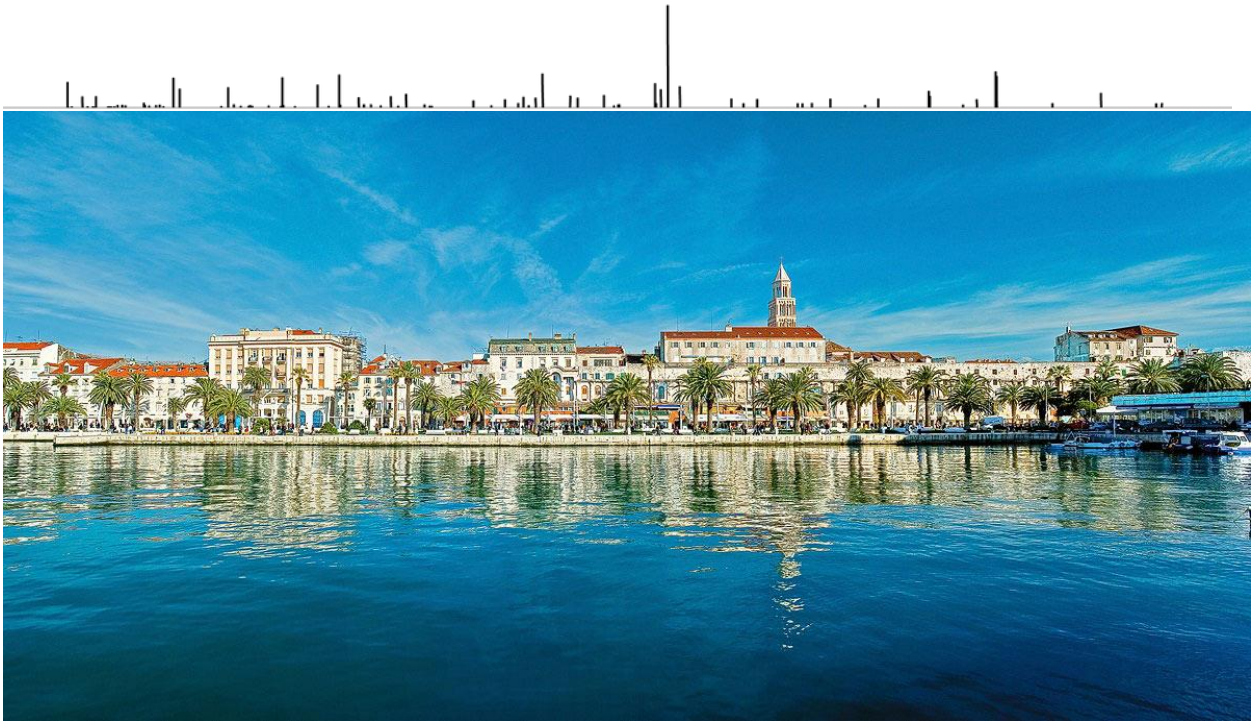
EuPA
EUROPEAN PROTEOMICS ASSOCIATION



EuPA School on Practical Proteomics

October 8 – 12th 2017
Split, Croatia

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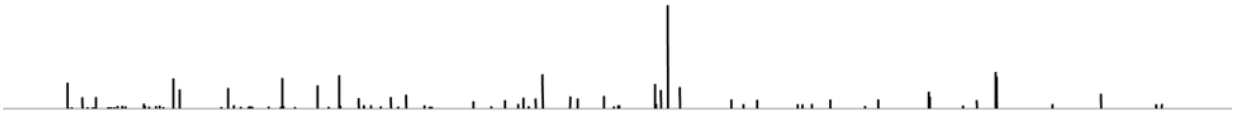


SUNDAY (October 8th)

16:00 - 17:00	Registration	
17:00 - 17:30	Opening Remarks by the Organizing Committee	
17:30 - 18:15	Plenary Lecture: Rolling Proteomics into the Clinical Practice	Andrea Urbani
18:15- 19:00	Plenary Lecture: Comprehensive Analysis of Human Proteomes	Jesper Velgaard Olsen
19:00 - 21:00	***Welcome mix***	

MONDAY (October 9th)

08:30 - 09:15	L1 Didactic Lecture: PTM Analysis and Interaction Proteomics	Michael Lund Nielsen
09:15 - 10:00	L2 Research Lecture: Systems-wide Analysis of SUMOylation in Mammalian Cells	Michael Lund Nielsen
10:00 - 10:30	***Coffee Break***	
10:30 - 11:15	L3 Didactic Lecture: Mass Spectrometry	Christian Kelstrup
11:15 - 12:00	L4 Didactic Lecture: Sample Preparation	Christian Kelstrup
12:00 - 13:00	***Lunch Break***	
13:00 - 18:00	Practical Session (Immunoprecipitation) Group A Group B	Klaus Herick, Christian Linke-Winnebeck (ChromoTek)
18:00 - 20:00	Poster Session	

TUESDAY (October 10th)

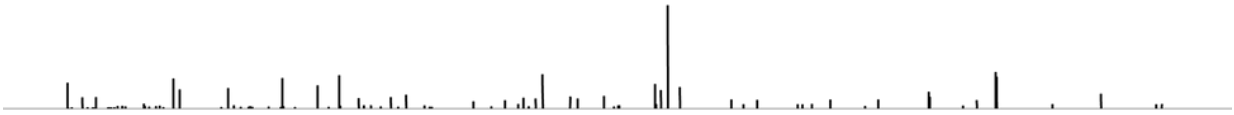
08:30 - 09:15	L5 Didactic Lecture: Glycomics	Jasna Peter-Katalinić
09:15 - 10:00	L6 Didactic Lecture: Glycoproteomics	Sergey Vakhrushev
10:00 - 10:30	***Coffee Break***	
10:30 - 11:15	L7 Didactic Lecture: Bacterial Proteomics and Metaproteomics	Boris Maček
11:15 - 12:00	L8 Didactic Lecture: Plant Proteomics	Karl Mechtler
12:00 - 13:00	***Lunch Break***	
13:00 - 18:00	Practical Session (Sample Preparation) Group A Group B	Garwin Pichler, Fabian Hosp (PreOmics)
18:00 - 23:00	***City Tour and Dinner*** ***(Bus transportation to the city center will be provided)***	

WEDNESDAY (October 11th)

08:30 - 09:15	L9 Didactic Lecture: Computational Proteomics	Jürgen Cox
09:15 - 10:00	L10 Research Lecture: Computational biology for large-scale omics data analysis	Jürgen Cox
10:00 - 10:30	***Coffee Break***	
10:30 - 11:15	L11 Didactic Lecture: Statistical Analysis of MS Data	Lennart Martens
11:15 - 12:00	L12 Didactic Lecture: Protein Interactions: Databases and Representation	Pablo Porras Millan
12:00 - 13:00	***Lunch Break***	
13:00 - 14:00	Participant Talks (3 short talks)	
14:00 - 17:00	Practical Session Group A (Data Analysis)	Jürgen Cox, Garwin Pichler, Fabian Hosp, Pablo Porras Millan
17:00 - 20:00	Practical Session Group B (Data Analysis)	Jürgen Cox, Garwin Pichler, Fabian Hosp, Pablo Porras Millan

THURSDAY (October 12th)

08:30 - 09:15	L13 Research Lecture: DNA Scissors to Simplify Glycoproteomics	Sergey Vakhrushev
09:15 - 10:00	L14 Research Lecture: Four Seasons of Proteomics Data Re-use, and How to Thrive in Each	Lennart Martens
10:00 - 10:30	***Coffee Break***	
10:30 - 11:15	L15 Didactic Lecture: Approaches to Quantify the Proteome	Kathryn Lilley
11:15 - 12:00	L16 Research Lecture: What Controls the Three Dimensional Proteome?	Kathryn Lilley
12:00 - 13:00	***Lunch Break***	
13:00 - 14:00	Participant Talks (3 short talks)	
14:00 - 19:00	Practical Session Group A (HPLC) Group B (HPLC)	Christopher Pynn (Thermo)
19:00 - 21:00	***Farewell Party***	



