BOOK OF ABSTRACTS

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OPENING KEYNOTE

INTRODUCTION of the Speaker by IGOR ŠTAGLJAR

Genotypic Variability and the Quantitative Proteotype

Ruedi Aebersold

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The question how genetic variability is translated into phenotypes is fundamental in biology and medicine. Powerful genomic technologies now determine genetic variability at a genomic level and at unprecedented speed, accuracy and (low) cost. Concurrently, life style monitoring devices and improved clinical diagnostic procedures generate an even larger amount of phenotypic information. To date, the effects of genomic variability on the expressed information of the cell, and thus on the phenotype, have been mainly studied by transcript profiling. Yet, most biochemical activities are catalysed by proteins, more specifically by protein complexes.

We are therefore aiming at the systematic determination of the effects of genomic variability on the proteotype (the acute state of the proteome and its organization of the cell). This is becoming feasible due to the development of new mass spectrometric methods, exemplified by SWATH-MS, that generate highly reproducible proteome maps from samples of (large) cohorts (1).

In this presentation, we will discuss the current state of the computational and quantitative aspects of SWATH-MS. We will additionally discuss selected applications of the technology using genetic reference strains, cancer affected tissues and cultured cells, to determine the effect of genetic variability on the quantitative proteome, thus functionally connecting the genome, the proteome and complex phenotypes (2,3,4).

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Session I

Emerging phenotypes in ageing and age-related diseases: a point of view

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Phenotypes, including disease phenotypes, are due to changes in functional homeostasis of proteome activities. Until recently, medicine considered gene mutations and epigenetic modifications as the sole source of phenotypic alterations. However, protein function can be affected directly by protein damage without genome alteration; such phenotypic change should be reversible. The reversibility of aging and age-related diseases (ARD) was demonstrated recently in heterochronic parabiosis experiments with mice and becomes a basis for day dreaming a new medicine for prevention and cure of all ARD.

The impact of such medicine on ARD would be comparable to the cumulative effect of vaccination and antibiotics upon infectious diseases. The arguments, the project and its underlying consequences will be presented, e.g.,:

(1) Identification of the common root cause of cell death, ageing and ARD (with clear separation from complex consequences of ageing and diseases).

(2) Diagnostic identification of "silent" mutations – specific protein polymorphisms – that increase protein susceptibility to oxidative damage resulting in delayed, but reversible, cellular phenotypes (with some irreversible consequences, mutations and epimutations, still subject to phenotypic suppression).

(3) Perspective of a targeted pharmacological action upon the common cause of ARD (e.g., oxidative protein damage) in order to: (a) avoid diseases (prevention), (b) extend the latency period in emergence (phenotypic expression) of ARD and (c) reverse the already manifested diseases to the pre-disease state (healing) by correcting the phenotype rather than genome.

The necessity for new methods will be discussed.

How endosomes tether, fuse and signal

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Rab5 is a master regulator of early endosome biogenesis. It regulates the specificity and directionality of endosome fusion via the recruitment of tethering effectors that lead membranes to dock and fuse. EEA1 is a Rab5 effectors that tethers endosomes leading to membrane fusion. Using a combination of biochemistry, electron microscopy and biophysics, we discovered that Rab5 induces an allosteric conformational change on EEA1, from extended to flexible, generating an entropic collapse force that helps pulling the membranes together. The mechanism we have discovered may be widespread in biology (not only vesicle tethering) given the large number of coiled coil proteins in multiple functions. Furthermore, endosome tethering and fusion responds to tyrosine kinase receptors to regulate their packaging in endosomes and determine the amplitude, lifetime and robustness of the signalling response. We are now applying quantitative imaging and functional genomics approaches to explore the mechanisms underlying endosome function in signal transduction.

Receptor Tyrosine Kinase-Interactomes, Drug Action & Genome-Informed Medicine

Zhong Yao, Jamie Snider, Punit Saraon, Shivanthy Pathmanathan, Farzaneh Aboualizadeh, Luka Drecun, Ingrid Grozavu, Victoria Wong, Attila Szabo, Igor Stagljar

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During tumor progression, cancer cells acquire characteristic features, due to signaling pathways that significantly differ compared to normal cells. These pathways involve numerous membrane proteins that transmit signals via tightly regulated protein-protein interactions (PPIs), resulting in conversion of signals into a biological response. Understanding how these signaling networks function *in vivo* and how they are altered in cancer represents a major scientific challenge crucial to the development of new cancer therapies. However, since membrane proteins are extremely difficult to study using current genomics technologies due to their complex biochemical features, research into this area has lagged behind, with membrane proteins screened to a significantly lower degree than other protein classes.

During my talk, I will report on our previously developed cell based high-throughput proteomics screening technology, called the Mammalian Membrane Two-Hybrid (MaMTH) assay and its application to dissect signaling pathways in normal and cancer cells. In addition, I will discuss how our recent application of MaMTH to various human Receptor Tyrosine Kinases (RTKs), an important family of membrane proteins involved in the variety of cell signaling processes, identified several novel therapeutically important interactors of these RTKs of a therapeutical importance. Lastly, I will also demonstrate how MaMTH can efficiently be used as a drug discovery assay for identification of inhibitory compounds that change the phosphorylation status of the human Epidermal Growth Factor Receptor (EGFR) in the context of living cells and in the low nanomolar range, an advance which may open up a whole new approach to drug development and lead to more effective treatments for lung cancer patients.

Toward a reasoned classification of diseases using physics-based criteria

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Background: Diseases and health conditions have been classified according to anatomical site, etiological and clinical criteria. Physical mechanisms underlying the biology of diseases, such as the flow of energy through cells and tissues, have been often overlooked in classification systems.

Objective: We propose a conceptual framework towards the development of an energy-oriented classification of diseases, based on the principles of physics.

Methods: A review of literature on the physical-biological interactions in a number of diseases is traced from the appoint of view of the fluid and solid mechanics, electricity, and thermodynamics.

Results: We found consistent evidence in literature of decreased or/and increased physical forces intertwined with biological processes of numerous diseases, which allowed the identification of mechanical, electric and thermodynamic phenotypes of diseases.

Discussion: Biological mechanisms of diseases need to be evaluated and integrated into more comprehensive theories that should account with principles of physics. A hypothetical model is proposed relating the natural history of diseases to mechanical stress accumulation, electric forces and free energy (ATP) changing. The present perspective towards an innovative disease classification may improve drug-repurposing strategies in the future.

Session II

The CFTR Interactome

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A component to understanding biological processes involves identifying the proteins expressed in cells as well as their modifications and the dynamics of processes. Several major technologies, but especially mass spectrometry, have benefited from large-scale genome sequencing of organisms. The sequence data produced by these efforts can be used to interpret mass spectrometry data of proteins and thus enables rapid and large-scale analysis of protein data from experiments. Advances in multi-dimensional separations as well as mass spectrometry have improved the scale of experiments for protein identification. This has improved the analysis of protein complexes, and more complicated protein mixtures. Quantitative mass spectrometry can be used to study biological processes such as protein-protein interactions, development or the effects of gene mutations on pathways. Recent studies on the loss of function mutant form of the Cystic Fibrosis Transport Regulator (□F508) as it progresses through the folding pathway will be presented. Through the study of protein-protein interactions and modifications that regulate maturation of CFTR, we are beginning to understand the critical interactions regulating pathways for export or destruction[1].

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[1]Pankow et al *Nature* 2015, 528, 510-6.

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From intracellular antibody fragments to small molecule inhibitors

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Our aim was to develop methods to employ intracellular antibody fragments for intracellular immunotherapy and as lead macromolecules for small compound selections. The method of intracellular antibody capture (IAC) has been developed to isolate single domain antibody fragments (iDAbs) that fold in the reducing environment of the cell and engage with their target antigens. IAC was used to isolate iDAbs that block protein-protein interactions (PPIs); an iDAb binding mutant RAS in a variety of tumour types and an iDAb binding to LMO2 in T-cell acute leukaemia.

We have further developed methods to use iDAbs to identify chemical compounds that can replace iDAbs for drug development. Methods have been developed for chemical library screening in-cell or *in vitro*. In this way, we have identified small molecules binding to mutant RAS with potential to inhibit the RAS-effector interactions.

Mutation in RAS family members is among the most frequent in human cancer and the mutant RAS proteins are tumour-specific proteins for therapy. We have previously selected an intracellular antibody single domain fragment that binds to mutant forms of KRAS and HRAS and used this antibody fragment to demonstrate that blocking RAS-effector interaction-dependent signal transduction prevents tumour initiation and overt tumour growth in mouse preclinical models. The antibody fragment binds to GTP-bound RAS with high pM affinity and we have used this binding property to isolate compounds from a fragment library using a competitive SPR method that places the compounds in the region of the binding site of the antibody fragment. Using a combination of X-ray crystallography and medicinal chemistry, we have obtained a family of compounds that bind adjacent to the KRAS switch I region with nM affinity. These compounds inhibit the interaction of RAS with effectors and inhibit downstream phosphorylation of AKT and ERK that results from RAS signaling. The chemical evolution and interaction characteristics of the KRAS-binding compounds will be described and their biochemical and cell-based properties presented.

Session III

Learning molecular mechanisms from high-throughput metabolomics

Sauer Uwe

Institute of Molecular Systems Biology, ETH Zurich, Switzerland

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Our recent developments in mass spectrometry-based high-throughput metabolomics have overcome the data generation problem in two ways: i) high speed detection of several hundred metabolites in a sample per minute[1] and ii) the ability to measure responses in near real time as a resolution of few seconds[2]. Large-scale applications include mapping of the so far uncharted gene-metabolite associating network[1] or genome-wide discovery of novel enzyme activities[3]. Here I will illustrate the potential of high-throughput measurements for discovery of metabolic microbe-host interactions in the gut. The talk will be concluded with so far unpublished real-time metabolomics to identify the regulation processes that determine the decision to grow in bacteria.

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[2] Link H, Fuhrer T, Gerosa L, Zamboni N & **U. Sauer**. 2015. Real-time metabolome profiling reveals dynamics and regulation of the metabolic switch between starvation and growth. **Nature Methods** 12: 1091-1079.

[3] Sevin DC, Fuhrer T, Zamboni N & U **Sauer U**. 2016. Nontargeted *in vitro* metabolomics for proteome-scale identification of novel enzymes in *E. coli.* **Nature Methods** 14:187-194.

