B.1
Development of a Proximity Labeling Method to Identify the Protein Targets of Bioactive Small Molecules
Zachary Hill, Min Zhuang, James Wells
University of California, San Francisco, CA, USA

Identifying the direct protein targets of a bioactive small molecule gives insight into the compound’s mechanism of action, its efficacy, and possible toxicity. Target identification is becoming an increasingly important part of the drug-development process. However, given the transient and heterogeneous nature of interactions between small molecules and proteins, this step is often difficult, greatly slowing the development of new therapeutics. For this reason, new methods to rapidly identify the direct protein targets of bioactive small molecules are of great importance. Enrichment strategies coupled with quantitative mass spectrometry have shown great promise in target identification. Here we will present our progress toward developing an engineered enzymatic tagging method that enables specific labeling and enrichment of protein targets from complex lysates. This method couples the binding of a small molecule to a proximity-based labeling event. Labeled target proteins are enriched and subsequently identified using quantitative LC-MS/MS. We will discuss several variations of this method, and highlight our progress towards applying proximity labeling to small-molecule target identification and validation.

B.2
Modelling Atherosclerosis: Molecular Changes in the Ascending Aorta of Cholesterol-fed Rabbits
Jingshu Xu1,2, Mia Jüllig1,2, Martin J. Middle ditch1,2, Garth J.S. Cooper1,2,3,4
1School of Biological Sciences, University of Auckland, New Zealand; 2Maurice Wilkins Centre for Molecular Biodiscovery, Faculty of Science, University of Auckland, New Zealand; 3Department of Pharmacology, Medical Sciences Division, University of Oxford, Oxford, UK; 4Centre for Advanced Discovery and Experimental Therapeutics, NIHR Manchester Biomedical Research Centre, the University of Manchester, Manchester, UK

The cholesterol-fed rabbit is commonly used to study the effect of hypercholesterolaemia and the associated atherosclerotic lesions. Here we maintained New Zealand White rabbits on a diet containing 2% (w/w) cholesterol (HC diet) for 12 weeks, after which their ascending aortas were excised and subjected to proteomic analysis. Extracts from ten individually obtained ascending aorta samples were labelled with isobaric (iTRAQ) tags and analyzed by LC-MS/MS to profile the proteomic changes in response to the HC diet (n=11005) in comparison with non-HC, standard diet (n=11005). ProteinPilot was used to search the LC-MS/MS output against the NCBI rabbit protein sequence database, leading to identification of 453 unique proteins. Of these, 74 showed significant differences in relative abundance (p<0.05), with 69 proteins higher and five lower in ascending aorta from HC diet-fed rabbits compared to controls.

Many of the observed protein changes are consistent with molecular perturbations within the ascending aorta in response to the HC diet in rabbits, e.g. elevation of apolipoproteins, extracellular matrix adhesion proteins, collagens, glycolytic enzymes, heat shock proteins, proteins involved in immune defence, and proteins regulating the polymeric state of actin.

We also made a number of novel observations, including an extreme (16-fold) elevation of a protein previously linked to angiogenesis but not atherosclerosis. Numerous other proteins not previously associated with atherosclerosis were also increased in ascending aorta from HC-fed rabbits. These novel observations merit further investigation as these perturbations may play important and yet undiscovered roles in the pathogenesis of atherosclerosis.
B.3

