2023 Biomacromolecular Structure Special Interest Group Meeting

Programme and Abstract Book

Friday 10th November 2023

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Programme - Friday 10th November 2023

9:00 – 9:45	**Conference Registration**
Session 1 (Chair: Aneika Leney, University of Birmingham)	
9:40 – 9:45	Welcome and Introduction to BMS-SIG
9:45 – 10:30	**Keynote Lecture** **Idlir Liko (Omass Therapeutics): Native MS takes the central stage - Enabling the development of novel medicines**
10:30 – 10:45	Ikhlas Mohamed Mohamud Ahmed (University of Strathclyde): Ion mobility mass spectrometry unveils conformational effects of drug lead-EPI-001 on the intrinsically disordered N-terminal domain of the Androgen receptor
10:45 – 11:00	Sarah Vickers (University College London/Birkbeck College London): Ion mobility mass spectrometry reveals oligomerisation intermediates in alpha-1 antitrypsin deficiency
11:00 – 11:45	**Coffee Break**
Session 2 (Chair: Kish Adoni, University College London)	
11:45 – 12:00	Niklas Geue (University of Manchester): Lessons from native ion mobility mass spectrometry applied to supramolecular complexes
12:00 – 12:15	Jaspreet Sound (University of Birmingham): Native mass spectrometry is a powerful tool to probe the evolution of photosynthesis in cyanobacteria
12:15 – 12:30	Anthony Devlin (Rosalind Franklin Institute): Structural elucidation of glycosaminoglycans (GAGs) using trapped ion mobility spectrometry (TIMS)
12:30 – 12:45	Poster flash talks (Chair: Joseph Gault, AstraZeneca)
12:45 – 2:00	**Lunch and Posters**
Session 3 (Chair: Kelechi Uleanya, University of York)	
2:00 – 2:15	Dan McGill (Rosalind Franklin Institute): Cold argon plasma for non-enzymatic digestion of proteins and peptides
2:15 – 2:30	Emma Sisley (University of Birmingham): Tissue washing improves native ambient mass spectrometry detection of membrane proteins directly from tissue.
2:30 – 2:45	Glenn Masson (University of Dundee): Structural basis of small molecule PI3kalpha activators via HDX-MS
2:45 – 3:30	**Coffee and Posters**
Session 4 (Chair: Alice Colyer, University of Leeds)	
3:30 – 3:45	Cameron Baines (University of Nottingham): Carbene footprinting of EF-Tu in complex with contrasting Elfamycin antimicrobials
3:45 – 4:15	**Keynote Lecture** **Frank Sobott (University of Leeds): Molecular Footprints of Proteins**
4:15 – 4:30	Closing address and prizes

Oral Presentations

Ion mobility mass spectrometry unveils conformational effects of drug lead EPI-001 on the intrinsically disordered N-terminal domain of the Androgen Receptor

Ikhlas Mohamed Mohamud Ahmed[1], Adam Rofe[2], Martyn C. Henry[1], Craig Jamieson[1], Iain J. McEwan[2], Rebecca Beveridge[1]

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Drug discovery techniques are heavily reliant on the existence of a well-folded protein structure which renders investigating the druggability of intrinsically disordered proteins (IDPs) difficult. The androgen receptor's disordered N-terminal domain (AR-NTD) has recently emerged as a target for prostate cancer therapy via small molecule drugs. In this project, we use native ion mobility mass spectrometry (IMMS) to characterise the effects of drug binding to this protein.
We characterised constructs containing important functional domains of the disordered N-terminal of AR, and determined how their shape is affected by the binding of a drug lead, EPI-001. This interaction causes preference for a conformation that is only populated to a low extent in the absence of the drug. We hypothesised that this effect would be a useful readout for the screening of new drugs. Indeed, we were able to screen and identify molecules that (i) bound to AR-NTD and (ii) affected the conformation of the protein. We therefore demonstrate that IMMS is a useful tool for identifying new small molecules that affect the conformational distribution of flexible drug targets, thereby increasing the druggability of IDPs.

