7th Central and Eastern European Proteomics Conference

October 13-16, 2013 Jena, Germany

Organized by:

Mass Spectrometry Laboratory of the
Max Planck Institute of Chemical Ecology

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The 7th Central and Eastern European Proteomics Conference has been kindly supported by

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Program

Sunday, October 13, 2013

15:00-18:00		Arrival and registration at Abbe Centre Beutenberg, Hans-Knoell-Str.1, D-07745 Jena
18:00-19:00		Opening ceremony (**Aleš Svatoš, Hana Kovářová**)
19:00-22:00		Mixer (Abbe Centre, outdoor/indoor)
20:00-22:00		CEEPC Scientific committee meeting with invited speakers

Monday, October 14, 2013

Clinical proteomics and Interactomics

09:00-09:40	**T01**	*Proteomics changes its image: history, status and vision* **Corinna Henkel**, Ruhr-University Bochum, Germany
09:40-10:10	**T02**	*Perturbed protein network analysis in human disease* **H. Alexander Ebhardt**, ETH Zürich, Switzerland
10:10-10:25	**T03**	*Elucidation of a mislocalization-based pathomechanism* **Jörg Reinders**, University Regensburg, Germany
10:25-10:40	**T04**	*Radiotherapy-related changes in serum proteome patterns of head and neck cancer patients; the evidence for effects of low radiation doses* **Piotr Widlak**, Maria Skłodowska Curie-Memorial Cancer Center and Institute of Oncology, Gliwice, Poland
10:40-11:10		Coffee break
11:10-12:00	**T05**	*How can chemical cross-linking/mass spectrometry contribute to protein 3D-structure analysis?* **Andrea Sinz**, Martin Luther University Halle-Wittenberg, Germany

12:00-12:20 **T06** *Which biomarker would you like to discover today?*
Karl Mechtler, Research Institute of Molecular Pathology, Vienna, Austria

12:20-13:00 **T07** *The COP9-Signalosome as a model to study the structure/function relationship of large protein complexes*
Gili Ben-Nissan, Weizmann Institute of Science, Rehovot, Israel

13:00-14:30 Lunch at the Abbe Centre Casino

Clinical proteomics and Interactomics (continued)

14:30-15:00 **T08** *Epithelial-mesenchymal cross–talk: contractile and cytoskeletal protein involvement in transition towards myofibroblasts and cancer-associated fibroblasts in wound healing and tumorigenesis*
Hana Kovářová, Czech Academy of Sciences, Libechov, Czech Republic

15:00-15:20 **T09** *Immobilized monolithic enzymatic reactors for online digestion of proteins secreted by developing human-embryos*
Wei-Qiang Chen, Medical University of Vienna, Austria

15:20-15:40 **T10** *Characterisation of the proteomic response of the human-pathogenic fungus Aspergillus fumigatus to low oxygen levels (hypoxia)*
Olaf Kniemeyer, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Jena, Germany

15:40-16:00 **T11** *Protein signaling in spinal parenchyma and dorsal root ganglion: key to future therapy approaches in spinal cord spasticity*
Suresh Jivan Gadher, Life Technologies, Frederick, MD, USA

16:00-16:40 Coffee break

New technology for high throughput clinical proteomics

16:40-17:30 **T12** *Proteomic approaches in nutrition research*
Sascha Sauer, Max Planck Institute for Molecular Genetics, Berlin, Germany

17:30-17:50 **T13** *Defensin level determination by targeted proteomic approach*
Gergo Kalló, University of Debrecen, Hungary

17:50-18:20 **T14** *An automated, high-throughput method for targeted quantification of intact insulin and its therapeutic analogues in human serum or plasma coupling mass spectrometric immunoassay with high resolution, accurate mass detection (MSIA-HRAM)*
Michaela Scigelova, ThermoFisher Scientific, Germany

18:20-18:40 **T15** *Glycoprofiling of Human Plasma Proteins*
László Drahos, Hungarian Academy of Sciences, Budapest, Hungary

Tuesday, October 15, 2013

Plant, insect and bacterial proteomics

09:00-09:50 **T16** *Into the unknown: Systems biology approaches in exploring novel protein functions in plant signaling pathways*
Waltraud Schulze, University of Hohenheim, Stuttgart, Germany

09:50-10:10 **T17** *Molecular dissection of nuptial food gifts in a cricket*
Yannick Pauchet, Max Planck Institute for Chemical Ecology, Jena, Germany

10:10-10:30 **T18** *Proteome and metabolome profiling of cytokinin action in Arabidopsis identifying both distinct and similar responses to cytokinin down- and up-regulation*
Martin Černý, Mendel University Brno, Czech Republic

10:30-10:50 **T19** *Identification of secretory proteins in defensive secretions from juvenile leaf beetles*
Antje Burse, Max Planck Institute for Chemical Ecology, Jena, Germany

10:50-11:10 **T20** *CYCAM – a main hub for the perception of beneficial microbe- and pathogen-associated molecular patterns leading to the cytosolic Ca^{2+} signaling, growth promotion and defense in Arabidopsis thaliana*
Joy Michal Johnson, Friedrich Schiller University Jena, Germany

11:10-11:40		Coffee break

Quantitative proteomics

11:40-12:10	**T21**	*Ion Mobility Enabled Data Independent Proteomics Approaches* **Alexander Muck**, Waters GmbH, Eschborn, Germany
12:10-12:30	**T22**	*Automated native sample preparation for biomarker search* **Heidrun Rhode**, University Hospital Jena, Germany
12:30-12:50	**T23**	*Quantitative N-Glycan Analysis using µLC-PGC-ESI-qTOF-MS* **Claudia Michael,** University of Vienna, Austria
12:50-13:10	**T24**	*Phosvitin as a standard protein for optimization of enrichment of multiply phosphorylated proteins* **Rudolf Kupcík**, University of Pardubice, Czech Republic
13:10-14:30		Lunch at the Abbe Centre Casino

Poster Session

14:30-15:00	Poster talks (1 min)
15:00-17:00	Poster Session at the Max Planck Institute for Chemical Ecology

Conference Dinner

18:30-22:00	Conference Dinner at Zeiss Planetarium / Café Bauersfeld, Am Planetarium 5, 07743 Jena

Wednesday, October 16, 2013

Spatially-resolved proteomics, imaging of biomarkers

09:00-09:40	**T25**	*Imaging mass spectrometry: Searching for the invisible, a new tool for disease investigations* **Marc Baumann**, University of Helsinki, Finland
09:40-10:10	**T27**	*Accurate mass MALDI imaging at 25 µm pixel size for proteins after on-tissue digestion* **Andreas Römpp**, Justus Liebig University Giessen, Germany
10:10-10:30	**T28**	*Identification of Biomarkers in MALDI imaging experiments* **Sören Deininger**, Bruker Daltonics, Bremen, Germany
10:30-10:50	**T29**	*3D MALDI imaging of larynx carcinoma* **Ferdinand von Eggeling**, Universitätsklinikum Jena, Germany
11:00-11:30		Coffee break

Concluding Lecture

11:30-12:20	**T30**	*Proteolipidomics by High Resolution Mass Spectrometry* **Andrej Shevchenko**, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
12:20		Closing of 7th CEEPC

Complete List of Posters

P01 *PilE4/OmpA:ICAM:1 – The potential candidates for Francisella adhesion to brain endothelium*
E. Bencurova, A. Kovac, L. Pulzova, M. Bhide

P02 *Adhesion of predicted binding site of OspA (Borrelia garinii) to CD40 of brain microvascular endothelial cells*
M. Bhide, P. Mlynarcik, L. Pulzova, A. Kovac

P03 *How TFA effects protein conformation and separation in RP-HPLC*
B. Bobály, E. Tóth, J. Fekete, K. Vékey, L. Drahos

P04 *Proteomic profile of human chorion derived mesenchymal stem cells by using ion trap mass spectrometry*
M. Chmelová, I. Talian, I. Géci, P. Bober, V. Kováčová, P. Urdzík, J. Sabo

P05 *Proteomic analysis of the cross talk between plant immunity inducing factors*
A. M. Egorova, V. G. Yakovleva, I. A. Tarchevsky

P06 *Peptide separation on a normal phase column under HILIC separation conditions*
G. Mitulović, A. Fichtenbaum, Mikhail Gorshkov, Anna Lobas, Marina Pridatchenko, R. Schmid

P07 *The non-model approach. Transcriptome guided identification of secretory proteins from leaf beetle defense secretions via LC-MSE*
René R. Gretscher, Natalie Wielsch, Magdalena Stock, Wilhelm Boland, Antje Burse

P08 *A porin-like protein from oral secretions of Spodoptera littoralis larvae induces defense-related early events in plant leaves*
Huijuan Guo, Natalie Wielsch, Jens B. Hafke, Aleš Svatoš, Axel Mithöfer, Wilhelm Boland

P09 *Proteomic study of cycloheximide effect on memory caused by exposure to elevated plus maze*
V. A. Hernández, R. G. Torres, C. A. Conde

P10 *Development of the method for quantitative determination of hepcidin - significant marker for diagnosis of anemia*
D. Holub, J. Přichystal, L. Sulovská, J. Houda, D. Pospíšilová, P. Džubák, M. Hajdúch

P11 *Changes in proteomic profile of blood plasma of rats after low-level laser therapy*
V. Kováčová, P. Bober, M. Chmelová, I. Talian, J. Hrubovčák, D. Petrášová, I. Géci, J. Sabo

P12 *Development and validation of targeted proteomic method for examination of oral cancer biomarkers*
P. Lábiscsák , V. Bácsik, A. Szabó, M. Fera, I. Márton, J. Tőzsér, É. Csősz

P13 *Identification of protein antigens from the secretome of Candida albicans for fungal sepsis diagnostics*
Ting Luo, Ilse Jacobsen, Bernhard Hube, Michael Bauer, Andreas Kortgen, Evangelos J. Giamarellos-Bourboulis, Natalie Wielsch, Aleš Svatoš, Axel A. Brakhage, Olaf Kniemeyer

P14 *Multi-Component Complexes Releasing Glycosylphosphatidylinositol-Anchored and Other Cell Surface Proteins into Body Fluids as Source for Novel Diabesity Biomarkers*
Günter Müller, Susanne Wied

P15 *Functionalized bis-enol acetates as specific molecular probes for esterases*
P. Richter, J. Weißflog, N. Wielsch, Aleš Svatoš, Georg Pohnert

P16 *Relative quantification analysis of O-glycans using ESI-qTOF MS/MS*
S. Sić , N. Maier, A. Rizzi

P17 *Proteomic workflow for the analysis of human serum low or medium abundance proteins*
Viorel I. Suica, Elena Uyy, Luminita Ivan, Raluca M. Boteanu, Felicia Antohe

P18 *Detergent Resistant Membranes proteomics changes under high fat stress*
Elena Uyy, Luminita Ivan, Raluca M. Boteanu, Viorel I. Suica, Felicia Antohe

P19 *Combining proteomics and transcriptome sequencing to identify active plant-cell-wall-degrading enzymes in a leaf beetle*
Roy Kirsch, Natalie Wielsch, Heiko Vogel, Aleš Svatoš, David G Heckel and Yannick Pauchet

P20 *The sweet side of proteomics: Nicotiana attenuata's floral nectar proteins*
Natalie Wielsch, Pil Joon Seo, Danny Kessler, Yvonne Hupfer, Aleš Svatoš, Chung-Mo Park, Ian T. Baldwin and Sang-Gyu Kim

P21 *Short-term proteomic dynamics reveal metabolic factory for active extrafloral nectar secretion by Acacia cornigera ant-plants*
Domancar Orona-Tamayo, Natalie Wielsch, María Escalante-Pérez, Aleš Svatoš, Jorge Molina-Torres, Alexander Muck, Enrique Ramirez-Chávez, Rosa-María Ádame-Alvarez and Martin Heil

P22 *Chemically mediated plankton interactions – molecular probes for investigating mechanisms of action of oxylipins*
Stefanie Wolfram, Natalie Wielsch, Yvonne Hupfer, Bettina Mönch, Oliver Werz, Aleš Svatoš, Georg Pohnert

P23 *Automated native sample preparation for biomarker search*
Sindy Wendler, Sabine Nemitz, Thomas Krüger, Jelena Pesek, Stefan Opitz, Bärbel Tautkus, Heidrun Rhode, Nadine Krieg, Tanja Illig, Johannes Norgauer, Michael Schiel, Julian Großkreutz, Steffen Richter, Thomas Hähnel, Heiko Oehme, Lutz Schmidt, Benedikt Hanf

P24 *Proteome analysis of Schizophyllum commune during black slate degradation*
J. Kirtzel, N. Wielsch, Y. Hupfer, S. Madhavan, M. Gube, A. Svatoš, E. Kothe

Talks

Proteomics changes its image: history, status and vision

Corinna Henkel, Hanna Diehl, Julian Elm and Helmut E. Meyer

Medical Proteom-Center (MPC), Ruhr-University Bochum, Germany

Proteomics as a technological platform in the field of biomarker discovery consist of several disciplines such as 2D gel electrophoresis, LC-MS approaches, array technologies and new MS methods like MRM for biomarker verification. Mass spectrometry imaging (MSI) has become a powerful and successful tool in the context of biomarker detection especially in recent years. This emerging technique is based on the combination of histological information of a tissue and its corresponding mass spectrometric information. The range of samples to be analyzed is wide and includes besides proteins, peptides, lipids, drugs and their metabolites also glycans and other posttranslational modifications. For data acquisition a tissue section covered with matrix is moved in two dimensions within the mass spectrometer whereas a mass spectrum is recorded for each position. The obtained spatially resolved information of for example protein or peptide abundances can be used to detect differences between healthy and diseased tissue. Different ionization techniques like matrix assisted laser desorption ionization (MALDI) imaging, desorption electrospray ionization (DESI) imaging or secondary ion mass spectrometry (SIMS) exist and allow diverse approaches on various tissues. Additionally the usage of specific analyzers allows high resolution and accuracy in mass and the visualization of very small histological features up to single cell level. An overview of the development of MALDI imaging within the last years together with different examples out of our own research will be presented.

