RE FEYN
WEIGHING MOLECULES WITH LIGHT

KAVLI INSTITUTE
FOR NANOSCIENCE DISCOVERY

Mass photometry
symposium and user meeting

14 June 2022

Kavli Institute for Nanoscience Discovery

AGENDA

Registration & Coffee
9.00 - 9.30 am

SESSION 1

9.30 - 9.40 am
Welcome
Anthony Fernandez, Refeyn

9.40 - 10.10 am
Mass Photometry - past, present and future
Philipp Kukura, University of Oxford

10.10 - 10.30 am
Tackling non-specific binding in single molecule microscopy using the fluorous effect
Carlos Bueno Alejo, University of Leicester

10.30 - 10.50 am
A ring or not a ring? Looking into how RAD52 promotes homologous recombination
Maria Kharlamova, Eberhard Karls Universität Tübingen/ Max Planck Institute for Biology

10.50 - 11.05 am
Automated mass photometry - easing the path to biomolecular characterisation
Gareth Rogers, Refeyn

Break
11.05 - 11.30 am

SESSION 2

11.30 - 11.50 am
Combination of mass photometry and native mass spectrometry reveals unbiased co-assembly in highly heterogeneous proteins
Dominik Saman, University of Oxford

11.50 am - 12.10 pm
First-in-class deubiquitylase inhibitors reveal new enzyme conformations
Francesca Chandler, University of Leeds

12.10 - 12.30 pm
The influence of stabilising mutations upon SARS-CoV-2 spike glycan processing, dynamics and function
Sean Burnap, University of Oxford

12.30 - 12.45 pm
Samux MP - Mass Photometry for AAV characterisation
Emily Spencer, Refeyn

Lunch & poster session
12.45 - 2.00 pm

SESSION 3

2.00 - 2.30 pm	Software discussion	MP Performance/Applications discussion	Instrument demos
2.30 - 3.00 pm	Software discussion	MP Performance/Applications discussion	Instrument demos
3.00 - 3.30 pm	Software discussion	MP Performance/Applications discussion	Instrument demos

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Poster & talk prizes
3.30 - 3.45 pm
Teja Sirec, Refeyn

Closing remarks
3.45 - 4 pm
Justin Benesch, University of Oxford

ABSTRACTS

Carlos Bueno Alejo

University of Leicester

Tackling non-specific binding in single molecule microscopy using the fluorous effect

Single molecule spectroscopy enables unique insight into the analysis of biomolecular interactions. Underpinning its application across all facets of biomolecular analysis is the need to selectively immobilize a biomolecule of interest, and concomitantly, minimize non-specific binding of a myriad of possible biomolecules present. Here we describe the preparation and testing of new surfaces that, due to the fluorous effect, prevent any non-specific binding, what makes them perfect for single molecule microscopy. To check this antifouling behaviour we used a fairly new single molecule technique called Mass Photometry that allow us to assess the binding and unbinding of single molecules happening on the surface1. We tested an array of different biomolecules including single proteins, heterodimers and liposomes, demonstrating the high versatility of the surfaces. The behaviour observed indicates that these surfaces are ideal to avoid non-specific binding in single molecule studies.

Maria Kharlamova

Eberhard Karls Universität Tübingen/Max Planck Institute for Biology

A ring or not a ring? Looking into how RAD52 promotes homologous recombination

RAD52 is a single-strand annealing protein that participates in DNA double strand break repair via homologous recombination. The exact annealing mechanism is yet unknown. Truncated RAD52 forms undecameric rings and stacks thereof. The current model suggests that stacks of rings run the annealing process. This model utilizes crystallography studies of truncated RAD52 obtained at micromolar concentrations. However, various studies have shown that in vivo RAD52 concentrations are within the nanomolar range. Due to protein self-oligomerization, it is probable that the oligomeric state is highly dependent on the protein concentration and is significantly different at nanomolar and micromolar concentrations. At high concentrations, RAD52 inhibits DNA annealing. An additional question is whether this model is valid for the full-length RAD52. It is known that the full-length forms heptameric rings and its C-terminus is intrinsically disordered. Therefore, no crystal structures exist. Our mass photometry measurements show that the full-length RAD52 is not a heptamer, rather a set of rings varying in the number of subunits. Additionally, it is not prone to stacking as strongly as the truncated RAD52. Upon addition of ssDNA molecules, ring stacks of the truncated RAD52 dissociated. These findings question the current annealing model and give insights into alternative possibilities.