Ion Mobility Mass Spectrometry Reveals Oligomerisation Intermediates in Alpha -1 Antitrypsin Deficiency

Sarah Vickers,[1,2,3] Ibrahim Aldobiyan,[2,3] Aisha Ben-Younis,[1,3] Sarah Lowen,[2,3] Riccardo Ronzoni,[2,3] James Irving, [2,3] David Lomas, [2] Konstantinos Thalassinos, [1,3]

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Alpha-1 antitrypsin (AAT) is a serine protease inhibitor essential to the control of proteolytic pathways. AAT has a complex mechanism for inhibition involving a conformational change, leaving it vulnerable to misfolding. Misfolded AAT can oligomerise and aggregate in the liver, eventually leading to cirrhosis. AAT thus fails to reach the lungs and inhibit elastase, leading to emphysema. Understanding the mechanism of this oligomerisation has been the subject of many studies. Here, we use a combination of native mass spectrometry techniques to elucidate the structure of an oligomerisation intermediate that can only be captured using this technique. Using a cyclic ion mobility mass spectrometer, we were able to isolate and activate this species using electron capture dissociation (ECD) to suggest the intermediate has an ejected c-terminus that is highly disordered. This provides novel evidence for the proposed C-terminal mechanism of oligomerisation of AAT. Further mobility investigations involved the conformational selection of this highly heterogeneous intermediate for collisional activation and ECD experiments. AAT oligomer specific antibodies were used to confirm that this intermediate is largely structured and is polymer-like. Overall, the investigation of this antitrypsin intermediate is highly novel and the technique can be applied to other aggregating proteins associated with disease, for example Alzheimer's, Parkinson's and Huntington's disease.

Lessons from Native Ion Mobility Mass Spectrometry Applied to Supramolecular Complexes

N. Geue [1], T. S. Bennett [2], A. A. Arama [2], L. A. I. Ramakers [1], G. A. Timco [2], G. F. S. Whitehead [2], E. J. L. McInnes [2], P. B. Armentrout [3], N. A. Burton [2], R. E. P. Winpenny[2] and P. E. Barran [1]

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The interest in complex supramolecules has rapidly grown in the last decades, however their structural characterisation is difficult. [1] Native ion mobility mass spectrometry (nIM-MS) is well-known for its potential in structurally characterising biomacromolecules, but their use for supramolecular complexes and other labile synthetic molecules remains underexplored.

We have been studying a range of metallosupramolecular complexes, which are interesting targets for materials applications and in quantum computing. We evaluated their stability using tandem mass spectrometry, and we used this to systematically examine how the substitution of d-metals, ligands and charge carriers alter properties. [2] Our results provided new insight to the contested criteria a threaded supramolecular assembly must fulfil to be considered a rotaxane, i.e. when the ammonium cation in the centre of the ring cannot slip off. We used DFT and MD calculations coupled with IM-MS experimental data to propose atomically resolved structures, and to demonstrate how different charge carrying ions can measure the cavity size of polymetallic complexes. [3] Our work also exemplifies how ion mobility data can inform on the packing density and conformational flexibility of metallosupramolecular complexes and we developed a unique toolbox to synthesise and characterise novel polymetallic rings and oligomers. [4] Taken together, our observations provide an empirical framework for the future design and characterisation of supramolecules using native ion mobility mass spectrometry.

[1] Geue et al., Structural characterisation methods for supramolecular chemistry that go beyond crystallography, Chem. Soc. Rev. 2022, 51, 8-27.
[2] Geue et al., Disassembly Mechanisms and Energetics of Polymetallic Rings and Rotaxanes,
J. Am. Chem. Soc. 2022, 49, 22528 – 22539.
[3] Geue et al., Adduct Ions as Diagnostic Probes of Metallosupramolecular Complexes using Ion Mobility Mass Spectrometry, Inorg. Chem. 2023, 62, 2672–2679.
[4] Geue et al., Formation and Characterisation of Polymetallic {CrxMy} Rings in vacuo, Nat. Synth. 2023.

Native mass spectrometry is a powerful tool to probe the evolution of photosynthesis in cyanobacteria.

J. K. Sound, A. C. Leney

University of Birmingham, Birmingham, UK

Cyanobacteria are ancient organisms, existing for billions of years. As a result, thousands of species have evolved to a variety of environments such as freshwater lakes and oceans and have subsequently adapted to endure conditions surrounding extreme high and low temperature, pH and light levels. Cyanobacteria rely on their highly adaptive photosynthetic machinery, termed the phycobilisome, to survive. How this mega-Dalton large protein complex has maintained its photosynthetic efficiency over the course of cyanobacterial evolution is yet to be understood. Here we use native mass spectrometry to investigate the structural dynamics of how phycobilisome protein complexes from different cyanobacterial species interact with each other. To do this, ~ 100 kDa hexameric phycobiliprotein complexes, allophycocyanin (APC) and phycocyanin (PC), which form the core and rods of the phycobilisome, were extracted and analysed by native mass spectrometry. An Orbitrap Eclipse mass spectrometer (Thermo Fisher Scientific) equipped with a nano-electrospray ionisation source was used to perform all analysis. Upon mixing APC and PC from different cyanobacterial species, heterogenous complexes formed consisting of subunits from both species. Despite minor differences in the molecular weight of these complexes, with advancements in the high resolution of the Eclipse mass spectrometer, we were able to readily distinguish the dynamics between APC and PC from closely related cyanobacterial species. These dynamics could unlock our understanding of how phycobilisomes have evolved over time.