Perturbed protein network analysis in human disease

H. Alexander Ebhardt

Institute of Molecular Systems Biology, Aebersold group, ETH Zürich, Switzerland

Novel Aspects: Targeted proteomics, human whole cell lysate, perturbed network analysis.

Quantifying perturbed protein networks in human disease requires highly accurate methods for peptide quantification, as physiological and morphological changes in human tissue might be caused by subtle changes in protein abundance. As robust method, selected reaction monitoring mass spectrometry (SRM-MS) has proven to provide robust quantification of proteins originating from plasma samples across laboratories with a large dynamic range of detection [1]. However, analyzing peptides in very complex samples using SRM-MS also provides challenges [2]. Here, I will provide a robust pipeline for targeted proteomics approach of characterizing perturbed protein networks in human disease using SRM-MS and/or SWATH-MS.

Acknowledgements: H.A.E. would like to acknowledge funding from the Marie Curie International Incoming Fellowship program (FP7).

References:
[1] T.A. Addona et al. Nat Biotechnol, 27, 633-41, (2009)
[2] H.A. Ebhardt et al. Proteomics, 12, 1185-93, (2012)

Elucidation of a mislocalization-based pathomechanism

N. Aßmann[1], C. Broeker[2], E. Klootwijk[3], K. Dettmer[1], P. J. Oefner[1], R. Kleta[3], R. Warth[2], M. Reichold[2], J. Reinders[1]

1) Functional Genomics, University Regensburg, Germany
2) Medical Cell Biology, University Regensburg, Germany
3) Center for Nephrology, University College London, UK

Novel Aspects: Mislocalization of a peroxisomal protein into mitochondria causes a mitochondriopathy.

Renal Fanconi-syndromes are caused by a dysfunction of the proximal tubulus of the kidney, thus leading to aminoaciduria, glucosuria, phosphaturia, small molecular weight proteinuria, and metabolic acidosis. In this study a new, autosomal dominantly inherited form of a renal Fanconi-syndrome was investigated. The causative mutation is located in a gene coding for a peroxisomal protein, leading to the generation of a mitochondrial targeting sequence in its N-terminus. Since a knock-out of this gene did not result in a corresponding phenotype, we hypothesized that mislocalization of the originally peroxisomal protein to the mitochondria is the causative mechanism.

Through the combination of proteomic, metabolomic and biochemical approaches, the underlying molecular mechanism of this disease was elucidated. Analysis of the localization, the interactions and modifications of the mislocalized protein revealed a disturbance of mitochondrial fatty acid oxidation resulting in a reduced transport activity of the proximal tubulus cells of the kidney.

To our current knowledge this disease is the first reported mislocalization of a protein to the mitochondria causing a mitochondrial pathology. Only the combination of various different analytical approaches facilitated the clarification of the molecular mechanism underlying this pathogenesis.

Acknowledgements: This work was funded by David and Elaine Potter Charitable Foundation, St Peter'sTrust for Kidney, Bladder & Prostate Research, Kids Kidney Research, Lowe SyndromeTrust, European Union (FP7), BayGene, Deutsche Forschungsgemeinschaft (SFB699), Intramural Research Program of the National Human Genome Research Institute.

Radiotherapy-related changes in serum proteome patterns of head and neck cancer patients; the evidence for effects of low radiation doses

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2) Silesian University of Technology, Gliwice, Poland

Keywords: dose effects; mass spectrometry; radiation toxicity; serum proteome;

Background: Conformal radiotherapy (RT) involves irradiation of large volume of normal tissue with low and medium doses, biological relevance of which is not clear yet. Serum proteome features were used here to study the dose-volume effects in patients irradiated with IMRT due to head and neck cancer.

Methods: Blood samples were collected before and during RT, and also about one month and one year after the end of RT in a group of 72 patients who received definitive treatment. Serum proteome profiles was analyzed using MALDI-ToF mass spectrometry in 800-14,000 Da range.

Results: Major changes in serum proteome profiles were observed between pre-treatment samples and samples collected one month after RT. Radiation-related changes in serum proteome features were affected by low-to-medium doses delivered to a large fraction of body mass. Proteome changes were associated with intensity of acute radiation toxicity, indicating collectively that RT-related features of serum proteome reflected general response of patient's organism to irradiation.

Conclusions: The effects of low and medium doses of radiation have been documented at the level of serum proteome, which is a reflection of the patient's whole body response.

How can chemical cross-linking/mass spectrometry contribute to protein 3D-structure analysis?

Andrea Sinz

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Martin Luther University Halle-Wittenberg, Germany

Detailed knowledge of three-dimensional protein structures is critical for understanding cellular processes at the molecular level. However, applying conventional methods of structural biology is challenging when analyzing membrane proteins, transient complexes or very large protein assemblies. Chemical cross-linking in combination with mass spectrometry (MS) and computational modeling has emerged as an alternative strategy to obtain three-dimensional structural information of proteins and protein complexes [1-3]. The chemical cross-linking/MS approach can be used in combination with complementary low-resolution structural methods to study proteins and protein assemblies, which are otherwise not amenable to the high-resolution structural techniques of X-ray crystallography or NMR spectroscopy. Chemical cross-linking relies on the introduction of a covalent bond between functional groups of amino acids within one protein, to gain insight into the conformation of a protein, or between interaction partners to elucidate interfaces in protein complexes. Based on the distance restraints derived from the chemical cross-linking data, three-dimensional structural models of proteins and protein complexes can be constructed.

We employ the chemical cross-linking/MS strategy to study proteins and protein complexes covering a wide range of biological activities. Currently, we are exploring the incorporation of unnatural photo-reactive amino acids into the proteins of interest followed by UV-induced cross-linking and MS analysis. In my talk I will give an overview of the cross-linking strategies that can be employed to derive 3D-structural information of proteins. With the continual improvements in mass spectrometric equipment and bioinformatics tools, the chemical cross-linking strategy can be expected to contribute valuable structural information of proteins that are otherwise not amenable to analysis.

References:
[1] Sinz, A., Mass Spectrom. Rev., 25, 663, (2006)
[2] Leitner, A. et al., Mol. Cell. Proteomics., 9, 1634, (2010)
[3] Rappsilber, J., J. Struct. Biol., 173, 663, (2011)

Which biomarker would you like to discover today?

Peter Pichler[1], Ludwig Wagner[2], Michael Schutzbier[1], Karl Mechtler[1]

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2) Department of Internal Medicine III, General Hospital and Medical University of Vienna, Austria

Acute Kidney Injury (AKI) is one of many clinical challenges where new biomarkers would be highly desirable to help improve patient care and clinical outcome. The KDIGO guidelines [1] (Kidney Disease: Improving Global Outcomes) emphasize the importance of proper risk assessment in patients with potential Acute Kidney Injury (AKI). However, risk assessment is unsatisfactory if based on clinical assessment and currently available laboratory parameters alone, and imaging studies are also frequently unrevealing in this situation.

Risk factors for AKI include hypovolemia, e.g. due to cardiovascular disease or surgery, inflammatory diseases, renal or liver disease, diabetes mellitus, radiologic contrast media and age > 75 years. Criteria for clinical assessment such as RIFLE allow stratification of AKI patients according to risk (***R***isk, ***I***njury, ***F***ailure) and outcome (***L***oss, ***E***nd Stage Renal Disease), based on the extent of decrease in the glomerular filtration rate (GFR) *or* the rise in serum creatinine level *or* the decrease in urinary output over prolonged duration. However, these criteria are not perfect [2,3,4]

We studied patients with AKI associated with sepsis. This is a particularly important setting, as mortality in sepsis was shown to increase from about 20% to more than 50% if both conditions are present. Samples were also stratified according to the severity of renal injury as indicated by a post-hoc analysis of the maximum RIFLE stage. In general, it is advantageous to conduct biomarker studies in well-defined patient populations, otherwise clinical entities such as AKI might comprise a too heterogeneous group of conditions which would make the detection of common hallmarks difficult.

Aim of the case-study was to determine the feasibility of establishing a suitable method for the quantification of proteins in patients' urine samples. The method should be simple, reliable, and rapid because any biomarker study of sufficient power requires the analysis of samples from a large number of patients. We believe that the main reasons for previous failures in biomarker discovery are due to inadequate study design, lack of proper clinical classification of patients, failure to establish time courses, and most of all inadequate number of analyzed samples.

Acknowledgements: The research leading to these results has received funding from the European Community's 7th Framework Programme (FP7/2007-2013) / grant agreement No. 241548 – MeioSys and 262067 – PRIME-XS, and from the Austrian Science Fund SFB F3402, P 22570-B09 (DBT) and TRP 308-N15.

References:

[1] KDIGO Clinical Practice Guideline for Acute Kidney Injury. Kidney Int Suppl. 2(1), 1–138, (2012)
[2] R. Bellomo et al., Critical Care, 8(4), R204-R212, (2004)
[3] P. D. Winterberg and C.Y. Lu, Am J Med Sci, 344(4), 318-325, (2012)
[4] R. Thadhani et al., N Engl J Med, 334(22), 1448-1460, (1996)

The COP9-Signalosome as a model to study the structure/function relationship of large protein complexes

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3) Cambridge Centre for Proteomics, Cambridge Systems Biology Centre, Department of Biochemistry, Cambridge, UK
4) Weizmann Institute of Science, Israel National Center for Personalized Medicine, Rehovot, Israel

Novel Aspects: Mass spectrometry of intact proteins can provide insight into the structural and functional properties of protein complexes

Many of the cellular processes are regulated and governed by proteins. However, proteins do not act alone and today, it is generally accepted that the majority of proteins act as part of multimeric assemblies. Such protein complexes are very dynamic and often interconvert between forms in a spatial and temporal manner. Therefore, a major challenge towards understanding the function of protein complexes is to elucidate the link between their structural architecture and function. Studying the structural organization of protein assemblies, however, is not a trivial task, mainly due to their large sizes, their heterogeneous composition, flexibility and asymmetric structure. In recent years, mass spectrometry has proven to be a valuable tool for analyzing such non-covalent protein complexes [1,2]. In my talk the COP9-Signalosome complex [3,4] will be used as a model for demonstrating the integration of various mass spectrometry approaches, combined with biochemical and live imaging methods to elucidate the structure/function relationship of a heterogeneous protein complex.

References:
[1] M. Sharon, J Am Soc Mass Spectrom 21, 487-500 (2010)
[2] M. Sharon and C. Robinson, Annu Rev Biochem 76, 167-193 (2007)
[3] M. Sharon, et al., Structure 17, 31-40 (2009)
[4] S. Rozen, et al., Methods 59, 270-277 (2013)

Epithelial-mesenchymal cross–talk: contractile and cytoskeletal protein involvement in transition towards myofibroblasts and cancer-associated fibroblasts in wound healing and tumorigenesis

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4) Life Technologies, Frederick, MD, USA

Novel Aspects: Epithelial-mesenchymal cross-talk; contractile proteins; wound healing; tumorigenesis progression

Tissue stroma, composed of cells and extracellular matrix (ECM), plays a crucial role as a structural bioactive scaffold during the prenatal development and in maintaining tissue homeostasis in the adult organism. Stromal cells, not only play a highly important role during tissue injury as judged by increased proliferation and production of ECM molecules during wound healing but also during tumor development and progression. Tumor stromal cells are known to play an active supporting role which significantly influences the biological properties of malignant cells including their aggressiveness and metastasizing ability. The well established *in vitro* co-culture models of communication between normal fibroblasts and keratinocytes (HF/HK) representing wound healing and cross-talk between normal fibroblasts and squamous cell carcinoma cell line FaDu (HF/FaDu) representing cancer development were utilised in this study to examine the effects of normal or malignant epithelial cells on normal fibroblasts at overall protein level. Using this approach, we were able to select co-regulated proteins in both the above models in order to evaluate possible biological functional similarities between wound healing and cancer development. Additionally, proteins altered differently in individual models may also highlight cellular phenotypes during transition of HFs toward MFs or CAFs. Our study showed that amongst the most pronounced co-regulated changes were the proteins implemented in contractile activity and formation of actin cytoskeleton such as caldesmon, calponin-2, myosin regulatory light chain 12A and cofilin-1.