Post-translational Modification Networks

Vera van Noort

Katholieke Universiteit Leuven, Leuven, Belgium

Protein post-translational modifications (PTMs) allow the cell to regulate protein activity and play a crucial role in the response to changes in external conditions or internal states. Advances in mass spectrometry now enable proteome wide characterization of PTMs and have revealed a broad functional role for a range of different types of modifications (1). We have systematically investigated the interplay of protein phosphorylation with other post-transcriptional regulatory mechanisms in the genome-reduced bacterium Mycoplasma pneumoniae (2). Systematic perturbations by deletion of its only two protein kinases and its unique protein phosphatase identified not only the protein-specific effect on the phosphorylation network, but also a modulation of proteome abundance and lysine acetylation patterns, mostly in the absence of transcriptional changes. Reciprocally, deletion of the two putative N-acetyltransferases affects protein phosphorylation, confirming cross-talk between the two PTMs. The measured M. pneumoniae phosphoproteome and lysine acety-lome revealed that both PTMs are very common, that (as in Eukaryotes) they often co-occur within the same protein and that they are frequently observed at interaction interfaces and in proteins that can be part of multiple protein complexes (3). The results imply previously unreported hidden layers of post-transcriptional regulation intertwining phosphorylation with lysine acetylation and other mechanisms that define the functional state of a cell. Aiming at a more global view of the interplay between PTM types, we collected modifications for 13 frequent PTM types in 8 eukaryotes, compared their speed of evolution and developed a method for measuring PTM co-evolution within proteins based on the co-occurrence of sites across eukaryotes (4). We found that PTM types are vastly interconnected, forming a global network that comprise in human alone >50,000 residues in about 6000 proteins.


B.4

Extracellular Phosphorylation in the Murine Synaptosome

Jonathan C Trinidad¹, Ralf Schoepfer², Alma L Burlingame³, Katalin F Medzihradszky²*¹

¹Department of Chemistry, Indiana University, Bloomington, IN, USA; ²Department of Pharmacology, University College London, England, UK; ³Department of Pharmaceutical Chemistry, School of Pharmacy, University of California San Francisco, CA, USA

Post-translational modifications (PTMs) play key regulatory roles in cellular localization and/or biological function of proteins. The site of modification within a protein; their fixed or transient nature; the stoichiometry and potential crosstalk between modifications have been the focus of numerous large-scale studies. Most of this research is focused on PTMs involved in intracellular processes, such as phosphorylation, methylation, GlcNAcylation, acetylation, and ubiquitination. Of these, phosphorylation is the most studied due to the biological importance of this modification as well as the availability of tools for such studies.

While the majority of protein phosphorylation occurs on intracellular proteins, phosphorylation of secreted proteins is well established. Well-characterized examples include the secreted milk protein, beta-casein, and the serum protein fetuin. Our current understanding of the biological role of extracellular phosphorylation, as well as knowledge regarding the process by which they are modified, is incomplete. A kinase known as FAM20 has been identified and is localized to the Golgi as well as secreted. It has been shown to phosphorylate extracellular proteins with a SXE motif.

We recently conducted extensive studies on the interplay between intracellular phosphorylation and GlcNAcylation on proteins isolated from murine synaptosomes. This data also allowed us to identify specific glycan structures on more than 500 secreted or transmembrane proteins. We have subsequently examined the extent to which phosphorylation was present in our sample on secreted proteins or extracellular regions of transmembrane proteins. Our goal was to determine the extent to which such phosphorylation can be explained by the known motif of Golgi-resident kinases.

We analyzed the sequences around the extracellular phosphorylation sites, and the spatial relationship on the linear amino acid sequence between glycosylated and phosphorylated residues. This work was supported by NIH grant NIGMS 8P41GM103481, and by the Howard Hughes Medical Institute.
B.5
Using Selective Reaction Monitoring (SRM) Mass Spectrometry To Unmask Regulatory Feedback Loops Controlling Adipogenesis

Robert Ahrends1,2, Asuka Ota2, Kyle M. Kovary2, Takamasa Kudo2, Byung Ouk Park2, Mary N. Teruel2
1ISAS, Dortmund, Germany; 2Clinical and Systems Biology, Stanford University, Stanford, CA, USA

Background: Due to modern lifestyle changes, obesity has a worldwide impact on human health. The obesity epidemic is now recognized as one of the most important public health problems facing the world today. Understanding adipogenesis is crucial to understanding obesity; failure of adipogenesis was shown to be a key factor in the development of diabetes.

In earlier work using single-cell imaging, we demonstrated that there is a distinct decision made during the time course of adipogenesis. Thereby positive feedback loops between PPARg and other transcription factors (TFs) in the differentiation network are regulating this decision. We identified a positive feedback loop between PPARg and C/EBPb that plays a critical role in regulating adipogenesis. Since multiple feedback loops with different timing and strengths can sharpen the decision process and control the number of cells which are differentiating, we wanted to gain a better understanding of how many other proteins could be involved in the decision process.

Objective: The objective of this work is to search for feedback loops that could play a key role in the commitment decision.

