in research and in publishing – including at the popular poster session on Monday evening with beer and pizza and the public exhibition and reception on Tuesday.

I hope that you will enjoy your time with us and that we will see you again in 2020!

With my best wishes,

Professor Sheena Radford FRS

OUR SPEAKERS

Richard Bayliss / University of Leeds

Dynamics and disorder in kinases and their binding partners

Protein phosphorylation is an important aspect of cell signalling and cell cycle regulation. Perhaps the best understood mechanism is the regulation of protein-protein interactions involving reader modules such as SH2 or BRCT domains that directly recognize the phosphorylated side chain. Phosphorylation has a distinct role in the stimulation of catalytic activity of protein kinases, most commonly through phosphorylation of the activation loop to stabilize a catalytically-competent state. However, the structural and functional roles of most cellular protein phosphorylation events are unknown. The capability to genetically encode phosphorylated amino acids using orthogonal aminoacyl-tRNA synthetase/tRNA pairs opens up new avenues to explore the structural mechanisms of protein phospho-regulation. Recent examples from my group illustrate how phosphorylation acts as a reversible switch for regulating enzyme function and protein-protein interactions.

Richard Bayliss joined the University of Leeds in 2016 as a Professor in the Faculty of Biological Sciences. He graduated from the University of Cambridge with a first degree in Natural Sciences, and a PhD based on his research at the MRC Laboratory of Molecular Biology. After periods as a Research Fellow at Trinity College and an EMBO Fellow at EMBL in Heidelberg, Germany, he returned to the UK as a postdoctoral researcher at Birkbeck College, London.

In 2005, he was awarded a Royal Society University Research Fellowship to establish his own research group at the Institute of Cancer Research in London. He moved to the University of Leicester in 2011, and was awarded a Personal Chair in 2014.

Richard works at the interface of structural, cell, chemical and cancer biology, studying cell division and signalling pathways involving protein kinases and intrinsically disordered proteins. He collaborates extensively with cancer biologists, clinicians and cancer drug discovery groups across the UK and internationally.

Elizabeth Morris / The Francis Crick Institute, London

Allostery and dynamics in cellular dNTP regulation by HIV-1 restriction factor SAMHD1

The cellular dNTP pool is highly controlled throughout the cell cycle. Two regulatory enzymes sterile-alpha-motif and HD-containing protein 1 (SAMHD1) and ribonucleotide reductase (RNR), counteract one another and respond to cellular dNTP concentrations via allosteric feedback mechanisms. SAMHD1, hydrolyses dNTPs into their constituent 2'-deoxynucleoside (dN) and triphosphate and is up-regulated by GTP- and dNTP-binding at adjacent allosteric sites. RNR reduces nucleoside 5'-diphosphate (NDP) into 2'-deoxynucleoside 5'-diphosphate (dNDP) and is regulated by two (d)NTP-binding allosteric sites that modulate the protein's activity and specificity.

Our work focuses on SAMHD1, which, in addition to its dNTP regulatory function, is also a HIV-1 restriction factor that in differentiated myeloid cells depletes cellular dNTPs to a level that prevent the viral reverse transcription and block infection. Moreover, mutations to SAMHD1 are associated with the autosomal recessive developmental disorder Aicardi-Goutieres Syndrome, and with chronic lymphocytic leukaemia. Through a combined structural and biophysical approach, we have previously shown how GTP- and dNTP-binding stimulates SAMHD1 catalytic activity and how Threonine 592 phosphorylation in the SAMHD1 C-terminal lobe allosterically down-regulates activity.[1]

We have now used this same structural and biophysical approach, additionally employing a variety of nucleotide analogues, to determine just how SAMHD1 catalyses dNTP hydrolysis. The catalytically active form of SAMHD1 is a GTP-/dNTP-induced homo-tetramer and using AUC and a novel fluorescence-based dNTP triphosphohydrolase assay we have determined the activation constants for GTP- and dNTP-binding that locate to adjacent allosteric sites. Further, NMR studies and co-crystal structures of SAMHD1 with nucleotide-analogue inhibitors reveal the dNTP substrate binding mode in the catalytic site and show that SAMHD1 uses an unusual Fe-O-Mg binuclear centre to position a hydroxide nucleophile for in-line attack on the αP-5'O bond in a dNTP substrate. These structures and proposed mechanism are validated using a number of catalytic site point mutants to probe substrate hydrolysis and X-ray absorption spectroscopy to probe the binuclear metal site.

These studies now reveal, in atomic detail, the mechanism by which SAMHD1 hydrolyses dNTPs and how SAMHD1 activity is fine-tuned by allosteric regulation. Our observations provide a basis to enable more accurate prediction of whether new and existing antiviral and anticancer drugs, impact on, or are hydrolysed by SAMHD1.

[1] Arnold et al (2015) PLoS Pathog 11(10):e1005194

Lotte van Beek / University of York

Tandem domains go a long way: Repetitive domains form an elongated stalk in a biofilm-forming protein

Staphylococcus aureus is an opportunistic Gram-positive bacterium.[1] It can colonise the surface of in-dwelling medical devices,[2] forming a biofilm.[3] These infections are often less susceptible to treatment with antibiotics.[4] Cell wall-anchored (CWA) proteins such as *S. aureus* surface protein C (SasC)[5] mediate both biofilm formation and accumulation,[6] independent of other mechanisms.[7] These multi-domain proteins feature a similar domain organisation[8] with the exposed N-terminal domain mediating adhesion,[5] while the C-terminus is covalently attached to the cell wall. The central repetitive region is partly buried in the cell wall[9] and the function is unknown. We hypothesise that the repetitive region forms an elongated structure with the necessary rigidity to project the functional N-terminus away from the cell wall.

We redefined the domain boundaries of the structural units, Domains of Unknown Function (DUF1542), in the repetitive region of SasC using bioinformatics approaches. The structure was determined by X-ray crystallography and showed elongated helical bundles, connected intricately in tandem. Importantly, the new domain boundaries for DUF1542 were verified. This enabled the biophysical characterisation of the repetitive region to determine whether it forms an elongated and rigid conformation.

The biophysical stability of repetitive domains was characterised by circular dichroism, thermal denaturation and ^{1}H-NMR. The interface between tandem domains was essential for the stability and a stable fold. Buried and putatively exposed domains showed different stabilities. The shape in solution was measured by size exclusion chromatography with multi-angle laser light scattering (SEC-MALLS). The radii of gyration (Rg) were larger than expected from the relationship between molecular weight and Rg reported for globular proteins,[10] suggesting an elongated shape. The oligomeric state of the full-length repetitive region was monomeric, indicating that the dimerisation observed for buried DUF1542 domains was non-physiological. The approximate size in solution measured by Small Angle X-ray Scattering (SAXS) for 4 DUF1542 domains showed elongation to the maximum length expected from the crystal structure, indicating rigidity.

Taken together, the biophysical characterisation of the central repetitive region provides structural insight into the mechanism of biofilm formation of SasC. The biofilm-mediating domain is displayed at the tip of a monomeric stalk, which is separated from the cell wall by an elongated and putatively rigid repetitive region. The biophysical properties of buried and exposed repetitive domains are tailored to their local environment. Knowledge of the structure and function of domains in cell-wall anchored proteins contributes to a better understanding of the mechanism of proteinaceous biofilm formation.

[1] Zapotoczna, M. et al, PLOS Pathogens, 1-6 (2016).
[2] Lora-Tamayo, J. et al, Clin. Inf. Dis. 56 (2), 182-194 (2013).
[3] Hall-Stoodley, L. et al, Nat. Rev. Microbiol. 2, 95-108 (2004).
[4] Mah, T.F., O'Toole, G.A. Trends Microbiol. 9, 34-39 (2001).
[5] Schroeder, K. et al, PLOS One, 4 (2009).
[6] Fitzpatrick et al, J. Clin. Microbiol. 43 (4), 1973-1976 (2005).
[7] Maira-Litrán, T. et al, Inf. Imm. 70 (8), 4433-4400 (2002).
[8] Foster, T.J. et al, Nat. Rev. Microbiol. 12, 49-62 (2014).
[9] Kim, S. J. et al. BBA – Biomembranes, 1860, 749-756 (2018).
[10] Smilgies, D. M., Folta-Stogniew, E. J. Appl. Cryst, 48 1604-1606 (2015).

Carol Robinson / University of Oxford

From peripheral proteins to membrane motors - mass spectrometry comes of age

What can we learn from the study of a protein complex in the gas phase of the mass spectrometer? Aside from the compositional information that is available from obtaining the molecular mass of an intact protein complex, many additional properties can be deduced: these include polydispersity and heterogeneity, which are some of most challenging properties of protein complexes, making them almost impossible to study by conventional structural biology approaches.

Mass spectrometry is providing significant insight into protein composition within assemblies by uncovering the effects of post translational modifications, the dynamics of subunit exchange and changes in interactions in response to small molecule binding.

Recent results, which combine the study of intact membrane complexes with ion mobility mass spectrometry, bring together compositional data with conformational information. By probing the stoichiometry and location of lipid binding sites we reveal different mechanisms in molecular motors and membrane pumps.

Carol Robinson holds the Chair of Dr Lee's Professor of Chemistry at the University of Oxford. She is recognised for using mass spectrometry to further research into the 3D structure of proteins and their complexes.

During her early research Carol developed and applied mass spectrometry to show how protein folding could be monitored in the presence of molecular chaperones. This research prompted her to find new ways to preserve mega Dalton complexes in the gas phase and led her to uncover the heterogeneity and dynamics of numerous multi protein complexes. In recent work she demonstrated the numerous roles played by lipids in regulating the structure and function of membrane protein assemblies. Her current interest is in uncovering the synergy of lipid and drug binding. With this information she is exploring new ways to characterise receptor-signalling complexes.

Carol's graduate education was completed whilst working full-time in industry. She was subsequently admitted to the University of Cambridge where she completed her PhD in two years. Following an eight-year career break to begin raising her three children, she returned to research at Oxford. In 2001 she became the first female Professor in Chemistry at the University of Cambridge, returning to Oxford in 2009 to take up the Chair of Dr Lee's Professor of Chemistry.

Her research has attracted international awards and prizes including the Anfinsen Award from the Protein Society, the Biemann Medal from the American Society of Mass Spectrometry, the Davy Medal and the Rosalind Franklin Award from the Royal Society, the HUPO Award for Distinguished Achievement, the Anatrace Award from the American Biophysical Society, the Kaj Lindström-Lang Prize from the Carlsberg Research Centre and the Torbern Bergmann Award from the Swedish Chemical Society. Carol also holds four honorary doctorates and received a DBE in 2013 for her contributions to science and industry.

David Agard / University of California, San Francisco

Protein Folding as an Allosteric Strategy: The Yin and Yang of Hsp90-mediated Client Activation

The Hsp90 molecular chaperone is required for the function of a vast array of signal transduction networks in eukaryotic cells. Unlike many other chaperones, Hsp90s preferentially interact with near native states facilitating their folding and remodelling for protein-protein and protein-ligand signalling.

Our past work has mapped the primary client binding site and elucidated much of the conformational ATP cycle and defined an asymmetric closed state for the mitochondrial Hsp90, TRAP1. Recent studies on TRAP1 reveal that the ATP hydrolysis is sequential and deterministic, providing a unique opportunity to harness the energy of the first ATP hydrolysis for client remodelling or cochaperone rearrangement, while the second is used to progress through the chaperone cycle, releasing client and cochaperones.

By developing a complete reconstitution of the multi-chaperone machinery, we have shown that folding and activation of the glucocorticoid receptor is inactivated by the Hsp70 system and then reactivated by Hsp90. Careful coordination of the ATP cycles of the chaperones and the transfer process is orchestrated via an Hsp90:Hsp70:Hop:GR complex.

Using the awesome power of cryoEM, we have solved the first atomic structure of an Hsp90:client complex- Hsp90:Cdc37:Cdk4. This reveals that the kinase is pulled apart and threaded through the lumen of a closed Hsp90, while Cdc37 wraps around the outside. The previously uncharacterized Cdc37-N terminus forms a novel coiled-coil structure that stabilizes kinase C-lobe:Hsp90 interactions. Cdc37 S13 is phosphorylated and the phosphate makes interactions stabilizing both Cdc37 N-terminal structure and interactions with Hsp90. To be discussed are insights into Hsp90 function resulting from these structures.

Professor David Agard: A US biophysicist, David joined the faculty of the department of Biochemistry and Biophysics at the University of California, San Francisco in 1983. He is currently a Professor of Biochemistry & Biophysics, Professor of Pharmaceutical Chemistry, and an Investigator with the Howard Hughes Medical Institute.

David was the founding Director of the California Institute for Bioengineering, Biotechnology and Quantitative Biomedical Research in 2001, and was its UCSF Scientific Director from 2002-2006. He received his PhD in Biological Chemistry from the California Institute of Technology working with Robert Stroud, did a brief postdoc with John Sedat at UCSF and his main postdoctoral work at the MRC Laboratory of Molecular Biology in Cambridge with Richard Henderson.

Having a strong background in structural biophysics, David's current work focuses on elucidating the mechanisms of assisted folding by the Hsp90 molecular chaperone system and the mechanism of microtubule nucleation. David has been instrumental in the development of direct phasing methods for SAXS, three dimensional deconvolution and Structured Illumination light microscopies, cryo-electron tomography, the K2 Summit single electron counting direct detector, and second-generation beam-induced motion correction software.

His work has been recognized by his election to the National Academy of Sciences in 2007 and American Academy of Arts and Sciences in 2009. Beyond numerous advisory boards, David served on the National Advisory General Medical Sciences Council at the NIH.

Anastasia Zhuravleva / University of Leeds

Conformational and functional flexibility of the molecular chaperone BiP

BiP is the only Hsp70 chaperone in the endoplasmic reticulum (ER). Similar to other Hsp70s, its activity relies on nucleotide- and substrate-controllable docking and undocking of its nucleotide-binding domain (NBD) and substrate-binding domain (SBD). However, little is known of specific features of the BiP conformational landscape that tune BiP to its unique tasks and the ER environment. We present methyl NMR analysis of the BiP chaperone cycle that reveals surprising conformational heterogeneity of ATP-bound BiP. This conformational heterogeneity of BiP enables gradual regulation of its chaperone activity by subtle local perturbations at SBD allosteric 'hotspots'. In particular, we found that BiP inactivation by AMPylation of its SBD results in redistribution of the BiP conformational ensemble and stabilization the domain-docked conformation in presence of ADP and ATP, whilst it does not disturb Hsp70 inter-domain allostery and preserves BiP structure.

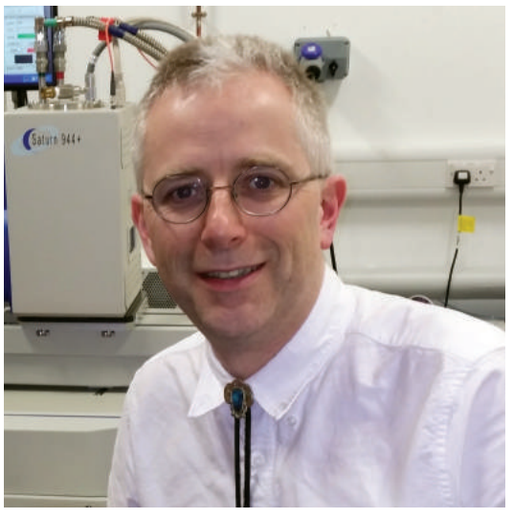
Lisa Jones / University of Maryland

Development of In-Cell and *In Vivo* Footprinting Coupled with Mass Spectrometry for the Structural Analysis of Proteins in their Native Environment

In recent years, protein footprinting coupled with mass spectrometry has been used to identify protein interaction sites and regions of conformational change. Hydroxyl radical-based footprinting (HRBF) approaches utilize hydroxyl radicals to oxidatively modify the side chains of solvent accessible amino acids. The solvent accessibility changes upon ligand binding and changes in protein conformation. By comparing the oxidation pattern of a protein modified in two states (i.e. ligand-bound and ligand-free), HRBF can identify protein-ligand and protein-protein interactions sites as well as regions of conformational change. One HRBF method, fast photochemical oxidation of proteins (FPOP), generates hydroxyl radicals via excimer laser photolysis of hydrogen peroxide. To date, HRBF methods have been used *in vitro* on relatively pure protein systems. We have further extended the FPOP method for both in-cell and *in vivo* analysis of proteins which will allow for the study of proteins in their native environment. These methods would be especially useful for analysis of membrane proteins which can be difficult to purify for *in vitro* studies. For in-cell studies, we have optimized FPOP conditions for several cell types including the commonly used cell lines HEK and CHO as well as both gram-negative and gram-positive bacteria. Using actin as a model system, we have demonstrated the utility of in-cell FPOP (IC-FPOP) for probing protein structure in the native cellular environment. HEK cells were subjected to FPOP in two conditions, when the monomeric form of actin was predominant and when the polymerized form of actin was predominant. IC-FPOP was able to identify interacting regions in the polymeric form of the protein. For *in vivo* studies, FPOP is carried out in *C. elegans*. These members of the nematode family are widely used as models for various human diseases such as cancer, Parkinson's disease, and diabetes. The development of *in vivo* FPOP (IV-FPOP) allows for the analysis of protein structure directly in an animal model for disease. Calmodulin was oxidatively modified by IV-FPOP in its calcium-bound and calcium-free state. IV-FPOP identified regions of conformational change upon calcium binding which were consistent with the crystal structure of calmodulin. These results indicate the efficacy of using both in-cell and *in vivo* FPOP to study proteins in their native environment.

Jim Naismith / University of Oxford

Enzymatic manipulation of peptides: making what is hard look easy



Bacteria make much better chemists than humans. Peptides are nature's open source chemicals, they connect via a common standard (peptide bond). As chemists we find it hard to target individual specific amino acids within a peptide. In the lecture I will discuss our insights into how very unusual and complex transformations are carried out by enzymes and what the application of this might be.

Jim Naismith, FRS FRSE FMedSci is a structural and chemical biologist. He left school aged 16 in 1985, graduated from Edinburgh University in 1989 with a BSc in Chemistry and a PhD in 1992 from the University of Manchester. He spent two years in the lab of Steve Sprang, Dallas Texas, as a NATO Fellow.

In January 1995 he came back to the UK and took a lectureship at St Andrews, where he was Director of the Biomedical Science Research Complex, doubling its size (2009-2016). His research focus is enzyme function and carbohydrate synthesis and translocation in bacteria. At the core of his work is the use of structures to illuminate molecular transformations.

He has served the wider community through Chairmanship of CCP4, SFX (the UK contribution to building a beamline at the European X-ray Free election laser) and for nearly 20 years in different roles as advisor to Diamond. He moved to Oxford in 2017 and is Director of the Research Complex at Harwell.

Babis Kalodimos / University of Minnesota

Allosteric interactions in protein kinases

The activity of protein kinases is often regulated in an intramolecular fashion by signalling domains, which feature several phosphorylation or protein-docking sites. How kinases integrate such distinct binding and signalling events to regulate their activities is not well understood, especially in quantitative terms. I will discuss recent data demonstrating how a multitude of structural elements within the Abl regulatory module (RM) form synergistically a multi-layered allosteric mechanism that enables Abl kinase to function as a finely-tuned switch. We have dissected the structure and energetics of the regulatory mechanism to precisely measure the effect of each one of the various stimuli, activating or inhibiting, on Abl kinase activity. The findings provide the mechanistic basis for explaining genetic observations and the effect of drug-resistant mutations.

Charalampos Babis Kalodimos is the Chair of the Structural Biology Department at St Jude Children's Research Hospital and holds the Joseph Simone Endowed Chair in Basic Research. He obtained his bachelor degree from University of Ioannina in Greece and his PhD from the Institut Curie in Paris. From 1999-2003 he worked as a postdoctoral fellow in the group of Robert Kaptein in Utrecht, The Netherlands, where he was introduced in the world of biomolecular NMR.

His group works on two main directions: first, the determination of the structural and dynamic basis for the function and assembly of large protein machineries; and second, the determination of the role of internal protein dynamics in regulating protein activity and allosteric interactions.

He has received numerous awards including the Young Investigator Awards from the Protein Society, the Biophysical Society, and the New York Academy of Sciences, the Stig Sunner Award and the Raymond and Beverly Sackler International Prize in the Physical Sciences.

Anna Higgins / University of Leeds

Determining the mechanism of BAM-assisted OMP folding

Outer membrane proteins (OMPs) mediate the survival and pathogenicity of Gram negative bacteria. The biogenesis of these proteins however, presents problems as they must be transported to, inserted and folded correctly in the outer membrane in the absence of ATP. This problem is resolved by the β-barrel assembly machinery (BAM) complex: a ~203 kDa complex of five proteins (BamA-E) that enables the membrane insertion and folding of substrate OMPs on a physiological timescale. Despite available crystal structures the mechanism of this vital protein complex remains poorly understood. We have used structural and biochemical tools to probe the nature of BAM-assisted OMP folding. We obtained the first cryo-electron microscopy structure of the complex, at a resolution of 4.9 Å. This reveals the intact BAM complex with BamA in a laterally-open conformation opening between the first (β1) and last (β16) strands of the barrel.

In addition, our biochemical assays provide the first *in vitro* evidence of the functional importance of BamA lateral gating. These assays demonstrate that in a reconstituted system utilising the BAM complex, inhibiting the lateral gating of BamA diminishes the ability of BAM to assist substrate folding. This is shown with two different assays, for different OMP substrates. In synthetic lipids, however, the presence of prefolded BamA shows increasing catalytic effect in longer lipid chains and prefolded BamA is always sufficient to aid substrate folding. Inhibition of lateral gating in this case does not diminish the catalytic effect, indicating that BamA likely adopts different roles depending on substrate and lipid.

Furthermore, we have begun work determining the significance of the β-signal: a conserved sequence found towards the C-terminus of OMPs hypothesised to be important for recognition by BamA. We have shown that while some mutations may slow the protein's intrinsic folding in the membrane they will not affect their apparent BamA-catalysed rate. However other single amino-acid mutations appear to incur an impossible energy penalty, rendering the protein incapable of folding, with or without BamA/BAM assistance. Combined our data allows us to begin dissecting the mechanism of BAM-assisted folding of outer membrane proteins, particularly the role of BamA in passive membrane destabilization or active lateral opening.

Jeremy Tame / Yokohama City University

Photoactivation of a light-regulated adenylate cyclase

The photo-activated adenylate cyclase (PAC) from the photosynthetic cyanobacterium Oscillatoria acuminata (OaPAC) detects light through a flavin chromophore within the N-terminal BLUF domain. BLUF domains have been found in a number of different light-activated proteins, but with different relative orientations. The two BLUF domains of OaPAC are found in close contact with each other, forming a coiled-coil at their interface. Crystallization does not prevent the activity switching of the enzyme, but flash-cooling the crystals to cryogenic temperatures blocks the signature spectral changes that occur on photo-activation/deactivation. High resolution crystallographic analysis of OaPAC in the activated state has been achieved by cryo-cooling the crystals immediately after light exposure. Comparison of the isomorphous light- and dark-state structures shows that the active site undergoes minimal changes, yet enzyme activity may increase up to 50-fold depending on conditions.

Katrin Rittinger / The Francis Crick Institute, London

Protein ubiquitination: there's more than one way to get modified

Ubiquitination regulates a wide variety of cellular processes, including immune and inflammatory signalling pathways. The TRIM subfamily of RING E3 ligases contains more than 70 members and nearly half of all TRIM proteins identified have been shown to enhance innate immune responses. Not surprisingly, given their role in immune signalling, pathogens have evolved mechanisms to target TRIM ligases to interfere with their function and suppress an immune response.

TRIM family ligases are characterized by a conserved tripartite domain structure that consists of a canonical RING domain, one or two B-box domains and a coiled-coil region ("RBCC") that mediates dimerization of TRIM proteins. We have recently shown how binding of an ubiquitin-loaded E2 (E2~UB) promotes dimerization of the RING domain of TRIM25, which in turn stabilizes the E2~Ub conjugate in a "closed" conformation in which ubiquitin is activated for transfer onto a target protein.

In this talk I will present novel structural and mechanistic data showing how TRIM25 function is targeted by viral pathogens to prevent signalling downstream of the pattern recognition receptor RIG-I and the production of interferon.

Katrin Rittinger is a senior group leader at the Francis Crick Institute in London. She obtained a degree in chemistry from the University of Heidelberg, Germany and went on to do a PhD at the Max Planck Institute for Medical Research in Heidelberg.

After a short postdoc at the Max Planck Institute for Molecular Physiology in Dortmund, Germany, she obtained an EMBO Long-term and a Marie Curie Fellowship to join the MRC-NIMR in London for a second postdoc, working on the structural characterisation of 14-3-3/ligand complexes and the regulation of Rho family GTPases.

In 2000 she established her own research group and has since studied a number of protein assemblies that regulate multiple aspects of signal transduction using biochemical and structural approaches. Her current work is focussed on the role of ubiquitination in the regulation of immune and inflammatory signalling pathways, with an emphasis on the function and mechanism of E3 ubiquitin ligases.

MEET THE EDITORS
panel discussion

Monday evening 6:00 pm

Neil Hammond
Royal Society of Chemistry

Neil Hammond is an Executive Editor at the Royal Society of Chemistry, the UK's professional body for chemical scientists, and the not-for-profit publisher of 44 academic journals. He is specifically responsible at the RSC for the development of a number of journals that span traditional boundaries between physics, biology, chemistry and engineering – including one of its newest titles, *Molecular Systems Design & Engineering*. He has held various roles in academic publishing over the last 14 years, and has worked with a diverse range of academic communities and learned societies.

Neil holds a physics degree and a PhD from Liverpool University. After leaving Liverpool he completed a postdoc at Argonne National Laboratory, just outside Chicago, Illinois, before leaving research to begin a career in publishing. He has co-authored more than 50 articles in peer-reviewed journals.

Karin Kuehnel
Nature Communications / Associate Editor

Karin Kühnel studied biochemistry at the FU Berlin and in Cambridge, where she also did her PhD studies. Karin carried out postdoctoral work at the Max-Planck-Institute (MPI) for Molecular Physiology in Dortmund and the MPI for Medical Research in Heidelberg. She then worked as a group leader at the MPI for Biophysical Chemistry in Göttingen, where she studied the structure of autophagy-related proteins. Karin joined *Nature Communications* in September 2016 and handles submissions on protein folding and all areas of structural biology.