Structural elucidation of glycosaminoglycans (GAGs) using trapped ion mobility spectrometry (TIMS)

A. Devlin [1], A. Simmonds [1,2], F. Green [1], C. Wooton [3], Z Takats [1,4]

1: Rosalind Franklin Institute, 2: Francis Crick Institute, 3: Bruker, 4: Imperial College London

Glycosaminoglycans (GAGs) are ubiquitous, vital polysaccharides. GAGs have roles in many important biological systems, including development, tissue macrostructure, are involved in pathogenesis and cancer progression and are also vital pharmaceutical agents. Unfortunately, GAGs represent a significant analytical challenge as they are structurally homogenous, very large (15kDa to 5mDa), possess isomeric backbones, and are covered in isomeric, labile sulphate groups. Here, we demonstrate the use of trapped ion mobility spectrometry (TIMS) as a tool to combat many of these analytical roadblocks; including the separation of isomers from small standards to large chains, the differentiation of isobaric charge states and the potential for semiquantitative analysis of sulphation. We also demonstrate the prevention of labile sulphate loss with a new, high transmission TIMS-FTICR instrument and simultaneously, a reduction in information loss by examining hitherto unreported ring conformer fingerprints found in the TIMS profiles of in-source fragmented ions. Finally, we demonstrate the use of these discoveries in situ, in a spatially resolved manner through examination of ape retina sections using MALDI.

Cold argon plasma for non-enzymatic digestion of proteins and peptides

D McGill, D Simon, Z Takats

Rosalind Franklin Institute

Proteins and peptides are ubiqutious in biological samples. Deconvoluting their chemical structures is crucial to understanding their role in endogenous and exogenous metabolism - however, this is often hindered by large molecular masses and the relatively small dynamic range of mass spectrometers. While this problem has previously been tackled with the use of enzymatic digests as 'bottom-up' proteomics, this approach has several drawbacks - including not only the logistical burden of sample preparation (such as time, expense, and expertise), but also the delocalisation of solid phase analytes caused by applying an enzymatic solution.

We present here a novel application of electrochemistry, utilising a radiofrequency-generated cold argon plasma to non-enzymatically digest condensed-phase small molecules, peptides, and proteins with minimal sample preparation. This reagentless method requires a fraction of the time needed for tryptic digestion, and can be applied to solid phase samples to generate analytes that are more easily detectable than their parent compound in a manner which is consistent, predictable, and spatially resolved, without loss of resolution.

Tissue Washing Improves Native Ambient Mass Spectrometry Detection of Membrane Proteins Directly from Tissue

E. K. Sisley [1], O. J. Hale [1], H. J. Cooper [1]

1: University of Birmingham, Birmingham, UK

Nanospray-desorption electrospray ionisation (nano-DESI) is an ambient MS imaging technique which can analyse native (folded) proteins from biological substrates such as tissue sections. Until recently the majority of proteins analysed using this technique have been soluble cytoplasmic proteins. The detection of less soluble proteins (including membrane proteins) has up until now proven challenging. Here, we demonstrate native ambient MSI of membrane proteins, including beta barrel and alpha-helical integral membrane proteins from rat brain, by integrating a simple washing protocol to remove soluble proteins. Membrane-associated proteins with lipid anchors were also observed with this technique. MSI revealed that washing did not disrupt the spatial distributions of the membrane and membrane-associated proteins while some delocalisation of the remaining soluble proteins was observed.

