8th Symposium on Structural Proteomics

October 10th-12th in Berlin, Germany

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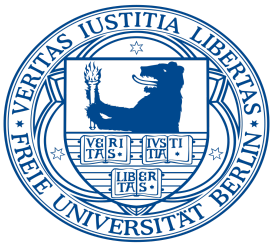
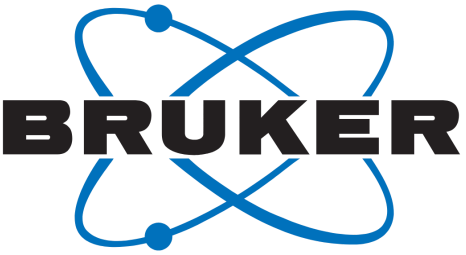




8th Symposium on Structural Proteomics (8SSP) in Berlin

October 10th-12th in Berlin, Germany

TU Berlin, Straße des 17. Juni 135, 10623 Berlin

Topics:

Cross-linking/MS
Surface labelling
Ion mobility/ native MS
Data-assisted modelling
Reporting standards
New chemistry, instruments, approaches
Software in structural proteomics

Organising Committee:

Juri Rappsilber, TU Berlin, Germany
Kevin Pagel, FU Berlin, Germany
Christoph Borchers, University of Victoria, Canada
Evgeniy Petrotchenko, University of Victoria, Canada

programme overview

October 10th, 2018 (Wednesday)

12:00	13:00	Registration
13:00	13:15	Welcome
13:15	14:00	Keynote: Martin Beck
14:00	14:30	Break for registration and Poster hanging
Session 1: Cross-linking/mass spectrometry proteome-wide		
14:30	15:00	Carla Schmidt
15:00	15:30	Richard Scheltema
15:30	16:00	Fan Liu
16:00	16:30	Francis O'Reilly
16:30	16:45	Sponsored talk: Thermo Fisher Scientific
16:45	17:15	Coffee
Session 2: Surface labelling		
17:15	17:45	David Schriemer
17:45	18:15	Lisa Jones
18:15	18:45	Kasper Rand
19:00	19:15	Poster Talk - Marie Barth
18:45	19:00	Poster Talk - Christian Stieger
19:15		Buffet Dinner

October 11th, 2018 (Thursday)

Session 3: Ion mobility/native MS		
9:00	9:30	Michal Sharon
9:30	10:00	Charlotte Uetrecht
10:00	10:10	Sponsored talk: MS Vision
10:10	10:20	Poster Talk - Claudio Iacobucci
10:20	10:50	Poster Session (even numbers)
Session 4: New Directions		
10:50	11:20	Ilaria Piazza
11:20	11:50	Andre Mateus
11:50	12:20	Henning Urlaub
12:20	12:50	Alfredo Castello
12:50	13:50	Lunch and posters (all numbers)
Session 5: Data-assisted modelling		
13:50	14:20	Daniel Saltzberg
14:20	14:50	Maya Topf
14:50	15:20	Panagiotis Kastritis
15:20	15:50	Jan Kosinski
15:50	16:20	Oliver Brock
16:20	17:00	Poster Session (odd numbers)
17:00	18:00	Standards (wwPDB: Vallat; proposal of reporting guidelines)
18:00	19:00	Meeting of the Society of Structural Proteomics
20:00		Dinner

October 12th, 2018 (Friday)

Session 6: Novel instrumentation		
9:00	9:15	Sponsored talk: Bruker
9:15	9:45	Petr Novak
9:45	10:15	Konstantinos Thalassinos
10:15	10:30	Poster Talk - Sarah Sipe
10:30	11:00	Coffee
11:00	11:15	Poster Talk - John Mark Skehel
11:15	11:30	Poster Talk - Therese Dau
11:30	11:45	Poster Talk - Nick Brodie
11:45	12:00	Poster Talk - Eugen Netz
12:00	12:15	Poster Talk - Andreas Linden
12:15	12:30	Poster Talk - Daniele Fabris
12:30	12:45	Poster Talk - Michael O. Glocker
12:45		**Closing Remarks**

information

conference website:
Is available via www.ssp2018.de.

attendance certificates:
Are available at the registration desk.

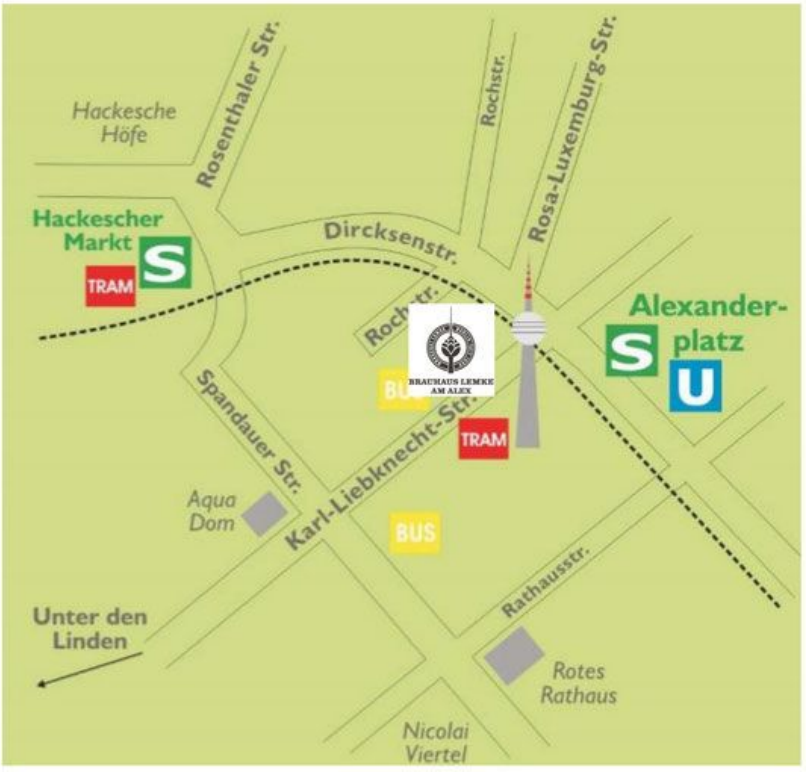
wlan:
Education Roaming (eduroam) is available as well as a personal login to the TUB-GUEST network.

conference schedule:
Can also be viewed in a google calendar:
https://calendar.google.com/calendar/ical/0rv0ukjivqq05b7j1rkpaik5rc%40group.calendar.google.com/public/basic.ics

posters:
Can be put up on Wednesday and collected after the conference is over. To find the right poster wall check the poster abstracts section or the quick reference page.

dinner:
The dinner will take place at Brauhaus Lemke which is located at Alexanderplatz. The easiest way to get to the dinner is by using Metro U2 direction S+U Pankow till Alexanderplatz and then to go on feet 400 meters till Karl-Liebknecht Str. 13, 10178 Berlin.

BRAUHAUS LEMKE
AM ALEX



Stadtbahn = S7, S5, S75 bis Alexanderplatz

Tram = M2, M4, M5, M6 bis S+U Bahnhof Alexanderplatz/Gontardstraße

programme abstracts

Keynote: Structural proteomics of protein complexes

Martin Beck
Structural and Computational Biology Unit, European Molecular Biology Laboratory

Session 1: Cross-linking/mass spectrometry proteome-wide

Heterogeneous protein interaction networks in synaptic vesicles mediate signal transduction in neurons

Carla Schmidt

HALOmem, Martin Luther University Halle-Wittenberg

Abstract:

Synaptic vesicles are storage organelles for neurotransmitters. They are densely packed with proteins and pass through a trafficking cycle in the nerve terminal. This includes neurotransmitter import, fusion with the presynaptic membrane, neurotransmitter release as well as vesicle recycling. These processes are governed by non-covalent protein interactions that assemble and dissociate on demand. Although the major components of synaptic vesicles are identified we have only little knowledge about their interactions. We set out to unravel these interactions by employing chemical cross-linking combined with mass spectrometry.

For this, synaptic vesicles were purified from rat brain and vesicle proteins were identified by LC-MS/MS. Proteins within synaptic vesicles were then chemically cross-linked using BS3 cross-linker, which specifically reacts with lysine side chains. We identified first interaction networks in synaptic vesicles which reveal many protein interactions with Synaptobrevin. To study this protein in detail we purified three variants, the full-length protein, the cytosolic domain as well as a shorter version. Combining chemical cross-lnking with native mass spectrometry and ion-mobility we found that Synaptobrevin oligomerises in solution presumably due to its disordered structure. We then targeted Synaptobrevin in its natural environment, the synaptic vesicle membrane. To unravel its role in complex formation, we followed three approaches: (i) we removed the cytosolic part of Synaptobrevin from the vesicle membrane by cleavage with Botulinum toxin, (ii) we fused synaptic vesicles with empty liposomes and thereby diluted the proteins in the vesicle membrane, and (iii) we incubated synaptic vesicles with the soluble DeltaN-SNARE complex to activate Synaptobrevin and, at the same time, engaged it in SNARE complex formation. We used chemical cross-linking to identify protein interactions under these conditions. Our results pave the way to unravel the heterogeneous protein interaction networks in synaptic vesicles which are key to our understanding of signal transduction in the neuronal synapse.

Fan Liu
FMP

Abstract:

Proteins are involved in almost all cellular processes. They are organized through extensive networks of interactions, regulating the working mechanisms of the cell with high fidelity and precision. However, characterization of the protein interactome has been an extremely challenging task due to the lack of highly confident and efficient technology. To address this issue, we describe a leap forward in cross-linking mass spectrometry demonstrating a new integrated workflow that robustly identifies cross-links from proteome samples. Our approach is based on the application of a cleavable cross-linker, sequential CID and ETD MS2 acquisitions of peptide fragmentation spectra of cross-linked peptides, and a dedicated search engine termed XlinkX. We applied this novel XL-MS strategy to several highly complex samples, including various cell lysates as well intact organelles such as the mitochondrion. The XL-MS-based method not only reveals protein partnerships systematically but also provides insights into subcellular protein localizations, protein conformations, and binding interfaces within protein complexes. These diverse capabilities were demonstrated in detail by our recent study on the protein interactome of mouse heart mitochondria.

In situ Structural Biology in Mycoplasma Pneumoniae using Cross-linking/Mass Spectrometry

Francis J. O'Reilly
Technische Universität Berlin, Germany,

Abstract:

Mycoplasma pneumoniae is a human pathogen that causes atypical bacterial pneumonia. Besides, it is known for its reduced genome, which makes it the simplest known self-replicating organism and as such, it is a prime candidate in the hope to model an entire cell. Up to now, a large amount of quantitative data has been collected on its genome, transcriptome, proteome and metabolism. Most information on the organisation of the proteome so far has been inferred from affinity-pulldown mass spectrometry working with cell lysates.

Here we present the first in-cell cross-linking mass spectrometry data for this organism, which provides unprecedented detail on the organisation of the proteome in situ. Addition of a cross-linking reagent covalently links proximal residues within the cell's proteins which are subsequently identified by mass spectrometry. These identified cross-links provide distance restraints that allow us to structurally model proteins and their interactions. This is the most comprehensive cross-linking data set ever reported with cross-links identified across 98% of the expressed proteome. The identification of higher-order complexes linking different cellular processes and the modelling of their interaction interfaces will be discussed.

Concepts and strategies for improving the performance of labeling methods in MS-based structure/function analysis of proteins

Dr. David C. Schriemer
Dept. of Biochemistry & Molecular Biology, University of Calgary, Calgary Canada

Abstract:

Integrative methods in structural biology use data from various sources to generate accurate models of large multi-protein assemblies. Mass spectrometry has enormous potential to serve as a key provider of structural restraints, and push structural biology towards a cell-based activity. In our lab, we develop chemical labeling methods (H/D exchange, covalent labeling and crosslinking) that anticipate structural biology at this scale, and we investigate ways in which the data can be used for assembling complex structures. In this presentation, I will present the key concepts behind our reagent development program, and highlight recent accomplishments in covalent labeling in particular. I will pay particular attention to what we are learning about photolytic labeling methods, and how to design and use a better "protein paint" for topographical analysis. I will also discuss computational challenges arising from mining large sets of "aggressively-modified" protein sequences that we encounter when using H/D exchange, covalent labeling and crosslinking methods. A significant new update to the Mass Spec Studio (www.msstudio.ca) will be presented that provides freely available plug-ins for all of these methods in one framework.

Lisa Jones
Department of Pharmaceutical Sciences, University of Maryland, BALTIMORE, United States

Abstract:

In recent years, protein footprinting coupled with mass spectrometry has become a valuable tool for studying protein structure. One such technique, fast photochemical oxidation of proteins (FPOP), utilizes hydroxy radicals to oxidatively modify solvent accessible amino acids in proteins. Hydroxyl radicals are generated via photolysis of hydrogen peroxide by an excimer laser at 248 nm. We have extended the use of FPOP for in-cell labeling. In-cell FPOP (IC-FPOP) allows for the analysis of protein structure directly in the cellular environment. IC-FPOP can oxidatively modify over 1300 proteins within the cell. The labeling of multiple proteins allows for proteome wide structural biology. We have used IC-FPOP for proteome wide structural biology to study the effects of the anti-cancer drug Gleevec on triple negative breast cancer cells. By comparing the oxidative labeling pattern of cells treated with Gleevec and non-treated cells, we were able to determine on and off targets of the drug. In addition, we saw differences in drug interactions between breast cancer cells from women of European ancestry and women of African ancestry highlighting the differences in drug efficacy between the two populations.

Analysis of the dynamics and ligand-binding of membrane transporters by HDX-MS

Kasper Rand
Department of Pharmacy, University of Copenhagen

Abstract:

The talk will highlight some of our recent work in which we apply HDX-MS to better understand the dynamic conformation and ligand binding of neurotransmitter:sodium symporter proteins. This class of membrane transporters are critical for regulation of neurotransmission and represent attractive drug targets yet insufficient information is available about their conformational dynamics during substrate transport and drug binding.

Chemical modification of proteins for structure elucidation

Marie Barth[1], Julian Bender[1], Andy Lau[2] , Argyris Politis[2], Carla Schmidt[1]

(1) Interdisciplinary Research Center HALOmem, Charles Tanford Protein Center, Martin Luther University Halle-Wittenberg, Halle/Saale, Germany

(2) Department of Chemistry, Kings College London, London, United Kingdom

Abstract:

Structural mass spectrometry (MS) includes various techniques, for instance covalent labelling or cross-linking. Covalent labelling is used to identify individual residues on the surface of proteins or protein-ligand complexes and cross-linking provides insights into direct contact sites of proteins in protein assemblies.

We used yeast alcohol dehydrogenase (ADH) and rabbit pyruvate kinase (PK) as model protein complexes to establish a (quantitative) protein labelling workflow for investigating protein dynamics under different conditions. Labelling was performed with (i) N-Succinimidyl Acetate (NHS-Ac), which labels lysine, tyrosine, serine and threonine, and (ii) diethyl pyrocarbonate (DEPC), which labels histidine, lysine, tyrosine, serine, threonine and cysteine. After chemical labelling, the protein was digested with trypsin and generated peptides were analysed by tandem-LC-MS. Obtained raw data were analysed using MaxQuant software to identify and quantify modified residues. Individual modified residues were visualised in the available crystal structures. We found that these are mostly localised in the solvent accessible surface area and, therefore, NHS-Ac and DEPC represent promising labelling agents for structure analysis. Quantification of labelling sites deduced from proteins under different conditions will highlight structural changes of the proteins. Currently, we are extending our labelling workflow with other labelling reagents targeting different amino acid residues. We further plan to integrate both chemical labelling and cross-linking into computational modelling approaches.

Stepped-Collision Energy HCD improves MS2-based identification of DSSO Cross-Linked Peptides

Christian E. Stieger[†], Philipp Doppler[†], Karl Mechtler[†¶§*]

† Institute of Molecular Pathology (IMP), Vienna BioCenter (VBC), Vienna, Austria ¶ Institute of Molecular Biotechnology, Austrian Academy of Sciences, Vienna BioCenter (VBC), Vienna, Austria § Gregor Mendel Institute (GMI), Austrian Academy of Sciences, Vienna BioCenter (VBC), Vienna

Abstract:

Cross-linking mass spectrometry (XLMS) is becoming increasingly popular, and current advances are widening the applica-bility of the technique so that it can be utilized by non-specialist laboratories. Specifically, the use of novel mass spectrome-try-cleavable (MS-cleavable) reagents dramatically reduce complexity of the data by providing i) characteristic reporter ions and ii) the mass of the individual peptides, rather than that of the cross-linked moiety. However, optimum acquisition strat-egies to obtain the best quality data for such cross-linkers with HCD (high energy collisional induced dissociation) alone is yet to be achieved. Therefore, we have carried out careful optimization of MS parameters to facilitate the identification of disuccinimidyl sulfoxide (DSSO)- based cross-links (XLs) on HCD-equipped mass spectrometers. From the comparison of 9 different fragmentation energies we developed several stepped- HCD fragmentation methods that were evaluated on a variety of cross-linked standard proteins. The optimal stepped-HCD-method was then directly compared with previously described methods using an Orbitrap Fusion Lumos instrument using a high-complexity sample. The final results indicate that our stepped-HCD method is able to identify more XLs than other methods, mitigating the need for MS3 enabled in-strumentation and alternative dissociation techniques.