Christian Matheou
BMC Biology / Senior Editor

Christian received his PhD from Queen Mary University of London in 2015, where he investigated the biophysical mechanismsunderlying Alzheimer's disease. He joined BMC Biology soon after, and is based in the London, UK office.

Stephen Royle
Warwick Medical School

Stephen Royle is a Senior Cancer Research UK Fellow and Reader at the Centre for Mechanochemical Cell Biology, Warwick Medical School, UK. Following a PhD at University of Cambridge, he was a post-doc at MRC-LMB where he discovered a new mitotic function for clathrin. He set up his lab at University of Liverpool in 2006 before relocating to Warwick in 2013. His research group continues to work on cell biology at the molecular level, focussing on the processes of mitosis and endocytosis in human cells. Steve is also an Ambassador for ASAPbio and an Affiliate of bioRxiv the preprint server for biology.

Brian Kobilka / Stanford University

Structural insights into the dynamic process of G protein coupled receptor activation

G protein coupled receptors (GPCRs) conduct the majority of transmembrane responses to hormones and neurotransmitters, and mediate the senses of sight, smell and taste. The ?2 adrenergic receptor (?2AR), the M2 muscarinic receptor and the mu-opioid receptor are prototypical Family A GPCRs. We have obtained three-dimensional structures of these receptors in inactive and active conformations, as well as a structure of the ?2AR in complex with the G protein Gs. Comparison of these structures provides insights into common mechanisms for propagation of conformational changes from the agonist binding pocket to the G protein coupling interface. We have also used fluorescence, EPR and NMR spectroscopy to study the dynamic properties of the β2AR. I will discuss what we these studies have taught us about allosteric regulation of GPCR structure by G proteins and ligands.

Brian Kobilka, MD is Professor of Molecular and Cellular Physiology, and Hélène Irwin Fagan Chair in Cardiology at Stanford University School of Medicine. He received a Bachelor of Science Degree in Biology and Chemistry from the University of Minnesota, Duluth in 1977. He graduated from Yale University School of Medicine in 1981, and completed residency training in Internal Medicine at the Barnes Hospital, Washington University School of Medicine, St. Louis, Missouri in 1984. From 1984-1989 he was a postdoctoral fellow in the laboratory of Robert Lefkowitz at Duke University. In 1990 he joined the faculty of Medicine and Molecular and Cellular Physiology at Stanford University.

Research in the Kobilka lab focuses on the structure and mechanism of action of G protein coupled receptors (GPCRs), which constitute the largest family of receptors for hormones and neurotransmitters in the human genome. GPCRs are the largest group of targets for new therapeutics for a very broad spectrum of diseases. In 2012, Kobilka was awarded the Nobel Prize in Chemistry for his work on GPCRs. He is a member of the National Academy of Sciences, the National Academy of Medicine, and the American Academy of Arts and Sciences.

Paul Varley / Medimmune

Structural Biology and BioPhamaceutical Development

The last 20 years or so has seen the emergence of biological molecules as the leading class of medicinal products providing life changing treatments for diseases such as cancer, asthma and inflammatory diseases. Understanding the structure and function of all biological medicines is a critical part of their development, both from a technical and Regulatory perspective. Advances in structural biology continues to provide the potential to greatly enhance the development of current and future medicines to significantly impact on the treatment of disease

Paul Varley, PhD, is Vice President, Science and Collaborations, Biopharmaceutical Development at MedImmune in Cambridge (formerly Cambridge Antibody Technology, CAT), having joined the company in 1998 as Director of Pharmaceutical Sciences. Previously, Paul was Head of Protein Science at British Biotech, and has also served as a staff scientist at the UK's National Institute of Biological Standards and Control (NIBSC). Paul holds a degree in Biomolecular Sciences from Portsmouth Polytechnic and a doctorate from the University of Newcastle upon Tyne, and has also undertaken post-doctoral research at both the University of Newcastle upon Tyne and the US National Institutes of Health

Paul is a member of the Advisory Board of the CPI UK National Centre for Biologics Manufacture and is Chair of the University College London EPSRC Centre for Innovative Manufacturing of Emergent Macromolecular Therapies. His involvement with many other biopharmaceutical/bioprocessing industry initiatives includes the European Biotech Enterprises (EBE) Biomanufacturing Committee, the BioProcess UK steering group, the International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) Biotherapeutics group, the UK BioIndustry Association Manufacturing Advisory Committee and the Bioprocessing Industry Research Club. In addition, Paul is a member of the British Pharmacopeia (BP) Commission, Chair of BP Expert Advisory Group on biological and biotechnological products, a member of the EP Expert Committee on Monoclonal Antibodies and a member of BP panel of experts for Blood Products.

Dr Zara Sands / Principal Scientist, UCB BioPharma

The discovery of a novel dual acting A2A/NR2B antagonist and how it has helped to further chart A2A conformational space

We have recently shown that combined use of an A2A antagonist with an NR2B antagonist boosts the anti-Parkinsonian efficacy of each individual drug on the motor symptoms.[1] Considering these very promising results, we decided to investigate whether a dual-acting compound that selectively antagonises both A2A and NR2B may have enhanced therapeutic potential in the treatment of Parkinson's disease. Rational design of dual compounds is a considerable challenge in medicinal chemistry and there are very few successful examples of this approach, in particular for CNS diseases.[2]

I will present the successful application of an integrated structure- and ligand-based approach for the rational identification of novel dual acting A2A/NR2B antagonists. Further to this, I will describe the A2A structure co-crystallized with one of our lead series compounds, which revealed a previously unseen conformation of A2A.[3] I will then conclude with opportunities and challenges that must be addressed to further enhance our GPCR rational drug discovery capabilities.

[1] Michel, A.; Downey, P.; Nicolas, J.-M.; Scheller, D. Unprecedented therapeutic potential with a combination of A2A/NR2B receptor antagonists as observed in the 6-OHDA lesioned rat model of Parkinson's disease. PLoS ONE, 9(12): e114086. doi:10.1371/journal.pone.0114086

[2] Morphy, R.; Rankovic, Z. Designed multiple ligands. An emerging drug discovery paradigm. J. Med. Chem. 2005, 48, 6523–6543. (b) Patyar, S.; Prakash, A.; Medhi, B. Dual inhibition: a novel promising pharmacological approach for different disease conditions. J. Pharm. Pharmacol. 2011, 63, 459–471.

[3] Sun, B.; Bachhawat, P.; Chu, M. L.; Wood, M.; Ceska, T.; Sands, Z. A.; Mercier, J.; Lebon, F.; Kobilka, T. S.; Kobilka, B.K. Crystal structure of the adenosine A2A receptor bound to an antagonist reveals a potential allosteric pocket. Proc. Natl. Acad. Sci. 2017, 114, 2066-2071.

After graduating from Manchester University with a 1st class BSc Hons degree in Chemistry Zara Sands embarked upon a PhD in the Cancer Research School, at Nottingham University where she successfully completed her doctoral training in Computational Medicinal Chemistry. In 2003 she took a Welcome Trust Fellowship position at the University of Oxford and under the direction of Prof Mark Sansom developed an expertise in membrane protein (MP) structural biology & biophysics. In 2006 Zara joined AstraZeneca where she applied and developed cutting edge *in silico* technologies for studying complex MP CNS targets. In 2009, she was recruited by UCB BioPharma to support and strengthen their CNS drug discovery pipeline through the application and development of *in silico* technologies towards membrane protein targets. She has been instrumental in developing UCB BioPharma's MP drug discovery capabilities and through the judicious application of computational approaches has been able to successfully drive UCB's GPCR drug discovery projects towards clinical candidates. She currently serves on the Scientific Advisory Board of BioExcel, a Centre of Excellence for Computational Biomolecular Research, and is a visiting researcher at the University of Oxford.

Adam Nelson / University of Leeds

Nature-inspired approaches for bioactive small molecule discovery

Natural products continue to inspire both drug discovery and chemical biology. Natural products are necessarily biologically-relevant because they arise through the evolution of biosynthetic pathways, driven by functional benefit to the host organism. In this lecture, two complementary and unified approaches for the discovery of bioactive small molecules will be described that have taken some inspiration from nature.

First, the design and synthesis of natural product-inspired scaffolds will be described. To demonstrate biological relevance, a set of fragments based on the scaffolds was screened against a disparate range of protein targets using high-throughput protein crystallography. It is demonstrated that the fragments can provide distinctive starting points for the discovery of modulators of epigenetic protein targets.

Second, our novel discovery approach – activity-directed synthesis (ADS) – will be described. The approach is structure-blind and function-driven, and takes inspiration from the evolution of biosynthetic pathways. Unlike traditional medicinal chemistry workflows, ADS deliberately harnesses the promiscuity of reactions that can yield alternative products. Crucially, ADS can exploit adventurous and powerful synthetic methods in the discovery of bioactive molecules in parallel with associated syntheses.

Adam Nelson has been Professor of Chemical Biology at the University of Leeds since 2005. He obtained his first degree (Natural Sciences; 1993) and PhD (in synthetic organic chemistry; 1996) from the University of Cambridge. He joined the University of Leeds as a lecturer in organic chemistry in 1998, and was Director of the Astbury Centre for Structural Molecular Biology at Leeds between 2009 2011.

His highly collaborative research programme focuses on the application of synthetic chemistry to problems ranging from the discovery of chemical probes of biological mechanisms to the directed evolution of synthetically-valuable enzymes. He currently holds an EPSRC Established Career Fellowship (2016-2021) focusing on the realisation of an autonomous approach to functional small molecule discovery. Adam currently supervises 11 PhD students and 9 postdoctoral fellows, and holds a range of local, national and international leadership roles.

Meni Wanunu / Northeastern University

Studying Biomolecules using Force and Temperature Control in Nanopores

In recent years, nanopores have gained attention as molecular counters, able to detect various species from the smallest of ions to large macromolecules. Nanopores have particularly enjoyed the spotlight due to their ability to linearly scan information content encoded in nucleic acids, and currently, nanopores are an integral part of the MinIon, a device commercialized by Oxford Nanpore Technologies for single molecule DNA and RNA sequencing. In this talk, I will discuss two other uses of nanopores in the biophysics and biotechnology space: First, I will describe our discovery of an optothermal effect that occurs in solid-state nanopores, in which a laser can induce a rapid temperature change localized to the nanopore. Using this effect, individual biomolecules can be subjected to a temperature ramp within millisecond timescales, allowing us to probe their thermal stability under a nanopore-induced electromotive force.[1] Second, I will discuss how introducing nanopores into optical waveguide cavities results in orders of magnitude enhancement of DNA capture of various lengths, and further, removes short read bias by attracting long DNAs into the waveguides with equal or better efficiency. Removing the length bias, allows us to optically sequence long DNA fragments at picogram DNA levels.[2]

[1] Yamazaki et al., Label-Free Single-Molecule Thermoscopy Using a Laser-Heated Nanopore, Nano Letters, 17, 7067–7074 (2017)

[2] Larkin et al., Length-independent DNA packing into nanopore zero-mode waveguides for low-input DNA sequencing, Nature Nanotechnology, 12, 1169–1175 (2017)

Jeroen van Dyck / University of Antwerp

The activation and oligomerization of BAX after treatment with different activating compounds

Bcl-2 associated X protein (BAX) belongs to the Bcl-2 protein family and plays a major role in the regulation of the apoptotic pathway in the cell. BAX is a pro-apoptotic protein which is in constant translocation between the cytosol and the outer mitochondrial membrane (OMM). BAX becomes activated through pathways initiated by intrinsic cell death signals, and subsequently translocates from the cytosol to the OMM where it disrupts the mitochondrial function by presumably forming pores. It is known to induce leakage of proteins such as cytochrome-C, leading to irreversible initiation of cell death by activation of the caspase pathway through the apoptosome.

In this study, we investigate the putative mechanisms of conformational transition (activation), membrane insertion and oligomerization of full length Bax using Ion mobility (IM) coupled with “native” mass spectrometry (MS). Purified human full length Bax was measured on a Synapt G2 (Waters) and a high-mass modified Micromass Q-TOF-2 (MS-Vision). Bax is treated with different agents including nonionic detergent Triton X-100 and the drug BAM-7. IM-MS analysis provides information on the stoichiometry and oligomeric size, average collisional cross sections and possibly the conformational state of the protein.

Results so far showed BAX forming oligomers after treatment with different molecules. Detergents DDM and Triton x100 were compared to the drug BAM-7 treated BAX, and showed dimers and tetramers with triton, while the DDM did not reveal the intensity in which triton x100 lead to oligomerization. The compound BAM-7 also revealed additionally trimers which were not detected with the detergent treated samples. Furthermore the dimers and tetramers had heterogeneous conformations, while the trimers seen in the BAM-7 treated BAX only revealed one conformation. This trimer might indicate that BAM-7 treated BAX sample is a second oligomeric activation process next to the dimer to tetramer oligomeric steps, which were also detected for triton x-100 treatment. The smallest collisional cross sections (CCS) for the monomer BAX extracted from ion mobility data was found to be close in size to the cytosolic crystal structure of BAX which represents the inactive state. The dimer CCS were identical, whether treated with triton x-100 or BAM-7. The observations indicate alternative oligomerization pathways, which may be linked to different assemblies within the membrane. These results provide evidence for symmetrical or asymmetrical growth of oligomers, which has been postulated before.

Riki Eggert / King's College, London

A chemical approach to understanding cell division

How cells regulate and execute cytokinesis, the final step in cell division, remain major unsolved questions in basic biology. It has been challenging to study cytokinesis by traditional methods because it is a very rapid and dynamic process that occupies only a small portion of the cell cycle. New approaches are needed to overcome these barriers to deeper understanding, one of which is to develop probes that act rapidly and with high temporal control. We are in the process of creating a toolbox of small molecules that inhibit different proteins and pathways in cytokinesis.

I will focus on our work to understand how membranes and membrane trafficking participate in cytokinesis. Although it is known that membranes are needed to seal daughter cells after severing, very little is known about whether (and how) specific lipids are involved in cytokinesis. Massive membrane rearrangements occur during cell division, suggesting that lipids play specific roles. We used mass spectrometry to determine if the lipidome changes in dividing cells and at a division site (the midbody) and found that only very specific lipids with specific side chains accumulate (Atilla-Gokcumen, Muro et al., Cell 2014). In parallel, we systematically used RNAi to knock down lipid biosynthetic enzymes and identified enzymes required for division, which highly correlated with lipids accumulated in dividing cells. Having determined the nature of lipids involved in cell division and their biosynthetic enzymes, the next steps are to understand their functions. To further investigate the lipids' biological roles, we are using chemical biology and cell biology approaches.

Ulrike Eggert is a chemical biologist who has been a Professor of Chemical Biology at King's since 2015. She received her first degree in chemistry from the University of Oxford and her PhD in chemistry from Princeton University, working on vancomycin resistance with Professor Daniel Kahne. She then moved to Harvard Medical School as a Helen Hay Whitney Foundation postdoctoral fellow in the laboratory of Professor Tim Mitchison, where she first started research on cell division.

In 2006, she started her independent career as Assistant Professor at the Dana-Farber Cancer Institute and Harvard Medical School and moved to King's College London in 2011. Riki's group uses chemical biology and cell biology approaches to study cell division at the process, pathway, protein and metabolite levels. A recent focus has been on understanding the roles lipids, and especially their side chains, play in processes driven by the cytoskeleton.

Riki serves on several editorial and advisory boards and is an associate editor for Biochemistry. Her lab is currently funded by a Welcome Investigator Award and an ERC Consolidator Grant.

Matt Iadanza / University of Leeds

All the same but completely different: A new cryoEM fibril structure illustrating the diverse structural underpinnings of a common amyloid architecture

Amyloid fibrils are formed by the misfolding and aggregation of a wide variety of unrelated proteins and are implicated in an equally diverse list of diseases including Alzheimer's, Parkinson's, and type II Diabetes. Pathologic aggregation of the protein β-2-microglobulin (B2M) into cross-beta amyloid fibrils is associated with dialysis related arthritis, a debilitating condition affecting patients undergoing kidney dialysis. Here we present the 3.9Å resolution structure of a B2M amyloid fibril determined using cryo electron microscopy (cryoEM) and solid state nuclear magnetic resonance (ssNMR) data. The fibril is composed of two parallel in-register protofilaments assembled from identically folded B2M subunits. This B2M fibril shares some commonalities with other amyloid fibrils; an ordered core surrounded by more disordered termini, convoluted subunit structure, and stabilisation of the subunit fold by 'steric zipper' interactions. Despite the gross morphological similarities, the specific interactions stabilising the subunit fold and interprotofilament interface are very different than those in the structures of fibrils determined previously. The most striking feature of the fibril is the stabilisation of the subunit fold and, to a lesser degree, the interprotofilament interface by π-stacking interactions between tyrosine sidechains, a feature not present in previously determined fibril structures, and uncommon in protein structures in general. When taken in context with other known amyloid fibrils this structure demonstrates the diversity of structure that can form a common fibril architecture, as well as highlighting features that may give insight into the mechanisms of fibril formation and toxicity.

Claire Friel / University of Nottingham

How sequence specifies function across the kinesin superfamily: one engine, many machines

Kinesins are crucial engines of eukaryotic self-organisation. Kinesins are found in all eukaryotes, where they perform vital roles in regulation of microtubule dynamics, transport of cellular cargo, development and maintenance of cilia, function of axons, and in cell division. Proteins of the kinesin superfamily are characterized by a common highly-conserved motor domain. However, different kinesin motors display different behaviours. Our aim is to understand structure-function relationships across the kinesin superfamily and how sequence variation relates to the different behaviours observed within the superfamily.

Kinesin activity can be broadly divided into two classes 1) translocating activity: moving directionally along the microtubule lattice and 2) microtubule-regulating activity: altering microtubule growth and shrinkage dynamics, with some families displaying activity from each class. These two broad classes of behaviour require kinesins to possess different properties. For example, microtubule lattice recognition and motor domain coordination, in the case of a translocating kinesin; or severing of inter- or intra-microtubule protofilament interactions and microtubule end recognition in the case of a microtubule depolymerase. We use protein engineering and single molecule TIRF microscopy to determine which sequence motifs within the kinesin motor domain are responsible for these properties.

An example of a microtubule-regulating kinesin is the microtubule depolymerizing kinesin, MCAK, which has the ability, in common with other kinesins that regulate microtubule dynamics, to specifically recognize the microtubule end. We have shown that the α4 helix of the motor domain is crucial to microtubule end recognition. Mutation of residues located in the α4 helix, disrupts the ability of MCAK to discriminate between the microtubule lattice and the microtubule end. Using single molecule TIRF microscopy we have been able to measure the residence time of MCAK and mutants either on the microtubule lattice or at the microtubule end. Mutation of specific residues in the α4 helix reduce the microtubule end residence time 4-fold whilst leaving the microtubule lattice residence time unchanged.

It will be interesting to discover if this role of the α4 helix in allowing a kinesin to discriminate between microtubule lattice and end is shared across other kinesins which regulate microtubule dynamics.

Matthias Rief / Technical University of Munich

Single molecule mechanics of proteins

Proteins are amazing molecular machines that can fold into a complex three dimensional structure. Even though powerful structural methods have allowed us taking still photographs of protein structures in atomic detail, the knowledge about the folding pathways and dynamics as well as material properties of those structures is still limited.

Over the past 15 years, our group has developed single mechanical methods to study the dynamics and mechanics of protein structures. In my talk I will discuss how these methods can be used to investigate and control the conformational mechanics of individual proteins. Examples include protein folding as well as protein-protein interactions and enzyme mechanics.

Matthias Rief obtained his PhD in Physics in 1997 at the Ludwig-Maximilians-Universität München, Germany, He continued his studies with a DFG-sponsored postdoctoral fellowship at Stanford University in the laboratory of J. A. Spudich focussing on the structure and function of molecular motors.

Since 2003, Matthias Rief has been a full professor of Biophysics at the Technische Universität München. Matthias Rief is an expert in single molecule force spectroscopy of biomolecules. He has made contributions to the understanding of the mechanics of molecular motors and folding and unfolding mechanics of proteins. Prof. Rief has been recognized with a number of awards, including the Jahrespreis of the German Biophysical Society and the Heinz Maier-Leibnitz Prize from the DFG.

He is a member of the German National Academy of Sciences Leopoldina and of the Bavarian Academy of Sciences.

Hagan Bayley / University of Oxford

Translocation of biopolymers through pores

When polymers move from one cellular compartment to another, they pass through protein pores. Nucleic acids, polypeptides and polysaccharides are all transported in this way, stimulating questions about the nature of the transported polymer (diameter, stiffness, branching, charge, charge distribution), the driving force (DV, DpH, refolding, binding) and how that driving force is coupled (direct coupling v diffusion/ ratchet). We have been investigating all three classes of biopolymer by current recording through individual transmembrane pores.

We have not only made interesting fundamental discoveries about the translocation processes, but also found useful applications of our work, for example in the nanopore sequencing of nucleic acids and the discovery of antibacterial agents. Recent work has focused on the translocation of folded proteins through pores, and the application of our findings in proteomics.

Hagan Bayley is the Professor of Chemical Biology at the University of Oxford. Major interests of his laboratory are the development of engineered pores for stochastic sensing, the study of covalent chemistry at the single molecule level, ultra-rapid DNA sequencing and the fabrication of synthetic tissues.

In 2005, Dr Bayley founded Oxford Nanopore to exploit the potential of stochastic sensing technology. The company has developed the MinION portable DNA sequencer. In 2014, he founded OxSyBio to build synthetic tissues for regenerative medicine.

Yuji Goto / Osaka University

Revisiting supersaturation as a factor determining amyloid fibrillation

Amyloid fibrils involved in various diseases are formed by a nucleation-growth mechanism, similar to the crystallization of solutes from solution. To study amyloid fibrils, we developed several types of unique techniques. First, to visualize amyloid fibrils, we combined total internal reflection fluorescence microscopy (TIRFM) with amyloid-specific thioflavin T fluorescence[1]. Second, we showed that ultrasonication is one of the best means of accelerating amyloid nucleation and thus the formation of fibrils[2]. By combining a water bath-type ultrasonicator and a microplate reader, we constructed a HANdai Amyloid Burst Inducer (HANABI), which enables a high-throughput analysis of ultrasonication-forced amyloid formation of proteins[3]. Third, calorimetry, one of the most powerful methods used to study the thermodynamic properties of globular proteins, has not played a significant role in understanding protein aggregation. We succeeded with β2-microglobulin in direct heat measurements of the formation of amyloid fibrils using isothermal titration calorimeter[4].

Our results with various unique approaches indicate that the solutions of denatured proteins are often supersaturated above the solubility limit and ultrasonic agitations release the supersaturation effectively, excluding solvated monomers to form fibrils. We suggest that amyloid fibrils and amorphous aggregates are similar to the crystals and glasses of solutes, respectively, and supersaturation is required to form crystal-like amyloid fibrils. We propose a general view of how the structures of protein and peptide precipitates vary dramatically from single crystals to amyloid fibrils and amorphous aggregates, in which “solubility” and “supersaturation” play critical roles[5], [6].

[1] Ban T, et al. (2006) Acc Chem Res 39:663-670.

[2] Ohhashi Y, et al. (2005) J Biol Chem 280:32843-32848.

[3] Umemoto A, et al. (2014) J Biol Chem 289:27290-27299

[4] Ikenoue T, et al. (2014) Proc. Natl. Acad. Sci. USA 111:6654-6659.

[5] So M, et al. (2016) Curr Opin Struct Biol 36:32-39.

[6] Nitani A, et al. (2017) J Biol Chem 292:21219-21230.

John Briggs / University of Cambridge

Understanding the structures of viruses and vesicles using cryo-electron tomography

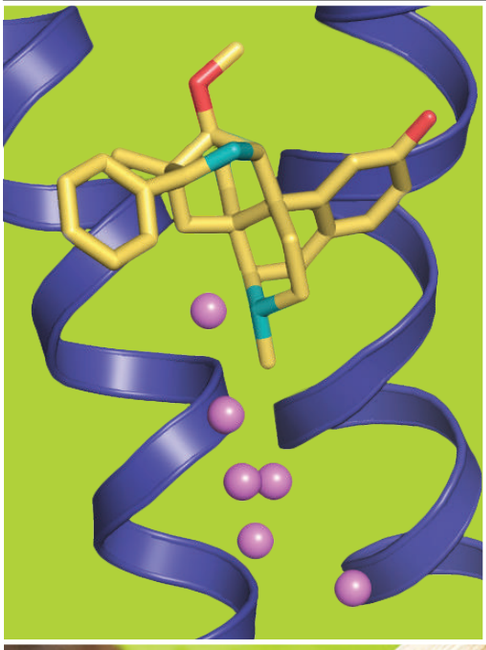
It is now possible to use cryo-electron tomography combined with computational image processing to determine the structures of protein complexes from within complex environments such as irregular viruses or cells. We are applying these methods to understand the assembly mechanisms of viruses such as HIV and influenza, as well as of membrane trafficking vesicles.

John Briggs is a Group Leader at the MRC Laboratory of Molecular Biology in Cambridge. Briggs completed his DPhil in Structural Biology at Oxford University, UK in the Lab of Stephen Fuller, where he performed cryo-electron microscopy of retroviruses. After a short postdoc in Munich, in 2006 he set up his own research group at the European Molecular Biology Laboratory, Heidelberg.

His group has worked on understanding the structure and the assembly/disassembly mechanisms of COPI, COPII and clathrin coated vesicles and of viruses including HIV-1 and influenza. Briggs' group has developed and optimized methods for cryo-electron tomography and for correlative light and electron microscopy. This work has been recognized by honours including the Royal Microscopical Society Medal for Life Sciences, the Ernst-Ruska Prize for Electron Microscopy, and election to EMBO membership. His lab moved to the LMB in January 2017.

