OMICs in Biomedical Research

BOOK OF ABSTRACTS

Mediterranean Institute for Life Sciences

June 8th - 12th 2015

Split, Croatia

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OMICs in Biomedical Research

Mediterranean Institute for Life Sciences
June 8th - 12th 2015

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OMICs in Biomedical Research

Mediterranean Institute for Life Sciences
June 8th - 12th 2015

Dear Colleagues and Friends,

On behalf of the Organizing Committee, it is my pleasure to extend a warm welcome to the OMICs in Biomedical Research Conference, which will be held at the Mediterranean Institute of Life Sciences in Split from June 8th through June 12th, 2015. We have assembled an exciting scientific program addressing important topics such as OMICs and Aging/Neurodegenerative diseases, OMICs and Networks, OMICs and Drug Discovery, OMICs and Cancer, and more.

The proposed meeting will bring together prominent scientists from the "OMICs" field including leading cell and molecular biologists, for the main purpose of discussing scientific issues arising at the interface of these two complementary fields, and their applications in human disease-related research. The general aim of this meeting is to develop and discuss new strategies in order to maximize the impact of the results of the "OMICs" revolution on the understanding of biological systems and processes.

This conference will bring together researchers that are developing and applying novel "OMICs" approaches to interrogate system-level networks with an ultimate goal of understanding the functions of therapeutically important proteins. Because these large-scale projects involve multidisciplinary teams from both academic and biotech/pharma labs, which are situated in different institutions all over the world, this meeting will provide a unique opportunity for diverse research groups to get together and discuss new ideas, approaches, and novel results.

We hope that you will take time to explore the ancient and vibrant city of Split, which offers plenty of restaurants, bars, shopping, and sightseeing, all by the beautiful Adriatic Sea.

We thank you for your participation, and we hope that you will enjoy the First "OMICs in Biomedical Research Conference"!

ORGANIZINIG COMMITTEE

Prof. dr. sc. Mladen Merćep
Prof. dr. sc. Miroslav Radman
Prof. dr. sc. Igor Štagljar



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Scientific program

June 8 (Monday), 2015

11:00 – 17:00	**Arrival & Registration**
18:15 – 18:30	Opening Keynote **INTRODUCTION of the Speaker by IGOR ŠTAGLJAR**
18:30 – 19:20	***Stan Fields*** (University of Washington, Seattle, USA) "Deep mutational scanning to analyse protein function*"*
19:30 – 21:00	*Songs, Mediterranean food and wine*

June 9 (Tuesday), 2015

09:00 - 13:00	**Morning session**
OMICs and Aging/Neurodegenerative diseases (30' talk+5minQs) **SESSION CHAIR: Erich Wanker** (MDC Berlin, Germany)	
09:00 - 9:35	**Adriano Aguzzi** (University of Zurich, Switzerland) "Molecular biology of prions"
09:35 - 10:10	**Gaia Novarino** (Institute for Science & Technology, Austria) "The HSPome: a journey in the genetic landscape of hereditary spastic paraplegias"
10:10 -10:30	**Christoph H. Borchers** (University of Victoria, Canada) "MRM-based assay kits for quantitative metabolomics in biomedical and clinical research"
10:30 - 11:00	*Coffee break*
11:00- 11:35	**Paul Muchowski** (KynuRex, USA) "Yeast As A Screening Tool For Protein Misfolding Diseases"
11:35 - 12:10	**Wolfgang Baumeister** (Max Plank, Martinsried, Germany) "The Challenge of doing Structural Biology *in situ*"
12:10 - 12:30	**Maciej Lalowski** (University of Helsinki, Finland) "Drafting the Neuronal Ceroid Lipofuscinosis 1 Interactome in the brain"
13:00 – 14:00	*Lunch*
14:00 – 15:00	*1st WORKSHOP "Publishing high profile papers in OMICs research"* Moderator: **Igor Štagljar** **Natalie de Souza** (Editor in Chief, Nature Methods) **Mirella Bucci** (Senior Editor, Nature Chemical Biology)
15:00 – 17:00	*Free activities*
17:00 – 20:00	**Evening session**
OMICs and Networks (30' talk + 5 min Qs) **SESSION CHAIR: Stan Fields** (University of Washington, USA)	
17:00 - 17:35	**Charlie Boone** (University of Toronto, Canada) "Modelling the Cell with Global Genetic Interaction Networks"

17:35 - 18:10	**Anne-Claude Gavin** (EMBL, Heidelberg, Germany) "Protein-lipid networks"
18:10 - 18:45	**Marc Vidal** (Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA) "Interactome Networks and Human Disease"
18:45 - 19:00	**Berend Snijder** (CeMM Research Centre, Vienna, Austria) "Functional mapping of the lipid landscape in innate immunity"
19:00 - 19:15	*Coffee break*
19:15 - 19:50	**Erich Wanker** (MDC Berlin, Germany) "Interactome maps for proteins involved in neurodegenerative diseases and synapse function"
19:50 - 20:25	**Patrick Aloy** (IRB, Barcelona, Spain) "A network biology approach to therapeutics"
20:30 - 21:30	*Diner*

June 10 (Wednesday), 2015

09:00 - 13:00	**Morning session**
	OMICs and Drug Discovery (30' talk + 5 min Qs) **SESSION CHAIR: Paul Muchowski** (KynuRex, USA)
9:00 - 9:35	**Gerard Drewes** (Cellzome/GSK, Heidelberg, Germany) "Tracking drugs in living cells by thermal profiling of the proteome"
09:35 - 10:10	**Klaus Seuwen** (Novartis, Basel, Switzerland) "Orphan G protein-coupled receptors in the human genome"
10:10 -10:45	**Domagoj Vučić** (Genentech, South San Francisco, USA) "Ubiquitination profiling reveals sensitivity factors in cancer and inflammatory diseases"
10:45 - 11:15	*Coffee break*
11:15 - 11:50	**Slobodan Vukičević** (University of Zagreb, Croatia) "Bone morphogenetic proteins in regenerative medicine"
11:50 - 12:25	**Jörg Hoheisel** (German Cancer Research Center, Heidelberg) "Affinity-based assays for personalised proteomics in cancer research"
12:25 - 12:40	**Marija Tadin-Strapps** (Merck,USA) "siRNA and CRISPR tools for target validation: Impact on early drug discovery"
12:40 – 13:00	**Volker Kruft** (AB SCIEX, Darmstadt, Germany) "Creating a digital image of any sample in the MS2 space: Discussing data-independent acquisition strategies"
13:00 - 14:00	*Lunch* Small conference room *(13:00-14:00) Alpha Chrom Satellite Symposium*
14:00 - 17:00	*Free activities* Small conference room (*16:00-17:00) SCIEX Workshop OneOmics and Swath*

17:00 - 20:00	**Evening session**
	OMICs & Cancer (30' talk + 5 min Qs) **SESSION CHAIR: Marc Vidal** (Dana-Farber Cancer Institute, Harvad Medical School, Boston, USA)
17:00 - 17:35	**Siniša Volarević** (University of Rijeka, Croatia) "Ribosome biogenesis stress and p53 regulation"
17:35 - 18:10	**Igor Štagljar** (University of Toronto, Canada) "Membrane Protein Interaction Networks in Cancer"
18:10 - 18:45	**Kristijan Ramadan** (University of Oxford, UK) "Dynamic and reorganisation of p97/VCP -proteome after ionising radiation"
18:45 - 19:15	*Coffee break*
19:15 - 19:50	**Thomas Kislinger** (UHN, Toronto, Canada) "Systematic development of SRM-MS assays for the detection of aggressive prostate cancers"
19:50 - 20:05	**Alexsander Buntru** (Max Delbrueck Center, Berlin, Germany) "DULIP: a dual luminescence-based co-immunoprecipitation assay for interactome mapping in mammalian cells"
20:05 - 21:00	*Dinner*
21:00 - 23:00	Poster session

June 11 (Thursday), 2015

09:00 - 13:00	**Morning session**
	Emerging "OMICs" technologies (30' talk + 5 min Qs) **SESSION CHAIR: Patrick Aloy** (IRB, Barcelona, Spain)
09:00 - 9:35	**Brenda Andrews** (University of Toronto, Canada) "Yeast proteome dynamics from single cell imaging and automated analysis"
09:35 - 10:10	**Gavin Wright** (Sanger Center, Hinxton, UK) "Using systematic extracellular protein interactions screens to identify and interactions that are essential for cellular recognition processes"
10:10 - 10:45	**Hana Kovarova** (Institute of Animal Physiology and Genetics, Czech Republic)"A Neural Progenitor differentiation Patterns of Surface and secreted proteins for Cell-Replacement therapies of Neuronal Disorders"
10:45 - 11:15	*Coffee break*
11:15 - 11:50	**Uwe Sauer** (ETH Zurich, Switzerland) "Metabolomics as a hypothesis generator"
11:50 - 12:25	**Ola Söderberg** (Uppsala University, Sweden) "Molecular tools for evaluation of cellular activity status"
12:25 - 12:45	**Lovorka Grgurević** (University of Zagreb, Croatia) "Utilizing osteogenic potential of BMP6 in developing new treatment options"

12:45 – 13:00	**Jolanda van Leeuwen** (University of Toronto, Canada) "Mapping genetic suppression interactions on a global scale"
13:00 - 14:00	*Lunch*
14:00 - 20:00	*Excursion*
20:00 - 21:00	*Dinner*

June 12 (Friday), 2015

09:00 - 13:00	**Morning session**
	OMICs & Microbes (30' talk + 5 min Qs) **SESSION CHAIR: Uwe Sauer** (ETH Zurich, Switzerland)
09:00 - 09:35	**Henning Walczak** (University College, London) "Linear Ubiquitin: at the crossroads of gene activation, cell death, inflammation and cancer"
09:35 - 10:10	**Miroslav Radman** (MedILS, Split, Croatia) "Are aging and age-related diseases phenotypes of protein damage?"
10:10 - 10:45	**Michel Desjardins** (University of Montreal) "Understanding antigen presentation and the immune response in the"OMICs"Era"
10:45 - 11:15	*Coffee break*
11:15 - 11:50	**Eric Brown** (McMaster University, Hamilton, Canada) "Small molecules as probes of complexity in microbial systems"
11:50 - 12:10	**David Duffy** (University College Dublin, Ireland) "MYCN Integrative Omics Enables Network-Based Therapeutic Target Discovery and Patient Stratification in Neuroblastoma"
12:10 - 12:30	**Maria Pires Pacheco** (University of Luxembourg, Luxembourg) "Integrated metabolic modelling reveals cell-type specific control of the macrophage metabolic network"
12:30 - 12:50	**Gordan Lauc** (University of Zagreb, Croatia) "Patient stratification beyond individual genes: Glycans as integrators of genes and environment"
12:50 - 13:10	**Shaun Bilsborough** (Agilent Technologies, UK) "Addressing the challenges of integrating data in the multi-omics laboratory"
13:10 - 14:00	*Lunch*
14:00 - 16:00	*Free activities*
16:00 - 17:00	2nd WORKSHOP "Careers in OMICs-based disciplines" Moderator**: Mladen Merćep** **Brenda Andrews** (University of Toronto, Canada), **Marija Tadin-Strapps** (Merck, USA)
17:00 -17:20	**Martin Beck** (EMBL, Heidelberg, Germany) "Integrated genome and proteome-wide analysis reveals organ-specific proteome deterioration during aging in rat"
17:20- 17:30	*Coffee break*

17:30 - 17:45	**Closing Keynote** **INTRODUCTION of the Speaker by Miroslav Radman**
17:45 – 18:20	**Ron Kopito** (Stanford University, USA) “Proteomic and functional genomic dissection of protein quality control in the endoplasmic reticulum”
20:00 - 23:00	*Conference Banquet*

June 13 (Saturday), 2015

Departure

OPENING KEYNOTE

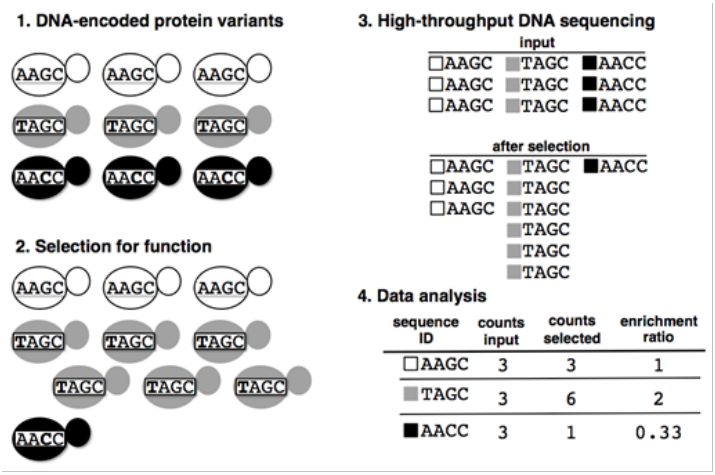
INTRODUCTION of the Speaker by IGOR ŠTAGLJAR

Deep mutational scanning to analyze protein function

Stan Fields

Howard Hughes Medical Institute
Departments of Genome Sciences and Medicine
University of Washington
Seattle, Washington 98195-5065, USA

Deep mutational scanning uses high-throughput DNA sequencing and a coupled genotype–phenotype platform to quantify the activity of up to about one million variants of a protein in a single experiment. This approach requires an appropriate selection system for the protein function of interest, a library of variants that is subjected to selection, and deep sequencing of library DNA from input and selected populations. A functional score (or enrichment ratio) for the activity of each variant can be calculated from the change in a variant's frequency over the course of the experiment. This method has been used with selections dependent on such properties as binding of a protein to another macromolecule, catalytic activity, and protein stability, and it has been used with diverse procedures to separate variants of differing activity that include growth-based selections and fluorescence sorting. I will discuss uses of this approach toward understanding the effects of human genetic variation and in analyzing protein properties.



OMICs and Aging/Neurodegenerative diseases

SESSION CHAIR: Erich Wanker

Molecular Biology of Prions

Adriano Aguzzi

(MD PhD DVM hc FRCPath)
Institute of Neuropathology, University Hospital of Zurich, Zurich, Switzerland

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Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases of humans and many animal species caused by prions. The main constituent of prions is PrP^{Sc}, an aggregated moiety of the host-derived membrane glycolipoprotein PrP^{C}. Prions were found to encipher many phenotypic, genetically stable TSE variants. The latter is very surprising, since PrP^{C} is encoded by the host genome and all prion strains share the same amino acid sequence. Here I will review what is known about the infectivity, the neurotoxicity, and the neuroinvasiveness of prions. Also, I will explain why I regard the prion strain question as a fascinating challenge - with implications that go well beyond prion science. Finally, I will report some recent results obtained in my laboratory, which is attempting to address the strain question and some other basic issues of prion biology with a "systems" approach that utilizesorganic chemistry, photophysics, proteomics, and mouse transgenesis.