Andrea Urbani

University of Rome, Department of Internal Medicine, Rome, Italy

Biography

Andrea Urbani is the Head of the Analytical Biochemistry and Proteomics facility in the Center of Study on Aging at the University Foundation "G. D'Annunzio" of Chieti-Pescara where he investigates Proteomics and Metabonomics by mass spectrometry. He is also Head of Proteomics and Metabonomics Laboratory at the IRCCS Fondazione Santa Lucia – Rome which supports the research of CNR Institute for Neurobiology & Molecular Medicine as well as the European Brain Research Institute – Rita Levi Monatalcini Foundation. During his PhD training he worked in the Merck, Sharp and Dohme laboratories of Pomenzia on the functional and structural characterization of the NS3 serine protease from the Hepatitis C Virus. Upon gaining his PhD title he moved to Heidelberg at the European Molecular Biology Laboratory (EMBL) in the Structural Biology Program where he pursued structural and functional investigation on integral membrane protein complexes. Following this experience, he moved as a visiting scientist to the German Cancer Research Centre in the Analytical Protein Chemistry unit where he dedicated most of the investigations in the proteomics area. In his scientific career he has been involved in protein characterisation by a number of different techniques. Andrea Urbani is the president of the Executive Committee of the European Proteomics Association (EuPa). Since his PhD in 1998, he has authored over 100 papers in the field of proteomics and metabonomics investigations.

Jesper Velgaard Olsen

NNF Center for Protein Research / University of Copenhagen, Health and Medical Sciences, Copenhagen, Denmark

Biography

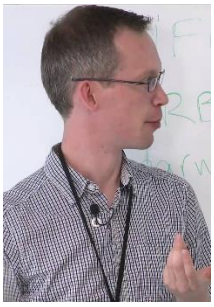
Jesper V. Olsen is the vice director for Novo Nordisk Foundation Center for Protein Research at the Faculty of Health Sciences at University of Copenhagen. He studied analytical chemistry at the University of Southern Denmark in Odense, and obtained his Ph.D. in biochemistry and molecular biology in the laboratory of Matthias Mann. During his Ph.D. he was involved in developing high-resolution mass spectrometry-based proteomics. He spent four years as a post-doctoral fellow at the Max Planck Institute for Biochemistry in Munich, where he developed a quantitative phosphoproteomics technology that was applied to global time-resolved analyses of cell signaling pathways in human cells. In 2009, Jesper moved back to Denmark to head a group at the newly established Novo Nordisk Foundation Center for Protein Research (CPR) at University of Copenhagen. In 2012, he was promoted to vice director of CPR and in 2014 to full professor. Jesper has received a number of research awards including the Max Planck Institute for Biochemistry Junior Research Award and HUPO Young Investigator Award in Proteomic Sciences. He is one of the most cited young scientists in the field of quantitative proteomics and mass spectrometry.

Michael Lund Nielsen

NNF Center for Protein Research / University of Copenhagen, Health and Medical Sciences, Copenhagen, Denmark

Biography

Michael Lund Nielsen is a Professor at the Faculty of Health and Medical Sciences at the University of Copenhagen and a group leader in Proteomics Technology Development and Application at the Novo Nordisk Foundation Centre for Protein Research. He obtained his master's degree in chemistry and applied mathematics from the University of Southern Denmark. After his Ph.D. at the University of Uppsala in 2007, Michael Lund Nielsen worked as a postdoctoral fellow at the department of Proteomics and Signal Transduction at the Max-Planck-Institute for Biochemistry in Martinsried. In 2009, Michael moved back to Denmark to lead a group of Proteomics Technology Development and Application at the newly established Novo Nordisk Foundation Center for Protein Research at the University of Copenhagen. Since 2013, he is a Professor at the Faculty of Health and Medical Sciences at the University of Copenhagen, Denmark. The Nielsen group is heavily involved in mass spectrometry technology development using high resolution mass spectrometry, with a strong interest in developing quantitative proteomics methods for unbiased analysis of novel and unexpected PTMs and mutations. Michael Lund Nielsen is the author of numerous peer-reviewed publications in the field of PTM analysis and quantitative proteomics.



Christian D. Kelstrup

NNF Center for Protein Research / University of Copenhagen, Health and Medical Sciences, Copenhagen, Denmark

Biography

Christian Kelstrup is Associate Professor at the Novo Nordisk Foundation Center for Protein Research at the Faculty of Health Sciences at University of Copenhagen, where he investigates proteomics with a specific focus on peptide sequencing and big MS data analysis. He received his master degree in biotechnology from the Technical University of Denmark, after which he worked as a research assistant in the Biomarker Research Center of the Bio-Rad Laboratories. Dr. Kelstrup was trained as a Ph.D. student and postdoctoral researcher in laboratories of Prof. Matthias Mann and Pof. Jesper Velgaard Olsen at the Max-Planck-Institute for Biochemistry in Martinsried, Germany, and University of Copenhagen in Denmark. Since 2016, he is leading his own group at the Faculty of Health and Medical Sciences at the University of Copenhagen, Denmark. Christian Kelstrup coauthored over 20 highly cited in the field of peptidomics and mass spectrometry method development with a strong interest in targeted, data-independent and data-dependent, as well as hybrid approaches.

Sergey Vakhrushev

Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark

Biography

Sergey Vakhrushev is Associate Professor at the Department of Cellular and Molecular Medicine at the University of Copenhagen, Denmark. He received his Ph.D. from the University of Muenster in Germany in the group of Prof. Dr. Jasna Peter-Katalinić and Prof. Dr. Harald Fuchs, where he developed mass spectrometry approaches and bioinformatics for glycomics. Sergey Vakhrushev was trained as a postdoctoral researcher at the at the Institute for Medical Physics and Biophysics at the University of Muenster and at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany. In 2010, he moved to Copenhagen and worked as a research assistant professor in the group of Prof. Henrik Clausen at the Copenhagen Center for Glycomics, University of Copenhagen, where he pursued research in the field of Glyoproteomics and Glyobiology. Since 2012, he is an Associate Professor at the University of Copenhagen.

Boris Maček

University of Tuebingen, Proteome Center Tuebingen, Tuebingen, Germany

Biography

Boris Maček is Chair of Quantitative Proteomics and Director of Proteome Center Tuebingen at the University of Tuebingen in Germany. He graduated from Molecular Biology Program of the University of Zagreb, Croatia in 1999 and joined the group of Jasna Peter-Katalinić at the University of Muenster, Germany. With Prof. Katalinić he characterized the composition and localization of a novel type of protein modification (O-fucosylation) by mass spectrometry and performed extensive biochemical studies to propose existence of a new type of the modifying enzyme, O-fucosyltransferase. After obtaining Ph.D. in Biology at the University of Muenster he joined the group of Matthias Mann at the University of Southern Denmark, Odense, where he was a member of the team that performed the first time-resolved phosphoproteomics study of the receptor tyrosine kinase (EGFR) signaling. In 2005, he moved with the Mann lab from Odense to the Max Planck Institute for Biochemistry in Martinsried, Germany, where he pursued research on Ser/Thr/Tyr phosphoproteomes of model bacteria, *E. coli* and *B. subtilis*, and published the first comprehensive Ser/thr/Tyr phosphorylation maps of these organisms. Since 2008, he is leading his own group at the University of Tuebingen, Germany and in 2014 he was promoted to full professorship.

Karl Mechtler

Research Institute of Molecular Pathology (IMP), Protein Chemistry Faculty, Vienna, Austria

Biography

Karl Mechtler is the Head of Protein Chemistry Facility at the Research Institute of Molecular Pathology in Vienna. His main area of research is mass spectrometric analysis of peptides and proteins with focus on posttranslational modifications. After his work at Hoechst AG, he worked in the group of Prof. Ernst Wagner and Prof. Gotthold Schaffner at the Research Institute of Molecular Pathology in Vienna, where he pursued research on peptide synthesis and bio-chromatography. In 1998, Karl Mechtler was a visiting scientist in the group of Mathias Mann. Since 1999, he is leading his own group for proteomics, peptide synthesis and protein purification at the Research Institute of Molecular Pathology in Vienna, Austria. His lab is involved in developing new methods to increase the sensitivity, accuracy and precision of protein identification/quantification and detection of PTMs. Karl has received the Outstanding Scientist Technology Award from the Association of Biomolecular Resource Facilities (ABRF) in 2010. He is a member of the Executive Committee of the European Proteomics Association (EuPa) and President of the Austrian Proteomics Society.