G-PROTEIN-COUPLED RECEPTORS: CHALLENGES FOR DRUG DISCOVERY

by Nobel Laureate Brian Kobilka MD,
Professor of Molecular and Cellular Physiology



Tuesday 17th April 4:30pm
The Great Hall

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Structural Biology

A transformational opportunity for biologic medicines

Paul Varley, Vice President, Biopharmaceutical Development, MedImmune

Modern science demands vision: following the science and anticipating the future is vital for bringing innovative and life-changing medicines to patients. During the last 20 years or so, biological molecules have emerged as the leading class of medicinal products providing life changing treatments for diseases such as cancer, asthma and inflammatory diseases. Historically this success has been delivered through therapeutic monoclonal antibodies. However, the industry is now looking to an increasingly diverse range of biological medicines including more complex engineered proteins, viral, nucleic acid and cell based therapies.

Understanding the structure of these increasingly complex biological medicines is critical for their successful development. Recent advances in structural biology techniques and applications have created the potential for this exciting area of science to make a significant impact on the discovery and development of this next generation of medicines ultimately meeting significant unmet medical need.

Industry challenges

For a company such as MedImmune – focussed on therapeutic proteins, monoclonal antibodies and other protein-based molecules – the critical challenges that structural biology can help to solve exist across the whole research and development process; from drug design and selection through to the manufacturing and formulation stages of biopharmaceutical development. Central to this is the need for state-of-the-art analytical science which has the potential to provide a comprehensive understanding of drug structure in relation to its manufacturing process, biological activity, quality, consistency and, ultimately, pharmaceutical performance. In addition to this, demonstrating that a new potential medicine is understood and can be consistently manufactured is a key expectation for the regulatory approval of such products.

Key areas of opportunity for structural biology to greatly enhance this process include:

- Selecting the right molecular entity (working with research and discovery colleagues): defining the requirements of the drug and designing in the desired biological and pharmaceutical attributes.
- Developing the method of manufacture, which is reproducible, scalable, economically feasible: Unlike 'small' molecule drugs, biologic medicines are manufactured using biological systems. This means that, unlike chemically synthesised drugs, their structure and composition is highly dependent upon the process by which they are manufactured. Structural analysis of products and related variants allows manufacturing processes to be developed that are consistent and productive, producing the desired product.
- Formulating the molecule so it's stable enough for manufacture and with sufficient shelf-life: The increased complexity of new biological medicines means that developing them in stable formulations, consistent with their use as a pharmaceutical product is a continual challenge. Using structural analysis to identify and understand any mechanism of degradation enables the development of formulations and methods of drug delivery that enable the use of the product in an effective way.

Academia and industry – a synergistic approach?

As referenced above, the recent advances in structural biology have significantly improved the tools available for studying biology at the molecular level. At the same time, biologics have emerged as the most successful and promising class of medicinal products.

However, the impact of modern structural biology has yet to be fully realised in the research and development of biologics medicines, and it's clear that real opportunity exists for advances in structural biology to be applied by biotechnology companies such as MedImmune to support the development of current and next generations of biological medicines. Comparatively little is known about the structure and pharmaceutical performance of these future, more complex therapeutic products, such as complex engineered proteins, viral, nucleic acid and cell based therapies, so it's here that structural biology advances will be have the most impact.

Ultimately though, it's the patients who will potentially gain the most.

MedImmune is the global biologics research and development arm of AstraZeneca with candidate biologics and vaccines that currently comprise approximately 50 per cent of AstraZeneca's overall R&D pipeline. It has one of the most robust and promising pipelines in the biologics industry, with more than 120 biologics in research and development and over 40 projects in clinical stage development. MedImmune is pioneering innovative research and exploring novel pathways across key therapeutic areas, including oncology; respiratory; and cardiovascular and metabolic disease. In addition, the company is opportunistic in infectious disease and vaccines. MedImmune is headquartered in Gaithersburg, Maryland, US – one of AstraZeneca's three global R&D centres, along with Cambridge, UK and Molndal, Sweden

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We would like to express our appreciation to the following organisations for their sponsorship of the 2018 Astbury Conversation.

Main event sponsors

Medimmune pioneers innovative research across key therapeutic areas including RIA, cardiovascular & metabolic disease, oncology, neuroscience, and more. We develop medicines for unmet medical needs worldwide. We're an industry leader in personalized medicine – developing new products that may allow doctors to prescribe a specific, customized treatment for each patient's individual needs.

We are the global biologics research and development arm of our parent company AstraZeneca, a recognized global leader in biopharmaceutical research and development. By combining AstraZeneca's worldwide presence with our distinct and unique culture, processes, and standards, we continue to solve global healthcare challenges, redefine science, deliver better medicines, and help people live longer, healthier lives.

UCB aspires to be the patient-centric global biopharma leader, making a difference to the lives of people living with severe diseases. UCB is a global biopharmaceutical company, with a focus on neurology and immunology. Looking ahead, we have a solid platform for continuous growth through 2022 with our core products Cimzia®, Vimpat® and Neupro® and Keppra®, and we are preparing the launches of our next wave of potential medicines to help patients with epilepsy, lupus and osteoporosis. At the same time, we are very excited about the progress in our early pipeline.

The key element of our evolution is to focus on the delivery of increased patient value. There is no such thing as an "average patient". We want to use all the tools, channels and scientific advances at our disposal to develop a better understanding of the various expressions of a disease and embed the real needs of specific patient populations in our science and innovation process. Rather than starting researching any new drug with the science alone, we want to better connect patients with science and science with patients. Better understanding the reality of patients living with neurological and immunological disorders will enable us to take a more holistic approach to care, ensuring that ultimately the right drug and the right care reaches the right patients in order for them to live the lives they choose.

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ABSTRACTS

01 | Nate Adams

The common active site of Magnesium and Cobalt Chelatase

Two of the most vital cofactors for life on the planet, chlorophyll and vitamin B12 require the insertion of the metal ion in challenging reactions. The vital importance of these cofactors in life cannot be understated, chlorophyll is responsible for all food and the majority of the energy on the planet while vitamin B12 is an essential cofactor in many biological processes.

These non-trivial reactions are performed in an aqueous environment within the cell, whereas synthetic methods involve anhydrous oxygen free environments at high temperatures.

The Class I chelatases, which includes magnesium chelatase and cobaltochelatase are large enzyme complexes composed of a minimum of three proteins. The class I chelatase complex is split into two functional regions, the metal ion insertion site, and the AAA+ molecular motor which not only powers the reaction, but controls the reaction by allostery. Using crystallography, electron microscopy, computational modelling and enzymology, we have determined the location of the conserved binding site for the porphyrin in ChlH (magnesium chelatase) and the corrin in CobN (cobaltochelatase). The binding site consists of a constellation of residues forming sidechain and backbone interactions in the active site of the magnesium and cobalt chelatase, which on mutation can cause either negative or positive effects on chelatation.

02 | Hope Adamson

Non-antibody Molecular Recognition Technology for Infection Diagnostics

The aim of our current research is to develop a rapid Point-of-Care (PoC) test to differentiate between bacterial and viral infections, and improve antibiotic stewardship. A number of studies provide evidence that measuring protein biomarkers produced by the body in response to infection can positively impact on antimicrobial prescribing. We are developing multiplexed sensors to detect the biomarkers CRP, IP-10, TRAIL, procalcitonin and IL-6 in blood samples, as this combination of biomarkers has been shown to have a high accuracy for differentiating viral and bacterial respiratory tract infections (a major source of inappropriate antibiotic prescribing)[1]

Our sensing technology is underpinned by small protein affinity reagents (Affimers), which are selected to bind to target biomarkers with high affinity and offer advantages over antibodies in terms of size, stability, ease of production and manipulation.[2] Affimers are used as recognition / capture elements in our sensing devices and we are developing a range of detection strategies. Our established electronic biosensors indirectly detect biomarkers by measuring a change in the electronic properties at a conducting surface upon binding of biomarkers to Affimers.[3] They are proven to provide a degree of sensitivity that exceeds currently available PoC tests, which is required for detection of TRAIL and IP-10 viral biomarkers that are only present at extremely low levels in blood. In addition to progressing this established technology we are developing new “active” recognition elements, which bind biomarkers and simultaneously give a direct signal for detection. Nanoparticles functionalised with Affimers are being developed, which exhibit changes in fluorescence upon binding of the biomarker. Affimers are also being incorporated into enzymatic detection systems. Here we will showcase our latest developments in these novel technologies and discuss how they can be translated into PoC tests to improve antibiotic stewardship.

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03 | Mohd Syed Ahanger

Structural and functional characterisation of the N-acetylglucosamine-6-phosphate deacetylase from Mycobacteria

Mycobacterium tuberculosis (*Mtb*) is the causative agent of tuberculosis (TB) and results in over 1.7 million deaths each year. *Mtb* encodes an N-acetylglucosamine-6-phosphate deacetylase enzyme, NagA (Rv3332) that belongs to the amidohydrolase superfamily and is predicted to be essential from transposon mutagenesis studies. NagA enzymes catalyse the deacetylation of N-acetylglucosamine-6-phosphate (NAcGlcN6P) to glucosamine-6-phosphate (GlcN6P) which is the key enzymatic step to generate essential amino-sugar precursors required for cell wall biosynthesis and influence recycling of cell wall peptidoglycan fragments.

In these studies, we report on the structural and functional biochemical characterisation of NagA from *Mycobacterium smegmatis* (MSNagA) and *Mycobacterium marinum* (MMNagA), close relatives of the Mtb pathogen. We have elucidated detailed events of substrate binding through a combination of x-ray crystallography, site-directed mutagenesis and biochemical/biophysical assays. We show that mycobacterial NagA enzymes have a preference for the NAcGlcN6P substrate and our site-directed mutagenesis studies indicate the crucial role of conserved amino-acids that comprise the active-site that result in the stereo-selective recognition of substrates for catalysis to occur. We have determined the crystal structure of MSNagA in both ligand-free form at 2.6 Å resolution and in complex with the NAcGlcN6P substrate at 2.0 Å resolution which has enabled us to determine the active site frame-work. We have identified the presence of two divalent metals located in the α/β binuclear site which have been analysed further using ICP-MS. Interestingly, the structure reveals that a mycobacterial specific cysteine residue is located on a flexible loop region that occludes the active-site and may represent a unique subsite to target in vitro to probe the mechanistic function of mycobacterial NagA enzymes. Combined, these studies provide new insights into the structural and mechanistic properties of mycobacterial NagA enzymes that have an essential role in amino sugar and nucleotide metabolism that may open up new avenues for the development of new anti-tubercular agents.

04 | Zainab Ahdash

Mechanistic insight into the assembly of the HerA-NurA helicase-nuclease DNA end resection complex using native mass spectrometry

DNA double-stranded breaks are one of the most deleterious forms of DNA damage. The HerA-NurA helicase-nuclease complex cooperates with Mre11 and Rad50 to coordinate the repair of double-stranded DNA breaks. However, little is known about the assembly mechanism and activation of HerA-NurA complex. To investigate the oligomeric formation of HerA and to understand the mechanism of nucleotide binding to the HerA-NurA from thermophilic archaea, we combined native mass spectrometry with electron microscopy, molecular dynamics simulations and biochemical analyses. We reveal that ATP-free HerA and HerA-DNA complexes predominantly exist in solution as a heptamer, and act as a DNA loading intermediate. The hexameric HerA is stabilised by the binding of either NurA or ATP suggesting that HerA-NurA is activated by substrates and complex assembly. To study the role of ATP in DNA translocation and processing, we investigated how nucleotides interact with the HerA-NurA complex. We show that while the hexameric HerA binds six nucleotides in an “all-or-none” fashion, HerA-NurA harbors a highly coordinated pairwise binding mechanism and permits the translocation and processing of double-stranded DNA. Using molecular dynamics simulations, we uncover novel inter-residue interactions between the internal DNA binding sites and the external ATP. Overall, we propose a stepwise assembly mechanism which details the synergistic activation of HerA-NurA by ATP, which allows efficient processing of double-stranded DNA. Our finding that the helicase HerA predominantly exists as heptamer prior to hexamer formation and NurA nuclease recruitment may serve as a model for DNA end resection in eukaryotes (*Z.Ahdash et al., 2017, NAR*).

05 | Natalie Al-Otaibi

Determining the structure and oligomerisation of the flagellum tip protein FliD in Campylobacter jejuni using Cryo-Electron Microscopy

The bacterial flagellum is a fascinating molecular motor, present at the surface of many pathogenic bacteria, which allows motility through the rotation of a long filament protruding from the bacterial cell. In *Campylobacter jejuni* it also facilitates adhesion to various surfaces making it invaluable for infection[MOU1] of the intestinal tract in humans and transmission through contaminated poultry. FliD is a protein that localizes at the end of the filament, and has been proposed to play an essential part in filament assembly, as well as in adherence. Currently we have high resolution view for many parts of the flagellum[MOU2] , but in isolation.

Crystal structures of truncated FliD domains in several species have revealed a range of crystallographic symmetries, from tetramers to hexamers. However, Cryo-EM studies of intact FliD in *Salmonella* indicate that it is pentameric. Based on these, we hypothesize that the different oligomeric states are crystallographic artifacts and FliD forms a pentamer in its native state across different bacterial species.

To address this, we have purified the intact *C.jejuni* FliD and used negative stain EM and size-exclusion chromatography to confirm that it forms a large, monodispersed oligomeric complex. We have imaged this complex by Cryo-EM, and we are in the process of determining the structure of this complex. Further work will be done to determine the interactions between FliD and the filament itself, in various bacterial species.

06 | Alex Bateman

When is a protein not a protein?

The protein sequence databases contain over 100 million proteins and are rapidly growing. It seems likely that we will have over 1 billion protein sequences to contend with in the near future. These protein sequences are generated by computational gene predictions which sometimes make mistakes. This means that an unknown fraction of protein sequences are probably never translated. This work attempts to estimate the number of spurious proteins that exist in the databases. These studies are based on two tools that we have created: AntiFam and database of spurious protein families that we have identified, and secondly using Spurio a new tool that can predict the likelihood that a protein sequence is not a true protein sequence.

07 | Travis Beddoe

Natural genetic variation in galectin-11 correlates with differential anti-parasitic activity

Mammalian innate immunity carries a substantial burden of the defence against pathogens. The first step of innate immunity is pattern recognition performed by pattern recognition receptors (PRRs) that sense the presence of infection and activate immune responses. Here we describe the structure and functional consequence of polymorphisms in the PRR galectin-11. Galectin-11 is specifically expressed by epithelial cells in ruminants upon parasitic infection as part of the innate immune response. The crystal structure of galectin-11 revealed head-to-tail dimer formation with two critical residues important for dimerization residues. One of these residues was shown to be polymorphic resulting in two natural variants termed NV-1 and NV-2. The resulting natural variation had effect on the oligomerisation state of galectin where NV-1 exists as tetramer in solution while NV-2 is in dimer-monomer state. We further observed that quaternary structure of galectin-11 effected the development of *Haemonchus contortus* larvae f in vitro. Co-culture of NV-1 galectin-11 with parasitic larvae limited larval motility and development with obvious tegumental damage observed. In contrast, NV-2 galectin-11, had no anti-parasitic effect. Additional sequencing of galectin-11 gene of the natural nematode resistant sheep carina hair breed was shown to contain only natural variant 1. This study demonstrates for the first time, that natural genetic variation in pattern recognition receptor galectin-11 resulted differential anti-parasitic activity that have potential outcomes in selective breeding of sheep to increase resistance to nematodes.

08 | David Bode

Ion Channel Formation by Amyloid-β42 Oligomers but not Amyloid-β40 in Cellular Membranes

A central hallmark of Alzheimer's disease (AD) is the presence of extracellular amyloid plaques A central hallmark of Alzheimer's disease (AD) is the presence of extracellular amyloid plaques chiefly consisting of amyloid-β (Aβ) peptides in the brain interstitium. Aβ largely exists in two isoforms, 40 or 42 amino acids long, while a large body of evidence points to Aβ(1-42) rather than Aβ(1-40) as the cytotoxic form. One proposed mechanism by which Aβ exerts toxicity is the formation of ion channel pores that disrupt intracellular Ca2+ homeostasis. However, previous studies using membrane mimetics have not identified any notable difference in the channel-forming properties between Aβ(1-40) and Aβ(1-42). Here, we tested whether a more physiological environment-membranes excised from HEK293 cells of neuronal origin-would reveal differences in the relative channel-forming ability of monomeric, oligomeric, and fibrillar forms of both Aβ(1-40) and Aβ(1-42). Aβ preparations were characterized with transmission electron microscopy and Thioflavin-T fluorescence. Aβ was then exposed to the extracellular face of excised membranes and transmembrane currents were monitored using patch-clamp. Our data indicated that Aβ(1-42) assemblies in oligomeric preparations form voltage-independent, non-selective ion channels. In contrast, Aβ(1-40) oligomers, fibres and monomers did not form channels. Ion channel conductance results suggested that Aβ(1-42) oligomers – but not monomers and fibres – formed 3 distinct pore structures with 1.7, 2.1, and 2.4 nm pore diameters. Our findings demonstrate that only Aβ(1-42) contains unique structural features that facilitate membrane insertion and channel formation, now aligning ion channel formation with the differential neurotoxic effect of Aβ(1-40) and Aβ(1-42) in AD.

09 | Alex Borodavka

'These can go up to eleven': shedding light on the molecular mechanism of genome segment counting in rotaviruses

Genome segmentation offers certain evolutionary benefits to a number of RNA viruses, including common human and animal pathogens such as rotaviruses and influenza virus. However, as the number of genomic segments per virus particle increases, segmented RNA viruses must maintain the integrity of their genomes by selecting a complete genomic set of distinct RNA segments during the virus replication. While some segmented RNA viruses can randomly co-package 2-4 segments per virion, such non-selective packaging would be extremely inefficient for maintaining a full genomic set of eleven distinct RNA segments in rotaviruses. Understanding the specificity of the RNA selection process would offer new insights into the evolution of these viruses, as well as open opportunities for creating new rationally designed rotavirus vaccine strains.

Here, we have employed multi-colour cross-correlation spectroscopy (FCCS) to detect stable, specific RNA-RNA interactions between rotavirus segment RNAs. In order to identify the genomic sequences involved in the formation of such contacts, we have developed an RNA-RNA SELEX methodology. Using both approaches we have shown that the formation of inter-segment contacts requires binding of the rotavirus-encoded RNA-binding protein that promotes annealing of the exposed complementary RNA sequences present in each genomic segment. This RNA remodelling brought about by the viral protein NSP2 results in the stabilisation of intermolecular RNA-RNA contacts between segments. We hypothesise that these newly identified sequence-specific contacts may underpin the RNA selection and assembly processes in rotaviruses. We are building a complete genomic structural profile for rotaviruses using high-throughput RNA probing technique SHAPE-MaP. Our SHAPE-directed structural models recapitulate some of the previously predicted RNA structures and provide new insights into the structures of individual genome segments at single-nucleotide resolution. Finally, we are developing new tools for super-resolved imaging of distinct genomic RNA segments in rotavirus-infected cells. We will use this approach to directly visualise the assembly of multiple RNA segments, and to investigate the effects of various small molecule inhibitors on this process.

10 | Sam Bunce

Aβ16-22 increases the aggregation rate of Aβ40 through a surface mediated 2° nucleation

The formation of amyloid deposits from usually soluble peptides and proteins is implicated in a wide range of debilitating and prevalent diseases and may also be a fundamental property of polypeptide chains.[1],[2] Understanding at the molecular level the structural changes that occur in these highly complex and heterogeneous systems is both a necessary and difficult task facing the scientific community. Due to the challenging nature of amyloids, in vitro research often focuses on a single highly pure peptide sequence, however, in vivo there are often a number of different amyloidogenic peptides that can interact during the aggregation process.[3],[4] In the present work, we explore the aggregation of two widely studied peptides from the Aβ sequence, Aβ40 and Aβ16-22, when incubated as a mixture. Using a combination of biochemical and biophysical assays, such as ion-mobility mass spectrometry and photo induced cross-linking (Figure 1.), we demonstrate that Aβ16-22 increases the aggregation rate of Aβ40 through a surface catalysed secondary nucleation pathway.[5]

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11 | Emma Cawood

Identifying small molecule modulators of amyloid formation

Amyloid fibrils arise as a consequence of transient interactions between specific protein conformers. While these fibrils are associated with a range of human diseases, including Alzheimer's and Parkinson's, the mechanisms of amyloid formation are generally poorly understood. This is due to the complexity and heterogeneity of these aggregation pathways, which makes them challenging targets. However, small molecules which modulate specific protein-protein interactions during fibril formation can be used as tools to provide new insight into amyloid formation. By promoting or inhibiting certain events, such molecules provide an opportunity to study the role of particular species/interactions on the rate and outcome of aggregation.

We are developing small molecules which can be used to probe the amyloid formation pathway of a highly aggregation-prone variant of β2-microglobulin (β2m-ΔN6), a natively folded amyloid protein. Using various computational approaches, we have identified "ligandable" pockets in regions which are thought to play important roles during early oligomer formation[1]. Molecular docking against these target sites has been used to design a small fragment library, which has been prepared by solid phase synthesis. Using a site-directed method of ligand discovery, we can specifically screen these fragments against the target regions. This screening method (called "tethering" or "disulfide trapping") [2] demands the functionalisation of all library members with thiol groups, but is highly sensitive, even at low protein and fragment concentrations. Due to the location of these target sites, relative to proposed protein-protein interaction surfaces, small molecules which bind to this region are likely to influence the aggregation kinetics and/or conformational ensemble of β2m-ΔN6, and therefore can be used to understand the contribution of specific microscopic events towards β2m aggregation.

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12 | Anu V Chandran

Preliminary characterisation of HSP-related mutants on Yop1

Yop1, a prototypical member of DP1 family of proteins, is an integral membrane protein of the endoplasmic reticulum of yeast. Yop1 is involved in maintaining the characteristic tubular shape of the endoplasmic reticulum. All members of this family possess a Reticulon Homology Domain (RHD) consisting of two transmembrane regions. Point mutations in human homologue of this protein, REEP1, is implicated in inherited neurological disorder called Hereditary Spastic Paraplegia (HSP) and these amino acids are conserved from yeast to humans. The characteristic feature of the protein is tubulation of lipids. Yop1 is known to form higher order oligomers consisting of 5-7 monomers and this oligomerisation has been shown to be important for the protein to function. The present study investigates the effects of some of the HSP related point mutations using Yop1 as a model. Three mutations in the second transmembrane helix were characterised. Our study indicates that the mutations affected the protein stability, oligomerisation and tubulation. Two of the three mutants, P71L and S75F, could not be solubilised from the membrane as efficiently as wild type and showed polydispersity in solution. The third mutant, A72E solubilised much effectively than the wild type and is monodisperse in solution. All the three proteins tubulate lipids in in vitro tubulation experiments although P71L showed a slight variation in the pattern of tubulation. Thus, our preliminary study has shown that P71 and S75 is vital for protein stability. No defect at the protein level could be seen for A72E from our studies on Yop1.

13 | Joseph Cockburn

Structural studies reveal a universal mechanism for kinesin-1 activation

Kinesin-1 transports numerous cellular cargoes along microtubules. The kinesin-1 light chain (KLC) mediates cargo binding and allosterically regulates kinesin-1 motility. To investigate the molecular basis for kinesin-1 recruitment and activation by cargoes, we solved the crystal structure of the KLC2 tetratricopeptide-repeat (TPR) domain bound to the cargo JIP3. This, combined with biophysical and molecular evolutionary analyses, reveals a new cargo-binding site on kinesin-1, located on KLC TPR1, which is strictly conserved in homologs from sponges to humans. In the complex, JIP3 cross-links two KLC2 TPR domains via their TPR1s. We show that TPR1 forms a dimer interface that mimics JIP3 binding in all crystal structures of the unbound KLC TPR domain. We propose that cargo-induced dimerization of the KLC TPR domains via TPR1 is a general mechanism for activating kinesin-1, and relate this to activation by other cargoes. The ability to transport a diverse repertoire of unrelated cargoes is a key aspect of kinesin-1 function. This work suggests how different cargoes "hotwire" the kinesin-1 molecule through related molecular mechanisms.

Joseph J. B. Cockburn[1],[2], Sophie J. Hesketh[1],[2], Peter Mulhair[3], Mary J. O'Connell[3] & Michael Way[4]

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14 | Owen Cornwell

Hydrogen Deuterium Exchange and FPOP probe the structure and dynamics of aggregation prone variants of B2-microglobulin

Wild-type β2-microglobulin (β2m) is found on the surface of all nucleated cells as the light chain component of the Major Histocompatibility Complex, an essential part of the adaptive immune system. In patients with renal failure undergoing long-term hemodialysis, β2m aggregates into amyloid fibrils resulting in Dialysis Related Amyloidosis (DRA). Despite this, wild-type β2m does not form amyloid *in vitro* under physiological conditions in the absence of external factors.

Recently, a naturally occurring variant of β2m was identified containing a single D-N mutation at position 76 in the E-F loop. This variant is highly amyloidogenic and readily forms fibrils under physiological conditions *in vitro*, as well as causing a hereditary systemic form of β2m amyloidosis - distinct from DRA. ΔN6 is an amyloidogenic truncation variant of β2m missing the N-terminal six residues which is known to co-fibrillate with the wild-type protein in DRA and accounts for ≈30% of protein extracted from *ex vivo* fibrils.

Here, we utilise Fast Photochemical Oxidation of Proteins (FPOP) and Hydrogen-Deuterium exchange (HDX) to highlight differences in the solvent accessibility and conformational dynamics between these three variants which may be related to their aggregation propensity. Furthermore, we show comparative data between the two methods and demonstrate that FPOP and HDX are effective complementary techniques that offer additional structural information when used together.