A The HSPome: a journey in the genetic landscape of hereditary spastic paraplegias

Gaia Novarito

Genetic and Molecular Basis of Epilepsy and Cognitive Disorders
Institute of Science and Technology Austria

Hereditary spastic paraplegias (HSPs) are neurodegenerative motor neuron diseases characterized by progressive age-dependent loss of corticospinal motor tract function. Although the genetic basis is partly understood, only a fraction of cases can receive a genetic diagnosis, and a global view of HSP is lacking. By using whole-exome sequencing in combination with network analysis, we identified 18 previously unknown HSP genes and generated a HSP interactome, demonstrating that many of known and candidate HSP genes are highly interconnected. Our network analysis links HSP to other neurodegenerative disorders and can facilitate gene discovery and mechanistic understanding of disease

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MRM-based assay kits for quantitative metabolomics in biomedical and clinical research

Christoph H. Borchers

UVic - Genome BC Proteomics Centre and Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia, Canada

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LC/multiple-reaction monitoring (MRM)/MS has been the gold standard technique in targeted bioanalysis. To facilitate quantitation of various unconjugated, glycine-conjugated and taurine-conjugated bile acids in different biological samples, we developed a “Bile Acids MRM Assay Kit” for simultaneous determination of 45 bile acids by LC-MRM/MS. The LC-MRM/MS method combined phospholipid depletion solid-phase extraction as an improved sample preparation procedure, and showed low-femtomole analytical sensitivities. With 14 deuterium-labeled bile acids as internal standards for calibration, excellent linearity ($R2 \geq 0.9991$) has been observed for all the bile acids. The use of this kit for assay of the bile acids in human plasma and mouse serum indicated high precision (intra- and inter-day CVs ≤ 8.8%) and high accuracy (80% to 114% for most analytes) of the quantitation. Validation of this kit by multiple users demonstrated consistent quantitation results for all of the low-, medium- and high-abundance bile acids detected in the blood samples. Other kits including the “Tricarboxylic Acid (TCA) Cycle Assay Kit”, the “Short-Chain Fatty Acids Assay Kit”, and the “Sugars and Sugar Phosphates Assay Kit” are under development.

Yeast As A Screening Tool For Protein Misfolding Diseases

Paul Muchowski

KynuRex, USA

Genome-wide genetic screens were performed in yeast with five different amyloid-forming proteins, including a mutant huntingtin fragment, full length huntingtin, α-synuclein, β-amyloid and a yeast prion, to identify mutants that enhance or suppress the toxicity of these misfolded proteins. Hierarchical clustering and gene annotation enrichment analyses indicated that the genetic modifiers of toxicity that were identified for the five amyloid-forming proteins participate in largely non-overlapping cell biological functions and pathways. Genetic and pharmacological approaches in a mouse model of Huntington's disease were used to successfully validate several of the modifiers of mutant huntingtin toxicity identified from our yeast screens. Our results provide additional evidence that genetic screens in model organisms, such as yeast, can successfully identify disease-modifying pathways that are conserved in lower and higher eukaryotes. Such screens may also yield novel therapeutic targets for neurodegenerative disorders.

The Challenge of doing Structural Biology *in situ*

Wolfgang Baumeister

Max Plank Institute of Biochemistry,
Martinsried, Germany

Traditionally, structural biology takes a reductionist or 'divide and conquer' approach: molecules are isolated and purified, or made recombinantly, and then subjected to structural studies by X-ray crystallography, NMR or cryo EM. On one hand, this approach has been enormously successful; on the other hand, awareness has grown that only rarely biological functions can be attributed to individual molecules.
Higher order functions rely on the interactions of proteins in 'functional modules' or protein networks. Some of these functional modules are deeply rooted in their cellular environments making it difficult, if not impossible, to isolate them without violating their structural integrity. Other interactions occur only fleetingly; in fact, entire biochemical pathways are likely to be organized in a non-random manner.
Hence there is a strong incentive to develop technology and methodology enabling structural studies in situ, i.e. in unperturbed cellular environments. Electron cryotomography has unique potential to study the 'molecular sociology' of non-repetitive biological structures, such as cells or organelles. It combines the power of three-dimensional imaging with the best structural preservation that is physically possible.

Drafting the Neuronal Ceroid Lipofuscinosis 1 Interactome in the brain

Enzo Scifo [1], Agnieszka Szwajda[2], Rabah Soliymani[1], Saara Tikka[1], Francesco Pezzini[3], Alessandro Simonati[3], Marc H. Baumann[1] and Maciej Lalowski[1].

[1]Medicum, Meilahti Clinical Proteomics Core Facility, Institute of Biomedicine / Biochemistry and Developmental Biology, Uni. of Helsinki, Helsinki, Finland

[2]Institute for Molecular Medicine (FIMM), Uni. of Helsinki, Helsinki, Finland

[3]Department of Neurological and Movement Sciences, Uni. of Verona, Verona, Italy

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Introduction. Neuronal ceroid lipofuscinoses (NCL) are a group of inherited progressive childhood disorders, encompassing 13 known genes characterized by early accumulation of autofluorescent storage material in lysosomes of neurons or other cells. Clinical symptoms of NCL include: progressive loss of vision, mental and motor deterioration, epileptic seizures and premature death. CLN1 disease (MIM#256730) is caused by mutations in the *CLN1* gene, which encodes *P*almitoyl *P*rotein *T*hioesterase 1 (PPT1). Our recent studies on CLN3-CLN5 and CTSD interactomes, pinpointed their inter-connections with proteins involved in neurodegeneration, mental retardation and epileptic seizures, as well functional modules, which can be targeted pharmaceutically[1,2]. In this study, we utilised single step affinity purification coupled to mass spectrometry to unravel the putative *in vivo* substrates of human PPT1 in the brain neuronal cells. **Materials and methods**. Full length PPT1 was shuttled in pES-CTAP-Puro [2] for the AP-MS experiments. Retroviral production in HEK293T cells, processing of viral particles for infection of low passage SH-SY5Y cells, cell culturing and verification of stably infected cells, were done as previously described [2]. We employed a modified filter-aided sample preparation protocol [3], for processing of protein complexes prior to MS analysis on a Q Exactive Hybrid Quadrupole-Orbitrap acquiring in data dependent mode. Functional annotations of PPT1 IP were performed with ClueGO (http://www.ici.upmc.fr/cluego/), FunCoup (http://FunCoup.sbc.su.se), Gene*Mania* (www.genemania.org) and Ingenuity Pathways (https://analysis.ingenuity.com/). **Results**. A total of 23 PPT1 interacting partners were identified from label free quantitation of the MS data by SAINT platform [4]. The PPT1 IP comprised of mostly neurodegenerative disease proteins and constituents of the pyruvate dehydrogenase and mitochondrial ATP synthase complexes. Validation of

a subset of the PPT1 IP was performed using Protein G affinity pulldowns/colocalization in the neuroblastoma cells. Three of the identified PPT1 IP, namely CRMP1, DBH and MAP1B are predicted to be palmitoylated, while four of them are differentially altered in their expression in the *Ppt1*$^{-/-}$ brain (ATP5B, CRMP1, MAP1B, PDHA1). Our proteomic analysis confirmed previously suggested roles of PPT1 in axon guidance and lipid metabolism, yet implicates the enzyme in novel roles including: involvement in neuronal migration and dopamine receptor mediated signalling pathway.

Acknowledgements/Literature

1 Koch, S. *et al. Neurobiol Dis* **50**, 107-119 (2013).
2 Scifo, E. *et al. J Proteome Res* **12**, 2101-2115 (2013).
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4 Choi, H. *et al. Nat Methods* **8**, 70-73 (2011).

This work was supported by Finnish Academy of Science (ML), EU 7th Framework Program (FP7/2007-2013/281234) (ML) and Kliinisen kemian tutkimussäätiö (ES).

OMICs and Networks

SESSION CHAIR: Stan Fields

Global Genetic Interaction Networks

Charlie Boone

The Donnelly Centre,
University of Toronto, Canada

Genetic interactions can play an important role in determining the relationship between genotype and phenotype and may underlie a significant component of the "missing heritability" in current genome-wide association (GWAS) studies. To explore the general principles of genetic networks, we've taken an unbiased and global approach to map digenic interactions in the budding yeast Saccharomyces cerevisiae. We developed Synthetic genetic array (SGA) analysis, which automates yeast genetics, enabling the combinatorial construction of defined mutants and the mapping of genetic interactions quantitatively. We are generating a comprehensive genetic landscape for yeast, examining networks derived from an analysis of all gene pairs, covering both nonessential and essential genes. Constructing a higher order network driven by genetic interaction profiles reveals the roles of specific genes and traces a global functional wiring diagram of the cell. Ultimately, our findings in yeast may allow us to explore genetic interactions underlying the heritability of traits in other organisms, including humans, and the design of synthetic lethal cancer therapies.

Protein-lipid networks

Anne -Claude Gavin

Bimolecular Networks
EMBL, Heidelberg, Germany

Many cellular processes require the recruitment of proteins to specific membranes which are decorated with distinctive lipids that act as docking sites. In particular, the phosphoinositides form signalling hubs, yet the underlying mechanisms remain elusive. We describe a novel method that measures protein recruitment to membranes in a quantitative, multiplexed and high-throughput manner. The liposome microarray-based assay (LiMA) integrates biochemical principles – that is the formation of giant liposomes (surrogates of biological membranes) on a thin agarose layer – with quantitative fluorescence microscopy-based imaging and microfluidics. The assay can reveal the complex, cooperative binding mechanisms that are frequently involved in targeting signaling proteins to biological membranes. LiMA also readily captured discrete changes in the lipid-binding affinities of proteins containing mutations associated with human disease. Miniaturization and parallelization of the assay makes it readily applicable to proteome– and lipidome–wide studies. We illustrate the use of LiMA with a systematic screen that reveals cooperativity as a key mechanism for membrane recruitment and, by enabling the interpretation of disease-associated mutations, offers a new approach for the design of small molecules targeting PH domains.

Interactome Networks and Human Disease

Marc Vidal

Center for Cancer Systems Biology (CCSB) and Department of Cancer Biology
Dana-Farber Cancer Institute & Department of Genetics
Harvard Medical School
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For over half a century it has been conjectured that macromolecules form complex networks of functionally interacting components, and that the molecular mechanisms underlying most biological processes correspond to particular steady states adopted by such cellular networks. However, until a decade ago, systems-level theoretical conjectures remained largely unappreciated, mainly because of lack of supporting experimental data.
To generate the information necessary to eventually address how complex cellular networks relate to biology, we initiated, at the scale of the whole proteome, an integrated approach for modeling protein-protein interaction or “interactome” networks. Our main questions are: How are interactome networks organized at the scale of the whole cell? How can we uncover local and global features underlying this organization, and how are interactome networks modified in human disease, such as cancer?

Functional mapping of the lipid landscape in innate immunity

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Pathogen recognition by Toll-like receptors (TLRs) and the subsequent cellular responses involve distinct membrane-dependent processes including receptor trafficking and signaling, cytokine secretion, and cell polarization. To study the role of membrane lipids in innate immunity, we combined systematic genetic perturbations of the sphingolipid metabolic network with quantitative lipidomics and functional characterization of TLR-related processes across different stimuli. Integrative analysis positioned sphingolipid metabolism in distinct feedback loops differentially regulating plasma membrane and endosomal TLR signaling pathways. Perturbing sphingolipid metabolism affected the entire lipid landscape, revealing a circular lipid co-regulatory network, reflecting lipid metabolism, subcellular localization, and adaptation mechanisms. A computational framework coupled with experimental validation assigned distinct functional roles for individual lipid species organized across the lipid landscape, correctly predicting cellular inflammatory phenotypes based solely on their lipid composition. This data-driven approach empowers the unbiased annotation of lipid function using a method broadly applicable to other complex biological systems.

Interactome maps for proteins involved in neurodegenerative diseases and synapse function

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Mapping of protein-protein interactions (PPIs) is critical for understanding protein functions, disease mechanisms and complex biological processes. Using an automated yeast two-hybrid (Y2H) screening technology, we have generated a focused interactome network for proteins involved in various neurodegenerative diseases (NDs), termed NeuroNet. Our map connects ~3,100 human proteins via ~13,700 high-quality binary PPIs, linking a large number of proteins known to be involved in neurodegeneration such as huntingtin, α-synuclein or TDP-43 to novel partners. Recently, we have validated NeuroNet Y2H PPIs using a systematic dual luminescence-based co-immunoprecipitation assay (DULIP), which allows the quantitative profiling of PPIs in mammalian cells. NeuroNet Y2H pairs exhibited validation rates that were equal to positive reference sets reported in the literature, demonstrating the quality of the data set. The overall functional relevance of PPIs in the network was confirmed by computational investigations, indicating functional relationships at levels seen for literature-based interaction maps. Finally, we validated the quality of PPIs using cell-based assays, facilitating the identification of multiple modulator proteins that influence abnormal protein aggregation, a key pathogenic event in neurodegenerative disease processes. Network analysis of NeuroNet PPIs also revealed potential "disease modules" for neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS) or Parkinson's disease (PD). Based on available interaction information, novel molecular relationships between neurodegenerative diseases were defined and modulator proteins that influence protein misfolding and aggregation in various diseases were predicted. Our studies indicate that focused interactome maps are valuable resources that enable the elucidation of common disease mechanisms and provide new targets for therapy development.

A Network Biology Approach to Therapeutics

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Network and systems biology offer a novel way of approaching therapeutics by developing models that consider the global physiological environment of protein targets, and the effects of modifying them, without losing the key molecular details. In this talk, I will discuss two recent projects developed in the lab that exploit global properties of complex systems. In particular, I will present a computational network biology strategy, based on the quantification of pathway crosstalk inhibition in therapeutic networks, to discover synergistic drug combinations for breast cancer treatment. In addition, I will show how taking a chemo-centric view of human health, which does not require detailed mechanistic information, we can build networks of human conditions able to predict disease comorbidities, as well as identifying potential drug side effects and opportunities for drug repositioning.

