Affinity MS workshop at the DGMS 2019 conference

Date: Sunday, 10th of March, 2019
Time: 14:00 – 17:00 p.m.
Location: Seminar room 126, Ulmencampus, University of Rostock
Organizers: M.O. Glocker and M. Przybylski

time	speaker	title	abstract
14:00 – 14:10	M.O. Glocker	Welcome and Opening of the Workshop	
14:10 – 14:45	M. Larsen	Affinity-Enrichment Methods in PTMomics	
14:45 – 15:20	S.M. Meier-Menches	Affinity-Mass Spectrometry to Determine Target Proteins of Reactive Small Molecules	
15:20 – 15:40	break		
15:40 – 16:15	L. Lupu	Molecular Epitope Determination of Aptamer Complexes of the Multi-domain Protein C-Met by Proteolytic Affinity-Mass Spectrometry	
16:15 – 16:50	M. Bantscheff	Studying Drug Effects on the Proteome using Affinity Methods	
16:50 – 17:00	M. Przybylski	Closing Remarks	

Affinity-Enrichment methods in PTMomics

Martin R. Larsen

Department of Biochemistry and Molecular Biology, University of southern Denmark

Over the years we have developed a large number of methods for enrichment of post-translationally modified (PTM) peptides, from complex biological matrices, in order to make it possible to study these in nature. A PTM is the attachment of a chemical group to a protein after or during protein synthesis and the global analysis of PTMs is termed PTMomics. Many PTMs are reversible and numerous enzymes for their controlled attachment and removal exist, in fact about 5% of the total amount of proteins in a cell is controlling PTMs. Often a complex interplay between these enzymes modulates the PTM level of a protein to fine-tune the protein activity, function and interaction, allowing a level of delicate control of signaling pathways not possible in any other way.

In normal proteomics proteins are digested with specific enzymes and subsequently analyzed by LC-MSMS in order to identify or quantify the peptide in the sample. However, if peptides are carrying a PTM, very often they are not identified by LC-MSMS analysis as they are either low abundant (low stoichiometry modification), hydrophilic so they do not bind to the LC column or they are not ionizing in the MS analysis. As a consequence PTMomics is not possible without robust and efficient methods for enrichment of peptides carrying PTMs. In the presentation a number of unique enrichment methods that are used widely in the proteomics society will be illustrated and their application in large scale comprehensive PTMomics strategies to characterize cellular signaling will be shown.

Affinity-Mass Spectrometry to Determine Target Proteins of Reactive Small Molecules

Samuel M. Meier-Menches [1,2], Christopher Gerner [2]

1: Institute of Cancer Research, Medical University Vienna, Austria.;
2: Department of Analytical Chemistry, University of Vienna, Austria.

Anticancer agents of the platinum-class including cisplatin, carboplatin and oxaliplatin are successful therapeutic small molecules that alkylate DNA and interfere with replication. Together with arsenic trioxide they are administered in roughly 50% of all cancer chemotherapies. In contrast, next-generation metal-based drug candidates are being discovered that may engage with protein targets, but their identification and validation proved to be a challenging task, partly due to the preconception that such reactive metal-based therapeutics would be highly unspecific.
Thus, a two-dimensional proteomics approach was established combining affinity purification and response profiling as a hypothesis-generating procedure to elucidate the cellular targets of such metal-based anticancer drug candidates [1,2]. The resulting target-response networks allow selecting the most probable target proteins due to their connection to the cellular response upon drug treatment.
We found that an organometallic ruthenium drug candidate selectively targets plectin, a scaffold protein and cytolinker. The organometallic compound seems to affect the protein-protein interaction between plectin and non-mitotic tubulins with considerable effects on cell shape and migration.

References:
[1] Samuel M. Meier and Christopher Gerner et al., An Organoruthenium Anticancer Agent Shows Unexpected Target Selectivity For Plectin, ***Angewandte Chemie Int. Ed.***, 2017, *56*, 8267–8271.
[2] Samuel M. Meier-Menches and Christopher Gerner et al., Time-Dependent Shotgun Proteomics Revealed Distinct Effects of an Organoruthenium Prodrug and its Activation Product on Colon Carcinoma Cells, ***Metallomics***, 2019, *11*, 118–127.

Molecular Epitope Determination of Aptamer Complexes of the Multi-domain Protein C-Met by Proteolytic Affinity- Mass Spectrometry

Loredana Lupu[1], Pascal Wiegand[1], Nico Hüttmann[1,2], Stephan Rawer[1], Wolfgang Kleinekofort[1,3], Alexander Lazarev[4], Maxim V. Berezovski[2], and Michael Przybylski[1*]

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C-Met protein is a glycosylated receptor tyrosine kinase of the hepatocyte growth factor heterodimer (HGF).C-Met has been found to be aberrantly activated leading to tumorigenesis and several other diseases and has been recognized as a biomarker in cancer diagnosis. C-Met aptamers have been recently considered a useful tool for detection of cancer biomarkers. Aptamers are single-stranded DNA or RNA oligonucleotides that can be easily produced and show stabilities and affinities comparable to monoclonal antibodies. Here we report a molecular interaction study of human C-Met protein with two DNA aptamers (CLN0003 and CLN0004), derived using the SELEX procedure. Epitope peptides of the aptamers on C-Met were identified by proteolytic affinity- mass spectrometry in combination with SPR biosensor analysis (PROTEX-SPR-MS), with the use of a high pressure proteolysis as an efficient tool for digestion and proteolytic-epitope extraction mass spectrometry. High affinities were determined for aptamer-C-Met complexes, with a two-step binding pathway suggested by kinetic analyses. A linear epitope peptide, C-Met (381-393) (NSSGCEARRDEYR) was identified for CLN0004. Subsequently, the interaction of C-Met with the CLN0003 aptamer revealed an assembled epitope comprised of two specific peptide sequences. Structure modelling studies of the protein and the two aptamers were consistent with the identified epitopes. The high affinities of aptamers to C-Met, and the specific epitopes revealed should render them of high interest for cellular diagnostic studies. In addition, the molecular epitopes identification of DNA aptamer-protein complexes could be used to characterize therapeutic antibodies and to neutralize the pathophysiological responses.

Studying drug effects on the Proteome using Affinity Methods

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Over the past decade, our ability to identify molecular mechanisms underlying disease and study drug action by mass spectrometry-based proteomics has progressed remarkably. Improved instrumentation and new experimental approaches allow studying cellular and tissue phenotypes in a disease context, as well as upon modulation by bioactive molecules, with unprecedented resolution and dimensionality. Furthermore, the diversification of direct and indirect target identification methodologies allows for comprehensive analysis of cellular targets of bioactive compounds in live cells and the correlation between target engagement and proteotype effects. This talk focusses on the emerging role of mass spectrometry based proteomics in drug discovery and highlights experimental approaches and applications with impact for understanding efficacy and safety of drug candidates.