9th Symposium on Structural Proteomics 2019

Nov 3rd
Nov 6th

GÖTTINGEN
GERMANY

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organizing committee
Henning Urlaub | Evgeniy Petrotchenko | Christoph Borchers

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Organization

GEORG-AUGUST-UNIVERSITÄT GÖTTINGEN

Max Planck Institute for Biophysical Chemistry

Conference Venue

Tagungs- und Veranstaltungshaus Alte Mensa
Wilhelmsplatz 3
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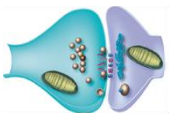

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doors open 6.15 pm **– admission only with name card!**
welcome and buffet opening 6.45 pm

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Workshop 1:00-3:15 pm

Adam-von-Trott Hall

Crosslinking Mass-Spectrometry with xi - Going from Raw-Files to Confident Identifications

Lutz Fischer (1,2)

(1) Bioanalytics, Institute of Biotechnology, Technische Universität Berlin, 13355 Berlin, Germany; (2) Wellcome Centre for Cell Biology, University of Edinburgh, Edinburgh EH9 3BF, United Kingdom

Crosslinking is more and more becoming an important tool to elucidate structural and functional insights into protein structures, protein conformations and protein interactions - from single proteins over complexes to proteomic scale interaction networks.
The first part of the workshop will start with a set of raw files and will walk you through all the steps to getting a list of confident identifications. Explaining along the way the usage MSConvert (http://proteowizard.sourceforge.net/download.html) of xiSEARCH, xiFDR (https://rappsilberlab.org/ software/).
To follow along with this workshop we recommend that you have a window notebook.

xiVIEW: Interactive visualisation and exploration of crosslinking data

Martin Graham

Wellcome Centre for Cell Biology, University of Edinburgh, Edinburgh EH9 3BF, United Kingdom

This workshop centers on the usage of the freely usable online tool xiVIEW (https://xiview.org). Here, attendees will be shown how to manipulate multiple representations of cross-linking data, along with synchronised filtering capabilities. The ability to use an integrated PDB viewer (NGL) to view and filter by crosslink distances will be of interest to many. Time permitting, attendees may use xiVIEW on their own datasets (requires prior xiview registration and data upload). Finally, the ability to export filtered data sets and publication quality figures will be practiced. Prerequisites: A computer with a modern HTML5-compatible browser is necessary, Chrome is recommended.

Workshop 1:00-3:00 pm

Emmy-Noether Hall

Xlink Analyzer: software for integrative analysis of cross-links and other restraints in the context of three-dimensional structures

Karol Kaszuba, Jan Kosinski

CSSB/EMBL Hamburg c/o DESY, Notkestraße 85, 22607 Hamburg, Germany, E-mail: kkaszuba@embl-hamburg.de, jan.kosinski@embl.de

Xlink Analyzer software allows for visualization and analysis of cross-links and other restraints in the context of three-dimensional structures or structural models from hybrid methods. Xlink Analyzer automatically visualizes the cross-links, distance restraints and interacting residues, calculates restraint violation statistics, and allows for several interactive manipulations that facilitate analysis of subsets of restraints. It provides a flexible interface to manage subunits and subcomplexes, which greatly facilitates analysis of large complexes. Xlink Analyzer is implemented as a plugin to UCSF Chimera, a standard structural biology software tool, and thus enables seamless integration of the restraints with, e.g. fitting of X-ray structures to EM maps, analyzing multi-scale integrative models and modeling simulations, and performing interactive modeling based on the restraints. The tutorial will give an overview of the interface and its functionality using various examples.

Xlink Aanylzer is available for download at

https://www.embl-hamburg.de/XlinkAnalyzer/XlinkAnalyzer.html

(For the tutorials, please install the UCSF Chimera and the Xlink Analyzer plugin beforehand following the instructions at
https://www.embl-hamburg.de/XlinkAnalyzer/documentation.html)

Workshop 3:30-5:30 pm

Emmy-Noether Hall

RNPxl: Identifying peptide-nucleic acid cross-links using the OpenMS tool RNPxl

Alexander Wulf (1), Timo Sachsenberg (2, 3)

(1) Bioanalytical Mass Spectrometry Group, Max Planck Institute for Biophysical Chemistry, 37077 Göttingen, Germany; (2) Institute for Bioinformatics and Medical Informatics, University of Tübingen, 72076 Tübingen, Germany; (3) Applied Bioinformatics, Dept. for Computer Science, University of Tübingen, 72076 Tübingen, Germany

Nucleic acids play a vital role in a multitude of cellular processes such as DNA replication, RNA transcription, and mRNA translation. To accommodate this wide range of different functions, nucleic acids in general form different types of protein-nucleic acid complexes. Studying these complexes by cross-linking coupled with mass spectrometry enables researches to answer questions about nucleic acid binding positions within a protein. Addressing this question involves a three step workflow: Sample preparation, data acquisition, and data evaluation. Sample preparation involves complex reconstitution, cross-linking, digests, and clean-up processes to generate samples for high-resolution mass spectrometric analyses. In data acquisition, instrument parameters need to be adjusted for cross-linked samples. Lastly, data evaluation relies on dedicated software to search for, identify, and annotate spectra containing peptide-nucleic acid heteroconjugates that would indicate a protein-nucleic acid binding site. A special focus is placed on how to use the software, including setting up a workflow, adjusting parameters, and evaluate potential hits. In this workshop, participants will learn how to approach the three individual steps to protein-nucleic acid cross-linking coupled with mass spectrometry, and thus how to identify protein-nucleic cross-linking sites based on spectrometric data. The dedicated software tool RNPxl (implemented in OpenMS) has been successfully utilized over the years to identify protein-nucleic acid cross-links and will be used in this workshop to demonstrate all important aspects of data evaluation and cross-link identification.

Workshop 3:30-5:30 pm

Adam-von-Trott Hall

Optimal use of MS cross-linking data in modelling biomolecular complexes by combining DISVIS and HADDOCK

J. Schaarschmidt (1, 2), G.C.P. van Zundert (3), M. Trellet (1), A.M.J.J. Bonvin (1)

(1) Bijvoet Center for Biomolecular Research, Faculty of Science - Chemistry, Utrecht University, Netherlands; (2) Institute of Nanotechnology, Karlsruhe Institute of Technology, Germany; (3) Schrödinger LLC, United States

In order to study macromolecular interactions where no experimental structures of the complex of interest are available, computational methods can be a powerful tool to generate structural models of the system. HADDOCK is a widely-used integrative platform that supports a large variety of experimental data for the modelling of biomolecular complexes. A powerful method providing experimental restraints is chemical crosslinking of reactive residues coupled to the identification of linked residues by mass spectrometry. The workshop will cover an approach that combines DisVis, another software developed in our lab, and HADDOCK to efficiently use the crosslink data in prediction of the complex. First DisVis performs an exhaustive search of the six-dimensional interaction space allowing to identify possible false positive restraints in the dataset. It also enriches the information from MS cross-links by mapping the most contacted residues on the surface of the molecule. The workshop will demonstrate, how this augmented information (the combination of MS-derived distance restraints and mapped interfaces from DISVIS) leads to better docking results.

Keynote 6:00-7:00 pm

Adam-von-Trott Hall

Integrative modeling of biomolecular assembly structures

Andrej Šali

Department of Bioengineering and Therapeutic Sciences, Department of Pharmaceutical Chemistry, and California Institute for Quantitative Biosciences (QB3), University of California, San Francisco, San Francisco, California 94143, United States

The networks and spatial structures of biomolecular interactions provide insights into their function and thus help us to understand the workings of living cells. Detailed structural characterization of large and often dynamic assemblies and their networks is generally impossible by any single existing experimental or computational method. This challenge can be overcome by hybrid approaches that integrate data from diverse biophysical experiments (eg, X-ray crystallography, NMR spectroscopy, electron microscopy, chemical cross-linking, yeast-two hybrid system, and various chemical genetics and proteomics approaches). We formulate the hybrid approach to structure and/or network determination as an optimization problem, the solution of which requires three main components: the representation of the assembly or network, the scoring function, and the optimization method. The ensemble of solutions to the optimization problem embodies the most accurate characterization given the available information. The key challenges remain translating experimental data into restraints on the structure, combining these spatial restraints into a single scoring function, optimizing the scoring function, and analyzing the resulting ensemble of solutions. The approach will be illustrated by several applications to specific biological systems, including the integrative modeling of the structure, function, and assembly of the nuclear pore complex

Keynote 9:00-9:50 am

Adam-von-Trott Hall

Developing Cross-linking Mass Spectrometry to Define Protein Interactions and Structures

Lan Huang

Department of Physiology & Biophysics, University of California, Irvine, USA

Protein-protein interactions (PPIs) play a key role in defining protein functions in biological systems. Perturbations of PPIs fundamental to the structure and function of protein complexes can cause deleterious effects on cellular activities and thus lead to various human diseases. Thus, detailed characterization of PPIs is not only critical to unraveling molecular details that underlie human pathologies, but also important for identifying potential targets for better therapeutics. In recent years, cross-linking mass spectrometry (XL-MS) have become a powerful structural tool for mapping PPIs and elucidating architectures of large protein complexes. In comparison to standard structural methods, XL-MS offers distinct advantages due to speed, accuracy, sensitivity and versatility, especially for the study of heterogeneous and dynamic protein complexes. Despite its great potential, XL-MS analysis remains challenging due to the difficulty in unambiguous identification of cross-linked peptides. To advance XL-MS studies, we have developed a series of sulfoxide-containing MS-cleavable cross-linkers to enable simplified and accurate identification of cross-linked peptides1,2. In addition, we have developed new sample preparation strategies to facilitate XL-MS analysis of protein complexes. Moreover, quantitative XL-MS methods have been established to define conformational changes. Here, we will present new developments in sulfoxide-containing MS-cleavable reagents based XL-MS approaches and their integration with structural modeling to uncover protein structural dynamics that is inaccessible to other structural tools. The analytical platform described here can be adopted to study other protein complexes. This work is supported by NIH grants R01GM074830 and R01GM130144 to L.H. Ref 1. Kao, A. et, al. MCP, 2011; 2. Yu, C. et al, Anal. Chem. 2018.

Talk 9:50-10:20 am

Adam-von-Trott Hall

Integrating protein-RNA cross-linking into hybrid structural biology projects

Alexander Leitner (1), Chris P. Sarnowski (1), Michael Götze (1), Ruedi Aebersold (1), Georg Dorn (2), Julien Boudet (2), Sébastien Campagne (2), Christine von Schroetter (2), Ahmed Moursy (2), Frédéric H.-T. Allain (2)

(1) Department of Biology, Institute of Molecular Systems Biology, ETH Zürich, Zurich, Switzerland; (2) Department of Biology, Institute of Molecular Biology and Biophysics, ETH Zürich, Zurich, Switzerland

The study of protein-RNA complexes by conventional structural biology methods is frequently complicated by the flexibility of such ribonucleoproteins (RNPs). Crystallography and electron microscopy are therefore not always suitable techniques, and NMR spectroscopy is typically limited to smaller targets. For this reason, UV-induced photochemical cross-linking is a promising technique to apply to the structural characterization of RNPs.
To enable the precise localization of cross-linking sites at the protein and RNA level, we make use of stable isotope labeling in a method we have called cross-linking of segmentally isotope-labeled RNA coupled to MS, or CLIR-MS in short. By specifically labeling portions of the RNA (segments down to individual nucleotides), we introduce a unique signature that allows the determination of the binding site with up to single amino acid and nucleotide resolution. As we will show, CLIR-MS is applicable to both in vitro transcribed or chemically synthesized RNA. Moreover, if an exact localization of the binding site on the RNA is not required, isotope labels can be introduced later in the workflow for enhanced data analysis.
Protein-protein cross-linking has been used in hybrid structural modeling strategies for a number of years. The integration of protein-RNA contacts has not been explored in a similar way because of the difficulties of localizing the cross-linking sites on both types of biomolecules. With the help of CLIR-MS, we can now combine this type of spatial restraints with other structural biology methods. I will present data from our ongoing effort to model the structure of the complex of polypyrimidine tract-binding protein 1 and its interacting RNA, the encephalomyocarditis virus internal ribosomal entry site, with data from CLIR-MS, NMR spectroscopy, electron paramagnetic resonance spectroscopy, and small-angle neutron scattering. For this purpose, mechanistic studies on UV-induced cross-linking on model complexes help to better define spatial restraints for hybrid modeling.

Selected Talk 10:50-11:05 am

Adam-von-Trott Hall

Using XL-MS to map the interaction of the oncogenic transcription factor N-Myc with a kinase

J. Pitts (1), E. Leen (1), C. Iacobucci (2), A. Sinz (2), F. Sobott (1), R. Bayliss (1), M. Wright (1)

(1) Astbury Centre for Structural Molecular Biology and School of Chemistry, University of Leeds, Leeds, LS2 9JT, UK; (2) Department of Pharmaceutical Chemistry & Bioanalytics, Institute of Pharmacy, Charles Tanford Protein Center, Martin Luther University Halle-Wittenberg, Kurt-MothesStr. 3a, D-06120 Halle (Saale), Germany

The pleotropic oncogenic transcription factor Myc is deregulated or amplified in the majority of cancers. Myc regulates 15 % of human genes, and most notably controls cell division and apoptosis. It has proven difficult to obtain crystal structures of the N-Myc transactivation domain (TAD) in complex with potential drug target kinases. Here we use crosslinking mass spectrometry (XL-MS) to explore how Aurora Kinase A (AURKA) and Polo-like Kinase-1 (PLK1), which are known to cooperate and are critical to mitosis, interact with N-Myc. XL-MS can give insights into the structure and dynamics of proteins and complexes. The nucleophile-reactive chemical cross-linker disuccinimidyl dibutyric urea (DSBU) was used in-combination with XL-MS analysis tool MeroX to build a model of the interactions of Myc TAD with Aurora A and PLK1. Using recombinant protein, we validate the XL-MS workflow against a crystal structure of AURKA and a small fragment of N-Myc. Our data show the potential for Myc to directly activate PLK1. PLK1 is known to cooperate with Myc to drive tumorigenesis. XL-MS gives a novel structural basis of this outcome.

Talk 11:05-11:35 am

Adam-von-Trott Hall

Membrane proteins and their environments

Nina Morgner, Khanh Vu Huu, Oliver Peetz, Nils Hellwig

Institute for physical and theoretical chemistry, Goethe-University Frankfurt/Main, Germany

Membrane proteins are of high interest, but still underrepresented in research due to the inherent difficulties for many investigation methods, arising from their hydrophobic nature. Depending on the feature of the membrane protein complex the researcher is interested in (protein stoichiometry, lipid affinity, annular lipids…), different means of solubilisation can enable MS analysis.
LILBID (Laser Induced Liquid Bead Ion Desorption) is an ionization method which employs a droplet generator to produce analyte droplets of 30-50um diameter at a frequency of 10Hz. These droplets are irradiated by a mid-IR laser leading to the explosive expansion of the droplet. The solvated ions are set free and are mass analysed.
The method is especially suited for the analysis of membrane protein complexes as for example an ATPase, for which we can analyse not only the composition, but as well the assembly process itself and the conditions, which are required for correct assembly.
For some questions different means of solubilisation are required. We investigated the usability of artificial membranes for the study of membrane protein complexes with LILBID-MS. This allows for example the study of lipid dependent oligomerization. LILBID-MS is well suited for the analysis of membrane proteins complexes solubilized by detergent or different lipid bilayer mimics, such as nanodiscs, liposomes or SMALPs. This allows analysis of the complexes' constituting proteins, stoichiometries, oligomerization states as well as their dependence on specific lipids or ligands.

Selected Talk 11:35-11:50 am

Adam-von-Trott Hall

Intact Transition Epitope Mapping - Thermodynamic Weak-force Order (ITEM - TWO)

Bright D. Danquah (1), Yelena Yefremova (1), Kwabena F.M. Opuni (2), Claudia Röwer (1), Cornelia Koy (1), and Michael O. Glocker (1)

(1) Proteome Center Rostock, University Medicine Rostock, Rostock, Germany; (2) School of Pharmacy, University of Ghana, Legon, Ghana

We have developed an electrospray mass spectrometry method which is capable to determine antibody affinity in a gas phase experiment. A solution with the immune complex is electrosprayed and multiply charged ions are translated into the gas phase. Then, the intact immune-complex ions are separated from unbound peptide ions. Increasing the voltage in a collision cell results in collision induced dissociation of the immune-complex by which bound peptide ions are released. When analyzing a peptide mixture, measuring the mass of the complex-released peptide ions identifies which of the peptides contains the epitope. A step-wise increase in the collision cell voltage difference changes the intensity ratios of the surviving immune complex ions, the released peptide ions, and the antibody ions. From all the ions´ normalized intensity ratios are deduced the apparent Gibbs energies of activation over temperature from which the apparent gas phase thermodynamic quasi equilibrium dissociation constants are calculated. The order of the apparent gas phase dissociation constants of four antibody – epitope peptide pairs matched well with those obtained from in-solution measurements. The determined gas phase values for antibody affinities are independent from the source of the investigated peptides and from the applied instrument.

Selected Talk 11:50 am-12:05 pm

Adam-von-Trott Hall

A synthetic peptide library for benchmarking crosslinking mass spectrometry search engines

Rebecca Beveridge (1), Johannes Stadlmann (2), Josef M. Penninger (2, 3), Karl Mechtler (1, 2)

(1) Research Institute of Molecular Pathology (IMP), Vienna, Austria; (2) Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna, Austria; (3) Department of medical Genetics, University of British Columbia, Vancouver, Canada

We have created synthetic peptide libraries to benchmark crosslinking mass spectrometry search engines for different types of crosslinker. The unique benefit of using a library is knowing which identified crosslinks are true and which are false. Here we have used mass spectrometry data generated from measurement of the peptide libraries to evaluate the most frequently applied search algorithms in cross-linking mass-spectrometry. In our analysis, false crosslink identifications ranged from 1.1% to a surprising 23%, despite being filtered to an estimated 1% false discovery rate. Remarkably, the use of MS-cleavable crosslinkers did not reduce the false discovery rate compared to non-cleavable crosslinkers, results from which have far-reaching implications in structural biology. We anticipate that the datasets acquired during this research will further drive optimisation and development of search engines and novel data-interpretation technologies, thereby advancing our understanding of vital biological interactions.

Industry talk 12:05-12:20 pm

Adam-von-Trott Hall

Evaluation of FAIMS technology for mass spec analysis of chemical cross-linked peptides

Rosa Viner (1), Michael W. Belford (2), Michal Nadler-Holly (2), Lennart Schnirch (2), Fan Liu (2)

(1) Thermo Fisher Scientific, San Jose, USA; (2) FMP Berlin, Berlin, Germany

Chemical cross-linking in combination with mass spectrometry is a powerful method to determine protein-protein or nucleic acid-protein interactions. This method has been applied to recombinant and native protein complexes and, more recently, to whole cell lysates or intact unicellular organisms in efforts to identify protein-protein interactions on a global scale. However, this method suffers from low identification rates as the typical yield of cross-linked peptides is less than 1 % of total identified peptides. In this study, we compared widely used enrichment/fractionation techniques and the newly developed High Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) device for cross-linked peptides.

Amine-reactive, homo-bifunctional crosslinkers, including DSS, DSSO, and DSBU were used to crosslink protein and protein complex standards. Cross-linked samples were reduced, alkylated and digested with trypsin for MS analysis. Cross-linked peptides were pre-fractionated using SCX. Samples were analyzed by LC-MS/MS on the Orbitrap mass spectrometers with/out the Thermo Scientific™ FAIMS Pro™ Interface. Data were analyzed using Thermo Scientific™ Proteome Discoverer™ 2.3 software and XlinkX node 2.0.

The identification of cross-linked peptides by LC-MS/MS presents significant analytical challenges due to their low abundance and higher charge state distribution compared to tryptic peptides. Selective enrichment/fractionation of cross-linked peptides by SCX fractionation using an offline LC approach is widely used for improved interaction sites identification; however, identification rates only increase by a maximum of 2-3 folds. To further enhance the detection of cross-linked peptides, we evaluated the use of a FAIMS device for gas-phase fractionation. Different compensation voltages (CV) between -40 and -90V were tested with 5V resolution. Analysis of the identical samples with a FAIMS device in place, using optimized methods, produced the same number of identified cross-linked peptides as after enrichment/fractionation.