Jürgen Cox

Max-Planck-Institute for Biochemistry, Computational Systems Biochemistry, Martinsried, Germany

Biography

Jürgen Cox heads the Computational Systems Biochemistry group at the Max-Planck-Institute for Biochemistry in Matrinsried. He obtained his master's degree from the University of Aachen (RWTH). He obtained Ph.D. degree from the Massachusetts Institute of Technology (MIT) in 2001, working on computational modelling in statistical and particle field theory. His work involved optimization of quantum mechanical calculations, which for the first time allowed the simulation of classes of quantum mechanical spin models relevant for the physics of quark gluon plasmas. After his time at the MIT, Jürgen Cox decided to switch fields to bioinformatics, working both as a post-doctoral fellow in structural bioinformatics at the Technical University of Munich and at a bioinformatics company, GeneData. In 2006, he joined the department of Proteomics and Signal Transduction at the Max-Planck-Institute of Biochemistry, where he is currently a tenured group leader. At the Max-Planck-Institute for Biochemistry he performed ground-breaking research in computational proteomics, which significantly impacted the entire field. Specifically, he developed MaxQuant, an innovative and comprehensive set of algorithms that enable highly precise analysis of proteomics data in an automated and statistically rigorous way. Since 2013, he is an Honorary Professor of Proteomics at the University of Copenhagen, Denmark. Dr. Cox is the author of numerous peer-reviewed publications in the field of proteomics.

Lennart Martens

Ghent University, Systems Biology, Ghent, Belgium

Biography

Lennart Martens is Professor of Systems Biology in the Department of Biochemistry at the Faculty of Medicine and Health Sciences at Ghent University, and Group Leader of the Computational Omics and Systems Biology group in the Department of Medical Protein Research at the VIB Research Institute. He has been working in proteomics bioinformatics since his Master's degree, which focused on computational interpretation of peptide mass spectra. After that worked as a software developer and framework architect for a software company, before returning to Ghent University to pursue a Ph.D. focused on proteomics and proteomics informatics. During this time, he worked on development of high-throughput peptide centric proteomics techniques and on bioinformatics tools to support these new approaches. In 2003 he started the PRIDE proteomics database at the EBI as a Marie Curie fellow of the European Commission. After obtaining his Ph.D. in Sciences: Biotechnology from Ghent University, he rejoined the PRIDE group at EBI, which he coordinated for several years before moving back to Ghent University to take up his current position. Dr. Martens serves on the Board of the Belgian Proteomics Association, and has been elected to the HUPO Council in 2016, and to the HUPO Executive Committee in 2017. He authored on more than 200 research papers and on two textbooks, and serves on Editorial Boards of several journals.

Pablo Porras Millan

EMBL-EBI – The European Bioinformatics Institute, Scientific Database Curator, Cambridge, United Kingdom

Biography

Pablo Porras Millan is Scientific Database Curator at the European Molecular Biology Laboratory located at the European Bioinformatics Institute (EMBL-EBI) in Cambridge. Pablo received his Ph.D. in 2006 at the University of Córdoba, Spain, performing research about transmembrane protein translocation and redox homeostasis. After that, he moved to Berlin to work in the Neuroproteomics group of the Max Delbrueck Center, getting involved in projects dealing with interactomics, neurodegenerative diseases and the ubiquitin-proteasome system. During this postdoc, he faced the problem of how to represent and analyze molecular interactions data. This experience proved to be of great value once he joined the EBI to work as a scientific curator in the molecular interactions database IntAct in 2011.

Jasna Peter-Katalinić

University of Rijeka, Department of Biotechnology, Rijeka, Croatia

Biography

Jasna Peter-Katalinić is professor emeritus of biophysics at the University of Muenster, Germany, and professor of Biotechnology at the University of Rijeka, Croatia. Born and educated in Zagreb, Croatia, she obtained Ph.D. from University of Zuerich, Switzerland and performed postdoctoral research at the Texas A&M University. Upon return to Germany, she worked as a scientist at the University of Bonn, where she pioneered the introduction of mass spectrometry to structural glycobiology/glycomics. In 1996 Jasna moved to the University of Muenster, where she was the founder of the laboratory „Biomedical Analysis“ at the Medical School and its director until 2008. She was the board member of the German Society for Mass Spectrometry from 2000-2008 and a member of the HUPO Human Glycoproteome Initiative (HGPI) from 2004-2008. In 2002 she received the 1st Life Science Award from the German Society of Mass Spectrometry (DGMS). She was visiting professor at Virginia Commonwealth University, Richmond, Va., Medical College of Georgia, Augusta, Ga., USA, and ETH Zuerich, Switzerland. Since 2001, she is the chair organizer of the ongoing, presently annual, series of “Summer Courses on Mass Spectrometry in Biotechnology and Medicine” in Dubrovnik, Croatia. She is the founding member and the first president of the Croatian Proteomics Society (CroProt). Her current interests are integrated instrumental platforms for high throughput analytics and bioinformatics of glycoconjugates.

Kathryn Lilley

University of Cambridge, Cambridge Centre for Proteomics, Cambridge, United Kingdom

Biography

Kathryn Lilley is a Professor in the Department of Biochemistry and the Cambridge Systems Biology Centre. She is also Director of the Cambridge Centre for Proteomics, and a Director of Studies at Jesus College. Kathryn received her Ph.D. in Biochemistry from the University of Sheffield in 1990. She continued her scientific career as a laboratory manager for 11 years at the University of Leicester where she ran the Protein and Nucleic Acid Laboratory core facility. In November 2000, she established the Cambridge Center for Proteomics (CCP) at the University of Cambridge, which she is currently directing. Her laboratory is developing technologies which enable measurement of proteome dynamics in a high throughput manner, resolved in space and time during critical cellular processes such as signaling and differentiation. She was appointed to a Professorship in Cellular Dynamics in 2012 and more recently has been awarded a Welcome Trust Senior Investigator Award.

P1 Revealing the proteomic biomarkers of stress response in Baikal endemic amphipods

Daria Bedulina

Irkutsk State University, Irkutsk, Russia

Eco-proteomics is the novel and emerging field of proteomics and eco-physiological researches, which helps to reveal new biomarkers of the negative impact to ecosystems. Applying proteomic approaches to nonmodel organisms is always a challenge, which faces with difficulties in protein identification. However, this approach can significantly expand our knowledge about the diversity and evolution of cellular processes, especially when applied to inhabitants of ancient ecosystems. Lake Baikal is one of the unique ancient ecosystem, which is one of the oldest (25-30 mln.y.o.) and deepest (1642 m) lake in the World with the highest level of biodiversity (up to 3000 species of animals) and endemism of the fauna. Among many Baikal endemics, amphipods (Amphipoda, Crustacea) constitute the most diverse taxonomic group, comprising 354 described species and sub-species (Takhteev et al., 2015). Current ecological crisis and the climate change are the substantial threat for this unique ecosystem in the current century. Thus, developing of the monitoring bioassays for this lake if of the great importance. In this pursuit, our work herein attempts to understand the molecular responses of Baikal endemic amphipod Eulimnogammarus cyaneus to thermal stress. We used the method of LC-MS and a transcriptome assembly of the respective species for protein identification. This proteogenomic approach allowed us to identify key proteins of stress response in this species and to describe for a first time a sexual proteomic dimorphism in Baikal amphipods. All identified thermal stress-responsive proteins could be assigned to several main groups according to their functions: proteins of the cytoskeleton, proteins of energy metabolism, molecular chaperones, ribosomal proteins, vitellogenins and antioxidant enzymes. The identified proteins may be useful as candidates for biomarker studies on the thermal stress condition of the studied amphipod species.