Structural Basis of Small Molecule PI3kalpha Activators via HDX-MS

Grace Q Gong [1], Benoit Bilanges [1], Ben Allsop [2], Glenn R Masson [3 4], Victoria Roberton[5], Trevor Askwith [2], Sally Oxenford [2], Ralitsa R Madsen [1], Sarah E Conduit [1], Dom Bellini [3], Martina Fitzek [6], Matt Collier [6], Osman Najam [7], Zhenhe He [7], Ben Wahab [8], Stephen H McLaughlin [3], A W Edith Chan [9], Isabella Feierberg [10], Andrew Madin [11], Daniele Morelli [1], Amandeep Bhamra [12], Vanesa Vinciauskaite [4], Karen E Anderson [13], Silvia Surinova [12], Nikos Pinotsis [14], Elena Lopez-Guadamillas [1], Matthew Wilcox [5], Alice Hooper [2], Chandni Patel [2], Maria A Whitehead [1], Tom D Bunney [15], Len R Stephens [13], Phillip T Hawkins [13], Matilda Katan [15], Derek M Yellon [7], Sean M Davidson [7], David M Smith [16], James B Phillips [5], Richard Angell [2 8], Roger L Williams [3], Bart Vanhaesebroeck [17]

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The development of small-molecule drugs is often reliant on determining the basis of their interaction with target proteins through structural biology. In the case of small molecule inhibitors, these compounds typically "freeze" proteins in a certain inactive conformation which reduces the inherent flexibility of the protein - something which is typically highly beneficial for structural biology. Therefore, the development of small molecule activators poses a challenge, as these often break autoinhibitory interactions and increase protein flexibility. Here I describe how hydrogen deuterium exchange mass spectrometry (HDX-MS) was used to determine the modality and allosteric nature of a first-in-class small molecule PI3Ka activator, which has unique biochemical and therapeutic potential.

Carbene Footprinting of EF-Tu in Complex with Contrasting Elfamycin Antimicrobials

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University of Nottingham, Nottingham, UK

The prokaryotic elongation factor, EF-Tu, is an essential and ubiquitous component of mRNA translation, responsible for transporting aminoacyl-tRNA to the actively translating ribosome. To accomplish this, EF-Tu undergoes a significant conformational change, couped to the hydrolysis of GTP to GDP and subsequent release from the ribosome. The elfamycin class of antimicrobials specifically target EF-Tu, either preventing aminoacyl-tRNA binding or EF-Tu release from the ribosome, causing a cessation of protein synthesis. We employed carbene footprinting to investigate the interactions between EF-Tu and two contrasting elfamycins, the polyketide enacyloxin IIa and cyclic thiazolyl peptide GE2270A. Carbene footprinting is a protein labelling method, utilising in situ generation of a highly reactive carbene species to covalently insert into the accessible surface of the protein. This is followed by a typical bottom-up proteomic workflow composed of proteolytic digestion and HPLC-MS analysis of the resulting peptides. Through differential footprinting experiments, performed on proteins with or without their binding partners, conformational changes and binding sites can be identified through changes in the amount of protein labelling. Carbene footprinting on either apo- (nucleotide bound) or holo- (elfamycin bound) EF-Tu identified both ligand binding sites, and captured the large conformational switch associated with enacyloxin IIa binding, represented as localised and global reductions in labelling respectively. These global reductions correlate with calculated reductions in SASA between the apo and holo states, providing confidence that we captured such a largescale event through this methodology. As such a dynamic protein, EF-Tu presented itself as an interesting system to interrogate using carbene footprinting.

Posters

1) Conformational diversity in Huntingtin, the protein implicated in the progression of Huntingtons disease is revealed by IM-MS

T. Hoare[1], X. Wang[1] , X. Lund[2], P. Bernado[2],P. Barran[1]

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We have synthesised a protein system containing super folder green fluorescent protein (sfGFP) linked to the N-terminus of Huntingtin, the protein which is implicated in the progression of Huntington's disease. We have employed nESI coupled with TWIM-MS on a Synapt G2 to examine the conformational spread of this system. The protein was sprayed from solutions of varying salt concentration and composition (ranging from 100-1000 mM ammonium acetate) in both ionization polarities. There are clear shifts in the charge state distribution of the protein due to changes in both ionic strength and ionisation polarity. In positive mode, the protein sequesters up to 42 salt adducts and occupies a wide charge state distribution ($\Delta z = 30$) where most of the charge states are above the theoretical limit for a globular protein. Ion mobility experiments confirm this with CCS values ranging from 3000-11,000 Å2. We have compared our experimental data to theoretical values using a toy model (1) and remarkably, this suggests that for much of the presented form of this protein even the sfGFP tag is partially unfolded. In negative mode the protein ionises much less efficiently and presents with a far lower average z, primarily within a charge state range where the construct is globular. These results are compared to the behaviour of sfGFP without the N-Terminus of Huntingtin attached.