Methods: Using Selected Reaction Monitoring (SRM) mass spectrometry combined with perturbations, we analyzed OP9 cells to detect peptides of TFs which can serve as probes. We validated these probes with isotopically coded internal peptide standards and established a SRM library of transcriptional key regulators. These probes were subsequently used to quantitatively profile different stages of adipogenesis to obtain time courses of different TFs. To achieve our major goal to elucidate the TF control network in more detail, we furthermore searched for hidden feedback loops in this differentiation system. To do so we chemically manipulated the activity level of PPARg and its potential feedback partners individually. If a protein was a component of one or several feedback loops and was experimentally manipulated, all the other components of feedback loops associated with this protein should display a relative change in abundance and vice versa.

Results: We developed a SRM methodology to monitor the concentration changes of TFs during adipogenesis. Using this SRM methodology together the perturbation of PPARg, and single cell analysis we were able to validate known feedback loops (C/EBPα, C/EBPβ) and to identify several new feedback Loops.

Conclusions: PPARg is the master regulator of adipogenesis. To successfully differentiate preadipocytes into adipocytes, its activity needs to be tightly regulated by a network of feedback loops. Overall, the study provides a new SRM MS-based method to uncover novel feedback loops regulating TFs. Based on this method; we have identified 7 new proteins which are fundamental regulators of PPARg and the fat cell commitment decision.

B.6
Application of Quantitative and Functional Phosphoproteomics In Study of Ethylene Signaling

Ning Li1
1The Hong Kong University of Science and Technology, Hong Kong, China

Ethylene is a major plant hormone that regulates a diverse aspect of plant growth and development. The regulatory roles of ethylene in plants include promotion of leaf and flower petal senescence, yellowing and abscission, as well as promotion of fruit abscission and ripening. This key hormone is also involved in regulation of a number of plant biotic and abiotic stress responses. A dramatic effect of ethylene on tropic response is the dual-and-opposing effect of ethylene on stem negative gravitropic response, in which short-term ethylene treatment (0.5 hour) appears to inhibit stem bending up following re-orientation of inflorescence of Arabidopsis. In contrast, a long-term treatment (12 hours) stimulates gravitropic response and promote stem curve up faster. This time-dependent and dose-independent dual-and-oppositing effect of ethylene on stem gravitropism may involve multiple signaling pathways. Stable isotope metabolic labeling-based quantitative phosphoproteomics performed on ein2–5, ctr1–1 and rcn1–1 ethylene signaling mutants indeed confirmed the time-dependent protein phosphorylation changes and some of phosphorylation events are independent to ein2 loss-of-function gene in response to ethylene treatment. Functional studies on the phosphorylated transcription factor ERF110 isoform suggest that it is required for the control of flowering time via multiple ethylene signaling pathways.
B.7

Intact N- and O-linked Glycopeptide Identification from HCD Data Using Byonic

Katalin F. Medzihradszky¹, Jason Maynard¹, Krista Kaasik¹, Marshall Bern²

¹University of California, San Francisco, CA, USA; ²Protein Metrics, San Carlos, CA, USA

The importance of high quality analysis of glycosylated proteins is steadily increasing. Both the regulatory and signaling functions of the intracellular GlcNAc modification are widely documented, and different enrichment strategies for GlcNAcylated peptides have been developed. Extracellular glycosylation has been linked to a wide variety of diseases and both N- and O-glycosylation play important roles in providing the structural integrity of certain proteins, controlling protein clearance, protein-protein interaction, and enzymatic processing. Furthermore, most protein pharmaceuticals are glycosylated, and thus, batch to batch characterization of these drugs also involves glycosylation analysis.