Interestingly, our data showed that both types of epithelia induced protein changes in normal fibroblasts that may contribute to phenotype conversion of fibroblasts to myofibroblasts. Such events, despite the absence of smooth muscle actin, may be responsible for wound contraction in the healing process. Cancer-associated fibroblasts including myofibroblasts shared at least part of the protein changes with wound healing myofibroblasts induced by

epithelial cells. These findings provide strong support for commonality of the processes involved in wound healing and tumor progression based on the epithelial – mesenchymal cell – cell communication. We conclude that normal and malignant keratinocytes start the program of myofibroblast formation in both wound healing and tumor growth with many similarities at protein level. However, some selective protein alterations participating in cytoskeletal regulation were typical for myofibroblast formation in wound healing. Simulation of such changes using silencing or overexpression tools in tumour myofibroblasts or cancer associated fibroblasts may help to convert a tumour into a wound that may be encouraged to heal with highly beneficial outcome.

Acknowledgements: This work was supported in part by , TACR (TA01011466; HK), and Institutional Research Concept RVO67985904 (IAPG, AS CR, v.v.i). Infrastructuralpart of this project was supported from the Operational Program Research and Development for Innovations (CZ.1.05/2.1.00/03.0124).

Immobilized monolithic enzymatic reactors for online digestion of proteins secreted by developing human-embryos

Wei-Qiang Chen[1], Philipp Obermayr[1], Urh Černigoj[3], Jana Vidič[3], Miloš Barut[3], Tanja Panić[1], Mikhail Gorshkov[4], Goran Mitulović[1,2]

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2) Proteomics Core Facility, Medical University of Vienna, Vienna, Austria
3) BIA Separations, Ajdovščina, Slovenia
4) Institute for Energy Problems of Chemical Physics, Russian Academy of Sciences, Moscow, Russia

Novel Aspects: Clinical application, online digest, in-vitro fertilization

The process of IVF (In-Vitro Fertilization) involves implantation of fertilized oocytes following long medical preparation upon optical investigation of developing oocytes. The physician has to make the decision on which oocyte will be transferred based on their morphology, which is a highly subjective "judgment" prone to failure. During the cultivation and growth of the fertilized oocytes, proteins are secreted into surrounding medium. The proteins secreted are not identical for all oocytes and the art and the amount depends on oocytes' condition and health status. Analysis of those proteins can help providing a more accurate prediction of the implantation success and embryo development.

Classical proteomics approaches involve enzymatic hydrolysis of a protein (either separated by polyacrylamide gels or in solution) followed by peptide identification using LC-MS/(MS) analysis. Enzymatic digestions can take up to several hours to complete, although several methods based on high-intensity focused ultrasound have been shown to accelerate the digestion process. In case of clinical analysis, it is of utmost importance to provide fast and reproducible analysis with a minimum of manual sample handling.

Herein we report the method development and application of immobilized enzymatic reactor (IMER) to accelerate enzyme digestion, reduce manual sample handling, and provide reproducibility to the digestion process in clinical laboratory.

By sequentially developing and improving the methods applied, we were able to establish an automate sample digestion method and reduce the time for protein digestion from overnight to one hour (including all preparation steps) and analyze and detect proteins secreted from developing embryos.

Acknowledgements: This research has been funded by European Union within FP7 Project Prot-HiSPRA, 282506

Characterisation of the proteomic response of the human-pathogenic fungus *Aspergillus fumigatus* to low oxygen levels (hypoxia)

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Aspergillus fumigatus is a ubiquitously occurring filamentous fungus which can be found in soil and organic matter. However, it is also an opportunistic pathogen which may cause a broad spectrum of disease in the human host, ranging from allergic or locally restricted infections to invasive mycoses. The most fatal *A. fumigatus* disease, invasive aspergillosis (IA) occurs in patients who are severely immunocompromised and is characterized by a high mortality. Infection is usually aerogen from aspirating *A. fumigatus* spores. During the course of the infection *A. fumigatus* has to cope with several kinds of stress condition including low oxygen levels (hypoxia). Just recently it was shown that hypoxia adaptation is an important virulence attribute of *A. fumigatus*. To identify novel hypoxia-sensing and adapting pathways we have characterized the changes of the *A. fumigatus* proteome in response to short (3-24 hours) and long periods (7-10 days) of hypoxia (1% O_2). To maintain reproducible culture conditions an oxygen-controlled fermenter was established. During long-term cultivation under hypoxia, proteins involved in glycolysis, the pentose phosphate shunt, amino acid biosynthesis, NO-detoxification and respiration showed an increased level. In contrast, proteins involved in sulfate assimilation and acetate activation were down-regulated. Strikingly, hypoxia also induced biosynthesis of secondary metabolites.

The proteomic response of *A. fumigatus* to short periods of hypoxia showed some similarities, but also marked differences: Here, enzymes involved in fermentation and other pathways involved in NADH regeneration significantly increased. To get a deeper knowledge about the specific role of metabolic pathways in adaptation to hypoxia, we have started to characterize candidate genes for their role in hypoxia by generating deletion mutants. First data about the phenotypic characterization will be presented.

Protein signaling in spinal parenchyma and dorsal root ganglion: key to future therapy approaches in spinal cord spasticity

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Keywords: Spinal cord trauma, Spasticity, Hyper-reflexia, Proteomic profiling, Dorsal root ganglia, Spinal gray matter

Development of progressive muscle spasticity and rigidity represents a serious complicationassociated with spinal ischemic or traumatic injury. Signaling proteins including phosphorylation status were analyzed in the spinal parenchyma below the Th9 spinal level of transection and in the corresponding dorsal root ganglion cells in rat model of spinal injury using Kinex™ antibody microarrays. The eIF4G Ser1107 hyperphosphorylation and pronounced decreases in protein levels in ventral spinal horn as well as direct protein interaction mediated cross-talk between angiogenesis and neurodegeneration pathways were highlighted and might significantly contribute to healing process in the damaged region. At 2 and 5 weeks after transection, up-regulation of several proteins including CaMKIV, RONalpha, MAPK3/ERK1 and PKCdelta was exclusively observed in the spinal lumbar ventral horns, where such proteins may play an important role in the initiation but also in the maintenance of spasticity states after spinal trauma. Hence, potential new treatment strategies such as gene silencing or drug treatment should primarily target dermatome-muscle-specific spinal segments to improve local inhibitory tone and/or decrease the primary afferent drive in prevention of spasticity development or treatment of spasticity states. We believe that this work will stimulate future translational research, ultimately leading to the improvement of quality of life of patients with spinal traumatic injury.

Acknowledgements: This work was supported in part by MEYS (ME10044; H.K.), NIH (NS051644; M.M.), (TA01011466; HK), and Institutional Research Concept RVO67985904 (IAPG, AS CR,v.v.i). Infrastructural part of this project was supported from the Operational Program Research and Development for Innovations (CZ.1.05/2.1.00/03.0124).

Proteomic approaches in nutrition research

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Novel Aspects: We developed and successfully applied new strategies for studying the systemic effects of dietary molecules and nutrition on key gene-regulatory networks.

The interaction of dietary molecules with gene-regulating proteins is a key molecular process in metabolism, including the regulation of various interconnected pathways. In this lecture we will highlight various approaches applied by us for understanding these complex processes and show the application of efficient methods for discovering small molecules with health-beneficial effects. We will further discuss our concept of analysing protein sets for detecting disease-related molecular networks and for providing potentially powerful diagnostic read-out for monitoring and treating complex diseases such as type 2 diabetes or cardiovascular disorders [1-4].

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Defensin level determination by targeted proteomic approach

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Novel Aspects: Defensin identification, method development, targeted mass spectrometry

Defensins represent an important group of antimicrobial peptides consisting of 16 – 50 amino acids, organized to a structurally conserved compact structure and associated with multiple functions to act in the first line of defense. The three subfamilies of defensins (α, β, θ) differ in their peptide length, location of disulphide bonds between cystein residues, their precursor structures and in the site of expression. They exhibit low immunogenicity, resistance to proteolysis, broad range of anti-microbial activities and based on these activities emerge also as low molecular weight immunomodulators with pharmaceutical potential [1]. We developed an LC-coupled Multiple Reaction Monitoring (MRM) based mass spectrometry method [2] for the analysis of β-defensin 2, β-defensin 3 and α-defensins aiming to have an alternative way for defensin identification and quantification. The multiplex feature has the advantage over classical ELISAs that the level of multiple proteins can be determined in the same time from one sample having relevance in biology and medicine where the amount of sample to be studied can be a limiting factor. In this study Caco-2 cell lines challenged with IL1β treatment as pro-inflammatory stimuli were used. In cell lysates the level of β-defensin 2 was significantly higher compared to the control samples, both at gene expression and protein level. In cell culture supernatant higher β-defensin 2 and β-defensin 3 amounts were found. Our results show that the developed targeted proteomics approach was sensitive and specific for both proteins and could be validated by independent methods serving as an alternative quantification method for further immunological studies.

Acknowledgements: This research was funded by TÁMOP 4.2.2.A-11/1/KONV-2012-0045

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An automated, high-throughput method for targeted quantification of intact insulin and its therapeutic analogues in human serum or plasma coupling mass spectrometric immunoassay with high resolution, accurate mass detection (MSIA-HRAM)

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Novel Aspects: sensitive, selective, semi-automated method for detection and quantitation of insulin variants

Recombinant insulins as well as a range of synthetic variants are now available as therapeutic agents. The detection and quantification of insulins is important for medical, sports doping and forensic applications. A simple universal analytical methodology has been developed for the simultaneous extraction of both endogenous and exogenous insulins, with subsequent detection and quantification. We present a multiplexed assay that couples immunoenrichment with high-resolution, accurate mass detection at concentrations across the clinical range. The assay described is sensitive, selective, semi-automated for simplicity, and potentially applicable to new insulin variants allowing their incorporation into second or third generation assays.

Glycoprofiling of Human Plasma Proteins

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Novel Aspects: Glycosylation database of human plasma proteins has been built

Glycosylation is the most common PTM, and shows variations in a given organism. Correlation between glycosylation and disease has often been found, but data are far from being systematic. Plasma glycoproteins show high promise to become disease biomarkers, and may become commonly used in clinical practice, provided their analysis can be improved.
We have developed a method which is capable for studying not only a purified glycoprotein sample, but is capable of dealing with glycoprotein mixtures, like blood plasma [1]. Plasma is fractionated using RP-HPLC; resulting in crude glycoprotein isolates. These are digested using trypsin, yielding peptide/glycopeptide mixtures. These are studied using nano-UHPLC-MS(MS) and evaluated by our home-built Glycominer [2] and Glycopattern softwares to determine glycosylation patterns.

Analysis at the glycopeptide level (in contrast to the more common oligosaccharide level) makes it possible to study glycosylation, even if the plasma fraction contains several glycoproteins. This is essential to obtain un-biased results [3-4]. The results will be used to build up a glycosylation library for future reference. Improved methodology makes it possible to determine site-specific glycosylation profiles of several glycoproteins. New glycoforms of several proteins have been identified and its application in biomarker research will be shown.

Acknowledgements: This work has been supported by the Hungarian Scientific Research fund (grant No. OTKA NK83857).

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Into the unknown: Systems biology approaches in exploring novel protein functions in plant signaling pathways

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Novel Aspects: Yet uncharacterized LRR-Receptor SIRK1 kinase regulates aquaporins.

The transmembrane receptor kinase family is the largest protein kinase family in Arabidopsis, and contains the highest fraction of proteins with yet uncharacterized functions. Here, we present functions of SIRK1, a receptor kinase that was previously identified with rapid transient phosphorylation after sucrose resupply to sucrose-starved seedlings. SIRK1 was found to be an active kinase with increasing activity upon external sucrose supply. In sirk1 T-DNA insertional mutants, sucrose-induced phosphorylation patterns of several membrane proteins were strongly reduced, particularly pore-gating phosphorylation sites in aquaporins were affected. SIRK1-GFP fusions were found to directly interact with aquaporins in affinity pull-down experiments on microsomal membrane vesicles. Furthermore, protoplast swelling assays of sirk1 mutants and SIRK1-GFP expressing lines confirmed a direct functional interaction of receptor kinase SIRK1 and aquaporins as substrates for phosphorylation. Lack of SIRK1 expression resulted in a failure of mutant protoplasts to control water channel activity upon change in external sucrose concentration. We propose that SIRK1 is involved in regulation of sucrose-specific osmotic responses by direct interaction with and activation of an aquaporin via phosphorylation and that the duration of this response is controlled by phosphorylation-dependent receptor internalization.

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Molecular dissection of nuptial food gifts in a cricket

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Sexual conflict occurs when the genetic interests of males and females diverge, and recent evidence suggests that the genomes of males and females are therefore in fact in active 'conflict'. However, we know little about the genes involved in this ongoing conflict or about how their gene products (proteins or small molecules) actually alter male or female fitness. Insects are perfect models with which to study such conflict, but much of what we know comes from the study of the fruit fly, Drosophila, which is not fully representative of the stunning array of sexual behavior shown by insects as a whole. Here, we will develop the decorated cricket, *Gryllodes sigillatus*, as a new model with which to begin a molecular dissection of sexual conflict in insects. Males of this cricket provide females with a nuptial gift (spermatophylax) packed with proteins that dramatically alter female behavior. We combined transcriptomics and proteomics to analyse the protein composition of spermatophylaces. We identified and isolated 17 different proteins, here termed SPX1a-e and SPX2-14. We also 454-pyrosequenced a normalised cDNA library prepared from male accessory glands to obtain the associated SPX cDNA sequences. Among these 17 proteins, we identified several small peptides, a pacifastin-like serine protease inhibitor, and an odorant binding protein-like. We are currently in the process of characterising several of these candidates in more details. This initial work provides the key to begin a molecular dissection of exactly how the male's gift alters female behavior and how this is affected by variation in the spermatophylax proteins.