(1) Beveridge, R. et al. ''A Mass-Spectrometry-Based Framework To Define the Extent of Disorder in Proteins.'' Anal. Chem. 2014, 86 (22), 10979–10991. https://doi.org/10.1021/ac5027435.

2) Structural MS of G protein-coupled receptors (GPCRs): Can HDX-MS provide sufficient information to identify ligand binding sites?

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1: Sosei Heptares

HDX-MS is widely employed to provide information relating to protein structure, dynamics, and ligand interactions. Although HDX-MS is recognised as a powerful tool for generating data on the majority of backbone amides in soluble proteins, GPCRs backbone characterisation has often proved to be significantly more challenging.

We successfully applied an optimised HDX-MS approach investigating ligand binding to various GPCRs and found that good data can also be obtained with moderate coverage (80%), provided sufficient peptide redundancy. In addition, changing buffers' compositions enabled use of LMNG to obtain highly reproducible and meaningful data for ligand-free wild-type receptors, usually suffering from stability issues in harsher detergents.
Stabilised (StaR) and wild type GPCRs were expressed in insect or mammalian cell and MS/MS spectra were obtained on an Orbitrap Fusion with HCD fragmentation. Proteins were digested online with either pepsin or dual-protease columns and obtained peptides were separated on a 100% water compatible C18 column.

It's sometimes difficult to interpret the nature of shielding observed in GPCR HDX, where observed kinetics shapes can be inconclusive. This bias could be mitigated by employing small molecule modifiers which covalently labelled amino acids in solvent-accessible areas. We will present examples where HDX alone and also where the combination of both HDX-MS and Covalent Labelling-MS was required to establish the ligand binding site. An HDX-MS case study, where ligand agonism was observed by engagement of a GPCR receptor with a miniG protein and the advantages and disadvantages of such approaches will also be discussed.

3) Native mass spectrometry for insights in enzyme interactions with substrates and inhibitors

Victor Mikhailov[1], Tika Mala[1], Faisal Alshref[1], Shuang Liu[2], James McCullagh[1], Christopher Schofield[1]

1: University of Oxford, UK, 2: Institute of Molecular and Cell Biology, Singapore.

Non-denaturing ('native') MS is now routinely used for screening non-covalent ligand binding to various protein targets, with a goal of finding drug candidates for regulating their function. In addition to the measurement of binding affinities of inhibitors to protein targets, important insights in the protein/ligand interactions can also be obtained by native MS. Here, we provide selected examples demonstrating how this method can be used to elucidate the stoichiometry and mechanism of binding (allosteric versus active site), and the mechanisms of activation/deactivation of oligomeric enzymes. Examples include insights in the binding of the oncogenic variant of human dehydrogenase IDH1 and SARS-CoV-2 virus main protease Mpro to their substrates and inhibitors, and the deactivation of Mpro and pseudomonal PiuC hydroxylase via their de-oligomerisation upon their interactions with non–covalently bound ligands.

4) Applications of native mass spectrometry using a Select Series Cyclic IMS

Eva Illes-Toth[1], Valentina Faustinelli1, [2], Francesca Robertson[1], Paul Dalby[2] and Christopher Hopley[1]

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Structure and function relationships, post-translational modifications, impurities and higher order structure are of great interest to many, in which the use of native mass spectrometry offers an unparalleled advantage. Here we describe assessment and benchmarking of a cyclic ion mobility (cIMS) mass spectrometer coupled to an Advion Triversa nESI source for various applications of native mass spectrometry.

We selected a range of proteins, including NT-proBNP variants, alpha-synuclein and a nanobody, for direct infusion nESI under native-like conditions and compared their mass spectra and collision cross section to data acquired on a Synapt G2S Si platform. Furthermore, we tested the binding characteristics of a camelid nanobody with its capsid protein on the cyclic instrument. Lastly, we performed collision induced unfolding (CIU) in the trap region of the cIMS instrument to probe the stability of IgF1 dimers in the gas-phase.

We show that the charge state distributions of proteins are similar for most proteins when comparing data acquired on both commercial platforms. The average collision cross sections of shared charge states correlate well with each other between the two instruments. We examine protein-protein interactions by describing the binding specifics of a diagnostic nanobody and derive its dissociation constant. Moreover, we demonstrate that CIU lends itself to probing the stability of low abundance multimers of IGF1.

We aim to expand and apply cIMS to other relevant biomarkers in the near future and establish the reproducibility of measurements across different laboratories to understand the associated uncertainties of these workflows of native mass spectrometry.