Towards the end of the last century mass spectrometry has become the method of choice for the analysis of post-translational modifications, and high throughput workflows have been developed for a number of different PTMs. Though mass spectrometry has been used for the characterization of N- and O-glycosylation of single proteins, its high-throughput application was prevented by a number of issues. These issues include the non-pattern based complex oligosaccharide structures, the isomeric building blocks, as well as the extensive carbohydrate fragmentation upon collisional activation. In-depth carbohydrate analysis still requires different tools: the released glycan pools are studied derivatized or unmodified using a wide variety of analytical methods, such as capillary electrophoresis, chromatography, exoglycosidase cocktails, and NMR. While such techniques will provide information on the identity of the sugar units and their linkages, the information on the localization of glycans within the protein sequence and of their site-specific heterogeneity is lost. Electron-transfer dissociation, which preserves peptide side-chain modifications, has enabled the MS/MS analysis of intact glycopeptides, and led to the successful assignment of thousands of GlcNAcylated sequences and Golgi-derived glycopeptides. This gave a boost to intact glycopeptide analysis, and search engines such as Protein Prospector and Byonic can handle even complex glycan mixtures. The most recommended acquisition workflow uses the diagnostic HexNAc oxonium ion produced by HCD analysis to trigger ETD analysis. Unfortunately, because extracellular glycosylation increases the peptide mass significantly without additional charge added, glycopeptides frequently produce low charge-density precursor ions that will yield only charge-reduced molecules upon ETD activation. However, properly acquired HCD data may contain sufficient information for glycopeptide identification. Byonic has been adjusted for the interpretation of such spectra. We will present the results from a complex N- and O-linked glycopeptide-containing mixture isolated from mouse brain synaptosome using WGA lectin weak affinity chromatography, highlighting the advantages and limitations of this approach.

This work was supported by NIH grant NIGMS 8P41GM103481, and by the Howard Hughes Medical Institute (to the Bio-Organic Biomedical Mass Spectrometry Resource at UCSF, Director: A.L. Burlingame).
B.9
Characterizing Qualitative and Quantitative Global Changes in the Aging Heart Using pSMART, a Novel Acquisition Method
Maryann S. Vogelsang, Amol Prakas, David Sarracino, Gouri Vadali, Scott Peterman
Thermo Fisher Scientific, BRIMS Center, Cambridge, MA, USA

The cardiovascular system has been shown to undergo significant changes as it ages. These changes range from genomic to structural. We have completed a label-free quantitative global profiling and targeted analysis of the cardiac proteome in aging mice using a novel data acquisition method, pSMART. Heart tissue was isolated and homogenized from both young (2 months old) and old (2 years old) mice. Solubilized and digested protein samples were spiked with the PRTC peptide retention time trainer kit and analyzed using unbiased data-dependent acquisition (DDA) method. Initial characterization experiments using unbiased DDA facilitated the building of detailed murine cardiac tissue spectral library. The spectral library records contain the relative retention time information based on the standard peptides as well as highly confident endogenous peptides, precursor and product ion information such as measured mass values and relative abundance used to create a consensus product ion spectrum. The spectral library information was used to create reference information to perform qual/quan determination in real-time. The pSMART method was used to acquire qualitative/quantitative data analysis using one HR/AM MS and a series of narrow DIA mass windows. Our pSMART strategy resulted in 30% more peptide identifications per run than a standard DDA run. Additionally, using pSMART, we were able to confirm MS1 quantitation at low abundance levels with MS/MS for each peptide. This novel acquisition enabled quantitation of previously identified peptides as well as novel putative targets of aging. By identifying and quantifying more targets, we were able to better characterize the dynamic proteomic changes of cardio-dysfunction in aging mice.

B.10
Quantitative Site-Specific Profiling S-glutathionylation in Macrophages in Response to Engineered Nanomaterial-induced Oxidative Stress
Jicheng Duan, Vamsi K. Kodali, Matthew J. Gaffrey, Jia Guo, Rosalie K. Chu, David G. Camp, Richard D. Smith, Brian Thrall, Wei-Jun Qian
Pacific Northwest National Laboratory, Richland, WA, USA