15 | Emma Cowan

UBE3A and UBE3A/Partner Protein Complexes

UBE3A is a human enzyme of roughly 100kDa, encoded by the UBE3A gene located in the 15q11-13 chromosomal region. The protein is also referred to as E6-Associated Protein (E6AP) for its interaction with the high risk HPV E6 protein in the oncogenesis of cervical cancers; although UBE3A is a ubiquitous protein, and it has many diverse targets throughout the cell, including many tumour suppressors, so disruption of UBE3A has since been associated with several different cancer types, including prostate cancer and breast cancers, both with and without E6. The E6AP protein was the founding member of the HECT family of E3 ligases, with HECT standing for Homologous to E6AP Carboxy-Terminus, as it was the first ubiquitin ligase to be identified and characterised as containing the 350 residue motif that allows the E3 to accept the ubiquitin moiety from an E2 ubiquitin carrier in the form of a thioester, before transferring the ubiquitin to the target protein. UBE3A is one of roughly 300 E3 ubiquitin ligases encoded in the human genome, and as such it is involved in regulation of the last step of the process of ubiquitination, which primarily targets proteins for degradation, but is also involved in regulating several cellular processes, including intracellular trafficking, endocytosis, or even stabilisation of the protein. The UBE3A gene is one of many genes that is imprinted in neuronal tissue, meaning that only one inherited copy of the gene is expressed within neurons in the brain, while the other copy is silenced. In the case of UBE3A it is the paternal copy of the gene that is silenced by a complex network of epigenetic marks, leaving only the maternal copy of the gene to be expressed in the brain. While it is currently unclear what the reason for this imprinting is, a consequence of it is that if the maternal copy of the gene is mutated or deleted, there is no functional UBE3A expressed in neurons, which results in the neurodegenerative disorder known as Angelman syndrome. Some research has been carried out into the structure of a UbcH7-p53-UBE3A complex active site, and some *in silico* modelling has been carried out to determine the order of events in UBE3A-mediated ubiquitin transfer, but there is currently no full structural model of the UBE3A protein or the multiprotein complexes it forms part of. X-ray crystallography is the predominant method of determining structures of proteins, especially large proteins over ~45kDa, although recent technological advances in the field of microscopy have led to cryo-EM emerging as an exciting new technique, able to overcome many of the difficulties associated with current methods. Utilizing both cyro-EM and x-ray crystallography techniques to determine a structure for UBE3A could assist in the development of drugs to treat various disorders, including Angelman syndrome and possibly many cancers.

16 | Nathan Cumberbatch

Monitoring native and aggregate structure of amino acids and human insulin with blue autofluorescence

Structural characterization of proteins, peptides and amino acids is key to understanding the mechanisms behind their aggregation to amyloid-like fibrillar structures. Blue autofluorescence stems from peptide carbonyls, enhanced by formation of zwitterionic NH3+-COO- pairs. It is known to increase in intensity when proteins aggregate. Here we show that different aggregated species exhibit different spectral characteristics. Excitation-emission matrices (EEM) of teh blue autofluorescence show distinct spectral shifts with aggregation state for both amino acids and human insulin. For cysteine and phenylalanine, we uncover that with increasing aggregate size both excitation and emission maxima shift to higher wavelengths by up to 50 nm. The tyrosine blue autofluorescent peak conversely does not shift significantly during aggregation. For human insulin, we find that fibrils formed at different pHs, which appear identical in Raman spectroscopy, exhibit distinct fluorescence EEM spectra. We suggest that the spectral shifts arise from highly localized changes in the environment of the peptide group, probably related to the hydrogen bond network. Blue autofluorescence excitation emission matrices are thus revealed as a useful assay to monitor aggregation of both proteins and amino acids.

17 | Elise Daems

Structure, stability and interactions of cocaine-binding aptamers using native MS

Aptamers are synthetic oligonucleotides with a well-defined 3D structure which allows them to specifically interact with their ligands. They can be used for various applications such as the treatment of diseases, targeted drug-delivery and as sensors for environmentally relevant compounds. With rapidly growing interest in the use of these designed oligonucleotides for specific functions and nano-structures, there is a need for efficient and fast methods for the characterization of their exact sequence, modifications, noncovalent interactions and 3D structure. Therefore, the present study seeks to elucidate the higher-order structure of these oligonucleotides and their conformational behaviour and stability. We investigate the molecular basis of ligand selectivity of cocaine-binding aptamers in order to understand their efficacy.

Using native ion mobility mass spectrometry (IM-MS), the three-dimensional structure and interactions of aptamers are characterized using a Synapt G2 HDMS (Waters) equipped with a nano-electrospray source. Moreover, collision induced dissociation and unfolding (CID/CIU) experiments are implemented to investigate the conformational stability of these oligonucleotides.

Cocaine-binding aptamers are designed to bind specifically to cocaine, but it was shown before that they also bind to analogous molecules such as quinine which was used here. Complex formation was observed and additional cocaine-binding aptamers with varying length, whose sequences only differ in their terminal parts and at one specific position, were examined. The findings reveal that the extent of complexation detected in the gas phase follows the order of known dissociation constants of the aptamer-cocaine complexes in solution. In a next step, a panel of quinine derivates was considered to test the ability of native MS to determine the ligand specificity of the aptamers, based on biophysical data reported previously by P. Johnson *et al.* for the cocaine-binding aptamer MN4.

Complementary to these experiments, information about the molecular basis of specificity and the mode of binding was obtained by performing ion mobility experiments. Changes in collision cross section (CCS) upon ligand binding are indicative of whether a structural transition occurs upon binding, or the ligand binds superficially or to a pre-formed pocket or site.

CIU experiments allowed us to obtain an indication of the stability of the aptamer fold by increasing the collision energy applied to the biomolecule while monitoring the CCS. The behaviour of all aptamers was comparable: none of them showed unfolding, while multiple base losses were observed. Contrary to our expectations, mainly guanine was lost independent of the sequence/structure of the aptamer.

18 | Janne Darell

Identification of novel interactions between Receptor Tyrosine Kinases proline rich motif and SH3 domain containing proteins

Receptor Tyrosine Kinases (RTKs) relay signals that regulate processes such as cell growth, differentiation and cell division. Perturbations of these signalling cascades can have serious consequences for the cell and are implicated in numerous disease states. It has previously been shown that proteins containing an SH3 domain can interact with a proline-rich motif on the intracellular region of the RTK fibroblast growth factor receptor 2 (FGFR2), which consequently either inactivates the receptor or relays a downstream signal without any extracellular stimuli. The majority of RTKs contain proline-rich sequences in their C-termini. Unlike other signalling through RTKs where tyrosine phosphorylation is required for transmission, proline-rich sequence-dependent interactions are dependent solely on the respective concentrations of protein containing SH3 domains, and can therefore be in competition with each other on interacting with the receptor tail. Recent studies have shown that numerous cancers have an imbalance in protein levels which can regulate receptor-initiated signalling, leading to increased cell motility and invasive behaviour of the cell.

Using a mass spectrometry-based approach peptides containing the proline-rich sequences from several RTKs have been used to identify direct or indirect interactions from with proteins containing an SH3 domain. One of the hits was selected for further validation and an interaction between LASP1 SH3 domain and FGFR2 proline rich motif has been shown by cell based methods. Additionally an interaction between ErbB2 and Src kinase has been shown and data suggests this is mediated by the proline rich motif of the receptor and the SH3 domain of Src kinase. The specificity and the biological outcome of some of these interactions will be further validated in this project.

19 | Mike Davies

Investigating the intracellular interactions of alpha synuclien

Parkinson's disease and dementia with Lewy bodies are neurological disorders characterised by the loss of motor function and the loss of cognitive ability respectively. Both diseases are linked by the presence of intracellular inclusion bodies known as Lewy bodies. A key component of the Lewy body is the intracellular protein α-synuclein, a protein whose physiological function is thought to be related to the trafficking and fusion of synaptic vesicles. Under normal conditions α-synuclein adopts a disorders or helically folded structure. However, under disease conditions, the protein can misfold adopting a beta sheet rich conformation that can then self-assemble into oligomeric and higher order fibrillar structures. Both oligomeric and fibrillar α-synucelin have been linked to *in vivo* toxicity by several mechanisms including pore formation, mitochondrial disruption and lysosomal disruption. Fibrillar synuclein has also been shown to spread between cells in a prion-like manner, seeding endogenous aggregation in recipient cells by an endocytosis dependent process. As the interaction of fibrils with endogenous proteins may be responsible for the observed toxicity, these intracellular interactions of α-synuclein following endocytosis are of interest. The aim of this project is to investigate these interactions by proteomics. Here it is demonstrated that synuclein fibrils in our hands are endocytosed by cells as previously shown and that fibrils can be efficiently isolated from the cells following endocytosis.

20 | Jemma Day

Malarial molecular chaperones: a network of drug targets

Malaria leads to 440,000 deaths per year with *Plasmodium falciparum* causing the majority of cases. There is still a great need for new therapeutics as resistance emerges, vaccines continue to remain challenging and mortality rates have stalled.

Molecular chaperones play a part in survival and proliferation of the *P. falciparum* parasite in times of stress. Additionally, a specific subsection of chaperones are exported into the host and are thought to contribute to red blood cell remodelling and thus pathogenicity. An understanding of the chaperone network could lead to new therapeutics through repurposing existing chaperone inhibitors or designing new inhibitors.

Here we present the structural and functional characterisation of exported malarial chaperones; PfHsp70-x, PFA00660w and PFE0055c, an Hsp70 and two Hsp40s respectively. An integrated approach utilising NMR and x-ray crystallography enables us to study the interaction between PfHsp70-x and its cochaperones in addition to potential inhibitors.

21 | Katie Day

Combinatorial Domain Hunting (CDH). Solving your protein production challenges

Are you experiencing challenges in expressing your protein domain(s) of interest to sufficient yield, solubility and crystallinity?

Do your proteins have poorly understood domain organisation or are they unrelated to known structures?

If so, Domainex may be able to provide you with a solution.

We provide a comprehensive protein characterisation service. At its heart is our patented Combinatorial Domain Hunting (CDH) technology which has been applied to over 50 targets from a range of cytoplasmic protein classes including kinases, epigenetic targets, transcription factors, proteases and deubiquitinylating enzymes (DUBs) with an overall success rate in excess of 90%. CDH couples random DNA gene fragmentation to efficient screening of resultant protein fragments to identify soluble protein domains for a range of applications including structural analysis and biochemical and biophysical screening. We produce high quality protein suitable for SBDD, FBDD, HTS and Antibody screening.

We present two cases studies: a DUB and Lysine Methyltransferase KMT2D (MLL4).

22 | Dario De Vecchis

The Band 3 membrane transporter in a lipid bilayer: Insights from molecular dynamics simulations

Band 3, the red blood cell chloride/bicarbonate anion exchanger (AE1/SLC4A1) is responsible for the rapid exchange of bicarbonate with chloride across the red blood cell membrane, a process necessary for efficient respiration. Human Band 3, a 911 amino acid glycoprotein, is comprised of a cytosolic domain and a membrane domain that contains 14 transmembrane helices. Although recent structural data provide some insights into Band 3 domain topology, the complete structure of Band 3 remains elusive. Modelling the complete Band 3 and determining its dynamics in a membrane environment will enable us to provide new knowledge about its transport mechanism as well as its organization in the red blood cell membrane.

By integrating molecular modelling and molecular dynamics simulations we propose a model of the complete Band 3 protein and investigate its dynamics in a red blood cell native-like membrane environment, containing a full complement of phospholipids, as well as cholesterol. This model shows the orientation of the cytosolic domain relative to the transmembrane domain and sheds light into the role of the linker region that connects these two domains. Residues that are involved in the association of the two domains and in the Band 3 dimer interface were also identified. Additionally, our studies explore the interactions of the cytoplasmic and the transmembrane region of Band 3 with specific lipids including PIP2 and cholesterol. We will use this membrane model to investigate the conformational changes in Band 3 associated with anion transport. The structural and dynamic information about Band 3 from this study enables us to increase our understanding for the mechanism of action of this very important transport protein.

23 | Ruth Dingle

Conformational sampling of arginine side chains

Amino acid side chains are fundamentally involved in all aspects of the biology and pathology of proteins. They have been shown to play critical roles in protein folding, catalysis and interactions with other proteins, nucleic acids, ligands and drugs. Despite their importance, understanding of the behaviour of side-chains is limited. Currently, there are only a few methods available to probe the relationship between their conformational sampling and protein function and these are mainly focused on those containing methyl groups.

To characterise side chain conformational sampling in a range of amino-acids (particularly those without methyl groups), we have adapted two NMR pulse sequences for measuring 3-bond J-couplings. We have measured coupling constants across all the side chain dihedral angles of the 13 arginine residues in the 18.6 kDa protein T4-lysozyme. From this, we have determined the conformation of these residues in a solution state. These results highlight correlations between dihedral angles and chemical shifts. Whilst this relationship has yet to be fully explored, it is a promising route for obtaining similar structural as well as dynamic information in systems not suited to extended, multi-dimensional J-coupling experiments, such as large proteins or minor state conformations.

24 | Ciaran Doherty

Pulling apart αSynuclein via single molecule AFM force spectroscopy

Protein aggregation is linked with the onset of various neurodegenerative disorders including Parkinson's disease (PD) that is hallmarked by the aggregation of alpha synuclein (αSyn). The pathways by which the protein aggregates however, are poorly understood. The toxic species in the aggregation cascade is still unknown, and so looking at the initial dimerisation of αSyn is of high importance, as this interaction offers a valid target for clinical intervention. In this study, the initial dimerisation step in the aggregation pathway is probed at the single molecule level using force spectroscopy.

In this work, we have interrogated the self-association of αSyn at a single molecule level by analysing the strength and conformation of self-association in different environmental conditions primarily by single molecule force spectroscopy (SMFS). The SMFS experiments show that force-resistant structure forms in the dimeric species of αSyn and that this structure is dependent on the environmental conditions. SMFS utilising different immobilisation regimes of αSyn have also allowed the location of a novel interaction interface involving the N-terminal region of the protein. Further SMFS experiments investigating the effects of salt and hydrophobicity have on dimerisation, alongside bioinformatics analyses of the protein sequence led to the hypotheses that the dimeric interaction is driven by hydrophobic stretches in the N-terminal region, but modulated by local electrostatics. In vitro aggregation assays and SMFS on non-aggregation-prone synuclein homologues (β- and γSyn) indicated that that this interaction is protective against aggregation, considering these finding with existing literature prompted speculation that the interactions observed in SMFS may indeed be physiologically relevant. This may therefore be an important finding in regards to targeting the aggregation process with disease modifying agents.

25 | Jessica Ebo

Using antibiotic resistance to select and evolve aggregation-resistant protein therapeutics

The production and formulation of biopharmaceuticals can be hindered by protein aggregation which can occur at every stage of the manufacturing process, ultimately jeopardising the successful development of promising candidates from becoming the next blockbuster biologic. Investigating protein aggregation and stability can be laborious, due to the difficulties inexpression and purification for *in vitro* analysis. To address this, we have developed an *in vivo* platform to characterise the aggregation propensity of biopharmaceuticals that circumvents the need for recombinant expression and downstream analysis. The system, based on a split ß-lactamaseenzyme assay, enables the identification of aggregation-prone sequences inserted between the two enzyme domains, via the survival rates of the bacteria in which they are expressed.

Applying the system to therapeutic proteins with known drug development issues, the antibody MEDI-1912 and granulocyte-colony stimulating factor (G-CSF), we demonstrate the system's ability to distinguish between aggregation and non-aggregation prone sequences, offering a powerful tool for assessing protein aggregation and stability earlier in the industrial pipeline. Furthermore, by developing a directed evolution methodology we show that this system can be used as a novel strategy to modulate the aggregation propensity of protein scaffolds enabling the identification of evolved variants with reduced aggregation propensity.

26 | Brendan Farrell

Structural Investigation of Fibroblast Growth Factor Receptor 3 Regulation

Fibroblast growth factor receptors (FGFRs) are receptor tyrosine kinases which in physiological settings regulate developmental pathways, angiogenesis and wound repair in response to fibroblast growth factor binding. These effects are mediated by stimulation of intracellular signalling cascades which control cell proliferation, differentiation and survival. It is essential, therefore, that FGFRs are tightly regulated to avoid aberrant signalling. This regulation is subverted in numerous cancers and skeletal dysplasias, achieved in a variety of fashions such as by point mutations or oncogenic gene fusions, anchoring FGFRs as a major therapeutic target. Despite extensive research, many facets of receptor tyrosine kinase activation and regulation are undefined. Here, we aim to use structural tools, a combination of NMR spectroscopy and cryo-EM, to gain a deeper, high-resolution, understanding of FGFR regulation which may inform future development of anticancer therapies.

As one aspect of this, using wild-type and oncogenic variants of FGFR3 kinase domain, we investigate FGFR3 as a client for the Hsp90 chaperone machinery of the cellular proteostasis network. Here, using NMR spectroscopy and other biophysical methods, we have furthered our understanding of the role of Hsp90 cochaperone Cdc37 and its recognition of kinase clients.

Additionally, to address the current lack of high-resolution structural information for full-length receptor tyrosine kinases, we aim to solve the structure of full-length, oncogenic point mutation variants or of intracellular gene-fusion constructs of FGFR3. While individual domains of FGFRs have been solved structurally in isolation, only in context of the full-length receptor will we be able to truly begin to understand how ligand binding and/or oncogenic variants induce receptor activation and transmission of signals across the plasma membrane.

27 | Asif Fazal

Understanding Acyltransferase Promiscuity in Polyketide Synthases

Microbial natural products underpin most clinically used drugs. Filamentous members of the phylum *Actinobacteria*, such as *Streptomyces* species are prolific producers of these diverse small molecules. Within natural products, those originating from non-ribosomal peptide synthetase (NRPS) and/or polyketide synthase (PKS) biosynthetic systems are particularly important. For example, the antibiotics erythromycin and tetracycline are polyketides and vancomycin and daptomycin are non-ribosomal peptides. Less common, but still prevalent, are hybrid NRPS/PKS biosynthetic systems.

Antimycins are the product of a hybrid NRPS/PKS biosynthetic system. Antimycins possess potent cytotoxic activity, as they inhibit the cytochrome c reductase, an enzyme involved in the terminal step in respiration. Recently, antimycins were found to be potent and selective inhibitors of the mitochrondrial Bcl-2/Bcl-XL-related antiapoptotic proteins, that are overproduced by cancer cells and confer resistance to chemotherapeutic agents whose mode of action is activation of apoptosis. Thus, there is interest in both understanding how these molecules are made and also bioengineering variants with improved properties.

The PKS involved in antimycin biosynthesis (AntD) contains an unusually promiscuous acyltransferase domain ($AntD^{AT}$). $AntD^{AT}$ utilises a range of 'atypical' malonyl-CoA-based extender units, which manifests in a broad chemical diversity of antimycins produced. The molecular basis of this promiscuity is not understood, but is believed to involve motif III, a four amino acid motif located in the active site of all acyltransferase domains, which has been shown to dictate, in part, substrate specificity. The native composition of $AntD^{AT}$ motif III is AAAH and using CRISPR/Cas9 genome editing we altered this motif to YASH and HAFH, which are signatures for utilisation of methylmalonyl-CoA and malonyl-CoA, respectively. High resolution electrospray ionisation mass spectrometry revealed a reduced portfolio of antimycins were present in chemical extracts generated from motif mutants and tandem mass spectrometry was indicative of antimycins where methylmalonyl-CoA and malonyl-CoA were utilised. Future work will be aimed at understanding the molecular basis of AntDAT promiscuity, as well as how biosynthesis is regulated and permitted. 3D structures will be produced for excised wild-type $AntD^{AT}$ and motif variants using X-ray crystallography. Additionally, crystallography structures will be obtained for the excised keotsynthase-acyltransferase di-domains, with a longer term aim of using cryo electron microscopy to obtain a complete structure of the entire AntD polyketide synthase. Interactions occurring between individual modules and domains will also be probed using a variety of biophysical and structural techniques.

28 | Jonathan Fenn

Structural and functional analysis of the solute-binding protein UgpB involved in glyco-lipid recognition from Mycobacterium tuberculosis

Tuberculosis (TB) is a global disease which kills 1.7 million people per year and is responsible for more deaths world-wide than HIV and malaria. *Mycobacterium tuberculosis* (*Mtb*), the aetiological agent of tuberculosis, has evolved to scavenge nutrients from the confined environment of host macrophages with mycobacterial ATP binding cassette (ABC) transporters playing a key role in nutrient acquisition.

Mtb UgpB (Rv2833c) is the solute binding protein of the essential UgpAEBC ATP-transporter, one of four Mtb ABC transporters implicated by homology in carbohydrate acquisition. Here, we report the structure and functional characterisation of *Mtb*-UgpB. To determine the substrate preference of *Mtb* UgpB a combination of approaches including membrane lipid arrays, microscale thermophoresis and thermal shift assays has revealed that *Mtb* has a preference for glycerol-3-phosphocholine (GPC) and phospholipids containing the inositol-phosphate and serine head groups and sulfoglycolipids.

Differential Epitope Mapping STD NMR spectroscopy (DEEP STD-NMR) has revealed the GPC ligand binding epitope to UgpB and has precisely identified the amino-acid residues that contact the GPC ligand. The glycerol moiety is the main recognition site and is oriented towards the aliphatic residue Leu205 whereas the choline moiety is facing the aromatic residues Tyr78 and Tyr345. Docking calculations are in good agreement with the DEEP STD-NMR data.

In addition, we have also determined the X-ray structure of *Mtb*-UgpB co-crystallised with GPC to 2.2 Å. Our co-crystal structure has enabled us to identify clear conformational changes upon ligand binding compared to the structure of UgpB with no ligand present, and to determine the molecular determinants involved in substrate binding and recognition.

Combined, these biochemical and biophysical approaches provide complementary information regarding the substrate preference of *Mtb*-UgpB and the mode of GPC ligand-protein interaction in binding pocket of the essential *Mtb* UgpB protein. These studies also suggest that UgpB has a role in the transport of additional glyco-lipids found in the *Mtb* cell wall.

Given the limited availability of carbohydrates within the phagosomal environmental niche during *Mtb* intracellular infection, our studies suggest that UgpB enables *Mtb* to optimise the use of scarce nutrients during intracellular infection, linking essentiality of this protein to a potential role in recycling components of cell wall glycolipids. This work lays the foundation for design of inhibitors of this *Mtb* transport system.

29 | Jodie Ford

Re-engineering of SAS-6: from spirals to rings

Centrioles template cilia and flagella and are important for cell division; as such aberrations in their structure or function can give rise to diseases such as microcephaly and ciliopathies. Centrioles have conserved 9-fold radial symmetry, yet the architecture of SAS-6 cartwheel scaffolds supporting this symmetry may differ between *C. elegans* and other species. In most organisms the protein SAS-6 oligomerises to form rings with characteristic 9-fold symmetry. However, electron microscopy and crystallographic studies suggest that *C. elegans* SAS-6 forms instead an intertwined 9-fold symmetric spiral. Thus, there exist two competing visions (rings vs spirals) for centriolar cartwheels in C. elegans. Given the role of *C. elegans* as a model system for centriole biology it is important to determine which SAS-6 oligomer architecture is valid, as this would impact other processes such as centriole elongation. Difficulties in electron microscopy of *C. elegans* centrioles have hampered direct visualisation of the SAS-6 oligomer. Here, we present a protein engineering approach aimed at altering the *C. elegans* SAS-6 oligomer architecture as an alternative way to probe the nature of centriolar cartwheels. We have designed *C. elegans* SAS-6 variants to form planar rings instead of spirals, and carried out molecular dynamics simulations to screen the oligomeric architecture of these variants prior to testing in vitro. We will discuss our latest results from the analysis of these variants by electron microscopy, AFM and crystallography.

30 | Ying Ge

Characterisation and Engineering of a Cyanobactin Heterocyclase

Cyanobactins are a class of natural products that have exhibited drug-like properties, including antibacterial, anti-malarial and anti-cancer activities. They are typically N-C macrocyclic and some have undergone additional chemical modifications such as heterocyclization and prenylation. The cyanobactin precursor peptide contains one or multiple hypervariable cores, which become the mature peptide, and conserved sequences that are recognised by its post-translational modification enzymes (PTMEs). By varying the core sequence, a library of natural and unnatural cyanobactins can be created, each one of which can then be modified differently to further expand the repertoire of compounds. One limitation of this approach is that only proteinogenic amino acids can be synthesised to start with, since precursor peptides are made by the ribosome. In addition, conserved recognition sequences are typically nearly five times longer than the final product, resulting in a large waste of material. We engineered one of the PTMEs, the heterocyclase, to remove the need for a recognition sequence on its substrate, the precursor peptide. The engineered enzyme enabled the introduction of azol(in)e moieties into short peptides (just above 10 residues) that simultaneously contain heterocyclizable residues and non-proteinogenic amino acids and/or non-amino acids. As the heterocyclase is capable of catalysing multiple residues on its substrate, we investigated the temporal and spatial preferences of the native and engineered enzymes. We discovered that the modified enzyme lost the reaction order observed for the native enzyme, and as a result produces a wider variety of products. The modified enzyme has also been shown to heterocyclize Cys/Thr/Ser-containing peptides that bear no homology to cyanobactins. In conclusion, our engineered heterocyclase improved the economy of the *in-vitro* synthesis of cyanobactins and derivatives, expanded the product diversity, and demonstrated potential for utilisation in the synthesis of other azol(in)e-containing peptides.

31 | Ashleigh Goodenough

Developing an *in vivo* probe to visualise perineuronal nets, a key structure for neuronal plasticity

Perineuronal nets (PNNs) are a condensed form of extracellular matrix that encase neurons in the central nervous system (CNS) and are instrumental for neuronal plasticity. Current research focuses on enhancing neuronal plasticity in various CNS pathologies, however there is no tool available to monitor plasticity and thus the potential treatment effect *in vivo*. *Wisteria floribunda* agglutinin (WFA) is a dimeric plant lectin used to visualise PNNs in fixed or post-mortem tissues via its high affinity binding to PNNs. WFA cannot be used to visualise PNNs *in vivo* due to its toxicity in animals via agglutination of haemocytes. Stable WFA monomers which lack the ability to crosslink haemocytes could be used *in vivo* to visualise PNNs in real time i.e. to assess disease progression and also potential treatments. We monomerised WFA by reducing the disulphide bond joining the two identical subunits, with the reducing agent TCEP. To prevent re-dimerisation of the WFA monomers, the WFA was further treated with iodoacetamide to create stable monomeric WFA. Histochemistry in P28 rat cortex showed that non-toxic WFA monomers were able to bind to PNNs. Thus we have identified monomeric WFA as a key candidate for further development into an *in vivo* probe for PNNs.

32 | Sandra Greive

Probing the function of a thermostable viral portal protein using single molecule electrical sensing

Double stranded DNA viruses, such as bacteriophages and herpes viruses package their genomes into preformed capsids through a pore in a unique vertex of the capsid using energy generated by a powerful ATP dependent motor. This pore is defined by the portal protein, is a circular dodecameric assembly that provides a scaffold onto which the motor is docked, and guides the DNA into the capsid during the packaging process. We have determined the stability of the WT dodecameric portal assembly from the G20c thermophage using thermofluor assays, fluorescence spectroscopy and negative stain TEM under various conditions. Additionally, we have also defined these characteristics for-, along with X-ray structural definition of- various mutant portal assemblies designed to confer specific properties to the original dodecamer.

We characterize here the electrical properties of lipid-embedded protein channels of a single-mutant porphyrin-anchored protein assembly, as well as a triple mutant in which we have engineered an increased tunnel diameter. Finally, we use a series of cyclo- dextrins to size the inner constriction of the pore, as well as to provide insight into the electroosmotic flux direction, revealing asymmetric transport that possibly originates from the portal's proposed DNA-ratchet function.

33 | Marie Grypioti

Development of tools to understand nutrient processing in mycobacteria

Mycobacterium tuberculosis (*Mtb*) is the causative agent of tuberculosis (TB). TB is the leading cause of death worldwide from a single-infectious agent, with 1.7 million deaths per year with a predicted one-third of the global population being latently infected with *Mtb*. *Mtb* can persist in the human host in a latent state for decades and has evolved specialised mechanisms for long-term intracellular survival. However, the nutrients that are available to *Mtb* intracellularly during infection are not fully understood. We have undertaken a multidisciplinary chemical biology approach to understand the nutrient sources that *Mtb* uses to survive and its mechanisms for doing so.

We have developed novel chemical probes of cell-envelope carbohydrates to enable the identification of *Mtb* proteins that interact with specific glycans. The modular design of the tri-functional probe consists of the carbohydrate of interest, a photo-reactive group for crosslinking and a biotin-tag for isolation of captured proteins. Mass spectrometry and proteomics analysis has enabled the identification of *Mtb* proteins that interact with specific carbohydrates at defined time points. This approach has enabled us to identify new enzymes in *Mtb* carbohydrate acquisition and metabolism pathways that provide new insights into key biochemical pathways in mycobacteria that have the potential to become novel drug targets.

34 | Nicolas Guthertz

Structural understanding of the molecular mechanism of amyloid formation, update on the β2m story.

Preventing protein aggregation is of paramount importance in the mission to alleviate some of the most prevalent diseases in the developed world, from the neurodegenerative disorders Alzheimer's disease and Parkinson's disease, to localized or systemic diseases such as type II diabetes mellitus and familial amyloid polyneuropathy. The key pathological hallmark of these diseases is the accumulation of aggregated proteins into large fibrillated structures known as amyloid plaques. Here we describe, a novel *in vivo* system capable of simultaneously assessing aggregation prone sequences and inhibitors that prevent the aberrant self-assembly.

35 | Collette Guy

Chemical tools to target Mycobacterium tuberculosis

Tuberculosis (TB) is a major global health threat as the world's leading cause of death from a single infectious agent, with 1.7 million deaths in 2016. The current treatment regime to treat drug sensitive TB requires a six-nine month course of the front line antibiotics isoniazid, rifampicin, ethambutol and pyrazinamide. In recent years, there has been a rise in resistant strains of TB leading to multidrug resistant TB (MDR-TB), extensively drug resistant TB (XDR-TB) and totally drug resistant TB (TDR-TB). As a result of this the next generation of antibiotic agents to treat TB are urgently required.

The causative agent of TB, *Mycobacterium tuberculosis*, possesses a cell envelope distinct from that of other bacterial pathogens. The cell envelope consists of a diverse range of complex carbohydrates and lipids that provide a highly efficient permeability barrier that prevents the intracellular access of many antibiotics.

The cell envelope and many of its components have been shown to have fundamental roles in pathogenesis and virulence, and as such enzymes and pathways involved in the biosynthesis of the mycobacterial cell wall are targeted by current TB drugs such as isoniazid and ethambutol, as well as being the focus of many drug development programmes. We hypothesised that the glycans present in the cell envelope could be targeted directly, rather than the pathways involved in their synthesis, as a novel route for targeting mycobacteria that has not previously been explored.

We have designed and synthesised a library of compounds that we have shown to be selectively active against a panel of mycobacteria with selectivity over Gram-negative bacteria and mammalian cells. Whole cell shotgun proteomics and biophysical measurements have been undertaken to elucidate the mechanism of action. Our results indicate that these compounds provide a new mode of action that can be further explored for the development of future TB therapeutic agents.

36 | Ashley Hancock

A Bottom-up Approach of Increasing the Absorption of a Light-Harvesting Protein-Based Nanomaterial

Light-Harvesting Complex II (LHC-II) acts as the primary antenna protein for all higher-order plants, collecting excitation energy at near quantum efficiency. Excitation is transferred through the antennae to photosystem proteins via the non-radiative process of Förster resonance energy transfer (FRET). The combination of chlorophyll and carotenoid pigments within the LHC-II protein provide relatively efficient absorption across the full visible spectrum aside from the 'green gap' of minimal absorption between 520-625nm.

Here, we show progress towards creating a lipid bilayer-based modular system where native LHC-II trimers are reconstituted into proteoliposomes containing lipid-linked chromophores to fill the absorption 'green gap' of LHC-II. Spectroscopy of proteoliposomes in solution has shown successful incorporation of LHC-II at a range of protein/lipid ratios by absorbance and the expected LHC-II+LHC-II interactions by steady state and lifetime based fluorescence. Donor-to-acceptor FRET from the additional chromophore is shown by quenching of donor fluorescence and increase in LHC-II emission. Atomic force and fluorescent microscopy reveal optical functionality of the proteoliposomes is maintained on a surface and the de-quenching of donors when LHC-II is damaged provides additional evidence of FRET taking place. In summary, we effectively increase the absorption cross section of the system with potential for improving biomimetic devices.

37 | Fruzsina Hobor

Understanding the basis of HIF-1α/p300 interaction using a biochemical approach

Hypoxia inducible factor (HIF)-1α regulates the hypoxic response in cells through a wide range of protein-protein interactions (PPIs). HIF-1α/p300 interaction plays an important role in tumour metabolism hence inhibiting this PPI represents a potential target for cancer chemotherapy[1].

This presentation will outline our latest efforts to exploit known structural data, together with *in silico* and *in vitro* methods to build an understanding of structure-binding relationships for HIF-1α/p300. These analyses will inform and underpin efforts to identify inhibitors of the HIF-1α/p300 interaction.

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38 | James Hodgkinson

Bridging the gap in the CoREST corepressor complex

Chromatin modifying enzymes play an integral role in the chemical makeup of the epigenome and subsequently the regulation of gene expression. In recent years it has become apparent such enzymes do not necessarily act in an isolated fashion but as subunits in much larger multiprotein complexes. The multiprotein CoREST complex consists of the corepressor CoREST and two enzyme subunits; lysine-specific histone demethylase 1A (LSD1) and Histone Deacetylase 1/2 (HDAC1/2). LSD1 and HDAC1/2 are important epigenetic drug targets for cancer in their own right and have received considerable attention from academia and the pharmaceutical industry alike.

Based on 2D class averages of the CoREST complex by negative stain electron microscopy we aimed to synthesise and evaluate dual functionalised peptides as chemical biology tools for structural studies of the CoREST complex. A series of five dual functionalised peptides were synthesised using SPPS. The dual functionalised peptides consisted of a HDAC inhibitor and LSD1 inhibitor separated by linkers of various lengths. All five peptides demonstrated dual enzymatic inhibitory activity against LSD1 and HDAC1 in the CoREST complex from mid to low nanomolar concentrations. It is hoped such chemical biology tools can give an estimate of the distance between the two enzymatic sites in the complex, aid in future drug design and they will also be used in further structural experiments such as cryo-electron microscopy.

Key words: Epigenetics; Chemical biology tools; CoREST complex; Peptide synthesis.

39 | Francis Hopkins

Ribonucleoprotein Structure in Pathogenic Orthobunyaviruses

The Orthobunyavirus genus within the Bunyavirales order contains many pathogens of humans and livestock. Their genome consists of three segments of negative-sense, single-stranded RNA. These are encapsidated with polymers of nucleocapsid protein (NP) which binds RNA in a sequence-independent manner to form ribonucleoproteins (RNPs). Formation of RNPs protects the viral genome from the host immune system and has roles in transcription, genome replication and the correct packaging of segments into new virions. Thus, the RNP is an attractive target for the development of new antivirals, which is of particular importance as such therapies do not currently exist for any of the human pathogenic orthobunyaviruses.

Using a combination of structural and biophysical techniques including cryo-EM, this project aims to determine high resolution structures of orthobunyavirus RNPs to shed light on their overall architecture, assembly and mechanisms of RNA binding.

Recombinant NP from different orthobunyaviruses has been used to reconstitute RNPs for analysis by negative stain EM, allow visualisation of full length RNPs and different oligomeric states of NP which are potential assembly intermediates. Infectious Bunyamwera virus has also been propagated and purified by ultracentrifugation, allowing the extraction and purification of RNPs from virions which have been visualized by negative stain EM. Work is ongoing to optimize these and reconstituted RNPs for analysis by cryo-EM, and to investigate the arrangement of RNPs within Bunyamwera virions by cryo-electron tomography.

The work presented here will aid in the development of small molecules which inhibit formation of the orthobunyavirus RNP and which could be invesitgated further for their therapeutic potential.

40 | Jim Horne

A novel crosslinking method for the rapid and unbiased analysis of protein-protein interaction interfaces *in vitro*

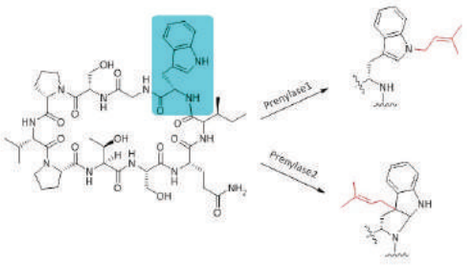
Current crosslinking approaches for investigating protein-protein interactions *in vitro* tend to suffer from three major drawbacks: slow rates of crosslinking, residue bias in their targets, and challenging enrichment and analysis of modified proteins/peptides. We have developed a new methodology to overcome all these issues with crosslinking reactions completed in 15s, a photoactivatable crosslinker that inserts into any -CH, -NH, -SH, or -OH bonds, and a strategy for enrichment and residue-level identification of interactions.

The method uses a photoactivatable crosslinker which can be conjugated on a bait protein of interest via a native cysteine or one introduced by mutagenesis. A custom-built UV LED lamp can crosslink samples in a specially designed Perspex chip for maximum speed or in a cuvette or Eppendorf for convenience. The presence of a reversible disulphide bond to the bait protein allows this to be reduced leaving behind a free thiol (–SH) group on the target protein(s). This can be capped by a small molecule to allow identification of the modified residue by mass spectrometry or it can be used to bind to thiol affinity resin for further enrichment and/or on-resin digestion.

This methodology is demonstrated for dynamic chaperone:protein complexes between outer membrane proteins and their periplasmic chaperones from *E. coli* as well as for a tight protein-protein interaction between the apoptotic regulatory pair Mcl-1/Bid from *H. sapiens*.

41 | Wael Houssen

Insights into the chemoenzymatic tryptophan C and N-prenylation of peptides



Our recent work progress on the use of cyanobactin biosynthetic enzymes to catalyse prenylation of tryptophan residues in linear and cyclic peptides will be discussed. Cyanobactins are cyanobacterial ribosomal linear and cyclic peptides that are characterised by having heterocycles, oxidised heterocycles, epimerised centres and, in many cases, prenylated and geranylated residues. They are part of the rapidly growing class of ribosomally-synthesized and post-translationally modified peptides (RiPPs). Like other RiPPs, they are biosynthesised on the ribosome as linear precursor peptide which is modified by a set of processing enzymes. We used genome mining, bioinformatics and biochemical testing to identify *C- and N-* prenyltransferases and to study their substrate promiscuity. These enzymes are invaluable tools to expand the diversity in cyclic peptide libraries.

42 | Ashley Hughes

Development of pump-probe Circular Dichroism at B23 beamline, Diamond Light Source

Presented here is the recently developed time-resolved pump-probe Circular Dichroism (CD) at Diamond Light Source B23 beamline for Synchrotron Radiation Circular Dichroism (SRCD). CD is a highly sensitive known technique used to characterise the conformation of biologically important molecules such as protein and DNA and study their ligand binding interactions in solution. For protein refolding, stopped-flow CD is the ideal technique to monitor and measure the refolding dynamic under relevant buffer and temperature conditions. However, only after a mixing time of 4-5ms time scale can be accurately measured.

The unique B23 capability of conducting pump-probe SRCD experiments has been successfully extended to the 0.5ms time scale resolution using a flat capillary of 0.03cm pathlenth that enabled the photo-irradiation of sub microliter sample solutions. We have validated the B23 pump-probe system studying the known photo-switchable and reversible process of the bacterial phytochrome construct CBD-PHY1 using 671 nm and 780 nm laser wavelengths. The data recorded with a 0.25ms integration time coupled with a 1ms excitation pulse from a CW 671 nm laser source were consistent with the results obtained by X-ray1 and recent FTIR work.

Diamond B23 pump-probe facility is now available to users for the study of photo-induced processes and of photo-induced reactions of photo-labile ligand systems. These can include a range of light harvesting proteins vital for vision, photosynthesis and the emerging field of ontogenetic. The system can also be utilised together with photo labile systems such as caged metals, protons and drug delivery systems allowing for the recording of fast (<5 ms) interaction kinetics not available with current techniques such as stopped flow CD.

43 | Ashley Hughes

Photon-induced protein stability and ligand binding using Diamond B23 beamline for SRCD

Diamond B23 beamline for synchrotron radiation circular dichroism (SRCD) has been built to provide a high UV photon flux at the sample in the region of 3.2 x 10^{12} photons s^{-1} (0.1% bandwidth)$^{-1}$[1]. Although high UV photon flux leads to protein denaturation in the far-UV region [2], implementation of a set of effective measures enables the photon flux at the sample to be controlled greatly mitigating or eliminating the denaturation of biomolecules. Protein UV photo denaturation, however, has been exploited as a novel assay to assess biopolymer photostability using B23 beamline.

Like thermal denaturation, photo-denaturation in the far-UV region varies from protein to protein showing different amount of conformational change that correlates with the degree of protein stability. In general biopolymers denaturation induced by vacuum- and far-UV light is radiation power and dose dependent and has been observed with SRCD beamlines operating at 1 nm or higher bandwidth[3].

It has been suggested that water radiolysis is the likely cause of protein UV denaturation[4] although another interpretation has been put forward with the heating of bound water molecules to proteins[5] [6] as the possible culprit. This latter suggestion is based on the apparent similarity of the CD spectra of protein under UV irradiation or heating. The fact that under the same conditions – number of repeated scans and beamline configuration in the far UV region – the protein UV denaturation does not occur in the near-UV region (250-330nm) even though the thermal denaturation can still be observed is an indication that the origin of the protein UV denaturation is more likely due to water radiolysis than thermal effects.

The good news is that protein UV denaturation using B23 can be controlled and that the rate of protein UV denaturation can be used to quantify the photostability of proteins.

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44 | Julia Humes

Understanding how the ATP-independent periplasmic chaperone SurA prevents OMP aggregation

Outer membrane proteins (OMPs) in Gram negative bacteria, which are required for many processes, must be transported from their site of synthesis in the cytoplasm to the outer membrane, via the periplasmic space. This requires a network of molecular chaperones to prevent aggregation or premature folding. My project focuses on SurA, the primary chaperone in OMPs transport through the periplasm.

The mechanism of how SurA recognises OMPs and allows them to be inserted into the membrane by the ß-barrel assembly machinery (BAM) complex, in the absence of ATP, is not fully understood. SurA is essential for the biogenesis of a number of OMPs such as LamB and its deletion results in reduced abundance of many other OMPs folded in the membrane.

I have been studying both full length SurA and SurA truncation mutants and their effects on OMP binding and delivery. The truncation mutants have one or both of the PPIase domains removed. My results have shown that SurA binds a range non-native OMPs, with a micromolar affinity agreeing with previous findings and that removing the PPIase domains does not reduce affinity, suggesting the core domain is responsible for binding. The removal of the PPIase domains however does effect the chaperone activity suggesting that these domains aid in preventing OMP aggregation.

45 | Nicholas Hurst

Structural characterisation of IRE1α's multi-step activation mechanism and how it is perturbed by cancer-associated mutations

The unfolded protein response is an important and multi-faceted pathway; it allows the cell to respond to ER stress caused by an overload of unfolded membrane and secreted proteins. The components of the response include stress sensors that transverse the ER membrane; these sensors initially elicit a pro-survival adaptive response before adopting a pro-apoptotic response following continued stress. Inositol-requiring enzyme 1α (IRE1α) is the most evolutionary conserved ER transmembrane sensor. IRE1α consists of an ER luminal domain which is regulated by interactions with chaperone proteins, and binds unfolded proteins to promote its oligomerisation upon ER stress. Luminal domain oligomerisation promotes the autophosphorylation and clustering of a cytoplasmic region of the protein, containing both kinase and endoribonuclease activities. The endoribonuclease activity of the cytoplasmic domain directs the cell to respond to the ER stress. The signal produced (adaptive or apoptotic) is dependent on the level of activation of IRE1α and through the selection of different RNA targets the cellular response is altered. Previous studies have only supplied a limited understanding of IRE1α's multistep activation, which appears to be a very complex mechanism involving multiple conformational changes alongside allosteric interactions for each IRE1α domain.

Aberrance in the unfolded protein response pathway causes a number of diseases and can aid viral replication as well as cancer growth. A number of cancer-associated mutations have been identified throughout both of IRE1α's domains. However, there is little functional information for how these mutations are able to promote cancer progression. Utilising a range of biophysical and structural techniques with the separate IRE1α domains, we aim to provide an insight into the conformational rearrangements that dictate the different stress responses, in order to aid therapeutic drug design for the system. This will include investigation of activation in *wild-type* IRE1α, its fine tuning by cancer-associated mutations and also through allosteric interactions.

46 | Alistair Jagger

Conformational dynamics of the disease-associated protein alpha-1-antitrypsin

α-1 antitrypsin (α1AT) is a 52 kDa serine protease inhibitor abundant in human plasma. Many naturally occurring single amino acid substitutions, the most common of which is the Z (Glu342Lys) mutation, promote misfolding and aggregation into long, ordered polymer chains at the site of synthesis in the liver endoplasmic reticulum. Accumulation of polymers leads to liver cirrhosis and the reduced protection of the lungs against proteolytic degradation predisposes individuals to early-onset emphysema. The molecular defect that promotes polymerisation remains incompletely understood: a crystal structure of the Z variant is essentially identical to that of wild-type (WT) α1AT and therefore fails to encapsulate this aberrant behaviour[1]. α1AT is intrinsically conformationally labile, and it is therefore the dynamic behaviour of the protein that is key to elucidating the molecular basis for the enhanced polymerisation. Here we have used NMR spectroscopy on patient-derived samples at natural isotopic abundance to investigate for the first time the effect of mutations and posttranslational modifications on the solution structure of α1AT. High-quality ^{1}H-^{13}C NMR correlation spectra have been acquired for two patient-derived aggregation-prone variants and these show long-range chemical shift perturbations, indicating that the solution-state structures of these variants are indeed different to that of the WT. Reference of these perturbations to the near complete assignment of the spectra of [^{2}H,$^{13}CH_3$–AILVM]-labelled WT α1AT also suggests that a long-range allosteric network may exist within the protein, and this is currently being investigated further using NMR measurements of dynamics across a range of timescales.

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47 | Thomas James

Perturbation of -Helix Mediated Protein-Protein Interactions

Protein-protein interactions (PPIs) have historically been considered challenging to 'drug' using small molecules due to the large, comparatively flat interface that mediates the interaction. We aim to address this grand challenge by designing novel PPI inhibitors using a combination of *in silico* design, synthetic methodology and experimental validation.

Initially focussing on a-helix mediated PPIs, our approaches to the development of small molecule inhibitors have been twofold: the screening of small molecule libraries and a rational design to dissect key determinants of protein-protein binding interactions. Using a predictive approach, coupled with synthesis, and subsequent determination of the binding affinities of a library of variant peptides, we have identified 'hot-spot' models for several PPIs. This has deepened our understanding of PPI topography, increased the robustness of our approach and will facilitate the identification of low molecular weight PPI inhibitors.

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48 | Kiani Jeacock

Synergistic Effects of Medin and Amyloid-Beta in Vascular Dysfunction

Medin is the main component of aortic medial amyloid (AMA) deposits in cardiovascular amyloid disease and may play a role in vascular dysfunction. Together with collaborators in the USA we have shown that amyloid-ß (Aß) and medin affect endothelial cell vasodilation and induce production of reactive oxygen species which may contribute to vascular dysfunction (Truran et al. 2016, Migrino et al. 2017). In addition, our recent findings demonstrated a striking resemblance between the sequences of both proteins, identifying aspartate and lysine residues in medin analogous to Asp^{23} and Lys^{28} of Aß, which influences the aggregation propensity of the protein by forming a salt-bridge capable of stabilising the fibrillar conformation of the protein (Davies et al. 2015). Owing to their structural similarities we aim to characterise a specific interaction between medin amyloid and Aß *in vitro* using nuclear magnetic resonance (NMR) spectroscopy. The synergistic effects of the proteins on their aggregation kinetics and fibril morphology will also be explored using Thioflavin T (ThT) fluorescence assays and electron microscopy. This study, supplemented by patient sample data, will begin to reveal the molecular mechanism underlying the potential involvement of medin in cerebrovascular and cardiovascular diseases.

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49 | Rachel Johnson

Cryo-EM studies of cytochrome bc1 to elucidate inhibitor binding

Parasitic protozoa are responsible for causing a wide range of devastating diseases. Diseases such as malaria, leishmaniasis, toxoplasmosis and sleeping sickness are a significant global burden with over ~500 million people affected each year. Malaria is caused by the protozoan parasite *Plasmodium falciparum (P. falciparum)* and predominately affects people in the developing world. However, resistance is emerging to all current treatments therefore there is an urgent need to develop new medicines. Cytochrome bc_1 is an established anti-parasitic drug target with a number of crystal structures which are bound to highly potent inhibitors. However, the design of compounds which are more selective to the parasitic targets over the model bovine system has been hindered by a lack of structural information of the parasite-derived protein. Elucidating structural information using X-ray crystallography requires large quantities of protein which cannot be obtained from the host parasite therefore alternative strategies, such as EM, are needed. As near-atomic resolution structures are more routinely being solved, EM could be used as a tool in drug discovery. In this project, cryo-EM has been used in a proof of principle approach to solve three structures of bovine bc_1 in the absence and presence of two different inhibitors (GSK932121 and SCR0911) to ~4.1 A resolution. The resolutions attained have enabled the inhibitors to be unambiguously positioned in the density thereby showing that cryo-EM could be used on the parasitic-derived protein.

50 | Kwan Ting Kan

Investigating the function and functional mechanism of mitochondrial i-AAA protease Yme1

Majority of mitochondrial proteins are synthesised in the cytosol and then imported into the mitochondria. The correct folding and assembly of imported proteins within the organelle is essential to their function. Indeed, accumulation of misfolded and non-native proteins would lead to increased toxicity, mitochondrial dysfunction, and would ultimately contribute to aging and various diseases. As a result, a network of protein quality control systems had been evolved to recognize and remove non-native proteins in order to maintain proteostasis. The i-AAA protease Yme1 (Yeast mitochondrial escape 1) was shown to play an important role in mitochondrial protein quality control. It is an inner membrane anchored hexametric protein complex consisting of three domains with the ATPase and protease domains located in the intermembrane space (IMS). Yme1 degrades misfolded or unassembled proteins of the inner membrane and the IMS selectively. For example, we showed previously that unassembled Tim10 is degraded by Yme1 but not Tim9. Furthermore, studies have also demonstrated the chaperone activity of Yme1 through the disaggregation of its substrates. Although the structure of Yme1 had been recently solved, the regulation and functional mechanism of Yme1 has yet to be fully understood. The overall aim of our study is to understand the function and functional mechanism of Yme1. Constructs with Yme1 ATPase domain alone, as well as both ATPase and protease domains together were expressed and purified in *E. coli*. Folding and function of these proteins, as well as how the WT and Yme1 mutants affect the folding and degradation of the IMS protein Tim9 and Tim10 are under investigation, using various biochemical and biophysical methods. Furthermore, how Yme1 affect mitochondrial proteostasis and yeast ageing *in vivo* have been studied using the WT and yme1 deletion yeast strains. Our results have shown that Yme1 plays an important role maintaining the chronological life span of yeast.

51 | Safi Kani Masandi

Structural and functional characterisation of the BRISC-SHMT2 and BRCA1-A complexes

Ubiquitylation is a post-translational key regulator of protein activity, function and stability. It plays a significant role in many cellular processes including signal transduction and proteasomal degradation. Ubiquitin-mediated signalling is controlled by the ubiquitin processing enzymes E1, E2 and E3. A single ubiquitin moiety can be conjugated to lysine residues on the surface of substrate proteins or further conjugated to lysine residues on the surface of ubiuitin resulting in a polyubiquitin chain. Seven lysine residues (K6, K11, K27, K29, K33, K48 and K63) and the free amino terminus (M1) on ubiquitin can be targeted for poly-ubiquitylation. Eight different ubiquitin chains can therefore be generated each with its own unique topology and signalling property. Ubiquitylation is a reversible process. Ubiquitin can be removed from a substrate protein or (poly)ubiquitin chain by deubiquitylating enzymes (DUBs). DUBs can be divided into six families: USP5, OUT, UCH, Josephins, MINDY and JAMM/MPN. The JAMM/MPN family require Zn^{2+} for catalysis and the remaining five are cysteine proteases. There are 14 members within the JAMM/MPN family: BRCC36, Abraxas1, Abraxas2, CSN5, CSN6, PSMD14, PSMD7, eIF3f and eIF3h. Seven out of 14 family members are catalytically active (denoted MPN^+) while the others, which bear substitutions to essential catalytic residues, are catalytically inactive (denoted MPN^-) and termed pseudo-DUBs. MPN family proteases typically function within larger multi-subunit complexes, which imparts great potential for multi-layer regulation.