Proteome and metabolome profiling of cytokinin action in Arabidopsis identifying both distinct and similar responses to cytokinin down- and up-regulation

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Novel Aspects: Plant hormone signaling, cytokinin, proteome and metabolome analysis.

In plants, numerous developmental processes are controlled by cytokinin (CK) levels and their ratios to levels of other hormones. While molecular mechanisms underlying the regulatory roles of CKs have been intensely researched, proteomic and metabolomic responses to CK deficiency are unknown. Transgenic Arabidopsis seedlings carrying inducible barley cytokinin oxidase/dehydrogenase (*CaMV35S*>GR>*HvCKX2*) and agrobacterial isopentenyl transferase (*CaMV35S*>GR>*ipt*) constructs were profiled to elucidate proteome- and metabolome-wide responses to down- and up-regulation of CK levels, respectively. Proteome profiling identified >1100 proteins, 155 of which responded to HvCKX2 and/or ipt activation, mostly involved in growth, development, and/or hormone and light signalling. The metabolome profiling covered 79 metabolites, 33 of which responded to HvCKX2 and/or ipt activation, mostly amino acids, carbohydrates, and organic acids. Comparison of the data sets obtained from activated *CaMV35S*>GR>*HvCKX2* and *CaMV35S*>GR>*ipt* plants revealed unexpectedly extensive overlaps. Integration of the proteomic and metabolomic data sets revealed: (i) novel components of molecular circuits involved in CK action (e.g. ribosomal proteins); (ii) previously unrecognized links to redox regulation and stress hormone signalling networks; and (iii) CK content markers. The striking overlaps in profiles observed in CK-deficient and CK-overproducing seedlings might explain surprising previously reported similarities between plants with down- and up-regulated CK levels.

Acknowledgements: This work was supported by grants 206/09/2062 and P305/12/2144 (Grant Agency of the Czech Republic), funds from the ERDF for 'CEITEC–Central European Institute of Technology' (CZ.1.05/1.1.00/02.0068) and ESF project COOPELIA- Support of international COOPEration – Multidisciplinary Themes in Life and Material Sciences (CZ.1.07/2.4.00/17.0045).

Identification of secretory proteins in defensive secretions from juvenile leaf beetles

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Insects have an outstanding virtuosity when it comes to producing defensive compounds for repellence their omnipresent enemies. These compounds frequently originate in exocrine glands to circumvent auto-intoxicative effects. The larvae of the leaf beetle subtribe Chrysomelina, for example, developed for the production of defensive secretions nine pairs of exocrine glands arranged segmentally along their back. The glands consist of small pouches replete with fluid, ordinarily kept withdrawn, but promptly everted when the larvae are disturbed. According to phylogenetic analyses, the juvenile Chrysomelina species evolved three different strategies to produce the defensive compounds: (*i*) *de novo* production of pre-toxins and conversion in the pouches into iridoids (cylopentanoid monoterpenoids) as the ancestral strategy, (*ii*) sequestration of plant-derived salicin which serves as precursors for the repellent salicylaldehyde, (*iii*) incorporation of a wide variety of glucosidically bound leaf alcohols which are further esterified in the secretions with butyric acids derived from the insects' internal pools of amino acids resulting in a cocktail of at least 70 different esters. The last activation steps from pre-toxins in the secretions require enzymatic conversions. Here we ask the question, how did the protein composition in the secretions of the different biosynthetic strategies adapt during the Chrysomelina evolution? Therefore we have carried out comprehensive proteome analyses by using LC-MS/MS of the secretions of the three synthesis strategies and compared the proteomes. Protein identification using homology-based searching (MS BLAST) revealed only strongly conserved proteins. To improve protein identification we implemented data-independent acquisition (DDI) that has been shown to improve protein coverage compared to conventional data-dependent acquisition (DDA). The results improve our understanding of the evolution of diversity in defensive secretions which has consequences for different trophic levels in an ecological network.

CYCAM - a main hub for the perception of beneficial microbe- and pathogen-associated molecular patterns leading to the cytosolic Ca^{2+} signaling, growth promotion and defense in *Arabidopsis thaliana*

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Key words: *Alternaria brassicae*, *Arabidopsis thaliana*, beneficial microbe associated molecular pattern, cytosolic calcium signaling, defense, growth promotion, *Piriformopsora indica.*

Evolutionarily conserved beneficial microbe- and pathogen-associated molecular patterns (bMAMP and PAMP) support microorganisms in their microbial life style as beneficial microbes and pathogens. bMAMP and PAMP are proteins/peptides, oligosaccharides, glycoproteins, lipids or small molecules [1]. Ca^{2+}, a unique intracellular second messenger in various signaling pathways, is involved in growth, development, symbiosis and defense. Early perception of microbes through the recognition of conserved bMAMPs or PAMPs by the respective pattern recognition receptors leads to changes in cytosolic calcium concentration ($[Ca^{2+}]_{cyt}$). The endosymbiotic beneficial interaction between the root colonising fungus, *Piriformospora indica* and *Arabidopsis thaliana* results in growth promotion, enhanced seed production and increased tolerance to biotic and abiotic stress [2,3], whereas the pathogenic interaction with the necrotrophic fungus *Alternaria brassicae* leads to disease development, inhibition of growth and finally death of *A. thaliana*. The model plant *A. thaliana,* expressing the bioluminescent Ca^{2+} reporter apoaequorin in the cytosol, was used to elucidate the role of cytosolic Ca^{2+} signaling in beneficial and pathogenic interactions.

The biologically active bMAMP from the *P. indica*-cell wall extract (Pi-CWE) which induces $[Ca^{2+}]_{cyt}$ elevation in Arabidopsis roots, was purified as a trisaccharide ($C_{18}H_{32}O_{16}$) with *m/z* of 505.1748 (Pi-504). Acid hydrolysis of Pi-504 cleaved the trisaccharide into glucose and galactose, which did not induce $[Ca^{2+}]_{cyt}$ elevation. Pi-504 promotes growth of Arabidopsis, tobacco and Chinese cabbage seedlings, as the fungus does, even though it did not completely replace the growth promotion induced by the fungus. High throughput screening of EMS mutagenised aequorin populations resulted in the isolation of *cycam* (cytosolic calcium mutants) which do not induce $[Ca^{2+}]_{cyt}$ elevation in response to Pi-504. The homozygous mutants also did not respond to CWE from another endophytic beneficial fungus, e.g. *Acremonium alternatum*, and pathogenic fungi e.g. *A. brassicae*, *Rhizoctonia solani*, *Fusarium solani* or *Phytophthora parasitica* var. *nicotianae*. Therefore, CYCAM mediates $[Ca^{2+}]_{cyt}$ elevation in beneficial and pathogenic interactions. The mutants responded normally to a CWE from

Mortierella hyalina and a toxin preparation from *A. brassicae*. The active component in the *M. hyalina*-CWE was purified as Mh-222 with *m/z* of 223.0599. The *CYCAM* mutants were impaired in both growth promotion and tolerance to biotic and abiotic stress. Pi-504- and *P. indica*-induced growth promotions are linked to enhanced nitrogen metabolism, phosphorus uptake and better photosynthetic performance. The mutants are highly sensitive to biotic (*A. brassicae* infection and *A. brassicae* toxin) and abiotic stress (salt, water, drought and oxidative stress). Hypersusceptibility of *cycam* to *A. brassicae* infection is correlated to low level of aliphatic glucosinolates and the bioactive (+)-7-*iso*-jasmonoyl-1-isoleucine ((+)-7-*iso*-JA-Ile), increased levels of abscisic acid (ABA), salicylic acid (SA) and reactive oxygen species (ROS). We propose that *cycam* is defective in a gene that is required for $[Ca^{2+}]_{cyt}$ elevation to establish growth promotion and stress responses.

Acknowledgements: SPP (1212) DFG, FSU Jena, IMPRS Jena, CMB Jena, IPB Halle.

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Ion Mobility Enabled Data Independent Proteomics Approaches

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This presentation will give an overview of some of the analytical challenges in shotgun proteomics, before introducing the LC-MSE data-independent aquisition method (DIA) and how it is further enhanced by the addition of ion-mobility separations (IMS). Technical aspects of such coupling of ion mobility to high resolution time-of-flight mass spectrometry will be discussed. Performance examples of IMS-MSE will be provided, along with comparisons with the more traditional data-dependent aquisition (DDA) strategies. In addition to providing protein identifications, these data independent acquisition protocols can also provide fully quantitative data using an accurate and efficient label-free strategy. The presentation will finish by giving updates on the latest Waters software release for proteomics; the Transomics 1.2 which brings significant enhancements and simplification in large scale complex proteome and metabolome studies.

Automated native sample preparation for biomarker search

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A multidimensional chromatographic method is presented which is adapted to fractionation of body fluids for proteomic analysis and biomarker search. This method combines native size exclusion (SEC, first dimension, 1D), followed by anion exchange (AEC, 2D) and lectin affinity (LAC, 3D) chromatography. After serial 1D fractionation the further procedures are performed in compatibility with microplate format. Thus beginning with 2D fractionation throughout parallelization and automation is realized with spectrophotometric read out, temporary storage, hit picking, medium exchange, digest, desalting, and finally ends within autosampler of LC-MS.

Central unit is an experimental setting realizing automated multichannel pipetting and robot handling of microplates, reservoirs, and column arrays. All, the fractionation process, hit picking and analytics are controlled by an adapted software packages. Some tools and tricks have been developed in order to improve sample preparation in between as for dialysis and desalting, re-concentration, and readout in microplates. Per working day for serial 1D runs or four synchronic 2D runs can be performed including spectrophotometric readout. Thus four samples may be processed comparable (1D) or actually in parallel (2D) within three days. Thereafter the majority of fractions run through tryptic digest and mass spec, the minority, still highly complex fractions has to be further separated parallel by LAC before digest.

Using commercially available columns the protein input of samples is scalable from 2 to more than 100 mg and from 2 to 1000 kDa molecular weights. Globally, derived from UV measurements protein recoveries from the corresponding load are 97.1 ± 11.2 and 88.8 ± 3.6% and best precisions are 3.8 and 5.1% CV with 2D and 3D fractionation, respectively.

For process control and all-round analytics a new software tool was developed accompanying the whole fractionation process. Their automatic interfaces collect entire data sets available including sample specification, location, and read out data of all fractions and identified

proteins into a central data-warehouse. Therewith, immediate data access, comparison, and visualization in versatile charts and reports are realized.

Besides automation, this method has several advantages: high proteome coverage, flexible dynamic range with respect to molecular weight and sample amount, optional enzymatic and immunological analytics additional to mass spectrometry. Without exception, all intrinsic components and information are preserved after fractionation, including natural complex formation, fragmentation, and biological activities. Such information is beneficial for comprehensive profiling, for biomarker search, as well as for efficient evaluation.

Preliminary applications show versatility with profiling plasma proteomes of humans, cattle, goat and mouse, and human cerebrospinal fluid (CSF) and with biomarker search in severe inflammation, nephropathies, neurodegenerative diseases, and with psoriatic arthritis.

Quantitative N-Glycan Analysis using µLC-PGC-ESI-qTOF-MS

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Novel Aspects: N-Glycan, Glycan reductive isotope labeling, porous graphitized carbon

Among all post-translational modifications, glycosylation is the most common one and plays crucial roles in various biochemical processes like cell adhesion, protein folding, receptor functioning or signal transduction. Though much effort has been invested in glycan analysis in the past decade, the detailed characterization of protein glycosylation remains still challenging due to the diversity of glycan structures regarding linkage, branching, length and composition of the polysaccharide chains. Such a detailed analysis of glycan structure and heterogeneity, however, is required for improving our understanding of disease-related changes in glycan expression levels as well as their specific biological functions[2].

High-Performance-Liquid-Chromatography in combination with mass spectrometry is nowadays the most powerful and versatile technique for structure elucidation of oligosaccharides, either still linked to peptides or present as neat glycans[4][5]. In this work, quantitative N-glycosylation patterns of selected glycoproteins were analyzed by HPLC-ESI-MS techniques using stable isotope coded labeling for allowing relative glycan quantitation in combined samples carried out in one LC-MS run[6].

Porous graphitized carbon (PGC) was used as stationary phase as it has been shown being suitable for size- and shape-selective separation of oligosaccharides. PGC columns are for instance capable to separate structural isomers of derivatized as well as underivatized glycans of high mannose and complex type distinguishing according branching, linkage and anomericity[3].