P2 Deciphering lysosome associated diseases by quantitative ubiquitinome analysis

Anshu Bhattacharya

Buchmann Institute for Molecular Life Sciences, Frankfurt, Germany

Lysosomes are acidic organelles commonly referred as stomach of the cell, although, recent evidences suggest that lysosomes are not merely degradative compartments but communicate with their environment playing important roles in secretion, plasma membrane repair, receptor recycling and antigen presentation. Lysosomal dysfunction is attributed to the pathogenic mechanism of several neurological disorders like hereditary spastic paraplegias, neurodegenerative neuronal ceroid lipofuscinoses and is the major cause of autosomal recessive hereditary disorders called Lysosomal Storage Diseases. Although some conditions are triggered by monogenic mutations, the underlying molecular and cellular mechanisms are not resolved completely. In this study we will try to see the altered ubiquitin regulatory networks on lysosome, caused by genetic mutations identified in disease conditions, via employing unbiased bottom up proteomics of lysosomes with a focus on the ubiquitinome. The ubiquitination status of the lysosomal and associated proteins is important as in some cases this post translational modification is crucial,

e.g. impaired ubiquitination of Rab7 by mutated Parkin can contribute to PD progression. Ubiquitination is also important for lysosomal membrane protein recycling and degradation of damaged lysosome as shown with the example of Ypq1 and p97. Taken altogether it is suggestive that the lysosomal ubiquitinome can serve as an axiom of pathology for several incidents. To understand the global picture of altered ubiquitinated status, we will employ proteomics. Wild type and cell lines from mice, bearing defect in lysosome function, will be used to enrich lysosome first and then differentially ubiquitined proteins will be analyzed quantitatively using SILAC coupled di*GLY* proteomics.

P3 Silver nanoparticles affect germination and induce oxidative stress in tobacco

Renata Biba

Faculty of Science, University of Zagreb, Zagreb, Croatia

Silver nanoparticles (AgNPs) are among the most widely used nanomaterials due to their excellent antimicrobial and antifungal properties. They can be found in various commercial products including textiles, paints and cosmetics. Increase in the production of AgNPs brings out concerns about their impact on the environment. Since plants have an important role in bioaccumulation of many environmentally released substances, they are likely to be influenced by AgNPs. In this study we have investigated the effects of three differently coated AgNPs [citrate, polyvinylpyrrolidone (PVP) and cetyltrimethylammonium bromide (CTAB)] and AgNO3 on germination and oxidative stress response of tobacco seedlings (Nicotiana tabacum L.). Five concentrations (25, 50, 75, 100 and 150 μM) of AgNPs and AgNO3, and two concentrations (10 and 25 μM) of surface coatings were tested. To evaluate the degree of oxidative damage, the content of malondialdehyde and protein carbonyls as well as the activity of antioxidant enzymes (pyrogallol peroxidase, ascorbate peroxidase, catalase and superoxide dismutase) was spectrophotometrically measured. Silver uptake in plant tissue was determined with inductively coupled plasma mass spectrometry (ICP-MS). The results showed that positively charged AgNPs-CTAB and CTAB coating have a negative effect on all the parameters of seed germination, while AgNPs-citrate and AgNPs-PVP as well as AgNO3 caused only a slight reduction of fresh weight and root length when applied in higher concentrations. Citrate and PVP surface coatings didn't show any significant impact on seed germination. Protein carbonyl content was increased after treatments with AgNPs-PVP, AgNO3 and CTAB surface coating, while AgNPs-CTAB exhibited the opposite effect. Lipid peroxidation was detected after treatments with AgNPs-PVP and AgNPs-CTAB. All of the investigated antioxidant enzymes showed changes in activity implying the existence of oxidative stress. Silver uptake was the highest in AgNPs-CTAB treatment thus demonstrating that phytotoxicity of AgNPs directly correlates with their coating and surface charge.

P4 In vitro assessment of cytotoxicity and anti-inflammatory activities of Romanian mud extracts

Elena Codrici

Victro Babes National Institute of Pathology, Bucharest, Romania

The use of mud extract contributes to a long term stability of therapeutic effects, thus avoiding common inconveniences of conventional drugs, like installation of therapeutic resistance and adverse effects. Cytotoxicity testing was performed in vitro using ATCC-CRL-9855 cell cultures and MTS (CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega). The anti-inflammatory action was evaluated by cytokine measurements using Milliplex MAP Human Cytokine/Chemokine Magnetic Bead Panel kit and analyzed using Luminex 200 system (Luminex Corp., TX, USA). We focused to establish if these mud extracts have cytotoxic effects and to what extent. The extracts were provided by Pell-Amar Cosmetics as spray-dried powders. For this purpose, we used different concentrations - ranging 3 to 75 mM, considering an "average" MW of 90 for extracts, at different cell densities (5000/10000 cells/well) and incubation times (48/72h). For 10000 cells incubated for 72 hours – IC50 were 247 mM for sample 1, 386 mM for sample 3, 410 mM for sample 5 and 373 mM for sample 7. For 5000 cells at 72 hours – IC50 were 440 mM for both samples 3 and 5. IC50 could not be calculated for 48 hrs exposure, although a dose-effect relation could be observed. Our results indicated the relatively low-cytotoxic effects of the mud extract analyzed. The results showed that cells treated only with mud extracts and unstimulated have not secreted pro-inflammatory cytokines. After that we treated cells with LPS and mud extracts in different concentration and were demonstrated that mud extracts modulate cytokine release, generating profiles that are characteristic to anti-inflammatory activities, respectively decrease of pro-inflammatory cytokines release depending on the concentration (IL-6, TNFa, MIP1a, IL-1a; $p<0.05$). Using a combination of in vitro assays, mud extracts could be classified and ranked for their cytotoxiciy and specific activity, providing an effective screening system for the discovery of potential therapeutic compounds.

Acknowledgment: Partially supported by the COP A 1.2.3., ID: P_40_197/2016, PNII 265/2014.

P5 Identification of LRRK2-associated functional protein networks at the vesicular membrane

Marita Eckert

German Center for Neurodegenerative Diseases (DZNE), Tuebingen, Germany

Leucine-rich repeat kinase 2 (LRRK2) is a multi-domain 286-kDa protein, which is linked to late-onset autosomal dominant Parkinson´s disease (PD). LRRK2 has seven domains, amongst others a kinase domain and a GTPase domain. Their functions can be altered by various mutations. The most common PD-associated mutation is G2019S, which activates the kinase two- to threefold. Only a few interactions and functions of LRRK2 are well defined so far. It is known that the monomer of LRRK2 localizes in the cytosol and the active dimer at membranes. Moreover, it interacts with many signaling pathways, such as ER and Golgi apparatus dynamics, endosomal and vesicle trafficking. Since LRRK2 is involved in many vesicular membrane related systems, the networks and their functions located at

the membrane are of particular interest in this work. Furthermore, the investigation of mutations may lead to a better understanding of LRRK2 dysfunction and interactions, which could be used as targets for therapeutic agents and potential pharmacologic markers. To detect these protein-interaction networks, especially at the membrane, various methods are applied on cellular models such as proximity labeling techniques namely BioID and in vivo crosslinking with cell fractionation.