Session 3: Ion mobility/native MS

Rapid Characterization of Secreted Recombinant Proteins

Gili Ben-Nissan, Shay Vimer, Jelena Cveticanin and Michal Sharon
Department of Biomolecular Sciences, Weizmann Institute of Science, Rehovot 7610001, Israel

Abstract:

Characterization of overexpressed proteins is essential for assessing their quality, and providing input for iterative redesign and optimization. This process is typically carried out following purification procedures, which are costly and time-consuming. In my talk, I will describe a native mass spectrometry method that enables characterization of recombinant proteins directly from culture media. Properties such as solubility, molecular weight, folding, assembly state, overall structure, post-translational modification and ability to bind relevant biomolecules can be immediately revealed. I will show the applicability of the method for in-depth characterization of secreted recombinant proteins from eukaryotic host systems such as yeast, insect, and human cells. This method, which can be readily extended to high-throughput analysis, considerably shortens the time gap between protein production and characterization, and is particularly suitable for characterizing engineered and mutated proteins, and optimizing the yield and quality of overexpressed proteins.

Hitting proteins with a sledgehammer - structural characterization with X-rays

Charlotte Uetrecht
Heinrich Pette Institute, Leibniz Institute for Experimental Virology and European XFEL GmbH

Abstract:

Native ion mobility mass spectrometry (MS) is a perfect tool to study protein complexes in a mass and conformation specific manner. Despite remarkable sensitivity and selectivity the structural resolution is limited in native MS. On the other hand, it allows monitoring structural transitions, which proteins and protein complexes undergo, e.g. during the viral lifecycle. However, such transient states cannot be purified and are inaccessible for crystallography.
The European XFEL, the world's most intense hard X-ray free-electron laser (XFEL), has just become operational and offers an opportunity to obtain high resolution structures of single particles. The benefits of native MS for single particle imaging of transient intermediates at European XFEL and initial feasibility studies on achievable ion flux will be presented.
Furthermore, we have tested native MS in conjunction with the soft XFEL FLASH and PETRA P04 soft X-ray synchrotron radiation. Our recent results show potential for soft X-rays protein complex fragmentation and dissociation for structural proteomics. The available intensity could alleviate proposed upper size limits for UV photo dissociation of protein complexes.

Turning Synapts into versatile platforms for structural analysis of large biomolecules

MS Vision

Abstract:

In close cooperation with various academic groups, MS Vision has developed a powerful ensemble of modifications for native MS of biomolecular compounds and complexes. Transferring the knowledge obtained to the Synapt generation of instruments seemed straightforward initially, however it wasn't long before users came back with observations that sent us back to the drawing board. In this talk we present two examples of analytical challenges that we were asked to solve, and how we managed to do so.

A Cross-linking/Mass Spectrometry Workflow Based on MS-Cleavable Cross-Linkers and the MeroX Software for Mapping Protein-Protein Interactions

Claudio Iacobucci, Michael Götze, Christian Ihling, Andrea Sinz

† Institute of Molecular Pathology (IMP), Vienna BioCenter (VBC), Vienna, Austria ¶ Institute of Molecular Biotechnology, Austrian Academy of Sciences, Vienna BioCenter (VBC), Vienna, Austria § Gregor Mendel Institute (GMI), Austrian Academy of Sciences, Vienna BioCenter (VBC), Vienna

Abstract:

Introduction: Mass spectrometry (MS)-cleavable cross-linkers are moving into the focus of the cross-linking/MS approach for studying 3D-structure of proteins and protein assemblies. They undergo specific fragmentations under collisional activation conditions generating characteristic product ion patterns. They have an enormous potential for a fast and reliable identification of cross-linked peptides even from highly complex samples, e.g. whole cells and organisms.

Methods: We developed a robust and widely applicable workflow that allows a facile identification of cross-links for deriving spatial constraints from proteins and protein complexes. Our protocol combines the synthesis of novel cross-linkers, protein cross-linking, bottom-up proteomics analysis, and data analysis based on the in-house MeroX software [1]. Two orbitrap mass spectrometers, Orbitrap Fusion Tribrid and Q-Exactive Plus, were employed.

Results: We designed and synthesized four novel MS-cleavable cross-linkers. 2,2'-Azobis(2-methylpropanimidate) is an innovative cross-linking principle that after collisional acitvation induces free radical-initiated sequencing (FRIPS) of connected peptides in positive ionization mode [2,3]. 1,1-carbonyldiimidazole is the first zero-length MS-cleavable linker for nucleophilic groups (amines and hydroxyls) in proteins [4]. 3,4-Diallylurea is a novel photo-activatable, MS-cleavable reagent to selectively target cysteines [5]. All cross-linkers were investigated to study several protein systems, such as the tumor suppressor p53, which is an intrinsically disordered protein, and the whole E. coli ribosome. We are currently extending our cross-linking/MS workflow to the in-vivo analysis of protein-protein interactions, exemplified for Drosophila embryos.

Conclusions: Our integrated cross-linking workflow allows to map protein 3D-structures and protein-protein interactions in-vitro and in-cell. We have synthesized innovative, MS-cleavable cross-linking principles that target different functional groups in proteins, such as amines, hydroxyls, thiols or react non-specifically with all 20 proteinogenic amino acids. The MeroX software is able to analyze cross-linked products in an automated fashion.

Novel Aspect: We present an integrated workflow based on novel MS-cleavable cross-linking principles and a fully automated analysis of cross-linked products by the MeroX software.

References.

1. Götze M., Pettelkau J., Fritzsche R., Ihling C. H., SchÃ¤fer M., Sinz A. Journal of The American Society for Mass Spectrometry, 26, 83-97 (2015).
2. Iacobucci C., Hage C., Schäfer M., Sinz A., Journal of The American Society for Mass Spectrometry, 28, 2039-2053 (2017).
3. Iacobucci C., Schäfer M., Sinz A., Mass Spectrometry Reviews, DOI: 10.1002/mas.21568 (2018).
4. Hage C., Iacobucci C., Rehkamp A., Arlt C., Sinz A. (2017). Angewandte Chemie International Edition, 56, 14551-14555 (2017).
5. Iacobucci C., Piotrowski C., Rehkamp A., Ihling C. H., Sinz A., Journal of The American Society for Mass Spectrometry, DOI: 10.1007/s13361-018-1952-8 (2018).

Session 4: New Directions

A systematic map of protein-metabolite interactions reveals principles of chemical communication

Ilaria Piazza
Institute of Molecular Systems Biology, Department of Biology, ETH Zurich (ETHZ), Zurich, Switzerland

Abstract:

Metabolite-protein interactions control a variety of cellular processes, thereby playing a major role in maintaining cellular homeostasis. Different types of functional interactions between proteins and metabolites have been reported and involve binding of metabolites to the active site of enzymes as substrates, cofactors, or products of enzymatic reactions. Metabolites comprise the largest fraction of molecules in cells, but our knowledge of the metabolite-protein interactome lags behind our understanding of protein-protein or protein-DNA interactomes, since most characterized protein- metabolite interactions have been discovered via hypothesis-driven experiments that rely on in vitro activity assays.

We devised LiP-SMap a proteomic workflow for the systematic identification of metabolite protein- interactions directly in their native environment. LiP-SMap combines limited proteolysis (LiP) with DIA (Data Independent Acquisition) mass spectrometry in the presence of unmodified metabolites to enable a systematic analysis, unbiased with regard to both metabolites and proteins. With LiP-SMap we identified a network of known and novel interactions and binding sites in Escherichia coli, and we demonstrated the functional relevance of a number of newly identified interactions. Our data enabled identification of new enzyme-substrate relationships and cases of metabolite-induced remodeling of protein complexes. Our metabolite-protein interactome consists of 1678 interactions and 7345 putative binding sites. Moreover the dataset reveals functional and structural principles of chemical communication, shed light on the prevalence and mechanisms of enzyme promiscuity, and enable extraction of quantitative parameters of metabolite binding on a proteome-wide scale. We will further present our latest applications for the study of protein-ligand interactomes and drug target deconvolution on a cell-wide scale.

Piazza, I., Kochanowski, K., Cappelletti, V., Fuhrer, T., Noor, E., Sauer, U., and Picotti, P. (2018). A Map of Protein-Metabolite Interactions Reveals Principles of Chemical Communication. Cell 172, 358–372.e23.

Probing protein state in bacteria by thermal proteome profiling

Mateus Andre, Bobonis J, Kurzawa N, Stein F, Helm D, Hevler J, Typas A, Savitski MM.
Genome Biology, EMBL Heidelberg

Abstract:

New technologies for studying microbes and antimicrobial mechanism of action are currently warranted due to the rise of antibiotic resistance. Thermal proteome profiling (TPP) is based on the principle that proteins become more resistant to heat-induced unfolding when bound to a ligand. Here, we used TPP in Escherichia coli to study the in vivo thermostability of its proteome, and to assess how genetic and chemical perturbations affect it.

We found that the E. coli proteome was more thermostable than the human one, which is consistent with the ability of this organism to grow at higher temperatures. Interestingly, protein thermal stability depended on subcellular location—forming a high-to-low gradient from the cell surface to the cytoplasm. Further, the subunits of protein complexes located in a single subcellular compartment (e.g., cytosol, inner membrane, or periplasm) melted in a similar manner, while protein complexes spanning multiple compartments had their subunits melting in a location-wise manner. This indicates that proteins thermally stabilize each other, but that the intrinsic stabilization conferred by protein localization (coded in the sequence) overrides this behavior. In agreement, knocking-out one of the members of a complex led to the destabilization of the remainder of the complex. Finally, by combining TPP in vitro and in vivo, we correctly identified targets of known antimicrobial drugs, the downstream effects of their inhibition, and possible resistance mechanisms.
In conclusion, TPP provides a novel way of systematically phenotyping the cell. This platform can be used to improve our understanding of basic bacterial biology by gaining insights into protein structure, protein complex formation, metabolic activity, and drug-protein interactions.

Multiple ways to cross-link proteins to RNA (and DNA)

Henning Urlaub
Max Planck Institute for Biophysical Chemistry, Göttingen, Germany; University Medical Center Göttingen, Germany

Abstract:

Protein-RNA and DNA interactions play a pivotal role in almost all cellular processes. Currently, much attention is devoted not only to the identification of proteins that bind to RNA and DNA, but also to the definition of the exact RNA/DNA interaction sites. Protein-nucleic acid cross-linking in combination with mass spectrometry (MS) has matured into a valuable tool that meets both requirements. There are general approaches: cross-linking can be performed photochemically using UV irradiation, resulting in a “zero length” cross-linking event where the interacting moieties directly form a covalent bond. Alternatively, the cross-link may be achieved by means of a chemical cross-linking reagent. Both approaches yield partially overlapping, but also complementary results depending on the availability of cross-linkable moieties. Here, I will summarize our recent developments using both photochemical and chemical cross-linking approaches, and exemplify our efforts by a range of examples from different highly relevant biomolecular complexes.

Alfredo Castello Palomares
Department of Biochemistry, University of Oxford, Oxford, United Kingdom

Abstract:

The compendium of RNA-binding proteins (RBPome) has been greatly expanded by the development of RNA-interactome capture (RNA-IC) [1, 2, 3]. However, it remains unknown how responsive is the RBPome and whether these responses are biologically relevant. To answer these questions, we created "comparative RNA-IC" to analyse RBP dynamics in cells challenged with an RNA virus, named sindbis (SINV). Strikingly, SINV infection regulated the activity of 245 RBPs, many of which discovered for the first time by RNA-IC. Mechanistically, alterations in RNA binding in infected cells are caused by changes in the subcellular localisation of RBPs and RNA availability. Importantly, "RBPome" responses are essential, as perturbation of dynamic RBPs modulates the capacity of the virus to infect the cell. For example, ablation of XRN1 causes cells to be refractory to infection, while GEMIN5 moonlights as a novel antiviral factor. Therefore, RBPome remodelling provides a mechanism by which cells can extensively rewire gene expression in response to physiological cues.

[1] Castello et al., 2012 Cell
[2] Castello et al., 2013 Nature Protocols
[3] Baltz et al., 2012 Mol Cell

Session 5: Data-assisted modelling

Integrative Modeling of Protein Perturbation.

Daniel Saltzberg[1], Seth Axen[1], Ben Webb [1], Howard Broughton[2], Patrick Griffin[3], Andrej Sali[1]

1 Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco; 2 The Eli Lilly Company; 3 Department of Molecular Medicine Scripps, Florida

Abstract:

Characterization of sub-cellular biological systems generally require observation by indirect biophysical methods that report parameters such as the magnetic spin of a particular nucleus, scattering at a certain angle or the mass of a protein fragment. To answer our biological questions, we somehow need to make sense of these sparse and disparate signals to construct a reasonable interpretation of what the actual system is. Integrative modeling is a method for precisely this, where modeling is cast as a computational optimization problem, for which all information about the system is encoded into a scoring function that evaluates candidate models. I will describe the process of integrative modeling, from developing the representation of the model, through encoding experimental information, sampling model configurations, validating and reporting models. We'll discuss this through the lens of developing a model for characterizing ligand and variant-induced perturbations in macromolecules using hydrogen exchange mass spectrometry (HDX-MS) data and other experimental information.

Modeling Protein Monomers and Complexes Using Restraints from Crosslinking Mass Spectrometry

Joshua ML Bullock, Konstantinos Thalassinos, Maya Topf

ISMB, Birkbeck and UCL, University of London

Abstract:

Crosslinking mass spectrometry (XL-MS) is becoming an increasingly popular technique for modeling protein monomers and complexes. The distance restraints gathered from these experiments can be used alone or as part of an integrative modeling approach, incorporating data from many sources. Recently, we identified that a combination of three different features inherent in crosslinking data can used successfully in the modeling process: expected distance between crosslinked residues; violation of the crosslinker's maximum bound; and solvent accessibility of crosslinked residues. Based on this, we have developed two scoring functions, MNXL [1] and cMNXL [2], for the modeling of protein monomers and complexes, respectively. We demonstrated that the new scores outperform the more commonly-used crosslink distance violation metrics. We also created a score that incorporates information from both 3D electron microscopy maps and XL-MS, which achieves, on average, better results than either information type alone.

References:
1. Matthew Allen Bullock J. Mol Cell Proteomics. 2016, 15(7):2491-500.
2. Bullock JMA, et al, Structure 2018, 26(7):1015-1024

Structural and computational biology of cellular homogenates

Jun.-Prof. Dr. Panagiotis L. Kastritis
Interdisciplinary Research Center HALOmem, Martin-Luther University Halle-Wittenberg,
Kurt-Mothes-Strasse 3a, Halle (Saale) 06120, Germany

Abstract:

Assemblies of macromolecular complexes participating in a specific cellular pathway are fundamental to cellular function. However, their sheer size, transient nature and intrinsic flexibility have prohibited biochemical and structural characterization on a systems-wide scale. I will present an integrative structural and proteomics approach that succeeds in recapitulating high-resolution features of transient assemblies directly from complex native cellular extracts. I will focus on a previously uncharacterized assembly intermediate in fatty acid synthesis.

keywords: cryo-EM, systems biology, protein-protein interaction networks, flexible docking

Integrative structure modeling based on crosslinking-MS and electron microscopy data

Jan Kosinski
European Molecular Biology Laboratory (EMBL), Center for Structural Systems Biology (CSSB), Hamburg

Abstract:

Crosslinking-mass spectrometry (XL-MS) remains a vital method for determining structures of protein complexes. Despite the resolution revolution in cryo-electron microscopy (cryo-EM), which enables solving structures at near-atomic resolution, many complexes still evade high resolution due to flexibility or problems with sample preparation. Moreover, structures from in situ electron tomography (cryo-ET) most of a time are limited to low resolution. XL-MS can provide complementary restraints that often enable interpreting such low-resolution EM maps, especially when combined with computational modeling.
During my talk, I will present our methodology for integrative modeling of protein complexes based on XL-MS and EM data. I will show how using this methodology we modeled two eukaryotic complexes: a 110 MDa human nuclear pore and 1 MDa Elongator based on 20-30 Å cryo-ET and negative stain EM maps and crosslinks. The NPC model shows how the entire scaffold of the NPC is organized. In particular, it reveals that seemingly very complex structure of the NPC is built from reoccurring interaction motifs and simple architectural principles. For the Elongator complex, I will show whether our published model agrees with the recently obtained high-resolution cryo-EM structure.

Where Data Live

Oliver Brock
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Abstract:

Integrative modeling has become a widely accepted necessity. But the integration of different sources of biological data is a challenge we are just beginning to understand. How can we most effectively integrate data? The most evident answer is: Bayes' theorem. And that is a great start. But just as this answer is somewhat obvious, so is the realization that much more remains to be discovered in data integration. In this talk, I present an algorithmic and a representational idea, both quite complimentary to Bayes' theorem, for making the most of data. The goal of these ideas is to maximally leverage data in conjunction our knowledge about it and our knowledge about the biological problem so as to most effectively integrate protein modeling data.

Session 6: Novel instrumentation

Ghazaleh Yassaghi[1]; Zdeněk Kukačka[1]; Petr Pompach[1,2], Dan Fabris[1] and Petr Novák[1,2]
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Abstract:

A common feature of covalent labeling techniques used in conjunction with mass spectrometry (MS) is the formation of novel covalent bonds upon treatment of the target biomolecule with a modifying chemical or photochemical agent. These experiments assume that the degree of exposure to the solvent correlates with the reactivity of each target site on the biomolecule's surface. Several different types of chemical probes have been developed to target the polypeptidic backbone (hydrogen/ deuterium exchange), or amino acid side chains (chemical probes, hydroxyl radicals) of protein substrates. The outcome of these experiments is the identification of solvent accessible and/or reactive areas of a protein under the selected conditions, which enables one to monitor the structure dynamics in solution and the effects of non-covalent ligand interactions. Therefore, the combination of covalent labeling and mass spectrometry contribute significantly to the understanding of protein function and activity.