Isotope Labeling was carried out *via* reductive amination using ^{12}C-aniline and ^{13}C-aniline. The main advantage of this method for comparative glycomics lies in the improved accuracy and precision of quantitation. With the used labels, structurally important information like sulfation and phosporylation can be preserved (unlike permethylation) and a robust mass difference between the labeling variants is attained without affecting the chromatographic separation of the glycans[1][6].

Acknowledgements: We would like to thank the University of Vienna for a PhD scholarship within the doctoral program BioProMoTION (Bioactivity Profiling and Metabolism).

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Phosvitin as a standard protein for optimization of enrichment of multiply phosphorylated proteins

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Novel Aspects: Phosvitin, multiply phosphorylated peptides, mass spectrometry

Phosphorylation of proteins is very important posttranslational modification in most intracellular biological processes [1]. In general, proteolytic digests contain phosphopeptides at low stoichiometry and corresponding ion signals are suppressed by the signal of more abundant nonphosphorylated peptides during the ionization process. That is why a selective enrichment of phosphopeptides before mass spectrometry (MS) analysis is necessary. Many applications, e.g. analysis of multiply phosphorylated peptides from hyperphosphorylated TAU protein, require a suitable enrichment procedure for isolation of multiply phosphorylated peptides. Most common techniques for phosphopeptides enrichment are metal oxide affinity isolation (MOAC, e.g. TiO_2) or immobilized metal affinity chromatography (IMAC). IMAC method is more suitable for analysis of multiply phosphorylated peptides because elution of these peptides from TiO_2 is difficult. That is why a combination of IMAC and MOAC called SIMAC is used [2].

Phosvitin from egg yolk could be a suitable standard for optimization of conditions for enrichment of multiply phosphorylated peptides (compared to commonly used α- or β-casein). Phosvitin is a phosphoglycoprotein that contains 216 amino acid residues and many of 123 serines present in the amino acid sequence are phosphorylated [3]. Tryptic digestion of this protein provides many multiply and monophosphorylated peptides and one of the aims of this work is to describe the detected phosphopeptides.

Phosvitin was digested with trypsin and analyzed by MALDI LTQ Orbitrap XL instrument. Tryptic digests were enriched either with TiO_2 resin or using SIMAC protocol [2]. Peptide digests were measured before and after enrichment procedure (employing the desalting step with POROS Oligo R3 resin). For better detection of phosphopeptides, phosvitin digests and elution fractions from enrichments were fractionated by microcolumn RP LC separation into 24 MALDI spots using nonlinear gradient of acetonitrile-water mixture containing 0.1% TFA. For determination of multiply phosphorylated peptides MS^n in linear ion trap was performed.

Dephosphorylation of phosphopetides was carried out by alkaline phosphatase in solution and also directly on MALDI target plate after RP LC fractionation.

All these techniques were used for determination large number of phosphorylated sites. Phosvitin can be used as a suitable standard protein containing multiply phosphorylated peptides for optimization of enrichment procedure.

Acknowledgements: This work was supported by EU project NADINE (No. 246513), project SGFCHT 07/2013, a long-term organization development plan no. 1011 from the Faculty of Military Health Sciences, University of Defence, Czech Republic.

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Imaging mass spectrometry: Searching for the invisible, a new tool for disease investigations

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Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF)-profiling and imaging mass spectrometry (MSI) are promising technologies for measuring hundreds of different molecules directly on tissues. For instance, small molecules, drugs and their metabolites, endogenous lipids, carbohydrates and complex peptides/proteins can be measured at the same time. In the most advanced instruments this is performed without significant disruption of sample integrity. It is a unique approach for assessing the spatial distribution of molecules providing graphical multidimensional maps of the constituent analytes to be correlated to histopathological changes found in the patient tissue.

In this presentation, the potential of MALDI-profiling/imaging technologies in disease proteomics, drug action and studies of cellular processes will be described. Spatial and sequence information obtained in tissue MALDI-profiling/imaging studies can be correlated with other mass spectrometry-based techniques, auxiliary imaging technologies and routine (immuno) histochemical staining.

Accurate mass MALDI imaging at 25 µm pixel size for proteins after on-tissue digestion

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The direct detection and identification of intact proteins in MALDI imaging remains a challenging task due to limited sensitivity and mass range. Here we present new approaches for on-tissue tryptic digestion of proteins. We focus on optimizing the spatial resolution and reliability of peptide identification.

Trypsin solution was deposited in several cycles on tissue with a spraying device [1]. An atmospheric pressure matrix assisted laser desorption (AP-MALDI) ion source coupled to a Q Exactive mass spectrometer (Thermo Fisher Scientific GmbH, Bremen) was used for imaging experiments [2]. Tryptic peptides were identified by matching imaged m/z peaks to peptides which were identified in complementary LC-MS/MS measurements of an adjacent tissue sections. All MS measurements were based on accurate mass (< 3 ppm RMS).

A coronal mouse brain section was measured at 50 µm pixel size. Peptide peaks were detected on tissue with a mass resolution of R=80000 (@m/z700). This measurement reveals detailed histological structures such as the ependyma (consisting of a single cell layer) which is clearly defined by several identified peptides. Highly reliable information about protein distribution was also obtained for clinical human tissue originating from brain and gastric cancer biopsies. This data is used to investigate intratumor heterogeneity on a molecular level. Additional measurements at 50 µm pixel size include the analysis of a whole body section of an infant mouse.

A coronal mouse brain section was imaged at a pixel size of 25 µm. The resulting ion images of tryptic peptides showed excellent correlation with myelin and H&E staining. Peptide peaks were detected on tissue with a mass resolution of R=40000 (@m/z700). The sensitivity could be significantly improved compared to previous experiments and about 150 tryptic peptides which show a clear spatial distribution were identified.

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Identification of Biomarkers in MALDI imaging experiments

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Novel Aspects: Isotopic fine structure as tool for identification of biomarkers in MALDI imaging experiments

MALDI imaging allows biomarker discovery directly on tissue. The compounds that can be detected range from proteins over peptides to lipids and endogenous metabolites. Usually a MALDI imaging based biomarker discovery workflow results in a list of statistically different masses. The identification of those masses usually requires additional analytical steps. These may include additional separation techniques and MS/MS experiments.

In the analysis of proteins it is possible to perform on-tissue tryptic digestions followed by MS/MS identification of peptides. This can be done directly on tissue, but a comprehensive analysis usually requires a separate LC-separation.

For the analysis of lipids and endogenous metabolites additional options for the identification exists. A common technique is the use of accurate mass measurements that can hint towards the molecular formula of the compound. Nevertheless, even accurate mass measurements usually leave some ambiguity and require at least some previous knowledge of the compound class. The identification of true unknowns may still require additional analytical dimensions. One new way to achieve this is the use of the isotopic fine structure in ultra-high mass resolution experiments. The isotopic fines structure contains multiply redundant information on the molecular composition and allows an unambiguous direct readout of the molecular formulas of unknown substances.

In this presentation we will show examples for the identification of different compound classes such as proteins, peptides and lipids. The first examples of unambiguous molecular formula characterization by isotopic fine structures will also be presented.

3D MALDI imaging of larynx carcinoma

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Novel Aspects: 3D MALDI Imaging, cancer, co-registration, segmentation

The localization of specific proteins in tissue on a cellular and subcellular spatial resolution is possible by immunohistochemistry (IHC). However, IHC is a targeted method, which means the identity of the proteins of interest must be known and protein-specific antibodies are necessary.
Mass spectrometry-based imaging techniques capable of elucidating molecular profiles directly from tissue are becoming increasingly popular. Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (MSI), shortly called as MALDI-imaging, is the most popular MSI technique for proteomic applications and allows to obtain proteomic profiles directly from thin tissue sections, e. g. from a tumor. Additionally, 3D MALDI MSI has the potential to combine consecutive tissue section of a tumor to a spatial model. However, 3D MALDI-MSI cannot tap its full potential due to the lack of efficient computational methods for constructing, processing, and visualizing large and complex 3D MALDI-MSI data.
To tackle this problem, we use an image registration based method to reconstruct the whole 3D structure of a human larynx carcinoma sample from 60 serial sections. The basic idea was to recapture the spatial correspondence between neighboring slides by means of affine image registration, followed by an elastic registration to correct nonlinear deformations from the slide to slide, that are introduced in the sectioning process. This new concept of multi-modal registration for 3D image reconstruction complements 3D MALDI-imaging and histochemically stained high-resolution optical images uniting molecular, anatomical and histological information. The fusion of modality dependent information helps in the correlation of anatomical and spectral features. The visualization of this concept (the registration of serial sections, reconstruction of a 3D MALDI-MSI model, co-registration with histological H&E stain section) will be presented.

Acknowledgements: This project was supported by the BMBF in the call Mobitec

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Proteolipidomics by High Resolution Mass Spectrometry

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Novel Aspects: Lipidomics and proteomcis at the single instrument platform

Developments in mass spectrometry technology, genomic sequencing, genetic engineering and bioinformatics have forged proteomics – a burgeoning branch of science aiming at both mechanistic and global understanding of the proteins role in living organisms. Despite being extraordinary powerful, this approach was also reductionist: particularly, in membranes the role of lipids was reduced to an unstructured hydrophobic carrier for specific proteins [1]. Remarkable efforts have been made to characterize proteins in lipid biosynthesis pathways, however the actual complexity of the lipidome composition was undervalued. Partly this was due to the limited ability of analytical technologies to decipher the lipidome composition in sufficient molecular details. Now the decisive move is to merge both "proteo-centric" and "lipido-centric" approaches by adopting a more systematic view on the collective behavior of proteins and lipids within biological membranes. This paper will discuss the technological implications of this motion and present a quantitative proteomics and lipidomics characterization pipeline relying on instruments of the Orbitrap family.

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Posters

PilE4/OmpA:ICAM:1 – The potential candidates for *Francisella* adhesion to brain endothelium

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Novel Aspects: Unfolding two crucial proteins involved in adhesion of *Francisella* to brain microvascular endothelial cells

Francisella tularensis, a causative agent of tularemia, are highly infectious Gram-negative bacteria, which causes serious disease in human and animals. First, we showed that *Francisella* can cross in vitro model of blood-brain barrier. Subsequently, we performed ligand capture assay to identify the brain microvascular endothelial cells (BMECs) receptor for *Francisella* adhesion, which was identified as ICAM-1. Modified pull down assays confirmed that on the surface of *Francisella* there are at least two proteins, which are interacting with ICAM-1 protein of brain microvascular endothelial cells. At first, we performed magnetic bead based immobilized metal ion affinity chromatography, wherein His-tagged ICAM-1 was fused on affinity beads and hybridized with whole cell lysate of LVS. After washings, protein complexes were eluted and fractionated on SDS-PAGE. Interacting protein partners of ICAM-1 observed on PAGE were excised and subjected for peptide mass fingerprinting which identified these protein candidates as PilE4, the major subunit of type IV pili and OmpA. We constructed truncated forms of PilE4 and OmpA, and used them in another round of pull down assay, wherein PilE4 or OmpA were immobilised on affinity beads and incubated with BMECs proteins. After elution, protein complexes were subjected for mass spectrometry, which confirmed interaction between PilE4 and OmpA with 55 kDa protein, which molecular weight corresponds with ICAM-1. In the presented work, we found and conformed interaction of two protein of *Francisella* interacting with BMECs. These findings could be important for the understanding of molecular basis of pathogenesis of franciselosis.

Acknowledgements: Work was supported by APVV-0036-10 and VEGA 1/0054/12 and EB and LP are funded by ITMS 26220220185, infrastructure was funded by ITMS 26220120002.

Adhesion of predicted binding site of OspA (*Borrelia garinii*) to CD40 of brain microvascular endothelial cells

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Novel Aspects: Identification of CD40 binding site of OspA protein

Objective: *Borrelia* is capable of penetrating the blood–brain barrier (BBB), either through transcellular and/or the paracellular route. In our previous study we showed that interaction between CD40 of rat brain microvascular endothelial cells (BMECs) and outer surface protein (OspA) of *B. garinii* (strain SKT-7.1) is crucial for the transient tethering of *Borrelia* to the endothelium. To this background, objective was set to predict and identify the domain/s of OspA that binds CD40 molecule.

Methods: Nucleotide sequence of OspA of SKT-7.1 was *in silico* translated and amino-acid sequence was subjected to search for endothelium binding sites, antibody binding pockets and hypervariable antigenically important regions in OspA based on database search (Uniprot, SMART) and data mining. Three putative endothelial cell binding sites were identified: 18 to 44 AA (putative tick gut endothelium binding site, TGEBS), 85 to 103 AA (putative TGEBS) and 144 to 183 AA (HUVEC binding site). Three his-tagged forms (N-terminal tag) of the proteins encompassing these three putative endothelial cell binding sites of OspA were overexpressed in *E. coli* (SG10003 strain) using pQE-30 vector (Qiagen) and isolated using affinity tag chromatography. Binding affinity of truncated forms of OspA was assessed with western-blotting in which BMEC proteins were immobilized on nitrocellulose membrane. To confirm the results of western-blotting, His-tagged proteins were immobilized on Talon beads (Clontech) hybridized with cell lysate of primary cultures of rat BMEC. Protein complex was eluted and candidate proteins were identified on MALDI-TOF.