P6 Ethylenediamine-disuccinate ([S,S]-EDDS) from Amycolatopsis japonicum: Production of a biodegradable EDTA analogon

Simone Edenhart

University of Tuebingen, Tuebingen, Germany

Chelating agents play a crucial role in the supply of microorganisms with trace elements. In addition to that they are widely used in paper, textile, and laundry industry and find application as a cosmetic, food or medical additive. One of the most abundantly used chelating agents is ethylenediamine-tetraacetate (EDTA). Due to its poor degradability, EDTA has become an environmental hazard. [S,S]-ethylene diamine-disuccinic acid (EDDS) is a chelating agent produced by the gram-positive soil bacterium Amycolatopsis japonicum. EDDS is a well degradable isomer of EDTA and has similar chelating properties. Therefore, a chemical process to produce [S,S]-EDDS has already been established. Nevertheless we are interested in developing a biotechnological way to economically produce [S,S]-EDDS as an alternative for chemically synthesized [S,S]-EDDS. We identified the putative [S,S]-EDDS biosynthesis genes in *A. japonicu*m and found out that the biosynthesis is regulated by a global zinc regulator (Zur) [1]. Already 2 µM zinc, a concentration occurring ubiquitously in standard media, inhibits the synthesis of [S,S]-EDDS. Therefore a biotechnological large scale production of [S,S]-EDDS was not possible until now. We developed a process to synthesize [S,S]-EDDS biotechnologically using an *A. japonicum* zur mutant, that produces [S,S]-EDDS in the presence of zinc and in rather cheap, complex media. By deleting the zur gene we created a mutant that synthesized EDDS even in the presence of high zinc concentrations.

[1] M. Spohn, W. Wohlleben, E. Stegmann (2016). Elucidation of the zinc-dependent regulation in Amycolatopsis japonicum enabled the identification of the ethylenediamine-disuccinate ([S,S]-EDDS) genes. Environ. Microbiol. 18:1249-1263

P7 Localization-specific interaction study reveals ESCRT-II-cargo interaction at the plasma membrane

Florian Fäßler

University of Tuebingen, Center of Plant Molecular Biology, Tuebingen, Germany

The plasma membrane (PM) forms the interaction surface between a cell and its environment. The proteins in the PM, namely translocators and receptors, allow for the transport of nutrients and information. Strict regulation of these proteins is necessary to avoid over-accumulation of micronutrients to toxic levels and to terminate signalling processes. One regulatory mechanism controlling PM proteins is poly-ubiquitination to initialize a sorting process, which ultimately leads into the degradative lumen of lytic compartments. The endosomal sorting complexes required for transport (ESCRTs) are integral components of this process. In yeasts and mammals ESCRT-0 and ESCRT-I mediate recognition and accumulation of ubiquitinated membrane proteins, while ESCRT-III induces formation of intra-luminal vesicles at endosomal membranes. ESCRT-II, however, is suggested to link these two basic functionalities by physically bridging ESCRT-III to ESCRT-I. It thereby allows for the sorting of ubiquitinated proteins to intra-luminal vesicles during the biogenesis of multivesicular endosomes, which ultimately results in transport into the lytic compartment. We now show that all three endogenous plant ESCRT-II subunits are not only present at endosomal structures, but mainly localize to the PM. Combining FRET-FLIM and a novel nanobody-based technique for in vivo precipitation (iVIP) of proteins, we analysed ESCRT complex assembly and composition in living plant cells. Furthermore, we demonstrate that ESCRT-II binds poly-ubiquitinated proteins, which are destined for endocytosis and vacuolar degradation. Together our results suggest that ESCRT-II can assemble in the cytosol according to a 1(VPS36):1(VPS22):2(VPS25) stoichiometry, is recruited en-bloc to the PM, where it binds ubiquitinated cargo and possibly establishes a link to the endocytic machinery.

P8 Evolutionary and spatial comparison of the brain N-glycomes

Ivan Gudelj

Genos Ltd., Zagreb, Croatia

Protein N-glycosylation is crucial for the development and function of the nervous system. Even though brain N-glycans were analyzed in several studies, these glycans were derived from a particular protein and murine brain. In this study, a spatial profile of the brain glycome was constructed by investigating N-glycan structures of four functionally distinct brain regions: the dorsal frontal cortex, the hippocampus, the striatum and the cerebellum. Moreover, in order to obtain an evolutionary perspective of the corresponding brain regions, a cross-species comparison was made between the human, the chimpanzee, the macaque and the rat. Two analytical approaches were used for glycosylation profiling of brain samples: ultra-performance liquid chromatography and matrix-assisted laser desorption/ionization - time of flight mass spectrometry. Significant difference was noticed between the regions and species in the analyzed N-glycomes. The difference between species was higher as they were evolutionary more distinct. More precisely, the difference between the primates and rats was the highest while between the chimpanzees and humans was the smallest. The cerebellar N-glycome was the most distinctive of all the analyzed brain regions. There was a decrease in the abundance of high-mannose and hybrid type N-glycans and an increase in the abundance of complex type N-glycans for each species between the rat and human as well as the regions were evolutionary younger.

P9 Mass spectrometry-based characterization of the aggregome in cutaneous malignant melanoma

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Tumor cells harbor many mutations in their genome including driver mutations and passenger mutations with a presumably unimportant role. It is however well characterized that many of these mutations reside in genes that are still being expressed as proteins. As a consequence, a substantial part of these proteins are predicted to be present in the tumor cell as misfolded and thereby aggregation-prone species that put cellular proteostasis under tremendous pressure. Here we want use mass spectrometry profiling of insoluble protein fractions to identify aggregated proteins (the 'aggregome') and integrate this with gene expression and genotype information from RNA-seq data in order to investigate the proteostatic imbalance in melanoma cells.

P10 Comprehensive proteomic characterisation of ontogenic changes in haematopoietic stem and progenitor cells

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Haematopoietic stem and progenitor cells (HSPCs) in the foetus and adult possess distinct molecular landscapes that regulate cell fate and change their susceptibility to initiation and progression of haematopoietic malignancies. The proteomic programs that govern these differences remain unknown. In this study, we have utilized mass spectrometry-based quantitative proteomics to comprehensively describe and compare the proteome of foetal and adult HSPCs. We found that the proteome of foetal HSPCs is of relatively low complexity, characterized by proteins involved in cell cycle and cell proliferation, while their adult counterparts are defined by a larger set of proteins that are involved in more diverse cellular processes. These adult characteristics include an arsenal of proteins important for viral and bacterial defence, as well as protection against ROS-induced protein oxidation. Our further analyses of Type I interferon signalling shows that foetal HSPCs are sensitive to Interferon alpha (FNa), which impairs their repopulation capacity, whereas stimulation with IFNa to the pregnant mother enhances the production of early progenitors from foetal HSCs. Our results provide new and important insights into the molecular landscape of foetal and adult haematopoiesis that advance our understanding of normal and malignant haematopoiesis during foetal and adult life.

P11 Interaction of human dipeptidyl peptidase III and Keap1

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Dipeptidyl peptidase III (DPP III) is a metallo-exopeptidase suspected to be involved in various physiological processes: protein catabolism, blood pressure regulation, pain modulation, inflammation, and oxidative stress response, and it is overexpressed in several different tumor types.1 Structural and enzymatic investigations have described the mechanism and mode of action of this enzyme.2 Substantial evidence on the physiological role of DPP III is lacking, and none of the proposed physiological roles have been proven. However, in the only well documented case of protein-protein interaction, the interaction with Keap1, the role of DPP III is independent from its peptidase activity.3 Keap1 is a cytoplasmic sensor for oxidative stress, regulating expression of hundreds of cytoprotective genes through the release of a transcription factor Nrf2.4 DPP III interacts with Keap1 through its Kelch domain, and the interaction is mediated through the "ETGE" motif of DPP III, located on a loop between two conserved regions that build the peptidase active site. We are currently using isothermal titration calorimetry (ITC) to determine the thermodynamic parameters of the interaction between DPP III and Kelch domain of Keap1. In the future, we plant to use ITC to confirm or disprove other putative interactors of DPP III. New candidates for interaction will be obtained using the SILAC mass spectrometry approach.

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P12 Tumor suppressor PP2A governs cancer cell signalling and drug responses

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Protein phosphatase 2A (PP2A) is a broad specificity seine/threonine phosphatase that functions as an antagonist of many oncogenic pathways and is implicated in tumor suppression. We have characterized PP2A-regulated phosphoproteome by label-free LC-MS/MS analysis and RNAi mediated depletion of PP2A subunits and endogenous inhibitor proteins, identifying a total of 7037 non-redundant phosphopeptides from whole cell lysates. PP2A activity manipulation altered the phosphorylation of significant fraction of quantified phosphorylation sites. To the extent that it may bias quantification. Therefore, we have developed a pairwise normalization method for label-free phosphoproteomics analysis (Kauko et al. Sci Rep 2015). This study provided several important general insights into organization of phosphosignaling. For example, subcellular localizations of PP2A and its inhibitors contribute to differential kinase/phosphatase balance in cytoplasm and nucleus that is likely to play a role in signal propagation. Phosphorylation stoichiometries are known to observe bimodal distribution. We show that as a functional consequence of this, activation and inhibition of a phosphatase or kinase largely affect different phosphosites, pathways and processes. PP2A-mediated regulation was consistent with previously identified factors associated with phosphorylation site occupancy (e.g. pT vs. pS, acidophilic vs. basophilic and proline-directed). The phosphoproteomics analysis was combined with sensitivity profiles to over 400 drugs. Combined analysis of these

two datasets demonstrated that deregulation of phosphatase activity is sufficient for activation of collateral resistance pathways. This is exemplified by PP2A-dependent MEKi sensitivity that was validated in multiple KRAS mutant lung cancer cell lines, and in xenografts using novel small molecule activator of PP2A. We utilized LC-MS/MS in combination with other methods to show that the resistance was mediated by activated AKT/mTOR and MYC. We are initiating a new large-scale phosphoproteomics project and I would be very eager to participate EuPA summer school to learn more about sample preparation, labeling, and prefractionation strategies.

P13 The study of the proteins of parasitic worm Triaenophorus nodulosus (Pallas, 1781) at different stages of the life cycle

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Ecological proteomics is a promising discipline exploring the proteins involved in the adaptations of organisms to environmental conditions. Exploration of specific molecular mechanisms of adaptation to different (sometimes extreme) habitats in the variety of existing organisms is of great interest. In our study, we investigated proteins of a tapeworm Triaenophorus nodulosus using 2D-electrophoresis and LC-MS/MS. All tapeworms are obligate parasites, which during their life undergo multiple metamorphoses associated with several subsequent changes of their hosts (i.e. worm environment). Thus, this unique genetic adaptability to very diverse conditions is presumably characteristic of this ancient taxon. T. nodulosus life cycle includes: planktonic crustaceans as first intermediate hosts, different freshwater fish (the larvae is localized in the liver) as a secondary host, and finally mature worms occupy intestine of pike Esox licius. Optical density of 45 protein spots on 2D-electroforegramms were found to differ in worms larvae (plerocercoid) in fish liver compared with mature individuals from pike intestine ($p<0.05$). Proteins identified among them included; immunosuppressive protein annexin, ornithineaminotransferase (the enzyme of the metabolism of amino acids), two forms of chaperones (stress response proteins), the signaling peptide (G-protein), carbohydrate metabolism enzymes as enolase, phosphoglycerate mutase, triosephosphate isomerase, enzyme of fatty acids metabolism propionyl-CoA carboxylase, two structural proteins (tubulin, titin), and the transitional endoplasmic reticulum ATPase. A comparative analysis for plerocercoids obtained from different host species (perca Perca fluviatilis, burbot Lota lota, ruffe Gymnocephalus cernuus) revealed a significant differences in the optical density of 24 protein spots on 2D-electroforegramms. The plerocercoids of all three species were found to have different content of tubulin, and the larvae from perch and burbot differ in the content of phosphoglycerate mutase, triosephosphate isomerase, and G-protein. Obtained results may help to understand mechanisms, involved in the parasite adaptation to a specific "environment" of the host.

P14 Signalling degradation by post-translational modifications

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Controlled protein degradation is necessary for cell survival. Especially in pathogenic organisms, Clp proteolytic complexes are essential for survival and/or pathogenicity and established as promising drug targets. These complexes are composed of a AAA+ (ATPase associated with various cellular activities) unfoldase that recognizes substrates and feeds them into the proteolytic core for degradation. The identity of the degraded substrates is the reason for the essential nature of these degradation system. The aim of my project is to identify Clp degradome substrates and how they are targeted to and recognized by the Clp proteolytic complex. I will establish model organisms for pathogenic bacteria and introduce a substrate-trapping variant of the proteolytic core to pull-out degradome substrates. I plan to identify these by mass spectrometry and analyze putative degradation signals. In particular, I will focus on the phosphorylation of arginine side chains, phospho-Arginine (pArg). So far, this novel protein modification has only been described in gram positive bacteria and targets proteins to the *B. subtilis* ClpCP complex. Our preliminary data show that pArg and pArg-modified proteins interact with Clp proteolytic complexes in bacteria lacking the McsB arginine kinase. This project therefore investigates the existence of pArg in different bacteria and aims to characterize the pArg phosphoproteome by mass spectrometry. Elucidation of Clp degradation targets and their signals will inspire further studies concerning the relevance of pArg mark in bacterial physiology and as a potential drug target.

P15 Proteomic biomarkers assessment for early diagnosis in chronic kidney disease

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Background: Chronic kidney disease (CKD) describes the gradual loss of kidney function that typically evolves over many years due to its clinically silent behavior. CKD is associated with increased inflammatory condition, which involves complex interactions among immune cells and soluble proteins. Accelerated vascular calcification (VC) is an important and devastating complication of CKD and contributes to high mortality in these patients. The present study aimed to assess a novel proteomic biomarkers panel, particularly useful for identification of VC early phases in CKD, having important consequences on therapeutic interventional strategies, prognosis, and life-expectancy of patients with CKD. Material and Method: Serum samples of 86 CKD patients (stages II-IV) and 20 normal controls were analysed to simultaneously measure the level of 6 biomarkers (IL-6, TNFα, OPG-osteoprotegerin, OC-osteocalcin, OPN-osteopontin, FGF-23 - Milliplex MAP Human Bone Magnetic Bead Panel) using Luminex xMAP technology. Fetuin-A serum level was assessed using Quantikine ELISA kit, R&D Systems. Results: Mineral metabolism candidate biomarkers and molecules that actively regulate VC process (OPG, OC, OPN, FGF-23, Fetuin-A) showed an increased level compared with control ($p<0.05$). The pro-inflammatory cytokines level was also increased in CKD patients compared with control ($p<0.05$). The association between inflammatory cytokines and bone disorders markers reflects the severity of vascular changes in CKD and could predict the disease progression.

It has also been observed a positive correlation between the circulating biomarkers and the stages inside the CKD group. Conclusion: Detecting VC through its severe clinical manifestations may turn to be too late for any therapy to halt its progression or to reverse it. Thus, it would be particularly useful to develop a specific biomarker panel for identification of early phases of VC and for scoring its severity in CKD patients, which would allow developing further strategies aiming to control and even reverse some of the processes.

P16 Large scale phosphoproteomics reveals Shp2 to be a master regulator of Pdgfr signaling

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Tyrosine phosphorylation (pTyr) regulates numerous cell signaling pathways in health and disease. However, its detection and quantitation remains challenging due to its low cellular abundance. We applied comprehensive phosphoproteomics to unravel differential regulators of receptor tyrosine kinase (RTK) initiated signaling networks upon activation by Pdgf-ββ, Fgf-2 or Igf-1, respectively, and identified over 40,000 phosphorylation sites. The analysis revealed RTK-specific regulation of hundreds of pTyr sites on key adaptor and signaling molecules. We found the tyrosine phosphatase Shp2 to be the master regulator of Pdgfr pTyr signaling. Application of a recently-introduced allosteric Shp2 inhibitor revealed global regulation of the Pdgf-dependent tyrosine phosphoproteome, which significantly impaired cell migration. Additionally, we present a list of hundreds of Shp2-dependent targets and putative substrates including Rasa1 and Cortactin with increased tyrosine phosphorylation, and Gab1 and Erk1/2 with decreased tyrosine phosphorylation. Our study demonstrates that large-scale quantitative phosphoproteomics can precisely dissect tightly regulated kinase/phosphatase signaling networks.