Although covalent labeling approaches were introduced two decades ago, the leading methods for identifying modified residues still rely on tryptic digestion of labeled proteins, followed by data-dependent LC-MS/MS analysis (bottom-up approach). However, it has been reported that the rather harsh environment present during chemical derivatization may introduce over-modification, protein degradation, and unwanted artifacts. As a result, heavily modified proteins may lose their native conformation, thus leading to incorrect interpretation of structural variations produced by dynamics or ligand binding. The ability to obtain a valid snapshot of the native protein structure is greatly enhanced by performing probing experiments under conditions that induce just one modification. In a typical bottom-up approach, identifying may require tedious and time-consuming iterations. A valid alternative consist of performing the analysis of probed material according to a top-down approach, in which protease digestion and mass mapping steps are replaced by direct tandem mass spectrometry. The top-down approach has been successfully utilized for protein sequencing, identification of post-translational modifications, and localization of intra-molecular cross-links. This talk will provide examples of the application of top-down techniques to the analysis of proteins treated with oxidizing agents. The talk will illustrate the pros and cons of this approach and discuss its potential in structural proteomics projects.

This work was supported by the Czech Science Foundation (grant numbers 16-24309S), the Ministry of Education of the Czech Republic (projects LH15010, LD15089; program "NPU II" project LQ1604; LM2015043 CIISB for CMS BIOCEV; LTC17065), COST Action (BM1403), European Regional Development Funds (CZ.1.05/1.1.00/02.0109 BIOCEV), and European Commission H2020 (European Network of Fourier-Transform Ion-Cyclotron-Resonance Mass Spectrometry Centers - project agreement No.731077).

XLIM-MS: Development of a Novel Approach to Crosslinking Mass Spectrometry

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Abstract:

Crosslinking Mass Spectrometry (XLMS) has emerged as an important technique for the structural elucidation of proteins that cannot be resolved by traditional methods. XLMS provides a set of distance restraints that can inform and guide structural modelling approaches.
The increased size and charge of crosslinked peptides compared to their un-crosslinked counterparts makes them ideal candidates for separation by Ion Mobility mass spectrometry. This extra degree of separation provides high resolution data for both precursor and fragment ions. QToF mass spectrometers offer higher resolution fragment ion spectra. In addition, longer scan times allow more of the ions to be used, improving sensitivity.
Bovine serum albumin was crosslinked with BS3 (d0 and d12 and versions). Samples were tryptically digested and enriched for crosslinked peptides via size exclusion chromatography performed on a AKTAmicro chromatography system. Samples were introduced via nano Electrospray Ionisation into a Synapt G2Si (Waters) Travelling Wave Ion Mobility Mass Spectrometer. Data were processed by a combination of in-house Python scripts and xQuest.
Here we present the initial development of Crosslinking Ion Mobility Mass Spectrometry (XLIM-MS). This method seamlessly integrates Ion Mobility separation into crosslink analysis using a QToF mass spectrometer. We show that crosslink identification rates from QToF data compares favourably with Orbitrap data. We also present further optimisations to enhance crosslink identifications using experimental methodology unique to the Synapt G2Si mass spectrometer.

Impact of Ligand Binding on Photodissociation of Protein Copmlexes

Sarah Sipe, Jennifer S. Brodbelt
The University of Texas at Austin

Abstract:

The ability of electrospray ionization to maintain non-covalent interactions as a protein is lifted from solution to the gas phase has advanced mass spectrometric studies of protein-protein and protein-ligand complexes. Many activation methods have been utilized to investigate dissociation patterns of these complex systems as it relates to their biological behavior. Ultraviolet photodissociation (UVPD) with 193 nm photons has demonstrated the capability of generating significant sequence ions directly from native protein complexes as well as fragment ions that maintain ligands or cofactors (i.e. holo-fragments). These attributes of UVPD have motivated our interest in evaluating its effectiveness for obtaining structural information of ligand-bound protein complexes. Here, UVPD and HCD are utilized to characterize oligomeric protein-ligand complexes using a prototype Thermo Q Exactive Plus Orbitrap instrument that was custom-modified to enable an ultra-high mass range (UHMR). The stabilizing or destabilizing effect of ligand binding was investigated for three model protein tetramers. Results obtained using UVPD demonstrate the increased characterization of model complexes that allow for inference of structural changes upon ligand binding and active site localization due to enhanced sequence coverage and distribution of holo-fragments.

Mechanism of Parkin activation by PINK1.

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Abstract:

Mutations in the E3 ubiquitin ligase parkin (PARK2) and the protein kinase PINK1 (PARK6) are linked to autosomal-recessive juvenile parkinsonism[1,2]. At the cellular level, these mutations cause defects in mitophagy, the process that organizes the destruction of damaged mitochondria[3,4]. Parkin is autoinhibited, and requires activation by PINK1, which phosphorylates Ser65 in ubiquitin and in the parkin ubiquitin-like (Ubl) domain. Parkin binds phospho-ubiquitin, which enables efficient parkin phosphorylation; however, the enzyme remains autoinhibited with an inaccessible active site[5,6]. It is unclear how phosphorylation of parkin activates the molecule. Here we follow the activation of full-length human parkin by hydrogen-deuterium exchange mass spectrometry, and reveal large-scale domain rearrangement during the activation process. Our data show how autoinhibition in parkin is resolved, and suggest a mechanism for how parkin ubiquitinates its substrates via an untethered RING2 domain.

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Sequential digestion with trypsin and elastase in cross-linking/mass spectrometry

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Abstract:

In cross-linking/mass spectrometry, protein proximities and conformations are preserved through cross-linker and translated into distance constraints. Using only trypsin is often insufficient, as the amino acid composition of some protein regions is incompatible with trypsin digestion. Several complementary proteases like AspN, GluC and chymotrypsin have been successfully used in combination with cross-linking/mass spectrometry. Still, some protein regions remain elusive and can only be analysed with broader specific proteases like elastase.

In this study, we show that the activity of elastase is not only dependent on the targeted amino acid, but also on substrate length. We exploit this property of elastase activity through the sequential digestion using trypsin prior to elastase. The cleavage by trypsin reduces the complexity typically associated with elastase digestion. Furthermore, it introduces peptides with a positive C-terminal charge. As a result, we detect cross-links in the previously inaccessible N-terminal region of TAF4.

Nicholas I. Brodie[1,2], Konstantin I. Popov[3], Evgeniy V. Petrotchenko[1], Andrew G. Cairns[4], Fredrik Almqvist[4], Nikolay V. Dokoholyan[3], Christoph H. Borchers[1,2,5,6]

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Abstract:

Parkinson's disease is characterized by the death of dopaminergic neurons in the substantia nigra of the brain caused by an oligomeric species of the protein α-synuclein. The early-forming oligomer of this protein has thus far been difficult to fully characterize. Using short distance crosslinking constraints we have obtained using a $^{14}N/^{15}N$ crosslinking strategy in discrete molecular dynamics simulations, we will determine a structure of the early synuclein oligomer generated by FN075. This structure will be validated using other structural proteomics techniques, including surface modification, HDX and affinity labelling. Analysis of the EDC crosslinking data yielded a total of 19 non-redundant zero-length distance constraints. 4 constraints were interprotein, and 15 were intraprotein constraints which were identified by the $^{14}N/^{15}N$ labelling strategy. These zero-length crosslinks, in addition to those from the DSG and SDA crosslinking experiments, are the most relevant to modelling, and are used to generate a structure both for the individual monomers within the oligomer and of the overall structure of the multimeric oligomer complex. The interprotein crosslinks found using EDC indicate an inter-molecular interaction between the N-terminus of one monomer of the oligomer with the C-terminus of an adjacent monomer. These interactions may indicate an alternating head-to-tail arrangement of monomers within the oligomers. Additionally, many of the intraprotein crosslinks found with EDC were different than those found previously on the native synuclein protein, indicating a change in structure between the two forms. Using these crosslinking data, a synuclein oligomer structure will be determined using crosslinking-constraint driven discrete molecular dynamics simulations (CL-DMD). This model will be verified by using a combination of HDX, surface modification and additional long-distance crosslinking data.

OpenPepXL: a versatile and sensitive XL-MS identification tool

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Abstract:

Introduction: Cross-linking coupled with mass spectrometry (XL-MS) has been recognized as an effective source of information about protein structures and interactions. Many methods and tools have been developed and reported for XL-MS identification through the last decade. Every tool applies different heuristics to cope with the quadratic search space inherent in XL-MS data analysis and uses their own model to estimate the False Discovery Rate (FDR). As part of the release of OpenMS 2.4 we introduce version 1.0 of the tool OpenPepXL and compare it to other commonly used tools for identification of non-cleavable cross-linkers on a diverse set of XL-MS experiments.

Methods: OpenProXL is a protein-protein cross-linking identification tool implemented using the open-source and well-documented OpenMS library (Röst et al. 2016). Like xQuest (Rinner et al., 2008) it can make use of labeled linkers to denoise spectra by comparing the spectra containing the light and heavy linkers. It can be used efficiently without any heuristics to reduce the search space for small datasets and has an optional fast pre-scoring algorithm to make analysis of large datasets feasible. OpenPepXL is part of the OpenMS proteomics pipeline that includes tools for labeled and label-free quantification. It can be installed on Windows, OSX and Linux and is compatible with most computing clusters and cloud services for large scale data analysis.

Results: “Kojak”, “pLink 2”, “StavroX”, “XiSearch”, “xQuest” and our own tool “OpenPepXL” were compared on several datasets from different laboratories on the same computational setup. Some of these datasets were from XL-MS experiments with proteins or protein complexes with known structures and were used for structural validation of the results. We found that the overlap of identified cross-links among the compared tools with an FDR of 5% is less than 50% for some datasets, but it increases with more stringent FDR cutoffs. A consensus approach using multiple tools could be promising in extracting a set of very confident identifications. We also found that it can pay off to analyze datasets without using heuristics to reduce the search space, if the dataset is small enough to make such an analysis feasible with the available computing power. OpenPepXL makes an exhaustive analysis more feasible for larger datasets through its efficient implementation and by being compatible with computing clusters and cloud services. Without using heuristics to filter out spectra and cross-link candidates before a full scoring, OpenPepXL reports about 10% more cross-links than the other tools without losing specificity.

Conclusion: OpenPepXL is an efficient and versatile XL-MS search engine with broad applicability and better sensitivity than other available tools.

The mitochondrial interactome in baker's yeast: a snapshot taken by cross-linking mass spectrometry

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Abstract:

Protein-protein interactions (PPIs) play a crucial role in living cells for signal transduction, protein transport and metabolism. For the investigation of PPIs, chemical cross-linking combined with mass spectrometric analyses (XL-MS) emerged as a powerful approach in the recent years. Here, a reactive ingredient, the cross-linker, covalently binds two amino acid residues that are in close proximity to each other. The distance constraint imposed by the length of the used cross-linker gives evidence for interactions between proteins and supports structural analysis of a protein on a low-resolution scale.

Mitochondria are known as the "powerhouses" in most of the eukaryotic cells. The electron transport chain (ETC) within mitochondria is a major pathway for ATP production and forms supercomplexes for more effective electron shuttling. By cross-linking mitochondria isolated from Saccharomyces cerevisiae grown under different conditions, we would like to dig deeper into the interaction partners of the ETC and other protein complexes. The mitochondria from baker's yeast grown on glycerol, a non-fermentable carbon source, show different interaction patterns as those grown on glucose, a fermentable carbon source. Especially, the interactions of proteins located in the inner membrane seem to be affected. Our goal is to shed light on structural organization of mitochondrial proteins under different growing conditions, eventually also in a quantitative manner.

Daniele Fabris

University at Albany and Institute of Microbiology of the Czech Academy of Sciences

Abstract:

The broader availability of advanced ion mobility spectrometry (IMS) instrumentation has promoted the development of a variety of strategies for studying the structure and dynamics of biopolymers in the gas phase. Structure information can be obtained, for example, by matching experimental collision cross sections (CCSs) with values calculated from corresponding high-resolution structures, if available, or obtained by advanced computational methods. However, a major challenge is posed by remaining questions about the actual structures assumed by biopolymers in solvent-free environment and their correlation with the structures observed in solution. The structure dynamics can be instead investigated by determining the conformational stability of a sample, which is achieved by monitoring the variations of experimental CCS as a function of selected experimental conditions. To this effect, we evaluated the ability to carry out the controlled unfolding of complex nucleic acid structures and protein-nucleic acid assemblies by varying the source temperature, as well as the cone and collision voltage on our Waters Synapt G2 HDMS. Additionally, we have built a feedback-controlled heating block to vary the temperature of the nanospray emitter, which allows us to induce the controlled perturbation of the system directly in solution, rather than in the gas phase. This presentation will report the results afforded by the different experiments and discuss the similarities/discrepancies between the effects observed in solution by heating the nanospray emitter, or in the gas phase by increasing the source temperature, cone voltage, or collision energy. The presentation will also compare the results obtained from nucleic acid substrates in the absence/presence of bound proteins or small molecule ligands. These experiments offer the ability assess whether specific binding may be capable of stabilizing or destabilizing the substrate conformation. This type of information will be thus expected to provide new valuable insights into the mechanism of binding between proteins and nucleic acids, which could lead to a better understanding of conditions caused by their malfunction. Conversely, the ability to assess the effects of ligands on conformation could help the development of more powerful inhibitors targeting essential protein-nucleic acids assemblies.

ITEM-TWO: Nano-Electrospray Ionization MS Enables Simultaneous Characterization of Specificities and Affinities of Epitope-Antibody Complexes in the Gas Phase

Bright D. Danquah and Michael O. Glocker
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Abstract:

Most important characteristics of antibodies are that they typically strongly bind to specific epitopes, thereby expressing low dissociation constants (KD s) and high Gibbs free binding energies (ΔG0s) [1-2]. Simple but accurate methods for elucidating such relevant antibody properties are therefore imperative. As desired [3], mass spectrometric methods are to be developed that - ideally simultaneously - allow both, epitope mapping and direct determination of the binding strengths of antibody-epitope interactions. We have previously shown that native electrospray mass spectrometry provides a means of determining dissociation energies and apparent dissociation constants of protein-protein complexes in gas phase, termed ITEM [4]; now ITEM-ONE. Here, we use our previously developed concept to simultaneously identify epitopes and obtain characteristic dissociation constants in a single experiment, termed ITEM-TWO [5].
To develop the method, interaction studies between an antiHis-tag antibody and its epitope peptide were engaged. In brief, a mixture of solution 1 (tryptic digest of a His-tag containing recombinant human ß-actin in 30 mM ammonium bicarbonate) and solution 2 (antiHis-tag antibody in 200 mM ammonium acetate) in which the specific immune complex forms was prepared. Without any purification this mixture (solution 3) was then electrosprayed. With the aid of ion filtering devices (quadrupole mass analyzers and/or ion-mobility separation chambers) the immune complex ions were separated from unbound peptide ions. Increasing the energy in the subsequent collision cell resulted in collision induced dissociation (CID) by which the intact epitope peptide(s) was(were) released from the immune complex. The mass(es) of the complex-released peptide(s) was(were) then measured in a ToF analyzer which identified the epitope. A step-wise increase in the collision cell energy allowed the simultaneous determination of the intensities of the surviving ionized immune complexes together with released epitope peptide ions. From the ions´ normalized intensity ratios were deduced the apparent dissociation energies (〚ΔG〛m0g/T) of the gas phase dissociation processes and the calculated apparent gas phase dissociation constants $K^{\#}_{D\,m0g}$. Application examples of this method encompassed determination of known epitope peptide – antibody complexes (antiRA33, antiTRIM21, or antiFLAG antibody) in which synthetic epitope peptides and mutated peptides, respectively, were investigated. The order of the apparent gas phase dissociation constants of all investigated cases matched very well with to that from corresponding in-solution values. Thus, we foresee that binding strengths determined by ITEM-TWO may become as useful as those currently determined in-solution.

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posters abstracts

quick reference:

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Abstract:
Top- and middle-down mass spectrometry (MS) have emerged as promising alternatives to the classical bottom-up approach for protein characterization, with limited sample handling, artifacts and reduced analysis time. Intact and middle level experiments after enzymatic digestion are routinely used for monoclonal antibody (mAb)-related compounds in most biopharma companies. We report here the combination of intact native and middle-down MS analysis of a site-specific antibody-drug conjugate (ADC) performed on high-resolution Orbitrap MS platforms. While native MS allowed assessment of drug load distribution and average drug-to-antibody ratio, middle-down MS afforded primary sequence assessment along with unambiguous drug conjugation and glycosylation sites identification. ADC subunits (LC, Fd and Fc/2) were analysed by middle-down MS with multiple fragmentation techniques, comprising high energy collisional-, electron-transfer and ultraviolet photo-dissociation (HCD, ETD and UVPD). The ion activation techniques were evaluated either as standalone fragmentation techniques or in combination, in terms of optimal sequence coverages, drug conjugation and PTM site identifications. Primary sequence assessment (62%, 44%, and 50% for Fc/2, Fd, and LC subunits, respectively) along with unambiguous conjugation site identification were obtained with unraveled performances within a unique 10 min UVPD LC-MS/MS run for the ADC. Finally, complementarity of UVPD, ETD and HCD techniques is highlighted with ~80-90% sequence coverage of all subunits along with simultaneous identification of drug conjugation and glycosylation sites. We envision that middle-down MS strategies with multiple fragmentation techniques will facilitate the in-depth characterization of empowered next generation mAb-based formats.