The Target Space of Clinical Kinase Inhibitors

Susan Klaeger[1], Stephanie Heinzlmeir[1], Mathias Wilhelm[1], Bernhard Kuster[1]

[1]*Chair of Proteomics and Bioanalytics, Technical University of Munich, Munich, Germany*

Kinase inhibitors have developed into important cancer drugs because de-regulated protein kinases are often driving the disease. Efforts in biotech and pharma have resulted in more than 30 such molecules being approved for use in humans and several hundred are undergoing clinical trials. As most kinase inhibitors target the ATP binding pocket, selectivity among the 500 human kinase is a recurring question. Polypharmacology can be beneficial as well as detrimental in clinical practice; hence, knowing the full target profile of a drug is important but rarely available. We have used a chemical proteomics approach termed kinobeads to profile 240 clinical kinase inhibitors in a dose dependent fashion against a total of 320 protein kinases and some 2,000 other kinobead binding proteins. In addition, we have charted the phosphoproteomes of many cancer cell lines providing insight into the signaling pathways that drive tumors. In this presentation, I will outline how this information can be used to identify molecular off-targets of efficacy or toxicity, to help understand the cellular mode of action of kinase inhibitors, to re-purpose existing drugs or combinations for new indications and to provide starting points for new drug discovery campaigns. We believe that the integrated use of drug target profiling with molecular phenotyping at the post-translational level will greatly inform tumor biology and drug discovery in the future.

Acknowledgements/Literature

The authors wish to thank the following colleagues for a fantastic collaboration:

Huichao Qiao, Dominic Helm, Harald Polzer, Binje Vick, Katrin Reiter, Maria Reinecke, Benjamin Ruprecht, Svenja Petzoldt, Heiner Koch, Melanie Schoof, Giulia Canevari, Elena Casale, Stefania Re Depaolini, Annette Feuchtinger, Chen Meng, Zhixiang Wu, Jana Zecha, Tobias Schmidt, Lars Rueckert, Wilhelm Becker, Jan Huenges, Bjoern-Oliver Gohlke, Anne-Kathrin Garz, Paul-Albert Koenig, Hannes Hahne, Juergen Ruland, Robert Preissner, Katharina Goetze, Gian Kayser, Tonu Vooder, Neeme Tonisson, Philipp A. Greif, Judith Schlegl, Hans-Christian Ehrlich, Stephan Aiche, Eduard Rudolf Felder, Karl Kramer, Sabine Schneider, Axel Walch, Guillaume Medard, Irmela Jeremias, Karsten Spiekermann, Bernhard Kuster

Dopamine oxidation mediates a human-specific cascade of mitochondrial and lysosomal dysfunction in Parkinson's disease

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Both mitochondrial and lysosomal dysfunction have been implicated in substantia nigra dopaminergic neurodegeneration in Parkinson's disease (PD), but how these two pathways are linked in human neurons remains unclear. Here, we used dopaminergic neurons derived from patients with idiopathic PD or familial PD and identified a time-dependent pathological cascade beginning with elevated mitochondrial oxidant stress leading to oxidized dopamine accumulation, ultimately resulting in reduced glucocerebrosidase enzymatic activity, lysosomal dysfunction and alpha-synuclein accumulation. Surprisingly, this toxic cascade was observed only in human, but not in mouse dopaminergic PD neurons, due in part to species-specific differences in dopamine metabolism. However, increasing dopamine synthesis or mutant alpha-synuclein levels in mouse midbrain neurons was sufficient to recapitulate pathological phenotypes observed in human neurons. Our study thus identifies several key factors contributing to species-specific dopaminergic neuronal vulnerability in PD, and identifies dopamine oxidation as an important link between mitochondrial and lysosomal dysfunction in human neurons.

Make no mistake: chromosomal instability and genomic heterogeneity in intestinal tumor organoids

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Genomic heterogeneity in human tumors is thought to underlie many aspects of tumor evolution and clinical response. Heterogeneity at the karyotype level is proposed to arise from ongoing mitotic errors, also known as chromosomal instability (CIN). Indeed, whole chromosome copy number variations, or aneuploidies, are among the most common genomic alterations in human cancers. It is not know however, if aneuploid karyotypes represent remnants of past segregation errors in presently stable populations, or if they are indicative of true CIN, and if so, to what extent CIN contributes to karyotype heterogeneity in human cancers.

I will describe our efforts using long-term live imaging and single-cell sequencing on various panels of healthy and diseased human intestinal organoids to examine if and to what extent CIN occurs in human cancers; if the level of CIN correlates with karyotype heterogeneity; when a CIN phenotype appears during tumor evolution; and what the molecular causes of CIN are. I will furthermore present newly developed conditional mouse models in which different levels of CIN can be induced to study their impact on tumor development.

Motor proteins driving chromosome segregation in human cells

Kruno Vukušić[1], Renata Buđa[1], Ana Milas[1], Iva Tolić[1]

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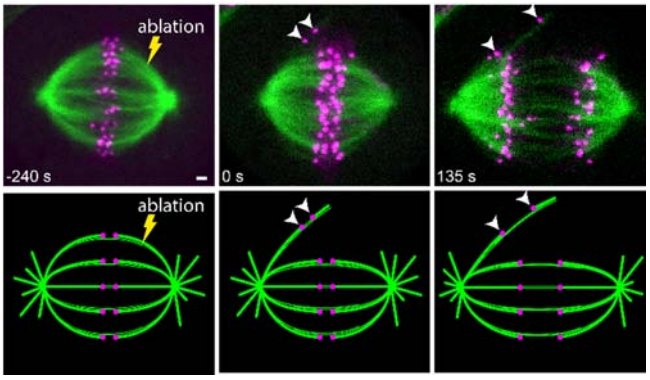
The critical task of the mitotic spindle is accurate segregation of sister chromatids. Spindle microtubules generate forces on the chromosomes through a multi-protein complex called the kinetochore. The main question is what forces drive chromosome segregation. In spite of intense research on mitosis over many decades on a variety of model organisms, the mechanisms relevant for chromosome segregation in human cells and the required motor proteins are largely unknown. By developing an assay for kinetochore dynamics based on laser ablation (Buđa et al., 2017), we show that kinetochores can separate without attachment to the spindle pole. This separation requires the bridging fiber, which connects sister kinetochore fibers (Kajtez et al., 2016; Milas and Tolić, 2016; Polak et al., 2017). Kinetochore separation is slower after depletion of MKLP1/KIF23 (kinesin-6), faster after depletion of KIF4A (kinesin-4), and unaffected by reduction of Eg5/KIF11 (kinesin-5) or KIF15/Hklp2 (kinesin-12). Thus, the sliding of microtubules in the bridging fiber, driven mainly by kinesin-6, pushes kinetochore fibers poleward to segregate chromosomes.



Kajtez, J., Solomatina, A., Novak, M., ..., Pavin, N., and Tolić, I.M. (2016) Overlap microtubules link sister k-fibres and balance the forces on bi-oriented kinetochores. *Nat Commun 7*, 10298.

Milas, A., Tolić, I.M. (2016) Relaxation of interkinetochore tension after severing of a k-fiber depends on the length of the k-fiber stub. *Matters (Zür.)*

Polak, B., Risteski, P., Lesjak, S., Tolić, I.M. (2017) PRC1-labeled microtubule bundles and kinetochore pairs show one-to-one association in metaphase. *EMBO Rep.* 18(2): 217–230.

Buđa, R., Vukušić, K., and Tolić, I.M. (2017) Dissection and characterization of microtubule bundles in the mitotic spindle using femtosecond laser ablation. *Methods Cell Biol.* 139: 81–101.

Session IV

“BBRC lecture”

INTRODUCTION of the Speaker by Mladen Merćep

The Integral Membrane Protease ZMPSTE24, Lamin A Processing, and the Premature Aging Disease Progeria

Michaelis, Susan

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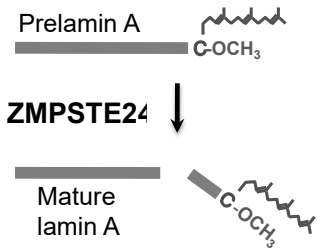
Our studies focus on ZMPSTE24, a fascinating integral membrane zinc metalloprotease important for human health and longevity. ZMPSTE24 plays a critical role in the proteolytic processing of farnesylated prelamin A, the precursor of the nuclear scaffold protein lamin A. Mutations in the genes encoding either prelamin A or ZMPSTE24 that impede prelamin A cleavage cause the devastating premature aging disorder Hutchinson-Gilford Progeria Syndrome (HGPS) and related progeroid diseases. In these diseases an aberrant and permanently farnesylated form of prelamin A is the “molecular culprit” that promotes premature aging. **Notably, growing evidence suggests that diminished ZMPSTE24 processing of prelamin A is also a factor in normal physiological aging**.

Our studies have been galvanized by the recently published structure of human ZMPSTE24 (Quigley et al. 2013, *Science* 339:1604) which reveals a novel and surprising structure, never heretofore seen. The seven transmembrane spans of ZMPSTE24 form a voluminous, enclosed, water-filled intramembrane chamber that is capped at both ends. Notably, the ZMPSTE24 metalloprotease domain faces the chamber interior, so that substrate access is restricted. Prelamin A must be threaded into the chamber through one of several side portals. I will discuss our newly developed “humanized yeast system” which will facilitate high throughput approaches to define precisely how substrate recognition and cleavage occur and to determine the step at which ZMPSTE24 disease alleles malfunction. Ultimately a basic mechanistic understanding of how ZMPSTE24 functions, and how to regulate its function, will have significant implications not only for premature aging disorders, but also for normal physiological aging.



Regulation of inflammatory and tissues injury diseases by RIP1 kinase

Domagoj Vucic

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Proper cell death regulation is critical for tissue homeostasis with impaired or excessive cell death contributing to numerous pathologies. Necroptosis is a regulated form of cell death that occurs when caspases are inhibited and involves activation of kinases RIP1 and RIP3. Studies with RIP1 kinase-inactive/dead (KD) mice have demonstrated that RIP1 kinase activity is instrumental for TNF induced necroptotic cell death and TNF stimulated hypothermia in vivo. We have used RIP1 KD, and RIP3 and MLKL KO mice to investigate physiological role of necroptosis in inflammatory and ischemia reperfusion injury mediated disease models and find that inhibiting RIP1 kinase activity has a great benefit is some but not in all inflammatory diseases. We have further investigated the role of necroptosis and apoptosis in various disease models by using MLKL and caspase-8 knockout mice and found that both pathways often contribute to severity of disease in physiological settings.

Given that RIP1 is prominently ubiquitinated, and in some instances phosphorylated, in the TNFR1-mediated signaling complexes, we investigated the spatial and temporal pattern of endogenous phosphorylation and ubiquitination of RIP1 and other signaling proteins during necroptosis. Our studies reveal that RIP1 ubiquitination is critically involved in the regulation of necroptotic cell death in vivo and the assembly of necrosome complexes. In addition, our findings reveal the intricate inter-dependency of phosphorylation and ubiquitination of RIP1 and validate their importance for the development of predictive biomarkers in in vivo disease models. Collectively, these studies define major events regulating cell death and inflammatory signaling, and contribute to development of anti-inflammatory and tissue protective treatments.

Functional heterogeneity of the triple-negative breast cancer microenvironment

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Breast cancer heterogeneity is one of the principal obstacles both to predicting outcome and to determining an effective course of treatment for this disease. Although genomic technologies have been used to gain a better understanding, by identifying gene expression signatures associated with clinical outcome and breast cancer subtypes, relatively little is known about heterogeneity in the tumor microenvironment. It is now accepted that changes in the normal cells that constitute the tumor microenvironment (TME) play important roles in determining cancer progression and ultimate outcome. Some of these changes impact the immune microenvironment. Understanding the immune microenvironment promises to be key for optimal cancer therapy, especially in hard-to-treat triple-negative breast cancer (TNBC). Using laser capture microdissection and gene expression profiling we define distinct tumor immune microenvironments that stratify TNBC patients.

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mTOR signaling in growth and metabolism

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TOR (target of rapamycin) is a highly conserved serine/threonine kinase that controls cell growth and metabolism in response to nutrients, growth factors, and cellular energy. TOR was originally discovered in yeast but is conserved in all eukaryotes including plants, worms, flies, and mammals. The discovery of TOR led to a fundamental change in how one thinks of cell growth. It is not a spontaneous process that just happens when building blocks (nutrients) are available, but rather a highly regulated, plastic process controlled by TOR-dependent signaling pathways. TOR is found in two structurally and functionally distinct multiprotein complexes, TORC1 and TORC2. The two TOR complexes, like TOR itself, are highly conserved. Thus, the two TOR complexes constitute an ancestral signaling network conserved throughout eukaryotic evolution to control the fundamental process of cell growth. As a central controller of cell growth, TOR plays a key role in development and aging, and is implicated in disorders such as cancer, cardiovascular disease, obesity, and diabetes.

While the role of TOR in controlling growth of single cells is relatively well understood, the challenge now is to understand the role of TOR signaling in disease and in coordinating and integrating overall body growth and metabolism in multicellular organisms. This will require elucidating the role of TOR signaling in individual tissues. Omics data on the role of mammalian TORC1 (mTORC1) and mTORC2 in controlling cellular processes and in specific tissues will be presented.

Cell-specific functions of the EGFR in inflammation and cancer

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The epidermal growth factor receptor (EGFR) is involved in the development of several epithelial and glial tumors and EGFR targeted inhibition is employed for the treatment of several cancer types such as colorectal and lung cancer. So far the oncogenic function of the EGFR has always been attributed to its expression in tumor cells. Recent results from our laboratory have challenged this concept, as we were able to demonstrate that in certain tumors the EGFR is oncogenic when expressed in tumor-associated myeloid cells (1,2). We found that in hepatocellular carcinoma (HCC) as well as in colorectal cancer (CRC), the EGFR is upregulated in tumor-infiltrating myeloid cells, where it plays a tumor-promoting function by regulating the expression of cytokines such as IL-6. The presence of EGFR-positive myeloid cells in HCC and CRC correlated with poor outcomes in patients. By employing genetically engineered mouse models (GEMMs), we demonstrated that deletion of EGFR in macrophages dramatically reduces liver as well as colorectal cancer development, whereas EGFR deletion in tumor cells had no effect or even accelerated tumor growth. These findings have important implications for the mechanism of action of anti-EGFR cancer drugs and might provide better stratification parameters for predicting anti-EGFR therapy responses.

(1) EGFR has a tumour-promoting role in liver macrophages during hepatocellular carcinoma formation. Lanaya H et. al. **Nat Cell Biol.** 2014 Oct;16(10):972-81, 1-7.

(2) EGFR in Tumor-associated Myeloid Cells Promotes Development of Colorectal Cancer in Mice and Associates With Outcomes of Patients.

Srivatsa S, et. al. **Gastroenterology**. 2017.

Session V

Omics of CFTR: Translating Science into Treatment

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Cystic Fibrosis (CF) is the most common genetic disease in Caucasians, caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel expressed at the plasma membrane of epithelial cells which also regulates ENaC, the major epithelial Na+ channel. Defective CFTR thus originates impaired epithelial sodium and chloride transport which severely impacts on dehydrated epithelia in several organs, most notably the airways.
The F508del is the most common CF-causing mutation, occurring in ~85% of CF patients, and it leads to protein misfolded which is recognized by the endoplasmic reticulum (ER) quality control (ERQC) and targeted for proteasomal degradation, thus being precluded from trafficking to the cell surface.
Our goal is to approach CF by systems biology using functional genomics. We thus developed high-throughput microscopy screens to identify novel ENaC regulators and novel factors affecting the traffic of normal CFTR and those rescuing mutant F508del-CFTR to the cell surface. The latter constitute potential drug targets for CF.
Overall, our results point to a complex involvement of several cellular functions in the regulation of the CF physiopathology, leading to the identification of potential drug targets whose relevance in the context of basic cell and molecular biology and CF will be discussed.

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Adhesion G protein-coupled receptors

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Adhesion GPCRs represent a subfamily of GPCRs with a particular structure. These receptors exhibit a classical transmembrane domain with 7 helices spanning the lipid bilayer, but also an unusually large N-terminal domain featuring a variety of structural motifs suggesting interaction with extracellular matrix elements. There are 33 adhesion GPCRs encoded in the human genome. The majority of these receptors are considered orphan, i.e. a physiologically relevant activating ligand or activation mechanism is not yet known.

A common feature of adhesion receptors is the presence of a GAIN (GPCR autoproteolysis-inducing) domain proximal to the 7TM region facing extracellular space. GAIN domains appear essential for correct membrane localization and function of adhesion receptors, and for a subgroup of receptors it could recently be demonstrated that autoproteolysis can expose a tethered ligand peptide which is able to activate the receptor and trigger signal transduction through calcium flux or cAMP formation. This activation mechanism resembles that observed for protease-activated receptors, such as the thrombin receptor PAR1. However, the exact mechanisms that lead to unmasking of the tethered ligand remain at present unclear.

We have set out to study in detail the physiological role and the regulation of the receptor GPR116/ADGRF5. This receptor is expressed prominently in lung, and knockout of the Adgrf5 gene in mice leads to a phenotype of pulmonary surfactant accumulation, which is due in large parts to an increased production and secretion by alveolar type II epithelial cells. Available evidence indicates that GPR116/ADGRF5 is a master regulator of surfactant homeostasis in mice and most likely in humans. Pulmonary surfactant consists of a mixture of lipids and proteins which is required for effective gas exchange in lung alveoli and for maintenance of alveolar structure. Infant respiratory distress syndrome (IRDS) is caused by a lack of surfactant and can be treated with surfactant replacement therapy.

We show that GPR116 can be activated by a tethered ligand peptide. In primary rat ATII cells receptor activation leads to inhibition of surfactant secretion. In *ex vivo* perfused murine lung, application of the tethered ligand peptide is sufficient to suppress surfactant secretion. Our data suggest that GPR116/ADGRF5 represents a therapeutic target to treat pulmonary diseases associated with surfactant dysfunction.

Proteometabostasis in safeguarding cellular longevity

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Selection for survival led to the evolution of maintenance of cellular homeostasis to coordinate and integrate biological processes and regulate cell fate. Complex gene networks are needed for biological processes, such as development, physiological homeostasis, as well as aging.
Cell-level symptoms of proteostasis failure are often intertwined with those of metabolic stress. We posit that the changes in the protein folding environment quality and metabolic activity are concerted/coupled, joined by a common task of safeguarding cellular fitness and survival: protein folding environment modulates metabolic activity, thus relying on it to provide conditions for efficient protein maintenance. Using budding yeast, *Saccharomyces cerevisiae*, as a model system, we have accumulated growing evidence that support our hypothesis: the metabolic response to improved protein maintenance phenocopies the glucose starvation response[1]. We have identified Target of Rapamycin (TOR) pathway as a hub of this intriguing signaling network, itself being responsive of the protein folding environment (via Hsp82 expression level) and responsible for relaying the message to glucose metabolism and mitochondrial activity by acting as a negative regulator of Snf1/AMPK (5' AMP-activated protein kinase)[1]. The opposite also stands: cell-wide metabolic reprogramming mitigates compartmentalized proteotoxicity[2]. Regardless of the cellular compartment of proteotoxic stress origin (mitochondria, endoplasmic reticulum, cytosol), a common cross-organelle response (CORE) is induced[2]. In addition to protein maintenance machineries activation in the cytosol, mitochondria and the ER, CORE features metabolic reprogramming and modulation of mitochondrial activity with a likely role of reporting on and assisting the management of proteoxicity. Finally, as a *par excellence* example of proteostasis-metabolism crosstalk, our results demonstrate that depositing misfolded proteins into aggregates during acute metabolic stress, and their return into the soluble phase following stress provide a fitness advantage promoting cell survival. The fact that amyloidogenic proteins are compartmentalized during glucose deprivation provides new insights into the molecular basis of protein folding diseases, as well as in aging and age-related changes.
We have termed this crosstalk proteometabostasis and we will discuss its contributions to understanding aging and the progression of age-related diseases.

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CLOSING KEYNOTE

INTRODUCTION of the Speaker by MIROSLAV RADMAN

The EMBO Keynote Lecture

Protein phosphorylation and cell signaling; histidine phosphorylation

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Posttranslational modification (PTM) increases the complexity of the proteome, and reversible PTMs are commonly used in the transmission of signals within cells in response to external stimuli. Protein phosphorylation is involved in the majority of cellular processes, and thousands of distinct phosphorylation events can be detected in a single cell type. The human kinome comprises >550 protein kinases of which 480 are typical eukaryotic protein kinases (ePKs), and the remainder are atypical protein kinases (aPKs); most are Ser/Thr kinases, but there are 90 Tyr kinases. In addition to Ser, Thr and Tyr, six other amino acids can be phosphorylated, including the three basic amino acids, His, Lys and Arg. Histidine phosphorylation of proteins has been recognized for over 60 years, but relatively little is known about its function as a PTM in mammalian cells or the enzymes that catalyze phosphorylation and dephosphorylation of histidine. Histidine is unique in having two phospho-isoforms - N1-pHis and N3-pHis, which may have distinct functions. To study global histidine phosphorylation events we generated monoclonal antibodies (mAbs) against non-cleavable 1-pHis and 3-pHis analogues, and are using these mAbs to study His phosphorylation in normal and transformed cells. Using immobilized mAbs for affinity purification of pHis proteins from 293 cells, we identified ~800 proteins that bound selectively under denaturing conditions, and putatively contain 1-pHis (~250) or 3-pHis (~160). By developing improved methods to enrich pHis tryptic peptides and analyze them by MS, we have identified 196 sites of His phosphorylation. We are characterizing some of the function of His phosphorylation of some of these proteins. We have obtained crystal structures of 1-pHis and 3-pHis mAbs bound to their cognate pTza antigen peptides, which will allow us to refine their properties. Staining of HeLa cells and primary macrophages with anti-1-pHis mAbs revealed staining on the outside of phagocytic vesicles; anti-3-pHis mAb staining of proliferating HeLa cells gave a striking pattern in mitotic cells, with strong signals at spindle poles (and centrosomes in interphase cells) and the midbody, suggesting that N3-His phosphorylation may regulate the mitotic process. In collaboration with Ed Skolnik's group (NYU), we have found that PGAM5 acts as a 1-pHis phosphatase in T cells to dephosphorylate and inactivate the NME2 His kinase. In the absence of PGAM5, NME2 phosphorylation of the calcium-regulated KCa3.1 potassium channel at the N3 position of H358 is increased in T cells; His358 phosphorylation promotes channel opening by preventing coordination of an inhibitory copper ion with H358, thus enhancing T cell activation.

SOP - Short Oral Presentations

Moving proteomics into personalized health: a route to individualized monitoring and intervention in early arthrosclerosis

Qin Fu[1*], Sarah Parker[1*], Zongming Fu[2*], Irene van den Broek[1*,] Vidya Venatraman[1*], Ronald Holewinski[1], Yue Wang[3^], David Herrington[4^], Jennifer E. Van Eyk[1,2^]

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An underlying premise of precision health is that continuous monitoring of an individual will allow detection of early disease and provide the potential for intervention thus, increasing quality of life and productivity of the individual while reducing overall health costs. Cardiovascular disease (CVD) remains a leading cause of mortality worldwide. Atherosclerosis, a primary risk of CVD, can begin in childhood although clinical signs of major adverse cardiovascular events (e.g. heart attacks, stroke, etc.) occur later. The goal is to develop both diagnostic markers of early indications of atherosclerosis and to identify the early regulatory mechanisms that drive vulnerable fibrous plaque.

In the initial phase, we analyzed 100s of aortic and left anterior descending coronary arteries obtained from individuals (<50 years old) using both DIA and DDA workflows. From this data, we were able to defined the proteome composition, network re-wiring and likely regulatory features of the protein networks specifically associated with early atherosclerosis. Furthermore, using Convex Analysis of Mixtures, which performs high-dimensional deconvolution of data from mixed sources whose individual features are *a priori* unknown, we identified 4 distinct proteomic signatures (2 healthy, fatty streak and fibrous plaque) implying that fatty streaks present an entirely distinct proteomic profile and are not a continuum between healthy and fibrous plaque.

From these in the second phase, early atherosclerosis linked tissue-secreted proteins were quantified using a 72 protein multiplex mass spectrometry assay in plasma of individuals with varying degrees of CVD. Using automated 96 well sample preparation workstation (Beckman Coulter) that carries out protein denaturation, reduction, alkylation and trypsin digestions in less than 2.5 hrs along with online desalting prior to LC/MS/MS we obtained reproducible and precise measurement (CV%< 20%) on 200 consecutive plasma samples.

In the third phase, we are carrying out continuous patent-centric health screening of patient populations. To do so, we adopted the volumetric absorptive microsampling device, Mitra device (Neoteryx) that allow remote blood collection by an individual and adapted our robust automated mass spectrometry sample preparation workflows (that includes system suitability and quality control measures). Here, data on 200 individuals at mid-risk for major adverse cardiovascular events were assessed over 4 consecutive months and comparing the ability of the same 72 protein biomarkers to predict a major adverse cardiac event in a real life setting.

PARTNERS IN CRIME – Signaling through Raf heterodimers

Cheree Fitzgibbon[1], Nora Rauch[1], Brendan McCann[1], Tapesh Santra[1], Angela Baljuls[1], Ruth Pilkington[1], Susan Kennedy[1], Alex von Kriegsheim[1], Jens Rauch[1] & Walter Kolch[1,2]

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Melanoma is considered one of the most aggressive and treatment resistant human cancers, approximately 60% of melanoma have the activating B-Raf V600E mutation present. B-Raf is one of three Raf kinases (A-Raf, B-Raf and C-Raf) which play a key role in the EGFR signalling network. Small molecular inhibitors targeted against mutated Raf have been used clinically. However, many patients relapse and develop resistance to the therapy. The main resistance mechanism in melanoma is believed to be a bypass of the drug blockade through Raf heterodimerization. This work is focused on determining the qualitative and quantitative differences of Raf heterodimers signalling compared to monomers or homodimers. We have established an inducible dimerization system to generate stable Raf heterodimers. Using co-immunoprecipitation in combination with quantitative mass spectrometry, we were able to identify dimer-specific interaction partners of Raf proteins, either in the absence or presence of Raf inhibitors. We identified approximately 2000 bona fide protein interactions including components of several novel molecular processes associated with Rafs, e.g the mediator complex, the RISC complex, and chromatin remodellers. Many of these proteins are altered in cancer or have been linked to drug resistance. The observed dimer-specific interaction partners indicate that Raf heterodimers affect different targets when compared to their monomer counterparts. We found novel crosstalk between the MAPK and other important cellular pathways that may play a role in tumour chemotherapy resistance.

Haploinsufficient loss of ESCRT components as new drivers of cancer

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Receptors located at the surface of the cell integrate the extracellular environment towards cellular functions such as cell survival, proliferation and migration. The Endosomal Sorting Complexes Required for Transport (ESCRT) efficiently controls cell surface receptor levels and turnover. Impairment in ESCRT function leads to receptor stabilization, persistent signalling, and potentially cancer promotion. We recently established a potent haploinsufficient TSG function of the ESCRT member, HD-PTP/PTPN23. Hemizygous deletion of HD-PTP predisposes mice to spontaneous lung adenoma, B-cell lymphoma and promotes oncogene-driven lymphoma onset and dissemination. Importantly, HD-PTP is frequently deleted in many human tumours including lung and lymphoma, which correlated with poor survival. HD-PTP depletion affects every step of tumorigenesis, including tumour initiation progression, dissemination and modulation of the tumour microenvironment. Interestingly, we have shown that UBAP1 and Endofin, two novel ESCRT components and HD-PTP binding partners, share HD-PTP functions. Therefore, we hypothesize that HD-PTP and its binding partners exert tumour suppressor functions. Moreover, since ESCRTs play a central role in a wide range of signalling pathways activated downstream of numerous cell surface receptors this pathway might have significant implications in the development of new therapeutic strategies that would be applicable to a wide range of cancers.

Studying bone healing induced by glycosaminoglycan-coated implants *in vitro* and *in situ* using proteomics

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To deal with age-related diseases that impair bone healing new implant materials, which support and accelerate active healing are of high interest. One promising therapeutic approach involves the coating of implants with modified glycosaminoglycans (GAGs) that partially mimic the native bone environment and actively promote osteogenesis and bon remodeling.

We investigated the effect of several GAGs on bone resorbing osteoclast as well as on bone forming osteoblast by comparative proteomics, activity assays, immunofluorescence staining, and on the distribution of marker proteins. Interestingly, sulfated GAGs were found to reduce the activity of bone-resorbing osteoclasts and to induce the activity of the bone-forming cells. (1)

To unravel the mechanism of increased bone formation we investigated whether GAGs influence only the osteoblasts or whether they also directly affect the formation, composition, activity, and distribution of osteoblast-released matrix vesicles (MV). Thus, MVs were enriched and the MV proteome as well as the proteome of the MV-releasing osteoblasts were analyzed with and without contact to sulfated GAGs. Interestingly, the proteome of these matrix vesicles, which are supposed to be the active machinery for bone mineralization, is much more strongly regulated by GAGs than the cellular proteome. Sulfated GAGs caused alteration of proteins mainly being involved in vesicle-ECM interaction and matrix vesicle activity, leading to stronger ECM formation and mineralization. Thus, the regulation of MV activity is one important mode of action the increased bone formation induced by implants coated with sulfated GAGs. (2)

To evaluate which proteins and metabolites are involved in early stages of bone healing, wound-fluid samples were collected *in situ* by microdialysis and quantified by mass spectrometry. This approach allows us to asses bone specific cytokine responses and distinct protein and metabolite profiles at several time points during the first 24 h of wound healing. (3,4)

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Regulation of catalytic activity of Nedd4 family ubiquitin ligases in cells and tissue organoids

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The Nedd4 family of E3 ubiquitin ligases comprises a C2-WW(n)-HECT domain architecture and plays pivotal roles in cell metabolism and homeostasis, cell proliferation, animal growth and cancer.

We had previously developed a proteome array screen to globally identify substrates for E3 ligases (*Mol.Syst.Biol.* 2007;2009), and identified the FGFR1 as a substrate for Nedd4 (Nedd4-1). We then showed that Nedd4 binds and ubiquitinates the FGFR1, leading to its endocytosis and termination of signalling, an effect required for normal animal development (*EMBO J*. 2011). Surprisingly, while Nedd4 can directly bind the FGFR1 even in the absence of receptor activation, such activation is required for Nedd4 to ubiquitinate and downregulate this receptor. Our subsequent studies showed that FGFR1 activation leads to Tyr-phosphorylation of Nedd4 via Src activation. Phosphoproteomic analysis then revealed that Src Tyr-phosphorylates Nedd4 on two sites: one on the HECT and one of the C2 domain, leading to dissociation of an inhibitory C2:HECT interaction, thus activating the catalytic activity of Nedd4 downstream of FGFR1 (or EGFR) activation (*Science Signal* 2014). These results suggest that receptor Tyr kinases that activate Src can lead to Nedd4 phosphorylation and activation, which in turn results in Nedd4-mediated ubiquitination and downregulation of at least some of these receptors. In a modified phosphoproteomic screen, we recently identified candidate Tyr phosphatases for Nedd4 and we are currently investigating their role in inactivating Nedd4, as will be described in my presentation.

In parallel studies, our collaborator G. Prag (Tel Aviv Univ.) identified another mode of Nedd4 regulation, mediated by self-ubiquitination and trimerization of the HECT domain, which results in its inactivation; as part of these studies, we showed that blocking this trimerization results in a constitutively active Nedd4 and enhanced ubiquitination of the FGFR1 (*EMBO J.* 2017). We are now studying the relationship between the different modes of regulation of catalytic activity of Nedd4, to evaluate if they are coordinated or operate as separate switches. Interestingly, our analysis of cancer mutations in Nedd4 identified various mutations predicted to modify Nedd4 phosphorylation or trimerization, thus inhibiting catalytic activity. These results suggest that Nedd4 has tumour suppressive function, and support our recent observation of the suppressive role of Nedd4 in colorectal cancer (*Oncogene* 2016).

To further analyze regulation of catalytic activity of Nedd4 proteins, we have been studying their regulation by ubiquitin variants (UBVs). In collaboration with D. Sidhu (Univ of Toronto), we recently showed that UBVs specifically targeting Nedd4-2 (Nedd4L) can modify its function in cells and in tissue (colonic) organoids (*Mol. Cell* 2016, and unpublished data), in agreement with our recent results following Nedd4-2 knockout in the colon. These studies, as well as additional unpublished studies using ileal and lung organoids, will be described in my presentation.

E. coli based genetic system for ubiquitylation cascade and drug discovery

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Two major challenges impede scientists from the completion of drawing the interactome of the associations between ~32 E2s, ~670 E3s and nearly 40,000 ubiquitylation targets of the human proteome:

i) ~100 deubiquitylases rapidly reverse the ligases' function making ubiquitin signals highly transient.

ii) Multiplex – the high redundancy of the E3:Substrates associations.

We will present a genetic selection tool that utilizes *Escherichia coli*, which lack deubiquitylases, to identify interactions along ubiquitylation cascades. Coexpression of split antibiotic resistance protein tethered to ubiquitin and ubiquitylation target along with a functional ubiquitylation apparatus results in a covalent assembly of the split resistance protein, giving rise to bacterial growth on selective media.

The system performance was carefully validated. We constructed and screened a yeast fusion library, discovering Sem1 as a novel ubiquitylation substrate of Rsp5 E3 ligase.

We employed the selection system to uncover novel E3-ligases that is injected by a molecular syringe of the pathogenic bacteria EHEC into the host. We screened our yeast library and identified one of the potential targets of the pathogenic ligases.

In a different study we demonstrate the system's ability to identify novel ultraweak Ub-receptors and we showed a strong correlation between ubiquitylation and growth efficiency.

We recently identified a novel allosteric restraint mechanism of HECT ligases. High resolution detailed characterization of the mechanism allowed us to generate unrestrained HECT E3 mutants which present significant increased activity in eukaryotic cells and provide an excellent tool to validate the associations found in the baterial genetic selection system.

Novel Approaches in Structural Proteomics Towards Determining Protein Conformational Changes and de-novo Protein Structure

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Structural proteomics, which may be defined as a combination of protein chemistry techniques with modern mass spectrometry, can provide detailed information on protein structure. Here, we present our recent technology developments in structural proteomics methods such as crosslinking, hydrogen-deuterium exchange, and surface modification. We have developed procedures for *de-novo* protein structure determination based on the incorporation of short-distance crosslinking constraints into discrete molecular dynamics simulations for modeling unknown protein structures. We have also developed new non-selective photo-reactive crosslinking reagents, and we will present here our first homo-bifunctional diazirine crosslinker. We will also present our experimental quantitative crosslinking and surface modification pipeline for the characterization of conformational changes in protein systems. We also describe an application of UVPD fragmentation for the top-down HDX analysis of protein secondary structure. We will also show how these new techniques have been used to solve structural problems such as determining the native α-synuclein structure in solution, determining the structures of prion and α-synuclein oligomers, and for the characterizing structural changes in disordered proteins upon ligand binding.

Detection of ovarian cancer recurrence using a novel integrated proteomics approach

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Epithelial ovarian cancers (EOCs) are the leading cause of mortality from gynecologic malignancy. Based on histology, EOCs are classified into endometrioid, mucinous, clear cell, and serous ovarian cancers. Most EOC patients present with advanced disease of the high-grade serous (HGSC) sub-type, which also has the worst outcome. Surgical debulking and chemotherapy significantly increase the survival of HGSC patients, but most relapse within 5 years of diagnosis and die of their disease. CA-125 is a FDA approved biomarker that is clinically used to monitor response to treatment and recurrence. Using a chemical proteomics approach we aim to identify novel biomarkers for improved detection of HGSC recurrence. Tumor tissue and serum were obtained from HGSC patient-derived xenograft models PDXs (n=12) and serum of un-engrafted animals (n=15) served as negative control. TFE-assisted protein extraction and magnetic-bead hydrazide-based glycopeptide enrichment identified 3,743 N-glycopeptides. To identify peptides for the development of targeted proteomics assays, a multi-step bioinformatics approach was applied. Since PDX tissues contain both mouse stroma and engrafted human cancer cells, we initially performed species assignment to all identified peptides. Only peptides that unambiguously mapped to a human peptide sequence were considered for future quantification. Based on these criteria, we identified 394 N-glycopeptides in both the PDX tumor and serum samples that were also never detected in the control samples (*i.e.* un-engrafted serum controls). This suggests that these peptides are secreted from the surgically engrafted human HGSC tissue and detectable in mouse serum background using our proteomics pipeline. All 394 peptides were synthesized as stable isotope labeled standards and used for the systematic development of Parallel Reaction Monitoring (PRM-MS) assays. All assays were initially tested in HGSC ascites to rapidly identify fragment ions that contain isobaric interference. Next, all peptides were quantified in longitudinal HGSC serum samples with matching CA-125 data. This richly annotated cohort includes serum collected at various time-points throughout HGSC progression (diagnosis, post surgery, during chemotherapy, post chemotherapy, recurrence). Our goal is to rapidly identify biomarkers that demonstrate improved sensitivity compared to CA125. As previously described (1) we will apply machine-learning approaches to identify N-glycopeptide signatures for improved detection of HGSC recurrence and validate these in independent patient cohorts.

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1) Kim Y, Jeon J, Mejia S, Yao CQ, Ignatchenko V, Nyalwidhe JO, Gramolini AO, Lance RS, Troyer DA, Drake RR, Boutros PC, Semmes OJ, Kislinger T. Targeted proteomics identifies liquid-biopsy signatures for extracapsular prostate cancer. *Nat Commun*. 2016 Jun 28;7:11906

Targeting type I interferon activity to the tumor microenvironment or to dendritic cells as a novel, generic and safe cancer immunotherapy

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Recently, immunotherapy was put forward as a fourth pillar for cancer treatment, next to surgery, chemo- and radiotherapy. Immunotherapeutics include antibodies, cellular therapies and cytokines. However, cytokines also cause severe systemic toxicities. To curtail toxicity, we are developing AcTakines, Activated-by-Targeting Cytokines, improved (mutated) immunocytokines fused to cell-specific targeting domains.

Type I IFN is approved for the treatment of several hematological and solid cancers, but its toxic side effects are dose-limiting. CD20 cell-targeted AcTaferon (type I IFN based AcTakine) using an anti-CD20 single domain antibody displays full IFN activity on $CD20^+$ cells, such as B lymphocytes or A20 lymphoma, but is inactive on other cell types. *In vivo* treatment of A20 or B16-$CD20^+$ tumors with CD20-targeted AcTaferon drastically reduced tumor growth, similar to high dose wild-type mIFN immunocytokine. In sharp contrast to the latter, however, tumor-targeted AcTaferon did not cause any systemic toxicity (evaluated via body weight, temperature, and blood cell counts). The AcTaferon antitumor effect was lost in IFNAR-deficient, Batf3-deficient or CD8-depleted animals, as well as in mice lacking IFNAR1 on CD11c+ cells, indicating involvement of cross-presenting DC (cDC1). Furthermore, selective targeting of AcTaferon to cDC1 was sufficient to induce tumor stasis in both melanoma and breast carcinoma models. Additionally, when combined with chemotherapy, low-dose TNF, or immune checkpoint blockade, complete tumor regressions and long-lasting tumor immunity were observed, still without adverse effects. Collectively, these findings indicate that AcTaferons can provide a safe and generic addition to current cancer (immuno)therapies.

"Omics approaches combined with screening in mammalian and yeast systems to target protein-protein interactions, globular proteins and GPCRs."

Philip M. Kim

The Donnelly Centre for Cellular and Biomolecular Research
Departments of Molecular Genetics and Computer Science
University of Toronto

I will present our advances in combining computational and experimental techniques to develop novel inhibitors. We have developed an integrated pipeline that first computationally designs large libraries of potential inhibitors and can then screen these using phenotypic, fluorescence-based or affinity selections. I will showcase our technology on a number of application examples, including large-scale inhibition of protein-protein interactions, cell surface receptors and globular proteins.

Dissecting intra and extra-mitochondrial metabolic interactions by large-scale RNAi-metabolomics

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Empowered by genome sequencing and mass spectrometry, over the last decade we acquired a detailed catalogue of the molecular components of human cells in terms. The next grand challenge is to map when and how these components interact to determine cellular phenotypes. This is a daunting task, in particular because of the lack of generally applicable, high-throughput, and broad scope methods to characterize cellular networks in action.

Metabolism is a prime example. In spite of exhaustive knowledge about enzymes and metabolite that compose the metabolic network, it remains prohibitive to infer e.g. fluxes from molecular data, the metabolic response to external perturbations, or predict the consequence of genomic mutations at network level. The relationship between genetic mutations and metabolite levels is often studied by classical genome-wide association studies, but a comprehensive interaction network has remained elusive.

Over the recent years, our lab developed a worldwide unique high-throughput metabolomics platform that allows to profiles hundreds of metabolites in thousands of samples per day[1]. Building on this capacity, we set out to systematically chart the association between genes and metabolites. We have already systematically mapped these gene-metabolome relationships in *E.coli*[2]. Here we present the first analysis of this kind in human cells.

We profiled the metabolome changes in normal human keratinocytes upon knock-down the genes included in the druggable genome panel[3]. This is a subset of about 10'000 protein-coding genes which are the most prominent therapeutic candidates. Almost 50% of this human genome subset are enzymes, hence having a direct impact on the metabolism. This resource supports systematic analysis of gene function, cellular regulation, and target identification. Alltogether, we performed ~ 70’000 non-targeted metabolomics runs to analyze metabolome differences in each single gene knock-down by siRNA.

In our initial analysis, we have focused on the silencing of mitochondrial genes according to the MitoCarta panel[4]. We have started with the enzymes and its effect on the mitochondrial (TCA cycle intermediates) and cellular metabolism. Apart of metabolome changes in immediate vicinity of the knocked-down enzyme (eg. accumulation of succinate in SDH silencing), we observed substantial distal effects. This indicate that metabolic changes propagate widely to non-mitochondrial pathways. Conversely, there are few non-mitochondrial knock-downs that show high enrichment in the mitochondrial metabolism. These initial results are only the tip of an iceberg, pointing out to the divergent landscape of cross-talk between human genome and metabolism.

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[1] Fuhrer *et al* (2011) Anal Chem

[2] Fuhrer *et al* (2017) MSB

[3] Hopkins and Groom (2002) Nat Rev Drug Discov

[4] Calvo *et al* (2015) Nucleic Acids Res

Systematically cracking the glycocode

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Glycans both encode cellular information, such as cell-cell interactions and cell state (Signal), and must avoid being targeted by pathogens (Noise). This leads to a system in which the sugar code (i.e. the glycan motifs controlling function) is hidden within the noisy milleu of larger heterogenous glycan structures. This talk focuses on use of our high-throughput analytical methods, including our lectin microarray technology and newly developed miRNA-proxy approach, in tandem with data integration, to decode structure-function relationships in the glycome. Our work is identifying glycan drivers of biological function including those involved in melanoma metastasis and host-response to pathogens (e.g. HIV-1 and influenza), providing a host of new targets for small molecule intervention in these disease states.

Leveraging human genetic variation to build better drugs

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Development of novel therapeutics is risky: It may take 15 years to advance a new molecular entity from therapeutic hypothesis to approval, with developing costs averaging 2.5 billion dollars and a chance of only 10% that a new drug that is tested in humans will reach the market. Two reasons stand out to explain the high failure rate of clinical trials and receding return on R&D investment across the pharmaceutical industry: A lower efficacy of the compound in the targeted disease population than anticipated from preclinical studies; and the occurrence of unintended drug effects, particularly adverse drug events. Human genetics has been proposed to offer attractive opportunities for improving trial success rates and reducing failures.

Here, I will discuss our insights gained from Mendelian Randomization and Phenome-wide association studies (PheWAS) for nominating targets and decision-making during drug development. I will present how studying the impact of naturally occurring genetic variation on the plasma proteome in >3,000 individuals can help to prioritize new drug targets for common diseases, and match existing drugs with new disease indications. I will further show how testing the association of drug targets nominated through genome-wide association studies against a rich array of phenotypes in >800,000 individuals from large population cohorts can pinpoint risks for adverse drug events. Our results demonstrate that human genetics is a powerful addition to the toolkit of drug discovery that should help reduce the unsustainably high attrition rates of drugs in pharmaceutical pipelines.

Integrated Omics Analysis Reveals Candidate Polygenic Lung Cancer Drivers With Prognostic Impact

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Cancer results from processes prone to selective pressure and dysregulation acting along the sequence-to-phenotype continuum DNA-RNA-protein-disease. However, the extent to which cancer is a manifestation of the proteome is unknown. Here we present an integrated omic map representing non-small cell lung carcinoma. Dysregulated proteins not previously implicated as cancer drivers are encoded throughout the genome including, but not limited to regions of recurrent DNA amplification/deletion. The proteome was largely unpredicted based on analysis of gene copy number and mRNA levels in patient-matched normal lung, and primary and patient-derived xenograft (PDX) tumors. Herein SHMT2 (mitochondrial serine hydroxymethyl transferase) is implicated as a driver of recurrent 12q14 amplification. SHMT2 converts serine into glycine, fueling folate-dependent one carbon metabolism. Loss of SHMT2 in the absence of extracellular glycine creates a state of glycine auxotrophy in transformed cells. Another mitochondrial protein, encoded by CHCHD2, consistently co-amplified next to EGFR on 7p11, promotes cell proliferation, migration and mitochondrial function. The ability of early stage primary NSCLC to engraft immune deficient mice is prognostic of poor outcome[1]. Metabolism proteins (enzymes and transporters) represent a segment of the proteome found to be most highly recapitulated between PDX tumors and the aggressive primary tumors from which they were derived. Clustering reveals signatures comprising subsets of metabolism proteins that co-vary in expression across NSCLC. Interrogation of The Cancer Genome Atlas revealed cohorts of patients with lung and other cancers that have DNA alterations in genes encoding the signatures, and this was accompanied by differences in survival. Therefore, proteomics-defined clusters encoding sets of metabolism genes, have features of polygenic cancer drivers. A “folate signature,” comprising 13 proteins/genes including SHMT2, is associated with worse outcome in 22% of lung adenocarcinoma (HR 2.8, Logrank P 0.0003). The recognition of genome and proteome alterations as related products of selective pressure driving the disease phenotype may be a general approach to uncover and group together cryptic, polygenic disease drivers.

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[1] John T et al. (2011) Clin Cancer Res. 2011;17:134-41

Identification of Novel Inhibitors of Activating EGFR Mutations in Non-Small Cell Lung Cancer using the Mammalian Membrane Two Hybrid Assay (MaMTH)

Punit Saraon

University of Toronto

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related mortality worldwide. Activating mutations in the tyrosine kinase domain of epidermal growth factor receptor (EGFR) in lung adenocarcinoma have been shown to display 'oncogenic addiction' to the EGFR pathway, and are sensitive to anti-EGFR small molecule inhibitors such as Erlotinib and Gefitinib. In about 60% of patients treated with these compounds, a secondary mutation (T790M) arises, conferring drug resistance. Recently, the compound AZD9291 has been approved as a T790M mutation-specific inhibitor. However, a third EGFR mutation arises in patients receiving this treatment, C797S, which confers resistance to AZD9291. Currently there are no treatments for patients that display the C797S mutation. Recently, we developed a new method to detect membrane protein-protein interactions (PPIs) in mammalian cells called Mammalian Membrane Two-Hybrid (MaMTH), and using this approach we were able to detect drug-sensitive interactions of oncogenic EGFR, and monitor the inhibition of these interactions in response to small molecule treatment. Using MaMTH, we screened over 2900 small molecules as inhibitors for the EGFR-C797S mutation and identified compounds that displayed preferential inhibition of the activating mutation while not affecting EGFR-wt. Subsequent validation in cell model systems identified one compound that could attenuate EGFR signalling in activating mutations. Taken together, we have shown that MaMTH can be used as a powerful drug discovery platform for the identification of small molecule inhibitors of activating mutations in cancer.

The ups and downs of protein expression regulation

Christine Vogel

New York University, USA

Gene expression is regulated by four major processes: transcription, translation, and RNA and protein degradation. These processes are adjusted, in different ways, when the cells respond to a stimulus. Many pathways are known, but their precise interaction over time is not well understood. In our lab, we use multiple time series datasets — on protein and mRNA expression changes and changes in the binding of ribosomes and other proteins — in combination with mass action models and other approaches to disentangle the contributions of the different levels of regulation and generate hypotheses on regulatory mechanisms. We focus on yeast and mammalian cells responding to stress of the endoplasmic reticulum, but have expanded these studies in a variety of directions.

Identifying kinase interactions relevant for cancer development

Marija Buljan[1], Anton Vychalkovskiy[1], Audrey van Drogen[1], Rodolfo Ciuffa[1], George Rosenberger[1], Matthias Gstaiger[1], Ruedi Aebersold[1]

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Human kinases are a large evolutionarily related protein family with 518 genes essential for signal transduction and regulation of metabolism, cell cycle, apoptosis and other cellular processes. It is hence not surprising that they are also associated with a number of diseases, including developmental and metabolic disorders and cancer. In this work, we searched for the interaction partners of 315 soluble human kinases, and used the obtained 'Kinome' data to identify interaction modules relevant for cancer development and progression. For this, we affinity purified the tagged kinases together with their stably bound interaction partners and we used mass spectrometry-based proteomics for identifying the bound proteins. In this way, we detected nearly 7,000 unique interaction pairs, 80% of which have not been reported previously. The compendium of interaction partners was enriched in proteins with the Kinase, Ras, 14-3-3 and scaffold domains which are all commonly found in kinase interaction networks. We further focused on the kinase interactions that could be of importance in cancer. For this, we used cancer genomics data from the TCGA [1] and ICGC [2] initiatives, which encompassed more than a 1,000,000 mutations and included more than 10,000 cancer patients. Specifically, we developed novel analytical approaches in order to (i) identify interaction modules that are enriched in known cancer-associated proteins, (ii) detect confident cancer mutation hotspots within kinases and their interaction partners, and (iii) uncover interaction interfaces that accumulate cancer mutations at a high rate, in those interaction pairs for which it was possible to build structural models, and to (iv) recognize novel kinase-substrate pairs and detect mutation clusters in the mapped phosphorylation sites. This highlighted a number of instances that are as yet not classified as cancer census genes but that exhibit mutational patterns typical of cancer drivers. An example is the Braf homolog Araf with a hotspot mutational residue and an interaction neighborhood enriched in known cancer proteins ($p < 0.01$, when compared to a random network). Instances that were also highlighted are Map2k3 and Map2k6 proteins that have mutation clusters ($p < 0.05$, logistic regression model) at the homologous interaction interfaces. Map2k6 is auto-inhibited through homodimer formation, and in this study we found that it can also form dimers with Map2k3. In addition to these observations, we identify a large number of cancer mutations that could rewire kinase-kinase signaling. Cancer genomics analyses allow for detecting genes whose mutation patterns indicate their likely role in the disease. However, how these genes and mutations connect to cancer pathways is not readily evident. Here we show that by combining different resources it is possible to prioritize novel cancer gene candidates and contextualize their roles in the cell.

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1. TCGA (The Cancer Genome Atlas) https://cancergenome.nih.gov/
2. ICGC (International Cancer Genome Consortium) http://icgc.org/

A Map of Functionally Coherent Binding for Snail and Twist Transcription Factors in Fly Mesoderm Development Informs Regulation of Epithelial Remodelling and Oncogenic Notch

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Cell identity is governed by gene expression, regulated by transcription factor binding at cis-regulatory modules (CRMs). Genome-scale assignment of functional targets is challenging and many CRMs likely remain uncharacterised. We developed and rigorously benchmarked network biology approaches to address these key limitations in understanding gene expression control. Novel software included the NetNC algorithm, which was applied to predict functional targets for the transcription factors Snail and Twist. Analysis of nine datasets in early fly development, including modENCODE 'HOT' regions, found considerable unanticipated direct regulation and illuminated approaches for transcription factor target discovery. Neutral binding estimates for these datasets were between 50% and 80% of statistically significant candidate target genes. Predicted Snail and Twist functional targets substantially overlapped with modifiers of the Notch pathway identified by genetic, RNAi screens and also identified novel factors in processes that control Notch signalling. Notch is critical for cell fate decisions across development, linking to several cancers and genetic disorders. The NetNC results offer a global representation of the mechanisms by which Snail and Twist exert tissue-specific regulation in early embryogenesis.

Snail and Twist are canonical Epithelial to Mesenchymal Transition (EMT) transcription factors; reactivation of a programme resembling EMT is a credible mechanism for key aspects of metastasis and drug resistance. Unsupervised clustering of microarray data for orthologues of functional Snail and Twist targets predicted by our algorithm stratified 2999 primary breast cancers by subtype. This classification had significantly better resolution of subtypes than clustering using randomly drawn gene lists. The aggressive, basal/triple-negative subtype has EMT characteristics and is driven by Notch signaling. Our integrative analysis identified conserved functional targets of EMT transcription factors in fly development as new players in basal-like breast cancers, including poorly characterised transcription factors, membrane receptors, splicing factors, vesicle trafficking proteins and chromatin modifiers. Ectopic expression of four predicted functional targets showed significant novel effects on invasion in an inducible Snail breast cancer cell line, validating *in silico* results.

Acknowledgements: IMO is grateful to Prof. Jeremy Gunawardena and Prof. Peter Sorger for hosting his Marie Curie Actions visits to HMS and for helpful discussions. Thanks also to Prof. PS Thiagarajan, Prof. Wendy Bickmore, Prof. Mike Levine and Prof Andrew Millar for invaluable advice.

High-throughput metabolomics identifies mode of action for uncharacterized antimicrobial compounds.

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[5] University of Basel, Basel, Switzerland.

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Despite rapid technological progress, the discovery of novel antibiotics has been stalled for the past 50 years. To combat the growing burden of antibiotic resistance, innovative drug discovery paradigms are required to improve and expedite the antibiotic discovery process. A crucial bottleneck in drug discovery is the identification of compounds' Mode of Action (MoA). To address this problem we developed a rapid and systematic metabolome profiling strategy to classify the MoA of bioactive compounds. In contrast to existing methods based on phenotypic drug profiling, mostly on the basis of growth assays, we exploit here the intracellular response of about 1000 metabolites as a truly multiparametric readout of the cellular response. The specific advance over existing omics platforms is a higher throughput of 1-2 orders of magnitude, allowing our combined MS-based metabolomics and computational workflow to scale with the size of typical compound libraries. We successfully identified MoA-specific dynamic metabolite responses for a large variety of antibiotics, in *Mycobacterium smegmatis*, regardless of whether the drug targets were metabolic or not. We then analyzed an open access set of ~200 novel anti-tuberculosis compounds with unknown MoAs. More than 70% of these compounds exhibited metabolic responses indicative of known MoAs, of which several were experimentally validated. Only 8% (16) of the compounds appeared to target non-conventional cellular processes, illustrating the difficulty in finding new antibiotic MoAs among compounds used as monotherapies in phenotypic drug screens. Overall, our metabolome-based screening approach is directly applicable to extract multiple quantitative signatures indicative of functional properties of MoAs in large compound libraries, beyond antimicrobial agents.

Poster Presentations

Circadian genes and oxidative stress regulate neuroplasticity to psychostimulants in *Drosophila*

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Addiction to psychostimulants (PS), cocaine (COC) and methamphetamine (METH), can be studied in model organisms by analyzing endophenotypes relevant for addiction, such as behavioral sensitization (BS). A group of circadian genes were previously identified as required for development of BS, but their mechanism of action on PS-induced neuronal plasticity is not known. We hypothesize that a potential mechanism involves interaction between PAS domain contained in circadian proteins and elevated levels of reactive oxygen species (ROS). PS increase ROS through oxidation of PS-induced monoamine release, while PAS domain is sensitive to redox potential. Here we present evidence for circadian and redox modulation of BS in *Drosophila*.

We are using BS phenotype to investigate interaction between drug-induced ROS levels and modulation by circadian genes. BS is measured using new high throughput test that allows administration of volatilized PS to a population of individually housed *Drosophila* and quantification of locomotor activity before and after drug exposure. Repeated administration of the same dose of PS leads to increased locomotor response (BS), but with different time interval between doses for COC or METH. We have confirmed the importance of circadian genes *period*, *Clock* and *cycle*, and we show the requirement for functional dopamine transporter and D1-type dopamine receptor in BS to COC and METH. Size of sensitized response depends on time of the day, indicating circadian modulation of the drug induced response. Single administration of COC or METH increase catalase (CAT), while decrease superoxide-dismutase (SOD), two enzymes responsible for regulating redox state of the cell. Exogenous suppression or induction of oxidative stress abolished BS to COC and METH, indicating that redox status interferes with neuronal plasticity.

Our data shows that *Drosophila* can develop BS to either COC or METH, and that BS is controlled and modulated by functional circadian genes, involves dopaminergic system and is influenced by the redox status. By combining different manipulations in the same animal, such as PS and exogenous pro- and anti-oxidant administration in transgenic animals that allows for spatial and temporal control of relevant genes, we plan to define the interaction between circadian genes and redox state on neuronal plasticity.

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Omic Network Modules as tools for Personalized cancer treatment in Non Small Cell Lung Cancer (NSCLC)

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Non small cell lung cancer constitutes the most common type (80%-85%) of lung cancer. Even though there are targeted chemotherapies existing for the treatment, the hospital admissions due to adverse drug reactions are on the rise. The range of individual therapeutic window varies greatly due to high variability in drug response for the given fixed dose of the drug. Hence a need for the personalized cancer therapy achieved through synergistic integration of germ line genetics decreases the risk of severe ADR's .

we hypothesized that genetic variants in genes encoding proteins that regulate the elimination and distribution of drugs are likely to correlate with both drug exposure and the occurrence of ADR's. 93% of the disease associated SNV's from Genome wide association studies lie within the noncoding sequences and they do possess a modest effect on disease onset and progress. powerful hypothesis generation from whole-genome sequencing analysis require synergistic and collective analysis of SNV using their combined effect, which could be performed using network and systems biology. Omic data of SNVs was derived from whole genome-sequencing of 96 lung cancer patients treated with gemcitabine/carboplatin, where about 50 suffered from induced leukopenia (leu), thrombocytopenia (thr), and neutropenia (npk) respectively and they were mapped to their closest gene for the downstream analysis and thereby identified 896, 995 and 936 genes for npk, tpk and lpk. From these we constructed disease modules which is a topologically & functionally interconnected network of genes using clique based clustering method for each of the traits and thereby identified gene modules of size 357, 320, and 347. Interestingly, 245 of those genes were shared across at least two modules, which hereafter referred to as the *shared* toxicity *module*. Simultaneously , we analysed Gene expression data of human cells from 300 micorarrays treated with Carboplatine and Gemcitabine respectively filtered for bone marrow expression , which corresponded to 120 Carboplatin and 109 Gemcitabine genes. We then performed enrichment analysis of the module genes and expression gene lists, which showed no enrichment for any of the trait lists, but significant enrichments (Fisher test $P<0.05$) for each of the modules on both lists (odds ratio (OR) = 2.5- 3.2). However, the shared module showed higher significant overlaps than each of the traits.(OR=4.1- 4.5, P= 2.2-3.7 x 10^{-3}).

Following major significant pathways were significantly enriched : Non-small cell lung cancer (hsa05223) , RAS signaling pathway (hsa04014) , Calcium signaling pathway (hsa04020) , ErbB (hsa04012) and estrogen signaling pathways(hsa04915)(Fig. 4B.). Most of the signaling pathways stated above are known to be affected in case of the K-RAS mutated or EGFR mutated non-small cell lung cancers. we found non-small cell lung cancer genes to be highly enriched in the modules, which supported the modules and suggests that the cancer genes interact highly with the toxicity genes.

Comprehensive analysis of the DNA binding topology of transcription factors from more than 1500 human ChIP-seq experiments

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Transcription factors (TF) are proteins, which recognize specific regions on the genome and influence the expression of nearby genes. This influence make them directly involved in the development of the cells. Due to their role in cellular activity, their dysfunction can lead to diseases. The role of these factors can be investigated by analysing ChIP-seq experiments. ChIP-seq is a powerful technique to determine the primary and indirect binding sites and the binding affinity of transcription factors. Earlier we showed (Nagy et al. BMC Genomics, 2016) that the exact positions of DNA binding proteins on the DNA can be extracted from the ChIP-seq data by identifying the peak summit positions. These summits correlate with the accurate contact positions of the proteins on the DNA and can be used to determine the topological arrangements of the binding proteins relative to the strand specific transcription factor binding sites.
Our goal is to analyse representative ChIP-seq sequencing data from the SRA database and to create a genome wide transcription factor binding site database with combination of the JASPAR non-redundant TF binding profile set. The database will contain position information (distance information measured in base pair) about the surrounding ChIP-seq summits (targeted different factors from several cell lines) around each identified transcription binding sites. The users will be able to download position and overlap data about two or more factors of interest from any (previously studied) cell line.
Our primary results already show some interesting examples of the power of this high-resolution motif position based ChIP-seq analysis approach. We found for example several cases where the CTCF motif was co-occupied with numerous other transcription factors which showed strand specific orientation in respect with the CTCF motif. Considering the ChIP-seq signal intensities and the location of summit positions, we hypothesize that the detected signals derive from indirect connection between CTCF and distally bounded transcription factors (in promoters). We expect that our results will help to better understand the overall mechanisms of transcription regulation and thus to associate regulatory variations to certain phenotypes (e.g. diseases)

Predicting Physiologic Effects of Downstream Rewiring Mutations in Cancer

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Kinases are key players in cellular signal transduction, and as such, kinase mutations can disrupt normal signaling pathways and contribute to diseases such as cancer. Kinase mutations in cancer are most often associated with two general categories: activating mutations in oncogenic kinases (e.g. EGFR, PI3K), and inactivating mutations in tumor suppressor kinases (e.g. LKB1, LATS). Recently, however, additional types of kinase mutations outside of the prototypical gain- or loss-of-function have been described. For example, so-called downstream rewiring mutations (DRMs) instead result in a substrate specificity switch of the kinase, theoretically causing a loss of affinity for some substrates while gaining completely new ones, and leading to a novel output resulting from the same input. A large number of such mutations have been predicted computationally, and only a few specific examples validated in *in vitro* kinase assays with peptide substrate libraries. However, the physiologic effects of these mutations on cellular signaling pathways and the mechanisms by which they contribute to disease phenotypes have not been elucidated. Here we develop an approach to validate the ability of DRMs to cause real substrate switches *in vivo*, using promiscuous biotin ligation to monitor global changes in substrate interaction and phosphorylation. We further use molecular modeling to assess alterations in kinase-substrate binding affinity and gain structural insights into how these mutations physically alter interactions with known substrates. Finally, we use computational approaches to predict physiologic substrate gains/losses and model the effects on downstream signaling pathways that contribute to the tumorigenic phenotype. Ultimately, this work will shed light on the underlying mechanisms of an understudied class of kinase mutations in human disease, and may help predict effective mutation-specific treatments for personalized medicine.

Membrane traffic modulating lipid PI3P regulates platelet production

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Platelets are the smallest of blood cells that play essential role in hemostasis but also contribute to diverse disease processes including inflammation, atherosclerosis and thrombosis. Platelets derive from precursor cells called megakaryocytes (MKs) that develop from hematopoetic stem cells in the bone marrow. Development of MKs implies enlargment of the cell size with formation of elaborate internal membranes called demarcation membrane system (DMS) and finaly extension of branched cytoplasmic structures, proplatelets, that will release mature platelets. Phosphoinositides (PIs) are short-lived minor membrane phospholipids implicated in cell signaling, membrane traffic, cytoskeletal dynamics, and motility among other functions. Phosphatidylinositol 3-monophosphate (PI3P), mainly produced by VPS34 kinase, is confined to endosomal compartments and regulates endolysosomal membrane trafficking. We hypothesized that constant dynamic exchange and traffic of membranes regulated by PI3P could contribute to platelet formation.

In our study we analyzed localization and levels of PIs in mouse fetal liver and bone marrow derived MKs by confocal microscopy. We stained MKs with recombinant probe GFP-2xFYVE to localize PI3P or expressed different PI binding domains from retriviruses to visualize diverse PIs. We found that PI3P levels and localization changes during MK development. PI3P levels were significantly higher in immature small compared to mature large MKs. In immature MKs, PI3P was confined to large vesicles, mostly colocalizing with early endosomes (EEA1), while in mature MKs PI3P resembled descrete vesicles colocalized with early and late endosomal markers (e.g. LAMP1). Furthermore, only expression of PI3P binding domains (YFP-PX and GFP-2xFYVE), but not YFP/EGFP, mutated GFP-2xFYVE, or domains detecting other types of PIs (PI(4,5)P2, PI(3,4,5)P3) decreased proplatelet formation from MKs. In addition, inhibition of PI3P production by pharmacological inhibitors significantly decreased proplatelet formation in a concentration dependent manner, and if applied at earlier stages, decreased size of MKs. Moreover, in mature MKs, PI3P was found to colocalize with both plasma membrane (PI(4,5)P2) and late endosomes/lysosomes (LAMP1), indicating close proximity/interaction of PI3P positive late endosomes/lysosomes and DMS. Collectively, our data indicate possible contribution of PI3P-dependent endolysosomal system in membrane growth during megakaryocyte maturation and platelet formation.

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Identification of differentially oxidized protein in psoriatic skin with 2D OxiDIGE

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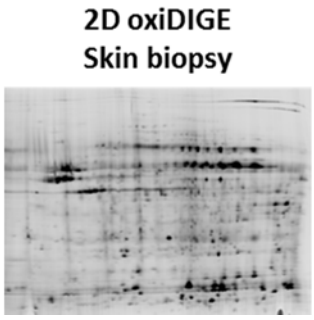
Psoriasis is an autoimmune skin disease involving oxidative stress through inflammation. This pathology was extensively studied but the proteomic redox aspect (carbonylome) of the disease was not described. Using 2D OxiDIGE method we investigated the proteome and carbonylome profile of skin biopsy from 11 psoriatic patient and 13 control donors in order to identify the differentially expressed and oxidized proteins in this pathology.

The 2D OxiDIGE method was improved and used in MedILS since 2014. and represents a state of the art method in the study of protein oxidation. It is used as a screening method for the detection of differentially oxidized proteins in complex samples. Simultaneous detection of carbonyl groups and protein peptide bonds in a 2D electrophoresis method allows precise quantification of the oxidative damage for all proteins detected on the 2D pattern.

Each spot on the 2D map is quantified for protein expression and oxidation. Using statistical approach we are able to select the spots differentially oxidized between tested conditions. Afterwards selected spots are sent to mass spectrometry analysis for identification of the proteins.

The experiment resulted in the identification of 20 proteins differentially oxidized and give us a new angle to understand and treat this pathology.



Characterization of the human immune-metabolic network associated to Age-related Macular Degeneration

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Age related macular degeneration (AMD) is a neurodegenerative retinal disease affecting around 30 million of individuals in worldwide and leading to severe vision loss in older people. Geographic atrophy (GA) is the advanced atrophic form of AMD for which no effective treatment is currently available. The concept that inflammation and immune cells play a role in AMD has arisen as a major mechanism of disease persistence and progression. While compelling evidences indicate that monocytes, key cells of host's innate immune response, display an activated phenotype and accumulate in the retinal tissue from patients with AMD, their potential contribution to the disease progression and the molecular mechanisms underlying their activation remain unknown. Emerging studies reveal the immune cells require specific changes to metabolic processes to ensure an appropriate immune response. In this project, we integrated literature information as well as AMD-associated genomic data to reconstruct metabolic changes occurring during the pathogenesis. We designed a network in which metabolic pathway changes are closely associated to innate immune response alterations. We propose now to provide a multi-omics approach to comprehensively characterize signaling and metabolic processes occurring within monocytes from patients with AMD in order to specifically identify signaling pathways and molecular targets that became accentuated during this advanced form of the disease. Finally, based on our metabolic network models, targeted molecular players will be evaluated on their ability to modulate monocyte functions and the disease outcome toward GA. Altogether, these results will increase our understanding on how metabolic pathways control cell functions and finally should allow us to identify promising molecular targets that could treat or delay AMD progression.

Characterization of spiral ganglion neurons *in vitro* cultured on 3D complementary metal-oxide-semiconductor chips

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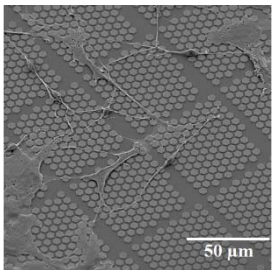
Cochlear implant (CI) is currently prevailing neuro-prosthetic treatment for partial restoration of hearing in deaf people. Despite its significant success, it is rather limited in terms of full recovery of sensorineural hearing loss (SNHL), caused by loss or damage of either hair cells or spiral ganglion neurons (SGN), or both, which affects majority of impaired patients[1]. Complementary metal–oxide–semiconductor (CMOS) chip with different diameters (1.4-4.8 μm) of micro-pillars and with maximal 3 μm spacing between pillars and a pillar height of 1 μm, was investigated as biocompatible material that can be used as permissive environment for organization and guidance of spiral ganglion neurons (SGN) as primary target for electrical stimulation of the auditory nerve. SGN extracted from cochleae of P5-P7 rat pups and adult guinea pigs were cultured 1, 4 and 7 days *in vitro* on glass coverslips (control) and CMOS chips and were analyzed for SGN presence, outgrowth, neurite alignment, axonal length as well as interaction with glial cells. Topography of isotropic substrate (CMOS) was shown to enhance growth, survival and morphology of SGN *in vitro* compared to control. Shorter spacing between 3D pillars on CMOS developed structured and guided neurites oriented along three topographical axes at 30°, 90° and 150°. Neurites in these areas grow in straight lines on top and between pillars and mostly followed a single direction by occasionally branching in the perpendicular direction, which is observed in SEM images that also highlight formation of nerve growth cone which filopodia encounter CMOS pillar and use it as anchoring point from where axon is repositioning. CMOS with 3D pillars can be used in neural engineering of auditory system, because it can guide neurites toward electrodes by means of structured pillar organization and it can produce electrical stimulation of neurons as well as to monitor their neural activities once neurites are in the vicinity of electrodes.



SEM image of spiral ganglion neurons on CMOS chip

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[1]Shepherd, R. K. & Hardie, N. A. Deafness-induced changes in the auditory pathway: implications for cochlear implants. *Audiol Neurootol* 6, 305-318 (2001).

Combined computational and rational design of α-helical antimicrobial peptides active against opportunistic pathogens

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Given the current global problem of multidrug resistance in human opportunistic pathogens, antimicrobial peptides (AMPs) show significant potential as novel anti-infective agents[1]. Notably, they show broad spectrum antibacterial activity and their most common mode of action, namely the disruption of bacterial membrane and/or the formation of pores or lesions, makes it hard for bacteria to gender permanent resistance. The toxicity towards host cells is however a key limitation factor, which imposes the necessity to design artificial AMPs *de novo*, or redesigning/mutating known natural AMPs to have an increased selectivity index (SI) and reduce side effects. We report four novel Gly and Lys rich peptides which were computationally designed based on a set of training natural peptides with low reported MIC values against *E.coli* and taking into account a defined set of parameters; net charge, hydrophobicity, number of Lysines, etc. One additional peptide was designed by creating the specular image of a previously designed sequence. All peptides were named kiadins, from the first three residues in the 'parent' peptide sequence[1].

Susceptibility testing was performed on clinically relevant, drug-resistant bacteria chosen among the ESKAPE pathogens; carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa,* 3rd generation cephalosporin-resistant *E. coli* and *Klebsiella pneumoniae*, as well as meticillin-resistant *Staphylococcus aureus* (MRSA). The results show different range of antibacterial activity with respect to Gly substitutions, where the peptides with higher proportion of Gly showed significantly weaker bactericidal effects. The best antibacterial activity was observed for Kiadin-2, which exhibited MIC values from 0.25 to 8 μM, a promising SI values and low cytogenotoxicity on HPBLs, making it a suitable candidate for further development.

Acknowledgments: Authors acknowledge funding from Croatian Science Foundation project 8481 and 4514. Department of Life Sciences (Trieste) acknowledges support from Beneficentia Stiftung, Lichtenstein, and Fondazione Stock-Weinberg, Italy.

1. Rončević, T. *et al.* PGLa-H tandem-repeat peptides active against multidrug resistant clinical bacterial isolates. *Biochim. Biophys. Acta BBA - Biomembr.* **1859,** 228–237 (2017).

“Thermofisher Lunch seminar”

Speeding up Biomarker Discovery: Advanced Translational Proteomics Workflow with High Resolution Accurate Mass (HRAM) MS

Claire Dauly, Omics Sales Support Manager Europe

ThermoFischerScientific

Diseases like cancer are complex and require another level of discipline in analytical proteomics measurements. Having set the standards for discovery research with deep comprehensive proteome profiles, new advances in Orbitrap technology and workflows allow for large scale analysis across large sample numbers.

Here we present a highly robust novel capillary-flow LC-MS platform that combines a capillary-flow chromatographic system (capLC), a new 150 µm column and a new Thermo Scientific™ Q Exactive™ HF-X mass spectrometer. The Q Exactive HF-X encompasses a high capacity transfer tube (HCTT), an electrodynamic ion funnel for increased ion flux and a scan rate of 40 Hz.

The new capLC-MS platform performances were assessed for shotgun and targeted high-resolution accurate-mass (HRAM) proteomics experiments demonstrating high sensitivity and robustness for the analysis of complex samples including bio-fluids.

A novel high resolution MS1 based data independent acquisition (DIA) method was also developed for comprehensive proteome profiling. The capLC-MS platform identifies and quantifies more than 4800 protein groups and 46000 peptide precursors in a 1-hour total run time (1% FDR and median CV<10%) delivering the analytical reproducibility and scalability required for rigorous results that matter.

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July 4th 2017

1st WORKSHOP – Behind the Scenes of Scientific Publishing

14:00 – 15:00	*Moderator:* ***Igor Stagljar*** *(University of Toronto, Canada)* **Ruth Zearfos -** Scientific Editor, Cell Reports **Maria Polychronidou -** Scientific Editor, Molecular Systems Biology **Natalie de Souza -** Chief Editor, Nature Methods

July 5th 2017

2nd WORKSHOP - Career workshop

15:00 - 16:30	*Moderator:* ***Iva Tolić*** *(Ruđer Bošković Institute, Croatia)* ***Uwe Sauer*** *(ETH Zurich, Switzerland)* ***John Yates III*** *(The Scripps Research Institute, USA)* ***Anita Kriško*** *(MedILS, Croatia)* ***Susan Michaelis*** *(Johns Hopkins University, USA)* ***Tony Hunter*** *(Salk Institute, USA)* ***Geert Kops*** *(Hubrecht Institute, Netherlands)*

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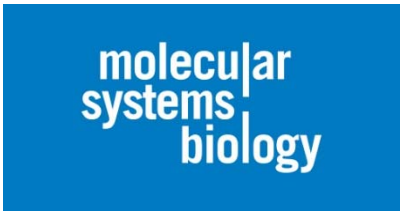

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