OMICs and Drug Discovery

SESSION CHAIR: Paul Muchowski

Tracking drugs in living cells by thermal profiling of the proteome

Gerard Drewes

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Changes in the thermal stability of proteins are frequently utilized to study ligand binding, and the recent development of the CETSA method enables the monitoring of ligand-induced temperature shifts even in living cells (Martinez-Molina et al., Science, 2013). Using quantitative mass spectrometry we determined the thermal stability of more than 7000 soluble proteins in live K562 cells, comprising the first “meltome” of a human cell (Savitski et al, Science, 2014). Treatment of cells with drugs enabled the unbiased assessment of drug-target binding by identifying proteins which show shifts in melting temperature. This approach delineated more than 50 targets for the kinase inhibitor staurosporine. Notably, we identified ferrochelatase (FECH) as a kinase inhibitor target and suggest that the photosensitivity side effect caused by the clinical drugs vemurafenib and alectinib is caused by FECH inhibition. Thermal shifts can also be observed for downstream effectors. Treatment of K562 cells with the CML drug dasatinib yielded a shift in several BCR-ABL effector proteins including CRK/CRKL, which was only observed in live cells but not in cell extracts, allowing a clear distinction from direct physical interactions. Our data indicate that the meltome can provide an unbiased measure for drug-target engagement and the identification of markers for drug efficacy as well as the large scale mapping of protein-ligand interactions.

G protein-coupled receptors: Target identification for drug discovery

Klaus Seuwen

Novartis, Basel, Switzerland

G protein-coupled receptors (GPCRs) represent one of the largest protein families encoded in mammalian genomes. GPCRs sense the environment and execute command and control functions in multicellular organisms. Their biological relevance, their localization at the cell membrane, and their accessibility for specific small molecule modulators has made these receptors targets of choice for the development of medicines. Despite intensive efforts in academia and industry ca. 100 'non-sensory' receptors that were identified through genomics research remain without a precisely annotated physiological function and without a known ligand. I'll describe strategies to identify ligands for these 'orphan receptors' and discuss specific examples where ligand identification has led to new insights in physiology.

Ubiquitination profiling reveals sensitivity factors in cancer and inflammatory diseases

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Ubiquitination is one of the most prevalent posttranslational modifications in eukaryotic cells with functional importance in protein degradation, subcellular localization and signal transduction pathways. Immunoaffinity enrichment coupled with quantitative mass spectrometry enables the in depth characterization of protein ubiquitination events at the site-specific level. We have applied this strategy to investigate global cellular responses triggered by several anti-cancer and pro-inflammatory stimuli. Temporal profiling of protein ubiquitination events across a series of time points covering the biological response permits interrogation of signaling through thousands of quantified proteins, of which only a subset display significant and physiologically meaningful regulation. In cells treated with a combination of tumor-targeting MEK and PI3K inhibitors we found differential ubiquitination of MEK within the first hour after treatment and a series of mitochondria proteins at later time points. We also profiled ubiquitination sites on thousands of proteins upon initiation of cell death by IAP antagonists in IAP antagonist-sensitive and -resistant breast cancer cell lines. Our analyses identified hundreds of proteins with elevated levels of ubiquitin-remnant (K-GG) peptides upon activation of cell death by the IAP antagonist BV6. The majority of these were observed in BV6-sensitive, but not -resistant cells. Among these were known proapoptotic regulators including cytochrome C, RIP1 and a selection of proteins known to reside in the mitochondria or regulate NF-□B signaling. For pro-inflammatory stimuli we used prominent cytokines TNF and IL-17. In the IL-17 signaling pathway, ubiquitination events on several signaling proteins including HOIL-1 and TOLLIP were observed, which allowed us to functionally implicate novel components of IL-17 mediated inflammation. Finally, TNF was examined in the context of proliferative and cell death signaling to reveal distinct pattern of protein modifications depending on the cellular context and survival outcome. Together, these data validate proteomic profiling of protein ubiquitination as a viable approach for identifying dynamic signaling components in response to anti-cancer and pro-inflammatory cellular perturbations.

Bone morphogenetic proteins in regenerative medicine

Slobodan Vukicevic

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Bone morphogenetic proteins (BMPs) are involved in the development and homeostasis of kidney, liver, joint and heart development. However, the total extent of the function/s and specificity of individual members of the BMP family is yet to be fully delineated. BMP2 and BMP7 are used in patients for bone regeneration when physiological mechanisms of bone union fail. Recently, we showed that BMP6 circulates in human plasma, increases bone volume in ovariectomized rats, accelerates fracture repair in rabbits and orchestrating glucose homeostasis via a direct role on the liver, adipocytes and pancreas. Bmp6-/- mice are hyperglycemic and have reduced circulating insulin levels, which is directly related to a decrease in the number of Langerhans islets (LI). Treatment of Bmp6-/- mice with rhBMP6 normalized blood glucose levels and increased the number of LIs. In non-obese diabetic (NOD) mice, a model of type 1 diabetes, a single injection of BMP6 reduced blood glucose for an extended period of 7 days. Similarly, treatment of ob/ob mice with BMP6 for seven days improved the glucose excursion during an OGTT, and resulted in reductions of circulating lipid levels. In vitro, BMP6 inhibited gluconeogenesis and the glucose output in hepatocytes, stimulated the insulin secretion in islets isolated from Sprague Dawley rats, and prevented the apoptosis in INS1 cells.

In parallel, BMP6 is a key endogenous regulator of hepcidin and iron metabolism. Although supraphysiologic dose of exogenous BMP7 increased the hepcidin expression and reduced the serum iron in Bmp6-/- mice, the amount of endogenous liver BMP7 following iron administration was not sufficient to substitute for the loss of BMP6.

This novel role of BMP6 in the pancreas, liver, adipocytes and the systemic glucose homeostasis suggests that BMP6 has a unique endocrine function that can be utilized for the treatment of diabetes, hemochromatosis, osteoporosis and fracture repair.

Affinity-based proteomics; personalised diagnostics and therapy optimisation with fully synthetic molecules

Jörg D. Hoheisel

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Research at the Division of Functional Genome Analysis aims at the analysis of the realisation and regulation of cellular functions from genetic information. Studies on tumour material are at the centre of attention with an emphasis on pancreatic cancer and personalised approaches. Beside the creation of knowledge, we aim at the establishment of means for early diagnosis, patient stratification, accurate prognosis, monitoring of treatment results and lead validation for new therapeutic avenues. Particularly for the last objective, we complement the molecular analyses with functional studies for the elucidation of relevant cellular mechanisms. One focus is the analysis of protein interactions at a comprehensive scale, in particular for the identification of variations, such as protein isoforms, that occur in tissues or body fluids of individual patients. We have established affinity-based processes that permit analyses on several thousand molecules simultaneously, with a robustness and reproducibility that meet the requirements of clinical applications. Another activity is the creation of a map of protein-mediated communication between the different cell types of the tumour microenvironment.
In a separate but related approach, we aim at the fully synthetic, in vitro implementation of complex biochemical processes. Motivation is their utilisation for the production of biomedically active molecules, such as non-immunogenic agents, and the establishment of entirely artificial molecular systems. Cell-free biosynthetic production will become important for many biotechnological and pharmacochemical challenges. Artificial experimental systems are meant to complement current Systems Biology. Eventually, this may lead to the establishment of a fully synthetic self-replicating system and – in the long run – an archetypical model of a cell.
www.dkfz.de/funct_genome/

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siRNA and CRISPR tools for target validation: Impact on early drug discovery

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The ability to interrogate targets in the early stages of drug discovery is necessary to improve probability of success in the clinic. At Merck, we are using RNA interference (RNAi) tools successfully across multiple disease areas for target validation, biomarker development, target de-risking and mechanism of action studies. By use of in-house developed algorithms and oligonucleotide chemical modifications, we generate siRNAs that are highly specific, stable, and non-immunostimulatory. We can also select siRNAs that are active across species with tunable durability of effect. Until recently, we focused primarily on hepatic targets using lipid nanoparticle (LNP) delivery and generating siRNAs for numerous targets with >90% success rate. We are successfully using LNP-delivered siRNA platform across preclinical species, including lean and dyslipidemic non-human primates and have shown them to be safe with dose-dependent pharmacological effects. More recently, we are leveraging CRISPR genome editing technology for functionalization of human genetic findings in cellular systems as well as to expedite generation of genetically engineered animal models. The use of state-of-the-art disruptive technologies such as RNAi and CRISPR have allowed us to optimally and rapidly interrogate target and pathway biology and reach faster decisions about our early stage discovery pipeline.

Creating a digital image of any sample in the MS2 space: Discussing data-independent acquisition strategies

Volker Kruft
AB SCIEX, Darmstadt, Germany

Recent advances in high resolution mass spectrometry, specifically QqTOF technology, have made it possible to acquire qualitative and quantitative information simultaneously from highly complex samples. The extreme speed and sensitivity of current instrumentation allows near complete analysis in information dependent (IDA) experiments. However, the concept of data independent acquisition (DIA) can now also be realistically applied for the first time. This will avoid the bias introduced by precursor selection and thus increase the reproducibility and comprehensiveness of data collection. In this data independent workflow – called MS/MS^{ALL} with SWATH™ acquisition – the Q1 quadrupole is stepped at defined mass increments across the mass range of interest, for example passing a 25 amu window into the collision cell independent of the number of precursors. Fragments – so called product ions - are analyzed in the TOF MS analyzer at high resolution. Due to the high speed of the QqTOF, these experiments can be done in a looped fashion at a cycle time compatible with LC separations. Post-acquisition MRM-like analysis can be performed on such datasets by the generation of large numbers of high resolution XIC´s. These are identified and quantified by comparison to the available proteomic or MRM spectral databases.
Recent developments of the data-independent SWATH™ workflow will be explained and compared to other quantitative techniques in systems biology like quantification via SRM/MRM. These include the introduction of variable window sizes and increasing the number of programmable windows to add even more selectivity to the workflow – as well as the automatic removal of interferences, a SWATH™ specific feature that further increases the specificity and accuracy of the quantitative results. Finally, the OneOMICS™ cloud environment, that allows fast data processing and comparison of proteomics data with genomic and metabolomic datasets in the public domain, will be introduced.
Recently published investigations will be highlighted, including studies of kidney disease, histone modifications and the proteome of *M. tubercolosis*.

OMICs & Cancer

SESSION CHAIR: Marc Vidal

Ribosome biogenesis stress and p53 regulation

Siniša Volarević

Department of Molecular Medicine and Biotechnology, University of Rijeka School of Medicine, Croatia.

Impairment of ribosome biogenesis can result in quantitative or qualitative defects in protein synthesis and consequently lead to improper execution of the genetic program and the development of specific diseases. Evidence has accumulated over the last decade suggesting that perturbation of ribosome biogenesis triggers a p53-activating checkpoint signaling pathway through inhibitory interactions of ribosomal proteins L5 and L11 with the p53 negative regulator, Mdm2. It was originally suggested that this pathway has a prominent role in preventing diseases by monitoring the fidelity of ribosome biogenesis. However, recent evidence suggests that p53 activation upon impairment of ribosome biogenesis might also be responsible for specific pathological manifestations in mammals. Our laboratory is focused on trying to understand the molecular basis of this checkpoint response and determining its role in pathogenesis of various diseases, including developmental abnormalities and cancer.

Membrane Protein Interaction Networks in Cancer

Igor Štagljar

University of Toronto,Toronto, Canada

My lab is focused specifically on understanding how the interactions of membrane proteins contribute to cellular disease states at a systems level. Despite extensive proteomics research in the past decade, there is a lack of in-depth understanding of protein networks associated with integral membrane proteins because of their unique biochemical features, enormous complexity and multiplicity. This is a major obstacle to understanding the biology of deregulation of integral membrane proteins which leads to numerous human diseases, and consequently hinders our development of improved and more targeted therapies to help treat these diseases.

To address this problem, we developed two unique technologies specifically suited for the study of full-length integral membrane proteins in their natural cellular context; the classic Membrane Yeast Two-Hybrid (MYTH) [1-5] and the newly created Mammalian Membrane Two-Hybrid (MaMTH)[6]. Our ultimate goal is to uncover a wealth of information about protein interactions for the majority of "druggable" human membrane proteins, which should in turn greatly facilitate the discovery of new truths about diseases like cancer, schizophrenia, cystic fibrosis, hypertension and Parkinson's disease.

During my talk, I will discuss our recent findings indicating that the application of MaMTH to the human Epidermal Growth Factor Receptor (EGFR) [6,7] resulted in the identification of CRK II protein as a novel interactor of oncogenic EGFR (L858R), and showed that CRKII promotes persistent activation of aberrant signaling in non-small cell lung cancer (NSCLC) cells6. I will also illustrate how MaMTH is a powerful tool for drug discovery as well as for investigating dynamic interactomes of human integral membrane proteins, and why it promises significant contributions to therapeutic research.

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Dynamics of p97 Interactome after Ionizing Radiation

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p97, also known as valosin-containing protein (VCP), is a central molecule of the ubiquitin-proteasome system, and a prominent molecular marker that is significantly over-expressed in many cancers correlated with poor prognosis and increased recurrence. However, the underlying mechanistic roles of p97 contributing to cancer biology and subsequent exploitation for cancer therapy are far from being understood. p97 is a molecular segregase implicated in many cellular pathways, whose activities are independently modulated by assembling subsets of interacting cofactors/adapters and ubiquitynated or sumoylated substrates. We have recently established an essential role of p97 in genome stability and DNA double strand break (DSB) repair after ionizing radiation (IR). Soon after cell exposure to IR p97 is recruited to sites of DSBs and by its segregase activity removes Lys-48 ubiquitinated substrates. p97- processing of Lys-48 ubiqutinated substrates at sites of DNA damage orchestrates a proper recruitment of downstream DNA repair molecules such as BRCA1, Rad51 and 53BP1. In addition, the hypersensitivity of p97-inactivated human cells to IR further implicates p97 as a potential druggable target for cancer therapy in combination with radiotherapy (synthetic lethality).

To identify p97 substrates at sites of DSBs after IR, and to better understand the role of p97 in radiation damage response we have performed quantitative proteomics (SILAC-MS), and analysed p97-interactome before and after IR. This approach isolated several p97-substrates, which dynamics are essential for DSB repair after IR, and partially uncovered how p97 promotes cell survival after radiotherapeutic dose of IR.

Systematic development of SRM-MS assays for the detection of aggressive prostate cancers

Thomas Kislinger

Princess Margaret Cancer Center, Toronto, Canada

Current prostate cancer (PCa) prognostic factors stratify patients into risk groups, but are inaccurate in predicting outcome, resulting in over-treatment of many men with indolent disease. In addition, men on active surveillance are required to undergo repeated needle biopsies, subjecting them to associated risks. A pressing need in PCa management is the development of improved prognostic factors that enable follow-up of men with low-risk disease in a non-invasive manner. We have systematically developed a proteomics strategy utilizing non-invasively collected prostate-proximal fluids, known as expressed prostatic secretions (EPS), as a novel source of PCa biomarkers. Initially, clinically annotated EPS samples were analyzed by various shotgun proteomics strategies to develop an atlas of detectable proteins. Selected candidates (232) were then systematically quantified using Selected Reaction Monitoring Mass Spectrometry (SRM-MS) in two independent cohorts of > 300 post-DRE urines (test and verification cohort). Statistical analyses have identified diagnostic and prognostic signatures for the potential use as liquid biopsies in the context of active surveillance.