P01

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Abstract: Chemical modification of proteins for structure elucidation

Structural mass spectrometry (MS) includes various techniques, for instance covalent labelling or cross-linking. Covalent labelling is used to identify individual residues on the surface of proteins or protein-ligand complexes and cross-linking provides insights into direct contact sites of proteins in protein assemblies.

We used yeast alcohol dehydrogenase (ADH) and rabbit pyruvate kinase (PK) as model protein complexes to establish a (quantitative) protein labelling workflow for investigating protein dynamics under different conditions. Labelling was performed with (i) N-Succinimidyl Acetate (NHS-Ac), which labels lysine, tyrosine, serine and threonine, and (ii) diethyl pyrocarbonate (DEPC), which labels histidine, lysine, tyrosine, serine, threonine and cysteine. After chemical labelling, the protein was digested with trypsin and generated peptides were analysed by tandem-LC-MS. Obtained raw data were analysed using MaxQuant software to identify and quantify modified residues. Individual modified residues were visualised in the available crystal structures. We found that these are mostly localised in the solvent accessible surface area and, therefore, NHS-Ac and DEPC represent promising labelling agents for structure analysis. Quantification of labelling sites deduced from proteins under different conditions will highlight structural changes of the proteins. Currently, we are extending our labelling workflow with other labelling reagents targeting different amino acid residues. We further plan to integrate both chemical labelling and cross-linking into computational modelling approaches.

P02

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Abstract: Protein Structures by Mass Spectrometry

Cross-linking/mass spectrometry (CLMS) has advanced as a powerful method for yielding low-resolution data regarding protein-protein interactions, protein conformational changes and protein tertiary structure. In our lab, we showed that with high-density cross-linking data with controlled FDR rates, combined with conformational space search, we were able to determine the structure of human serum albumin (HSA) domains, with an RMSD to X-ray structure of up to 2.53 Å. To further test our approach, the lab embarked on the 11th Critical Assessment of protein Structure Prediction (CASP) experiment. CASP is a community-wide, worldwide experiment, taking place every two years since 1994, for protein structure predictors to test their computational algorithms on proteins of unpublished structure. In a blind study, we provided cross-linking data for four CASP targets. The results of CASP11 confirmed that CLMS data can assist de novo structure prediction. Most significantly however, CASP11 revealed some of the limitations of the methodology at the time, such as uneven sequence coverage caused by a bias against β -sheets and uneven distribution of proteolytic cleavage sites. Concurrently, we were providing actual experimental data for use by structure predictors in CASP for the first time in the 22 years of CASP history. We followed this in CASP12, providing CLMS data on a 170-kDa protein and a small complex. In the latest iteration, CASP13, we provided data on two heteromeric complexes and a further two protein targets.

P03

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Abstract:
Parkinson's disease is characterized by the death of dopaminergic neurons in the substantia nigra of the brain caused by an oligomeric species of the protein α-synuclein. The early-forming oligomer of this protein has thus far been difficult to fully characterize. Using short distance crosslinking constraints we have obtained using a $^{14}N/^{15}N$ crosslinking strategy in discrete molecular dynamics simulations, we will determine a structure of the early synuclein oligomer generated by FN075. This structure will be validated using other structural proteomics techniques, including surface modification, HDX and affinity labelling. Analysis of the EDC crosslinking data yielded a total of 19 non-redundant zero-length distance constraints. 4 constraints were interprotein, and 15 were intraprotein constraints which were identified by the $^{14}N/^{15}N$ labelling strategy. These zero-length crosslinks, in addition to those from the DSG and SDA crosslinking experiments, are the most relevant to modelling, and are used to generate a structure both for the individual monomers within the oligomer and of the overall structure of the multimeric oligomer complex. The interprotein crosslinks found using EDC indicate an inter-molecular interaction between the N-terminus of one monomer of the oligomer with the C-terminus of an adjacent monomer. These interactions may indicate an alternating head-to-tail arrangement of monomers within the oligomers. Additionally, many of the intraprotein crosslinks found with EDC were different than those found previously on the native synuclein protein, indicating a change in structure between the two forms. Using these crosslinking data, a synuclein oligomer structure will be determined using crosslinking-constraint driven discrete molecular dynamics simulations (CL-DMD). This model will be verified by using a combination of HDX, surface modification and additional long-distance crosslinking data.

P04

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Abstract: Monitor complement activation in serum by quantitative cross-linking/mass spectrometry
Protein dynamics plays a key role in the activation and regulation of the sophisticated human complement system. Unfortunately, experimentally following such dynamic structural events, especially in their native context, are often hampered by lack of tools. In the last decade, cross-linking/mass spectrometry (CLMS) has matured as a standard tool to determine protein folds and interactions even in complex protein backgrounds. Its recent merger with quantitative proteomics allows for capturing dynamics of protein conformations and interactions. This has been successfully applied to interrogate the structure of C3(H2O) (Chen et al. MCP 2016) and to characterize in-solution structural dynamics of factor H, suggesting how factor H can be kidnapped by a bacterial protein to suppress complement activation (Herbert et al. J.Immunol. 2015). Here we show that by combining discovery type of QCLMS analysis and targeted proteomics, we could monitor the activation of C3 in serum. In a previous QCLMS analysis, inactive C3 and its two active products C3(H2O) and C3b were compared pairwise. This study confirmed that relocation of the TED domain is a signatural structural rearrangement accompanying the activation of C3 (Chen et al. MCP 2016). From 116 quantified cross-links we choose five between the TED and the shoulder of the molecule to represent the inactive conformation and five cross-links between the TED and the foot of the molecule to represent the active conformation of C3. Target lists were generated directing the mass spectrometer to selectively detect these cross-links in a cross-linked serum sample. Isolated C3 and C3b were cross-linked, each with a heavy isotope-labeled analogue of the cross-linker. To target cross-links representative of the inactive conformation, cross-linked C3 was spiked in the serum sample with equal total amount of C3 in serum. The proportion of inactive C3 in serum was quantified by comparing signals of the heavy isotope-labeled and the non-labeled versions of each cross-link. The proportion of active C3 in the serum sample was similarly quantified by spiking in cross-linked C3b and targeting active conformation representative cross-links. With this work we demonstrate that QCLMS can be applied to discover structural biomarkers of events in complement response and regulation. Targeted detection of these biomarkers can be applied to monitor corresponding complement events in situ with good selectivity and sensitivity.

P05

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Abstract: Sequential digestion with trypsin and elastase in cross-linking/mass spectrometry
In cross-linking/mass spectrometry, protein proximities and conformations are preserved through cross-linker and translated into distance constraints. Using only trypsin is often insufficient, as the amino acid composition of some protein regions is incompatible with trypsin digestion. Several complementary proteases like AspN, GluC and chymotrypsin have been successfully used in combination with cross-linking/mass spectrometry. Still, some protein regions remain elusive and can only be analysed with broader specific proteases like elastase.

In this study, we show that the activity of elastase is not only dependent on the targeted amino acid, but also on substrate length. We exploit this property of elastase activity through the sequential digestion using trypsin prior to elastase. The cleavage by trypsin reduces the complexity typically associated with elastase digestion. Furthermore, it introduces peptides with a positive C-terminal charge. As a result, we detect cross-links in the previously inaccessible N-terminal region of TAF4.

P05.1

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Abstract:
Eukaryotic precursor messenger RNAs (pre-mRNAs) are processed at their 3′ ends by the Cleavage and Polyadenylation Factor. CPF cleaves pre-mRNAs, adds a polyadenylate tail and triggers transcription termination. It is unclear how the complex is assembled and coordinated. A Cryo-EM investigation of CPF aided by nanoESI-MS and XL-MS put new light on its assembly and activity. This provides a new example of the powerful XL-MS support in structural investigations.[1-4]

XL-MS experiments were performed on purified complex of the Poly(A) polymerase module of CPF (with and without Pap1). This is composed by four or five proteins: Cft1, Yth1, Fip1, Pfs2 and Pap1. The isotopically-coded NHS ester BS3 was used as cross-linker. Trypsin digests were fractionated by peptide-level SEC and fractions analysed by nano-scale LC-MS/MS on an Orbitrap Velos. Cross-links were searched using xQuest and spectra were manually validated.

NanoESI-MS of CPF complex revealed an interaction network organized into three modules: nuclease, phosphatase and polymerase. Poly(A) polymerase module of CPF was expressed in insect cells. The ~200-kDa recombinant four-subunit complex (Cft1, Pfs2, Yth1, Fip1) was imaged using cryo-EM at a 3.5 Å resolution. Cryo-EM maps display only three ordered subunits (Cft1, Pfs2 and Yth1). A XL-MS investigation was performed to validate the structural model and to determine where Pap1 and Fip1 bind. Cross-links map agree with the atomic models, and the crystal structure of Pap1. Fip1 cross-links to the C-terminal part of Yth1 and the polymerase domain of Pap1. Pap1 also cross-links the C-terminal helical domain of Cft1, ZnF1 of Yth1, and the C-terminal region of Pfs2. Together, these data suggest that the flexible C-terminal half of Yth1 binds the intrinsically disordered protein Fip1, which in turn flexibly tethers Pap1 to the complex, allowing conformational freedom to add long poly(A) tails onto diverse RNA substrates.
These data, combined with in-vitro reconstitution experiments, show that the polymerase module brings together factors required for specific and efficient polyadenylation, to help coordinate mRNA 3′-end processing.

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P06

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Abstract: Anti-apoptotic Bag-1 Isoforms are Involved in Endoplasmic Reticulum Associated Degradation

Bag-1 is an anti-apoptotic adaptor protein, which involves in the regulation of transcription, cell proliferation, tumorigenesis, apoptosis, and several cellular signaling pathways via interacting with heat shock proteins, E3 ligases, nuclear hormone receptors, Raf-1 and Bcl-2. Bag-1 enhances cell survival and has high expression levels in many types of cancer, especially in breast cancer. In human cells, Bag-1 has three major isoforms as Bag-1S, Bag-1M and Bag-1L, which are derived from alternative translation initiation of a single mRNA transcript. Bag-1 isoforms have different cellular localizations and may also have different interacting partners. In this study, we aimed to detect interacting partners of each isoform to determine their role in breast cancer.

Bag-1 isoforms with an N-terminal TAP-tag were cloned in an expression vector and further used for the transfection of MCF-7 breast cancer cells. After successful overexpression of Bag-1 isoforms with their interacting proteins, each isoform was purified as complexes from the cells. Purified interactomes were digested to their peptides and then peptide mapping analyses were performed using LC-MS/MS (Waters Synapt G2-Si HDMS). Further identified proteins in the complexes were confirmed by immunoblotting and PRISM, a protein-protein interaction prediction tool was used to map the interaction surfaces of proteins in complexes.
Interacting partners of Bag-1S, Bag-1M and Bag-1L were identified by LC-MS/MS analyses. Among all identified proteins, some heat shock proteins, endoplasmic reticulum resident chaperones, lectins, protein disulfide isomerases, ubiquitin ligases, and transitional endoplasmic reticulum ATPase found as common interacting partners for all Bag-1 isoforms and those mainly participate in endoplasmic reticulum-associated protein processing. Immunoblotting assays confirmed those interactions for all isoforms and protein-protein interaction predictions showed the possibility of existence of a large complex near the endoplasmic reticulum that formed by Bag-1 isoforms via BAG domain and UbL (ubiquitin-like) domain.

Considering the mechanisms that Bag-1 is involved, known partners and newly identified interacting proteins whose functions are well known in protein homeostasis pointed out the role of Bag-1 in endoplasmic reticulum associated degradation (ERAD). Prediction of interaction surfaces suggested that Bag-1 is capable of interacting with identified proteins simultaneously and forms a large complex that functions in the degradation of unfolded proteins and locates between endoplasmic reticulum and proteasome.

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P07

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Abstract:
Biogenesis of peroxisomes requires the import of nuclear-encoded matrix proteins into the organelle, which involves the peroxisomal matrix protein import machinery. A central component of this complex machinery is the cytosolic receptor protein Pex5p, which binds matrix proteins carrying a peroxisomal targeting signal (PTS) 1 in the cytosol. Cargo-loaded Pex5p then binds to the docking complex, which is composed of Pex14p, Pex17p, and Pex13p, at the peroxisomal membrane. Subsequently, Pex5p and Pex14p form a highly dynamic PTS1 import pore by which the cargo is translocated across the peroxisomal membrane and released into the lumen of the organelle by a not yet defined mechanism. In this work, we have analyzed dynamic protein interactions of yeast Pex14p and Pex5p. To this end, we affinity-purified Pex14p complexes from membrane fractions and performed chemical cross-linking and high-resolution mass spectrometry (XL-MS) to identify homo- and hetero-multimeric interactions of Pex14p and its core interaction partners Pex17p and Dyn2p. The analysis indicated that Pex14p forms distinct homooligomers in vivo. To obtain further information, we performed XL-MS analyses of recombinantly expressed and purified Pex14p and truncated forms thereof, showing a predominant trimeric association dependent on the presence of its predicted coiled-coil domains. We used quantitative XL-MS to identify oligomer-specific linkages and confirmed the predominant formation of Pex14p trimers by native MS. Furthermore, we reconstituted Pex5p receptor-cargo complex and analyzed its formation and stability using native MS and collisional activation experiments. To further reveal regulatory mechanisms, we used phosphomimicking mutations of Pex14p and Pex5p at in vivo phosphorylated sites. Native MS analyses suggest that site-specific phosphorylation events modulate the formation and stability of their dynamic protein interactions.

P08

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Abstract: The impact of a post-translational modification on glycan binding to the norovirus capsid P domain

Infection with noroviruses is the predominant cause of viral gastroenteritis in humans with the genogroup II strains causing most outbreaks worldwide. Histo-blood-group antigens (HBGAs) on the cell surface are considered to be the major attachment factors for noroviruses. Attachment to these glycan factors is thought to be the prerequisite for infection and is then supposedly followed by conformational changes, which allow the virus to bind a receptor and enter the cell. The norovirus capsid is composed of 90 dimers of the major capsid protein VP1 encoding the shell (S) and the protruding (P) domains. The P domain is important for glycan recognition and therefore provides a future vaccine or drug target. Previous analysis of HBGA B and fucose binding to P dimers by NMR and crystallography revealed the important role of a single post-translational modification (PTM) in this process. Here, we used hydrogen deuterium exchange mass spectrometry (HDX MS) to analyze the impact of this PTM on glycan binding and potential allosteric effects in the protein.

For HDX MS experiments native and post-translationally modified P dimers were labelled with deuterium in presence and absence of glycan ligands for several time intervals. Quenched, flash frozen samples were directly loaded onto a cooled HPLC system for online pepsin digestion and separation of the resulting peptides. Peptide deuteration was measured in an Orbitrap Fusion Tribrid mass spectrometer (Thermo Scientific). Peptide identification and mapping of PTM sites was done on non-deuterated and fully reduced samples using HCD for fragmentation.

Optimization of the HDX MS workflow for the Saga P domain yielded sequence coverage of 97%. In order to address the conformational effects of glycan binding on the native P dimer structure, HBGA B trisaccharide, fucose and as non-binding control galactose were compared. HDX data in presence and absence of glycans revealed deuteration differences in the supposed glycan binding pocket, which had been identified by crystallization earlier. Interestingly, a single PTM introduces higher flexibility in the binding pocket and attenuates glycan binding to a high extent.

The results suggest that glycan binding occurs on a well-defined site on the P dimer surface and is strongly dependent on the native structure. We have extended the work to virus-like particles to consolidate these findings.

P09

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Abstract: Towards a protocol to enrich muscle cytoskeletal proteins and intermediate filaments for protein-protein interaction studies using cross linking mass spectrometry"

Mammalian skeletal muscle compromises about 40 % of total body mass. Skeletal muscles consist of different muscle fibers. These fibers contain numerous myofibrils, which are divided into segments called sarcomeres. Each sarcomere is bound by the so called Z-discs and is linked by Intermediate filaments (IF). IF and Z-disc associated proteins interact with each other to maintain the structural and mechanical integrity of the muscle cytoskeleton by strongly interacting with each other.

In various neuromuscular diseases like Myofibrillar Myopathies (MFM) mutations in these proteins lead to specific phenotypes. MFM's are defined by desmin-positive aggregates and the destruction of myofibrillar structures, leading to a severe impairment of muscle function. Early symptoms are still unknown and no curative treatment is possible so far. On molecular level, mutations in the desmin gene cause highly dysfunctional networks and disable proper protein-protein interaction. Desmin is a muscle specific IF protein and is forming a three dimensional network connecting the contractile apparatus with mitochondria and other organelles. More than 70 mutations are currently known causing desminopathy one major subgroup of MFM.

To study the influence of desmin mutation on protein-protein interactions a protocol for the isolation of cytoskeletal proteins and intermediate filaments from differentiated myotubes was established. These isolated proteins were used for cross link studies with BS3 to identify potential interaction partners in this complex network. Several hundred proteins could be identified after fractionation and cross linking events. In total over 100 cross links were identified using StravoX among them 4 cytoskeletal proteins were assigned. Currently, the study is ongoing to enhance the identification of cross linked proteins and to apply our protocol to a cell culture system harboring the most common mutation causing desminopathy in patients.

P10

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Abstract: Structural basis for ligand and inhibitor binding in cytochrome bd-oxidases revealed by HDX-MS

Introduction
Cytochrome bd-oxidases are membrane embedded enzymes exclusively found in bacteria and archaea. They reduce molecular oxygen to water and play critical roles in oxidative stress protection and the colonization of pathogenic bacteria in O_2-poor environment. Bd-oxidase deficient strains of human pathogens show hypersensitivity towards known antibiotics, making bd-oxidases promising drug targets.

Recently, our group solved the first structure for this enzyme family, the bd-oxidase from Geobacillus thermodenitrificans. Although this structure increased our understanding of this protein family, only limited data are available on the electron donor binding site and the mechanistic details of the catalytic cycle. Here we investigate substrate and inhibitor binding in bd-oxidase using HDX-MS.

Methods
Bd-oxidase was homologously produced and the detergent solubilized enzyme was purified via multiple chromatographic steps. The purified protein was incubated with substrate mimicking quinones and inhibitory aurachines, respectively. All samples including ligand-free controls were incubated at elevated temperatures to promote ligand binding.

For subsequent HDX-MS analysis, the Waters HDX-Automation V2 was utilized. Samples were diluted in an excess of D_2O for various times before the reaction was quenched by shifting pH and temperature (0°C, pH 2.5). Bottom-up analyses were performed via online peptic digestion followed by RP UPLC and ESI IMS-QTOF MS (Waters Synapt G2Si). Peptide specific uptake differences were mapped onto the protein structure to elucidate substrate and inhibitor binding sites and resulting structural changes.

Preliminary data
We achieved exceptionally high sequence coverage for membrane embedded complexes, with an average of 80%. This high sequence coverage allowed us to generate comprehensive uptake difference maps of the enzymes in the presence and absence of the tested compounds.

We successfully elucidated the quinone binding site using coenzyme-Q1 as a substrate mimic. Our results show that the quinone binds in proximity to the electron accepting heme b558 and is kept in place by a short antiparallel β-sheet of an otherwise mostly unstructured soluble domain, the Q-loop. In addition to the binding site, our HDX-data revealed conformational changes along two transmembrane spanning α-helices proposed to be involved in proton pumping. Moreover, we collected HDX data in the presence of aurachine D3-11, a known inhibitor of bd-oxidases. Our results suggest that the inhibitor binds irreversibly to the quinone binding site, inhibiting the electron transfer and locking the enzyme in an occluded conformation.

Currently, we perform biophysical and docking experiments to further elucidate the catalytic mechanism of bd oxidases. Our results thereby may facilitate rational design of drugs directed against terminal oxidases present in human pathogens such as Mycobacterium tuberculosis.

P11

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Abstract:
Ion mobility mass spectrometry (IM-MS) allows separation of native protein ions into 'conformational families'. Increasing the IM resolving power would allow finer structural information to be obtained, and can be achieved by increasing the length of the IM separator. This, however, increases the time that protein ions spend in the gas phase and previous experiments have shown that the initial conformations of small proteins can be lost within tens of milliseconds. Here, we report on investigations of protein ion stability using a multi-pass travelling wave (TW) cyclic IM (cIM) device. Using this device indicate minimal structural changes were observed for Cytochrome C after hundreds of milliseconds, while no changes were observed for a larger multimeric complex (Concanavalin A). The geometry of the instrument (Q-cIM-ToF) housing this device allows complex tandem IM experiments to be performed which were used here to obtain new, detailed collision induced unfolding pathways for Cytochrome C. Our data show that extended states of Cytochrome C can collapse after activation, rather than refold in the gas phase as previously suggested, a phenomenon that would be difficult to probe using typical, single stage IM instruments. The novel instrument geometry provides unique capabilities with the potential to expand the field of protein analysis via IM-MS.

P12

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Abstract: Development of a Novel Multiplexing Proteomics Platform for the Identification of Oxidative Modifications in Caenorhabditis elegans

Caenorhabditis elegans, part of the nematode family, are used as model systems for many human diseases including protein misfolding diseases such as Parkinson's and Alzheimer's. Structural studies on C. elegans strains expressing the aggregate forms of the proteins involved in these diseases would shed light on how these aggregate structures lead to disease. Preliminary studies of the hydroxyl radical-based footprinting method fast photochemical oxidation of proteins (FPOP) has shown the potential for protein structural analysis in C. elegans. FPOP is especially suited for these in vivo studies because of the irreversible nature of the modification. However, there is an increase interest to reduce analysis time and cost. We have used the enhanced multiplexing strategy combined Precursor Isotopic Labeling and Isobaric Labeling and Isobaric Tagging (cPILOT) to enhance sample multiplexing of traditional isotopic labeling and isobaric tagging approaches. Here, we present an 18-plex analysis using light and heavy dimethylation combined with ten-plex isobaric reagents to analyze 18 samples from IV-FPOP in a single analysis. Initial results indicate the feasibility in coupling IV-FPOP with multiplexing to reduce instrument time thus expanding the use of hydroxyl radical based footprinting for in vivo studies.

P13

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Abstract:
The broader availability of advanced ion mobility spectrometry (IMS) instrumentation has promoted the development of a variety of strategies for studying the structure and dynamics of biopolymers in the gas phase. Structure information can be obtained, for example, by matching experimental collision cross sections (CCSs) with values calculated from corresponding high-resolution structures, if available, or obtained by advanced computational methods. However, a major challenge is posed by remaining questions about the actual structures assumed by biopolymers in solvent-free environment and their correlation with the structures observed in solution. The structure dynamics can be instead investigated by determining the conformational stability of a sample, which is achieved by monitoring the variations of experimental CCS as a function of selected experimental conditions. To this effect, we evaluated the ability to carry out the controlled unfolding of complex nucleic acid structures and protein-nucleic acid assemblies by varying the source temperature, as well as the cone and collision voltage on our Waters Synapt G2 HDMS. Additionally, we have built a feedback-controlled heating block to vary the temperature of the nanospray emitter, which allows us to induce the controlled perturbation of the system directly in solution, rather than in the gas phase. This presentation will report the results afforded by the different experiments and discuss the similarities/discrepancies between the effects observed in solution by heating the nanospray emitter, or in the gas phase by increasing the source temperature, cone voltage, or collision energy. The presentation will also compare the results obtained from nucleic acid substrates in the absence/presence of bound proteins or small molecule ligands. These experiments offer the ability assess whether specific binding may be capable of stabilizing or destabilizing the substrate conformation. This type of information will be thus expected to provide new valuable insights into the mechanism of binding between proteins and nucleic acids, which could lead to a better understanding of conditions caused by their malfunction. Conversely, the ability to assess the effects of ligands on conformation could help the development of more powerful inhibitors targeting essential protein-nucleic acids assemblies.

P14

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Abstract:
"Lytic Polysaccharide Monooxygenases (LPMOs) are industrially important enzymes used in cellulose saccharification enzyme cocktails and are promising enzymes to use in mass-production of second generation biofuels. Unlike standard cellulases and glycosidases, they degrade polysaccharides oxidatively instead of hydrolytically. Their active site harbours copper ion, which upon its reduction from Cu2+ to Cu1+ generates reactive oxygen species (ROS). Active site is located on a flat surface surrounded with aromatic amino acids facilitating substrate binding enabling created ROS to precisely attack β-1-4 glyosidic bond in polysaccharides. Owing to the shape of the catalytic site, they have the ability to depolymerize recalcitrant crystalline cellulose structures not degradable by standard glycosidases, which is a bottleneck in current biofuel production. LPMOs are unfortunately notoriously known for their low stability during the reaction caused by auto-oxidative damage and therefore difficult to control kinetics. In this study we use structural mass spectrometry, namely Hydrogen/Deuterium Exchange Mass Spectrometry, to uncover the dynamics and structural aspects of Neurosporra crassa LPMO9c unfolding and degradation induced by its reduction and subsequent auto-oxidation and recently reported stabilization of the enzyme by substrate binding. We also used mass spectrometric techniques to elucidate precise location and nature of oxidative modifications of LPMO leading to its structural changes and degradation. Beside the oxidation-related amino acid side chain modifications already found in different LPMO, we also observed previously unnoticed oxidative peptide bond cleavages. Polysaccharide substrate was shown to have slight protective role delaying the inevitable structure unfolding during LPMO reaction. These observations can potentially aid in rational design of LPMO variants with higher oxidative resistance, better substrate binding and thus increased stability and reaction yields.

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P15

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Abstract: Xi - A unrestricted software pipeline for identifying Cross-links from complex samples

Crosslinking mass spectrometry has become powerful tool to study topology of protein/protein complex and protein interaction network. However identifying cross-links from complex protein samples remain challenging. Software tools have been developed often relying on use of isotope labelled cross-linkers or MS cleaveable cross-linkers.
Here we introduce Xi a software package and pipeline for identifying cross-links from samples containing a single protein to several thousand proteins.
Xi software package is developed in our group. The current development happens in an open repositories on github (https://github.com/Rappsilber-Laboratory/) and binaries are available from https://xi3.bio.e.d.ac.uk/downloads/. It place no restriction on either structure or chemistries of the Cross-linker, or the proteases used. It can deal with search-strategies involving digestion with multiple proteases, both when applied separately or in a sequential manner.
Here we present the core search software (XiSearch) and the software to filter the results to a set of residue pairs with a given confidence (XiFDR).

P16

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Abstract: Exploring proteoliposomes for mass spectrometry

Liposomes are phospholipid bilayer vesicles which resemble cellular organelles and membranes. Due to their variability in size, composition and amphiphilic character they are promising mimics of natural membranes and can therefore be used as ideal drug-carrier systems.

For liposome preparation, lipids were first dissolved in chloroform and mixed in different proportions. The solvent was then evaporated using a rotary evaporator and the resulting lipid film was hydrated by adding an aqueous solution. Liposome-protein solutions were prepared by mixing different model proteins (Atg18, Syb (49-96)) with the prepared liposomes. Finally, single liposomes and liposome-protein solutions were analysed by native mass spectrometry.

The goal of this work was to use liposomes as artificial membrane systems for the analysis of membrane proteins. First results showed, that liposome fragmentation is independent on size, composition and concentration. However, at higher collision energies larger lipid clusters dissociate and smaller clusters are obtained providing the basis for mass spectrometric analysis of proteoliposomes. By using different model proteins (Atg18, Syb(49-96)), we were also able to show that liposomes and proteins can be analysed together in solution. However, the analysis of transmembrane proteins in their native-like environment still remains difficult. Following this, new experiments are currently developed to optimise the analysis of membrane proteins in liposomes under non-denaturing conditions.

P17

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Abstract: Chemical cross-linking for the characterization of retinoid acid receptor-retinoid X receptor: correlation between crosslinker distance and 3D structure

Mass spectrometry (MS) has been needed for years as a complementary method to other analytical approaches such as electron microscopy, and nuclear magnetic resonance (NMR) in the structural study of non-covalent biological structure. [1] Indeed, this technique is known to carry out effective structural characterization due to its speed of analysis, its sensitivity, its resolution, its mass accuracy, its range of unlimited mass. This so-called supramolecular MS is then mainly used to determine the stoichiometry of the complex and to analyze the dynamics of the interactions.

However, some difficulties require optimization and can be limiting such as the preparation of samples in a medium compatible with the 3D conformation of the proteins. To push back these limits, approaches like cross-linking have been developed [2]. This technique involves covalently linking the subunits of the complexes upstream of the MS analysis and then characterizing the zones of proximity of the bridged complex by identifying the peptides or the amino acids bridged using bio-tool software.

In our case, we chose to use and optimize this strategy to obtain information on the zones in interaction between several biological partners and thus to complete the results obtained by biochemical technique. We worked on the RAR-RXR complex of retinoic acid receptors RAR and retinoid X receptors. [5] [6] During this work, the goal was to optimize the cross-linking reaction. to freeze the structure of the complex, then to locate the protein contacts between the two nuclear receptors using the tools of the proteomic analysis.

However, all crosslinkers have a fixed spacer arm length despite the possibility for them to extend over. After identifying crosslinks, we could observe on a 3D structure of our complex, the placement and distances of all the crosslinks. Some distances that we could observed on the structure corresponded to the maximum extension distance of the crosslinker, but other peptides selected and observed by mass spectrometry have far greater distances than the maximum length of the crosslinker. A question then came to us: what correlation can be made between the data obtained by XL-MS and identification of crosslinks on the 3D structure?

P18

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Abstract: ITEM-TWO: Nano-Electrospray Ionization MS Enables Simultaneous Characterization of Specificities and Affinities of Epitope-Antibody Complexes in the Gas Phase"

Most important characteristics of antibodies are that they typically strongly bind to specific epitopes, thereby expressing low dissociation constants (KD s) and high Gibbs free binding energies (ΔG0s) [1-2]. Simple but accurate methods for elucidating such relevant antibody properties are therefore imperative. As desired [3], mass spectrometric methods are to be developed that - ideally simultaneously - allow both, epitope mapping and direct determination of the binding strengths of antibody-epitope interactions. We have previously shown that native electrospray mass spectrometry provides a means of determining dissociation energies and apparent dissociation constants of protein-protein complexes in gas phase, termed ITEM [4]; now ITEM-ONE. Here, we use our previously developed concept to simultaneously identify epitopes and obtain characteristic dissociation constants in a single experiment, termed ITEM-TWO [5]. To develop the method, interaction studies between an antiHis-tag antibody and its epitope peptide were engaged. In brief, a mixture of solution 1 (tryptic digest of a His-tag containing recombinant human ß-actin in 30 mM ammonium bicarbonate) and solution 2 (antiHis-tag antibody in 200 mM ammonium acetate) in which the specific immune complex forms was prepared. Without any purification this mixture (solution 3) was then electrosprayed. With the aid of ion filtering devices (quadrupole mass analyzers and/or ion-mobility separation chambers) the immune complex ions were separated from unbound peptide ions. Increasing the energy in the subsequent collision cell resulted in collision induced dissociation (CID) by which the intact epitope peptide(s) was(were) released from the immune complex. The mass(es) of the complex-released peptide(s) was(were) then measured in a ToF analyzer which identified the epitope. A step-wise increase in the collision cell energy allowed the simultaneous determination of the intensities of the surviving ionized immune complexes together with released epitope peptide ions. From the ions´ normalized intensity ratios were deduced the apparent dissociation energies (〚ΔG〛m0g/T) of the gas phase dissociation processes and the calculated apparent gas phase dissociation constants $K^{\#}_{D\,m0g}$. Application examples of this method encompassed determination of known epitope peptide – antibody complexes (antiRA33, antiTRIM21, or antiFLAG antibody) in which synthetic epitope peptides and mutated peptides, respectively, were investigated. The order of the apparent gas phase dissociation constants of all investigated cases matched very well with to that from corresponding in-solution values. Thus, we foresee that binding strengths determined by ITEM-TWO may become as useful as those currently determined in-solution.

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Abstract: XiView - Visual Interface to Cross-Linking Mass Spectrometry Data

Due to the volume, variety and uncertainty of data that CLMS experiments produce, an accessible and interactive interface for scientists to query experimental results is required. xiView provides a common platform for the downstream analysis of Cross-Linking / Mass Spectrometry data. It is independent of the search software used and its input is compliant with the relevant mass spectrometry data standards. It uses established visualisation techniques, notably Multiple Coordinated Views, to help the user explore the data and is designed to facilitate comparisons between different datasets. XiView's functionality has been driven by real-world scientific needs and can be summarised as follows:

1. Multiple Views – Shows the dataset simultaneously from multiple, linked perspectives. Selections and highlighting are coordinated across active views.
2. Filtering – Reduce the dataset to cross-links of interest, which again is mirrored across all active views.
3. Drill Down – Explore the dataset at multiple levels of abstraction.
4. Data & Image Export – Export filtered datasets and publication quality images.
5. Metadata Integration – See the dataset in the context of PDB structures.

P20

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Abstract:
Monitoring changes in protein higher order structure (HOS) is an important regulatory requirement for reasons of safety, efficacy and potency of the resultant therapeutic. As monoclonal antibodies (mAbs) emerge rapidly as a dominant class of therapeutics, so does the need for suitable analytical technologies to monitor for changes in HOS of these complex large biomolecules. Reference materials (RM) serve a key analytical purpose of benchmarking the suitability and robustness of both established and emerging analytical procedures for both drug producers and regulators. Here, two simple enzymatic protocols for generating Fc-glycan variants from the NISTmAb RM have been developed and both global and localised changes in HOS between the RM and these Fc-glycan variants have been characterised using both hydrogen deuterium exchange-mass spectrometry (HDX-MS) and ion mobility spectrometry-mass spectrometry (IMS-MS) measurements. Relative to the native RM conformation, changes associated with deglycosylation were larger than those resulting from degalactosylation. Measurements revealed decreases in structural stability as a result of loss of Fc-glycan structure. These data promote the use of these Fc glycan variants for establishing the sensitivity of and validating analytical methods for the detection of HOS measurements of mAbs and also the suitability of HDX-MS and IMS-MS as valuable analytical methods within the biopharma toolbox.

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Abstract:
Fast photochemical oxidation of proteins (FPOP) utilize hydroxyl radicals to oxidatively modify solvent accessible sites in proteins. Coupling FPOP with mass spectrometry (MS) has been a technique used to structurally characterize proteins. As is, this technique does not take in consideration the different conformational states of a protein. By adding Ion Mobility Separation (IMS), another element is included to better understand higher order protein structure and interactions. Using a SYNAPT G2 HDMS system, the dominant charge state of cytochrome c (+7) was selected. Protein conformers were separated by their collision cross sections in the ion mobility cell and subjected to fragmentation in the transfer cell. Using the extracted drift times, cytochrome c fragmentation patterns are analyzed and residues showing oxidation are determined. Differences in FPOP modification can be seen between the drift time conformers. This suggests combining FPOP and IM-MS can give structural information that is over-looked in bottom up analysis. Combining FPOP, IMS, and MS will provide a technique to expand the understanding of higher order protein structures.

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Abstract:
The obligate anaerobic Dehalococcoides mccartyi strain CBDB1 conserves energy only by using halogenated compounds as a terminal electron acceptor. This organohalide respiration takes place in the membrane-associated organohalide respiration complex (OHR complex) which also includes the key enzyme the reductive dehalogenase (RdhA). Due to low biomass yields, oxygen sensitivity and the challenge in heterologous expression, a NMR and/ or X-ray structure of RdhA from strain CBDB1 could not be obtained yet.

To clarify its structure and location in the OHR-complex, an in-silico model of RdhA was predicted by I-TASSER. The precision of the model was checked experimentally by chemical modifications and crosslinking of the native protein surface in combination with mass spectrometry. Under appropriate conditions, the reactivity of amino acid residues depends on their surface accessibility so that their reactions can be correlated with their position in their tertiary structure of the protein. In addition to the crosslinking reactions, the not-solvent accessible surface areas of the proteins in the OHR complex are supposed to give information about the protein interaction sites within the complex.

The results of the study indicate that, the surface of the protein complex can be modified with various detergents and despite the low amount of biomass some crosslink reactions took place. Protein labeling was performed by nitration of tyrosine residues with tetranitromethane (TNM), the acetylation of lysine residues with Sulfo-NHS-Acetate and crosslinking reactions with bissulfosuccinimidyl suberat (BS^3), disuccinimidyl suberate (DSS) and Leiker (bAL2) respectively. After the modification reaction the proteins were separated by SDS-PAGE and subsequently enzymatic digested. The labeled residues were identified using mass spectrometry by observing the mass shift of the peptides.

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Abstract: Analysis of SNAP25 using structural mass spectrometry techniques

The SNARE complex mediates signal transmission in neurons. The trimeric complex is formed by SNAP25, Synaptobrevin-2 and Syntaxin-1A and is responsible for the fusion of synaptic vesicles with the presynaptic membrane. Composed of two SNAP25 alpha-helices as well as one Syntaxin-1A and one Synaptobrevin-2 alpha-helix the SNARE proteins assemble into a four-helix bundle. While SNARE proteins are well-structured in this four-helix bundle they are natively unstructured in the absence of other SNARE proteins or lipids.

We study the stepwise assembly of the SNARE complex by combining cross-linking and native mass spectrometry (MS). For this, we set out with isolated SNAP25 in solution. Cross-linking was performed using bis(sulfosuccinimidyl)suberate (BS3), which preferentially links lysine residues, as well as serine, threonine and tyrosine residues to a minor extent. Gel electrophoresis and western blotting of cross-linked SNAP25 indicated the presence of oligomers up to heptamers. Following tryptic digestion in solution and analysis of the cross-linked peptides by LC-MS/MS, we identified intra- and intermolecular interactions between both lysine and serine residues. Verified cross-links are mostly located outside the SNARE-motif. Performing native MS using SNAP25 at different protein concentrations the presence of oligomers up to hexamers could be confirmed. Combining cross-linking and native MS is therefore well-suited to study oligomerisation of SNAP25 and will help to investigate SNARE complex intermediates in future studies.

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Abstract: A Cross-linking/Mass Spectrometry Workflow Based on MS-Cleavable Cross-Linkers and the MeroX Software for Mapping Protein-Protein Interactions"

Introduction.
Mass spectrometry (MS)-cleavable cross-linkers are moving into the focus of the cross-linking/MS approach for studying 3D-structure of proteins and protein assemblies. They undergo specific fragmentations under collisional activation conditions generating characteristic product ion patterns. They have an enormous potential for a fast and reliable identification of cross-linked peptides even from highly complex samples, e.g. whole cells and organisms.

Methods.
We developed a robust and widely applicable workflow that allows a facile identification of cross-links for deriving spatial constraints from proteins and protein complexes. Our protocol combines the synthesis of novel cross-linkers, protein cross-linking, bottom-up proteomics analysis, and data analysis based on the in-house MeroX software [1]. Two orbitrap mass spectrometers, Orbitrap Fusion Tribrid and Q-Exactive Plus, were employed.

Results.
We designed and synthesized four novel MS-cleavable cross-linkers. 2,2'-Azobis(2-methylpropanimidate) is an innovative cross-linking principle that after collisional acitvation induces free radical-initiated sequencing (FRIPS) of connected peptides in positive ionization mode [2,3]. 1,1-carbonyldiimidazole is the first zero-length MS-cleavable linker for nucleophilic groups (amines and hydroxyls) in proteins [4]. 3,4-Diallylurea is a novel photo-activatable, MS-cleavable reagent to selectively target cysteines [5]. All cross-linkers were investigated to study several protein systems, such as the tumor suppressor p53, which is an intrinsically disordered protein, and the whole E. coli ribosome. We are currently extending our cross-linking/MS workflow to the in-vivo analysis of protein-protein interactions, exemplified for Drosophila embryos.

Conclusions.
Our integrated cross-linking workflow allows to map protein 3D-structures and protein-protein interactions in-vitro and in-cell. We have synthesized innovative, MS-cleavable cross-linking principles that target different functional groups in proteins, such as amines, hydroxyls, thiols or react non-specifically with all 20 proteinogenic amino acids. The MeroX software is able to analyze cross-linked products in an automated fashion.

Novel Aspect.
We present an integrated workflow based on novel MS-cleavable cross-linking principles and a fully automated analysis of cross-linked products by the MeroX software.

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P25

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Abstract:
Vimentin belongs to type III intermediate filament proteins that is a major constituent of mesenchymal cells such as fibroblasts, lymphocytes, lens epithelial and lens fiber cells. A major functional role of IFs is to establish cell elasticity. However, IFs are involved in various other activities such as cell division, motility, organelle positioning and signal transduction 1. Several of these specific functions of IFs have been demonstrated to depend on the cells ability to dynamically assemble and disassemble them in a time-specific and spatially controlled manner, for which a multitude of posttranslational modifications (PTM) 2.
Using hydrogen-deuterium exchange monitored by mass spectrometry we gained unique insights into structural properties of subdomains within full length vimentin, in particular in regions of contact in α-helical and linker segments that stabilize different oligomeric forms such as tetramers, ULFs, and mature filaments 3. Moreover, we investigate the role of redox modifications of the single, evolutionarily conserved cysteine (Cys328). We analyze how both nitrosylation and glutathionylation of vimentin at Cys328 affect the assembly kinetics of vimentin tetramers into unit length filaments (ULFs) and their further elongation to filaments.

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Abstract: LinX - a Software Tool for Analysis of Protein/Nucleic Acids CXMS Data

Chemical cross-linking in combination with mass spectrometry (CXMS) has developed into a powerful method for mapping low-resolution protein structures and for characterisation of molecular interfaces in protein-protein and protein-nucleic acid complexes. Despite the excellence of CXMS as an analytical tool, the analysis of high complex cross-linking data presents demanding task. Therefore in last two decades, several tools for analyzing cross-linked products has been reported.

In this study, we report a new software tool called LinX which is a Java language-based program designed for fast assignment, evaluation, and validation of large mass spectrometric datasets. It has been primarily developed for high resolution mass spectrometric data. However, the simple parameters of input file enable to use data from almost any mass spectrometry instrument. LinX is highly suitable for wide range of users for its simple and intuitive graphical user interface as well as for its application for protein-nucleic acid cross-linking.

This work was supported by the Czech Science Foundation (grant numbers 16-24309S), the Ministry of Education of the Czech Republic (projects LH15010 and LD15089; program "NPU II" project LQ1604), COST Action (BM1403), European Regional Development Funds (CZ.1.05/1.1.00/02.0109 BIOCEV), and European Commission H2020 (European Network of Fourier-Transform Ion-Cyclotron-Resonance Mass Spectrometry Centers - project agreement No.731077).

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Abstract: The mitochondrial interactome in baker's yeast: a snapshot taken by cross-linking mass spectrometry

Protein-protein interactions (PPIs) play a crucial role in living cells for signal transduction, protein transport and metabolism. For the investigation of PPIs, chemical cross-linking combined with mass spectrometric analyses (XL-MS) emerged as a powerful approach in the recent years. Here, a reactive ingredient, the cross-linker, covalently binds two amino acid residues that are in close proximity to each other. The distance constraint imposed by the length of the used cross-linker gives evidence for interactions between proteins and supports structural analysis of a protein on a low-resolution scale.

Mitochondria are known as the "powerhouses" in most of the eukaryotic cells. The electron transport chain (ETC) within mitochondria is a major pathway for ATP production and forms supercomplexes for more effective electron shuttling. By cross-linking mitochondria isolated from Saccharomyces cerevisiae grown under different conditions, we would like to dig deeper into the interaction partners of the ETC and other protein complexes. The mitochondria from baker's yeast grown on glycerol, a non-fermentable carbon source, show different interaction patterns as those grown on glucose, a fermentable carbon source. Especially, the interactions of proteins located in the inner membrane seem to be affected. Our goal is to shed light on structural organization of mitochondrial proteins under different growing conditions, eventually also in a quantitative manner.

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Abstract: Binding affinity of TEAD transcription factor and its DNA response M-CAT elements studied by structural mass spectrometry

Transcription factors mediate gene expression regulation through interactions with DNA and other regulatory proteins. TEAD transcription factors are active mainly during growth and development and induce expression of wide range of genes most of which encode proteins involved in cell proliferation, differentiation or apoptosis prevention. TEADs and many of their target proteins are also known to be upregulated in several types of cancer cells. Thus TEADs are considered as a possible target for anti-cancer therapy. Due to their aforementioned properties, strict regulation of TEAD proteins activity is required to prevent tumorigenesis or developmental disorders. To date, known ways of TEAD proteins regulation include mostly interaction with other regulatory proteins - coactivators such as YAP, TAZ or Vgll. Nevertheless, the information on TEAD transcription factors activity regulation through interaction with DNA is still limited.

To study the interaction of DNA binding domain of TEAD1 (TEAD1-DBD) with 15bp long DNA duplexes containing the M-CAT motif and originating from regulatory regions of different human genes, first, dissociation constant of each complex was determined using fluorescence anisotropy-based binding assay. To make sure, that the changes in fluorescence anisotropy occurring during titration of DNA by the protein were caused by complex formation, native complexes presence was confirmed using nano-ESI ionization coupled with FT-ICR MS. According to KD assay results, tested M-CATs could be divided into two groups one with approximately ten times higher affinity to TEAD1-DBD than the other depending on the M-CAT motif orientation. On top of that, the ratio of bound and unbound protein observed while using native MS agreed well with dissociation constants measured by fluorescence anisotropy.

For further investigation of the structural basis of this effect, structural mass spectrometry techniques, H/D exchange and chemical cross-linking, were utilized. These experiments provided information about the interaction interface, which was for all M-CATs located to H3 helix, and distance restrains needed for homology modelling and molecular docking used to explain the different binding affinities of each oligonucleotide to TEAD1-DBD. During the molecular docking, the lower affinity inverted M-CATs preferred to bind to TEAD1-DBD in 180° rotated orientation, where fewer and weaker amino acid-base interaction could form, which leads to weaker binding affinity and thus higher dissociation constant.

This work was supported by the Czech Science Foundation (grant numbers 16-24309S), the Ministry of Education of the Czech Republic (projects LH15010 and LD15089; program "NPU II" project LQ1604), COST Action (BM1403), European Regional Development Funds (CZ.1.05/1.1.00/02.0109 BIOCEV), and European Commission H2020 (European Network of Fourier-Transform Ion-Cyclotron-Resonance Mass Spectrometry Centers - project agreement No.731077).

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Abstract:
Native mass spectrometry (MS) enables the ionization and transfer of intact non-covalent protein complexes into the gas phase. As such, it is a perfect tool to study proteins and their assemblies in a mass and conformation specific manner. This enables MS to probe structural transitions which proteins and their complexes undergo, e.g. during a viral lifecycle. Such transient states are of high importance for structural biology, but most often cannot be purified and are inaccessible for crystallography.

Despite its remarkable sensitivity and selectivity, the structural resolution in native MS alone is limited. The amount of structural information could be vastly increased by its combination with powerful hard X ray free electron lasers (XFELs) such as the already established LCLS in Stanford or the European XFEL, the worldâ€™s most intense light source so far, which has just become operational in Hamburg. These instruments promise an opportunity to obtain high resolution structures of single particles. Reciprocally, native MS could solve some of the issues with delivering sample into the beam and could also add another dimension of possibilities by manipulating and selecting charged molecules in the gas phase prior to their imaging.

This contribution will highlight the benefits of native MS for single particle imaging of transient protein intermediates at XFELs. It will also describe our plans and ongoing work to bring native MS to European XFEL single particle beamline as well as present our initial feasibility studies on achievable ion fluxes.

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Abstract: An integrated workflow for cross-linking/mass spectrometry

Cross-linking/mass-spectrometry (CLMS) has become a standard tool for the topological analysis of multi-protein complexes and has begun delivering high-density information on protein structures, insights into structural changes and the wiring of interaction networks in situ. Advances in the methodology have been made due to the development of new cleavable cross-linkers and the introduction of new mass spectrometers that resulted in a considerable amount of protocols and software that can't be integrated. Furthermore, challenges like cross-linked peptide identification due to size and low abundance need still to be further addressed. We present a 12-fraction concise workflow using standard cross-linkers and fragmentation methods and introduce a sequential digestion step to decrease the size of cross-linked peptides and enhance their detection avoiding over-digestion. We used our software Xi to perform cross-linked peptide search as it supports any type of cross-linker, protease or protease combination and fragmentation methodology. QCLMS was also performed to demonstrate the applicability of the combined protocols. We benchmarked our protocol in samples that ranged from low to high complexity. We increased cross-linked peptide identification by 1.5 fold without compromising data quality. QCMLS was successfully combined with sequential digestion suggesting a bottom to bottom orientation for the C3b dimerization. We were able to differentiate, in solution, between different states of the proteasome and point towards the existence of more alternative states and found data that support the existent cryoEM-based model. Finally, we identified an essential interaction for the OCCM complex formation that could represent an ideal target for the development of inhibitors with potential as anti-cancer therapy.This simple and concise 12-fraction approach, in solution, increases cross-link coverage, reveals dynamic protein-protein interaction sites, which are accessible, have fundamental functional relevance and are therefore ideally suited for the development of small molecule inhibitors.

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Abstract:
Multiple oligomerization domains along RAGE sequence.
Oligomerization code of RAGE receptor involves the C-terminal TM domain.
Enhanced oligomerization of full-length RAGE
Synergistic effect of different domains in oligomerization of full-length RAGE receptor.

Oligomerization is common among proteins, it is believed that even 2/3 of cellular proteins form oligomers. Oligomer formation often appears to be critical for the interaction of membrane proteins with ligands and signal transduction. RAGE (Receptor for Advanced Glycation End Products) is a pattern recognition receptor, transmitting inflammatory signals. Oligomerization of the receptor is believed to play an important role in its manner of functioning, however, the structure and stoichiometry of these complexes remain unclear. Different modes of oligomerization of RAGE have been proposed during previous studies. In all these studies different truncated versions of extracellular parts of RAGE were used. Here, we provided a basic characterization of the oligomerization states of full-length form of RAGE (including transmembrane (TM) and cytosolic region (CT)) and compare it with the oligomerization modes of its four fragments, containing domains V_C1, V_C1_C2, C2, and C2_TM_CT. For this purpose, we used native mass spectrometry, analytical ultracentrifugation, and size exclusion chromatography coupled with multi-angle light scattering. Our results confirm known oligomerization tendencies of separated domains while highlighting the enhanced oligomerization properties of full-length RAGE. In addition, mutational analyses within the GxxxG motif of the TM region confirm the sensitivity of oligomeric distributions from the TM sequence. Therefore, our data provide experimental evidence for the role of the TM domain in oligomerization, indicating a synergy of different oligomerization hot-spots along the RAGE sequence, crucial for shaping the native oligomeric distribution. This results also explain the variability of obtained oligomerization modes in RAGE fragments. Using HDX-MS we also mapped regions involved in TM-dependent oligomerization of RAGE.

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Abstract: Data-independent acquisition improves quantitative cross-linking/mass spectrometry

Quantitative cross-linking/mass spectrometry (QCLMS) reveals structural detail on altered protein states in solution. On its way to becoming a routine technology, QCLMS could benefit from data independent acquisition (DIA), which generally enables greater reproducibility than data dependent acquisition (DDA) and increased throughput over targeted methods. Therefore, here we introduce DIA to QCLMS by extending a widely used DIA software, Spectronaut, to also accommodate cross-link data. A mixture of seven proteins cross-linked with bis[sulfosuccinimidyl] suberate (BS3) was used to evaluate this workflow. Out of the 414 identified unique residue pairs, 292 (70%) were quantifiable across triplicates with a coefficient of variation (CV) of 9.8%, with manual correction of peak selection and boundaries for PSMs in the lower quartile of individual CV values. This compares favorably to DDA where we previously quantified only 63% of the identified cross-links across triplicates with a CV of 14%, for a single protein and complete manual data curation. In conclusion, the DIA software Spectronaut can now be used in cross-linking and DIA is indeed able to improve QCLMS.
The structure of proteins can change with time or as part of a molecular process and depends on their chemical and physical environment such as ionic strength, temperature or pH. To study protein structural changes under these conditions we paired DIA-QCLMS with the photoactivatable cross-linker sulfo-succinimidyl 4,4'-azipentanoate (sulfo-SDA). Our study applied seven pH conditions in a range from pH4 to pH10 to our model protein human serum albumin (HAS). Photo-DIA-QCLMS enabled to distinguish the fast (F-form) (pH 2.7-4.5) and the basic form (B-form) (pH 8-10) from the native conformation (pH 7) and demonstrate that conditional independent UV cross-linking using sulfo-SDA is capable to distinguish different pH conditions based on differential abundances of unique residue pairs. Our workflow simplifies studying structural changes induced by environmental parameters and allow researchers to determine how protein structures behave in dynamic systems.

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Abstract: OpenPepXL: a versatile and sensitive XL-MS identification tool

Introduction: Cross-linking coupled with mass spectrometry (XL-MS) has been recognized as an effective source of information about protein structures and interactions. Many methods and tools have been developed and reported for XL-MS identification through the last decade. Every tool applies different heuristics to cope with the quadratic search space inherent in XL-MS data analysis and uses their own model to estimate the False Discovery Rate (FDR). As part of the release of OpenMS 2.4 we introduce version 1.0 of the tool OpenPepXL and compare it to other commonly used tools for identification of non-cleavable cross-linkers on a diverse set of XL-MS experiments.

Methods: OpenProXL is a protein-protein cross-linking identification tool implemented using the open-source and well-documented OpenMS library (Röst et al. 2016). Like xQuest (Rinner et al., 2008) it can make use of labeled linkers to denoise spectra by comparing the spectra containing the light and heavy linkers. It can be used efficiently without any heuristics to reduce the search space for small datasets and has an optional fast pre-scoring algorithm to make analysis of large datasets feasible. OpenPepXL is part of the OpenMS proteomics pipeline that includes tools for labeled and label-free quantification. It can be installed on Windows, OSX and Linux and is compatible with most computing clusters and cloud services for large scale data analysis.

Results: “Kojak”, “pLink 2”, “StavroX”, “XiSearch”, “xQuest” and our own tool “OpenPepXL” were compared on several datasets from different laboratories on the same computational setup. Some of these datasets were from XL-MS experiments with proteins or protein complexes with known structures and were used for structural validation of the results. We found that the overlap of identified cross-links among the compared tools with an FDR of 5% is less than 50% for some datasets, but it increases with more stringent FDR cutoffs. A consensus approach using multiple tools could be promising in extracting a set of very confident identifications. We also found that it can pay off to analyze datasets without using heuristics to reduce the search space, if the dataset is small enough to make such an analysis feasible with the available computing power. OpenPepXL makes an exhaustive analysis more feasible for larger datasets through its efficient implementation and by being compatible with computing clusters and cloud services. Without using heuristics to filter out spectra and cross-link candidates before a full scoring, OpenPepXL reports about 10% more cross-links than the other tools without losing specificity.

Conclusion: OpenPepXL is an efficient and versatile XL-MS search engine with broad applicability and better sensitivity than other available tools.

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Abstract:
The structural organization of the highly specialized synaptosome membranes and underlying proteinous active zone matrix represent challenge for biochemical and proteomic characterization. Main critical points are (1) the extraction and preservation of integral proteins and their complexes in a native state using membrane mimetics (e.g. detergents), (2) the biochemical separation and component identification of such complexes and (3) the deciphering of the biological meaning behind the multiple intra- and intermolecular interactions. These experimental difficulties accompanied the isolation of native integral and/or peripheral protein complexes from synaptosomes and synaptosomal subfractions for decades and were addressed in our study. Firstly, little is known about the extractability of synaptic proteins and the preservation of their protein-protein interactions upon use of detergents. Therefore, we successfully conducted detergent screening with surfactants from different classesto assess their solubilization properties in regard to presynaptic proteins. Secondly, due to existing technical limitations for separation of protein complexes in the molecular weight range of few hundred kilodaltonds (kDa) to megadaltons (MDa), we implemented a protocol with optimized of size exclusion chromatography conditions for the separation of membrane-derived extracts upon preservation of the protein-protein interactions. Thirdly, up to date no systematic study evaluated the effect of the centrifugation step post-solubilization on the protein composition of the extracts. Thus, we performed quantitative immunoblotting analysis was performed under two different centrifugation conditions to address the extractability of synaptic proteins. Subsequently, in the context of synaptic research and interactomics, we screened different affinity matrices for a model integral presynaptic protein (SNARE protein syntaxin-1) to find optimal immunopurification and buffer stringency conditions for its enrichment. Additionally, we developed a robust workflow for the LFQ proteomic and data analysis to filter non-specifically bound proteins from true positive hits and validated biochemically the findings. Importantly, we demonstrated that the tandem use of non-denaturing protein extraction in combination with LFQ AP-MS represents a powerful tool for the quantitative characterization of (synaptic) membrane protein complexes under various experimental conditions. Additionally, by the introduction of a chemical cross-linking (XL) step for affinity purified fractions, for the first time we are able to show that the intramolecular protein networks differ in detergent-dependent manner. Thus, we concluded that critical validation procedures including chemical XL should be implemented into standard analytical proteomic workflow of protein-protein interactions identified under surfactant-containing conditions to carefully depict true positive results.

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Abstract: A peptide-focused XL-MS approach to identify protein-protein interactions in complex samples

Cross-linking mass spectrometry (XL-MS) as a tool in structural biology is steadily expanding its range of application from purified protein complexes to organelles and entire cells. One main challenge is the so-called "n^2 problem", describing the exponentially increasing search space for the identification of two covalently linked peptides per spectrum. One solution is the usage of MS-cleavable cross-linkers, which break in the gas phase and enable the identification of each peptide separately. However, such a strategy requires longer cycle times and ETD and/or MS3 capable MS instruments. For non-cleavable cross-linkers, the n^2 problem has to be solved computationally. Thus, more and more algorithms are introduced with ever increasing search speed and faster scoring. Nonetheless, it is advantageous to restrict the protein database to the most abundant proteins in the sample. Here, we report an alternative search strategy by rather focusing on those peptides, which likely cross-link under experimental conditions. By using a thiol-cleavable cross-linker in a parallel experiment, we identify peptides containing a cleaved cross-link-tag after reduction and generate a peptide database for the subsequent database search of the actual cross-linking experiment with a non-cleavable cross-linker. Thereby, the search space and false positive rate are significantly decreased.
Applied on protein complexes of moderate complexity and known crystal structure, a peptide-focused approach had a high overlap with a conventional database search without substantially increasing the number of over-length cross-links. An analysis of in vivo cross-linked E. coli was ten times faster and led to significantly more identifications than a search against the entire E. coli proteome. Finally, we applied the strategy onto synaptosomes, a model system for brain synapses, to identify novel protein interactions involved in key functions like synaptic vesicle docking, fusion and recycling.

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Abstract: CaM/bMunc13-2 Interaction Studies by Cross-linking/Mass Spectrometry

Munc13 proteins are essential for vesicle docking and priming during synaptic short-term plasticity[1]. They are not only regulated by DAG, phorbol ester or calcium, but also by the highly conserved calcium-binding protein calmodulin (CaM)[2]. The complex between CaM and Munc13 is involved in the formation of the SNARE complex that drives synaptic vesicle fusion with the membrane[3]. So far, only sparse structural information is available for the CaM/Munc13 interaction and complex conformation has so far exclusively been observed with short Munc13 peptides comprising the known or proposed CaM binding sites. Here, we focus on the bMunc13-2 isoform located in the brain, which contains two putative CaM binding sites. Segments containing either one or both CaM binding sites were employed for cross-linking/MS and native MS experiments to obtain more detailed structural information on the CaM/bMunc13-2 complex.
Cross-linking experiments between CaM and the bMunc13-2 relied on the incorporation of photo-methionine[4] as well as on a variety of external cross-linkers. Cross-linked amino acids were identified by LC/MS/MS on an Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific). Cross-linking/MS revealed an interaction between CaM und bMunc13-2 and confirmed the expected 1:1 stoichiometry of the protein complex, which was confirmed by native MS (High-Mass Q TOF 2, MSVision/Micromass) of the intact complex. For gaining deeper insights into the interaction, a bMunc13-2 peptide containing the C-terminal CaM binding site with N-terminally flanking amino acids was docked to CaM by Rosetta[5]. The resulting two models of the CaM/bMunc13-2 complex displayed a helical structure of the bMunc13-2 peptide interrupted by a loop, allowing a slight flexibility of the peptideÂ´s N-terminus. In further cross-linking/MS and surface plasmon resonance experiments the focus was set to the N-terminally elongated CaM binding site. Our results give first hints on a CaM binding mode of bMunc13-2 that differs from the three other Munc13 isoforms Munc13 1, ubMunc13-2, and Munc13-3.

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P37

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Abstract:
S100A8 and S100A9 proteins exert a variety of important roles in human physiology and pathology. They make up to 60% of the protein fraction of human neutrophils. Inflammatory mediators or oxidative stress may induce very high expression of S100A8 and S100A9 in every human cell type. The proteins are laboratory markers used in diagnostics of rheumatoid arthritis, ulcerative colitis, Crohn's disease and established biomarkers of many types of human cancers including breast, prostate, pancreatic, liver or skin cancer. S100A8 and S100A9 play important roles in innate immunity as antibacterial and antifungal molecules produced by the human host.
The functional diversity of human S100A8 and S100A9 relies on binding of metal ions, oligomeric status and post-translational modifications of the proteins.
Our poster presents the application of hydrogen/deuterium exchange mass spectrometry (HDex-MS) to study differences in conformational dynamics between several S100A8 and S100A9 proteoforms.

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P38

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Abstract: Elucidation the structure of plasmid partitioning protein IncC using XL-MS

P39

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Abstract: Transcription factor limited proteolysis on MALDI compatible biochip

Proteolysis plays an irreplaceable role in most proteomic studies. Whereas complete digestion of studied protein is required in complicated proteomics bottom up workflows, an analysis of limited proteolysis products provides a valuable structural information immediately. Limited proteolysis enables to describe the most protease exposed protein regions, while the protected regions resist the protease activity for a longer time. Uncovering the proteolysis sensitive protein regions allow us to determine structured and unstructured part of proteins or to localize binding interfaces in protein complexes. In case of protein-nucleic acid complexes, represented by transcription factor in this work, the limited proteolysis allows to localize a nucleic acid binding site. Commonly used approaches to analyze limited proteolysis products are a traditional polyacrylamide gel electrophoresis, and HPLC separation of resulting peptides coupled usually with the ESI MS detections. Both approaches practically allow to analyze only a one part of reaction mixture - either high mass protein fragment with short missing sequences or low mass resulting peptides. Our approach for analysis combining in situ proteolysis with MALDI MS detection enables to gain maximum available information from each time step.
In this approach, an ambient soft-landing approach has been used for protease molecule immobilization to the MALDI compatible ITO (Indium Tin Oxide) glass. The ambient soft landing is an electrospray based technique operates under very mild atmospheric conditions and perform noncovalent and nondestructive, but very strong interaction between immobilized protease and conductive surface. Since it is a very difficult to study protein-nucleic acid interaction by conventional mass spectrometry techniques, protein-DNA complexes were selected to test a potential of our technology. Studied transcription factors were directly applied on proteolytical biochips spots, where the proteolysis was discontinued at defined time by the acidic water solution. An acidic pH irreversibly inhibits used serine proteases. After rapid vacuum drying, all spots were overlaid by acidic MALDI matrix and analyzed using MALDI-TOF MS instrument in linear and reflectron mode. A combined information obtained from both MS modes allow us to localize the protein-DNA interaction interface.

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Abstract:
Deregulation of the ubiquitin ligase E6AP is causally linked to the development of human disease, including cervical cancer. In complex with the E6 oncoprotein of human papillomaviruses, E6AP targets the tumor suppressor p53 for degradation, thereby contributing to carcinogenesis. Moreover, E6 acts as a potent activator of E6AP by a yet unknown mechanism. However, structural information explaining how the E6AP-E6-p53 enzyme-substrate complex is assembled, and how E6 stimulates E6AP, is largely missing. We therefore developed and applied different approaches in structural mass spectrometry. Using qualitative and quantitative XL-MS approaches, we show that binding of the E6 oncoprotein induces conformational changes within full-length E6AP, which result in the positioning of E6 and p53 in the immediate vicinity of the catalytic centre of E6AP. Since conventional XL-MS cannot readily distinguish between intra-subunit crosslinks and inter-subunit crosslinks between individual protomers within a homo-oligomer, we combined XL-MS with stable isotope labeling with amino acids in cell culture (SILAC) and termed this approach SILAC-XL-MS. Data obtained by this approach indicate that E6AP is capable of engaging in homo-oligomeric contacts via the HECT domain.

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Abstract:
Mycoplasma pneumoniae is a human pathogen that causes atypical bacterial pneumonia. Besides, it is known for its reduced genome, which makes it the simplest known self-replicating organism and as such, it is a prime candidate in the hope to model an entire cell. Up to now, a large amount of quantitative data has been collected on its genome, transcriptome, proteome and metabolism. Most information on the organisation of the proteome so far has been inferred from affinity-pulldown mass spectrometry working with cell lysates.

Here we present the first in-cell cross-linking mass spectrometry data for this organism, which provides unprecedented detail on the organisation of the proteome in situ. Addition of a cross-linking reagent covalently links proximal residues within the cell's proteins which are subsequently identified by mass spectrometry. These identified cross-links provide distance restraints that allow us to structurally model proteins and their interactions. This is the most comprehensive cross-linking data set ever reported with cross-links identified across 98% of the expressed proteome. The identification of higher-order complexes linking different cellular processes and the modelling of their interaction interfaces will be discussed.

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Abstract:
Native mass spectrometry has emerged as a powerful technique to study protein-ligand interactions and elucidate the structure of macromolecular assemblies, including both soluble and membrane protein complexes. Top-down studies of intact protein complexes have been reported since the early 1990's, but their characterization using MS3 have only recently been reported and most work has been done on homomeric assemblies. However, poor fragmentation into subunits and stripped complexes in the front end of the MS limits the use of current MS instrumentation for native top-down analysis using a pseudo-MS3 approach. In this work we examine this limitation and explore new ways for extending native top-down performance to allow interrogation of heteromeric protein assemblies like proteasome by top-down pseudo-MS3. During such analysis the intact protein complex is initially transferred through the mass spectrometer without fragmentation using only moderate desolvation energy, yielding the MS1 spectrum. Dissociation of the protein complex into its composing subunits in the inject flatapole region gives the MS2 spectrum, and it is followed by quadrupole selection and fragmentation of individual subunits in the HCD cell enabling sequence analysis through the MS3 spectrum. In this work we demonstrate outstanding performance of the new Q Exactive UHMR mass spectrometer for structural characterization of homomeric and heteromeric protein assemblies using native MS and native top-down analysis.

P43

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Abstract: Impact of Ligand Binding on Photodissociation of Protein Copmlexes

The ability of electrospray ionization to maintain non-covalent interactions as a protein is lifted from solution to the gas phase has advanced mass spectrometric studies of protein-protein and protein-ligand complexes. Many activation methods have been utilized to investigate dissociation patterns of these complex systems as it relates to their biological behavior. Ultraviolet photodissociation (UVPD) with 193 nm photons has demonstrated the capability of generating significant sequence ions directly from native protein complexes as well as fragment ions that maintain ligands or cofactors (i.e. holo-fragments). These attributes of UVPD have motivated our interest in evaluating its effectiveness for obtaining structural information of ligand-bound protein complexes. Here, UVPD and HCD are utilized to characterize oligomeric protein-ligand complexes using a prototype Thermo Q Exactive Plus Orbitrap instrument that was custom-modified to enable an ultra-high mass range (UHMR). The stabilizing or destabilizing effect of ligand binding was investigated for three model protein tetramers. Results obtained using UVPD demonstrate the increased characterization of model complexes that allow for inference of structural changes upon ligand binding and active site localization due to enhanced sequence coverage and distribution of holo-fragments.

P44

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Abstract: Mechanism of Parkin activation by PINK1.

Mutations in the E3 ubiquitin ligase parkin (PARK2) and the protein kinase PINK1 (PARK6) are linked to autosomal-recessive juvenile parkinsonism[1,2]. At the cellular level, these mutations cause defects in mitophagy, the process that organizes the destruction of damaged mitochondria[3,4]. Parkin is autoinhibited, and requires activation by PINK1, which phosphorylates Ser65 in ubiquitin and in the parkin ubiquitin-like (Ubl) domain. Parkin binds phospho-ubiquitin, which enables efficient parkin phosphorylation; however, the enzyme remains autoinhibited with an inaccessible active site[5,6]. It is unclear how phosphorylation of parkin activates the molecule. Here we follow the activation of full-length human parkin by hydrogen-deuterium exchange mass spectrometry, and reveal large-scale domain rearrangement during the activation process. Our data show how autoinhibition in parkin is resolved, and suggest a mechanism for how parkin ubiquitinates its substrates via an untethered RING2 domain.