BRCC36, an active MPN protein, forms two protein complexes with the catalytically inactive proteins Abraxas1 and Abraxas2. BRCC36 together with Abraxas1, BRCC45, MERIT40 and RAP80 forms the BRCA1-A complex in the nucleus. BRCC36 and Abraxas2 on the other hand along with BRCC45, MERIT40 and SHMT2 constitute the BRISC-SHMT2 complex in the cytoplasm. The BRCA1-A and BRISC-SHMT2 complexes are involved in DNA damage repair and interferon signalling respectively. We are using crystallography and cryo-EM techniques in combination with biochemical and biophysical analyses to investigate the structure, function and regulation of BRCC36 DUB complexes.

52 | Anastasia Kantsadi

Structural insights into the molecular mechanism behind centriolar cartwheel assembly

Centrioles are cylindrical organelles which are essential for forming cilia, flagella and centrosomes in animal cells. In this capacity centrioles are important for mitotic spindle formation, correct chromosome segregation and cell polarity during division[1]. The wide-reaching contributions of these organelles are best appreciated when errors in centriole assembly occur leading to a broad range of human diseases such as primary microcephaly, male sterility and cancer[2] [3].

Structurally, the best studied region of centrioles is the cartwheel, which is the first region forming during centriole and basal body assembly. The cartwheel is 9-fold symmetric and, thus, it is seen as critical for imparting 9-fold symmetry to the entire organelle. The evolutionary conserved protein SAS-6 is essential for cartwheel formation and it is recruited to the site of centriole assembly at the onset of their duplication. The cartwheel consists of a circular hub from which nine spokes emanate and radiate towards the cartwheel periphery, where spokes originating from two superimposed rings merge. The joined spokes then connect to a pinhead that bridges them with peripheral-most microtubules[4] [5] [6]. However, we currently do not understand the molecular basis of cartwheels stacking or how do cartwheels connect via pinheads to peripheral microtubules to form the complete organelle.

Here we present our progress combining biophysical and structural approaches with the aim to study the oligomerisation properties of SAS-6 and elucidate how SAS-6 ring oligomers stack along the length of centrioles, thereby providing an initial scaffold for subsequent recruitment of further centriole components. The proposed work will provide robust framework for understanding biogenesis of the centriole organelle.

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53 | Sarah King

Increasing FLImP resolution using cryogenic conditions: a feasibility study

Fluorophore Localisation Imaging with Photobleaching (FLImP) is a single molecule localisation method used to measure pairwise lateral separations between identical dye molecules with a ~5-7nm resolution, returning normalised distributions in the 0-60nm range. FLImP analysis can be used as a technique to determine the dimeric and oligomeric status of the Epidermal Growth Factor Receptor (EGFR) with a bound fluorophore-conjugated ligand in cancer cells lines and cell models. The FLImP method requires cells to be fixed before taking measurement with reagents such as glutaraldehyde and formaldehyde, which involves cross-linking of molecules that has the possibility of inducing artefactual oligomerisation. Vitrification of samples in fully hydrated conditions has been shown to alleviate the formation of artefacts in ultrastructural studies. This mode of imaging can be applied to improve super-resolution FLImP method if the stability of the microscope can be validated. The aim is to test the improvement in resolution for a set of dyes and to label EGFR in cells to validate the cryo-FLImP method. Using cyro-fluorescence to achieve higher signal to noise ratios for FLImP will thereby increase the localisation accuracy of single molecules and hence separation resolution.

54 | Patrick Knight

Surface Induced Dissociation Mass Spectrometry (SID-MS) for the investigation of native protein complexes

The formation of higher order protein structures is a vital part of many life processes and diseases. Native mass spectrometry can be used to investigate the composition and structures of these complexes. Top down analysis of protein complexes can reveal information about the constituent subunits of a complex, however, traditional MS fragmentation techniques such as collision induced dissociation (CID) typically result in the unfolding and ejection of a monomeric protein yielding little information about the topology of the intact structure. Surface induced dissociation causes the ejection of folded subunits from the complex of interest. These subunits may be protein complexes in their own right.

Here we develop an SID workflow to investigate the structure of protein complexes. A Waters Synapt G2S HDMS was modified for SID in the trap region resulting in an instrument capable of running in SID-IMS-MS mode. In this set up the post-fragmentation sub-complexes can be examined by ion mobility spectrometry in order to observe the effect of ejection upon the sub unit conformation. Several protein complexes were examined by CID and SID.

Our results show that CID of a complex, *n*, results primarily in the ejection of highly charged monomer and a lowly charged subcomplex, *n* - 1. This asymmetric distribution of charges is typical of CID of protein complexes. In contrast, the SID of a protein complex typically results in the ejection of subcomplexes with symmetrically distributed charge. This is evidence for the ejected subcomplexes retaining native like structural information. It is also noted that SID appears to result in more efficient fragmentation of the precursor than CID.

The maintenance of native like structure for subcomplexes will allow for greater understanding of the topology and formation of protein:protein complexes. This can be of particular use in the investigation of complexes in heterogeneous samples or that occur at low abundance in solution. Future work will apply this technique to a wider range of protein complexes, in particular aberrant higher orders structures involved in diseases states.

55 | Amit Kumar

Understanding extensional flow induced protein aggregation in microfluidic device

The aggregation of biopharmaceuticals during their expression, purification and formulation presents a significant, often unsurmountable barrier to the translation of a promising candidate sequence to a blockbuster therapeutic. While the application of hydrodynamic force to proteins is widely accepted to trigger aggregation, the relationships between the type and magnitude of flow, protein structure and stability and aggregation propensity is poorly understood. To address this issue we have designed and developed an instrument which fitted with the microfluidic chip that mimics the extensional flow conditions found in bioprocess steps (e.g. nano-filtration) and analysed the effects of flow on the biophysical properties (structure and dispersity) of several test proteins (monoclonal antibodies and single chain variable fragment (scFvs)). Our results suggest that the developed device can produce flow conditions analogous to those found in industry and induces amorphous protein aggregation at concentrations as low as 1 mg/ml within 5 – 10 minutes.

56 | Qiuhong Beatrice Li

Structural insights into the interaction mechanism between Cep135 and CPAP

Centrioles are conserved organelles essential for cell organisation, division and motility through its capacity to organise microtubules. A broad range of human diseases, such as primary microcephaly (MCPH), male sterility and cancer, have been associated with defects in centriole formation, making the full characterisation of its assembly process of great interest. Centrioles are miniature cylinders of characteristic nine-fold symmetry, diameter and length; yet, the molecular mechanism behind the function of these centriolar proteins remains poorly understood.

Our group is interested in the study on how centriole organelles assemble and how their functions are related to human diseases. My research focus is on the structural and biophysical insights into the essential centriolar proteins and their disease related variants. MCPH is a genetically neurodevelopmental disorder characterized by microcephaly present at birth. Intriguingly, MCPH is strongly connected to defects in centrosomes. The key centriolar proteins CEP135 and CPAP essential for centriole assembly are two of the MCPH-causing genes. Cep135 is suggested to bind SAS-6, microtubules and CPAP, bridging the central hub to the outer microtubules, and further stabilizes the cartwheel structure for CPAP-mediated centriole elongation.

Here, we present the recent progress in unravelling the interactions between Cep135 and CPAP by integrating structural biology and biophysical techniques. We determined the CPAP binding domain on Cep135 and further determined its X-ray structure. We also investigated their variants on the protein-protein interaction. The proposed work will further our understanding of the centriole formation process and how it is perturbed in centriole dependent diseases.

57 | Ryan Lithgo

Investigating SilP from the sil family proteins involved in bacterial resistance

The use of silver (Ag^+) within medicine as an antimicrobial agent has been common practice for many years, with extensive use lining bandages within burn wards. However the extensive use of Ag^+ has led to the emergence of silver resistance within bacterial species such as *Salmonella enterica*, enabling the bacteria to survive in conditions up to six times the normal lethal dose of Ag^+. Within these resistant bacteria the plasmid pMG101 was discovered containing the *sil* operon, this operon encodes nine proteins *sil*-PGABFCRSE all involved in the exportation of Ag^+ of which SilP is of particular interest.

SilP is a heavy metal exporting P_{1B}-ATPase that is involved in exporting cytoplasmic Ag^+ into the periplasm at the expense of ATP, after which the Ag^+ is exported out of the cell entirely. The mechanism in which SilP works is thought to follow the Post-Albers cycle of events, whereupon the bind of Ag^+ results in conformational changes in the protein that lead to the Ag^+ being exported out of the cytoplasm. The activity of SilP is the main aim of this investigation, whereby activity assays of SilP where conducted in order to determine several parameters such as rate of Ag^+ exportation, the amount of silver exported per molecule of ATP, and the identification of motifs related to the activity of SilP through homology models and subsequent mutagenesis. The results of the investigation will enable a better understanding of SilP and how it forms an integral part of the silver resistance mechanism.

58 | Yixin Liu

An *in-vitro* study on the interaction between Eph8rinA5 and RET receptor tyrosine kinase

The receptor tyrosine kinase RET is involved in neuronal cell maintenance. A number of proteins have been reported to signal through RET, including ephrinAs (efn-As) and glial cell derived neurotrophic factor (GDNF) family ligands (GFLs)/ GDNF family receptor αs (GFRαs). Efn-As were reported to interact with RET *in cis* for reverse signalling. Such association, mediated by GDNF and EphA receptors ensured correct axonal guidance in the lateral motor column (LMC). However, there are yet no detailed functional studies for RET/efn-As *in vitro* and neither is the mechanism of their interaction available. This study focuses on the *in vitro* interaction of purified extracellular domains of zebrafish RET and efn-A5 to pioneer further structural studies to unveil the molecular mechanism of the activation RET-involved efn-As reverse signalling. Here, we report that zebrafish efn-A5b and mouse EphA4 interact with each other with a K_d of 0.18 ± 0.01 μM, supporting the evolutionary conservation of EphA/efn-A signalling. Contrary to previous discoveries from *in cellulo* studies, we found no apparent interaction between zebrafish efn-A5b and zebrafish RET *in vitro* at high concentrations, regardless of the presence of zebrafish GDNF or mouse EphA4 or not, using a combination of biochemical and biophysical assays. The results show that zebrafish efn-A5b and zebrafish RET do not interact directly and our finding indicates how complex EphA/efn-A and RET signaling is. It also suggests that the reverse signalling of efn-As may not be evolutionarily conserved, or other, yet unknown, binding partners are involved in mediating the reverse signalling of efn-A5 *via* RET.

59 | Harry Mackenzie

A 13C-detected 15N double-quantum NMR experiment to probe arginine side-chain guanidinium chemical shifts

Arginine side-chains are often key for enzyme catalysis, protein-ligand and protein-protein interactions. The importance of arginine stems from the ability of the terminal guanidinium group to form many key interactions, such as hydrogen bonds and salt bridges, as well as its perpetual positive charge. We present here an arginine $^{13}C^{\zeta}$-detected NMR experiment in which a double-quantum coherence involving the two $^{15}N^{\eta}$ nuclei is evolved during the indirect chemical shift evolution period. As the precession frequency of the double-quantum coherence is insensitive to exchange of the two $^{15}N^{\eta}$; this new approach is shown to eliminate the previously deleterious line broadenings of $^{15}N^{\eta}$ resonances caused by the partially restricted rotation about the C^{ζ}-Nε bond. Consequently, sharp and well-resolved $^{15}N^{\eta}$ resonances can be observed. The utility of the presented method is demonstrated on the L99A mutant of the 19 kDa protein T4 lysozyme, where the measurement of small chemical shift perturbations, such as one-bond deuterium isotope shifts, of the arginine amine $^{15}N^{\eta}$ nuclei becomes possible using the double-quantum experiment.

60 | Najet Mahmoudi

Extended law of corresponding states for γ-crystallin phase behaviour

Understanding protein stability and behaviour is of central importance in biology, medicine, and chemistry. While solvent isotope effects have already provided important clues about the structural and thermal stability of disparate proteins, the effects of H/D substitution on protein phase behaviour and interactions remain to be elucidated. Here, we report the large effect of solvent isotope substitution on the phase behaviour and interactions of γB-crystallin, a globular eye lens protein interacting via a short-range attractive interaction potential. Using a combination of scattering techniques (small-angle x-ray scattering and static light scattering), we show that the liquid-liquid phase separation critical temperature Tc increases linearly from 276 K in H_2O to 292 K in D_2O[1]. Furthermore, we demonstrate that the phase boundaries and the osmotic compressibility of γB-crystallin scale with the reduced second virial coefficient b_2, quantifying protein–protein interactions, through the extended law of corresponding states (ELCS). This thermodynamic scaling confirms the applicability of the ELCS to the equilibrium properties of colloids with short-range attractions and provides an extension of its predictive power to systems with varying hydrogen isotope content.

[1] S. Bucciarelli, N. Mahmoudi, L. Casal-Dujat, M. Jéhannin, C. Jud and A. Stradner, *JPCL* 7, 1610 (2016).

61 | Chloe Martens

The role of lipids in the molecular mechanism of secondary transporters

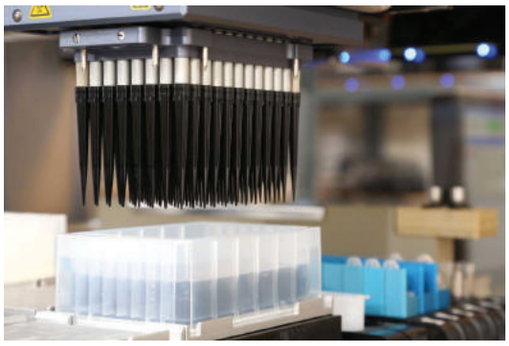
The recent progress of structural biology techniques is now providing a continuous stream of membrane proteins structures. However, linking a protein's static snapshots to its cellular function is a difficult task that requires a comprehensive understanding of complex molecular mechanisms. Two key aspects underlying the molecular mechanism of membrane proteins are still poorly understood: their intrinsic conformational dynamics and the role of their interactions with the lipid bilayer. Here, we address these challenges by studying the molecular mechanisms of three transporters from the widespread Major Facilitator Superfamily (MFS); XylE, LacY and GlpT. These transporters share a similar fold but perform different functions. By mapping their transition from outward-open to inward-open conformations, we aim to understand how lipid-protein interactions regulate transport.

In order to identify molecular details of specific functionality, we carried out mutations in conserved motifs and monitored the changes in the conformational equilibrium using Hydrogen-Deuterium Exchange mass spectrometry (HDX-MS). In parallel, we performed similar experiments in the presence of substrate and inhibitors to understand the complete sequence of events leading to transport. Our results show that despite differences in function and energetics, there is a remarkable conservation in the conformational role of specific motifs. The perturbation of conserved polar networks on the cytoplasmic side systematically triggers opening of the extracellular side, indicating a conserved mechanism of alternating-access within the structural fold of MFS.

This first set of experiments was performed on detergent-solubilized proteins. We then set out to optimize the applicability of the HDX-MS workflow to more heterogeneous membrane systems, namely nanodiscs. We reconstituted the transporter XylE in nanodiscs of different lipid compositions, one similar to the native composition, where the main component is phosphatidylethanolamine (PE) and the other with a non-native lipid phosphatidylcholine (PC). We observed that phosphatidylethanolamine is involved in stabilizing the inward-open conformation. Indeed, replacing the phospholipid PE by PC shifted the conformational equilibrium towards the outward-open conformation. This finding is supported by molecular dynamics simulations, in which PE is shown to favour the inward-open conformation compared to PC. Interestingly, these simulations show that PE, and not PC, interacts with residues involved in the aforementioned polar network, thus preventing its formation and the conformational transition. This study provides novel molecular insights into the role of lipids in the transport mechanism of a prominent family of membrane proteins.

62 | Andre Matagne

Robotein®: A robotic platform dedicated to high-throughput protein production and analysis



Robotein® is a technological platform built on competences and infrastructures available in the academic setting of two labs that offer a complete structural biology portfolio: the Centre for Protein Engineering (CIP, Université de Liège, Belgium) and the Structural Biology and Bioinformatics Centre (SBBC, Université Libre de Bruxelles, Belgium).

Equipped with two Hamilton workstations (one EasyPick Microlab STARlet and one Microlab STAR), one system for biomolecular interactions analysis (Octet HTX by *forté*BIO – Pall Life Sciences), one system for automated electrophoretic separation of RNA, DNA and proteins (LabChip GXII by Perkin Elmer), one high-throughput protein arrayer (Marathon Classic Microarrayer by Arrayjet) combined with an infrared imager (128x128 focal plane array detector FTIR imaging microscope by Agilent) and two microplate readers (Infinite M200 PRO by TECAN), we offer automated screening for optimal cloning and gene expression in both bacteria and yeast, protein purification, refolding and stabilization (formulation), and protein biochemical and biophysical characterization, on either a collaborative or service basis. (http://www.robotein.ulg.ac.be)

63 | Daumantas Matulis

Database of CA protein-ligand binding Gibbs energies, enthalpies, entropies, and crystallographic structures

Target-based drug design is often based on the discovery and selection of a most-strongly binding compound to a target protein. However, the binding affinity and the binding mechanism is usually interplay of highly compensating enthalpic and entropic contributions. Even homologous compounds that exhibit similar affinities may have significantly different enthalpies and entropies of binding. When high-resolution crystallographic structures are available for all compound complexes with the target protein, sometimes it is possible to assign these significant enthalpy and entropy differences to the behavior of the water molecules located at the compound-protein binding interface.

We have designed, synthesized and determined the binding thermodynamics of over 700 aromatic sulfonamides to the family of 12 human carbonic anhydrase (CA) isoforms. The proteins were cloned and expressed in bacterial and human cell cultures and affinity-purified in large quantity sufficient for ITC and crystallography. The binding affinities were determined by the thermal shift assay (FTSA, also termed ThermoFluor or differential scanning fluorimetry, DSF), a high-throughput method. The enthalpies and entropies of binding were determined by ITC, a medium throughput method, for a selection of compounds and CA isoforms. A correlation map between the compound chemical structure and the binding ΔG and ΔH was drawn. The map showed which structural features of the compounds generated the highest increments in exergonicity and exothermicity of compound binding. Furthermore, only some structural features were most useful in generating compounds that would selectively bind to cancer-expressing CA isoforms, but would not bind to essential for life human CA isoforms. Over 60 X-ray crystal structures showed the position of compounds bound in the enzyme active center.

ITC was essential technique that enabled the dissection of unknown contributions from linked reactions such as buffer protonation to the binding reaction. Only after the subtraction of pH-dependent buffer contribution to the enthalpy of binding, the *intrinsic* Gibbs energies and enthalpies of binding were obtained. All methods that determine the binding reaction, such as FTSA, ITC, SPR, thermophoresis, and enzymatic inhibition methods would provide only the *observed* thermodynamics of binding that is pH and buffer-dependent. It was important to calculate the true (intrinsic) parameters and use them in the structure-thermodynamics correlation maps.

Vaida Linkuvienė, Asta Zubrienė, Vaida Paketurytė, Alexey Smirnov, Vytautas Petrauskas, John E. Ladbury, Daumantas Matulis

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64 | Fairolniza Mohd Shariff

Simultaneous Mutations at the N- and C-Terminal Regions of Thermostable L2 Lipase

Lipase is one of the most favoured enzyme in various industrial processes due to its diverse range of catalytic activity. Thermostable lipase in particular has garnered attention for years for its stability not only at elevated temperature, but also in wide range of pH and organic solvent. L2 lipase is a thermostable lipase from *Bacillus* sp. isolated from a hot spring in Malaysia. Previous study has introduced a single residue substitution at respective N- and C-terminal of the lipase. Single mutants of L2 exhibit an improvement in optimum temperature and half-life. In this work, the potential of simultaneous residue substitution at both N- and C-terminal is explored. Based on *in silico* studies, through screening of three different residue substitution of the respective terminals, a combination of Valine at position 8 (N-terminal) with Glutamic acid at position 385 (C-terminal) showed RMSD of 1.5076 A when superimposed single mutant A8V and an RMSD value of 1.5083 A was shown when it was superimposed with single mutant S385E. Structurally, the terminal ends of the double mutant deviates 1.50 A relative to single mutant. Further studies will be carried out to understand effects of residue substitution at both terminal of the lipase structure and consequently its stability in catalytic activity.

65 | Sophie Meredith

Lipid bilayer patterning for the investigation of photosynthetic arrays and model thylakoid membranes

Photosynthetic membranes rely on complex ordering and dynamic reorganization in order to induce functional responses to physiological stimuli. Specialised membranes form multilamellar stacks, a property that is ubiquitous to all land-plants, in order to increase the surface area available for light absorption and to enhance the efficiency of energy transfer between antenna proteins and the reaction centres of photosynthesis.

The major light-harvesting complex (LHC-II) is the most abundant membrane protein on earth and is a major component in photosynthetic membranes. It acts to absorb light and funnel energy to photosystems PSI and PSII within the cell membrane. The complex is also believed to play a structural role in facilitating the membrane stacking by providing contact points between neighbouring thylakoid bilayers. In addition to this, the structural conformation of the LHC-II is key to protect the system from a potentially harmful build-up of excitation energy when subject to high intensity incident light. Multiple studies have shown the ability of

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LHC-II to reversibly change between a quenched and an unquenched state in a process called Non Photochemical Quenching (NPQ). This property facilitates the dynamic response of the membrane to excess incident light, where a conformational change in LHC-II causes grana unstacking and the decoupling of LHC-II and PSII complexes; allowing for the excess energy to be dissipated as heat and preventing any photo-oxidative damage.

Using a combination of lipid bilayer patterning techniques, surface chemistry and the modulation of physicochemical conditions, this project aims to develop models to test the properties of self-assembly, light absorption and non-photochemical quenching in biological membranes. By constructing two dimensional photosynthetic arrays in supported lipid bilayers, it will be possible to investigate these effects using an array of surface specific techniques, such as AFM and fluorescence methods ranging from epifluorescence to combined FLIM-FRET measurements. Furthermore, methods used for 2D patterning of bilayers, may also be combined with protein-protein contacts (or other multilayer tethering molecules) to induce protein segregation and self-organization of multilayers, allowing for the formation of a piecewise model of energy transfer, structural relationships and the self-assembly of complexes within multilamellar membranes.

Success in these efforts will represent a major advance in the controlled design of 3-D complex, functional biomaterials. Other membrane proteins, e.g. signalling receptors, could be incorporated, allowing investigation of varied biological processes. The development of model membranes has the potential to provide insight into the intimate relationship between the structural arrangement of LHC-II, or other photosynthetic proteins, and their functionality, as well as the possibility of manipulating the conformational self-quenching of LHC-II to produce tuneable photosynthetic arrays. Future applications could include artificial photosynthetic devices with enhanced absorption and biosensors with high-protein-density with improved recognition capability. More generally, the ability to selectively pattern LHC-II may also have applications for creating patterns and arrays of other membrane proteins.

66 | Rani Moons

From IDP to fibril: Effect of small drug-like molecules and metal ions on a-synuclein conformation and aggregation

The intrinsically disordered protein (IDP) α-synuclein is prone to aggregation, which is linked with Parkinson's disease (PD). Despite numerous efforts to develop a treatment, at the moment symptoms can only be temporarily moderated in earlier stages of the disease. Major questions regarding the conformational space of the protein, oligomerisation intermediates and their link with fibril morphology and disease etiology remain unanswered, as does the mechanism by which some small molecules and metal ions can modulate α-synuclein monomer structure in a way which impacts aggregation and toxicity. We study the effect of structure-modulating interactors, and use a combination of MS-based approaches towards characterization of species along the protein aggregation pathway which are "toxic" and potentially druggable. Structure-modulating compounds are able to affect the species distributions in a way that alters α-synuclein toxicology. The stoichiometry of binding of various biologically relevant metal ions and small molecules with promising drug-like effects is investigated using native electrospray ionisation mass spectrometry (ESI-MS). Ion mobility (IM; Synapt G2) approaches which are based on native ESI-MS are optimised and applied to study the conformational space of α-synuclein monomers and oligomers in the presence of these structural modulators. Top-down native ETD fragmentation is used to map binding sites or regions in the soluble monomers or oligomers. We link these data with the fibril morphology observed by EM and with peptide fingerprints generated by limited proteolysis and analysed by ESI-MS.

By examining two important compounds with proven drug-like properties, epigallocatechin gallate (EGCG) and dopamine (DA), our results show they bind to different conformers (more extended/compact) and have opposite conformational effects on the α-synuclein monomer ensemble. While binding of EGCG leads to compaction of the α-synuclein monomer, binding of DA gives the protein a more extended conformation. This opposite effect indicates that the molecular structure, the stoichiometry of binding as well as the mode and place of binding influences α-synuclein conformation on the monomer level.

Recently the physiological role of some metal ions, in particular Ca^{2+}, has caused considerable interest. We study the effect of a variety of different mono-, di- and trivalent cations including Ca^{2+} on α-synuclein conformation, oligomerization and aggregation. In ion mobility, electrostatic (Coulombic) effects can be separated from specific, structural effects by applying charge-reduction ETnoD to different charge states and comparing the resulting CCS with the "native" charge state. Upon titration into α-synuclein, Ca^{2+} as well as some other multivalent cations and their respective counterions show distinct binding stoichiometries and mobility shifts. We find that up to ten calcium ions can bind to α-synuclein resulting in a strong compaction of the monomer.

We show how structure-modulating compounds such as small molecules with proven drug-like properties and metal ions relevant in a biological context, affect the conformation of AS monomers. Through the combination of IM-MS with a newly developed limited proteolysis method, we aim at bridging the information gap between molecular studies of AS monomers and the morphology of in vitro formed aggregates.

67 | Ethan Morgan

E6 induced STAT3 phosphorylation is mediated by NFκB-dependent IL-6 signalling in HPV positive cervical cancer

Human Papillomaviruses (HPV) are small DNA viruses that are the leading etiological cause of cervical cancer. We have previously demonstrated that the HPV E6 oncoprotein upregulates the activity of the transcription factor STAT3 in primary human keratinocytes; however, the mechanism of this upregulation in HPV mediated cervical cancer is currently unclear. Here, we show that STAT3 phosphorylation induced by HPV is primarily mediated by the cytokine Interleukin 6 (IL-6). Treatment of HPV negative cervical cancer cells with recombinant IL-6 or conditioned media from HPV positive cervical cancer cells induced Janus kinase 2 (JAK2) and STAT3 phosphorylation, and inhibition of IL-6 signalling by use of a neutralising antibody against IL-6 or the IL-6 co-receptor, gp130, inhibits JAK2 and STAT3 phosphorylation. Mechanistically, HPV E6 induces IL-6 transcription and secretion in HPV negative C33A cells. To identify upstream regulators of IL-6 production, we demonstrate that the transcription factor NFkB, a known mediator of IL-6 production, leads to a reduction in STAT3 phosphorylation due to a loss in IL-6 transcription and secretion. Finally, using clinical biopsy samples, we demonstrate that IL-6 expression correlates with cervical disease progression. Together, these data identify a potential therapeutic avenue for HPV-mediated cervical cancer, using clinical available inhibitors.

68 | Jens Preben Morth

Functional characterisation of the first primary active magnesium transporter

Three classes of Magnesium transporters have been identified in Bacteria; CorA, MgtE and MgtA/MgtB[1]. While CorA and MgtE are both magnesium channels. Active influx is believed to mediated by MgtA The magnesium transporter A (MgtA) is a specialized P-type ATPase, that import Mg(II) into the cytoplasm. In both *Salmonella typhimurium* and *Escherichia coli*. This study demonstrates, for the first time, that MgtA is highly depended on anionic phospholipids and in particular, cardiolipin, the *in vitro* kinetic experiments performed on detergent solubilized MgtA suggest that cardiolipin act as a magnesium chaperone. We further show that MgtA is highly sensitive to free Mg(II) (Mg(II) free) levels in the solution. MgtA is activated when the Mg(II) free concentration is reduced below 10 uM and is strongly inhibited above 1 mM, indicating that Mg(II) free acts as product inhibitor. Colocalization studies confirm that MgtA is found in the cardiolipin lipid rafts in the membrane. Combined, our findings indicate that MgtA may act as a sensor as well as a transporter of Mg(II)[2]. With the present functional data, we now hypothesize that regulation of ion transport in the MgtA might be fundamentally equivalent to that of the Na+/K+ -ATPase. The discovery that MgtA acts as a receptor in addition to being an ion transporter, is a major breakthrough.

[1] Groisman, E.A et al. *Annu Rev Genet* 47, 625-46, 2013,

[2] Subramani, S. et al. *Elife*, 5, 2016

69 | James Murphy

Does KSHV induce virus specific specialised ribosomes during infection?

Historically, ribosomes have been viewed as unchanged homogeneous units with no intrinsic regulatory capacity for mRNA translation. Recent research is shifting this paradigm of ribosome function to one where ribosomes may exert a regulatory function or specificity in translational control. Emerging evidence has identified heterogeneity of ribosome composition in specific cell populations, leading to the concept of specialised ribosomes. Viruses manipulate many cellular pathways to enhance their replication, therefore we have examined whether Kaposi's sarcoma associated herpes virus (KSHV) alters host cell ribosome composition to enhance the production of viral proteins.

Using a B cell line containing latent KSHV episomes, we have produced eight cell lines that stably express different ribosomal associated proteins containing tandem affinity purification (TAP) tags. Using TAP we have isolated precursor ribosome complexes from latent and lytically replicating KSHV infected cells. TMT-labelling coupled to LC-MS/MS was used to identify changes in the stoichiometry and composition of the precursor ribosomal complexes during lytic vs latent KSHV infection. Using a variety of molecular biology techniques, we are currently confirming these changes and detailing how they affect virus replication. We are also using cryo-EM to solve the structure of isolated precursor ribosome complexes and modeling these structures against high-detail cryo-EM ribosome maps to help further identify loss or gain of proteins from these complexes throughout KSHV infection.

Our research will demonstrate how viruses can engineer specific specialised ribosomes that could preferentially translate viral mRNAs over host mRNAs by altering ribosome composition.

70 | David Nicholson

A structural investigation into ribosome-targeting antibiotics

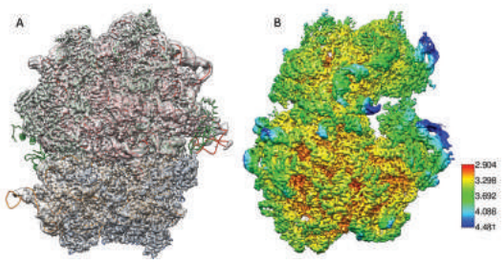

Figure 1 Cryo-EM density of the *E. coli* ribosome solved to 3.2 Å resolution. A) PDB 5DMZ rigid-body fitted to the density map. Grey = CryoEM density map; Red = 23S/5S rRNA; Green = 50S ribosomal proteins; Orange = 26S rRNA; Blue = 30S ribosomal proteins. B) Density map coloured by local resolution, from red (2.9 Å) to blue (4.5 Å).

Antibiotics are crucial to modern medicine, allowing treatment of life-threatening bacterial infections and making surgeries like transplantations possible. However, pathogenic bacteria are rapidly evolving to resist their effects. To effectively combat the threat of antibiotic resistance, a detailed structural and molecular understanding of both the mechanisms by which antibiotics work, and by which bacteria acquire resistance, must be gained.

Many antibiotics target the bacterial ribosome. High resolution structures of ribosome:antibiotic complexes have been solved previously, however most of these contain ribosomes from non-pathogenic bacteria. Here, high-resolution cryo-electron microscopy (EM) and single-particle image processing methods are used to solve structures of whole ribosomes from pathogenic bacteria commonly associated with antibiotic resistance, as well as these ribosomes in complex with clinically relevant antibiotics or novel natural products with antimicrobial properties.

This structural information provides understanding of how ribosome-targeting antibiotics bring about their effects on pathogenic bacteria, which could aid in the development of new antibiotics that more effectively and specifically target these organisms. Additionally, solving novel structures of ribosomes from pathogenic bacteria will improve our understanding of species-specific translation regulation mechanisms, including antibiotic resistance.

Following optimisation of ribosome purification and sample preparation, a structure of the *Escherichia coli* ribosome was solved to 3.2 A resolution by cryo-EM and single particle analysis (figure 1). This was used as a standard to compare with the structure of the ribosome in complex with antibiotics including oxydifficidin. The structure of the ribosome from *Enterococcus faecium*, a notorious ESKAPE pathogen, was also solved.

71 | Kristina Paraschiv

Predictive and Experimental Analyses of the Key Determinants of β-Sheet Mediated PPIs

Protein-protein interactions (PPIs) play a central role in cellular processes relevant to health and disease; hence the ability to selectively control PPIs is of great significance in chemical biology. The design of selective and high affinity small molecule inhibitors for β-sheet mediated interactions is comparatively unexplored, which, unlike α-helix mediated interactions show a more complex mode of binding[1]. The identification of the key determinants that contribute the majority of the binding energy in a PPI will aid in the design of peptidomimetic structures and lead to the identification small molecule scaffolds that are able to mimic the composition and 3D orientation of recognition functionality.

In silico methods have widely been used to identify important molecular features at PPI interfaces, however little experimental data is available to validate the comprehensive application of this approach. In our studies we use and contrast the results of *in silico* predictions for β-sheet mediated interactions, determined using peptide/protein co-crystal structures, and experimentally validate the predictions through the synthesis of multiple mutant peptides which were tested using biophysical assays. The experimental results are then used to refine the predictions.

Ultimately, experimental identification of recognition functionality will help increase reliability and confidence of *in silico* predictions. This could further ease inhibitor design and identification of small molecule compounds able to bind to challenging protein targets.

[1] A. M. Watkins and P. S. Arora, ACS Chem. Biol., 2014, 9, 1747–1754.

72 | Chinar Pathak

Molecular mechanism of β2-microglobulin amyloid formation and its inhibition using affimers

Aggregation of β2-microglobulin into amyloid fibrils is associated with the dialysis related amyloidosis disorder. Studies on these mechanisms involves the understanding of structure and dynamics of the intermediates in amyloid assembly leading to fibril formation. Such intermediates or interfaces involved in β2m amyloid formation can be mapped, enabling the development of protein affimers that are able to inhibit amyloid formation. Kinetic assembly reactions in conjunction with NMR, negative stain EM and other biophysical methods was employed in studying such molecular events, and the effect of affimers was tested in vitro. The inhibition of β2m fibril formation by the different affimers is discussed in detail.

73 | Lisa Patrick

Explaining allosteric modulation of protein function using Principal Components Analysis and Information Theory

Although allostery was first discovered over 50 years ago, the molecular determinants underlying signal transduction are not yet completely understood. The ability to predict the activity of allosteric small molecules could have a huge therapeutic impact, as targeting allosteric sites in proteins potentially presents significant benefits over active site inhibitors, in both selectivity and efficacy. While some systems undergo fairly well understood structural changes, there is no overall model that satisfactorily describes how allostery works. Molecular dynamics simulations provide a tool to study protein dynamics at the atomistic level, however traditionally employed analysis methods have been proved inadequate to deliver a mechanistic description of allostery.

In this work, we present our approach to tailor MD simulation analysis methods to identify motions which may be significant to signal transmission (or interactions which are important to conformational selection), in the case study of PDK1 (Phosphoinositide-dependent kinase-1). Long MD trajectories were run for PDK1 in complex with covalent activator and inhibitor small molecules[1], using the software Sire/Somd[2]. A geometrical analysis using the Kullback-Leibler divergence allowed comparison of probability distributions of various descriptors. Cartesian and torsional angle PCA was carried out to find dominant motions. Subsequently, an energetic comparison was performed using a per-residue decomposition of the interaction energy between the protein and the substrate. Mutual information was then used to determine whether particular structural changes (distances, principal components) correlate with changes in energetics, to identify motions which are important for the allosteric signal.

Preliminary results are also presented for a new protein target (Protein-tyrosine phosphatase 1B: PTP1B), where similar analysis methods have been employed in order to understand allosteric conformations for two distinct allosteric sites[3].

[1] Sadowsky, J. D. *et al., Proc. Natl. Acad. Sci.* 2011, 108 (15), 6056–6061.

[2] Woods, C. J. and Michel, J., Sire/*OpenMM*. 2014.

[3] a) Wiesmann, C. *et al., Nat. Struct. Mol. Biol.*, 2004, 11(8), 730-7. b) Fraser, J. et al., Preprint: www.biorxiv.org, New routes for PTP1B allosteric inhibition by multitemperature crystallography, fragment screening and covalent tethering, 2017.

74 | Michael Plevin

Conserved asymmetry underpins homodimerization of Dicer-associated double-stranded RNA-binding proteins

Double-stranded RNA-binding domains (dsRBDs) are commonly found in modular proteins that interact with RNA. Two varieties of dsRBD exist: canonical Type A dsRBDs interact with dsRNA, while non-canonical Type B dsRBDs lack essential RNA-binding residues and have instead evolved to interact with proteins. In higher eukaryotes, the microRNA biogenesis enzyme Dicer forms a 1:1 association with a dsRNA-binding protein (dsRBP) that contains a conserved Type B dsRBD. In humans, Dicer associates with HIV TAR RNA-binding protein (TRBP) or protein activator of PKR (PACT), while in Drosophila, Dicer-1 associates with Loquacious (Loqs). The Type B dsRBD in each of these proteins interacts with the RNA helicase domain of Dicer. All three dsRBPs are also reported to homodimerize, while yeast-two-hybrid studies suggest that TRBP and PACT form heterodimers.

We will present data that show that the Type B dsRBDs of Loqs, PACT and TRBP self associate to form homodimers that have significant structural asymmetry. We have elucidated the 3D structures of the Type B dsRBDs of TRBP and PACT, which are consistent with a previous structure of Loqs. Together, these structures reveal an asymmetric self-association mechanism that involves a parallel β-strand at the homodimer interface. This interaction motif means that each dsRBD has two non-equivalent self-association modes. NMR analysis of the Type B dsRBDs of Loqs, PACT and TRBP reveal that asymmetric self-association is conserved from flies to humans. However, the three dsRBDs have different self-association affinities, which can be attributed to evolutionary divergence of their homodimerization interfaces. Mutation of a single conserved leucine residue on this interface abolishes self-association in all three dsRBPs. Moreover, mutating the TRBP homodimer interface to render it more PACT-like enhances self-association, whereas the reciprocal mutations in PACT reduce self-association.

We have also determined that the Type-B dsRBDs of TRBP and PACT preferentially form a heterodimer. Unlike the two modes of interaction that are observed in self-association, heterodimerisation of these Type B dsRBDs involves only a single mode of interaction. Finally, these data show that dsRBD-dsRBD interactions involving Loqs, PACT and TRBP utilize the same surface that is required for binding Dicer, which suggests that dissociation of dsRBD-dsRBD interactions may be a key step in the assembly of a functional Dicer complex.

75 | Christos Pliotas

Allosteric mechanical activation of an ion channel by chemical modification of pressure sensitive nano-pockets

Mechanical activation of ion channels is caused by changes in membrane tension and occurs through sites accessible to the lipid bilaye [1] [2]. Pore residues control channel conductance by forming narrow constriction points, not accessible to the mediators of tension changes, the lipids. Amongst all known ion channels the mechanosensitive (MS) channel MscL presents the highest-pressure activation threshold[3].

We have identified highly pressure-sensitive nano-pockets within MscL's transmembrane region, not directly exposed, but accessible to lipid acyl-chains. Similar nano-pockets identified in MscS[1], have been proposed to be associated with mechanical activation, caused by acyl-chain disruption within these pockets. It still remains unclear though, whether such disruption could initiate an allosteric response that drives through a conformational change for MS channels.

We coupled PELDOR (DEER) spectroscopy with SDSL[4] [5] and promoted allosteric mechanical activation on MscL. By using cysteine modification we altered the degree of acyl-chain availability within the pockets and observed global structural changes by PELDOR in solution. A single residue substitution at the entrance of the pockets had a similar effect on channel's conformation. PELDOR distance measurements in lipid reconstituted MscL showed that the channel undergoes a reversible conformational rearrangement, adopting its original closed state. Single channel recordings in lipid bilayers supported the PELDOR findings. NMR and lipid ES-MS lipid analysis suggested that MscL has preference but not specificity, to endogenous lipids, consistent with a previous study[6]. Specific spin label conformers restricted lipid-chain access to the nano-pockets, as evidenced by atomistic MD simulations of modified MscL within lipids. Mutations deficient of lipid headgroup binding, not associated with the nano-pockets, had no effect on channel's conformation.

We propose it is the degree of acyl chain penetration within these highly pressure sensitive TM domains, rather than specific lipid binding, responsible for initiating mechanotransduction. We demonstrate manipulation of MscL channel pores in the absence of pressure, via specific cysteine modification. De novo design of molecular regulators with properties accounting for the unique landscape of MS channel nano-pockets could potentially facilitate optimal disruption of lipid-chain penetration and allosterically modulate MS channels in lipid membranes.

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76 | Christina Redfield

Structure and dynamics of the CheY response regulators from Rhodobacter sphaeroides

The chemotaxis signalling network of *E. coli* depends on autophosphorylation of a histidine protein kinase (HPK) in response to a signal from a sensor domain, with subsequent transfer of the phoshoryl group to an aspartate on response regulator (RR) proteins that bind to the flagellar motor and alter its rotation. CheY is a 14kDa single domain RR that is conserved across motile species. It is formed by 5 α-helices and 5 β-strands surrounding a conserved phosphoryl accepting aspartate residue, and once phosphorylated diffuses to the flagellar motor, binding to its FliM component to cause switching of rotational direction. The photosynthetic bacterium *Rhodobacter sphaeroides* has multiple chemosensory pathways formed by homologues of the E. coli chemosensory proteins. It has six CheY homologues with different effects on chemotaxis. Only $CheY_6$ is able to stop the flagellar motor but either $CheY_3$ or $Chey_6$ are also required for chemotaxis.

NMR and computational methods have been used to answer questions about the structure, dynamics and function of two of these CheYs, $CheY_3$ and $CheY_6$. NOEs, chemical shifts and residual dipolar couplings are used to define the structures of $CheY_3$ and $Chey_6$ in their inactive and active states in solution, where phosphorylation is mimicked by BeF_3-. We have investigated fast timescale backbone dynamics using the $\{^1H\}$-^{15}N heteronuclear NOE and have used CPMG relaxation dispersion experiments to detect low populations of alternative 'excited-state' conformations. $CheY_6$ differs from the other R. sphaeroides CheYs and *E. coli* CheY by the insertion of a ten-residue loop before the C-terminal helix. We have deleted this loop region from $CheY_6$ in order to determine, using *in vivo* and *in vitro* assays, if it plays a role in the unique function of $CheY_6$ in *R. sphaeroides*.

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77 | Inbal Riven

Correlated motions in an allosteric machine at the single-molecule level

Critical for the function of many proteins, allosteric communication involves transmission of the effect of binding at one site of a protein to another through conformational changes. Yet the structural and dynamic basis for allostery remains poorly understood. This work aims to explore time-dependent coordination of allosteric transitions of multiple units within a large protein machine. Our model protein is ClpB, a disaggregation machine from *Thermus thermophiles*. This protein is made of six identical protomers that form a ring structure. Its functional cycle is driven by binding and hydrolysis of ATP, as well as by binding of the co-chaperone DnaK. The six-fold symmetry of *ClpB* is broken during its cycle due to the non-concerted nature of its conformational transitions.

Our methodology relies on single-molecule FRET spectroscopy with multiple labels on the same protein and advanced analysis techniques. This framework enable us to explore fundamental issues in protein dynamics like the relative motions of domains within subunits, the propagation of conformational change between subunits, and the synchronization of these motions by effector molecules. In *ClpB* each monomer of the protein is made of four domains. By putting three fluorescent labels on three different domains within the same subunit we aim to explore their relative motions during function. And by inserting two different FRET pairs into two subunits we can measure the time delay involved in the conformational spread.

Labeling the proteins in a site specific manner is a critical step. To this end we combine the classical method of labeling two cysteine residues with two different fluorescent dyes with the famous unnatural amino acid (UAA) technology of Schultz and co-workers to introduce a third, orthogonal labelling site that can be exploit by click chemistry.

78 | Iosifina Sampson

Characterising how chemokines move through the extracellular matrix

The extracellular matrix (ECM) is a highly dynamic network which not only provides structural support to cells but also helps regulate intracellular signalling cascades and biochemical reactions. The ECM is composed of a plethora of structural components and signalling molecules[1] [2] and we aim to probe individual elements of this in order to gain an insight into the molecular mechanisms that can lead to diseases such as cancer metastasis or chronic inflammation.

Our intention is to characterise how chemokines (signalling proteins) move and interact within the ECM in relation to glycosaminoglycans (GAGs). GAGs are extracellular polysaccharides that harbour signalling proteins such as chemokines and cytokines and control their distribution and availability to help attract immune cells as part of the inflammatory response[3].

Using well defined bio-mimetic surfaces[4] (model matrices) we can reproduce structural, biochemical and biological features of the ECM, fine-tuned to enable the presentation of GAGs in a particular orientation, density and concentration to mimic selected aspects of tissue specific conditions. By applying a range of biophysical techniques to these bio-mimetic surfaces we can probe the dynamics of chemokines in relation to GAGs in well-defined environments and identify the main molecular and physical mechanisms that define chemokine mobility and gradient formation in the ECM.

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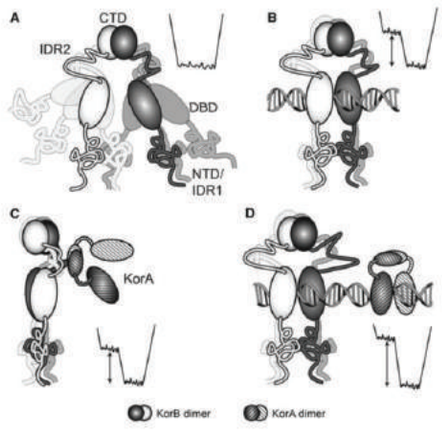
79 | Ashlea Rowley

Mechanisms of centrosome clustering regulated by the Nek6-Hsp72 pathway

Here, we have explored the mechanism through which the Nek6-Hsp72 pathway promotes centrosome clustering in cancer cells with amplified centrosomes. We specifically tested the hypothesis that this pathway regulates localization of microtubule-based motor proteins known to contribute to centrosome clustering. Using antibodies against dynein intermediate chain (IC) and p150Glued as markers of dynein and dynactin, respectively, we showed that localisation of these proteins to the spindle apparatus was markedly reduced in MDA-MB-231 cancer cells by Hsp70 inhibition, or depletion of Hsp72 or Nek6. However, localization of these proteins to the mitotic apparatus in non-cancer-derived RPE1 cells was unaffected, while their overall expression was unchanged in both MDA-MB-231 and RPE1 cells. Localization of dynein IC and p150Glued at kinetochores overlapped with localization of pHsp72-T66 as detected with a phosphospecific antibody against the site phosphorylated by Nek6, and a PLA assay revealed close association of these proteins at kinetochores. Interestingly, the outer kinetochore kinesin-related motor protein, CENP-E, was also reduced upon Hsp70 inhibition and showed PLA interaction with pHsp72-T66. On the other hand, the inner kinetochore proteins, CENP-A and CENP-I, were not affected by Hsp70 inhibition. PLA assays revealed reduced association of microtubules with kinetochores upon Hsp70 inhibition consistent with defects in both K-fibre stabilization and kinetochore-microtubule attachment. Finally, co-precipitation followed by mass spectrometry analysis revealed association in mitotic cells between dynein and not only Hsp70/Hsp90 complexes, but also with specific myosin isoforms with co-precipitation of dynein and myosin reduced upon Hsp70 inhibition. Together, this suggests that the Nek6-Hsp72 pathway may be required for assembly and recruitment of selected outer kinetochore proteins involved in microtubule attachment, including dynein/dynactin and CENP-E, while also potentially playing a role in other mitotic processes that contribute to centrosome clustering, including cortical cytoskeletal organization.

80 | Dave Scott

Dynamic Allostery gives rise to cooperative interactions in a protein/ nucleic acid interaction



The ParB protein, KorB, from the RK2 plasmid is required for DNA partitioning and transcriptional repression. It acts co-operatively with other proteins, including the repressor KorA. Like many multifunctional proteins, KorB contains regions of intrinsically disordered structure, existing in a large ensemble of interconverting conformations. Using NMR spectroscopy, circular dichroism and small-angle neutron scattering, we studied KorB selectively within its binary complexes with KorA and DNA, and within the ternary KorA/KorB/DNA complex. The bound KorB protein remains disordered with a mobile C-terminal domain and no changes in the secondary structure, but increases in the radius of gyration on complex formation. Comparison of wild-type KorB with an N-terminal deletion mutant allows a model of the ensemble average distances between the domains when bound to DNA. We propose that the positive co-operativity between KorB, KorA and DNA results from conformational restriction of KorB on binding each partner, while maintaining disorder.

81 | Lucas Siemons

Determining isoleucine rotamer populations from chemical shifts

We present a method for determining isoleucine rotamer population distributions from carbon chemical shifts. Here each rotamer is defined by both X_1 and X_2 angles providing a near-complete description of the side-chain. This gives a more detailed description of the side-chain dynamics than what is available using scalar-couplings. The readily available nature of chemical shifts allows this method to be applied in situations where long-range scalar-couplings are impossible to obtain. To demonstrate the method's utility isoleucine rotamer distributions were determined from chemical shifts for the DsbB–DsbA complex, a 41 kDa membrane protein, and the L24A FF domain's 'invisible' folding intermediate that exchanges with the ground state.

82 | Hugh Smith

Understanding the aggregation mechanism of the familial β_2m variant

D76N was recently identified as the first naturally occurring amyloidogenic mutant of Beta 2-microglobulin (β_2m), a 99 residue, seven stranded Ig domain protein. The D76N variant of β_2m causes a hereditary, late onset and fatal systemic amyloid disease[1]. Affected individuals are heterozygous for the D76N mutation and thus also express wild-type (WT) β_2m in the serum. Co-aggregation of D76N and WT β_2m is not observed in vivo, and D76N amyloidosis follows a distinctive disease progression from the WT β_2m-associated disease, Dialysis Related Amyloidosis (DRA). DRA occurs in patients undergoing long term haemodialysis where WT β_2m is not effectively cleared from the serum, resulting in elevated plasma β_2m levels and eventually deposition of β_2m amyloid fibrils in the bones and joints. Individuals affected by D76N amyloidosis have both normal kidney function and plasma β_2m levels.

The aim of this work is to understand the mechanism by which the single amino acid substitution, D76N, results in a dramatically more aggregation-prone protein, whilst maintaining the same overall native structure as WT β_2m[1]. In this work the structure, stability and dynamics of the D76N variant are studied with Nuclear Magnetic Resonance (NMR) spectroscopy and a range of complimentary biophysical techniques. The folding intermediates of D76N and WT β_2m are also probed with real time NMR and Circular Dichroism (CD) spectroscopy, revealing structural, dynamic and kinetic insights into the folding intermediates present within the D76N and WT β_2m folding pathways. These results begin to unravel the effects of the D76N substitution on the conformational landscape of the protein.

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83 | Nicolae Solcan

Conformational stabilisation as an aid for structure determination of GPCRs bound to allosteric modulators

G-protein coupled receptors (GPCRs) are the largest and most diverse family of eukaryotic membrane proteins, and serve as targets for many commonly used drugs, the majority targeting the receptors' orthosteric binding site. Allosteric modulators, which modulate GPCR signalling by binding at other sites, are increasingly being seen as attractive alternatives. They are often more selective for their targets, and in some instances are intrinsically more easily druggable, particularly in the case of receptors with large natural orthosteric ligands. However, insufficient structural information has made modelling allosteric binding particularly difficult, and there are currently only a few FDA-approved allosteric drugs targeting GPCRs.

Heptares Therapeutics uses the StaR® technology to create an SBDD platform for targeting GPCRs. StaR® proteins have been evolved to possess defined, stable conformations that can be co-purified with a wide range of ligands. This unique technology has allowed us to solve X-ray crystal structures of ligand-bound receptors from different GPCR classes, revealing different binding modes and assisting in the process of designing better, more selective drug candidates.