Results: Truncated OspA candidate encompassing amino acid residues 144 to 183 AA showed affinity to BMECs in western-blot analysis, while other two truncated forms of the OspA did not show binding ability to any of the BMEC proteins. As expected, in the Talon based MALDI assay, peak at 11 kDa corresponding to truncated OspA protein and its binding partner at ~30 kDa were found. 30 kDa protein was identified as CD40 with peptide mass fingerprinting.

Conclusion: Results indicate that interaction between CD40 and OspA is mediated through the OspA domain encompassing 144 to 183 AA. Thus this domain may be the crucial for transient adhesion of borreliae to BMECs and may be essential for subsequent BBB translocation of *Borrelia*.

Acknowledgements: Work was supported by APVV-0036-10 and VEGA 1/0054/12 and LP is funded by ITMS 26220220185, infrastructure was funded by ITMS 26220120002.

How TFA effects protein conformation and separation in RP-HPLC

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Novel Aspects: TFA-protein adduct, protein conformation, RP-HPLC, protein separation mechanism

RP-HPLC of intact proteins is a promising separation technique in proteomics, in isolation tasks, in the pharmaceutical field, and related research areas. Gradient RP-HPLC provides powerful enrichment, when studying small amount of proteins and serves the reduction of the matrix in complex samples. In reversed phase separation both the stationary phase and mobile phase have a primary function. In addition to, in reversed phase intact protein chromatography ion pairing agents and their concentration in the mobile phase play an important role.

Trifluoroacetic acid (TFA) is a widely used ion pairing additive in RP separations of proteins. We observed unusual chromatographic features on the retention properties of proteins as the function of TFA concentration in the mobile phase. These findings are assumed to be related to structural change of proteins. Chromatographic behavior of model proteins lysozyme and transferrin was investigated by UPLC-UV-MS. Unfolded and compact conformation of lysozyme and transferrin were observed, determined by TFA concentration level in the mobile phase. At TFA concentrations below 0.01 v/v% for lysozyme and 0.001 v/v% for transferrin, proteins showed unfolded conformation and excluded from the pores (200 Å) of the stationary phase. This and the applied eluent strength (30% acetonitrile) resulted the elution of the proteins before dead time. Above these TFA concentrations, the proteins showed structural change to a more compact, TFA adducted conformation, which were able to diffuse into the pores of the stationary phase and eluted after dead time.

Critical TFA concentrations related to the described conformational change significantly depend on the type of protein. This phenomenon could be advantageously used in the fast purification of proteins from each other. Additionally, pore exclusion of the macromolecules enables the separation of intact proteins from small molecules, which diffuse into the pores, therefore elute in dead time or later. In this poster we present the conformational exclusion based purification of lysozyme from transferrin.

Acknowledgements: This work was supported by Chemical Works of Gedeon Richter Plc. under Gedeon Richter Talent Foundation and by the Hungarian Research Found under OTKA NK83857.

Proteomic profile of human chorion derived mesenchymal stem cells by using ion trap mass spectrometry

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Key words: placenta, mesenchymal stem cells, ion trap mass spectrometry, proteome

The human placenta and fetal membranes are reservoirs of mesenchymal stem cells. Stem cells from fetal tissues have significantly higher plasticity in comparison to adult stem cells [1]. Therefore, stem cells derived from placenta are promising candidates for the development of the future strategies in cell therapy.

The chorionic membranes was obtained from healthy female donors (n = 6) after elective caesarean section. The populations of mesenchymal stem cells were enzymatic isolated from tissues. On proteomic analysis were used cells from fourth passages. Whole cell protein fractions were identified utilizing a bottom-up approach. The tryptic peptides were separated by two-dimensional high-performance liquid chromatography and identified by ion trap mass spectrometry.

A total of 760 protein sequences were identified from whole cell fractions. The list of identified proteins has showed also proteins which higher expression is typical for placenta or fetal membranes and which are related to cell growth, maintainance and to cytoskeletal organization. Other identified proteins include chaperones, metabolic enzymes, transporters, kinases, proteases, cytoskeletal, adhesion and cellular defense proteins, transcription and translation initiation factors.

Knowledge about proteome of chorion derived mesenchymal stem cells are highly relevant for understanding properties and changes during cell processes like proliferation, differentiation and reaction on changes of experimental conditions, thereby giving highly relevant information in future developments of desired therapeutic methods.

Acknowledgements: This study was supported by Scientific Grant Agency of the Ministry of Education of Slovak republic (VEGA1/1109/11) and Agency of Slovak Ministry of education for the Structural Funds of the EU under project ITMS: 26220220143 for the financial support of the research.

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Proteomic analysis of the cross talk between plant immunity inducing factors

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Novel Aspects: Salicylic acid, jasmonic acid, azelaic acid, cross talk

Evolutionally plants have developed effective defenses against pathogens. Plant stress phytohormones - salicylic acid (SA), jasmonic acid (JA) play an important role in realizing these defense responses. It is known that SA is mainly effective against biotrophic pathogens whereas JA - against necrotrophes. Recently a new component of plant immunity azelaic acid (AzA), mediator for the induction of systemic acquired resistance was revealed [1]. Much attention was given to the interaction of SA and JA-induced signaling pathways in plants. Features of cross talk between these compounds have been mainly studied on the expression of marker genes. It is considered that between them are competitive relationships i.e. SA signaling pathway inhibits JA and vice versa. However, it was later revealed that there may be a synergistic effect in their relationships. Almost nothing is known about interaction AzA with these phytohormones.

In our work we studied the interaction between SA and methyl jasmonate (MeJ), and the SA and the AzA, their influence on the proteome of pea roots. Proteomic analysis revealed that SA and MeJ caused significant changes in protein content, while under the action of AzA protein spectra changed to a lesser extent. Both the SA and MeJ induced defense proteins involved in the protection of plants against pathogens. They are chitinases, glucanases induced by SA, and protease inhibitors induced by MeJ. Under the action of the AzA the induction of defense proteins wasn't observed.

Studying simultaneous action of SA and MeJ the summation of the effects characteristic for the separate action of SA and MeJ on proteomes was found. If SA was preceded MeJ mainly SA-induced proteins were revealed. If the roots were first treated by MeJA, the MeJA-induced as well as SA-induced proteins decreased. Studying the interaction of SA and AzA mainly SA-induced proteins were revealed. This may be due to the fact that the AzA itself has no significant effect on the pea roots proteins change.

Thus, the specificity of the proteomes reaction on SA and MeJ cross talk depends not only on the concentrations of the studied phytohormones, but also on the sequence of their actions. In interaction between the AzA and SA the latter has the advantage.

Acknowledgements: This work was supported by RFFI grant No. 13-04-00324 and grant of Leading Scientific Schools LS – 825.2012.4.

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Peptide separation on a normal phase column under HILIC separation conditions

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Novel Aspects: Normal phase separation, added selectivity

The separation of peptides containing many basic and acidic residues has always been troublesome when RP-HPLC has been used. One possible solution is the gradient adjustment or switching to using polar stationary phase and the HILIC separation conditions Further, the combination of RPLC with HILIC would lead to an orthogonal separation system, and would possibly enable higher identification rates in comparison to the current separation approaches.
Synthetic peptides were used to set up and optimize the separation on a normal phase HPLC system. The sequences of synthetic peptides were designed as (WAGG-#*-SGK), where (#) was either N or Q, and (*) in each set was V, I, G, A, E or S. Three different mobile phases were used for peptide separation by applying identical separation gradient.
The effect of buffer composition of the eluting aqueous phase, its pH-value, the ionic strength, and the effect of the mixture ratio with the organic mobile phase were examined.
Peptide retention is significantly stronger when MPh buffer was used in comparison to buffers containing AmF or TEAP.
Due to a lower polarity of the glutamine side chain, peptides containing glutamine eluted later compared to peptides which contained asparagine. Therefore, it can be speculated that the MPh buffer promotes the orientation of peptides with their polar site towards the stationary phase while the apolar site of the MPh molecule would stronger interact with the apolar side chain of the glutamine.
It is assumed that TEAP interacts with the stationary phase and competitively prevents peptide interaction with binding sites. Therefore, the more TEAP molecules occupy polar binding sites on the stationary phase the weaker is the retention of polar peptides on normal phase columns.
As expected, polar peptides are retained stronger on polar stationary phase with lesser amount of the aqueous mobile phase.

Acknowledgements: This research has been funded by European Union within FP7 Project Prot-HiSPRA, 282506

The non-model approach. Transcriptome guided identification of secretory proteins from leaf beetle defense secretions via LC-MSE

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Novel Aspects: identification of proteins stabilizing the "bioreactor" of the defense secretions of chrysomeline species and revealing the molecular base of deterrent biosynthesis.

Leaf beetle larvae (Coleoptera: Chrysomelina) prohibit predator attacks and microbial infections by releasing defensive secretions, which they store in nine pairs of dorsal accessory glands. These organelles consist of a cuticular coated reservoir which is yielded by secretory gland cells. The gland cells not only secrete the precursors of later deterrent chemicals into the reservoir, but also all enzymes necessary for their further conversion. Further transformations, such as cyclisation or esterification are known [1]. In order to elucidate these putative enzymes proteins from different defense secretions were separated by 1D-SDS-PAGE and analyzed using LC-MS/MS. Protein identification using homology-based searching (MS BLAST) revealed only strongly conserved proteins, found also in other insect taxa, namely an extracellular Superoxide dismutase and a member of the juvenile-hormone-binding-protein-family. To improve protein identification we implemented data-independent acquisition (DDI) that has been shown to improve protein coverage compared to conventional data-dependent acquisition (DDA) [2]. Combined with recently sequenced in house transcriptomes our approach enabled identification of 45 unique putative proteins in the defense related secretome of the poplar-leaf-beetle (*Chrysomela populi*) and 24 in case of the mustard-leaf-beetle (*Phaedon cochleariae*). Here we discuss and compare proteins involved in biochemical activities of the defense secretion in both species.

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A porin-like protein from oral secretions of *Spodoptera littoralis* larvae induces defense-related early events in plant leaves

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Novel Aspects: Oral secretion; Planar lipid bilayer; Proteomics; Porin-like protein; Calmodulin-like protein; Cytosolic Ca^{2+} concentrations; Plant electrical signals

Insect herbivory on plants is a complex incident consisting of at least two different aspects, namely mechanical damage and chemical challenge, as feeding insects introduce oral secretions (OS) into the wounded tissue of the attacked plant. Mechanical wounding alone is sufficient to induce a set of defense-related reactions in host plants, but some early events such as membrane potential (*V*m) changes and cytosolic Ca^{2+}-elevations can be triggered only by herbivores suggesting that OS-derived molecules are involved in those processes. Following an assay-guided purification based on planar lipid bilayer membrane technique in combination with proteomic analysis, a porin-like protein (PLP) of most likely bacterial origin was determined from collected OS of *S. littoralis* larvae. PLP exhibited channel-forming activity. Further, early defense-related events in plant-insect interaction were evaluated by using a purified fraction and α-hemolysin (α-HL) as a commercial pore-forming compound. Both up-regulated the calmodulin-like *CML42* in *Arabidopsis thaliana*, which only responds to oral secretion and not to wounding. An elevation of *in vivo* $[Ca^{2+}]_{cyt}$ was not observed. Because membrane channel formation is a widespread phenomenon in plant–insect interactions, this PLP might represent an example for microbial compounds from the insect gut which initially involve in plant-insect interactions.

Acknowledgements: We thank Dr. Jyothilakshmi Vadassery for providing the *CML42* and *RPS18B* primer, Dr. Rita Büchler, Dr. Heiko Maischak, and Andrea Lehr for technical support. We thank Angelika Berg for rearing insects, and the greenhouse team for taking care of *A. thaliana* plants. This work was funded by the International Leibniz Research School for Microbial and Biomolecular Interactions (ILRS), as part of the excellent graduate school "Jena School for Microbial Communication" (JSMC).

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Proteomic study of cycloheximide effect on memory caused by exposure to elevated plus maze

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Keywords: Learning, memory, hippocampus, 2D SDS-PAGE electrophoresis

Memory formation originates neurophysiological alterations requiring protein synthesis [1]. Rats re-exposed to the elevated plus maze (EPM) exhibit behavioral changes due to the recovery of aversion learning associated with the initial exploration [2]. Inhibition of protein synthesis in the dorsal hippocampus promotes recovery drugs anxiolytic effect [3]. This study evaluated cycloheximide effect both behavioral evaluating acquisition and consolidation processes, and protein synthesis in the rats hippocampus re-exposed to the EPM. Rats were injected with cycloheximide (3 mg/kg, i.p.) 30 min before or immediately after the first experience in the maze. The re-exposure was done in two time intervals, 3 h or 24 h after the first event. Rats were decapitated, the hippocampus was removed and homogenized in lysis buffer. The proteins were quantified by the Bradford method and separated by 2D SDS-PAGE electrophoresis. The images were analyzed using the PD-Quest software, proteins showed expression variations were identified by MALDI-TOF/TOF MS. Differentially expressed proteins were determined in the left and right hemispheres, we found increased expression of proteins in re-exposure intervals of 24 hours correlated with an amnestic effect of cycloheximide. In addition, new syntheses were found linked to the consolidation of the acquired information, which shows that protein synthesis is required for consolidation of learning associated with the initial exploration of the EPM.