P17 Assessment of cytokines level in management of patients with prostate cancer

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BACKGROUND: Prostate cancer (PCa) is a major health problem in modern society, and its prevalence is continuously increasing. Furthermore, PCa is currently the second most common cause of cancer death in men. To improve the outcome prediction for patients treated with radical prostatectomy, a personalized medicine strategy, starting from proteomic and genomic approach for tumor and patient must be applied. METHODS:We determined cytokine levels in sera from 23 patients with prostate cancer, 5 with benign prostate hyperplasia and 9 healthy controls. Using Milliplex MAP Human Cytokine/Chemokine Magnetic Bead Panel (Merck-Millipore) on a Luminex 200 system, we analyzed 12 analyte-specific bead sets: IL-2, IL-10, IL-1β, IL-12, IL-4, IL-6, IL-8, TNFα, VEGF, FGF-2, G-CSF, IFNγ. Multiplex data acquisition and analysis were performed using xPONENT 3.1 software.

RESULTS: The assessed serum level of cytokines/chemokines/angiogenesis factors was increased in Pca patients compared to the control group (IL-6 – 3.5, IL-8 – 3.9, TNF-α – 1.2, FGF-2 – 4.1, VEGF –4,5; GM-CSF –6,7; IFNɣ - 3,7 fold higher). It has also been observed increases in level expression of these biomarkers in benign prostate hyperplasia versus control. CONCLUSIONS: Proteomics represents a promising approach for the discovery of new biomarkers able to improve the management of PCa patients, an important tool to identify new molecular targets for PCa tailored therapy. Identification of markers associated with multi-stage PCa will provide greater scientific understanding of possible causes and underlying mechanisms, and important insights needed for improving life expectancy. Acknowledgment: Partially supported by the grant COP A 1.2.3., ID: P_40_197/2016, grants PNII 192/2014, PN 16.22.05.03.

P18 Interplay of recombination, plasmid stability, and CRISPR-Cas immunity in Escherichia coli

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CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats; CRISPR-associated) system is a prokaryotic adaptive immune system against foreign genetic elements. Immunity is acquired through insertion of small fragments of invader DNA into a CRISPR array. This process of fragment (spacer) insertion is called adaptation, and can be mediated solely by the protein complex Cas1-Cas2 (“naïve CRISPR adaptation”). In E. coli, naïve adaptation is helped by RecBCD enzyme to generate single-stranded DNA (ssDNA) intermediates. We report here that cells lacking recD gene and transformed with Cas1-Cas2 expressing plasmid (pCas1-Cas2) could not acquire new spacers, as expected, but that this also corresponded with moderate loss of pCas1-Cas2. The adaptation could be re-established by simultaneous deletion of recA. Since RecA loading is constitutive in recD mutant, the presence of bound RecA onto 3’ ssDNA protects and prevents generation of ssDNA fragments that are required for spacer preparation. We suggest that RecA has an inhibitory role in spacer acquisition by preventing spacer formation from self DNA coated by RecA, while at the same time foreign DNA is unprotected by RecA and available for DNA fragmentation and spacer preparation.

P19 Purification, characterization and thermodynamic studies of an α-amylase from a newly isolated thermoactinomyces, Laceyella sacchari TSI-2

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Thermoactinomyces, Laceyella sacchari TSI-2 (GeneBank no. KC989946), isolated from a hot spring located in TulsiShyam, Gir Forest, Gujarat (India) was found to secret α-amylase optimally at 50 °C and pH 7. The effect of various carbon sources, nitrogen sources and metal ions were assessed on the production of amylase. The amylase

was purified by gel permeation chromatography up to 54.66 fold with 12.23% yield having specific activity 4215.91 U/mg. The molecular weight, Km, Vmax and Kcat were 31 kD, 4 mg ml-1, 2000 µgm min-1 and 20 s-1 respectively. The stability and catalysis of the enzyme at different temperature and pH was studied. The metal ions, chelators and inhibitors did not significantly affect the amylase catalysis. In the presence of surfactants at 10 and 50 mM concentration, the amylase was stable up to 5 hours retaining 100% activity. The thermodynamic parameters; Kd, t1/2, ΔH, ΔS and ΔG* supported high thermal stability and affinity for the starch. The substrate cleavage specificity of the amylase based on the end product analysis, by HPTLC, revealed the enzyme type as α-amylase. The structural study of the amylase was carried out using FT-IR and CD spectroscopy. The α-amylase with high thermal, alkaline and surfactant stability would have significant role in various applications.

P20 Growth and phosphorylation profiles in S. rimosus, oxytetracycline producer strains

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Streptomyces are prokaryotic filamentous bacteria predominantly found in soil that exhibit a tightly regulated and complex life cycle. It has been estimated that 2/3 of clinically relevant antibiotics are produced by Streptomycetes. During their multicellular development Streptomyces species produce many natural products, amongst which are the best known bioactive compounds such as broad-spectrum antibiotics (tetracyclines, streptomycins and β-lactams), immunosuppressants (rapamycin) and anticancer drugs (doxorubicin). Among these natural products, tetracyclines (TCs) belong to one of the most clinically and commercially significant class of antibiotics. Streptomyces rimosus is best known strain of oxytetracycline (OTC) producers. It has been reported that phosphorylation plays an important regulatory role in metabolism and antibiotic production in Streptomyces. Analysis of the S. rimosus genome has predicted 33 eukaryotic-like protein kinases and 27 eukaryotic type protein phosphatases in this species. We hypothesized that OTC production in S. rimosus may be triggered by posttranslational modifications in some regulatory proteins. In order to identify these key proteins we measured the production of OTC in various strains of Streptomyces using HPLC. Additionally, we examined the phosphorylation pattern during growth of these strains by Western blot, whilst the morphology of mycelia was observed by fluorescence microscopy. Our observations indicated that the Streptomyces strains had variations in OTC production, whereas Western blot analyses showed different phosphorylation patterns for each strain. Images from confocal microscopy revealed different viability and morphological features. From these strains, we selected the most consistently optimal producer of OTC and used this to perform phosphoproteomic analyses using high accuracy mass spectrometry. Our preliminary results revealed a differential expression of the proteome/phosphoproteome at different stages of Streptomyces growth. Further analysis of these proteomic results is still in progress.

P21 Haptoglobin Glycopeptide Analysis by nano-LC-ESI-MS/MS

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Haptoglobin (Hp) is a positive acute phase protein synthesized by hepatocytes. It is a tetramer, which always contains 2 β and 2 α chains, connected via disulfide bonds. Hp has four N-glycosylation sites at Asn184, Asn207, Asn211 and Asn241, located on the β chain and occupied by complex-type N-glycans. Its glycosylation, as one of the most complex post-translation modifications, is associated with different kinds of diseases. In addition, Hp glycosylation is a promising target in cancer research. Our aim was to analyze Hp N-glycosylation in a site-specific manner on glycopeptide level by nano-LC-ESI-MS/MS. Thus, we optimized method with Hp standard, which was treated with trypsin and a combination of trypsin and endoproteinase Glu-C, in order to obtain glycopeptides containing a single glycosylation site. Data analysis showed that we obtained four glycopeptides with each one glycosylation site, when combining both enzymes. However, to obtain a quantitative digestion with only singly glycosylated species, sample preparation before nano-LC-ESI-MS/MS analysis will be further optimized.

P22 Structural and functional characterization of myosin folding

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Muscle contraction, but also cytokinesis, endocytosis and various other cellular and organismal functions rely on the actin-dependent motor protein myosin that creates force by utilizing ATP-hydrolysis-derived energy. Myosin is an intricately folded protein that requires the help of several, partially still unknown, chaperones, which assist during protein folding and protect myosin under stress conditions. Besides the unspecific heat-shock proteins Hsp70 and Hsp90, which are involved in numerous cellular processes, myosin folding is facilitated by the specific chaperone UNC-45. This chaperone is highly conserved among metazoans and can assemble into linear chains that orchestrate myosin folding and myofilament formation. Disruption of the UNC-45-myosin complex is assumed to promote the development of certain myopathies. While UNC-45 has been characterized structurally and functionally, insight into the UNC-45-myosin complex is limited. To dissect the interactions between UNC-45 and myosin, we initially aim to apply cross-linking mass spectrometry. This approach will be complemented by further structural and biochemical studies and an integrative modeling approach, all together shedding light on the UNC-45-myosin complex. For our studies, we have developed an insect cell-based, recombinant expression system yielding high quantities of functional and properly folded C. elegans and D. melanogaster myosin. As a second step, we plan to not only study known myosin chaperones, but also apply proteomics tools to complete the picture of the chaperone network that guides myosin folding. We are driven by the question why recombinant expression of myosin fails in E. coli, but instead requires an insect cell expression system and therefore hypothesize that additional folding factors are essential. By obtaining potential chaperone candidates, we aim to gain insight into the myosin folding pathway and eventually unravel the ‘chaperome’ that is necessary for myosin folding.