Engineered nanoparticles are emerging functional materials with unique physicochemical properties, which make them desirable for commercial and medical applications. It is important to assess the toxicity of nanomaterials and recognize the underlying mechanisms of their toxicity. Oxidative stress is known to play important roles in nanomaterial-induced cellular toxicity, which leads to the generation of reactive oxygen species and alteration of protein activities and functions in cells. However, the knowledge about proteins and signaling pathways associated with nanomaterial-induced oxidative stress and nanotoxicity is still limited. Reversible cysteine-based protein modifications, such as S-glutathionylation (SSG) and S-nitrosylation (SNO), represent an important mechanism that modulates diverse cellular pathways in response to the disturbance of redox balance in cells. These redox modifications would be a potential regulatory mechanism in response to nanomaterials-induced oxidative stress and nanotoxicity. Recently, we have developed an effective proteomic approach for site-specific identification and quantification of different cysteine-based redox modifications by integrating selective reduction of oxidized cysteines, resin-assisted enrichment of thiol-containing proteins, and isobaric labeling to enable LC-MS/MS-based quantification. Herein, we present the preliminary results about the alteration of protein SSG modifications in mouse macrophages after exposure to different nanoparticles (CoO, Fe3O4 and SiO2 nanoparticles) by quantitative site-specific profiling. We observed that among these nanoparticles, CoO nanoparticles led to the most significant dose-dependent cytotoxicity and increase of protein SSG modifications in macrophages. Our site-specific SSG data highlighted redox sensitive proteins and their specific Cys residues potentially implicated in oxidative stress response. Functional analysis revealed that the most significantly enriched molecular function categories for SSG-modified proteins were free radical scavenging and cell death/survival. This preliminary result provides some insights on protein SSG modification as a potential regulatory mechanism of nanomaterial-induced oxidative stress.
**B.11**

O-GlcNAc Regulates SOX2 Activity in Embryonic Stem Cells by Altering Protein-SOX2 Interactions

Samuel Myers1, Sailaja Pedadda, Tara Freidrich, Sean Thomas, Gregor Krings, Michael Lopez, Marena Trinidad, Barbara Panning, Al Burlingame

University of California, San Francisco, CA, USA

SOX2 is a versatile transcription factor that maintains embryonic stem cell (ESC) pluripotency and self-renewal, and is important for proper lineage specification and adult stem cell maintenance. This versatility is likely due to post-translational modifications (PTMs) as SOX2 has been reported to be modified by numerous chemical moieties in a variety of cell types. One such PTM is O-GlcNAc, the dynamic and regulatory glycosylation of intracellular proteins. Global O-GlcNAc is essential for ESC self-renewal though the function of SOX2 O-GlcNAcylation in ESC is not understood. Here, we show that SOX2 is O-GlcNAc modified in the transactivation domain and alterations of self-renewing signals induce changes in SOX2 O-GlcNAc stoichiometry. Replacement of wild-type SOX2 with an O-GlcNAc-deficient mutant SOX2 in ESCs increases the pluripotency transcriptional network while down-regulating genes involved in differentiation. Analysis of SOX2-interacting proteins from ESCs revealed that the WT and mutant SOX2 interact with distinct subsets of transcriptional regulatory complexes. Thus, SOX2 O-GlcNAcylation modulates the transcriptional landscape of ESCs by modulating SOX2 activity and interactions with epigenetic regulatory complexes.

**B.12**

Development of Multiplexed Assays for Oral Cancer Biomarker Verification by Peptide Immunoaffinity Enrichment and Targeted Mass Spectrometry

Yung-Chin Hsiao1, Lang-Ming Chi2, Kun-Yi Chien1, Yi-Ting Chen1, Yu-Sun Chang1, Jau-Song Yu1

1Chang Gung University, Tao-Yuan, Taiwan; 2Chang Gung Memorial Hospital, Tao-Yuan, Taiwan