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Development of the method for quantitative determination of hepcidin - significant marker for diagnosis of anemia

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Novel Aspects: Hepcidin, SPE-LC-MRM, anemia

Hepcidin is a peptide hormone that affects the homeostasis of iron metabolism in the human body. It is the main negative regulator of iron release from duodenal enterocytes, macrophages and hepatocytes. Hepcidin blocks iron export from these cells by binding to ferroportin, its unique surface receptor. The generated complex of hepcidin - ferroportin is internalized and afterwards degraded in lysosomes. Subsequently, ferroportin loses the ability to export iron to extracellular space. Affecting and disruption of this regulatory axis leads to various pathological states. Therefore, hepcidin could be one of the potential markers for differentiation of various iron metabolism disorders.

Our method for determination of hepcidin level uses a single-step adsorption to the solid phase. The subsequent separation of the extract is carried out on a reverse phase column C18 by liquid chromatography (UHLPC). The level of hepcidin expression is detected by SRM on QQQ mass spectrometer. Internal standard and calibration curve is obligatory for exact quantification of the molecule. The automatized method is much faster and robust than commercially available ELISA, with higher specificity.

The determination of hepcidin levels can enable more accurate diagnosis of anemia by providing information on the current status of iron metabolism.

Acknowledgements: This project was supported by an internal grant Faculty of Medicine and Dentistry, Palacky University in Olomouc (LF_2012_018); infrastructural part of this project (Institute of Molecular and Translational Medicine) was supported from the Operational Programme Research and Development for Innovations (project CZ.1.05/2.1.00/01.0030).

Changes in proteomic profile of blood plasma of rats after low-level laser therapy

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Keywords: blood plasma, two- dimensional gel electrophoresis, proteomics, laser therapy, mass spectrometry.

The laser radiation absorbed by living cells induces the production of reactive oxygen species (ROS). Proteins are major targets for ROS due to of their abundance in biological systems and they are primarily responsible for the most functional processes in cells.

We monitored changes of acute phase proteins (APP) in blood plasma of rats after application of low-level laser therapy (LLLT). The rats were divided in two groups. One group was the control group without irradiation. Rats of second group were irradiated from dorsal side during 9 days by laser diode (wavelength 830 nm , power density 450 mW/cm2, dose 60 J/cm^2 per day). The animals were anesthetized and blood samples were isolated from heart. Blood was separated into individual fractions. Proteins from top fraction of blood (the plasma) were analysed using bottom up approach . Proteins from blood plasma were first separated by two dimensional gel electrophoresis. Gels from electrophoresis were analysed by program PDQuest and individual proteins were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Proteomic analysis of blood plasma showed changes in abundant proteins such as haptoglobin , hemopexin, α-1-antitrypsin and fibrinogen gamma, fetuin A, fetuin B. The increase of positive APP hemopexin (20-70 %) and alpha 1-antitrypsin (40-100 %) was found in irradiated rats in comparison with non-irradiated rats. The biggest increase was shown in case of haptoglobin (520-570 %). At the same time the decrease of the negative phase proteins was observed, namely fibrinogen gamma decreased by (20-40 %), fetuin A by (10-30 %) and fetuin B by (40-50 %). APP are potential variables for monitoring the changes induced by LLLT.

Acknowledgments: This work was supported by Agency of Slovak Ministry of education for the Structural Funds of the EU under project ITMS: 26220220143 and VEGA grant no. 1/1109/11.

Development and validation of targeted proteomic method for examination of oral cancer biomarkers

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Novel Aspects: proteomics oral cancer biomarker research

Oral squamous cell carcinoma (OSCC) accounts for about 90% of malignant oral lesions which is the 6th most common malignancy with increasing incidence and mortality rate especially in the younger generation. The 5-year survival rate is considerably lower than for cancers of colorectal, cervix and breast origin [1]. Hungarian population occupies the top places of statistics regarding OSCC incidence and mortality figures with 5-fold elevation of overall mortality rate in Hungary since the sixties.

Current diagnostic and screening tools for the OSCC are scalpel biopsy and histopathological evaluation. These techniques require more advanced technical training and skill to prevent false-positive and false-negative results. Delayed detection is likely to be a primary reason for the discovery of the high mortal rate and this supports the need for biomarkers to improve early detection.
The human saliva seems to be an alternative, non-invasive, low-cost source for oral cancer biomarkers and so far different types of genes, proteins, RNAs, miRNAs were examined as potential biomarkers [2, 3].

Using proteins already published as biomarkers our aim is to develop and validate an MRM (Multiple Reaction Monitoring) based method suitable for parallel examination of 14 cancer-related salivary proteins from one sample. Analysis of tumor-specific proteomics provides an opportunity for early diagnosis, and detection of OSCC and for a better understanding of behavior of this disease.

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Identification of protein antigens from the secretome of *Candida albicans* for fungal sepsis diagnostics

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Novel Aspects: *Candida albicans*, secretome, diagnostic biomarkers, immunoproteomics, mass spectrometry

Candida albicans is the most important fungal pathogen of nosocomial bloodstream infections. Especially among immunocompromised individuals and patients in intensive care units, *C. albicans* can cause superficial candidasis and even disseminated systemic candidemia. Noteworthy, it has been shown recently, that the late phases of bacterial sepsis are always associated with a significant re-infection with opportunistic pathogenic fungi, particularly *Candida albicans*. Morover, fungal infections have much higher morbidity and mortality compared with bacterial infection in sepsis. Until now, the diagnosis of Candida infection remains difficult and the antifungal therapy is still far from being efficient.

By applying an immunoproteomics approach we aimed to identify secreted, immunoreactive *Candida albicans* proteins which may be used as diagnostic markers. Firstly, we analysed the secretomes of *Candida albicans* yeast and hyphal cells by performing 2D-gel electrophoresis. After gel staining all visible protein spots were excised and analysed by MALDI-TOF-TOF-MS and ESI-TOF-MS/MS. For the detection of protein antigens, secreted proteins were blotted on PVDF-membranes and incubated with serum of candidemia patients and patients without any fungal infection. Subsequently, human IgG-binding antibodies were detected by a HRP conjugated secondary antibody for chemiluminescent detection.

From our 2D-maps, 46 secreted yeast and 114 secreted hyphal proteins were identified. They included not only classical secreted proteins, but also many cell wall-associated metabolic enzymes, whose secretion mechanism has not been elucidated yet. The hyphal secretome of *C. albicans* showed a higher number of proteases, e.g SAP4-SAP6, APE2-3, ECM14, whereas

in the yeast secretome more lipases and chitinases were found. In addition, we tested the serological response against *C. albicans* secreted yeast and hyphal proteins in sepsis patients with Candida infections. The IgG antibody pattern in serum of patients against *C. albicans* yeast and hyphal cells differed significantly. Furthermore, sepsis patients were distinguishable from human control groups without sepsis. However, the immunodominant mannoprotein MP65, enolase and β-glucanases were detected in all cases, although with different intensity. The detected immuodominant protein antigens of *Candida albicans* may present a core set to be used as diagnostic biomarkers for Candida infections.

Acknowledgements: This study was supported by funding from the Federal Ministry of Education and Research (BMBF) in the framework of the Center for Sepsis Control and Care (CSCC), Jena, Germany.

Multi-Component Complexes Releasing Glycosylphosphatidylinositol-Anchored and Other Cell Surface Proteins into Body Fluids as Source for Novel Diabesity Biomarkers

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Novel Aspects: Cell surface protein transfer, extracellular nucleotidases, glycosyl-phosphatidylinositol, exosomes, microvesicles, diabetes, obesity, serum biomarkers

The paracrine and endocrine transfer of biological information between cells of the same or distinct tissues by secretory proteins, such as cytokines, hormones and growth factors, for the control of proliferation, differentiation and metabolism in multicellular eukaryontes has been studied for decades in great detail. Recently the use of systematic proteome analysis nourished the hope for a complete description of the secretome and understanding of the physiology of the individual members as well as of their functional interactions.

However, due to the nature of the proteomics procedures subsets of transmembrane and glycosylphosphatidylinositol (GPI)-modified cell surface proteins which become released from the plasma membrane of a donor cell in response to certain nutritional or hormonal stimuli upon incorporation into phospholipid (mixed) micelles (e.g. folate-binding protein, sonic hedgehog), lipoproteins (e.g. CD59, wingless) or small vesicles (Gce1, CD73), such as exosomes and microvesicles (EMVs), routinely escape detection and elucidation of their assembly states [1].

In a first step to study the putative transfer of the GPI-anchored extracellular nucleotidase, CD73, from donor adipocytes to acceptor brain cells and vice versa and its physiological roles for the (dys)regulation of glucose and lipid metabolism in the normal and diabetic/obese state [2], a novel biosensor relying on surface acoustic waves technology has been introduced for the discrimination of GPI-modified CD73 assembled into phospholipid micelles as multimers, lipoprotein particles and EMVs or CD73 lacking the GPI modification. Importantly, biosensing of the culture medium from primary mouse adipocytes and astrocytes as well as of mouse adipose tissue and brain extracts revealed the presence of CD73 in the various multi-component complexes in differential quantitative and qualitative fashion depending on the stimulus exerted onto the cells (e.g. fatty acids, reactive oxygen species, cytokines) and diet administered to the mice (chow vs. high fat), respectively.

In future experiments the relationship between the differential release of CD73 and its putative appearance in various body fluids in normal and diabesity probands will be tested and the protein composition will be analyzed. Correlation would hint to the usefulness of cell surface proteins released into multi-component complexes as functional biomarkers.

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Functionalized bis-enol acetates as specific molecular probes for esterases

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Novel Aspects: We designed new esterase specific probes modeled after the natural product Caulerpenyne. These probes follow a genuinely new strategy for specific ABPP of lipolytic enzymes.

The sesquiterpene bis-enol acetate caulerpenyne (CYN) is found in many green algae of *Caulerpa spp.* in high amounts.[1] As a response to injury of the algae, CYN is enzymatically deacetylated into the 1,4-dialdehyde oxytoxin 2.[2] This intermediate reacts with nucleophilic moieties of cytoplasmic proteins to form a polymer wound plug.[3] Here we introduce molecular probes that use this biochemical mechanism for the selective identification of esterases. Like their natural prototype, these compounds are deacetylated by esterases to form highly reactive 1,4-dialdehydes. These intermediates immediately react with nucleophilic residues of the deacetylating enzymes, resulting in labeled esterases without formation of crosslinked proteins. This reaction is highly selective towards esterases even in the presence of other proteins like ovalbumin. Sequencing of labeled porcine liver esterase revealed, that eight of 35 lysines are covalently modified after incubation with the probe. Remarkably all labeled lysines surround the active side of the esterase supporting the need of an active transformation of the probe.

Acknowledgements: We thank the Dyomics GmbH, Jena, Germany for the generous supply of DY-490 and acknowledge the Volkswagen-Foundation for funding within the framework of a Lichtenberg Professorship.

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Relative quantification analysis of *O*-glycans using ESI-qTOF MS/MS

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Novel Aspects: mass spectrometry, *O*-glycans, 1-phenyl-3-methyl-5-pyrazolone, stable isotope labeling.

Glycosylation is one of the most common post-translational modifications of proteins. It has gained increasing interest due to its role in many biological processes, particularly cell adhesion and immunity as well as signal transduction. Glycosylation patterns and changes therein depend more or less specifically on cell type and cell status and are influenced by stress conditions. Qualitative and quantitative analysis of glycan structures attached to proteins (glycan fingerprinting) has thus gained increasing importance with respect to their potential as biomarkers. *O*-glycosylation, however, is underrepresented in these investigations up to now, probably due to the lack of enzymes catalyzing their release and due to the rather low ionization yield attained for small oligosaccharides.

The focus in this poster is to establish a sensitive and robust platform for the analysis of mucin-type *O*-linked glycan profiles. In this work an analytical procedure is established involving the release of *O*-glycans from (standard) glycoproteins by a chemical method together with stable-isotope coded labeling in a one-step reaction. The chosen method combines a condensation under alkaline conditions with a Michael addition using 1-phenyl-3-methyl-5-pyrazolidone (PMP) as labeling reagent (attaching two PMP molecules per one glycan). For the "heavy"-version of the label penta-deuterated PMP was synthesized, introducing *in toto* a mass difference of 10 Da which can easily be distinguished (even for highly charged oligosaccharides) using HRMS. The derivatisation of sugars improves the detection by ESI-MS and impacts retention with all types of stationary phases.

By using chip-nano-ESI in combination with HRMS (ESI-Qq-oaTOF) the method is aimed for the fast quantitative screening of *O*-glycan profiles. In addition, on-line HPLC-ESI-MS/MS was developed and established for qualitative and quantitative analysis of the derivatized *O*-glycans. Reversed Phase-HPLC offers a limited potential of separating isobaric oligosaccharide isomers, however, it is most beneficial as precleaning step for avoiding Na clusters.

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Proteomic workflow for the analysis of human serum low or medium abundance proteins

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Novel Aspects: human serum, mass spectrometry, ProteoMiner

Introduction: The analysis of human serum can provide essential information about the presence and level of secreted or circulating protein molecules that can assess the progression of certain pathologies. Still, a complete proteomic dataset is hampered by the very wide dynamic range of serum constituents; the improvement of current strategies for detecting low abundance proteins is an on-going process.