Talk 1:20-1:50 pm

Adam-von-Trott Hall

Marvels of enzyme catalysis at true atomic resolution: Distortions, bond elongations, hidden flips and protons in flight

Kai Tittmann

Georg-August-Universität Göttingen, Göttingen, Germany

Although general principles of enzyme catalysis are fairly well understood nowadays, many important details of how exactly a given substrate is bound and processed in an enzyme remain often invisible and as such elusive. In fortunate cases, structural analysis of enzymes can be accomplished at true atomic resolution (≤1 Å) thus making possible to shed light on otherwise concealed fine-structural traits of bound substrates, intermediates, cofactors and protein groups. I will present recent structural studies of several enzymes using ultrahigh-resolution X-ray protein crystallography showcasing its enormous potential as a tool in the elucidation of enzymatic mechanisms and in unveiling fundamental principles of enzyme catalysis. This includes the observation of seemingly hyper-reactive, physically distorted cofactors and intermediates with elongated scissile substrate bonds, stable carbene intermediates, the detection of “hidden” conformational and chemical equilibria and the analysis of protonation states with surprising findings including low-barrier hydrogen bonds in communication proton wires.

Selected Talk 1:50-2:05 pm

Adam-von-Trott Hall

Quantitative cross-linking mass spectrometry of rat brain synaptosomes

Parfentev Iwan (1), Ninov Momchil (1), Jahn Reinhard (2), Urlaub Henning (1, 3)

(1) Bioanalytical Mass Spectrometry, Max-Planck-Institue for Biophysical Chemistry, Goettingen, Germany; (2) Laboratory of Neurobiology, Max-Planck-Institue for Biophysical Chemistry, Goettingen, Germany; (3) Institute for Clinical Chemistry, University Medical Center Goettingen, Germany

Synapses are essential structures for inter-cellular communication in the central nervous system between neuronal cells. They form highly-specialized compartments that convert electrical to chemical signals, i.e. neurotransmitter release by synaptic vesicle fusion. A highly dynamic network of interacting proteins facilitates the cycle of synaptic vesicle recruitment, docking, priming, Ca2+-triggered exocytosis and subsequent retrieval and reconstitution of fused vesicles. So far, protein interactions in synapses were studied individually and often under artificial conditions. Here, we applied quantitative chemical cross-linking mass spectrometry (XL-MS) to identify and quantify the protein interaction dynamics in synaptosomes, which are pinched-off enriched synapses that are still physiologically active, in resting and excited state.

A cross-linking protein interaction network of resting and excited synaptosomes was obtained involving numerous known and novel protein interactions, e.g. ion transporting ATPases, synapsins, 14-3-3 scaffold proteins, G-proteins and Stxbp1/Munc18-1. Furthermore, quantitative XL-MS allowed the quantification of significant changes in protein conformations and interactions upon stimulation of synaptosomes. Significantly changed cross-linked residues were observed in Ca2+- and Ca2+/calmodulin-binding proteins, e.g. synaptotagmin, Anxa6, alpha spectrin, and Camkv. Ion channels like PMCA, Na+/K+ ATPase, SERCA, I3PR and VDAC exhibited significantly changed cross-linked residues under excited conditions. The implied conformational changes agreed with the respective ion channel function. Moreover, previously unknown conformational changes were observed, e.g. a major domain movement in I3PR that might turn the channel inactive, a possible monomerization of Cend1, and an enhanced interaction between CamkII and neutral ceramidase.

A quantitative XL-MS analysis of changing protein interactions in complex samples like stimulated synaptosomes was not attempted before. This study therefore analyzed the most complex and transiently changing system by quantitative XL-MS, to date.

Talk 2:05-2:35 pm

Adam-von-Trott Hall

How to get more from your XL-MS experiments

Richard A. Scheltema

Utrecht University, Utrecht, The Netherlands

Cross-linking mass spectrometry (XL-MS) is a powerful tool to uncover structural features from proteins and protein-complexes; especially when combined with structural biology approaches like cryo-EM. XL-MS is however still limited in analytical depth, resulting in high variability in experimental success. To illustrate, at an estimated 0.01 - 0.1% reaction efficiency of current NHS-based cross-linking reagents the identification of cross-linked peptides remains difficult. Compounding this, variations in sequence and/or the presence of PTMs make the already complicated data close to impossible to analyze.
With this in mind, we apply a two-step strategy. The first part of this strategy consists of use of a novel cross-linking reagent PhoX integrating a phosphonic acid as enrichment handle. Harnessing the incredible efficiency of IMAC brings the low abundant cross-linked peptides into sharp focus of the mass spectrometer, leading to superior data quality and analytical depth in a single measurement. The second part consists of an integrative approach, which combines top-down mass (TD-MS) with XL-MS. The results from TD-MS are used to uncover the full proteoform complement (i.e. sequence variants and PTMs), ultimately informing the XL-MS data analysis. This leads to a vastly reduced search space making the data analysis straightforward.
In this talk I will exemplify the power of this approach on the light harvesting complex PSII-LHCII, which dimerize into super complexes responsible for stacking of thylakoid membranes into discs. Each copy of this complex consists of a highly conserved core of 12 proteins and an antenna system of 6 proteins. Our TD-MS measurement uncovered 46 distinct proteoforms (driven by N-terminal truncations and high levels of acetylation), which were fed into the XL-MS data analysis pipeline. In total, this uncovered over 300 unique cross-linked peptides (present in at least 2 out of 3 replicates) from which we were able to uniquely place proteins inside the available cryo-EM density at 14 Å resolution and uncover a unique biological mechanism with which the super complex is held together.

Selected Talk 2:35-2:50 pm

Adam-von-Trott Hall

Enhanced oligomerization of full-length RAGE by synergy of the interaction of its domains and full-length RAGE S100b binding

Alexander Moysa (1), Dietmar Hammerschmid (2), Roman H. Szczepanowski (3), Frank Sobott (2, 4), Michal Dadlez (1)

(1) Institute of Biochemistry and Biophysics, Poland; (2) University of Antwerp, Belgium; (3) International Institute of Molecular and Cell Biology, Poland; (4) Astbury Centre for Structural Molecular Biology and School of Molecular and Cellular Biology, University of Leeds

The pattern recognition receptor RAGE (receptor for advanced glycation end-products) transmits proinflammatory signals in several inflammation-related pathological states, including vascular diseases, cancer, neurodegeneration and diabetes. Its oligomerization is believed to be important in signal transduction, but RAGE oligomeric structures and stoichiometries remain unclear. Different oligomerization modes have been proposed in studies involving different truncated versions of the extracellular parts of RAGE. Here, using native MS, we characterize the oligomerization patterns of full-length RAGE (including the transmembrane (TM) and cytosolic regions (CT)) and compare the results with oligomerization modes of its four truncated fragments. Our results confirm known oligomerization tendencies of separate domains and highlight the enhanced oligomerization properties of full-length RAGE. Mutational analyses within the GxxxG motif of the TM region show sensitivity of oligomeric distributions to the TM sequence. Using hydrogen–deuterium exchange, we mapped regions involved in TM-dependent RAGE oligomerization. Collision induced unfolding experiments showed increased conformation stability of full-length RAGE dimers compared to dimers of an extracellular truncated protein. Our data provide experimental evidence for the major role of the C2 and TM domains in oligomerization, underscoring synergy among different oligomerization contact regions along the RAGE sequence. These results also explain the variability of obtained oligomerization modes in RAGE fragments.
The Investigation of full-length RAGE-S100b complexes demonstrated that they form by dimer or trimer of RAGE. Such observation is evidence of importance for ligand binding. HDX experiment of complex RAGE-S100b and RAGE comparison revealed significant increasing protection in TM domain. Chemical cross-linking experiment of full-length RAGE monomer, dimer, and dimer of full-length RAGE-S100b complex revealed changes in the

cytoplasmic domain of RAGE-S100b complex. Changes in dynamics of the TM and CT regions may be related to the process of signal transduction after ligand binding.

Selected Talk 2:50-3:05 pm

Adam-von-Trott Hall

Structural Insights into Full-Length Retinal Guanylyl Cyclase 1 (ROS-GC1) by Cross-linking/Mass Spectrometry and Homology Modeling

Anne Rehkamp (1), Dirk Tänzler (1), Christian Tüting (2), Panagiotis Kastritis (2), Claudio Iacobucci (1), Christian H. Ihling (1), Andrea Sinz (1)

(1) Department of Pharmaceutical Chemistry and Bioanalytics, Institute of Pharmacy, Charles Tanford Protein Center, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany; (2) Interdisciplinary Research Center HALOmem, Charles Tanford Protein Center; Institute of Biochemistry and Biotechnology and Biozentrum, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

The retinal guanylyl cyclase 1 (ROS-GC1) is a transmembrane protein that is regulated by guanylyl cyclase-activating proteins (GCAPs) on the intracellular site in response to changes in intracellular Ca2+ concentration. Dysfunction and mutations in ROS-GC1 correlate with different retinal diseases, such as Leber's congenital amourosis (LCA) and Cone-Rod-Dystrophies (CORD), which often lead to blindness.

To date, no structural data are available for ROS-GC1. To obtain first 3D-structural information of full-length ROS-GC1, we conducted cross-linking/MS studies of cell lysates containing ROS-GC1. So far, we have obtained cross-linking data of the ROS-GC1 intracellular domain using different cross-linking principles. The cross-links identified are located within the kinase homology domain (KHD) and between the KHD and the catalytic domain of ROS-GC1, providing evidence that the catalytic domain is located in close neighborhood to the KHD. Moreover, the cross-links identified give strong hints on the existence of a ROS-GC1 homodimer in its natural cellular environment. Based on the cross-linking constraints, structural models of the intracellular domain of ROS-GC1 are currently being established via integrative structural biology methods.

Workshop 3:30-5:30 pm

Emmy-Noether Hall

HDX-MS: what is it and what can it do for you?

Kasper Rand

Department of Pharmacy, University of Copenhagen, Denmark

A wide range of biophysical tools are available to study protein conformation, dynamics and interactions. Whilst X-ray crystallography and Nuclear Magnetic Resonance (NMR) remain gold standards, Hydrogen Deuterium Exchange coupled with Mass Spectrometry (HDX-MS) has emerged as powerful, sensitive biophysical tool for the study of protein structure and dynamics in solution.
This workshop will introduce the fundamental aspects and considerations of performing a successful HDX-MS experiment – based on the recent community-based white paper (Masson et al. Nature Methods 2019). Advanced methodology such as use of statistics, ion mobility separation and fragmentation techniques will also be discussed.

Workshop 3:30-5:30 pm

Adam-von-Trott Hall

Cross-linking mass spectrometry data analysis with XlinkX embedded in Proteome Discoverer

Richard A. Scheltema (1), Rosa Viner (2), Kai Fritzemeier (2), and Bernard Delanghe (2)

(1) Utrecht University, Utrecht, The Netherlands; (2) Thermo Fisher Scientific

In this workshop you will learn the details of setting up the data analysis for XL-MS data with XlinkX integrated into Proteome Discoverer 2.4. The cross-linking data analysis pipeline is very versatile, supporting both cleavable as well as non-cleavable cross-linking reagents recorded with many different strategies. The analysis pipeline also includes an editor where any cross-linking reagent can be defined, including different gas-phase cleavable features, amino acid reactivities and others. Combined with the ability to specify your own protein sequence databases and modifications, many biological applications can be tackled.
The XL-MS results are not the end-point though. Based on the detected cross-linked peptides and the distance constraints they represent we demonstrate how to leverage structural modeling and lift a tip of the veil for protein docking with HADDOCK (also a workshop by the Bonvin lab) to determine the final structure of the full complex.
Part of the workshop you will receive a USB stick with the following materials:
Proteome Discoverer 2.4;
XlinkX nodes for PD 2.4;
RAW data of DSSO cross-linked BSA (cleavable cross-linking);
RAW data of PhoX cross-linked RAP-LRP1 protein complex (non-cleavable cross-linking);
Manual with the exercises.
Bring your laptop for an optimal experience, although the entire workshop is displayed on large screen as well.

Keynote 9:00-9:50 am

Adam-von-Trott Hall

Molecular mechanisms underlying phase separation in functional compartmenlization and disease

Tanja Mittag

St. Jude Children's Research Hospital

Biomolecular condensates coordinate a variety of important functions in cells including stress responses, RNA metabolism and membrane receptor clustering. Here, I will focus on discussing our work on two systems: the RNA-binding protein hnRNPA1, which associates with stress granules and mutations in which drive familial forms of neurodegenerative diseases; and the tumor suppressor SPOP, a substrate adaptor of a ubiquitin ligase which targets substrates in biomolecular condensates and mutations in which lead to a variety of solid tumors. I will use our work on these systems to address the following questions: (1) Which interactions mediate LLPS and can we device protocols to identifying them in an unbiased manner? (2) If we know the interaction strengths of adhesive elements in proteins, can we develop models to predict their full phase behavior? (3) Is phase separation required for function or can smaller complexes mediate function? Our results are transferable to other phase-separating proteins and provide mechanistic insights into the contributions of structured and disordered domains to phase separation, enzymatic activity inside liquid organelles, and disruption of phase separation by disease mutations.

Talk 9:50-10:20 am

Adam-von-Trott Hall

Sugars in the Gas Phase – Novel Techniques to Unravel the Glycocode

Kevin Pagel

Institute of Chemistry and Biochemistry, Freie Universität Berlin, Takustraße 3, 14195 Berlin and Fritz Haber Institute of the Max Planck Society, Department of Molecular Physics, Faradayweg 4-6, 14195 Berlin

Sugars are the most abundant biopolymers on earth. Most of them occur in the form of highly regular polysaccharides as in cellulose or starch. However, in biology it is often smaller, more complex structures which play a crucial role for example when attached to proteins. Currently, the vast majority of these glycans are characterized using mass spectrometry-based techniques (MS). Measuring the molecular weight of a sugar, however, immediately poses a fundamental problem: entire classes of monosaccharide building blocks exhibit an identical atomic composition and, consequently, an identical mass. Therefore, glycan MS data can be highly ambiguous and often it is not possible to clearly assign a particular structure.

A promising approach to overcome this limitation is to implement an additional gas-phase separation step using ion mobility-mass spectrometry (IM MS). Here, ions travel through a gas-filled cell aided by an electric field and are separated according to their collision cross section (CCS). Proof of principle experiments showed that linkage- and stereoisomers, which are difficult to distinguish using established techniques, can be separated and unambiguously identified on basis of their CCS.1 Further analyses revealed that also glycopeptides, which merely differ in the regiochemistry of the attached glycan can be distinguished using fragmentation and subsequent IM-MS analysis.2

Furthermore, we recently assessed the potential of cold-ion IR spectroscopy for oligosaccharide analysis.3 Gas-phase IR spectra of a series of synthetic oligosaccharide standards were recorded. For each of these oligosaccharides, unique and well-resolved absorption patterns which allow a simple, fingerprint-based discrimination between isomers where obtained. Encouraged by these results, we used cold-ion IR spectroscopy to study short-lived cationic intermediates that are relevant in sugar synthesis and fragmentation.4 From the obtained fingerprints, high-resolution structures of elusive cationic species can be generated, which in turn provide a novel level of mechanistic understanding of sugar chemistry.

1. J. Hofmann et al. Nature 2015, 256, 241-244.
2. H. Hinneburg and J. Hofmann et al. Chem. Commun. 2016, 52, 4381-4384.
3. E. Mucha et al. Angew. Chem. Int. Ed. 2017, 56, 11248–11251.
4. E. Mucha et al. Nature Comms. 2018, 9, 4174.

Selected Talk 10:50-11:05 am

Adam-von-Trott Hall

Microfluidic chip for sub-second HDX analysis of challenging proteins

Eleanor R. Dickinson (1), Rasmus R. Svejdal (1), Drago Sticker (2), Thomas Ötzen (3), Jörg P. Kutter (2) and Kasper D. Rand (1)

(1) Protein Analysis Group, Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark; (2) Microscale Analytical Systems Group, Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark; (3) Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark

The interrogation of transient protein dynamics and weak affinity interactions in solution is a challenge for current biophysical techniques. Sub-second hydrogen deuterium exchange coupled to mass spectrometry (HDX-MS) can reveal structural and conformational changes in proteins currently inaccessible with other methods. Here we have developed and validated a microfluidic mixing device (fastHDX chip) capable of HDX labelling and quenching on a millisecond timescale.
FastHDX chip design and set-up: The fastHDX chip is fabricated entirely by Thiol-ene polymer and contains µm-width channels for protein sample, deuterated buffer and quench buffer. Efficient on-chip mixing at channel junctions is achieved by spatially defined in-channel monolith plugs. The fastHDX chip is interfaced with a commercial LC-MS system by a custom 3D-printed holder.
Back-exchange: The loss of deuterium label (back-exchange) during on-chip quenching and sample collection was quantitated using the reference peptides Bradykinin and Angiotensin II. On-chip HDX yielded comparable back-exchange to that observed for manual HDX reactions. Reproducibility and robustness: The reproducibility of the fastHDX chips was investigated by performing triplicate HDX labelling of Hemoglobin on four fastHDX chips. The robustness was assessed by comparing deuterium uptake from three chips; a new chip, a chip after heavy use (18 sample injections) and chip stored for 2 months. We observe no significant differences in the deuterium uptake, indicating the reproducibility of the fastHDX chips and their stability for long-term use.
On-chip HDX labelling: HDX labelling was first performed on human Hemoglobin at labelling times ranging from 140 ms – 1.1 s. We observe structurally relevant deuterium uptake, including protection in helices, fraying of helices and evidence of salt bridge

stabilization. In ongoing work, the fastHDX chip is used to probe the HDX of a-synuclein, an intrinsically disordered protein the monomer of which lacks stable structure under native conditions.

Talk 11:05-11:35 am

Adam-von-Trott Hall

Estimating false-discovery rates of protein-protein interactions (PPI FDR) detected by crosslinking mass spectrometry

Juri Rappsilber

Bioanalytics, Institute of Biotechnology, Technische Universität Berlin, Germany

Crosslinking mass spectrometry, like any other new bioanalytical field thrives by rapidly expanding application areas and providing novel dimensions in types and volumes of data on biological systems. This initial breakthrough phase when lasting too long can damage the fields reputation, however. As part of maturing our field it must commit towards acknowledging limitations and challenges and establishing standardised procedures in conduct and reporting. Error is an essential component of scientific measurement. Together with reproducibility it is a defining element of science. Crosslinking mass spectrometry papers with great enthusiasm report data without tested error estimation methods. We as a field must come together and define tests of error estimation and on the basis of this choose a method to standardise error estimation and reporting. In this talk I will examine tests and PPI FDR approaches in hope of homing in on a conclusion that might allow us to arrive at a consensus.

Selected Talk 11:35-11:50 am

Adam-von-Trott Hall

Structural analysis of interaction between lecithin:cholesterol acyltransferase bound to the apolipoprotein A-I belt of high density lipoprotein particles

Lolita Piersimoni (1), Kelly A. Manthei (2), Dhabaleswar Patra (3), Christopher J. Wilson (4), Maria V. Fawaz (5), Jenny Shenkar (5), Wenmin Yuan (4), Philip C. Andrews (1), John R. Engen (4), Anna Schwendeman (5), Melanie D. Ohi (2), John J.G. Tesmer (3)

Chemical crosslinking of proteins coupled with mass spectrometry (XL-MS) has become a major tool in integrative structural biology for elucidation conformations of protein complexes in solution. XL-MS provides distance constraints, relative subunit orientations, and can validate structures from computational or other structural biology methods.
We used XL-MS to refine the orientation of Lecithin:cholesterol acyltransferase (LCAT) bound to the apolipoprotein A-I (ApoA-I) belt of recombinant high density lipoprotein particles (rHDL). The LCAT-rHDL complex was covalently stabilized with the CID-cleavable crosslinker DC4 and analyzed on an Orbitrap Lumos Tribrid Mass Spectrometer. LCAT catalyzes cholesterol esterification on HDL and is activated by ApoA-I. Structural analysis of LCAT-rHDL interactions will provide a snapshot of a phospholipase engaged with its physiological target, clarifying how LCAT mutations influence the HDL interface in genetic diseases.
We observed 15 unique crosslinks between LCAT and ApoA-I, with most LCAT crosslinks occurring on a common hydrophobic surface proposed as the HDL interaction site. We also observed a crosslink between identical LCAT lysyl residues, suggesting that some LCAT-rHDL complexes have the corresponding LCAT surfaces facing each other. Hyperconnected lysyl residues, diagnostic for flexible domains, were also observed, suggesting some LCAT structural flexibility. However, most crosslinks are found in discrete regions of the ApoA-I belt on either side of the helix 4/6 double-belt segment, indicating the preferred LCAT binding site on HDL.
Crosslinking data reflects the dynamic nature of the LCAT–HDL complex which hampered high resolution crystallographic analysis, but the integration of XL-MS, with negative stain electron microscopy and hydrogen-deuterium exchange MS, allowed refinement of the LCAT–rHDL complex structure. They revealed that LCAT preferentially interacts at the edge of HDL in a manner consistent with direct interactions with ApoA-I helix 6 and with LCAT gaining access to lipids at the edge of the protein delimited lipid bilayer.