The StaR® technology has also proven useful in generating GPCR structures with allosteric compounds, as in the case of the human glucagon receptor (GCGR), a class B receptor stabilised and co-crystallised with small molecule MK-0893, a negative allosteric modulator (NAM). The 2.5A structure reveals a novel, intracellular binding site, pointing to a modulatory mechanism involving locking the receptor in an inactive conformation, thereby counteracting the effect of glucagon binding. More recently, the structure of the human complement receptor C5aR1 was determined at 2.7A resolution in the presence of non-peptide inverse agonist NDT9513727. The ligand binds at an allosteric extra-helical site shared by TM's 3, 4 and 5 on the side of the receptor. The binding mode is again different, largely based on hydrophobic interactions, the ligand acting as a molecular wedge preventing a helical reorientation typically associated with the active conformation of class A receptors. These structures reveal the diversity of binding modes and mechanisms by which compounds can allosterically control GPCR function, but they also underscore the challenges inherent to applying SBDD approaches to allosteric binders.

84 | Luke Souter

Understanding the composition and biophysical properties of a specialised neuronal surface

Perineuronal nets are large molecular assemblies of extracellular matrix molecules tethered to the surface of a defined population of neurons. They are involved in regulating plasticity in the central nervous system (CNS). Enzymatic removal of perineuronal nets reactivates plasticity, which improves recovery from spinal cord injury, and improves memory retention in mouse model of Alzheimer's disease and in ageing. This makes them an interesting therapeutic target for enhancing neuronal repair and regeneration.

This project aims to understand how the formation of perineuronal nets changes the microenvironment and local mechanics on the neuronal surface, therefore affecting cell behaviour. Interestingly, cells in the CNS are sensitive to mechanical changes in the extracellular matrix.

Initially, regional differences in the composition of perineuronal nets have been characterised using immunohistochemistry. This has demonstrated region-specific differences in perineuronal net composition which may be relevant to manipulating the biophysical properties of the perineuronal net surface.

Using an acoustic surface measurement technique called quartz crystal microbalance with dissipation monitoring (QCM-D) an artificial perineuronal net surface can be produced to examine how the components of perineuronal nets interact to form the compact perineuronal net structure. The interactions between components can be studied and their effect on the biophysical properties of the surface can also be analysed.

We aim to produce a biomimetic surface that simulates perineuronal nets with tuneable features. This will allow neuronal behaviour to be studied in relation to changes in the biophysical properties of their surfaces. The artificial net could also be used as a method for screening potential drug candidates that interact with perineuronal nets, which may help produce translational therapies for spinal cord injury and Alzheimer's disease.

85 | Sonja Srdanovic

Small-Molecule Stabilisation of hDMX / 14-3-3 Interaction

Protein-Protein Interactions (PPIs) are of remarkable significance given their role in the vast majority of cellular processes [1]. p53, the "guardian of the genome", regulates the cell cycle as a tumour suppressor. hDMX primarily acts as a negative regulator of a p53, inhibiting its function in cell cycle repair or apoptosis[2]. hDMX also binds to the hub protein 14-3-3[3] upon phosphorylation. Subsequently, hDMX is prevented from binding to p53, thus not supressing levels of p53. Stabilization of the hDMX/14-3-3 interaction by a small molecule is one approach toward restoring p53's function. Firstly, to understand the interaction of hDMX and 14-3-3, several hDMX peptides with key phosphorylated residues for binding to 14-3-3 were synthesized. Binding affinity between hDMX and six isoforms of 14-3-3 was determined by fluorescence anisotropy (FA) assays and ITC. In addition, X-ray crystallography was used to obtain structural information on the hDMX/14-3-3 interface. Future efforts will focus on using FA to screen small molecule stabilizers of this interaction, and thermal shift assays to screen fragments. Modulating PPIs in the p53 pathway by stabilizing hDMX/14-3-3 interaction may represent an initial step towards developing cancer therapeutics targeting the p53 pathway.

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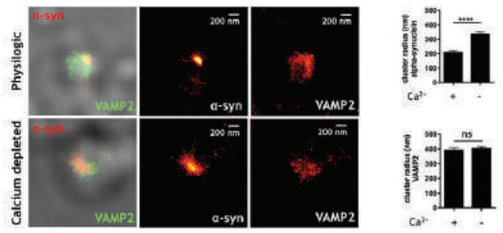
86 | Alice Stelfox

Structural basis for divergent receptor tropism for emerging unclassified paramyxoviruses

Novel paramyxoviruses continue to be identified worldwide and are responsible for a diverse range of diseases across many species. These viruses, termed orphan paramyxoviruses, do not cluster within the well characterised paramyxovirus genera, such as morbilliviruses and henipaviruses. The initial stage of paramyxoviral infection is dependent upon the interaction between a viral attachment protein and a cell surface receptor. The molecular specificity underlying this recognition event is a key determinant of cell and species tropism. However, the high level of sequence diversity amongst paramyxoviral attachment proteins impedes predictions of receptor usage when considering amino acid sequence alone. Here, we study number of rodent-borne paramyxoviruses using X-ray crystallography to probe the diversity of attachment glycoproteins and aid the characterisation of these orphan paramyxoviruses. We solved the attachment glycoprotein structures for the Novel paramyxoviruses continue to be identified worldwide and are responsible for a diverse range of diseases across many species. These viruses, termed orphan paramyxoviruses, do not cluster within the well characterised paramyxovirus genera, such as morbilliviruses and henipaviruses. The initial stage of paramyxoviral infection is dependent upon the interaction between a viral attachment protein and a cell surface receptor. The molecular specificity underlying this recognition event is a key determinant of cell and species tropism. However, the high level of sequence diversity amongst paramyxoviral attachment proteins impedes predictions of receptor usage when considering amino acid sequence alone. Here, we study number of rodent-borne paramyxoviruses using X-ray crystallography to probe the diversity of attachment glycoproteins and aid the characterisation of these orphan paramyxoviruses. We solved the attachment glycoprotein structures for the Mossman, Nariva, Beilong and J paramyxoviruses (MosPV, NarPV, BeiPV and JPV, respectively). Using structural phylogenetic analysis, the distinct clustering of MosPV and NarPV attachment glycoproteins, and the BeiPV and JPV attachment glycoproteins. Furthermore, motifs implicated in binding the morbilliviral or henipaviral receptors were not present. These data expand our appreciation of the diversity of paramyxoviral receptor-binding architectures and indicate a new paradigm for paramyxovirus host-cell recognition and entry.

87 | Amberley Stephens

Calcium binding at the C-terminus of synuclein modulates synaptic vesicle interaction



Alpha-Synuclein (aSyn) is a major component in familial and sporadic forms of Parkinson's disease (PD). It is a structurally disordered protein with unknown function, primarily localised in the pre-synapse. In PD the selective decline of dopaminergic neurons in the substantia nigra pars compacta may be due in part to their increased cytoplasmic calcium fluctuations. We aim to investigate how calcium may influence aSyn function/dysfunction.

We show that multiple calcium ions bind to the C-terminus of aSyn, revealed by NMR and MS. Using isolated synaptic vesicles (SV) from rat brains and NMR we reveal not only the N-terminus interacts with SV, but also the C-terminus upon calcium binding in an extended-double anchor mechanism. Using super-resolution microscopy we see addition of calcium leads to increased clustering of SV and localisation to the plasma membrane in a subpopulation of SV. However, an excess of calcium leads to an increase in aggregation rate of aSyn. Using isolated neurons we see that both calcium and aSyn are needed for toxicity to occur, as only use of both siRNA knock down of aSyn and use of the calcium channel blocker isradipine could rescue cells.

To conclude, calcium binding is likely important in the function of aSyn. It enhances aSyn-lipid interactions, potentially bridging two membranes, either tethering vesicles together or to the presynaptic membrane. This further supports the role of aSyn in SV homeostasis and endo/exocytosis, but in a calcium dependent manner. However, calcium also increases the aggregation rate of aSyn, suggesting there is a fine balance between physiological and pathological function and a dysregulation of both aSyn and calcium leads to disease.

88 | Xiaofan Tang

Investigation of yeast Erv1 downstream substrates with liposomes

Disulfide bond formation is an important post-translational modification for folding and function of many proteins. Introduction of disulfides in the intermembrane space of mitochondria is catalysed by Mia40-Erv1 disulphide relay system. The sulfhydryl oxidase Erv1 generates disulfide bond de novo using either molecular oxygen or cytochrome c (cyt *c*). Whilst a study on the ALR (human homologue of Erv1) showed that cyt *c* is a better substrate than oxygen[1]; Erv1 of Trypanosoma brucei (TbErv1) was shown to reduce oxygen and cytochrome c simultaneously[2]. So far no study has been carried out to assess the relative substrate specificity of yeast Erv1 for oxygen and cyt *c*. On the other hand, although cyt *c* is highly water-soluble, it is found associated with the inner membrane in mitochondria, and cardiolipins (the signature lipid of mitochondrial inner membrane) were proven to be good binding partners for cyt *c*. In this study, we performed enzyme kinetic study to understand the relative substrate specificity of yeast Erv1 for oxygen and cyt *c*. The experiments were performed using oxygen consumption assay for Erv1 oxidase activity[3], and stopped-flow absorption measurement under both aerobic and anaerobic conditions systematically to understand how oxygen affects cyt *c* reductase activity of Erv1. Furthermore, we used liposomes modified with cardiolipin to investigate whether liposomes affect catalytic properties of Erv1 towards oxygen and cyt *c*. Taken together, our results demonstrate that cyt *c* is a better substrate than oxygen for Erv1, and liposomes alter the substrates affinity of Erv1 indicating the mitochondrial inner membrane play a role in Erv1-cyt *c* interaction.

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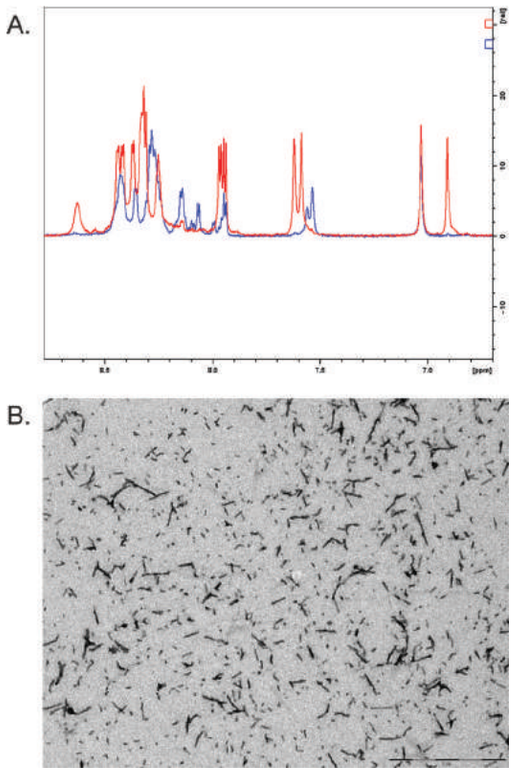
89 | James Torpey

The inhibition of alpha synuclein aggregation using a novel peptide-based inhibitor



Alpha synuclein (aSyn) is a 140-residue protein localized at the presynaptic terminals. ASyn's physiological function remains poorly understood, yet it is a key player in Parkinson's disease (PD) and other neurodegenerative diseases. Disease progression is caused by the progressive loss neurons in the brain. Cell death appears to be associated with the aberrant aggregation of aSyn, and thus the inhibition of aggregation represents an enticing therapeutic strategy. Here we employ NMR to probe interactions between aSyn and a 10-residue peptide that has been previously shown to reduce aSyn fibril formation and associated cell toxicity (Cheruvara et al. 2015). Using a series of NMR experiments over time we show that a structural rearrangement of the peptide occurs (Figure 1A), and this is a pre-requisite for binding to higher-order aSyn species. Electron microscopy shows the presence of more numerous yet much shorter fibrils when aSyn is incubated in the presence of the peptide (Figure 1B), implying that the peptide prevents fibril extension. We hypothesize that binding of the peptide prevents the formation of the aSyn species responsible for cell toxicity. We now aim to elucidate the cellular pathways involved through the use of NMR metabolomics, using our previously published SH-SY5Y neuroblastoma metabolic profiles (Phelan et al. 2017).

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90 | Keni Vidilaseris

Studies of the membrane-bound pyrophosphatase from protist parasites as a potential drug target

Membrane-bound pyrophosphatases (mPPases) are homodimeric integral membrane proteins which hydrolyse pyrophosphate into orthophosphates, coupled with the active transport of protons or sodium ion across membranes. They occur in bacteria, archaea, plants, and protist parasites, but no homologous proteins found in human and animals. mPPases are also important for the growth of protist parasites causing disease. Because of their absence in animals and humans, mPPase represent a potential candidate for rational drug design for treating diseases caused by protist parasites, such as malaria, leishmaniasis, toxoplasmosis and African sleeping sickness. Aim of the research is to study and characterise mPPases from different protist parasites (e.g. *Plasmodium falciparum*, *Toxoplasma gondii*, *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania donovani*) and using the solved structure of *Thermatoga maritima* mPPase as a starting model, our research is also focusing on understanding the binding, inhibition, and specificity of the drug candidates against protist parasite's mPPases. We currently manage to express the protist mPPases in the baculovirus-insect cell expression system to milligram levels. We also have identified some hit compounds which inhibit the activity of mPPase from *T.* maritima and P. falciparum at the micromolar level. X-ray crystallography and other complementary approaches will be employed to analyse the molecular basis of drug inhibition and to improve its specificity. Enzymatic activity and ion transport experiments will be employed to generate a model of the catalysis and ion transport mechanisms of the protist mPPases. Overall, this work has the potential not only to yield a molecular-level understanding of mPPase catalysis coupled with ion pumping in protist parasites, but also lead to breakthroughs in a variety of diseases, both in terms of molecular understanding and in terms of the development of potential drug candidates for widespread and debilitating diseases such as malaria, toxoplasmosis, Chagas' disease, sleeping sickness, and visceral leishmaniasis.

91 | John Viles

Ion channel formation and Co-fibrillisation of truncated isoforms of Amyloid-β in Alzheimer's disease

A small peptide, amyloid-β (Aβ), self-assembles into cyto-toxic oligomers and fibers that are thought to be essential contributors to the cascade of events in Alzheimer's disease etiology. The process by which amyloid-β (Aβ) disrupts synaptic activity, and causes neuronal cell death in Alzheimer's disease remains poorly understood. A potential mechanism of toxicity is in the ability of Aβ to form, membrane-spanning ion channels. However, there has been a mismatch between the channel forming properties of Aβ isoforms, 40 and 42 amino acids long, and their known relative pathogenicity. We observe ion channel formation by oligomeric $A\beta_{42}$, but also show $A\beta_{40}$ does not form ion channels in cellular membranes. This makes a strong link between ion channel formation and the pathology of Aβ isoforms. Molecules that block these ion channels may represent therapeutic targets.

Amyloid-β peptide (Aβ) isoforms of different lengths and aggregation propensities coexist in vivo. These different isoforms are able to nucleate or frustrate the assembly of each other. N-terminal truncated $A\beta_{(11\text{-}40)}$ and $A\beta_{(11\text{-}42)}$ make up one fifth of plaque load yet nothing is known about their interaction with full-length $A\beta_{(1\text{-}40/42)}$. Here we show that in contrast to C-terminal truncated isoforms which do not co-fibrillise, deletions of ten residues from the N-terminus of Aβ have little impact on its ability to co-fibrillise with the full-length counterpart. As a consequence N-terminal truncated Aβ will accelerate fibre formation and co-assemble into short rod-shaped fibres with its full-length Aβ counterpart. Furthermore we show Cu2+ forms a very tight tetragonal complex with truncated $A\beta_{(11\text{-}40)}$ with a femtomolar affinity. These observations have implications for the assembly kinetics, morphology and toxicity of all Aβ isoforms.

Ion Channel Formation by Amyloid-β42 Oligomers but not Amyloid-β40 in Cellular Membranes, DC Bode, MD Baker, JH Viles, (2017) J of Biol Chem 292, 1404-1413

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Truncated Amyloid-β (11-40/42) from Alzheimer's Disease Binds Copper2+ with a Femtomolar Affinity and Influences Fibre Assembly J D Barritt, J H. Viles, (2015) J of Biol Chem, 290, 27791-27802

92 | Martin Walko

New protein labels for efficient photocrosslinking

Biochemical studies rely with increasing frequency on the exploitation of state-of-the-art organic chemistry. A wide array of synthetic labels and hundreds of synthetic unnatural amino acids and nucleotides can surpass the limited toolbox of 20 natural amino acids and 5 nucleobases. Chemical biology provides tools allowing development of new techniques to identify isolate and study the structure, dynamics and function of biomolecules.

Photocrosslinking is a unique technique to study interactions in complex biomolecular systems: it is exploits a photosensitive group in one of the interacting partners. Upon irradiation, the photocrosslinking group produces a highly reactive species that can react with the other interacting partner, thus turning the transient non-covalent interaction into a more stable covalent link. Photocrosslinking can thus trap, isolate and identify interacting molecules. Due to indiscriminate reactivity of the photogenerated species, excellent spatial selectivity can be achieved, allowing identification of the exact interaction site.

We have developed new reagents for efficient and robust diazirine based photocrosslinking that facilitate studies of protein-protein interactions. Since the diazirine moiety must be in close contact with the molecule it crosslinks to, it is usually proximal to, or even directly in, the interaction site. Therefore, the design of our new labels is minimalistic to prevent disruption of the studied interaction. Despite the small size, we have imparted additional functionality to the label by choosing the methanethiosulfonate as a cysteine specific labelling group. This leads to attachment of the photocrosslinker through a disulphide bridge allowing transfer of the label from the photocrosslinker tagged protein to interacting partners following reduction of the disulphide. The tag-transfer photocrosslinking process leaves the interacting partner protein labelled with a free thiol group that can be further functionalizationed with mass, affinity or fluorescent tags to facilitate structural proteomics analyses.

93 | Alice Webb

Mutations in PPIL1 cause a novel syndrome of microcephaly and pontocerebellar hypoplasia: insights from structural biology

We report a novel brain malformation syndrome in six individuals from four families. Patients present with profound microcephaly (OFC 7-9 SD < mean), severe cortical dysplasia, profound cerebellar hypoplasia of both vermis and hemispheres and brainstem hypoplasia. Autozygosity mapping and whole exome sequencing revealed variants in peptidyl-prolyl isomerase 1 (PPIL1.) Previous work has shown that PPIL1 is expressed ubiquitously in humans[1] and is part of the spliceosome, a complex RNA and protein enzyme responsible for splicing introns from pre-mRNA[2]. To gain an insight into the mechanism of this disease, PPIL1 WT and mutant proteins have been expressed and purified from E. coli. This work has shown that two PPIL1 patient mutations affect protein stability. Using SPR a further patient mutation has been shown to abolish an interaction with SKIP, a protein which is thought to recruit PPIL1 to the spliceosome[3]. PPIL1 is also a peptidyl prolyl-isomerase enzyme and using NMR we have shown that a further patient mutation reduces this enzymatic activity on a model substrate. Currently the biological target of PPIL1 peptidyl prolyl-isomerase enzyme activity in the spliceosome is unknown. By studying published cryo-EM data[4],[5] and using NMR experiments we have shown that PRP17, is a plausible biological target of PPIL1 in the spliceosome. Taken together these results confirm pathogenicity of the identified patient mutations and provide novel insights into the role of PPIL1.

This work is as yet unpublished and we would appreciate your treating this data as confidential.

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94 | Leon Willis

Towards understanding the mechanisms of flow-induced monoclonal antibody aggregation

Over 60 monoclonal antibody (mAb)-based biopharmaceuticals have been approved to date, with a market value exceeding $80bn/year. These powerful protein-based medicines combat serious diseases such as cancer and chronic inflammation. Like all proteins, they are prone to unfolding, misfolding and aggregation. This can hinder the progression of mAbs to market, as aggregates may harm patients. The hydrodynamic forces found in biopharmaceutical manufacturing have long been linked to causing aggregation. However, an understanding of how fluid flow induces protein aggregation has been unclear, in part due to confusion around the specific fluid fields (e.g. extensional or shear flow) present during certain process steps (e.g. filtration).

We have recently developed and characterised a bespoke device which subjects proteins to defined fluid fields. Whilst we have found extensional flow to be a key driver in the aggregation of BSA, the role this type of flow plays in mAb aggregation, at the molecular level, has remained unknown. In this work, several model IgG1 mAbs were subjected to defined fluid stresses and the subsequent aggregates analysed with an array of biochemical and biophysical techniques. We have shown that the flow conditions under which aggregation is initiated is different for IgGs, depending on their sequence. These data, in addition to bioinformatics analysis, allow us to propose a mechanism by which mAbs aggregate under flow. This understanding could facilitate the identification of robust IgG1 biopharmaceutical candidates, as well as the flow conditions with which to manufacture them.

95 | Wei-Feng Xue, University of Kent

The Division of Amyloid Fibrils

Amyloid fibrils are filamentous protein assemblies associated with a number of human disorders. Breakage of amyloid fibrils is a key factor for amyloid function and pathogenicity, as the division of amyloid particles through fibril fragmentation is a key process in their lifecycles in the same way as microbial proliferation through cellular divisions. However, the molecular mechanism and biological impact of fibril division in terms of the normal role of functional amyloid as well as the disease association of pathological amyloid, including transmissibility of prions, is not understood. Because protein filaments formed from different precursors show a variety of suprastructures and size distributions, the stability of amyloid fibrils towards breakage is also not well characterized and subsequently not compared. Here, to address these knowledge gaps, we combine kinetic nano-scale imaging experiments that detect the fibril division processed in isolation with a mathematical model of amyloid fibril division based on a continuous partial differential equation, which uniquely results in an analytical solution that fully describes the length dependent stabilities, division rates and time-independent characteristic length distributions when applied to the experimental data of any filament divisions. Subsequently, through application of our analysis to the fragmentation of dissimilar amyloid fibrils, we show that the stability of protein filaments towards breakage can be determined and compared for fibrils of different types independently of each of their distinctive suprastructures and size distributions. Our results establish how amyloid fibrils divide, and therefore, enables the comparison of the protein filaments' stabilities that are key to unravel the toxic and infectious potential of amyloid and prions.

96 | Maria Zacharopoulou

Alpha-synuclein conformers formed during storage of recombinantly produced protein affect aggregation kinetics

Alpha-synuclein (aSyn) is an intrinsically disordered protein abundantly distributed at the presynaptic nerve terminals of neurons. However, its aggregation into amyloid fibrils is considered a molecular hallmark of Parkinson's disease. In order to understand the mechanisms behind amyloid protein aggregation, in vitro assays are used to study the progress of the disease on a molecular level and to potentially contribute to the development of novel therapeutic strategies. In the scope of protein aggregation diseases, protein damage, misfolding or presence of oligomers during purification and storage is unwanted, as it can influence biophysical assays. In the present work we demonstrate that chromatographic buffers and storage conditions can induce the formation of structural conformers of aSyn, which can affect protein aggregation kinetics. Using thioflavin T (ThT)-based aggregation assays we show that lyophilised compared to frozen aSyn samples display a higher variance in their aggregation kinetics. Dynamic light scattering (DLS) and atomic force microscopy (AFM) also reveals that aSyn conformers display a wider size distribution and an increased heterogeneity after lyophilisation than after freezing. Hydrogen-Deuterium exchange mass spectrometry (HDX-MS) was introduced as a powerful tool to investigate the sub-molecular structure of the aSyn frozen and lyophilised samples in order to begin to rationalise the findings described above. HDX-MS shows that the lyophilised sample is significantly less solvent exposed/ more hydrogen bonded throughout the protein sequence, suggesting different structural conformers of aSyn are being formed. Interestingly, we also observed the most solvent exposure in the NAC region and the least exposure at the C-terminus of the monomeric protein. We conclude that the effect of protein storage protocols must be taken into consideration, as it may hamper our goal to reliably measure amyloid protein conformation and aggregation kinetics.

97 | Zacharchenko, T, Kalverda, A.P. and Wright S,C.

Structural determinants of BCL6 corepressor interactionskinetics

BCL6 is a transcription factor that is dysregulated in germinal centre-derived Diffuse Large B Cell Lymphoma (DLBCL). It represses gene expression by the recruitment of corepressors such as SMRT or NCOR1. A conserved corepressor BCL6 Binding Site (BBS) of SMRT and NCOR1 interacts with the N-terminal BTB (Bric-à- brac, Tram-track and Broad complex) domain of BCL6 at a site known as the lateral groove. We have solved the structure of the NCOR1BBS-BCL6 complex and demonstrate its structural similarity to the SMRTBBS-BCL6 complex. Bioinformatic analysis of NCOR1 revealed a potential second site of BTB engagement, and structural elucidation of this region in complex with BCL6 revealed a previously uncharacterised binding site.

Our data exemplifies a new mechanism of BTB domain-protein interactions. Its structural novelty may provide the basis for a further understanding of BCL6 function.

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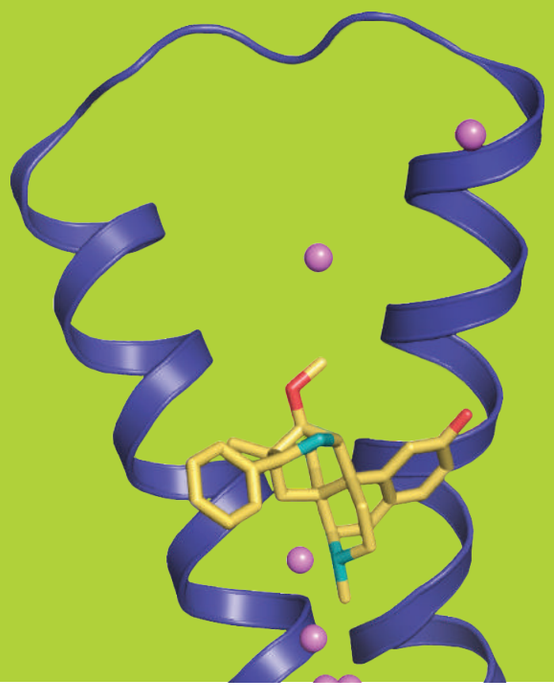

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