5) Transient structural dynamics of glycogen phosphorylase from non-equilibrium hydrogen/deuterium-exchange mass spectrometry

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It remains a major challenge to ascertain the specific structurally dynamic changes that underpin protein functional switching. High resolution models of protein structure need to be complemented with the determination of how these structures change as proteins function. Glycogen phosphorylase (GlyP), which catalyses the release of glucose from glycogen, is regulated both by phosphorylation and a myriad of allosteric effectors. The exploitation of GlyP as a drug target in type II diabetes and metastatic cancer is hindered by a lack of understanding of the dynamic structural changes that mediate its complex regulation. We have developed a time-resolved millisecond non-equilibrium hydrogen/deuterium-exchange mass spectrometry method, capable of precisely locating dynamic structural changes of GlyP during allosteric regulation. We resolved transient changes in localized structure that are absent when directly comparing active/inactive states of the enzyme and show that they are common to allosteric activation by adenosine monophosphate and inhibition by caffeine, which bind at different sites. This indicates that opposing allosteric regulation by inhibitor and activator ligands is mediated by pathways that intersect at a common structurally dynamic motif. This mass spectrometry approach uniquely stands to discover local transient structural dynamics and could be used broadly to identify features that influence structural transitions of proteins.

6) Native Mass Spectrometry Guided Optimisation of 14-3-3 based Molecular Glues as Cancer Therapeutics

Hadeeqa Raza[1], Paulo Pitasse-Santos[1], Richard G. Doveston[1], Aneika C. Leney[2]

1: University of Leicester, 2: University of Birmingham

14-3-3 proteins are crucial hub proteins that participate in various cellular processes through an extensive network of interactions with partner proteins. A significant number of these protein-protein interactions (PPIs) are disrupted in human diseases like cancer and neurodegeneration. Using drug-like 'molecular glues' to stabilize specific 14-3-3 PPIs is a promising therapeutic approach. However, the discovery of novel PPI stabilizers has been challenging due to the lack of tools for monitoring PPI stabilization. We have shown that native mass spectrometry (Native MS) can bridge this technology gap. Here, we expand the use of native MS to investigate the mechanism of novel designed small molecular glues.

A structure-activity relationship study was carried out on the WR-1065, an FDA approved radioprotector drug, to create a library of new potential molecular glues. These molecules were screened for their ability to stabilise the PPI between the 14-3-3σ and the estrogen receptor (ERα). An acrylamide derivative of WR-1065 displayed similar stabilization to that observed for FC-A, a well-established and characterized 14-3-3 molecular glue. However, differently from the prototype WR-1065, we found no evidence of covalent binding between the acrylamide derivative and 14-3-3σ. Interestingly, native MS analysis revealed a large stoichiometric change upon the addition of the acrylamide derivative that was not visible upon adding FC-A, suggesting stabilisation might occur via an alternate mechanism. Together our results show how native MS can reveal insight into the mechanism of molecular glues and highlight innovative mechanisms of stabilisation that can be exploited for the further design of cancer therapeutics.

7) HDX-MS reveals novel interaction partner involved in Alzheimer's disease progression.

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Hydrogen-deuterium exchange mass spectrometry (HDX-MS) is a powerful and widely used tool to investigate ligand binding. Here, we utilise a home-built HDX-MS to investigate a novel protein-ligand interaction between Pin1, a peptidyl prolyl isomerase enzyme, and collapsin response mediator protein-2 (CRMP2), a phosphoprotein, both of which are implicated in the pathogenesis of Alzheimer's disease (AD). Despite the substantial functional and pathological overlap, whether CRMP2 directly binds Pin1 in a phosphorylation-dependent manner remains to be deciphered.

The CRMP2 present in Alzheimer's patients is hyper-phosphorylated at its C-terminus. We hypothesised that Pin1 may bind specifically to CRMP2's phosphorylated C-terminus acting as a feedback mechanism to prevent CRMP2 hyperphosphorylation in vivo. Pin1 was incubated with unmodified and a doubly phosphorylated peptide corresponding to the C-terminus of CRMP2. Native mass spectrometry showed the formation of a 1:1 complex between CRMP2 and Pin1 but only when CRMP2 was phosphorylated. Next, the binding site of CRMP2 on Pin1 was probed by HDX-MS. The data show that CRMP2 binds specifically to the WW domain of Pin1.

Together the data highlights Pin1 as a novel binding partner of CRMP2. Moreover, further characterisation of this interaction in vivo may open novel therapeutic avenues to prevent Alzheimer's disease progression.