Selected Talk 11:50am-12:05 pm

Adam-von-Trott Hall

Mass spectrometry study of formaldehyde cross-linking

Tamar Tayri (1, 2), Moriya Slavin (1), Joanna Zamel (1), Uri Raviv (2), Nir Kalisman (1)

(1) Institute of Life Sciences, The Hebrew University, Jerusalem, Israel; (2) Institute of Chemistry, The Hebrew University, Jerusalem, Israel

Formaldehyde is a widely used fixative in biology and medicine. The current chemical model for formaldehyde cross-linking of proteins is the formation of a methylene bridge that incorporates one carbon atom into the link. We present mass spectrometry data that largely refute this model. Instead, the data show that cross-linking of structured proteins mainly involves a reaction that incorporates two carbon atoms into the link. Under MS/MS fragmentation, the link cleaves symmetrically to yield unusual fragments with a modification of one carbon atom. This new understanding of the underlying cross-linking chemistry is applied to the structural approach of cross-linking coupled to mass spectrometry. On a cross-linked mixture of purified proteins with formaldehyde, our new analysis readily identified tens of cross-links. On a more complex scenario of in situ cross-linking of human cells in culture, we identified 469 intra-protein and 90 inter-protein cross-links. These cross-links fit well with available atomic structures. Interestingly, some cross-links could not be mapped onto a known structure and thus provide new structural insights. We highlight an example in which formaldehyde cross-links localize the binding site of βNAC on the ribosome. We also find several interactions of actin with auxiliary proteins. Our findings not only shed light on formaldehyde reactivity and toxicity, but also clearly demonstrate how to use this potent reagent for structural studies.

Industry talk 12:05-12:20 pm

Adam-von-Trott Hall

Reproducible and accurate CCS using TIMS as a tool for structural biology

Lucy Woods (1), Yoshihiko Takinami (2), Michael Greig (3), Blaine Roberts (4)

(1) Bruker Daltonik GmbH, Bremen, Germany; (2) Bruker Japan K.K., Yokohama-shi, Japan; (3) Bruker Scientific, LLC, San Jose, CA, US; (4) The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Australia

Bruker`s timsTOF Pro provides the fastest and deepest analysis for bottom-up proteomics and is also proven to be exceptional for a wide variety of other applications, such as PTM or cross-linking analyses. CCS values are a physical attribute of molecules that are measured in the gas phase in the timsTOF Pro. Because they are measured in the gas-phase, CCS values are independent of the introduction source, making them an important value for characterizing composition, topology and connectivity of multimeric protein complexes. Structural characterization of native proteins benefits from accurate and reproducible measures of the collision cross section, which is key for a comparison to NMR and crystallographic structures. Here, we used Trapped ion mobility spectrometry (TIMS) couple with native mass spectrometry to clearly differentiate oligomeric protein complexes with reproducible and precise CCS values.

Talk 1:20-1:50 pm

Adam-von-Trott Hall

Study of the largest and most heterogeneous macromolecular complex by HDX-MS, bringing new important mechanistic insights in proteasome regulation

Jean Lesne (1, †), Julien Parra (1), Marie Locard-Paulet (1, ‡), Dušan Zivković (1), Matthieu Chavent (1), David Bouyssie (1), Thomas Menneteau (1, ϕ), Marie-Pierre Bousquet-Dubouch (1), Odile Burlet-Schiltz (1) and Julien Marcoux (1)

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Introduction

The 20S proteasome is a multi-catalytic protease composed of 4 heptameric rings. It degrades proteins in a controlled fashion, thereby directly regulating intracellular concentration of cytokines and hub proteins. Alteration of its activity can lead to pathologies including cancers, heart and auto-inflammatory diseases. Its activity can be regulated by replacing its constitutive catalytic subunits and/or by interacting with different activators. However, whether its catalytic subunit composition favors the interaction with a particular regulator is still unclear.

Methods

Here, we utilized Hydrogen-Deuterium eXchange coupled to Mass Spectrometry (HDX-MS) to investigate the impact of the catalytic subunit composition of the 20S proteasome on its structure and association to specific activators. Human standard (std20S) and immuno (i20S) proteasomes were deuterated alone of after incubation with the PA28αβ or PA28γ activators. Samples were analyzed on a commercial Waters setup (Twin HTS PAL coupled to SynaptG2Si).

Results

We successfully optimized the classical HDX-MS workflow in terms of sample preparation, chromatography and MS acquisition to work on both poorly concentrated and very heterogeneous protein complexes. We developed a web application called HDX-Viewer [1] to instantly visualize the raw data of these large complexes, directly from DynamX outputs. Deuteration rates of the three PA28 monomers (forming the PA28αβ or PA28γ heptamers)

indicated both common features suggesting a similar mode of activation but also local discrepancies.The std20S and i20S clearly showed a faster deuteration on the solvent-exposed surface of the α-ring compared any other ring-interface. Furthermore, we identified flexible regions that are available for interaction with the ~200 Proteasome-Interacting-Proteins described so far. Comparison of the std20S Vs. i20S deuteration highlight subtle but meaningful discrepancies. Similarly, binding of the PA28 regulators influences the deuteration of the 20S proteasomes.

Conclusions

Deuteration of the std20S Vs. i20S suggests a first “inner to outer ring allosteric change”. Changes in the inner β-rings upon regulator binding were interpreted as an “outer to inner ring allosteric change”. Altogether, the α-ring region that was more dynamic in the std20S Vs. i20S was also the most protected by activators. Our results thus highlight how the incorporation of different catalytic subunits can alter the proteasome affinity to different regulators.

References

[1] Bouyssié D*, Lesne J*, Locard-Paulet M, Albigot R, Burlet-Schiltz O, Marcoux J “HDX-Viewer: interactive 3D visualization of Hydrogen-Deuterium eXchange data” 2019 Bioinformatics, In Press

Selected Talk 1:50-2:05 pm

Adam-von-Trott Hall

Radical labeling by fluoro-alkyl radicals as novel tool for structural proteomics

Lukas Fojtik, Zdenek Kukacka, Jan Fiala, Daniel Kavan and Petr Novak

Biocev-Institute of Microbiology, The Czech Academy of Sciences, Prague, Czech Republic (2) Faculty of Science, Charles University, Prague, Czech Republic

Structural proteomic techniques such as chemical cross-linking, hydrogen/deuterium exchange, native electrospray, ion mobility or covalent labeling have been established as powerful tools for structural studies of biomolecules in general. Although covalent labeling and especially radical labeling techniques provide structural information about amino acid side chains that are complementary to HDX data, its usage is still very rare. Since FPOP technique requires an expensive instrumentation and therefore it is unavailable for most of users, we focused on development of foot-printing technique not requesting powerful photon source.

In this study, we tested a potential of new radical labeling method by using fluoro-alkyl radicals that does not require photon activation. Instead, the simple activation is performed by reducing agent in conditions that are close to the native ones. The first results on several model proteins show the reaction takes only a few seconds and resulting radicals preferentially modify the aromatic amino acid side chains. Our data clearly demonstrate the potential of the Fluor-alkyl radical labeling for fast and inexpensive analysis of protein surface accessible area.

This work was supported by the Czech Science Foundation (grant numbers 19-16084S), The Czech Academy of Sciences (PPLZ for Zdenek Kukacka), the Ministry of Education of the Czech Republic (program “NPU II” project LQ1604), 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).

Talk 2:05-2:35 pm

Adam-von-Trott Hall

Analysis of the lysosomal interactome by cross linking

Jasjot Singh (1), Fatema Akter (1), Srigayatri Ponnaiyan (1), Edgar Kaade (1), Jan Muntel (2), Roland Bruderer (2), Lukas Reiter (2), Melanie Thelen (1), Volkmar Gieselmann (1), Dominic Winter (1)

(1) Institute for Biochemistry and Molecular Biology, University of Bonn, Germany; (2) Biognosys, Schlieren, Switzerland

The lysosome is the main cellular organelle for the degradation of macromolecules and the recycling of their building blocks. Correct lysosomal function is essential for mammalian cells, which is demonstrated by the pathogenic relevance of lysosomal proteins: mutations in almost every of the ~ 50 known lysosomal hydrolases result in serious diseases called lysosomal storage disorders. Furthermore, it is becoming more apparent in the recent years, that alterations in lysosomal membrane proteins and their interaction partners play key roles in a variety of common diseases such as cancer. Therefore, there is an increasing interest in the characterization of the lysosomal proteome and interactome.
Due to the low abundance of lysosomes, their enrichment is a prerequisite for in-depth proteomic analysis. We compared four enrichment approaches using DIA and determined optimal conditions for the generation of lysosome enriched fractions using superparamagnetic iron oxide nanoparticles. With this approach, we generated lysosome-enriched fractions from HEK cells and cross-linked them in two states (mechanically disrupted and intact) with the MS cleavable cross linker DSSO. After proteolytic digestion, peptide samples were fractionated by strong cation exchange chromatography and analyzed by LC-MSMS. We identified several thousand cross-links originating from lysosomes and other organelles. For a shortlist of proteins with confirmed lysosomal localization, we identified several hundred cross links. This covered inter and intra cross-links of lysosomal and lysosome-associated proteins, as well as inter cross-links of lysosomal and cytosolic proteins. We identified interactions for members of the currently best characterized lysosomal membrane complexes BORC, mTORC1, and vATPase confirming the validity of the approach. Additionally, we found novel potential protein interactions. To further evaluate these proteins for their localization at the lysosome, we applied a SILAC based approach to discriminate between lysosomal and background proteins. These data present the first draft of the lysosomal protein interactome determined by cross-linking.

Selected Talk 2:35-2:50 pm

Adam-von-Trott Hall

Studying the assembly of the kinetochore structure by chemical crosslinking and mass spectrometry

Franz Herzog, Victor Solis, Götz Hagemann, Sylvia Singh

Gene Center, Department of Biochemistry, Ludwig-Maximilians Universität München, Munich, Germany

The timely and precise assembly of the macromolecular kinetochore complex is essential for accurate chromosome segregation. Although the kinetochore has been studied intensively, the molecular mechanism that generates a stable cellular structure linking DNA and microtubules has not been comprehensively understood. The combination of chemical crosslinking and mass spectrometry (XLMS) facilitates the characterization of large protein complexes and has emerged as a versatile tool for the identification of protein connectivity and complex topology at the domain level. Recent advances in the detection of crosslinks have been used to study structural rearrangements or dynamics within proteins and protein networks. XLMS has the potential to capture the stabilization of protein complexes through cooperative protein interactions. Crosslink-derived distance restraints identify interactions at peptide resolution and allow to pinpoint binding interfaces and to investigate the contribution of post-translational modifications to the stabilization of protein complexes. Here, we introduce a XLMS workflow to characterize binding interfaces building up macromolecular complexes. We studied the interactions of several yeast kinetochore subunits and revealed their interdependency for kinetochore stabilization. The in vitro reconstitution of CENP-C/Mif2, CENP-U/Ame1 and CENP-Q/Okp1 interacting with CENP-A/Cse4 containing nucleosomes provided us first insights into the assembly of a Cse4 binding complex.

Industry talk 2:50-3:05 pm

Adam-von-Trott Hall

ECD - a powerful tool for the fragmentation of intact proteins under denaturing & native conditions

Nico Wortel, Remco Swart, Jan Commandeur

Spectrometry Vision B.V.

Electron capture dissociation (ECD) has proven to be an excellent tool for top-down proteomic analysis, showing efficient fragmentation of intact proteins. Most studies reported on ECD data thus far have been performed on FT-ICR instrumentation. In our presentation, we will present two studies, where a novel ECD device has been implemented on both a Q-Exactive as well as a Synapt series instrument. Both studies reveal the huge potential of utilising ECD fragmentation for top-down proteomic applications, while keeping the instrument transmission, resolution and CID performance unaffected. Q-Exactive ECD MS-MS data will be presented on an intact protein under denaturing conditions, showing highly efficient fragmentation and excellent sequence coverage of >93%. Synapt ECD MS-MS data will be presented on intact proteins under native conditions. Combining ECD and ion mobility data provides information on the topological changes in proteins during collisional unfolding.

Workshop 3:40-5:40 pm

Adam-von-Trott Hall

MeroX 2.0 - MS/MS-cleavable protein-protein XL-MS data analysis

Michael Götze (1), Claudio Iacobucci (2), Andrea Sinz (2)

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Chemical cross-linking in combination with a mass spectrometric analysis of the created cross-linked products is a powerful tool to derive valuable structural information of proteins and protein complexes. We have developed an integrated workflow for cross-linking/mass spectrometry (XL-MS) based on protein cross-linking with the MS-cleavable reagent DSBU (disuccinimidyl dibutyric urea), followed by enzymatic digestion, enrichment of cross-linked peptides by size-exclusion chromatography, and LC/MS/MS analysis.
To exploit the full potential of MS-cleavable cross-linkers, we developed an updated version of the freely available MeroX software for automated data analysis (www.StavroX.com). In this workshop, we will give an introduction into our integrated workflow. In particular, we will focus on the MeroX 2.0 software by sharing all elements to get the most out of your data, ranging from protein assemblies to whole proteome analyses.

Keynote talk 9:00-9:50 am

Adam-von-Trott Hall

From single ion proteomics to protein complexes: The denaturing and native modes of Top-Down mass spectrometry

Neil Kelleher

Departments of Chemistry and Molecular Biosciences, The Chemistry of Life Processes Institute, The Proteomics Center of Excellence at Northwestern University, Evanston, Illinois 60208, United States

While top down mass spectrometry has become synonymous with the direct analysis of intact proteins and their complexes via mass spectrometry, the term more generally denotes an approach to measurement that recognizes the value of retaining as much information as possible about a system prior to analysis. By avoiding proteolytic digestion, proteoform-specific identifications can be made - directly. Notably, the top down philosophy is equally applicable to the level of protein-protein and protein-ligand interactions. A series of vignettes will focus on both modes of Top Down Proteomics mentioned in the title. I will also describe a few fundamentals and most recent advances top down MS, like the most recent breakthrough of multiplexed single ion mass spectrometry in orbitraps (joint work with the group of Mike Senko at Thermo Fisher Scientific). I will also describe an approach that enables the direct analysis of protein interactions in both targeted and untargeted fashion using multistage native mass spectrometry (MS2 and MS3). Providing information from intact complex mass (MS1) to subunits and their backbone fragment ions, native top-down MS even enables identification and characterization of unknown protein interactions. By more faithfully preserving post-translational modifications and non-covalent interaction throughout the measurement process, top down mass spectrometry is positioned to make basic and translational proteomics more efficient, particularly in the detection and assignment of function to proteoforms and their PTMs underlying human wellness and disease.

Talk 9:50-10:20 am

Adam-von-Trott Hall

From molecular recognition to cellular function: Pex5p receptor phosphorylation regulates import of proteins into peroxisomes

Sven Fischer (1), Shiran Maskit (2), Jerome Buergi (3), Thomas Mastalski (4), Renate Maier (1), Ralf Erdmann (5), Matthias Wilmanns (3), Harald W. Platta (4), Maya Schuldiner (2), Einat Zalckvar (2), Silke Oeljeklaus (1, 6), Friedel Drepper (1, 6), Bettina Warscheid (1, 6)

(1) Biochemistry and Functional Proteomics, Institute of Biology II, Faculty of Biology, University of Freiburg; (2) Department of Molecular Genetics, Weizmann Institute of Science; (3) Hamburg Unit c/o DESY, European Molecular Biology Laboratory (EMBL); (4) Biochemistry of Intracellular Transport, Medical Faculty, Ruhr-University Bochum; (5) Systems Biochemistry, Medical Faculty, Ruhr-University Bochum; (6) Signalling Research Centres BIOSS and CIBSS, University of Freiburg

Peroxisomes are dynamic organelles of eukaryotic cells that fulfill a large variety of essential metabolic functions including fatty-acid beta-oxidation and the degradation of hydrogen peroxide. The vital importance of peroxisomes is emphasized by the occurrence of severe, if not lethal disorders in humans with dysfunctional peroxisomes. Peroxisomal functions rely on the posttranslational import of nucleus-encoded matrix proteins. The vast majority of peroxisomal matrix proteins contain a carboxy-terminal peroxisomal targeting signal (PTS) 1, which is recognized by the cytosolic receptor protein Pex5p. Most PTS1 proteins bind to Pex5p via conserved tetratricopeptide repeat (TPR) domains located in the carboxy-terminal region of the receptor. At the peroxisomal membrane, cargo-loaded Pex5p binds to Pex14p of the docking complex, followed by the formation of a dynamic import pore through which the cargo is released in the peroxisomal matrix. In this presentation, I will address the question whether and how the import of peroxisomal matrix proteins is posttranslationally regulated. The approach that we followed is integrative and includes data from phosphoprotein analysis, fluorescence microscopy screening, native MS and collisional activation experiments, as well as biophysical measurements. Our data show that Pex5p is phosphorylated in its TPR domains in Saccharomyces cerevisiae. We demonstrate that the binding affinity between PTS1 protein and a phosphomimetic Pex5p mutant is reduced and identify the extended PTS1 sequence as the region exhibiting reduced binding. Through in vivo analysis, we discovered that peroxisomal import of a subset of proteins is affected to different extent, whereas a specific set of PTS1 proteins is not. This physiological effect of the phosphomimetic mutations correlates with changes in binding affinity of the corresponding extended PTS1-

sequences. We therefore conclude that phosphorylation of Pex5p in its TPR domains constitutes a so far unknown mechanism for modulating the flux of specific matrix proteins into peroxisomes.

Talk 10:50-11:20 am

Adam-von-Trott Hall

Optimized parameter settings enhance proteome-wide formation of crosslinks on low-abundant proteins

Julius Fürsch (1, 2, 5), Kai-Michael Kammer (1, 2, 5), Stefan G. Kreft (1), Martin Beck (3, 4, #) and Florian Stengel (1, 2, #)

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Studies using crosslinking coupled to mass spectrometry (XL-MS) on the proteome-wide level have spurred great interest as they facilitate structural probing of protein interactions in living cells or even organisms. As exciting as these recent breakthroughs are and even though an impressive number of crosslinks were identified from complex samples, the vast majority of cross-linked peptides that were identified arise from proteins that are known to be highly abundant in cells and expressed in large copy numbers.
We show, by using both an in-vitro mimic of a crowded cellular environment and eukaryotic cell lysate, that there is indeed a nearly exclusive detection of crosslinks within high abundant proteins in current proteome-wide crosslinking experiments. We demonstrate both experimentally and by a kinetic model that this bias is also caused by the propensity of crosslinks to preferentially form on high abundant proteins and not by the inability to detect crosslinks that have formed on low abundant proteins due to limitations in current MS technology. We further show that parameter settings optimized towards a pseudo 1st order kinetics model results in a significant 3 to 10-fold overall increase in the detection of lower abundant proteins within cellular lysates on a proteome-wide scale.
Our study therefore explains the cause of a major limitation in current proteome-wide crosslinking studies and demonstrates a way forward how to redesign or repurpose XLMS studies in the future in order to address a larger part of the proteome.

Talk 11:20-11:50 am

Adam-von-Trott Hall

Proteomes in 3D

Paola Picotti

Institute of Biochemistry, Department of Biology, ETH Zurich, Zurich, Switzerland; Institute of Molecular Systems Biology, Department of Biology, ETH Zurich, Zurich, Switzerland

Protein structural changes induced by external perturbations or internal cues can profoundly influence protein activity and thus modulate cellular physiology. Mass spectrometry (MS)-based proteomic techniques are routinely used to measure changes in protein abundance, post-translational modification and protein interactors, but much less is known about protein structural changes. In my talk, I will present a structural proteomics method that enables analysis of protein structural changes on a proteome-wide scale and directly in complex biological extracts. The approach relies on the coupling of limited proteolysis (LiP) tools and MS. LiP-MS can detect subtle alterations in secondary structure content, larger scale movements such as domain motions, and more pronounced transitions such as the switch between folded and unfolded states. I will describe how we are applying this approach to study the molecular bases of protein aggregation diseases and to the identification of protein-small molecule interactions (e.g drug targets). I will also propose that monitoring protein structural states on a proteome-wide scale can serve as a new powerful readout to pinpoint altered protein functional states and the (de)regulation of biochemical pathways. Last, I will discuss the power and limitations of the new approach.

Poster Presentation P1

Novel Methods for Chemical Crosslinking Based Protein Complex Analysis

Qun Zhao, Lili Zhao, Yuxin An, Hang Gao, Lihua Zhang, Yukui Zhang

Dalian Institute of Chemical Physics, Chinese Academy of Sciences

Chemical cross-linking combined with mass spectrometry (CXMS) has emerged as a powerful tool to assist traditional technologies to study protein structure, conformation, and protein–protein interaction with advantages of less time-consuming and less demanding on sample purity. However, application of CXMS is still limited by the high complexity of CXMS samples, the low abundance and the huge data searching space of cross-linked peptides. In addition, limited methods were developed targeting to the in-situ protein complex analysis, especially the subcellular organelle-targeted in-situ protein complex analysis, and there is no report respected to the systematical evaluation on the cross-linking conditions to the cell disturbance, as well as the coverage of cross-linking and the space resolution of the protein complex.

In response to the above scientific problems, methods to improving the identification coverage of cross-links and developing in-vivo cross-linking strategy were imperative. Our research team has carried out a series of research work to achieve accurate characterization of the dynamic structure of protein complexes by improving the depth of chemical crosslinking, increasing the enrichment selectivity of cross-linked peptides and reducing the complexity of mass spectrometry data. Briefly, various kinds of functional chemical cross-linking agents with membrane permeability and membrane impermeability were developed, and these linkers have features of that with different cross-linking arm lengths; with group that can be enriched or groups have fluorescence probe tracer groups to perform in-vivo monitoring. Then, the click-chemistry based cross-linked peptides enrichment methods were developed. Furthermore, new crosslinked data analysis algorithms and multiple crosslinked data quantification algorithms, as well as novel subcellular organelle-targeted in-situ protein complex analysis methods were developed.

Poster Presentation P2

Establishing proteoliposomes for the analysis of membrane proteins directly from lipid bilayer

Melissa Frick, Julian Bender, Carla Schmidt

Interdisciplinary research center HALOmem, Charles Tanford Protein Center, Institute for Biochemistry and Biotechnology, Martin Luther University Halle-Wittenberg, Kurt-Mothes-Straße-3a, 06120 Halle (Saale)

Liposomes are phospholipid bilayer vesicles which resemble cellular organelles and membranes. Due to their variability in size, composition and amphiphilic character they are promising mimetics of natural membranes. However, due to their heterogeneity and the ability of lipids to form large clusters in the gas phase, liposomes were not employed for mass spectrometric analysis to-date. To analyze proteins in a native-like membrane environment, we explored liposomes for the mass spectrometric analysis of lipids and proteins directly from phospholipid bilayers. We envision that, in the gas-phase of the mass spectrometer, proteins can be released intact from lipid bilayers allowing their structural analysis.

To reach this goal we first analysed “empty” liposomes under denaturing and non-denaturing conditions. For this we prepared liposomes varying in size, composition and concentration. Mass spectrometric analysis showed that liposomes dissociate independent of their size, composition or concentration. Using shotgun lipidomics we were able to identify and quantify different phospholipid species from various classes directly from the lipid bilayer. Employing a mass spectrometer modified for transmission of high masses revealed the presence of lipid clusters, which dissociate at higher collision energies. Next, we mixed proteins and liposomes in solution and analysed them together by denaturing and non-denaturing mass spectrometry. We obtained mass spectra showing both proteins and lipids. Finally, we reconstituted transmembrane proteins and encapsulated soluble proteins into liposomes. We are currently optimizing their structural analysis by mass spectrometry. Using both, denaturing and non-denaturing conditions, we aim to release soluble and transmembrane proteins from the liposomes to study their structures in the gas phase of the mass spectrometer.

Poster Presentation P3

Analysis of SNARE complex intermediates by structural mass spectrometry

Julia Hesselbarth, Sabine Wittig, Carla Schmidt

Interdisciplinary Research Centre HALOmem, Charles Tanford Protein Centre, Institute for Biochemistry and Biotechnology, Martin Luther University Halle-Wittenberg, Germany

The SNARE complex mediates signal transmission in neurons and is responsible for the fusion of synaptic vesicles with the presynaptic membrane. The trimeric complex is formed by SNAP25, Synaptobrevin-2 and Syntaxin-1A. SNAP25 contributes two alpha-helices and Syntaxin-1A and Synaptobrevin-2 both contribute one alpha-helix forming a stable four-helix bundle. While SNARE proteins are well-structured in this four-helix bundle they are natively unstructured in the absence of interaction partners.
We study the stepwise assembly of the SNARE complex by combining cross-linking and native mass spectrometry (MS). For this, we set out with the isolated SNARE proteins. Cross-linking was performed using bis(sulfosuccinimidyl)suberate (BS3). Gel electrophoresis and western blotting of isolated cross-linked proteins indicated oligomerisation. Following tryptic digestion, we analyzed the cross-linked peptides by liquid chromatography-coupled tandem MS (LC-MS/MS) to identify specific interactions. Verified cross-links are located across the entire protein sequence confirming their intrinsic disorder. Using native MS, we approved the presence of oligomers.
Following the same procedure, we further analyzed SNARE complex intermediates by incubating the individual proteins to form binary complexes. In contrast to the isolated proteins we could show that, in the presence of their physiological interaction partners, the proteins preferentially form SNARE complex intermediates. In particular, SNAP25 and Syntaxin-1A form a complex with a 1:2 stoichiometry, imitating the four-helix bundle of the SNARE-complex.
In conclusion, combining cross-linking and native MS is well-suited to study oligomerisation as well as complex formation and will help investigating the assembly of the full SNARE complex in future studies.

Poster Presentation P4

Protein interaction networks within synaptic vesicles examined by chemical cross-linking

Sabine Wittig (1), Marcelo Ganzella (2), Susann Kostmann (1), Angel Perez-Lara (2), Reinhard Jahn (2), and Carla Schmidt (1)

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Synaptic vesicles (SVs) are small storage organelles for neurotransmitters. They are densely packed with proteins and pass through a trafficking cycle in the nerve terminal. Available models assume random distribution of the proteins in the membrane, however, there is evidence that they form functionally active assemblies. We set out to unravel these assemblies by combining chemical cross-linking and biophysical approaches.

Synaptic vesicles were purified from rat brain and major proteins were identified by LC-MS/MS and database searching. Subsequently, a database including the 400 most abundant proteins of 5 independent SV preparations was generated. Proteins within intact SVs were then chemically cross-linked using BS3 cross-linker. After tryptic digestion, cross-linked di-peptides were enriched by size exclusion chromatography and analyzed by LC-MS/MS and database searching. Applied in this way, chemical cross-linking revealed first interaction networks in synaptic vesicles. These networks reveal many protein interactions with Synaptobrevin-2. Local networks, for instance between Synaptophysin, SV2A and subunit a of the V-type ATPase, were also observed. In addition, we identified Synaptobrevin-2 and Synaptophysin homomultimers.

To unravel the role of Synaptobrevin-2 in complex formation, we followed three approaches: (i) we cleaved the cytosolic domain of Synaptobrevin-2 from SVs with Botulinum toxin, (ii) we fused SVs with liposomes to 'dilute' the proteins in the membrane, and (iii) we incubated SVs with the soluble ΔN-SNARE complex to engage Synaptobrevin-2 in SNARE complex formation. Again, we used chemical cross-linking to identify protein interactions under these conditions.

We found that protein interactions between Synaptobrevin-2 and other proteins are reduced, while interactions of Synaptophysin, SV2A and subunit a of the V-type ATPase remain, suggesting their importance for the SV trafficking cycle.

Poster Presentation P5

Data-independent acquisition improves quantitative cross-linking mass spectrometry

Fränze Müller (1), Lars Kolbowski (1, 2), Oliver M. Bernhardt (3), Lukas Reiter (3), Juri Rappsilber (1, 2)

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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 10%. We found DIA-QCLMS to be capable of detecting changing abundances of cross-linked peptides in complex mixtures, despite the ratio compression encountered when increasing sample complexity through the addition of E. coli cell lysate as matrix. In combination with photoactivatable crosslinkers (photo-DIA-QCLMS), the workflow can increase data density and capture better protein dynamics due to short reaction times. Additionally this can reveal conformational changes caused by environmental influences that would otherwise affect crosslinking itself, such as changing pH conditions.

Poster Presentation P6

Top-down proteomics applied to human CSF

Marina Gay (1), Ester Sánchez-Jiménez (1), Laura Villarreal (1), Mar Vilanova (1), Romain Huguet (2), Gianluca Arauz-Garofalo (1), Antonio Lorenzo (1), Mireia Díaz-Lobo (1), Daniel López-Ferrer (2), Marta Vilaseca (1)

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Cerebrospinal fluid (CSF) is the fluid of choice to study disorders of the central nervous system (CNS). Its composition holds the promise to reflect the pathological state of an individual. Traditionally, proteins in CSF have been analysed using bottom-up proteomics in the search of high proteome coverage. However, information regarding the connectivity between post-translational modifications (PTMs), mutations, truncations or alternative splice variants is lost. As an alternative, top-down proteomics offers low to medium proteome coverage, but high protein coverage, thereby enabling almost a full characterization of the primary structure of the proteins. This capacity thus allows the identification of multiple proteoforms, as well as naturally occurring bioactive peptide fragments that could be of significant biological relevance.

We describe various top-down proteomics methods that can be used as a guide to perform a complete and rapid end to end top-down proteomics analysis of CFS. These workflows are an excellent analytical companion in the search for new protein biomarkers in neurodegenerative diseases. The workflows comprise all the steps required for the analysis, including sample preparation protocols, LC-MS/MS analysis methods, and data analysis pipelines.

We compared various prefractionation protocols based on three basic principles: molecular weight separation, affinity enrichment, and liquid chromatography.

We tested several chromatographic regimes and a range of columns and traps (reversed phase: C4, C18, polymeric) and MS fragmentations techniques (CID, ETD, ETDhCD, UVPD). Parameters such as the pressure within the ion guides and resolution settings at the MS1 and MS2 level were adjusted using orbitrap technology.

Each workflow provided different proteome coverages. Preliminary results indicate that CSF can be easily analysed without extensive prefractionation with ACN depletion, immunoaffinity kits or SAX tips. Approximately 50 to 200 proteoforms can be identified depending on the sample preparation protocols. Seven hundred peptides were identified in the peptide fraction.

Poster Presentation P7

In-depth analysis of UV induced cross-linking in a model protein-RNA complex using CLIR-MS

Chris P. Sarnowski (1), Anna Knörlein (2), Michael Götze (1), Ruedi Aebersold (1), Jonathan Hall (2), Alexander Leitner (1)

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RNA binding by proteins is more complex than previously thought, with RNA binding activity facilitated by many structural features other than canonical RNA binding motifs (e.g. RRMs). Cross-linking of protein RNA complexes, using 254 nm UV light to induce zero-length covalent links, has become a popular way of studying protein-RNA interactions, allowing scientists to discover more about where and how proteins bind RNA. Several techniques take advantage of this reaction, such as CLIP (cross-linking and immunoprecipitation) and its derivatives for localisation of protein binding on RNA, or mass spectrometry-based methods that localise RNA binding on proteins. Pioneers in the latter field focussed on localisation of the cross-linked RNA on a peptide, but localisation on the RNA component of the complex is more challenging. By using Cross-Linking of Isotope-labelled RNA and tandem Mass Spectrometry (CLIR-MS), we demonstrate the utility of segments of stable isotope labelled RNA to retain localisation information about the cross-link from the RNA component of the complex. We selected a well characterised protein-RNA complex, Fox1 RRM and its canonical “Fox binding element” (FBE) RNA sequence motif (UGCAUGU), as a model system for detailed studies. We applied a variety of labelling schemes to the RNA, ranging from all bases in the 7mer to just a single base. Characteristic doublet peaks from the labelled bases within the sequence provide RNA localisation information, whilst the site of the cross-link on the peptide is still obtained by traditional peptide fragmentation during tandem mass spectrometry. This approach allows us to explore the cross-linking behaviour of the Fox1-RRM/FBE complex at single amino acid or single ribonucleotide base resolution, providing novel insights into the UV-induced protein-RNA cross-linking behaviour in this complex, and new information about the utility of this approach for structural biologists.

Poster Presentation P8

Photo-induced cross-linking of unmodified proteins (PICUP) for structure elucidation of protein complexes

Susann Kostmann, Amelie Dier, Sabine Wittig and Carla Schmidt

Interdisciplinary research center HALOmem, Charles Tanford Protein Centre, Martin Luther University Halle-Wittenberg, Halle, Germany

Protein cross-linking is a valuable tool in structural biology complementing traditional techniques such as X-ray crystallography, NMR spectroscopy or electron microscopy. There are many strategies that can be followed to cross-link proteins. Most commonly employed is chemical cross-linking using amine-reactive N-hydroxysuccinimide esters. However, these reagents usually contain a comparably long and flexible linker inducing rather long-range linkages. We therefore evaluated an alternative, 'zero-length' approach, namely photo-induced cross-linking of unmodified proteins (PICUP), using tris(bipyridyl)Ru(II) (Ru(Bpy)3) and ammonium persulfate (APS).

To establish PICUP, we used homo-oligomeric alcohol dehydrogenase and pyruvate kinase as model protein complexes. The cross-linking reaction was performed using 10 or 20 uM protein and 0.125 mM Ru(Bpy)3 / 2.5 mM APS. The cross-linking reaction was induced with visible light using a flashlight (Mini Maglite AA) located 15 cm above the reaction tube. The cross-linking yield was reviewed by gel electrophoresis using the NuPAGE system (Thermo Fisher Scientific). For identification of cross-links, the proteins were digested with trypsin in solution using RapiGest surfactant (Waters). Cross-linked peptides were enriched by size exclusion chromatography using an Äkta purifier chromatography system (GE healthcare) followed by LC-MS/MS analysis on a Q Exactive plus hybrid quadrupole-mass spectrometer coupled with an Ultimate 3000 HPLC system (both Thermo Scientific). Potential cross-links were identified by database searching using pLink2 software followed by manual validation of the mass spectra.

We first optimised the exposure time ranging from 1 up to 60 s and found that exposure times of 20 to 30 s yield sufficient cross-linking. For exposure times >30 s, broad protein bands and protein degradation were observed by gel electrophoresis. Analysis of the cross-linked peptides obtained from 20 s exposure delivered intra- and inter-molecular cross-links for both alcohol dehydrogenase and pyruvate kinase. These cross-links were projected onto the available crystal structures and C(alpha)-C(alpha) distances were evaluated.

Poster Presentation P9

Characterizing Circadian Rhythms in Red Blood Cells

Gad Armony (1, 2), Esther A. Zaal (1, 2), Brigitte B.A. van Oirschot-Hermans (3), Richard H.A. van Wijk (3), Albert J.R. Heck (1, 2)

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Circadian rhythms are present in almost all light-sensitive organisms from cyanobacteria through plants, flies, and mammals. Human hormone levels and metabolism are tied to circadian rhythms and affect sleep and wakefulness timings. Disruption of the synchronous action of circadian clocks leads to several human diseases, mainly sleep and metabolic disorders. Most known circadian clocks stem from transcription-translation feedback loops. Protein and mRNA levels oscillate with a 24 hour period, matching the day-night cycle. An intriguing exception is circadian oscillations in red blood cells (RBCs); the circadian rhythms of RBCs cannot involve a transcription-translation feedback loop since RBCs are anuclear and thus not capable of transcription. Therefore, the circadian rhythms in RBCs must rely on another mechanism of action. This mechanism probably involves changes in proteins such as post-translational modifications and complex formation and disassembly. Not much is known about this circadian mechanism and what cellular features take part, only a few cellular features have been identified as circadian. We are using several mass spectrometry methods: proteomics, phospho-proteomics, metabolomics, and cross-linking to investigate the circadian rhythms in RBCs. These methods provide quantitative information about many biomolecules in parallel, ideal for identifying unknown circadian biomolecules. Human RBCs are incubated at physiological temperature and in a medium that allows incubations over several days, during which we sample the RBCs every four hours. We measure the levels of proteins and metabolites at these time points and identify periodic patterns. These patterns indicate that sugar metabolism and protein ubiquitination behave in a cyclical manner. Using these high-throughput methods we start unveiling the players of the circadian rhythms in the red blood cells.

Poster Presentation P10

Studying the Interactions between the C-Terminal Domain of the Tumor Suppressor p53 with S100ß and Human Sirtuins

Alan An Jung Wei, Christian Arlt, Andrea Sinz

The tumor suppressor p53, known as the "guardian of the genome," plays a significant role in DNA repair, cell cycle control, and apoptosis in human cells. Gaining insights to the full-length, wild-type three-dimensional structure of p53 upon binding to different proteins proves to be challenging due to p53's flexibility from its intrinsically disordered domains located on the N- and C-termini. With the lack of a stable structure in solution, the conformation of an intrinsically disordered protein (IDP) is difficult to characterize. A promising approach is through chemical cross-linking combined with mass spectrometry (XL-MS) as no crystallization is required and low protein concentration is sufficient to obtain structural information.

The regulatory domain located on the C-terminus of p53 plays an important role in transcriptional activity. We are interested in studying the binding of S100ß and human sirtuins to p53. They are known to bind specifically to the regulatory domain and inhibit the transcriptional activity of p53. IDPs often undergo a disorder-to-order transition upon binding. By applying geometric constraints between p53 and its interactors, S100ß and human sirtuins, via chemical cross-linking, these protein-protein interactions can be further investigated and more details on conformational changes will be gained.

Poster Presentation P11

Mapping the differences in structural dynamics of Coil2B region in WT and S-glutathionylated vimentin by HDX-MS with on-line nepenthesin-2 digestion

Magdalena Kaus-Drobek (1) and Michal Dadlez (2)

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Vimentin belongs to the type III intermediate filaments proteins (IFs) being a major cytoskeletal component of mesenchymal cells. Besides its basic mechanical function in maintaining cell flexibility, vimentin is important in cell division, wound healing and cell signaling. It has been implicated in many aspects of cancer initiation and progression, including tumorigenesis and epithelial-mesenchymal transition, making this protein an attractive potential target for cancer diagnosis and therapy. To fulfill its roles, vimentin undergoes highly dynamic disassembly/assembly cycles that can be regulated by post-translational modifications e.g phosphorylation or cysteine modification.

Due its coiled-coil structure and unstructured head and tail regions, vimentin is difficult target for high-resolution protein structure determination methods like X-ray crystallography or NMR. Hydrogen-deuterium exchange study monitored by mass spectrometry (HDX-MS) enabled to follow the structural changes of full-length vimentin during transition from tetramers to ULFs, and upon further annealing of ULFs into filaments.

HDX-MS studies have shown that S-glutathionylated vimentin (Vim-SSG) does not form filaments, whereas the ULFs formation is not affected. The results were confirmed by electron microscopy (EM) and atomic force microscopy (AFM). Vimentin contains the single cysteine residue present in Coil2B region. This region was not covered during pepsin digestion (80% sequence coverage). Thus, we started to investigate other acidic proteases to obtained the highest sequence coverage. We successfully applied nepenthesin-2 (nep-2) column (AfiiPro, Czech Republic) that enable us to obtain almost 100% sequence coverage of Coil2B. We have performed HDX-MS study on full length vimentin with on-line nep-2 digestion and track structural changes upon cysteine modification. We identified differences in coil2B structural dynamics between WT-Vim and Vim-SSG, that can help us to understand the consequences of such modification.

Poster Presentation P12

Structural and interacting analysis of the human Peptidyl-Prolyl-cis/trans-Isomerases Par14 and Par17 by photo-amino acids

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Human Parvulin14 and Parvulin17 result from alternative transcription initiation of the PIN4 gene and share the same catalytic PPIase domain. The N-terminus of Par17 is elongated by 25 amino acids and both N-terminis seem to be unfolded and flexible. Par14 is involved in biogenesis of ribonucleoprotein-complexes, RNA processing and DNA repair [1]. Its elongated isoform Par17 participates in protein transport/translocation and in cytoskeleton organization. The polymerization of Tubulin by Par17 was already demonstrated [2]. We try to narrow down the cellular roles of the PPIases. In a first attempt, the interaction of the N-terminus with the PPIase domain was investigated by NMR analysis. When the N-terminus is attached to the PPIase domain changes of the structure near the catalytic cleft could be demonstrated. Intramolecular cross-linking of photo-amino acid labeled proteins and DSSO crosslinking experiments complement these NMR results. In addition, the photo-labeled parvulin proteins were cross-linked with HeLa lysate. Evaluation by MS/MS spectrometry resulted in the identification of already known, but also new interactors of the PPIases. Among these interactors was b-actin, whose polymerization was accelerated in the presence of Par17.

References:
[1] Matena, Anja; Rehic, Edisa; Hönig, Dana; Kamba, Bianca; Bayer, Peter (2018): Structure and function of the human parvulins Pin1 and Par14/17. In: Biological chemistry 399 (2), S. 101–125. DOI: 10.1515/hsz-2017-0137.
[2] Burgardt, Noelia Inés; Schmidt, Andreas; Manns, Annika; Schutkowski, Alexandra; Jahreis, Günther; Lin, Yi-Jan et al. (2015): Parvulin 17-catalyzed Tubulin Polymerization Is Regulated by Calmodulin in a Calcium-dependent Manner. In: The Journal of biological chemistry 290 (27), S. 16708–16722. DOI: 10.1074/jbc.M114.593228.

Poster Presentation P13

Toward an Optimized Strategy for Direct Measurement of Nascent Proteomes

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Regulation of gene expression is controlled by mRNA translation and thus defines the functional proteome. Experimental methodologies are needed that can rapidly capture and reveal actual nascent proteomes. In addition, such methods must be able to measure changes in nascent protein abundances quantitatively in response to cellular states, and pharmacologic or environmental cues.

We have reported a protocol using an analog of puromycin, a tyrosine-tRNA mimetic, called O-propargylpuromycin (OPP), to identify nascent proteomes rapidly. OPP is cell permeable and catalytically incorporated into nascent polypeptide chains as a specific covalent label during elongation resulting in premature termination. These polypeptides are biotinylated using click chemistry, then isolated and measured by tandem mass spectrometry [1].

Recently we have made significant changes in our protocol aimed at optimization of the detection and identification of a nascent proteome by minimizing the number of steps in the procedure and by incorporating a cleavable biotin linker. Thus, with the use of a cleavable biotin linker, on-bead digestion is avoided and thus the problem of distinguishing actual nascent proteins from lysate proteins adsorbed into the beads (the crapome).

This optimized OPP-ID strategy provides significantly increased extent of sampling of the nascent proteome and reveals an accurate experimental view of changes in gene expression at the nascent protein level. These changes provide enhanced sensitivity for the determination of the actual proteomic changes occurring in response to a variety of cues that direct cellular fates such as proliferation or differentiation.

Our OPP-ID strategy has the potential to ask innumerable questions on the composition and rapid dynamics of proteomic networks at the organismal level.

1. Forester C., et al., Proc Natl Acad Sci., 2018, doi: 10.1073/pnas.1707514115

Poster Presentation P14

A Simple Cross-Linking/Mass Spectrometry Workflow to Study System-Wide Protein Interactions

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We present a cross-linking/mass spectrometry (XLMS) workflow for performing proteome-wide cross-linking analyses within one week. The workflow is based on the commercially available MS-cleavable cross-linker disuccinimidyl dibutyric urea (DSBU) and can be employed by every lab having access to a mass spectrometer with tandem MS capabilities. We provide an updated version 2.0 of the freeware software tool MeroX, available at www.StavroX.com, that allows conducting fully automated and reliable studies delivering insights into protein-protein interaction networks and protein conformations at the proteome level. We exemplify our optimized workflow for mapping protein-protein interaction networks in Drosophila melanogaster embryos on a system-wide level. From cross-linked Drosophila embryo extracts, we detected 29,931 cross-link spectrum matches corresponding to 7,436 unique cross-linked residues in biological triplicate experiments at 1% FDR. Among these, 1,611 inter-protein cross-linking sites were identified that yield valuable information on protein-protein interactions. The remaining 5,825 intra-protein cross-links yield information on conformational landscape of proteins in their cellular environment.

Ref.:
Goetze M, Iacobucci C, Ihling CH, Sinz A. Analytical Chemistry 91:10236-10244, (2019).

Poster Presentation P15

Integrative structural modeling of the type III secretion system

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The type III secretion system (T3SS) is a macromolecular transmembrane protein complex used by many pathogenic Gram-negative bacteria (e.g. Shigella, Salmonella, Pseudomonas, Yersinia) to initiate infection by injecting virulence proteins (effectors) from the bacterial cytoplasm directly into their eukaryotic host cells. It is composed of a needle complex that spans through both bacterial membranes and intracellular components of the sorting platform, responsible for effector recruitment. With antibiotic resistance on the rise and the resulting need for novel therapeutics, the T3SS displays as a potential new drug target, to combat infections caused by Gram-negative bacteria. With an integrative modeling approach combining cryo-electron microscopy with cross-linking mass spectrometry, we hope to gain further structural insights into the function of the Shigella T3SS.

Poster Presentation P16

Can we detect local conformational changes with XLMS?

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Our project aims to use the potential of mass spectrometry in coupling with a cross-linking strategy (XLMS) in a context of structural analysis of a nuclear receptor system from the retinoic acid family. However, even if experimental approach becomes better controlled, we still face many difficulties in choosing the most appropriate cross-linker or in the choice of key criteria for a reliable interpretation of MS/MS fragmentation spectra of cross-linked peptides. Different cross-linkers have been tested on our system like classical (BS3) or MS-Cleavable (DSBU, CDI) cross-linker. The XLMS strategy was applied in absence and in presence of a nucleotidic interacting partner in order to evaluate whether the spatial resolution obtained makes it probable to describe the possible conformational changes upon DNA binding. Monitoring and optimization of the cross-linking reaction was carried out using MALDI-TOF. The cross-linked amino acids were then characterized by proteomic analysis using dedicated bioinformatic tools. A comparison of different XLMS dedicated software demonstrated that scoring cannot be directly related to interpretation's confidence making thus manual validation of crosslinked MS/MS spectra crucial. In a second step, XLMS was applied in presence of various oligonucleotides partners to assess whether the obtained spatial resolution makes it possible to describe an eventual conformational change upon DNA binding. One of the big questions on the XLMS strategy is whether the addition of the cross-linker agent leads to a conformational change of the complex. For this, studies by Infrared spectroscopy (ATR-FTIR) were carried out to see if a conformational change of the secondary structure would be visible in presence of the cross-linker. With this technique, it was also possible to study the complex in the presence and absence of the oligonucleotides in order to observe whether a conformational change of the complex is visible and therefore complete the results obtained by XLMS.

Poster Presentation P17

Identification of Coomassie-stained lipids separated by thin-layer chromatography

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The main components of biological membranes are phospholipids, a diverse group of molecules characterized by the nature of their head groups as well as the composition of their fatty acyl chains. The interactions of phospholipids and membrane proteins is only poorly understood. Limited insights have been gained by co-purifying proteins together with their associated lipids followed by mass spectrometry (MS)-based identification. However, the analysis of complex lipid mixtures and co-extracted detergents requires separation by liquid chromatography which is not always possible. Thin-layer chromatography (TLC) is a straighforward alternative, however, commonly used dyes modify lipids and complicate their identification by MS. We therefore developed a TLC-based workflow including Coomassie staining and subsequent extraction of separated lipids for the identification of phospholipids by MS.

Samples containing phospholipids were separated on standard TLC plates using Chloroform:Methanol:Water (65:25:4) as mobile phase. After staining with 0.3 (m/v) % Coomassie Brilliant Blue in 20 (v/v) % methanol, lipids were extracted from TLC plates according to Bligh and Dyer followed by additional washing steps. Extracts were then subjected to shotgun lipidomic experiments using a Q Exactive Plus Orbitrap mass spectrometer.

We found that Coomassie staining is suitable for most phospholipid classes including lipid species with acyl chains > 14:0/14:0. Extraction of lipids from TLC plates is sufficient for MS-based identification with recovery rates > 30 %. Utilizing an E. coli lipid extract as a benchmark, we could show that preceding TLC separation yields identification of more lipid species when compared with simple shotgun analysis. The proposed workflow therefore enables the identification of low abundant lipids by decreasing spectral complexity in shotgun experiments. Currently, we are extending this workflow to identification of lipids co-purified with membrane proteins by separating lipids and detergent molecules.

Poster Presentation P18

ClpCP in the act: Degradomics investigation of ClpCP-substrates

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In Gram-positive bacteria, the AAA+ protease ClpCP acts as driving force in protein quality control, regulating protein homeostasis under cellular stress conditions. In resemblance to the eukaryotic proteasome-ubiquitin system, protein degradation via ClpCP can be initialized by introduction of a substrate recognition tag. In addition to conventional protein targeting, phosphoarginine (pArg) acts as such degradation-initializing tag. Aberrant proteins that need to be degraded are labeled by McsB, a protein kinase unique in phosphorylating arginine residues. The protease associated unfoldase ClpC specifically recognizes pArg via a binding pocket located at its N-terminal domain (NTD) and then translocates the captured substrate protein into the proteolytic cage formed by ClpP. However, knowledge on the native ClpCP substrates is very limited so far.
In this project, a combined biochemical, proteomics and structural biology approach is used to characterize ClpCP's substrate specificity and degradation efficiencies. By application of the novel degradomics approach MAPP (mass spectrometric analysis of proteolytic peptides), ClpCP substrate degradation is monitored on a proteome-wide scale. In a proof of principle experiment MAPP is tested at small scale on a system containing the model substrate beta-casein as well as the native substrates Kre and CtsR. Degradation products of those substrates are captured, using DSP to crosslink proteolytic peptides in the active ClpP chamber, and measured by LC-MS/MS. The optimized workflow and mass spectrometric measurement of proteolytic peptides is then applied in-vitro as well as in-vivo on cell lysates of the model organism B. subtilis to map ClpCP substrates and to develop a better understanding of ClpCP function.

Poster Presentation P19

Targeting protein-protein interactions in cancer by mass spectrometry

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The chaperone HSP90 is essential for protein homeostasis and cell survival, particularly in cancer where high growth rates cause major cellular perturbations. In tumoral cells, HSP90 has been found significantly overexpressed associated with the co-chaperone HSP70-HSP90 organising protein (HOP). The HOP-HSP90 interaction is characterised by a 'carboxylate clamp' present on the TPR2A domain of HOP, which binds to the conserved C-terminal MEEVD motif of HSP90. The main challenge faced by conventional HSP90 inhibitors is the compensatory action of other chaperones that is induced upon treatment. Recent evidence suggests that inhibiting the HOP-HSP90 interaction does not induce this compensatory response, constituting an attractive approach for inhibiting HSP90.
Here we present our latest results on the biophysical characterisation of the TPR2A-MEEVD interaction and the screening of potential inhibitors of the TPR2A-HSP90 interface. To that end, several mass spectrometry techniques are applied, including collision-induced unfolding ion mobility MS and chemical cross-linking MS. In addition, different small molecule inhibitors and synthetic peptides mimicking the chemistry and/or structure of the C-terminal motif of HSP90 are studied. These results may provide some insights to the design of optimised ligands in later screening assays.

Poster Presentation P20

Application of DSBU cleavable cross-linker to study IFT-B complex interactions

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Cilia play central roles in cellular signaling, fluid movement and locomotion. As cilia lack protein synthesis machinery, their generation (ciliogenesis) largely relies on the delivery of ciliary proteins from the cytoplasm into the cilium. This active protein transport process is called intraflagellar transport (IFT) and is mediated by two large, highly conserved protein complexes, IFT-A and IFT-B. Defects in the IFT machinery cause various genetic diseases collectively called ciliopathies. These include Bardet–Biedl syndrome (BBS), Joubert syndrome (JBTS), Meckel syndrome, polycystic kidney, retinal degeneration, obesity, infertility, and brain and skeletal malformation. However, the molecular basis of these phenotypes is largely unknown. A detailed understanding of the structure and interactions of these complexes is critical towards understanding their biological functions. In this work we have combined chemical cross-linking with mass spectrometry (XL-MS) to map protein−protein interactions between a subset of proteins from the IFT-B core subcomplex, the hexamer IFT81/74/27/25/22 and RabL2. Our results support previous studies that have shown interaction between RABL2 and the IFT74–IFT81 heterodimer, and provide additional evidence of the recently published association between IFT22 and IFT74/81. Overall, we have been able to identify 882 crosslinked peptide pairs at 1% FDR, which correspond to 357 unique crosslinking sites. We demonstrate that XL-MS is a powerful approach that enables structural biology studies by the identification and mapping of protein-protein interactions. This valuable spatial information will help to elucidate the function and regulation of different proteins complexes as well as the processes in which they are involved.

Poster Presentation P21

Using quantitative crosslinking mass spectrometry to investigate the structural dynamics of ubiquitin ligase E6AP

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Chemical crosslinking in combination with mass spectrometry (XL-MS) has emerged as a powerful new technique in structural biology over the past decade [reviewed in 1]. The ability to covalently link specific reactive groups in proteins like the ε-amino group of lysine residues allows to reveal information on the structure of single proteins as well as on interaction of protein complexes. Using XL-MS, our project aims to investigate the structural dynamics of the E3 ubiquitin ligase E6AP ("E6 associated protein") upon activation. The E3 ligase E6AP is an important component of the ubiquitin-proteasome system which was initially discovered through its interaction with the high-risk HPV protein E6. Upon activation by the oncoprotein E6 the E6AP-E6 complex targets the tumor suppressor p53 for degradation, leading to the formation of tumors as for example cervical carcinoma. A dysfunctional E6AP regulation is involved in other diseases as well: a functional loss of the maternal UBE3A allele in neuronal cells causes the neurodevelopmental disorder Angelman syndrome while an overexpression of E6AP was shown to be connected to autism spectrum disorders [reviewed in 2]. Since to this date little structural information on E6AP is known, the application of crosslinking in combination with quantitative mass spectrometry (qXL-MS) to investigate the structure-function relationship of E6AP is a highly promising and intriguing approach. Quantification of crosslinks can depict structural changes, for example induced by the activating protein E6 or by target substrate p53 [3]. Nevertheless, the challenge in qXL-MS remains that crosslinks are of lower abundance than non-crosslinked peptides resulting in a potentially less reliable quantification if data-dependent acquisition is used. Therefore, we and others [4] have recently started to establish DIA as well as targeted acquisition for a more sensitive identification and a more reproducible quantification of crosslinks. The combination of XL-MS and DIA or targeted proteomics is a highly promising approach for a more efficient quantification of crosslinked peptides.

[1] Leitner, A. et al., Trends in Biochemical Sciences, 2016. 41(1): 20-32
[2] Beaudenon S., Huibregtse JM., BMC Biochemistry, 2008. 9(Suppl 1): S4
[3] Sailer C., Offensperger F. et al., Nature Communications, 2018. 9(1): 4441
[4] Müller F. et al., Mol Cell Proteomics, 2019. 18(4): 786-795

Poster Presentation P22

S-gluthationylation in the calprotectin complex – an HDX MS analysis

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Calprotectin (CP) is a noncovalent heterodimer of S100A8 and S100A9 proteins. The complex exerts a variety of important intra- and extracellular roles in human physiology and pathology. [1] It is released from different cell types in sites of acute and chronic inflammation where it interacts with receptors and acts as a crucial pro-inflammatory mediator. It is most abundant and constitutively expressed in neutrophils, composing ~40% of the total cytoplasmic proteins [2]. CP plays also important antibacterial and antifungal role in innate immunity as a metal-chelating protein, which sequester metal nutrients essential for patognes [2]. Functional diversity of CP depends on formation of different proteoforms through oligomerization, metal ion binding and post translational modifications.

Most studies on CP structures were performed for either truncated CP variants or CP with mutated cysteine residues, whereas it is known that oxidative modifications of cysteines in S100A8 and S100A9 modify their biological functions. [3, 4, 5, 6].

We will presents the application of HDX-MS to study structural consequences of S-glutathionylation in full-length, wild-type CP.

1. Donato R., Cannon B.R., Sorci G., Riuzzi F., Hsu K., Weber D.J., Geczy C.L. Functions of S100 Proteins. Curr Mol Med. 2013 January; 13(1): 24–57.

2. Zygiel E, Nolan E. Transition Metal Sequestration by the Host-Defense Protein Calprotectin, Annual Review of Biochemistry, 2018.

3. Lim SY, Raftery MJ, Goyette J, Geczy CL. S-glutathionylation regulates inflammatory activities of S100A9. J Biol Chem. 2010;285(19):14377-88.

4. Lim SY, Raftery M, Cai H, Hsu K, Yan WX, Hseih HL, Watts RN, Richardson D, Thomas S, Perry M, Geczy CL. S-nitrosylated S100A8: novel anti-inflammatory properties. J Immunol. 2008;181(8):5627-36.

5. Berthier S, Paclet MH, Lerouge S, Roux F, Vergnaud S, Coleman AW, Morel F.Changing the conformation state of cytochrome b558 initiates NADPH oxidase activation: MRP8/MRP14 regulation. J Biol Chem. 2003;278(28):25499-508

6. Hoskin TS, Crowther JM, Cheung J, Epton MJ, Sly PD, Elder PA, Dobson RCJ, Kettle AJ, Dickerhof N. Oxidative cross-linking of calprotectin occurs in vivo, altering its structure and susceptibility to proteolysis. Redox Biol. 2019 Jun;24:101202. doi: 10.1016/j.redox.2019.101202. Epub 2019 Apr 13.

Poster Presentation P23

Cross-linking mass spectrometry on P-glycoprotein

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The ABC transporter P-glycoprotein (Pgp) has been found to be involved in multidrug resistance in tumor cells. Conformations of Pgp are extensively investigated, but these studies do not take into account the effect of lipids and cholesterol on the membrane protein, although they were shown to play an important role in Pgp's conformations.
In order to maintain the lipid environment, we used cross-linking mass spectrometry (XL-MS) technology to map exta- and intracellular regions on Pgp. Experiments were carried out using BS2Gd0/d4 and DSSO cross-linkers on living cells, membrane separation was performed with Mem-PER™ Plus Membrane Protein Extraction Kit. Enrichment of the cross-linked complexes was carried out by means of monoclonal anti Pgp antibodies on magnetic beads, followed by on-bead trypsin digestion. LC-MS/MS measurements were carried out on Orbitrap Fusion™ Lumos™ Tribrid™ Mass Spectrometer. Collected data was processed with Protein Prospector, Merox, Hekate and XlinkX software tools.
Several proteins, that had been known to be in proximity with Pgp, were identified in different samples. Our preliminary results revealed information about accessibility on the protein by means of monolinks. Some cross-links were recognized between neighboring proteins and residues from the same protein. Our further aim is to detect antibody binding sites and to set up 3D structures using the information cross-links can disclose.

Poster Presentation P24

Tyrosine-specific nitration of influenza hemagglutinin proteins by selective covalent labeling and mass spectrometry

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Vaccination is the most effective means of preventing seasonal influenza epidemics and mitigating pandemics. Hemagglutinin (HA), the primary antigenic component of influenza vaccine, forms various complexes (trimers, rosettes, micelles) and vaccine potency is determined by quantification of immunologically active HA. Additionally, in vivo, HA undergoes an irreversible prefusion to postfusion structural transition, mediating viral entry. Thus, investigation of how HA's structure and conformation align with function is important when evaluating infection and vaccine efficacy.Amino acid-specific labeling coupled with mass spectrometry (AAL-MS) identifies HA's prefusion and postfusion states to assess conformational changes. A/Guangdong/17SF003/2016 (H7N9), a potentially pandemic strain, was evaluated. Native and low-pH stressed rH7 samples were prepared. Non-stressed samples were reconstituted in dH2O and used without further treatment. Low-pH samples were treated with 50 mM citrate for reduction and diluted with 1M Tris for neutralization. Tyrosine-specific nitration of native and pH-stressed rH7 was performed using tetranitromethane as the covalent labeling reagent, filtered to remove excess nitrating reagent and tryptically digested.

Peptide identification and labeling confirmation was performed using a quadrupole-orbitrap mass spectrometer (MS) and a triple quadrupole tandem MS (ThermoScientific). Peptide transitions were identified using Skyline v 4.2.0 (MacCoss Lab). Peptide coverage was evaluated using Mascot v 2.5.1 (Matrix Science). Data analyses were achieved by LC-ESI-MS/MS using an Agilent 1200 system and a triple quadrupole tandem MS (ThermoScientific).

Data indicate tetranitromethane selectively labels and successfully differentiates native from low-pH denatured rHA. In HA's native immunogenic form, unlabeled tyrosine is predominantly seen. In contrast, a substantial decrease in unlabeled tyrosine in combination with a significant increase in labeled tyrosine represents low-pH stressed, inactive HA. In summary, tyrosine-specific HA nitration distinguishes prefusion from postfusion states, has the capability to be accurately quantified, and is an innovative approach, providing insight into HA's conformation and its influence on measurements of influenza vaccine potency.

Poster Presentation P25

HaDeX: tool for analysis of HDX-MS data

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Hydrogen-deuterium mass spectrometry (HDX-MS) is an analytical tool for monitoring dynamics and interactions of proteins. In the glaring opposite to crystallography-based methods, HDX-MS allows a unique insight into the dynamics of the protein structure. Such data is larger and more complicated than static structure, thus requires a dedicated software suite which is provided by a few vendors. However, the majority of existing tools do not cover a satisfying analytic workflow, especially on the level of data presentation and proper statistical interpretation. We propose HaDeX, a novel tool for processing, analysis, and visualization of output data from existing search engines used in HDX-MS experiments. HaDeX features functions supporting the whole analytical process, including preliminary data exploration, ISO-based uncertainty, quality control, and generation of customizable publication-quality figures. The reproducibility of the entire process is ensured with advanced reporting functions. HaDeX is available primarily as a web-server (http://mslab-ibb.pl/shiny/HaDeX/), but his all functionalities are also accessible as the R package (https://github.com/hadexversum/HaDeX or https://cran.r-project.org/web/packages/HaDeX/index.html) and standalone software.

Poster Presentation P26

Charting the structural organization of higher-order protein interaction networks in cells and organelles by cross-linking MS

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Structural analysis methods like X-ray crystallography (XRC), nuclear magnetic resonance spectroscopy (NMR) and cryo-electron microscopy (Cryo-EM) are well established and powerful tools for the examination of macromolecular structures and interactions, as well as their dynamics. However, those methods currently not easily allow the investigation of proteins in their larger biological context. To achieve this feat, in our laboratory, cross-linking mass spectrometry (XL-MS) has gained considerable interest as a complementary method, enabling us to study protein structures as well as interactions in a more complex – and biologically more relevant environment (Klykov et al., 2018; Fasci et al., 2018; Liu et al., 2018). Using cross-linking mass spectrometry, we aim to capture protein complex structures and computationally model their structural organization, to reveal new complex structures and to further refine published structural models. We further aim to use cross-linking data to unravel structure-function relationships by identifying higher-order protein interaction networks, ultimately to better understand the physiological functions exerted by proteins and protein complexes in-vivo. In this poster I will highlight recent developments in our work-flow, based on cleavable and/or enrichable cross-linker reagents, advanced hybrid fragmentation methods and software suite XlinkX, and how I apply that to better understand the mitochondrial organization.

Poster Presentation P27

XiView: A common platform for the downstream analysis of Crosslinking Mass Spectrometry data

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XiView provides a common platform for the downstream analysis and visualisation of Crosslinking Mass Spectrometry (CLMS) data. It is independent of any search software and its input is compliant with the HUPO-PSI mzIdentML 1.2.0 mass spectrometry data standard but also accepts common CSV formats.

Existing interfaces for CLMS data focus on static presentations or strictly sequential and separate views of the datasets under analysis. In XiView, once uploaded, the experimental data can be investigated in a suite of tightly-coordinated simultaneous views that support dynamic and coordinated filtering, selection and highlighting. View types include a network view, a circular view, a contact map, a scatterplot, and a histogram for summary statistics. The NGL Viewer is also integrated as a first-class component to show a 3D structure if a relevant PDB file is known, and allows export of structures and selected crosslinks to PyMol. The underlying spectra that support any given cross-link can be opened and inspected. All the views can be exported in their current states as SVG or PNG graphic files, and filtered data exported in CSV format.

Each individual view within XiView accentuates and reveals a different aspect of the underlying data, and the coordination between them aids in understanding the relationships between these aspects, which ultimately leads to a better understanding of the dataset as a whole.

XiView is available at xiview.org. Registration is needed to provide the fullest utility of the website, but this is free, and an example dataset is available through the XiView home page that requires no registration to view.

Poster Presentation P28

Formation of transcription factor·DNA complexes studied by native MS

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Transcription factors (TF) mediate gene expression regulation through interactions with DNA and other regulatory proteins. Therefore, they play a crucial role in many biological processes including growth and development, metabolic pathways or tumorigenesis. Nevertheless, strict regulation of TF's activity is required and one of the possible ways how they are regulated, is through differences in their affinity to different DNA sequence motives. In our work, we have studied the effect of DNA sequence adjacent to the binding motif together with orientation of the motif on interaction of DNA binding domain (DBD) of TEAD1 with its DNA response motifs.

First, we checked the ability of TEAD1-DBD to form complexes with all selected oligonucleotides and estimated KD of each complex. Since methods usually used for protein-DNA KD determination are sample consuming or require labelled DNA or protein, they are not convenient for evaluation of multiple complexes in short time. Thus, we have tested the potential of native MS (nESI coupled to FT-ICR) for KD estimation. For that purpose, TEAD1-DBD·DNA complexes with different previously determined KD were used. The ratio of signal intensities of TEAD1-DBD in its apo- and holo-forms were used to calculate bound fraction of TEAD1-DBD. It was proportional to the known KD and thus proved this technique suitable for evaluation of various protein-DNA complexes.

Since interaction with other regulatory proteins is also important for TF regulation, we have tested binding two different TFs (TEAD1 and FOXO4) to several oligonucleotides with binding sites for both proteins. Using gel shifts together with native MS we have observed, that the trimer was preferentially formed if the binding motives partially overlapped.

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Poster Presentation P29

Protein-DNA binding characterization using FPOP protein footprinting

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FPOP protein footprinting method (Fast Photochemical Oxidation of Proteins) is a covalent labelling method able to detect solvent-accessible regions on proteins by comparing reactivity of amino acid residues to hydroxyl radicals produced by laser photolysis of hydrogen peroxide. In comparison to hydrogen-deuterium exchange, the covalent nature of the label ensures the stability of the information until MS-detection and easy handling of the sample. This also means that broader range of proteases can be used for digestion to obtain optimal sequence coverage, which can be difficult for some proteins in hydrogen-deuterium exchange without using specialized low-pH proteases. The resolution of the method is dependent on both protease digestion and amino acid composition, since not every amino acid is reactive to hydroxyl radicals. Amino acids readily reacting with hydroxyl radicals are Met, Cys, Trp, Tyr, His, Phe and Pro, with several other such as Leu and Ile also reported as reactive with lower yields.
In our current project, we aim to expand the use of FPOP technique for study of protein-DNA binding. We chose known system of FOXO4 transcription factor and its DNA binding motif as a model. Samples of FOXO4 alone and FOXO4 in complex with DAF16 DNA were subjected to FPOP and the extent of oxidation was compared using bottom-up analysis. During the analysis, several proteases in solution or immobilized to the beads were tested and significant differences in the rate of oxidation were detected on FOXO4 DNA binding helix, with higher oxidation being present in samples without DNA.
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Poster Presentation P30

Identification of protein-DNA interaction sites with chemical cross-linking mass spectrometry in chromatin samples

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Protein-DNA interactions play important role in various cellular processes such as transcription or replication. To have a better understand in the underlying processes the protein-DNA complexes need to be studied. Among several methods, cross-linking mass spectrometry is one of the suitable tools to identify these interaction sites. In this method as a first step a chemical bond is formed between the close proximity protein and DNA moieties, followed by enzymatic digestion on both protein and DNA too. The resulting peptide-oligonucleotide cross-links are enriched, measured with LC-MSMS and analyzed by database approach. With this method, we can assign DNA-binding protein domains, we can narrow down the interactions to the cross-linked amino acids, and we can also identify the cross-linked nucleotides.
In this study, we mainly focused on in-vitro reconstituted oligonucleosomal arrays as chromatin model. We identified the cross-linked peptide-oligonucleotides heteroconjugates of all four core histones as well as the cross-linked nucleotides. Overall, our data proofs that chemical cross-linking mass spectrometry is a very efficient tool for characterization of protein-DNA complexes.

Poster Presentation P31

Conformational differences in human BAG-1 isoforms revealed by hydrogen/ deuterium exchange mass spectrometry

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BAG-1 is a potent anti-apoptotic molecule and its aberrant expression is prevalent in human malignancies. BAG-1 is expressed as multiple isoforms (-1L, -1M, -1S, -XS), each functioning in an array of distinct key cellular events including proliferation, cell survival, transcription, apoptosis and cell motility. This multi-functionality renders BAG-1 a potential therapeutic target to blockade signal transmission in cancer cells. However, the lack of resolved tertiary structure of BAG-1 isoforms limits the development of molecularly-targeted approaches for therapeutic use. In this study, to define higher-order structure and to understand in more depth the molecular architecture of two major BAG-1 isoforms (BAG-1L, BAG-1S), we utilized hydrogen-deuterium exchange mass spectrometry (HDX-MS) as a structural tool. First, His6TEV-BAG-1S and His6TEV-BAG-1L plasmids were constructed, and MCF-7 cells were transiently transfected with the constructs. Cells were lysed, and BAG-1L and BAG-1S were purified from total cell lysates of transfectants with a tandem Ni-NTA purification approach through bind/elute and flow-through mode, respectively. The purified BAG-1L and BAG-1S showed an apparent 33-kDa and 50-kDa band in gel electrophoresis, respectively. Sample purity of both proteins was estimated as >90% using ImageJ analysis of SDS-PAGE gel. To monitor the level of deuteration and to analyze conformational variations among human BAG-1L and BAG-1S isoforms, HDX-MS experiments with five time-points that ranged from 12 s to 24 h were carried out. Subsequent HDX-MS experiments revealed the identification of ~150 peptides of BAG-1L and BAG-1S with a >99% sequence coverage. Based on three-dimensional structural model, we found that nuclear localization signal sequence and the more number of copies of acidic-rich repeats located at the N-terminus of BAG-1L resulted in some structural variations at its N-terminus compared to BAG-1S. We report here the first isoform-specific structural variations of BAG-1.

Poster Presentation P32

Fast photochemical oxidation of protein/DNA complexes coupled to Top down mass spectrometry

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Fast photochemical oxidation of proteins (FPOP) coupled with mass spectrometry has become powerful method of structural proteomics. Wide range of the approaches including hydrogen deuterium exchange and chemical crosslinking are used to study protein structures and protein-protein interactions. Among these techniques, hydroxyl radical footprinting is currently evolving in recent years. FPOP utilizes KrF excimer laser (wavelength 248 nm) to dissociate hydrogen peroxide and produce reactive hydroxyl radicals. In the presence of reactive hydroxyl radicals, solvent accessible amino acids of protein are oxidized. Labelled protein is subsequently submitted to bottom-up analysis.
In this study, an alternative Top down approach was tested to identify oxidized residues on a full-length DNA binding domain (DBD) of FOXO4 in the presence or absence of its DNA binding element (DAF16). Native electrophoresis and native nano-electrospray ionization were used to confirm the presence of complex FOXO4-DBD/DAF16. Then, the Apo (FOXO4-DBD) and Holo (FOXO4-DBD/DAF16) forms were submitted to FPOP. At a protein level, most of the modifications appeared as a +16 Da and +32 Da. To identify oxidized residues, singly and doubly oxidized Apo and Holo forms were fragmented in the gas phase by electron-capture and collision-induced dissociations. Comparing the oxidized fragment ions intensities of Apo and Holo forms pointed out the resistance to oxidation of helix H3 which is responsible for DNA binding. Such results demonstrate the potential of Top down approach for FPOP of protein/DNA complexes.
This work was supported by the Czech Science Foundation (grant numbers 19-16084S), the Ministry of Education of the Czech Republic (program “NPU II” project LQ1604), 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).

Poster Presentation P33

Infrared Reflection Absorption Spectroscopy reveals Structural Transitions in Membrane Proteins

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Infrared Reflection Absorption Spectroscopy (IRRAS) is a versatile technique to study the organization of interfacial films on a molecular or even sub-molecular level. It provides important information to understand macroscopic properties of interfacial layers, such as lipid monolayers including peripheral membrane proteins. IRRAS combines the film balance technique with IR reflectivity and spectroscopy, which supplies structural information, reveals the presence of molecules, their interactions, their conformation and their phase state. In addition, and in contrast to solution IR spectroscopy the orientation of molecules or molecular moieties at the interface can be determined by IRRAS. This is due to the perfectly flat geometry of the model system and the possibility to control polarization and incidence angle of the IR light.
We will present examples for the value of IRRAS in studying lipid - protein interactions. Such, it was used to determine conformation and orientation in which fusion peptides bind to lipid membranes [1]. Furthermore, the orientations of membrane binding proteins could be determined [2] and even structural rearrangements within large proteins during the binding process [3] were revealed in nearly natural conditions, i.e. physiological temperature, desired buffer composition and low protein concertation.

[1] M. Rabe et al., Langmuir, 2014, 30, 7727
[2] C. Schwieger et al., Polymers 2017, 9, 612
[2] M. Hoernke et al., PNAS, 2017, 214 (22), E4360

Poster Presentation P34

HypG and its Role in Diatomic Ligand Biosynthesis of NiFe-Hydrogenases Studied by Native Mass Spectrometry

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[NiFe]-hydrogenases have a bimetallic active-site cofactor, in which the iron ion carries a CO and two CN- as diatomic ligands (Fe(CN)2CO). While biosynthesis of the CN- ligands seems to be clear, the biosynthesis of the CO ligand remains unresolved. Six Hyp proteins are involved in cofactor biosynthesis, but only the FeS-cluster-containing HypD protein is redox-active. HypD is assumed to be required to transfer the CN- ligands from the HypE protein to the iron, and circumstantial evidence also suggests that the CO ligand is generated by HypD from endogenous CO2 already bound to an iron ion on a HypD-HybG (HypC paralogue) sub-complex. The assembly of the Fe(CN)2CO co-factor was known to happen in the HybG/HypD complex where HypD acts as a scaffold for assembly. The interaction sites for the CN-, Fe and CO2 within the HybG/HypD complex remained elusive. We were able to determine not only the binding site for the CN—ligand, but also the dependency of CN--binding for CO2 attachment by native mass spectrometry. We discovered that HybG binds the CN- ligand at position C2 by analyzing a C2A mutant of HybG. Impairing this interaction also hinders CO2 binding of HybG which is required to form the CO-ligand for the assembly of Fe(CN)2CO. A further analysis of HybG derived from a HypE-deficient E.coli strain yielded identical results. This finding indicates an alternative source for the CN- ligand besides the assumed HypE/F maturation factors. These investigations give new insights into the mechanisms of the maturation process of [NiFe]-hydrogenases.

Poster Presentation P35

Chemical cross-linking: Polarity matters

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Nowadays many cross-linkers with different chemistry mechanisms are used for mapping structure of proteins or proteins complexes. However, often due to their poor solubility in water (hydrocarbon chain spacers, hydrophobic leaving group) non-polar solvents such as DMSO, DMF must be used for their dissolution. To prevent addition of non-polar solvent to sample, water-soluble cross-linkers with a polar leaving group such as sulfosuccinimidyl group have been synthesized and are commonly available on the market.
This study comparing the polarity effect of leaving groups of cross-linking agents while maintaining the spacer distance (7.7 Å). As crosslinking agents disuccinimidyl glutarate (DSG), bis(sulfosuccinimidyl) glutarate (BS2G) and equimolar mixtures composed of DSG/d6-BS2G and d6-DSG/BS2G were used for crosslinking of BSA protein. Then, standard bottom-up approach was used, and data were evaluated by StavorX software.
Despite the equimolar ratio used for crosslinking reaction with DSG/d6-BS2G yields of light and heavy cross-linked peptides were inconsistent from expected ratio 1:1. Moreover, opposite ratios were observed for a mixture composed of d6-DSG/BS2G. According to this observation relative quantification of all found crosslinked peptides was performed. Due to the known origin of light and heavy cross-linker preferential cross-links were assigned for DSG and BS2G.
Basically, this experiment has shown that polarity of cross-linker can highly affect obtained results even if the cross-linkers have the same spacer length. Thus, consideration should be given on using cross-linkers in the mixture (nonpolar/polar) instead of using one cross-linker with the desired length.
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Poster Presentation P36

Cross-Linking Mass Spectrometry and Evolutionary Couplings; A Hybrid Approach for Interaction Prediction and Structure Elucidation of Protein-Protein Complexes

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Almost all biological processes are effectively mediated by protein-protein interactions (PPIs). Understanding PPIs requires 3D information about protein-protein complexes and interfaces. High-resolution experimental approaches still have comparatively low throughput and computational approaches still have robustness issues. Hybrid approaches may offer immediate solutions that outperform standalone approaches. Combining sparse data from cross-linking mass spectrometry and evolutionary coupling might be an effective solution for a large-scale interaction prediction and structural elucidation of PPIs, as the methods are comparatively fast and cost-effective. The information generated by these two approaches is thought to be complimentary. For instance, evolutionary couplings can provide short-range constraints between the key interacting residues at the interface while cross-linking mass spectrometry can provide long-range constraints about the topological arrangement of the interacting domains. This project aims to conduct a proof of concept study combining cross-linking mass spectrometry and evolutionary coupling towards the better de novo prediction, structure elucidation and modeling of protein-protein complexes.

Poster Presentation P37

Interaction interface of matrix protein and cytoplasmic tail of polyprotein Env from Mason-Pfizer monkey virus

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The recruitment of the retroviral envelope glycoproteins (Env) into the newly formed retroviral particle is crutial to maintain the infectivity of the retrovirus. Env is membrane protein complex initially incorporated into host-cell membrane. There it must be recognized by other retroviral components to ensure that the virus will be enveloped with the Env-containg host-cell membrane during the viral budding process. Specific incorporation of HIV-1 Env was shown to be facilitated by the interaction of its cytoplasmic tail (CT) with matrix domain (MA) of the retroviral Gag polyprotein precursor. However, the mechanism of such interaction has not been investigated in the case of other retrovires.

Our aim was to clarify the interaction between MA and CT of Mason-Pfizer monkey virus (M-PMV). Since both proteins are supposed to form trimers, we produced M-PMV CT in fusion with yeast trimerization domain (GCN4V-CT) on its N-terminus and MA was produced in concentration ensured its trimerization. Both proteins were mixed and analyzed by the use of chemical crosslinking with mass-spectrometric detection (XL-MS) and NMR spectroscopy. As a result of XL-MS we detected nine cross links derived from both studied proteins. Five crosslinks were formed from peptides localized in the loop between MA helices I and II and N-terminal part of CT sequence indicating that cross-linked regions of both proteins could be located close to the plasmatic membrane of the host cell. Using NMR spectroscopy, the chemical shift perturbations in both the MA and the CT spectra confirmed the close interaction between regions of helices III and IV in MA and C-terminal part of CT which lack of lysine residues and could not be maped by th used BS3 crosslinker. Based on the XL-MS and NMR results, the model of MA interacting with CT was designed.

Poster Presentation P38

Structural investigation of the peroxisomal docking complex by quantitative crosslinking-MS and native MS

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Peroxisomes emerged in recent years as versatile and dynamic organelles, which fulfill important functions in cell metabolism. Peroxisomal matrix proteins are nucleus-encoded and thus need to be imported into the organelle upon their synthesis in the cytosol. The import is facilitated by a complex and highly dynamic protein import machinery, also termed the peroxisomal translocon. Cytosolic matrix proteins, comprising a peroxisomal targeting signal (PTS) 1, are captured by the soluble receptor protein Pex5p, which in turn interacts with the membrane associated docking complex for subsequent import. This complex comprises Pex14p, for which it is known that homo-oligomerization is essential for complex formation, Pex13p and in the yeast Saccharomyces cerevisiae additionally contains Pex17p and Dyn2p. However, beside these basic steps many aspects of peroxisomal matrix protein import remain elusive.
In this work, we identified essential oligomerization domains of Pex14p by subjecting recombinant truncated constructs to chemical crosslinking and native MS as a complementary method. We further established a label-free quantitative XL-MS approach for the analysis of cross-linked products of specific oligomeric states of Pex14p. By this, we were able to identify interaction sites which are unique for the respective oligomeric state of Pex14p. Furthermore, we used native MS to examine the dynamic nature of Pex14p homo-oligomerization as well as its dependency on the sample concentration and functional domains. To also obtain some insights into the so far unknown role of Dyn2p, we acquired native MS and XL data for the dimeric wildtype form of Dyn2p and analyzed its dimerization behavior using specific site mutants. Finally, we compare our structural proteomic data with in silico prediction data and discuss them in terms of possible structural arrangements of the individual components of the peroxisomal docking complex.

Poster Presentation P39

Protocol for high-yield production of photo-leucine-labeled proteins in Escherichia coli

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Cross-linking mass spectrometry is an emerging field to obtain structural and dynamic information of biomacromolecules and their complexes in cells and in vitro. In the past decade, several new cross-linkers have become available for this purpose (1). A highly interesting class among these for short-distance structural information are photo-reactive amino acids, such as photo-leucine and photo-methionine (2,3). Leucine residues are frequently found in the hydrophobic core of proteins and therefore, photo-leucine can provide information complementary to soluble cross-linkers which have a preference for surface-accessible sites. For structural biology applications, it is desirable to obtain photo-leucine-labeled proteins in milligram quantities at minimal costs.
Here, we present a protocol for the incorporation of photo-leucine in recombinantly expressed proteins in Escherichia coli. The protein is expressed at high cell densities, which reduces the amount of added photo-reactive amino acid by a factor of 10 compared to standard protocols, while achieving both high yields and high incorporation rates. We tested our protocol with the two chaperones Trigger Factor and SecB and determined label incorporation rates by label-free quantification. Our protocol is suitable to produce up to 3 mg of photo-leucine labeled protein from 100 ml of high cell density culture with incorporation rates of up to 34%. To further validate the successful incorporation of photo-leucine, in-cell cross-linking experiments were analyzed by SDS-page and Western Blot, as well as by MS/MS experiments. For the E. coli chaperone Trigger factor, 13 unique UV-cross-links were identified, which are all in agreement with published three-dimensional structures. The accessibility of milligram amounts of photo-leucine-labeled proteins at low costs will be highly useful to address structural biology questions with cross-linking methods.

1. Sinz A., Angew. Chem. Int. Ed. 2018, 57, 6390–6396.
2. Suchanek M. et al., Nat. Methods 2015, 2, 261–268.
3. Piotrowski C. et al., Methods 2015, 89, 121–127.

Poster Presentation P40

Azurin (electron transport protein) oligomerization study

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Countless electron transport (ET) processes occur in living organisms every day. Their study is a crucial field of modern structural and functional proteomics. In many cases model proteins like azurin from P. aeruginosa are utilised in experiments. This small cupredoxine exhibits absorbance maximum at 630 nm in 2+ redox state of the central Cu atom. During its oxidation to 1+ state the A630 value decreases allowing UV-VIS detection of ET reaction. We have introduced a structural photoinducible analogue of canonical amino acid Met – L-2-amino-5,5-azi-hexanoic acid (photo-Met) – into azurin structure to study oligomerization in solution via photo-induced cross-linking (PIXL). Using previously optimised protocols for recombinant expression in E. coli B834 we have inserted photo-Met into two types of azurin: wild-type azurin and Az2W mutant where two adjacent W residues with confirmed role in electron hopping across protein-protein interface (1) are present. The incorporation percentage of photo-Met in analysed samples was determined via MALDI-TOF MS. Four different concentrations of protein (in range of 5-180 μM) were analysed employing PIXL to study azurin-azurin interaction. The samples were exposed to intense UV light and the results were evaluated via SDS-PAGE and UV-VIS spectrophotometry. Two SDS-PAGE protein bands corresponding to dimer and intramolecularly linked monomer were observed and analysed employing MALDI-TOF MS (to confirm protein moiety) and LC-MS/MS (to determine XL identity) after in-gel proteolysis. We have observed higher dimerization yield of Az2W mutant compared to wild-type azurin.

Our findings support the role of two additional W residues on the interacting surface (formed by a b-sheet close to Cu1+/2+ centre) not only during ET hopping (1) but also in azurin oligomerization. Analytical ultracentrifugation (sedimentation velocity experiment) will also be employed.

(1) Takematsu, K. et al. (2019). J.Phys.Chem. B, 123(7), 1578-1591.

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Poster Presentation P41

Native ESI of Human Insulin - multimerization under different pH and buffer composition

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Insulin activity relies on monomeric molecule interacting with the insulin receptor. It is well described that insulin forms natively not only monomers but also dimers and hexamers. These multimeric structures are well known for several years and the assembly and disassembly formation phenomena is in the centre of clinical research of several pharma companies and many academic laboratories. Formulation of insulin that includes buffers containing several ions and small molecules led to different versions of insulin preparations with important clinical outcomes.
We present the results of experiments with native electrospray ionization that was applied to map and describe the formation of insulin multimeric structures dependent on pH and buffer composition.

Poster Presentation P42

MS Annika: A New Search Tool for the Identification of Cross-Linked Peptides from Tandem Mass Spectrometry Data

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Numerous chemical cross-linkers linkers have been developed over the last years, each with their own physical and chemical properties [1]. The development of MS-cleavable linkers has further boosted the popularity of cross-linking mass spectrometry. These cross-linkers can be fragmented in MS/MS analysis to yield characteristic doublet peaks that correspond to the two cross-linked peptides [2].
Several software packages have been developed for the identification of peptides from spectra containing cross-link information. Most of these tools are designed to identify non-cleavable cross-linkers. Tools aimed at cleavable cross-linkers are often specialised for use with one particular cross-linker, or rely on MS3 data, requiring a specific instrument to measure such data. Many cross-link spectra are chimeric spectra and contain fragment ions of both peptides, hampering peptide identification. Furthermore, since only a fraction of measured spectra contains cross-link information, selection of these spectra is crucial.
Here, we present MS Annika, a new cross-linking search engine. The MS Annika algorithm consists of three stages: (1) Cross-link spectra selection based on several different identification modes; (2) Peptide identification using a modified version of the MS Amanda peptide identification algorithm [3]; and (3) Cross-link validation based on FDR calculation. We integrated MS Annika into Proteome Discoverer 2.3. An exporter utility enables the export of found cross-links to xiView to visualise the results [4]. MS Annika can select cross-link spectra, identify, and verify the contained peptide sequences.
MS Annika requires MS2 data only, therefore eliminating the need for MS3 capabilities in mass spectrometers. The search engine can be adapted in Proteome Discoverer to use a wide variety of cross-linkers, and is therefore very versatile. First results show that the algorithm can compete with comparable tools, such as MeroX [5] and XLinkX [6]. On a sample E. coli data set, MS Annika found 3775 cross-link spectrum matches (CSMs) at 1% FDR. MeroX and XlinkX found 1459 and 3381 at the same false discovery rate, respectively.

[1] A. Sinz, “Chemical cross-linking and mass spectrometry to map three-dimensional protein structures and protein-protein interactions,” Mass Spectrometry Reviews, vol. 25, no. 4, pp. 663–682, 2006;
[2] C. E. Stieger et al., “Optimized Fragmentation Improves the Identification of Peptides Cross-Linked by MS-Cleavable Reagents,” J. Proteome Res., vol. 18, no. 3, pp. 1363–1370, Mar. 2019; [3] V. Dorfer et al., “MS Amanda, a Universal Identification Algorithm Optimized for High Accuracy Tandem Mass Spectra,” J. Proteome Res., vol. 13, no. 8, pp. 3679–3684, Aug. 2014; [4] M. J. Graham et al., “xiView: A common platform for the downstream analysis of Crosslinking Mass Spectrometry data,” bioRxiv, p. 561829, Feb. 2019;
[5] M. Götze et al., “Automated Assignment of MS/MS Cleavable Cross-Links in Protein 3D-Structure Analysis,” J. Am. Soc. Mass Spectrom., vol. 26, no. 1, pp. 83–97, Jan. 2015;
[6] F. Liu et al., “Proteome-wide profiling of protein assemblies by cross-linking mass spectrometry,” Nature Methods, vol. 12, no. 12, pp. 1179–1184, Dec. 2015.

Poster presentations P43

Optimized parameter settings enhance proteome-wide formation of crosslinks on low-abundant proteins

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Studies using crosslinking coupled to mass spectrometry on the proteome-wide level have spurred great interest as they facilitate structural probing of protein interactions in living cells or even organisms. Even though numerous studies have demonstrated a quite remarkable number of protein-protein interactions, all studies have in common that crosslinks are only identified on a small subset of the very high abundant proteins. To this day this was mainly traced back to the incapability to detect these crosslinks due to insufficient enrichment or fractionation. In this study we focused on the so far ignored crosslink formation in cell lysates. We show, by using both an in-vitro mimic of a crowded cellular environment and eukaryotic cell lysates, that crosslink formation depends strongly on the protein abundance leading to a bias for high abundant proteins in current protocols. We demonstrate that this bias can be explained by kinetics that govern the formation of a crosslink between two polypeptides. We further show that parameter settings optimized towards a pseudo 1st order kinetics model lead to a significant 3 to 10-fold overall increase in the detection of lower abundant proteins within cellular lysates on a proteome-wide scale. Our study therefore explains the cause of a major limitation in current proteome-wide crosslinking studies and demonstrates a way forward how to address a larger part of the proteome by crosslinking.

Poster presentations P44

Towards a comprehensive landscape of 60S ribosomal biogenesis

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Biogenesis of the eukaryotic ribosome, a macromolecular ribonucleoprotein complex, starts in the cell nucleolus with the transcription of 5S and 35S pre-rRNA. About 200 protein assembly factors and many small nucleolar RNAs are involved in processing these pre-RNAs to assist their correct modification, cleavage, folding, association with r-proteins and export into the cytoplasm [1]. Determining the structural changes of highly dynamic complexes is challenging due to the low abundance of these protein assemblies. Cryo-EM structures of select 60S pre-ribosomal particles helped to uncover the site of action of many assembly factors and to describe principles of ribosome assembly [2]. However, some assembly factors evade structural analysis and the molecular dynamics of rRNP interaction networks remain not fully understood.
Here, we used large scale biochemical enrichment of pre-ribosomal particles in order to obtain a comprehensive landscape of 60S ribosomal biogenesis. The affinity enriched pre-ribosomal particles were analyzed by label free quantitative mass spectrometry. In addition, crosslinking coupled to mass spectrometry (XL-MS) was applied to the purified particles to capture transient protein interaction sites on the amino acid residue level.The 60S ribosomal biogenesis dataset consists of around 36 pulldowns which allowed us to capture 163 known assembly factors and potentially novel ones. The identified crosslinks were used to determine a high-resolution protein interaction network for the purified pre-ribosomal particles showing additional information compared to the published cryo-EM structures. Furthermore, the dataset allowed us to reconstruct a detailed timeline for 60S ribosome biogenesis.

[1] Woolford, J.L., Jr. and S.J. Baserga, Ribosome biogenesis in the yeast Saccharomyces cerevisiae. Genetics, 2013. 195(3): p. 643-81.
[2] Konikkat et al., Biochemical Journal, 2017. 474(2): p.195-214

Poster presentations P45

Beaming up proteins - is there a XID?

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Native mass spectrometry has been developing into a powerful tool to study native-like proteins and their assemblies while keeping crucial non-covalent interactions intact. Time-honored methods as collisional activation or electron attachment for deliberate dissociation of the precursor ions as well as newly emerging ones like photodissociation are available to obtain information about components, stoichiometry and internal organization in addition to overall mass and conformation. However, none of these approaches is entirely suited to disassemble very large protein complexes as, for instance, virus shells conveniently.
With this in mind, we coupled native MS with highly energetic soft X-ray radiation provided by PETRA III synchrotron's P04 beamline in Hamburg. The results hint at alternative subunit dissociation and backbone fragmentation mechanisms alike.

Poster presentations P46

Just give me a p-value: statistical analysis of deuterium uptake curves in HDX-MS

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The misuse of statistical testing is one of the pitfalls of contemporary science (Krueger and Heck, 2019). It is especially prevalent in rapidly growing fields as the HDX-MS, where the development of experimental techniques precedes the formalization of good practices in data analysis.
We have proposed and investigated 30 statistical models on several HDX-MS datasets, partially based on models proposed elsewhere (Houde et al., 2011; Hourdel et al., 2016). As the theoretical model of deuterium uptake distributions is still unclear, we compare our models to empirical measures used to compare deuterium uptake curves as the area under the curve (Mazur and Weber, 2017). We point out that tests based on non-aggregated deuterium uptake are more sensitive, especially to the differences of shapes of deuterium uptake curves. In consequence, such tests can find not only significant differences in absolute deuterium uptake but also in the speed of hydrogen-deuterium exchange.
Currently, statistical models for HDX-MS data are limited to the analysis of specific peptides instead of the regions of the protein. The exact coverage of peptides is an artifact resulting from the experimental procedure. Thus, it might introduce bias to further data analysis and requires the usage of multiple testing corrections as many peptides are separately tested at the same time (Strutzenberg et al., 2019). We propose a novel ROI (region of the interest) framework. Here, results from peptides belonging to the region specified by a researcher are gathered together to reflect changes in deuterium uptake relevant to this region.
Concluding, our framework allows more specific identification of protein regions with an altered deuterium uptake. It evades problems associated with multiple testing and allows an in-depth investigation of the shape of the deuterium uptake curves.

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Poster presentations P47

Macromolecular structure and dymamics based on cross-links

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The majority of proteins function when associated in macromolecular assemblies. To understand their function, we need to describe their structures and dynamics. However, no experimental method is universally applicable for direct structure determination. Integrative modeling is often used to characterize the structures of complexes by relying on multiple types of input information. The individual proteins or domains are often determined by x-ray crystallography or modeled by comparative modeling and cross-linking mass spectrometry data is used to assemble the subunits.

We have developed two novel computational methods that rely on cross-links for determination of structure and dynamics, respectively. The main challenge in integrative modeling of complexes is sampling the complete ensemble of models consistent with the input information. Due to the large size and number of subunits, the sampling is often necessarily stochastic and does not guarantee obtaining a complete ensemble. Our deterministic method uses pairwise protein-protein docking to assemble the complexes in an hierarchical manner. We successfully benchmark the method on complexes consisting of 10-15 subunits and apply it to assemble the yeast chromatin remodeling complex (RSC) which consists of 17 proteins.

A single structure often does not satisfy a large fraction of the cross links. This can be explained by conformational heterogeneity of the protein or assembly. The challenge is to determine the multi-state models (two or more conformations) starting from a single input structure, a list of flexible residues, and a list of cross-links. The second method addresses this problem in two steps. First, we sample the input structure by exploring the space of the ϕ and ψ dihedral angles of the user-defined flexible residues with a Rapidly exploring Random Trees (RRTs) algorithm. Second, we enumerate the multi-state models that are consistent with the cross-links. We successfully applied the method on several heterogeneous systems.

Poster Presentation P48

MS Annika: A New Search Tool for the Identification of Cross-Linked Peptides from Tandem Mass Spectrometry Data

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Due to their sessile lifestyle, plants are sensitive to very distinct types of stress compared to animals. When it comes to stress response this implies that they had to evolve unique ways to cope with environmental treats. A central transcription factor (TF) involved in regulating DNA damage response pathway (DDR) in plants is the TF Suppressor Of Gamma Response (SOG1), that belongs to the plant specific NAM (no apical meristem), ATAF1,2 and CUC2 (cup-shaped cotyledon) (NAC) family. As such SOG1 takes on the role of the mammalian p53, which is absent in plants. SOG1 has a compound organization consisting of a folded NAC domain surrounded by N- and C-terminal IDP domains. Therefore an integrative approach is required to gain insight into SOG1 structure, which has not been described so far. We used native MS and XL-MS to complement small angle X-ray scattering (SAXS), synchrotron radiation circular dichroism (SRCD) and electrophoretic mobility shift assay (EMSA) data. We found that SOG1 binds DNA in a non-specific manner, but that structure is gained upon binding DNA. We confirmed that the functional unit of SOG1 is a dimer and that it heterodimerizes in solution with the closely related NAC protein ANAC044. XL-MS allows mapping close interactions within a SOG1 dimer. Crosslinking experiments enabled us to gain insight into the dimerization orientation. Moreover XL-patterns were compared to SOG1-ANAC044 heterodimers. This study is a first step towards integrating MS structural data into a DNA-binding model of SOG1.

Poster Presentation P49

Detection of Aberrant Protein Conformations in Alzheimer Disease with Covalent Protein Painting

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The structures of aberrant protein folds have been visualized to atomic resolution in vitro, however methods that measure protein folds across a proteome in vivo have been missing. We recently developed Covalent Protein Painting (CPP), a mass spectrometry-based chemical footprinting approach to quantify relative conformer and protein-protein interaction abundances at low structural resolution but with high throughput. CPP employs dimethylation of amines with isotope defined methyl moieties in order to quantify the solvent exposure of lysine sites in proteins. When applied to post-mortem brain tissue, CPP was used to determine the relative contribution of misfolded fibrillar amyloid-beta in patients with Alzheimer disease (AD). Further, CPP indicated alternate folding or protein-protein interactions of specific proteins involved in cell adhesion, local inflammation, and glucose catabolism, suggesting broader structural perturbation of the proteome. It revealed that the relative contribution of fibrillar amyloid-beta and the pattern of additional structurally altered proteins differentiates patients with Lewy Body disease (LBD) from AD and from those with both LBD and AD. In summary, CPP opens up an opportunity for conformational diagnostics of neurodegenerative diseases and enables users to look at the molecular biology of protein misfolding diseases from a structural proteomics perspective.

Poster Presentation P50

PhoX - an IMAC-enrichable Crosslinking Reagent

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Due to the low crosslink reaction efficiency (below 1%), crosslinked peptides are extremely difficult to detect, especially with high backgrounds of non-modified peptides. Here we present a novel lysine-reactive crosslinking reagent, PhoX (Phosphonic acid X-linker), which addresses this issue. PhoX incorporates a phosphonic acid functionality that makes it amenable to automated IMAC enrichment in a 96-well-plate format, a unique approach for XL-MS.

We estimate a best-case 300x enrichment efficiency and 97% enrichment specificity when enriching for PhoX by IMAC. This is exemplified on BSA, where we show an increase from seven identified crosslinks in a non-enriched sample to 101 in the IMAC-enriched samples. Most of the unmodified peptides were removed from the enriched samples resulting in highly reliable crosslink identifications. Further support for the identification of crosslinked peptides is given by PhoX-specific diagnostic crosslink ions. Furthermore, we crosslink a standard intact protein mix (IGF-1 LR3, thioredoxin, Protein G, Carbonic Anhydrase II, Protein A/G fusion protein, and DNA Polymerase Exo Klenow), show that over-crosslinking is not occurring and were able to detect 134 crosslinks in a single measurement.

Application of PhoX to human cytosolic lysates resulted in 1156 linked lysine pairs and 140 crosslinks on the 80S ribosome. These numbers of crosslinks on the ribosome were obtained with a single LC MS run of 3 h, whereas for previous efforts, extensive fractionation was required.

Application of PhoX to the complex of the lipoprotein receptor-related protein 1 (LRP1) and receptor associated protein (RAP) reveals unexpected interactions. Furthermore, data on other exciting protein complexes such as the human signal peptidase complex in will be shown.

Poster Presentation P51

MHC class I peptide binding monitored by native MS

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Major Histocompatibility Complex (MHC) class I molecules selectively bind peptides for presentation to cytotoxic T cells. There are many peptide-bound structures of MHC class I molecules, but the peptide-free state is not explored well due to its instability. We characterized a disulfide-engineered version of the human class I molecule HLA-A*02:01 (dsA2) that is stable without peptide by means of native mass spectrometry.
Successful refolding of the heterogenic protein complex was verified, whereas dipeptide used for refolding was detected neither free nor bound to dsA2, which confirms that the protein complex is indeed empty and stable in absence of any peptide. In striking contrast, only a minimal amount of folded wild type A2 (wtA2) was detected in the absence of dipeptide, but wtA2 did remain stable when 0.5 mM dipeptide was present. We conclude that while wtA2 is dependent on the dipeptide at high concentration to maintain its folded conformation, dsA2 is conformationally and thermally stable in the absence of dipeptide.
Furthermore, dsA2 was observed to bind a variety of peptides resulting in distinct signals with respective ratios based on their different affinities even when measured from a peptide pool. Thus, we introduce native MS analysis of disulfide-stabilized MHC class I molecules as a versatile tool for peptide screening approaches.

Poster Presentation P52

Improving RNA-protein cross-link identification by heavy/light ATP-labeling

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RNA-protein interactions mediate a vast number of intracellular processes. Different RNA-binding domains and folds have been known for a long time but recently many non-canonical binding motifs have been discovered using for example mass spectrometry-based methods or deep sequencing approaches. Most methods are either protein- or RNA-centric and therefore lack information on the other binding partner. With CLIR-MS (cross-linking of segmentally isotope labeled RNA and tandem mass spectrometry) the RNA- as well as protein cross-linking site can be determined in a single experiment. The isotopic label allows filtering and preprocessing steps during data analysis and pinpoints the initially labeled segment within the RNA. By segmentally labeling different parts of an RNA the protein-RNA interaction can be investigated thoroughly.
The most critical step in this workflow is the synthesis of segmentally isotope-labeled RNA. For initial experiments as well as optimization experiments, a straightforward approach to isotopic labeling would simplify the screening for potentially interesting cross-links. Here we introduce an extension of the CLIR-MS workflow that can be applied to unlabeled RNA by adding a label during sample preparation for mass spectrometric analysis. RNA-peptide adducts are labeled using the enzyme T4-PNK and a 1:1 mixture of heavy and light ATP before TiO2-based enrichment via the RNA-phosphate backbone. The γ-phosphate of the heavy ATP contains four heavy oxygen atoms (γ-18O4-ATP). The label is introduced at the 5'-hydroxy group of the RNA-part and leads to a 6-Da shift between heavy and light products. Due to oxygen labeling, heavy and light RNA-peptide adducts also perfectly co-elute.
As an example, we applied this approach to the well-studied FOX1-RRM in complex with its cognate binding substrate FBE-RNA (FOX-binding element). The presented extension allows a quick investigation of novel protein-RNA interactions using the existing CLIR-MS workflow.

Poster Presentation P53

Structural analysis of breast cancer biomarker Her2 bound to its specific nanobody A10 by cross-linking/mass spectrometry

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Cross-linking/mass spectrometry (XL-MS) is a well-established tool for the structural elucidation of proteins and protein complexes. XL-MS makes use of chemical cross-linkers that covalently connect residues of proteins, which are in close proximity. Therefore, analysis of cross-linked peptides by high-resolution MS and their identification by MeroX software provides useful distance constraints for 3D-structural studies.
In this work, a structural study by XL-MS has been performed on the complex formed by the extracellular domain of the membrane receptor Her2, fused with the purification tag Fc2 (an antibody constant fragment) and the miniaturized antibody (nanobody) A10. Her2 is currently employed as biomarker and therapy target for specific variants of breast cancer. A10 is a nanobody that was previously obtained by biopanning procedures from a preimmune VHH (variable domain of camelid heavy chain-only antibodies) library for the antigen Her2.
15 N-HSQC-NMR experiments were carried out in parallel in order to identify the nanobody residues involved in the binding. The cross-linking constraints and the A10 interacting residues obtained by NMR have been used to guide the docking analysis with HADDOCK2.2 software. The model of the complex so obtained was further refined with molecular dynamics simulations.

Poster Presentation P54

Glycan binding induces strain dependent dynamics in the human norovirus capsid protein

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Infection with noroviruses is the predominant cause of viral gastroenteritis in humans with the genogroup II strains causing most outbreaks worldwide. Attachment to certain glycan factors, called Histo-blood-group antigens (HBGAs), 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 comprised 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 occupation of a conserved glycan binding pocket, but did not provide information on potential structural changes induced by glycan binding. Here, we used hydrogen deuterium exchange mass spectrometry (HDX MS) to analyze the conformational response of the P dimer to glycan binding and compare dynamics in different virus strains.

In order to address the conformational effects of glycan binding on the native P dimer structure, binding of HBGA B trisaccharide and fucose, as the minimum binding element, were compared for three different virus strains (GII.4, GII.10 and GII.17). HDX data revealed protection of the conserved glycan binding pocket in all strains for both HBGA B trisaccharide and fucose. Interestingly, the GII.4 and GII.17 strains showed only small structural responses to glycan binding, whereas the GII.10 strain displayed protection of large parts of the protein, even far away from the conserved binding site.

The results suggest that glycan binding occurs on a well-defined site on the P dimer surface for the tested GII.4 and GII.17 strains. The GII.10 strain shows larger conformational changes potentially due to additional binding sites or allostery.

Poster Presentation P55

Combining cross-linking mass spectrometry and hydrogen/deuterium exchange mass spectrometry to study the receptor interaction of monoclonal antibodies

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Immunoglobulin G antibodies (IgG) are one of the most abundant proteins in the blood, due to their extensive production and an unusually long serum half-life for up to 22 days. The extraordinary half-life of IgGs is caused by a pH-specific interaction of IgGs with the neonatal Fc receptor (FcRn) in the endosome. Here, FcRn rescues IgGs from degradation and instead releases them into the bloodstream. In the literature, it has been suggested that the interaction between IgGs and FcRn is only mediated via the Fc domain of the IgG. However, lately it has been observed that IgGs with identical Fc domains, but different Fab domains, experience different half-lives in the human body due to either increased or diminished interaction of the Fab domains with FcRn. Here, we employed a combination of cross-linking mass spectrometry (XL-MS) and hydrogen/deuterium exchange mass spectrometry (HDX-MS) to characterize the interaction of a full-length IgG with FcRn.
The XL-MS and HDX-MS results confirmed the primary interaction site between FcRn and the Fc-domain of IgGs, as three cross-links were identified between FcRn and the Fc-part of the IgG. Furthermore, a reduction of HDX was observed in both the Fc domain and FcRn in presence of the respective binding partner. Highly interesting, a single XL was also observed between the Fab domains and the tip of FcRn structurally confirming the presence of the Fab-FcRn interaction. The Fab-FcRn interaction was further supported by a decrease in HDX in the Fab arms in the presence of FcRn.
By combining the obtained HDX-MS and XL-MS results, we have developed the first experimentally verified model of the binding of full-length IgG to FcRn. The model clearly supports a participation of the Fab domains in the IgG-FcRn interaction. We envision that this new knowledge will advance engineering of IgGs with tailored pharmacokinetics and enhanced drug efficacy.

Poster Presentation P56

A novel, highly efficient protocol for affinity-based enrichment of DSBSO cross-linked peptides

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Cross-linking mass spectrometry is increasingly used as powerful technique to study protein-protein interactions and to uncover questions in structural biology. Due to sub-stochiometric reaction efficiencies, cross-linked peptides are, however, usually completely overwhelmed by linear peptides. This results in challenging data evaluation and the need for an effective enrichment for cross-linked peptides from a potentially complex matrix like a cell lysate. Here we describe a novel and improved one-step method to enrich azide-tagged, acid-cleavable disuccinimidyl bis-sulfoxide (DSBSO) cross-linked peptides. Using homemade sepharose beads covalently coupled to a dibenzocyclooctyne functionality, enables a fast and effective enrichment via click chemistry. We probed this method using recombinant Cas9 or a ribosomal protein complex. The number of detectable cross-links (XLs) was thereby increased by factor 5 compared to non-enriched samples for a pure protein and up to factor 292 when the sample is spiked 1:2 into a background of tryptic peptides from HEK293 cells. Furthermore the enrichment efficiency was significantly improved (by factor 4 based on XL numbers) compared to the originally published 2 step method1. We were still able to detect XLs on the ribosomal complex from an estimated amount of 5 ng cross-linked material in a background of 100 µg tryptic HEK peptides, indicating the high enrichment sensitivity. In contrast to conventional enrichment techniques, like SEC, the needed preparation and MS measurement time is significantly reduced as no fractionation is needed. Azide tagged cross-linkers, like DSBSO, in combination with this robust, fast and selective enrichment method will contribute to map protein-protein interactions and investigate protein architecture more in depth, which helps to understand biological processes, especially in complex environments like human cells. Ref1: Kaake, R. M. et al. Mol. Cell. Proteomics 13, 3533–3543 (2014).

Poster Presentation P57

OpenPepXL: Sensitive, comprehensive identification and quantification of protein-protein cross-links

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Many methods and tools have been developed for XL-MS identification in the last decade. Every tool applies different heuristics to cope with the quadratic search space inherent in XL-MS data analysis.
We introduce 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.
OpenPepXL is a protein-protein cross-linking identification tool for non-cleavable cross-linkers implemented using the open-source library OpenMS. It can make use of labeled linkers to denoise spectra and reduce the search space. OpenPepXL can be installed on Windows, OSX and Linux and supports the MzIdentML 1.2 format for XL-MS identification results.
We compared OpenPepXL to several existing tools on a complex cell lysate dataset with a database of 128 target proteins. At 5% FDR, OpenPepXL finds from 7% to over 50% more unique residue pairs (URPs) than other tools. On additional datasets with available high resolution structures for cross-link validation OpenPepXL reports from 7% to over 40% more structurally validated URPs than the other tools.
OpenPepXL searches the entire search space of an XL-MS experiment without using heuristics or linear peptide pre-scoring. Due to efficient data structures and built-in parallelization it achieves very good speedups on compute clusters and cloud services while maintaining its slim memory footprint. The increased computational effort can thus be compensated and results in the increased sensitivity.
OpenPepXL is free to use for private, academic and commercial applications under the three clause BSD license. It is freely available as part of OpenMS at https://www.openms.de/openpepxl/.

Poster Presentation P58

Chemical modification of proteins for structure elucidation and analysis of protein orientation in lipid bilayers

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Structural mass spectrometry (MS) includes a variety of techniques, which are advantageous when traditional methods like protein crystallography or nuclear magnetic resonance prove difficult. One such technique is covalent labelling which identifies solvent accessible residues on the surface of proteins.
We used yeast alcohol dehydrogenase and rabbit pyruvate kinase as model proteins to establish a (quantitative) labelling workflow for investigating protein dynamics under different conditions. Labelling was performed using (i) N-Hydroxysuccinimidyl Acetate (NHS-Ac), acetylating lysine, tyrosine, serine and threonine, and (ii) diethyl pyrocarbonate (DEPC), which modifies histidine, lysine, tyrosine, serine, threonine and cysteine. The labelled proteins were digested with trypsin and generated peptides were analysed by LC-MS/MS. Acquired raw data were analysed using MaxQuant software and an R script to identify and quantify modified residues. Close inspection of the mass spectra obtained from DEPC-labelling revealed neutral loss of the label from histidine, lysine, serine and threonine residues. Including these neutral losses in the database search increased identification scores. Modified residues are mostly localised at the solvent accessible surface of the proteins and showed quantitative differences in labelling efficiencies depending on their localisation in the protein structure. We therefore conclude that both, NHS-Ac and DEPC, are promising labelling agents for structural analysis of proteins and their complexes.
Currently, we are extending our labelling workflow to investigate the orientation of membrane proteins in a lipid bilayer. For this, we utilise membrane permeable NHS-Ac and non-membrane permeable Sulfo-NHS-Ac. The labelling ratio of the modified residues using the two reagents provides insights into the orientation of membrane proteins in a lipid bilayer.
A valuable application of this approach is the quantitative description of the orientation of membrane proteins incorporated into liposomes (i.e. right-side-out versus inside-out orientation), which usually proves difficult and requires purification tags or fluorescence labels.

Poster Presentation P59

Developments in the analysis of membrane proteins on orbitrap platforms

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Analysis of membrane proteins by mass spectrometry has come a long way in recent years. From intact mass spectrometry of denatured alkyloid membranes years ago, we now have seen the first reports of analysis of native membrane protein complexes from lipid vesicles derived directly from cell membranes. Supporting these developments has been the recent introduction of the orbitrap analyzers for native mass spectrometry providing researches with access to its superior desolvation capabilities and sensitivity, while working at unprecedented resolution.

In this presentation we will discuss recent developments in instrumentation for native mass spectrometry of membrane proteins and discuss the challenges in analyzing them. Although detergents are still the most popular carrier system for membrane protein MS, new platforms such as nanodiscs and SMALPS are rapidly gaining popularity, especially due to their compatibility with other structural biology techniques. Through various examples we will discuss critical parameters in optimizing membrane protein spectra for various experiments ranging from native MS to top-down sequencing and ligand/lipid characterization using new MSn capabilities of Orbitrap tribrid instrument.

Poster Presentation P60

CyaA adenylate cyclase domain (B. pertusis)-human calmodulin: protein-protein interaction study

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The Gram-negative, aerobic, pathogenic, encapsulated coccobacillus, Bordetella pertussis, is a causative agent of pertussis or whooping cough. One of the key virulence factors of B. pertussis is the bifunctional adenylate cyclase toxin (CyaA, 178 kDa) that consists of a calmodulin-sensitive adenylate cyclase enzyme and a pore-forming hemolysin. During bacterial pathogenic invasion the direct penetration of host cell membrane and translocation of N-terminal adenylate cyclase domain (dAC, 40 kDa) into the host cell cytosol prepare the stage for dAC activation by binding of cytoplasmic calmodulin (Cam, 15 kDa) followed by unregulated conversion of ATP to cAMP. The results of our study should map the interaction of Cam (after binding of calcium ions) with dAC employing PIXL-MS experimental approach (Photo-Induced Cross-Linking - Mass Spectrometry)[1].
We have introduced a structural photoinducible analogue of canonical amino acid Met – L-2-amino-5,5-azi-hexanoic acid (photo-Met) – into dAC using recombinant expression in Escherichia coli B834 (DE3), but no protein band corresponding to cross-linked heterodimer was observed on SDS-PAGE in the bimolecular system with Cam in the presence of Ca^{2+}, Mg^{2+} and ATP in vitro. On the other hand, the specific interaction Cam-dAC was detected employing EDC XL (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide). Therefore, two dAC mutants (mutL240M and mutL241M) with predicted calmodulin contacts were prepared by site directed mutagenesis and recombinant proteins with partial incorporation of pMet into the sequence were expressed and purified as described previously. Although no interaction was detected by PIXL experiment for mutL240M, the mutL241M revealed positive result that confirmed the proposed role of W242 and its close vicinity in contact with calmodulin[2].
The project was supported by GACR 19-12695S.
1 Jecmen, T. et al. (2015). Methods, 123(7), 1578-1591.
2 PDB (2COL).

Poster Presentation P61

Probing the structural landscape of α-synuclein in cells and tissues using LiP-MS

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Alpha-synuclein (α-Syn) is a central protein in the development of Parkinson's disease (PD). It was found to constitute a major component of Lewy bodies (LB), a clinical hallmark of PD and other neurodegenerative diseases. Numerous studies have demonstrated the exceptional conformational plasticity of this protein in vitro, though little insights have been gained on the structure-function relationship in vivo, due to the lack of techniques that can assess the structure of α-Syn in native environments. To address this challenge, we employed a new method that combines limited proteolysis (LiP) and mass spectrometry (MS) to probe the structural features of α-Syn directly in cells and tissues. In the LiP-MS method, we measure the accessibility of peptides to cleavage by a structure-specific protease, which reflects the folding state of the given peptide, where low accessibility is an indication for a folded or aggregated state and high accessibility indicates an unfolded state. Using this approach, we generated a library of structure-specific proteolytic signatures using well-characterized in vitro structures, which we used as references in a mathematical framework to predict the structural composition of α-Syn in a given in situ sample. We measured the proteolytic pattern of α-Syn in different cell systems. Interestingly, we observed high accessibility values for peptides corresponding to the amyloid core of α-Syn when expressed in both, yeast and mammalian cells, which suggests that α-Syn is not aggregated. Overall, these results demonstrate the applicability of our method to different biological systems. Ultimately, we will use the method to assess the structural landscape of α-Syn in tissues and biological fluids of PD patients.

Poster Presentation P62

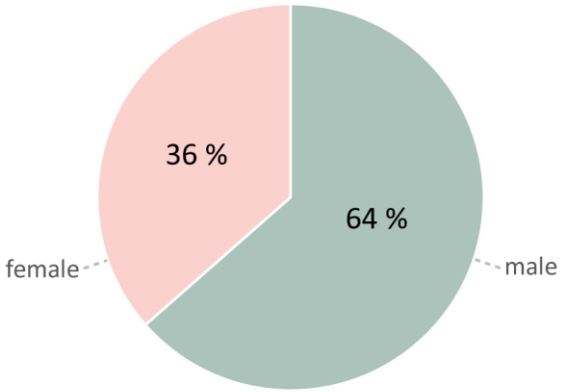
Impact of immunoglobulin G1 Fc sialylation on backbone amide H/D exchange

Lea Bonnington, Felix Kuhne, Patrick Bulau

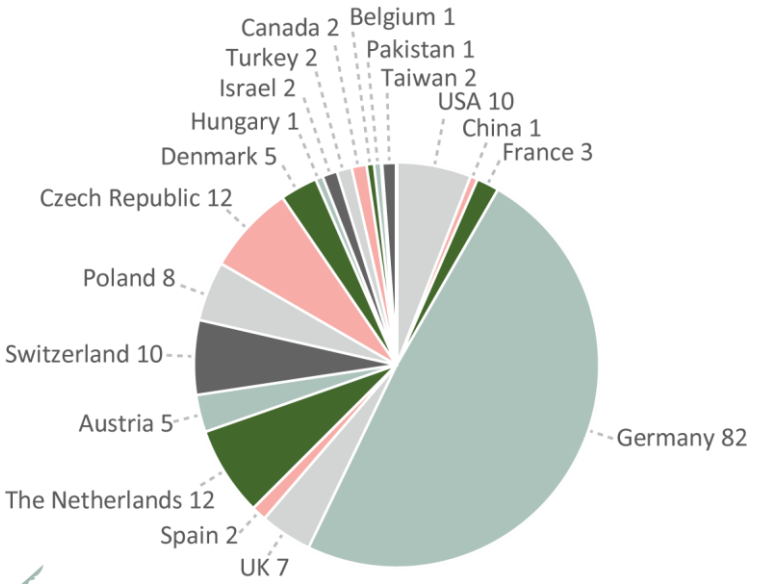
Development Analytics Roche Pharmaceuticals

The usefulness of higher-order structure information provided by hydrogen/deuterium exchange-mass spectrometry (H/DX-MS) for the structural impact analysis of chemical and post-translational antibody modification has been demonstrated in various studies. However, the structure-function assessment for protein drugs in biopharmaceutical research and development is often impeded by the relatively low-abundance (below 5%) of critical quality attributes or by overlapping effects of modifications such as glycosylation with chemical amino acid modifications, e.g. oxidation or deamidation. We present results demonstrating the applicability of the H/DX-MS technique to monitor conformational changes of specific Fc glycosylation variants produced by in vitro glyco-engineering technology. A trend towards less H/DX in Fc Cγ2 domain segments correlating with larger glycan structures could be confirmed. In contrast, significant deuterium uptake differences and corresponding binding properties to Fc receptors (as monitored by SPR) between α-2,3- and α-2,6-sialylated Fc glycosylation variants were verified at sensitive levels.

Participants – Gender



Participants – Country of Affiliation



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SUN 3rd

REGISTRATION

12:30-17:30 Registration

13:00-15:15 **Lutz Fischer** Workshop **xi** — Adam-von-Trott Hall

Martin Graham Workshop **xiVIEW** — Adam-von-Trott Hall

13:00-15:00 **Karol Kaszuba** Workshop **Xlink Analyzer** — Emmy-Noether Hall

15:00-15:30 Coffee Break — Upstairs Foyer

15:30-17:30 **Alexander Wulf** **Timo Sachsenberg** Workshop **RNPxl** — Emmy-Noether Hall

15:30-17:30 **Jörg Schaarschmidt** Workshop **HADDOCK** — Adam-von-Trott Hall

17:30-18:00 Coffee Break — Upstairs Foyer

18:00-19:00 **Andrej Šali** Opening Remarks Keynote — Adam-von-Trott Hall

19:00-22:00 Welcome Reception — Upstairs Foyer

MON 4th

REGISTRATION

8:00-9:50 Registration

9:00-9:50 **Lan Huang** Keynote — Adam-von-Trott Hall

9:50-10:20 **Alexander Leitner** — Adam-von-Trott Hall

10:20-10:50 Coffee Break — Upstairs Foyer

10:50-11:05 **James Pitts**

11:05-11:35 **Nina Morgner** — Adam-von-Trott Hall

11:35-11:50 **Michael Glocker**

11:50-12:05 **Rebecca Beveridge**

12:05-12:20 Thermo Scientific

12:20-13:20 Lunch Break — Upstairs Foyer

13:20-13:50 **Kai Tittmann** — Adam-von-Trott Hall

13:50-14:05 **Iwan Parfentev**

14:05-14:35 **Richard Scheltema** — Adam-von-Trott Hall

14:35-14:50 **Alexander Moysa**

14:50-15:05 **Anne Rehkamp**

15:05-15:30 Coffee Break — Upstairs Foyer

15:30-17:30 **Kasper Rand** Workshop **HDX-MS** — Emmy-Noether Hall

15:30-17:30 **Richard Scheltema** Workshop **XlinkX** — Adam-von-Trott Hall

17:30-18:00 Snacks — Upstairs Foyer

18:00-22:00 Poster Session and Snacks — Hannah-Vogt & Emmy-Noether Hall

TUE 5th

9:00-9:50 **Tanja Mittag** Keynote — Adam-von-Trott Hall

9:50-10:20 **Kevin Pagel** — Adam-von-Trott Hall

10:20-10:50 Coffee Break — Upstairs Foyer

10:50-11:05 **Eleanor Dickinson**

11:05-11:35 **Juri Rappsilber** — Adam-von-Trott Hall

11:35-11:50 **Lolita Piersimoni**

11:50-12:05 **Nir Kalisman**

12:05-12:20 Bruker

12:20-13:20 Lunch Break — Upstairs Foyer

13:20-13:50 **Julien Marcoux** — Adam-von-Trott Hall

13:50-14:05 **Zdenek Kukacka**

14:05-14:35 **Dominic Winter** — Adam-von-Trott Hall

14:35-14:50 **Franz Herzog**

14:50-15:05 MSVision

15:05-15:40 Coffee Break — Upstairs Foyer

15:40-17:40 **Andrea Sinz** **Claudio Iacobucci** **Michael Götze** Workshop **MeroX** — Adam-von-Trott Hall

18:15 Doors Open

18:30-22:00 Conference Dinner — Hannah-Vogt & Emmy-Noether Hall

WED 6th

9:00-9:50 **Neil Kelleher** Keynote — Adam-von-Trott Hall

9:50-10:20 **Bettina Warscheid** — Adam-von-Trott Hall

10:20-10:50 Coffee Break — Upstairs Foyer

10:50-11:20 **Florian Stengel** — Adam-von-Trott Hall

11:20-11:50 **Paola Picotti** — Adam-von-Trott Hall

11:50-12:20 Community Meeting

12:20-13:00 Closing Remarks — Adam-von-Trott Hall