Materials and Methods: Human serum was fractionated based on the different interactions developed between its constituent protein molecules and the hexapeptide combinatorial peptide libraries of the ProteoMiner enrichment kit (Bio-Rad Laboratories). Modifying the standard serum preparation workflow by using 4 different elution buffers, we could obtain four fractions that were purified by precipitation. The resulting proteins were reduced, alkylated and digested (sequencing grade modified trypsin- Promega). The peptides were purified by SPE and separated using RPLC (Easy nLC II) coupled to the LTQ Orbitrap Velos PRO mass spectrometer (Thermo Scientific). A Top6 data dependent analysis was performed along a 90 min 2-35% acetonitril gradient. The raw data were deconvoluted for protein inference using Proteome Discoverer 1.4 software (Thermo Scientific) and Mascot search engine and the human non redundant SwissProt database.

Results and Discussions: The Principal Component Analysis performed in the Sieve 2.0 (Thermo Scientific) software revealed a good reproducibility of the technical replicates and also a statistical variance between the different groups (serum and 4 fractions). This was confirmed by the lists of identified proteins showing that the 4 different elution fractions contained on average (4 replicate groups) 547 unique protein groups that were not identified in the whole serum.

Conclusions: Using ProteoMiner enrichment kit we could demonstrate that the fractions eluted from the processed serum contained not only a decreased level of high abundance proteins (HSA, A2M, IGG, haptoglobulin, etc) but also provided the means to enrich medium abundance proteins (clusterin, selenoprotein P, ficolin-3, lumican, etc.) or identify new medium or low abundance proteins (lactotransferrin, angiogenin, von Willebrand factor, talin-1, girdin, etc.).

Acknowledgements: Ministry of Education and Research grant PN-II-PCCA-2011-3 no. 135, 153/2012 and CARDIOPRO Project ID: 143, ERDF co-financed investment in RTDI for Competitiveness.

Detergent Resistant Membranes proteomics changes under high fat stress

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Novel Aspects: hyperlipidemia, DRMs proteomics, heat shock proteins, caveolin-1, cavin-1

A high-lipid diet is one of the main risk factors in atherosclerosis and can induce changes in the composition of plasma membrane microdomains. In response, important functions such as vesicle trafficking, protein docking, signaling, and receptor recognition are significantly altered. In particular, interactions of heat-shock proteins (Hsps), acting as danger signals, with components of the membrane microdomains can influence signaling pathways and the inflammatory response of cells. Our study focused on the composition of detergent-resistant membrane (DRM) isolated from ApoE–/– mice fed a standard or high-fat diet with (At) and without (A) fluvastatin treatment versus appropriate controls (WT). Liquid chromatography mass spectrometric analysis, biochemical studies and immunoblotting were performed to investigate whether the structural components (such as caveolin and cavin) of the detergent-resistant microdomains were correlated with the expression and secretion of stress inducible Hsps (Hsp70 and Hsp90) and AKT phosphorylation in experimental atherosclerosis. ApoE-/– mice challenged with a high-fat diet developed extensive atherosclerotic plaques in the lesion-prone areas. DRM harvested from hyperlipidemic animals showed a modified biochemical composition with cholesterol, glycerolipids, caveolin-1, and phospho-AKT being up-regulated, whereas cavin-1 and dynamin were down-regulated. The mass spectrometry analysis demonstrated different amounts of identified proteins in the three experimental groups (WT, A and At), underlining the modified biochemical composition of the cell membrane. The data from mass spectrometry proteomic analysis of DRM also demonstrated the co-fractionation of Hsps with caveolin-1, the expression being positively correlated with their secretion into blood serum. Statin therapy significantly attenuated the processes induced by the development of atherosclerosis in ApoE–/– mice under a high-fat diet. Thus, high-lipid stress induces profound changes in DRM biochemistry and modifies the cellular response, supporting the systemic inflammatory onset of atherosclerosis.

Acknowledgements: This work was supported by POSDRU project no. [89/1.5/S/60746] and the Romanian Academy and Ministry of Education and Research grant CNCSIS-UEFISCSU [PN-II-PCCA-2011-3 nos. 135 and 153/2012]. The MS experiments were performed in Luxembourg Clinical Proteomics Department, Centre de Recherché Public de la Santé, funded by project [346 PN-II-ID-PCE, code no. 159] CNCSIS-UEFISCSU.

Combining proteomics and transcriptome sequencing to identify active plant-cell-wall-degrading enzymes in a leaf beetle

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Novel Aspects: Combining proteome and transcriptome sequencing analyses proved to be a powerful tool for the discovery of active PCWDEs in a non-model species. Our data represent the starting point of an in-depth functional and evolutionary characterization of PCWDE gene families in phytophagous beetles and their contribution to the adaptation of these highly successful herbivores to their host plants.

The primary plant cell wall is a complex mixture of polysaccharides and proteins encasing living plant cells. Among these polysaccharides, cellulose is the most abundant and useful biopolymer present on earth. These polysaccharides also represent a rich source of energy for organisms which have evolved the ability to degrade them. A growing body of evidence suggests that phytophagous beetles, mainly species from the superfamilies Chrysomeloidea and Curculionoidea, possess endogenous genes encoding complex and diverse families of so -called plant cell wall degrading enzymes (PCWDEs) [1-3]. The presence of these genes in phytophagous beetles may have been a key element in their success as herbivores [4-5]. Here, we combined a proteomics approach and transcriptome sequencing to identify PCWDEs present in larval gut contents of the mustard leaf beetle, *Phaedon cochleariae*.

Using a two-dimensional proteomics approach, we recovered 11 protein bands, isolated using activity assays targeting cellulose-, pectin-and xylan-degrading enzymes. After massspectrometry analyses, a total of 13 proteins putatively responsible for degrading plant cell wall polysaccharides were identified; these proteins belong to three glycoside hydrolase (GH) families: GH11 (xylanases), GH28 (polygalacturonases or pectinases), and GH45 (β-1,4-glucanases or cellulases). Additionally, highly stable and proteolysis-resistant host plant-derived proteins from various pathogenesis-related protein (PRs) families as well aspolygalacturonase-inhibiting proteins (PGIPs) were also identified from the gut contents proteome. In parallel, transcriptome sequencing revealed the presence of at least 19 putative PCWDE transcripts encoded by the *P. cochleariae* genome. All of these were specifically expressed in the insect gut rather than the rest of the body, and in adults as well as larvae. The discrepancy

observed in the number of putative PCWDEs between transcriptome and proteome analyses could be partially explained by differences in transcriptional level [6].

Acknowledgements: We thank Angelika Berg for maintenance of the insects. We thank Bianca Wurlitzer, Domenica Schnabelrauch and Yvonne Hupfer for technical assistance. Dieter Ruder and Martin Niebergall are acknowledged for providing IT resources for data analysis. Financial support was provided by the Max Planck Society.

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The sweet side of proteomics: *Nicotiana attenuata*'s floral nectar proteins

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Novel Aspects: Natural variation of identified FN proteins in the ecological model plant *N. attenuata* suggests that nectar chemistry may have a complex function in plant-pollinator-microbe interactions.

Floral nectar (FN) contains not only energy-rich compounds to attract pollinators, but also defense chemicals and several proteins [1-4]. However, proteomic analysis of FN is hampered by the lack of public available sequence information from nectar-producing plants. Here we used next-generation sequencing and advanced proteomics to profile FN proteins in the opportunistic outcrossing wild tobacco, *Nicotiana attenuata*. We constructed a transcriptome database of *N. attenuata* and characterized its proteome using LC-MS/MS. The FN proteins of *N. attenuata* contained nectarins, sugar-cleaving enzymes (glucosidase, galactosidase, and xylosidase), RNases, pathogen-related proteins, and lipid transfer proteins. Natural variation of FN proteins in eleven *N. attenuata* accessions revealed a negative relationship between the accumulation of two abundant proteins, nectarin1b and nectarin5. In addition, microarray analysis of nectary tissues revealed that protein accumulation in FN is not simply correlated with the accumulation of transcripts encoding FN proteins and identified a group of genes that were specifically expressed in the nectary [5].

Acknowledgements: This work was supported by European Research Council advanced grant ClockworkGreen (No. 293926) to ITB, the Global Research Lab program (2012055546) and the Leaping Research Program (20110016440) from the National Research Foundation of Korea, and the Max Planck Society.

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Short-term proteomic dynamics reveal metabolic factory for active extrafloral nectar secretion by *Acacia cornigera* ant-plants

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Novel Aspects: Our results demonstrate that the nectary represents a metabolically independent organ, and that most synthetic processes that are required for production of important nectar components occur in the nectary itself.

Despite the ecological and evolutionary importance of nectar, mechanisms controlling its synthesis and secretion remain largely unknown. It is widely believed that nectar is 'secreted phloem sap', but current research reveals a biochemical complexity that is unlikely to stem directly from the phloem [1-2]. We used the short daily peak in production of extrafloral nectar by *Acacia cornigera* to investigate metabolic and proteomic dynamics before, during and after 2 h of diurnal secretion. Neither hexoses nor dominating nectar proteins (nectarins) were detected in the phloem before or during nectar secretion, excluding the phloem as the direct source of major nectar components. Enzymes involved in the anabolism of sugars, amino acids, proteins, and nectarins, such as invertase, b–1,3–glucanase and thaumatin-like protein, accumulated in the nectary directly before secretion and diminished quantitatively after the daily secretion process. The corresponding genes were expressed almost exclusively in nectaries. By contrast, protein catabolic enzymes were mainly present and active after the secretion peak, and may function in termination of the secretion process. Thus the metabolic machinery for extrafloral nectar production is synthesized and active during secretion and degraded thereafter. Knowing the key enzymes involved and the spatio-temporal patterns in their expression will allow elucidation of mechanisms by which plants control nectar quality and quantity [3].

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Chemically mediated plankton interactions – molecular probes for investigating mechanisms of action of oxylipins

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Novel Aspects: novel probes for activity-based protein profiling (ABPP) are used to discover and identify target proteins of polyunsaturated aldehydes (PUAs)

Diatoms are unicellular algae of crucial importance as they belong to the main primary producers in aquatic ecosystems. Some of these algae release biologically active metabolites such as oxylipins that mediate inter- and intraspecific interactions. A major class of diatom-derived oxylipins are polyunsaturated aldehydes (PUAs), which are known to interfere with reproduction of copepods, the main predators of diatoms, and to inhibit growth of certain competing algae, bacteria, and fungi.[1,2]
Despite the well-researched biological effects of PUAs on planktonic organisms and communities their mechanisms of action are still poorly understood.[3] Here, a novel activity-based protein profiling (ABPP) probe that mimics the PUA 2,4-decadienal was developed. The probe enables specific binding to protein targets in living organism and their analysis based on LC/MS.
The probe was applied to the alga *Phaeodactylum tricornutum*, which was previously shown to undergo cell death after exposure to the PUA 2,4-decadienal.[4] In *in vitro* experiments *P. tricornutum* was incubated with the reactive probe, labeled protein targets were visualized by 2D difference gel electrophoresis, isolated and identified by LC/MS under data independent aquisition. Analysis of the function of labeled proteins will enable us to identify affected biochemical pathways and to gain deeper understanding of PUAs' mode of action.

Acknowledgements: We thank the Studienstiftung des deutschen Volkes and the Volkswagen Foundation for funding.

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Proteome analysis of *Schizophyllum commune* during black slate degradation

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Novel Aspects: The proteome analysis of the fungus *S. commune* reveals the complex molecular mechanism behind the black slates degradation.

Schizophyllum commune is a filamentous basidiomycete fungus, which has been in the focus of research for many decades. Because of its short life cycle it is a model organism for physiological and genetic studies. As a white-rot wood degrader, *S. commune* can decompose lignin and other complex organic molecules with its numerous enzyme excretions [1]. Some of these exoenzymes have wide substrate specificities and are so capable of degrading other substrates including organic matter from rocks such as low grade metamorphic black slates [2]. Excreted laccases may take part in this process and effect the carbon mobilization from black slates. Due to the fact that many other enzymes are released during wood decay, it is thought that they also influence rock degradation [3].

To determine all proteins involved in this biomolecular mechanism, a proteome analysis is being performed. For this, a monokaryon and dikaryon of *S. commune* was cultivated in liquid media with and without black slates to allow a direct comparison of excreted proteins using shotgun proteomics. First results identified many proteins known to have a significant involvement in wood decay. Their frequency and occurrence changes when considering the different strains and treatments. Enzymes of glucose-methanol-choline oxidoreductase have a contribution in lignin degradation and this family was, in contrast to the control sample, detected in black slate samples of both strains. Contrary to this, glycoside hydrolases, involved in hemi- and cellulose degradation, were more often found in the control sample. In the monokaryon a higher amount of laccases was detected in the black slate sample but no laccases were found in the dikaryon. The proteins involved in the degradation process seem to be a complex network in which the detailed role of some proteins for *S. commune* is still not clear. Thus, overexpressions of some genes are planned to gain an explicit insight into their function.

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