Dominik Saman

University of Oxford

Combination of mass photometry and native mass spectrometry reveals unbiased co-assembly in highly heterogeneous proteins

The small heat-shock proteins are a family of ATP-independent chaperones. Some of them have a highly polydisperse quaternary structure. Here, we combine mass photometry (MP) and native mass spectrometry (MS) to study the co-assemblies between the two most highly expressed, and polydisperse, human small heat-shock proteins, HspB1 and HspB5. First, we show how we can optimise the analysis of MP movies to obtain mass spectra of highly polydisperse systems. We then use higher resolution (but biased for lower masses) tandem MS results and combine them with MP mass envelopes (for which the higher mass envelopes are practically unbiased) to obtain detailed (full) mass envelopes of these mixtures that show hundreds of different sub-stoichiometries formed between the pair of proteins. We finally then extract energy differences between hetero- and homo- dimerisation of these two proteins to reveal that their co-assembly is practically unbiased for either interaction even after hundreds of millions of years after their corresponding gene duplication event.

Francesca Chandler

University of Leeds

First-in-class deubiquitylase inhibitors reveal new enzyme conformations

Cells maintain protein homeostasis by adding a small protein, ubiquitin, to regulate a variety of cellular processes, dictating protein activity, localisation or degradation. The addition of ubiquitin, known as ubiquitylation, is a reversible process making it a versatile post-translational modification aptly suited for cell signalling. Removal of ubiquitin is catalysed by deubiquitylating enzymes, commonly referred to as DUBs. BRCC36 isopeptidase complex (BRISC) is a multi-protein DUB complex which hydrolyses lysine-63-linked ubiquitin chains on Type I interferon receptors (IFNAR1/2), thus regulating interferon-dependent signalling. Therefore, BRISC-mediated inflammatory signalling amplification is a promising target for autoimmune disease drug development. We performed a high-throughput screen to identify small molecules which inhibit BRISC enzymatic activity. Employing an integrative structural biology approach (cryo-electron microscopy, mass photometry, native mass spectrometry, hydrogen-deuterium exchange mass spectrometry), complemented with biochemical assays, we have uncovered new enzyme conformations, revealing a remarkable mode of action for BRISC inhibitors. Exploring these key mechanisms will expand current knowledge of inflammatory signalling pathways and establish the use of DUB inhibitors as therapeutics to combat autoimmune disease and hyperactive cytokine signalling.

Sean Burnap

University of Oxford

The influence of stabilising mutations upon SARS-CoV-2 spike glycan processing, dynamics and function

The severe acute respiratory syndrome (SARS)-coronavirus (CoV)-2 is decorated with trimeric spike glycoproteins that mediate binding to host cells. The structural study of these spike complexes is hindered by proteolytic cleavage and inherent instability, resulting in the transition from a prefusion to the more stable postfusion state. The introduction of several proline substitutions within spike have enabled a dramatic stabilisation of the prefusion state, increasing protein yield, to act as a superior immunogen. Furthermore, the glycosylation state of spike greatly influences antibody recognition and is therefore of paramount importance for vaccine design. However, it remains unclear how stabilisation modulates glycan processing, and the extent to which these alterations influence spike function. Utilising mass photometry we reveal that the extent of proline substitutions used for stabilisation directly hinders the ability of SARS-CoV-2 spike to bind its host receptor angiotensin-converting enzyme 2 (ACE2), which was consistent among Wuhan and Omicron variants. Omicron spike had increased affinity for ACE2, with a greater propensity to bind three ACE2 monomers when compared to Wuhan spike. Glycomics and glycoproteomic analyses highlighted no major change in glycan processing upon spike stabilisation, suggesting that differences in ACE2 binding are not driven by alterations in glycan structure. Finally, the influence of temperature on the interaction between spike and ACE2 was explored. Spike protein pre-incubated at 37oC prior to ACE2 interaction showed reduced binding capacity when compared to spike that remained at 4oC. In conclusion, we reveal proline stabilisation and temperature to directly alter spike structural dynamics and ACE2 binding.