Oral cancer, one of the common cancers in Taiwan and other areas of Southern Asia, has become an increasing burden on the health care system in this region. Although numerous potential oral cancer biomarkers have been discovered in the past decades, very few of them have been verified and validated in parallel to compare their clinical utility. Recently, a multiplexed, targeted proteomics assay platform, termed SISCAPA-MRM-MS (stable isotope standards and capture by anti-peptide antibodies combined with multiple reaction monitoring mass spectrometry) has been shown to be a feasible approach for verifying multiple protein biomarker candidates in body fluid samples. Therefore, we sought to prioritize biomarker candidates from published literature and our in-house database and develop a high-throughput/multiplexed SISCAPA-MRM-MS assay for quantifying potential oral cancer biomarker candidates. We produced ~400 clones of anti-peptide mAbs against 50 selected targets and effectively sieved out the high quality anti-peptide mAbs against 24 targets according to their binding affinity to peptide antigens (using peptide-immobilized SPR system) and immuno-capture capability (using SISCAPA-MRM MS assay) and applied to preliminarily evaluation of these 24 candidates in pooled saliva samples obtained from oral cancer patients and healthy controls. Eight of the 24 candidates were found to be drastically increased in pooled saliva samples from oral cancer patients as compared with healthy controls. The promise of this 24-plex SISCAPA LC-MRM MS assay allows us to systematically evaluate the abundance of targets in clinical samples for oral cancer biomarker discovery in the near future.
B.13
Characterisation of Glycosylation of Paramyxovirus Surface Glycoproteins by Mass Spectrometry
Cassandra L. Pegg1, C. Hoogland1, S.M. Johnson2, C.C. Gonzalez2, M.E. Peeples2, J.J. Gorman1
1QIMR Berghofer Medical Research Institute, Herston, Australia; 2Center for Vaccines & Immunity, The Research Institute at Nationwide Children’s Hospital, Columbus, OH, USA

The family Paramyxoviridae (paramyxovirus) contains a number of significant human and animal pathogens. Represented within this family are human respiratory syncytial virus (hRSV), human metapneumovirus (hMPV) and Newcastle disease virus (NDV). The former two cause severe respiratory tract disease in infants, children and immunocompromised individuals. At present, safe and effective vaccines are not available for hRSV and hMPV. NDV is the causative agent of Newcastle disease (ND) afflicting a wide range of avian species. The desire to study NDV is due not only to the significant economic impact it has on the poultry industry worldwide but also its potential use as an oncolytic agent and vaccine vector for human and animal use. Additionally, findings on NDV may be translated to closely related viruses that cause disease in humans, such as parainfluenza viruses. Of great importance to paramyxoviruses are the variable attachment glycoproteins, hemagglutinin (H), hemagglutinin-neuraminidase (HN) and major surface glycoprotein (G) along with the fusion (F) glycoprotein. Glycoproteins H, HN and G are involved in viral attachment to the host cell, while F is responsible for viral entry by means of fusion with host cell membranes. Research has shown that the glycosylation sites present on these proteins can modulate the ability of the virus to infect host cells and stimulate the host immune system. Characterisation of site-specific glycan heterogeneity remains one of the few unexplored areas related to hRSV, hMPV and NDV surface glycoproteins. Previous research has been conducted to determine glycan heterogeneity, but not glycan site specificity, of NDV F. As yet, glycan site occupancy and glycan heterogeneity for glycoproteins G and F of hRSV and hMPV and HN of NDV, have not been defined at a chemical level. Revealing the glycosylation profile of these proteins may help elucidate mechanisms of viral attachment, replication and immune evasion within paramyxoviruses. Additionally, accurate identification and characterisation of protein glycosylation is required for producing glycoprotein therapeutics and for the development of targeted treatments. Liquid chromatography-MS/MS strategies utilising ETD, HCD and CID fragmentation were implemented to structurally characterise the digested glycoproteins. Initial research has revealed complex N-linked and mucin-like O-linked glycosylation of recombinant RSV G. Analysis of NDV revealed high-mannose N-linked glycans of F glycoprotein as well as high mannos and sialylated and sulphated complex N-linked glycans and a novel sialylated O-linked glycan of NDV HN.

B.14
Developing A New In Vivo Cross-linking Mass Spectrometry Platform to Define Protein-Protein Interactions in Living Cells
Robyn M. Kaake1, Xiaorong Wang1, Anthony Burke1, Clinton Yu1, Wynne Kandur1, Yingying Yang1, Eric J. Novtisky1, Tonya Second2, Jicheng Duan1, Athit Kao1, Shenheng Guan3, Danielle Vellucci1, Scott D. Rychnovsky1, Lan Huang1
1University of California, Irvine, CA, USA; 2Thermo Fisher Scientific, Waltham, MA, USA; 3University of California, San Francisco, CA, USA