8) Combining Experimental Crosslinking Mass Spectrometry with AlphaFold2 to Improve Protein Structure Prediction for Multiconformational Proteins

Karen Manalastas-Cantos[1,2], Kish R. Adoni[3], Matthias Pfeifer[2,5], Birgit Märtens[2,5], Kay Grünewald[2,6], Konstantinos Thalassinos[3,4], Maya Topf [2,5]

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AlphaFold2 (AF2) has revolutionized structural biology with unprecedented accuracy in protein structure prediction, even on sequences for which related structures are unavailable. It has been shown that AF2 predicts ordered protein domain structures well, but performs less well on proteins with predicted flexibility or disorder. A possible cause is that AF2 and other protein structure prediction approaches have been trained with the assumption that one protein sequence corresponds to one structure, something we know to be untrue for a wide variety of proteins, such as molecular switches which transition between two different conformations as part of their function, as well as proteins that are either fully or partially disordered. We propose a pipeline that combines AF2 with experimental crosslinking mass spectrometry (XL-MS) to model the structure of multi-conformational proteins. Using a shallow Multiple Sequence Alignment to generate multiple ensembles for a protein conformer, the ensembles were then scored against experimentally verified residue depths and distance constraints, from the crosslinks and monolinks of experimental crosslinking MS. In 5/6 cases, the most accurate model within the AF2 ensembles, or a conformation within 1 Å of this model, were identified using crosslinks. For the remaining case, monolinks that were exclusively identified in the open conformation of the protein (QBP) were required to distinguish conformational changes between the open and closed conformation of the protein. Our results highlight the complementarity of AF2 with XL-MS, with monolink/crosslink scores providing reliable metrics to assess the quality of the predicted models.

9) Improved detection of tryptic peptides from tissue sections using Desorption electrospray ionisation mass spectrometry imaging (DESI-MSI)

Heather Bottomley [1], Jonathan Phillips [2], Philippa Hart [3]

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DESI-MSI is an ambient ionisation technique used frequently for the detection of lipids, small molecules, and drug targets. Previously, DESI had only limited use for the detection of proteins and peptides in tissue, due to the setup and needs around deconvolution of data resulting in a small number of species being detected at lower spatial resolution. Here, we present the use of DESI for the detection of large numbers of tryptic peptides from mouse and rat brain tissue sections, with enhanced spatial resolution when compared to previous DESI-MSI studies.
The images were obtained with DESI using a Waters pre-commercial heated inlet (approx. 450°C) to the mass spectrometer (Waters, Synapt G2-si). Further, ion mobility separation was applied in a traveling wave ion guide with nitrogen gas to resolve spectral overlap of peptide ions and improve the detection of multiply charged species. The images acquired had a resolution of 100 µm for the rat brain sections and 50 µm for the mouse brain sections.
Improved detection of tryptic peptides in tissue using DESI-MSI at higher spatial resolution than shown previously, has been achieved. These peptides were tentatively assigned with the addition of a proteomic target list. DESI-MSI can be used alongside MALDI-MSI for tryptic peptide confirmation, allowing drug targets to be detected in the tissue with greater certainty. The benefit of using DESI-MSI to find a greater number of localised tryptic peptide ions has been demonstrated, due to the improved detection of multiply charged species.

10) Mapping the Photodegradation Pathways of the Antibiotic, Oxytetracycline with Laser Interfaced Mass Spectrometry

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Oxytetracyline (OTC), a common antibiotic found in the environment because it is not well absorbed by the gastrointestinal tract and are excreted partially un-metabolized into environmental waters. To gain a comprehensive understanding of the photophysics and photodegradation pathways of oxytetracycline, the protonated and deprotonated forms of OTC were studied using gas-phase photolysis experiments via laser interfaced mass spectroscopy (LIMS), higher energy C-trap dissociation experiments and solution phase photolysis. The results obtained indicated fragmentation dominantly produced anionic photoproducts via photochemical processes likely to involve reactive species in solution. The research highlighted the value of LIMS as a technique for conducting gas-phase photolysis experiments, as well as the importance of characterizing photofragments detected via deprotonation.