DULIP: a dual luminescence-based co-immunoprecipitation assay for interactome mapping in mammalian cells

Philipp Trepte[a], Alexander Buntru[a], Konrad Klockmeier[a], Lindsay Willmore[a], Anup Arumughan[a], Martina Zenkner[a], Lydia Brusendorf[a], Christopher Secker[a] and Erich E Wanker[a]

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Mapping of protein-protein interactions (PPIs) is critical for understanding protein functions and complex biological processes. Here, we present DULIP, a dual luminescence-based co-immunoprecipitation assay, for systematic PPI mapping in mammalian cells. DULIP is a second-generation luminescence-based PPI screening technology that allows the systematic and quantitative analysis of co-immunoprecipitations using two different luciferase tags. Benchmarking studies with positive and negative PPI reference sets revealed that DULIP allows the detection of interactions with high sensitivity and specificity. Furthermore, using the well-characterized interaction between Syntaxin-1 and Munc18, we found that DULIP is capable of detecting the effects of point mutations on interaction strength. Taken together, our studies indicate that DULIP is a sensitive and reliable method of great utility for systematic interactome research. It can be applied for interaction screening as well as for the validation of PPIs in mammalian cells. Furthermore, DULIP allows the specific analysis of mutation-dependent binding patterns.

Emerging “OMICs” technologies

SESSION CHAIR: Patrick Aloy

Yeast proteome dynamics from single cell imaging and automated analysis

Brenda Andrews

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Proteomics has proved invaluable in generating large-scale quantitative data; however, the development of systems approaches for examining the proteome in vivo has lagged behind. To evaluate protein abundance and localization on a proteome scale, we exploited the yeast GFP-fusion collection in a pipeline combining automated genetics, high-throughput microscopy, and computational feature analysis. We developed an ensemble of binary classifiers to generate localization data from single-cell measurements, and constructed maps of ~3,000 proteins connected to 16 localization classes. To survey proteome dynamics in response to different chemical and genetic stimuli, we measure proteome-wide abundance and localization and identified changes over time. We analyzed >20 million cells to identify dynamic proteins that redistribute among multiple localizations in hydroxyurea, rapamycin and in an *rpd3* mutant background. Because our localization and abundance data are quantitative, they provide the opportunity for many types of comparative studies, single cell analyses, modeling and prediction.

Using systematic extracellular protein interactions screens to identify receptor-ligand interactions that are essential for cellular recognition processes

Gavin Wright

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Extracellular interactions between membrane-embedded cell surface receptor proteins play important roles in cellular recognition events that are central to many basic biological processes. Experimentally identifying these interactions is technically challenging due to the largely intractable biochemical nature of membrane proteins, as well as the weak interaction affinities that typify this class of protein interactions. We have developed methods to identify these highly transient interactions in a systematic and scalable way based around creating soluble recombinant highly avid oligomerised ectodomain probes. We have applied these approaches to several important unanswered problems of cellular recognition including the invasion of human erythrocytes by the malaria parasite, and more recently, identified the receptor-ligand pair required for mammalian fertilization.

A Neural Progenitor differentiation Patterns of Surface and secreted proteins for Cell-Replacement therapies of Neuronal Disorders

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The pluripotent cells with their ability to differentiate into various cell types, present a promising source of cells for curing many human disorders that have so far eluded medical science including spinal cord injury and neurological diseases. In order to gain comprehensive view of neural progenitor cell (NPC) differentiation, surface N-glycoproteome patterns of HUES-7 derived NPC were examined. Quantitative changes detected in cell surface protein levels reveal a set of proteins which highlight the complexity of neuronal differentiation process. Several of them including cell adhesion proteins ICAM1, CHL1, and astrotactin1 as well as LAMP1 were validated by SRM. Immunofluorescence staining of CAM1 combined with flow cytometry indicated a possible direction for future scrutiny of such proteins as targets for enrichment of neuronal subpopulation from mixed cultures after differentiation of neural precursor cells.
Additionally, cell secreted proteins, called secretome, reflecting cellular stages were investigated. Amongst the detected molecules, chemokines of CXCL and CCL families were observed and these proteins may play potential roles in the maintenance of the neural niche, differentiation of primary neural progenitor cells and regulation of synaptic transmission.
Altogether, surface and secreted proteins hold an important key for development of safe strategies in cell-replacement therapies of neuronal disorders.

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Key words: neuronal differentiation; surface N-glycoproteome; cell adhesion proteins; chemokines; growth factors.

Metabolomics as a hypothesis generator

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The omics revolution has vastly expanded our ability to capability to monitor and quantify molecular events in cells and tissues. This technological advance has typically not been matched with conceptual advances in our ability to interpret and understand such data or to systematically generate hypotheses from them. In particular the more recently developed metabolomics technologies generate data that are harder to interpret mechanistically than transcript and protein abundances because the direct connection to the genome is lost. Here I will focus on illustrating recent advances in generating hypotheses on molecular functions from high-throughput metabolomics, a technology that allows to detect 300-700 metabolites in a given sample per minute (Sevin & Sauer 2014, Nature Chem Biol 10: 266). Specifically I will cover two so far unpublished aspects: i) identifying allosteric regulation in microbial biosynthesis through dynamic, real-time metabolomics and ii) metabolic host-pathogen interactions of Mycobacterium tuberculosis during early infection in human macrophages and during latent tuberculosis of non-growing cells in granulomas.

Molecular tools for evaluation of cellular activity status

Ola Söderberg

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The activity status of a protein or signaling networks can be visualized with in situ Proximity Ligation Assays (in situ PLA) using a pair of antibodies equipped with DNA oligonucleotides (proximity probes) to target interacting proteins. Proximal binding of such probes template the creation of a circular DNA molecule, which is a surrogate marker for the interaction. I will describe how different versions of in situ PLA may be used to also analyze protein interactions, post-translational modifications and protein-DNA interactions. I will also describe a multiplexed version of in situ PLA, in which unique tags are introduced in each different proximity probe. The combinatorial events generating an in situ PLA signal will harbor a unique identifier tag for each protein interaction. By combining in situ PLA with padlock probes, analysis of signaling activity can be achieved together with genotyping expressed mRNA in fixed tissue sections, retaining the architectural information while providing single-molecule resolution. This "next generation pathology" will enable not only analysis of molecular profiles throughout a tissue section, but might be used to evaluate how cellular communications affects the cellular programs.

Utilizing osteogenic potential of BMP6 in developing new treatment options

Grgurevic L

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In patients with osteoporosis non-unions of bone following fractures still present an unmet medical need. Current BMP based bone devices use high doses of BMPs to achieve spinal fusion and long bone unions. We found that BMP6 is less sensitive to endogenous inhibitors, particularly noggin. Recently, we discovered that patient's blood coagulum could be used as a BMP6 carrier due to high binding affinity to specific blood components. The BMP6 carrier is a whole blood derived coagulum (WBCD) from the peripheral blood which acts as an endogenous biocompatible material (OSTEOGROW). More than 80% of BMP6 added to the full blood remains incorporated, bound mainly to its extracellular matrix components. Pharmacokinetic studies showed that BMP6 is rapidly cleared from the blood of mice and rats after IV dosing. Presence of BMP6 in circulation is minimal after systemic application and BMP6 is not distributed into the deep tissue compartment. Release of BMP6 from the coagulum in *in vitro* conditions showed slow discharge from the coagulum with a mean residence time of approximately 7 days. In animal models the osteogenic biological activity of newly produced BMP6 was confirmed without inducing inflammation and oedema in the surrounding tissues. In a model of critical size defect of rabbit ulna WBCD containing BMP6 fully re-bridged the bone defect at a significantly accelerated rate as compared to commercially used BMP7. We found that WBCD with 50 µg of BMP6 compared to commercially used device containing 3.5 mgs of BMP7 was 2 orders of magnitude more potent in *in vivo* rabbit ulna critical size defect. Clinical grade of BMP6 will be shortly tested clinically in two indications for regeneration of the metaphyseal bone, compartments where BMP2 and BMP7 have not been effective. Safe, affordable and non-toxic BMP6 based autologous carrier OSTEOGROW will promote faster bone healing and reduce the need for secondary interventions. This novel therapy for bone induction and acceleration of repair will significantly decrease the incidence of non-unions in osteoporotic patients following long bone fractures and successfully support spinal fusion procedures.

Mapping genetic suppression interactions on a global scale

Jolanda van Leeuwen[1], Carles Pons[2], Joseph Mellor[1], Takafumi Yamaguchi[1], Anastasia Baryshnikova[1], Michael Costanzo[1], Brenda Andrews[1], Chad Myers[2], Frederick Roth[1], and Charles Boone[1]

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Genetic suppression analysis is a powerful approach for identifying new and unexpected functional relationships between genes. Moreover, in the case of human disease genes, suppressors often represent strong candidates for therapeutic intervention. While genetic suppressors provide rich information about biological pathways, we do not understand the general principles that underlie genetic suppression networks. Here, we describe a large-scale study to identify spontaneous extragenic mutations that suppress fitness defects associated with deletion or conditional alleles in the model eukaryote, *Saccharomyces cerevisiae*. We used Synthetic Genetic Array (SGA) analysis to map the location of suppressor mutations to within ~200 kb. The identity of the suppressors is further characterized by next-generation sequencing. Thus far, we have identified and confirmed ~200 extragenic suppressor mutations, which encompass the full spectrum of adaptive mutations, including change- and gain-of-function mutations. The identified suppression interactions involve gene pairs that span a wide range of functional categories and include mutations in genes encoding mitochondrial ATPase subunits that suppress the growth defect associated with loss of mitochondrial translation. Most of the suppressor interactions we identified occur between functionally related genes and do not overlap with previously identified genetic or physical interactions. We are combining our new dataset with literature-curated suppression interaction data to construct a large-scale suppressor network and to compare the properties of the literature-curated subnetwork to the unbiased network mapped in this study. We are using the combined experimental and literature-curated networks to explore general rules that apply to genetic suppression.

OMICs & Microbes

SESSION CHAIR: Uwe Sauer

Linear Ubiquitin: at the crossroads of gene activation, cell death, inflammation and cancer

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Signalling complexes induced by immune cell receptors, including TNF receptor (TNFR), toll-like-receptor (TLR), NOD-like receptor (NLRs) and IL-1 receptor families employ phosphorylation and ubiquitination to regulate their composition and signalling output. So far the role of ubiquitin in signalling was thought to be mediated almost exclusively by K63-linked ubiquitin chains. However, we and others recently found that linear, also known as M1-linked, ubiquitin chain formation is crucial for the physiological response to various cytokines and immune stimuli including TNF, IL-1 and CD40L as well as various TLR and NLR ligands. The linear ubiquitin chain assembly complex (LUBAC), at present the only protein complex known to generate M1 linkages, consists of three components, SHARPIN, HOIL-1 and HOIP. We found that non-proficiency in linear ubiquitination resulted in decreased TNF-induced gene activation and, at the same time, increased apoptosis and necroptosis in response to TNF. Because co-deletion of TNF resulted in complete correction of the inflammatory phenotype that characterises mice deficient for SHARPIN, the cell-death-favouring deregulation of TNF signalling was responsible for inflammation. Hence, TNF-induced inflammatory disease cannot only arise as a consequence of increased gene-activatory activity of this cytokine but also when it aberrantly induces cell death. We generated a conditional knockout for the central component of LUBAC, HOIP, in mice and our most recent results on the role of linear ubiquitination in development as well as in regulating immune signalling via different receptors will be presented.

Are aging and age-related diseases phenotypes of protein damage?

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Here I review research in MedILS on biology of aging and death at cellular level. We (ref. below) find that cellular morbidity and mortality correlate with oxidative protein damage, rather than with incurred DNA damage, in standard and robust microbial and animal species. Lethal and mutagenic DNA damage remaining after repair, appears as one of many consequences of direct damage to proteome (here damage to the DNA repair proteome). Whereas short-term survival depends on a functioning proteome, the perpetuation of life requires also an intact genome to provide for a regulated proteome renewal.

We show that selective damage to the proteome generates phenotypic changes akin to those observed during aging and therefore speculate that the root cause of aging and age-related diseases is (oxidative) protein damage. We consider aging as an increasingly complex phenotypic consequence of proteome malfunction correlating with protein oxidative damage. This concept predicts that aging should be reversible upon increased protein turnover (except for acquired mutations and stable epigenetic changes). Reversibility of aging was demonstrated by heterochronic parabiosis in mice.

We present an improved method for quantitative detection of oxidative damage to individual proteins and propose that:

(i) Aging is the snowballing phenotype of oxidative damage to the proteome that accumulates exponentially with age.

(ii) Individual age-related diseases are specific phenotypes of excessive oxidative damage to particular proteins. (Most native proteins are found to be resistant to oxidation, but « silent » mutations (polymorphisms) can increase protein susceptibility to oxidative damage and become progressively phenotypic (« loud ») with age. We support this hypothesis by the study of the a-synuclein mutations predisposing to early onset Parkinson's disease.)

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S. Dukan et al., Protein oxidation in response to increased transcriptional and translational errors. PNAS 2000; 97: 5746.
D. Slade, A.B. Lindner, G. Paul and M. Radman, Recombination and replication in DNA repair of heavily irradited Deinococcus radiodurans. Cell 2009; 136: 1044.

Proteomics analyses helped to define a new antigen presentation pathway involved in the onset of Parkinson's disease

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Antigen presentation allows the immune system to distinguish self-proteins from non-self and aberrant form of proteins. In the last decade proteomics analyses have significantly contributed to the understanding of the mechanisms regulating antigen presentation. Interestingly, as it is often the case with proteomics, data led us to identify a new pathway of antigen presentation that might be implicated in the onset of Parkinson's disease. Mutations in two genes coding for proteins involved in mitophagy, Parkin and PINK1, are linked with the autosomal form of Parkinson's disease. The role played by mitophagy in the progression of this disease, however, is still under debate. In the present study we demonstrate that unlike other autophagic pathways, which have been shown to actively participate in antigen presentation, mitophagy is a poor inducer of this immune surveillance process in macrophages. We show, instead, that the presentation of mitochondrial proteins is driven by the generation of vesicles regulated by two molecules involved in vesicle formation elsewhere, sorting nexin 9 and rab9. Remarkably, inhibition of mitophagy in cells expressing lower levels of ATG5, Pink1 and Parkin promotes vesicle formation and antigen presentation, while its activation inhibits these processes. These results suggest that mitophagy provides a way by which cells can get rid of damaged mitochondria without triggering antigen presentation response, a feature that might limit autoimmune reaction. By promoting mitochondria-derived vesicle formation, alteration of the mitophagic pathway would enhance mitochondrial antigen presentation leading to the establishment of a strong T cell response that might, ultimately, participate in the destruction of dopaminergic neurons observed during Parkinson's disease.

Small molecules as probes of complexity in microbial systems

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Biologically active small molecules are finding increasing use in a research paradigm that emphasizes the value of these as probes of biology. This genre of discovery follows from the tenet that in order to understand a system we must perturb it. Despite the advantages of small molecules as probes of biology, efforts to discover and characterize the interactions of chemical compounds with complex biological systems have been narrowly focused. I will describe on-going efforts in my laboratory to discover new chemical probes of complex biology. We are working to build a repertoire of novel bioactive compounds by screening large chemical libraries using model microbes. We are further characterizing these new probes using genome-scale clone sets and highly parallel chemical-genetic interaction analyses. We are also using libraries of active compounds to learn more about uncharted networks, pathways and proteins. Finally, we have been using these approaches to exploit a rich knowledge on small molecules of known biological activity to understand the action of novel compounds.

MYCN Integrative Omics Enables Network-Based Therapeutic Target Discovery and Patient Stratification in Neuroblastoma

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Introduction: Neuroblastoma is the most common extracranial childhood cancer and is primarily diagnosed in the first year of life. It is responsible for more deaths annually than any other paediatric cancer. MYCN gene amplification occurs in 20% of cases, and is the primary driver of poor outcome. MYCN, being a transcription factor, has proved difficult to therapeutically target. In addition, recurrent somatic mutations are rare in neuroblastoma. This means that current therapeutic strategies and classical genetic approaches which rely on frequently altered oncogenic drivers to identify novel therapeutic options have been largely unsuccessful. Therefore, there exists an urgent clinical need to identify novel therapeutic options for high-risk neuroblastoma.

Methodology: In order to overcome this difficulty and to address oncogenic MYCN functioning we employed a variety of omic technologies: standard- and 4sU-RNA-seq, miRNA-seq, ChIP-

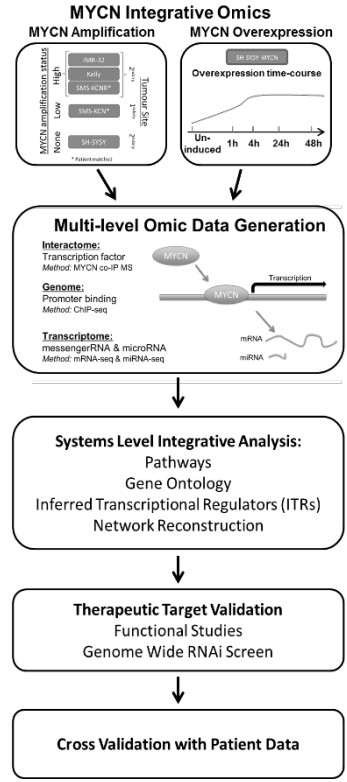

Figure 1. Schematic outline of the experimental and analysis approach.

seq and interaction proteomics (Fig. 1). We examined a number of biological conditions MYCN overexpression, MYCN amplification, therapy induced differentiation and inhibitor mediated cell death.

Results: By mapping and integrating MYCN regulated gene and protein networks we identified vulnerable nodes amenable to therapeutic interventions. These were validated by siRNA knockdown screens, functional studies and patient data. In addition, the response of these targets to induction of differentiation or apoptosis (further omics) was assessed. We focused our validation on MAPK, Estrogen and Wnt/β-catenin signaling pathways, as druggable vulnerabilities of MYCN-amplified neuroblastoma.

Summary: We describe a systems-level approach to systematically uncovering network-based vulnerabilities and therapeutic targets by integrating disparate but complementary omic data types. We employed this approach to identify existing small molecule therapeutics with novel potential for high-risk neuroblastoma treatment.

INTEGRATED METABOLIC MODELLING REVEALS CELL-TYPE SPECIFIC CONTROL POINTS OF THE MACROPHAGE METABOLIC NETWORK

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Fast reconstruction of cell type-specific metabolic models and understanding the epigenetic regulatory mechanisms underlying the observed differences will be increasingly important at the age of personalized medicine. To enable fast creation of high-quality metabolic models from gene expression data, we have developed a new workflow named FASTCORMICS. It is devoid of heuristic parameter settings, has a low computational demand and outperforms its competitors in speed and accuracy. Applying FASTCORMICS, we have generated metabolic models for 63 primary human cell types based on microarray data, thus revealing significant differences between metabolic networks of the different cell types. To better understand the cell type-specific regulation of the alternative metabolic pathways we built multiple microarray-based metabolic models at different time points of differentiation of primary human monocytes to macrophages and performed ChIP-Seq experiments in macrophages for histone H3 K27 acetylation (H3K27ac) in order to map the active enhancers. Focusing on the metabolic genes under high regulatory load from multiple strong enhancers, we identified these genes to show the most cell type-restricted and abundant expression profiles within their respective metabolic pathways. Importantly, the high regulatory load genes are associated to reactions significantly enriched for transport reactions and other pathway entry points, suggesting that they are the critical regulatory control points for cell type-specific metabolism. Finally, we provide examples of selected macrophage-specific pathways and their regulators.

Patient stratification beyond individual genes: Glycans as integrators of genes and environment

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Glycosylation is an essential posttranslational modification [1] generated by a complex biosynthetic pathway comprising hundreds of glycosyltransferases, glycosidases, transcriptional factors, ion channels and other proteins [2]. This process results in the creation of branched oligosaccharide chains, called glycans, which become integral part of proteins and significantly contribute to their structure and function [3]. Since glycans are created without the genetic template, alternative glycosylation creates an additional layer of protein complexity by combining genetic variability with past and present environmental factors[4]. Individual variability in glycome composition is very large [5, 6, 7], but glycosylation of an individual protein seems to be under strong genetic influence, with the heritability of the IgG glycome being up to 80%[8]. Structural details of the attached glycans are of great physiological significance and many pathological conditions are associated with various types of glycan changes [9, 10, 11]. Since the onset of genome wide association studies (GWAS), thousands of genetic loci have been associated with different diseases and traits. However, in the last few years, and particularly after recent publication of the results from the ENCODE project, it is becoming increasingly clear that GWAS studies are only a beginning of the understanding of complex human diseases. Hypotheses generated in these studies have to be put in the context of complex biology of life and a more elaborate approach that combines different 'omics phenotypes is needed to understand disease mechanisms and perform patient stratification that transcends genomics. Glycomics, as by far the most complex epiproteomic modification, has an immense potential in this respect, which is only beginning to be investigated [12, 13, 14]

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Addressing the challenges of integrating data in the multi-omics laboratory

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The acquisition of large data sets from multiple platforms and analytical techniques in the multi-omics laboratory provides a major challenge in the interpretation of experimental outcomes. For example, non-targeted metabolomics requires the analysis of many biological replicates in order to introduce enough biological variance to obtain statistically significant results. The data from these replicates is further multiplied through the requirement for different analytical conditions to ensure maximum coverage of the metabolome. To address these challenges, software has been developed to allow the reduction, integration and statistical analysis of large data sets. Although this provides some insight into the statistical outcome of the study, it does not provide an understanding of the changes in the biological system. This final step may be achieved through visualization following mapping of results onto biological pathways. In this presentation, an overview of the workflow with a practical example of integration of transcriptomic and metabolomic data sets is shown, together with visualization of results after mapping to the relevant biological pathways.

Integrated genome and proteome-wide analysis reveals organ-specific proteome deterioration during aging in rat

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Aging is a multifaceted process that is associated with the decline of cell and organ function. How molecular events occurring at the genomic and proteomic levels integrate within complex biological systems to cause tissue-specific deterioration remains poorly understood. Here we measured changes in transcript abundance and translation rates by RNA sequencing and ribosome profiling, and determined alterations of protein abundance and post-translation modifications by shotgun proteomics, in the brain and liver of aging rats. We found that the majority of age-related changes in protein abundances are a consequence of altered translation output. However, we also identified changes in protein localization, phosphorylation, and alternative splicing to affect proteome integrity in old cells. The integration of different types of system-wide data allowed us to identify common as well as organ-specific alterations during aging, such as those related to neuronal communication and protein kinases in brain, and modification of metabolic processes in liver.

CLOSING KEYNOTE

INTRODUCTION of the Speaker by Miroslav Radman

Proteomic and functional genomic dissection of protein quality control in the endoplasmic reticulum

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The ERAD system recognizes and destroys a highly diverse array of secreted and integral membrane proteins including folding-defective mutants, unassembled subunits of oligomeric complexes and correctly folded ER resident proteins that are turned-over in response to metabolic signals. How the ERAD system can effectively degrade these diverse substrates while sparing folding-proficient proteins and stable ER-resident components is not well understood, but requires molecular chaperones and progressive trimming of N-linked glycans followed by recognition of these modified sugars by dedicated ERAD lectins. Substrates that become committed to ERAD must be reduced and dislocated across the ER membrane to the cytoplasm where they become de-glycosylated, ubiquitylated, and ultimately degraded by the proteasome. The ERAD system thus is comprised of a complex network of proteins that exist in three subcellular compartments ER lumen, membrane and cytoplasm. Although genetic studies in yeast have identified a set of ER and cytoplasmically localized proteins required for destroying misfolded or unassembled proteins, the ERAD system in mammalian cells is far more complex and appears to be organized into different functional modules We have undertaken to construct a comprehensive map of the ERAD system in mammalian cells using a combination of proteomic and functional genomic analyses. I will discuss the functional and dynamic organization of the mammalian ERAD network and present a novel genetic strategy that uses retrotranslocated toxins in order to generate an ERAD genetic interaction map..

POSTER SESSION

Proteomic Data From Human Cell Cultures Refine Mechanisms of Chaperone-Mediated Protein homeostasis

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Cellular functions are overwhelmingly carried by proteins and quantitative informations from proteomics, on the true physiological concentrations and ratios between polypeptides in cells, are of great value to experimental biology and medicine. Mass spectrometry (MS) analysis coupled with stable isotope labeling with amino acids in cell culture (SILAC) has become a method of choice to analyze the human proteome. In unstressed cells, the members of the chaperone network contributes up to 10.4% of the total protein mass (Finka and Goloubinoff 2013). Molar ratios between the most prominent members of the Hsp60, Hsp70, sHSPs and Hsp90 chaperone families and their main co-chaperones provided new insights on their molecular mechanisms in the cytosol, mitochondria and ER lumen of human cells. Quantitative high-throughput proteomics showed that during a mild four hours heat shock at 41°C, many house-keeping proteins, such as ribosomal proteins, become mildly degraded in an across-the-board manner, summing up into a net protein mass loss of 2%. Reciprocally, only a few HSPs become massively synthetized, summing up into an equal, net protein mass gain of 2%. When protein copy numbers after the heat-shock were classically expressed in relative “fold change” values, the heat-inducible isoform Hsp70 (HSPA1A) was expectedly 2.7 fold more abundant than before the heat-shock, whereas the presumably non-heat-inducible isoform Hsc70 (HSPA8) was only 1.08 fold more abundant than before the heat-shock (Finka et al. 2015). However, in terms of net copy numbers, the heat-treated cells contained only 15’000 more Hsp70 molecules, as compared to 1’300’000 additional Hsc70 molecules, implying that under heat-shock, each cell generated 86 times more non-heat-inducible Hsc70s than bona fide heat-inducible Hsp70s molecules. This strongly argues that in the case of protein and mRNA copy numbers, absolute quantitative values should be used to describe cellular concentrations and differences between conditions, rather than “fold changes”, which are less informative and may be plainly misleading.

Finka A, Goloubinoff P (2013) Cell Stress and Chaperones 18 (5):591-605. doi:10.1007/s12192-013-0413-3
Finka A, Sood V, Quadroni M, Rios P, Goloubinoff P (2015) Cell Stress and Chaperones:1-16. doi:10.1007/s12192-015-0583-2

Targeting the "SLC-ome"

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The SoLute Carrier proteins (SLCs), with some 400 members, constitute the major group of membrane transporters *(Schlessinger, Matsson et al. 2010; Hediger, Clemencon et al. 2013)* and the second largest gene family in the human genome *(Hoglund, Nordstrom et al. 2011)*. They are responsible of essential physiological functions such as nutrient uptake, waste removal, and intracellular pH, volume and ion homeostasis, and therefore play a central role in cellular metabolism. SLCs are further highly relevant for pharmacology, with several families being implicated in drug transport (including SLC22, SLCO, SLC47, SLC35) *(Nigam 2015)*, at least 26 different SLCs being direct targets of known drugs or drugs in development *(Rask-Andersen, Masuram et al. 2013)*, and several examples mediating drug-drug and nutrient-drug interactions *(Zhang, Zhang et al. 2014)*. Finally, about a fourth of SLC genes have already been associated with disease *(Amberger, Bocchini et al. 2015)*. Despite ample evidence for their druggability, more than three fourths of SLCs with an already identified disease link have no active compounds associated with them.

Yet, in spite of their importance, the majority of SLCs is functionally uncharacterized and no systematic, integrative studies have been reported. Analysis of the literature reveals that SLCs are the group of genes in the human genome with the largest publication asymmetry, even when compared with other membrane protein or transporter groups. While some SLC members have thousands of publications, most of them are barely studied, making SLCs the most neglected gene family in the genome. The interrogation of publicly available large datasets coming from high-throughput technologies makes it possible to undertake a systems-level analysis of SLCs that can provide a clearer insight into the coordinated function and interdependencies of these proteins within the

functional networks of the cell. Understanding how different conditions, such as a disease, or external stimuli, such as a drug treatment, relate to the environment-dependent plasticity of the SLC circuitry is of extraordinary importance in order to explore metabolic vulnerabilities which can be worth targeting in the context of diseases such as cancer. Integration of different "omics" to study the "SLC-ome" can thus help to untangle the relationship between SLC expression, metabolism, and drug sensitivity.

Protein profiling of extracellular vesicles upon NPC1-dysfunction and cholesterol accumulation Implications in Alzheimer' s disease

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Cells release into the extracellular environment diverse types of membrane vesicles of endosomal and plasma membrane origin called exosomes and microvesicles, respectively. These extracellular vesicles (EVs) represent an important mode of intercellular communication by serving as vehicles for transfer between cells of membrane and cytosolic proteins, lipids, and RNA. EVs have been suggested as potential carriers in the intercellular delivery of misfolded proteins associated to neurodegenerative disorders, supporting their role in spreading of the disease throughout the brain tissue. Exosome secretion was found to be increased in a rare inherited neurodegenerative disorder Niemann-Pick type C (NPC) [1] that shares several similarities with Alzheimer's disease (AD). **The goal of this work is to identify molecular details of increased exosome secretion in NPC.**

NPC is caused by mutations in either *NPC1* or *NPC2* genes, that code for cholesterol transport proteins involved in the egress of free, unesterified cholesterol from late endosomal/lysosomal compartments. Increased exosome secretion in NPC could serve as a rescue mechanism against intracellular cholesterol accumulation and neurodegeneration. So far in our studies we have been using NPC cellular models as well as NPC mice

(*NPC1*$^{-/-}$) to underpin molecular mechanisms of an AD-like phenotype in NPC disease [2-4].

In this study we show an increased secretion of EVs by CHO-*NPC1*-null (NPC) cells compared to CHOwt cells as well as in human neuroblastoma SH-SY5Y cells treated with U18666A-compound, an inhibitor of cholesterol trafficking commonly used to mimic NPC disease. EVs were pelleted by differential centrifugation at 100.000 x g and were further processed by a differential sucrose densitiy gradient ultracentrifugation. Protein profiling of exosome fractions was performed using MALDI-TOF MS..

In conclusion, according to our results, impairment of cholesterol metabolism causes an increase in the secretion of extracellular vesicles (mainly exosomes) and an alteration in exosomal protein composition. This effect suggests a link between lipid metabolism, extracellular vesicle secretion and spreading of neurodegenerative disorders throughout the brain tissue that will be further characterised by extensive proteomic and lipidomic analysis.

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2-Kosicek et al. *Biochem. Biophys. Res. Commun.* (2010) 393(3):404-9
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Expression biomarkers of Crohn's disease: characterization of inflammatory and apoptotic molecular markers

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Crohn's disease (CD) remains an expanding global health problem, particularly in most Caucasian populations, including Serbian. Intestinal mucosal damage, the hallmark of CD, occurs as a result of the deregulation of inflammatory and apoptotic processes, which influence T cell longevity and epithelial barrier integrity. Transcriptional factor NF-κB has a key role in the transcription of genes that mediate these events. The

aim of this study was to elucidate molecular patterns that underlie these processes and to define potential diagnostic biomarkers of CD.

We analyzed proinflammatory *IL-6* and *TNF-α*, and apoptotic *Bcl2*, *Bax*, *Fas* and *FasL* mRNA levels in intestinal mucosa, as well as in the peripheral blood mononuclear cells of 24 CD patients and 21 controls using qRT-PCR methodology. Additionally, among these participants, we selected 10 CD patients and 5 controls in order to conduct EMSA analysis of the DNA binding activity of NF-κB from the nuclear extracts of donors' intestinal mucosal samples.

Results of the expression analyses have shown that levels of *IL-6* and *TNF-α* mRNAs were significantly increased, while level of *Bcl2* mRNA was significantly decreased in ileal inflamed mucosa of CD patients compared with healthy mucosa of controls. Our results also revealed that the level of *FasL* mRNA in peripheral blood mononuclear cells of CD patients was significantly decreased compared with controls, but only in male patients. In addition, levels of intestinal *IL-6* and *Bcl2* mRNAs, as well as *Fas*/*FasL* mRNA ratio in the blood samples of male patients had predictive significances and therefore, they could be used as diagnostic biomarkers of the CD active phase.

The analysis of the DNA binding activity of NF-κB from the nuclear extracts of CD patients' intestinal mucosal samples revealed an association of decreased level of NF-κB binding activity and increased level of *TNF-α* mRNA with intestinal mucosal fragility. In contrast, increased level of NF-κB binding activity and decreased level of *TNF-α* mRNA were associated with intestinal strictures. Therefore, we propose the status of NF-κB binding activity as a marker of apoptotic and inflammatory processes that cause these two types of CD clinical phenotypes.

Our results demonstrated that the expression profiles of selected proinflammatory and apoptotic genes, as well as the DNA binding activity status of one of the main transcription factors that is involved in the expression of these genes are signatures of active CD and can be considered as potential CD diagnostic and prognostic biomarkers.

Acknowledgements

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Pharmacogenomics of childhood acute lymphoblastic leukemia

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Childhood acute lymphoblastic leukemia (ALL) represents one of the best examples of progress in disease treatment based upon the incorporation of the principles of pharmacogenomics. Administering of mercaptopurine (6-MP) in patients with variants in the genes involved in the drug metabolism leads to severe hematologic toxicity.

We have investigated genetic variants of TPMT, ITPA, MDR1 and MRP4 genes, relevant for metabolism of mercaptopurine drugs in 150 Serbian childhood ALL patients and 100 healthy controls. We detected genetic variants using PCR and sequencing methodology. TPMT gene expression and 6-MP toxicity in vitro and in vivo was analyzed using functional CAT and Real Time PCR assays.

We showed that genetic variants in TPMT exons accounted for 7.5%. Our research points out that promoter VNTR region of TPMT gene is a new candidate pharmacogenomic marker. The therapy for pediatric ALL patients with these genetic markers was modified, which contributed to the efficiency of treatment. Administering reduced 6-MP dosages in the initial phase of maintenance, allowed TPMT-deficient and heterozygous patients to later receive full protocol doses of 6-MP. Study of TPMT gene expression in childhood ALL patients before and after administering 6-MP therapy, revealed that 6-MP has a positive effect on transcription of TPMT gene. Increase of median 280% in TPMT gene transcription was detected in the maintenance phase of therapy, Analysis of variants in ITPA, MDR1, MRP4 genes indicates their pharmacogenomics potential.

Acknowledgements

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Genetic Analysis of the ERAD Network using Ribosome-Inactivating Toxins

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The ER-associated degradation (ERAD) system identifies terminally misfolded polypeptides in the ER, dislocates these polypeptides across the ER membrane, and targets them for degradation in the cytosol by the ubiquitin-proteasome system (UPS). Degradation of misfolded secretory proteins requires the coordinated action of ERAD machinery in three spatially separated cellular compartments - the ER lumen, membrane, and cytosol. Recent proteomic and genetic studies suggest that ERAD is composed of large, membrane-spanning multisubunit protein complexes that form a flexible and dynamic network capable of accommodating a broad range of substrates and cellular environments. However, the functional organization of this complex molecular network is poorly understood.

We have exploited the ribosome inactivating A chain (RTA) of the plant cytotoxin ricin as a selective agent to perform functional genetic analysis of the ERAD system in *Saccharomyces cerevisiae*. Ricin is composed of two subunits (RTA and RTB) linked by a single disulfide bond. In the normal route of intoxication, RTB binds to sugar moities on the surface of the target cell to initiate endocytic uptake of the toxin. Internalized ricin trafficks through the secretory system in a retrograde direction to the ER, where RTA and RTB are separated through reduction of the disulfide bond and free A chain escapes into the cytoplasm by exploiting ERAD machinery. Once in the cytoplasm the A chain kills cells by inactivating ribosomes. To enable genetic analysis of the ERAD system, we have expressed an ER-targeted variant of RTA, or ssRTA, under the control of a galactose-inducible promoter. This strain grows normally in a non-inducing carbon source but is nonviable in galactose-containing media. To identify genes required for RTA dislocation, we screened the yeast homozygous diploid and non-essential mutant libraries and a partial library of yeast temperature-sensitive mutants for growth in the presence of induced ssRTA expression. This selection identified many known ERAD components, including Hrd1, Hrd3, Der1, Ubc7, and Usa1, as well as several genes not previously implicated in ERAD. We are currently characterizing screen hits using traditional biochemical approaches and genetic interaction (GI) analysis.

The RTA selection performed in budding yeast strongly supports the feasibility of applying genome-wide tools to probe ERAD function in metazoans. We are adapting this strategy to study the ERAD system in mammalian cells by expressing an ER-targeted variant of RTA (ssRTA) under the control of a doxycycline-inducible promoter. Genes and GIs involved in ssRTA toxicity will be identified through high-throughput screening of ultracomplex genome-wide shRNA and sgRNA libraries. This information will be used to construct the first GI network map of the ERAD system.

Distribution of protein poly(ADP-ribosyl)ation systems across all domains of life

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Poly(ADP-ribosyl)ation is a post-translational modification of proteins involved in regulation of many cellular pathways. Poly(ADP-ribose) (PAR) consists of chains of repeating ADP-ribose nucleotide units and is synthesized by the family of enzymes called poly(ADP-ribose) polymerases (PARPs). This modification can be removed by the hydrolytic action of poly(ADP-ribose) glycohydrolase (PARG) and ADP-ribosylhydrolase 3 (ARH3). Hydrolytic activity of macrodomain proteins (MacroD1, MacroD2 and TARG1) is responsible for the removal of terminal ADP-ribose unit and for complete reversion of protein ADP-ribosylation. Poly(ADP-ribosyl)ation is widely utilized in eukaryotes and PARPs are present in representatives from all six major eukaryotic supergroups, with only a small number of eukaryotic species that do not possess PARP genes. The last common ancestor of all eukaryotes possessed at least five types of PARP proteins that include both mono and poly(ADP-ribosyl) transferases. Distribution of PARGs strictly follows the distribution of PARP

proteins in eukaryotic species. At least one of the macrodomain proteins that hydrolyse terminal ADP-ribose is also always present. Therefore, we can presume that the last common ancestor of all eukaryotes possessed a fully functional and reversible PAR metabolism and that PAR signalling provided the conditions essential for survival of the ancestral eukaryote in its ancient environment. PARP proteins are far less prevalent in bacteria and were probably gained through horizontal gene transfer. Only eleven bacterial species possess all proteins essential for a functional PAR metabolism, although it is not known whether PAR metabolism is truly functional in bacteria. Several dsDNA viruses also possess PARP homologues, while no PARP proteins have been identified in any archaeal genome. Our analysis of the distribution of enzymes involved in PAR metabolism provides insight into the evolution of these important signalling systems, as well as providing the basis for selection of the appropriate genetic model organisms to study the physiology of the specific human PARP proteins.

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High resolution RNAseq profiling of primary human endothelial cells – resource for regenerative medicine target identification and validation

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Vertebrate organisms developed an elaborate system that enables transport of nutrients, gases, cells and metabolites throughout the body - the task carried out by the blood vascular and the lymphatic vascular systems. Both vascular networks are comprised of vessels lined by endothelial cells with differences in gene expression profiles underlying the differences in functional roles of these two systems. Substantial

remodeling of blood and lymphatic vasculature (lymph/angiogenesis) is a hallmark of various inflammatory disorders as well as cancer, and it is a requirement for tissue regeneration *(1)*. Therefore, the ability to influence the growth and function of blood and lymphatic vessels should have significant therapeutic benefits.

We used RNA sequencing to profile the transcriptome of primary human adult dermal blood and lymphatic endothelial cells stimulated with a pro-angiogenic cocktail of factors at different time points. Out of an average 139 million reads per sample from a paired-end sequencing assay, approximately 80-90% of the reads were aligned to over 16,000 genes in the reference human genome. Differentially expressed genes were identified using the limma voom method developed by Law *et al (2).* The analysis enabled a distinction between common genes and pathways, and highlighted novel cellular mechanisms required for both vascular systems. While 25% of genes regulated by the pro-angiogenic stimulation are in common to the two cell types, a high proportion of the differentially expressed genes are unique, confirming the distinct functional roles of both vascular systems.

A phenotypic lymphangiogenesis screen was also performed with well-studied, well-annotated LMW compounds. This allowed us to incorporate the molecular targets of the hit compounds with the transcriptome profiling data, further corroborating our findings through an integrated chemical biology analysis.

Further in-depth analysis of the transcriptional regulation reported in this study will shed light on molecular mechanisms underlying the lymph/angiogenesis process and provide new potential therapeutic targets.

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MAPPIT and KISS, two complementary methods to detect protein-protein and protein-small molecule interactions in living mammalian cells

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For the detection and study of protein-protein interactions (PPIs) in living mammalian cells we developed two complementary technologies, MAPPIT (MAmmalian PPI Trap) and KISS (KInase Substrate Sensor). Both are based on cytokine receptor signaling. While in MAPPIT the bait is fused to a signaling-deficient receptor, in KISS the bait is coupled to a tyrosine kinase. In both cases the prey is fused to a receptor chain domain containing STAT recruitment motifs. Upon bait-prey interaction endogenous STAT molecules are activated and translocate to the nucleus to initiate reporter gene expression.

Similar to MAPPIT, KISS can detect 34% of a positive reference set with a threshold allowing a 1% random reference pairs detection rate. 10% is detected only by either of the two methods, revealing their complementarity. While the advantage of MAPPIT is that the read-out is ligand-dependent facilitating an extra control, in KISS the bait-prey interaction can occur at its physiological-relevant subcellular localization and it enables the use of full-length transmembrane proteins.

To increase the screening throughput of both assays, a library of 15.000 preys was generated using the CCSB human ORFeome collection. These preys are spotted on micro-array slides in triplicate and a fluorescent reporter gene enables an easy and fast read-out. Comparison with the classical luciferase read-out revealed an identical sensitivity.

Variants of both methods were developed to allow the study of protein-small molecule interactions. Here, the small molecule of interest is fused to methotrexate via a PEG linker and will bind to DHFR which is coupled to the tyrosine kinase or signaling deficient receptor. While the original methods can be used to discover compounds that inhibit PPIs, these variants allow the study of mechanism of action of the compounds and can reveal off-target effects.

Acknowledgements
We thank the group of Marc Vidal (CCSB, Boston, Massachusetts 02115) for providing the human ORFeome8.1 collection and their assistance in generating the prey library.

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GENE VARIANTS IN *PTEN* AND *SMO* MIGHT INTERFERE WITH THE OUTCOME IN PATIENTS WITH PRIMARY DIFFUSE LARGE B CELL LYMPHOMA OF CENTRAL NERVOUS SYSTEM: THE NEXT GENERATION SEQUENCING ANALYSES

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Our purpose was to identify the genetic changes involved in primary central nervous system lymphoma (PCNSL) oncogenesis through next generation sequencing analyzes (NGS) and evaluate their clinical relevance. We investigated a series of 19 (12F/7M) newly diagnosed, HIV-negative, PCNSL patients using the MiSeq and TruSeq Amplicon Cancer Panel (TSCAP) for 48 cancer-related genes. Obtained molecular results were correlated with prognosis. The median overall survival (OS) was 41 months while the median event free survival (EFS) was 36 months. Overall treatment response was achieved in 16 patients (84.2%). After total tumor resection, median OS was not reached, comparing to partial tumor resection or biopsy only (Log Rank χ^2=4.34, p=0.037) when median OS was 28 months. NGS analyses have revealed that over 80% of potentially protein-changing mutations were detected in 8 genes: *CTNNB1, PIK3CA, PTEN, ATM, KRAS*, *PTPN11*, *TP53* and *JAK3*, pointing to potential role of these genes in lymphomagenesis. *TP53* was the only gene harboring mutations in all 19 PCNSL patients, while the presence of mutated *ATM*

gene correlated with higher total number of mutations in other analyzed genes. Moreover, mutated *PTEN* and *SMO* genes were in correlation (Fisher's Exact Test p<0,045 and p<0,018, respectively) with earlier disease relapse. *TP53* and *ATM* genes could be involved in molecular pathophysiology of PCNSL whereas mutations in *PTEN* and *SMO* genes might be of importance for the disease outcome regardless the initial treatment.

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The Mammalian Membrane Two-Hybrid (MaMTH): Principles and Applications

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Membrane proteins comprise nearly 30% of the proteome of most organisms and are involved in a diverse range of important cellular functions. Dysfunction of membrane proteins is responsible for many diseases, including cancer, cardiovascular disease, 'channelopathies' such as cystic fibrosis, and peroxisomal disorders, among others, making them targets of great therapeutic relevance to human health.

Understanding the mechanisms associated with membrane protein function is critically dependent on knowledge of their physical interactions and complex organization. Unfortunately, the unique biochemical properties and complexity of this class of proteins make them notoriously difficult to study in a high-throughput format using traditional methods, limiting our knowledge of their detailed molecular roles and hindering our ability to develop new targeted therapies.

To help overcome the difficulties associated with the study of mammalian integral membrane proteins, our lab recently developed a powerful new genetic-based assay called the Mammalian Membrane Two-Hybrid (MaMTH). MaMTH allows the high-throughput mapping and

characterization of mammalian membrane protein interactions directly in the native context of the mammalian cell. Additionally, the assay allows the highly sensitive detection and monitoring of 'dynamic' protein-protein interactions which change in response to conditions such as phosphorylation state, mutation and the presence or absence of various hormones, agonists and inhibitors.

Here we describe the principles of MaMTH, highlighting the latest improvements and modifications to the technology, and its adaptation for use in large-scale small molecule and library screening. We also demonstrate the direct application of the MaMTH technology to screen for interactions of human ABC transporters, an important class of human integral membrane proteins with key roles in human disease.

MaMTH represents a significant advance in biomedical research, allowing high-throughput screening of full-length membrane proteins in human cells under multiple conditions for comparison, and provides a much-needed solution to a known technology barrier associated with the study of membrane proteins.

Molecular characterization of mutations in Serbian patients with glycogen storage diseases – an NGS approach

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Glycogen storage diseases (GSD) are group of rare metabolic monogenic diseases. They include defects in glycogen degradation, glycolysis, glucose release and glycogen synthesis, therefore having heterogeneous genetic base. Symptoms in patients vary based on the enzyme that is deficient, and usually nine different types of GSD are recognized. GSD Ia and GSD Ib, the most frequent types, are characterized by accumulation of glycogen and fat in the liver and kidneys. Neutropenia is additional symptom of GSD Ib.

In this study, we analyzed 16 Serbian patients diagnosed as GSD according to biochemical data and clinical symptoms. We used Sanger sequencing for targeted analysis of *G6PC* and *SLC37A4* genes. We identified c.247C>T and c.441T>C mutations in *G6PC* gene of two patients, on 3 and 1 alleles respectively. Interestingly, in majority of patients mutations in *SLC37A4* gene were found: c.1042_1043delCT (66.7%) and c.81T>A (33.3%). Further, we used an NGS panel with 4813 genes to detect disease-causing mutations in two patients with ambiguous symptoms. In the first patient, mutations in *AGL* gene (c.655A>G and c.3980G>A) indicated GSD III type. Surprisingly, in the second patient with characteristic neutropenia, we detected mutations in *SBDS* gene (c.258+2T>C and c.184A>T) responsible for Shwachman-Diamond syndrome.

This study provided the first data about molecular genetics of Serbian patients presenting with GSD clinical symptoms, thus enabling molecular genetic diagnostics and genetic counseling of this disease in the country. Furthermore, reaching 100% of mutation detection by high-throughput resequencing approach, set the base for research and clinical utility of NGS in Serbia.

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Mutated HUWE1 Contributes to an Impaired DNA Repair in X-linked Intellectual Disability

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X-linked intellectual disability (XLID) is a clinically complex and genetically heterogeneous disease that originates from mutations along the X chromosome. A recent screen revealed two missense mutations and a submicroscopic duplication of the *HECT, UBA and WWE domain-containing 1* (*HUWE1*) gene in fifteen independent XLID families [1]. *HUWE1* encodes an E3 ubiquitin ligase regulating different DNA repair proteins, including DNA polymerases (Pols) β and λ as well as the DNA glycosylase MutY homolog (MutYH), by mediating their proteasomal degradation. The inability to repair oxidative DNA lesions and their subsequent accumulation in the genome contributes to the onset of neurological disorders [2]. Though mutations in *HUWE1* are clearly associated with XLID, the underlying pathomechanism by which mutated HUWE1 contributes to the development of this disease remains unknown. Analysis of genomic instability in the XLID patient cells revealed an increased mutation frequency, hypersensitivity to oxidative stress and a decreased DNA repair capacity. Screening for proteins involved in the DNA damage response demonstrated an XLID-specific down-regulation of the important base excision repair (BER) protein Pol λ in mutant cells. Pol λ plays a major role in the repair of the frequent oxidative lesion 7,8-dihydro-8-oxo-guanine (8-oxo-G), thus counteracting transversion mutations [3]. Ubiquitination experiments with mutated HUWE1 showed an increased ubiquitination activity toward Pol λ that potentially results in overshooting proteasomal degradation and lower Pol λ levels in XLID cells. Together, our findings suggest that inefficient BER of oxidative DNA damage and subsequent accumulation of mutations could contribute to HUWE1-driven XLID.

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Maintenance therapy of childhood ALL patients induces *TPMT* gene expression in VNTR dependent manner

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Thiopurine S-methyltransferase (TPMT) is the most important enzyme involved in metabolism of thiopurine drugs, such as 6-mercaptopurine (6-MP). Great variability of TPMT activity cannot be explained considering just coding region variations. Variation of regulatory elements, such as VNTR architecture (i.e. number and types of repeats) in promoter, as well as chemotherapy drugs are shown to influence TPMT enzyme activity. However, it is not known whether these factors influence TPMT gene expression in ALL patients.

In this study, for the first time, TPMT gene expression was measured in hematopoietic tissue of 57 childhood ALL patients, both before chemotherapy and during the maintenance therapy when ALL patients are given 6-MP and methotrexate.

Our results show that maintenance therapy strongly induces TPMT expression, more than 3 times, on average. For each ALL patient, TPMT expression was higher during maintenance therapy, than before chemotherapy ($p<10\text{-}10$). An interaction of maintenance therapy with VNTR region in TPMT promoter modified TPMT gene expression. Specifically, VNTR*5a/*5a carriers were found to be the highest expressers during the therapy ($p=0.045$), even though they were low expressers before chemotherapy. Our results confirm negative correlation between “A” repeats number of VNTR and TPMT gene expression ($rs=-0.35$).

In conclusion, maintenance therapy strongly induces TPMT expression. This effect is modified by architecture of VNTR region of TPMT gene. It could be of great importance to consider TPMT genetic variations at the very beginning of the maintenance therapy for childhood ALL patients, especially for carriers of less expressed VNTR alleles.

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Systematic Interaction Network Filtering identifies CRMP1 as a Novel Suppressor of Huntingtin Misfolding and Neurotoxicity

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Assemblies of huntingtin (HTT) fragments with expanded polyglutamine (polyQ) tracts are a pathological hallmark of Huntington's disease (HD). The molecular mechanisms by which these structures are formed and cause neuronal dysfunction and toxicity are poorly understood. Here, we utilized available gene expression datasets of selected brain regions of HD patients and controls for systematic interaction network filtering in order to predict disease-relevant, brain region-specific HTT interaction partners. Starting from a large protein-protein interaction (PPI) data set, a step-by-step computational filtering strategy facilitated the generation of a focused PPI network that directly or indirectly connects 13 proteins potentially dysregulated in HD with the disease protein HTT. This network enabled the discovery of the neuron-specific protein CRMP1 that targets aggregation-prone, N-terminal HTT fragments and suppresses their spontaneous self-assembly into proteotoxic structures in various models of HD. Experimental validation indicates that our network filtering procedure provides a simple but powerful strategy to identify disease-relevant proteins that influence misfolding and aggregation of polyQ disease proteins.

Proteomic analysis of a multidrug-resistant and β-lactamases-producing environmental strain of *Enterobacter cloacae* complex

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The continuous emergence of antibiotic-resistant pathogens seriously threatens the effectiveness of antimicrobial treatment leading to increased morbidity, mortality and healthcare expenditure worldwide, and according to WHO [1] "is about to become a public health emergency of yet unknown proportions". Among many mechanisms by which bacteria render ineffective almost all antibiotic families currently in use, one of the most important is the resistance of Gram-negative bacilli to extended-spectrum β-lactam antibiotics, like cefotaxime (CTX). *Enterobacter hormaechei,* a member of *Enterobacter cloacae* complex and a part of human endogenous flora, has now become clinically significant as emerging nosocomial pathogen associated with septicaemia outbreaks [2]. *E. hormaechei* strain 51, isolated in 2012 from seawater, showed multidrug resistant phenotype including resistance to CTX as a result of simultaneous production of several β-lactamases. To understand the mechanisms involved in CTX induced stress response, Differential In-Gel Electrophoresis (DIGE) followed by LC-MS/MS analysis and bioinformatics, was employed to investigate protein changes of this resistant strain when challenged with sub-MIC of CTX. Total of 1072 protein spots were detected, out of which 35 were differentially expressed (p-value ≤0.05, max fold change ≥1.5). Twelve spots were up- and 23 were down-regulated, when compared with control. Protein expression changes at different subcellular levels were detected in CTX-resistant strain when exposed to antibiotic stress. In this aspect, coherent genomic and proteomic approaches might help us understand the specific traits associated with persistence of antibiotic-resistant strains in order to gain control over further spread of antibiotic resistance and possibly even uncover the drug resistance-associated proteins that could serve as novel drug targets.

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Comprehensive relative quantification of the cytochromes P450 using SWATH™ acquisition

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Introduction and Objectives
Metabolism of drugs by the Cytochrome P450 superfamily is pivotal in determining their disposition, safety and efficacy. Since drugs may induce expression of several isoforms of Cytochrome P450, they may enhance their own turnover, increasing the risk of toxic metabolite formation or adverse interactions with co-ingested compounds. Thus P450 profiling is a fundamental aspect of drug safety evaluation. The Cytochromes P450 share extensive sequence homology, so that antibodies are incapable of discriminating every isoform, plus mRNA levels do not correlate well with protein. SWATH™ acquisition is a data-independent MS acquisition method for label-free quantification which enables closely-related proteins to be quantified retrospectively through post-acquisition extraction of specific peptide ions, and is thus perfectly suited to P450 profiling.

Methods
Mice were exposed to inducers of the Cytochromes P450, and pooled microsomal fractions were prepared from the livers. Following protein extraction and digestion, a database of microsomal proteins was generated by 2D-LC-MS/MS using information-dependent acquisition on a TripleTOF 5600 (SCIEX, Framingham, USA). Individual samples were then processed and LC-MS data were acquired using the SWATH™ approach. PCA analysis was performed using MarkerView™ software (SCIEX) to identify differentially expressed proteins.

Results and Discussion
PCA analysis separated induced and non-induced mice based on their overall protein expression pattern, and that of the P450s. Relative quantification of uniquely discriminatory P450 peptides enabled the induction profile of each compound to be ascertained in unprecedented detail. For instance, it was possible to identify and quantify peptides unique to Cyp2C50 and Cyp2C54 despite the fact that the proteins share 92% sequence identity.

Novel Aspect
SWATH™ technology will facilitate even highly homologous proteins to be discriminated, it may also refine our understanding of enzyme function.

TARGETED NEXT GENERATION SEQUENCING (NGS) IN PARALLEL ANALYSES OF CHILDHOOD (cAML) AND ADULT ACUTE MYELOID LEUKEMIA (aAML) PATIENTS

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The aim of this study was to facilitate our understanding of the similarities and differences in molecular pathogenesis between cAML and aAML by application of parallel targeted NGS technology. We analyzed DNA isolated from the bone marrow mononuclear cells from 20 childhood (cAML) and 20 adult AML (aAML) unselected patients, using TruSeq Cancer Panel. Filtering and variant calling were performed using GATK UnifiedGenotyper and VariantFiltration tools. Resulting data were mapped against human genome b37. FLT3/ITD and IDH2 mutations were screened using PCR followed by direct sequencing. We identified 939 (467 cAML, 472 aAML) different mutations in both coding and non-coding targeted regions, with substantial variation from patient to patient. A total of 527 (260 cAML, 267 aAML) mutations in the non-coding regions and 412 (207

cAML, 205 aAML) mutations in the coding regions was detected, out of which only 122 (62 cAML and 60 aAML) were potentially protein-changing, i.e. nonsense (N), frameshift (F) and missense (M) mutations. Seven genes were found to have more than 5 NFM mutations, namely *NRAS*, *KIT*, *KDR*, *NPM1*, *MET*, *FLT3*, and *TP53*. Out of these, *TP53* and *KDR* were found to have more than 20 mutations in over 50% of AML patients. Finally, the number of patients who harbored at least one mutation in *TP53* gene was 17 of 20 in cAML and 19 of 20 in aAML. As expected, the prevalence of the most AML associated mutations including *FLT3/ITD*, *NPM1*, *IDH1* and *IDH2* gene, differed in cAML and aAML patient cohorts. *IDH1* (0% cAML, 5% aAML), *IDH2* (0% cAML, 10% aAML), *NPM1* (10% cAML, 35% aAML), *FLT3/ITD* (0% cAML, 10% aAML). This discrepancy in prevalence in leukemia associated genes highlights the differences in the pathogenesis of cAML versus aAML at the genetic level. Our results confirm that AML contains relatively small number of genetic alterations, suggesting that for the development of AML fewer genetic alterations are required than for other malignancies. Our results merely confirmed existing prevalence AML specific mutations in aAML. Many of them are well known (*FLT3*, *NPM1*, *CEBPA*) and their detection has already entered standard clinical practice. Given that AML is extremely heterogeneous in its clinical and genetic characteristics, more definite results regarding similarities and differences in pathogenesis of cAML and aAML will be obtained in a parallel NGS analysis of the morphological and cytogenetic homogeneous groups of cAML and aAML patients.

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Predictive genetic markers of coagulation, inflammation and apoptosis in Perthes disease - Serbian experience

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Perthes disease is one of the most common forms of pediatric femoral head osteonecrosis with an unknown etiology. Coagulation factors were the first genetic factors suspected to have a role in the pathogenesis of this disease, but studies showed inconsistent results. It is described that inflammation is present during early stages of Perthes disease, but its genetic aspect has not been studied extensively. Little is known regarding the status of apoptotic factors during the repair process that leads to the occurrence of hip deformity in patients. Therefore, the aim of this study was to analyze major mediators involved in coagulation, inflammation and apoptotic processes as possible causative factors of Perthes disease.

The study cohort consisted of 37 patients. Gene variants in TNF-α, FV, FII and MTHFR genes were determined by PCR-RFLP, while IL-3 and PAI-1 were genotyped by direct sequencing. The expression level of Bax, Bcl-2, Bcl2L12, Fas and FasL was analyzed by qRT-PCR technique. Our results showed a significantly increased level of expression of pro-apoptotic factor Bax along with significantly higher Bax/Bcl-2 ratio in the patient group.

The results presented indicate that apoptosis could be one of the factors contributing to the lack of balanced bone remodeling process in Perthes patients.

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STUDY THE FUNCTION OF UBIQUITIN B^{+1} PROTEIN IN ALZHEIMER'S DISEASE MODEL

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Alzheimer disease (AD) is the most common form of dementia, and it is a progressive, incurable and fatal disease. It affects approximately 36 million people worldwide, and will become the world's leading cause of death by 2050 due to increasing longevity. The key pathological hallmark of AD is the accumulation of insoluble plaques in the brain, which are preferentially composed of aggregated amyloid-β protein (Aβ). The exact mechanism of Aβ accumulation is poorly understood, and thus it is difficult to develop treatments. Yeast *Saccharomyces cerevisiae* can provide profound insights into cellular mechanisms and perturbations in cell attained by Aβ. We modelled the Aβ localization and toxicity in yeast by directing the peptide to the secretory system. The cells constitutively producing Aβ exhibited a lower growth yield and shorter chronological life span. This model provides a new tractable system for the screening for inhibitors of the toxicity caused by Aβ.

The ubiquitin-proteasome system (UPS) is a principal proteolytic pathway which is responsible for degradation of damaged and/or misfolded proteins. Dysfunctional UPS has been involved in several neurodegenerative disorders. UBB^{+1} is a frameshift version of ubiquitin B (UBB) produced by molecular misreading. It has been found to accumulate in the neuritic plaques and tangles in patients with AD. Studies showed UBB^{+1} accumulation induced a proteasome inhibition and contributed to AD pathogenesis when it was at high expression levels. Interestingly, our recent findings in yeast have shown that constitutive low level of UBB^{+1} induced a protective response that assists cells to cope better with misfolded proteins during chronological aging. To confirm and extend these preliminary findings, we investigated the effect of low UBB^{+1} expression on Aβ pathology *in vivo*. Present results showed that constitutively expression of UBB^{+1} significantly increased life span of Aβ expressing yeast. Cellular roles and functions of UBB^{+1} will be further investigated and discussed.

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Exploring Impact of Dynamic Accumulation for Improving MS/MS Quality of QqTOF Data

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Introduction & Objectives

Recent innovations in QqTOF instrumentation has resulted in a large increase in MS and MS/MS acquisition speed providing deeper coverage of complex proteomes. Some workflows, such as iTRAQ® reagent quantitation or PTM characterization, benefit more from higher spectral quality than traditional data-dependent workflows. Here, a QqTOF acquisition strategy that uses precursor intensity to adapt the MS/MS accumulation time (dynamic accumulation) was explored for its utility in improving these proteomic datasets.

Materials & Methods

Analysis of complex protein digests was performed using nanoflow LC/MS analysis on a TripleTOF® system. Data collection was done in data dependent mode with prototype acquisition software to explore a range of acquisition rates and precursor intensity combinations for optimal coverage and spectral quality. Protein identification data was processed using ProteinPilot™ Software and results assessment was performed using Excel tools. A number of areas of improvement were investigated, impact on MS/MS quantitation for iTRAQ reagents, effect on number of acquired spectra and therefore subsequent processing time, impact on protein identification rates, and impact on the generation of SWATH™ acquisition spectral ion libraries.

Ecoli lysate was labeled with 8plex iTRAQ® regents and mixed with equal loading in all channels. The sample was analyzed using three different acquisition strategies and the identification yields were characterized for both ID and quantitation.

Results & Conclusion

The dynamic accumulation approach provided a small increase in total protein/peptide identifications and significant improvements in the quantitation quality. The median reporter ion intensity was shifted higher by 34%, and the variance of protein ratio distributions was reduced (16% improvement in quality). The peptide variation about the protein was

constant across the peptide intensity range, indicating improved quantitation of lower signal peptides.

Increasing Depth of Coverage in Data Independent Acquisition

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Introduction & Objectives

Data independent acquisition (DIA) strategies have been used to increase the comprehensiveness of data collection while maintaining high quantitative reproducibility. In DIA, larger fixed-width Q1 windows are stepped across the mass range in an LC timescale, transmitting populations of peptides for fragmentation, and high resolution MS and MS/MS spectra are acquired. Previous work has shown that using more narrow Q1 windows can improve peptide detection and increase sample coverage. Here both method and instrumentation advancements will be explored to continue to increase depth of sample coverage.

Materials & Methods

The MS analysis was performed on a modified quadrupole time of flight instrument equipped with an ADC detection system. DIA data collection was done using SWATH™ acquisition with prototype acquisition software to explore a variety of acquisition strategies. The DIA data was interrogated using a comprehensive yeast spectral library created from many data dependent experiments. Results assessment was performed using Excel tools.

Results & Conclusion

Original work exploring variable window size and more narrow windows demonstrated that increasing the number of total Q1 windows from 24 to 60 windows provided an increase in confident peptide detections with good quantitative reproducibility of ~15%. To enable higher sample loads, the dynamic range of the detection system was extended by switching from a TDC based detection system to an ADC based system on a modified

TripleTOF system. At the higher sample loads, we next applied increasingly narrow Q1 windows during SWATH acquisition to continue to improve the S/N in MS/MS. The number of windows was extended from 60 to 100 windows across the 400-1250 precursor m/z range while maintaining a cycle time of 3.2 secs. This provided a 20% increase in confident peptide detections with 20% or better CVs across replicates. Further optimization of longer chromatography and investigations of impact on other proteomes will be discussed.

Investigating Biological Variation in Human Hepatocytes of Phase I and II drug Metabolism Enzymes

Joerg Dojahn[4], Xu Wang[1]; Hui Zhang[2]; Christie Hunter[3]

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Introduction & Objectives

Measurement of drug metabolizing enzymes responsible for phase I and II biotransformations is a fundamental aspect of assessing drug-drug interactions, and evaluating drug safety and efficacy. In this work, we used SWATH Acquisition, a data independent acquisition method, to analyze protein expression levels of many of the enzymes involved in the drug metabolism.

Materials & Methods

A spectral ion library containing more than 2000 proteins was generated from data dependent analysis of a pooled sample. In the SWATH data generated using a TripleTOF® 5600+ system, an average of 1987 proteins, including 19 CYP proteins, 12 UGT proteins, and 7 GST proteins, were quantified across the 13 samples. The quantitative interpretation of SWATH data was achieved by automatic extracting characteristic fragment ions for each identified peptide from high resolution TOF MS/MS spectra. A set of protein/peptides of interest obtained from SWATH acquisition was then further analyzed with MRM using a QTRAP® 6500 System.

Results & Conclusion

Quantitative comparison of two phase II metabolism enzymes, EST1 - liver carboxylesterase 1 and EST2 - cocaine esterase, showed 40% variations

across 13 samples. The relative intensities of 4 individual peptides of each protein showed very good agreement (<12% CV), which highlight the reproducibility of quantitation. Principal component analysis (PCA) was applied to discover proteins differing between the samples. Here, multiple proteins were found to be correlated, such as CYP3A4 and CYP3A5, which are two major phase I drug metabolism enzymes in cytochrome P450 superfamily. Previously published data also demonstrated the positive correlation between these two proteins [1]. Finally, very good correlation (<15% CV) was seen between the MRM and the SWATH acquisition data.

1. Lin Y. S. et al., Mol. Pharmacol. 62: 162-172, 2002.

Using SWATH™ Acquisition for Characterization and Quantification of the Epigenetic Histone Modifications

Joerg Dojahn[3], Marcus Macht[3], Benjamin A. Garcia[1], Eric Johansen[2], Sean Seymour[2], Zuo-Fei Yuan[1], Kelly R. Karch[1], Christie L Hunter[2], Sahana Mollah[2]

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Introduction & Objectives

Histone post-translational modification (PTMs) sites regulate gene transcription, thus making reliable quantification a high priority in epigenetic studies. Quantification of histone PTMs is performed by shotgun proteomics favoring discovery of novel/low level PTMs, or targeted analyses (SRM) of known PTM sites. Each workflow has strengths and weaknesses for PTM quantification. Data independent acquisition (DIA) for comprehensive data generation combined with targeted data processing has recently been demonstrated to provide very high quality quantitative data. The advantages of this approach for targeted PTM quantification include no upfront assay development, quantitative data on all analytes and no dynamic exclusion of isobaric peptides. In this study, we develop a SWATH™ acquisition platform for quantitating histone.

Materials & Methods

Histones were acid extracted from HeLa cells. A custom-made synthetic heavy labeled modified peptide library was spiked into the sample. SWATH™ acquisition of the sample was performed using a QqTOF

system interfaced to a nanoflow source and a chip based LC system operated at various flow rates. Replicate analysis was performed for these samples to obtain characterization and quantitation statistics. Data was processed using either commercial or in-house software.

Results & Conclusions

Results from both the asynchronous non-treated and butyrate treated samples run in a normal data-dependent mode (DDA) was used to generate a spectral ion library for the histone samples. A key issue in analyzing histone peptide mixture is the presence of various isoforms of peptides, primarily from PTMs on the same peptide, but localized on different amino acids. Quantitation of these isoforms in a data-dependent mode using MS1 quantitation has been very challenging due to co-elution of isobaric forms. This made it difficult to distinguish and quantify each isoform based on MS1. However, with SWATH acquisition, all the MS/MS fragments are collected in one data set, so unique/combination of MS/MS fragments of each isoform were extracted post-acquisition and used for sequence assignment and quantification even if they co-eluted. Narrowing Q1 Window thru Variable Window SWATH™ Acquisition helps to increase specificity, which leads to even better quantitation, especially for low abundant compounds. Based on the SWATH analysis, several peaks co-eluted or partially overlapped, would make it impossible to be quantified by MS1 analysis. Replicate SWATH analysis resulted on average a 10% CV for the peptide fragments, quality rivaling an MRM approach. We will present results from more of these histone isoforms. This work illustrates the advantage of using SWATH analysis for characterization of histone modifications.

Using SWATH™ acquisition for unambiguous sequence assignment and quantification of isobaric histone peptides.

WORKSHOPS

June 9th 2015

14:00 – 15:00	*1st WORKSHOP "Publishing high profile papers in OMICs research"* Moderator: **Igor Štagljar** **Natalie de Souza** (Editor in Chief, Nature Methods) **Mirella Bucci** (Senior Editor, Nature Chemical Biology)

June 10th 2015

13:00 - 14:00	Small conference room *(13:00-14:00) Alpha Chrom Satellite Symposium*
14:00 - 17:00	Small conference room (*16:00-17:00) SCIEX Workshop OneOmics and Swath*

June 12th 2015

16:00 - 17:00	2nd WORKSHOP "Careers in OMICs-based disciplines" Moderator**: Mladen Merćep** **Brenda Andrews** (University of Toronto, Canada), **Marija Tadin-Strapps** (Merck, USA)

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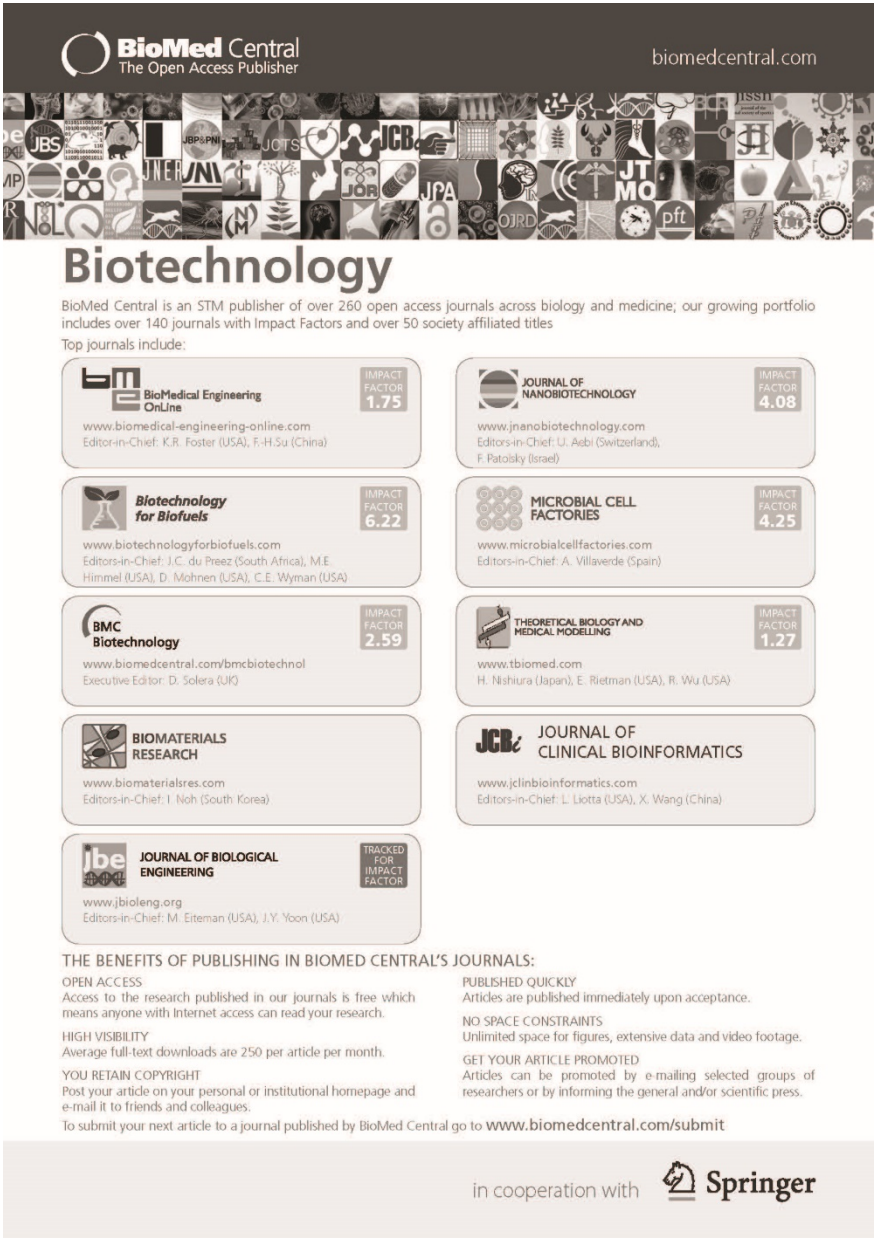

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Notes

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