Protein-protein interactions (PPIs) are fundamental to the structure and function of protein complexes. Resolving the physical contacts between proteins as they occur in cells is critical to uncovering the molecular details underlying various cellular activities. To advance the study of PPIs in living cells, we have developed a new in vivo cross-linking mass spectrometry platform that couples a novel membrane permeable, enrichable and MS-cleavable cross-linker with multistage tandem mass spectrometry. This strategy permits the effective capture, enrichment, and identification of in vivo cross-linked products from mammalian cells, and thus enables the determination of protein interaction interfaces. The utility of the developed method has been demonstrated by profiling PPIs in mammalian cells at the proteome scale and at the targeted protein complex level. Our work represents a general approach in studying in vivo PPIs in mammalian cells, and thus enables the determination of protein interaction interfaces. The utility of the developed method has been demonstrated by profiling PPIs in mammalian cells at the proteome scale and at the targeted protein complex level. Our work represents a general approach in studying in vivo PPIs, and provides a solid foundation for future studies towards the complete mapping of PPI networks in living systems.
B.15
High-resolution Orbitrap Characterization of Preferential Chain Pairing in Co-expressed Bispecific Antibody Production by MS Under Native and Acidic Conditions


Departments of Protein Chemistry and Antibody Engineering, Genentech, Inc., South San Francisco, CA, USA

Bispecific antibodies possess the characteristics and binding specificity of two distinct monoclonal antibodies, and as such can bind to two targets or epitopes simultaneously. Bispecific antibodies have recently received great attention for their promising results in clinical trials or potential new modes to deliver therapeutics. Generation of a bispecific antibody by co-expression of two light and heavy chains, would result in several mispaired species. While the "knobs-into-holes" technology enables efficient hetero-dimerization of the two heavy chains, the presumed random mispairing of the light chains has not been studied in detail as technologies to readily characterize and quantify the heterodimer species were missing. Using an anti-IL-4/IL-13, a bispecific antibody, which targets the IL-4 and IL-13 cytokines involved in type 2 cytokine-induced inflammation, we describe a mass spectrometry characterization assay under native and acidic conditions for co-expressed bispecific antibodies using an Exactive Plus Extended Mass Range (EMR) Orbitrap instrument. The high mass resolving power of the EMR Orbitrap allows unambiguous identification of all light and heavy chain pairing variants in a mixture of bispecific antibodies randomly assembled in vivo upon co-expression. Using the EMR Orbitrap technology, we identify and characterize the preferential pairing of the anti-IL-13 light chain to its cognate heavy chain. This unexpected, non-random pairing may be leveraged to guide the design of a single-cell solution for the production of bispecific antibodies.

REFERENCES:

B.16
Controlling Low Rates of Cell Differentiation through Noise and Ultra-high Feedback

Robert Ahrends, Asuka Ota, Kyle M. Kovary, Takamasa Kudo, Byung Ouk Park, Mary N. Teruel

Dept. of Chemical and Systems Biology, Stanford University, Stanford, CA, USA

The tissue size of adult mammals is maintained by replacement of aging or damaged cells by slow, ongoing cell differentiation. Disruption of this rate of ongoing differentiation results in serious disease. For example, acute myeloid leukaemia (AML) is caused by a block in differentiation which results in precursor cells proliferating uncontrollably rather than differentiating into a more terminal state. Adipocytes, the key regulators of glucose and lipid metabolism, make up 10–40% of human body mass and are renewed at a rate of approximately 10% per year [1]. The adipocyte system that will be used has the unique advantage that the terminal differentiation transition is relatively short and experimentally accessible using single cell microscopy, Understanding how cells regulate such very slow differentiation rates may enable better treatments of metabolic diseases and obesity. Here we combine quantitative mass spectrometry [2–4], computational modeling, and single-cell microscopy [5] to identify the network architecture that enables pre-adipocytes to differentiate at a rate of only 0.5% every 4 days. We show that that cell-to-cell variability, or noise, in protein abundance acts within a network of more than six positive feedbacks to permit pre-adipocytes to differentiate at very low rates. This system architecture resolves two fundamental opposing requirements: to irreversibly lock cells in the differentiated state and to create large cell-to-cell signal variability to enable differentiation at very low rates. The resolution of this optimization problem by noise and ultra-high feedback connectivity provides a generalizable mechanism for mammalian tissue size control.

REFERENCES: