A.1

Dynamic proteomic profiling of the Salmonella-host interplay reveals new modes of action for known and novel virulence factors

Jennifer Geddes-McAlister[1], Stefanie Vogt[2], Jennifer Rowland[2], Sarah Woodward[2], Arjun Sukumaran[1], Lilianne Gee[1], Baerbel Raupach[3], Brett Finlay[2], Felix Meissner[4]

[1]University of Guelph, Guelph, Canada, [2]University of British Columbia, Vancouver, Canada, [3]Max Planck Institute for Infectious Biology, Berlin, Germany, [4]Max Planck Institute of Biochemistry, Martinsried, Germany

Intracellular bacterial pathogens cause a diverse array of diseases in humans and represent a significant threat to global health. These pathogens have evolved sophisticated strategies including the secretion of virulence factors to interfere with host cell functions and to perturb immune responses. However, interplay between the host and pathogen at the protein level in the context of infection has not been systematically investigated. Our 'infectome' analysis aims to identify previously undescribed proteins involved in bacterial virulence and host immune defense, representing an opportunity to elucidate molecular mechanisms of host-pathogen interplay during disease. Here, we investigate the host-pathogen interplay between the pathogenic bacteria, *Salmonella enterica* serovar Typhimurium, and primary macrophages. We performed quantitative proteomics of the host cells infected with wild-type (SL1344) or the type 3 secretion system (T3SS) mutant strains (Dspi-1 and Dspi-2) in single runs using high resolution mass spectrometry on a Quadrupole Orbitrap instrument. Our results provide a comprehensive and dynamic view of both pathogen and host proteins during infection. In the host cells, we observed the upregulation of proinflammatory and lysosomal proteins, representing host defense mechanisms to initiate immune responses and combat bacterial invasion. For *S. Typhimurium*, integration of proteome and infectome data identified eight proteins not encoded on SPI-1 or SPI-2 as being co-regulated with known virulence factors, suggesting a co-functional role in virulence and infection. Additionally, murine model competitive index assays revealed virulence-associated phenotypes of five proteins and defined their roles in bacterial cell regulation, as well as their impact on the host proteome. Overall, we provide an innovative strategy for profiling infection from dual perspectives in a single assay and characterizing novel virulence factors.

A.2

Identification of urine-derived diagnostic biomarkers for Tuberculosis

Bridget Calder[1]

[1]University of Cape Town, South Africa

Tuberculosis remains a leading cause of death worldwide, driven in part by the lack of sufficiently decisive diagnostic tools in the clinical setting. In South Africa, the incidence of TB/HIV co-infection is high, and co-infected individuals have particularly bad clinical outcomes. Some of the available diagnostics have a sensitivity as low as 50% in HIV positive individuals, and sputum-based testing is not possible in a high proportion of TB positive patients. An alternative TB diagnostic test should have high sensitivity and specificity in TB/HIV co-infected individuals, and be applicable in a biofluid that is obtained non-invasively. Urine has been proposed as an ideal biofluid for these purposes, and previous studies have found biomarkers for renal or GIT diseases in human urine. Since disseminated or extrapulmonary TB is often found in HIV positive individuals post mortem, we theorised that it should be possible to find a signature for TB in human urine that is either of TB or human origin. To that end, we employed discovery mass spectrometry-based proteomics to survey the urine of individuals who had been classified into four clinical groups: TB+/HIV-, TB+/HIV+, TB-/HIV+, and TB-/HIV-. This is the largest human urinary proteome-based study to date, comprising 120 individuals. Using Random Forest machine learning, TB status could be predicted using only four human proteins with a sensitivity and specificity of 95% and 85%, respectively in a one third hold-out set of the total data. We propose these human-derived biomarkers as a potential diagnostic panel for TB, which warrants further validation in a larger cohort.

This is an Open Access article under the CC BY license.

A.3

Microscaled Proteogenomic Methods for Precision Oncology

Shankha Satpathy[1], Eric Jaehnig[2], Karsten Krug[1], Michael Gillette[1], Alexander Saltzman[2], Kimberly Holloway[2], Meenakshi Anurag[2], Chen Huang[2], Purba Singh[2], Beom-Jun Kim[2], Goerge Miles[2], Noel Namai[2], Anna Malovannaya[2], DR Mani[1], Chuck Perou[3], Bing Zhang[2], Steven Carr[1], Matthew Ellis[2]

[1]The Broad Institute of MIT and Harvard, [2]Baylor College of Medicine, [3]University of North Carolina

Cancer proteogenomics combines genomics, transcriptomics and mass spectrometry-based proteomics to gain insights into cancer biology and treatment responsiveness. While proteogenomics analyses have shown great potential to deepen our understanding of cancer tissue complexity and signaling, how a patient's tumor changes upon treatment has largely been the province of genomics. This is due to technical difficulties associated with doing proteogenomic analysis on clinic-derived core-needle biopsies. To address this critical need, we have developed a "microscaled" proteogenomics approach for tumor-rich OCT-embedded core needle biopsies. Tissue-sparing specimen processing ("Biopsy Trifecta EXTraction", BioTExt) and microscaled proteomics (MiProt) methodologies allowed generation of deep-scale proteogenomics datasets, with copy number and transcript information for >20,000 genes and mass spectrometry-based identification and quantification of nearly all expressed proteins in a tumor (>10,000 proteins) and more than >20,000 phosphosites starting with just 25 micrograms of peptides per sample. In order to understand the capabilities and limitations of our approach relative to conventional deepscale proteomics requiring >10X more starting material, we compared preclinical patient derived xenograft (PDX) models at conventional scale with data obtained by core-needle biopsy of the same tissues. Comparable depth and biological insights were obtained from the cores relative to surgically resected tumors. As a proof-of-concept for implementation in clinical trials, we applied microscaled proteogenomic methods to a small-scale clinical study where biopsies were accrued from patients with ERBB2+ advanced breast cancer before and 48 to 72 hours after the first dose of neoadjuvant Trastuzumab-based chemotherapy. Multi-omics comparisons were conducted between samples associated with residual disease versus samples associated with complete pathological response. Integrative proteogenomic analyses efficiently diagnosed the molecular bases of diverse candidate treatment resistance mechanisms including: 1) absence of ERBB2 amplification (false-ERBB2+); 2) insufficient ERBB2 activity for therapeutic sensitivity despite ERBB2 amplification (pseudo-ERBB2+); 3) resistance features in true-ERBB2+ cases including androgen receptor signaling, mucin expression and an inactive immune microenvironment; 4) lack of acute phospho-ERBB2 down-regulation in non-pCR cases. In summary, we have developed a proteogenomics pipeline well suited for large-scale cancer clinical studies to identify potential resistance mechanism in patients. We conclude that microscaled cancer proteogenomics could improve diagnostic precision in the clinical setting.

A.4

Reduced proteasome activity in the aging brain results in ribosome stoichiometry loss and aggregation

Joanna M. Kirkpatrick[1], Erika K. Sacramento[1], Mariateresa Mazzetto[1], Simone Di Sanzo[1], Cinzia Caterino[1], Aleksandar Bartolome[1], Michele Sanguanini[3], Nikoletta Papaevgeniou[4], Maria Lefaki[4], Dorothee Childs[5], Eva Terzibasi-Tozzini[2], Natalie Romanov[5], Mario Baumgart[1], Wolfgang Huber[5], Niki Chondrogianni[4], Michele Vendruscolo[3], Alessandro Cellerino[1,2], Alessandro Ori[1]

[1]Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Jena, Germany, [2]Scuola Normale Superiore, Pisa, Italy, [3]Centre for Misfolding Diseases, Department of Chemistry, University of Cambridge, Cambridge, UK, [4]Institute of Biology, Medicinal Chemistry and Biotechnology, Athens, Greece, [5]European Molecular Biology Laboratory, Heidelberg, Germany

A progressive loss of protein homeostasis is characteristic of aging and a driver of neurodegeneration. To investigate this process quantitatively, we characterized proteome dynamics during brain aging by using the short-lived vertebrate Nothobranchius furzeri and combining transcriptomics, proteomics and thermal proteome profiling. We found that the correlation between protein and mRNA levels is progressively reduced during aging, and that post-transcriptional mechanisms are responsible for over 40% of these alterations. These changes induce a progressive stoichiometry loss in protein complexes, including ribosomes, which have low thermal stability in brain lysates and whose component proteins are enriched in aggregates found in old brains. Mechanistically, we show that reduced proteasome activity occurs early during brain aging, and is sufficient to induce loss of stoichiometry. Our work thus defines early events in the aging process that can be targeted to prevent loss of protein homeostasis and age-related neurodegeneration.

A.5

Affinity Proteomics Reveals Assembly of PPP-type Phosphatase Holoenzyme by PPM1G-B56δ

Parveen Kumar[1,2], Prajakta Tathe[1,2], Subbareddy Maddika[1]

[1]Laboratory of Cell Death & Cell Survival, Centre for DNA Fingerprinting and Diagnostics, INDIA, [2]Graduate studies, Manipal Academy of Higher Education, Manipal 576104, INDIA

Serine/threonine phosphatases form distinct holoenzymes to achieve substrate specificity. PPP serine/threonine phosphatase family members such as PP1 and PP2A are well known to assemble and function as holoenzymes, but none of the PPM family members was so far shown to assemble holoenzymes. Here, we performed a systematic proteomic analysis of human Ser/Thr protein phosphatases associated protein complexes using tandem affinity purification coupled with mass spectrometry (TAP-MS). Our interaction screen revealed an assembly of a holoenzyme by PPM1G, a member of PPM family of serine/threonine phosphatases. We identified that PPM1G interact with a regulatory subunit B56δ to form a distinct holoenzyme complex. B56δ alters the localization of PPM1G to the cytoplasm where PPM1G can act on a discrete set of substrates. Further, we identified α-Catenin, a component of adherens junction, as a novel substrate for PPM1G-B56 phosphatase in the cytoplasm. B56δ-PPM1G dephosphorylates α-Catenin at Serine 641 and prevents aberrant cell migration. Collectively, we identified a new phosphatase holoenzyme with PPM1G-B56δ as integral components, in which the regulatory subunit regulates its cellular localization to target distinct substrates.

A.6

Targeted Quantification of Incomplete Prohormone Processing Products in Type 1 Diabetes

Yinyin Ye[1], Adam C. Swensen[1], Lian Yi[1], Yuqian Gao[1], Emily K. Sims[2,3], Tujin Shi[1], Carla J. Greenbaum[4], Carmella Evans-Molina[2,5,6,7,8]

[1]Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA 99354, [2]Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN 46202, [3]Center for Diabetes and Metabolic Diseases, Indiana University School of Medicine, Indianapolis, IN, [4]Diabetes Clinical Research Program, Benaroya Research Institute, Seattle, WA 98101, [5]Department of Cellular and Integrative Physiology, Indiana University School of Medicine, Indianapol, [6]Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202, [7]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapoli, [8]Richard L. Roudebush VA Medical Center, Indianapolis, IN 46202

Type 1 diabetes (T1D) is an autoimmune disease marked by the loss of insulin production in pancreatic islet β-cells. In healthy β-cells, proinsulin processing results in the cleavage of the C-peptide fragment thereby forming mature insulin. At diagnosis, C-peptide is usually still detectable at low levels but over time it is lost completely. However, low-level detectable proinsulin secretion was recently shown to be retained years after the initial diagnosis of T1D. This suggests that incomplete proinsulin processing may be associated with T1D etiopathology. Accurate quantitative measurements of proinsulin and C-peptide help us better understand the pathophysiology of T1D. The most common current measurement methods rely on antibody-based affinity assays. These assays may not be sufficiently specific and may fail to capture the subtle variations within proinsulin and C-peptide. To overcome these issues, mass spectrometry (MS) can be utilized to achieve confident detection specificity. In this presentation, we aim to develop a targeted MS method (i.e., liquid chromatography-selected reaction monitoring) in order to characterize incomplete hormone processing in islets and serum from T1D subjects. In addition to our initial classical trypsin-digestion-based proteomics technique, we have included two additional proteases, GluC and AspN, which were optimized using mouse macrophages RAW264.7. These two proteases were selected primarily because their cleavage sites are located outside of the important enzymatic processing regions used by proinsulin processing enzymes (i.e., PC1/3, CPE, etc.). Whether proinsulin is partially processed or not can be identified. Our preliminary results demonstrate that the optimized GluC and AspN digestions achieved digestion specificities of nearly 80% and 50%, respectively. We are further optimizing the method with clinical samples, and will apply the assays to longitudinal T1D samples. The targeted MS method can be easily expanded in future work to characterize other prohormone forms in human islets, such as proglucagon, pro-islet amyloid polypeptide (pro-IAPP), and pro-somatostatin.

A.7

Novel methods and reagents for characterization of protein biotinylation sites by peptide-based immunoaffinity enrichment

Yiying Zhu[1], Matthew D. Fry[1], Alissa J. Nelson[1], Jian Min Ren[1], Vicky Yang[1], Michael C. Palazzola[1], Charles L. Farnsworth[1], Matthew P. Stokes[1], Kimberly A. Lee[1]

[1]Cell Signaling Technology INC

Biotin labeling in combination with LC-MS/MS has been widely applied in large-scale analysis of protein-protein interactions, subcellular localization, and post-translational modifications. Direct characterization of protein biotinylation sites is challenging due to the low recovery of biotinylated peptides using conventional streptavidin-based purification methods. Previous published studies demonstrated that anti-biotin antibodies are superior capture reagents for biotinylated peptides compared to streptavidin. In this study, we establish an immunoaffinity enrichment method using a monoclonal anti-biotin antibody compared to previously published approaches using commercially available polyclonal antibodies.

Trypsin digested mouse liver peptides labeled with NHS-biotin and mixed with unlabeled liver peptides were used as the test sample for method optimization and comparison. Enrichment with the rabbit monoclonal anti-biotin antibody yielded more biotinylated peptide identifications than any polyclonal antibody tested. Over 3,400 unique biotinylated peptides were identified using the monoclonal antibody, with other available antibodies ranging from 200 to 3,200 unique peptides.

We then used this optimized enrichment method to characterize protein biotinylation sites from APEX proximity labeling in living cells. HEK 293T cells stably expressing fused β2AR and APEX were cultured, incubated with biotin phenol, and treated with the agonist BI167107 for 10 min. Protein biotinylation was activated by adding H_2O_2. In total 1,354 unique biotinylated peptides from 858 proteins were identified and quantified. Among them, the levels of 148 biotinylated peptides from 125 proteins were responsive to agonist treatment.

A.8

Exploring the glycosylation levels of snake venom proteins by mass spectrometry: microheterogeneity determination of sweet spots in toxins of Bothrops snake genus

Débora Andrade Silva[1], Lívia Rosa-Fernandes[2], Giuseppe Palmisano[3], Silvia R. Travaglia-Cardoso[4], Solange M. Toledo Serrano[1], Martin R. Larsen[2]

[1]Laboratório Especial de Toxinologia Aplicada, Instituto Butantan, São Paulo, Brazil, [2]Centre of Clinical Proteomics, Department of Biochem. and Mol. Biology, SDU, Odense, Denmark, [3]Laboratório de GlicoProteômica, Instituto de Ciências Biomédicas, USP, São Paulo, Brazil, [4]Museu Biológico, Instituto Butantan, São Paulo, Brazil

Differently from proteomic studies based on non-modified peptide identification, the analysis of post-translational modifications (PTMs), such as glycosylation, faces challenges in all analytical steps. The first key step to identify glycopeptides using mass spectrometry involves their enrichment from a complex mixture to overcome the ion suppression and abundance of non-modified peptides. In this study, we used two strategies to enrich and fractionate glycopeptides of Bothrops venom proteins: TiO_2 beads and HILIC. It has recently been shown that the N-glycome of Bothrops venoms contains structures belonging to high mannose and hybrid/complex types (with and without sialic acid); therefore, the enrichment step using TiO_2 beads allowed the isolation of sialylated glycopeptides, while the non-binding fraction was submitted to HILIC to further capture the remaining glycopeptides. To help in peptide backbone identification, part of each glycopeptide fraction was submitted to enzymatic deglycosylation with PNGase F. Then, the fractions were submitted to LC-MS/MS fragmentation using stepped HCD fragmentation. These enrichment steps proved to be efficient to separate the sialic acid-containing from other glycopeptides, as observed from the different profiles of oxonium ion reporters in their MS2 spectra. This strategy allowed the identification of the primary structures of deglycosylated peptides, which, otherwise, is not straightforwardly obtained from the fragmentation of the intact glycopeptides. To identify the glycosylation profile of Bothrops venoms, we combined information from a database of N- glycans from eight Bothrops venoms together with the peptide sequence identified in the deglycosylated peptide fraction, using the GlycReSoft software. The number of identified deglycosylated peptides (1500–3500 per venom) was significantly higher than that of identified intact glycopeptides (90–230 per venom) confirming the difficulty in assigning intact glycopeptides. Nevertheless, an important aspect of these findings is the view of toxin glycosylation microheterogeneity and profile, especially in snake venom metalloproteinases, which appear to display specific patterns of N-glycan structures on their different structural domains. As a general view, the data illustrate that N-linked glycosylation is not only a common PTM but also an important component of venom phenotype variability.

Financial Support: FAPESP (2013/07467-1; 2018/08794-0; 2016/16935-7); the Villum Center for Bioanalytical Sciences at SDU.

A.9

Expression of PNPLA3 I148M Variant Alters Lipid Droplet Proteome

Mara Monetti[1], Jeffrey Culver[1], Sharath Sasi[1], Liang Xue[1], Gregory Tesz[1], Collin Crowley[1], Trenton Ross[1], Thomas Magee[1], Melissa Miller[1], Bei Zhang[1], Kendra Bence[1]

[1]Pfizer, Internal Medicine Research Unit, Cambridge, MA, 20139, USA

Liver cirrhosis as a consequence of fatty liver disease is a leading cause of liver transplant in the Unites States. A variant (I148M) of the lipid droplet protein patatin-like phospholipase domain-containing protein 3 (PNPLA3) is associated with hepatic steatosis. However, the mechanisms of liver lipid accumulation due to PNPLA3 mutation are unclear.

To investigate the biological function of wildtype and variant PNPLA3, we used an unbiased and systematic proteomic approach, analyzing the protein composition of lipid droplets (LD) isolated from livers of mice expressing either variant of human PNPLA3. We isolated lipid droplets by sucrose gradient (20 to 55% sucrose) centrifugation and analyzed their proteome using a label free quantification approach. Using high resolution mass spectrometry-based proteomics, we identified ~800 proteins in the lipid droplet fraction. Among the most abundant of these proteins were well-known lipid droplet proteins, including perilipins, hormone sensitive lipase and CGI58. Bioinformatics analyses of these datasets show that PNPLA3 accumulates to a higher level on lipid droplets isolated from mice expressing PNPLA3 I148M as compared to those from mice expressing the wild-type protein. Interestingly, some lipid droplet proteins, such as CGI58, increased in the lipid droplet fraction upon expression of I148M, while other lipid droplet proteins, such as Lipe, did not change between the two genotypes, consistent with published observations (Smagris et al, 2015). When challenging murine liver with a Western diet, PNPLA3 I148M induced extensive changes in the lipid droplet protein composition, consistent with data published by the laboratory of Drs. Cohen and Hobbs. Among the striking changes, we detected fewer proteins of the ubiquitin/proteasome system in murine liver lipid droplets from PNPLA3 I148M expressing animals, supporting the hypothesis of differential turnover regulation due to PNPLA3 expression.

A.10

Vascular Cell Surface Proteomics In Vivo

Dirk M. Walther[1], Elizabeth Gordon[1], Benjamin Smith[1], Thomas O. Cameron[1], Ru Wei[1], Peter Juhasz[1]

[1]Biogen, Cambridge, MA 02142

Therapeutic antibodies have great potential for the treatment of neurological diseases. However, only a small fraction of drug molecules cross from the bloodstream into the central nervous system (CNS) because they are excluded by of the blood brain barrier (BBB). Receptor mediated transport (RMT) is a mechanism which relies on cell surface proteins shuttling between the luminal (blood) and the abluminal (brain) side of endothelial cells to facilitate the transport of cargos across the BBB. Most known RMT receptors, such as Transferrin receptor (TfR1), are expressed in multiple organs, resulting in a rapid target-mediated drug disposition and thus unfavorable pharmacokinetics. This limitation could be overcome if suitable brain-specific RMT receptors were known.

To better understand tissue-specific differences in the microvasculature, we developed an in vivo approach to catalogue the luminally accessible proteome of different organs in rats and non-human primates (cynomolgus monkeys). We optimized a chemical cell surface labeling protocol by cardiac perfusion of anesthetized animals ($n \geq 6$) using a lysine-reactive reagent with a biotin affinity handle. A vehicle perfused group of animals was included as a control. After tissue collection and lysis, labeled proteins were enriched with streptavidin beads and eluted by reductive linker cleavage under mild conditions. After digestion proteins were quantified by label-free proteomics using a QE HF mass spectrometer. Additionally, proteomes of total lysates were acquired to determine enrichment factors of labeled proteins with regards to total expression levels, resulting in the identification of more than 14,000 proteins in each species.

With this workflow, we were able to label the vascular bed in the CNS and all seven peripheral tissues analyzed. The corresponding cell surface proteomes show reproducible tissue-specific expression patterns. In non-human primates, most of the cell surface proteins enriched in the CNS over the periphery were detected consistently and at similar levels in the five different brain regions covered in our study. Among those proteins were most known RMT receptors, including TfR1, insulin receptor, IGF-I receptor, and GLUT-1 In summary, studying vascular cell surface proteomes in vivo provides a valuable starting point for the development of bispecific therapeutic antibodies with improved CNS exposure.

A.11

Application of 4C Proteomics and Interactomics in Study of PTM Proteins Involved in Regulation of Arabidopsis Flowering

Ning Li[1,2]

[1]Division of Life Science, Hong Kong University of Science and Technology, Hong Kong SAR, [2]The HKUST Shenzhen Research Institute, Shenzhen, [3]Energy Institute, Hong Kong University of Science and Technology, Hong Kong SAR, [4]Institute for the Environment, Hong Kong University of Science and Technology, Hong Kong SAR

Ethylene and force signals are well-known for their roles in regulation of bolting. The underlying molecular mechanisms are important issues in plant biology. To understand these molecular mechanisms, we have applied proteomics to study the post-translational modification (PTM) of proteins that play an important role in regulation of numerous cellular events, protein-protein interactions and enzymatic activities in Arabidopsis, a model plant organism. A stable isotope labeling-based 4C quantitative PTM proteomics and interactomics were therefore established to investigate the roles of key proteins in both ethylene and force cell signaling. The SILIA- and iTRAQ-based quantitative PTM proteomics have revealed that TREPH1 and ERF110 proteins are phosphorylated in response to mechanical and hormonal signals, respectively. Molecular genetics and cell biology studies confirmed that phosphorylation of the transcriptional factor, ERF110, and the cytoskeleton protein, TREPH1, are required for regulating Arabidopsis flowering.(Funding Supports: 16101819, 16100318, 16103817, 1613615, AOE/M-403/16, 31570187, 31370315, C020406). Author's website for publication: https://life-sci.ust.hk/team/ning-li/.

A.12

Characterization of Symptomatic Aortic Valve Stenosis Subtypes by DIA-MS Proteome Profiling

Christof Lenz[1,2], Lisa Neuenroth[2], Soeren Brandenburg[3], Stephan Lehnart[3,4,5], Henning Urlaub[1,2]

[1]Bioanalytical Mass Spectrometry, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany, [2]Core Facility Proteomics, University Medical Center Göttingen, Germany, [3]Heart Research Center, University Medical Center Göttingen, Germany, [4]German Centre for Cardiovascular Research (DZHK), Göttingen, Germany, [5]DFG Collaborative Research Center 1002-A09

Introduction: The success of proteomics in precision medicine rests on the availability of experimental protocols that provide sufficient analytical depth, reproducibility, throughput and turnaround for relevant tissue and body fluid samples. Left ventricular (LV) biopsies from patients with aortic valve stenosis (AVS) promise to be a highly valuable source of information to deepen the molecular understanding and, potentially, differential diagnosis of presumed classes and/or pathophysiological stages of heart disease. We have successfully developed and demonstrate a rapid analytical workflow consisting of pressure-cycling tissue lysis and label-free data-independent acquisition mass spectrometry (DIA-MS) that enables the medium throughput analysis of small LV biopsy samples.

Materials & Methods: Small volumes (1–3 mm^3) typical for bioptic samples of human heart tissue obtained during a clinical study from patients diagnosed with severe AVS were lysed and digested using Pressure Cycling Technology (PCT: Barocycler 2320; Pressure Biosciences), and analyzed by DIA-MS on a hybrid quadrupole/time-of-flight mass spectrometer (TripleTOF 5600+, Sciex). Neutral pH Reversed Phase (nPH-RP) pre-fractionation was used to build a tissue-specific spectral library.

Results: nPH-RP separation of digested peptide samples provided a spectral library consisting of 2,951 proteins @ 1% FDR. Data-independent analysis (DIA) by SWATH-MS on a hybrid quadrupole/time-of-flight instrument showed that small LV biopsies could be profiled to a depth of 2,273 protein across 25 samples each representing one idividual patient. Our workflow enabled high reproducibility, a sample throughput of up to 12 samples/day and a turnaround time of 1.5 days. Hence, the use of parallelized pressure cycling technology allows for straightforward upscaling of sample handling. Exploratory statistical analysis shows that different classes of AVS (type I-IV) correlate with their corresponding proteome profiles, opening avenues to explore potential diagnostic biomarkers.

A.13

Cell-surface proteomic landscape of developing and mature olfactory projection neurons

Jiefu Li[1], Shuo Han[2], Hongjie Li[1], Namrata D. Udeshi[3], Tanya Svinkina[3], D. R. Mani[3], Chuanyun Xu[1], Ricardo Guajardo[1], Qijing Xie[1], Tongchao Li[1], Bing Wu[1], Anthony Xie[1], David J. Luginbuhl[1], Pornchai Kaewsapsak[2], Stephen R. Quake[4], Steven A. Carr[3], Alice Y. Ting[2], Liqun Luo[1]

[1]Department of Biology, Howard Hughes Medical Institute, Stanford University, [2]Departments of Genetics, Biology, and Chemistry, Chan Zuckerberg Biohub, Stanford University, [3]The Broad Institute of MIT and Harvard, [4]Departments of Bioengineering and Applied Physics, Chan Zuckerberg Biohub, Stanford University

Intercellular signaling governs the development and physiology of multicellular organisms. Delineating the principles of cell-surface signaling is thus a crucial step to understand the organization and function of any multicellular system, including the intricately wired nervous system. We developed a cell-type-specific, spatiotemporally-resolved, proximity-labeling proteomic approach to profile the cell-surface proteome in intact tissues. Applying it to the Drosophila olfactory circuit, we observed proteome-wide temporal evolution of cell-surface molecules in coordination with the developmental timeline. Multi-omic analysis revealed a broad impact of post-transcriptional regulation on the dynamics of cell-surface proteins, especially the ones playing central roles in neural development and synaptic transmission. Unbiased genetic screen of developmentally enriched cell-surface proteins identified many new molecules required for wiring specificity, the majority of which belong to unexpected molecular families.

A.14

Breast cancer quantitative proteome and proteogenomic landscape

Henrik J. Johansson[1], Fabio Socciarelli[1], Nathaniel M. Vacanti[1,2], Mads H. Haugen[3], Yafeng Zhu[1], Ioannis Siavelis[1], Alejandro Fernandez-Woodbridge[1], Miriam R. Aure[4], Bengt Sennblad[5], Mattias Vesterlund[1], Rui M. Branca[1], Lukas M. Orre[1], Mikael Huss[6], Erik Fredlund[1], Elsa Beraki[7], Øystein Garred[7], Jorrit Boeke[1], Torill Sauer[8,9], Wei Zhao[10], Silje Nord[4], Elen K. Höglander[4], Daniel C. Jans[11], Hjalmar Brismar[11,12], Tonje H. Haukaas[13], Tone F. Bathen[13], Ellen Schlichting[14], Bjørn Naume[9,15], OSBREAC [16], Torben Luders[9,17], Elin Borgen[7], Vessela N. Kristensen[4,9,17], Hege G. Russnes[4], Ole Christian Lingjærde[4,18], Gordon B. Mills[10], Kristine K. Sahlberg[4,19], Anne-Lise Børresen-Dale[4,9], Janne Lehtiö[1]

[1]Science for Life Laboratory, Department of Oncology-Pathology, Karolinska Institutet, 171 21, Solna, [2]Cornell University, Division of Nutritional Sciences, Ithaca, NY 14853, USA, [3]Dept of Tumor Biology & Dept of Cancer Genetics, Inst for Cancer Research, Oslo University Hospital, [4]Dept of Cancer Genetics, Inst for Cancer Research, Oslo University Hospital, 0424, Oslo, [5]Dept. of Cell and Molecular Biology, NBIS Sweden, Science for Life Laboratory, Uppsala University, [6]Dept of Biochemistry and Biophysics, NBIS Sweden, Science for Life Laboratory, Stockholm University, [7]Department of Pathology, Oslo University Hospital, 0424, Oslo, Norway, [8]Department of Pathology, Akershus University Hospital, 1478, Lørenskog, Norway, [9]Institute for Clinical Medicine, University of Oslo, 0318, Oslo, Norway, [10]Department of Systems Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA, [11]Department of Applied Physics, KTH Royal Institute of Technology, 171 21, Solna, Sweden, [12]Department of Womens's and Children's Health, Karolinska Institutet, 171 21, Solna, Sweden, [13]Dept. of Circulation and Medical Imaging, The Norwegian University of Science and Technology – NTNU, [14]Section for Breast- and Endocrine Surgery, Dept of Cancer, Oslo University Hospital, [15]Dept of Oncology, Division of Surgery & Cancer & Transplantation Medicine, Oslo University Hospital, [16]www.osbreac.no, Oslo, Norway, [17]Dept of Clinical Molecular Biology & Laboratory Science (EpiGen), Akershus University Hospital, [18]Centre for Cancer Biomedicine, University of Oslo, 0424, Oslo, Norway, [19]Department of Research, Vestre Viken Hospital Trust, 3004, Drammen, Norway

We present a proteome-centric multi-omics examination of the breast cancer (BC) molecular landscape. Unbiased analyses of deep tumor proteomes recapitulate PAM50 BC subtypes while further distinguishing poor-prognosis basal-like and luminal B tumors by immune component infiltration, suggesting the current classification is incomplete. Proteome-based networks distinguishes BC subtype-specific functional protein modules with co-expression of known drug targets marking ductal carcinoma in situ regions of normal-like tumors, lending to a more accurate classification of this poorly defined subtype. We find effects of copy number alterations to be dampened dependent on protein-level gene regulation, and transcripts within prognostic mRNA panels to be reliable protein surrogates, underscoring the value of proteome quantification for prognostication and phenotypic classification. Furthermore, protein products mapping to "non-coding" genomic regions were identified; highlighting a potential new class of tumor-specific immunotherapeutic targets.

A.15

Native Mass Spectrometry Study on the Stoichiometry of Proteasome AAA+ ATPase Nucleotide Binding

Yadong Yu[1,4], Haichuan Liu[2,5], Zanlin Yu[1], H. Ewa Witkowska[2], Yifan Cheng[1,3]

[1]UCSF, Dept. Biochemistry and Biophysics, San Francisco, CA 94143, [2]UCSF, Dept. OBGYN & Reproductive Sci, Sandler-Moore MS Core Facility, San Francisco CA 94143, [3]Howard Hughes Medical Institute, University of California San Francisco, CA 94143, [4]LakePharma, 201 Industrial Rd, San Carlos CA 94070, [5]Thermo Fisher Scientific, 355 River Oaks Pkwy, San Jose, CA 95134

AAA+ ATPases constitute a large family of proteins that are involved in a plethora of cellular processes including DNA disassembly, protein degradation and protein complex disassembly. They typically form a hexameric ring-shaped structure with six subunits in a (pseudo) six-fold symmetry. In a subset of AAA+ ATPases that facilitate protein unfolding and degradation, six subunits cooperate – in a yet unknown fashion – to translocate protein substrates through a central pore in the ring. The number and type of nucleotides in an AAA+ ATPase hexamer is inherently linked to the mechanism that underlies cooperation among subunits and couples ATP hydrolysis with substrate translocation. We conducted a native mass spectrometry study of a monodispersed form of PAN, an archaeal proteasome AAA+ ATPase, to determine the number of nucleotides bound to each hexamer of the wild-type protein. We utilized ADP and its analogues (TNP-ADP and mant-ADP), and a non-hydrolyzable ATP analogue (AMP-PNP) to study nucleotide site occupancy within the PAN hexamer in ADP- and ATP-binding states, respectively. Throughout all experiments we used a Walker A mutant that is impaired in nucleotide binding as an internal standard to mitigate the effects of residual solvation on mass measurement accuracy and to serve as a "reference protein" to control for non-specific nucleotide binding. This approach led to the unambiguous finding that a wild-type PAN hexamer carried – from expression host – six tightly bound ADP molecules that could be exchanged for ADP and ATP analogues. While the Walker A mutant did not bind ADP analogues, it did bind AMP-PNP, albeit at multiple stoichiometries. We observed variable levels of hexamer dissociation and an appearance of multimeric species with the overcharged-like molecular ion distributions across repeated experiments. We posit that these phenomena originated during ESI process at the final stages of ESI droplet evolution.

A.16

Towards Elucidation of Muscle-Specific Receptor Tyrosine Kinase (MuSK) Signaling Pathway by Differential Agonists

Hanna G. Budayeva[1], Arundhati Sengupta Ghosh[1], Lilian Phu[1], Donald S. Kirkpatrick[1]

[1]Genentech Inc., South San Francisco, CA 94080

Muscle-Specific receptor tyrosine Kinase (MuSK) is essential for neuromuscular junction (NMJ) formation. MuSK is activated upon binding to Lrp4 co-receptor in complex with agrin, a motor neuron signaling molecule. In response to MuSK activation, acetylcholine receptors begin clustering on the muscle surface to guide neuronal attachment. Molecular players involved in propagation of MuSK signaling are not yet fully defined. We employed mass spectrometry-based quantification by TMT to elucidate ubiquitination- and phosphorylation-mediated signaling pathways activated in response to treatment with agrin or MuSK agonist antibody. We observed that majority of MuSK signaling was mediated by phosphorylation. Significant increases in phosphotyrosine levels were detected on MuSK and its adaptor protein Dok7 upon treatment with each agonist. Several AchR subunits were detected with increased phosphorylation, in line with previously reported observations that phosphorylation plays an important role in AchR clustering. Profiling of phosphorylation and ubiquitination events revealed that proteins with functions in clathrin-mediated endocytosis are regulated by ubiquitination events downstream of MuSK. Overall results point at a significant overlap in signaling processes initiated by MuSK natural agonist agrin and MuSK agonist antibody.

A.17

Multiplexed and Quantitative Assessment of the Cellular Reactive Cysteinome in T cell activation

Liang Xue[1], Uthpala Seneviratne[2]

[1]Simulation and Modeling Science, Pfizer, [2]I&I Medicinal Chemistry, Pfizer

Over the past few years, a broad variety of chemoproteomic methods on targeting cysteine residues have been reported. One such technology, isotopic tandem orthogonal proteolysis-activity based protein profiling (isoTOP-ABPP) uses iodoacetamide alkyne probe for cysteine targeting and isotope coded cleavable azide for click chemistry mediated-enrichment for greater peptide and protein identifications in a complex proteome. In here we describe a much simpler platform that uses a broad spectrum cysteine-reactive desthiobiotin iodoacetamide (DBI) probe in combination with tandem mass tags (TMT-10plex) to uncover in-depth of the reactive cysteinome. By employing the multiplexed-cysteine profiling platform we identified and quantified more than 13000 probe labeled-peptides that correspond to more than 4500 proteins in the human T cell proteome. The method facilitates a high throughput chemoproteomics by comparing multiple samples at once and enables the interrogation of low abundant cysteine activated proteins with greater depth.

A.18

Enzyme toolkit for selective enrichment and analysis of mucin-domain glycoproteins

Stacy A. Malaker[1], Judy Shon[1], Kayvon Pedram[1], Nicholas M. Riley[1], Carolyn R. Bertozzi[1,2]

[1]Stanford University, Stanford, CA 94305, [2]Howard Hughes Medical Institute, Stanford, CA 94305

Mucin domains are densely O-glycosylated modular protein domains that are found in a wide variety of cell surface and secreted proteins. Mucin-domain glycoproteins are known to be key players in a host of human diseases, especially cancer, wherein mucin expression and glycosylation patterns are altered. Mucin biology has been difficult to study at the molecular level in part because methods to manipulate and structurally characterize mucin domains are lacking. One major issue is that these domains are resistant to degradation by trypsin, meaning the majority of their sequence space is often left unanalyzed. Selective mucin degradation or enrichment, especially in a sequence- and glycan-specific manner, can facilitate study of these proteins by mass spectrometry.

Previously, we expressed and characterized a bacterial mucinase, StcE, and used its unique properties to improve sequence coverage, glycosite mapping, and glycoform analysis of recombinant human mucins by mass spectrometry. To expand on this work, we expressed and characterized several other bacterial mucinases to generate a mucin-selective enzymatic toolkit. Their activities were confirmed using a panel of O-glycoproteins by mass spectrometry. We manually validated peptide sequences from MS/MS spectra to elucidate all cleaved peptides present in the mucinase-digested samples but not in the control samples, revealing that each enzyme has a slightly different cleavage motif. Interestingly, all of the enzymes rely on a combination of peptide sequence and glycosylation status. Together with StcE, we have characterized a total of five bacterial mucinases capable of digesting mucins into peptides amenable for mass spectrometric analysis.

Further, given the enzymes' selectivity for mucin-domain glycoproteins, we reasoned that they could be employed to purify mucins from protein mixtures. Thus, inactivated mucinases were conjugated to aldehyde beads using reductive amidation. Using the enzyme-conjugated beads, we demonstrate that we can selectively enrich for mucin-domain glycoproteins from lysate and crude cancer patient ascites fluid. We are thus defining the "mucinome", as a comprehensive list of mucin-domain glycoproteins does not exist. Future experiments will be devoted to isolation, digestion, and characterization of mucins from human cancer patient ascites fluid, with the ultimate goal of identifying diagnostic and/or prognostic markers of disease states.

A.19

Functional Metabolomics uncovering the role of Trp–KYN–KA Axis in Intestinal Injury and Repair

Di Wang[1], Huimin Guo[1], Zunjian Zhang[1], Fengguo Xu[1]

[1]China Pharmaceutical University, Nanjing 210009, P. R. China

Drug-induced disease has become one of the major causes of death in clinical. Gastrointestinal dysfunction which accounts for about 20% of all kinds of adverse drug reactions (ADRs) cause gastrointestinal mucosa damage, reduce mucosal barrier function and led to inflammatory bowel disease (IBD). Targeted metabolomics studies of Trp metabolic profile in vincristine-induced rat ileus, irinotecan-induced rat diarrhea and DSS-induced rat IBD models we found that Trp–KYN–KA axis metabolism was significantly increased in rat damaged intestinal. Besides, damaged intestinal was significantly recovered after drug was terminated, hypothesizing that Trp–KYN–KA axis might be plays an important role in intestine injury and repair. Following studies we found that colon formed IL-6–IDO1–AHR positive feedback loop at pathologic condition, not at normal condition, accelerate KYN and KA accumulation. Besides, we also found that IL-6 which came from macrophages plays a leading role in the accumulation of KYN and KA, whereas positive feedback loop only works as a role of auxiliary acceleration. Unexpectedly, KYN and KA inhibited LPS-induced IL-6 production by activating AHR in macrophages, reminding us that the following studies need to consider the whole role of AHR in different organizations. Crucially, G protein-coupled receptor 35 (GPR35), which significantly high expressed in intestine, negative feedback regulated intestinal injury and inflammation to promote colon repair and maintain intestinal homeostasis through sensing KA level selectively. Taken together, This study provides a promise insight about the body feedback regulation of intestinal damage and inflammation to maintain intestinal homeostasis through sensing KA level, suggesting regulate Trp–KYN–KA axis and combine with AHR and GPR35 agonist may be play a synergistic effect in reducing ADRs and treating IBD.

A.20

Metabolic Control of OGT Interactome in Hepatocytes

Krista Kaasik[1], Chin Fen Teo[1], Robert Chalkey[1], Alma L. Burlingame[1]

[1]University of California San Francisco

O-linked-β-N-acetylglucosamine transferase (OGT) post-translationally catalyzes the addition of a single N-acetylglucosamine in O-glycosidic linkage to serine and threonine residues and is required for stem cell viability. Remarkably, hepatocytes are resistant to the loss of OGT in hepatocyte specific OGT knockout mouse. O-GlcNAcylation is drastically reduced in mutant liver tissue compared to wild type littermates during postnatal development analyzed by wheat germ agglutinin (WGA) lectin affinity chromatography. We have applied proximity labeling based proteomics coupled to label-free mass spectrometry to identify dynamic OGT interactions in vivo. The short labeling time based on an engineered ascorbate peroxidase (APEX2) labeling enables capture of more transient interactions while reducing non-specific interactions. We have made stably expressed OGT-APEX2 and its mutants in hepatocyte cell lines. We have identified 724 proteins from OGT full-length sample, 983 proteins from OGT catalytically inactive cell lines, after removal background proteins. Molecular function analyses show that ~45% of proteins identified by APEX2 labeling have enzymatic activity and ~38% with scaffolding activity. Many of them were identified O-GlcNAc modified representing macromolecular complexes in multiple cell signaling pathways. Catalytically inactive OGT is unaffected in substrate binding supporting OGT scaffolding role in addition to its enzymatic function. Proteins from adherent junctions, proteasome, DNA replication complexes, RNA degradation, splicing are among identified. For example focal adhesion proteins - talin, vinculin, paxillin, zyxin all identified modified by OGT in adherence junctions that controls cell adhesion and mechanotransduction. Currently we are validating novel targets from proximity labeling to characterize the loss of OGT hepatocyte specific liver phenotype.

A.21

Integrating Phosphoproteomics and Transcriptional Classifiers Reveals "Hidden Signaling" in Multiple Myeloma Including Differential KRAS and NRAS Mutant Effects

Yu-Hsiu T. Lin[1], Gregory P. Way[2], Benjamin G. Barwick[3], Margarette C. Mariano[1], Makeba Marcoulis[1], Ian D. Ferguson[1], Christoph Driessen[4], Lawrence H. Boise[3], Casey S. Greene[2], Arun P. Wiita[1]

[1]University of California, San Francisco, [2]University of Pennsylvania, [3]Emory University, [4]Kantonsspital St. Gallen

Introduction: Multiple myeloma (MM) is a complex disease that requires a sophisticated treatment strategy. Currently, no kinase inhibitors have been approved for MM despite their potential for supplementing current combination therapies. Previous functional studies have explored kinase dependency in MM by either a small molecule inhibitor library or RNA interference. However, owing to their off-target effects, these approaches are imprecise at dissecting signaling networks driving MM growth and survival. Here, we aim to improve prognostic measures and recommend small molecule-based treatments for MM patients by identifying vulnerable signaling patterns in disease using integrated transcriptome- and phosphoproteome-based predictive models.

Results: We inferred the activities of 297 kinases across eight MM cell lines from mass spectrometry-based quantitative phospho-proteomic data by performing a kinase-substrate enrichment analysis (KSEA). Initially, we were surprised to find greater predicted activity in *KRAS*^{G12}-mutant cell lines compared to *NRAS*-mutant cell lines. We further explored this disparity with our machine learning-based Ras classifier built on transcriptional data from CoMMpass, a longitudinal study of >1000 MM patients. We identified 311, 405, and 390 genes whose expressions are characteristic of the WT *RAS*, *KRAS* mutant, and *NRAS* mutant genotype, respectively, with surprisingly limited overlap between *KRAS* and *NRAS* transcriptional signatures. Building on our KSEA analysis, we next performed a kinase inhibitor screen to evaluate the predictive value of the inferred kinase activities for drug sensitivity. Of 12 screened compounds, mTOR inhibitor INK128 displayed the strongest correlation between drug response and predicted kinase activity. Furthermore, we probed the potential of using pathway activity signatures as prognostic markers. To this end, we applied a gene expression-based signaling pathway prediction model to RNAseq data derived from CoMMpass patients and found that the MAPK signature stratifies patient survival with statistical significance, while the presence and absence of RAS mutations carry no prognostic value. Conclusion: Both phosphoproteomics and a machine learning-based transcriptional classifier highlight a striking difference in the pattern of signaling between *NRAS* and *KRAS* mutants. Taken together, uncovering the cellular signaling networks dysregulated in MM may lead to improved precision medicine, particularly in stratifying patients who may benefit most from kinase inhibitor therapy.

A.22

Characterization of KRAS 4B C-Terminal Hypervariable Region using LC/MS

James Wilkins[1], A.L. Burlingame[1]

[1]UCSF

Aberrant KRAS 4B activity has been shown to be linked to 30 percent of all cancers. The C-terminus of the molecule contains a region (termed the Hypervariable Region) with extremely high positive charge and the mature form of the molecule is prenylated. It shares this property with a number of other membrane-associated RAS-related proteins that are involved in cellular vesicle targeting, cytoskeletal and other functions. In order to understand better the behavior of the KRAS 4B polybasic region, modified and unmodified C-terminal synthetic peptides were studied using online LCMS approaches. A peptide consisting of the C-terminal 22 amino acids of KRAS 4B was synthesized and used for these studies. The native peptide (containing a cysteine-linked farnesyl group) was prepared by chemical methods and its behavior along with the peptide containing a free cysteine was studied using online LCMS. The nonfarnesylated peptide has an isoelectric point of 11.27 and exhibits charge states ranging from +3 to +7 when examined in an Orbitrap instrument at pH 2. Its solubility properties were surprising and we found that the peptide was more soluble at basic pH values as demonstrated by its ability to ionize in nanospray MS experiments. Chromatographic behavior of the unmodified peptide using conventional reversed phase approaches on silica stationary phases yielded poor results. We also looked at separation on a polymeric stationary phase (PLRP-S) and found behavior similar to that obtained with C18. The peptide was cleaved using ASP-N protease resulting in 2 fragments that were also both poorly behaved in chromatography. We found dramatic improvement in the peptide's chromatographic behavior on the same C18 stationary phase after blocking lysine ε-amino groups with propionyl moieties. For this reaction, we employed succinimidyl propionate and found that all 12 lysine side chains were blocked along with the N-terminal amino group. These studies have been done to enable the use of high sensitivity characterization of the KRAS molecule and its modifications in normal and diseased tissues using LC/MS.

A.23

Rapid, Sensitive and Multiplexed Ubiquitylation Profiling in Cells and Tissues

Deepak C. Mani[1], Namrata D. Udeshi[1], Philipp Mertins[1,2], Shaunt Fereshetian[1], Jessica A. Gasser[1,3], Shankha Satpathy[1], Tanya Svinkina[1], Hasmik Keshishian[1], Benjamin L. Ebert[1,3,4], Steven A. Carr[1]

[1]Broad Institute of MIT and Harvard, Cambridge, MA, USA, [2]Max Delbrück Center for Molecular Medicine, Germany, [3]Division of Hematology, Brigham and Women's Hospital, Boston, MA, USA, [4]Dana-Farber Cancer Institute, Department of Medical Oncology, Boston, MA, USA

The study of ubiquitin systems is of great interest as they play an important role in numerous cancers and diseases. Global ubiquitylation profiling by mass spectrometry has been a key method for identifying and understanding how protein ubiquitylation sites are regulated in cellular systems. Almost all analyses of ubiquitylation to date have been carried out in cells grown in culture with quantification using SILAC. This approach has been enormously successful, but is limited by the requirement that samples be amenable to metabolic labeling, the need for relatively large amounts of sample (multi-milligram/sample), and that SILAC can only routinely be multiplexed to three. Here, we present a new method that allows for deepscale, quantitative and highly multiplexed ubiquitylome analyses in any biological system. The method is much faster and requires far less sample than prior approaches and permits the comparison of up to 11 conditions without a significant loss in total numbers of identified ubiquitylation sites. Using this method we identify $>$10,000 ubiquitin sites in tissue samples across 10 states in a TMT 10-plex using only 500 ug tissue per state.

A.24

Characterization of a hybrid insulin peptide as an autoantigen in human type 1 diabetes

Timothy A. Wiles[1], Rocky L. Baker[2], Maki Nakayama[3], Thomas Delong[1]

[1]University of Colorado Skaggs School of Pharmacy and Pharmaceutical Sciences, [2]University of Colorado School of Medicine, [3]Barbara Davis Center for Childhood Diabetes

Relatively little is known about the primary peptide epitopes targeted by the autoimmune response during the development of type 1 diabetes (T1D) in humans. We have shown in the non-obese diabetic (NOD) mouse model of type 1 diabetes that insulin peptides within the pancreatic beta cell become covalently linked via a peptide bond to other beta cell peptides, leading to the generation of hybrid insulin peptides (HIPs). Using mass spectrometry, we recently confirmed that HIPs are present in both mouse and human islets. We established that HIP-reactive CD4 T cells can trigger disease in NOD mice, indicating that HIPs are major autoantigens in this animal model. Furthermore, we determined that HIP-reactive CD4 T cells are present in the peripheral blood of recent onset T1D patients and in the residual islets of organ donors with T1D. Here, we demonstrate the presence of a specific insulin C-peptide HIP in the islets of human donors by mass spectrometry. CD4 T cells specific for this peptide can be detected in the peripheral blood of T1D patients, and from one of these patients we have isolated T cell clones that respond to the HIP at low nanomolar concentrations. Our evidence suggests that this HIP may be an important antigen in the autoimmune pathogenesis of human T1D.

A.25

Improved reproducibility of enrichment and site-assignment of biotinylated peptides using new anti-biotin antibody and its use to investigate redox signaling

Meagan Olive[1], Namrata D. Udeshi[1], Samuel A. Myers[1], Steven A. Carr[1]

[1]Broad Institute of MIT and Harvard, Cambridge, MA 02142

Affinity purification of biotinylated proteins with a standard streptavidin-based enrichment is a powerful tool, but it is limited in its ability to provide site-specific information due to difficult recovery of biotin-modified peptides. Previous studies have shown that enrichment of biotin-modified peptides with an anti-biotin antibody allows for large-scale identification of biotinylated sites by tandem mass spectrometry, making it a potentially useful tool for the study of various post-translational modifications. Application of antibody-based methods to broadly purposed enrichment strategies necessitates interbatch reproducibility of antibody, leading us to test a new monoclonal anti-biotin antibody from Cell Signal Technologies. Here, we evaluate the depth and reproducibility of enrichment of biotinylated peptides using this antibody and compare the results to those obtained using the prior ImmuneChem antibody. We then utilize the new antibody to investigate the prevalence and potential biological roles of redox signaling in immune cells. The ability of this new antibody to purify biotin-labeled peptides will contribute to the development of robust strategies to study post-translational modifications and their biological implications.

A.26

Proteome-wide analysis of protein stability in E. coli using pulse proteolysis

Liang Zhao[1], Giulia Vecchi[2], Michele Vendruscolo[2], Roman Körner[1], Manajit Hayer-Hartl[1], Ulrich Hartl[1]

[1]Max-Planck Institute of Biochemistry, Martinsried, Germany,
[2]University of Cambridge, Centre of Misfolding Diseases, UK

Molecular chaperones play an essential role for maintaining proteins in native states, but how they affect proteome-wide protein stability under native conditions is not well understood. Here, we used pulse proteolysis and quantitative proteomics to screen protein folding states globally under different growth conditions in Escherichia coli and characterized the effects of the DnaK (Hsp70) chaperone system on proteome stability. During a 1 min. short incubation of cells upon lysis, accessible protein regions got cleaved by thermolysin. We then determined the percentage of cleavage for each protein and identified the degraded regions by SILAC-labeling, gel-separation and direct identification of cleaved peptides by mass spectrometry. Comparison of cleavage patterns between stressed and unstressed cells at normal, increased (overexpression mutant) and decreased (deletion mutant) levels of the DnaK (Hsp70) system gave inside into the effects of heat stress and the protective role of the Dnak (Hsp70) chaperone system. We found ~500 proteins (~25% of total by mass) to be protease-sensitive under normal growth conditions, indicating that conformationally dynamic proteins make up a large fraction of the cytosolic proteome. These metastable proteins tend to be larger than average in size, with a high degree of connectivity in protein interaction networks. Upon acute heat stress, not resulting in upregulation of the major chaperone systems, an additional ~200 proteins unfolded, exposing hydrophobic amino acid residues to the solvent that are buried in the native state. These thermosensitive proteins are enriched in large, abundant, and hetero-oligomeric proteins as well as proteins with the CATH fold domain c.37, which is among the most ancient classified folds. Heat shock also resulted in a further destabilization of proteins which were protease sensitive already under normal growth conditions, increasing the fraction of cleaved proteins to ~33% by mass. Overexpression of the DnaK (Hsp70) chaperone system revealed its potential to markedly stabilize numerous thermo-sensitive proteins, including ribosomal proteins as well as large multi-domain, hetero-oligomeric proteins. These results reveal a strong capacity of DnaK (Hsp70) to stabilize proteins in their folded states under denaturing stress conditions.

A.27

The case for mass spectrometry-based proteomics and phospho-proteomics in personalized cancer medicine

Sophia Doll[1], Fabian Coscia[2], Alberto Santos[2], Philipp Nuhn[3], Philipp Geyer[1], Matthias Mann[1,2]

[1]Department of Proteomics and Signal Transduction, Max Planck Institute of Biochemistry, Martinsried, [2]Novo Nordisk Foundation Center for Protein Research, Faculty of Health Sciences, University of Cope, [3]Department of Urology, University Medical Centre Mannheim, University of Heidelberg, Mannheim, Germ

Recent advances in mass spectrometry (MS) based proteomics together with progresses in computational biology are transforming translational MS-based cancer proteomics from an idea to a practice. Mindful of the time constraints in the clinic, we developed a rapid and robust proteomic workflow for the analysis of cancer tissues, including FFPE tissues. It allows the quantification of thousands of tumor proteins in several hours of measuring time and a total turnaround of currently only a few days from obtaining the sample to interpreted result. Here, we applied our pipeline to several 'case studies' of single patients, a well-established paradigm in medicine. In a first metastatic case study - of the extremely rare urachal carcinoma - we uncovered the epigenetic regulator lysine specific histone demethylase 1 as a potential therapeutic target. This protein is an epigenetic regulator and a therapeutic target of new drugs in clinical trials. Thus clinical cancer proteomics can rapidly and efficiently identify actionable therapeutic options. Complementing the proteomic data with NGS and a newly developed 'clinical knowledge graph' that integrates vast amounts of proteomic, genomic and clinical information, helped to guide the therapy decision. In another end-stage cancer patients, we uncovered an up-regulation of a mutated form of the androgen receptor. Finally, we integrated the analysis phosphorylation sites along with somatic mutations, combining genomics with proteomics to uncover additional and personalized treatment options for cancer patients. We envision that our MS-based proteomic workflow can be broadly applied to cancer patients.

A.28

Characterization of the Sin3 HDAC complex interaction network

Mark K. Adams[1], Charles A. Banks[1], Janet L. Thornton[1], Cassandra G. Kempf[1], Sayem Miah[1], Laurence Florens[1], Michael P. Washburn[1,2]

[1]Stowers Institute for Medical Research, Kansas City, MO, [2]Department of Pathology & Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS

The efficacy of HDAC inhibitors (HDACis) as chemotherapeutic agents is a focus of many ongoing clinical studies. Despite the current existence of 4 FDA-approved HDACis, the molecular mechanisms that mediate their beneficial and off-target effects are poorly defined. Among HDAC complexes that are targeted by HDACis, Sin3 complexes have important roles in the regulation of transcriptional activity and may mediate many the effects associated with the application of these compounds. Sin3 complexes are named for the scaffolding proteins of the complexes and have forms conserved from yeast to humans. However, the acquisition of complex components by humans that are not present within the well characterized yeast forms of the complex contributes to our poor understanding of the functional attributes of the Sin3 complexes in humans. Using MudPIT mass spectrometry, we characterize the human Sin3 interaction network. We show that the interaction networks of the two human Sin3 protein paralogs, SIN3A and SIN3B, only partially overlap and that the identity of the Sin3 protein paralog within a complex influences complex composition. Through the comparison of SIN3A and SIN3B protein features, we identify shared and divergent attributes that influence the functional properties of these proteins. Our results reveal the presence of mutually exclusive components of the Sin3 interaction network and provide definition to the heterogeneous population of Sin3 complexes. These findings highlight the need for future studies to assess the biological consequences of diversity within populations of HDAC complexes.

A.29

Proteogenomics of melanoma cell lines and xenografts identifies amino acid variants with a potential to rewire signal transduction networks

Marisa Schmitt[1], Nicolas Nalpas[1], Tobias Sinnberg[2], Heike Niessner[2], Claus Garbe[2], Boris Macek[1]

[1]Quantitative Proteomics, University of Tuebingen, Tuebingen, Germany, [2]Division of Dermatooncology, University of Tuebingen, Tuebingen, Germany

Malignant melanoma is characterized by somatic mutations in BRAF and NRAS in the MAPK pathway, which strongly correlate with poor prognosis of the disease. Targeted inhibition with kinase inhibitors shows a promise in melanoma treatment; however, treated tumours inevitably develop resistance. Although several mechanisms of resistance have been proposed, key phosphoproteins and associated mutations responsible for therapy responses are largely elusive. Here, we reconstruct the disturbed cellular signalling networks upon establishment of melanoma resistance using individualised genomic, proteomic and PTM data. To study the impact of mutations on signal transduction networks, we have established a bioinformatics workflow to predict non-synonymous single nucleotide variants and applied it to exome sequencing data of different drug-resistant and drug-sensitive cell lines as well primary tissues of patients. This led to incorporation of about 13,000 amino acid variants into human proteome database, resulting in around 20,000 novel protein sequence entries. We classified the mutations based on their potential to attack signaling networks for example assignment to a reported cancer-relevant protein. This stratification allowed for further mutation ranking and selection of highest effect scoring mutation. The resulting proteogenomic databases were applied to phosphoproteomics data from several melanoma cell lines and xenografts. Across cell lines and xenografts, we covered about 14,000 protein groups and 16,000 phosphosites, of which 1,300 were localized on peptides containing single amino acid variants. Notably, we identified a number of phosphopeptides resulting from knock-in of a phosphorylated residue and we detected multiple instances of phosphosite loss due to mutations. Most of them were unique to a specific phenotype, cell line or xenograft, which calls for personalized approaches to cancer understanding and treatment. Several proliferation and signaling pathways (PI3K-Akt pathway) were over-represented in mutated proteins. We are currently validating a number of interesting candidates such as the transcription factor RUNX1 via CRISPR/Cas9 strategy followed by MS-based proteomic. Future work will include interactome studies of wild-type and mutated proteins under different conditions.

A.30

Integration of the deep learning prediction tool Prosit into Skyline for high-accuracy, on-demand fragment intensity and iRT prediction

Tobias Rohde[1], Tobias Schmidt[2], Bernhard Kuster[2,3], Michael J. MacCoss[1], Mathias Wilhelm[2], Brendan MacLean[1]

[1]Department of Genome Sciences, University of Washington, Seattle, WA 98195, [2]Chair of Proteomics and Bioanalytics, Technical University of Munich, Freising, Germany, [3]Bavarian Center for Biomolecular Mass Spectrometry, Freising, Germany

Mass spectrometry-based proteomics employs a variety of acquisition schemes. When aiming for high reproducibility and quantitative accuracy, targeted (SRM/MRM and PRM) and data-independent acquisition (DIA and SWATH) are commonly used because of the lower missing values they produce in comparison to data-dependent acquisition methods. For both acquisition schemes, the measured fragment intensities are subsequently matched against MS/MS spectra stored in a library to determine the presence and quantity of peptides, while spectral libraries are often used to choose transitions for SRM. Knowledge of peptide elution time is used for acquisition scheduling, efficient chromatogram extraction, and peak picking. Skyline is a popular open-source tool for building and analyzing such methods, but like most other tools, requires empirically measured spectral libraries. These libraries are usually acquired by DDA experiments which might require extensive offline fractionation or synthetic peptides. While publicly available spectral libraries can be used as well, they are often incomplete and may have been acquired using different LC/MS settings. Recently, a deep neural network named Prosit has been developed to predict MS/MS fragment ion intensities and retention time indices (iRT) with high accuracy. Although Prosit was trained on ~460.000 human tryptic peptides synthesized in the ProteomeTools project, it generalizes to other organisms and even proteases, allowing the prediction of MS/MS spectra and retention times for any precursor of interest. This motivated the integration of Prosit into Skyline. Because Prosit requires GPUs for prediction, we decided to use the Google Remote Procedure Call system (gRPC) to request spectra directly from GPUs hosted by ProteomicsDB. This allows on-demand generation of high quality spectral libraries in Skyline within seconds. Skyline is the first tool to use this Prosit interface for spectrum and RT prediction. The implementation provides a reference for other developers to integrate Prosit predictions into their tools. Comparison of RT prediction in Skyline between SSRCalc and Prosit show a 5-fold decrease in regression residuals with Prosit. We found that using the Prosit spectral libraries in Skyline produced detection of almost the same number of peptides as through experimental libraries in a benchmark DIA experiment (−3%).

A.31

Exploring mechanisms of immune suppression promoted by cancer-associated fibroblasts in lung squamous cell carcinoma

Carlo P. Ramil[1], Handan Xiang[2], Josephine Hai[3], Chunsheng Zhang[4], Huijun Wang[5], Amanda A. Watkins[2], Roshi Afshar[2], Peter Georgiev[2], Xuelei S. Song[3], Dongyu Sun[3], Andrey Loboda[4], Yanlin Jia[3], Lily Y. Moy[3], Philip E. Brandish[2], An Chi[1]

[1]Chemical Biology, Merck & Co., Inc., 33 Avenue Louis Pasteur, Boston, MA, 02115, [2]Discovery Oncology, Merck & Co., Inc., 33 Avenue Louis Pasteur, Boston, MA, 02115, [3]Pharmacology, Merck & Co., Inc., 33 Avenue Louis Pasteur, Boston, MA, 02115, [4]Informatics, Merck & Co., Inc., 33 Avenue Louis Pasteur, Boston, [5]Modeling & Informatics, Merck & Co., Inc., 2000 Galloping Hill Rd, Kenilworth, NJ 07033

Cancer-associated fibroblasts (CAFs) are activated fibroblasts that constitute the stromal component in the tumor microenvironment (TME). Although CAFs have been shown to promote tumor growth and mediate resistance to chemotherapy, their role and potential mechanisms by which they may contribute to immune suppression in lung squamous cell carcinoma (LSCC) remain largely unexplored. Here, we used discovery proteomics to identify potential mechanisms of CAF-promoted immune suppression. We established a patient-derived co-culture model system and showed that CAF polarizes monocytes to adopt a myeloid-derived suppressor cell (MDSC) phenotype characterized by robust suppression of autologous CD8+ T cell proliferation and IFNγ production. We measured the total proteome of CAF-induced MDSCs and compared to non-suppressive immature dendritic cells derived from the same monocyte population. One of the highly enriched pathways in MDSCs is the activation of NADPH oxidase. Pharmacological inhibition of NOX2 activity in CAF-induced MDSCs restored CD8+ T cell proliferation. This study highlights a pivotal role of CAFs in regulating monocyte differentiation and demonstrates that NOX2 inhibition abrogates the CAF-MDSC axis, illuminating a potential therapeutic path to reversing the CAF-mediated immunosuppressive microenvironment.

A.32

Changes in prooncogenic and immune response proteins during development of cervical cancer through quantitative proteomics

Gonzalo A. Soto-Fuenzalida[1], Rosa C. Lopez-Sanchez[1], Sergio Encarnacion-Guevara[2], Juan E. Martinez-Ledesma[1], Rocio Ortiz-Lopez[1], Luis M. Villela-Martinez[3,4], Victor M. Treviño-Alvarado[1], Jose A. Hernandez-Hernandez[1]

[1]Tecnologico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Campus Monterrey, 64710, [2]Programa de Genomica Funcional de Procariotes, Centro de Ciencias Genomicas-UNAM, 62210, [3]Universidad del Valle de México, Escuela de Medicina, Hermosillo, Sonora, 83165, [4]Centro médico Dr. Ignacio Chávez, ISSSTESON, Hermosillo, Sonora, Mexico, 83000

Currently, cervicovaginal cancer (CaCu) is one of the most common cause of gynecological cancer worldwide. Nearly 99% CaCu cases are associated with Human papilloma virus (HPV) infection. The progression of this disease is slow, and it has different and sequential precancerous stages until reach cancer. A differential proteomic study in Cervical mucus samples was set up. We studied CaCu mucus using an iTRAQ approach combined with high-resolution mass spectrometry to describe protein patterns found in mucus during cancer development. Cervix fluid samples were obtained from healthy and precancerous patients (CIN1–3) and analyzed. HPV genotype presence was evaluated. We identified and reported 1731 different proteins quantified, with high confidence, that are common for all disease stages. The proteomic analysis showed that during disease progression, different protein change patterns are shown in cervical mucus. In our study, the most important changes found was down-regulation in proteins as H2AFX, LYPD3, S100A9, SPRR3 and FLG. In contrast, proteins up-regulated showed in protein related with cancer process AGT, OSTF1, GADPH and SERPINA1. Several proteins found in our study are dysregulated and present in mucus during progression of HVP infection to CaCu. They have potential to be used as biomarkers and/or therapeutic targets but validation studies about those proteins are needed.

A.33

Elucidating Changes in O-GlcNAcylation in Pancreatic Cancer

Talieh Zomorrodinia[1], Jason Maynard[1], Krista Kaasik[1], Alma Burlingame[1]

[1]University of California, San Francisco

Cancer cell growth, survival, and proliferation are linked to a metabolic shift from oxidative phosphorylation to glycolysis and as such requires increased glucose uptake. Glucose is used by the hexosamine biosynthetic pathway to create UDP-GlcNAc, the donor substrate required for intracellular protein O-GlcNAcylation. O-GlcNAcylation is a post-translational modification found mainly on serine and threonine residues of nuclear and cytoplasmic proteins. This dynamic modification is controlled by two enzymes, O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA); the former adding a GlcNAc moiety and the latter removing it. Studies have shown that cancer cells have an increase in the occurrence of O-GlcNAcylation (Ma, Vocadlo, & Vosseller, 2013). Pancreatic cancer is one of the most common forms of cancer in the United States. Previous studies have linked the observation of hyper-O-GlcNAcylation with NF-κB activity in the pancreatic cancer cell line, MiaPaCa-2. This cell line possesses the KRASG12C mutation. It is known that the ARS-1620 inhibitor selectively targets this KRASG12C mutation resulting in inhibition of KRASG12C activity. In this study, we aim to elucidate the broader proteomic and posttranslational changes revealed upon treatment of MiaPaCa-2 cells with ARS-1620. SILAC and lectin weak affinity chromatography (LWAC) was employed to compare and enrich GlcNAc containing glycopeptides. EThcD based mass spectrometry was used to identify specific sites of protein O-GlcNAcylation. Changes to the proteome and phosphoproteome were also analyzed and the results will be presented.
Financial acknowledgement: Dr. Miriam And Sheldon G. Adelson Medical Research Foundation and UCSF Program for Breakthrough Biomedical Research (PBBR).

A.34

In vivo investigation of kigelia africana leaf as possible therapeutic option for gastric ulcer disease

Oladayo E. Apalowo[1], Babatunde M. Adekola[3], Funke T. Asaolu[1], Vincent O. Oriyomi[4], Gbenga S. Ogunleye[1], Oladayo J. Areola[2], Olusegun O. Babalola[1]

[1]Department of Biochemistry and Molecular Biology, Obafemi Awolowo University, Ile-Ife, [2]Department of Medical Biochemistry, Faculty of Basic Medical Science, Obafemi Awolowo University, [3]Department of Environmental Management and Toxicology, Federal University of Agriculture, Abeokuta, [4]Institute of Ecology and Environmental Studies, Obafemi Awolowo University

The study investigated the antiulcer potentials of kigelia africana leaf using aspirin-induced model of gastric ulcer in wistar albino rats. Fresh leaves of Kigelia africana was extracted with 70% (v/v) ethanol and subjected to liquid-liquid partitioning using solvents of different polarities. In vitro tests comprising of DPPH radical scavenging activity, FRAP assay, 5-Lipoxygenase and Xanthine oxidase inhibitory activity of different fractions obtained revealed ethyl acetate fraction as possible lead fraction and was used for the antiulcer study. GC-MS fingerprinting of the lead fraction was carried out to identify active chemical constituents. Pre-treatment of experimental animals with varying doses of the lead fraction lasted for 30 days. Thereafter, gastric ulcer was induced in wistar rats with a single dose of aspirin. A control was set up which consisted of wistar rats that received only standard animal pellet and water. Several biochemical markers for gastric ulceration were determined from the plasma, stomach tissue and gastric content. Results from the in vivo study revealed a significant increase ($p \leq 0.05$) in defensive factors like mucin content, total cholesterol and triglyceride concentrations when compared to the control while pepsin activity, myeloperoxidase activity, nitric oxide and malondialdehyde levels were significantly reduced. The study revealed that neutrophil infiltration, an index of myeloperoxidase activity, and inflammation are key factors in gastric ulcer pathogenesis. However, increased total cholesterol and fatty acid concentration may serve as defensive mechanism during an offensive onslaught leading to gastric ulcer. GCMS analysis of lead fraction revealed the presence of several constituents with anti-inflammatory properties, some of which have not been previously investigated.

A.35

Formation of N-GlcNAc proteins is upregulated upon inhibition of proteasome activity in Ngly1-KO cells

Jason C. Maynard[1], Haruhiko Fujihira[2], Gabby E. Dolgonos[1], Tadashi Suzuki[3,4], Alma L. Burlingame[1]

[1]Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA, [2]Division of Glycobiologics, Juntendo University, Tokyo, Japan, [3]Glycometabolic Biochemistry Laboratory, RIKEN Cluster for Pioneering Research, Saitama, Japan, [4]Suzuki Project, T-CiRA discovery, Kanagawa, Japan

NGLY1 is a widely conserved eukaryotic cytosolic deglycosylase. Recently, a human genetic disorder called NGLY1 deficiency was reported, indicating the functional importance of NGLY1 in humans. NGLY1 is involved in the endoplasmic reticulum-associated degradation (ERAD) process, which eliminates misfolded proteins through retrograde translocation and proteasomal degradation. NGLY1 is also reported to be involved in the activation of a transcription factor, making the disease mechanism of NGLY1 deficiency complicated. Recent evidence also suggests that *Ngly1*-KO in the C57BL/6 mouse strain is embryonic lethal, while additional deletion of the Engase gene, encoding another cytosolic deglycosylating enzyme (endo-β-*N*-acetylglucosaminidase; ENGase), partially rescued lethality in mice. It was suggested that upon compromised NGLY1 activity, ENGase-mediated deglycosylation of misfolded glycoproteins may cause excess formation of N-GlcNAc proteins in the cytosol, which can somehow be detrimental to mice. Whether endogenous N-GlcNAc proteins are really formed in *Ngly1*-KO cells/animals or not remains unclarified.

In this study, comprehensive identification of O- and N-GlcNAc proteins was carried out using the cytosol fraction of mice embryonic fibroblasts from wild type, *Ngly1*-KO, *Engase*-KO and *Ngly1*/*Engase* double KO mice in the presence or absence of proteosomal inhibition. It was revealed that, while there is no dramatic change in the level of O-GlcNAc proteins among conditions examined, there was a vast increase of N-GlcNAc proteins in *Ngly1*-KO fibroblasts upon proteasome inhibition. Importantly, few N-GlcNAc proteins were observed in *Engase*-KO or *Ngly1*/*Engase* double-KO cells, clearly indicating that ENGase is responsible for the formation of cytosolic N-GlcNAc proteins. The excess formation of N-GlcNAc proteins may at least in part account for the pathogenesis of NGLY1 deficiency. This work was supported by the Dr. Miriam And Sheldon G. Adelson Medical Research Foundation (AMRF), the UCSF Program for Breakthrough Biomedical Research (PBBR), the Grace Science Foundation, RIKEN Pioneering Project (Glycolipidologue Initiative), and Grants-in-Aid for Scientific Research (grant no. 16K18520).

A.36

Unbiased Proteomics and Network Propagation Reveals Cancer Drug Targets

Mehdi Bouhaddou[1], Neil Bhola[1], Rachel O'Keefe[1], Margaret Soucheray[1], Hua Li[1], Tian Zhu[1], Kelechi Nwachuku[1], Toni Brand[1], Gordon Mills[1], Dan Johnson[1], Danielle L. Swaney[1], Jennifer Grandis[1], Nevan J. Krogan[1]

[1]University of California San Francisco, San Francisco, CA 94158

Head and neck cancer is the seventh most common malignancy worldwide with few treatment options. The only FDA-approved targeted kinase inhibitor to treat the disease is cetuximab, a monoclonal antibody against EGFR to which patients often develop lethal resistance. There is a critical need to understand the mechanisms of drug resistance and discover novel targets whose inhibition could provide synergy with current therapy. Here, we integrate proteomic data from cell lines and patients to reveal novel factors underlying cetuximab resistance. To collect proteomics data in cell lines, we first cultured head and neck cancer cell lines with cetuximab for several months until resistance developed. We then performed global phosphoproteomics and abundance proteomics on both the drug-naïve and resistant models. In addition, for each model, we performed affinity purification mass spectrometry (AP-MS) for 28 of the most commonly mutated proteins in head and neck cancer (including several protein mutants). High confidence interacting proteins were identified and quantified using a multi-step bioinformatics pipeline. The result is a comprehensive map of changes in protein-protein interactions between the drug-naïve and resistant cell contexts. Phosphoproteomics revealed increased activation of several signaling pathways. Abundance proteomics revealed enhanced recruitment of metabolic pathways. AP-MS data reveals several novel oncogene interactions—many of which drastically change upon drug-induced rewiring. Lastly, an integrative network propagation technique, which incorporates all layers of proteomics data including reverse phase protein array (RPPA) data from head and neck cancer PDX models, reveals a subnetwork of $\sim$100 genes underlying drug resistance and sensitivity. Ongoing studies aim to perform CRISPRa/i screens of identified targets in combination with cetuximab to assess synergistic potential. We create a resource map of altered protein-protein interactions and reveal a protein subnetwork signature of drug resistance using a network propagation procedure to overlay distinct datatypes and extract overlapping features. Potential applications from this study span to other cancer types and drug targets.

A.37

Tuning residence time with lysine-targeted, reversible covalent probes

Tangpo Yang[1], Adolfo Cuesta[1], Xiaobo Wan[1,2], Jack Taunton[1]

[1]Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA 94158, [2]Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94158

Irreversible or reversible covalent modification, often applied to cysteine residues, can enhance the on-target residence time of small-molecule drugs and probes. However, many protein targets lack an accessible cysteine, and alternative strategies for covalent modification are therefore needed. Here, we report a series of benzaldehyde-based chemoproteomic probes that rapidly and reversibly engage the catalytic lysine of up to 167 protein kinases in cells and in mice. We demonstrate that probe-kinase residence time can be tuned by appending a hydroxyl group ortho to the aldehyde moiety. Chemoproteomic studies revealed that the intracellular selectivity of otherwise promiscuous salicylaldehyde-based probes increased dramatically upon washout due to distinct kinase-specific residence times. Finally, co-crystal structures of salicylaldehyde-bound AurA and Src kinases provided insight into the basis of prolonged residence time. We anticipate this approach can be applied more generally to the design of reversible covalent probes with sustained target engagement.

A.38

Characterizing and Targeting the Hypoxic T Cell Surfaceome to Promote Immune Function in Cancer

James R. Byrnes[1], Lisa Kirkemo[1], Amy M. Weeks[1], James A. Wells[1,2]

[1]Department of Pharmaceutical Chemistry, University of California, San Francisco, [2]Department of Cellular and Molecular Pharmacology, University of California, San Francisco

Recent cancer treatment research efforts have focused on developing T cell-based immunotherapies. However, these therapies have minimal efficacy in solid tumors. One characteristic of the solid tumor microenvironment is low oxygen availability, or hypoxia. Previous studies investigating the effects of tumor hypoxia on T cell function suggest that hypoxia suppresses the anti-tumor immune response. We therefore hypothesize that hypoxia alters the T cell surface protein profile (the “surfaceome”) and T cell function in a manner consistent with a net immunosuppressive effect. Using proteomics-based approaches, we aim to identify targets for antibody tools designed to increase the anti-tumor function of hypoxic T cells. To characterize hypoxia-induced surfaceomic changes, we first examined how hypoxia affects T cell surfaceomes in vitro. Primary $CD8^+$ or $CD4^+$ effector T cells (Teffs), as well as immunosuppressive regulatory T cells (Tregs), were isolated from peripheral blood and expanded for two weeks in heavy or light lysine/arginine containing media to ensure complete isotope labeling. Cells were then stimulated with anti-CD3/CD28 and cultured for 3 days in either normoxia (20% O_2) or hypoxia (1% O_2). Surfaceomes were profiled using an established biocytin hydrazide surface glycoproteomics pipeline. LC-MS/MS of surface-enriched proteins from either $CD4^+$ or $CD8^+$ Teffs cultured in normoxia or hypoxia identified over 900 surface proteins, many of which were significantly repressed or induced by hypoxia. Overall, $CD4^+$ and $CD8^+$ Teffs responded similarly to hypoxia ($R=0.7$, $P<0.0001$, $N=3$ donors), but the magnitude of hypoxia-induced surfaceomic changes was greater in $CD4^+$ versus $CD8^+$ cells. Consistent with previous reports suggesting hypoxia is immunosuppressive, hypoxia significantly downregulated numerous Teff stimulatory proteins (cytokine receptors, co-stimulatory proteins). In addition to observing previously reported hypoxia-induced proteins, many proteins involved in protein glycosylation and carbohydrate metabolism were upregulated on hypoxic Teffs. Preliminary Treg experiments showed this T cell subtype was more resistant to hypoxia-induced surfaceomic changes than Teffs, suggesting these cells may function better in the hypoxic tumor microenvironment. Collectively, these data suggest hypoxia induces surfaceomic changes consistent with reduced Teff function. Future proteomic and functional studies will aim to validate these findings and identify new antibody-based strategies for enhancing the anti-tumor function of hypoxic Teffs.

A.39

Twins Labeling Derivatization-based LC-MS/MS Strategy for absolute quantification of modified metabolites

Wei Li[1,2], Zunjian Zhang[1,2], Fengguo Xu[1,2]

[1]Key Laboratory of Drug Quality Control and Pharmacovigilance, China Pharmaceutical University, China, [2]State Key Laboratory of Natural Medicine, China Pharmaceutical University, Nanjing 210009, China

Epigenetic modifications and regulation of DNA, RNA and proteins have been thoroughly investigated these days with various detection techniques. Accordingly, there are also diverse forms of modifications in metabolites such as bases, nucleosides and amino acids. However, the biological function of these modified metabolites has not been well illustrated owing to their obstacles in identification and quantification. In the current study, a sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed for the simultaneous quantification of eighteen metabolites including cytosine bases, nucleosides, amino acids and their different forms of modification. For the purpose of improving quantification sensitivity and accuracy, two structure analogs named N-dimethyl-amino naphthalene-1-sulfonyl chloride (Dns-Cl) and N-diethyl-amino naphthalene-1-sulfonyl chloride (Dens-Cl) were used for twins labeling derivatization. With the introduction of naphthalene and easily ionizable moiety of tertiary ammonium, this method notably improved the chromatography retention and detection sensitivity of these polar metabolites. In allusion to the problem of wide concentration range between these unmodified metabolites and their modified forms in biological samples, a wide range of concentration was tested and met the requirements with good accuracy and precision. The lower limit of quantification (LLOQ) was in the range of 1–100ng/mL. The validated method was successfully applied to quantify monomethyl, dimethyl, trimethyl, and acetyl modifications of metabolites and their ratios in human lung adenocarcinoma cell line A549 and its cisplatin resistant derivative A549/DDP. The results demonstrated significant reduction of 1-methyladenosine, 1-methyladenine, symmetric dimethylarginine in A549/DDP compared with A549. These modified metabolites could potentially act as biomarkers or have underlying effects on the epigenetic regulation in the process of cisplatin resistance.

A.40

A Chemoproteomics Workflow for the Global Analysis of Acyl-CoA Signaling Networks

Michaella J. Levy[1], David C. Montgomery[2], Mihaela E. Sardiu[1], Abigail Thorpe[2], Steve Fox[3], Qishan Lin[4], Thorkell Andresson[3], Laurence Florens[1], Michael P. Washburn[1,4], Jordan L. Meier[2]

[1]Stowers Institute for Medical Research, Kansas City, MO 64110, [2]Chemical Biology Laboratory, Center for Cancer Research, NCI, NIH, Frederick, MD 21702, [3]Laboratory of Proteomics and Analytical Technologies, Leidos, Inc, Frederick, MD 21702, [4]Department of Pathology and Laboratory Medicine, KUMC, Kansas City, KS 66160

Acyl-CoAs are essential for life. These metabolites serve as fundamental cellular building blocks in the biosynthesis of lipids, intermediates in energy production via the TCA cycle, and essential precursors for reversible protein acetylation. Each of these functions are physically dependent on acyl-CoA/protein interactions, which can regulate protein activity as enzyme cofactors, competitive or allosteric inhibitors, or through covalent modification of proteins. These examples illustrate the ability of acyl-CoA signaling to influence biology and disease. However, the global scope and selectivity of these metabolite-governed regulatory networks remains unknown. To this end, we used a previously reported resin-immobilized CoA analogue (Lys-CoA) to capture CoA-utilizing enzymes from unfractionated biological samples. To understand the global profiling or discovery of novel lysine acetyltransferase (KAT) enzymes in an unbiased, high throughput manner, we implemented Multidimensional Protein Identification Technology (MudPIT) mass spectrometry, integrating online multidimensional liquid chromatographic separation and quantitative tandem mass spectrometry analysis of complex peptide mixtures. Proteomes competed with acetyl-CoA at three concentrations were analyzed in triplicate. The 1700 proteins identified by at least 4 spectral counts in the control were separated into 8 distinct clusters by k-means clustering. Next, the binding profiles the proteins in each of the 8 clusters were determined and showed which clusters contained proteins that were susceptible to competition with acetyl-CoA. Three clusters contained proteins with profiles showing moderate, competitive, or hyper-competitive binding profiles with acetyl-CoA. Further, gene ontology analysis of the clusters revealed the highest percentage of CoA annotated proteins were in the three competed clusters. Analyzing the data using this pipeline highlighted the ability to identify CoA binding proteins in an unbiased manner from a whole proteome. We further demonstrated the strengths of this approach when proteomes were competed with various concentrations of CoA or CoA metabolite analogues at a single concentration. We term this approach CATNIP (CoA/AcetylTraNsferase Interaction Profiling) and demonstrate its ability to globally enrich and analyze acyl-CoA/protein interactions in endogenous human proteomes. Overall, our studies illustrate the power of integrating chemoproteomics and systems biology analysis methods and provide a novel resource for understanding the diverse signaling roles of acyl-CoAs in biology and disease.

A.41

Proteomics insights into the role of PknG in mycobacterial physiology and pathogenesis

Analía Lima[1,2], Magdalena Gil[1,3], Bernardina Rivera[1,2], Jessica Rossello[1,2], Annemarie Wehenkel[3], María N. Lisa[4], Pedro M. Alzari[3], Rosario Durán[1,2]

[1]Institut Pasteur de Montevideo, [2]Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, [3]Institut Pasteur, Paris, [4]Instituto de Biología Molecular y Celular de Rosario, Argentina

Mycobacterium tuberculosis, the causative agent of tuberculosis, is a major public health problem, being the first cause of death due to a single infectious agent. The success of *M. tuberculosis* as a human pathogen lies mainly in its ability to switch lifestyles to survive in the different conditions found in the host. A key player in facilitating bacterial survival within host macrophages is the Ser/Thr kinase PknG, an autophosphorylated multi-domain protein. Although the central role of PknG in mycobacterial physiology and virulence is well documented, the molecular mechanisms underlying these effects, as well as the protein partners involved, are still poorly characterized. To contribute to a better understanding of the signaling pathways of PknG we have carried out proteomics and interactomics studies. We developed a tailored interactomic approach that combines the use of different constructions of PknG with specific sequential elution steps to identify kinase mediated protein complexes *in vitro*, and to discriminate those interactions relying on PknG's autophosphorylated docking sites[1]. We report a list of kinase substrates and interactors that suggest its involvement in the regulation of a wide range of cellular processes including protein translation, nitrogen assimilation and cell wall biosynthesis. The interaction with the kinase, and/or the phosphorylation by PknG, was further confirmed for selected candidates. Moreover, we carried out quantitative proteomic approaches to compare *M. tuberculosis* wild type and a mutant derivative lacking PknG. The results showed that the expression of as much as 6.8% of the predicted *M. tuberculosis* proteome is altered in the bacteria lacking PknG, and indicated that a battery of proteins that are relevant for the adaptation to host's environment and induction of a mycobacterial persistent state are underrepresented in this strain. Altogether, our results suggest that the effect of PknG on mycobacterial survival inside macrophages could be mediated by a general metabolic fitness to the conditions encountered in the host.

[1]Gil et al, J Proteomics. 2019;192:321–333

A.42

Pushing the limits: Boosting sensitivity of PRM assays for the detection of very low abundant proteins in complex samples

Emmanuelle Lezan[1,2], Erik Ahrné[1,3], Thomas Bock[1], Alexander Schmidt[1]

[1]Biozentrum, University of Basel, Switzerland, [2]F. Hoffmann-La Roche Ltd, Basel, Switzerland, [3]Novartis, Basel, Switzerland

Even though several DIA approaches have been developed to date, parallel reaction monitoring (PRM) provides the most promising approach to detect specific proteins of low abundance within a complex sample. While providing superior sensitivity, very low abundant proteins being expressed with less than a few hundred copies per cell remain challenging to confidently detect and quantify in higher eukaryotes when applying a standard PRM setup. Here, we evaluated the impact of several critical MS parameters on PRM sensitivity and compared the data to other DIA approaches. We performed a dilution series experiment comprising twenty unmodified synthetic heavy labeled peptides spiked into a complex human cell digest to determine limits of detection (LOD), quantification (LOQ) and identification (LOI) of the different methodologies. We further employed different parent ion mass windows, resolutions and ion fill times and determined their impact on LOD, LOQ and LOI. For shotgun and SWATH/HRM LC-MS analysis we identified detection limits of about 1 fmol on column. As expected, PRM analysis provided the highest sensitivity with LODs being in the high attomole range using standard settings (resolution 30,000, fill time 50 ms). We next evaluated the impact of different parent isolation mass windows on LODs. In the initial PRM studies, a mass window of 2 Th was applied, however, for our Q Exactive HF LC-MS platform, we found smaller mass windows (0.4 – 0.7 Th) to considerably improve the sensitivity of our PRM assays. Interestingly, we found large mass windows, like utilized in SWATH/HRM-MS, to have a considerable negative impact on detection limits. We further evaluated the impact of increased resolution and fill time of the Orbitrap analyzer and found both parameters to boost overall sensitivity of the PRM assays up to 20-fold. This allowed peptide monitoring in the low attomole range within this complex human sample, albeit with a reduced number of peptide targets of around 10. We finally demonstrate the power of this highly sensitive PRM assays by robust quantification of centrosomal proteins that are expressed at extremely low levels and that were not identified in previous large-scale LC-MS studies.

A.43

Discovery of a common target of natural products through combination of chemical genomics and proteomics

Haijun Guo[1,2], Yang Yang[1], Jieren Deng[1], Mankin Wong[1,2], Qian Zhao[1,2]

[1]Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, HK SAR, [2]State Key Laboratory of Chemical Biology and Drug Discovery, Hong Kong SAR, China

Chemproteomics that seeks to design small molecule probes to study protein function, is a powerful tool to understand the binding targets and related mechanisms of functional small molecules. As challenges lie in probe synthesis as well as throughput of target identification and validation, an approach that offers high efficiency without extensive synthesis is desired. We have developed a new method by introducing chemical genomics into chemical proteomics-based target identification. First, natural products Cel, WA, Au were classified to have similar effects on gene expression patterns by using the chemical genomics platform L1000. Next, we synthesized a chemical probe C1a based on Cel structure as it is the most well studied and easiest for chemical derivatization among all three natural products. As we expected, probe C1a remained the same biological activities of its parent compound Cel, including inhibition on cancer cell proliferation and anti-inflammation. By using C1a, we were able to monitor the cellular localization of Cel as well as its binding proteins. In a competition experiment where WA and AU served as competitors, a common protein target of all the three natural products was identified, which may also explained their biological activities in common. In summary, our study revealed a novel protein target of several natural products, indicating that the similarities in genomic profiles could result from common binding targets. Meanwhile, the novel method that we have developed through combining chemical genomics with proteomics will be useful for multiplexing target identification and mechanism studies of structurally distinct small molecules.

A.44

Developing a reversible covalent protein/ peptide capture technology for low abundance proteomics

Brendan M. Floyd[1], Cecil J. Howard II[1], Jagannath Swaminathan[1], James L. Reuther[1], Edward M. Marcotte[1], Eric V. Anslyn[1]

[1]University of Texas at Austin

A major challenge in proteomics is the handling of extremely low abundance samples spurring the development of new sample handling technologies. Here we present the development of a bead-based covalent capture method for low abundance sample handling. This method uses a physiological pH covalent reaction to specifically capture proteins and peptides by the amino terminus. Attachment of the reactive agent to a solid-support has allowed for capture and manipulation of linked peptides and proteins including proteolysis and covalent modification. Peptides can subsequently be released with high efficiency and analyzed using mass spectrometry or single-molecule protein sequencing. This new method offers a novel way to tackle the contemporary proteomics problem of low abundance sample handling.

JS, EMM, and EVA are cofounders and hold equity of Erisyon, Inc.