P23 Proteomic analyses of Calreticulin mutants in Myeloproliferative Neoplasms

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Myeloproliferative neoplasms (MPNs), a group of non-Bcr-ABL mutated myeloid cancers, are to a large extent characterized by tumor-driving mutations in specific genes including Jak2, MPL, and CALR. In contrast to the vast majority of currently known tumor-driving oncogenes and tumor suppressors, CALR does thus not belong to the classes of receptors or kinases yet is best known for its ER-resident glycoprotein chaperone function. Moreover, mutations in CALR can drive the occurrence of tumors in mouse models and are restricted to the MPNs. Since a mutation in a chaperone protein will not solely affect a single protein but rather result in changes in the global cellular proteome, an unbiased approach is required to study the biology of its tumorigenic mutations and the effect on myeloid cells. In this project, various proteomic approaches will therefore be applied, including cellular proteomics, glycoproteomics, structural proteomics using limited proteolysis (LiP), and interactomics using BioID, for which technical experience is present in the lab of prof. dr. Bernd Wollscheid and collaborating labs at ETH Zürich. We will apply these techniques to cellular cell lines carrying various clinically relevant CALR mutants, and compare them between to each other to determine functional differences between different mutants, to the parental cell lines possessing the wildtype gene, and to full knockout cell lines. Furthermore given the intrinsic collaborative nature of this project with the lab of dr. med. Alexandre Theocharides of the University Hospital Zürich (USZ), we have full access to patient material for validation of our findings. Using the regular proteomics, we want to get an unbiased overview of cellular responses to the CALR mutation. In a first trial experiment using a megakaryocytic cell line, we discovered that a large amount of ER unfolded protein response (UPR) proteins is significantly upregulated. This led us to the hypothesis of an impaired chaperone function of CALR mutants. To follow up on this, we are planning to decipher the chaperone clients of CALR. Firstly, since CALR chaperones N-linked glycosylated proteins and incorrectly folded clients are likely to show different abundances, we will perform a glycoproteome enrichment to enable a deeper investigation of abundance of cellular glycoproteins. Secondly, in order to directly determine incorrect folding of these potential chaperone clients, we will collaborate with the lab of Paula Picotti to perform LiP, enabling elucidation of structural protein differences in CALR-mutated and wildtype cells. Lastly, we will investigate direct interaction partners of CALR using BioID, thereby determining not only client proteins but also differences in all other cellular interactions between mutant and wildtype CALR. Using multiple proteomic approaches on both cell lines and patient material, we hope to elucidate the effects of CALR mutations on the cell and decipher the tumor-driving properties of the mutants. This can potentially result in the determination of specific vulnerabilities, including the currently determined ER UPR, that can be exploited using therapies.

P24 Fc-linked N-glycosylation of IgG subclasses in three mouse strains analyzed with nanoUPLC-ESI-MS

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Biological activity of immunoglobulin G (IgG) is heavily influenced by the biantennary N-glycans attached to the heavy chains of the fragment crystallizable (Fc). The aim of the project is to compare N-glycosylation profiles of the Fc-region of IgG subclasses in three commonly used mouse strains. IgG was isolated from the serum of individual sex- and aged-matched mice of BALB/c, C57BL/6 and C3H strains kept in the same environment. The obtained IgG tryptic glycopeptides corresponding to the Fc region of the four mouse IgG subclasses were separated with nano ultra-performance liquid chromatography and quantified with electrospray ionization mass spectrometry (nanoUPLC-ESI-MS) method. We compared several derived N-glycan traits, describing the relative abundance of N-glycan structural features. We observed differences in Fc-linked N-glycosylation profiles both between mouse strains and between IgG subclasses. Interestingly, the C57BL/6 mice carrying a rare IgG1 allotype characterized by a Phe -> Ile substitution in the tryptic Fc-glycopeptide showed increased agalactosylation and reduced sialylation of IgG1 compared to BALB/c and C3H strains. Our next goal is to develop a technique for relative quantification of the IgG allotypes in our experimental setup to determine if they are expressed on equal levels in the heterozygous individuals and if their N-glycosylation profiles differ. We plan to further investigate the connection between the abundance of known IgG allotypes and their N-glycosylation profiles, with a particular focus on known IgG1, IgG2b and IgG2c allelic polymorphism in mice and IgG3 in humans, which will require integration of proteomics, genomics and glycomics data.

P25 High-throughput immunoaffinity isolation and variability of transferrin N-glycosylation in population study of Croatian Korčula island

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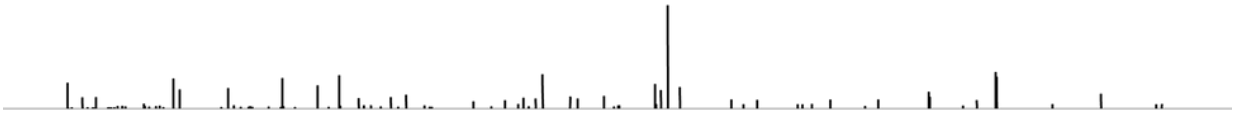
Transferrin (Tf) is an iron-binding glycoprotein, produced mainly by hepatocytes, that regulates the level of free iron in biological fluids. Tf has been associated with different diseases, e.g. cardiovascular diseases, inflammation, atransferrinemia and allergic reactions. Additionally, changes in Tf glycosylation are a common marker for congenital disorders of glycosylation and alcoholism, and are seen in hepatocellular carcinoma patients. In spite of this, biological interindividual variability of Tf N-glycosylation as well as genes involved in glycosylation regulation are not known. To address these unknowns our aim was to analyze Tf N-glycosylation in a large population cohort of Croatian Korčula island. We used our recently developed immunoaffinity-based monolithic 96-well plate for specific high-throughput isolation of Tf from human blood plasma samples. Isolated Tf was denatured and deglycosylated with PNGase F and released N-glycans labeled with fluorescent dye, 2-aminobenzamide (2-AB). Labeled glycans were cleaned-up on a hydrophilic GHP filter plate and subsequently analyzed by hydrophilic interaction liquid chromatography (HILIC) on ultra performance chromatography system (UPLC) with 100 mM ammonium formate and acetonitrile as mobile phases. Here we present the variability of Tf N-glycosylation between healthy human individuals related to their age and sex. To our knowledge, this is the first population study of Tf N-glycosylation.



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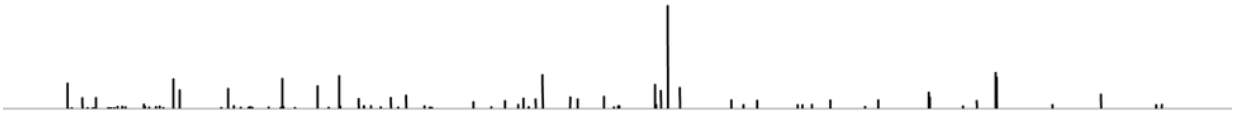

Meeting Location

University of Split, School of Medicine
Šoltanska ulica 2,
21000 Split, Croatia

Restaurant Location

(Gala Dinner on Tuesday, Oct. 10th at 20:00 h, preceded by city tour)

Restaurant Apetit
Ulica Pavla Šubića 5,
21000 Split, Croatia

Bus will leave on Tuesday, Oct. 10th at 18:00 h from the parking lot of the Medical School

Organizing Committee

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