11) Unravelling the Mechanism of Rotavirus Viral Factory Formation using Structural Mass Spectrometry

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The formation of viral factories (VFs), a type of biomolecular condensate, is an obscure process involving the formation of transient, heterogeneous protein / RNA assemblies. In this work, we demonstrate how the structural mass spectrometry (MS) toolkit can be employed to investigate the structure, dynamics and interactions of proteins within a condensate forming environment, to bridge the gap between high- and low-resolution biophysical and structural techniques. Here, we have utilised Rotavirus (RV) as a model system to study the mechanism of VF formation. Two non-structural proteins, NSP2 (an RNA chaperone) and NSP5 (an intrinsically disordered protein which undergoes hyperphosphorylation in infected mammalian cells), along with viral RNA, drive VF formation. Native MS has been utilised to characterise the oligomeric state of NSP2 and NSP5. This has revealed that NSP2 exists as an octamer and NSP5 as a decamer. Hydrogen-deuterium exchange MS (HDX-MS) has additionally revealed that the N-terminus of NSP5 is least protected from deuterium exchange and therefore likely to be disordered while the C-terminal region of NSP5 is most protected from exchange and thus likely to be more structured. Furthermore, native MS has also implicated this region in the formation of higher order structures. This has provided the first glimpses into the structure of this IDP and interactions which mediate oligomerisation. Next, we sought to understand the interaction between NSP2 and NSP5 mediating VF formation. We developed a HDX-MS workflow to uncover key interacting regions of NSP2 and NSP5 within a condensate forming environment. This has revealed NSP5 binding within the C-terminal region of NSP2 (previously identified to promote RNA annealing) induces a conformational change which could contribute to the allosteric regulation of RNA annealing. Finally, using label free quantification, comparing infected and uninfected mammalian cells, we have studied the host proteins/pathways that are modulated upon infection. These insights are vital for understanding VF function and for developing therapies that interfere with the assembly of mature VFs.

12) Native LESA Mass Spectrometry of Membrane Proteins from Microorganisms

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Native mass spectrometry (MS) enables the exploration of tertiary and quaternary protein structures by introducing proteins from specially formulated solutions that mimics their natural environment. This method conserves the non-covalent interactions present within and between protein molecules, even when they transition into the gas phase. The analysis of membrane proteins via MS presents notable challenges due to their hydrophobic characteristics, intricate post-translational modifications, and their relatively low abundance in biological systems. To overcome the solubility difficulty, membrane mimetics are used to solubilize membrane proteins. In our ongoing research, we are striving to create a methodology that combines native ambient MS with various techniques to directly identify intact membrane proteins sourced from microorganisms

13) Elucidating the Oligomeric States of Highly Heterogeneous Small Heat-Shock Proteins using CDMS

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The small heat-shock proteins is a group of ATP-independent molecular chaperones that prevent protein aggregation during cellular stress by binding unfolded substrates. Contrary to most known proteins, many of the small heat-shock proteins have a highly dynamic quaternary structure, where they exist in a large polydisperse ensemble of oligomeric states. Additionally, certain different small heat-shock proteins have been shown to co-assemble with each other when mixed, further increasing the number of different states present in the system. This surprising heterogeneity have long made these biomolecules challenging to study using traditional structural method. One such method is native mass spectrometry, since the different oligomers cause sigificant overlap in the mass-to-charge domain. This problem can be circumvented by utilising charge detection-mass spectrometry, an emerging biophysical technique where the charge is measured simultaneously with the mass-to-charge ratio. Here, we present a framework for quantitatively analysing highly heterogeneous systems, such as small heat-shock proteins, using Orbitrap charge detection-mass spectrometry.

14) Unravelling Downstream Dynamics: Conformational Changes in Avian Cryptochromes

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It has been suggested that night-migratory birds utilise the putative magnetoreceptor cryptochrome 4a to navigate using the Earth's magnetic field. This process involves the generation of a radical pair through an established electron hopping pathway, and subsequent chemical and biochemical reactions influencing the singlet and triplet yield of the resultant radical pair depending upon the magnetic field's inclination. This triggers downstream conformational changes within the protein and may help to initiate crucial interactions with other proteins in the bird's retina.

To investigate these changes in conformation and dynamics, we have employed native mass spectrometry and hydrogen-deuterium exchange mass spectrometry (HDX-MS). Our analysis revealed light-dependent changes in three different bird species, pinpointing specific areas of the protein that become protected when exposed to light. Additionally, investigations of mutants where the electron pathway has been shortened has highlighted the importance of these residues in mediating these light-dependent changes.

Furthermore, we have begun to explore potential interactions between cryptochrome 4 and two candidate partners: a G protein and an opsin loop The application of mass spectrometry methodologies offers a promising avenue to elucidate the existence of these binding interactions and may provide insights into the downstream pathways integral to the mechanisms of magnetoreception.