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Abstract: Stepped-Collision Energy HCD improves MS2-based identification of DSSO Cross-Linked Peptides

Cross-linking mass spectrometry (XLMS) is becoming increasingly popular, and current advances are widening the applicability of the technique so that it can be utilized by non-specialist laboratories. Specifically, the use of novel mass spectrometry-cleavable (MS-cleavable) reagents dramatically reduce complexity of the data by providing i) characteristic reporter ions and ii) the mass of the individual peptides, rather than that of the cross-linked moiety. However, optimum acquisition strategies to obtain the best quality data for such cross-linkers with HCD (high energy collisional induced dissociation) alone is yet to be achieved. Therefore, we have carried out careful optimization of MS parameters to facilitate the identification of disuccinimidyl sulfoxide (DSSO)- based cross-links (XLs) on HCD-equipped mass spectrometers. From the comparison of 9 different fragmentation energies we developed several stepped- HCD fragmentation methods that were evaluated on a variety of cross-linked standard proteins. The optimal stepped-HCD-method was then directly compared with previously described methods using an Orbitrap Fusion Lumos instrument using a high-complexity sample. The final results indicate that our stepped-HCD method is able to identify more XLs than other methods, mitigating the need for MS3 enabled instrumentation and alternative dissociation techniques.

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Abstract: Cross-linking of SCML2 Polycomb Protein to Nucleosomal DNA

Protein-DNA interactions are at the center of numerous cellular processes ranging from structural to catalytic activities that are mediated by DNA-containing protein complexes. In recent years, we have established UV-irradiation based CX-MS as a tool to analyze nucleic-acid-protein interactions [1]. We have successfully cross-linked histone proteins to DNA in nucleosomal context. Here, we used human SCML2, an MBT-repeat containing Polycomb protein that is part of PRC2, to increase the complexity of our model system. SCML2 binds methylated and ubiquitinated histones [2,3] and interacts with RNA [4] and DNA via its SLED domain [5]. We found SCML2 to bind to unmodified nucleosomes independent of their DNA length. When incubated with nucleosomes and exposed to UV-irradiation, we detected a number of protein-DNA cross-linking sites. All sites were histidine or cysteine residues cross-linking to the deoxyribose moiety of DNA. Next, we irradiated nucleosomes containing linker histone H1 together with SCML2. We detected the same cross-liking sites, and in addition found cross-linking sites in the SLED domain and in a putatively unstructured region that did not display DNA cross-links in the absence of linker histones. In contrast to the cross-links in the SCML2-nucleosome complex, these new cross-linking sites were established to thymine (via Lys and Tyr) and not to deoxyribose, indicating a change in SCML2 binding behavior in nucleosome+H1 complexes.

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P47

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Abstract:
BioMS is a nationally distributed infrastructure for biological mass spectrometry and proteomics. Each site offers specialized analytical techniques for the Swedish life science community. BioMS also performs education and outreach activities in order to increase the awareness of Biological mass spectrometry. Today BioMS offers the following technologies: proteogenomics, targeted proteomics, glycoproteomics, structural MS, glycomics, lipidomics, and chemical proteomics. This poster describes BioMS activities and support for structural proteomics.

Hydrogen deuterium mass spectrometry (HDX)
Two different platforms are used for HDX MS in Lund a Q Exactive plus equipped with liquid handling robot on a LEAP H/D-X PAL™ platform is dedicated for HDX MS analysis. At Karolinska Institutet, the HDX MS system consist of a CTC PAL Liquid Handling System connected to a custom made Peltier-cooled box containing Pepsin and LC columns. Automation is controlled via Chronos Software (Axel Semrau), and data acquisition is perform on an Orbitrap Q Exactive mass spectrometer. For both system, data analysis and visualization is performed with HDExaminer from Sierra Analytics.

Chemical cross linking mass spectrometry (CXMS)
Samples in CXMS projects are processed with 1-D Page analysis followed by in gel digestion or in solution digestion after chemical crosslinking with different crosslinkers. Enrichment of type 2 crosslinked peptides is performed by a single step method (1). Samples are then analysed on a Fusion MS tribid system and data analysis is made with Stavrox (2) or Merox (3).

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P48

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Abstract: Feasibility of succinimidyl-based crosslinking at slightly acidic conditions

Introduction
Cross-linking (XL) mass spectrometry (MS) is an emerging biophysical technique able to probe structures of protein and protein complexes by generating structural constraints between amino acids. The most commonly used XL reagents are succinimidyl-based (e.g. BS3, DSS, DSSO or DSBU) and primarily cross-link lysine side chains. So far, these linkers have predominantly been used to investigate protein structures at neutral to slightly basic pH (7-8) to ensure reactivity of the lysine side chain. However, disease-related molecular processes are not limited to such pH ranges, e.g. in acidic intracellular compartments or in case of viral entry into a host cell. Here, we investigated the ability of DSS to probe protein structures at slightly acidic conditions (pH 6.0) to increase the versatility of the XL-MS technique.

Methods
A standard mixture of eight proteins was prepared in either 20 mM MES pH 6.0 buffer or 20 mM HEPES pH 7.5 buffer. To the protein mixtures, 1 mM DSS H12/D12 (Creative Molecules Inc.) was added and samples were incubated for 30 min at 25°C, the reaction was then quenched with NH4HCO3 to a final concentration of 50 mM. The cross-linked protein mixture was digested by trypsin and the resulting peptides were separated by size-exclusion chromatography. The top three XL-containing peptide fractions were analyzed by LC-MS. Here, the peptides were separated by nano-flow reverse-phase liquid chromatography prior to mass analysis on an Orbitrap Elite (Thermo) system. The mass spectrometer was operated in DDA-CID mode with precursor and fragment ions detected in the orbitrap and the linear Ion trap, respectively.

Preliminary data
A potential concern of lowering the pH of the reaction mixture is the possibility of increasing the amount of side reactions. To check for possible side reactions the acquired data were searched by the MODa algorithm, which performs an unrestricted search for peptide modifications. No substantial difference in the distribution of modified residues was observed for DSS specific mass increases (+156 Da and +168 Da), when comparing the reactions at pH 7.5 and pH 6.0.
Cross-linked peptides were identified by the software xQuest using a score cut-off of 25. A total of 80 and 76 non-redundant protein cross-links were observed at pH 6.0 and pH 7.5, respectively, and 64 of the identified XLs were identified at both pH conditions. The distance distributions of the identified cross-links (Euclidean $C\alpha$-$C\alpha$ distances as determined by PyMOL) were highly similar with a slight increase in the mean distance of XLs, from 16.4 Å to 17.2 Å when the pH was decreased from pH 7.5 to pH 6.0. Furthermore, a small increase (from two to four) in the number of XLs with non-allowed distances (>35 Å) was observed when the pH was decreased. However, no statistical difference between the two distance distributions was detected, as compared by a two-sample Kolmogorov-Smirnov test (p=0.996). This further shows that the structural integrity of the investigated proteins was preserved even at pH 6.0.
In conclusion, the presented results highlight the potential of succinimidyl-based XL reagents to cross-link proteins and protein complexes not only at neutral to slightly basic conditions, but also at slightly acidic conditions (pH 6.0). An ability of succinimidyl-based XL reagents, which enables structural characterization of proteins and protein complexes found at acidic conditions, e.g. molecular machines in acidic intracellular compartments or protein complexes implicated in viral entry into a host cell.

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Abstract: Regulation of Tomm34 - chaperone interaction by 14-3-3 proteins

Maintenance of stable protein homeostasis is ensured by ubiquitous complex chaperone network cooperation in the sense of proper protein folding, prevent their aggregation or mediate degradation. The chaperone protein specificity is determined by interaction with its co-chaperones and one of such modulator of chaperone action is Tomm34. This protein contains a conserved solvent exposed linker sequence between its TPR domains which is engaged in the regulation of Tomm34 ATP-dependent binding to Hsp70. Simultaneously the linker comprises overlapped binding motifs for Protein Kinase A and 14-3-3 protein family. The increased levels of chaperone - co-chaperone complexes were identified in cancer cells and thus represent interesting targets in cancer therapy.
The proteins were designed, expressed in E. Coli and purified. We have verified that Tomm34 can be phosphorylated in vitro and showed that the phosphorylation is crucial for binding to 14-3-3 proteins by combination of size exclusion chromatography and structural mass spectrometric techniques. To study this interaction in more detail we prepared selected Tomm34 serine mutants and showed that Ser160 located in the interdomain linker is the key residue for the interaction. Interestingly, Ser93 from the first TPR bundle had also some role in the efficient binding. By employing HDX-MS measurements we then further studied the influence of 14-3-3 protein in the context of Tomm34-Hsp70/Hsp90 interaction.

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Abstract: Relative Quantification of TMT-labeled, cross-linked proteins using XlinkX node in Proteome Discoverer software

Introduction: To study structural and interaction dynamics of protein complexes, multiple quantitative cross-linking mass spectrometry (QXL-MS) strategies based on isotope-labeled cross-linkers or isobaric-labeled cross-linked peptides have been developed. Quantitation of Multiplexed, Isobaric-labeled cross(X)-linked peptides or QMIX1 is a multiplexed QXL-MS strategy that combines MS-cleavable cross-linkers with isobaric labeling reagents and novel hybrid mass spec acquisition methods. Although it was possible to identify TMT-labeled, cross-linked peptides using XlinkX 1.0 node in Thermo Scientific™ Proteome Discoverer 2.2, quantification was not implemented. In this study, we describe a new, complete analytical workflow in Proteome Discoverer 2.3 for identification and multiplex quantitation of cross-linked peptides, which can be directly applied to study conformational dynamics of protein complexes and protein-protein interactions at the proteome scale.
Methods: Cytochrome C and rabbit 20S proteasome complex in PBS buffer (pH 7.4) or HEPES buffer (pH 8.0) were reacted with DSSO in a molar ratio of 1:5 (protein:cross-linker) for 1 h at room temperature and quenched with excess hydroxylamine. Cross-linked proteins were then pelleted via TCA precipitation, resuspended, and digested. The resulting peptide mixtures were de-salted, fractionated by peptide size exclusion and TMT labeled according to manufacturer's instructions. Samples were separated using a 50cm Thermo Scientific™ EASY-Spray™ column and a Thermo Scientific™ EASY-nLC™ 1200 UPLC system in 60 min gradient, followed by detection on the Thermo Scientific™ Orbitrap Fusion™ Lumos™ mass spectrometer. Data were analyzed using Proteome Discoverer 2.3 and XlinkX 2.0 node to automatically identify and quantify TMT-labeled, cross-linked peptides.
Preliminary Data: Multiplexed protein quantitation using TMT isobaric tags currently provide the highest level of sample multiplexing. Using MSn analyses such as SPS-MS3 has been shown to greatly reduce peptide interference which can affect isobaric tag quantitation. MS2-MS3 methods are also some of the most preferable methods for identification of MS-cleavable cross-linked peptides.
We established a new QMIX workflow that addresses the major difficulties in cross-linking data analysis, identification and quantification. Our novel approach comprises three essential features: the use of MS-cleavable cross-linkers, a novel hybrid MS2-MS3- SPS fragmentation strategy and a dedicated cross-link search engine for data analysis (XlinkX 2.0) implemented into Proteome Discoverer software.
For TMT quantification, reporter ion abundances were corrected for isotopic impurities based on the manufacturer's data sheets. Signal-to-noise (S/N) values were used to represent the reporter ion abundance with an co-isolation threshold of 75% and an average reporter S/N threshold of 10 and above required for quantitation spectra to be used. The S/N values of peptides, which are summed from the S/N values of the peptide spectral matches (PSMs), are finally summed to represent the abundance of the proteins. In the case of cross-linked peptides, crosslinked quantitation ratios are calculated from the S/N values of the cross-linked spectral matches (CSMs). Only SPS spectra were used for quantification.
Initial experiments using Cytochrome C confirmed that cross-linked peptides labeled with TMT reagents can be quantified using the SPS method and identified using a single notch in MS3. We successfully tested this workflow to measure changes in the structural dynamics of rabbit 20s proteasome complex in different buffer systems.
Refs:1. C.Yu et al, Anal.Chem, 2016, 10301-10308
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Abstract: Epitope Mapping of monoclonal VEGF antibodies by HDX-MS

Hydrogen/deuterium exchange (HDX) monitored by mass spectrometry (MS) is an emerging powerful analytical method for investigation of protein conformation, dynamics and intermolecular interactions. HDX-MS monitors the exchange of hydrogen isotopes in protein backbone amides and thus serves as a sensitive method along the entire protein backbone. It allows an in-depth characterization of proteins in the native state and therefore contributes to a better understanding of proteins and their functions.
In pharmaceutical research HDX is an established technology to study antibody-antigen interactions to better understand mode of action in early project phases. Here, we present our results on epitope mapping studies of the Vascular Endothelial Growth Factor (VEGF) in interaction with anti-VEGF antibodies AB1 and AB2.

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Abstract: Chemical RNA-protein cross-linking – A detailed characterization of bifunctional reagents: 2-iminothiolane, 1,2,3,4-diepoxybutane and mechlorethamine

A considerable number of common human diseases result from errors during gene expression which is a complex process underlying manifold protein-protein, DNA-protein and RNA-protein interactions. Numerous studies have been performed contributing to the elucidation of the thereof arising complicated network of these biopolymers. Owing to massively increased resolution power and sensitivity of mass spectrometry (MS) instruments, MS-based approaches have become today's gold-standard for protein-protein, DNA-protein as well as RNA-protein interaction site determination within peptide sequences at amino acid resolution. The stabilization of these interactions is prerequisite for MS analysis to keep them stable in the gas phase. For the analysis of protein-protein and DNA-protein interactions, chemical cross-linking has widely been used, while RNA-protein cross-linking is still commonly performed by UV-irradiation which works grossly inefficient. Our work provides detailed characterization of three chemical RNA-protein cross-linking agents, namely 2-iminothiolane (2-IT), 1,2:3,4-diepoxybutane (DEB) and the nitrogen mustard prototype mechlorethamine (NM). By first performing denaturing polyacrylamide gel electrophoresis (PAGE) of [Î^{3}32P]-labeled RNA cross-linked with the known RNA-binding protein (RBP) Hsh49 from yeast, we could i) rapidly determine optimal experimental conditions for 2-IT-, DEB- and NM-based RNA-protein cross-linking and ii) inexpensively estimate 2-IT, DEB and NM cross-linking efficiencies. In subsequent MS/MS analyses of cross-linked RNA oligonucleotides with the protein Hsh49, we evaluated the specificity of the cross-linking agents. We set up individual parameters for 2-IT, DEB and NM database searches to automate cross-link assignment after MS/MS analysis. Additionally, we investigated the suitability of 2-IT, DEB and NM in an application-oriented context by cross-linking Dnmt2 methyltransferase from Schizosaccharomyces pombe with its cognate tRNAAsp and compared UV-light induced with 2-IT-, DEB- and NM-based cross-linking. In summary, 2-IT-based RNA-protein cross-linking performance did not outrace UV-light induced cross-linking. However, the results of this study show, that DEB-, NM-based and UV-light induced cross-linking deliver complementary information and may be used in combination for a comprehensive, MS-based interaction site analysis.

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Abstract: CID, ETD and ECD Fragmentation to Study Protein Modifications Using Hydroxyl Radicals

Chemical-labeling methods of protein (protein footprinting) have proven to be vastly successful to gain insights into the protein structure. Most of the approaches include hydrogen deuterium exchange (H/DX), stable chemical labeling and hydroxyl radical footprinting. Among these, hydroxyl radical labeling applications are currently fast developing technique. Hydroxyl radicals can be generated by various methods, including Fenton chemistry, electrochemistry, synchrotron radiolysis of water and fast photochemical oxidation of proteins (FPOP). FPOP relays on dissociating hydrogen peroxide using excimer laser (248 nm) and represents an efficient way of the fast protein labeling. To investigate products of labeling reaction, bottom up approach is mainly used to identify modified amino acids.
In this study, we tested the potential of Top Down mass spectrometry to characterize the oxidized ubiquitin. FPOP labeling was performed for 250 ms using 248 nm KrF excimer laser to dissociate the hydrogen peroxide (3 mM, $H_2O_2 \rightarrow 2HO\bullet$) that modify the protein. We analyzed the results of protein footprinting at both - the protein and the amino acid residue levels. At the protein level, comparing the native and oxidized protein spectra, the most of the modifications appeared as +16 Da. Subsequently, different fragmentation techniques were applied to identify the site of modification: collision-induced dissociation (CID), electron-capture dissociation (ECD) and electron-transfer dissociation (ETD). Different ion types can provide complementary information for the structural characterization of protein. Met1, Phe4, lle44, Phe45 and His68 were found to be modified for ubiquitin when comparing the CID and ETD. Moreover, it had been found that ECD generates more sequence ions than ETD. Oxidation was observed for Leu73, Arg74 and Gly76 as well. Thus, such comprehensive Top Down experiment determined oxidized amino acid residues of single modified protein.

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